Carbohydrate Polymers 79 (2010) 1117–1123

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Structure and digestibility of crystalline short-chain amylose from debranched waxy wheat, waxy maize, and waxy potato starches

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ARTICLE INFO

Article history:
Received 24 August 2009
Received in revised form 18 October 2009
Accepted 22 October 2009
Available online 29 October 2009

Keywords:
Waxy starch
Short-chain amylose
Crystalline structure
Resistant starch
Digestibility

ABSTRACT

The structure and digestibility of crystalline short-chain amylose (CSCA) from debranched waxy wheat, waxy maize, and waxy potato starches were investigated and compared. The starches (5%, w/w) were cooked in acetate buffer (pH 4.0) and debranched by isoamylase at 50 °C. After 24 h, the mixture was cooled and held at 25 °C for another 24 h. Debranched waxy potato starch had a longer average chain length than debranched waxy wheat and waxy maize starches, resulting in a higher yield of crystallized product. All CSCA products displayed a B-type X-ray diffraction pattern, indicating that low solids concentration (5%) favored the formation of B-type crystals. 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 and waxy maize starches, suggesting that waxy potato starch is a preferred starting material to prepare products with high resistant starch content by debranching and crystallization.

1. Introduction

Starch, an abundant natural polysaccharide, normally consists of two types of α-D-glucose polymers: amylose and amylopectin. Amylose is an essentially linear polymer with few branches, whereas amylopectin has a highly branched structure in which branch chains are linked to the linear chains by α-(1,6)-linkages (Hizukuri, Abe, & Hanashiro, 2006; Tester, Karkalas, & Qi, 2004). The ratio of amylose to amylopectin contents in starch varies depending on botanical source. Normal starches consist of 20–30% amylose and 70–80% amylopectin, but waxy (amylose-free) starches contain essentially 100% amylopectin.

Under specific conditions, amylopectin can be debranched in the presence of debranching enzymes (e.g. isoamylase and pullulanase). The chain length (CL) distribution of debranched amylopectin is highly related to its crystalline polymorphs (Hizukuri, 1985). In general, B-type starches have long branch chains of amylopectin, whereas A-type starches have a large amount of short chains with a degree of polymerization (DP) of 6–12 (Hanashiro, Abe, & Hizukuri, 1996). The distinct branch CL distributions of amylopectin from A- and B-type starches affect their functional properties, such as gelatinization (Jane et al., 1999), retrogradation (Silverio, Fredriksson, Andersson, Eliasson, & Aman, 2000), pasting properties (Jane & Chen, 1992; Srichuwong, Sunarti, Mishima, Isono, & Hisamatsu, 2005b), and acid and enzyme hydrolysis (Fredriksson et al., 2000; Srichuwong, Sunarti, Mishima, Isono, & Hisamatsu, 2005a).

Compared with waxy maize, waxy wheat (Chibbar & Chakraborty, 2005; Graybosch, 1998; Guan, Seib, Graybosch, Bean, & Shi, 2009) and waxy potato (Jeffcoat, Mason, Emling, & Chiu, 2002; Visser, Suurs, Bruinenberg, et al., 1997; Visser, Suurs, Steeneken, et al., 1997) are relatively new and of great interest to the food and starch industries. However, the crystallization behaviors of debranched waxy wheat and waxy potato starches have not been reported. In contrast, waxy sorghum (Shin et al., 2004), waxy rice (Guraya, James, & Champagne, 2001a, 2001b), and waxy maize (Berry, 1986; Miao, Bo, & Zhang, 2009; Shi, Cui, Birkett, & Thatcher, 2005a, 2005b, 2006) starches have been debranched and crystallized to prepare slowly digestible starch (SDS) and resistant starch (RS). Waxy wheat and waxy maize starches are cereal starches with short branch chains and an A-type X-ray diffraction pattern whereas waxy potato starch contains long branch chains and displays a B-type pattern (McPherson & Jane, 1999). The objective of this work was to investigate the effects of CL and starch source on the properties of short-chain amylose derived from waxy wheat, waxy maize, and waxy potato starches. The three starches were completely debranched and crystallized. The CL distributions, crystalline structure, thermal properties, and digestion behaviors of the crystalline short-chain amylose (CSCA) products were determined. This information will be useful for better designing starch-based food ingredients with improved health benefits.

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doi:10.1016/j.carbpol.2009.10.057

2. Materials and methods

2.1. Materials

Waxy wheat starch was isolated from hard waxy wheat flour as described by Guan et al. (2009). Waxy maize starch was obtained from National Starch Food Innovation (Bridgewater, NJ, USA). Waxy potato starch was provided by Penford Food Ingredients Company (Centennial, CO, USA). Isoamylase (EC 3.2.1.68) was obtained from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan) and had an enzyme activity of 1.41×10^6 isoamylase activity units (IAU)/g. The enzyme activity was determined by incubating the enzyme with soluble waxy maize starch as a substrate in the presence of iodine for 30 min at pH 3.5 and 40 °C and measuring the absorbance of the reaction mixture at 610 nm (Joint FAO/WHO Expert Committee on Food Additives., 2007). One IAU is defined as the amount of isoamylase that increased absorbance of the reaction mixture by 0.008 in 30 min under the conditions of the isoamylase assay.

2.2. Debranching of starch and preparation of CSCA

Starch (5 g, dry basis) was mixed with 95 mL acetic acid buffer (0.01 M, pH 4.0) in a sealed glass bottle and cooked in a boiling water bath with stirring for 1 h. After the mixture was cooled to 50 °C, 1% isoamylase based on the dry weight of starch was added. The mixture was kept at 50 °C with stirring. After 24 h, the mixture was cooled to 25 °C and held for another 24 h to increase the yield of crystallites. The precipitates (CSCA products) were filtered, washed with water, and dried at 40 °C in an oven overnight. The yield was calculated by dividing the weight of the precipitates over the weight of the starting starch.

In separate experiments, each starch was debranched as described as above and the whole debranched product including solubles and precipitates was freeze-dried. To confirm that the starch was completely debranched, the freeze-dried debranched starch (20 mg) was weighed into a 10-mL vial, mixed with 2 mL of a mixture of DMSO and water (9:1, w/w), and cooked in a boiling water bath with magnetic stirring for 15 min. After the mixture was cooled to room temperature, acetic acid buffer (6.98 mL, 0.01 M, pH 4.0) and isoamylase (2 μL) were added (Shi, Capitani, Trzasko, & Jeffcoat, 1998). The mixture was then kept at 50 °C with stirring for 24 h. After that, 2 mL of the mixture was taken and mixed with 2 mL DMSO, and analyzed by gel permeation chromatography (GPC).

2.3. GPC

Each starch sample (4 mg) was mixed with DMSO (4 mL) and stirred in a boiling water bath for 24 h. The sample was filtered through a 2 μm filter and then injected by an autosampler into a PL-GPC 220 instrument (Polymer Laboratories, Inc., Amherst, MA, USA) with three Phenogel columns (Phenomenex, Inc., Torrance, CA, USA), a guard column (Phenomenex, Inc., Torrance, CA, USA), and a differential refractive index detector. The eluent system was DMSO containing 0.5 mM $NaNO_3$ at a flow rate of 0.8 mL/min. The column oven temperature was controlled at 80 °C. Standard dextrans (American Polymer Standards Co., Mentor, OH, USA) with different molecular weights (MW) were used for MW calibration.

2.4. High-performance anion-exchange chromatograph (HPAEC)

CL distributions of debranched waxy starches and CSCA products were quantitatively analyzed using a HPAEC (Dionex ICS-3000, Dionex Corp., Sunnyvate, CA, USA) equipped with a pulsed amperometric detector, a guard column, a CarboPac™ PA1 analytical column and an AS-DV autosampler. The eluents were prepared as described previously (Shi & Seib, 1992). Eluent A was 150 mM NaOH and eluent B was 150 mM NaOH containing 500 mM sodium acetate. The gradient program was as followed: 40% of eluent B at 0 min, 50% at 2 min, 60% at 10 min, and 80% at 40 min. The separations were carried out at 25 °C with a flow rate of 1 mL/min. The concentration of debranched starches and CSCA products was 2 mg/mL in 1 M NaOH solution. Maltohexaose and maltoheptaose (Sigma–Aldrich, Inc., St. Louis, MO, USA) were used as standards.

2.5. Average CL determination

Average CL was determined by the Nelson/Somogyi reducing sugar method (Nelson, 1944; Robyt & Whelan, 1968; Somogyi, 1952). Because the CSCA products could not be completely dissolved in boiling water, each starch sample was dissolved in 1.25 N sodium hydroxide, neutralized by hydrochloride acid, and analyzed for reducing sugar.

2.6. Wide angle X-ray diffraction

X-ray diffraction was conducted with a Philips X-ray diffractometer with Cu Kα radiation at 35 kV and 20 mA, a theta-compensating slit, and a diffracted beam monochromator. The moisture of all samples was adjusted to about 18% in a sealed dessicator at room temperature before analysis. The diffractograms were recorded between 2° and 35° (2θ). Relative crystallinity was estimated by the ratio of the peak areas to the total diffractogram area (Komiya & Nara, 1986).

2.7. Differential scanning calorimetry (DSC)

A 25% (w/w) suspension of solid sample in water was prepared and sealed in a DSC pan and analyzed with a TA Q5000 (TA Instruments, New Castle, DE, USA). An empty pan was used as a reference. Samples were heated from 10 to 150 °C at 10 °C/min. The onset (T_o), peak (T_p), and conclusion (T_c) temperatures and enthalpy (ΔH) were obtained from the DSC endotherm with DSC software (TA Instruments, New Castle, DE, USA). Experiments were conducted in duplicate.

2.8. In vitro digestion method

The *in vitro* digestion of starch was determined by a modified Englyst procedure (Englyst, Kingman, & Cummings, 1992; Sang & Seib, 2006). Percentages of rapidly digestible starch (RDS), SDS, and RS were calculated by (% digestible starch at 20 min), (% digestible starch at 120 min − % digestible starch at 20 min), and (100% − % digestible starch at 120 min), respectively.

2.9. Statistical analysis

Data were analyzed by an analysis of variance (ANOVA) procedure with Tukey's studentized range (HSD) test using SAS version 9.1 (SAS Institute, Inc., Cary, NC, USA). Mean values from the duplicated experiments were reported.

3. Results and discussion

3.1. MW distribution by GPC

The MW distributions of debranched waxy wheat, waxy maize, waxy potato starches and their CSCA products as determined by

GPC are shown in Fig. 1. A bimodal distribution of low and high MW peaks, designated Peak 1 and Peak 2, respectively, was observed for all debranched starches and CSCA products. The area and percentage of each peak were calculated and reported in Table 1. Waxy wheat starch had a large proportion of the low MW fraction (i.e. short side chains in amylopectin) (Fig. 1A). Debranched waxy wheat and waxy maize starches had a similar ratio of area of Peak 1 to Peak 2, In contrast, debranched waxy potato starch had a distinct MW distribution (Fig. 1A), and the proportion of the low MW fraction was much lower compared with debranched waxy wheat and waxy maize starches (Table 1). These results are consistent with the fact that side chains of amylopectin in a B-type starch are longer than those in A-type starch (Hanashiro et al., 1996; Hizukuri, 1985; Jane et al., 1999).

Compared with parent debranched starches, the MW distribution curves of all CSCA products shifted to higher molecular weight (Fig. 1B) and the proportion of the low MW fraction (Peak 1) decreased (Table 1). CSCA products from debranched waxy wheat and waxy maize starches showed a similar MW distribution, whereas CSCA product from debranched waxy potato starch had a distinct MW distribution (Fig. 1B) and a small proportion of the low MW fraction (Table 1).

To confirm that the three waxy starches were completely debranched, the freeze-dried whole debranched starch samples were further debranched by isoamylase. No change in MW distribution was observed (data not shown), indicating that the whole debranched starch molecules were linear.

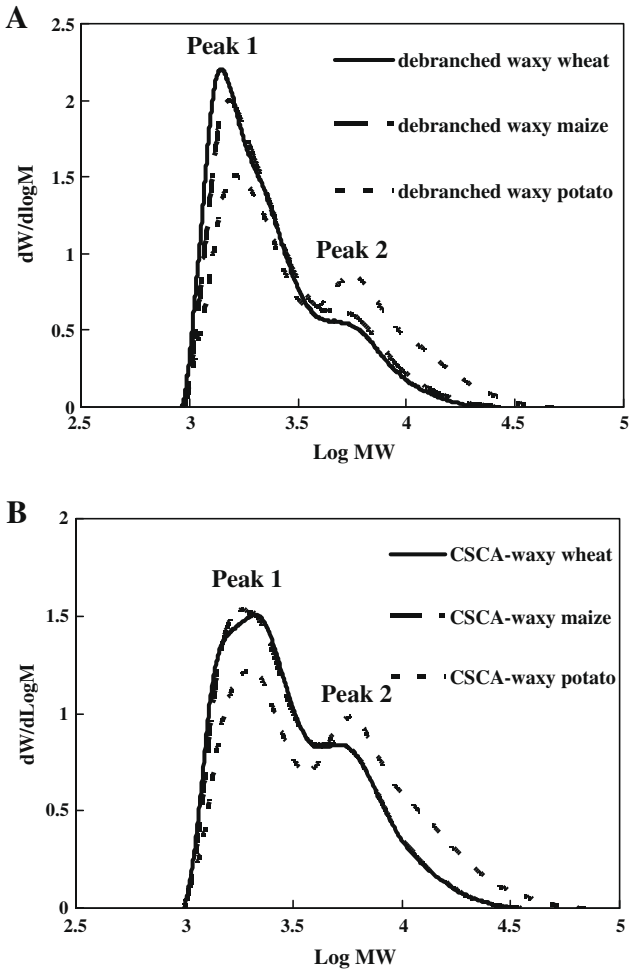

Fig. 1. Molecular weight distributions of (A) debranched waxy wheat, waxy maize, and waxy potato starches and (B) crystalline short-chain amylose (CSCA) products from debranched waxy wheat, waxy maize, and waxy potato starches.

Table 1
Molecular weight distributions of debranched waxy wheat, waxy maize, waxy potato starches and their crystalline short-chain amylose (CSCA) products.

Starch samples	Waxy wheat		Waxy maize		Waxy potato	
	Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2
Peak area (%)						
Debranched starch	79.8	20.2	74.2	25.8	56.5	43.5
CSCA	63.9	36.1	64.4	35.6	45.6	54.4
DP_{max}[a]						
Debranched starch	8		9		10	35
CSCA	13		11		12	35

[a] DP_{max} = degree of polymerization at the peak.

3.2. CL distribution

The CL distributions of debranched starches and their CSCA products as determined by HPAEC are shown in Fig. 2. The debranched waxy wheat starch had a higher proportion of chains with DP 6–19 and a lower proportion of chains with DP 20–67 than its CSCA product (Fig. 2A). Similar trend was observed for debranched waxy maize and waxy potato starches and their corresponding CSCA products (Fig. 2B and C). These results suggested that the chain length of the CSCA products were longer than that of their parent debranched starches. This increase in chain length of CSCA was attributed to the preferential crystallization and precipitation of long chains. According to Gidley and Bulpin (1987), the minimum CL required to form starch double helices is 10. During debranching and crystallization, a fraction of low molecular amylose (mostly DP < 10) was too short to form the stable double helices and remained in the solution. In this study, the CL distribution of the CSCA was peaked at around DP 16 (Fig. 2).

3.3. Yield and average CL

The yields and average CLs of debranched starches and their corresponding CSCA products are listed in Table 2. Debranched waxy potato starch gave a higher yield (72.6%) than debranched waxy wheat (58.7%) and waxy maize (60.7%) starches. The average CLs of debranched waxy wheat, waxy maize, and waxy potato starches were 21.8, 24.1, and 32.1 glucose units, respectively. Our results suggest that B-type starch with a longer CL yields more CSCA than A-type starches. Using amylose with different average DPs ranging from 40 to 610, Eerlingen, Deceuninck, and Delcour (1993) reported that the yield of enzyme RS was correlated with the DP of amylose. However, the isolated RS fractions consisted of short chains (average DP between 19 and 26) and were independent of the CL of the starting amylose used.

The average CLs of CSCA products from debranched waxy wheat, waxy maize, and waxy potato starches were 28.1, 29.2, and 35.5 glucose units, respectively, larger than those of the whole starches. The results were in agreement with the MW distribution (Fig. 1 and Table 1) and CL distribution (Fig. 2) data.

3.4. Crystalline structure

Native waxy wheat and waxy maize starches showed the A-type crystalline structure, whereas waxy potato starch displayed the B-type X-ray diffraction pattern (Fig. 3). The degree of crystallinity of all three native starches was around 40%. Among the three CSCA products, the one produced from debranched waxy potato starch gave a stronger peak at 2θ of 5° and the two peaks between 13° and 15° at 2θ were better resolved (Fig. 3), suggesting that long chains form a stronger crystalline structure more readily than short chains.

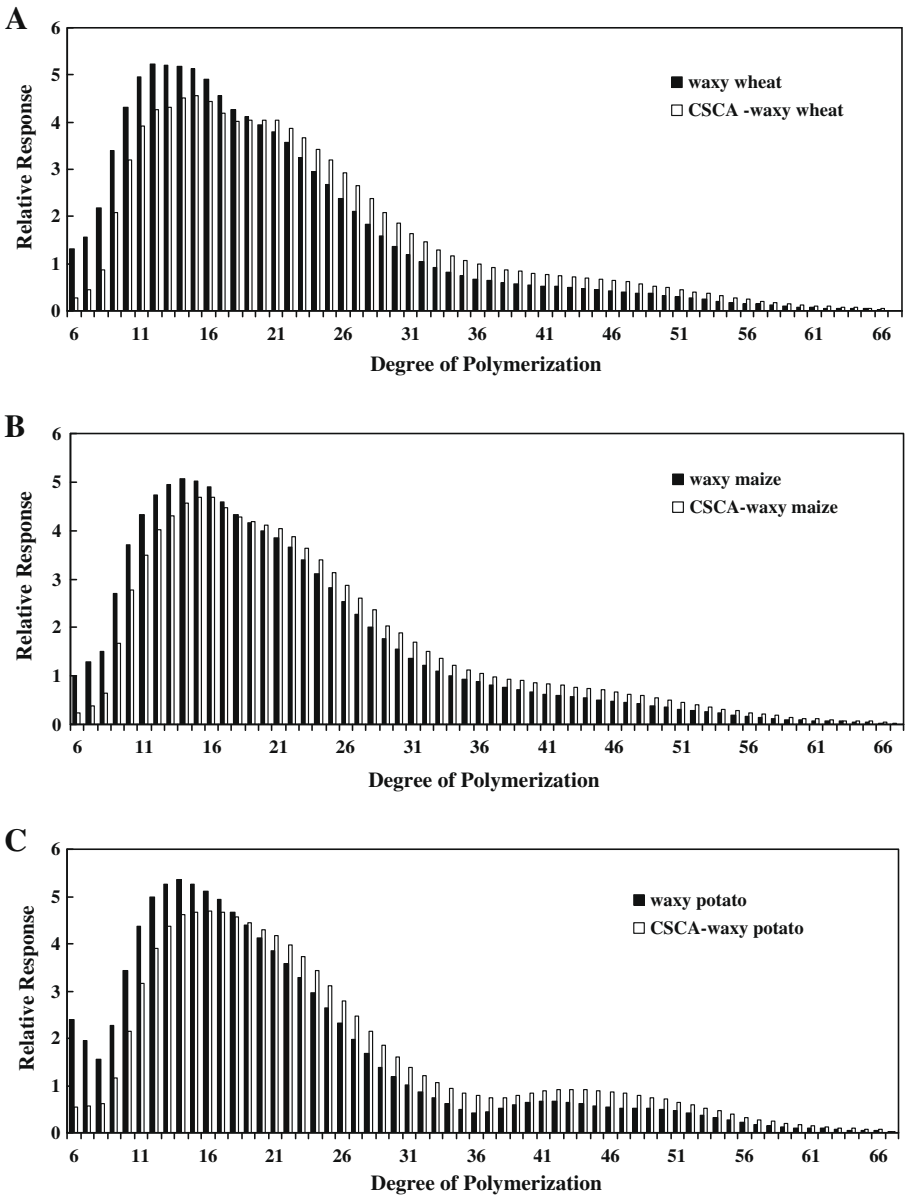

Fig. 2. Chain length distributions of (A) debranched waxy wheat starch and its CSCA product, (B) debranched waxy maize starch and its CSCA product, and (C) debranched waxy potato starch and its CSCA product.

Table 2
Yields and average chain lengths (CL) of debranched waxy wheat, waxy maize, waxy potato starches and their corresponding crystalline short-chain amylose (CSCA) products.[A,B]

Debranched starch samples	Average CL (whole starch)	Average CL (CSCA products)	Yield of CSCA (%)
Waxy wheat starch	21.8[b]	28.1[b]	58.7[b]
Waxy maize starch	24.1[b]	29.2[b]	60.7[b]
Waxy potato starch	32.1[a]	35.5[a]	72.6[a]

[A] Yield of CSCA (%) = $(W_{Precipitate}/W_{Starch}) \times 100$.
[B] Values with the same letter in the same column are not significantly different ($p < .05$).

After debranching and crystallization, a typical B-type structure was observed for all CSCA products. The results followed the general "rules" of short-chain amylose crystallization, namely, shorter CL, higher concentration, and higher temperature favor the formation of A-type crystallites, whereas the reverse conditions induce B-type crystallization (Buleon, Veronese, & Putaux, 2007; Gidley & Bulpin, 1987; Lebail, Bizot, & Buleon, 1993; Pfannemuller, 1987; Ring, Miles, Morris, Turner, & Colonna, 1987; Whittam, Noel, & Ring, 1990). In this study, a dilute starting concentration (5% solid) was used, which led to the formation of B-type crystalline structure. In another study, short-chain amylose from debranched waxy maize starch formed an A-type crystalline structure when debranched at 25% solids and crystallized at 50 °C (Liming Cai, personal communication). Continued research is being conducted to investigate the debranching and crystallization of these waxy starches at a high solid content and to manipulate temperature, CL, and starch solid content to produce highly pure A- and B-type crystallites and then determine their digestibility.

3.5. Thermal properties

Native waxy wheat, waxy maize, and waxy potato starches were characterized with a sharp endotherm peak at 67.8, 73.5,

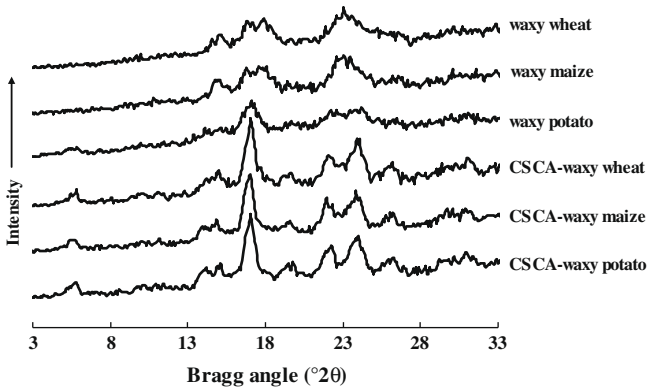

Fig. 3. X-ray diffraction patterns of waxy wheat, waxy maize, waxy potato starches and debranched crystalline short-chain amylose (CSCA) products.

and 70.4 °C and an enthalpy of 15.6, 18.9, and 18.3 J/g, respectively (Fig. 4 and Table 3). An endothermic peak that ranged from 80 to 140 °C was observed for all three CSCA products, revealing the formation of crystalline structure during the starch debranching. The large melting temperature range is due to the broad chain length distributions in the CSCA products. In a study by Moates, Noel, Parker, and Ring (1997), the dissolution temperature of short-chain amylose crystals increases from 57 to 119 °C with increasing CL from 12 to 55 residues. In this study, the average CL of the CSCA products from waxy wheat, waxy maize and waxy potato starches was 28.1, 29.2, and 35.5, respectively (Table 2). The CSCA from debranched waxy potato starch displayed a higher peak melting temperature (116.2 °C) than those from debranched waxy wheat (99.7 °C) and waxy maize (99.9 °C) starches (Table 3). The difference in melting properties was due to the longer chains generated from debranched waxy potato starch, which formed stronger double helices than the shorter chains in debranched waxy wheat and waxy maize starches.

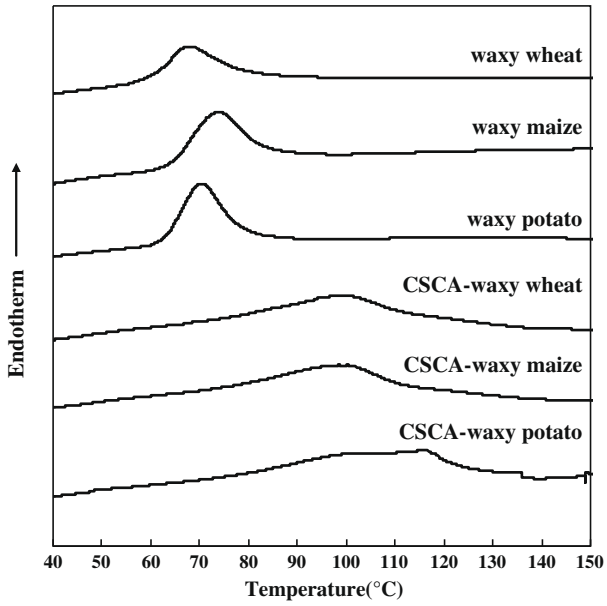

Fig. 4. Differential scanning calorimetry thermograms of waxy wheat, waxy maize, waxy potato starches and debranched crystalline short-chain amylose (CSCA) products.

3.6. In vitro digestion

The *in vitro* digestion profiles of waxy wheat, waxy maize, and waxy potato starches and their corresponding CSCA products are given in Table 4. Native waxy wheat starch and waxy maize starch had high RDS (33.1% and 29.0%, respectively) and low RS content (0.4% and 4.3%, respectively). In contrast, native waxy potato starch contained very low RDS (1.2%) and high RS content (88.5%). It is well known that native normal potato starch and high-amylose maize starches, both having B-type X-ray diffraction patterns, are more resistant to α-amylase digestion than those starches with an A-type pattern (Dreher, Dreher, & Berry, 1984; Evans & Thompson, 2004; Gallant, Bouchet, & Baldwin, 1997; Gerard, Colonna, & Buleon, 2001; McCleary & Monaghan, 2002; Oates, 1997). In this study, it was observed again that native waxy potato starch, which had a B-type X-ray diffraction pattern, was more resistant to enzyme digestion than cereal waxy starches with an A-type pattern. The underlying reasons of the above observations, however, are still not well understood. Gallant et al. (1997) noted that granules of normal potato starch and high-amylose maize starch appear to have a thick peripheral layer of large stacked "blocklets". The enzyme resistance of these B-type starches may be linked to their large blocklet size. The "blocklets" comprises of both crystalline and amorphous lamellae of amylopectin that are organized in larger, more or less spherical structures. However, as pointed out by Gallant et al. (1997), wrinkled pea starch has a smaller blocklet size than smooth pea, but it is more resistant to enzyme digestion (Gallant, Bouchet, Buleon, & Perez, 1992), indicating that other factors besides blocklet size determine the resistance to α-amylase. Zhang, Ao, and Hamaker (2006) suggested that the pores and channels in

Table 4
Levels of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) in waxy wheat, waxy maize, waxy potato starches and debranched crystalline short-chain amylose (CSCA) products.[A,B]

Samples	Native starches			CSCA products		
	RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)
Waxy wheat starch	33.1[a]	66.5[a]	0.4[c]	18.6[a]	13.7[a]	67.7[b]
Waxy maize starch	29.0[a]	66.7[a]	4.3[b]	17.5[a]	14.4[a]	68.1[b]
Waxy potato starch	1.2[b]	10.3[b]	88.5[a]	13.5[b]	8.7[b]	77.8[a]

[A] RS% = 100% − RDS% − SDS%.
[B] Values with the same letter in the same column are not significantly different ($p < .05$).

Table 3
Thermal properties of waxy wheat, waxy maize, waxy potato starches and debranched crystalline short-chain amylose (CSCA) products as determined by differential scanning calorimetry.[A,B]

Samples	Native starches				CSCA products			
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
Waxy wheat	59.7[c]	67.8[c]	92.9[b]	15.6[b]	83.4[a]	99.7[b]	136.4[a]	18.3[b]
Waxy maize	64.3[a]	73.5[a]	94.6[a]	18.9[a]	76.7[b]	99.9[b]	135.9[a]	18.6[ab]
Waxy potato	62.5[b]	70.4[b]	89.1[c]	18.3[a]	80.8[ab]	116.2[a]	133.8[a]	19.2[a]

[A] The ratio of sample (dry basis) and water was 1:3.
[B] Values with the same letter in the same column are not significantly different ($p < .05$).

waxy and normal cereal starches are large enough for enzymes to enter into the starch granules and allow enzymes to digest starch granules in a side-by-side mechanism. B-type starches without pores are more resistant to enzyme digestion. In addition, waxy wheat and waxy maize starches had a high level of SDS content (Table 4). The supramolecular A-type crystalline structure, including both the crystalline lamellae and the amorphous lamellae, has been linked to the slowly digestion property of A-type cereal starches (Zhang et al., 2006).

Comparing the digestibility of the native starches and the CSCA products (Table 4) reveals interesting results. The CSCA products from debranched waxy wheat and waxy maize starches contained a much higher RS content (67.7% and 68.1%, respectively) than the native cereal starches, which were not resistant to enzyme digestion. In the case of waxy potato starch, both native waxy potato starch and its CSCA product had high RS content but they belong to different types of RS (Englyst et al., 1992) and have different thermal stability (Table 3 and Fig. 4). Native potato granular starch is classified as a type 2 RS whereas CSCA has no granular structure and is considered a type 3 (cooked and crystallized) RS product. The enzyme resistance was due to the granular structure in native waxy potato starch but largely attributed to the dense crystalline structure in the CSCA products. It is known that even though native normal potato starch has greater than 70% RS content, its RS content reduces to less than 1% after boiling (Evans & Thompson, 2004). Similarly results would be expected for waxy potato starch because its gelatinization temperature was below 90 °C and the granular structure would be lost during cooking. In contrast, the CSCA from waxy potato starch had a much higher peak melting temperature (116.2 °C), suggesting that the product would have better thermal stability and more potential applications because of its high melting temperature (Fig. 4 and Table 3).

Among the three CSCA products, the one from debranched waxy potato starch had the lowest RDS content and highest RS content (Table 4). Those results were consistent with the yield and chain length data (Table 2), indicating that double helices formed from longer chains were more resistant to enzyme digestion.

Lopez-Rubio, Flanagan, Shrestha, Gidley, and Gilbert (2008) reported that the average characteristic dimension of the RS crystals was about ≈5 nm, suggesting that enzyme resistant crystals are formed from chains with a maximum DP of ≈13 for double helices (2.2 helix turns) with potential amorphous fringed ends. In this study, the CL distribution of the CSCA was peaked at around DP 16 (Fig. 2). As a result, a highly dense crystalline structure was formed and became resistant to enzyme digestion.

4. Conclusions

CSCA prepared from debranched waxy wheat, waxy maize, and waxy potato starches were studied and compared for the first time in this study. Waxy potato starch is the preferred starch to make a product with high resistant starch content by debranching and crystallization. Debranched waxy potato starch had a higher average CL than debranched waxy wheat and waxy maize starches, which resulted in a higher yield of crystallized product with stronger crystalline structure, higher peak melting temperature, and higher RS content. These differences suggest that the double helices formed from the longer chains in waxy potato starch are stronger, more resistant to enzyme hydrolysis, and have better thermal stability.

Acknowledgments

We thank Kansas Agricultural Experiment Station for support and are grateful to Dr. Paul Seib for helpful discussions and reviewing the manuscript, Dr. Michel Ransom and Paul Hartley for their assistance with X-ray analysis and Dr. Sajid Alavi for the use of DSC instrument.

This is Contribution No. 10-027-J from Kansas Agricultural Experiment Station, Manhattan, Kansas.

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