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Effect of controlled gelatinization in excess water on digestibility of waxy maize starch

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ABSTRACT

An aqueous dispersion of waxy maize starch (5%, w/w) was controlled gelatinized by heating at various temperatures for 5 min. The treated samples were analysed using *in vitro* Englyst assay, light microscopy, differential scanning calorimetry, X-ray diffraction, and Fourier-transform infrared spectroscopy. When heated, SDS and RS levels were decreased inversely with RDS. A high SDS content (>40%) was kept prior to the visible morphological and structural changes (before 60 °C). Swelling factor began to increase slightly at 50–60 °C and continued to maximum value at 80 °C. A large decrease in ΔH, crystallinity, and ratio of 1047/1022 cm^{-1} attributed to partially dissociation of crystalline clusters and double helices occurred at 65–80 °C. These changes showed that controlled gelatinized starch with slow digestion property occurred in the molecular rearrangement process before granule breakdown and SDS mainly consists of amorphous regions and a small portion of less perfect crystallites.

1. Introduction

Starch is the main source of digestible carbohydrates in the human diet and contributes a substantial amount of calories for human metabolism. The slow rate of glucose release from food products inducing low glycemic responses is considers to be beneficial for dietary management of metabolic disorders, including diabetes, prediabetes, glycogen storage disease, cardiovascular disease, and obesity (Björck & Asp, 1994; FAO/WHO, 1998; Miao, Jiang, & Zhang, 2008). The concept of glycemic index (GI) describing the level of the postprandial glucose rise in blood as compared to a reference food (white bread or glucose), was introduced to classify carbohydrate-based foods (FAO/WHO, 1998). Low-GI meals (GI $\leqslant$ 55) yield a more stable diurnal profile, reducing postprandial hyperglycaemia and hyperinsulinemia, as well as attenuating late postprandial rebound in circulating non-esterified fatty acids, all of which are factors that exacerbate these metabolic syndromes (Björck, Liljeberg, & Östman, 2000; FAO/WHO, 1998; Ludwig, 2002). A food product with a low-GI is preferable, not only in individuals with hypoglycaemia or hyperglycaemia, but also in healthy individuals.

It is well known that starch is composed of a mixture of two distinct macromolecules with α-D-glucopyranosyl unit, a linear fraction linked by the α-1,4 bonds, amylose, and a highly branched fraction linked by α-1,4 and α-1,6 linkages, amylopectin. From a nutritional physiological point of view, starch is generally classified into three major fractions depending on the rate and extent of digestion *in vitro*: rapidly digestible starch (RDS), the portion of starch digested within the first 20 min of incubation, slowly digestible starch (SDS), the portion of starch digested from 20 to 120 min, and resistant starch (RS), the remaining portion that cannot be further digested (Englyst, Kingman, & Cummings, 1992). RDS induces a fast increase of postprandial blood glucose and insulin level and is correlated to high GI, whereas SDS provides a slow and extended release of glucose into the blood stream and a low glycemic response (Björck et al., 2000; Englyst et al., 1992; Miao et al., 2008). Foods containing a substantial amount of SDS result in a diet with a low-GI, which may be advantageous to satiety, physical performance, improved glucose tolerance, as well as reduced blood lipid levels and insulin resistance through lessening the stress on regulatory systems related to glucose homoeostasis (Björck et al., 2000; Ludwig, 2002). Therefore, much attention is being given to SDS as a new functional material in novel food product development.

There have been reports on making SDS by chemical, physical, enzymatic, genetic, or multiple modifications (He, Liu, & Zhang, 2008; Miao et al., 2008). However, most of the reported SDS materials are also sensitive to thermal processing which is most often used in food preparation and SDS-based product is not commercially available in the current food market. Thus a challenge for the food industry is to develop new technologies to make heat-stable SDS. Zhang, Ao, and Hamaker (2006) reported that native cereal starches are ideal SDS (about 50%) and the semicrystalline A-type structure determines the slow digestion property. When crystalline structure is destroyed with processing, such as cooking, baking, and autoclaving, starch is more easily digested than raw

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starch (Holm, Lundquist, Björck, Eliasson, & Asp, 1988; Noda et al., 2008; Slaughter, Ellis, & Butterworth, 2001; Zhang et al., 2006). The objective of the present study is to determine the effect of controlled gelatinization in excess water on digestibility of waxy maize starch and to investigate the slow digestion property and structural characteristics for SDS. It is demonstrated that different digestibility of starch can be achieved by controlling the moist heat processing and containing rich-SDS diets for practical applications may be provided.

2. Materials and methods

2.1. Materials

Waxy maize starch was obtained from Changchun Dacheng Industrial Group Co., Ltd. (Changchun, Jilin, China). α-Amylase type VI-B from porcine pancreas and amyloglucosidase Dextrozyme® GA from *Aspergillus niger* were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO) and Novozymes (Tianjin, China), respectively. Glucose assay reagents were from Megazyme International Ireland Ltd. (Wicklow, Ireland). All chemicals were of reagent grade and were obtained from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China.

2.2. Preparation of starch samples

Starch samples, having different degree of gelatinization, were prepared by controlling moist heat treatment. A dispersion of starch (5 g) in distilled water (100 ml) was treated in a water bath at specific temperatures (ranging from 50 to 80 °C at 5 °C increments) for 5 min with shaking (200 rpm). The samples were filtered through Whatman Grade No.1 filter papers, and the residue was subjected to dry in an air oven at 50 °C for 24 h.

2.3. In vitro digestibility of starch samples

The digestibility of starch was analysed according to the procedure of Englyst et al. (1992) with a slight modification. To prepare enzyme solution I, amyloglucosidase solution (0.14 ml) was diluted to 6.0 ml with deionized water. Enzyme solution II was prepared by suspending porcine pancreatic α-amylase (12.0 g) in water (80.0 ml) with magnetic stirring for 10 min, centrifuging the mixture for 10 min at 1500*g*, then transferring a portion (54.0 ml) of the supernatant into a beaker. Enzyme III was prepared immediately before use by mixing water (4.0 ml), enzyme solution I (6.0 ml), and enzyme solution II (54.0 ml).

A starch sample (200 mg) was dissolved in phosphate buffer (15 ml, 0.2 mol/l, pH 5.2) by vortexing. After equilibrated at 37 °C for 5 min, seven glass balls (10 mm diameter) and enzyme solution III (5.0 ml) were then added, followed by incubation in a water bath at 37 °C with shaking (150 rpm). Aliquots of hydrolysed solution (0.5 ml) were taken at different time intervals and mixed with 4 ml of absolute ethanol to deactivate the enzymes. The glucose content of the hydrolyzates was determined using glucose oxidase/peroxidase assay kits. Percentage of hydrolysed starch was calculated by multiplying a factor of 0.9 with the glucose content. Each sample was analysed in triplicate.

The values of different starch fractions of RDS, SDS and RS were obtained by combining the values of G20 (glucose released after 20 min), G120 (glucose released after 120 min), FG (free glucose) and TG (total glucose) and using the following formulas:

$$\%\mathrm{RDS} = (\mathrm{G120} - \mathrm{FG}) \times 0.9 \times 100$$
$$\%\mathrm{SDS} = (\mathrm{G120} - \mathrm{G20}) \times 0.9 \times 100$$
$$\%\mathrm{RS} = (\mathrm{TG} - \mathrm{FG}) \times 0.9 \times 100 - (\mathrm{RDS} + \mathrm{SDS})$$

2.4. Light microscopy

Diluted starch samples (1 g in 25 ml of water) in a glass Petri dish on a hot plate for 5 min. After reaching the experimentally specified temperature (between 50 and 80 °C at 5 °C intervals, i.e., 55, 60, and 65 °C, etc.) the Petri dish was promptly viewed using an XP-201 light microscope (Shanghai Caikon Optical Instruments Factory, Shanghai, China) and observed at a 10×40 magnification.

2.5. Swelling factor (SF)

The swelling factor (SF) of the starches was measured according to the blue dextran dye exclusion method of Tester and Morrison (1990). The SF is reported as a ratio of the volume of swollen granules to the volume of dry starch. Starch (100 mg) in water (5.0 ml) was heated in a water bath at the required temperature for 30 min with constant shaking. The tube was then cooled rapidly to 20 °C, 0.5 ml of blue dextran (Pharmacia, M_r 2×10^6, 5 mg/ml) was added, and then contents mixed by gently inverting the closed tubes several times. After centrifuging at 2000*g* for 10 min, the absorbance of the supernatant (A_S) was measured at 620 nm. The absorbance of reference tubes (A_R) that contained no starch was also measured.

Calculation of SF was based on starch weight corrected to 12% moisture, assuming a density of 1.4 g/ml. Free or interstitial-plus-supernatant water (FW) is given by:

$$\mathrm{FW}\ (\mathrm{ml}) = 5.5(A_R - A_S) - 0.5$$

The initial volume of the starch (V_0) of weight W (in mg) is

$$V_0(\mathrm{ml}) = W/1400$$

The volume of absorbed intragranular water (V_1) is

$$V_1 = 5.0 - \mathrm{FW}$$

The volume of the swollen starch granules (V_2) is

$$V_2 = V_0 + V_1$$

The SF can be calculated as:

$$SF = V_2/V_0$$

This can also be expressed by the single equation:

$$SF = 1 + (7700/W)[(A_\mathrm{S} - A_\mathrm{R})/A_\mathrm{S}]$$

2.6. Differential scanning calorimetry (DSC)

The thermal properties of each starch sample were examined using differential scanning calorimetry (Pyris-1, Perkin Elmer Inc., Norwalk, CT, USA). Approximately 3 mg anhydrous starch sample was mixed with 6 mg deionized water and hermetically sealed in an aluminIum pan. The samples were allowed to equilibrate for 12 h at room temperature, then scanned at a heating rate of 5 °C/min from 40 to 120 °C. The differential scanning calorimetry analyser was calibrated using indium as a standard and an empty aluminIum pan was used as the reference. The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and enthalpy of gelatinization (ΔH) were calculated automatically.

2.7. X-ray diffraction (XRD)

X-ray diffraction analysis was performed with an X' Pert PRO X-ray powder diffractometer (PANalytical, Almelo, The Netherlands) operating at 40 kV and 40 mA with Cu Kα radiation (λ = 1.5406 Å). The starch powder was packed tightly in a rectangular

glass cell (15 × 10 mm, thickness 0.15 cm) and scanned at a rate of 2°/min from the diffraction angle (2θ) 5° to 35° at room temperature. The crystallinity was calculated according to the equation below:

$$X_c = A_c/(A_a + A_c)$$

where X_c is the crystallinity, A_c is the crystalline area and A_a is the amorphous area on the X-ray diffractogram.

2.8. Fourier-transform infrared spectroscopy (FT-IR)

All infrared spectra were obtained on a Nicolet Nexus 470 spectrometer (Thermo Electron Corporation, Waltham, MA, USA) equipped with a deuterated triglycine sulphate (DTGS) detector using the Digilab attenuated total reflectance (ATR) accessory at 4 cm^{-1} resolution by 64 scans. An ATR cell with a Ge crystal was used. The ATR cell was allowed to equilibrate at room temperature before each measurement. Each spectrum recorded against an empty cell as background and was subtracted from the spectrum of air. Spectra were baseline-corrected and deconvoluted by drawing a straight line at 1200 and 800 cm^{-1} (using Omnic version 6.2 software). A half-width of 26 cm^{-1} and a resolution enhancement factor of 2.4 were employed. The ratio of absorbance height at 1047 cm^{-1} to the height at 1022 cm^{-1} was obtained on the deconvoluted spectra.

2.9. Statistical analysis

All numerical results are average of at least three independent replicates. The mean differences were determined by Tukey's HSD test ($p < 0.05$) using SigmaStat® version 2.0 software (Jandel Scientific/SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. In vitro digestibility

The amounts of RDS, SDS, and RS in treated waxy maize starch samples are presented in Fig. 1. Based on the Englyst method, percentages of RDS, SDS, and RS in waxy maize starch at 25 °C were 32.4%, 49.2%, and 18.4%, respectively. Native waxy maize starch containing a large portion of SDS belongs to an ideal SDS material, which support previous finding by Englyst et al. (1992), and Zhang et al. (2006). During controlled gelatinization in excess water, RDS content increased as the heat temperature (degree of gelatinization) increased, reaching maximum (72.7%) at completely gelatinization (80 °C) accompanied by a corresponding decrease of SDS, whereas RS content was kept fairly constant. The process taking place in waxy maize starch just before gelatinization is characterised as annealing. Annealing as one of hydrothermal treatments is performed in excess water or at an intermediate water content (⩾40% w/w) and modifies the binding forces between the crystallites and the amorphous matrix (Jayakody & Hoover, 2008). Before at 65 °C heat treatment, the increase in RDS levels of starch is probably attributed to annealing may create pores or fissures, which alter the pattern of α-amylase hydrolysis from surface to internal erosion as reported by Jayakody and Hoover (2008). This suggests that changes to the granule surface on annealing could negate the effect of glucan chain interaction and crystallite perfection on α-amylase hydrolysis and thereby facilitate the entry of hydrolytic enzymes into the amorphous and crystalline domains of granule interior. The little change of RS levels on annealing reflects unaffected interactions between amylose–amylose and/or amylose–amylopectin chains (extent of crystalline perfection). According to Ratnayake & Jackson, 2006, starch gelatinization is a more complex process than the previously suggested order-to-disorder transition. Energy absorbed by granules not only unfolds amylopectin double helices during gelatinization but also facilitates rearrangement or formation of new bonds amongst molecules at lower temperatures before gelatinization takes place. An array of new amylopectin crystallites having different stabilities is formed during this structural reordering process.

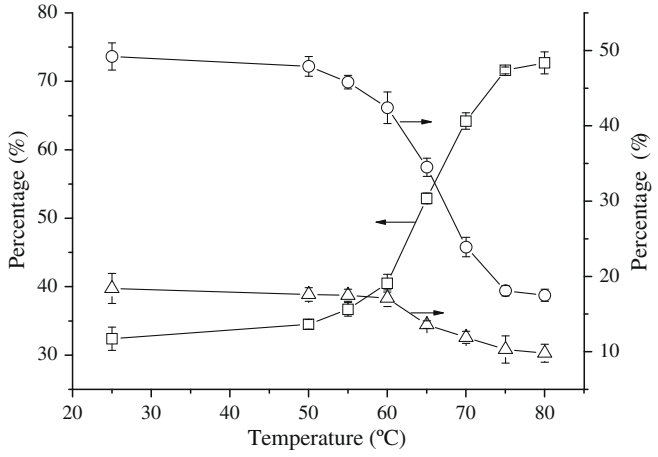

Fig. 1. RDS, SDS, and RS content on the basis of Englyst assay of waxy maize starch granules in excess water heated to specific temperature. □, RDS; ○, SDS; △, RS.

When cooked starch at a range of 60–80 °C, the granules undergo a process known as gelatinization, and there were a dramatically decrease the content of SDS together with a huge increase of RDS. Chung, Lim, and Lim (2006) reported that the partially gelatinized starch samples displayed hydrolysis rate between those of gelatinized starch and native starch, indicating that the hydrolysis rate was proportionally increased by the degree of gelatinization (melting). In the excess water environment, water is absorbed preferentially into the background region up to temperatures of about 55 °C. Above this temperature, the background region appears to have reached saturation, and more water is absorbed into the amorphous lamellae resulting in a dramatic drop in crystalline order. This changeover point could be due to the dissociation of double helices and could mark the change from radial to tangential swelling (Cameron & Donald, 1993). Waxy maize starch heated at 80 °C led to a composition of 72.7% RDS, 17.5% SDS, and 9.8% RS, respectively, showing the highest RDS and being consistent with the finding that cooking leads to a great loss in SDS (Miao et al., 2008). When starch granules in water are exposed to heat, the inter- and intra-molecular bonds between starch chains are disrupted, allowing the granules to swell and then disintegrate. The availability of starch chains to the digestive enzymes is thus increases as gelatinization progresses. Zhang et al. (2006) reported that cooking process completely destroyed the semicrystalline structure of A-type native starch granules, which is critical for their slow digestion property. Benmoussa, Moldenhauer, and Hamaker (2007) also showed that the amylopectin fine structure affected *in vitro* cooked starch digestion property. The fully gelatinized starch exhibited a plateau (maximum hydrolysis) at approximately 20 min of hydrolysis (Chung et al., 2006). This result indicates that fully gelatinized starch belongs to RDS. The suspension of native wheat starch was heated, and the catalytic efficiency of amylolysis reached a maximum at a pre-treatment temperature of 75 °C and then fell sharply if the treatment was conducted at higher temperatures, which means starch structure properties influence enzyme kinetic parameters (Slaughter et al., 2001). For an *in vivo* study of

Holm et al. (1988), gelatinized starch produced higher postprandial glucose and insulin response than raw starch. Plasma glucose and insulin responses as well as the rate of *in vitro* hydrolysis with α-amylase were strongly correlated to the degree of starch gelatinization; lower the degree of gelatinization, the lower glucose response. Bornet et al. (1989) suggested that amylose content becomes an important factor in determining α-amylase susceptibility after starch gelatinization. In the meantime, an inverse relationship appears between amylose content and the degree of glycemic response to processed starchy food. In our study, a high SDS level (>40%) was kept in heated aqueous solution of waxy maize starch by controlled gelatinization before 60 °C. During the heating process, SDS and RS decreased inversely with RDS.

3.2. Granules swelling

Fig. 2 shows the results of size and shape of treated waxy maize starches obtained by optical microscopy. The granules size of waxy maize starch was variable and ranged from 5–25 μm whereas starch granules had polygonal or round shapes at 25 °C. During heating in excess water, starch granules remained intact until 55 °C and stared to swell and slightly fold at 60 °C, this observation is consistent with the studies of Liu and Zhao (1990), and Ratnayake and Jackson (2006). It appears that the outermost layers (or skin) of the granules tend to maintain granular integrity during heating to 60 °C, before they disintegrate as the internal structure and free movement of starch polymers destabilize the granule's internal structure. Folding becomes more pronounced at higher

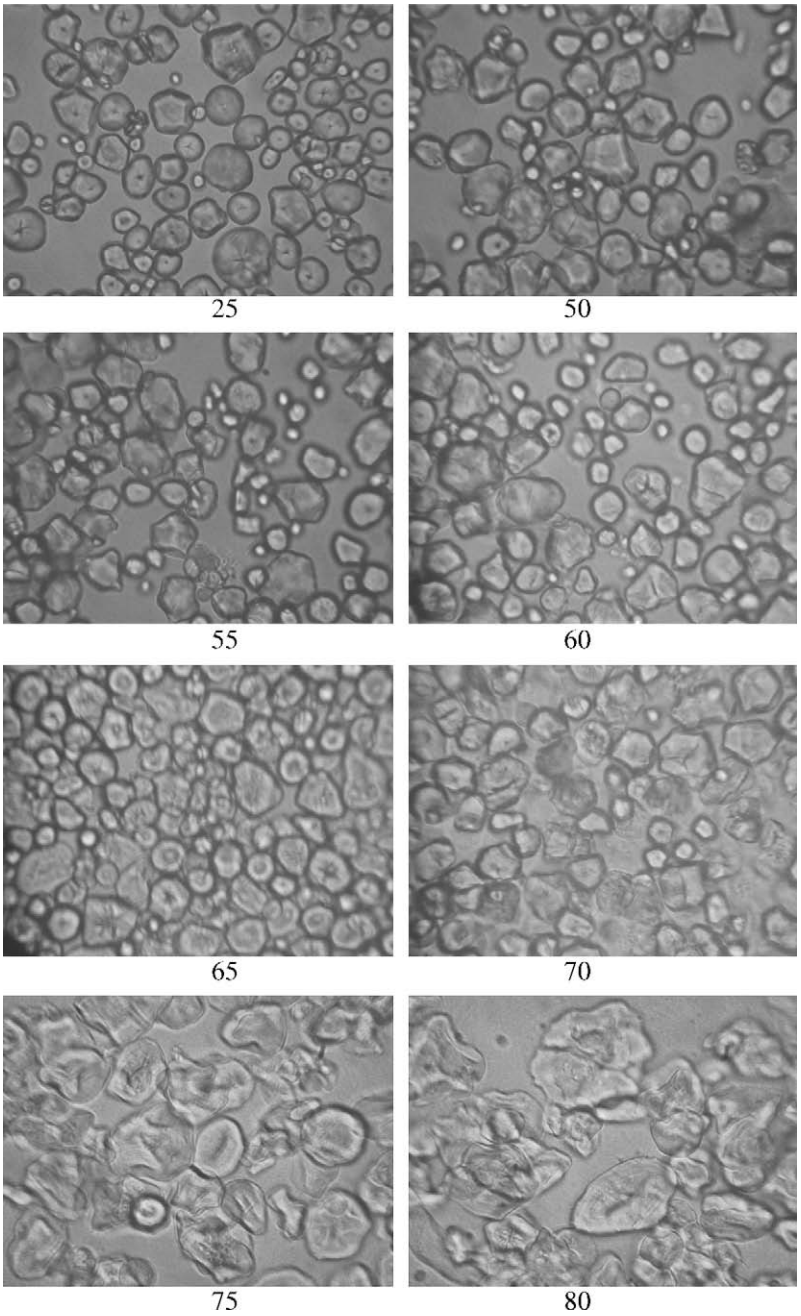

Fig. 2. Light microscopic images of waxy maize starch granules in excess water heated to specific temperature. The number below each figure represents the treatment temperature (°C). Magnification: 400×.

temperatures. At 80 °C, starch granules were extensively swollen (approximately 200% in size or a 25-fold volume incease) and grossly deformed. As observed by light microscopy, the large starch granules swelled first and began to break apart at around 55–60 °C; small granules did not start to disintegrate until 65 °C. Almost all the granules were irreversibly disrupted at or before 80 °C. According to Atkin, Abeysekera, Cheng, and Robards (1998), during the process of gelatinization, radial and tangential swelling expansion displaces amylopectin units from granular rings as a gradual centrifugal process concluding at the granular surface, as well as the external layers of starch granules form granule envelopes which degrade into ghost remnants composed of amylopectin. Liu and Zhao (1990) reported that corn starch granules heated at 64 °C lost their integrity and polarisation crosses, as well as formed a sponge-like structure. In contrast, our results showed that complete granular disruption and the formation of a gelatinized solution did not occur below 70 °C. The differences might be due to concentration differences, botanical and genetic origin (i.e., variety and cultivar) (Miao, Zhang, & Jiang, 2009). In the fully gelatinized starch, most of swollen starch granules were completely disrupted by excess heat, resulting in transformation to a continuous amorphous structure, whereas partially gelatinized starch samples may contain swollen starch granules which were not completely disrupted as suggested by Chung et al. (2006). Tester and Morrison (1990) showed that starch granular swelling is primarily a property of amylopectin because waxy starch swelled much more than normal starch did.

The SF of starch granules expressed on a starch basis and in terms of amylopectin content and was investigated over the temperature range 50–80 °C (Fig. 3). Swelling curves were characterised by an initial phase of slight swelling, a second phase of rapid swelling, and a final stage of maximum swelling. Starch granule swelling is known to begin in the bulk, relatively mobile amorphous fraction and in the more restrained amorphous region immediately adjacent to the crystalline regions. Significant granule swelling was evident only at temperatures beyond 60 °C, which confirmed the light microscopy observations (Fig. 2). The curve reached a plateau value (maximum SF) at 75–80 °C with a SF range of 30–32. A similar trend has also been observed for waxy rice starches (Tester & Morrison, 1990). High swelling capacities were associated with relatively disordered arrangements of polymers within granules (Wong & Lelievre, 1982). The SF has been shown to be influenced by amylose–lipid complexes, amylose content, long amylopectin chain, and granule crystallinity (Tester & Morrison, 1990; Chavan, Shahidi, Hoover, & Perera, 1999; Miao et al., 2009). Based on the study on starches from different wheat cultivars, Sasaki and Matsuki (1998) have shown that starches with higher swelling power tend to contain higher proportion of long chains in amylopectin, and the difference in amylopectin structure is related to variations in starch swelling properties. Association between long amylopectin chains could result in the formation of a large number of crystallites, which could increase granular stability thereby reducing the extent of granular swelling. Thus, the SF of the waxy maize starch is most likely influenced by amylopectin molecular structure (extent of interaction between starch chains in the native granule) and/or by the amount of lipid complexed amylose chains.

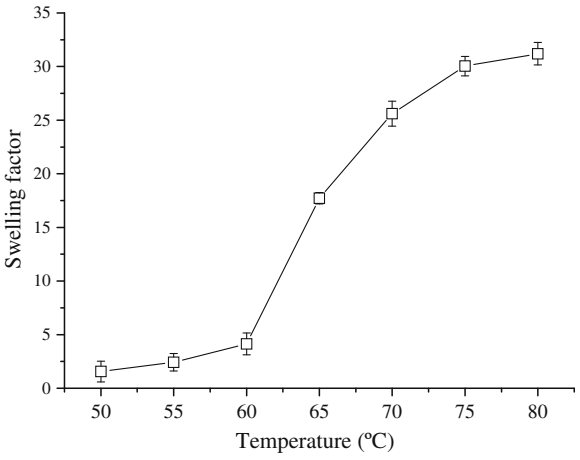

Fig. 3. Swelling factor curves of waxy maize starch granules in excess water heated to specific temperature.

3.3. Gelatinization characteristics

The gelatinization transition temperatures (T_o, T_p, and T_c), gelatinization transition temperature range (T_c–T_o), and gelatinization enthalpy (ΔH) of starch samples are presented in Table 1. There were significant differences in T_o, T_p, T_c, and ΔH between treated starches ($p < 0.05$). The changes that take place during gelatinization have been attributed to water availability, crystal stability within granules, difference between amorphous and crystalline regions, and glass transition related progressive melting. The differences in gelatinization transition temperatures have been shown to be attributed to the differences in amylose content, distribution of amylopectin chains, and lipid complexed amylose chains (Chavan et al., 1999; Miao et al., 2009). The gelatinization temperatures of the waxy maize starch ranged from 58.0 to 80.0 °C. T_o, T_p, and T_c of waxy maize starch heated at 25 °C were 59.942, 69.079, and 78.211 °C, respectively. Moreover, the gelatinization parameters were within the range reported for waxy maize cultivars (Jayakody and Hoover (2008), Miao et al., 2008). Annealing treatment narrowed gelatinization temperature ranges and increased gelatinization peak temperatures of annealed starches as compared to their native counterpart (Jayakody & Hoover, 2008). The similarity between waxy maize starches treated at the range of 50–60 °C, such as T_o, T_p, and T_c increased whilst $T_c - T_o$ decreased. These changes have been attributed to perfection of pre-existing crystallites. The extent of increase in T_o, T_p, and T_c were more pronounced at 50 °C, which mainly induces by interaction between amylose and the outer branches of amylopectin (polymer rearrangement). Ratnayake and Jackson (2006) reported that the energy provided before the initiation of gelatinization causes the formation of new, but less stable, molecular interactions within granules that result in progressively lower DSC peak temperatures between 50 and 60 °C treatments. Annealing has a greater influence on T_o, since T_o represents melting of the weakest crystallites, which are more susceptible to crystallite perfection on annealing than crystallites that have a higher stability (represented by T_c). The gelatinization transition temperatures also increased as the heat temperature (60–80 °C) increased. Upon further heating, at increased temperature, starch polymers become more mobile, reduce or lose their interpolymer interactions, and starch granules disrupt (no thermal transitions at 80 °C, figure not shown), as granular morphological characteristics shown in Fig. 2. T_o, T_p, and T_c are influenced by the molecular architecture of the crystalline region, which corresponds to the distribution of amylopectin short chains (DP 6–11), and not the proportion of the crystalline region, which indicates the amylose–amylopectin ratio (Miao et al., 2009). In Tester and Morrison (1990) study, the low-gelatinization temperature starches had less crystallinity and less perfect crystallites than the high-gelatinization temperature starches due to minor structural differences in their amylopectins. Differences in T_c–T_o may be due to the presence of crystallites, which are composed of small crystallites, each possessing slightly different crystal strength. Therefore, the extent of decrease in $T_c - T_o$ at 60–80 °C could be disrupted crystallites by heating treatment.

Table 1
Gelatinization parameters of waxy maize starch granules in excess water heated to specific temperature.

Temperature treatment (°C)	T_o [A] (°C)	T_p [A](°C)	T_c [A] (°C)	T_o–T_o [B] (°C)	$\triangle H$ [C] (J/g)
25	59.942 ± 0.680[a]	69.079 ± 0.272[a]	78.211 ± 0.405[a]	18.269	11.193 ± 0.281[a]
50	62.516 ± 0.128[b]	68.923 ± 0.184[b]	78.129 ± 0.253[b]	15.613	11.546 ± 0.672[b]
55	64.345 ± 0.154[c]	68.885 ± 0.336[c]	79.530 ± 0.191[c]	15.185	11.656 ± 0.354[c]
60	64.002 ± 0.529[d]	67.516 ± 0.253[d]	79.582 ± 0.646[d]	15.580	11.763 ± 0.175[d]
65	68.409 ± 0.501[e]	69.451 ± 0.359[e]	81.841 ± 0.255[e]	13.432	9.341 ± 0.415[e]
70	71.351 ± 0.417[f]	70.408 ± 0.680[f]	82.993 ± 0.302[f]	11.642	4.006 ± 0.290[f]
75	75.988 ± 0.192[g]	71.916 ± 0.463[g]	84.211 ± 0.177[g]	8.223	1.898 ± 0.302[g]

All data reported on dry basis and represent the mean of three replicates. Values followed by the same letter in each column are not significantly different ($p < 0.05$) by Tukey's HSD test.
[A] T_o, T_p, T_c indicate the temperature of the onset, peak, conclusion of gelatinization, respectively.
[B] T_o–T_o indicates the gelatinization temperature range.
[C] $\triangle H$ indicates enthalpy of gelatinization.

DSC enthalpies of heat-treated samples kept little changes until 60 °C and then disappearing gradually between 60 and 80 °C. No enthalpies were observed at 80 °C (Data not shown), indicating the absence of crystalline domains. In a literature review by Jayakody and Hoover (2008), annealing significantly decreased ΔH in DSC profiles, which are not consistent with the ΔH results of annealed starches in Table 1. These results may suggest that there were no considerable molecular rearrangements occurring before they started to swell at 60 °C without viewable light microscopic changes. According to Cooke and Gidley (1992), ΔH reflects the loss of double helical order rather than the loss of crystallinity. In excess water, gelatinization is a primarily swelling driven process. Water uptake by the bulk amorphous and the intercrystalline amorphous areas is accompanied by swelling within these regions. The swelling acts to destabilize the amylopectin crystallites within the crystalline lamella, which are ripped apart. Consequently, the unraveling and melting of the double helices forming the crystallites would require a higher input of thermal energy. Our data on DSC (Table 1) and SF (Fig. 3) suggested the formation of double helical order is due to interaction between amylose chains and amylopectin chains, which may be loosely associated, and hence prone to disruption at the temperature range of 60–80 °C.

3.4. X-ray diffraction pattern and crystallinity

The X-ray diffractograms and crystallinity of treated starches are presented in Fig. 4. For the A-type spectrum, the most intense peaks corresponded to Bragg angles (2θ): 15, 17, 18.1, and 23.5°. The raw waxy maize starch powder showed the strongest diffraction peak at 16.97° 2θ, and a few small peaks at around 2θ with values of around 15.17, 18.07, 22.91° 2θ. These results indicated that the crystal type of waxy maize starch is a characteristic A-type (Miao et al., 2008). The treated starches at 50–70 °C showed X-ray diffraction patterns of the A-type. Heated starch at 80 °C did not show any peaks, mostly due to the fact that it is mainly amorphous. Annealing did not have an influence on the wide angle X-ray diffraction pattern of waxy maize starches, but only give a small increase in intensity. Based on the small angel X-ray scattering study, Vermeylen et al. (2006) reported that annealing the 9 nm scattering intensity was more pronounced at 51 °C (closed to T_o) than at 44 °C or 47 °C. The enhanced intensity (density contrast between the crystalline and amorphous lamellae) was attributed to more efficient packing of double helices within the crystalline domains of the granule. In Waigh, Gidley, Komanshek, and Donald (2000) report where a chiral side-chain polymeric liquid crystalline approach was used, the two-stage process involved in the gelatinization of starch in excess water. The first stage involves a slow dissociation of the helices side-by-side. Immediately a helix coil transition occurs as a secondary effect. Thus, the structure changes that take place during gelatinization include a simultaneous crystallite melting and double-helix unwinding, absorption of water in the amorphous growth ring, changes in shape and size of granules, dispersion of blocklet-like structures, and leaching of amylose from granules.

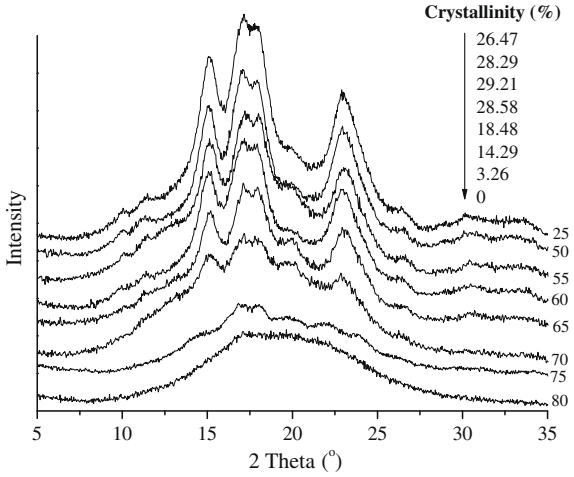

Fig. 4. XRD spectra of waxy maize starch granules in excess water heated to specific temperature. Numbers to the right of each profile represent the corresponding treatment temperature (°C).

Starch crystallites are due to sequential packing of double helices that are found between the flexible 'A' chains of amylopectin. The crystallinity of waxy maize starch increased from 26.47% at 25 °C to 28.58% at 60 °C and disappeared over increasing temperature (Fig. 4). Generally differences in crystallinity between starches could be attributed to the following: (1) crystal size, (2) amount of crystalline regions which is influenced by amylopectin content and amylopectin chain length, (3) orientation of the double helices within the crystalline domains, and (4) extent of interaction between double helices (Miao et al., 2009). The results indicated that the double helices of treated starches at 60–80 °C were less compactly packed and/or less well arranged to diffract X-rays than those of annealed starches. From the results in Fig. 1 and Fig. 4, it may be concluded that SDS consists of amorphous regions and a small portion of ordered double helix structure. Crystallinity of treated starch is lost in the temperature region roughly corresponding to DSC gelatinization.

3.5. FT-IR spectroscopy

The FT-IR spectrum of starch has been shown to be sensitive to changes in structure on a molecular level (short-range order), such as starch chain conformation, crystallinity, and retrogradation (van

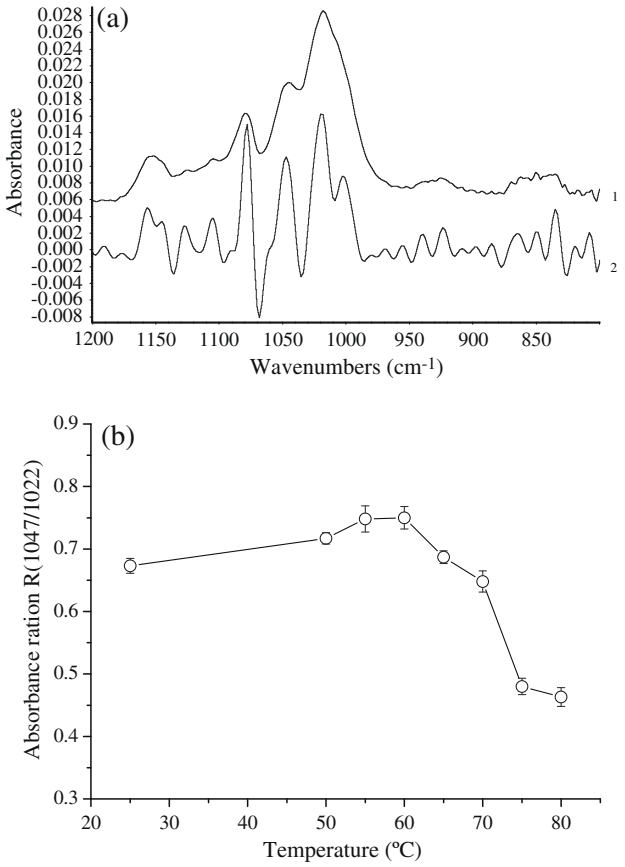

Fig. 5. FT-IR spectrum (a), and IR ratio of the absorbance 1047/1022 cm^{-1} (b) of waxy maize starch granules in excess water heated to specific temperature. 1 – Original spectrum; 2 – deconvoluted spectrum.

Soest, Tournois, de Wit, & Vliegenthart, 1995). The spectral region, 800–1200 cm^{-1}, investigated was assigned to C–O, C–C, and C–H stretching modes in alcoholic COH moieties. In Fig. 5a the original and deconvoluted FT-IR spectra of treated starches are given. The band at 1047 cm^{-1} appears to be composed of two overlapped bands at 1040 and 1053 cm^{-1} in original spectrum. The IR absorbance bands at 1047 and 1022 cm^{-1} are sensitive to ordered or crystalline structures and amorphous structures in starch, respectively, and the ratio of 1047/1022 cm^{-1} from deconvoluted FT-IR spectrum has been used to express the amount of ordered crystalline to amorphous domains in starches (van Soest et al., 1995). The changes in short-range molecular order of the waxy maize starch during heating were measured and the ratios of 1047/1022 cm^{-1} are presented in Fig. 5b. Annealing increased the ratio of 1047/1022 cm^{-1} in the waxy maize starch heated at 50–60 °C, which show a similar increasing intensity of the band at 1047 cm^{-1}. This increase can be attributed to crystalline perfection that occurs during hydrothermal treatment. The diffractograms of treated starch (Fig. 4) also showed an increase in crystallinity and thus in long-range ordering. van Soest et al. (1995) reported that the changes in intensity of bands at 1047 and 1022 cm^{-1} show a first-order relationship with the crystallinity as determined by wide-angle X-ray diffractometry. However, FT-IR is not able to differentiate between the A and B polymorphs and thus the long-range packing (Sevenou, Hill, Farhat, & Mitchell, 2002). Li, Shoemaker, Ma, Moon, and Zhong (2008) showed that short-range order correlated positively with amylopectin content at 60 °C (before gelatinization). The short chains of amylopectin are thought to be arranged in crystallites responsible for the crystallinity of the native starch granule. The short-range order of waxy maize starches decreased when temperature increased from 60 to 80 °C, which indicated that the loss of order was significant as soon as the temperature reached the pasting onset temperature. Vermeylen et al. (2006) found that the melting of amylopectin crystallites during gelatinization was accompanied by the formation of amorphous networks. At temperatures above 70 °C, a relatively significant decrease of short-range order was observed. This was due to the higher T_o of thermal treated starch in Table 1, indicating FT-IR results strongly supported the DSC findings.

4. Conclusions

Controlled gelatinization of waxy maize starch in excess water displays different starch digestibility according to their heat treatment. During the temperature range before gelatinization takes place (60 °C), a high proportion of SDS (>40%) was obtained. This study also showed that treated starch wildly differ in morphological granular characteristic, swelling factor, gelatinization transition temperatures, enthalpy of gelatinization, X-ray diffraction intensities, crystallinity and short-range order. These differences are probably influenced by differences in the magnitude of interaction between and amongst starch chains within the amorphous and crystalline regions (double helices or crystallites), and by amylopectin molecular structure (distribution pattern of branches and chain length). The results also conclude that SDS is mainly consists of amorphous regions and a small portion of ordered double helix structure.

In food products, starch granules are subjected to different thermal condition and other unit operations that result in granules with differing stages of partial and complete gelatinization; these collectively influence the product's physicochemical properties. Studies are underway to obtain products with rich-SDS and low GI properties from the above starches. This will enable us to obtain a more precise insight into how starch structure influence functionality.

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References

Atkin, N. J., Abeysekera, R. M., Cheng, S. L., & Robards, A. W. (1998). An experimentally-based predictive model for the separation of amylopectin subunits during starch gelatinization. *Carbohydrate Polymers, 36*, 173–192.

Benmoussa, M., Moldenhauer, K. A. K., & Hamaker, B. R. (2007). Rice amylopectin fine structure variability affects starch digestion properties. *Journal of Agricultural and Food Chemistry, 55*, 1475–1479.

Björck, I., & Asp, N.-G. (1994). Controlling the nutritional properties of starch in foods – A challenge to the food industry. *Trends in Food Science & Technology, 5*, 213–218.

Björck, I., Liljeberg, H., & Östman, E. (2000). Low glycaemic-index food. *British Journal of Nutrition, 83*, 149S–155S.

Bornet, F. R. J., Fontvieille, A.-M., Rizkalla, S., Colonna, P., Blayo, A., Mercier, C., & Slamma, G. (1989). Insulin and glycemic responses in healthy humans to native starches processed in different ways: Correlation with in vitro α-amylase hydrolysis. *American Journal of Clinical Nutrition, 50*, 315–332.

Cameron, R. E., & Donald, A. M. (1993). A small-angle X-ray scattering study of starch gelatinization in excess and limiting water. *Journal of Polymer Science. Part B: Polymer Physics, 31*, 1197–1203.

Chavan, U. D., Shahidi, F., Hoover, R., & Perera, C. (1999). Characterization of beach pea (*Lathyrus maritimus* L.) starch. *Food Chemistry, 65*(6), 1–70.

Chung, H.-J., Lim, H. S., & Lim, S.-T. (2006). Effect of partial gelatinization and retrogradation on the enzymatic digestion of waxy rice starch. *Journal of Cereal Science, 43*, 353–359.

Cooke, D., & Gidley, M. J. (1992). Loss of crystalline and molecular order during starch gelatinization: Origin of the enthalpic transition. *Carbohydrate Research, 227*, 103–112.

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition, 46*, 30–50.

FAO/WHO. (1998). Carbohydrates in human nutrition. (FAO Food and Nutrition Paper-66). *Report of a joint FAO/WHO expert consultation*. Rome, Apr. 14–18.

He, J., Liu, J., & Zhang, G. (2008). Slowly digestible waxy maize starch prepared by octenyl succinic anhydride esterification and heat–moisture treatment: Glycaemic response and mechanism. *Biomacromolecules, 9*, 175–184.

Holm, J., Lundquist, I., Björck, I., Eliasson, A.-C., & Asp, N.-G. (1988). Degree of starch gelatinization, digestion rate of starch *in vitro*, and metabolic response in rats. *American Journal of Clinical Nutrition, 47*, 1010–1016.

Jayakody, L., & Hoover, R. (2008). Effect of annealing on the molecular structure and physicochemical properties of starches from different botanical origins – A review. *Carbohydrate Polymers, 74*, 691–703.

Li, Y., Shoemaker, C., Ma, J., Moon, K. J., & Zhong, F. (2008). Structure–viscosity relationships for starches from different rice varieties during heating. *Food Chemistry, 106*, 1105–1112.

Liu, J.-M., & Zhao, S.-L. (1990). Scanning electron microscope study on gelathization of starch granules in excess water. *Starch/Stärke, 42*, 96–98.

Ludwig, D. S. (2002). The glycemic index: Physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. *Journal of the American Medical Association, 287*, 2414–2423.

Miao, M., Jiang, B., & Zhang, T. (2008). *Effect of pullulanase debranching and recrystallization on structure and digestibility of waxy maize starch*. Carbohydrate Polymers, in press (doi:10.1016/j.carbpol.2008.10.007)

Miao, M., Zhang, T., & Jiang, B. (2009). Characterizations of kabuli and desi chickpea starches cultivated in China. *Food Chemistry, 113*, 1025–1032.

Noda, T., Takigawa, S., Matsuura-Endo, C., Suzuki, T., Hashimoto, N., Kottearachchi, N. S., Yamauchi, H., & Zaidul, I. S. M. (2008). Factors affecting the digestibility of raw and gelatinized potato starches. *Food Chemistry, 110*, 465–470.

Ratnayake, W. S., & Jackson, D. S. (2006). A new insight into the gelatinization process of native starch. *Carbohydrate Polymers, 67*, 511–529.

Sasaki, T., & Matsuki, J. (1998). Effect of wheat structure on swelling power. *Cereal Chemistry, 74*, 525–529.

Sevenou, O., Hill, S. E., Farhat, I. A., & Mitchell, J. R. (2002). Organisation of the external region of the starch granule as determined by infrared spectroscopy. *International Journal of Biological Macromolecules, 31*, 79–85.

Slaughter, S. L., Ellis, P. R., & Butterworth, P. J. (2001). An investigation of the action of porcine pancreatic α-amylase on native and gelatinised starches. *Biochimica et Biophysica Acta, 1525*, 29–36.

Tester, R. F., & Morrison, W. R. (1990). Swelling and gelatinization of cereal starches II. Waxy rice starches. *Cereal Chemistry, 67*, 558–563.

van Soest, J. J. G., Tournois, H., de Wit, D., & Vliegenthart, J. F. G. (1995). Short-range structure in (partially) crystalline potato starch determined with attenuated total reflectance Fourier-transform IR spectroscopy. *Carbohydrate Research, 279*, 201–214.

Vermeylen, R., Derycke, V., Delcour, J. A., Goderis, B., Reynaers, H., & Koch, M. H. J. (2006). Gelatinization of starch in excess water: Beyond the melting of lamellar crystallites. A combined wide- and small-angle X-ray scattering study. *Biomacromolecules, 7*, 2624–2630.

Waigh, T. A., Gidley, M. J., Komanshek, B. U., & Donald, A. M. (2000). The phase of transformation in starch during gelatinization: A liquid crystalline approach. *Carbohydrate Polymers, 328*, 165–176.

Wong, R. B. K., & Lelievre, J. (1982). Comparison of crystallinity of wheat starches with different swelling capacities. *Starch/Stärke, 34*, 159–161.

Zhang, G., Ao, Z., & Hamaker, B. R. (2006). Slow digestion property of native cereal starches. *Biomacromolecules, 7*, 3252–3258.