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Structural characteristics and glucose response in mice of potato starch modified by hydrothermal treatments

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ABSTRACT

The structural properties and digestibility of slowly digestible hydrothermally treated potato starch (SDS) were investigated. The potato starch with 20, 30 or 40% moisture content was heated at 100 °C for 30 min, and then kept at 30 °C or 70 °C for 12 h. The sample with 30% moisture content, heated and kept at 30 °C, produced the highest SDS content (37.5%). The modified products were analysed with scanning electron microscopy, X-ray diffraction, and differential scanning calorimetry (DSC). The cross-section showed a large hollow area and X-ray patterns were altered from B- to a mixture of B- and A-type. DSC of the heated samples demonstrated a broader gelatinisation temperature range compared with the heated-and-stored samples. *In vivo* glucose responses with mice correlated with the *in vitro* starch digestibility experiments. This study showed that structural changes during hydrothermal treatment of potato starch significantly affected digestibility and blood glucose levels in mice.

1. Introduction

Potato starch is widely used in food and industrial applications and has significant economic importance in the United States and Europe (Mitch, 1984). It has been reported that potato starch shows a B-type X-ray diffraction pattern and contains a high proportion of resistant starch (Sajilata, Singhal, & Kulkarni, 2006).

Starch is a major source of energy and nutrition for humans. For nutritional purposes, starch is classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) according to the rate of glucose release and its absorption in the intestinal tract (Englyst, Kingman, & Cummings, 1992). RDS is the starch fraction that causes an immediate increase in blood glucose levels after ingestion, and SDS is the starch fraction digested completely in the small intestine at a lower rate than RDS. RS refers to the starch fraction that cannot be digested in the small intestine, but is fermented in the large intestine into short-chain fatty acids (Cummings, Beatty, Kingman, Bingham, & Englyst, 1996). SDS and RS have significant implications in human health (Jenkins et al., 2002). They both are correlated with a low glycaemic index, which is important for the treatment and prevention of several diseases such as obesity, diabetes, and cardiovascular disease (Englyst et al., 1992; Han et al., 2006). The potential health benefits of SDS are linked to stable glucose metabolism, diabetes management, mental performance, and satiety (Jenkins et al., 2002; Lehmann & Robin, 2007; Seal et al., 2003).

Recently, studies on the production of SDS based on chemical, physical, and enzymatic methods have been reported (He, Liu, & Zhang, 2008; Miao, Jiang, & Zhang, 2009; Shin, Kim, Ha, Lee, & Moon, 2005). Among these methods, physical and chemical modifications are commonly used to produce starches with special properties. Although chemically modified starches are available for industrial purposes, the food and pharmaceutical industries prefer starches that have not been chemically altered. In contrast, physically modified starch using moisture, heat, shear, or radiation produces no chemical reagent waste during the modification process and as a result has gained broader acceptance than chemically modified starches (Adebowale, Afolabi, & Olu-Owolabi, 2005). Hydrothermal treatments, including heat-moisture treatment (HMT) and annealing (ANN), are physical modifications that change the physicochemical properties of starch without destroying its granule structure. A few authors have reported the effect of ANN and HMT on the formation of SDS (Chung, Hoover, & Liu, 2009; Chung, Liu, & Hoover, 2009; Niba, 2003; Shin et al., 2005). SDS level in starches and flours increased, depending on the temperature and moisture content of the hydrothermal treatment. Crystalline disruption in HMT tuber and root starches from a B- or C-type to a partial A-type-like structure markedly influences digestibility (Hoover & Vasanthan, 1994). A higher susceptibility of A-type crystallites to enzymatic hydrolysis, compared with that of B-type crystallites, has been reported (Lehmann & Robin, 2007; Zhang, Venkatachalam, & Hamaker, 2006). Changes in starch structure and

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properties from hydrothermal treatment vary depending upon the treatment condition. For example, potato starch has been shown to be more susceptible than legume or cereal starches towards HMT (Hoover & Vasanthan, 1994). Jacobs and Delcour (1998) reported that ANN starch was resistant to enzyme digestion and HMT starch exhibited an increase in enzymatic susceptibility due to reduced relative crystallinity. HMT alters the crystallographic pattern of the starch, which has been attributed to double helical reorientation within crystalline regions and/or to disruption of original double helices. The effect of hydrothermal treatment on potato starch was studied by Vermeylen, Goderis, and Delcour (2006), who observed broadening of the differential scanning calorimetry (DSC) gelatinisation endotherms and a considerable decrease in the crystallinity and its intensity. Niba (2003) researched the effect of heat treatment, storage temperature, and time on the digestibility of maize, potato, cocoyam, plantain, yam, and rice flour. For all samples, the SDS content increased with autoclaving and parboiling and was significantly reduced by microwaving, compared with untreated flour. Shin et al. (2005) reported that the hydrothermal treatment of sweet potato starch changed the X-ray pattern, relative crystallinity, gelatinisation enthalpy, swelling factor, and contents of RDS, SDS, and RS. Additionally, hydrothermal treatment followed by storage at 50% moisture and 55 °C for 12 h increased the SDS level, from 15.6% to 31.0%. However, the increased SDS in granular starch by hydrothermal treatment was not heat-stable. The insufficient thermal stability of SDS restricts its use as a food ingredient.

Most studies on hydrothermal treatment have used starches from different sources, such as rice, potato, cassava, wheat, maize, and sorghum. However, to our knowledge, no study has reported SDS formation in potato using hydrothermal treatment. The objective of the present study was to examine the effects of heat treatment and storage temperature on SDS formation and digestibility in relation to structural properties of potato starch.

2. Materials and methods

2.1. Materials

Potato starch was purchased from KMC Ingredients (Brande, Denmark). The enzymes used in the starch digestion were pancreatin (P7545, Sigma, St. Louis, MO, USA) and amyloglucosidase (AMG 300L, Novozymes Inc., Bagsvaerd, Denmark). All other chemicals were analytical grade reagents.

2.2. Methods

2.2.1. Hydrothermal treatment

The original moisture content of the potato starch was measured at 12.5% according to the AACC 44-15A method (American Association of Cereal Chemists, 2000). The moisture content of the potato starch was brought to 20, 30 or 40% by adding distilled water. After mixing, the samples were equilibrated overnight at ambient temperature in sealed glass containers. They were heated at 100 °C for 30 min in a dry oven, and kept at 30 °C or 70 °C for 12 h in a water bath. The storage temperature mentioned above was set at a level lower and higher than the optimised SDS formation gelatinisation temperature from the pre-experiment in which SDS formation had been tested at 5, 20, 30, 50, 70 and 90 °C. Then, potato starch was deep-frozen (−70 °C) and lyophilised. For control starch, a lyophilised heated potato starch skipping the storing process was used. Additionally, amorphous starch was prepared by autoclaving a 10% starch solution at 121 °C for 30 min prior to the lyophilisation process. All the samples were ground and sieved with a 100-mesh screen after drying.

2.2.2. Scanning electron microscopy

The surface structures of hydrothermally treated starches were observed by scanning electron microscopy (SEM). Starch samples were mounted on circular aluminum stubs with double sticky carbon tape, coated with a thin film of gold under vacuum, and examined with a scanning electron microscope (JSM 5410LV, Jeol, Tokyo, Japan) at an accelerating potential of 20 kV. The cross-section of a starch granule was prepared with a 2-μm thick stainless blade (ST 300, Dorco Co., Ltd., Seoul, Korea). This sample was observed using a field-emission scanning electron microscopy (Supra 55VP, Carl Zeiss, Oberkochen, Germany) at an accelerating potential of 3 kV.

2.2.3. X-ray diffraction

X-ray diffraction analysis was performed with an X-ray diffractometer (Model D5005, Bruker, Karlsruhe, Germany) operating at 40 kV and 40 mA producing Cu K_α radiation of 1.54 Å wavelength, scanning through the 2θ range of 3–30° and step time of 4 s. Relative crystallinity of starches was calculated using the method of Nara and Komiya (1983) using a peak-fitting software (Origin-version 7.5, OriginLab Co., Northampton, MA, USA).

2.2.4. Gelatinisation parameters

Gelatinisation parameters were measured using a differential scanning calorimeter (Phyris Diamond DSC, Perkin-Elmer Inc., Waltham, MA, USA). The calorimeter was calibrated with an indium standard. Water (40 μL) was added with a micropipette to a sample (10 mg) in an aluminum DSC pan, which was then sealed, reweighed and allowed to stand for 4 h at room temperature to attain an even distribution of water. The sample pan was heated from 30 to 120 °C at 5 °C/min with an empty pan as reference. Onset (T_0), peak (T_p), and conclusion (T_c) temperatures of gelatinisation as well as gelatinisation enthalpies (ΔH, J/g) were determined with the Phyris software.

2.2.5. Solid-state ^{13}C CP/MAS NMR

High-resolution solid-state ^{13}C CP/MAS NMR experiments were conducted using a Bruker DSX-400 (Bruker Instrument Inc., Billerica, MA, USA) equipped with CP-MAS accessories. NMR spectra were observed under cross-polarization (CP), magic angle sample spinning (MAS), and high power decoupling conditions. The samples were spun at a rate of 5 kHz at room temperature in a 4-mm rotor with a spectral width of 3.1 kHz. The acquisition time was 35 ms, time domain points, 2.2 k, and line broadening 10 Hz. Spectra were referenced to the high-field resonance of adamantine (29.5 ppm). The double helix to amorphous ratio was obtained by comparison between spectra of sample and amorphous starches (Gidley & Bociek, 1985). The data processing and calculation of integrated peak areas were performed using the MestRe-C package software (Mestrelab Research, Santiago de Compostela, Spain).

2.2.6. Measurement of starch digestibility

Starch digestibility was determined following the method described by Englyst et al. (1992), as modified by Shin et al. (2007). Briefly, pancreatin (1 g) was added to 12 mL distilled water and stirred with a magnetic stirrer for 10 min. The pancreatin solution was then centrifuged (1500 × *g*, 10 min). A portion of the cloudy supernatant (10 mL) was transferred to a conical flask containing 0.2 mL of amyloglucosidase and 1.8 mL distilled water. The digestibility of the hydrothermally treated starch was measured as follows: sodium acetate buffer (0.1 M, pH 5.2, 0.75 mL) and a glass bead were added to a microtube (2 mL) containing starch (30 mg, wet basis). The enzyme solution (0.75 mL) was added to the sample and incubated in a shaking incubator (240 rpm) for 240 min. The tubes were removed at 10, 20, 60, 120, and 240 min, boiled to

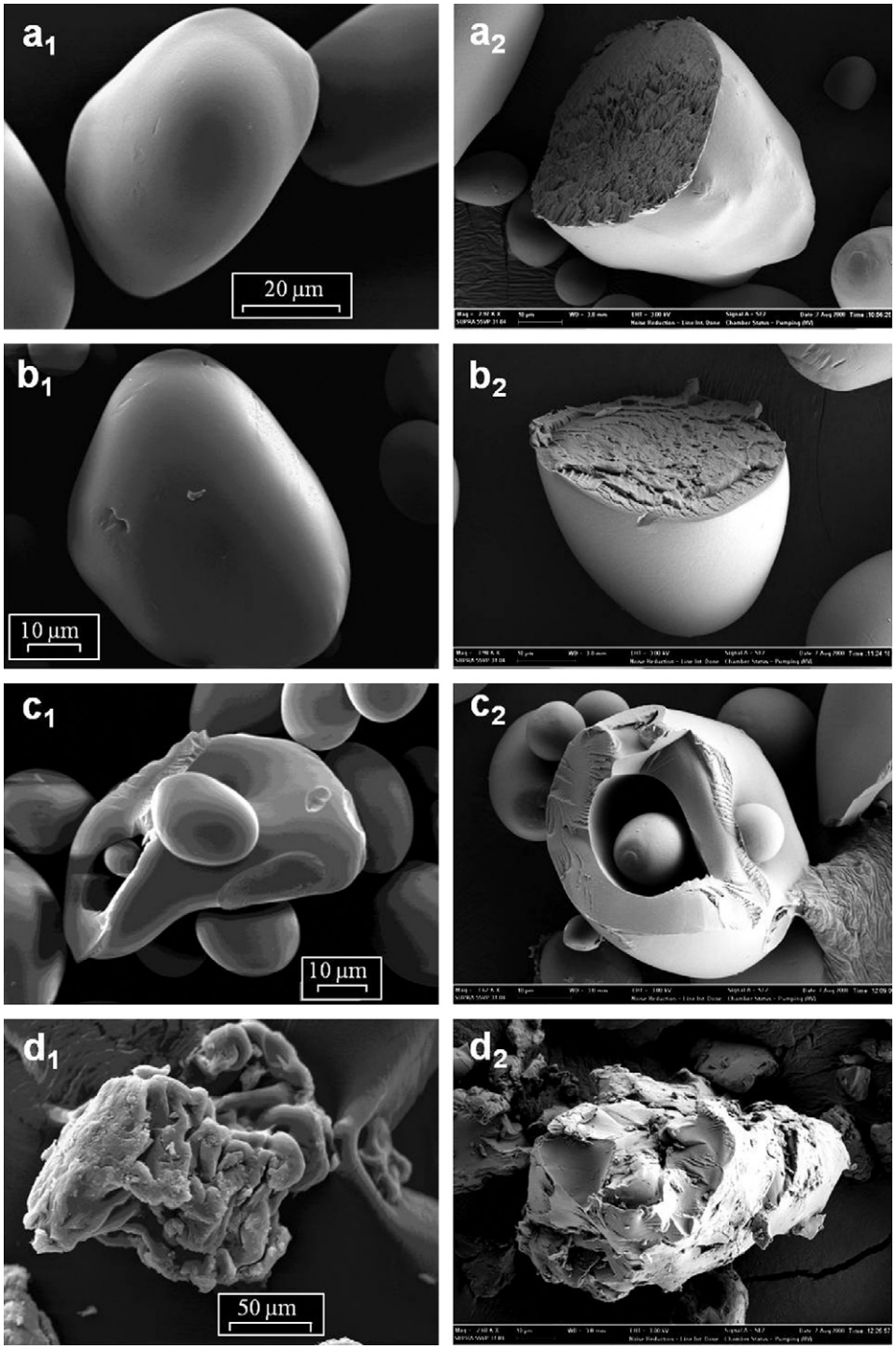

Fig. 1. Scanning electron micrographs (SEMs) of native and hydrothermally treated potato starches: (a) native, (b) 20% control, (c) 30% control and (d) 40% control. (1) SEM of surface of starch granules and (2) SEM of the cross-section of a starch granule.

stop the reaction, and centrifuged (5000 × *g*, 5 min). The glucose content of the supernatant was measured using a GOD-POD kit (BCS Co., Anyang, Korea). The RDS content was measured as the amount of glucose released after 10 min of digestion. The SDS fraction was defined as digested fraction between 10 min and 240 min of digestion. The unhydrolysed fraction after 240 min of digestion was measured as RS content.

2.2.7. Glucose responses in mice

Forty female mice (ICR mouse) were individually housed in an approved laboratory animal facility for a 7-day adaptation period. The mice were fasted for 12 h, and then given 0.5 mL sample suspension (7.5%, w/v) or glucose (7.5%) via an oral Zonde needle. Blood samples were taken from the tail vein of each mouse at 0, 30, 60, 90, 120, 150, 180, and 240 min. Blood serum glucose levels were measured with an Accu-Chek Active (Roche Ltd., Basel, Switzerland).

Glucose response was calculated by comparing the area under the blood glucose response curve of each sample as a percent of the response to the reference (glucose) using Origin 7.5 (Microbial, Northampton, MA, USA) based on the procedure described by the Food and Agriculture Organization (1998).

Table 1
Relative crystallinity of native and hydrothermally treated potato starches.

Sample	Relative crystallinity
Native	0.40 ± 0.02^{h}
20% Control	0.32 ± 0.00^{f}
20% 30 °C	0.31 ± 0.01^{f}
20% 70 °C	0.33 ± 0.00^{g}
30% Control	0.24 ± 0.00^{d}
30% 30 °C	0.22 ± 0.01^{c}
30% 70 °C	0.28 ± 0.01^{e}
40% Control	0.12 ± 0.01^{a}
40% 30 °C	0.13 ± 0.00^{ab}
40% 70 °C	0.14 ± 0.00^{b}

The values with different superscript are significantly different ($P < 0.05$) by Duncan's multiple range test.

2.2.8. Statistical analysis

All experiments were performed in triplicate, and mean values and standard deviations are reported. Analysis of variance (ANOVA) was conducted and the mean separations were analysed by Duncan's multiple range test ($P < 0.05$). A principal component analysis (PCA) of measured starch properties was performed to summarize the relationship between the sample and its digestibility. All statistical analyses were conducted using the SPSS software (ver. 12.0 for Windows; SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Scanning electron microscopy (SEM)

The surface and cross-section of starch granules of hydrothermally treated starches were investigated using SEM. The shape of native starch granules appeared to be elliptical with no evidence of cracks (Fig. 1a_1), and the cross-section of the starch granules showed no hollow area (Fig. 1a_2). Similarly, the granule shapes and cross-section of 20% moisture samples were not significantly different from those of native starch (Fig. 1b_1 and b_2). Lower moisture levels, which lessened the structural changes, explain these results. On the other hand, the surface of the samples treated at 30% moisture showed some cracks (Fig. 1c_1), and the cross-section of the starch granules displayed a large hollow region at the centre, which could have been caused by the hydrothermal treatment (Fig. 1c_2). It has been suggested that the hydrothermal treatment may cause transfer or arrangement of the molecular structure at the centre of the starch granules where the tissue structure is weak. At the same time, the crystallinity of the molecular chains in the outer tissue surrounding the hollow area is reinforced (Kawabata et al., 1994). The 40% moisture samples, however, exhibited aggregation of granules, and a distorted granular shape (Fig. 1d_1), and these samples did not alter the shapes during storage (data not shown). The original starch granule shape disappeared in most of the 40% moisture samples. Vermeylen et al. (2006) previously reported granule degradation in heat-moisture treated corn and potato starches, consistent with our observations.

3.2. X-ray diffraction and relative crystallinity

The X-ray diffraction patterns and relative crystallinity of the samples are displayed in Fig. 2 and Table 1, respectively. The diffraction pattern obtained for potato starch at 30% moisture appeared to be altered from a B-type to a combination of B- and A-type. Native potato starch showed the typical B-type X-ray pattern with reflection intensities at 5.5, 17, 19.3, and 22–24° 2θ angle. The 30% moisture samples displayed significantly changed X-ray diffractograms. The original peaks at 5.5 and 19.3° disappeared and the peaks at 22 and 24° shifted to 23°. This B-type to mixture of B- and A-type transformation in potato starch has been reported by other researchers, and the A- and B-type differs in their packing of double helices and water content (Vermeylen et al., 2006). All the control starches for each moisture level showed no significant difference in X-ray patterns compared to their corresponding sample starches that were stored at 30 °C and 70 °C.

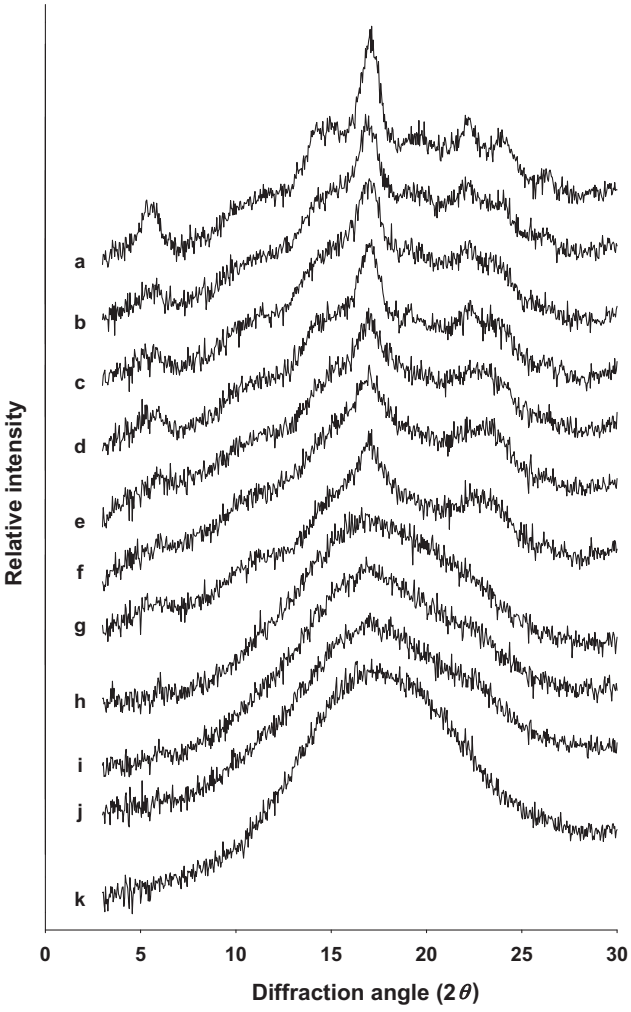

Fig. 2. X-ray diffraction patterns of native and hydrothermally treated potato starches: (a) native, (b) 20% control, (c) 20% 30 °C, (d) 20% 70 °C, (e) 30% control, (f) 30% 30 °C, (g) 30% 70 °C, (h) 40% control, (i) 40% 30 °C, (j) 40% 70 °C and (k) amorphous starch.

Hydrothermally treated starches showed a decrease in the intensities of the major peaks and thus their crystallinity. The noticeable changes from this process were that the peak intensities and crystallinity in the hydrothermally treated starches were influenced by the level of moisture content during the heat treatment (Fig. 2). More specifically, as the moisture content level increased, the level of crystallinity decreased. Regarding storage temperature, the level of crystallinity of the samples stored at 70 °C increased more than those that were stored at 30 °C, except the 40% moisture samples. Such results were presumably caused by more reorientation of double helices in the 70 °C samples than in the 30 °C samples. There was no significant difference within the 40% moisture samples at different storage temperatures because most of the crystallinity had been destroyed. However, intensity changes in the 20% samples were only marginal. The 20% moisture samples with 30 °C and 70 °C storage exhibited a weak B-type diffraction pattern. In contrast, 40% moisture samples showed the disappearance of peaks and a large decrease in relative crystallinity (Fig. 2and

Table 2
Thermal properties of native and hydrothermally treated potato starches.

Sample	T_0 (°C)	T_p (°C)	T_c (°C)	T_c–T_0 (°C)	ΔH (J/g)
Native	54.3 ± 0.1^{d}	58.6 ± 0.1^{d}	62.5 ± 0.2^{a}	8.2 ± 0.3^{bc}	14.4 ± 0.4^{g}
20% Control	56.8 ± 0.3^{f}	60.1 ± 0.1^{e}	64.3 ± 0.2^{c}	7.6 ± 0.4^{ab}	13.5 ± 0.1^{f}
20% 30 °C	56.5 ± 0.1^{f}	59.7 ± 0.1^{e}	63.6 ± 0.1^{b}	7.1 ± 0.1^{a}	13.1 ± 0.1^{f}
20% 70 °C	56.8 ± 0.1^{f}	59.9 ± 0.2^{e}	63.6 ± 0.2^{b}	6.8 ± 0.2^{a}	13.3 ± 0.1^{f}
30% Control	58.0 ± 0.4^{g}	62.2 ± 0.1^{f}	67.6 ± 0.2^{e}	9.6 ± 0.3^{d}	8.6 ± 0.1^{d}
30% 30 °C	55.2 ± 0.3^{e}	60.5 ± 0.2^{e}	64.0 ± 0.2^{bc}	8.8 ± 0.1^{cd}	9.6 ± 0.3^{e}
30% 70 °C	56.6 ± 0.1^{f}	62.2 ± 0.1^{f}	67.5 ± 0.3^{e}	10.9 ± 0.3^{e}	9.8 ± 0.2^{e}
40% Control	46.5 ± 0.7^{c}	55.6 ± 1.4^{b}	68.0 ± 0.6^{e}	21.5 ± 1.0^{f}	2.7 ± 0.1^{a}
40% 30 °C	44.7 ± 0.4^{a}	54.3 ± 0.5^{a}	66.0 ± 0.1^{d}	21.3 ± 0.4^{f}	4.3 ± 0.6^{c}
40% 70 °C	45.9 ± 0.5^{b}	56.9 ± 0.6^{c}	77.8 ± 0.9^{f}	32.0 ± 0.9^{g}	3.5 ± 0.2^{b}

The values with different superscripts within a column are significantly different ($P < 0.05$) by Duncan's multiple range test.

Table 1). The decrease in intensity and crystallinity indicated that the crystalline region of starch was disrupted by hydrothermal treatment. Some researchers have reported decreased intensities of X-ray diffraction for hydrothermally treated tuber starches (Gunaratne & Hoover, 2002; Hoover & Manuel, 1996; Lorenz & Kulp, 1982). It has been suggested that the double-helical movement during heat-moisture treatment could disrupt starch crystallites and change crystallite orientation (Gunaratne & Hoover, 2002; Lorenz & Kulp, 1982). This could explain the observed changes in crystallinity and peak intensity following hydrothermal treatment and storage at elevated temperatures.

3.3. Gelatinisation parameters

The gelatinisation parameters (T_0, T_p, T_c, ΔH) of various hydrothermally treated starches are shown in Table 2. The 20% moisture samples displayed increased T_0, T_p, T_c, and decreased gelatinisation enthalpies (ΔH) and gelatinisation temperature ranges (T_c–T_0) compared with native starch. Furthermore, T_0, T_p, T_c and T_c–T_0 in 30% moisture samples increased more than in 20% moisture samples and showed a greater decrease in ΔH. The storage of the samples at 70 °C appeared to have no effect on the T_p value. The decrease in T_c–T_0 in annealing could be attributed to crystalline perfection, whereas the increase in T_c–T_0 in HMT suggests melting of crystallites of different stability (Chung, Hoover, et al., 2009; Chung, Liu, et al., 2009). The increased gelatinisation parameters (T_0, T_p, T_c) of 30% moisture samples were consistent with previous studies on the HMT effect in tuber and root starches (Gunaratne & Hoover, 2002; Hoover & Manuel, 1996). Increased gelatinisation enthalpy suggested that new double helices were formed during the storing process. The 40% moisture samples had decreased T_0, T_p and ΔH and increased T_c and T_c–T_0, compared with those of the 20% and 30% moisture samples. At the 40% moisture level, the starch was almost totally converted from the ordered to the disordered form, as evidenced by the marked collapse of starch granules on micrographs (Fig. 1), the decrease in DSC enthalpy and the thermal transition peak, the disappearance of the B-type X-ray diffraction pattern, and the almost total loss of crystalline structure. Such changes in the starch samples occurred because starch granules were disrupted by the high moisture content during the hydrothermal treatment.

The total gelatinisation enthalpy change for the transition, determined from the area measurement of the endotherm, decreases from hydrothermal treatment (Donovan, Lorenz, & Kulp, 1983). The gelatinisation enthalpies of the heated samples decreased as the moisture level increased, whereas those of the stored samples increased. Such results may be caused by more loosely packed granules after hydrothermal treatment and it indicates that the extent of double-helical disruption was influenced by double-helical chain motions, which increased with the increase in moisture level (Hoover & Vasanthan, 1994). Storage after hydrothermal treatment at elevated temperatures produced a change in the endotherm, but the change was different when the storage was performed before rather than after hydrothermal treatment. The storage temperature contributed to altering the structure of the granule: in particular, the rearrangement and association of the A and B matrices. Thus, 30 °C-stored samples showed increases in ΔH and decreases in T_c–T_0 compared with controls. Jacobs and Delcour (1998) suggested that a change in the amorphous fraction by hydrothermal treatment was related to an increase in order with no increase in crystallinity. This implies that hydrothermal treatment changed the internal structure in the amorphous region of samples to a more rigid structure and enhanced the dense packing structure, compared with native starch.

The decrease in ΔH values in the hydrothermal treatment of potato starch reflects the loss of double helices and some crystallites. Lopez-Rubio, Flanagan, Gilbert, and Gidley (2008) recently suggested that ΔH is due to melting of imperfect amylopectin-based crystals, with potential contributions from both crystal packing and helix melting enthalpies. The changes in ΔH of heated-and-stored potato starch samples could be caused by the rigid form in granules resulting from the movement and reorientation of double helices.

In general, the extension of T_c–T_0 indicates that internal changes between the components of starch during the hydrothermal treatment may lead to the formation of crystallites with different stabilities (Hoover & Manuel, 1996). It has been reported that this phenomenon was due to the increase in the degree of heterogeneity of crystallite within the granules (Wongsagonsup, Varavinit, & BeMiller, 2008). Accordingly, a new type of helical organization was formed by the hydrothermal treatment. This result was greatly affected by the moisture content level and the storage temperature.

3.4. ^{13}C CP/MAS NMR

The ^{13}C CP/MAS NMR patterns for native and hydrothermally treated potato starches are shown in Fig. 3, together with comparative spectra from granular and amorphous potato starches. Resonance peaks in the regions 90–110, 67–90 and 58–67 ppm were assigned to C-1, C-2, -3, -4, -5, and C-6 sites, respectively (Gidley & Bociek, 1985). The lack of sharp signals is consistent with the relatively low level of crystallinity in the samples (Gidley & Bociek, 1988; Gidley & Bulpin, 1989). Gidley and Bociek (1985) showed that ^{13}C CP/MAS NMR spectra for granular starches can be interpreted as a composite of intensity features from ordered (double-helical) and non-ordered (amorphous single chain) materials. This was based primarily on chemical shift and line shape differences in C-1 and C-4 sites in double-helical and amorphous states. Resonance peaks in the regions 80–87 and 103–104 ppm are characteristic for non-double-helical (amorphous) material, while the signal at 99–102 ppm is characteristic for starch double helices (Gidley & Bociek, 1985; Gidley & Bociek, 1988; Gidley & Bulpin, 1989; Gidley et al., 1995). By comparison of the relative intensities

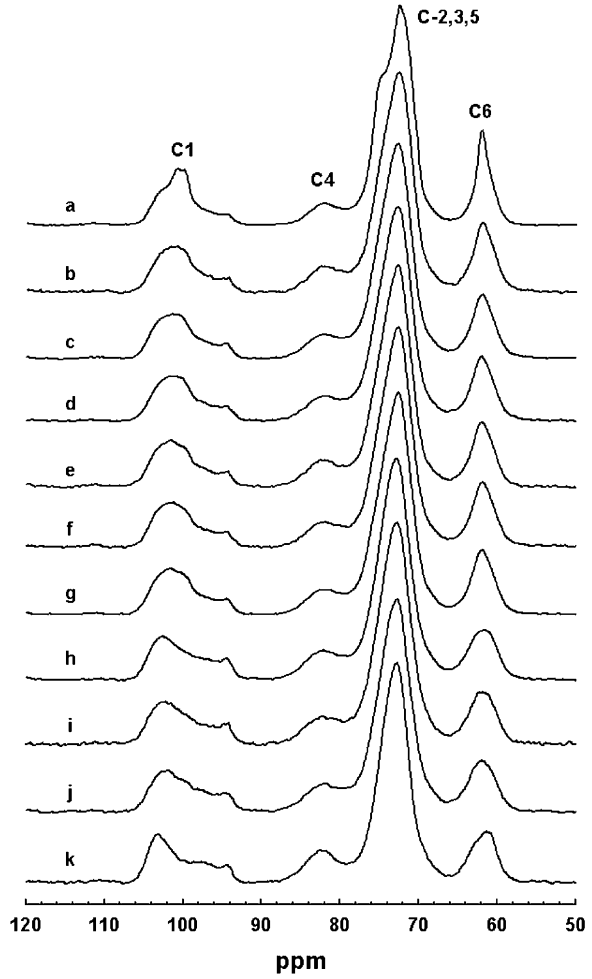

Fig. 3. ^{13}C CP-MAS NMR spectra of native and hydrothermally treated potato starches: (a) native, (b) 20% control, (c) 20% 30 °C, (d) 20% 70 °C, (e) 30% control, (f) 30% 30 °C, (g) 30% 70 °C, (h) 40% control, (i) 40% 30 °C, (j) 40% 70 °C and (k) amorphous starch.

of these signals for different samples, it is thus possible to compare the relative double-helix contents in starch samples. In hydrothermally treated potato starch samples (Fig. 3), relative C-1 intensity was lower in the range 99–102 and higher in the ranges 80–87 and 103–104 ppm. This indicates a lower double-helical content, compared with native starch. The double helix to amorphous ratio for native, 20, 30, and 40% moisture samples were estimated to be 57:43, 50:50, 48:52, and 41:59, respectively, indicating a decrease in the double-helical content of the hydrothermally treated samples with increasing moisture level. However, those of the stored samples were not significantly different from each other. On the basis of the work by Gidley and Bociek (1985), this indicated that there was either no change in double-helix content as a result of HMT or that it was below the detection limit.

3.5. Starch digestibility

The amounts of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) in native and hydrothermally treated potato starch are presented in Table 3. The RDS, SDS, and RS contents of native starch were 16.1, 5.4, and 78.5%, respectively. The RS of the hydrothermally treated starch decreased as the moisture level increased, whereas RDS and SDS increased, compared with native. The RDS, SDS, and RS in the 20% 30 °C sample were not significantly different from the control. However, the 20% 70 °C sample showed an increased RS content and a decreased SDS content, due to the association of amylose chains within the amorphous region. The value of the RDS in the 40% moisture samples suddenly increased, compared with that of other samples at different moisture levels; however, within the 40% moisture samples, the samples stored at 30 °C and 70 °C showed no significant difference from the control. The overall increase in RDS in the 40% sample was due to the increased enzyme susceptibility resulting from the disruption of the starch granules in the samples. The decrease in the relative crystallinities and the gelatinisation enthalpies followed the disruption of the starch granules (as shown in Tables 2 and 3), indicating disruption of crystallite and loss of double helices within the granule, respectively (Cooke & Gidley, 1992). Cooke and Gidley (1992) suggested that the initial step of α-amylase corresponds to the adsorption of α-amylase on the granule surface. Thus, crystallite disruption near the granule surface upon heat-moisture treatment of potato starch could facilitate the rapid entry of α-amylase into the granule interior (Gunaratne & Hoover, 2002).

Table 3
Starch fractions of native and hydrothermally treated potato starches.

Sample	RDS (%)	SDS (%)	RS (%)
Native	16.1 ± 0.8^{a}	5.4 ± 0.3^{a}	78.5 ± 1.0^{g}
20% Control	22.2 ± 0.8^{b}	13.2 ± 3.1^{d}	64.6 ± 2.6^{e}
20% 30 °C	23.8 ± 1.0^{bc}	12.3 ± 2.9^{cd}	64.0 ± 2.6^{e}
20% 70 °C	23.7 ± 1.7^{bc}	8.7 ± 3.1^{ab}	67.6 ± 3.5^{f}
30% Control	39.3 ± 0.6^{e}	28.0 ± 0.6^{e}	32.7 ± 3.0^{c}
30% 30 °C	35.2 ± 0.7^{d}	37.5 ± 2.4^{f}	27.4 ± 2.1^{b}
30% 70 °C	24.7 ± 1.9^{c}	35.1 ± 1.0^{f}	40.1 ± 1.1^{d}
40% Control	74.3 ± 1.1^{f}	10.3 ± 0.7^{bcd}	15.3 ± 0.4^{a}
40% 30 °C	75.6 ± 0.9^{f}	7.8 ± 0.9^{abc}	16.6 ± 0.5^{a}
40% 70 °C	75.8 ± 1.3^{f}	7.0 ± 1.1^{ab}	17.2 ± 0.3^{a}

The values with different superscripts within a column are significantly different ($P < 0.05$) by Duncan's multiple range test.

Most notably, the 30% samples exhibited higher SDS contents than 20% and 40% moisture samples. Increasing storage temperature of 30% moisture samples from 30 °C to 70 °C resulted in an increase in RS content, from 27.4 to 40.1%, and a slight decrease in SDS, from 37.5% to 35.1%. The SDS fraction of the 30% 30 °C sample showed the highest value of 37.5%. The increase in the RS fraction of 30% 70 °C sample may be a result of a partial association of amylose chains within the amorphous region, which would decrease accessibility to α-amylase. After all, depending on the temperature and the moisture conditions of the hydrothermal treatment, the SDS content of potato starch could be either increased or decreased as its enzyme susceptibility was affected. The influence of HMT on digestibility is determined by moisture content during HMT, temperature and duration of HMT, amylose–lipid interactions, and amylose–amylose and/or amylose–amylopectin interaction (Gunaratne & Hoover, 2002; Liu, Gu, Donner, Tetlow, & Emes, 2007). The increase in RDS and the decreases in SDS and RS levels from hydrothermal treatment suggest that crystallite disruption resulted from HMT, as evidenced by the decreased crystallinity (Fig. 2and Table 1), double helix (Fig. 3), and gelatinisation enthalpy (Table 2) (Chung, Hoover, et al., 2009; Chung, Liu, et al., 2009; Vieira & Sarmento, 2008). On the other hand, the decrease in RDS level and the increases in SDS and RS levels in the heated-and-stored samples were caused by the formation of rigid structures. The structural feature of SDS is an optimal mix of amorphous and semi-crystalline material (Lehmann & Robin, 2007). In most cases, A- or B-type crystallites markedly influence digestibility. A higher susceptibility of A-type crystallites to hydrolysis compared with that of B-type crystallites has been reported (Jane, Wong, & McPherson, 1997; Vieira & Sarmento, 2008; Zhang et al., 2006). Shorter double helices and interior crystallites in A-type starches are more readily digestible and exhibit a high amount of RDS and SDS, compared

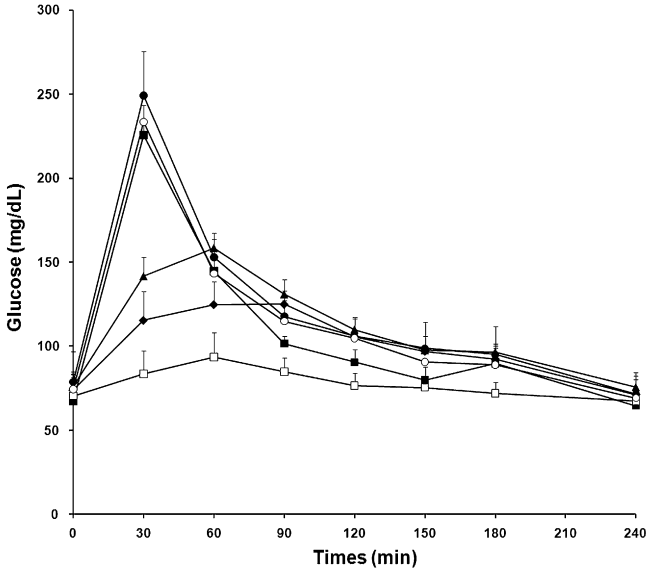

Fig. 4. Mean blood glucose concentration in mice after intake of glucose, native, 20% 30 °C, 30% 30 °C, 40% 30 °C and amorphous starches: ●, glucose; □, native; ♦, 20% 30 °C; ▲, 30% 30 °C; ■, 40% 30 °C; and ○, amorphous starch.

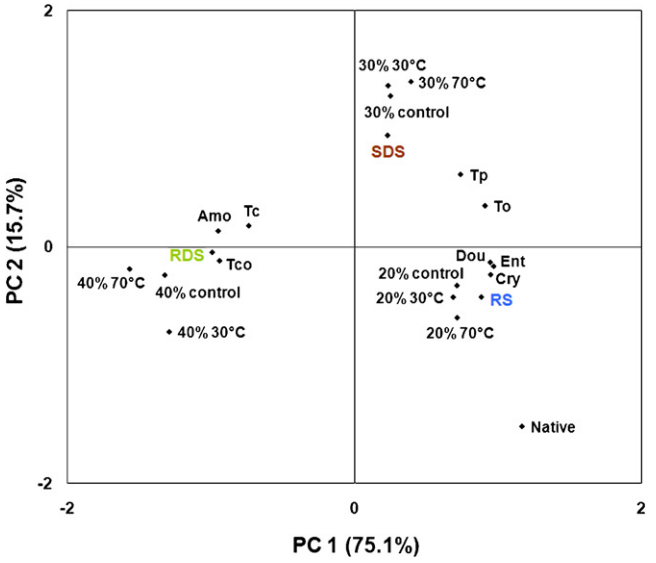

Fig. 5. Principal component analysis: loading plot of PC1 and PC2 describing the variation among properties of hydrothermally treated potato starch. Amo: amorphous starch; Cry: crystallinity; Dou: double helix; Ent: gelatinisation enthalpy; Native: native potato starch; RDS: rapidly digestible starch; RS: resistant starch; SDS: slowly digestible starch; Tc: conclusion temperature; Tco: gelatinisation temperature range; To: onset temperature; Tp: peak temperature.

with those of B-type starches, which often contain a high amount of RS (Jane et al., 1997). Accordingly, as the structure became closer to A-type than B-type, it showed slower digestibility. These results can be summarized as the effects of hydrothermal treatments on starch digestibility are complex and their potential to generate SDS depends largely on the treatment conditions and storage temperature.

3.6. Glucose responses in mice

The postprandial glucose concentrations in mice after uptake of glucose, amorphous, native, and hydrothermally treated starches are shown in Fig. 4. In this study, the area under the blood glucose response above the glucose (reference) baseline was compared according to the concept of the glycaemic response (Jenkins et al., 1981). Glucose responses for native, 20% 30 °C, 30% 30 °C, 40% 30 °C, and amorphous starch samples were estimated to be 20, 62, 77, 88 and 91, respectively. Glucose, amorphous and 40% 30 °C samples caused dramatic increases at 30 min and then sharp decreases in the postprandial blood glucose levels in mice (Fig. 4). However, in mice fed with the native starch, much lower blood glucose levels were obtained at 30 min due to the higher content of RS (78.5%) fraction found in the *in vitro* digestibility experiment (Table 3). The 20% 30 °C and 30% 30 °C samples displayed a much lower blood glucose level than the 40% 30 °C sample and amorphous starch sample due to a higher content of RS and SDS fractions. However, the decrease in blood glucose after 60 min was slower than that for the 40% 30 °C sample. The 30% 30 °C sample showed higher blood glucose levels at 60, 120, and 180 min, as compared with the amorphous starch sample. This meant that the 30% 30 °C sample contained a substantial amount of the slowly digestible fraction (Table 3), which continuously supplies the blood glucose during digestion at a slower pace, but is ultimately completely digested. The glucose response of the hydrothermally treated samples increased as the moisture level increased. The digestion patterns of hydrothermally treated potato starch from *in vitro* assay using digestion enzymes and from *in vivo* experiment using mice were not significantly different. Thus, the results from the *in vitro* assay can substitute for those from *in vivo* experiments.

3.7. Principal component analysis of digestibility in relation to hydrothermal treatment

The principal component analysis (PCA) of digestibility in relation to hydrothermal treatment is shown in Fig. 5. The PCA loading plots provide an overview of the similarities and differences among hydrothermal treatment conditions as well as the interrelationships between the fraction of digestibility and measured structural properties. Using PCA, the first and second principal components (PCs) could describe 75.1 and 15.7% of the variance, respectively, indicating that the first two PCs together explained 90.8% of the total variability. PC1 was characterized by crystallinity, T_0, T_p, T_c, T_c–T_0, ΔH, double helix, RDS, and RS attributes, while PC2 was characterized by T_0, T_p, and SDS. Crystallinity, double helix, ΔH, T_0, T_p, and RS were highly correlated with each other and highly loaded on PC1 in the positive direction where 20% moisture samples and native starch were closely located, indicating that these attributes were strong in 20% moisture samples and native starch (Tables 1–3). The 40% moisture samples were located close to the amorphous starch sample, T_c, and T_c–T_0 and RDS were loaded on PC1 in the negative direction, indicating that these attributes were strong in 40% moisture samples (Tables 2 and 3). The 30% moisture samples were located close to T_p, and T_0, and SDS were loaded highly on PC2 in the positive direction, demonstrating a relatively strong relationship with those three attributes (Tables 2 and 3). The principal components discriminated between hydrothermally treated starches with regard to digestibility, gelatinisation parameters, crystallinity, X-ray pattern, and double helix ratio.

4. Conclusions

Under the hydrothermal treatment conditions used in this study (100 °C, for 30 min at moisture contents of 20–40%, and then stored at 30 °C or 70 °C for 12 h), structural modifications led to different changes in the crystallinity, X-ray pattern, ^{13}C CP/MAS NMR pattern, gelatinisation parameters, and formation of a hollow region, as observed in the cross-section. These changes resulted in an increase in enzyme susceptibility. For this reason, hydrother-

mal treatment of potato starch resulted in a modified product that displayed much lower RS content and higher SDS content. The optimum conditions for the preparation of slowly digestible potato starch were 30% moisture level with heating at 100 °C for 30 min, followed by storage at 30 °C for 12 h. Starch with high SDS content was made up primarily of a mixture of B- and A-type crystals. Starch susceptibility during enzyme digestion was influenced not only by the hydrothermal levels, but also by the heating and storage conditions. Correlation analysis indicated a relationship between crystallinity, gelatinisation temperature, ΔH, double helix, and digestibility properties of hydrothermally treated potato starch. These findings suggest that structural changes within the starch granules were caused by hydrothermal treatment, which led to an increase in the SDS content of potato starch.

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