

## Effects of different heat–moisture treatments on the physicochemical properties of brown rice flour

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**We evaluated the effect of heat–moisture treatment (HMT) on the main chemical components, physical properties, and enzyme activities of two types of brown rice flour: high-amylose Koshi-nokaori and normal-quality Koshiibuki. Five different HMTs using brown rice (moisture content was 12.0%) were assessed: 0.1 MPa/120 °C for 5 or 10 min, 0.2 MPa/134 °C for 5 or 10 min and 0.3 MPa/144 °C for 10 min. HMT, decreased the  $\alpha$ -amylase and lipase activities, and fat acidity, and slightly increased the dietary fiber and resistant starch levels. After 2 months' storage at 35 °C, rice samples that were treated with 0.2 MPa/134 °C or 0.3 MPa/144 °C for 10 min had a lower fat acidity than untreated samples, which would be useful for long-term storage and export of rice flour. And HMT exhibited inhibition of retrogradation in the pasting and physical properties, which is profitable to promote the qualities of the rice products.**

**Key words:** heat – moisture treatment; fat acidity; retrogradation inhibition

Rice (*Oryza sativa* L.) is one of the main food crops in the world and a staple food for over half of the global population. Therefore, it is necessary to improve the quality of rice. 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) foods inhibit rapid increases in blood glucose and insulin secretion after meals.

Several studies have reported on the development of rice that is high in resistant starch rice,<sup>1,2)</sup> or amylose and dietary-fiber through physical or chemical mutation.<sup>3)</sup> Furthermore, both animal and clinical studies have shown that indigestible dextrin (ID)<sup>4)</sup> regulates

the functions of the intestines, moderates the postprandial blood glucose levels, lowers serum lipid levels, and reduces body fat.<sup>5)</sup>

The glycemic effect of food depends on numerous factors, including the structure of the amylose and amylopectin it contains.<sup>6)</sup> Kurahashi et al.<sup>7)</sup> and Taniguchi et al.<sup>8)</sup> reported that starch that has been subjected to heat–moisture treatment (HMT) is rich in RS, while Saito et al.<sup>9)</sup> and Itou et al.<sup>10)</sup> showed that the short-chain fatty acid concentration is higher in high-amylose corn starch and HMT high-amylose corn starch than in ordinary corn starch. HMT also appears to change the granules of wheat flour, causing them to have a flattened shape when viewed under a scanning electron microscope (SEM).<sup>11)</sup> Kawabata et al.<sup>12)</sup> reported that the swelling power and solubility of starch is considerably suppressed following HMT, while Takahashi et al.<sup>13,14)</sup> and Billiaderis et al.<sup>15)</sup> showed that amylose-lipid complexes are formed in rice flour during HMT by examining X-ray diffraction patterns. Cereal starch usually has lipids bound firmly in the granules.<sup>16)</sup> Furthermore, Fukui and Nikuni<sup>17)</sup> and Kitahara et al.<sup>18)</sup> reported that a new crystalline structure of helical starch complexes is formed in cereal starch, following HMT, which includes the lipid materials that are usually firmly bound in the starch granules. Legendijk and Pennings<sup>19)</sup> further reported that monoglycerides that consist of linear and saturated fatty acids easily form complexes with starches, whereas those that consist of unsaturated fatty acids such as oleic acid rarely do so because of the bending structure of the double bonds in the fatty acids. In rice, the main lipids are triglycerides in the bran, free fatty acids in the endosperm, and almost exclusively monoacyl lipids in the rice starch.<sup>20–22)</sup>

Fat acidity is one of the most important factors affecting the quality of rice during storage. Fat acidity

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Abbreviations: 0.1 MPa/5 or 10 min, heat-moisture treatment at 0.1 MPa for 5 or 10 min; 0.2 MPa/5 or 10 min, heat-moisture treatment at 0.2 MPa for 5 or 10 min; 0.3 MPa/10 min, heat-moisture treatment at 0.3 MPa for 10 min; AAC, apparent amylose content; Ad/Tou, adhesion/toughness; Break.d, break down; Cons, consistency; HMT, heat-moisture treatment; KSI, Koshiibuki rice cultivar; KSK, Koshihokaori rice cultivar; Max/Final, maximum/final viscosity; Max/Mini, maximum/minimum viscosity; Past. t, pasting temperature; RS, resistant starch; RVA, Rapid Visco Analyzer; Set/Cons, setback/consistency; Sti/Tou, stickiness/toughness.

generally increase as storage progresses, but the rate of increase is suppressed by heating, with higher temperature and longer periods of heating leading to lower fat acidity.<sup>23,24</sup> In addition, Shibuya et al.<sup>25</sup> reported that the fat acidity of milled rice increases more rapidly than brown rice, moreover, during storage, resulting in greater changes in the flavor and taste of milled rice.

In Japan, rice flour is classified into two types: raw and pre-gelatinized, which is prepared by heating (steaming or boiling), drying and pulverizing the rice grain.<sup>26</sup> HMT changes the physicochemical properties of starches by facilitating starch chain interactions within the amorphous and crystalline domains, and/or by disrupting starch crystallites.<sup>27,28</sup>

The brown rice contains many molecules with antioxidant activity, including phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols,  $\gamma$ -oryzanol, and phytic acid. Rice bran also contains various functional substances, such as  $\gamma$ -oryzanol, ferulic acid, sterol, wax, ceramide, phytin, inositol, and protein.<sup>29</sup> Furthermore, rice bran oil, which is the only domestic edible vegetable oil made from rice bran that is produced in Japan, is known to have high oxidative stability and serum cholesterol-lowering activity.<sup>30,31</sup>

HMT is a physical method that can be used to improve the functional properties of starch according to the required application, with the source of the starch and the treatment conditions, including time and temperature, affecting the final characteristics.

We aimed to develop new brown rice flour, of which quality would be improved, and its quality deterioration during the storage would be inhibited. Therefore, in the present study, we investigated the properties and storage stability of two types of brown rice flour under five different HMTs; 0.1 MPa/5 min, 0.1 MPa/10 min, 0.2 MPa/5 min, 0.2 MPa/10 min and 0.3 MPa/10 min. We then used the finding to determine the optimal HMT conditions for each of these rice cultivars. The present study would contribute to judge if we try to commercialize the HMT rice flour as a basic research.

## Materials and methods

**Materials.** The high-amylose rice cultivar Koshinokaori was given by Bourbon Co. Ltd. And the normal-quality rice cultivar Koshiibuki was purchased from a local market. All of the rice samples were stored at 4 °C prior to the experiments.

**Heat-moisture treatment of brown rice.** Brown rice samples (500 g) with an initial moisture content of 12.0% (on wet basis) were put in stainless sieve basket in stainless vessels with grass lid. The device made use of a vacuum steam heating apparatus (SP-20-S28, As on Inc, Manufactured by Stainless Labotech Inc., tank volume was 28 L) with through flow boiler (SZ60, Miura Co. Ltd). After reducing the pressure using vacuum pump, they were then applied pressure with steam at 0.1 MPa (added water vapor pressure) 120 °C, 0.2 MPa, 134 °C, 0.3 MPa, and 144 °C for 5 min or 10 min. The apparatus were reduced to atmospheric pressure rapidly, and brown rice in the sieve baskets

was then cooled to room temperature with air flow. After treating brown rice grains for 5 min or 10 min, steam was immediately evacuated to the normal pressure (0.1 MPa). Thus, brown rice samples were treated, in an oven set, at 0.1 MPa/120 °C, 0.2 MPa/134 °C, and 0.3 MPa/144 °C for 5 min or 10 min. In the present paper, values of pressure indicate added pressure onto normal pressure (0.1 MPa). The vessels were then cooled to room temperature, and opened, following which the HMT brown rice samples were dried in an oven to a moisture content of approximately 12.0%. HMT of each samples were carried out by National institute of technology, Nagaoka College.

**Preparation of brown rice flour.** Brown rice flour was prepared from each sample using a cyclone mill (SFC-S1; Udy, Corp., Fort Collins, Co, USA) with a screen containing 1-mm-diameter pores.

**Moisture content.** The moisture content was measured using an oven-drying method. Samples of 2 g were dried for 1 h at 135 °C.

**$\alpha$ -Amylase activity.** The  $\alpha$ -amylase activity of samples were determined with an enzyme kit (Megazyme Ltd, Wicklow, Ireland). Brown rice flour (0.5 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 1000 $\times$ g. The extraction solution (0.1 mL) and substrate (0.1 mL) were pre-incubated at 40 °C for 5 min, and then incubated at 40 °C for exactly 20 min, following which a stopping reagent (1.5 mL) was added. The absorbance was measured at 400 nm.

**Lipase activity.** The lipase activity of each sample was determined with an enzyme kit (Cayman Chemical Co, USA). Brown rice flour (0.1 g) was extracted with 0.3 mL of 80% ethanol for 1 h at room temperature and then centrifuged for 15 min at 3000 $\times$ g. The extraction buffer (170  $\mu$ L), fluorescent buffer (10  $\mu$ L), extraction solution (10  $\mu$ L), and substrate (10  $\mu$ L) were then added in order to a 96-well tissue culture plate (AS One Corporation), and pre-incubated at 37 °C for 15 min. The absorbance was measured at 380 nm (excitation) and 510 nm (emission) using a fluorescent microplate reader (Grating Based Multimode Reader SH-9000: Corona Electric Co, Ltd.).

**Starch damage ratio.** The starch damage ratio of each sample was determined using a starch damage kit (Megazyme, Ltd, Wicklow, Ireland). Each sample (corresponding to 0.1 g of dry matter) was placed in a tube and 1.0 mL of a pre-equilibrated fungal  $\alpha$ -amylase solution (50 U/mL) was added. The solution was then incubated at 40 °C for exactly 10 min. Following incubation, 8.0 mL of dilute sulfuric acid (0.2%, v/v) was added to solution. After 10 min of digestion, the acidified solution was centrifuged at 1000 $\times$ g for 5 min. The clear supernatant (0.1 mL) was mixed with 0.1 mL

of amyloglucosidase (20U/mL), stirred on a vortex mixer and incubated at 40 °C for 10 min. A glucose oxidase/peroxidase (GOPOD) reagent (4 mL) was then added to the solution and the mixture was incubated at 40 °C for 20 min. The absorbance of the solution was measured at 510 nm with a UV-1600 spectrophotometer (Shimadzu, Kyoto, Japan), and the glucose content was calculated and converted to the starch damage ratio according to the manufacturer's manual.

**Color difference.** The difference in color between the samples was measured using a color-difference meter (Color Meter NW-11; NIPPON DENSHOKU INDUSTRIES CO., LTD, Tokyo, Japan).

**Dietary fiber.** Measurement of the dietary fiber content of each sample was carried out by the Food Analysis Technology Center using a total dietary fiber assay kit (Megazyme, Ltd, Wicklow, Ireland).

**Glucose content.** The glucose content was measured by adding 1 mL of 60% ethanol to each brown rice flour sample (0.1 g) and subjecting the mixture to glucose extraction by rotating the tube at 20 °C for 1 h. The solution was then centrifuged 1500×g for 15 min, and the supernatant was collected as the sample solution. The glucose content of the sample solution was measured using the NADPH enzyme assay method with a glucose assay kit (Roche, Darmstadt, Germany).

**Resistant starch.** The RS contents of the samples were measured according to the AOAC method using an RS assay kit (Megazyme, Ltd, Wicklow, Ireland). Each sample (100 mg) was digested with pancreatin and amyloglucosidase at 37 °C for 6 h, and the glucose content was then measured with a spectrophotometer at 510 nm.

**Hydrophilic and lipophilic oxygen radical absorbance capacities.** The hydrophilic and lipophilic oxygen radical absorbance capacities (H-ORAC and L-ORAC, respectively) of samples were measured as described by Prior *et al.*<sup>32)</sup> Ito *et al.*<sup>33)</sup> and Watanabe *et al.*<sup>34,35)</sup> For the hydrophilic antioxidant assay, each sample (0.1 g) was extracted with 10 mL of hexane at room temperature for 2 min, and then centrifuged for 15 min at 3000×g. The hexane layer was then removed, and the process was repeated. Residual hexane was evaporated using a water bath at 70 °C for 10 min and the residue was extracted with 10 mL of acetone/water/acetic acid (70:29.5:0.5, v/v/v), following which the solution was sonicated (Ultrasonic Cleaner 3510J-MTH; Branson Ultrasonics Co, Ltd.) at 37 °C for 15 min. The tube was then kept at room temperature for 15 min, following which it was centrifuged for 15 min at 3000×g. The supernatant was removed and transferred to a volumetric flask and diluted to 25 mL total volume.

For the lipophilic antioxidant assay, each sample (0.1 g) was extracted with 1 mL of hexane at room temperature for 2 min and then centrifuged for 15 min at 3000×g. The hexane layer was then collected and the process was repeated. The hexane was evaporated using a water bath at 70 °C for 10 min, and the dried hexane extract was then dissolved in 250 µL of acetone and diluted with 750 µL of 7% randomly methylated β-cyclodextrin (RMCD; prepared by dissolving 0.7 g of methyl-β-cyclodextrin (Sigma Chemical Co.) in 10 mL of 50% acetone. Trolox calibration solutions (50, 25, 12.5, and 6.25 µmol/L in assay buffer solution) were prepared to obtain a standard curve. The trolox calibration solutions, blank (dilute buffer), diluted test samples (35 µL), fluorescein solution (115 µL, 110.7 nmol/L), and 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) solution (50 µL, 31.7 mmol/L: H-ORAC, 63.4 mmol/L: L-ORAC) were added in order to a fluorometer 96-well plate (AS One Corporation), and absorbance values were immediately measured at 37 °C for 90 min at 485 nm (excitation) and 530 nm (emission) using a fluorescent microplate reader (Grating Based Multimode Reader SH-9000: Corona Electric Co, Ltd.).

**Iodine absorption spectrum.** The iodine absorption spectrum of each sample was measured using a Shimadzu UV-1800 spectrophotometer. The apparent amylose content (AAC) of milled rice was estimated using the iodine colorimetric method, as described by Juliano *et al.*<sup>36)</sup> The iodine absorption spectrum<sup>37)</sup> was analyzed from 200 to 900 nm using a square cell with inner dimension of 1 cm × 1 cm. Distilled water was added to the same cell as a control. The absorbance was measured at λ<sub>max</sub>, i.e. the peak wavelength, in the visible light range of iodine staining of starch, which is highly correlated with the length of the glucan chain, the molecular size of amylose, and the super-long chain (SLC) of amylopectin.

**Pasting properties.** The pasting properties of each sample were measured using a RVA (model Super 4; Newport Scientific Pty Ltd, Warriewood, Australia). Each sample (4.0 g based on 14% moisture content) was combined with 25 mL of water. The sample cup was then fitted to the rotor of the RVA and heated at 50 °C for 1 min and then to 93 °C over 4 min. The sample temperature was held at 93 °C for 7 min, and then cooled to 50 °C over 4 min, where it was allowed to stand for 3 min. The programmed heating and cooling cycle followed that of Toyoshima *et al.*<sup>38)</sup> Novel indices such as the Set/Cons ratio and Max/Fin ratio are very strongly correlated with the proportion of intermediate and long chains of amylopectin: Fb<sub>1+2+3</sub> (DP ≥ 13).<sup>39)</sup>

**Physical properties of gelatinous brown rice flour.** Gelatinized samples were prepared using an RVA, and then immediately cooled in ice water for 1 h. The physical properties of retrogradation of the gelatinized samples (10 g) were measured based on bulk measurement



using a Tensipresser (My Boy System, Taketomo Electric Co., Tokyo, Japan) according to the method described by Okadome et al.<sup>40,41</sup> The bulk measurements were repeated six times, and the mean value was calculated.

**Fatty acid composition.** Measurement of the fatty acid composition of each sample was carried out by the Food Analysis Technology Center (using a gas chromatography method).

**Fat acidity.** The fat acidity of each sample was measured using a colorimetric method, as described by Ohtsubo et al.<sup>24</sup> Each sample (1.0 g) was extracted with 3.0 mL of toluene at 30 °C for 1 h and then filtered through filter paper (110 mm, ADVANTEC CO Ltd., Tokyo, Japan). Chloroform (4.0 mL), and copper reagent (2.5 mL) were then added to the filtrate solution (1.0 mL), following which the mixture was shaken well and centrifuged for 5 min at 3000×g. The supernatant (Cu-fatty acid compound) was then removed, and the remaining solution was used as the sample solution. The clear sample solution (3.0 mL) was mixed with 0.5 mL of coloring substance (0.1% sodium diethyldithiocarbamate with isobutyl alcohol), stirred on a vortex mixer and incubated at room temperature for 15 min. The absorbance of the solution was then measured at 440 nm with a UV-1600 spectrophotometer (Shimadzu, Kyoto, Japan). Linoleic acid solution (0, 0.3, 0.7, and 1.1) with toluene was used to produce a calibration curve.

**Thermal measurement by differential scanning calorimetry (DSC).** The gelatinization properties of each sample were analyzed using a differential scanning calorimeter (DSC8000; Perkin Elmer Japan, Yokohama, Japan). Each sample (15 mg) was placed in a stainless steel pan (Perkin Elmer Japan, Yokohama, Japan) and combined with 40 µL of deionized water, following which the pan was sealed hermetically and equilibrated overnight. The pan was then placed in the DSC instrument and scanned from 30 to 130 °C at a heating rate of 5 °C/min to measure gelatinization start, peak, and end temperatures ( $T_s$ ,  $T_p$ , and  $T_e$ , range of application : 60–90 °C and 90–115 °C, respectively), and endothermic enthalpy ( $\Delta H$ ).

**Storage test at 35 °C for 2 months.** To evaluate the characteristics of the various rice flour with storage, the HMT and untreated brown rice flour were stored at 35 °C for 2 months in a Bio Multi Incubator (MODEL LH-30-8CT; Nippon Medicals and Chemical Instruments Co., LTD, Osaka, Japan).

**Statistical analyses.** All results, including the significance of regression coefficients, were statistically analyzed using Student's *t*-test, one-way analysis of variance, and Tukey's test with Excel Statistics (ver. 2006; Microsoft Corp., Tokyo, Japan).

## Results and discussion

### Moisture content

The quality of preserved rice flour is greatly affected by its moisture content. As shown in Table 1, the moisture contents of the HMT samples (KSK: 12.3–12.8%, mean = 12.5%, KSI: 11.8–12.4%, mean = 12.2%) were almost identical to those of the untreated samples (KSK: 12.6%; KSI: 12.0%). It means that HMT does not change the moisture contents of the rice flour samples by the processing.

### $\alpha$ -Amylase activity

Unlike  $\alpha$ -glucosidase, which is mainly localized in the inner layers of rice grains,  $\alpha$ -amylase is predominantly localized mainly in the outer endosperm.<sup>42</sup> and so it would be affected by HMT more severely. As shown in Table 1, the  $\alpha$ -amylase activity was 1.4-fold higher in KSK than in KSI, and was significantly lower in the HMT samples (KSK: 0.000–0.038 CU/g, mean = 0.012 CU/g; KSI: 0.000–0.015 CU/g, mean = 0.007 CU/g) than in the untreated samples (KSK: 0.048 CU/g; KSI: 0.035 CU/g) ( $p < 0.05$ ), being completely inactivated with the 0.2 MPa/10 min and 0.3 MPa/10 min treatments, and decreased 0.8-fold for KSK and 0.3-fold for KSI with the 0.1 MPa/5 min treatment.  $\alpha$ -Amylase activity was significantly negative correlation with the starch damage ratio ( $r = -0.68$ ) and color difference ( $r = -0.68$ ) ( $p < 0.05$ ; data not shown).

### Lipase activity

The presence of lipase, galactolipase, and phospholipase has previously been confirmed, in rice grains, with an activity ratio of 100:64:9, respectively.<sup>43</sup> The free fatty acid content of grains is often employed as a measure of deterioration during storage with Katsuhara et al.<sup>44</sup> reporting that the amounts of free fatty acids increases during storage, as a result of lipase hydrolysis. In the present study, lipase activity was 1.1-fold higher in KSI than in KSK, and was lower in the HMT samples (KSK: 4.4–5.0 nmol/min/mL, mean = 4.8 nmol/min/mL; KSI: 4.6–6.1 nmol/min/mL, mean = 5.4 nmol/min/mL) than in the untreated samples (KSK: 5.3 nmol/min/mL; KSI: 6.0 nmol/min/mL), with a significantly difference being observed for both KSK and KSI with 0.3 MPa/10 min HMT ( $p < 0.05$ ; Table 1). It seems that the strong HMT tends to damage the lipase activity, which would be profitable to prevent the deterioration of rice flour quality during the storage.

### Starch damage ratio

Flour has a granular size of 25–85 µm when milled with an SM-250 mill and 90–110 µm when milled with an UDY mill,<sup>45</sup> and the milling of cereals causes partial physical damage to the starch granules they contain. In the present study, the starch damage ratio was 1.4-fold higher in KSI than in KSK, and was significantly higher in the HMT samples (KSK: 7.3–14.4%, mean = 9.5%; KSI: 9.8–17.8%, mean = 13.5%) than in the untreated samples (KSK: 7.0%; KSI: 9.8%) ( $p < 0.05$ ). Furthermore, the starch damage ratio of the HMT samples gradually increased with increase in

Table 1. Component analysis, enzyme activities, and anti-oxidative activity of HMT and untreated brown rice flour.

	Moisture		$\alpha$ -Amylase		Lipase activity		Starch damage		Color difference	
	(%)	SD	Activity(CU/g)	SD	(nmol/min/ mL)	SD	(%)	SD	$\Delta E^*(ab)$	SD
Untreated KSK	12.6a	0.1	0.048a	0.001	5.3a	0.1	7.0a	0.5	23.8a	0.6
0.1 MPa 5 min	12.5a	0.0	0.038b	0.002	4.9a	0.2	7.3ab	0.1	23.8a	1.0
0.1 MPa 10 min	12.7a	0.1	0.017c	0.003	4.9a	0.0	7.7ab	0.1	24.6ab	0.7
0.2 MPa 5 min	12.3a	0.0	0.007d	0.002	5.0a	0.1	8.1ab	0.2	24.9ab	1.7
0.2 MPa 10 min	12.8a	0.3	0.000d	0.000	4.7ab	0.1	9.7c	0.0	26.7b	0.8
0.3 MPa 10 min	12.4a	0.1	0.000d	0.000	4.4b	0.1	14.4 e	0.1	26.9b	0.4
Untreated KSI	12.0a	0.2	0.035a	0.000	6.0a	0.3	9.8a	0.1	27.1a	0.6
0.1 MPa 5 min	12.4a	0.2	0.010b	0.004	6.1a	0.2	9.8a	0.1	28.2a	1.3
0.1 MPa 10 min	12.4a	0.1	0.015b	0.001	5.5b	0.2	11.6b	0.3	28.8ab	0.6
0.2 MPa 5min	12.1a	0.2	0.008c	0.004	5.8a	0.3	12.8c	0.0	29.4b	1.4
0.2 MPa 10 min	12.2a	0.0	0.000c	0.000	5.1b	0.1	15.5d	0.3	31.2b	1.5
0.3 MPa 10 min	11.8a	0.0	0.000c	0.000	4.6b	0.2	17.8 e	0.3	31.4b	0.8
	Dietary fiber (g/100g)	SD	Glucose content (g/100g)	SD	Resistant starch (%)	SD	L-ORAC $\mu\text{mol TE}/$ 100gFW	SD	H-ORAC $\mu\text{mol TE}/$ 100gFW	SD
Untreated KSK	3.2a	0.1	0.017a	0.002	11.1a	0.1	87.1a	1.0	58.1a	1.5
0.1 MPa 5 min	2.9a	0.1	0.016a	0.001	11.3a	0.1	84.0a	3.3	51.8b	3.6
0.1 MPa 10 min	3.2a	0.2	0.016a	0.001	11.9b	0.1	74.1b	4.5	50.3b	2.2
0.2 MPa 5 min	3.2a	0.0	0.016a	0.001	11.4a	0.1	72.0b	1.5	49.4b	0.4
0.2 MPa 10 min	2.9a	0.2	0.016a	0.001	10.8c	0.1	49.5c	2.1	48.6b	0.1
0.3 MPa 10 min	3.2a	0.1	0.017a	0.000	9.5c	0.2	46.3c	1.3	37.2c	1.3
Untreated KSI	2.8a	0.1	0.024a	0.002	1.5a	0.0	66.3a	0.9	54.0a	2.1
0.1 MPa 5 min	3.2ab	0.1	0.026a	0.004	0.7b	0.0	56.4b	0.9	51.1a	0.1
0.1 MPa 10 min	3.1ab	0.1	0.025a	0.002	0.7b	0.0	38.1c	0.4	51.3 a	1.2
0.2 MPa 5 min	2.9a	0.2	0.026a	0.001	0.6b	0.0	39.0c	4.1	46.7b	1.1
0.2 MPa 10 min	3.0ab	0.2	0.022a	0.000	0.4c	0.0	32.8d	0.7	53.3a	2.1
0.3 MPa 10 min	3.5b	0.1	0.019b	0.001	0.3c	0.0	27.7d	0.6	44.1b	1.4

Notes: HMT, heat-moisture treatment at various pressures (0.1, 0.2, or 0.3 MPa) and time (5 or 10 min).

KSK, Koshinokaori rice cultivar, KSI, Koshiibuki rice cultivar.

Means with the same letter are not significantly different ( $p < 0.05$ ).

strength of the treatment, with samples treated with 0.3 MPa/10 min showing the highest values (Table 1). The starch damage ratio of was positively correlated with the color difference ( $r = 0.90$ ; data not shown). It would be necessary for us to select the suitable utilization even though the starch was damaged slightly.

#### Color difference

The color of the samples became more yellowish with HMT. Previous studies have indicated that the browning reaction is caused by the amino-carbonyl-reaction.<sup>15,46</sup> The color difference [ $\Delta E^*(ab)$ ] was increased 1.1-fold higher in KSI than in KSK, and was higher in the HMT samples (KSK: 23.8–26.9, mean = 25.4; KSI: 28.2–31.4, mean = 29.8) than in the untreated samples (KSK: 23.8; KSI: 27.1). Furthermore, the color difference [ $\Delta E^*(ab)$ ] of the HMT samples also increased with increase in strength of the treatment (Table 1). The discoloration seems to be caused by Maillard reaction. From this result, we should select the suitable processing condition, such as 0.1 MPa/5 min, to less the discoloration in the case of color of the final products is important.

#### Dietary fiber

Brown rice is more nutritious than white rice, particularly with respect to dietary fiber, vitamin, and mineral.<sup>47</sup> Short-chain fatty acids, which are the end products of the fermentation of dietary fibers by the

anaerobic intestinal microbiota, have been shown to exert multiple beneficial effects on mammalian energy metabolism.<sup>48</sup> The dietary fiber content was 1.1-fold higher in KSI than in KSK, and was similar or slightly higher in the HMT samples (KSK: 2.9–3.2; mean = 3.1, KSI: 2.9–3.5; mean = 3.2) than in the untreated samples (KSK: 3.2; KSI: 2.8), being significantly higher in KSI samples treated with 0.1 MPa/5 min, 0.1 MPa/10 min, 0.2 MPa/10 min, and 0.3 MPa/10 min ( $p < 0.05$ ; Table 1). High dietary fiber products would be promising to prevent obesity or diabetes or improve the microsphere of the intestine.

#### Glucose content

$\alpha$ -Amylase activity and  $\alpha$ -glucosidase decompose starch and generate glucose during the early stage of cooking. The glucose content was almost identical between all of the HMT samples and the untreated samples except KSI treated with 0.3 MPa/10 min HMT, which had significantly lower glucose content than the untreated sample ( $p < 0.05$ ) due to the almost complete inactivation of  $\alpha$ -amylase activity by HMT (Table 1). It seems to be promising for HMT brown rice flour that glucose content did not increase by processing.

#### Resistant starch

Yang *et al.*<sup>1</sup> have reported that mutant rice is rich in RS, and Nakamura *et al.*<sup>49</sup> showed that *ae* mutant rice

grains contain markedly higher amounts of RS than other rice grains. RS is expected to promote body fat consumption, and has been shown to reduce the GI and improve glucose tolerance in rats, indicating that its use over the long term could help to prevent and improve type 2 diabetes.<sup>50</sup> Furthermore, RS improves mineral availability through increased absorption in the large intestine.<sup>51</sup> It has previously been shown that HMT may be able to increase the RS content of wheat (*Triticum aestivum*), although the treatment of wheat starch was not as effective as that of corn starch.<sup>8</sup> In the present study, we examined the gelatinization properties of samples using DSC.  $\Delta H$  (first heating peak: 60–90 °C) was slightly lower in the HMT KSK samples (6.8–10.3, mean = 9.3) than the untreated sample (10.3), with the 0.1 MPa/5 min, 0.1 MPa/10 min, 0.2 MPa/5 min, 0.2 MPa/10 min, and 0.3 MPa/10 min treatments being 0.98-, 0.96-, 0.93-, 0.91-, and 0.65-fold lower, respectively (Supplementary Table 1). Similarly,  $\Delta H$  (first heating peak) was also slightly lower in the HMT KSI samples (8.1–11.0, mean = 9.9) than in the untreated (14.2) (data not shown). Thus, we consider that the gelatinization properties of the HMT samples gradually increased with increase in strength of HMT (Table 1).  $\Delta H$  (second heating peak: 90–115 °C) was also generally slightly lower in the HMT samples (0.0–1.2, mean = 0.6) than in the untreated sample (0.7), however, samples treated with 0.1 MPa/5 min and 0.1 MPa /10 min HMT showed higher values than the untreated sample, while no data were obtained for HMT KSI (data not shown). Therefore, we believe that  $\Delta H$  (second heating peak) represented the melting transition or complex formation between amylose and native lipids, as both  $\Delta H$  (first heating peak) and  $\Delta H$  (second heating peak) showed a significant positive correlation with the RS content ( $r = 0.89$  and  $0.82$ , respectively; data not shown). Similarly, Hoover and Vasanthan<sup>28</sup> showed that heat treatment induced complex formation between amylose and native lipids, with DSC indicating that the heat-treated samples exhibited a broadening of the gelatinization temperature range and a shifting of the endothermal transition toward higher temperatures.

The RS content was 7.4-fold higher in KSK than in KSI, and was similar or markedly lower in the HMT samples (KSK: 9.5–11.9, mean = 11.0; KSI: 0.3–0.7, mean = 0.5) than in the untreated samples (KSK: 11.1; KSI: 1.5). In particular, the KSK sample treated with 0.1 MPa/10 min had a significantly higher RS content, while those treated with 0.2 MPa/10 min and 0.3 MPa/10 min had significantly lower RS contents than the untreated sample ( $p < 0.05$ ; Table.1). Hoover<sup>52</sup> showed that HMT alters the extent of starch-chain associations within the amorphous regions and the degree of crystalline order. Consequently, we believe that the RS contents of HMT samples were derived from the amylose-lipid complexes.

#### Oxygen radical absorbance capacity (ORAC)

L-ORAC was 1.3-fold higher in KSK than in KSI, and was lower in the HMT samples (KSK: 46.3–84.0, mean = 65.2; KSI: 27.7–56.4, mean = 38.8) than in the untreated samples (KSK: 87.1; KSI: 66.3).

Furthermore, L-ORAC gradually decreased with increase in strength of HMT, with the 0.2 MPa/10 min and 0.3 MPa/10 min treatments showed very low values (Table 1). Similarly, H-ORAC was 1.1-fold higher in KSK than in KSI, and was significantly lower in the HMT KSK samples (KSK: 37.2–51.8, mean = 47.5) than in the untreated sample (58.1) ( $p < 0.05$ ; Table 1). By contrast, H-ORAC of the HMT KSI samples (KSI: 44.1–53.3, mean = 49.3) was more similar to that of the untreated sample, with only the 0.2 MPa/5 min and 0.3 MPa/10 min treatments resulting in significantly lower values ( $p < 0.05$ ). It was shown that strong conditions of HMT decrease oxygen radical absorbance capacity of the brown rice flour.

#### Iodine absorption spectrum

AAC is considered a useful parameter for estimating the cooking or eating quality of rice grains, and can be measured using the iodine colorimetric method at 620 nm, which was developed by Juliano et al.<sup>53</sup> AAC comprises a large amount of amylose and a small amount of SLC of amylopectin. In general, low amylose rice becomes soft and sticky after cooking, whereas high amylose rice becomes hard and separated.<sup>54</sup> AAC was 2.2-fold higher in KSK than in KSI, and was higher in the HMT KSK samples (28.3–29.6%, mean = 29.0%) than in the untreated sample (28.1%) with significant differences being observed for the 0.2 MPa/5 min, 0.2 MPa/10 min, and 0.3 MPa/10 min treatments [ $p < 0.05$ ; Table 2(A)]. By contrast, AAC was significantly lower in the HMT KSI samples (10.8–12.0%, mean = 11.3%) than in the untreated sample (12.7%) ( $p < 0.05$ ). We believe that these findings resulted from HMT causing the large amount of amylose and small amount of SLC of amylopectin in KSK to swell, but only having a small effect on KSI due to the small amount of amylose and lack of SLC of amylopectin it contains. Hoover and Vasanthan<sup>52</sup> similarly showed that the extent of changes as a result of HMT is influenced by the starch composition, moisture content and temperature during treatment, and by organization of amylose and amylopectin chains within native starch granules. AAC was positively correlated with Cons ( $r = 0.95$ ), Setback ( $r = 0.94$ ), Toughness ( $r = 0.96$ ), and fat acidity ( $r = 0.97$ ), but negatively correlated with the Max/Final ratio ( $r = -0.94$ ) and Ad/Tou ratio ( $r = -0.95$ ) ( $p < 0.01$ ; data not shown).

The molecular structures of many starches have been reported previously, including the molecular sizes of amylose and the amylopectin branch chain lengths.<sup>55–58</sup> High molecular-weight amyloses tend to have a longer wavelengths for  $\lambda_{\max}$ , and it has previously been shown that the glutinous rice cultivars have very low  $\lambda_{\max}$  values, while *indica* rice, *japonica-indica* hybrid rice cultivars have higher  $\lambda_{\max}$  values, with normal *japonica* rice cultivar having intermediate values.<sup>37</sup> In the present study, we found that the  $\lambda_{\max}$  values were slightly higher for KSK than or KSI [Table 2(A)]. Furthermore,  $\lambda_{\max}$  was almost identical in the HMT KSK samples (KSK: 574.0–579.0 nm, mean = 576.4 nm) and the untreated sample (576.5 nm), but were slightly lower in the HMT KSI samples (557.0–561.5 nm, mean = 559.0 nm) than in the untreated sample

Table 2. Analysis of iodine absorption curve and amylose content of HMT and untreated brown rice flour.

	$\lambda_{\text{max}}$ (nm)	SD	$A_{\lambda_{\text{max}}}$	SD	$\lambda_{\text{max}}/A_{\lambda_{\text{max}}}$	SD	Amylose (%)	SD
(A)								
Untreated KSK	576.5a	2.1	0.42a	0.010	1384.3a	16.1	28.1a	0.4
0.1 MPa 5 min	574.0a	1.4	0.42a	0.000	1361.9a	12.6	28.4a	0.4
0.1 MPa 10 min	575.0a	1.4	0.42a	0.000	1356.1a	1.2	28.3a	0.2
0.2 MPa 5 min	578.0a	2.8	0.44b	0.000	1327.2a	4.3	29.6b	0.0
0.2 MPa 10 min	579.0a	1.4	0.43b	0.000	1340.3a	1.1	29.3b	0.0
0.3 MPa 10 min	576.0a	1.4	0.44b	0.000	1321.2a	16.1	29.6b	0.3
Untreated KSI	562.5a	3.5	0.31a	0.001	1800.0a	15.4	12.7a	0.0
0.1 MPa 5 min	561.5a	0.7	0.30a	0.004	1844.1a	23.7	12.0b	0.1
0.1 MPa 10 min	557.0b	0.0	0.30a	0.002	1885.0b	13.5	10.8c	0.1
0.2 MPa 5 min	559.5b	2.1	0.30a	0.001	1877.6a	16.0	11.4b	0.1
0.2 MPa 10 min	558.0b	0.0	0.30a	0.000	1847.7a	0.0	11.5b	0.1
0.3 MPa 10 min	559.0b	2.8	0.29a	0.001	1907.8c	0.4	10.8c	0.1
(B)								
Untreated KSK	577.5a	0.7	0.39a	0.003	1469.5a	8.8	19.4a	0.2
0.1 MPa 5 min	576.5a	0.7	0.40a	0.001	1459.5a	3.4	19.5a	0.1
0.1 MPa 10 min	578.0a	2.8	0.40b	0.001	1432.5a	9.5	20.0a	0.1
0.2 MPa 5 min	576.5a	0.7	0.40a	0.001	1452.1a	3.4	19.7a	0.2
0.2 MPa 10 min	575.5a	3.5	0.41b	0.002	1415.8b	16.1	20.0a	0.2
0.3 MPa 10 min	575.5a	0.7	0.40a	0.002	1455.1a	6.0	19.0a	0.3
Untreated KSI	561.5a	2.1	0.31a	0.003	1835.1a	23.9	11.9a	0.2
0.1 MPa 5min	558.0a	1.4	0.30a	0.003	1872.6b	22.5	11.2b	0.2
0.1 MPa 10 min	559.0a	0.0	0.30a	0.001	1879.0b	4.5	11.2b	0.1
0.2 MPa 5 min	557.5b	0.7	0.30a	0.000	1846.0c	2.3	11.5a	0.1
0.2 MPa 10 min	556.5b	0.7	0.30a	0.001	1848.9c	11.0	11.5a	0.1
0.3 MPa 10 min	558.5a	2.1	0.30a	0.005	1846.5c	23.2	11.2b	0.1

Notes: (A): Initial value. (B): Value following 2 months' storage at 35 °C.

HMT, heat-moisture treatment at various pressures (0.1, 0.2, or 0.3 MPa) and time (5 or 10 min).

KSK, Koshinokaori rice cultivar, KSI, Koshiibuki rice cultivar.

Means with the same letter are not significantly different ( $p < 0.05$ ).

(562.5 nm), with significant differences being observed with the 0.1 MPa/10 min, 0.2 MPa/5 min, 0.2 MPa/10 min, and 0.3 MPa/10 min treatments ( $p < 0.05$ ).

Here, we found that  $A_{\lambda_{\text{max}}}$  was 1.4-fold higher in KSK than in KSI, and was almost identical in the HMT samples (KSK: 0.42–0.44, mean = 0.40; KSI: 0.29–0.31, mean = 0.30) as in the untreated samples (untreated KSK: 0.42; untreated KSI: 0.31) [Table 2(A)]. It means that HMT does not decompose starch molecule.

Nakamura *et al.*<sup>59</sup>) showed that AAC was negatively correlated with the  $\lambda_{\text{max}}/A_{\lambda_{\text{max}}}$  ratio. The  $\lambda_{\text{max}}/A_{\lambda_{\text{max}}}$  ratio was 1.3-fold higher in KSI than in KSK [Table 2(A)]. However, although this ratio was almost identical for the HMT KSK samples (1321.2–1361.9, mean = 1341.3) and the untreated sample (1384.3), it was slightly higher in the HMT KSI samples (1844.1–1907.8, mean = 1872.4) than in the untreated sample (1800.0) with significant differences being observed with the 0.1 MPa/10 min, and 0.3 MPa/10 min treatments ( $p < 0.05$ ).

#### Physical properties

The physical properties of gelatinous samples were measured based on bulk measurement using a Tensi-presser. Differences were detected in the values of hardness, toughness, adhesion, stickiness, the adh/Tou ratio, and the Sti/Tou ratio (overall balance), which are important indices when evaluating the palatability of

rice<sup>60</sup>) with toughness showing a similar tendency to hardness (Table 3).

HMT KSK samples had slightly lower values for hardness (0.12–0.22, mean = 0.17), stickiness (79.48–94.57, mean = 87.4), the Ad/Tou ratio (4.83–6.88, mean = 5.90), and the Sti/Tou ratio (7.88–11.48, mean = 9.89), and higher values for toughness (7.64–10.10, mean = 9.02) and adhesion (48.59–54.28, mean = 52.2) than the untreated sample. By contrast, HMT KSI samples had almost identical values for hardness, toughness, stickiness, and the Sti/Tou ratio as the untreated samples, but higher values for adhesion (63.90–86.33, mean = 71.4) and the Ad/Tou ratio (15.82–21.97, mean = 17.40). These findings indicate that the gel texture of the KSK samples became harder and less sticky following HMT, while that of the KSI samples became more adherent and elastic.

#### Pasting properties

As affecting the eating quality of rice, it is useful to test the gelatinization properties as quality indicators. Breakdown (Break.d) indicates the degree of ease with which the starch granules are disintegrated. It has been shown that high-amylose rice cultivars have higher final viscosities (Final.vis) than low-amylose cultivars, and that Final.vis is related to the degree of starch retrogradation during cooling.<sup>61</sup>

In a previous study,<sup>62</sup>) we developed a novel index for the ratios of Max/Mini and Max/Fin, which have

Table 3. Physical properties of gelatinous HMT and untreated brown rice flour.

	Hardness (gw/cm <sup>2</sup> )		Toughness (gw/cm <sup>2</sup> )		Adhesion (gw/cm <sup>2</sup> )		Stickiness (gw/cm <sup>2</sup> )		Adhesion/toughness		Stickiness/toughness		SD
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
(A)													
Untreated KSK	0.21a	0.07	8.51a	0.51	50.75a	2.54	92.40a	3.45	5.97a	0.12	10.88a	0.39	
0.1 MPa 5 min	0.19a	0.06	10.10b	0.39	48.59b	3.64	79.48b	6.67	4.83b	0.59	7.88b	0.87	
0.1 MPa 10 min	0.22a	0.05	9.16c	0.76	53.87a	1.54	94.57a	3.69	5.92a	0.58	10.37a	0.66	
0.2 MPa 5 min	0.12b	0.01	8.50a	0.44	54.28a	4.03	90.87a	2.77	6.39a	0.51	10.72a	0.85	
0.2 MPa 10 min	0.19a	0.01	9.71c	1.12	52.40a	2.46	85.92c	7.98	5.46c	0.67	9.01c	1.82	
0.3 MPa 10 min	0.14b	0.01	7.64a	1.01	51.84a	1.39	86.04c	4.74	6.88a	0.82	11.48d	2.07	
Untreated KSI	0.04a	0.01	4.15a	0.21	61.83a	2.03	49.48a	1.17	14.94a	0.59	11.96a	0.45	
0.1 MPa 5 min	0.05a	0.01	4.29a	0.23	67.47b	5.69	46.61b	0.58	15.82b	2.26	10.90b	0.71	
0.1 MPa 10 min	0.05a	0.01	4.24a	0.22	68.04b	1.21	49.83a	0.84	16.08b	0.67	11.78a	0.54	
0.2 MPa 5 min	0.04a	0.01	4.02a	0.11	63.90a	8.27	48.50a	2.21	15.88b	2.01	12.08a	0.62	
0.2 MPa 10 min	0.04a	0.01	4.15a	0.16	71.41c	1.13	50.44a	1.28	17.23c	0.57	12.18a	0.67	
0.3 MPa 10 min	0.04a	0.01	3.93b	0.12	86.33d	1.48	49.78a	0.59	21.97e	0.95	12.66a	0.28	
(B)													
Untreated KSK	0.08a	0.02	7.33a	0.82	68.67a	5.30	89.04a	2.76	9.52a	1.79	12.29a	1.47	
0.1 MPa 5 min	0.09a	0.01	6.55b	1.05	67.67a	3.19	86.43b	3.63	10.52b	1.36	13.34a	1.59	
0.1 MPa 10 min	0.09a	0.01	6.06b	0.93	66.63a	4.50	87.39b	3.64	11.17b	1.48	13.83a	0.89	
0.2 MPa 5 min	0.08a	0.01	5.42c	0.28	70.22b	1.58	85.94b	3.38	12.99b	0.92	15.86b	0.47	
0.2 MPa 10 min	0.09a	0.01	5.77c	0.19	64.13c	2.36	86.99b	1.59	11.12b	0.42	15.11b	0.84	
0.3 MPa 10min	0.07a	0.01	6.35b	0.68	56.79e	3.12	79.65c	3.21	9.09a	1.57	12.71d	1.74	
Untreated KSI	0.06a	0.02	5.01a	0.23	72.91a	4.89	67.58a	1.15	14.55a	0.51	0.89a	0.39	
0.1 MPa 5 min	0.05a	0.00	4.73b	0.36	65.38b	2.35	63.47b	0.88	13.82a	1.61	0.80a	0.87	
0.1 MPa 10 min	0.06a	0.01	4.54b	0.17	76.28c	2.74	62.31b	1.78	16.80b	0.99	0.49b	0.66	
0.2 MPa 5 min	0.05a	0.01	4.87b	0.59	70.85a	4.90	62.99b	2.03	14.55a	3.07	1.43c	0.85	
0.2 MPa 10 min	0.04b	0.01	4.63b	0.56	75.15c	4.76	56.27c	1.17	16.23b	2.25	1.84d	1.82	
0.3 MPa 10 min	0.02c	0.00	3.72c	0.07	82.59d	3.84	47.24d	2.06	22.20d	1.66	0.88a	2.07	

Notes: (A): Initial value. (B): Value after 2 months' storage at 35 °C.

HMT, heat-moisture treatment at various pressures (0.1, 0.2, or 0.3 MPa) and time (5 or 10 min).

KSK, Koshinokaori rice cultivar, KSI, Koshiibuki rice cultivar.

Means with the same letter are not significantly different ( $p < 0.05$ ).



stronger negative correlations with RS content than the conventional indexes obtained using RVA. Kiribuchi<sup>63</sup> and Kawashima<sup>64</sup> reported that the amylogram patterns for non-defatted and defatted rice show that the viscosity of rice is affected by the presence of lipids.

HMT KSK samples had slightly lower values for Max.vis (282.9–388.5, mean = 354.5), Mini.vis (129.6–167.5, mean = 155.5), Break.d (153.3–221.0, mean = 199.0), Final.vis (319.4–402.5, mean = 377.2), Cons (189.8–235.4, mean = 221.7), Max/Mini ratio (2.18–2.34, mean = 2.27), and Max/Final ratio (0.89–0.96, mean = 0.94), but slightly higher values for Setback (14.7–36.5, mean = 22.7), Past.t (75.1–76.1, mean = 75.7), and Set/Cons ratio (0.06–0.19, mean = 0.11) than the untreated sample [Table 4(A)]. By contrast, the HMT KSI samples had slightly higher values for Max.vis (330.2–449.9, mean = 417.3), Mini.vis (139.1–150.3, mean = 144.2), Break.d, (187.0–300.5, mean = 273.2), Final.vis, (256.0–280.3; mean = 268.5), Cons (116.9–135.0; mean = 124.3) and Max/Final ratio (1.19–1.67, mean = 1.56), and slightly lower values for Setback (–179.8 to –52.0, mean = –148.8), Past.t (62.2–68.4, mean = 66.9) and the Set/Cons ratio (–1.49 to –0.39; mean = –1.22) than the untreated sample.

These results indicate that HMT KSK and HMT KSI samples had opposite pasting properties from each other, and that samples that were treated with 0.2 MPa/5 min, 0.2 MPa/10 min and 0.3 MPa/10 min HMT significantly differed from the untreated samples. HMT affected to the opposite direction depending on the rice cultivars.

#### Fatty acid composition

Linoleic, oleic and palmitic acids are the major fatty acids contained in the lipids of brown rice.<sup>65</sup> The essential fatty acid contents of rice may increase with temperature during grain development, but this is at the expense of the total oil content, which is concurrently reduced.<sup>66</sup> Sato *et al.*<sup>67</sup> showed that glutinous rice appears to have quite similar fatty acid composition to nonglutinous rice with the exception of a slightly higher proportion of palmitic acid. Furthermore, Ike-moto and Naganuma<sup>68</sup> reported that the ratio of saturated/monounsaturated/polyunsaturated fatty acids was relatively constant between rice varieties, although the  $n - 6/n - 3$  ratio of polyunsaturated fatty acids showed higher diversity in *japonica* rice varieties. Barber<sup>69</sup> showed that saturated free fatty acids are shorter than unsaturated free fatty acids and can efficiently re-form amylose/free fatty acid complexes, whereas the unsaturated fatty acid linoleic acid does not form a complex in corn (*Zea mays*) based on the DSC reheating curve. Mano *et al.*<sup>70</sup> showed that the C14-20/C21-26 ratio of the hydroxyl fatty acids in cerebroside was 1.18 in *japonica* and 0.62 in *indica*.

The myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, arachidic acid, behenic acid and lignoceric acid contents, and the oleic acid/(linoleic acid +  $\alpha$ -linolenic acid) and linoleic acid/ $\alpha$ -linolenic acid ratios significantly differed between the KSK and KSI cultivars ( $p < 0.05$ ; data not shown).

The palmitic acid was slightly higher in the HMT KSK samples (23.50–23.70, mean = 23.60) than in the

untreated sample (23.50), with significant differences being observed with the 0.1 MPa/5 min, 0.1 MPa/10 min and 0.3 MPa/10 min treatments (Supplementary Table 2). However, overall, there was little difference in the composition of lipid classes between HMT and untreated samples.

It seems that fatty acid composition varies depending on rice cultivars, but fatty acid composition changes little except saturated fatty acid, such as palmitic acid, by HMT.

#### Correlation between fat acidity, fatty acid composition, pasting properties, physical properties and the iodine absorption curve

The pasting properties Final.vis, Setback, Past.t, Cons, and Set/Cons ratio were positively correlated with the fat acidity, palmitic acid content, oleic acid content, and oleic acid/(linoleic acid +  $\alpha$ -linolenic acid) ratio, but negatively correlated with the linoleic acid content and (linoleic acid/ $\alpha$ -linolenic acid) ratio ( $p < 0.01$ ; Table 5). By contrast, Break.d, and the Max/Mini and Max/Final ratios were negatively correlated with the fat acidity, palmitic acid content, oleic acid content, and oleic acid/(linoleic acid +  $\alpha$ -linolenic acid) ratio, but positively correlated with the linoleic acid content and (linoleic acid/ $\alpha$ -linolenic acid) ratio ( $p < 0.01$ ).

The physical properties hardness, toughness, and stickiness were positively correlated with the fat acidity, palmitic acid content, oleic acid content, and oleic acid/(linoleic acid +  $\alpha$ -linolenic acid) ratio, but negatively correlated with the linoleic acid content and linoleic acid/ $\alpha$ -linolenic acid) ratio ( $p < 0.01$ ; Table 5). By contrast, adhesion was negatively correlated with the fat acidity, palmitic acid content, oleic acid content, and oleic acid/(linoleic acid +  $\alpha$ -linolenic acid) ratio, but positively correlated with the linoleic acid content and (linoleic acid/ $\alpha$ -linolenic acid) ratio ( $p < 0.01$ ).

The  $\lambda_{\max}$ ,  $A_{\lambda_{\max}}$ , and AAC of the iodine absorption curve were positively correlated with the fat acidity, palmitic acid content, oleic acid content, and oleic acid/(linoleic acid +  $\alpha$ -linolenic acid) ratio, but negatively correlated with the linoleic acid content and (linoleic acid/ $\alpha$ -linolenic acid) ratio ( $p < 0.01$ ; Table 5). By contrast, the  $\lambda_{\max}/A_{\lambda_{\max}}$  was negatively correlated with the fat acidity, palmitic acid content, oleic acid content, and oleic acid/(linoleic acid +  $\alpha$ -linolenic acid) ratio, but positively correlated with the linoleic acid content and (linoleic acid/ $\alpha$ -linolenic acid) ratio ( $p < 0.01$ ).

Thus, the pasting properties (Final.vis, Setback, Past.t, and Cons), physical properties (hardness, toughness, and stickiness) and iodine absorption curve parameters ( $\lambda_{\max}$ ,  $A_{\lambda_{\max}}$ , and AAC) were significant positively correlated with the fat acidity, palmitic acid content, and oleic acid content, but correlated negatively with the linoleic acid content, and  $\alpha$ -linolenic acid showed a similar trend to linoleic acid.

#### Effect of 2 months' storage at 35 °C of fat acidity

Takano *et al.* showed that at the initial of stage, phospholipase D reacted with the phospholipid membrane of spherosome to damage its structure, which

Table 4. Pasting properties of HMT and untreated brown rice flours.

	Maxi.vis.		mini.vis.		Break.d		Final.vis		Setback		Past.t		Consis		Setback/consis		Max/mini		Max/Final		
	(RVU)	SD	(RVU)	SD	(RVU)	SD	(RVU)	SD	(RVU)	SD	(RVU)	SD	(RVU)	SD	(RVU)	SD	(RVU)	SD	(RVU)	SD	
(A)																					
Untreated KSK	389.8a	0.4	166.0a	3.0	223.7a	3.4	405.5a	2.2	15.7a	2.6	72.7a	0.0	239.4a	0.8	0.1a	0.0	2.3a	0.0	1.0a	0.0	0.0
0.1 MPa 5 min	385.0a	0.9	164.3a	1.6	220.7a	0.6	399.7a	2.1	14.7b	1.2	75.1b	0.2	235.4b	0.5	0.1a	0.0	2.3a	0.0	1.0a	0.0	0.0
0.1 MPa 10 min	388.5a	5.7	167.5a	2.8	221.0a	2.9	402.5a	3.6	14.0b	2.1	75.7b	0.1	235.0b	0.8	0.1a	0.0	2.3a	0.0	1.0a	0.0	0.0
0.2 MPa 5 min	351.5b	0.8	155.6b	1.4	195.9b	2.2	377.2b	0.6	25.7c	1.4	75.9b	0.0	221.6c	0.8	0.1b	0.0	2.3b	0.0	0.9a	0.0	0.0
0.2 MPa 10 min	364.8b	0.4	160.8a	1.1	204.0b	0.7	387.5b	2.8	22.8c	2.5	76.1b	0.2	226.8c	1.8	0.1b	0.0	2.3b	0.0	0.9a	0.0	0.0
0.3 MPa 10 min	282.9c	1.1	129.6d	0.2	153.3c	1.4	319.4c	1.2	36.5e	2.4	76.0b	0.0	189.8e	1.0	0.2d	0.0	2.2d	0.0	0.9c	0.0	0.0
Untreated KSI	396.4a	0.2	137.0a	1.4	259.4a	1.2	259.5a	1.0	-136.8a	0.8	67.5a	0.0	122.5a	0.4	-1.1a	0.0	2.9a	0.0	1.5a	0.0	0.0
0.1 MPa 5min	423.9b	3.8	139.1a	3.2	284.8b	0.6	256.0a	3.7	-167.9b	0.2	67.6a	0.4	116.9b	0.4	-1.4b	0.0	3.0a	0.0	1.7b	0.0	0.0
0.1 MPa 10 min	446.7c	1.0	146.3b	0.8	300.5c	1.8	266.9a	0.8	-179.8c	1.8	68.3b	0.1	120.7a	0.0	-1.5b	0.0	3.1a	0.0	1.7b	0.0	0.0
0.2 MPa 5 min	436.0b	4.5	142.0b	1.9	294.0c	2.5	261.1a	1.8	-174.9c	2.7	68.0b	0.0	119.2a	0.1	-1.5b	0.0	3.1a	0.0	1.7b	0.0	0.0
0.2 MPa 10 min	449.9c	0.8	150.3b	0.9	299.7c	1.8	280.3b	1.4	-169.6b	2.2	68.4b	0.0	130.1c	0.5	-1.3c	0.0	3.0a	0.0	1.6b	0.0	0.0
0.3 MPa 10 min	330.2d	0.4	143.2b	0.5	187.0d	0.1	278.2b	0.6	-52.0e	0.2	62.2c	0.9	135.0c	0.1	-0.4d	0.0	2.3b	0.0	1.2c	0.0	0.0
(B)																					
Untreated KSK	282.2a	2.6	145.8a	3.6	136.4a	1.0	483.0a	1.2	200.8a	3.8	77.0a	0.9	337.2a	4.8	0.6a	0.0	1.9a	0.0	0.6a	0.0	0.0
0.1 MPa 5 min	283.4a	4.9	138.5a	1.6	144.9b	3.4	434.5b	3.2	151.0b	1.7	77.7a	0.1	295.9b	1.6	0.5b	0.0	2.0a	0.0	0.7b	0.0	0.0
0.1 MPa 10 min	284.3a	0.3	140.3a	0.9	144.0b	1.2	415.4c	0.4	131.1c	0.6	78.0a	0.2	275.1c	0.6	0.5b	0.0	2.0a	0.0	0.7b	0.0	0.0
0.2 MPa 5 min	266.7b	0.6	140.2a	0.6	126.5c	1.2	419.3c	1.6	152.6b	2.2	78.4a	0.1	279.1c	1.0	0.5c	0.0	1.9a	0.0	0.6b	0.0	0.0
0.2 MPa 10 min	223.4c	0.1	125.0b	1.1	98.4d	0.9	315.6d	0.4	92.2d	0.5	78.7a	0.2	190.6d	1.5	0.5b	0.0	1.8b	0.0	0.7c	0.0	0.0
0.3 MPa 10 min	146.1d	7.4	90.5c	0.8	55.6e	6.5	199.0e	1.8	52.9e	5.6	79.3a	0.1	108.5e	0.9	0.5b	0.1	1.6c	0.1	0.7c	0.0	0.0
Untreated KSI	340.7a	1.5	128.7a	0.4	212.0a	1.9	300.2a	1.4	-40.5a	2.9	68.5a	0.0	171.5a	0.9	-0.2a	0.0	2.6a	0.0	1.1a	0.0	0.0
0.1 MPa 5 min	374.8b	1.5	130.6a	0.9	244.2b	0.6	285.5b	2.1	89.3b	0.6	69.5a	0.2	154.8b	1.2	-0.6b	0.0	2.9a	0.0	1.3b	0.0	0.0
0.1 MPa 10 min	373.5b	4.8	136.7b	1.3	236.8b	3.5	291.5b	0.1	-82.0b	4.7	70.6a	0.1	154.8b	1.2	-0.5b	0.0	2.7a	0.0	1.3b	0.0	0.0
0.2 MPa 5 min	364.6b	0.3	136.2b	0.1	228.5ab	0.2	292.0b	0.5	-72.6c	4.2	70.4a	0.0	155.9b	0.4	-0.5b	0.0	2.7a	0.0	1.2b	0.0	0.0
0.2 MPa 10 min	308.3c	1.8	130.3a	1.0	178.0c	2.8	273.4c	2.2	-34.9d	4.0	70.8a	0.1	143.1c	1.2	-0.2a	0.0	2.4b	0.0	1.1a	0.0	0.0
0.3 MPa 10 min	195.8d	5.3	113.3c	0.2	82.5d	5.5	232.5d	0.4	36.8e	5.7	69.0a	0.0	119.3d	0.1	0.3c	0.0	1.7c	0.1	0.8c	0.0	0.0

Notes: (A)Initial value. (B): Value after 2 months' storage at 35 °C.

HMT, heat-moisture treatment at various pressures (0.1, 0.2, or 0.3 MPa) and time (5 or 10 min).

KSK, Koshinokaori rice cultivar, KSI, Koshiibuki rice cultivar.

Means with the same letter are not significantly different ( $p < 0.05$ ).

Table 5. Correlation between fat acidity, fatty acid composition, pasting properties, physical properties and iodine absorption curve of HMT and untreated brown rice flour.

	Fat acidity	Palmitic acid	Oleic acid	Linoleic acid	$\alpha$ -linolenic acid	Oleic acid/Linoleic + $\alpha$ -linoleic	Linoleic acid/ $\alpha$ -linolenic acid
Fat acidity	1.00						
Palmitic acid	0.97**	1.00					
Oleic acid	0.86**	0.83**	1.00				
Linoleic acid	-0.97**	-0.98**	-0.91**	1.00			
$\alpha$ -linolenic acid	-0.59	-0.57	-0.45	0.55	1.00		
Oleic/Lino + $\alpha$ -lino	0.97**	0.97**	0.94**	-1.00**	-0.55	1.00	
Linoleic/ $\alpha$ -lino	-0.89**	-0.91**	-0.88**	0.94**	0.23	-0.94**	1.00
Maxi.vis.	-0.60	-0.59*	-0.51	0.58*	0.13	-0.58*	0.63*
Mini.vis.	0.64	0.58*	0.43	-0.56	-0.62*	0.54	-0.40**
Break.d	-0.76**	-0.74**	-0.62*	0.73**	0.29	-0.72**	0.73**
Final.vis	0.95**	0.92**	0.79**	-0.92**	-0.66*	0.91**	-0.80**
Setback	0.97**	0.94**	0.81**	-0.93**	-0.52	0.92**	-0.88**
Past.t	0.84**	0.90**	0.87**	-0.93**	-0.47	0.93**	-0.89**
Consis	0.98**	0.96**	0.84**	-0.96**	-0.64*	0.95**	-0.85**
Set/Consis	0.96**	0.93**	0.79**	-0.92**	-0.52	0.91**	-0.86**
Max/Mini	-0.90**	-0.87**	-0.69*	0.84**	0.45	-0.83**	0.79**
Max/Final	-0.96**	-0.94**	-0.79**	0.93**	0.52	-0.92**	0.87**
Hardness	0.93**	0.91**	0.87**	-0.93**	-0.58*	0.93**	-0.84**
Toughness	0.97**	0.95**	0.87**	-0.97**	-0.51	0.96**	-0.92**
Adhesion	-0.75**	-0.79**	-0.88**	0.85**	0.43	-0.86**	0.81**
Stickiness	0.96**	0.97**	0.88**	-0.98**	-0.70*	0.97**	-0.85**
Ad/Tou	-0.90**	-0.92**	-0.92**	0.96**	0.53	-0.96**	0.90**
St/Tou	-0.71**	-0.68*	-0.66*	0.70*	0.14	-0.69*	0.75**
$\lambda$ max	0.96**	0.96**	0.93**	-0.98**	-0.57	0.99**	-0.91**
$A\lambda$ max	0.96**	0.98**	0.91**	-1.00**	-0.55	0.99**	-0.93**
$\lambda$ max/ $A\lambda$ max	-0.96**	-0.98**	-0.91**	1.00**	0.56	-0.99**	0.93**
AAC	0.97**	0.98**	0.91**	-1.00**	-0.56	1.00**	-0.93**

Notes: Correlation significant at 5%\* or 1%\*\* by *t*-test.

then allowed triacylglycerol to get out through the spherosome membrane. After that lipase decomposed triacylglycerol to free fatty acids.<sup>71)</sup> Furthermore, Takano *et al.*<sup>72)</sup> reported that phospholipids, triacylglycerol, and glycolipids decreased during 2 months' storage at 37 °C, while lipolytic enzyme activities were retained 30–60%; and Yasumatsu and Moritaka<sup>73)</sup> showed that rice stored at room temperature had a higher oleic acid content and a lower linoleic acid content in the free fatty acid fraction than rice stored at 9 °C. Furthermore, Hikida *et al.*<sup>23)</sup> showed that the fat acidity of rice grains increased as storage progressed, but that rate of increase was suppressed in heated samples, due to the inactivation of lipid decomposing enzymes.

In the initial stage of storage, the fat acidity was slightly lower in the HMT KSK samples (45.1–50.3, mean = 48.3 mgKOH<sup>-1</sup> 100 g<sup>-1</sup>D.W.) than in the untreated sample (49.1 mgKOH<sup>-1</sup> 100 g<sup>-1</sup>D.W.), with a significant difference being observed with the 0.3 MPa/10 min treatment [ $p < 0.05$ ; Fig. 1(A)]. Moreover, the fat acidity of the HMT KSK samples was significantly positively correlated with  $\Delta H$  (first heating peak;  $r = 0.96$ ) and  $\Delta H$  (second heating peak;  $r = 0.94$ ) ( $p < 0.01$ ).

By contrast, the fat acidity was slightly higher in the HMT KSI samples (21.5–30.3, mean = 24.2 mg KOH<sup>-1</sup> 100 g<sup>-1</sup> D.W.) than in the untreated sample (22.2 mg KOH<sup>-1</sup> 100 g<sup>-1</sup>

D.W.), with a significant difference being observed with the 0.3 MPa/10 min treatment [ $p < 0.05$ ; Fig. 1(B)]. We think that non-enzymatic decompose,

namely phospholipid membrane of spherosome was damaged by treatment conditions including high pressure and long time. The freshness determine check result, the KSI sample treated with 0.3 MPa/10 min HMT showed a yellow color with the MB method: (pH indicator; methyl red and bromothymol blue; data not shown).

After 1 month of storage at 35 °C, the fat acidity was lower in the HMT samples (KSK: 46.4–61.9, mean = 57.2 mg KOH<sup>-1</sup> 100 g<sup>-1</sup> D.W.; KSI: 33.1–61.1, mean = 50.8 mg KOH<sup>-1</sup> 100 g<sup>-1</sup> D.W.) than in the untreated samples (KSK: 62.0 mg KOH<sup>-1</sup> 100 g<sup>-1</sup> D.W.; KSI: 62.0 mgKOH<sup>-1</sup> 100 g<sup>-1</sup> D.W.), with significant differences being observed with the 0.2 MPa/10 min, and 0.3 MPa/10 min treatments ( $p < 0.05$ ; Fig. 1). Moreover, fat acidity was positively correlated with lipase acidity in the HMT samples (KSK:  $r = 0.91$ ,  $p < 0.05$ ; KSI:  $r = 0.95$ ,  $p < 0.01$ ; data not shown).

After 2 months' storage at 35 °C, the fat acidity was lower in the HMT samples (KSK: 45.3–118.2, mean = 90.8 mg KOH<sup>-1</sup> 100 g<sup>-1</sup> D.W., KSI: 29.7–82.5, mean = 63.4 mgKOH<sup>-1</sup> 100 g<sup>-1</sup> D.W.) than in the untreated samples (KSK: 126.4 mg KOH<sup>-1</sup> 100 g<sup>-1</sup> D.W., KSI: 84.7 mgKOH<sup>-1</sup> 100 g<sup>-1</sup> D.W.), with significant differences being observed with the 0.2 MPa/10 min, and 0.3 MPa/10 min treatments ( $p < 0.05$ ; Fig. 1). Moreover, fat acidity was positively correlated with lipase acidity in the HMT samples (KSK:  $r = 0.95$ ,  $p < 0.01$ ; KSI:  $r = 0.95$ ,  $p < 0.01$  data not shown).

Thus, the fat acidity of samples treated with 0.1 MPa/5 min, 0.1 MPa/10 min, and 0.2 MPa/5 min

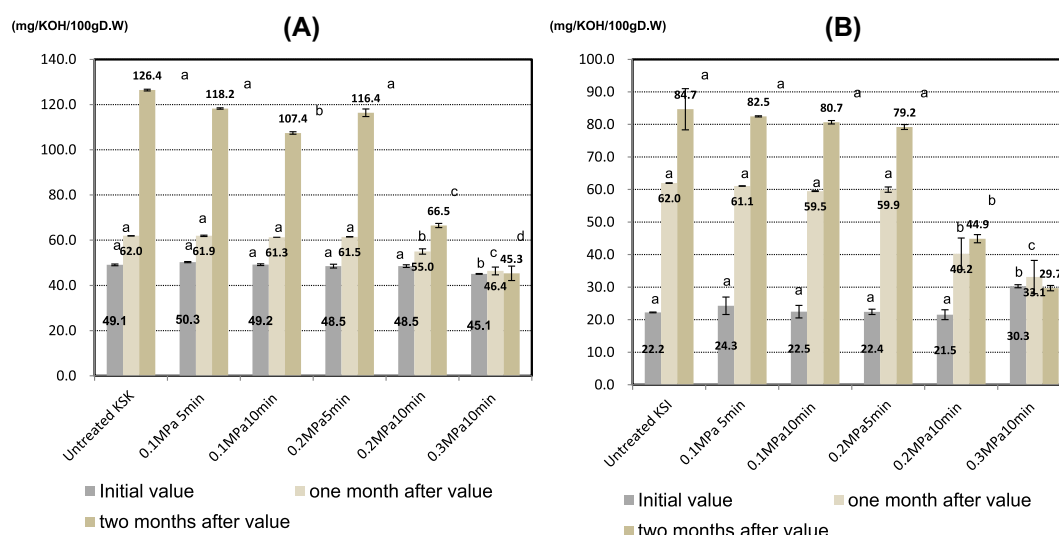


Fig. 1. Fat acidity of HMT and untreated brown rice flour during 2 months' storage at 35 °C.

Notes: (A) KSK samples. (B) KSI samples. HMT, heat-moisture treatment; KSK, Koshinokaori rice cultivar; KSI, Koshiibuki rice cultivar. The same letters indicate no significant difference.

HMT was almost identical to that of the untreated samples during 2 months' storage at 35 °C. However, samples that were treated with 0.2 MPa/10 min HMT decreased 0.89-fold, for KSK and 0.65-fold, for KSI, while those that were treated with 0.3 MPa/10 min HMT decreased by 0.75-fold for KSK and 0.53-fold for KSI compared with the untreated samples after 1 month of storage; and samples that were treated with 0.2 MPa/10 min HMT decreased 0.53-fold for both cultivars, while those that were treated with 0.3 MPa/10 min HMT decreased 0.36-fold for KSK and 0.35-fold for KSI compared with the untreated samples after 2 months' storage. There was no increase in the fat acidity of the samples treated with 0.3 MPa/10 min HMT during 2 months' storage at 35 °C, because the lipase activity decreased 0.83-fold for KSK, and 0.76-fold for KSI compared with the untreated samples.

As a result, strong HMT would be useful for the prevention of quality deteriorations of brown rice flour during the storage by inhibiting the increase of free fatty acids.

#### Effect of 2 months' storage at 35 °C of iodine absorption curve

Amylose is a non-branched  $\alpha$ -1,4-glucan, that is component of rice starch, and greatly affects the quality and gelatinization properties of cooked rice, with the starch of low-amylase rice not being easily retrograded.<sup>74)</sup> The high-amylase starch group includes two types of rice starches with similar AAC contents but different SLC contents of amylopectin. Hoover and Vasanthan<sup>28)</sup> previously demonstrated that the extent of starch-chain associations within the amorphous regions and the degree of crystalline order altered during HMT.

We measured the iodine absorption curve values after 2 months' storage at 35 °C, and found that HMT samples of both cultivars had almost identical  $\lambda_{\max}$  values [Table 2(B)]. However, KSK exhibited a slightly lower  $A_{\lambda_{\max}}$  values, a 1.1-fold higher  $\lambda_{\max}/A_{\lambda_{\max}}$  ratio, and a 0.7-fold lower AAC value than was

observed initially, while KSI exhibited almost no change in any of these values. Furthermore, AAC was positively correlated with fat acidity at the end of the 2 months storage period ( $p < 0.01$ ; data not shown). Whistler and Johnson<sup>75)</sup> and Foster and Paschall<sup>76)</sup> showed that alongside the retrogradation of amylose, the iodine reaction was also decreased.

As shown in Fig. 2(A), the fat acidity of retrograded brown rice flour was estimated with multiple regression analysis using fat acidity as the response variable and each of the parameters from the iodine absorption curve as predictor variables based on 12 samples (KSK or KSI that was untreated, or treated with 0.1 MPa/5 min, 0.1 MPa/10 min, 0.2 MPa/5 min, 0.2 MPa/10 min, or 0.3 MPa/10 min). The resulting equation was:

$$\begin{aligned} \text{Fat acidity (mgKOH}^{-1}\text{100g}^{-1}\text{D.W.)} \\ = 2.15 \times \lambda_{\max}/A_{\lambda_{\max}}\text{ratio} + 111.25 \\ \times \text{AAC} - 5202.78. \end{aligned}$$

and had a multiple regression coefficient of 0.90 based on the calibration. This formula for estimating fat acidity provides an easy and low-cost spectroscopic method for evaluating the quality of brown rice flour.

#### Effect of 2 months' storage at 35 °C of the physical properties

The textural properties of starch gels affect the quality of the final products. HMT leads to changes in the physical properties of starch by changing from the crystal structure to the semi-amorphous structure in the starch granules. In the case of potato starch, HMT causes the "B" pattern of X-ray diffraction to be changed to "A" or "C" pattern.<sup>77)</sup> Furthermore, the gel hardness increases as a result of HMT and treatment time significantly enhances this parameter.<sup>78)</sup> Kulp and Lorenz<sup>79)</sup> reported that HMT alters the physical order of starch granules and causes a certain degree of starch degradation, under stronger treatment conditions, which is greater in wheat than in tuber starch.



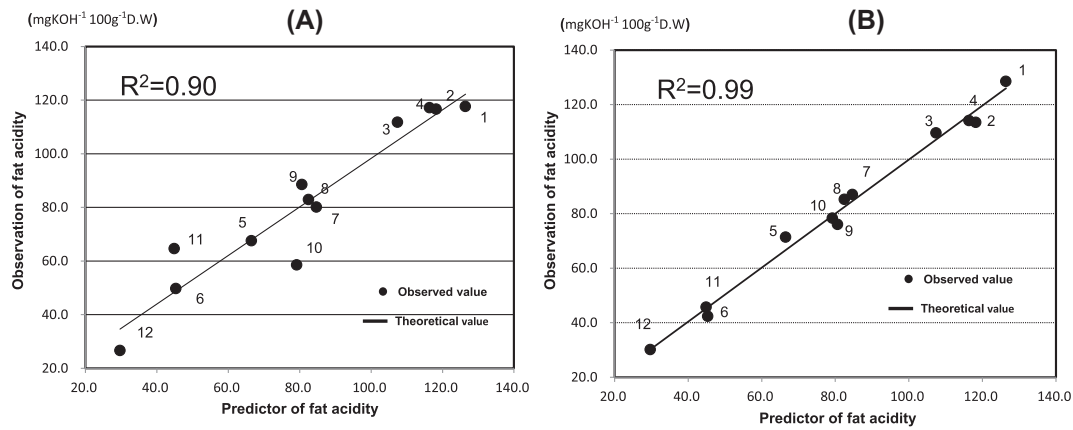


Fig. 2. Formula for estimating the fat acidity based on the iodine absorption curve and pasting properties of retrograded brown rice flour.

Notes: (A) Fat acidity values based on the iodine absorption curve. Estimation formula: Fat acidity ( $\text{mgKOH}^{-1} 100 \text{ g}^{-1} \text{D.W.}$ ) =  $2.15 \times \lambda_{\text{max}}/A_{\lambda_{\text{max}}} + 111.25 \times \text{AAC} - 5202.78$ . The equation has a determination coefficient ( $R^2$ ) of 0.90. (B): Fat acidity values based on the pasting properties. Estimation formula: Fat acidity ( $\text{mgKOH}^{-1} 100 \text{ g}^{-1} \text{D.W.}$ ) =  $4.74 \times \text{Setback} + 1071.77 \times \text{Max.vis} - 624.09 \times \text{Break.d} - 288.39 \times \text{Past.t} - 176.10 \times \text{Mini.vis} + 132.19$ . The equation has a determination coefficient ( $R^2$ ) of 0.99.

We measured the physical properties of gelatinous brown rice flour after 2 months' storage at 35 °C, and found that hardness and toughness were 0.5- and 0.7-fold lower, respectively, in KSK but 1.1-fold higher in KSI compared with the initial values [Table 3(B)]. By contrast, adhesion was 1.3-fold higher in KSK and 1.1-fold higher in KSI compared with the initial values, while stickiness was almost identical in KSK and 1.2-fold higher in KSI after 2 months' storage. Consequently, we believe that the physical properties of retrograded brown rice flour depended on the source of the starch, fat acidity content, fatty acid component ratio, and proportion of unsaturated fatty acids (i.e. the number of double bonds).

#### *Effect of 2 months' storage at 35 °C of the pasting properties*

Katsuhara *et al.* reported that the increase in free fatty acids that occurs during rice storage results in an increase in the maximum viscosity of cooked rice due to free fatty acids forming a helical structure with starch molecules.<sup>80)</sup> Furthermore, Dhaliwal *et al.*<sup>81)</sup> found that the peak viscosity of milled rice increases with storage time due to an increase in the free fatty acid content and a decrease in the diastatic activity. However, Shin *et al.*<sup>82)</sup> showed that the decrease in amylase activity that occurs during storage has very little effect on the total setback of undefatted brown rice flour.

We measured the pasting properties of brown rice flour after 2 months' storage at 35 °C, and found that the Final.vis, and Cons.vis values were significantly lower in HMT samples than in untreated samples [ $p < 0.05$ ; Table 4(B)]. By contrast, the Max.vis and Mini.vis values were similar to the untreated sample for KSK samples treated with 0.1 MPa/5 min and 0.1 MPa/10 min HMT, but significantly higher than the untreated sample for the HMT KSI samples ( $p < 0.05$ ). These findings demonstrate that retrogradation of the pasting properties after 2 months' storage at 35 °C was inhibited by HMT.

As shown in Fig. 2(B), the fat acidity of retrograded brown rice flour was estimated with multiple regression

analysis, using fat acidity as the response variable and each of the pasting property parameters as predictor variables using 12 samples (KSK or KSI that was untreated, or treated with 0.1 MPa/5 min, 0.1 MPa/10 min, 0.2 MPa/5 min, 0.2 MPa/10 min, or 0.3 MPa/10 min). The resulting equation was:

$$\begin{aligned} \text{Fat acidity (mgKOH}^{-1} 100 \text{ g}^{-1} \text{D.W.)} \\ = 4.75 \times \text{Setback} + 1071.77 \\ \times \text{Max.vis} - 624.09 \times \text{Break.d} - 288.39 \\ \times \text{Past.t} - 176.10 \times \text{Mini.vis} + 132.19. \end{aligned}$$

And had a multiple regression coefficient of 0.99 based on the calibration. This formula is the novel one which makes it possible to estimate fat acidity without dissolution of fatty acid by organic solvent and titration of free acid by KOH. This formula for estimating fat acidity provides an easy and rapid method for evaluating the quality of brown rice flour during the storage.

## Conclusions

HMT resulted in new functional properties in brown rice flour. The treatment conditions, i.e. the pressure, temperature and time, affected the physicochemical characteristics of the modified starch, including the structural characteristics, which were particularly affected by 0.3 MPa/10 min HMT.

HMT led to a decrease in  $\alpha$ -amylase activity, lipase activity, L-ORAC and H-ORAC, and an increase in the starch damage ratio, and color difference in both cultivars, as well as an increase in AAC of KSK and a decrease in AAC of KSI compared with untreated sample.

The pasting and physical properties of KSK samples treated with 0.1 MPa/10 min HMT were the same as for the untreated sample. However, the gelatinization enthalpy and  $\alpha$ -amylase activity decreased, while the RS content, AAC and gelatinization temperature slightly increased compared with the untreated sample. Furthermore, the fat acidity exhibited a 0.8-fold decrease compared with the untreated sample, after 2 months'

storage at 35 °C. By contrast, the pasting and physical properties of the KSI samples treated with 0.1 MPa/10 min HMT were almost the same as the untreated sample, but the dietary fiber content slightly increased 1.1-fold.

The starch gel texture became harder, and less sticky in the KSK samples, but more adherent and elastic in the KSI samples by HMT. Furthermore, the pasting properties of KSK and KSI were also opposite from each other, following HMT. The pasting properties (Final.vis, Setback, Past.t, and Cons.), physical properties (hardness, toughness, and stickiness) and iodine absorption curve parameters ( $\lambda_{\max}$ ,  $A\lambda_{\max}$ , and AAC) in HMT samples were also positively correlated (significant) with fat acidity, palmitic acid, and oleic acid contents, but negatively correlated with the linoleic acid content, and  $\alpha$ -linolenic acid.

After storage for 2 months at 35 °C, sample treated with 0.2 MPa/10 min and 0.3 MPa/10 min HMT showed lower AAC and fat acidity values, and inhibition of retrogradation of the pasting and physical properties compared with untreated samples. Although the fat acidity usually increase as storage progresses, in the present study, lipase activity decreased 0.8-fold in samples treated with 0.3 MPa/10 min HMT compared with the untreated samples, which resulted in no increase in fat acidity being observed in HMT samples during the storage for 2 months at 35 °C.

Furthermore, we propose estimation formulas for the fatty acid contents of retrograded brown rice flour based on the iodine absorption curve and pasting properties, which will enable the quality of brown rice flour to be evaluated with an easy and rapid iodine colorimetric method.

Based on our findings, we believe that the physical and pasting properties of retrograded brown rice flour changed or unchanged depended on the source of the starch, the fatty acid component ratio, and proportion of unsaturated fatty acids and the condition of HMT.

Finally, it would be necessary to continue and accelerate our research, for example, quality evaluation of the brown rice flour made by the enlarged pilot-scale manufacturing system, in the near future as a collaborative research.

## Authors contribution

Ken'ichi Ohtsubo, Sumiko Nakamura, and Masayoshi Sugawara conceived and designed the experiments. Sumiko Nakamura and Wataru Noro, Hisako Okumura, and Noriyuki Honma performed the experiments. Sumiko Nakamura wrote the manuscript with assistance from Ken'ichi Ohtsubo. All authors reviewed the results and approved the final version of the manuscript.

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## Disclosure statement

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## Supplemental materials

The supplemental material for this paper is available at <https://doi.org/10.1080/09168451.2017.1387047>.

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