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Physicochemical characteristics of waxy rice starch influencing the *in vitro* digestibility of a starch gel

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ABSTRACT

Starches were isolated from three waxy rice varieties: Koganemochi (Kog), Hakuchomochi (Hak), and Kantomochi 172 (K172). Forty percent starch gels were prepared and the extent of starch gel digestibility was determined by an *in vitro* method. The distribution of chain lengths of amylopectin was analyzed and differential scanning calorimetry was used to analyze gelatinization and retrogradation of waxy rice starch. The K172 gel had significantly higher resistance to hydrolysis than had the other gels. The K172 starch contained lower proportions of the short chains of amylopectin and showed higher gelatinization temperature and enthalpy. The retrogradation peak was measured using waxy rice starch gels stored for 1 and 7 days at 5 °C. The K172 gel was observed to retrograde more quickly and to have a greater extent of retrogradation than the other gels. The difference in amylopectin chain length distribution and recrystallinity contributed to the variation in the starch gel digestibility of waxy rice.

1. Introduction

Starch is a major component of rice and an important structural constituent in many rice products. Starch digestion is a highly important metabolic response and the rate and extent of starch digestibility and absorption in the small intestine are nutritionally important. The most desirable starch ingredient, from the nutritional point of view, is believed to be slowly digestible starch (SDS) (Jenkins et al., 1982; Lehmann & Robin, 2007). The development of new techniques to alter starch digestibility in food products and new materials resistant to digestion are nutritionally beneficial issues. The extent and rate of starch digestibility are measured by *in vitro* procedures, developed in attempt to mimic human digestion (Englyst, Kingman, & Cummings, 1992; McCleary & Monaghan, 2002). In such procedures, pancreatic α-amylase digestion is frequently used, followed by the measurement of released glucose. The susceptibility of native starch granules to amylolytic enzyme has been studied and starch with higher amylose content is known to have less susceptibility to enzymatic hydrolysis. When starch granules are heated in excess water, granules swell and gelatinize. During the gelatinization process, the molecular order of the starch granule is destroyed and starch is easily digested. When cooling a sufficiently concentrated suspension of gelatinized starch, starch retrogradation occurs, resulting in gel formation. Retrograded starch is common in the human diet, because it is formed by cooking or food processing. Therefore, the digestibility of retrograded starch is an essential property of food products. During the retrogradation process, the resistance to the enzymatic hydrolysis arises from a change in the physical state of the starch, including the crystallization of amylose and amylopectin (Berry, I'Anson, Miles, Morris & Russell, 1988). The resistance to starch digestibility after retrogradation has been extensively studied, with a focus on retrograded amylose (Eerlingen, Crombez, & Delcour, 1993; Sievert & Pomeranz, 1989).

Waxy rice, also known as sticky or glutinous rice, is widely used for food products, such as glutinous rice cakes (mochi) in East and Southeast Asia, and particularly Japan. Waxy rice starch consists almost entirely of amylopectin and the crystallization of amylopectin contributes to the resistance to the enzymatic digestibility of starch. The digestibility of a concentrated starch gel prepared from waxy rice could provide useful information on waxy rice products, because the water content of rice cake, a common waxy rice product, is ≈55% and the starch concentration is high. The objective of this study was to investigate the effects of starch characteristics on the *in vitro* digestibility of concentrated starch gels prepared from three characteristic waxy rice varieties.

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2. Materials and methods

2.1. Materials

Three waxy rice varieties, Koganemochi (Kog), Hakuchomochi (Hak), and Kantomochi 172 (K172), were used to isolate starch. Kog and Hak are common paddy waxy rice varieties for making rice cakes, in Japan, with different textures, whereas K172 is an upland waxy rice variety produced by crossing japonica waxy rice Tsukuba-hatamochi and tropical japonica non-waxy rice IRAT109 (Okamoto & Nemoto, 1998). Kog (Niigata, Japan) and Hak (Hokkaido, Japan) were obtained from farmers. K172 was grown at the National Institute of Crop Science (Tsukuba, Japan). Starch samples were isolated, based on the alkaline-steeping method of Patindol, Wang, Siebenmorgen, and Jane (2003), then freeze-dried.

2.2. Chemical analysis

The amylose content and α-amylase activity of isolated starches were determined using assay kits (Megazyme International Ireland Ltd., Ireland) by the methods of Gibson, Solah, and McCleary (1997), and McCleary and Sheehan (1987), respectively.

The chain length distribution of amylopectin in isolated starch was analyzed by high-performance anion-exchange chromatography on a CarboPac PA-100 column equipped with a pulsed-amperometric detector (HPAEC-PAD, Dionex, USA). The column was eluted at 1 ml/min with a linear gradient of 150–360 mM sodium acetate in 100 mM NaOH. Isolated starch from waxy rice (~50 mg) was weighed into a screw-cap test tube and dissolved in 2 ml of 90% dimethyl sulfoxide with gentle stirring overnight at room temperature. After adding 8 ml of 95% ethanol, the tube was allowed to stand for 1 h at room temperature and centrifuged at 1,500*g* for 10 min. The supernatant was decanted, and the residue washed five times with 95% ethanol, and then vacuum-dried. Pretreated starch (5 mg) was weighed into a screw-cap test tube and dissolved in 1.875 ml of distilled water by heating at 100 °C for 1 h. After cooling to room temperature, 0.125 ml of 1 M acetate buffer (pH 3.6) and 1 μl of isoamylase (I2758, Sigma, USA) were added. The tube was incubated at 37 °C overnight and heated at 100 °C for 5 min to inactivate the enzyme. After cooling to room temperature, the mixture was filtered through a Millex-GP filter unit (0.22 μm, Millipore, USA) and used for HPAEC-PAD analysis.

2.3. Preparation of starch gels

A 40% (w/w) starch suspension, on a dry basis, was stirred continuously, at 500 rpm for 30 min, using a magnetic stirrer, then heated in water bath at 52 °C for 1–2 min with continuous stirring at 400 rpm until the suspension became thick enough to prevent the starch from settling. The paste was placed between two glass plates with a 1 mm spacer, sealed in an airtight bag, and heated in a temperature chamber at 100 °C for 15 min, followed by storage at 5 °C for 1 and 7 days.

2.4. Differential scanning calorimetry measurement

Differential scanning calorimetry (DSC) measurement was conducted using a PerkinElmer DSC 7 RS fitted with an intracooler. The instrument was calibrated with octadecane and indium. For gelatinization studies, 30 mg amounts of 40% starch suspension (w/w) were weighed into aluminium pans. After sealing, the pans were scanned at 10 °C/min, from 10 to 95 °C. For retrogradation studies, 30 mg of 40% starch gels, stored at 5 °C for 1 day and 7 days, were weighed into pans, and then scanned at 10 °C/min from 5 to 95 °C.

2.5. In vitro digestibility of starch gel

Starch gel (1.0 g) was weighed into a glass tube and 5 ml of 0.1 M sodium acetate buffer (pH 6.0) containing 1 mM $CaCl_2$ was added, then milled at 10,000 rpm for 2 min using an Ace homogenizer (Nihonseiki Kaisha Ltd., Japan). Then, 15 ml of 0.1 M sodium acetate buffer (pH 6.0) containing 1 mM $CaCl_2$ was added to the milled sample and the sample solution equilibrated at 25 or 37 °C for 10 min. Enzymatic hydrolysis was started by adding 1000 U of α-amylase (porcine pancreas, A6255, Sigma, USA) in 5 ml of 0.1 M sodium acetate buffer (pH 6.0) and carried out at 25 or 37 °C in a shaking water bath (100 strokes/min) for 20 min, 2, 7, and 24 h, after which the samples were immediately put in a boiling water bath for 5 min to inactivate the enzyme and cooled to room temperature. The pH of the hydrolyzed solution (0.5 ml) was adjusted to 4.5 by adding 0.1 M acetic acid and it was incubated with 5 U of amyloglucosidase (A1602, Sigma, USA) at 37 °C for 30 min. After incubation, ethanol (99.5%) was added and the sample solution centrifuged at 1,500*g* for 10 min. The glucose content of the supernatant was measured using a Megazyme glucose assay kit (GOPOD method). The rate of digestion (%) was defined as the ratio of digested starch (released glucose by enzyme hydrolysis × 0.9) to total starch weight (db). The total starch content was determined by an assay kit (Megazyme International Ireland Ltd., Ireland).

2.6. Statistical analysis

All samples were analyzed in duplicate. The general linear model (SAS Institute, USA) was used to analyze data. Analysis of variance was conducted using Tukey's studentized test at the 5% significance level.

3. Results and discussion

3.1. Chemical analysis of isolated waxy rice starch

The amylose contents of starches isolated from three waxy rice varieties, Kog, Hak, and K172, were 1.1%, 1.3%, and 1.7%, respectively. The amylose content of K172 was significantly higher than that of Kog (Table 1). No isolated starches exhibited high amylase activity (<0.002 U/g).

Fig. 1 shows the differences in amylopectin chain length distribution among the three waxy rice varieties. The amylopectin from K172 had a lower proportion of short chains below the degree of polymerization (DP) 12, but higher proportions of DP17-29 compared with Kog and Hak. A distinct difference between Kog and Hak was not observed, compared to the difference from K172 (Fig. 1). Amylopectin branch chains are classified into chain type as follows: A chain (DP6-12), B1 chain (DP13-24), B2 chain (DP25-36), and B3+ chain (DP ⩾ 37) (Hanashiro, Abe, & Hizukuri, 1996). Table 2 shows the peak area ratios of each chain type. The difference of A chain (DP6-12) percentage between K172 and the other two varieties was remarkable. The specific structural charac-

Table 1
Amylose content and thermal analysis of native starch gelatinization[A].

Sample	Amylose (%)	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)
Kog	1.1b[B]	55.4b	66.7b	81.8b	15.0b
Hak	1.3ab	41.6c	56.9c	76.7c	12.6c
K172	1.7a	61.6a	75.8a	88.3a	16.7a

[A] To (°C), Tp (°C), Tc (°C), ΔH (J/g) = onset, peak, and final gelatinization temperature, and gelatinization enthalpy.
[B] Values with the same letter in the same column do not differ significantly at $P < 0.05$.

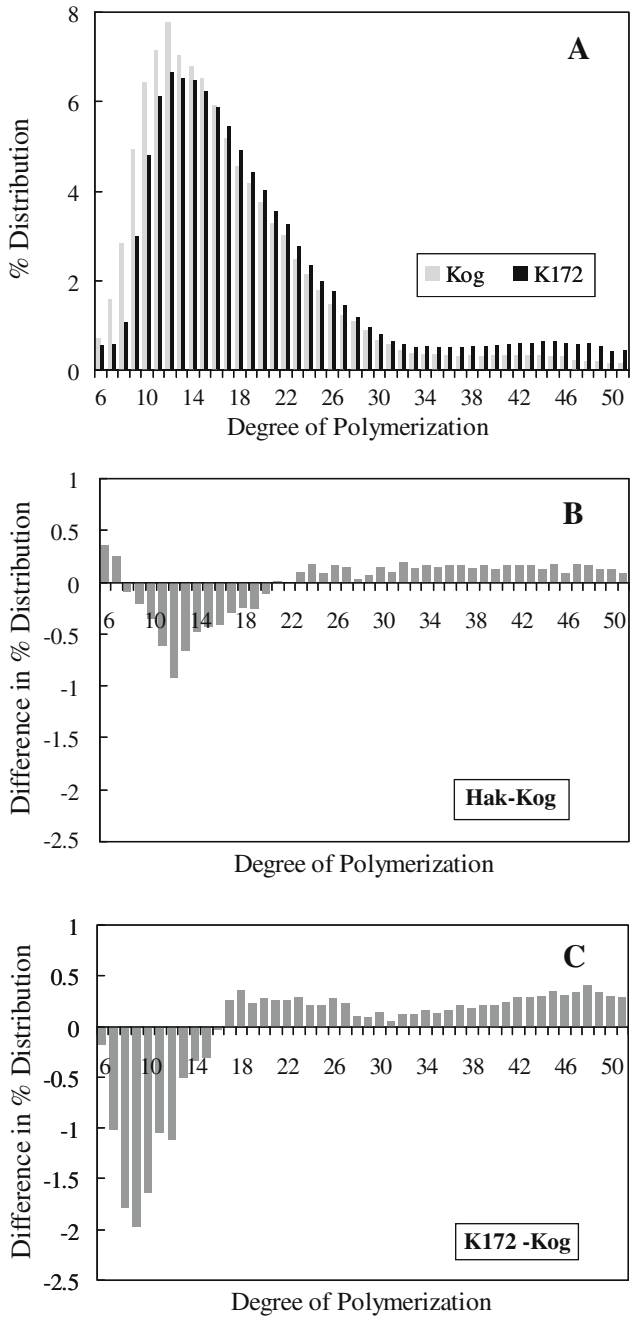

Fig. 1. Amylopectin chain length distribution of Kog and K172 (A), and the difference in the percentage distribution of amylopectin chain from Hak (B) and K172 (C) in comparison with Kog.

Table 2
Chain length distribution of amylopectin.

Sample	DP6-12 (A chain, %)	DP13-24 (B1 chain, %)	DP25-36 (B2 chain, %)	DP ⩾ 37 (B3+ chain, %)
Kog	31.5	54.8	9.5	4.2
Hak	30.1	52.5	11.1	6.4
K172	23.1	56.6	11.5	8.8

teristics of K172 amylopectin are consistent with the previous report (Okamoto, Kobayashi, Hirasawa, & Umemoto, 2002) that K172 had fewer short chains of amylopectin and more intermediate-length chains than had other Japanese waxy rice varieties. Okamoto et al. (unpublished work) observed no significant difference in the peak area ratios of longer chain (DP50-68) between K172 and other waxy rice varieties. Okamoto et al. (2002) suggested that the activity of starch synthase 2a is responsible for fewer short chains in amylopectin of K172 than in other varieties.

3.2. Gelatinization and retrogradation properties

Table 1 shows the gelatinization properties determined using DSC. DSC parameters recorded were onset (To), peak (Tp), final (Tc) gelatinization temperature, and gelatinization enthalpy (ΔH). Gelatinization temperature and enthalpies associated with gelatinization endotherms varied significantly among the three varieties; To was 41.6–61.6 °C, Tp was 56.9–75.8 °C, Tc was 76.7–88.3 °C, and ΔH was 12.6–16.7 J/g. Hak had markedly lower gelatinization temperatures and enthalpy, and a broader peak than had the other two varieties. On the other hand, K172 had much higher gelatinization temperatures and enthalpy, and a narrower peak. Kog showed characteristics of the DSC curve intermediate between Hak and K172 (Fig. 2A). The gelatinization enthalpy reflects both crystalline order and the level of amylopectin double-helical order (Cooke & Gidley, 1992). The relative amounts of amylopectin chain with DP6-9 were reported to be negatively correlated with the gelatinization temperature of rice starches (Vandeputte, Vermeylen, Geeroms, & Delcour, 2003a). The higher proportion of longer chains contributes to delayed gelatinization, because long double helices require a higher temperature to dissociate (Yuan, Thompson, & Boyer, 1993). Longer chains may form longer crystallites, resulting in more thermal energy to break the kinetic barrier due to the increased crystalline order (Sanders, Thompson, & Boyer, 1990), which means that the difference in amylopectin fine structure greatly influences gelatinization properties. The results of amylopectin chain distribution and DSC measurements suggest that the lower proportion of short chains and higher proportion of intermediate-length chains result in the higher gelatinization temperature and enthalpy of K172 starch. On the other hand, a marked difference in gelatinization properties was found between Kog and Hak starches. The difference in amylopectin chain length distribution cannot explain the much lower gelatinization temperature and enthalpy of Hak, because no marked difference in amylopectin chain length distribution was observed between Kog and Hak compared with the difference between K172 and each sample. Further studies on amylopectin structure and starch granule properties are needed to elucidate the difference in thermal characteristics.

Table 3 shows the gelatinization temperature and enthalpy in starch gels cooled for 1 and 7 days at 5 °C. Retrograded starch gels showed a much lower gelatinization temperature and enthalpy than did raw starches, meaning that they have weaker starch crystallinity, because the gelatinization enthalpy and temperature reflect the loss of molecular order and crystallite perfection (Cooke & Gidley, 1992). Starch gel prepared from K172 retrograded quickly and showed a greater extent of retrogradation than did Kog and Hak starch gels (Fig. 2B and C). For 1-day gels, K172 showed substantially higher enthalpy of melting of recrystallized starches and higher Tc than the other starch gels. Since Hak starch gel stored for 1 day showed an extremely shallow and broad peak, gelatinization temperature and enthalpy could not be accurately evaluated. For 7-day gels, K172 showed significantly higher values of ΔH, Tp, and Tc than did the other starch gels (Table 3). Starch retrogradation occurs when starch molecules begin to reassociate in an ordered structure. The chain length distribution of amylopectin influences the retrogradation rate. Shi and Seib (1992) demonstrated that gelatinization enthalpy, after retrogradation, was directly proportional to the mole fraction of DP14-24 unit chains and negatively to that of DP6-9 unit chains. Vandeputte, Vermey-

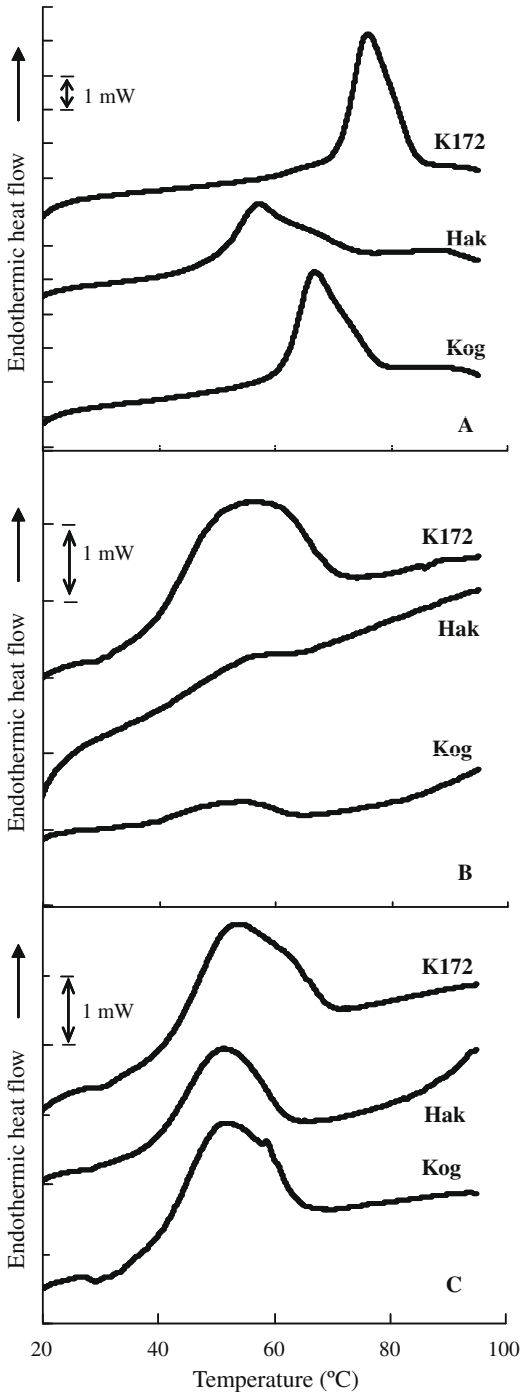

Fig. 2. Differential scanning calorimetry profiles of 40% waxy rice starch suspension (A), 40% starch gel stored for 1 day (B) and 7 days (C).

len, Geeroms, and Delcour (2003b) indicated that the relative amount of amylopectin chains with DP6-9 and DP > 25 decreased and the amount with DP12-22 increased amylopectin retrogradation enthalpy. The minimum chain length required for the formation of double helices was concluded to be DP10 by Gidley and Bulpin (1987), meaning that short chains in amylopectin retard retrogradation and inhibit the recrystallization of gelatinized starch. The results indicate that the amylopectin from K172 can be highly recrystallized for a short storage period of only 1 day. The analysis of amylopectin chain distribution demonstrated that the starch sample from K172 had much lower proportions of short chains than had Kog and Hak. The difference in amylopectin structure reflected the distinctly greater extent of recrystallization of K172 starch.

Table 3
Thermal analysis of retrogradation for starch gels stored at 5 °C for 1 and 7 days[A].

Sample	To (°C)	Tp (°C)	Tc (°C)	*H* (J/g)
1 day-stored gel				
Kog	30.8	53.4	66.0	3.1
Hak	–	–	–	–
K172	31.1	53.9	72.9	12.0
7 day-stored gel				
Kog	32.4a[B]	50.8b	66.7b	11.7b
Hak	29.3b	50.9b	64.0b	8.8c
K172	30.0b	53.0a	72.8a	15.2a

[A] To (°C), Tp (°C), Tc (°C), ΔH (J/g) = onset, peak, and final gelatinization temperature, and gelatinization enthalpy.
[B] Values with the same letter in the same column do not differ significantly at $P < 0.05$.

3.3. In vitro digestibility of starch gel

Starch gels stored for 1 and 7 days were employed in this study to compare the rates of digestion between waxy rice varieties. The rate of digestion by pancreatic α-amylase was determined at 25 °C in addition to the optimum temperature (37 °C), because the onset gelatinization temperature of starch gels by DSC was below 37 °C, except for the 1- day stored gel of Hak (Table 3), meaning that recrystallized amylopectin partially starts to melt at 37 °C. The results at 25 °C are useful for estimating the influence of recrystallized amylopectin, without melting, on enzyme accessibility. Table 4 shows the results of enzyme hydrolysis in 1- and 7- day stored starch gels when incubated at 25 and 37 °C, for comparison. Starch gels stored for 1 day were entirely digested after 7 h at both 25 and 37 °C, whereas 7- day stored gels were resistant to complete digestion after 24 h of enzymatic treatment. The hydrolysis rate of 7- day stored gels was slower than the 1- day stored gel, which shows that the retrogradation of amylopectin had a strong impact on the *in vitro* digestibility of a starch gel. For 1- and 7- day stored gels, K172 showed distinctly higher resistance to hydrolysis by α-amylase than did other waxy rice varieties during the course of 24 h hydrolysis. The rate of digestion of K172 starch gel stored for 1 day was significantly lower than those of Kog and

Table 4
The rate of digestion (%) of starch gels stored at 5 °C for 1 and 7 days.

Sample	Duration			
	20 min	2 h	7 h	24 h
1 day-stored gel				
At 37 °C				
Kog	77.1a[A]	95.9a	–	–
Hak	75.5a	87.5a	–	–
K172	57.7b	88.0a	–	–
At 25 °C				
Kog	37.8a	85.3a	–	–
Hak	43.2a	91.6a	–	–
K172	28.6b	67.9b	–	–
7 day-stored gel				
At 37 °C				
Kog	43.1ab	80.1a	92.0a	94.4a
Hak	49.9a	79.3a	86.8a	95.0a
K172	35.6b	63.0b	79.3a	87.6a
At 25 °C				
Kog	22.4b	45.8ab	52.8ab	87.2ab
Hak	36.0a	49.5a	54.0a	90.5a
K172	18.1b	36.5b	46.3b	73.8b

[A] Values with the same letter do not differ significantly at $P < 0.05$ among three varieties.

Hak after 20 min of hydrolysis at 25 and 37 °C and 2 h at 25 °C (Table 4). For 7- day stored gels, there were significant differences between K172 and the other two starch samples after 2 h of incubation at 37 °C, whereas marked differences in digestibility were not observed between Kog and Hak. The rate of hydrolysis at 25 °C was much slower than that at the optimum temperature (37 °C) for α-amylase, but 1- day stored gels were largely hydrolyzed in 2 h of enzymatic treatment. The gel prepared from K172 starch was found to be least susceptible to enzymatic digestion before and after the onset of melting of recrystallinity. For reference, the digestibility of native starch isolated from three varieties was also determined at 37 °C by the same method. The native starch was entirely digested after 7 h. The rates of digestion of Kog, Hak, and K172 were 88.8%, 87.4% and 55.1% after 20 min of enzyme treatment and 90.6%, 89.2%, and 87.1% after 2 h, respectively. K172 showed significantly higher resistance to enzyme hydrolysis in the native state as well as in starch gel.

In the case of starch gels, enzyme susceptibility depends on the diffusion rate into the gel network and penetration into the starch granules. The enzymatic susceptibility of starch gel decreases with increasing extent of retrogradation, because the entanglement of the molecules is reinforced and the increase in molecular order results in the formation of double helix and crystallites (Eerlingen, Jacobs, & Delcour, 1994). In this study, waxy rice starch gel prepared from K172 showed distinctly higher enzyme resistance (Table 4) and melting enthalpy of recrystallized starch (Fig. 2). For 7- day stored starch gels, the rate of hydrolysis (%) after 20 min incubation at 37 °C decreased linearly with the increase in enthalpy for melting recrystallized starches ($r = -0.999^{*}$). The observed relationship was consistent with the previous report by Eerlingen et al. (1994), using waxy maize starch, which showed that increased melting enthalpy of retrograded amylopectin caused a reduction in *in vitro* starch digestibility. This indicates that the increased crystallinity in starch gels might restrict both the diffusion and penetration of the enzyme. As shown in Table 4, the high resistance to enzymatic digestion of K172 was observed for both 25 and 37 °C incubation. The crystal structure of starch is considered to be partially disrupted at 37 °C, which is above the gelatinization onset temperature, and almost entirely maintained at 25 °C. Under both conditions, K172 displayed a lower degree of hydrolysis by α-amylase than did the other two waxy rice starches, which suggests that recrystallized amylopectin of K172 may have a fairly rigid structure. Waxy rice starch consists mainly of amylopectin and the retrogradation of amylopectin dramatically influences the properties of waxy rice starch gel. In the amylopectin retrogradation process, the formation of double helices of the outer branches induces the rigidity of the starch granule and recrystallized amylopectin reinforces the continuous starch network in the gel (Hug-Iten, Escher, & Conde-Petit, 2003). The greater rigidity of the recrystallized amylopectin was considered to induce the decrease in enzyme susceptibility of K172 starch gel.

Starch digestibility is influenced by the proportions of amylopectin unit-chains. The hydrolysis rate of starch by α-amylase was reported using starches from different botanical sources to be positively and negatively correlated with the proportions of DP8-12 and DP16-26, respectively (Srichuwong, Sunarti, Mishima, Isono, & Hisamatsu, 2005). Jane, Wong, and McPherson (1997) suggested that the more branch linkages and short double helices derived from the short chains (DP6-12) in the crystalline region increase the weak points of the starch crystalline structure, resulting in greater susceptibility to enzymatic hydrolysis. In this study, the starch isolated from K172, with higher resistance to enzymatic digestion, had lower proportions of the short chains (DP6-12) than did the other waxy rice varieties. This finding shows that more long chains could increase hydrogen bonds between chains, due to making long helices, and reinforce the recrystallized region in the K172 starch gel. Consequently, the results of this study demonstrate that distinct differences in recrystallinity and the extent of amylopectin retrogradation strongly influenced the differences in starch gel digestibility between waxy rice varieties, whereas a lower proportion of shorter chain in amylopectin contributed to the stable and higher recrystallinity, resulting in higher resistance to enzymatic digestion.

4. Conclusion

Starch gels were prepared from three waxy rice varieties and *in vitro* digestibility was determined using pancreatic α-amylase. The starch gel prepared from K172 had significantly higher resistance to enzymatic hydrolysis after 1 day and 7 day of storage than did the other two waxy rice varieties, Kog and Hak. The K172 starch gel was observed in DSC studies, to retrograde more quickly and have a greater extent of retrogradation than did the other two waxy rice varieties. The amylopectin from K172 starch had lower proportions of the short chains (DP6-12) and higher proportions of DP17-29. These results suggest that the differences in amylopectin chain distribution reflect the stability, perfection, and extent of recrystallinity in the starch gel, inducing a difference in digestibility of the starch gel. The unique waxy rice variety K172, with higher resistance to digestibility, may be useful in applications requiring altered rates of digestion in waxy rice products.

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