Carbohydrate Polymers 125 (2015) 200–205

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Structural characteristics of slowly digestible starch and resistant starch isolated from heat–moisture treated waxy potato starch

Chang Joo Lee, Tae Wha Moon*

Department of Agricultural Biotechnology, Center for Food and Bioconvergence and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Republic of Korea

ARTICLE INFO

Article history:
Received 6 November 2014
Received in revised form 25 January 2015
Accepted 13 February 2015
Available online 26 February 2015

Keywords:
Waxy potato starch
Slowly digestible starch
Resistant starch
Heat–moisture treatment

ABSTRACT

The objective of this study was to investigate the structural characteristics of slowly digestible starch (SDS) and resistant starch (RS) fractions isolated from heat–moisture treated waxy potato starch. The waxy potato starch with 25.7% moisture content was heated at 120 °C for 5.3 h. Scanning electron micrographs of the cross sections of RS and SDS + RS fractions revealed a growth ring structure. The branch chain-length distribution of debranched amylopectin from the RS fraction had a higher proportion of long chains (DP ≥ 37) than the SDS + RS fraction. The X-ray diffraction intensities of RS and SDS + RS fractions were increased compared to the control. The SDS + RS fraction showed a lower gelatinization enthalpy than the control while the RS fraction had a higher value than the SDS + RS fraction. In this study we showed the RS fraction is composed mainly of crystalline structure and the SDS fraction consists of weak crystallites and amorphous regions.

1. Introduction

Starch, the main plant carbohydrate, is an important plant derivative used by humans. Starch digestibility varies among different starchy foods due to various factors including botanical source, food processing (Tester, Karkalas, & Qi, 2004), amylose/amylopectin ratio (Hoover & Sosulski, 1985), A- and B-type X-ray patterns (Jane, Wong, & McPherson, 1997) and presence of amylose–lipid complexes (Hoover & Manuel, 1995). For nutritional purposes, starch is classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) according to the rate of glucose release and its absorption in the intestinal tract (Englyst, Kingman, & Cummings, 1992). RDS consists mainly of amorphous and dispersed starch and is found in high amounts in starchy foods that have gone through moist heat cooking, such as bread and potatoes. SDS consists of physically inaccessible amorphous starch and raw starch with A- and C-type crystalline structures, such as cereals, while B-type starch found in tuber and root is resistant to enzymatic digestion. RS has various structures according to the RS type and is not digested in the small intestine but can be fermented in the large intestine. Sajilata, Singhal, and Kulkarni (2006) reported a reason for the enzyme resistance is the crystallinity of native B-type starch granules as observed in the case of amylomaize starch. A number of physiological effects have been ascribed to the indigestible characteristics of RS (Haralampu, 2000). The reported health benefits of RS are as follows: prevention of colon cancer, hypoglycemic effects, substrate for growth of probiotic microorganisms, reduction of gall stone formation, hypocholesterolemic effects, inhibition of fat accumulation, and increased absorption of minerals (Sajilata et al., 2006). Nutritional characteristics of SDS include satiety, physical performance, glucose tolerance enhancement and blood lipid level reduction in healthy individuals and in those with hyperlipidemia (Jenkins et al., 2002). Therefore, SDS is receiving much attention as a new functional food component in innovative food product development. Several studies reported the production of SDS through chemical, physical, enzymatic, genetic, or multiple modifications (Lee, Shin, Kim, Choi, & Moon, 2011; Lee, Kim, Choi, & Moon, 2012; Miao, Jiang, & Zhang, 2009; Shin et al., 2007). Specifically, SDS was produced from debranched waxy sorghum starch by controlling the storage conditions and the degree of debranching. The structural properties of SDS were previously investigated and reportedly SDS contained a small proportion of imperfect crystallites (Guraya, James, & Champagne, 2001; Shin et al., 2004).

Heat–moisture treatment (HMT) has been used to modify starch digestibility. HMT of starch is a physical treatment in which starches are treated at various moisture levels (<35%) for a certain period of time at a temperature above the glass transition temperature but below the gelatinization temperature (Jacobs & Delcour, 1998). Many researchers have investigated the

* Corresponding author. Tel.: +82 2 880 4854; fax: +82 2 873 5095.
E-mail address: twmoon@snu.ac.kr (T.W. Moon).

http://dx.doi.org/10.1016/j.carbpol.2015.02.035
0144-8617/

formation and structural characteristics of RS using HMT (Chung, Liu, & Hoover, 2009; Haralampu, 2000; Sajilata et al., 2006). In all starches, HMT caused increases in both gelatinization temperatures and gelatinization temperature range as well as decreases in swelling factor and amylose leaching. In contrast, depending on the starch source, transition of crystalline type, formation of amylose–lipid complexes, disruption of crystallinity and an increase or a decrease in enzyme susceptibility have been observed in HMT (Gunaratne & Hoover, 2002; Hoover & Manuel, 1996; Hoover & Vasanthan, 1994). Crystalline disruption and a transition of crystalline type from B- or C-type to A-type in heat–moisture treated tubers and root starches significantly influenced starch digestibility (Lee et al., 2011; Vieira & Sarmento, 2008). Higher susceptibility of A-type crystallites to enzymatic hydrolysis compared with B-type crystallites has been reported (Lehmann & Robin, 2007; Zhang, Venkatachalam, & Hamaker, 2006). A- and B-types differ in their packing of double helices and water content. Shorter double helices and interior crystallites in A-type starches are more readily digestible and exhibit higher contents of RDS and SDS compared with B-type starches, which often contain high amounts of RS (Jane et al., 1997). Many techniques have been used to elucidate the starch structure. X-ray diffraction and nuclear magnetic resonance (NMR) spectroscopy were employed to investigate the difference in crystalline structure and crystallinity under different conditions (Gidley et al., 1995). Srichuwong, Sunarti, Mishima, Isono, and Hisamatsu (2005) reported chain-length distribution of amylopectin was associated with digestibility showing the proportion of amylopectin chain with degree of polymerization (DP) of 8–12 was positively correlated with hydrolysis rate and DP 16–26 was negatively correlated with hydrolysis rate. However, information on how HMT affects the formation and structural characteristics of SDS fraction is lacking. Chung et al. (2009) investigated the impact of annealing and HMT on the digestibility of corn, pea and lentil starches. For all granular native starches, the contents of RDS and RS increased but the content of SDS decreased by annealing and HMT, compared with the raw starch. Depending on the temperature and moisture levels during HMT, the RDS and SDS of potato starch increase, yet the RS content decreases as compared with the native starch (Lee et al., 2011). The HMT of normal potato starch has been extensively researched and is widely used in the food industry. However, few studies have focused on the waxy potato starch extracted from a recently developed amylose-free potato mutant. Thus, structural properties of SDS and RS fractions in waxy potato starch formed by the HMT are not well defined. Our previous article dealt with the optimal conditions for SDS production through HMT from waxy potato starch and the effects of HMT-induced structural changes on glucose response in mice (Lee et al., 2012). The purpose of the current study was to elucidate the structural characteristics of SDS + RS and RS fractions isolated from waxy potato starch subjected to HMT.

2. Materials and methods

2.1. Materials

Waxy potato starch was purchased from AVEBE (Veendam, The Netherlands). The enzymes used in the starch digestion were porcine pancreatin (activity 8 × USP/g, P7545, Sigma, St. Louis, MO, USA) and amyloglucosidase (activity 300 AGU/mL, AMG 300L, Novozymes, Bagsvaerd, Denmark), where USP and AGU stand for United States Pharmacopia and amyloglucosidase activity, respectively. Isoamylase was obtained from Megazyme (activity 1000 U/mL, Megazyme International, Bray, Ireland). Other chemicals and regents used in this study were of analytical grade.

2.2. HMT

The optimum conditions for forming high concentrations of SDS fraction in waxy potato starch formed by HMT were previously reported (Lee et al., 2012). The original moisture content of the waxy potato starch used was measured 12.7% according to the AACC 44-15A procedure (American Association of Cereal Chemists, 2000). The moisture content of the waxy potato starch was increased to 25.7% by adding the appropriate amount of distilled water. The starch sample was mixed thoroughly with the water in a glass container, sealed and left overnight at ambient temperature to equilibrate the moisture content. The heat treatment was performed in a forced air oven at 120 °C for 5 h 20 min. Then, the container was opened and the sample was air-dried to uniform moisture content (~10%).

2.3. Isolation of SDS and RS

The SDS + RS and RS fractions were isolated based on the method of Englyst et al. (1992) as modified by Shin et al. (2007). RDS was measured for 10 min based on the amount of glucose after the enzyme reaction. SDS was obtained as the amount digested between 10 min and 240 min. RS was the amount left undigested after 240 min. The contents of RDS, SDS, and RS obtained were very similar to the ones determined by the Englyst method. Porcine pancreatin (10 g) was added to 120 mL of distilled water and stirred with a magnetic stirrer for 10 min. The pancreatin solution was then centrifuged at 1500 × *g* for 10 min. The cloudy supernatant (100 mL) was transferred to a conical flask containing 2 mL (600 AGU) and 18 mL of amyloglucosidase and distilled water, respectively. The digestibility of the heat–moisture treated starch was measured by adding sodium acetate buffer (0.1 M, pH 5.2, 75 mL) and 5 glass beads to a glass container (500 mL) containing starch (3 g, wet basis). The enzyme solution (75 mL) was added to the sample and incubated in a shaking incubator (240 rpm) at 37 °C for 10 min to remove RDS and absolute alcohol was added to the sample solution to a final concentration of 80% to stop the reaction. The SDS + RS fraction was isolated by centrifugation (5000 × *g*, 10 min). The RS fraction was obtained by hydrolyzing the treated starch for 240 min to remove RDS and SDS, followed by the addition of alcohol and centrifugation. To prepare control starch, the native and heat–moisture treated starches were incubated without enzyme solution for 240 min, and the addition of alcohol and centrifugation were conducted under the same conditions described above. The residue was air-dried at room temperature. The degrees of starch hydrolysis of native SDS + RS, native RS, HMT-SDS + RS, and HMT-RS fractions were 16.1%, 24.2%, 14.2%, and 56.0%, respectively (Lee et al., 2012).

2.4. Scanning electron microscopy (SEM)

The surface structures and cross sections of SDS + RS and RS fractions were observed using field-emission scanning electron microscopy (SEM). Starch samples were mounted on circular aluminum stubs with double sticky carbon tape, coated with a thin film of platinum under vacuum and examined using a field-emission scanning electron microscope (Supra 55VP, Carl Zeiss, Oberkochen, Germany) at an accelerating potential of 3 kV. The cross section of a starch granule was prepared with a stainless blade approximately 2-μm thick (ST 300, Dorco Co., Seoul, Korea).

2.5. X-ray diffraction

X-ray diffraction analysis was performed using an X-ray diffractometer (Model D5005, Bruker, Karlsruhe, Germany) operating at 40 kV and 40 mA producing CuK_α radiation of 1.54 Å wavelength,

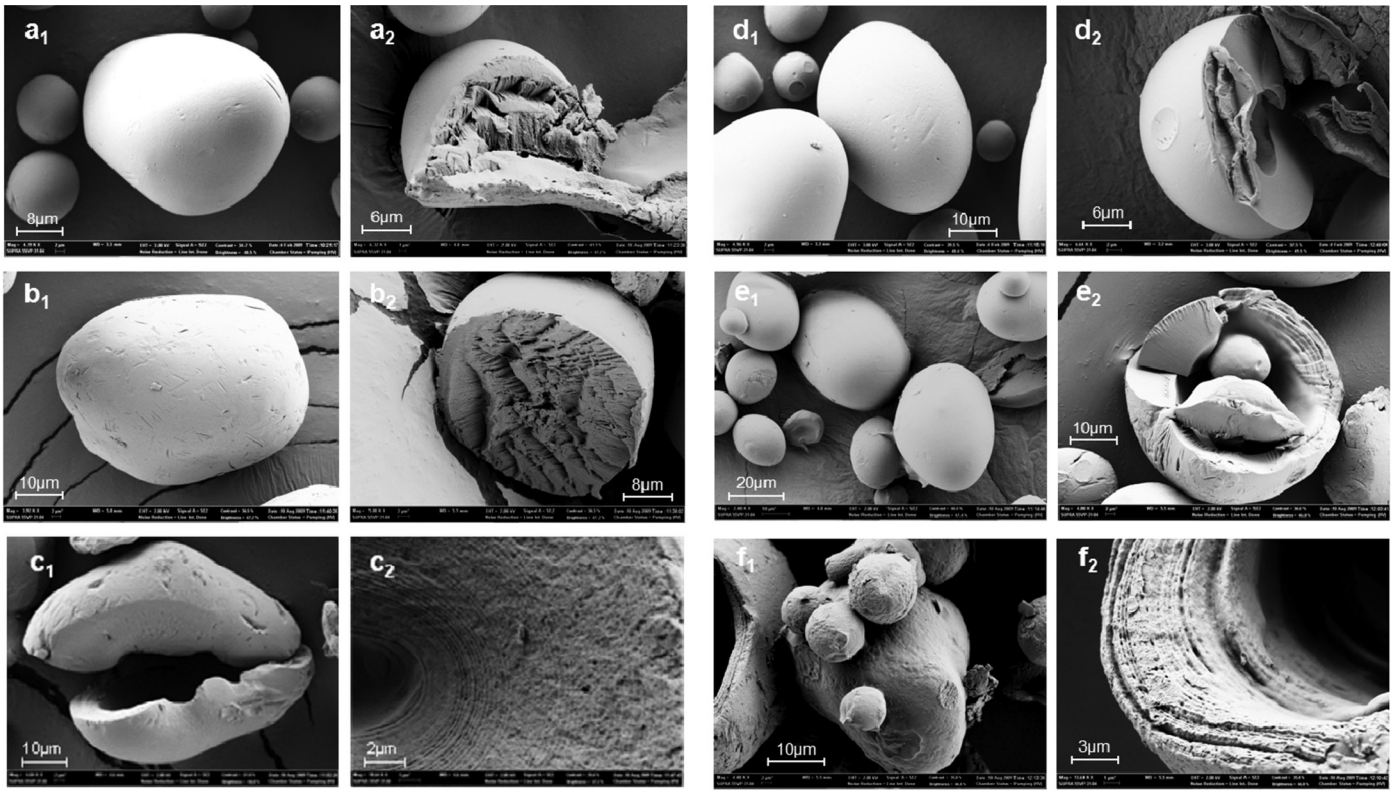

Fig. 1. Scanning electron micrograph (SEM) of heat–moisture treated waxy potato starch: (a) native control, (b) native slowly digestible starch plus resistant starch (SDS + RS), (c) native RS, (d) heat–moisture treatment (HMT) control, (e) HMT-SDS + RS, (f) HMT-RS, (1) SEM of the surface of starch granules (2) SEM of the cross-section of starch granules.

scanning through the 2θ range of 3–30° and step time of 4 s. Relative crystallinity of starches was calculated according to the method of Nara and Komiya (1983) using a peak-fitting software (Origin-version 7.5, OriginLab, Northampton, MA, USA).

2.6. Gelatinization parameters

Gelatinization parameters were determined using a differential scanning calorimeter (Pyris Diamond DSC, Perkin-Elmer, Waltham, MA, USA). The calorimeter was calibrated with an indium standard. Water (40 μL) was added with a micropipette to the sample (10 mg) in a DSC pan, which was then sealed, reweighed and allowed to stand for 4 h at room temperature to attain an even distribution of distilled water. The sample pan was heated from 30 °C to 120 °C at 5 °C/min with an empty pan as reference. During the scan, the space surrounding the sample chamber was flushed with dry nitrogen to avoid condensation. Onset, peak and conclusion temperatures of gelatinization as well as gelatinization enthalpies were determined using the Pyris software.

2.7. Determination of branched chain-length distribution using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

The branched chain-length distributions of SDS + RS and RS fractions were determined after debranching with isoamylase. Starch (15 mg) was solubilized in 90% DMSO (3 mL) and boiled for 15 min. Ethanol (15 mL) was added to the starch suspension to precipitate the starch. The suspension was centrifuged at 10,000 × *g* for 10 min twice. Distilled water (1.5 mL) was added to the pellet and boiled for 10 min. After boiling, 1.5 mL of 50 mM sodium acetate buffer (pH 4.3) and 30 μL of isoamylase (Megazyme International) were added and the sample was incubated in a water bath at 45 °C and 50 rpm for 2 h. The sample was boiled for 10 min to stop the reaction. The debranched sample was filtered through a 0.45-μm membrane filter and analyzed using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) on a CarboPac™ PA-1 anion exchange column (Dionex, Sunnyvale, CA, USA) with a pulsed amperometric detector (Dionex). This analysis was performed using 150 mM NaOH for column equilibration and 600 mM sodium acetate in 150 mM NaOH for sample elution with flow rate of 1 mL/min. The gradients of sodium acetate used were as follows: linear gradients from 0% to 20% for 0 to 5 min, from 20% to 45% for 6 to 30 min, from 45% to 55% for 31 to 60 min, from 55% to 60% for 61 to 80 min, from 60% to 65% for 81 to 90 min, from 65% to 80% for 91 to 95 min and from 80% to 100% for 96 to 100 min. The values of DP from 1 to 7 were designated using a mixture of maltooligosaccharides (DP 1–7, Sigma Chemical) as standard.

2.8. Statistical analyses

All experiments were performed in triplicate and mean values and standard deviations reported. Analysis of variance was conducted and the mean separations were analyzed using the Duncan's multiple range test ($p < 0.05$). The statistical analyses were conducted using the SPSS for Windows 12.0 software (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. SEM

The surface and cross sections of starch granules of SDS + RS and RS fractions were investigated using SEM. The surface of native starch granules was displayed as round or oval granular shapes with no evidence of cracks (Fig. 1a$_1$). The surface of starch granules

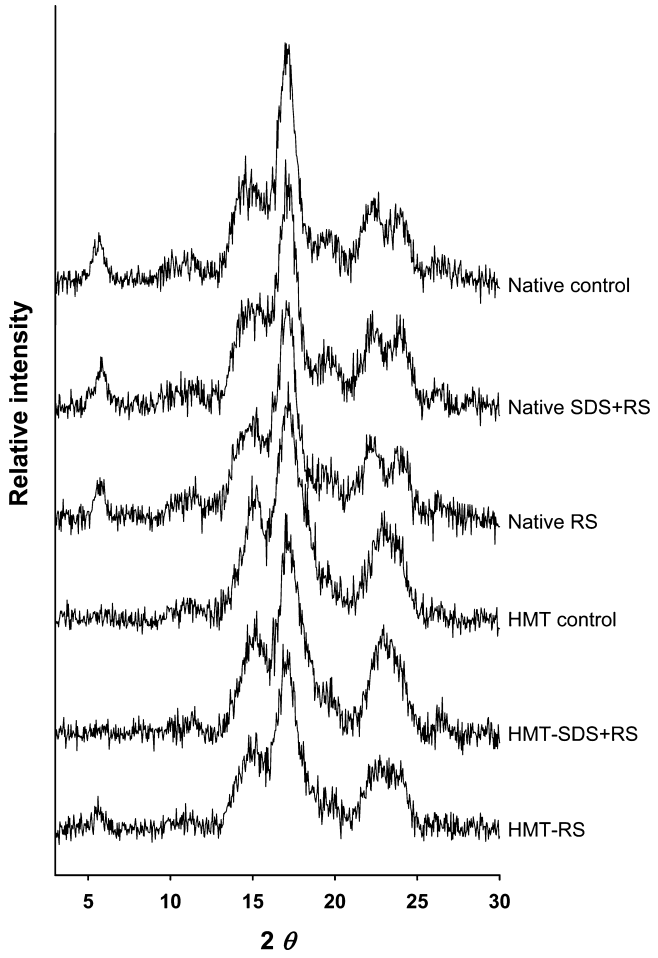

Fig. 2. X-ray diffraction patterns of slowly digestible starch plus resistant starch (SDS + RS) and RS fractions.

in the native SDS + RS fraction was similar to that of native starch. However, many cracks and granular degradation were observed in the native RS fraction (Fig. 1c_1). The cross section of the native starch granules showed no hollow areas (Fig. 1a_2). Growth ring structure was observed in the cross section of the native RS fraction (Fig. 1c_2), but was not observed in the native SDS + RS fraction. The surface of the heat–moisture treated sample showed signs of cracks (Fig. 1d_1) and more granular degradation occurred in the SDS + RS and RS fractions (Figs. 1e_1 and f_1). The cross section of each starch granule displayed a large hollow region at the center, possibly caused by the HMT (Fig. 1d_2). HMT may cause transfer or rearrangement of the molecular structure at the center of the starch granules where the tissue structure is weak (Kawabata et al., 1994). A growth ring structure was observed in the HMT-SDS + RS and HMT-RS fractions. In comparison with the HMT-SDS + RS fraction, more degradation of the amorphous growth ring occurred in the HMT-RS fraction. This result was due to the hydrolysis of starch molecules, which occurred to a greater extent in the amorphous region that was relatively more easily accessible by enzymes than in the semicrystalline growth ring. Reportedly, α-amylase attacks preferentially amorphous regions of the starch, and solid regions are less accessible and hydrolyzed at a slower rate (Vasanthan & Bhatty, 1996).

3.2. X-ray diffraction and relative crystallinity

The X-ray diffraction patterns and relative crystallinities of the samples are shown in Fig. 2 and Table 1, respectively. Native waxy potato starch showed the typical B-type pattern with diffraction intensities at 5.5°, 14.8°, 17°, 19.3°, 22° and 24° 2θ angle. Heat–moisture treated samples exhibited significantly changed X-ray diffractograms. The original peaks at 5.5° and 19.3° disappeared and those at 22° and 24° were replaced by the peak at 23°. Thus, the X-ray patterns were altered from a B-type to a combination of B- and A-types. The B- to B + A-type transformation in potato and waxy potato starches has been previously reported (Hoover & Vasanthan, 1994; Lee et al., 2012). In the HMT-SDS + RS fraction, the peaks at 22° and 24° were replaced by the peak at 23°, which showed the B + A-type pattern. In contrast, in the HMT-RS fraction, peaks appeared at 5.5°, 22° and 24°, thus displaying a B-type pattern. The B-type polymorph has been proposed to have more branch points in non-crystalline regions, leading to high-density amorphous regions and stable crystallites, which resist enzymatic hydrolysis compared to A-type polymorphs (Srichuwong & Jane, 2007). In the current study, heat–moisture treated starches showed a decrease in the intensity of the major diffraction peaks along with a decrease in crystallinity. The X-ray diffraction patterns in the native SDS + RS and native RS fractions were unchanged. However, small increases in the level of relative crystallinity in the native SDS + RS and native RS fractions were observed, and the increase in the latter was greater. The relative crystallinity increased in the HMT-SDS + RS and HMT-RS fractions and the increase was higher in the latter. The HMT-RS fraction displayed lower relative crystallinity compared with the native RS fraction. These results indicated that the RS fraction was mainly composed of semicrystalline regions, which was in agreement with the description by Sajilata et al. (2006).

Table 1
Relative crystallinity of slowly digestible starch plus resistant starch (SDS + RS) and RS fractions.

Samples	Relative crystallinity
Native control	0.50 ± 0.01^{c}
Native SDS + RS	0.52 ± 0.01^{d}
Native RS	0.56 ± 0.01^{e}
HMT control	0.42 ± 0.01^{a}
HMT-SDS + RS	0.47 ± 0.01^{b}
HMT-RS	0.52 ± 0.01^{d}

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

3.3. Gelatinization parameters

The gelatinization parameters of various samples are shown in Table 2. The HMT samples displayed slight increases in T_o and T_p, a significant increase in gelatinization temperature range (T_c–T_o), and a decrease in gelatinization enthalpies compared with the native control starch. The increased gelatinization parameters of HMT samples were consistent with the previous studies on the effects of HMT on tuber and root starches (Gunaratne & Hoover, 2002; Hoover & Manuel, 1996). Both the native SDS + RS and native RS fractions showed higher gelatinization temperatures and a lower T_c–T_o than the native control. There were no significant differences in T_o, T_p, T_c, and ΔH for the native SDS + RS and native RS fractions. T_o, T_p, and T_c increased more in both the HMT-SDS + RS and HMT-RS fractions than the HMT control. Compared with the HMT control, only the HMT-RS fraction showed a decrease in T_c–T_o. The decrease of ΔH was the greatest in the HMT-SDS + RS fraction, followed by the HMT-RS fraction. The differences in T_c–T_o are due to the degree of crystallite heterogeneity, which has slightly different crystal strengths (Gunaratne & Hoover, 2002; Vasanthan & Bhatty, 1996). The decrease in ΔH values after HMT of potato starch suggested several of the original double helices might be disrupted during the polymorphic transformation from B-type to the combination of A- and B-types. Shin et al. (2004) reported the

Table 2
Gelatinization parameters of slowly digestible starch plus resistant starch (SDS + RS) and RS fractions.

Sample	T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)	ΔH (J/g)
Native control	63.5 ± 0.4[a]	69.3 ± 0.2[a]	74.3 ± 0.3[a]	10.8 ± 0.8[c]	16.1 ± 0.2[d]
Native SDS + RS	66.8 ± 0.1[d]	71.8 ± 0.3[c]	76.4 ± 0.4[b]	9.6 ± 0.4[b]	15.2 ± 0.5[c]
Native RS	67.1 ± 0.3[d]	71.5 ± 0.1[c]	75.8 ± 0.2[b]	8.7 ± 0.2[a]	15.5 ± 0.1[cd]
HMT control	64.1 ± 0.4[b]	70.1 ± 0.2[b]	86.6 ± 0.2[d]	22.5 ± 0.1[e]	14.0 ± 0.5[b]
HMT-SDS + RS	66.1 ± 0.0[c]	71.9 ± 0.1[c]	89.1 ± 0.2[e]	22.9 ± 0.2[e]	10.9 ± 0.3[a]
HMT-RS	67.1 ± 0.4[e]	71.8 ± 0.5[c]	79.6 ± 0.4[c]	11.9 ± 0.4[d]	13.5 ± 0.1[b]

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

Table 3
The branched chain-length distributions of slowly digestible starch plus resistant starch (SDS + RS) and RS fractions.

Sample	Percent distribution			
	DP 6–12	DP 13–24	DP 25–36	DP ≥37
Native control	21.4 ± 0.4[c]	49.8 ± 0.7[b]	17.5 ± 0.4[b]	11.3 ± 0.3[a]
Native SDS + RS	21.0 ± 0.3[c]	49.4 ± 0.4[b]	17.7 ± 0.5[b]	11.9 ± 0.2[abc]
Native RS	21.1 ± 0.4[c]	49.1 ± 0.3[b]	17.8 ± 0.3[b]	12.0 ± 0.5[bc]
HMT control	21.1 ± 0.3[c]	49.6 ± 0.2[b]	17.6 ± 0.3[b]	11.6 ± 0.3[ab]
HMT-SDS + RS	20.4 ± 0.4[b]	48.2 ± 0.3[a]	19.0 ± 0.6[a]	12.5 ± 0.3[c]
HMT-RS	19.0 ± 0.5[a]	47.9 ± 0.6[a]	19.7 ± 0.3[a]	13.3 ± 0.3[d]

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

enzymatically hydrolyzed fraction from debranched waxy sorghum starch was inferred to be RDS, which was mostly amorphous. Additionally, double helices of the SDS + RS fraction had a less ordered structure than the RS fraction. In the present study, the ΔH of HMT control revealed a higher value than the heat–moisture treated waxy potato starch (12.7 J/g, Lee et al., 2012). This seemed due to the rearrangement of crystalline matrix during incubation for producing control sample. The HMT-SDS + RS fraction displayed a lower ΔH than the HMT-RS fraction (10.9 and 13.5 J/g, respectively). As the ΔH primarily reflects the loss of double helical order, the variation in ΔH corresponds to the number of double helices and the extent of interaction between adjacent amylopectin double helices within the crystalline domains (Cooke & Gidley, 1992; Gunaratne & Hoover, 2002). It is known that short chains of amylopectin molecules form double helices (short order), which organize into crystalline lamella (long order) (Roder, Ellis, & Butterworth, 2005). Therefore, the lower ΔH of HMT-SDS + RS than HMT control might be caused by a decrease in the proportion of short chains of DP < 25. The amorphous region and a less perfect crystalline region were hydrolyzed through enzyme digestion, as supported by a significant difference in enthalpy between the HMT-SDS + RS and HMT-RS fractions. This result indicated that double helices of the HMT-SDS + RS fraction were removed during the enzyme digestion and the HMT-SDS + RS fraction had a less ordered structure than the HMT-RS fraction. The HMT-SDS + RS fraction included SDS which could be consisted of weak crystallites and amorphous regions, supporting the SDS structure reported by Shin et al. (2004). Thus, the crystalline regions likely composed most of the HMT-RS fraction.

3.4. Branch chain-length distributions

The chain-length distributions of the SDS + RS and RS fractions debranched by isoamylase are presented in Fig. 3 and Table 3. The branch chain-length distribution was determined as a percentage of the total peak area and the variation in the detector response with DP was disregarded (Hanashiro, Abe, & Hizukuri, 1996). Grouping of DP numbers followed that of Hanashiro et al. (1996), who categorized branched chains into the following four fractions: DP 6–12, 13–24, 25–36, and ≥37, corresponding to A, B1, B2, and B3, respectively and longer chains of amylopectin. Reportedly, B-type starches have longer branch chains than A- and C-type starches

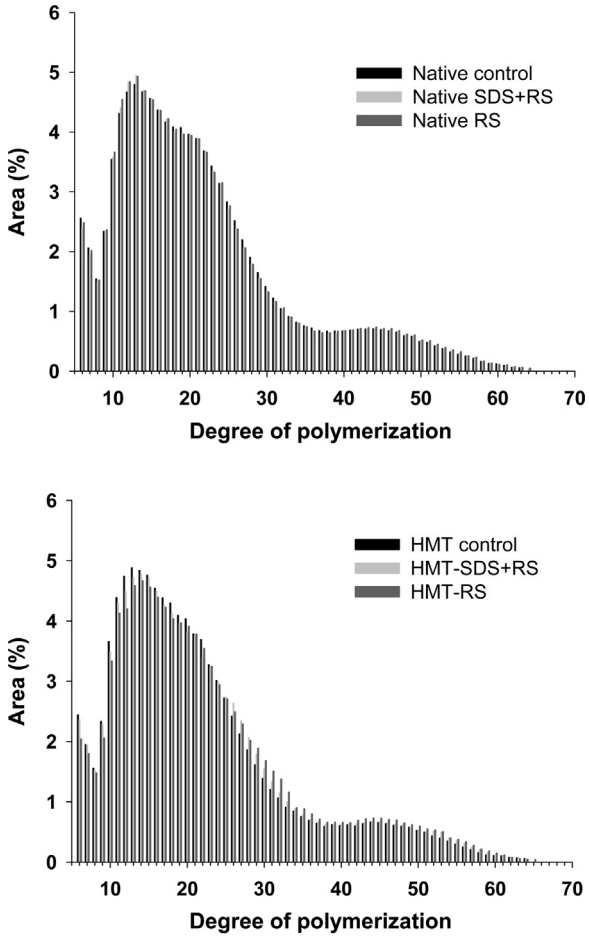

Fig. 3. The branched chain-length distributions of slowly digestible starch plus resistant starch (SDS + RS) and RS fractions.

(Jane et al., 1999; Hizukuri, Kaneko, & Takeda, 1983). Waxy potato amylopectin had a high proportion of DP 13–24 and a low proportion of DP ≥37. No significant differenences were observed in the SDS + RS and RS fractions of the native and HMT controls. However, the HMT-SDS + RS fraction had lower proportions of short branch chains (DP 6–12 and DP 13–24) and higher proportions of DP 25–36 and DP ≥37 compared with the native and HMT controls. Moreover, amylopectins of the HMT-RS fraction had a higher proportion of long branch chains (DP ≥37) but a lower proportion of short branch chains of DP 6–12 than the HMT-SDS + RS fraction.

The B-type starches have lower percentages of short chains (DP 6–12) and higher percentages of long chains (DP ≥37) than do A- and C-type starches (Hanashiro et al., 1996; Jane et al., 1999). The HMT-RS fraction, which showed the B-type X-ray pattern, had a higher proportion (13.3%) of long chains (DP ≥37) and a lower proportion (19.0%) of short branch chains (DP 6–12) than the HMT control (11.6% and 21.1%, respectively).

Using partial α-amylase hydrolysis, Han et al. (2006) developed a novel maize starch with a slowly digestible and resistant character compared with raw maize starch. They reported the proportion of short chain was reduced in the partially α-amylase-treated starch having higher SDS and RS contents than raw starch and the proportion of long chains progressively increased. A significant amount of short chains in amylopectin molecules interrupted the formation of crystallites perfectly resistant to digestion during retrogradation. Amylopectin molecules having a small amount of short chains and a large amount of long chains preferentially formed relatively perfect crystallites resistant to starch-hydrolysing enzymes.

Zhang et al. (2006) reported SDS is composed of undigested or partially digested amylopectin and a considerable amount of small molecules was detected after debranching. In addition to the short chains of DP 5–9, the long chains of DP > 30 greatly contributed to SDS. The fine structure of amylopectin is potentially related to the content of SDS (Zhang, Ao, & Hamaker, 2008).

4. Conclusions

HMT of waxy potato starch caused a change in the granular structure altering the digestion pattern. Cross-sections of starch granules displayed a more definite growth ring pattern in the SDS + RS fraction than in the RS fraction. The RS fraction exhibited increased crystallinity, gelatinization enthalpy and proportion of long chains (DP ≥37) but a decreased gelatinization temperature range compared with the SDS + RS fraction. The SDS + RS fraction showed increases in crystallinity and proportion of long chains (DP ≥25) but a decrease in gelatinization enthalpy compared with the control starch. These results indicated that the majority of RS was composed of crystalline structure and had a high proportion of long chains (DP ≥37). Finally, most of the SDS consisted of amorphous regions and weak crystallites and showed a high proportion of DP ≥25. This structural information could be used to develop a low-digestible food ingredient based on heat–moisture treated waxy potato starch.

Acknowledgements

This work was supported by both the BioGreen 21 Program (No. PJ007192) and the "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ008575)", Rural Development Administration, Republic of Korea.

References

American Association of Cereal Chemists. (2000). *Approved methods of the AACC* (10th ed.). St. Paul, MN: The American Association of Cereal Chemists.

Chung, H. J., Liu, Q., & Hoover, R. (2009). Impact of annealing and heat–moisture treatment on repidly digestible, slowly digestible and resistant starch levels in native and gelatinized corn, pea and lentil starches. *Carbohydrate Polymers*, *75*, 436–447.

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

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, *46*, 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*, 23–31.

Gunaratne, A., & Hoover, R. (2002). Effect of heat–moisture treatment on the structure and physicochemical properties of tuber and root starches. *Carbohydrate Polymers*, *49*, 425–437.

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*, *53*, 131–139.

Han, X. Z., Ao, Z. H., Janaswamy, S., Jane, J. L., Chandrasekaran, R., & Hamaker, B. R. (2006). Development of a low glycemic maize starch: Preparation and characterization. *Biomacromolecules*, *7*, 1162–1168.

Hanashiro, I., Abe, J., & Hizukuri, S. (1996). A periodic distribution of the chain length of amylopectin as revealed by high-performance anion-exchange chromatography. *Carbohydrate Research*, *283*, 151–159.

Haralampu, S. G. (2000). Resistant starch—A review of the physical properties and biological impact of RS_3. *Carbohydrate Polymers*, *41*, 285–292.

Hizukuri, S., Kaneko, T., & Takeda, Y. (1983). Measurements of the chain length of amylopectin and its relevance to the origin of crystalline polymorphism of starch granules. *Biochimica et Biophysica Acta—General Subjects*, *760*, 188–191.

Hoover, R., & Manuel, H. (1995). A comparative study of the physicochemical properties of starches from two lentil cultivars. *Food Chemistry*, *53*, 275–284.

Hoover, R., & Manuel, H. (1996). The effect of heat–moisture treatment on the structure and physicochemical properties of normal maize, waxy maize, dull waxy maize and amylomaize V starches. *Journal of Cereal Science*, *23*, 153–162.

Hoover, R., & Sosulski, F. (1985). Studies on the functional characteristics and digestibility of starches from *Phaseolus vulgaris* biotypes. *Starch*, *37*, 181–191.

Hoover, R., & Vasanthan, T. (1994). Effect of heat–moisture treatment on the structure and physicochemical properties of cereal, legume, and tuber starches. *Carbohydrate Research*, *252*, 33–53.

Jacobs, H., & Delcour, J. A. (1998). Hydrothermal modifications of granular starch, with retention of the granular structure: A review. *Journal of Agricultural and Food Chemistry*, *46*, 2895–2905.

Jane, J., Wong, K. S., & McPherson, A. E. (1997). Branch-structure difference in starches of A- and B-type X-ray patterns revealed by their Naegeli dextrins. *Carbohydrate Research*, *300*, 219–227.

Jane, J., Chen, Y. Y., Lee, L. F., McPherson, A. E., Wong, K. S., Radosavljevic, M., et al. (1999). Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chemistry*, *76*, 629–637.

Jenkins, D. J. A., Kendall, C. W. C., Augustin, L. S. A., Franceschi, S., Hamidi, M., Marchie, A., et al. (2002). Glycemic index: Overview of implications in health and disease. *American Journal of Clinical Nutrition*, *76*, 266S–273S.

Kawabata, A., Takase, N., Miyoshi, E., Sawayama, S., Kimura, T., & Kudo, K. (1994). Microscopic observation and X-ray diffractometry of heat/moisture-treated starch granules. *Starch*, *46*, 463–469.

Lee, C. J., Kim, Y., Choi, S. J., & Moon, T. W. (2012). Slowly digestible starch from heat–moisture treated waxy potato starch: Preparation, structural characteristics, and glucose response in mice. *Food Chemistry*, *133*, 1222–1229.

Lee, C. J., Shin, S. I., Kim, Y., Choi, H. J., & Moon, T. W. (2011). Structural characteristics and glucose response in mice of potato starch modified by hydrothermal treatments. *Carbohydrate Polymers*, *83*, 1879–1886.

Lehmann, U., & Robin, F. (2007). Slowly digestible starch—Its structure and health implications: A review. *Trends in Food Science & Technology*, *18*, 346–355.

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

Nara, S., & Komiya, T. (1983). Studies on the relationship between water-sutured state and crystallinity by the diffraction method for moistened potato starch. *Starch*, *35*, 407–410.

Roder, N., Ellis, P. R., & Butterworth, P. J. (2005). Starch molecular and nutritional properties: A review. *Advances in Molecular Medicine*, *1*, 5–14.

Sajilata, M. G., Singhal, R. S., & Kulkarni, P. R. (2006). Resistant starch—A review. *Comprehensive Reviews in Food Science and Food Safety*, *5*, 1–17.

Shin, S. I., Choi, H. J., Chung, K. M., Hamaker, B. R., Park, K. H., & Moon, T. W. (2004). Slowly digestible starch from debranched waxy sorghum starch: Preparation and properties. *Cereal Chemistry*, *81*, 404–408.

Shin, S. I., Lee, C. J., Kim, D.-I., Lee, H. A., Cheong, J.-J., Chung, K. M., et al. (2007). Formation, characterization, and glucose response in mice to rice starch with low digestibility produced by citric acid treatment. *Journal of Cereal Science*, *45*, 24–33.

Srichuwong, S., & Jane, J. (2007). Physicochemical properties of starch affected by molecular composition and structure: A review. *Food Science and Biotechnology*, *16*, 663–674.

Srichuwong, S., Sunarti, T. C., Mishima, T., Isono, N., & Hisamatsu, M. (2005). Starches from different botanical sources I: Contribution of amylopectin fine structure to thermal properties and enzyme digestibility. *Carbohydrate Polymers*, *60*, 529–538.

Tester, R. F., Karkalas, J., & Qi, X. (2004). Starch-composition, fine structure and architecture. *Journal of Cereal Science*, *39*, 151–165.

Vasanthan, T., & Bhatty, R. S. (1996). Physicochemical properties of small- and large-granule starches of waxy, regular, and high-amylose barleys. *Cereal Chemistry*, *73*, 199–207.

Vieira, F. C., & Sarmento, S. B. S. (2008). Heat-mositure treatment and enzymatic digestibility of Peruvian carrot, sweet potato and ginger starches. *Starch*, *60*, 223–232.

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*, 4686–4694.

Zhang, G., Venkatachalam, M., & Hamaker, B. R. (2006). Structural basis for the slow digestion property of native cereal starches. *Biomacromolecules*, *7*, 3259–3266.