Technical paper

Effects of Heat Moisture Treatments on the Digestibility and Physicochemical Properties of Various Rice Flours

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Heat moisture treatment (HMT) is a processing method used to improve the physicochemical properties of starch. Here, to inform technology with respect to increasing resistant starch (RS) in rice, three rice flours with different starch structures—namely, Koshihikari, Koshinokaori, and Goami2 (GA2)—were treated at 120 °C and 14 %–26 % humidity and compared with cornstarch. HMT increased the RS content of all specimens, with GA2 showing a marked RS increase up to 11.0 %. HMT also increased the gelatinization temperature (T_{gel}) of all samples, as shown by differential scanning calorimetry, and RS content tended to increase with increasing T_{gel} values. As shown by the increase in relative crystallinity by X-ray diffraction and change in the iodine absorption spectrum, HMT easily changed the starch structure in GA2, leading to increased RS. These results support GA2 as a suitable rice cultivar for bio-functional rice products such as low glycemic index foods.

Keywords: heat moisture treatment, resistant starch, amylopectin long chain rice

Introduction

Rice is one of the three most important food crops in the world, and more than 90 % of rice is processed and consumed in Asia. Although rice is usually prepared by boiling, it has recently started to be used as a raw ingredient in various foods such as rice bread (Nishita, 1977, Nakamura *et al.*, 2009) and coatings for fried food (Nakamura *et al.*, 2010). Rice is the only crop that can be supplied domestically in Japan; however, rice consumption has steadily decreased since the 1970s. Therefore, it is necessary to develop processing methods to expand the consumption of rice.

As of 2017, more than 400 million people worldwide are reported to suffer from diabetes (i). Thus, an increasing

percentage of the population is paying attention to postprandial blood glucose levels. Furthermore, the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) have recommended low glycemic index (GI) foods as an effective method to prevent diabetes (Nantel, 2003). Since starchy foods like processed rice are easily digested into glucose, and are thus classified as high GI foods, excess intake is not recommended for individuals suffering from insulin disorders.

Englyst *et al.* (1992) defined resistant starch (RS) as starch tolerant of α -amylase and which arrives at the large intestine undigested. RS is recognized as a functional ingredient for controlling postprandial blood glucose levels and reducing GI

Abbreviations: heat moisture treatment, HMT; resistant starch, RS; Goami2, GA2; gelatinization temperature, T_{gel} ; World Health Organization, WHO; amylopectin long chain, ALC; super-long chains, SLC; glycemic index, GI; Koshihikari, KSH; Koshinokaori, KSK; commercial cornstarch, CS; high amylose cornstarch, HCS; apparent amylose content, AAC; differential scanning calorimetry, DSC; endothermic enthalpy, ΔH

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values (Birt *et al.*, 2013). To meet modern nutritional needs, it is important to select a rice cultivar that contains more RS and to develop a suitable processing method to control its digestibility.

Digestibility of rice starch depends on the content of amylose and the structure of amylopectin. It has been reported that high amylose rice cultivars reduce the postprandial glucose response because of their high RS content (Ohtsubo *et al.*, 2010, Zenel *et al.*, 2015, Ohtsubo *et al.*, 2016). Nakamura *et al.* (2011) reported that amylopectin long chain (ALC) rice cultivars contain more RS than high amylose cultivars. ALC rice cultivars were developed *via* chemical mutation (Satoh, 1994) or crossbreeding with ALC rice cultivars such as EM10 (Nishi *et al.*, 2001) and Goami2 (GA2) (Choi *et al.*, 2008), which have super-long chains (SLC) in amylopectin (Takahashi *et al.*, 2001, Kubo *et al.*, 2010). It is reported that rice flour bread made from the ALC rice cultivar Konayukinomai contains more RS and reduces postprandial blood glucose levels (Noro *et al.*, 2016).

A processing technique for low-digestible food has been developed to increase indigestible carbohydrates in starch. Indigestible dextrin is made from hydrolyzed heat-treated cornstarch using amylase, removing the digestible components from the non-decomposed components, and is already used in various foods such as “low GI drinks” (Okuma and Matsuda, 2003). Heat moisture treatment (HMT) is a physicochemical method of heating at restricted moisture conditions (Sair, 1966) and is used for the physical modification of starches and cereal flours. Kawabata *et al.* (1994) reported that the swelling ability and starch solubility of starch is suppressed by HMT. Taniguchi *et al.* (2003) reported that HMT starch is high in RS, and which increased in samples with greater amylose content. Nakamura *et al.* (2017) demonstrated that the pasting properties of HMT brown rice inhibit retrogradation. The degree of these changes was dependent on the raw materials and moisture content during HMT. Most of the above studies were carried out using normal starch (i.e., starch with no mutant material). Few studies have reported on the effects of HMT on mutant rice cultivars such as the ALC rice GA2.

Aiming to develop a new high RS rice flour, we treated three rice cultivars (medium amylose rice, Koshihikari (KSH), high amylose rice, Koshinokaori (KSK), and ALC rice, GA2) under four different HMT conditions (moisture contents: 14 %, 18 %, 22 %, and 26 %). We compared the various HMT rice flours with HMT cornstarch and investigated the mechanism of the RS content increase. We anticipate that the present study will contribute to the commercialization of HMT rice flour and development of new rice cultivars as basic research.

Materials and Methods

Materials Three rice cultivars were used. The typical middle amylose rice cultivar Koshihikari (KSH) was purchased from a local rice store in Japan. The high amylose rice cultivar

Koshinokaori (KSK) was cultivated at the Crop Research Center, Niigata Agricultural Research Institute, Japan. The ALC rice Goami2 (GA2) was purchased from a rice market in Korea. Commercial cornstarch (CS) and high amylose cornstarch (HCS) were purchased from J Oil Mills Inc. (Tokyo, Japan).

Preparation of rice flours The brown rice was polished with a rice polisher (VP-32; Yamamoto Seisakusyo Co., Ltd., Tendo, Japan) to a milling yield of 90 %. Rice flour was prepared from the polished rice using a cyclone mill (Tecator Cyclotec 1093; FOSS JAPAN, Yokohama, Japan) with a screen with 0.5-mm diameter pores. The mean diameter of rice flour was measured with a laser diffraction-scattering analyzer (LMS-2000; Seishin Enterprise Co., Ltd, Tokyo, Japan).

Heat moisture treatment Each sample (rice flour or cornstarch) was stirred by a vertical mixer (KK81; Japan Kneader Co., Ltd., Kanagawa, Japan) and sprayed with distilled water so that the moisture was uniformly spread in the sample. Distilled water was added until the sample had a predetermined moisture content (14 %, 18 %, 22 %, or 26 %). The moist sample was sealed in a retort pouch and kept at room temperature for 12 h to equilibrate. HMT was performed using a retort machine (RCS-40RG; HISAKA WORKS, LTD., Osaka, Japan) under hydrostatic pressure of 0.2 MPa at 120 °C for 8–24 h. The HMT sample was removed from the retort bag and dried at 35 °C to a uniform moisture content (approximately 12 %).

Nutritional analysis The nutritional components of the rice flours and cornstarches were analyzed. Protein content was measured by the macro-Kjeldahl method, and a conversion factor of 5.95 (rice flour) or 6.25 (cornstarch) was used to convert nitrogen into protein. Lipid content was measured by Soxhlet extraction using diethyl ether as the solvent. Ash content was calculated by the difference in the mass of the sample before and after incineration in a furnace at 550 °C. Carbohydrate content was calculated by subtracting the weight of protein, lipid, and ash contents from the total weight. All nutritional components of the samples were calculated on a dry matter basis.

Apparent amylose content Apparent amylose content (AAC) was measured using the iodine colorimetric method (Juliano, 1971). Potato amylose type III (Sigma Chemical Co., St. Louis, MO, USA) and waxy rice starch (prepared from Kogamemochi) were used as the standards of amylose and amylopectin, respectively.

Resistant starch RS content was measured according to the AOAC method using an RS assay kit (Megazyme, Ltd., Wicklow, Ireland). The sample (100 mg) and 2 mL of distilled water were placed in a test tube and heated at 100 °C for 15 min. Next, the test tube was placed in a 37 °C water bath for 20 min. The boiled sample was digested with pancreatin and amyloglucosidase at 37 °C for 6 h. Lastly, the glucose content was measured with a spectrophotometer at 510 nm (Nakamura

Table 1. Nutritional contents of rice flour and cornstarch

	Protein(%)	Lipid (%)	Ash (%)	Carbohydrate(%)	AAC (%)	RS (%)
KSH	5.32 ± 0.07 a	0.74 ± 0.049 a	0.43 ± 0.026 a	93.5 ± 0.15 a	15.4 ± 1.68 a	0.03 ± 0.004 a
KSK	6.51 ± 0.02 b	0.71 ± 0.044 a	0.44 ± 0.005 a	92.3 ± 0.06 b	32.2 ± 0.40 b	0.10 ± 0.011 b
GA2	6.64 ± 0.03 c	1.56 ± 0.006 b	0.72 ± 0.016 b	91.1 ± 0.03 c	39.2 ± 1.68 c	0.22 ± 0.026 c
CS	0.35 ± 0.01	0.14 ± 0.016	0.02 ± 0.005	99.5 ± 0.03	41.1 ± 0.73	0.50 ± 0.142
HCS	0.90 ± 0.01	0.17 ± 0.015	0.04 ± 0.004	98.9 ± 0.02	92.0 ± 1.79	18.6 ± 0.628

The nutritional contents are calculated in terms of dry weight basis. Each value represents the average ± standard deviation (n = 3). Different letters indicate significant differences among three rice flours, as shown by Tukey's tests ($p < 0.05$). KSH, Koshihikari; KSK, Koshinokaori; GA2, Goami2; CS, commercial cornstarch; HCS, high amylose cornstarch.

and Ohtsubo, 2015).

Thermal measurement by differential scanning calorimetry (DSC) The thermophysical properties of each sample were analyzed using a differential scanning calorimeter (DSC8000; Perkin Elmer Japan, Yokohama, Japan). The sample (15 mg) and distilled water (45 µL) were encapsulated in a stainless-steel pan (Perkin Elmer Japan). The pan was then placed in the DSC instrument and scanned from 4 °C to 150 °C at a heating rate of 10 °C/min. The gelatinization temperature (T_{gel}) and endothermic enthalpy (ΔH) were determined using the supplied software. The ΔH was calculated in terms of dry matter (J/g).

X-ray diffraction patterns of flour samples X-ray diffraction patterns of each sample were measured using an X-ray diffractometer (Rint-Ultima III; Rigaku Holding Inc., Tokyo, Japan). Each sample was tightly packed in a sample holder and scanned at 2θ value from 5° to 40° at room temperature with a scanning rate of 2°/min. A diffraction pattern was obtained using Cu-K α radiation and a scintillation counter at 40 kV and 40 mA. Each sample was scanned three times, and the diffraction patterns were obtained by averaging and smoothing using the Savitzky–Golay method (Iwashita, 2003). Calcium fluoride (CaF₂) was used as an internal standard and mixed 5 % (w/w) relative to each sample. The relative crystallinity was measured as per the method of Hamanishi *et al.* (2000) and calculated as follows:

Relative crystallinity = [peak intensity of sample ($2\theta = 15.0^\circ$ or 17.9°) ÷ peak intensity of CaF₂ ($2\theta = 28.3^\circ$)]

Iodine absorption spectrum The iodine absorption spectrum of each sample (100 mg) was measured using a colorimetric method, as with ALC. The iodine absorption spectrum (Nakamura *et al.*, 2015) was analyzed from 250 to 750 nm using a spectrophotometer (UV-2700; Shimadzu Corporation, Kyoto, Japan) and a square cell with inner dimensions of 1 cm × 1 cm. Distilled water was used as a control. Absorbance was measured at λ_{max} , i.e., the peak wavelength in the visible light region of the iodine-starch reaction, which was highly correlated with the glucose polymerization degree, molecular size of amylose, and SLC of amylopectin.

Statistical analyses The data were analyzed using a Student's *t*-test, one-way analysis of variance, and Tukey's test

with Excel Statics 2010 (Social Survey Research Information Co., Ltd., Tokyo). Statistical significance was defined as a *p* value less than 0.05.

Results and Discussion

Nutritional composition of native samples The mean diameter of the rice flours used for analysis and HMTs was $97.5 \pm 1.0 \mu\text{m}$ for KSH, $117.2 \pm 1.9 \mu\text{m}$ for KSK, and $97.3 \pm 2.2 \mu\text{m}$ for GA2. Although KSK had a larger particle size compared to KSH and GA2, it was sufficiently fine for use in experiments.

The nutritional contents of the native samples are shown in Table 1. Protein content of KSH was significantly lower than that of KSK or GA2 ($p < 0.05$). Lipid and ash contents of GA2 were significantly higher than those of KSH or KSK ($p < 0.05$). On the other hand, carbohydrate content of GA2 was lower than that of KSH or KSK ($p < 0.05$). AAC of GA2 was the highest of the three rice cultivars and was the same as for CS. AAC is used as a parameter for estimating the cooking and eating qualities of rice grains (Nakamura *et al.*, 2016), and it is related to actual amylose content and the structure of amylopectin. Because iodine interacts strongly with longer glucans, the ALC rice cultivar GA2 should have a higher AAC due to the SLC in its amylopectin. Although the RS content of the three rice cultivars was very low (KSH: 0.03 %; KSK: 0.10 %; GA2: 0.22 %), a significant difference was observed ($p < 0.05$). HCS showed a very high AAC and contained the most RS (18.6 %) compared to the other samples. Samples with higher AACs tended to contain more RS. The difference in RS content was presumed to relate to the actual amylose content and SLC in amylopectin.

RS content of HMT samples Changes in the RS content after HMT are shown in Fig. 1. The RS content of all samples increased in accordance with treatment time. The RS content of HMT samples reached a maximum of 0.3 % for KSH, 4.0 % for KSK, 11.0 % for GA2, 9.4 % for CS, and 50.2 % for HCS. In samples with higher AAC, the RS content was greatly increased by HMT. It was reported that amylose and amylopectin in starch combine during HMT, which is promoted by the greater amylose content in starch (Kurahashi and Yoshino, 2000). In ordinary industrial processing, HMT is

Fig. 1. RS of rice flour and cornstarch prepared by different HMTs.

Resistant starch is calculated in terms of dry weight. Different letters indicate significant differences among native (0 h) and HMT (24 h) samples, as shown by Tukey's test ($p < 0.05$). (A) KSH, Koshihikari; (B) KSK, Koshinokaori; (C) GA2, Goami2; (D) CS, commercial cornstarch; (E) HCS, high amylose cornstarch. ○, 14%; ●, 18%; ◇, 22%; ◆, 26%.

carried out for 16 to 24 h. It seemed that the RS content for GA2 or HCS reached equilibrium by HMT under optimal moisture condition (18% or 22%) at 24 h. In cases of higher moisture conditions, the RS content tended to increase by HMT. It is assumed that starch containing more free water was changed more easily by HMT and became more resistant to

digestive enzymes. Interestingly, the moisture content where maximum RS was reached differed among the three rice cultivars (KSH and KSK: 26%; GA2: 22%). The RS content of GA2 treated at 26% moisture content was lower compared with 18% or 22% ($p < 0.05$). We presume that GA2 showed reduced RS content by partial gelatinization when HMT was

conducted under high moisture conditions. On the other hand, the RS of GA2 was increased under lower moisture conditions compared to the other rice cultivars.

Based on these results, we suggest that GA2 is suitable for increasing RS by HMT, since the RS content after HMT was about 50 times higher than in untreated samples. Moreover, treatment under lower moisture conditions is practical because it is easier to dry the rice to the appropriate moisture content after HMT.

Thermal properties of native and HMT samples DSC endotherm profiles of the native and HMT samples are shown in Fig. 2. All native samples gave a typical endothermic enthalpy peak showing gelatinization of the crystalline regions of the starch granules. The endothermic enthalpy peaks of HMT samples shifted to a higher temperature and became smaller and broader compared to those of the native samples. In the HMT sample of GA2, the endothermic peak of amylose-lipid complex was observed at 120 °C. We assume that GA2 had a lower actual amylose content, even though it has a higher AAC compared with the other rice samples.

The gelatinization temperatures (T_{gel}) of the native and HMT samples are shown in Table 2. T_{gel} values for the native samples were 66.5 °C, 76.4 °C, 75.5 °C, 70.0 °C, and 71.6 °C for KSH, KSK, GA2, CS, and HCS, respectively. KSK and GA2 had a higher T_{gel} than KSH. It seemed that their high AACs resulted in strong interactions of long chains in amylose and/or amylopectin. The T_{gel} values of all samples were shifted to a higher temperature by HMT, increasing to 86.0 °C, 93.8 °C, 100.9 °C, 94.9 °C, and 117.6 °C for KSH, KSK, GA2, CS, and HCS, respectively. T_{gel} tended to rise under higher moisture conditions during HMT, consistent with reports by Khunae *et al.* (2007) for glutinous and jasmine rices. In general, the increase in T_{gel} by HMT is due to interactions of starch polymers. Compared to other rice cultivars, GA2 had the largest increase in T_{gel} by HMT (KSH: 19.5 °C; KSK: 17.4 °C; GA2: 25.4 °C). We suggest that the T_{gel} increase for GA2 was due to interactions of SLC in amylopectin. In addition, the T_{gel} of cornstarch samples tended to increase more efficiently by HMT compared to the rice samples (CS: 24.9 °C; HCS: 32.0 °C). Further, HMT samples with a higher T_{gel} tended to have a greater increase in RS content. Therefore, we suggest that the changes in starch structure caused at a higher T_{gel} by HMT led to the generation of more RS in the rice samples.

The endothermic enthalpies (ΔH) of the native and HMT samples are shown in Table 3. The ΔH of the native rice flours were 10.0, 16.5, and 15.5 J/g for KSH, KSK, and GA2, respectively. KSH showed a lower ΔH than KSK and GA2. Although the high moisture content during HMT led to a decrease in ΔH for all samples, the ΔH s of GA2 and HCS easily decreased even at a low moisture content. We propose that hydrogen bonding in the starch granules was partially disrupted by HMT. The ΔH of GA2 treated under a 26 % moisture content was further reduced compared to other

Fig. 2. DSC endotherms of native and HMT samples.

HMT of all samples except GA2 was conducted at 120 °C for 24 h at a moisture content of 26 %. HMT of GA2 was conducted at 120 °C for 24 h at a moisture content of 22 %. The arrow shows the endothermic peak of the amylose-lipid complexes. KSH, Koshihikari; KSK, Koshinokaoi; GA2, Goami2; CS, commercial cornstarch; HCS, high amylose cornstarch.

moisture conditions. We thus propose that both the partial gelatinization and disruption of hydrogen bonding occurred simultaneously under a high moisture content.

X-ray diffraction patterns of native and HMT samples

X-ray diffraction patterns of native and HMT samples are shown in Fig. 3. Calcium fluoride was added as an internal standard for all samples, and was detected at a diffraction peak at 28.3°. Native KSH, KSK, and CS showed typical A-type crystalline patterns with diffraction peaks at 15.2°, 17.0°, 17.9°, and 23.2° (2 θ). On the other hand, native GA2 and HCS showed B-type crystalline patterns with diffraction peaks at 5.6°, 15.0°, and 18.0°. GA2 showed a B-type crystalline pattern due to the higher proportion of SLC in amylopectin, which was similar to that reported by Kim *et al.* (2005). The crystalline patterns of KSH, KSK, and CS were not changed by HMT, whereas those of GA2 and HCS changed from B-type to A-type, showing a reduction in the peak at 5.6°, a sharper peak at 15.2°, and a broader peak at 17° to 18°. Jiranuntakul *et al.* (2011) reported that the diffraction peaks of normal and waxy

Table 2. Gelatinization temperature (T_{gel}) of native and HMT samples

sample	moisuture (%)	native	HMT		
			8 h	16 h	24 h
KSH	14	66.5 ± 0.68 a	70.0 ± 0.14	73.1 ± 0.24	75.6 ± 0.38 b
	18		74.5 ± 0.25	76.3 ± 0.07	78.4 ± 0.34 c
	22		79.1 ± 0.24	81.8 ± 0.26	83.6 ± 0.11 d
	26		84.1 ± 0.19	85.8 ± 1.01	86.0 ± 0.92 e
KSK	14	76.4 ± 0.07 a	79.6 ± 0.23	81.8 ± 0.43	84.2 ± 0.30 b
	18		83.3 ± 0.09	85.6 ± 0.04	88.1 ± 0.10 c
	22		87.5 ± 0.15	91.3 ± 0.02	90.3 ± 1.10 d
	26		89.5 ± 0.06	91.7 ± 0.32	93.8 ± 0.54 e
GA2	14	75.5 ± 0.56 a	80.2 ± 0.50	81.1 ± 0.52	82.6 ± 1.07 b
	18		86.5 ± 0.71	88.8 ± 0.63	89.4 ± 0.37 c
	22		91.0 ± 0.63	92.9 ± 0.21	94.4 ± 0.36 d
	26		96.5 ± 0.28	98.7 ± 0.46	100.9 ± 0.18 e
CS	14	70.0 ± 0.28 a	72.6 ± 0.40	77.8 ± 0.39	82.0 ± 0.40 b
	18		78.0 ± 0.12	83.7 ± 0.07	90.0 ± 0.50 c
	22		83.5 ± 0.07	88.2 ± 0.16	93.5 ± 0.04 d
	26		84.7 ± 0.50	89.2 ± 0.25	94.9 ± 0.21 e
HCS	14	71.6 ± 0.39 a	79.1 ± 0.49	87.1 ± 0.33	91.3 ± 0.52 b
	18		87.1 ± 0.48	96.2 ± 0.45	100.6 ± 0.46 c
	22		92.7 ± 0.14	102.3 ± 0.16	105.3 ± 1.17 d
	26		117.6 ± 2.70	115.8 ± 2.47	113.6 ± 1.07 e

Each value represents the average ± standard deviation (n = 3). Different letters indicate significant differences among native and HMT (24 h) samples, as shown by Tukey's test ($p < 0.05$). KSH, Koshihikari; KSK, Koshinokaori; GA2, Goami2; CS, commercial cornstarch; HCS, high amylose cornstarch.

potato starches transformed from B-type to A-type by HMT. Takahashi *et al.* (2005) reported that increases in diffraction peaks at 13° and 19° in autoclave-treated rice flour were attributable to amylose-lipid complexes. Therefore, the peaks at 13.2° and 19.9° in all samples are thought to be amylose-lipid complexes.

The relative crystallinity of the native and HMT samples is shown in Table 4. In the native samples, GA2 had a lower relative crystallinity than KSH and KSK. A similar observation was reported by Kang *et al.* (2003), which might be attributable to SLC in GA2 amylopectin. KSH and KSK showed a decrease in relative crystallinity by HMT, whereas GA2 showed an increase. Gunaratne and Hoover (2002) reported that the relative crystallinity of potato and yam starches was reduced by HMT, while that of taro and cassava starches was unchanged. These results indicate that the effect of HMT on relative crystallinity differs depending on the structure of the starch, amylose content, and branched structure of amylopectin.

Iodine absorption characteristics The iodine absorption characteristics of native and HMT samples are shown in Table

5. In the native rice samples, the λ_{max} of KSK was longer than that of KSH or GA2. Fukahori *et al.* (1996) reported that high molecular weight amyloses tended to have a longer wavelength of λ_{max} , indicating that the actual amylose content of KSK was higher than that of KSH or GA2. In the native samples, the actual amylose content of rice samples tended to be lower than that of the cornstarch samples. The absorbance at λ_{max} for native GA2 was higher than that for KSH or KSK, which is due to the reaction of iodine and SLC in amylopectin of GA2. The λ_{max} and absorbance at λ_{max} for KSH and KSK did not change after HMT, whereas the λ_{max} of GA2, CS, and HCS was significantly shifted to a lower wavelength ($p < 0.05$) and the absorbance at λ_{max} became lower ($p < 0.05$). We propose that the reaction with iodine decreased because HMT caused the collapse of the helical structure of starch glucan and/or the invasion of linear fatty acid.

Given these results, we suggest that the increase in RS by HMT is related to the degree of change in gelatinization temperature, crystallinity, and structure of starch. However, the drastic changes also included gelatinization of starch, which

Table 3. Endothermic enthalpy (ΔH) of native and HMT samples

sample	moisuture (%)	native	HMT		
			8 h	16 h	24 h
KSH	14	10.0 $\pm$ 1.30 ab	10.2 $\pm$ 0.76	10.6 $\pm$ 0.57	11.5 $\pm$ 0.85 a
	18		11.7 $\pm$ 0.32	11.9 $\pm$ 0.52	8.6 $\pm$ 0.98 b
	22		6.7 $\pm$ 0.29	5.9 $\pm$ 0.31	4.5 $\pm$ 0.18 c
	26		1.5 $\pm$ 0.04	1.5 $\pm$ 0.18	1.2 $\pm$ 0.22 d
KSK	14	16.5 $\pm$ 1.04 a	15.2 $\pm$ 1.06	15.6 $\pm$ 1.02	15.3 $\pm$ 0.60 a
	18		15.5 $\pm$ 1.27	14.4 $\pm$ 0.64	13.2 $\pm$ 0.53 b
	22		9.7 $\pm$ 0.67	8.7 $\pm$ 0.33	8.4 $\pm$ 0.53 c
	26		5.4 $\pm$ 0.20	4.8 $\pm$ 0.42	3.7 $\pm$ 0.69 d
GA2	14	15.5 $\pm$ 1.21 a	8.8 $\pm$ 0.71	9.0 $\pm$ 0.33	9.0 $\pm$ 0.85 b
	18		9.2 $\pm$ 1.18	8.7 $\pm$ 0.67	9.2 $\pm$ 0.36 b
	22		8.3 $\pm$ 0.46	9.5 $\pm$ 0.96	8.7 $\pm$ 0.44 b
	26		6.4 $\pm$ 0.55	5.6 $\pm$ 0.10	4.9 $\pm$ 0.63 c
CS	14	16.6 $\pm$ 0.74 a	18.4 $\pm$ 0.97	18.5 $\pm$ 0.28	16.2 $\pm$ 0.32 a
	18		18.7 $\pm$ 0.84	15.8 $\pm$ 0.65	13.7 $\pm$ 0.42 b
	22		10.6 $\pm$ 0.76	10.8 $\pm$ 0.55	8.1 $\pm$ 0.27 c
	26		3.2 $\pm$ 0.20	3.4 $\pm$ 0.13	2.0 $\pm$ 0.11 d
HCS	14	13.5 $\pm$ 1.60 a	6.7 $\pm$ 0.42	5.3 $\pm$ 0.02	4.6 $\pm$ 0.36 b
	18		5.8 $\pm$ 0.91	8.2 $\pm$ 0.47	6.0 $\pm$ 0.38 b
	22		5.7 $\pm$ 0.60	5.6 $\pm$ 0.66	5.0 $\pm$ 0.30 b
	26		7.3 $\pm$ 0.87	5.7 $\pm$ 0.69	4.7 $\pm$ 0.12 b

Endothermic enthalpy (ΔH) is calculated in terms of dry weight basis. Each value represents the average $\pm$ standard deviation ($n = 3$). Different letters indicate significant differences among native and HMT (24 h) samples, as shown by Tukey's test ($p < 0.05$). KSH; Koshihikari, KSK; Koshinokaori, GA2; Goami2, CS; commercial cornstarch, HCS; High amylose cornstarch

did not always lead to an increase in RS.

In this study, we conducted HMT using four moisture conditions for three rice cultivars with different starch structures to identify the most suitable rice cultivar and processing condition to increase RS. The gelatinization temperature increased because of HMT, which was responsible for the increased RS. The RS content of the ALC rice cultivar GA2 increased by HMT at a lower moisture condition up to about 11.0%. Therefore, GA2 is promising as a bio-functional rice material. We propose that the increase in RS due to HMT was related to the susceptibility (changes in crystal structure and chain length of starch) to HMT as well as the original properties of the starch.

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Fig. 3. X-ray diffraction patterns of native and HMT samples. HMT of all samples except GA2 was conducted at 120 °C for 24 h at a moisture content of 26 %. HMT of GA2 was conducted at 120 °C for 24 h at a moisture content of 22 %. KSH, Koshihikari; KSK, Koshinokari; GA2, Goami2; CS, commercial cornstarch; HCS, high amylose cornstarch.

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Table 4. Relative crystallinity of native and HMT samples

	15.0°		17.9°	
	native	HMT	native	HMT
KSH	0.404 ± 0.005 a	0.357 ± 0.019 a	0.470 ± 0.010 a	0.404 ± 0.020 b
KSK	0.494 ± 0.025 a	0.417 ± 0.024 b	0.573 ± 0.016 a	0.480 ± 0.044 b
GA2	0.355 ± 0.026 a	0.374 ± 0.010 b	0.420 ± 0.022 a	0.464 ± 0.025 a
CS	0.460 ± 0.043 a	0.484 ± 0.027 b	0.549 ± 0.044 a	0.556 ± 0.043 a
HCS	0.383 ± 0.021 a	0.461 ± 0.014 b	0.446 ± 0.031 a	0.540 ± 0.023 b

HMT was conducted at 120 °C for 24 h at a moisture content of 22 % (all samples except GA2) or 26 % (GA2). Each value was calculated from sample peak (15.0° or 17.9°) and internal standard peak (CaF₂, 28.3°), and represents the average ± standard deviation (n = 3). Different letters indicate significant differences between native and HMT samples, as shown by *t*-test (*p* < 0.05). KSH; Koshihikari, KSK; Koshinokaori, GA2; Goami2, CS; commercial cornstarch, HCS; High amylose cornstarch

Table 5. Iodine absorption characteristics of native and HMT samples

	λmax(nm)		absorbance λmax	
	native	HMT	native	HMT
KSH	553.7 ± 0.6 a	552.7 ± 0.6 a	0.283 ± 0.006 a	0.275 ± 0.005 a
KSK	579.3 ± 1.5 a	579.7 ± 1.5 a	0.401 ± 0.009 a	0.389 ± 0.005 a
GA2	568.0 ± 1.0 a	563.3 ± 0.6 b	0.514 ± 0.002 a	0.493 ± 0.005 b
CS	593.7 ± 1.5 a	582.0 ± 1.0 b	0.484 ± 0.006 a	0.362 ± 0.006 b
HCS	601.0 ± 1.0 a	565.7 ± 1.5 b	0.870 ± 0.013 a	0.341 ± 0.009 b

HMT was conducted at 120 °C for 24 h at a moisture content of 22 % (GA2) or 26 % (all samples except GA2). Each value represents the average ± standard deviation (n = 3). Different letters indicate significant differences between native and HMT samples, as shown by *t*-test (*p* < 0.05). KSH; Koshihikari, KSK; Koshinokaori, GA2; Goami2, CS; commercial cornstarch, HCS; High amylose cornstarch

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