Carbohydrate Polymers 113 (2014) 91–96

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Debranching and temperature-cycled crystallization of waxy rice starch and their digestibility

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ARTICLE INFO

Article history:
Received 17 February 2014
Received in revised form 7 June 2014
Accepted 15 June 2014
Available online 10 July 2014

Keywords:
Crystallization
Pullulanse debranching
Short linear chain
Slowly digestible starch
Temperature-cycled

ABSTRACT

Slowly digestible starch (SDS) was obtained through debranched waxy rice starch and subsequent crystallization under isothermal and temperature-cycled conditions. Temperature-cycled crystallization of dual 4/−20 °C produced a higher yield of SDS product than isotherm crystallization. Crystal structure of SDS products changed from A-type to a mixture of B and V-type X-ray diffraction patterns. The relative crystallinity was higher in the temperature-cycled samples than that of isotherm. Attenuated total reflectance Fourier transform infrared spectroscopy suggested that the peripheral regions of isothermal storage starch were better organized than temperature-cycles. Temperature cycling induced higher onset temperature for melting of crystals than isothermal storage under a differential scanning calorimeter. The cycled temperature storage induced a greater amount of SDS than the isothermal storage.

1. Introduction

Starch is the principal carbohydrate in cereal grains and an important source of nourishment for humans. From a nutritional point of view, starch is generally classified as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) based on the rate and extent of digestion (Englyst, Kingman, & Cummings, 1992). RDS causes an increase in blood glucose level immediately after ingestion and SDS is digested completely in the small intestine but this process is slow. RS is not digested in the small intestine but fermented in the large bowel into short-chain fatty acids (Cummings, Beatty, Kingman, Bingham, & Englyst, 1996). SDS offers the advantage of a slow increase of postprandial blood glucose level and sustains blood glucose level over time which is helpful in controlling and preventing the hyperglycemia related diseases. Foods containing a substantial amount of SDS could be advantageous to satiety, physical performance, improve the glucose tolerance and reduce blood lipid levels in both healthy individuals and those with hyperlipidemia (Jenkins et al., 2002).

Recently, substantial efforts have been made to modify the starch to reduce its digestibility, including temperature-cycled crystallization (Park, Baik, & Lim, 2009; Tian et al., 2012; Zhang, Hu, Xu, Jin, & Tian, 2011), enzymatic modification by debranching (Miao, Jiang, & Zhang, 2009; Shin et al., 2004), hydrothermal treatment (Chung, Liu, & Hoover, 2010; Lee, Kim, Choi, & Moon, 2012; Lee, Shin, Kim, Choi, & Moon, 2011), retrogradation treatment (Chung, Lim, & Lim, 2006; Tian et al., 2013), chemical modification (Güzel & Sayar, 2010; Han & BeMiller, 2007), acid modification (Shin et al., 2007, 2009; Zhang, Huang, Luo, & Fu, 2012) and functional starch resources. Among the enzymatic methods, pullulanase debranching and crystallization treatment are cost-effective, safe and more suitable for commercial use (Miao et al., 2009; Shin et al., 2004).

Enzymatic modification by debranching generates short linear α-1,4-linked glucans, resulting from reforming of double helix structure by low temperature crystallization. A process for making SDS products by using debranching waxy rice starch, waxy maize starch and waxy sorghum have been reported (Guraya, James, & Champagne, 2001; Miao et al., 2009; Shin et al., 2004). A few authors have reported the impact of temperature-cycle on the formation of SDS in waxy rice starch (Tian et al., 2012; Zhang et al., 2011) and waxy maize starch (Park et al., 2009). A temperature near the glass transition temperature favors nucleation, whereas a higher temperature up to the melting temperature favors propagation (Baik,

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http://dx.doi.org/10.1016/j.carbpol.2014.06.057
0144-8617/

Kim, Cheon, Ha, & Kim, 1997; Durrani & Donald, 1995; Silverio, Fredriksson, Andersson, Eliasson, & Åman, 2000). When the storage temperature of gelatinized starch was cycled between the temperature for nucleation and the temperature for propagation, the rate of recrystallization could be accelerated (Bemiller, 2007; Slade, Oltzik, Altomare, & Medcalf, 1987). Temperature cycling induces a stepwise nucleation and propagation, promoting the growth of crystalline regions and perfection of crystallites (Silverio et al., 2000). These processes depend on the storage temperature, cycled interval and storage time.

Because of its wide-ranging food and industrial applications, waxy rice starch has been extensively studied. In our knowledge, no further report was found using a combination of pullulanase debranching and subsequent temperature-cycled crystallization treatment to produce SDS. It is important to understand its digestibility so that novel methods can be developed to produce SDS that has practical applications in the food industry. Englyst's method, which can be used to determine the portions of starch and starch degradation products, was developed to imitate the physiological conditions of starch digestion. The primary objective of this study is to investigate the digestibility and physicochemical properties of starch prepared by pullulanase debranching and subsequent temperature-cycled crystallization treatment.

2. Materials and methods

2.1. Materials

Waxy rice starch (0% amylose) was obtained from Jiangsu Baobao Group (Nantong, China). Porcine pancreas α-amylase (EC3.2.1.1, 16 U/mg) type-B and amyloglucosidase (EC 3.2.1.3, 300 U/mL) from *Aspergillus niger* were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Pullulanase (EC 3.2.1.41, 2800 ASPU/g) from *Bacillus licheniformis* was attained from Guangzhou Yulibao Biotechnology Co., Ltd. (Guangzhou, China). Megazyme glucose assay kit (GOPOD method) was bought from Megazyme International Ireland Ltd. (Wicklow, Ireland). Other chemicals and solvents were all of analytical grade.

2.2. Preparation of the SDS products

Waxy rice starch (15 g, dry basis) slurry (10%, w/v, in diluted pH 5.2 buffer solution containing 0.2 M sodium acetate and 0.2 M acetic acid) was cooked at 100 °C with continuous stirring for 30 min. The temperature of cooked starch gels was adjusted to 58 °C and debranched by pullulanse at 60 ASPU/g of starch for 12 h. The debranched starch gels were proved to be linear (Cai & Shi, 2010). Immediately after the reaction, the gels were heated at 100 °C for 30 min to deactivate the enzyme and cooled to room temperature. The gels were subjected to isothermal and temperature-cycled crystallization with different temperature cycles at the time intervals of 24 h for four days. The temperature cycles were designed as described in Table 1. The four days isothermal crystallization was describered as 4/4 4/4 °C. Temperature-cycled crystallization of 4/−20 4/−20 °C, 4/30 4/30 °C and 4/40 4/40 °C was describered as dual 4/−20 °C, dual 4/30 °C and dual 4/40 °C, respectively.

Table 1
The different temperature cycles used during four days of crystallization.

Temperature cycles	Storage temperature (°C)			
	Day 1	Day 2	Day 3	Day 4
4/4 4/4 °C	4	4	4	4
4/−20 4/−20 °C	4	−20	4	−20
4/30 4/30 °C	4	30	4	30
4/40 4/40 °C	4	40	4	40

Specimens were subjected to water bath (thawing dual 4/−20 °C samples) treatment at 45 °C after dual cycles. The precipitate was centrifuged, washed three times with distilled water and dried at 40 °C overnight then gently ground by a mortar and pestle to pass through a 100-mesh sieve.

2.3. ATR-FTIR analysis

ATR-FTIR analysis of starches was obtained with an FT-IR spectrometer (TENSOR27, BRUCK, Germany) equipped with a deuterated triglycine sulfate (DTGS) detector using an attenuated total reflectance (ATR) mode. For each spectrum, 16 scans were recorded at a resolution of 4 cm^{-1} at room temperature. Spectra were baseline-corrected and then deconvoluted over the range of 1200–800 cm^{-1}. A half- width of 22 cm^{-1} and a resolution enhancement factor of 2.2 were used. The amplitudes of absorbance for each spectrum at 1022 and 1047 cm^{-1} were noted and the ratio of amplitudes of absorbance at 1047 cm^{-1} and at 1022 cm^{-1} was calculated per sample to estimate the degree of order of the starch at the surface (Sevenou, Hill, Farhat, & Mitchell, 2002).

2.4. X-ray diffraction and relative crystallinity

X-ray diffraction analysis was performed with an X-ray diffractometer (D8 ADVANCE, Bruker, Germany) operated at 40 kV and 40 mA producing Cu Kα radiation of 1.5418 Å wavelength, scanning through the 2θ range from 3° to 35° at a rate of 2°/min. The moisture of a specimen was regulated to about 15% by storage in a sealed desiccator over water at 25 °C. Relative crystallinity was calculated by the ratio of the crystalline area to the total diffractogram area (Nara & Komiya, 1983).

2.5. Differential scanning calorimetry

The thermal transitions of starches were investigated with the use of a differential scanning calorimetry (DSC 8000, Perkin Elmer Inc., Norwalk, USA). A starch sample (3 mg) was weighed in a DSC pan and the excess water was added to obtain a starch/water ratio of 3:7. The pans were then sealed, equilibrated for 4 h at room temperature, then heated from 30 to 130 °C at the rate of 10 °C/min. Gelatinization onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), gelatinization range (ΔT) and enthalpy values (ΔH) were measured to characterize the thermal properties of starch.

2.6. High-performance size-exclusion chromatography (HPSEC) and multi-angle laser-light scattering (MALLS) with refractive index (RI) detector

Starch sample (12.5 mg) was stirred in 25 mL of dimethyl sulfoxide (DMSO) which contain 50 mM LiBr and heated it in a boiling water bath for 30 min. After that, the liquid system was stirred for 24 h at room temperature. The solutions were then filtered through a nylon filter (0.22 μm type membrane, Millipore, USA) before injection into the MALLS system (Wyatt Technology, Santa Babara, CA, USA) consisting of a pump (P2000, Spectra System, San Hose, CA, USA), an injector valve with a 1 mL loop, SEC column (P8514-806, Showa Denko, Tokyo, Japan), a MALLS fitted with an argon laser (488 nm), and an Optilab 903 refractive index detector (Wyatt Technology, Santa Barbara, CA, USA). The sample (1 mL) was injected into the system and ran at a flow rate of 0.3 mL/min. The mobile phase was DMSO and degassed under vacuum. The column oven temperature was controlled at 40 °C. The molecular weights were calculated using the ASTRA 6.1 software program (Wyatt Technology). Each sample was analyzed in duplicate.

2.7. In vitro digestibility

2.7.1. Before cooking

Starch nutrition fractions were analyzed according to the method described by Englyst et al. (1992) with minor modifications. Enzyme solution containing porcine pancreas α-amylase and amyloglucosidase was prepared immediately before use. Starch (200 mg) was dispersed in 15 mL of sodium acetate buffer (0.2 mol/L, pH 5.2) by vortexing. Then six glass balls and 10 mL mixtures of porcine pancreatic α-amylase (290 U/mL) and amyloglucosidase (15 U/mL) were added. Enzyme digestion was carried out at 37 °C and 0.5 mL aliquots of hydrolyzed solution was withdrawn at different time intervals and mixed with 4 mL of absolute ethanol to denature the enzymes. The glucose in the supernatant gained from centrifugation (4000 rpm, 10 min) was measured with the Megazyme glucose assay kit (GOPOD method).

The rapidly digestible starch (RDS) content was measured as the amount of glucose released in 20 min of incubation. The slowly digestible starch (SDS) fraction was defined as the fraction digested between 20 and 120 min of hydrolysis. The starch not hydrolyzed within 120 min was designated resistant starch (RS) content.

2.7.2. After cooking

Starch (200 mg) was dispersed in 15 mL of sodium acetate buffer (0.2 mol/L, pH 5.2) by vortexing. Six glass balls were added and capped, then placed in a boiling water bath for 20 min and vortexed to avoid agglomeration. The dispersion was equilibrated in a shaking water bath at 37 °C for 10 min. After that, 10 mL mixtures of porcine pancreatic α-amylase (290 U/mL) and amyloglucosidase (15 U/mL) were added. The same analysis of digestibility described in Section 2.7.1 was followed (Zhang et al., 2012).

2.8. Statistical analysis

Each sample was analyzed in duplicate. Mean values and standard deviations were reported. Duncan's least significant test was used to compare means at the 5% significance level. All the statistical analyses were conducted using the SPSS for Windows 12.0 software (SPSS, Chicago, IL, USA).

3. Results and discussion

3.1. The yield of SDS products under different storage conditions

The yield of SDS products is defined as the ratio (w/w) between crystallized starch (after modification) and native starch (before modification). The yield of SDS products under isothermal and temperature-cycled treatments followed the order: 4/−20 4/−20 °C (82.78%) > 4/4 4/4 °C (69.11%) > 4/30 4/30 °C (60.17%) > 4/40 4/40 °C (51.70%). The yield of SDS product was much higher in 4/−20 4/−20 °C than those of the other treatments. Tian et al. (2012) observed that temperature cycle of 4/25 °C, 14 days storage of waxy rice starch gel was available for increasing the yield of SDS which reached the maximum yield of 54.5%. It is generally recognized that the gelatinized amylose can be organized into a crystalline area completing in several hours (Miles, Morris, Orfopd, & Ring, 1985; Sievert & Wursch, 1993), while amylopectin crystallization needs several days or more than 1month (Eliasson, 1985; Gudmundsson, 1994; Tian et al., 2009). Similarly, short linear chains generated from debranched waxy rice starch showed a shorter time in organizing into crystalline than that of non-debranched waxy rice starch (Tian et al., 2012).

However, the yields of SDS products were lower in temperature-cycled 4/30 °C and 4/40 °C than that of isothermal 4 °C. Cai and Shi (2013) suggested that the recovery of short-chain amylose

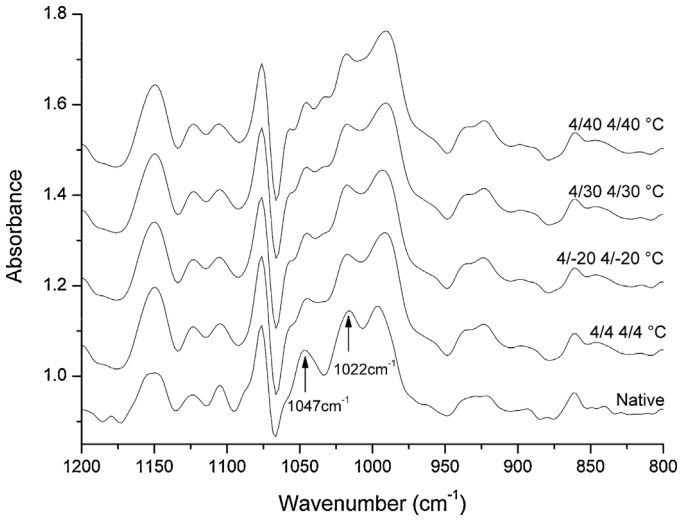

Fig. 1. ATR-FTIR spectra of starch samples.

spherulites decreased from 88 to 50% as the crystallization temperature increased from 4 to 50 °C. In this study, possibly because part short linear chains could not rearrange and crystallize at 30 °C and 40 °C. Thus, both cycles of dual 4/30 °C and dual 4/40 °C showed lower yield than isotherm storage. These results exhibit that a suitable crystallization temperature and cycle could lead to the interaction between short linear chains and result in the higher yield of SDS product. Thus, combination of debranching and temperature-cycled treatment (dual 4/−20 °C) exhibited higher yield of SDS products in shorter time than the results of Tian et al. (2012).

3.2. ATR-FTIR spectra

The deconvoluted FT-IR spectra of native and modified starches are presented in Fig. 1. FT-IR technique yields information on the structural organization of starch chains near the granule surface, since the IR beam penetrates only to a depth of 2 μm into the granule (Sevenou et al., 2002; van Soest, Tournois, de Wit, & Vliegenthart, 1995). The IR bands at 1047 and 1022 cm^{-1} have been revealed to be associated with ordered and amorphous structures of starch, respectively (Capron, Robert, Colonna, Brogly, & Planchot, 2007). The ratio of the heights of bands at 1047 and 1022 cm^{-1} expresses the amount of ordered starch to amorphous starch.

The modified waxy rice starches showed similar deconvoluted FT-IR spectra to native starch. The ratios of the absorbance of the bands at 1047 and 1022 cm^{-1} of starch samples followed the order: native (0.96) > 4/4 4/4 °C (0.95) > dual 4/30 °C (0.94) > dual 4/−20 °C (0.93) ~ dual 4/40 °C (0.93). This suggests that the external regions of native starch granules are better organized than in modified starches. The above ratio was higher in non-cycled sample (4/4 4/4 °C), but lower in cycled samples. The higher ratio of non-cycled sample suggested that crystallites at the granule surface were better organized, and were thus easily perfected on isothermal storage. The decrease in the ratio on temperature cycled specimens were more pronounced in dual 4/40 °C starch, since crystallite disruption on 40 °C would have a greater impact on crystallites that are highly organized at the granule surface.

3.3. X-ray diffraction and relative crystallinity

The X-ray diffraction patterns of starch samples are shown in Fig. 2. The relative crystallinity calculated from X-ray diffraction pattern ranged from 27.64 to 38.10% (Fig. 2). The native waxy rice

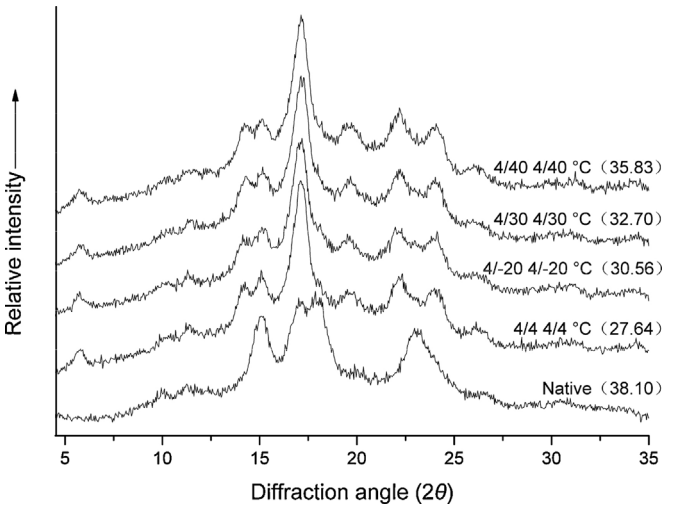

Fig. 2. X-ray diffraction patterns and relative crystallinity of starch samples.

starch displayed the typical A-type diffraction pattern with peaks at 15°, 17°, 18° and 23° (2θ).

After pullulanase debranching and stored for four days, the peaks at 5.5°, 17°, 22°, 24° (2θ) showed that the starch crystals formed in the crystallization process had the B-type. The B-type characteristic is typical for crystallized starch. The results were in agreement with the results of Eerlingen, Jacobs, and Delcour (1994) and Miles et al. (1985). Meanwhile, the modified starches all exhibited V-type diffraction pattern with peaks at 20° (2θ). Miao et al. (2009) suggested that pullulanase debranching waxy maize starch stored at 4 °C for up to four days, the X-ray diffraction pattern changed from B-type to B-type and V-type complex. There was no notable difference in X-ray diffraction patterns between isothermal and cycled samples, while an increase in relative crystallinity was observed after cycled storage. Park et al. (2009) claimed that no significant differences were observed in X-ray patterns among waxy maize starch gels stored for different periods of time and temperature-cycles.

3.4. Gelatinization parameters

Table 2 presents gelatinization parameters (T_o, T_p, T_c, ΔT, ΔH) for native and modified starches. The starch crystals formed under the cycled conditions melted at a higher T_o and T_c than that of constant temperature. Park et al. (2009) suggested that the temperature-cycled starch samples melted at a higher onset temperature than those formed under constant temperature storage at 4 °C. During storage at 30 °C and 40 °C, similar to annealing treatment, possibly some of the unstable crystals transformed to more stable crystals which would result in an increased melting T_o (Table 2). Zeleznak and Hoseney (1987) reported that starch crystallites were annealed to increase perfection at a high storage temperature. It is probable that the imperfect crystallites formed at 4 °C were melted which lowered the number of crystallites during storage at 30 °C and 40 °C, although the overall crystallinity increases with the repeated cycles storage.

The crystallites under the cycled conditions melted at a substantially greater ΔT and ΔH than that of constant temperature. Differences in ΔT could be resulted from the presence of crystallites which are composed of smaller crystallites, each possessing slightly different strength (Vasanthan & Bhatty, 1996). Thus, debranched starch under different storage conditions probably produced several different types of crystallites during storage and there was an increased variation in relative crystallinity, which was consistent with the results in Fig. 2. In this study, temperature-cycled induced higher ΔH as compared with isothermal condition. Park et al. (2009) suggested that the temperature-cycled waxy maize starch samples melted at a lower ΔH than those formed under constant temperature storage at 4 °C. Silverio et al. (2000) also suggested that a decreased melting ΔH was observed with waxy maize starch after a treatment at cycling temperature of 6 and 40 °C. The difference between our results and previous studies possibly due to differences in botanical variety, as well as the method employed to debranch starch molecule which generated a substantial amount of short linear chains. The results indicated that the temperature cycles storage appeared to induce the formation of stable crystals as compared to isothermally at 4 °C.

Table 3
Weight-average molar mass, radius of gyration and molecular density of starches before and after treatments.

Starch sample	$M_w \times 10^5$ (g/mol)	R_z (nm)	ρ (g/mol/nm^3)
Native	1037.75 ± 116.32^a	337.35 ± 32.74^a	2.83 ± 1.11^b
4/4 4/4 °C	3.69 ± 0.18^b	25.95 ± 0.49^b	21.10 ± 0.18^a
4/−20 4/−20 °C	3.57 ± 0.05^b	26.55 ± 0.07^b	19.05 ± 0.12^a
4/30 4/30 °C	3.92 ± 0.09^b	26.00 ± 0.42^b	22.34 ± 1.62^a
4/40 4/40 °C	4.02 ± 0.09^b	26.60 ± 0.28^b	21.38 ± 1.16^a

M_w, weight-average molar mass; R_z, z-average radius of gyration; ρ, density (M_w/R_z^3).
Values with different superscripts within a column are significantly different ($p < 0.05$).

3.5. Weight-average molar mass, gyration radius and molecular density

The weight-average molar mass (M_w), gyration radius (R_z) and molecular density (ρ) from the starch samples are shown in Table 3. The M_w of native waxy rice starch was larger than those of treated counterparts. This indicated that native waxy rice starch was composed of highly polymerized amylopectin. The M_w of native waxy rice starch obtained in our study was similar to that reported in Shin, Choi, Park, and Moon (2010).

The lower M_w of the treated starches resulted from the degradation of α-(1→6) glycosidic by pululanase debrabching. The radius of gyration (R_z) is related to the theoretical probability of finding the molecule at a given distance from the center. The larger proportion of long branch-chains of amylopectin of the waxy rice starch may result in higher R_z, since it is dependent on the volume occupied by

Table 2
Gelatinization parameters of starch samples.

Starch sample	T_o (°C)	T_p (°C)	T_c (°C)	ΔT (°C)	ΔH (J g^{-1})
Native	70.2 ± 0.0^c	75.0 ± 0.0^d	82.0 ± 0.0^c	11.8 ± 0.1^c	12.4 ± 0.3^a
4/4 4/4 °C	74.0 ± 0.4^b	82.5 ± 0.0^a	89.0 ± 0.9^b	9.0 ± 0.5^d	5.4 ± 0.1^b
4/−20 4/−20 °C	75.3 ± 0.7^b	81.3 ± 0.8^a	91.4 ± 1.3^{ab}	16.1 ± 1.2^{ab}	11.0 ± 1.6^a
4/30 4/30 °C	77.3 ± 0.3^a	83.3 ± 1.0^a	93.0 ± 0.5^a	15.7 ± 0.6^b	12.0 ± 1.0^a
4/40 4/40 °C	75.0 ± 0.4^b	82.8 ± 0.0^a	93.6 ± 0.2^a	18.4 ± 0.5^a	11.8 ± 1.0^a

T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔT, gelatinization temperature range; ΔH, gelatinization enthalpy.
Values with different superscripts within a column are significantly different ($p < 0.05$).

Table 4
The amount of RDS, SDS and RS of starch samples before and after cooking.

Starch sample	Before cooking			After cooking		
	RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)
Native	32.4 ± 1.4^{a}	45.5 ± 2.0^{ab}	22.1 ± 2.1^{b}	75.4 ± 2.5^{a}	13.2 ± 1.4^{b}	11.4 ± 2.6^{b}
4/4 4/4 °C	29.1 ± 0.6^{ab}	36.2 ± 0.8^{c}	34.7 ± 2.7^{a}	61.5 ± 0.7^{b}	27.6 ± 2.3^{a}	10.9 ± 2.9^{b}
4/−20 4/−20 °C	20.5 ± 2.2^{c}	48.4 ± 2.1^{a}	31.1 ± 1.5^{a}	47.6 ± 1.2^{c}	32.3 ± 1.2^{a}	20.1 ± 2.0^{a}
4/30 4/30 °C	26.5 ± 1.7^{b}	45.4 ± 0.7^{ab}	28.1 ± 0.9^{ab}	50.9 ± 0.6^{c}	30.9 ± 1.0^{a}	18.2 ± 0.8^{ab}
4/40 4/40 °C	29.4 ± 0.8^{ab}	39.7 ± 3.1^{bc}	30.9 ± 2.6^{a}	46.3 ± 2.1^{c}	29.4 ± 1.6^{a}	24.3 ± 1.5^{a}

RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch.
Values with different superscripts within a column are significantly different ($p < 0.05$).

the molecule in solution (Millard, Dintzis, Willett, & Klavons, 1997). A higher R_z and a lower dispersed molecular density ($\rho = M_w/R_z^3$) was found in native starch than those of treated starches. The changes in M_w, R_z and ρ between isothermal starch and temperature-cycled starches were not significantly different. These results indicated that the structure changes of starch molecules mainly depended on the process of pululanase debrabching.

3.6. *In vitro digestibility*

The amounts of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) are presented in Table 4. The SDS levels among the native and modified starches followed the order: dual 4/−20 °C (48.4%) > native starch (45.5%) > dual 4/30 °C (45.4%) > dual 4/40 °C (39.7%) > 4/4 4/4 °C (36.2%). The lower enzyme susceptibility of temperature-cycled starches could be attributed to their higher relative crystallinity than that of isotherm (Fig. 2). SDS content increase in some modified starches and, isotherm storage increased RS content mostly. Pullulanase debranching and arrangement of starch chains have been shown to decrease enzyme susceptibility in starch (Miao et al., 2009; Shin et al., 2004). Our result showed higher SDS content as compared with debranched waxy sorghum starch storage at 1 °C for three days which resulted in 40.9% RDS, 27.0% SDS and 32.1% RS (Shin et al., 2004). This difference is presumably due to the differences in the levels of crystallization of short linear chains and the starch source. Temperature-cycled crystallization has been shown to either increase or decrease enzyme susceptibility (Tian et al., 2012; Zhang et al., 2011). It could conclude that the decrease in enzyme susceptibility on pullulanase debranching and subsequent temperature-cycled crystallization has been attributed to crystallite perfection and the short linear chains interaction. In this study, crystalline perfection should have theoretically decreased RDS level and increased RS level.

After cooking, the inter- and intra-molecular hydrogen bonds between starch chains are disrupted and the swollen starch granules are completely disrupted by profuse heat and mechanical stir, leading to transformation to a continuous amorphous structure, which allows physical accessibility to the digestive enzymes (Chung et al., 2006). Gelatinization substantially increased RDS level and decreased SDS and RS levels in native and all modified starches (Table 4). It indicated that some RS transformed into SDS which become thermo-stable. For instance, the RDS, SDS and RS levels were 75.4%, 13.2% and 11.4% in gelatinized native starch, and 32.4%, 45.5% and 22.1% in ungelatinized native starch, respectively. Similar observations have been reported between ungelatinized and gelatinized corn, pea, and lentil starches (Chung, Liu, & Hoover, 2009, 2010). They suggested that increases in thermo-stable SDS and RS were likely owing to amylose–amylose interactions which resist disruptions during gelatinization. In this study, the pullulanase debranching resulted in short linear chains, and crystallized under temperature-cycled conditions, further decreased in RDS levels and increased in SDS and RS levels.

4. Conclusions

Debranching and temperature-cycled crystallization starches exhibited different physicochemical properties and in vitro digestibility. The maximum SDS products yield was observed when debranched starch exposed to the temperature-cycled of dual 4/−20 °C. Both isothermal and temperature-cycled samples showed B-type and V-type complex XRD pattern. Relative crystallinity was found higher in temperature-cycled specimens than that of isothermal. The gelatinization temperature and enthalpy of temperature-cycled starches increased as compared to that of isothermal starch. Pullulanase debranching and subsequent temperature-cycled crystallization changed the conformation of starch molecules which made the SDS proportion more thermostable to those of the native starch. These findings suggested that the pullulanase debranching combines temperature-cycled crystallization can utilize as health benefits of high SDS content.

Acknowledgements

The authors gratefully thank Ph.D. Rana Muhammad Aadil (South China University of Technology) for helpful proof reading the article. This study was supported by the Key Program of National Science Foundation of China (No. 31230057) and the ministry of science and technology in agriculture science and technology Achievements Transformation Fund Project (No.2013GB23600669).

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