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Effect of pullulanase debranching and recrystallization on structure and digestibility of waxy maize starch

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ABSTRACT

An optimal slowly digestible starch (SDS) content was obtained through assessment of variation in digestibility of pullulanase debranched waxy maize starch which was recrystallized for up to 8 days. The effects of debranching time, debranching enzyme concentration, storage time and temperature on digestibility and structural properties of waxy maize starch samples were investigated. When gelatinized starch was treated with higher enzyme concentration and less debranching time, higher SDS was formed while resistant starch (RS) increased with time. The maximum SDS content was obtained by debranching for 3–6 h with high pullulanase (20 or 40 ASPU/g) then storing at 4 °C for 2 days. X-ray diffraction pattern of treated starch at optimal conditions was similar to the B-type in which illuminated treated starches contain partially ordered crystalline structures. Scanning electron micrographs showed the treated starch had more irregular angular shapes with a higher crystallinity structure and pitted surface as debranching and recrystallization time increased. In differential scanning calorimetry thermograms, the melting temperature and enthalpy of treated starches were gradually enhanced, resulting from reforming of double helix structure by low temperature recrystallization following debranching. These changes suggested that SDS mainly consists of a small portion of partially ordered double helix crystalline components with short amylopectin chains and amorphous components.

1. Introduction

Starch is the basic source of energy for the majority of the world's population. In human nutrition, starch plays a major part in supplying the metabolic energy that enables the body to perform its different functions. According to the rate and extent of starch digestion *in vitro*, starch is generally classified into three major fractions: (1) rapidly digestible starch (RDS), the portion digested within the first 20 min in mouth and intestine, (2) slowly digestible starch (SDS), the portion digested from 20 to 120 min in small intestine, and (3) resistant starch (RS), the remaining portion that cannot be further digested in the small intestine but is mainly fermented in colon (Englyst, Kingman, & Cummings, 1992).

Recent studies suggest that slowly digestible starch and resistant starch have significant implications on human health (Björck & Asp, 1994; Englyst et al., 1992; Han et al., 2006; Miao, Jiang, Zhang, & Mu, 2007). They are both correlated with a low glycemic index, which is important for treatment and prevention of several diseases (Björck, Liljeberg, & Östman, 2000; Englyst, Veenstra, & Hudson, 1996; Han et al., 2006; Jenkins, Kendall, Marchie, & Augustin, 2004; Miao et al., 2007). Slowly digestible starch for example, provides sustained postprandial levels of glucose in blood that may be related with non-insulin diabetes (Brand et al., 1991; Ells, Seal, Kettlitz, Bal, & Mathers, 2005; Jenkins, Wolever, & Buckley, 1988; Ludwig, 2002), cardiovascular diseases (Ells et al., 2005; Leeds, 2002; Ludwig, 2002), satiety (Ball et al., 2003; Ludwig, 2002), physical and mental performance (Benton & Nabb, 2003; Lang, Degouy, & Champenois, 2003) and dental effect (Björck & Asp, 1994).

SDS is not commercially available, while RS product is sold. Two primary RS products are Novelose® of National Starch and Chemical Corporation and Crystalean® of Opta Food Ingredient. Recently, studies on production of SDS based on chemical, physical and enzymatic methods been published. A process for making SDS products by debranching waxy rice starch and waxy sorghum was reported by Guraya, James, and Champagne (2001b) and Shin et al. (2004). Han et al. (2006) attempted the production of SDS based on controlling hydrolysis of gelatinized maize starch by α-amylase. However, very little information exists regarding starch digestibility and structural properties during enzymatic treatment and storage. In this study, production of a starch with slow digestion property and the effect of debranching and recrystallization treatment of cooked waxy maize starch suspensions on the digestibility and accompanying structural changes were investigated.

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2. Materials and methods

2.1. Materials

Waxy maize starch was obtained from Changchun Dacheng Industrial Group Company Ltd. (Changchun, Jilin, China). Hi-maize™ (resistant starch) was from National Starch and Chemical Corporation (Shanghai, China). Porcine pancreas α-amylase (EC 3.2.1.1) type-B and guar gum were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). Pullulanase (EC 3.2.1.41) OPTIMAX L-1000 from *Bacillus licheniformis* and amyloglucosidase (EC 3.2.1.3) Dextrozyme® GA from *Aspergillus niger* were donated by Wuxi Genencor Bio-products Co., Ltd. (Wuxi, Jiangsu, China) and Nov Nordisk (Tianjin, China), respectively. Glucose assay reagents were from Megazyme International Ireland Ltd. (Wicklow, Ireland).

2.2. Preparation of starch samples

Waxy maize starch slurry (10% w/v in diluted pH 4.4 buffer solution containing 0.2 M NaH_2PO_4 and 0.2 M citric acid) was cooked in water bath at 90 °C for 60 min. The temperature of samples was adjusted to 58 °C thereafter, and debranched by pullulanse at 10, 20, and 40 ASPU/g of starch for 0, 3, 6, 9, 12, 18, and 24 h (ASPU is defined as the amount of enzyme that liberates 1.0 mg glucose from starch in 1 min at pH 4.4 and 60 °C). Immediately after the reaction, the solutions were autoclaved at 121 °C for 30 min to stop reaction and cooled to room temperature and 90% ethanol (v/v) was added to facilitate precipitation of retrograded starch. The mixtures were stored at 4 °C for 2 days.

Cooked 10% waxy maize starch slurry was also digested by pullulanase (20 ASPU/g) for 6 h, reheated (121 °C for 30 min), and stored at 4 °C for 3, 6, 12, and 24 h and 2, 4, 6, and 8 days or −20, 4, and 20 °C for 2 days. The precipitated starch was collected by centrifugation at 3000*g* for 10 min, washed with deionized water and collected by centrifugation twice, then freeze–dried.

2.3. In vitro digestibility of starch samples

The digestibility of starch was analyzed according to the method of Englyst et al. (1992) with a slight modification. Starch (200 mg) and guar gum (50 mg) were 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 5 ml of porcine pancreatic α-amylase (290 U/ml) and amyloglucosidase (15 U/ml) (U is defined as the amount of enzyme that liberates 1.0 mg glucose from starch in 1 min at pH 5.2 and 37 °C) were then added, followed by incubation in a water bath at 37 °C with shaking (150 rpm). Aliquots of hydrolyzed 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 (GOPOD method). Percentage of hydrolyzed starch was calculated by multiplying a factor of 0.9 with the glucose content. Each sample was analyzed in triplicate.

The values of different starch fractions of RDS, SDS and RS were obtained based on their definition by Englyst et al. (1992), which combines the values of G20 (glucose released after 20 min), G120 (glucose released after 120 min), FG (free glucose) and TG (total glucose) using the following formulas:

$$\%\mathrm{RDS} = (\mathrm{G120} - \mathrm{FG}) \times 0.9 \times 100$$
$$\%\mathrm{SDS} = (\mathrm{G120} - \mathrm{G20}) \times 0.9 \times 100$$
$$\%\mathrm{RS} = (\mathrm{TG} - \mathrm{FG}) \times 0.9 \times 100 - (\mathrm{RDS} + \mathrm{SDS}) \quad (1)$$

2.4. X-ray diffraction

X-ray diffraction analysis was performed with a X' Pert PRO X-ray powder diffractometer (PANalytical, Netherlands) operating at 40 kV and 40 mA with Cu Kα radiation (λ = 1.5406 Å). The starch powders scanned at a rate of 2°/min from 2θ 10° to 35° at room temperature. Analysis was conducted following the procedure reported by Song and Jane (2000). The degree of crystallinity was calculated according to the method of Nara and Komiya (1983) as follows:

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

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

2.5. Morphological properties

Structural properties of the starch sample were studied using a scanning electron microscope (Quanta-200, FEI company, Netherlands). Dried, finely ground samples were mounted on an aluminum stub using double-sided stick tape and coated with a thin film of gold (10 nm), then examined at an accelerating voltage of 10 kV.

2.6. Differential scanning calorimetry

The thermal properties of each starch sample were examined using a differential scanning calorimetry (Pyris-1, Perkin Elmer Inc., USA). Approximately 3 mg anhydrous starch sample was mixed with 6 ml deionized water and hermetically sealed in an aluminum pan. Samples were allowed to equilibrate for 12 h at room temperature, then scanned at a heating rate of 5 °C/min from 40 to 130 °C. The differential scanning calorimetry analyzer was calibrated using indium as a standard and an empty aluminum pan was used as the reference. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy of gelatinization (ΔH) were calculated automatically.

2.7. Statistical analysis

The averages and Student's *t*-test were performed by SPSS 13.0 for windows software (SPSS Institute Inc., Cary, NC, USA). A significance level of $p < 0.05$ was used throughout the study for statistical analysis.

3. Results and discussion

3.1. Effect of pullulanase debranching on the digestibility of starch

The raw waxy maize starch after being cooked was treated at three specific dosages and different debranching time. The SDS and RS rates of formation in debranched waxy maize starch suspensions are presented in Fig. 1. SDS content increased as the debranching time increased, reaching a maximum at about 3–6 h with high pullulanase, and decreasing thereafter, whereas the RS content increased. High enzyme concentration and less debranching time increased the amount of SDS, whereas longer and complete debranching time promoted the production of RS. This trend is similar to the result from waxy rice starch by Guraya et al. (2001b) in which most SDS was produced by debranching with 10 g of pullulanase per 100 g of starch for 4 h. Shin et al. (2004) also reported optimal conditions for SDS production with gelatinized waxy sorghum starch hydrolyzed by isoamylase for 8 h and the highest of SDS content was 27.0%.

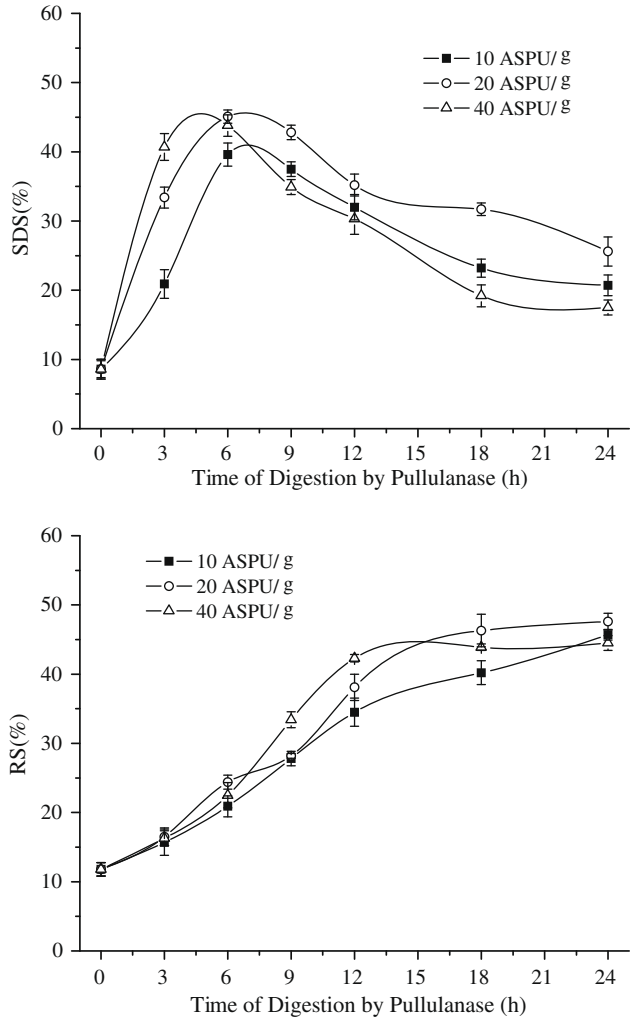

Fig. 1. Effect of pullanase debranching of cooked waxy maize starch on percentage of SDS and RS. [a]All samples were stored at 4 °C for 2 days after debranching cooked waxy maize starch. Values are means of three replications with corresponding standard deviations.

The differences in the digestibility of waxy starch are due to the differences in the amount of debranching and how it affects the retrogradation process during storage (Fig. 1). Gidley and Bulpin (1987,1989) described starch precipitation/aggregation and gelation in a phase diagram showing the effect of cooling aqueous solutions of synthetic amylose. In general, precipitation was favored by shorter chain lengths and gelation was favored by longer chain lengths. Thus, mechanisms of chain alignment and cross-linking in cooled aqueous solutions were formulated. For long chains, extensive cross-linking occurs which results in the formation of a macromolecular network eventually resulting in gelation. If the total chain length is not substantially longer than the interacting chain length, then extensive cross-linking will not occur and chain alignment will predominate, a process which, if followed by lateral aggregation, would eventually lead to precipitation (following extensive aggregation). These helices further aggregate leading to formation of ordered crystalline arrays which precipitate out of solution and are resistant to digestion. Conversely, if the chain length over which these interactions occur is substantially shorter than the total chain length, then more than two regions within a single chain could be involved in separate interactions, thereby leading to a cross-linked network structure. Guraya, James, and Champagne (2001a) and Guraya et al. (2001b) found that waxy debranched starches in aqueous solution formed particle sizes around 4 μm on cooling while solutions of non-waxy starches formed 45 μm size aggregates. He also reported that waxy starches would be a good source for making SDS products. In the case of waxy starch, due to absence of longer-chain amylose and greater abundance of smaller chains aggregation is favored resulting in precipitation, then formation of dense and perfect crystallites, which caused decrease in digestibility of starch (Hizukuri, 1985). When partially debranched starch is retrograded the packing of helices to form crystallites might be hindered leading to formation of less dense structures. In addition, Gidley and Bulpin (1987) and Shi and Seib (1992) found that the minimum chain-length required for the formation of double helices (crystallization) was 10. Chains of degree of polymerization (*DP*) 10–12 crystallized as A-form, whereas higher oligomers crystallized as B-form (Kalichevsky, Orford, & Ring, 1990). Zhang, Ao, and Hamaker (2006) and Zhang, Venkatachalam, and Hamaker (2006) found that supramolecular with A-type crystalline structures to be more susceptible to enzymatic hydrolysis compared to B-type starches. Their study further classified starch with A-type crystalline into the category containing a large portion of SDS.

Initially when starch is partially debranched and is allowed to retrograde, imperfect packing of helices in crystallites might take place leaving some tight amorphous regions which can only be digested slowly. By controlling the enzyme concentration in this study, the highest SDS content could be reached by high pullulanase (20 or 40 ASPU/g) debranched for 3–6 h. Samples debranched for more than 6 h displayed decreased SDS and increased RS contents at the three specific dosages. Furthermore, SDS and RS production decreased inversely with that of RDS.

3.2. Effect of recrystallization on the digestibility of starch

The term recrystallization (retrogradation) is used to describe the changes that occur upon cooling and storage of gelatinized starch. Through the process of recrystallization, the gelatinized starch is transformed from an amorphous state to a more ordered or crystalline state. Starch recrystallization occurs readily during the storage of heat-processed starchy foods, as a spontaneous process resulting into a metastable state of lower free energy.

In this study, the amount of SDS increased markedly with storage time at 4 °C up to 48 h, (Fig. 2(a) and (b)). When stored for more than 2 days, there was no marked change in the amount of SDS, although an increase in RS content were observed with increasing storage time. Recent studies (Fredriksson, Silverio, Andemon, Eliasson, & Åman, 1998; Miles, Morris, Orford, & Ring, 1985) have shown that retrogradation consists of two processes: (a) short-term development of crystallinity in a starch gel network which is attributed to the gelation and crystallization of the amylose fraction that happens in a few hours, (b) the long-term changes that occur during storage of starch gels have been attributed to the amylopectin fraction – a much slower process. Eerlingen, Jacobs, and Delcour (1994) reported the enzyme susceptibility of retrograded waxy maize starch depended on the time and temperature conditions of storage. Cui and Oates (1997) found that the digestibility of sago starch was highly sensitive to the retrogradation of amylose that occurred mainly in the early period of storage, whereas amylopectin recrystallization, which occurs more slowly, had little influence on the enzymatic digestibility. It was suggested that the amylose chain associations that contribute to the formation of a gel network provide more significant effects on the enzymatic digestion than amylopectin recrystallization.

Our experiments on waxy maize starch showed that storage at different temperatures resulted in differences in the compositions of the starches (Fig. 2(c)). Debranched (6 h) waxy starch stored for 2 days at 4 °C results in 45.1% SDS and 24.4% RS, showing the

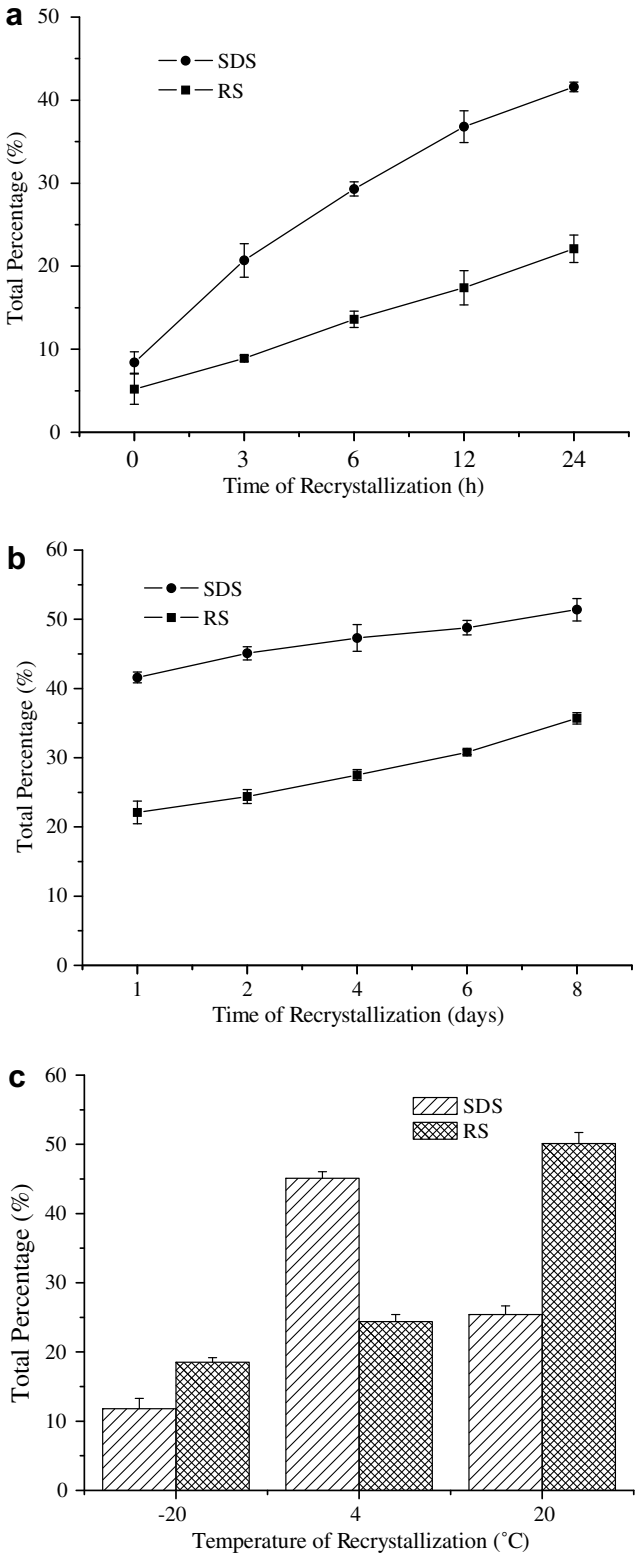

Fig. 2. Effect of recrystallization time at 4 °C (a, b) and recrystallization time for 2 days (c) on SDS and RS production. Values are means of three replications with corresponding standard deviations.

maximum SDS. Storage of debranched starch at −20 °C for 2 days led to a composition of 11.8% SDS and 18.5% RS. Results at 20 °C were 25.4% SDS and 50.1% RS which was the highest RS content. At −20 °C, it displayed lower amount of both RDS and RS contents, which also meant producing highest RDS. In general, crystallization consists of three steps: (1) nucleation – formation of critical nuclei, (2) propagation – growth of crystals from the nuclei formed, and (3) maturation – crystal perfection or slow crystal growth, which strongly depend upon temperature (Eerlingen et al., 1994). For a partially crystalline polymer system, the crystallization process can only occur within the temperature range between the glass transition temperature (T_g) of the system and melting temperature (T_m) of the rubbery state. Guraya et al. (2001a,b) found that making a starch with high amounts of SDS could be done through debranching followed by cooling at 15 °C, which would maximize the amount of RS in an SDS/RS product. In our study, maximum SDS was formed at 4 °C in debranched waxy maize starch aqueous solution, which indicates that storage at 4 °C accelerates nucleation more than propagation and maturation.

3.3. X-ray diffraction

The X-ray diffractograms of the starches from different treatment are presented in Fig. 3(a) and (b). Three different types of crystal structure have been identified and classified by Katz and Itallie in 1930 (Donald, 2004). A-type starch crystallites are from shorter chains and B-type from longer chains. C-type starches actually consist of a mixture of A-type and B-type. In our study, 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°. These results indicated that the crystal type of waxy maize starch is a characteristic A-type (Planchot, Colonna, & Buleon, 1997). The other small peaks were mainly caused by the small-molecules crystal components. Cooked starch did not show any peaks, mostly due to the fact that it is mainly amorphous. The cooked debranched starches stored at 4 °C gave X-ray diffraction patterns of the B-type, because the characteristic diffraction peaks appeared at around 17°, 22° 2θ and varied slightly with different debranching treatment. The results were in agreement with the reports of Miles et al. (1985) and Eerlingen et al. (1994). However, Shin et al. (2004) found the X-ray diffraction pattern of stored sorghum starch was similar to those of cooked and cooked debranched starches. The X-ray diffraction pattern therefore, may depend on the starch origin as well as the environmental growth condition and treated condition.

After storing cooked debranched starches, X-ray diffraction pattern was not comparably consistent (Fig. 3(b)). The cooked debranched starches stored at −20 °C showed a diffraction peak at 14.40°, 17.08°, 21.97° 2θ and were similar to B-type crystalline structure, whereas that stored at 20 °C gave a composite of the B-type crystalline structure and V-form of amylose–lipid complex at 2θ with values of 14.30°, 17.04°, 19.60°, 22.08°, 24.12°, which also is a typical crystal structure of Hi-maize™ resistant starch. When cooked debranched starches were stored at 4 °C for up to 4 days, the X-ray diffraction pattern changed from B-type to B-type and V-type complex. According to Han et al. (2006), lipid-complexed amylose in the amorphous region of starch can be partially hydrolyzed to form B-type crystal structure and ethanol used in the precipitation of pullulanase treated starches complexes with amylose and hence displays V-type diffraction pattern of amylose–lipid complex.

The calculated degrees of crystallinity of the starch samples in our study are shown in Table 1. Generally, differences in degree of crystallinity between starches could be attributed to the following: crystal size, amount of crystalline regions (influenced by amylopectin content and amylopectin chain length), orientation of the double helices within the crystalline domains, and extent of interaction between double helices (Song & Jane, 2000). The differences in degree of crystallinity (Table 1) could be due to an interplay of these factors. From the X-ray diffractions and degree of crystallinity, the SDS appeared to contain amorphous components and dou-

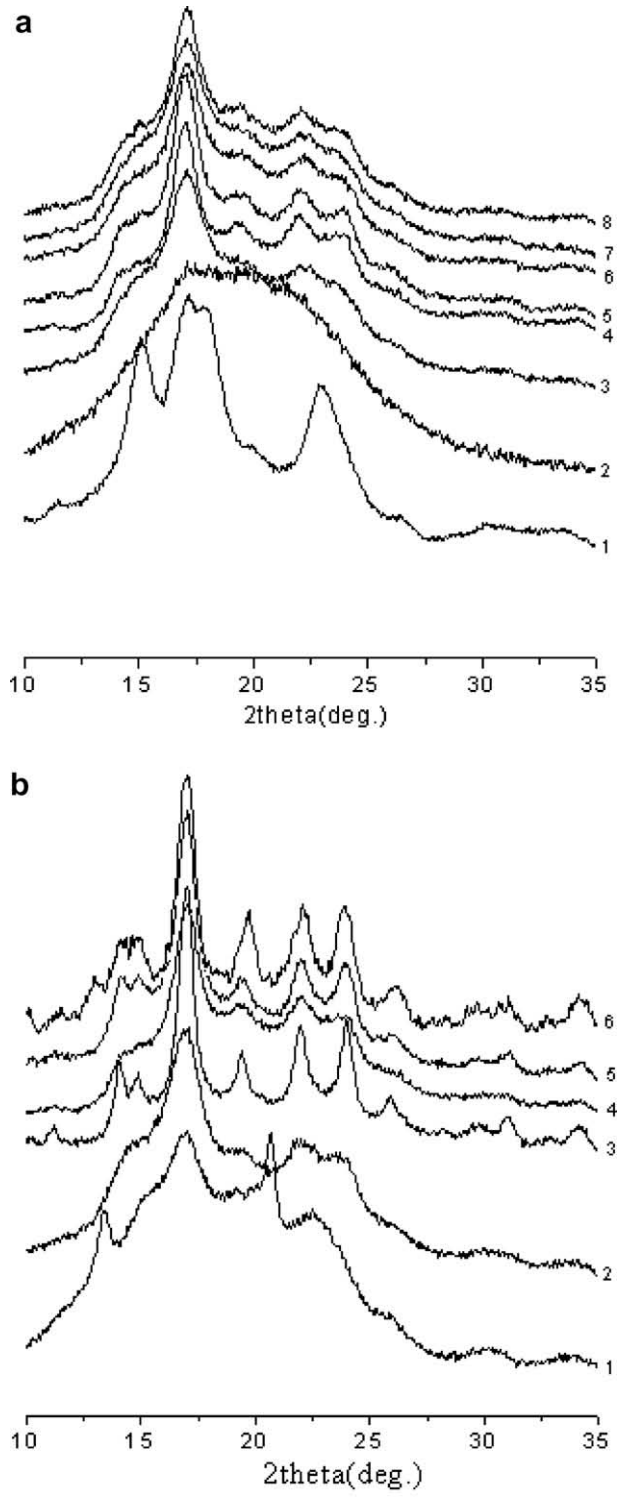

Fig. 3. X-ray diffraction patterns of debranching (a) and recrystallization (b) of cooked waxy maize starch. (a) 1. Raw waxy maize starch, 2. Cooked waxy maize starch, 3. Debranching 6 h at 10 ASPU/g pullulanase then storage at 4 °C for 2 days, 4. Debranching 6 h at 20 ASPU/g pullulanase then storage at 4 °C for 2 days, 5. Debranching 6 h at 40 ASPU/g pullulanase then storage at 4 °C for 2 days, 6. Debranching 3 h at 20 ASPU/g pullulanase then storage at 4 °C for 2 days, 7. Debranching 6 h at 20 ASPU/g pullulanase then storage at 4 °C for 2 days, 8. Debranching 9 h at 20 ASPU/g pullulanase then storage at 4 °C for 2 days. (b) 1. Debranching 6 h at 20 ASPU/g pullulanase then storage at −20 °C for 2 days, 2. Debranching 6 h at 20 ASPU/g pullulanase then storage at 4 °C for 2 days, 3. Debranching 6 h at 20 ASPU/g pullulanase then storage at 20 °C for 2 days, 4. Debranching 6 h at 20 ASPU/g pullulanase then storage at 4 °C for 4 days, 5. Debranching 6 h at 20 ASPU/g pullulanase then storage at 4 °C for 8 days, 6. Hi-maize™ resistant starch.

Table 1
X-ray diffraction data of raw and treated starches.

Samples	Degree of crystallinity (%)	Crystal pattern
Raw waxy maize starch	26.47	A
Cooked waxy maize starch	0	–
Debranching with pullulanase (10 ASPU /g)[a]	10.77	B
Debranching with pullulanase (20 ASPU /g)[a]	15.73	B
Debranching with pullulanase (40 ASPU /g)[a]	19.99	B
Debranching 3 h[b]	11.39	B
Debranching 6 h[b]	13.03	B
Debranching 9 h[b]	16.25	B
Storage at −20 °C[c]	4.27	B
Storage at 4 °C[c]	15.73	B
Storage at 20 °C[c]	44.03	B+V
Storage for 4 days[d]	20.42	B+V
Storage for 8 days[d]	35.02	B+V
Hi-maize™ resistant starch	41.50	B+V

[a] Debranching 6 h then storage at 4 °C for 2 days.
[b] Debranching at 20 ASPU/g pullulanase then storage at 4 °C for 2 days.
[c] Debranching 6 h at 20 ASPU/g pullulanase then storage for 2 days.
[d] Debranching 6 h at 20 ASPU/g pullulanase then storage at 4 °C.

ble helical components with partially ordered structure as suggested by Guraya et al. (2001b).

3.4. Morphological properties

The granular structure of raw starch and treated starches exhibited significant variations in size and shape when viewed by scanning electron microscopy (SEM). Scanning electron micrographs of the starch granules are illustrated in Fig. 4. The granule size of waxy maize starch varied from 5 to 25 μm. The granule surface appeared smooth, round or oval shaped with indentations. When starch was cooked in excess water at gelatinization temperature, molten starch granules connected with each other forming a net-like structure. Heating lead to further swelling and melting, ultimately the granules lost their identity, and beams of the neighbouring deformed granules joined together and to form a continuous sponge-like structure as reported by Liu and Zhao (1990) and Ratnayake and Jackson (2007). After freeze-drying treatment, the granular structure disappeared in cooked–debranched–stored samples, and smooth plate-like surfaces, bigger, irregularly shaped particles with a higher density structure were observed. The size of particles diminished as the recrystallizing time increased and more angular and pitted surfaces were appeared. Results obtained with changes in debranching time showed the same trend but were less clear (Figures not shown). According to a report of Shin et al. (2004), RDS, SDS and RS fractions were reformed into a single crystallite by low temperature storage following debranching with high concentration of enzyme. RDS had amorphous structure and appeared to be located in the outer regions. SDS was intermediate between RDS and RS and mostly consisted of amorphous regions and a small portion of imperfect crystallites. RS had an ordered double helix structure and located in inner regions. It could be speculated through combining these results with those obtained from X-ray diffraction and digestibility stated above, that the granular structure of SDS fraction was made up of imperfect crystallites with lower crystallinity.

3.5. Differential scanning calorimetry

The gelatinization transition temperature (T_o, T_p, T_c) and the enthalpy of gelatinization (ΔH) are presented in Table 2. Variations in T_o, T_p, T_c and ΔH have been shown by various researchers to reflect the cystallinity, structure and composition of starches. Native waxy

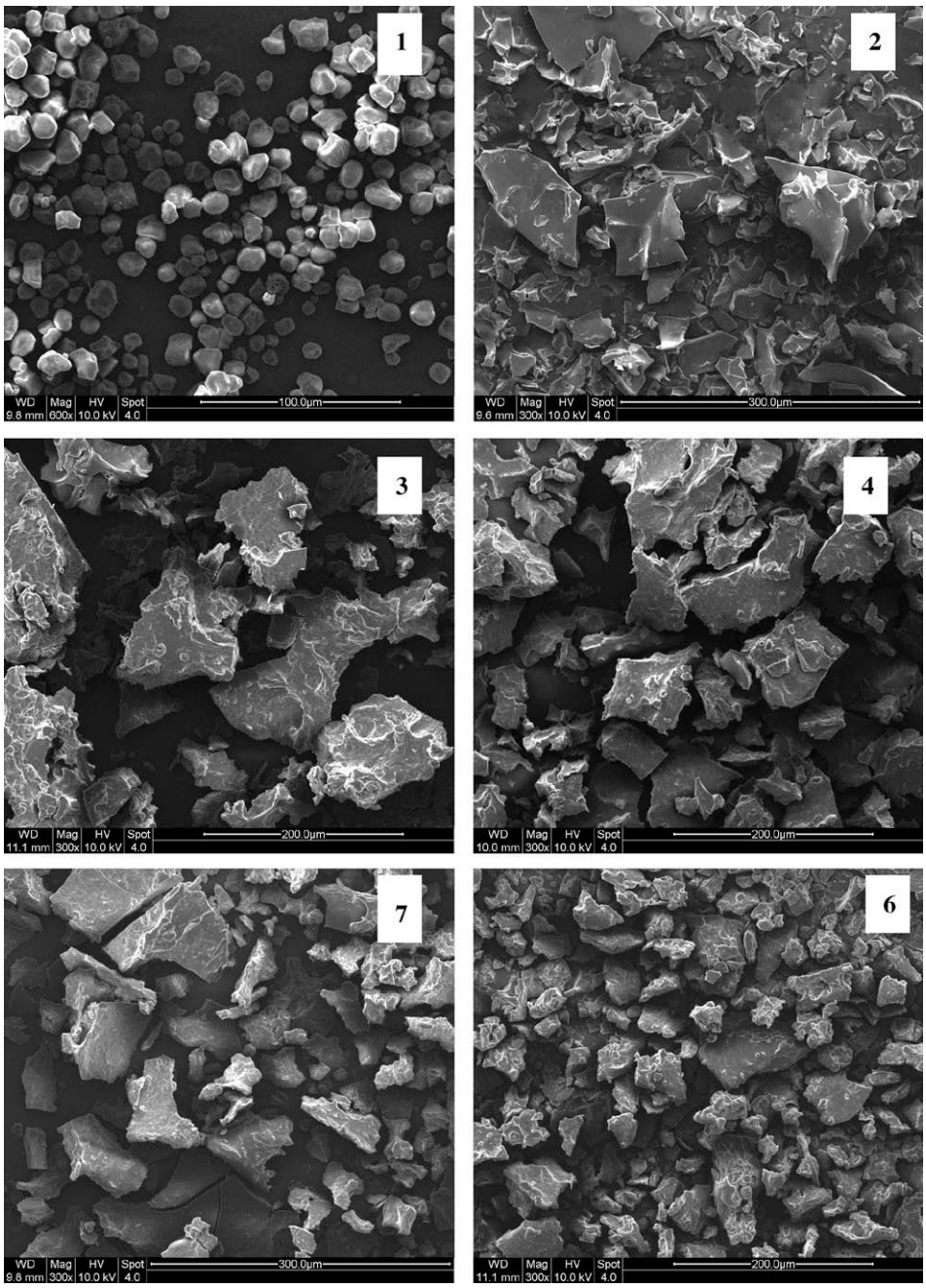

Fig. 4. Scanning electron micrograph of the raw and treated starches. 1. Raw waxy maize starch, 2. Cooked waxy maize starch, 3. Debranching 6 h at 20 ASPU/g pullulanase then storage at 4 °C for 24 h, 4. Debranching 6 h at 20 ASPU/g pullulanase then storage at 4 °C for 2 days, 5. Debranching 6 h at 20 ASPU/g pullulanase then storage at 4 °C for 4 days, 6. Debranching 6 h at 20 ASPU/g pullulanase then storage at 4 °C for 8 days.

maize starch showed two endotherms (Fig. 5) with T_p (56.590 and 103.735 °C). Similar biphasic endothermic were also observed in other waxy starches when gelatinized at low moisture levels (Eerlingen et al., 1994). The cooked starch did not show any thermal transitions. This indicated the gelatinization under the experimental conditions used was complete, and no recrystallization occurred. On storage of debranched starches, an endothermic transition was observed. This endothermic transition has been attributed to the recrystallization of amylopectin (Eerlingen et al., 1994). The T_o, T_p, T_c, and ΔH values of stored starch increased markedly as the storage time prolonged. 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. Low T_o, T_p and T_c reflect the presence of abundant short amylopectin chains (Noda et al., 1998). In the study by Tester and Morrison (1990), 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 (Vasanthan & Bhatty, 1996). Therefore, cooked–debranched starch with short amylopectin chains probably produced several different types of crystallites during storage and there was an increased variation in crystallinity and perfect

Table 2
Thermodynamic parameters of raw and treated starches.

Samples	T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)	ΔH (J/g)
Raw waxy maize starch	48.112	56.590, 103.735	115.071	66.959	7.858
Cooked waxy maize starch	0	0	0	0	0
Storage for 24 h[a]	48.660	56.583	69.573	20.913	2.146
Storage for 2 days[a]	71.100	80.072	90.239	19.139	3.812
Storage for 4 days[a]	77.582	86.172	97.662	20.08	4.154
Storage for 8 days[a]	80.603	90.079	98.226	17.623	6.287

[a] Debranching at 20 ASPU/g pullulanase then storage at 4 °C.

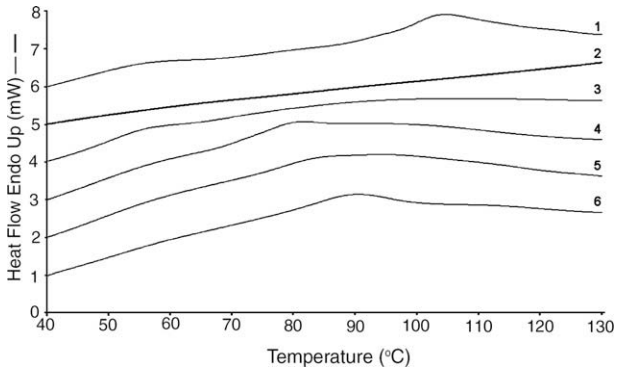

Fig. 5. Differential scanning calorimetry thermograms of the raw and treated starches. 1. Raw waxy maize starch, 2. Cooked waxy maize starch, 3. Debranching 6 h at 20 ASPU/g pullulanase then storage at 4 °C for 24 h, 4. Debranching 6 h at 20 ASPU/g pullulanase then storage at 4 °C for 2 days, 5. Debranching 6 h at 20 ASPU/g pullulanase then storage at 4 °C for 4 days, 6. Debranching 6 h at 20 ASPU/g pullulanase then storage at 4 °C for 8 days.

crystallites with increasing recrystallization time, which was consistent with the results in Table 1.

According to Cooke and Gidley (1992), ΔH reflects loss of double helical order rather than the loss of crystallinity. Tester and Morrison (1990), however, postulated that ΔH reflects the overall crystallinity (quality and amount of crystallites) of amylopectin. Eerlingen et al. (1994) also reported increased retrogradation extents (high melting temperatures, melting enthalpies, and higher crystallinity levels) caused reduced enzyme susceptibility to pancreatic α-amylase and amyloglucosidase at 37 °C, since these were associated with slow digestion property of starch. Based on the results from X-ray diffraction and DSC in the present study, we therefore can conclude that SDS probably mainly contains a small portion of double helical components with a partially ordered amylopectin structure which is essentially made up of short chains, in addition to amorphous components.

4. Conclusions

The proportion of SDS, RS and RDS varied after debranching and recrystallization treatment. Increasing pullulanase concentration and decreasing debranching time led to increased maximum SDS, while longer debranching time increase the proportion of RS. Alteration of enzyme concentration changed the amount of imperfect packing helices in crystallites, hence altering the proportion of amorphous and ordered packed regions associated with SDS. Recrystallization at 4 °C affects the proportion of SDS relative to RS and RDS due to increased nucleation during recrystallization as opposed to propagation and maturation steps. A higher SDS content was obtained with debranching 3–6 h at high pullulanase (20 or 40 ASPU/g) then storage at 4 °C for 2 days compared to at low pullulanase (10 ASPU/g). Starch with high SDS content was made up of mainly B-type crystals, but time and temperature alterations shift the crystal form to B- and V-type complex. Longer time and higher temperature increased degree of crystallinity. Scanning electron microscopic observation revealed that the treated starch was more irregular angularly shaped with a higher crystallinity structure and pitted surface as debranching and recrystallization time increased. Based on the distribution of amylopectin short chains, there were different crystallites formed after treatment. The formed crystallites with different recrystallization time showed the changes in the gelatinization transition temperature (T_o, T_p, T_c) and the enthalpy of gelatinization (ΔH) on a differential scanning calorimetric thermogram. These changes showed that SDS mainly consists of a small portion of double helical crystalline regions with a partially ordered structure and amorphous regions.

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