Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Polymers 69 (2007) 398–405
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Kinetics of hydrolysis and changes in amylose content during preparation of microcrystalline starch from high-amylose maize starches

Wende Li [a,b], Harold Corke [b], Trust Beta [c,*]

[a] Cereal Chemistry & Quality Analysis Laboratory, College of Chemistry & Chemical Engineering, Shanxi University, Taiyuan City 030006, PR China
[b] Cereal Science Laboratory, Department of Botany, University of Hong Kong, Hong Kong
[c] Department of Food Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

Received 26 October 2006; received in revised form 21 December 2006; accepted 21 December 2006
Available online 12 January 2007

Abstract

Two commercial sources of high amylose (~65% amylose) maize starch, Hi-maize and Hylon VII, were acid-hydrolyzed to produce microcrystalline starch and hydrolyzed starch sugar solution. The yield of microcrystalline starch was lower than 50% of the original starch weight when hydrolysis was carried for up to 8 days. The kinetics of hydrolysis was divided into three stages. The slope of the linear graph ranged from 11.11 to 11.36 mg/(ml days), 4.24 to 4.55 mg/(ml days), and 2.15 to 3.96 mg/(ml days) in the first, second and third stage and corresponded to a rapid, slow and very slow hydrolysis rates, respectively. HPAEC-ED analyses of the hydrolyzed starch solutions revealed 14 and four major sugar and oligosaccharide components when the hydrolysis was carried for 1 and 8 days, respectively. There was a good linear relationship between glucose content and hydrolysis time ($R > 0.992$). Oligosaccharide components ($dp \geqslant 2$) attained highest levels and then decreased with further increase in hydrolysis time. For hydrolyzed Hi-maize starch solution, the highest levels were 35.9, 12.4, 8.3, 7.6, 2.8, 2.3, and 2.0 mg/ml for glucose (dp_1), maltose (dp_2), maltotriose (dp_3), maltotetraose (dp_4), maltopentaose (dp_5), maltohexaose (dp_6), and maltoheptaose (dp_7), respectively. Similarly, hydrolyzed Hylon VII starch solution contained the highest levels at 31.8, 12.7, 7.2, 5.6, 4.3, 2.7, and 2.2 mg/ml for glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose, respectively. The critical times (rapid to slow transition) for hydrolysis of Hylon VII and Hi-maize starches should be between 5 and 6 days under the present conditions for preparation of microcrystalline starch. The hydrolysis process also significantly increased amylose content of microcrystalline starch.

Keywords: Starch polymers; High amylose starch; Microcrystalline starch; Acid hydrolysis; Sugars; Oligosaccharides; Carbohydrate analysis

1. Introduction

Starch is a natural, renewable, biodegradable polysaccharide polymer. Its major molecular components are amylose with predominantly linear α-(1 → 4)-linked D-glucose units and branched amylopectin with α-(1 → 4)-linked D-glucose units and α-(1 → 6)-linked branch points (Banks & Greenwood, 1975). Starch granules generally contain three zones, amorphous (Jenkins & Donald, 1997), semicrystalline and crystalline zones (Gérard, Colonna, Buléon, & Planchot, 2002). The three distinct zones of wheat starch granule are highly crystalline regions formed from double-helical starch chains, solid-like regions formed from lipid inclusion complexes of starch, and completely amorphous regions associated with the branching regions of the amylopectin component of starch and possibly the lipid-free amylose (Morgan, Furneaux, & Larsen, 1995). Grain amaranth is a unique resource of natural microcrystalline starch because of its very small starch granules of only 1–3 μm in diameter (Myers & Fox, 1994). Other microcrystalline starches are generally obtained from starches

* Corresponding author. Tel.: +1 204 474 8214; fax: +1 204 474 7630.
E-mail address: trust_beta@umanitoba.ca (T. Beta).

0144-8617/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2006.12.022

through the action of acid or enzyme hydrolysis (Dufresne, Cavaillé, & Helbert, 1996; Gross & Haralampu, 1999). These products have special characteristics and important uses including their use as biodegradable particulate fillers (Dufresne & Cavaillé, 1998), and in food formulations (Gross & Haralampu, 1999; Whistler, 1996). In fact, microcrystalline starch obtained through acid hydrolysis is a special type of acid-modified starch. Extent of hydrolysis depends on starch consistency, acidity of the medium, hydrolysis temperature and duration of hydrolysis (BeMiller, 1965). Acid attacks both amylose and amylopectin but preferentially attacks the amorphous zones of the starch granule in the early stages of hydrolysis, and then attacks the more crystalline sections at slower rate (Maniňgat & Juliano, 1979; Biliaderis, Grant, & Vose, 1981; Wang & Wang, 2001). In comparison to original starch, properties of common acid-modified starch include less hot-paste viscosity, a smaller degree of granule swelling in hot water, and increased solubility in warm water below the gelatinization temperature due to the alteration by acid of the gelatinization and retrogradation behavior of starch (Thirathumthavorn & Charoenrein, 2005). Microcrystalline starch exhibits a greater extent of hydrolysis compared to common acid-modified starch. Extensive hydrolysis of waxy maize starch granules in H_2SO_4 medium leads to a low yield of starch nanocrystals, a type of aqueous suspension of microcrystalline starch (Angellier, Choisnard, Molina-Boisseau, Ozil, & Dufresne, 2004). During preparation of microcrystalline starch, the amorphous zones of the starch granules are preferentially hydrolyzed into the sugar solution. Therefore, the yield and characteristics of microcrystalline starch have a close relationship with raw starch granular structure and its degree of acid hydrolysis.

The main objectives of the present work are to: (1) investigate the composition of sugar and oligosaccharide components in hydrolyzed starch solutions, and (2) study the kinetics of hydrolysis and changes in amylose content during preparation of microcrystalline starch from high-amylose maize starch. By understanding the oligosaccharide components and amylose composition, various functional properties of hydrolyzed starch solutions and microcrystalline starch can be further developed.

2. Materials and methods

2.1. Materials

Hi-maize starch was a gift from Starch Australasia Limited (Lane Cove, Australia), and Hylon VII starch was a gift from National Starch & Chemical Company (Singapore). Glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, maltoheptaose, and maltooligosaccharide were purchased from Sigma Chemical Co. (St. Louis, MO) as standards. Sodium hydroxide was purchased from BDH Laboratory Supplies (Poole, England). Sodium acetate anhydrous was purchased from Fluka BioChemika (Fluka Chemie GmbH, Germany). Hydrochloric acid (32%) was purchased from Merck (Darmstadt, Germany). Amylose/amylopectin assay kit and total starch kit were purchased from Megazyme International Ltd. (Co. Wicklow, Ireland). All other chemicals were analytical grade. Water (18 MΩ cm) used in all eluents and sample preparations for HPAEC-ED analysis was obtained from NanoPure Ultrapure Water System (Barnstead Ultrafiltered Type I Water, Hong Kong).

2.2. Preparation of microcrystalline starch

Microcrystalline starch samples were prepared according to the procedure described by Dufresne et al. (1996) with some modifications. Briefly, a starch suspension (10% w/w solids in 2.2 M HCl solution) was prepared and held at 40 °C in an orbital incubator (SI 50, Stuart Scientific) whilst continuously being shaken at 150 rpm during hydrolysis for the various periods. The supernatant was removed by centrifugation. The acid resistant starch sample was neutralized with the NaOH solution of 1.0 N and washed using deionized water by repeated centrifugation until the washings reached a pH of 7. Samples were either dried at 38 °C in air-draft oven or freeze-dried. The options of oven-drying or freeze-drying procedure depends on the extent of hydrolysis of the starch granules, because microcrystalline starch products that have undergone extensive hydrolysis will become colloid when using ambient air-draft oven-dried. However, freeze-drying is a suitable method for preparing powdered products of microcrystalline starches following extensive hydrolysis. After drying the microcrystalline starch, samples were left uncovered at room temperature to reach air equilibrium moisture content.

2.3. Determination of amylose content

Amylose content was determined using a commercial amylose/amylopectin assay kit from Megazyme (Ireland) according to the procedure described by Gibson, Solah, and McCleary (1997) and Gérard, Barron, Colonna, and Planchot (2001). The principle of this method involves removal of lipids in starch by precipitating the DMSO starch solution in ethanol and recovering the precipitated starch. Amylopectin was precipitated using Concanavalin A solution and removed by centrifugation. Pure amylose and total starch solutions were enzymatically hydrolyzed to glucose. The glucose levels were determined using the glucose oxidase/peroxidase (GOD–POD) kit according to the procedure described by Planchot, Colonna, and Saulnier (1997). Amylose content was deduced by calculating the ratio of supernatant concentration to that of the initial solution.

2.4. Kinetics of starch hydrolysis and sugar solution component assay

Kinetics of the starch hydrolysis process was followed by monitoring changes in total carbohydrate content (TCC) in the supernatant solution (hydrolyzed starch solution) during the preparation of microcrystalline starch. Total soluble carbohydrate content was measured by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) against maltose standard. The main methods of determining sugar components in hydrolyzed starch solution include high-performance anion-exchange chromatography with electrochemical detector (HPAEC-ED) (Hanashiro, Abe, & Hizukuri, 1996) or with pulsed amperometric detector (HPAEC-PAD) (Wong & Jane, 1997). Sugar components were determined using the former against glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose standards, according to the procedure described by Hanashiro et al. (1996) and with some modification. Other oligosaccharide components with degree of polymerization (dp) >7 were also qualitatively analyzed according to the peak area. Aliquots (1 mL) of supernatant were withdrawn at different time intervals, and then centrifuged for 10 min at 10,000 rpm for TCC and HPAEC-ED assays. The changes in total carbohydrate and sugar composition were used to follow the process of hydrolysis during preparation of microcrystalline starch.

2.5. Analysis conditions of HPAEC-ED

Glucose and oligosaccharide components in hydrolyzed starch solutions were analyzed using an HPAEC (Dionex, Sunnyvale, CA, USA) with an ED40 Electrochemical Detector System. Standards and hydrolyzed starch solutions were filtered through a 0.22 μm filter. If the glucose concentration in hydrolyzed starch solution was outside the range of calibration, it was diluted. An aliquot (20 μL) was injected onto a CarboPac PA-100 anion-exchange column (250 × 4 mm) coupled to a CarboPac PA-100 guard column. The sodium acetate gradient system included two eluents: 150 mM NaOH (eluent A) and 150 mM NaOH containing 600mM NaOAc (eluent B). The flow rate was 1 mL/min. The elution gradient was (1) 0–5 min with 100% eluent A, (2) 5–6 min with a linear gradient from 0 to 6% eluent B, (3) 6–12 min with 6% eluent B, (4) 12–12.2 min with a linear gradient from 6 to 14% eluent B, (5) 12.2–19 min with 14% eluent B, (6) 19–19.2 min with a linear gradient from 14% to 20% eluent B, (7) 19.2–30 min with 20% eluent B, (8) 30–30.2 min with a linear gradient from 20% to 30% eluent B, (9) 30.2–40 min with 30% eluent B, (10) 40–40.2 min with a linear gradient from 30% to 100% eluent B, (11) 40.2–55 min with 100% eluent B, (12) 55–55.2 min with a linear gradient to 100 eluent A, and (13) 55.2–60 min with 100% eluent A.

2.6. Statistical analysis

Data were reported as means of triplicate measurements and subjected to analysis of variance. Means were separated using Fisher's protected least significant difference (LSD) test at $P = 0.05$. Quantitative results were generally expressed on a dry weight basis (dwb).

3. Results and discussion

3.1. Starch selection for preparation of microcrystalline starch

High amylose starch of B-type X-ray pattern was selected for preparation of microcrystalline starch since high amylose Hylon VII starch (which is B-type) has been shown to exhibit a low degree of hydrolysis under the same conditions of acid concentration, hydrolysis time and temperature (Jane, Wong, & McPherson, 1997). For example under the same condition of mild acid hydrolysis, 60% final hydrolysis extent of *Ae* maize mutant starch containing 63% amylose was obviously lower than 90% hydrolysis extent of normal maize starch with 24% amylose (Gérard et al., 2002). The amylose contents of Hylon VII and Hi-maize starches were 65.10% and 66.30% (Table 1), respectively much higher than ~24% for wheat or other normal-amylose cereal starches (Li, Lin, & Corke, 1997). B-type potato starch of about 24% amylose has also been used for preparation of microcrystalline starch (Dufresne et al., 1996; Gérard et al., 2001). Potato and Hylon VII starches have the same B-type X-ray pattern (Jane et al., 1997). While it is generally considered that high-amylose starches show low susceptibility to mild acid hydrolysis, others have reported that some of the low-amylose starches are less susceptible to acid while some of the high-amylose starches are highly susceptible to acid (Inouchi, Glover, & Fuwa, 1987; Gérard et al., 2002). Biliaderis et al. (1981) found no correlation between the amylose content and initial rate of hydrolysis. Other workers have indicated a good correlation between hydrolysis parameters and the

Table 1
Amylose content (%) of unhydrolyzed starch[a]

Hydrolysis time (days)	Hylon VII starch (Amylose %)	Hi-maize starch (Amylose %)	Dried method
0	65.10b	66.30b	NA
1	99.15a	99.60a	Oven-dried
5	99.26a	99.04a	Freeze-dried
8	99.39a	99.44a	Freeze-dried
LSD	1.43	1.26	

NA, not applicable (since these are original starch materials).
[a] LSD, least significant difference at $P = 0.05$ level of probability. Mean values for samples having similar letters in the same column are not significantly different.

content of B-type starch (Gérard et al., 2002). As B-type crystallites increase, starch susceptibility to acid is lower and the number of residual structures is greater; however, crystallinity level has no direct influence on the intrinsic acid-induced behavior of starch to hydrolysis (Gérard et al., 2002). Therefore, high-amylose starches of complete B-type pattern with a relatively low hydrolysis rate could be considered as suitable raw materials for production of microcrystalline starch with relatively high yield.

3.2. Amylose content of microcrystalline starch

Amylose content of unhydrolyzed starch at different hydrolysis times is given in Table 1. Amylose content of Hylon VII and Hi-maize starches increased from 65.10% to 99.15% and from 66.30% to 99.60%, respectively after one day of acid hydrolysis. There was no significant difference ($P = 0.05$) in amylose content after one, five and eight days of acid hydrolysis. The results indicated that amylopectin of high amylose starches mainly existed in amorphous zones of starch granule and had been gradually hydrolyzed into sugar solution in the first stage of acid hydrolysis. Effect of hydrolysis of different acids on starch amylose was also reported, with native maize starch amylose content 24% increasing to 55%, 45%, and 29% after HCl, HNO_3, and H_2SO_4 hydrolysis, respectively and decreasing to 22% after H_3PO_4 hydrolysis (Singh & Ali, 2000). In this study, we did not investigate the effect of oven drying versus freeze-drying on amylose content of microcrystalline starch.

3.3. Yield of microcrystalline starch

The yield of unhydrolyzed starch at different hydrolysis times is given in Table 2. Hylon VII and Hi-maize starches gave yields lower than 50% of their original starch weight when hydrolysis was carried out for up to 8 days. Hi-maize starch hydrolyzed for 10 days gave a yield of only 36.3%. There were significant differences ($P = 0.05$) in yield at different durations of acid hydrolysis for Hylon VII and Hi-maize starches. The yield of nanocrystals from waxy maize starch was only 15 wt% after 5 days of H_2SO_4 hydrolysis, and an even lower yield of 0.5 wt% was obtained after 40 days of HCl treatment (Angellier et al., 2004), indicating extensive acid hydrolysis. Acid hydrolysis resulted in solubilization of some starch polymers during preparation of microcrystalline starch. Therefore, further analyses were conducted to determine the composition of the sugar and oligosaccharide components in hydrolyzed starch solutions.

Table 2
Yield (%) of unhydrolyzed starch[a]

Hydrolysis time (days)	Hylon VII starch (Yield %)	Hi-maize starch (Yield %)	Dried method
1	77.9a	74.7a	Oven-dried
5	55.3b	52.9b	Freeze-dried
8	47.2c	46.6c	Freeze-dried
10	NA	36.3d	Freeze-dried
LSD	7.19	5.73	

NA, not available.
[a] LSD, least significant difference at $P = 0.05$ level of probability. Mean values for samples having similar letters in the same column are not significantly different.

3.4. Kinetics of hydrolysis

The kinetics of hydrolysis for Hi-maize and Hylon VII starches are shown in Fig. 1a and b, respectively. Since Hylon VII starch is of B-type pattern and has high amylose content of 65.10%, its amorphous zones should be fewer and its hydrolysis rates under the same conditions slower than those of normal-amylose starches. Among waxy maize, normal maize, potato, and Hylon VII starches,

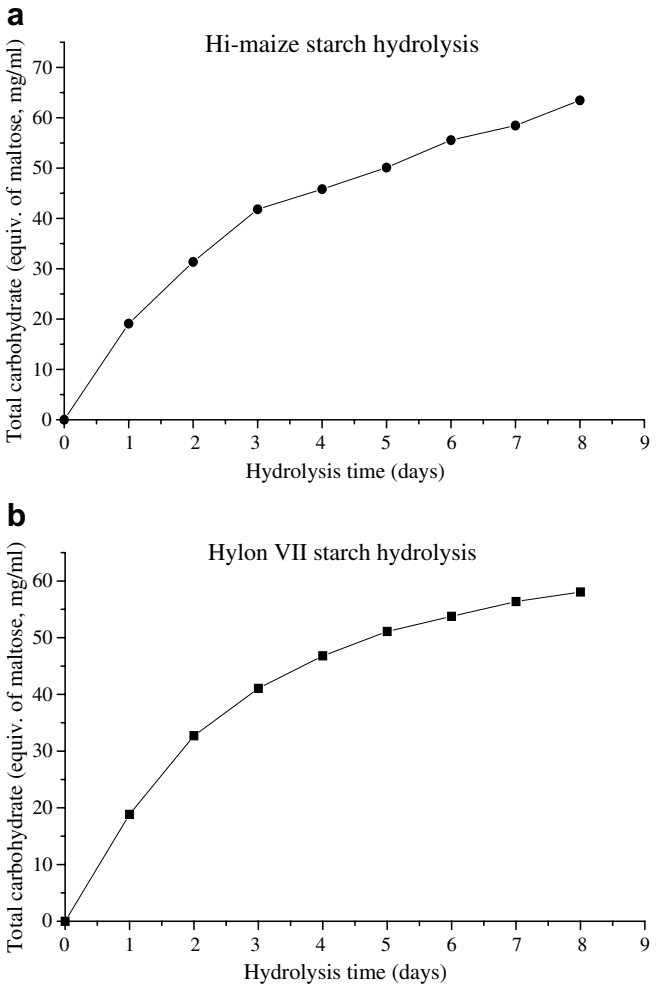

Fig. 1. Kinetics of hydrolysis of Hi-maize and Hylon VII starches (equiv. = equivalent).

Table 3
Linear correlation coefficient (R) and slope of graph for the different hydrolysis stages[a]

Hydrolysis stage	Hylon VII starch		Hi-maize starch	
	R	Slope (mg/(ml days))	R	Slope (mg/(ml days))
First stage	0.990	11.11	0.999	11.36
Second stage	0.987	4.24	0.997	4.55
Third stage	0.992	2.15	0.989	3.96

[a] Values are calculated from the plots of maltose concentration versus hydrolysis time. For the first stage, hydrolysis time = 1–3 days; second stage, hydrolysis time = 3–6 days; 3–6 day; and third stage, hydrolysis time = 6–8 days.

waxy maize starch had the greatest hydrolysis rate and Hylon VII starch had the least (Jane et al., 1997). Increasing the hydrolysis temperature obviously shortens the time needed for the same degree of starch hydrolysis. For microcrystalline starch made from potato starch of full B-type, Dufresne and Cavaillé (1998) reported that a 5% starch suspension hydrolyzed at 30 °C for 15 days had most of the amorphous zones removed without damaging the crystalline zones and deduced that 15 days was the critical time (rapid hydrolysis to slow hydrolysis transition) for potato microcrystalline starch. In order to shorten the critical times of Hylon VII and Hi-maize starches, a hydrolysis temperature of 40 °C was selected.

The hydrolysis process for Hylon VII and Hi-maize starches was divided into three stages in order to determine their critical times (Table 3). The linear correlation coefficient (R) between maltose concentration and hydrolysis time ranged from 0.987 to 0.999 for the three stages. The slopes of the graph ranged from 2.15 to 11.36 mg/(ml days) for the three stages, with high values corresponding to rapid rates of hydrolysis.

The first, second, and third stages of hydrolysis were carried out from 1 to 3 days, 3 to 6 days, and 6 to 8 days, respectively. The slopes of the graph were between 11.11 and 11.36 mg/(ml days), 4.24 and 4.55 mg/(ml days), and 2.15 and 3.96 mg/(ml days), corresponding to rapid, slow and very slow rates of hydrolysis in the first, second, and third stages, respectively. For hydrolysis times less than 15 days, the rate of hydrolysis for potato starch was rapid and corresponded to the hydrolysis of amorphous starch domains (Dufresne & Cavaillé, 1998). For hydrolysis times more than 15 days, the rate of hydrolysis for potato starch was slow and corresponded to the hydrolysis of crystalline starch domains (Dufresne & Cavaillé, 1998). We surmised that hydrolysis of mainly amorphous, semi-crystalline, and crystalline layers within the starch granule occurred in the first, second, and third stage, respectively.

According to these hydrolysis parameters, the critical time for Hylon VII and Hi-maize starches was between 5 and 6 days under the present conditions. Their critical times were much lower than the 15 days reported for potato microcrystalline starch.

3.5. Components of starch hydrolysis solution

In order to determine glucose and oligosaccharide components of hydrolyzed starch solutions produced during preparation of microcrystalline starch, seven standards ranging from dp1 to dp7 were used. The chromatogram of dp_1 glucose (A), dp_2 maltose (B), dp_3 maltotriose (C), dp_4 maltotetraose (D), dp_5 maltopentaose (E), dp_6 maltohexaose (F), dp_7 maltoheptaose (G) is shown in Fig. 2. Glucose had the shortest retention time (about 2.33 min) while maltoheptaose had the longest (about 24.75 min). Correlation coefficients (R) of the linear regression equations of the seven standards ranged from 0.991 to 0.999, a high value of R indicating a good linear relationship (mg/ml vs peak area) for the calibration curve of each standard. Accordingly the content of individual sugar and maltooligosaccharide could be determined in supernatants during preparation of microcrystalline starch using the standard curve generated.

Hydrolysis of Hi-maize and Hylon VII starches for 1, 4, and 8 days resulted in similar sugar components in their solutions for the same period. Chromatograms of hydrolyzed Hylon VII starch solutions at various hydrolysis periods are used for illustration in Fig. 3a, b, and c. Compared to the maltooligosaccharide standard (Fig. 4), better separation was observed in hydrolyzed starch solutions. Hydrolyzing Hi-maize and Hylon VII starches for 1 day resulted in more than 14 types of sugar and maltooligosaccharide components (Fig. 3a). Hydrolysis for 4 days however, gave seven components ranging from dp_1 to dp_7 (Fig. 3b). Only four sugar components of dp_1 to dp_4 were recorded following 8 days of hydrolysis (Fig. 3c). Chromatograms of hydrolyzed Hi-maize and Hylon VII starch solutions clearly indicated that high dp oligosaccharides were hydrolyzed to become low dp components with further increase in hydrolysis time.

Oligosaccharide components of hydrolyzed starch solution were comparable to that of maltooligosaccharide stan-

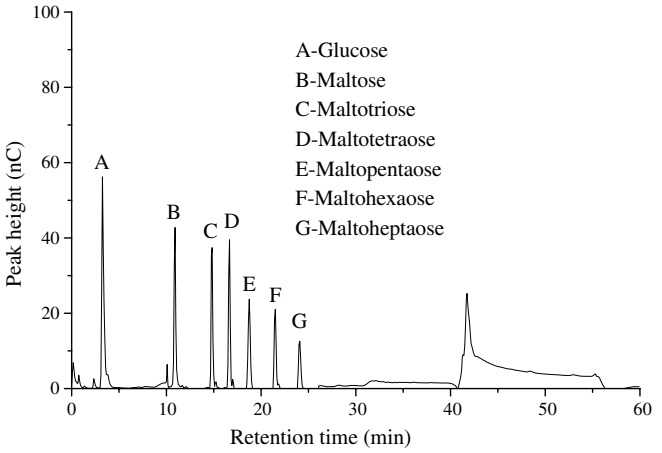

Fig. 2. Chromatogram of glucose, maltose, and maltooligosaccharide standards.

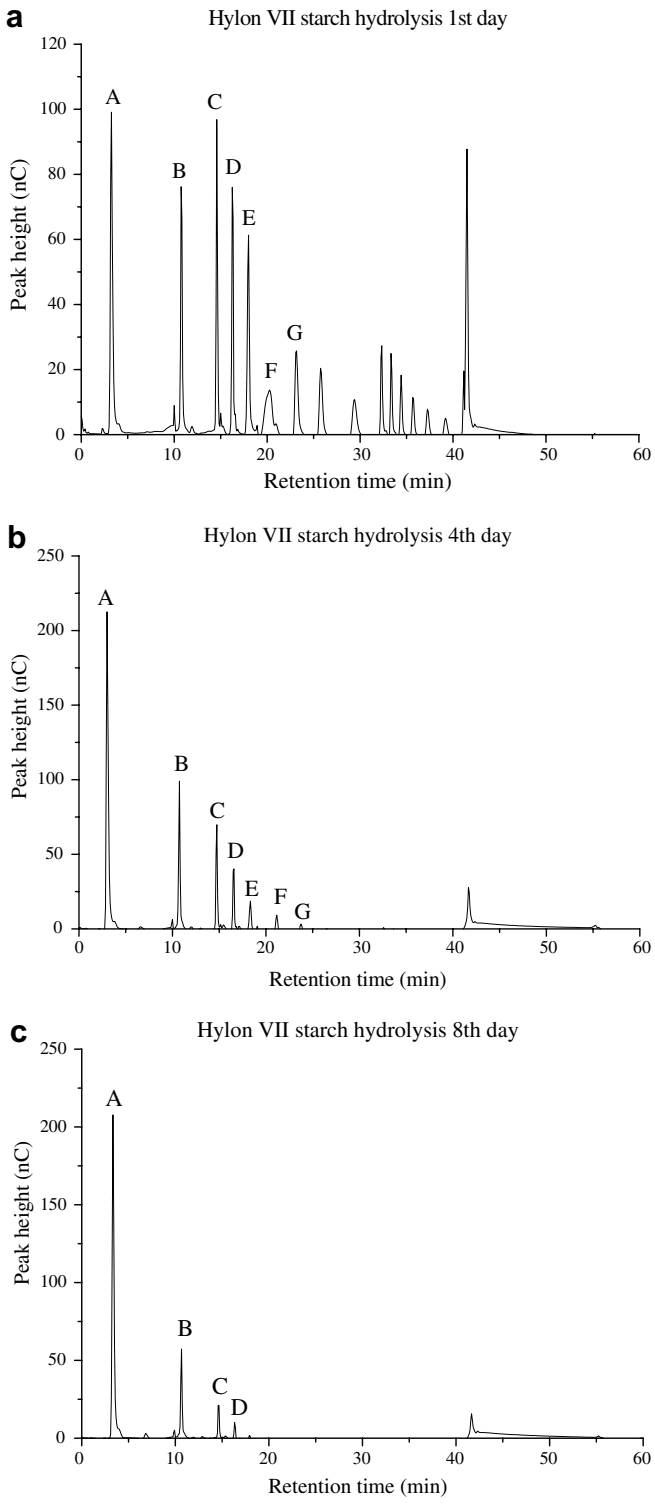

Fig. 3. Sugar and maltooligosaccharide composition in solutions of Hylon VII starch after hydrolysis for various periods (A, glucose; B, maltose; C, maltotriose; D, maltotetraose; E, maltopentaose; F, maltohexaose; G, maltoheptaose).

dard (Fig. 4). The known components in maltooligosaccharide standard were dp_4 maltotetraose (0.9 mg/ml), dp_5maltopentaose (0.9 mg/ml), dp_6 maltohexaose (2.2 mg/ml) and dp_7 maltoheptaose (2.9 mg/ml). Unknown oligo-

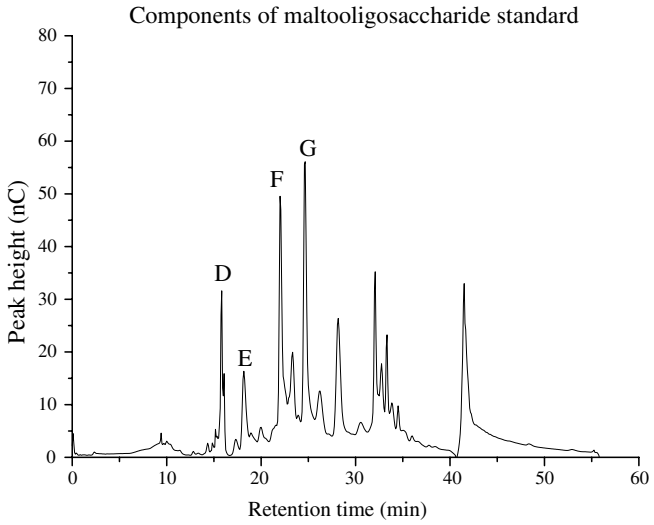

Fig. 4. Chromatogram of maltooligosaccharide standard (D, maltotetraose; E, maltopentaose; F, maltohexaose; G, maltoheptaose).

saccharide components of dp greater than 7 were also present in the maltooligosaccharide standard. Unknown components accounted for 28.76% of the total peak area of the maltooligosaccharide standard chromatogram. Differences in chromatograms were also found between hydrolyzed starch solution and maltooligosaccharide standard.

Changes in concentration of sugar and maltooligosaccharide components of dp_1 to dp_7 that occurred at a hydrolysis period of 8 days are shown in Fig. 5a for hydrolyzed Hylon VII starch solution. At any hydrolysis period for Himaize and Hylon VII starches, glucose content steadily increased with hydrolysis time. For maltose and other oligosaccharide components higher than dp_2, there was a peak in concentration during the hydrolysis process. Once the highest level was reached, there was a decrease in concentration with further increase in hydrolysis time.

For hydrolyzed Hi-maize starch solution, glucose content continuously increased and reached 35.9 mg/ml on the 10th day of hydrolysis. The concentration of maltose peaked at 12.4 mg/ml on the 8th day of hydrolysis, maltotriose and maltotetraose at 8.3 and 7.6 mg/ml on the 5th day of hydrolysis, respectively maltopentaose and maltohexaose at 2.8 and 2.3 mg/ml on the 3rd day of hydrolysis, respectively and maltoheptaose at 2.0 mg/ml on the 2nd day of hydrolysis. There was significant variation in the rate of hydrolysis for maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose after reaching their highest concentrations. The trend was that oligosaccharides of high dp rapidly decreased in concentration compared to those with low dp. Maltose concentration, for example, decreased very slowly from 12.4 mg/ml on the 8th day hydrolysis to 11.3 mg/ml on the 10th day hydrolysis. There was very little to nil left for maltopentaose after the 9th day of hydrolysis and for maltohexaose and maltoheptaose after the 6th day of hydrolysis.

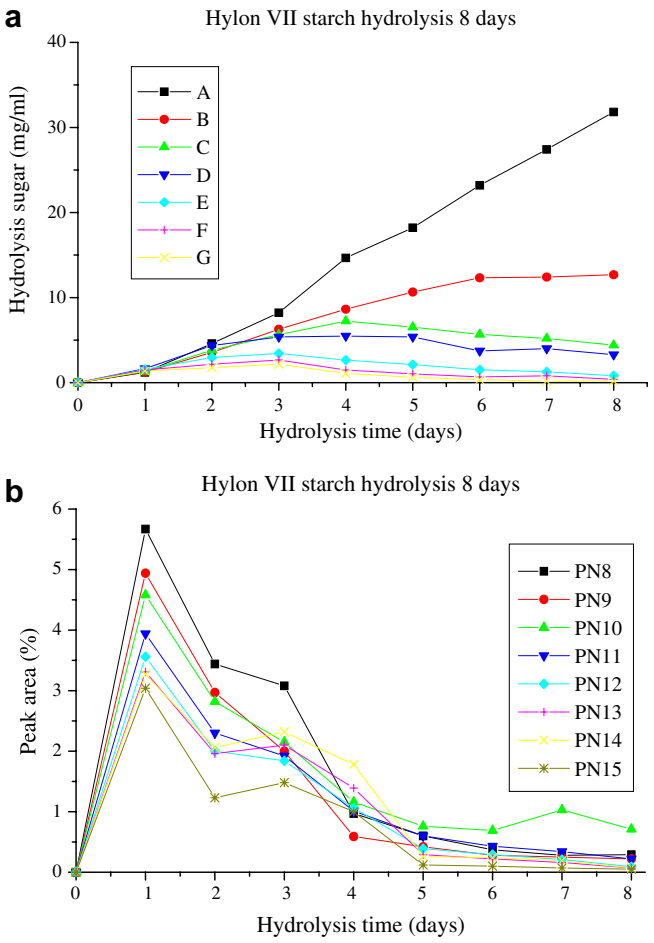

Fig. 5. Changes in concentration of sugar and maltooligosaccharide components (A, glucose; B, maltose; C, maltotriose; D, maltotetraose; E, maltopentaose; F, maltohexaose; G, maltoheptaose; and PN8 to PN15, maltooligosaccharides of dp greater than 7) in solutions of Hylon VII starch hydrolyzed over a period of 8 days.

The hydrolysis process for Hylon VII starch was very similar to that of Hi-maize starch. Glucose content reached 31.8 mg/ml after hydrolysis for 8 days. Maltose levels reached 12.3 mg/ml on the 6th day and then very slowly increased to 12.7 mg/ml on the 8th day of hydrolysis. The highest contents of maltotriose and maltotetraose were at 7.2 and 5.6 mg/ml, respectively on the 4th day of hydrolysis. Maltopentaose, maltohexaose, and maltoheptaose concentrations were highest at 4.3, 2.7, and 2.2 mg/ml, respectively on the 3rd day of hydrolysis. Maltohexaose and maltoheptaose were reduced to negligible levels after the 6th day of hydrolysis.

Due to difficulties in acquiring oligosaccharide standards of dp higher than 7, the hydrolysis trends of these unknown components in hydrolyzed starch solutions was explained in terms of their retention times and peak areas at different hydrolysis periods. Components with dp greater than 7 were assigned peak numbers (PN) (Fig. 5b). About 15 types of oligosaccharide components were recorded in hydrolyzed solutions of Hi-maize and Hylon VII starches. A higher peak number with longer retention time denoted a higher dp component with longer chain length. A high peak area corresponded to a high concentration of the oligosaccharide component in hydrolyzed starch solutions. While peak area of glucose steadily increased with hydrolysis time, the peak areas of the other 14 components attained their highest values and then decreased with further increase in hydrolysis time. Oligosaccharide components ranging from PN8 to PN15 all attained their highest peak area on the 1st day of hydrolysis (Fig. 5b). After the 1st day of hydrolysis, the peak area rapidly decreased with further increase in hydrolysis time.

Since the concentration (mg/ml) of glucose steadily increased during hydrolysis, linear regression analysis was conducted. A high correlation coefficient (0.992) was found between glucose content and hydrolysis time. We recommend that for control of the degree of hydrolysis and concentration of oligosaccharide components in hydrolyzed starch solutions during preparation of microcrystalline starch, the hydrolysis curve of glucose content versus hydrolysis time be used to monitor the process.

4. Conclusions

A significant amount of starch was hydrolyzed to yield a solution containing different sugar and oligosaccharide components during preparation of microcrystalline starch. Hydrolyzing starch for 8 days resulted in a yield of less than 50% microcrystalline starch. Hylon VII and Hi-maize starches were hydrolyzed in three stages, the first, second and third stages involving rapid hydrolysis of amorphous layers, slow hydrolysis of semi-crystalline layers and very slow hydrolysis of part of the crystalline layers, respectively. Amylose content of microcrystalline starch significantly increased in comparison to the levels found in the original high amylose starches. Major sugar components in hydrolyzed starch solutions differed significantly and their concentration increased with hydrolysis time. While over 14 types of sugar and oligosaccharide components were present after hydrolyzing starch for 1 day, only four components remained after 8 days of hydrolysis. Glucose content gave an excellent linear relationship with hydrolysis time. Maltooligosaccharide components increased to a peak and then decreased with further increase in hydrolysis time. High dp components were further hydrolyzed to low dp oligosaccharides. A thorough understanding of the nature of sugar components and changes in amylose content during preparation of microcrystalline starch is important for both process control and utilization of hydrolyzed starch solutions and microcrystalline starch.

Acknowledgments

We thank Dr. L. Ramsden and Miss Jessica Leung for their assistance in establishing the HPAEC-ED method. We thank the Shanxi Scholarship Council on Research Grants, the Shanxi Natural Science Fund, and Shanxi Science & Technology Office on Research Grants for their financial assistance.

References

Angellier, H., Choisnard, L., Molina-Boisseau, S., Ozil, P., & Dufresne, A. (2004). Optimization of the preparation of aqueous suspensions of waxy maize starch nanocrystals using a response surface methodology. *Biomacromolecules, 5*, 1545–1551.

Banks, W., & Greenwood, C. T. (1975). Fractionation of the starch granule, and the fine structures of its components. In W. Banks & C. T. Greenwood (Eds.), *Starch and its components* (pp. 5–66). Edinburgh: Edinburgh University Press.

BeMiller, J. N. (1965). Acid hydrolysis and other lytic reactions of starch. In R. L. Whistler, E. F. Paschall, J. N. BeMiller, & H. J. Roberts (Eds.). *Starch: chemistry and technology* (Vol. 1, pp. 495–520). New York: Academic Press.

Biliaderis, C. G., Grant, D. R., & Vose, J. R. (1981). Structural characterization of legume starches. II. Studies on acid treated starches. *Cereal Chemistry, 58*, 502–507.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substance. *Analytical Chemistry, 28*, 350–356.

Dufresne, A., & Cavaillé, J. Y. (1998). Clustering and percolation effects in microcrystalline starch-reinforced thermoplastic. *Journal of Polymer Science: Part B: Polymer Physics, 36*, 2211–2224.

Dufresne, A., Cavaillé, J. Y., & Helbert, W. (1996). New nanocomposite materials: microcrystalline starch reinforced thermoplastic. *Macromolecules, 29*, 7624–7626.

Gérard, C., Barron, C., Colonna, P., & Planchot, V. (2001). Amylose determination in genetically modified starches. *Carbohydrate Polymers, 44*, 19–27.

Gérard, C., Colonna, P., Buléon, A., & Planchot, V. (2002). Order in maize mutant starches revealed by mild acid hydrolysis. *Carbohydrate Polymers, 48*, 131–141.

Gibson, T. S., Solah, V., & McCleary, B. V. (1997). A procedure to measure amylose in cereal starches and flours with concanavalin A. *Journal of Cereal Science, 25*, 111–119.

Gross, A., & Haralampu, S.G. (1999). Microcrystalline starch-based product and use in foods. US Patent, 5962047.

Hanashiro, I., Abe, J. I., & 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.

Inouchi, N., Glover, D. V., & Fuwa, H. (1987). Properties of residual maize starches following acid hydrolysis. *Starch/Stärke, 39*, 284–288.

Jane, J. L., 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.

Jenkins, P. J., & Donald, A. M. (1997). The effect of acid hydrolysis on native starch granule structure. *Starch/Stärke, 49*, 262–267.

Li, W., Lin, R., & Corke, H. (1997). Physicochemical properties of common and tartary buckwheat starch. *Cereal Chemistry, 74*, 79–82.

Maniňgat, C. C., & Juliano, B. O. (1979). Properties of lintnerized starch granules from rice differing in amylose content and gelatinization temperature. *Starch/Stärke, 31*, 5–10.

Morgan, K. R., Furneaux, R. H., & Larsen, N. G. (1995). Solid-state NMR studies on the structure of starch granules. *Carbohydrate Research, 276*, 387–399.

Myers, D. J., & Fox, S. R. (1994). Alkali wet-milling characteristics of pearled and unpearled amaranth seed. *Cereal Chemistry, 71*, 96–99.

Planchot, V., Colonna, P., & Saulnier, L. (1997). Dosage des glucides et des amylases. In B. Godon & W. Loisel (Eds.), *Guide pratique d'analyses dans les industries des cereals* (pp. 346–398). Paris: Tec & Docs.

Singh, V., & Ali, S. Z. (2000). Acid degradation of starch. The effect of acid and starch type. *Carbohydrate Polymers, 41*, 191–195.

Thirathumthavorn, D., & Charoenrein, S. (2005). Thermal and pasting properties of acid-treated rice starches. *Starch/Stärke, 57*, 217–222.

Wang, L., & Wang, Y. J. (2001). Structures and physicochemical properties of acid-thinned corn, potato and rice starches. *Starch/Stärke, 53*, 570–576.

Whistler, R. L. (1996). Subgranular crystalline starch as fat substitute. US Patent, 5580390.

Wong, K. S., & Jane, J. (1997). Quantitative analysis of debranched amylopectin by HPAEC-PAD with a postcolumn enzyme reactor. *Journal of Liquid Chromatography & Related Technologies, 20*, 297–310.