Carbohydrate Polymers 84 (2011) 1151–1157

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Physicochemical properties, structure and in vitro digestion of resistant starch from waxy rice starch

Miao-miao Shi, Qun-yu Gao*

Carbohydrate Laboratory, College of Light Industry and Food Sciences, South China University of Technology, Wushan Road 381#, Guangzhou 510640, Guangdong, PR China

ARTICLE INFO

Article history:
Received 15 October 2010
Received in revised form
23 December 2010
Accepted 5 January 2011
Available online 12 January 2011

Keywords:
Waxy rice starch
Resistant starch
In vitro digestibility

ABSTRACT

Physicochemical properties, structure and in vitro digestibility of resistant starch (RS) from debranched waxy rice starch were investigated. Compared to native starch, all RS products have higher apparent amylose content. All RS products displayed a mixture of B and V-type X-ray diffraction (XRD) pattern while native starch showed A-type X-ray diffraction pattern. With increasing the RS content, the relative crystallinity was increased. RS products had a higher peak melting temperature and enthalpy than that of native starch in differential scanning calorimetry (DSC). The in vitro digestibility of products was decreased. The total carbohydrate digestion products and the average rate of digestion of resistant starches were decreased with increasing RS content.

1. Introduction

In a world of rapidly changing food habits and stressful life styles it is more and more recognized that a healthy digestive system is essential for overall quality of life. This recognition has led to the development of foods that are designed to contribute to a healthy digestive system and indirectly to the maintenance of general well being (Brouns, Kettlitz, & Arrigoni, 2002). For this reason, there is an immediate growing interest in the field of gut health related to the consumption of resistant starch (RS) (Asp, van Amelsvoort, & Hautvast, 1996). Resistant starch (RS) is defined as starch and products of starch degradation that cannot be absorbed in the small intestine of healthy individuals and, hence, might be fermented in the colon (Englyst, Kingman, & Cummings, 1992). There are four types: RS1, physically inaccessible to digestion by entrapment in a non-digestible matrix (Haralampu, 2000); RS2, native granule or ungelatinized starch as in raw potato and banana; RS3, starch that has been processed and partially recrystallized (retrograded) (Bird, Lopez-Rubio, Shrestha, & Gidley, 2009); RS4, which is formed by chemical modification of starch (Eerlingen & Delcour, 1995; Thompson, 2000). RS3 is of particular interest, because of its thermal stability. This allows it to be stable in most normal cooking operations and enables its use as an ingredient in a wide variety of conventional foods (Haralampu, 2000). Therefore, RS3 is the major type to be found in processed foods. It is classified as part of dietary fibers and has been widely studied by researchers (Zhao & Lin, 2009).

The method of enzyme hydrolysis was adopted in preparation of RS3. Pullulanase is the common debranching enzyme in starch processing. It is well documented that when starch slurry is heated and cooked, it becomes starch gel. Adding pullulanase, amylopectin molecules are cut into short chain amylose and amylose is re-associated which leads to a new and strong crystalline structure upon cooling, so RS3 is formed. Zhao and Lin (2009) reported that maize starch was treated by autoclaving–cooling cycles, coupled with pullulanase hydrolysis to prepare resistant starch (RS). When gelatinized maize starch was hydrolyzed by pullulanase at addition level of 3 PUN/g (E.C.3.2.1.41, 1.25 g/mL, 400 PUN/mL) (PUN is defined as the amount of enzyme that liberates 1.0 mmol glucose from starch in 30 min at pH 5 and 40 °C) starch for 12 h at 60 °C and then treated with two autoclaving–cooling cycles, RS yield increased to 23.5%. Cai and Shi (2010) investigated that waxy wheat, waxy maize, and waxy potato starches were debranched by isoamylase, and resistant starch content of samples was determined by a modified Englyst et al. (1992) procedure. Crystalline short-chain amylose (CSCA) from debranched waxy potato starch had a higher peak melting temperature (116.2 °C) and higher resistant starch content (77.8%) than that from debranched waxy wheat (67.7%) and waxy maize starches (68.1%).

Waxy rice starch almost entirely consists of amylopectin. After treatment with pullulanase, amylopectin becomes relatively short linear glucans which can promote the formation of resistant starch. The objective of this study was to prepare waxy rice resistant starch with different conditions. Resistant starch content was determined by Megazyme Resistant Starch Assay Kit (AOAC Method. 2002.02).

* Corresponding author. Tel.: +86 13660261703.
E-mail address: qygao@scut.edu.cn (Q.-y. Gao).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.01.004

The properties of resistant starch samples included digestibility, thermal property, X-ray diffraction pattern and apparent amylose. This research can provide important information about properties of waxy rice resistant starches.

2. Materials and methods

2.1. Materials

Waxy rice starches were purchased from Baby Group, JiangSu, China. Pullulanase (E.C.3.2.1.41, 1000 ASPU/g, 1.15 g/mL) (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) was obtained from Danisco Company (Diazyme® P10) (USA). Resistant starch assay kit was bought from Megazyme International Ireland Limited, including pancreatic α-amylase, amyloglucosidase and GOPOD reagent enzymes. Pancreatic α-amylase (E.C.3.2.1.1) (one unit will liberate 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20 °C) was bought from Sigma Chemical Company (St. Louis, MO, USA). Amylose and amylopectin standards were also obtained from Sigma Chemical Company. Other chemicals and solvents were all of analytical grade.

2.2. Preparation of resistant starch samples

2.2.1. Different quantity of pullulanases and reacting times

Waxy rice starch slurry (10%, w/w in diluted pH 4.5 buffer solution containing 0.2 M acetic acid and 0.2 M sodium acetate) was cooked in water bath at 95 °C for 30 min. The temperature of samples was adjusted to 58 °C thereafter, debranched by pullulanase at 30, 40, 50, 55, 60, 70 and 80 ASPU/g of dry starch for 12 h. After the reaction, the solutions were heated at 100 °C for 30 min to stop reaction and cooled to room temperature. The solutions were stored at 25 °C for 24 h to form retrograded starch. The precipitated starch was oven dried at 45 °C overnight. All the samples were ground and screened through 80 mesh sieve.

Cooked 10% waxy rice starch slurry was also treated by pullulanase (55 ASPU/g) for 1, 4, 8, 12, 16, 20 and 24 h. The following steps were the same with above.

Based on the different pullulanase concentrations, the samples treated by pullulanase at 30, 40, 55, 70 and 80 ASPU/g of dry starch will be referred to as S1, S2, S3, S4 and S5 respectively.

2.2.2. Different stored temperatures and times

Cooked 10% waxy rice starch slurry was treated by pullulanase (55 ASPU/g) for 12 h, reheated (100 °C for 30 min), and stored at −20, 4, 25, 35, 45, 55 and 65 °C for 24 h. The following steps were the same with above.

Cooked 10% waxy rice starch slurry was also treated by pullulanase (55 ASPU/g) for 12 h, reheated (100 °C for 30 min), and stored at 25 °C for 1, 6, 12, 24, 36, 48 and 72 h. The following steps were the same with above.

2.3. Resistant starch determination

Resistant starch content was determined by a Megazyme Resistant Starch Assay Kit (AOAC Method. 2002.02) (McCleary et al., 2002). The samples (100 mg) were incubated in a shaking water bath with 4 mL mixture of pancreatic α-amylase and amyloglucosidase for 16 h (at 37 °C, 200 strokes/min) to hydrolyze digestible starch to glucose. The reaction was terminated with 4 mL of 95% ethanol and the indigested resistant starch was recovered by centrifugation (1500 × *g*, 10 min). The residue was washed with 50% ethanol twice, and then solubilized with 2 mL of 4 M KOH solution in an ice bath. After 20 min, the solution was neutralized with 8 mL sodium acetate buffer (1.2 M, pH 3.8), incubated with amyloglucosidase (0.1 mL, 3300 U/mL) at 50 °C for 30 min. Aliquots (0.1 mL) of the diluent, 3 mL of glucose oxidase/peroxidase reagent (GOPOD) were added and the mixture was incubated at 50 °C for 20 min. Absorbance was measured using a spectrophotometer under 510 nm. Resistant starch was calculated as glucose × 0.9.

2.4. Apparent amylose contents

Apparent amylose contents in starches were assessed according to the colorimetric procedure of Kumari, Urooj, and Prasad (2007) All the samples – both native and debranched were analyzed for the amylose content by the colorimetric method based on the reaction between amylose and iodine. Starch (100 mg, dry basis) was wetted with 1 mL ethanol (95%) and gelatinized by treatment with 9.2 mL of 1 mol/L NaOH and storage for about 16–24 h at ambient temperature. After adjusting to 100 mL of distilled water, an aliquot of 5 mL of the solution was transferred to 100 volumetric flasks and 1 mL of 1 mol/L acetic acid and iodine solution (2 mL, 0.2% I_2 in 2% KI) were added. The volume made up to 100 mL with distilled water and mixed, after 20 min the absorbance was measured at 620 nm using as blank 5 mL 0.09 mol/L NaOH, to which acetic acid (1 mL) and iodine solution (2 mL) were added in 100 mL total volume (Juliano et al., 1981). Amylose and amylopectin (Sigma Company) were used as standards. The above analysis was carried out in 2 replicates.

2.5. Wide angle X-ray diffraction

X-ray diffractograms were obtained with D/Max-2200 X-ray diffractometer (Rigaku Denki Co., Tokyo, Japan) with Cu Kα radiation at 44 kV and 26 mA. The samples were adjusted to the equilibrium water content in a sealed desiccator at room temperature before analysis. The diffractograms were scanned between 4° and 35° (2θ) at the rate of 5°/min. Relative crystallinity was estimated by the ratio of the peak areas to the total diffractogram area (Chen, Zhang, Huang, & Lu, 2010).

2.6. Thermal analysis

The thermal properties of each starch sample were examined using a differential scanning calorimetry (DSC) (DSC8000, Perkin Elmer Inc., USA). A 30% (w/w) suspension of solid sample in water was prepared and sealed in a DSC pan. Samples were allowed to equilibrate for 2 h at room temperature, then heated from 30 °C to 150 °C at 10 °C/min. The differential scanning calorimetry analyzer was calibrated using indium as a standard and an empty DSC 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. In vitro digestion of starch samples

According to the methods of Jenkins et al. (1981) and Wen, Lorenz, Martin, Stewart, and Sampson (1996) with some modifications. Weigh 400 mg starch samples in beaker and add approximately 10 mL pH 6.9 phosphate buffer, then carefully transfer the entire contents into a 13 cm dialysis bag (width 4.5 cm, molecular weight cutoff 14,000, Japan). Wash the beaker thoroughly with phosphate buffer and add these washings to the dialysis bag to make the final volume of all samples 15 mL. Add 10 mL pancreatic α-amylase solution (520 U/mL), close and mix. Place the dialysis bag into a beaker containing 400 mL phosphate buffer at 37 °C with agitation. After 1–7 h of incubation, pipette 0.2 mL of dialysate into test tube, add 1 mL of 5% phenol and 5 mL of concentrated sulfuric acid, thoroughly mix the tube and allow to stand for 10 min, shake, place in the water bath at 25–30 °C for 20 min. Measure the absorbance at 490 nm. Make a calibration curve of micrograms of maltose vs. absorbance. Total carbohydrate

digestion products and average rate of digestion were calculated according to:

Total carbohydrate digestion products (mg): $CHO = C \times D \times (425 - S) \times 0.001$

Average rate of digestion (mg/g/h): $V = CHO/W/H$

where C, carbohydrates in 0.2 mL diluted dialysate by reference to the standard curve; D, multiple of dilution; CHO, total carbohydrate digestion products, mg; 425, the volume of whole system, mL; S, 0.2 mL from 400 mL diluted dialysate, mL; 0.001, conversion from milligrams to micrograms; W, weight of sample, g; H, reaction time, h.

2.8. Statistical analysis

The differences between the mean values of multiple groups were analyzed by one-way analysis of variance (ANOVA) with Duncan's multiple range test. ANOVA data with a $P < 0.05$ were classified as statistically significant. SPSS 13.0 software, Origin 75 and Microsoft Excel 2007 program were used to analyze and report the data. Mean values from the duplicated experiments were reported.

3. Results and discussion

3.1. Effect of pullulanase debranching on RS content

Pullulanase has the ability to debranch amylopectin molecules. The raw waxy rice starch after being cooked was treated with different pullulanase concentrations and debranching time. The resistant starch contents of native and debranched waxy rice starches are presented in Fig. 1(A) and (B). Resistant starch contents increased as the amount of enzyme increased, reaching a maximum (28.61%) at about adding 55 ASPU/g (base on dry starch) pullulanase, and decreasing thereafter to 21.59% and 18.55% upon 70 ASPU/g and 80 ASPU/g starch of pullulanase enzyme concentration. With increasing the debranching time, RS content of debranched starches increased greatly. Ozturk, Koksel, Kahraman, and Ng (2009) and Ozturk, Koksel, & Ng, (2009) also reported that among the debranched samples of Hylon V, the 48 h hydrolyzed sample had a significantly higher RS content than those with lower debranching times. When starches were debranched for 12 h, the yield of RS was the highest (28.61%). Too long and too short time were not beneficial to form RS.

Compared to the resistant starch content of native waxy rice starch (0.33%), the resistant starch content of all samples increased favorably, and the enhancement of the sample S3 was supreme. These results may be explained by analyzing the crystallization process and the chain length of substances. After debranching, amylopectin became to relatively short linear glucans which is ideal for RS3 formation (Schmiedl, Bäuerlein, Bengs, & Jacobasch, 2000). Gidley et al. (1995) also suggested that an approximate relative maximum at DP20-30 is suitable to form RSIII. Therefore, an appropriate chain length is required for crystallization and formation of double helices. Too short chains are known to inhibit retrogradation.

3.2. Effect of retrogradation on RS content

The term 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 (Miao, Jiang, & Zhang, 2009). Amylose recrystallization occurs rapidly and is called short-term retrogradation, whereas amylopectin molecules need a longer time to associate, a process called

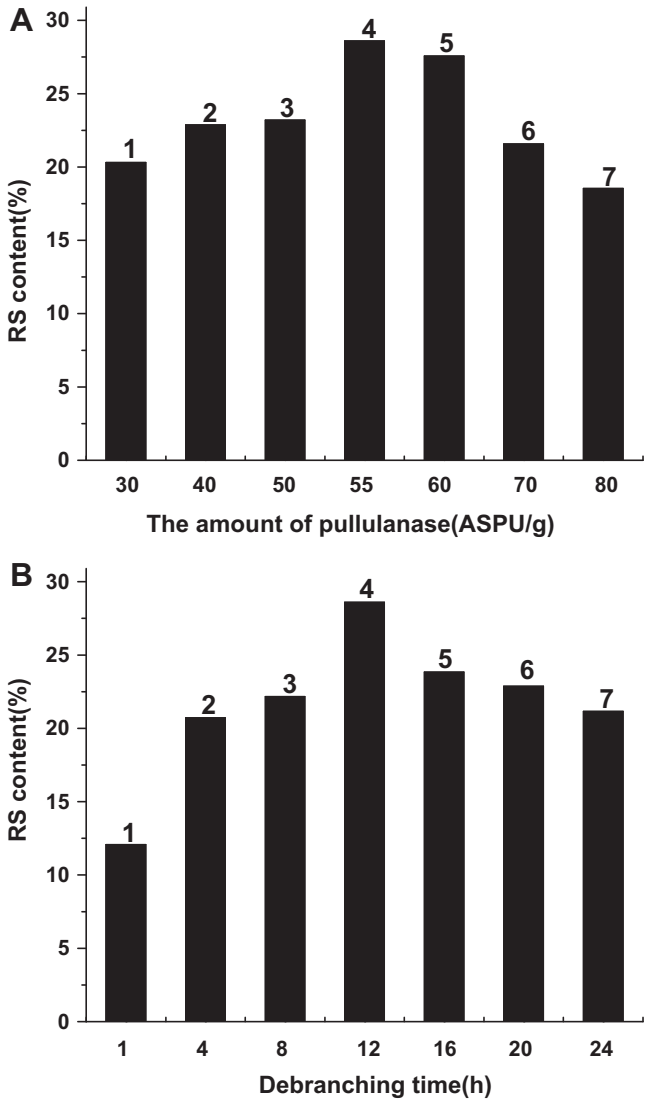

Fig. 1. Effect of pullulanase debranching on RS content. (A) 1 (20.31% RS), 2 (22.88% RS), 3 (23.20% RS), 4 (28.61% RS), 5 (27.57% RS), 6 (21.59% RS) and 7 (18.55% RS) respectively added 30, 40, 50, 55, 60, 70 and 80 ASPU/g pullulanase to debranch for 12 h, then stored at 25 °C for 24 h. (B) 1 (12.09% RS), 2 (20.73% RS), 3 (22.18% RS), 4 (28.61% RS), 5 (23.84% RS), 6 (22.88% RS) and 7 (21.17% RS) at 55 ASPU/g pullulanase respectively were debranched for 1, 4, 8, 12, 16, 20 and 24 h, then stored at 25 °C for 24 h.

long-term retrogradation (Zhang, Ao, & Hamaker, 2008; Zhang, Sofyan, & Hamaker, 2008). Fig. 2(A) showed the effect of storage time (1 h, 6 h, 12 h, 24 h, 36 h, 48 h and 72 h) on the formation of RS. In this study, the amount of RS increased markedly with storage time at 25 °C up to 24 h. RS content decreased slowly when storage time increased to a longer time. Fig. 2(B) showed the effect of different temperatures (−20 °C, 4 °C, 25 °C, 35 °C, 45 °C, 55 °C and 65 °C) on the formation of RS. RS content was affected by both chain length and recrystallization temperature (Robin, Merinat, Simon, & Lehmann, 2008). Debranched (12 h) waxy rice starch stored at 35 °C results in 31.84% RS, showing the maximum RS. The highest (65 °C) and lowest (−20 °C) storage temperature had a negative effect on RS formation. When starches stored at 25 °C, 35 °C and 45 °C, it showed no significant differences in the RS content. Therefore, the suited storage temperature of debranched waxy rice starch is approximately the room temperature and the temperature is propitious to develop products with appropriate RS levels. The result is in accord with Gonzalez-Soto, Mora-Escobedo, Hernandez-Sanchez,

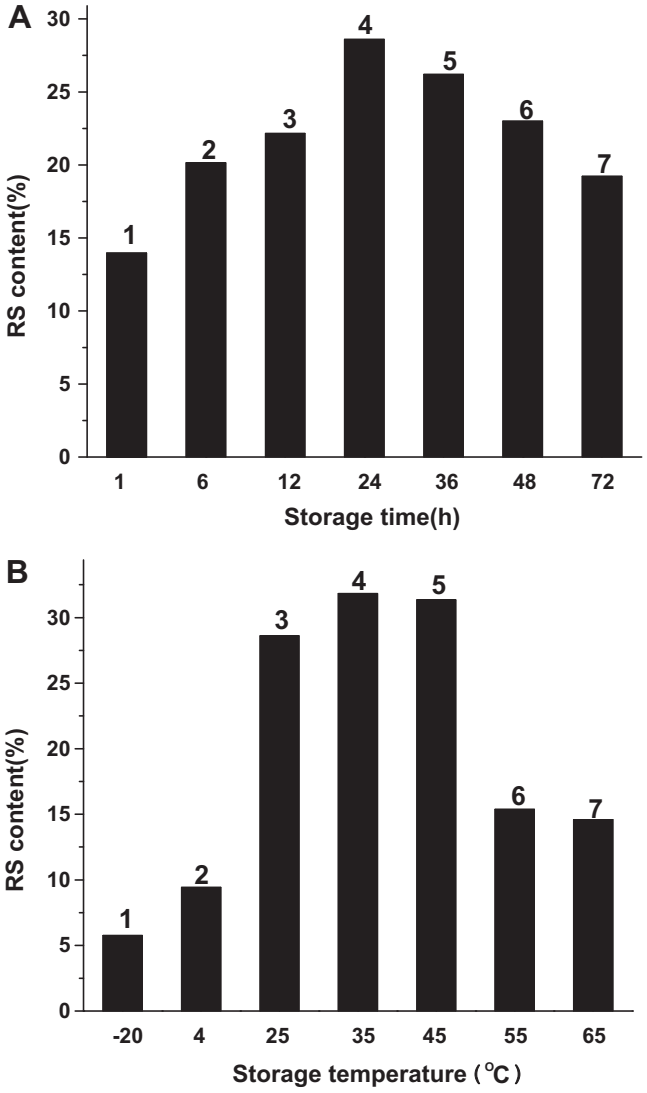

Fig. 2. Effect of recrystallization on RS content. (A) 1 (13.98% RS), 2 (20.15% RS), 3 (22.15% RS), 4 (28.61% RS), 5 (26.21% RS), 6 (23.00% RS) and 7 (19.22% RS) at 55 ASPU/g pullulanase was debranched for 12 h, then stored respectively at 25 °C for 1, 6, 12, 24, 36, 48 and 72 h. (B) 1 (5.77% RS), 2 (10.41% RS), 3 (28.61% RS), 4 (31.84% RS), 5 (31.35% RS), 6 (15.38% RS) and 7 (14.59% RS) at 55 ASPU/g pullulanase was debranched for 12 h, then stored respectively at −20, 4, 25, 35, 45, 55 and 65 °C for 24 h.

Sanchez-Rivera, and Bello-Perez (2007) and Guraya, James, and Champagne (2001). In addition, all the samples were oven dried at 45 °C overnight, therefore, the RS contents of samples should be higher than those of other ways dried samples (such as freeze dried samples) due to ongoing retrogradation of samples during oven drying at 50 °C (Ozturk, Koksel, Kahraman, et al., 2009; Ozturk, Koksel, et al., 2009).

3.3. Apparent amylose contents

The changes observed in the amylose content of native and resistant starch samples are given in Table 1. All the samples contained amylose in the range from 20% to 30%. The amylose contents of all samples were obviously higher than that of native waxy rice starch (0%). Among different samples, the sample of S3 had highest amylose content (30.26%). The results exhibited the enhancement trend of amylose content of native and starch samples matched resistant starch content. Amylose contents increased as resistant starch contents increased. It is coincident with Shu et al. (2006), RS111 (a high-resistant starch rice) contained more amylose than R7954 (a wild rice type) and a little less amylopectin than R7954, indicating that the increased RS in RS111 might mainly result from increased amylose content. Zhang, Ao, et al., 2008 and Zhang, Sofyan, et al., 2008 also reported that a significant positive relationship was shown between amylose content and RS content in the cooked maize starch samples. The presence of amylose in starch is also known to affect the formation of RS (Kumari et al., 2007). The well-known connection between high amylose content and resistant starch arises from the greater number of longer branches, which can form the small retrograded species (Witt, Gidley, & Gilbert, 2010). Generally speaking, amylose molecules combined easily to form solid crystal structure, so high amylose starches were conducive to formation of resistant starch (Jian, Gao, & Liang, 2002).

Table 1
Apparent amylose content of native starch and samples.

Samples	RS content (%)	Apparent amylose content (%)
Native starch	0.33 ± 0.00^{a}	0.00 ± 0.00^{a}
S1	20.31 ± 0.00^{c}	21.14 ± 0.47bc
S2	22.88 ± 0.51^{d}	23.03 ± 0.31d
S3	28.61 ± 0.54^{e}	30.26 ± 0.94e
S4	21.59 ± 0.18^{cd}	21.70 ± 0.71^{bc}
S5	18.55 ± 0.55^{b}	20.14 ± 0.55^{b}

Values with the different letters in the same column are significantly different ($P < 0.05$).

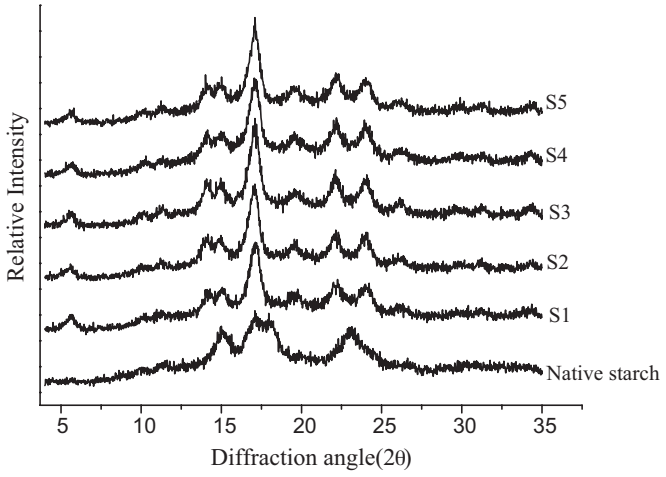

Fig. 3. X-ray diffraction patterns of native starch and samples. Native starch (0.33%RS); S1 (20.31%RS); S2 (22.88%RS); S3 (28.61%RS); S4 (21.59%RS); S5 (18.55%RS).

3.4. X-ray diffraction pattern

X-ray diffraction patterns and relative crystallinity of native and debranched starches are summarized in Fig. 3 and Table 2. Native waxy rice starch exhibited strong diffraction peak at 2θ with values

Table 2
The relative crystallinity of native starch and samples.

Samples	RS content (%)	The relative crystallinity (%)	The type of diffraction pattern
Native starch	0.33 ± 0.00^{a}	40.6 ± 0.21^{a}	A
S1	20.31 ± 0.00^{c}	44.3 ± 0.28^{b}	B + V
S2	22.88 ± 0.51^{d}	46.9 ± 0.28^{d}	B + V
S3	28.61 ± 0.54^{e}	47.5 ± 0.21^{d}	B + V
S4	21.59 ± 0.18^{cd}	45.1 ± 0.42^{c}	B + V
S5	18.55 ± 0.55^{b}	44.1 ± 0.28^{b}	B + V

Values with the different letters in the same column are significantly different ($P < 0.05$).

Table 3
Thermal properties of native starch and samples.

Samples	RS content (%)	T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)	ΔH (J/g)
Native starch	0.33	71.19	75.42	80.78	9.59	11.22
S1	20.31	74.03	87.10	96.57	22.54	14.32
S2	22.88	79.49	91.43	100.34	20.82	16.70
S3	28.61	82.31	94.06	104.40	22.09	17.18
S4	21.59	79.01	90.56	100.18	21.17	15.85
S5	18.55	73.56	86.88	95.07	21.51	13.84

of around 15.02°, 17.11°, 18.01° and 23.04°. These results indicated that the crystal type of native waxy rice starch is a characteristic A-type. However, after treated, X-ray diffraction patterns of all resistant starch samples formed new crystalline structure (Miao et al., 2009). The sample S4 showed strong diffraction peak at 2θ with values of around 5.62°, 14.16°, 14.95°, 17.10°, 19.55°, 22.11° and 24.02°, and the results of other samples were alike. B-type signature reflection at 2θ with values of 5.62° and V-type signature reflection at 2θ with values of 14.16° and 19.55° was absent. As a result, X-ray diffraction patterns of all resistant starch samples displayed a mixture of B and V-type. A low starch concentration (10%, w/w) was used, debranching of waxy rice starch at 58 °C followed by crystallization at 25 °C resulted in crystalline short-chain amylose with a B-type X-ray pattern. As Cai, Shi, Rong, and Hsiao (2010) said, B-type polymorph is favored by low concentration and temperature. Similarly, resistant starch isolated from cooked rice displayed a mixture of B- and V-type diffraction pattern that was more resistant to starch hydrolysis by alpha-amylase (Eerlingen, Deceuninck, & Delcour, 1993).

The relative crystallinity of starch samples was different. Compared to native waxy rice starch (40.6%), all samples have slightly higher relative crystallinity (Table 2). Overall, samples with higher resistant starch have higher relative crystallinity. The sample of S3 which had the highest resistant starch content (28.61%) had the highest relative crystallinity (47.5%). The relative crystallinity of the sample of S1 (44.3%) was least of all starches, accordingly, it had the lowest resistant starch content (20.31%). RS was formed through the rearrangement of amylose chains into enzyme-resistant structures of higher crystallinity (Lopez-Rubio, Flanagan, Shrestha, Gidley, & Gilbert, 2008). Generally, differences in degree of crystallinity between starches could be attributed to the following: crystal size, amount of crystalline (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 average chain length of waxy rice starch is 17–20 (Eliasson, 2004). Compared to native starch, the diffractogram of the RS samples displayed more intense and sharper peaks due to the B-polymorph formed during retrogradation (Leong, Karim, & Norziah, 2007).

3.5. Thermal analysis

Thermal properties of native waxy rice starch and the resistant products were determined by DSC analysis. The native waxy rice starch was characterized with a sharp endothermic peak at 75.42 °C and enthalpy of 11.22 J/g (Table 3). Compared to native waxy rice starch, the transition temperatures (T_o, T_p, and T_c) and enthalpies (ΔH) of samples were increased significantly. The resistant starch products exhibited very broad peaks and the endothermic peak that ranged from 73 °C to 105 °C was observed for all samples. The enthalpy of RS product increased from 13.84 J/g to 17.18 J/g with increasing RS content. The sample S3 displayed a higher peak melting temperature (94.06 °C) and the endothermic enthalpy (17.18 J/g) than others. This endothermic transition has been attributed to the recrystallization of amylose during the starch retrogradation (Eerlingen, Jacobs, & Delcour, 1994). The increase in ΔH can be explained in terms of increased ordering and stabilization of double helical structures through hydrogen bonds and other intermolecular forces (Mutungi, Rost, Onyango, Jaros, & Rohm, 2009). Accordingly, it can be argued that the transition temperatures and endothermic enthalpy increases with increased the degree of crystallite and the stabilization double helical structures.

3.6. In vitro digestibility of starch samples

The total carbohydrate digestion products and the average rate of digestion of resistant starches are shown in Fig. 4(A) and (B). With increasing incubation time, the total carbohydrate digestion products were increased and the average rates of digestion were decreased. The native starch has the most digestion products and the highest hydrolysis rate. With increasing RS content, the total carbohydrate digestion products and the average rate of digestion of resistant starches were decreased. In the first 3 h, the digestion products of all samples and the average rate of digestion were sharp increasing. This was mainly attributed to the high concentration and efficiency of pancreatic α-amylase solution in the former reaction. After 3 h, starch molecules were hydrolyzed

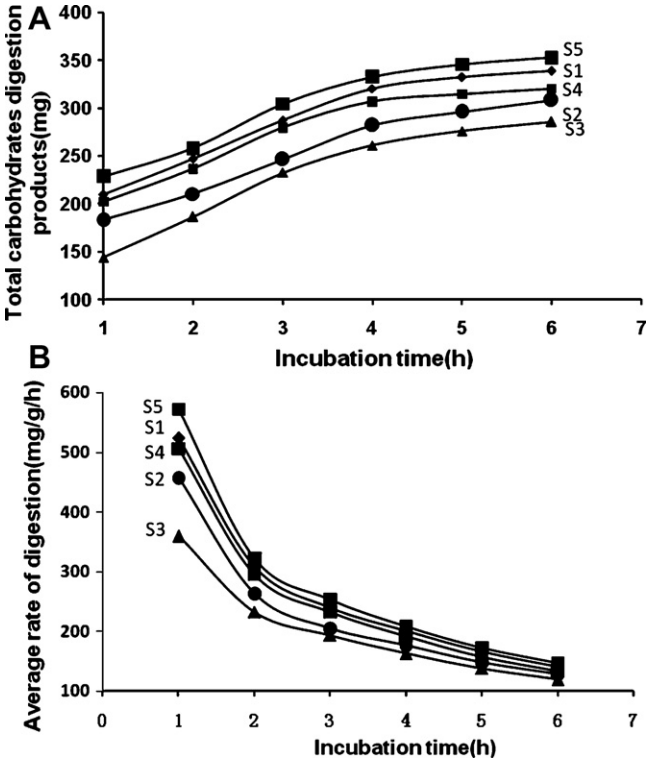

Fig. 4. In vitro digestibility of starch samples. (A) The total carbohydrate digestion products of RS samples. (B) The average rate of digestion of RS samples. S1 (20.31%RS); S2 (22.88%RS); S3 (28.61%RS); S4 (21.59%RS); S5 (18.55%RS).

into the too small molecular chain and the binding sites between starch molecules and α-amylase were decreased, so the digestion of resistant starch slowed down. The longer the substrate–enzyme contact time, the more starch was digested within the duration of the experiment (Dhital, Shrestha, & Gidley, 2010). More and shorter short chains that are difficult for the enzymes to digest (Zhang, Ao, et al., 2008; Zhang, Sofyan, et al., 2008). Generally speaking, with increasing the RS content, the digestion products and the average rate of digestion of resistant starches were consistently decreased. The digestion products of S3 with the highest RS content (28.61%) were the least and the reaction rate was also the slowest of all starch samples. These results indicate that the debranched starch samples were resistant to α-amylase digestion. The results were also accordance with Pongjanta, Utaipattanaceep, Naivikul, and Piyachomkwan (2009) who found the in vitro starch hydrolysis rate (0–180 min) of the debranched amylose rice starch with the highest RS content was lowest among the samples. The decrease in the enzymatic digestion of starch after retrogradation had been reported by Cui and Oates (1999). It can be attributed to the recrystallization of amylose during the starch retrogradation and the compact double helical structures through hydrogen bonds. Therefore, the debranched starch with higher RS content had less the total carbohydrate digestion products and the low average rate of digestion. That is to say, the samples with high RS content had low in vitro digestibility and more resistant to α-amylase digestion.

4. Conclusions

Waxy rice resistant starch products with various RS contents possessed differences in physicochemical properties, structure and in vitro digestibility. All RS products have higher apparent amylose content than that of native starch. All RS products displayed a mixture of B and V-type X-ray diffraction pattern. With increasing the RS content, the relative crystallinity was increased. RS products had a higher peak melting temperature and enthalpy than that of native starch in DSC. The in vitro digestibility of products was decreased. The total carbohydrate digestion products and the average rate of digestion of resistant starches were decreased with increasing RS content. It indicates that the debranched starch samples were resistant to α-amylase digestion. These results can be attributed to the recrystallization of amylose during the starch retrogradation.

Acknowledgements

The study was carried out with financially support of the National High-tech R&D Program of China (2007AA10Z309), and Guangdong Province Program of China (2009B090300274).

References

Asp, N.-G., van Amelsvoort, J. M. M., & Hautvast, J. G. A. J. (1996). Nutritional implications of resistant starch. *Nutrition Research Reviews*, *9*(01), 1–31.

Bird, A. R., Lopez-Rubio, A., Shrestha, A. K., & Gidley, M. J. (2009). Resistant starch in vitro and in vivo: factors determining yield, structure, and physiological relevance. *Modern biopolymer science: bridging the divide between fundamental treatise and industrial application*, 449–510.

Brouns, F., Kettlitz, B., & Arrigoni, E. (2002). Resistant starch and "the butyrate revolution". *Trends in Food Science & Technology*, *13*(8), 251–261.

Cai, L., & Shi, Y.-C. (2010). Structure and digestibility of crystalline short-chain amylose from debranched waxy wheat, waxy maize, and waxy potato starches. *Carbohydrate Polymers*, *79*(4), 1117–1123.

Cai, L., Shi, Y.-C., Rong, L., & Hsiao, B. S. (2010). Debranching and crystallization of waxy maize starch in relation to enzyme digestibility. *Carbohydrate Polymers*, *81*(2), 385–393.

Chen, F.-q., Zhang, B.-s., Huang, Q., & Lu, H.-f. (2010). Research progress of determination crystallinity of starch granular by X-ray diffraction. *Science and Technology of Food Industry*, *31*(1), 432–435.

Cui, R., & Oates, C. G. (1999). The effect of amylose–lipid complex formation on enzyme susceptibility of sago starch. *Food Chemistry*, *65*(4), 417–425.

Dhital, S., Shrestha, A. K., & Gidley, M. J. (2010). Relationship between granule size and in vitro digestibility of maize and potato starches. *Carbohydrate Polymers*, *82*(2), 480–488.

Eerlingen, R., Jacobs, H., & Delcour, J. (1994). Enzyme-resistant starch. 5. Effect of retrogradation of waxy maize starch on enzyme susceptibility. *Cereal Chemistry*, *7*(4), 351–355.

Eerlingen, R. C., Deceuninck, M., & Delcour, J. A. (1993). Enzyme-resistant starch. II. Influence of amylose chain length on resistant starch formation. *Cereal Chemistry*, *70*, 345–350.

Eerlingen, R. C., & Delcour, J. A. (1995). Formation, analysis, structure and properties of type III enzyme resistant starch. *Journal of Cereal Science*, *22*(2), 129–138.

Eliasson, A.-C. (2004). *Starch in food—Structure, function and applications*. Woodhead Publishing Limited.

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, *46*, S33–S50.

Gidley, M. J., Cooke, D., Darke, A. H., Hoffmann, R. A., Russell, A. L., & Greenwell, P. (1995). Molecular order and structure in enzyme-resistant retrograded starch. *Carbohydrate Polymers*, *28*(1), 23–31.

Gonzalez-Soto, R. A., Mora-Escobedo, R., Hernandez-Sanchez, H., Sanchez-Rivera, M., & Bello-Perez, L. A. (2007). The influence of time and storage temperature on resistant starch formation from autoclaved debranched banana starch. *Food Research International*, *40*(2), 304–310.

Guraya, H. S., James, C., & Champagne, E. T. (2001). Effect of enzyme concentration and storage temperature on the formation of slowly digestible starch from cooked debranched rice starch. *Starch-Stärke*, *53*(3–4), 131–139.

Haralampu, S. G. (2000). Resistant starch—a review of the physical properties and biological impact of RS3. *Carbohydrate Polymers*, *41*(3), 285–292.

Jenkins, D., Wolever, T., Taylor, R., Barker, H., Fielden, H., Baldwin, J., et al. (1981). Glycemic index of foods: A physiological basis for carbohydrate exchange. *The American Journal of Clinical Nutrition*, *34*(3), 362–366.

Jian, H., Gao, Q., & Liang, S. (2002). Effect of amylose content on resistant starch. *Cereals & Oils*, *10*, 5–7.

Juliano, B. O., Perez, C. M., Blakeney, A. B., Castillo, T., Kongseree, N., Laignelet, B., et al. (1981). International cooperative testing on the amylose content of milled rice. *Starch-Stärke*, *33*(5), 157–162.

Kumari, M., Urooj, A., & Prasad, N. N. (2007). Effect of storage on resistant starch and amylose content of cereal–pulse based ready-to-eat commercial products. *Food Chemistry*, *102*(4), 1425–1430.

Leong, Y. H., Karim, A. A., & Norziah, M. H. (2007). Effect of pullulanase debranching of sago (Metroxylon sagu) starch at subgelatinization temperature on the yield of resistant starch. *Starch-Stärke*, *59*(1), 21–32.

Lopez-Rubio, A., Flanagan, B. M., Shrestha, A. K., Gidley, M. J., & Gilbert, E. P. (2008). Molecular rearrangement of starch during in vitro digestion: Toward a better understanding of enzyme resistant starch formation in processed starches. *Biomacromolecules*, *9*(7), 1951–1958.

McCleary, B. V., McNally, M., Rossiter, P., Aman, P., Amrein, T., Arnouts, S., et al. (2002). Measurement of resistant starch by enzymatic digestion in starch and selected plant materials: Collaborative study. *Journal of AOAC International*, *85*(5), 1103–1111.

Miao, M., Jiang, B., & Zhang, T. (2009). Effect of pullulanase debranching and recrystallization on structure and digestibility of waxy maize starch. *Carbohydrate Polymers*, *76*(2), 214–221.

Mutungi, C., Rost, F., Onyango, C., Jaros, D., & Rohm, H. (2009). Crystallinity, thermal and morphological characteristics of resistant starch type III produced by hydrothermal treatment of debranched cassava starch. *Starch-Stärke*, *61*(11), 634–645.

Ozturk, S., Koksel, H., Kahraman, K., & Ng, P. (2009). Effect of debranching and heat treatments on formation and functional properties of resistant starch from high-amylose corn starches. *European Food Research and Technology*, *229*(1), 115–125.

Ozturk, S., Koksel, H., & Ng, P. K. W. (2009). Characterization of resistant starch samples prepared from two high-amylose maize starches through debranching and heat treatments. *Cereal Chemistry*, *86*(5), 503–510.

Pongjanta, J., Utaipattanaceep, A., Naivikul, O., & Piyachomkwan, K. (2009). Debranching enzyme concentration effected on physicochemical properties and alpha-amylase hydrolysis rate of resistant starch type III from amylose rice starch. *Carbohydrate Polymers*, *78*(1), 5–9.

Robin, F., Merinat, S., Simon, A., & Lehmann, U. (2008). Influence of chain length on alpha-1,4-D-glucan recrystallization and slowly digestible starch formation. *Starch-Stärke*, *60*(10), 551–558.

Schmiedl, D., Bäuerlein, M., Bengs, H., & Jacobasch, G. (2000). Production of heat-stable, butyrogenic resistant starch. *Carbohydrate Polymers*, *43*(2), 183–193.

Shu, X., Jiao, G., Fitzgerald, M. A., Yang, C., Shu, Q., & Wu, D. (2006). Starch structure and digestibility of rice high in resistant starch. *Starch-Stärke*, *58*(8), 411–417.

Song, Y., & Jane, J. (2000). Characterization of barley starches of waxy, normal, and high amylose varieties. *Carbohydrate Polymers*, *41*(4), 365–377.

Thompson, D. B. (2000). Strategies for the manufacture of resistant starch. *Trends in Food Science & Technology*, *11*(7), 245–253.

Wen, Q. B., Lorenz, K. J., Martin, D. J., Stewart, B. G., & Sampson, D. A. (1996). Carbohydrate digestibility and resistant starch of steamed bread. *Starch-Stärke*, *48*(5), 180–185.

Witt, T., Gidley, M. J., & Gilbert, R. G. (2010). Starch digestion mechanistic information from the time evolution of molecular size distributions. *Journal of Agricultural and Food Chemistry*, *58*(14), 8444–8452.

Zhang, G., Ao, Z., & Hamaker, B. R. (2008). Nutritional property of endosperm starches from maize mutants: A parabolic relationship between slowly digestible starch

and amylopectin fine structure. *Journal of Agricultural and Food Chemistry*, *56*(12), 4686–4694.

Zhang, G. Y., Sofyan, M., & Hamaker, B. R. (2008). Slowly digestible state of starch: Mechanism of slow digestion property of gelatinized maize starch. *Journal of Agricultural and Food Chemistry*, *56*(12), 4695–4702.

Zhao, X. H., & Lin, Y. (2009). The impact of coupled acid or pullulanase debranching on the formation of resistant starch from maize starch with autoclaving–cooling cycles. *European Food Research and Technology*, *230*(1), 179–184.