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Characterization of internal structure of maize starch without amylose and amylopectin separation

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ABSTRACT

Normal maize starch was used to characterize the internal structure of starch without separating amylose and amylopectin, and the result was compared with amylose-free waxy maize starch. The clusters in the whole starch were produced by partial hydrolysis using α-amylase of *Bacillus amyloliquefaciens*, and were subsequently treated with β-amylase to remove the linear amylose and to produce β-limit dextrins of clusters from amylopectin. The clusters were further hydrolyzed extensively with α-amylase to produce building blocks. The compositions of clusters in the form of β-limit dextrins and their building blocks were analyzed by gel-permeation chromatography and high-performance anion-exchange chromatography. The results showed that the structures of clusters and building blocks from whole starch of normal and waxy starches were similar. By number, each cluster contained 9–10 chains and 5–6 building blocks. The inter-block chain length in the clusters of whole starch was around six glucosyl residues. This study explored an alternate procedure to characterize the composition of branches in whole starch without separating amylose and amylopectin components.

1. Introduction

Starch as biopolymers of glucose may be regarded as a mixture and spectrum of structures with various molecular sizes (elongated by α-(1→4) bonds), degree of branching (by α-(1→6) bonds), average chain-lengths and number of chains (Matheson, 1990). Separation of starch components has been attempted by ultracentrifugation, gel-permeation chromatography, selective aqueous leaching, retrogradation, preferential precipitation with iodine or alcohol, and complexing with concanavalin A (Matheson, 1990). Among these methods, butanol precipitation of starch components (Schoch, 1942) is still most commonly employed. Butanol-precipitated fraction (amylose) contains some branches, which, however, are few by number compared with the non-precipitated fraction (amylopectin) (Takeda, Hizukuri, Takeda, & Suzuki, 1987; Takeda, Shitaozono, & Hizukuri, 1990). Depending on the separation methods and varied conditions of the same method, the amounts and branching density of branched amylose in the amylose fraction varied (Matheson & Welsh, 1988; Matheson, 1990; Vilaplana, Hasjim, & Gilbert, 2012). A method for separation of essentially linear amylose from slightly branched amylose remains to be developed (Buléon, Colonna, Planchot, & Ball, 1998), and the nature of the branched amylose in relation to specific separation methods used is still to be explored. Calculation based on previous studies (Takeda et al., 1987; Takeda, Shitaozono, & Hizukuri, 1988) showed that the branches from amylose fractions (isolated from butanol precipitation based method) may contribute to around 1–2% of the total branches in the whole starch. Overall, the methods used so far suggest that the amount of branched amylose fractions in normal starches is small compared to amylopectin fractions.

In many scenarios in research, starch from biological organisms like leaves or algae (Ball, Colleoni, Cenci, Raj, & Tirtiaux, 2011; Smith, 2012) or from breeding lines are only available in very small quantities (in milligrams) for structural characterization. Traditional ways of separating amylose and amylopectin, such as with butanol precipitation, is time and labour intensive, and usually requires relatively large amounts of sample (in grams) to get enough fractions to finally analyze structures at the building block levels. This limits the possibilities to explore structural features of interesting mutants or other research questions related to starch synthesis. A method of using whole starch to obtain a full structural analysis on starch branching patterns remains to be developed. Furthermore, in the broader picture, understanding structural features of whole starch might provide an alternative viewpoint of structure–synthesis and structure–function relationships.

α-Amylase of *Bacillus amyloliquefaciens* (Priest, Goodfellow, Shute, & Berkeley, 1987) has been a powerful tool to characterize the cluster and building block structure of amylopectin (Bertoft, 1990, 2007a, 2007b). α-Amylase of *B. amyloliquefaciens* has a higher propensity to act as an endo-acting enzyme compared to other

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α-amylases (Goesaert, Bijttebier, & Delcour, 2010). Thus it can be used to efficiently release clusters from starch with limited hydrolysis (Bertoft, 1990). The released clusters in the form of φ,β-limit dextrins (φ,β-LDs) or β-LDs can be structurally elucidated by diverse chromatographic techniques. The average size of the clusters from starches from diverse plant origins reported hitherto ranges from DP 50 to 90 and the number of chains per cluster ranges from 8 to 16 (Bertoft, Koch, & Åman, 2012a). When the clusters are further subjected to more extensive hydrolysis by α-amylase, building blocks with smaller size and tighter branching patterns than clusters are obtained (Bertoft, Koch, & Åman, 2012b). These building blocks are practically α-LDs. The internal chain length of the building blocks is between DP 1 and 3. The most common building blocks are of the smallest size (DP 5–9) and contain 2 chains per block.

Internal structure of starch molecules (mostly amylopectin) has been shown to be related to the biosynthesis of starch molecules, organization and packing of the granules, and eventually the functional properties for applications (Buléon et al., 1998; O'Sullivan, & Pérez, 1999; Vamadevan, Bertoft, & Seetharaman, 2013; Zhu, Corke, & Bertoft, 2011). Thus, the structure and organization of clusters and building blocks, which are the structural elements of the internal parts of the biomacromolecules, is likely one of the most informative aspects of starch structure.

In this study, whole normal maize starch from the endosperm was used for characterization of the structure of clusters and building blocks without separating amylose from amylopectin, and the result was compared with waxy maize starch (containing no amylose). The result reported here is a first step towards the development of small-scaled analytical methods for starch internal structural characterization that can be used in studies related to starches from diverse tissues, such as leaves and pericarp, when the samples are available in limited amounts.

2. Materials and methods

2.1. Starches and enzymes

Normal maize starch (Melojel) was obtained from National Starch and Chemical Company (Bridgewater, NJ, USA). The amylose content was 22.8% determined after debranching and gel-permeation chromatography (Sargeant, 1982). Waxy maize starch was from AVEBE, Belgium.

α-Amylase of *B. amyloliquefaciens* (EC 3.2.1.1), β-amylase of barley (EC 3.2.1.2, specific activity 705 U/mg), isoamylase of *Pseudomonas amyloderamosa* (EC 3.2.1.68, specific activity 210 U/mg), and pullulanase of *Klebsiella pneumoniae* (EC 3.2.1.41, specific activity 699 U/mg) were from Megazyme (Wicklow, Ireland). The activity of the α-amylase (413 U/mL at pH 6.5, 25 °C) was determined based on a previous description (Bertoft, Manelius, & Qin, 1993).

2.2. Time course analysis of α-amylolysis for cluster production from whole starch

Whole starch (20 mg) was dissolved in 400 μL of 90% dimethyl sulfoxide (DMSO) by heating in boiling water bath and then constant stirring for two days at room temperature (~21 °C). Hot double-distilled water (1.4 mL) was then added to the sample. After cooling to 25 °C, 200 μL of α-amylase (0.9 U/mL) in NaOAc buffer (0.01 M, pH 6.5) was added to start the reaction in a water bath with magnetic stirring. The concentrations for substrate and α-amylase in the reaction system were 10 mg/mL and 0.09 U/mL, respectively. Samples (200 μL) were taken from 10 min up to 150 min. If not analyzed immediately, 4 μL of NaOH solution (5.0 M) was added and mixed to destroy the α-amylase, and then stored at −18 °C. If analyzed immediately, the sample was diluted in 200 μL water and 40 μL of NaOH solution (5.0 M), and analyzed by GPC on Sepharose CL 6B as described below.

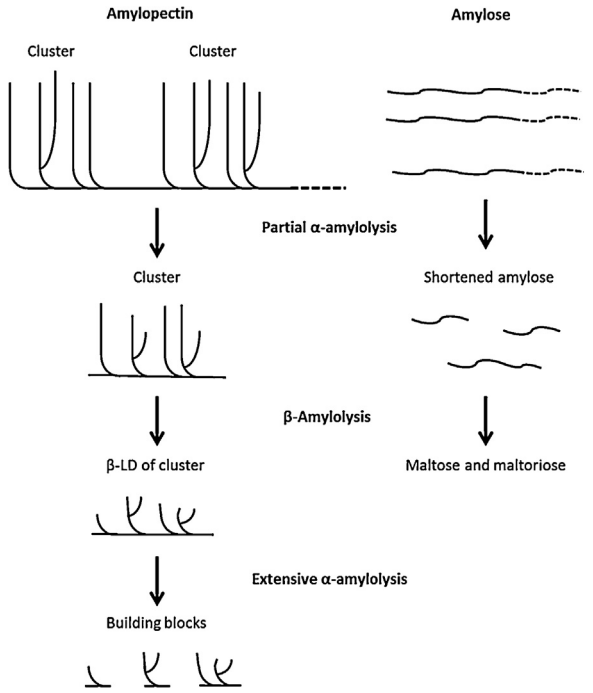

Fig. 1. Fate of amylose and amylopectin in the course of differential hydrolysis by α-amylase of *B. amyloliquefaciens* for cluster and building block production.

2.3. Production of clusters

Whole starch (80 mg) was dissolved in 1.6 mL of 90% dimethyl sulfoxide (DMSO) by gentle heating and then constant stirring for two days at room temperature. Hot double-distilled water (5.6 mL) was added and, after cooling to 25 °C, 800 μL of α-amylase (0.9 U/mL) in NaOAc buffer (0.01 M, pH 6.5) was added to start the reaction in a water bath (25 °C) with magnetic stirring. The reaction was continued for 100 min before being stopped by adding 160 μL NaOH (5.0 M) and standing at room temperature for 1 h to destroy the α-amylase. Pure methanol (40 mL) was added to precipitate the clusters in the form of α-dextrins. The sample was left at 4 °C for 3 h before the precipitate was recovered by centrifugation (4000 × *g*, 20 min) at room temperature. The precipitate was washed twice with pure methanol (10 mL) and let stand in fume hood overnight to remove the methanol through evaporation. The principle of the methods and fate of starch components are illustrated in Fig. 1. Amylose component is illustrated as linear since the number of branches in amylose fractions is rather limited compared with that of amylopectin as discussed in Section 1.

The α-LDs of clusters were re-dissolved in hot water and the carbohydrate content was adjusted to 10 mg/mL. NaOAc buffer (0.33 volumes, 0.01 M, pH 6.0) and β-amylase (3 μL) was added at 40 °C. The reaction continued for 3 h and was stopped by boiling for 10 min.

The clusters in the form of β-limit dextrins were separated from maltose and the buffer salt on two PD-10 columns (GE Healthcare Life Sciences, NJ, USA) coupled in tandem (Kong, Corke, & Bertoft, 2009). The filtration through the PD-10 columns was done twice before the clusters were freeze-dried.

2.4. Production of building blocks

β-LDs of clusters (5 mg) were dissolved in hot water (450 μL) before α-amylase (60 U/mL) in NaOAc buffer (0.01 M, pH 6.5) was added (50 μL) at 35 °C to start the reaction, which then continued for 3 h to produce the building blocks. The concentrations for substrate and α-amylase in the reaction system were 10 mg/mL and 6.0 U/mL, respectively. The reaction was stopped by boiling for 10 min. Then, β-amylase (5 μL) with NaOAc buffer (pH 6.0, 0.01 M, 150 μL) was added to remove any remaining external chain segments. The β-amylolysis was conducted at 40 °C for 3 h before being stopped by boiling for 10 min. The building blocks in solution were frozen and stored at −18 °C before analysis by GPC.

2.5. Analysis of molecular weight distribution by gel-permeation chromatography (GPC)

The clusters were analyzed on a column (1.6 cm × 90 cm) of Sepharose CL 6B (Pharmacia, Uppsala, Sweden). The eluent was NaOH (0.5 M) with a pumping speed of 1.0 mL/min. The building block mixtures were analyzed on a column (1.6 cm × 90 cm) of Superdex 30 (Pharmacia, Uppsala, Sweden) eluted with NaCl (0.05 M) at 1.0 mL/min. The injection volume was 500 μL with carbohydrate content of 2.0 mg. Collected fractions (1.0 mL) were analyzed for carbohydrate content by the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The columns were calibrated with commercial linear dextrins of DP 1–7 (Supelco, Bellefonte, PA) and with branched dextrins of known DP (DP > 11) prepared from starch α-hydrolysates (Bertoft & Spoof, 1989).

2.6. Analysis of unit chain length distribution by high-performance anion-exchange chromatography (HPAEC)

Lyophilized β-LDs of clusters (1.0 mg) were dissolved in warm water (225 μL) before adding NaOAc buffer (0.01 M, pH 5.5, 25 μL), isoamylase (1 μL) and pullulanase (1 μL). The concentration of substrate in the reaction system was 4.0 mg/mL. The reaction was terminated by boiling for 10 min after incubation for 20 h at room temperature. The unit chain distribution was analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) on a Dionex ICS 3000 instrument (Sunnyvale, CA, USA). The analytical column was CarboPac PA-100 (4× 250 mm), combined with a CarboPac PA-100 guard column (4× 50 mm). The flow rate was 1.0 mL/min and the injection volume was 25 μL with a carbohydrate concentration of 1 mg/mL. The eluent phase consisted of solutions A (0.15 M NaOH) and B (0.15 M NaOH, containing 0.50 M NaOAc) using the following gradient: 0–9 min, 15–36% eluent B; 9–18 min, 36–45% B; 18–110 min, 45–100% B. The column was equilibrated with 15% eluent B for 60 min between runs. PAD signal was converted to carbohydrate content (Koch, Andersson, & Åman 1998).

2.7. Statistical analysis

Tests were conducted in triplicate and analyzed using SPSS Version 19.0 software (IBM Corporation, USA). Differences between means of data were compared by least significant difference at a significance level $p < 0.05$.

3. Results and discussion

3.1. Time course analysis of α-amylolysis of whole starch

The chromatograms of whole starches hydrolyzed with α-amylase and the degraded products are shown in Fig. 2A and B. The hydrolysis kinetics, as expected, was rapid in the initial phase and then was slower (Bertoft, Källman, Koch, Andersson, & Åman, 2011; Kong et al., 2009; Zhu, Corke, Åman, & Bertoft, 2011a) and it appears to be a universal feature of starch hydrolysis. In this study, whole starch, with amylose present, showed a similar pattern as pure amylopectin (Fig. 2A and B, respectively), with some noticeable differences. From fractions of K_{av} ~0–0.3, a portion of dextrins from whole normal maize starch remained during α-amylolysis time course (Fig. 2A). This may be attributed to amylose that likely re-associated when the whole starch in DMSO solution was diluted in water and cooled as had been suggested by Hayashi, Kotani, and Cho (1984), thereby becoming resistant to α-amylase attack. Nevertheless, these remnant dextrins were hydrolyzed by subsequent β-amylolysis treatment as reported below (Section 3.2).

In fractions of K_{av} ~0.7–0.8, the carbohydrate content for normal maize starch was higher, particularly at longer incubation times, than for waxy maize starch. The boundary between linear dextrins (mostly found in fractions of K_{av} ~0.8–1.0) and branched dextrins (mostly in fractions of K_{av} ~0.0–0.8) thereby became less obvious. This may be attributed to the amylose fractions that were degraded by α-amylolysis, which would accumulate around fraction of K_{av} ~0.8. It has been reported that hydrolysis of gelatinized starch dispersions by α-amylase from other sources such as *Bacillus licheniformis* also causes simultaneous de-polymerization of both amylopectin and amylose (Poutanen, Lauro, Suortti, & Autio, 1996).

The relative molar amounts of apparently branched dextrins (DP > 25) were plotted against time (Fig. 2C), and it showed that the branched dextrins formed from either normal or waxy starch became more resistant to further attack by α-amylase after 100 min. Therefore, 100 min of α-amylolysis was chosen for the large-scale clusters production from both normal and waxy maize starches.

3.2. Characterization of clusters in whole starch

Clusters in the form of α-dextrins were further subjected to β-amylolysis to remove external chains that remained after α-amylolysis. The molecular weight distribution of the resulting β-limit dextrins (β-LDs) of clusters is shown in Fig. 2D. Previous studies (Bertoft et al., 2012a; Zhu et al., 2011a) mostly focused on the clusters in the form of φ,β-LDs, whose external chain stubs at average are 0.5 residues shorter than that of β-LDs (Bertoft, 1989, 2004) employed in this study.

The molecular weight distribution patterns of β-LDs of clusters from normal and waxy maize starches were similar. Average DP of clusters of normal and waxy maize starches were 74.2 and 67.4, respectively (peak-DP 120 and 100) (Table 1). This generally agreed with a previous report on clusters from maize amylopectin (Bertoft et al., 2012a). The β-LDs of clusters were debranched by pullulanase and isoamylase before further analysis with HPAEC to obtain the unit chain length distribution (Fig. 3A). The most abundant peaks were maltose and maltotriose. A series of structural parameters were calculated based on DP and CL (Table 1). Among them, number of chains per cluster (NC) was 9.7 and 9.4, average chain length (CL) was 7.2 and 7.6, and internal chain length (ICL) was 5.3 and 4.8 for normal maize starch (NM) and waxy maize starch (WM), respectively. The data of WM and NM are consistent with a previous report on waxy maize starch, and fit with the structural feature of clusters from group 2 amylopectins (Bertoft et al., 2012a). These parameters suggest similarities in the cluster structure for both NM and WM, and allows for the possible use of whole starch to reveal the internal structure of amylopectins.

For amylopectin, chains not carrying any other chains by α-(1 → 6) linkages are termed A-chains, whereas B-chains carry other

Table 1
Characterization of β-LDs of clusters from whole normal maize and waxy maize starch.

Sample	DP	Peak-DP	NC	Peak-NC	CL	ICL	TICL
NM	74.2a	120a	9.7a	15.7a	7.2a	5.3a	10.1a
WM	67.4b	100b	9.4a	14.0b	7.6a	4.8b	10.7a

DP, average degree of polymerization estimated by GPC; Peak-DP, estimated by GPC; NC, number of chains estimated as DP/CL; Peak-NC, peak number of chains estimated as peak-DP/CL; CL, average chain length estimated by HPAEC; ICL, internal chain length = (CL − ECL) × NC/(NC − 1) − 1, in which ECL is 2.0 for β-limit dextrin; TICL, total internal chain length = CL of b-chains in β-LD–1.5; NM, normal maize starch; WM, waxy maize starch. Different small-cased letters within a column indicate significant differences ($p < 0.05$).

chains (Peat, Whelan, & Thomas, 1952). In order to distinguish new chains produced in a cluster, as opposed to those that originally existed in amylopectin, the nomenclature of new chains not carrying any other chains in clusters are termed "a"-chains and new chains that carry other chains are "b"-chains (Bertoft et al., 2012a), compared to capital letters ("A" and "B") for the amylopectin. The amount of a-chains in clusters of waxy (48.0%) and normal (46.8%) maize starches was similar. The unit chain profile of b-chains in the clusters reflects their internal structure. The general profiles of b-chains of both normal and waxy maize starches, especially for b0-chains on molar basis, were similar to each other (Fig. 3B), and agreed with a previous study on the internal structure of waxy maize clusters (Bertoft et al., 2012a), suggesting a possible common feature of starch from the same botanical origin (maize) as reflected by the average values of different structural parameters. To better define the structure of clusters, unit chains of b-chains can be divided into different categories (Bertoft & Koch, 2000; Bertoft et al., 2012a) (Table 2). The amounts of certain b-chain categories in the clusters of both NM and WM starches were similar as is evident in Table 2.

Table 2
Molar distribution of diverse chain categories in β-LDs of clusters of whole normal maize and waxy maize starch.

Sample	Chain categories (%)				
	a	b0	b1	b2	b3
NM	46.8a	14.1a	29.7a	6.5a	2.8a
WM	48.0a	14.2a	30.1a	5.6b	2.0b

DP range of a = 2–3, b0 = 4–6, b1 = 7–18, b2 = 19–27, b3 ≥ 28 (Bertoft et al., 2012a). NM, normal maize starch; WM, waxy maize starch. Different small-cased letters within a column indicate significant differences ($p < 0.05$).

3.3. Characterization of building blocks in clusters of whole starch

Building blocks are small and tightly branched units in clusters and can be produced by extensive α-amylolysis of the clusters at high enzyme concentrations (Bertoft, 2007b). The traditional concept of "cluster" (Bertoft, 2007a) is therefore essentially a group of building blocks linked by linear inter-block segments. The composition of building blocks analyzed by GPC is shown in Fig. 4A. The

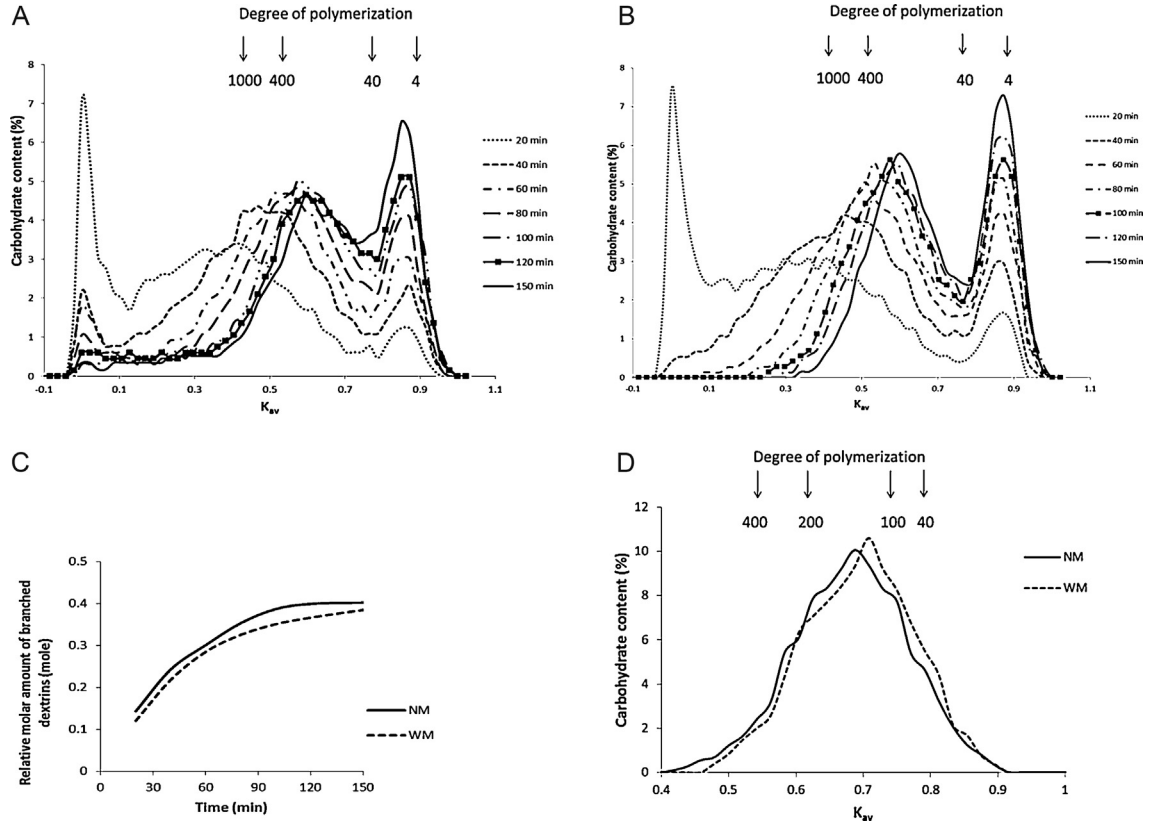

Fig. 2. (A) Time course for a-amylolysis of normal maize starch by gel-permeation chromatography on Sepharose CL 6B. (B) Time course for α-amylolysis of waxy maize starch by gel-permeation chromatography on Sepharose CL 6B. (C) Development of the relative molar amount of branched dextrins in the time course of α-amylolysis of whole normal maize (NM) and waxy maize (WM) starches for clusters production. (D) Molecular weight distribution obtained by gel permeation chromatography on Sepharose CL-6B of β-limit dextrins of clusters from whole starches of normal (NM) and waxy maize (WM).

Table 3
Building block structure of β-LDs of clusters of whole normal maize and waxy maize starch.

Sample	DP_{Bbl}	DBbl	NBbl	IB-CL	Distribution of building blocks in clusters (mole%)			
					Group 2	Group 3	Group 4	Groups 5 and 6
NM	11.5a	7.2a	5.3a	5.9a	53.7a	24.8b	10.4a	11.1a
WM	11.3a	7.4a	5.0a	6.0a	52.5a	27.0a	11.1a	9.3b

DP_{Bbl}, average DP of branched building blocks; DBbl, density of building blocks = NBbl/DP_{cltr} × 100, in which DP_{cltr} is the average DP of β-LDs of clusters; NBbl, number of building blocks = wt%$_{Bbl}$/100 × DP_{cluter}/DP_{Bbl}, in which wt%$_{Bbl}$ is the weight percent of branched building blocks; IB-CL, calculated according to equation (1); Categorization of groups 2–6 of building blocks based on their number of chains per block; NM, normal maize starch; WM, waxy maize starch. Different small-cased letters within a column indicate significant differences ($p < 0.05$).

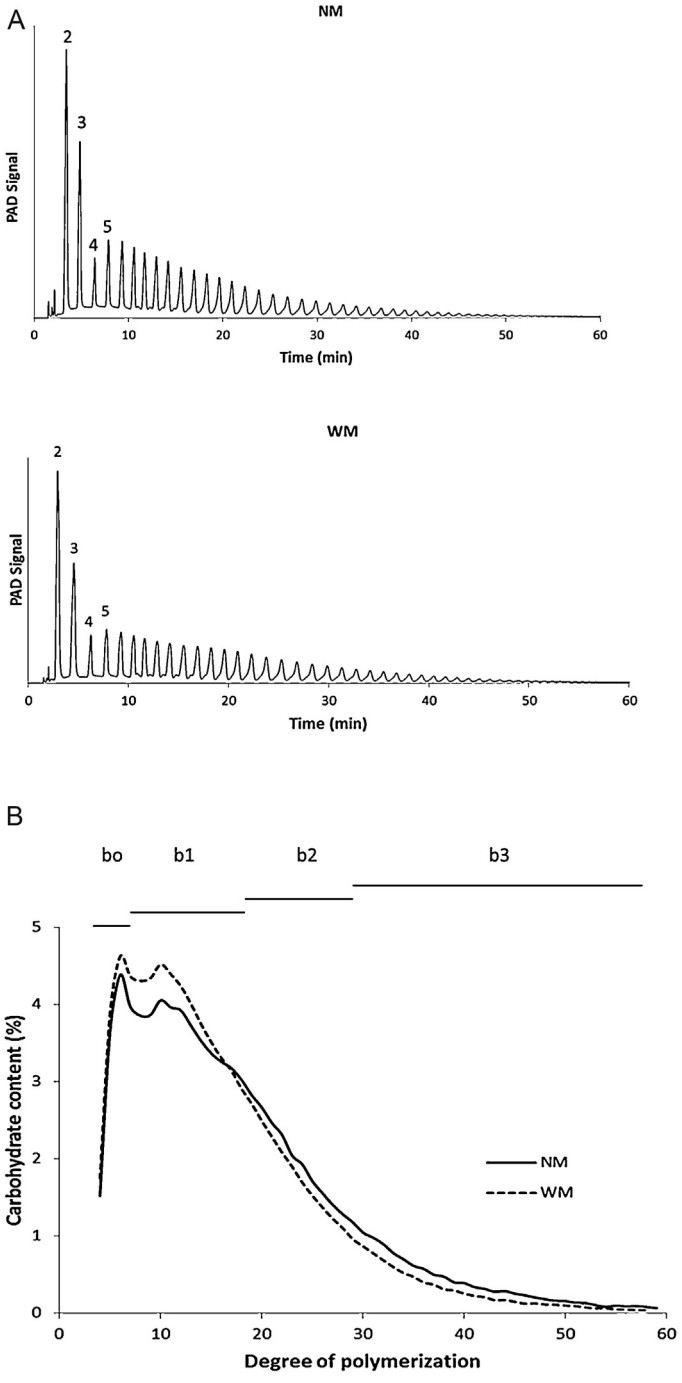

Fig. 3. (A) HPAEC chromatographs of unit chain length distribution of β-limit dextrins of clusters from whole starches of normal (NM) and waxy maize (WM). Unit chain length of DP 2, 3, 4, and 5 were indicated. (B) Internal unit chain length distribution of β-limit dextrins of clusters from whole starches of normal (NM) and waxy maize (WM). Only chains with DP ≥ 4 are shown and different b-chain categories are indicated.

profile of building blocks with two, three and four chains (groups 2–4) were somewhat similar in NM and WM starches. WM possessed somewhat more of group 3 building blocks, whereas NM had more of groups 5 and 6 (Table 3). Possibly, this difference reflected the larger size of the clusters in the latter starch. The fact that the clusters of the normal starch also possessed more of the longer b2- and b3-chains (Fig. 2B) could suggest differences in the fine structure of the amylopectin components in the two starches, even though the extent of the influence of amylose on the results remained unclear. The inter-building block linear segments are released by extensive α-amylolysis and are reduced to DP 1–3 by subsequent β-amylase addition (group 1 in Fig. 3A) (Bertoft et al., 2012b), and the amount of these small carbohydrates was similar in both samples.

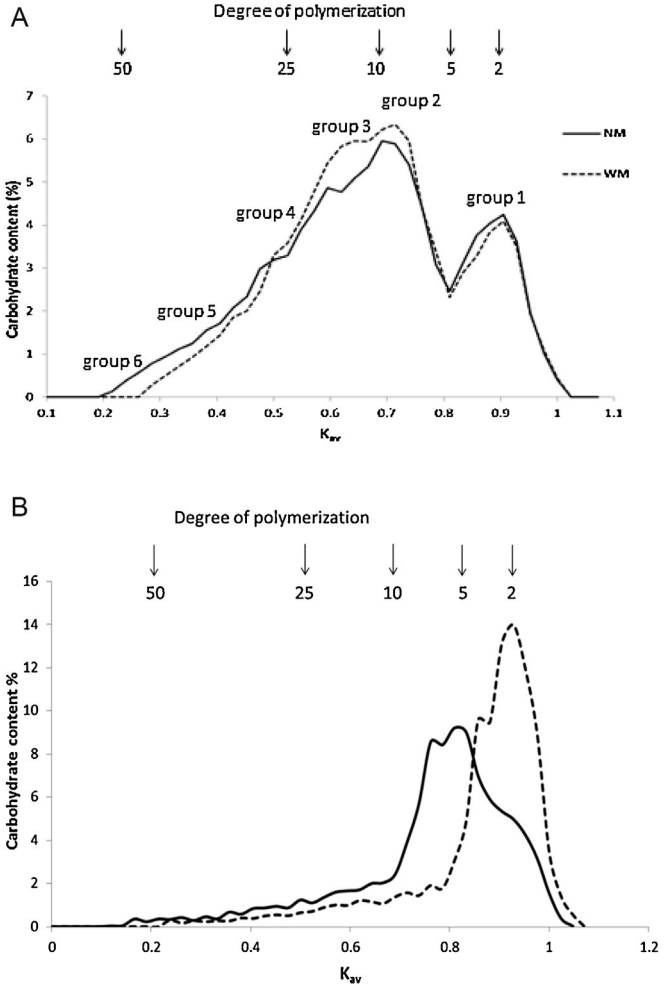

Fig. 4. (A) Molecular weight distribution of building blocks in β-limit dextrins clusters of whole maize starches obtained by GPC on Superdex 30. (B) Molecular weight distribution of building blocks directly produced from whole common maize starch before and after β-amylolysis obtained by GPC on Superdex 30.

The linear segments originally linking building blocks in the clusters in the form of DP 1–3 (group 1) can be used with the following equation to calculate the inter-block chain length (IB-CL): an attribute that has been correlated to functional properties of starch (Vamadevan et al., 2013).

$$\text{IB-CL} = \text{mole\%}_{\text{linear}} \times \frac{\text{DP}_{\text{linear}}}{\text{mole\%}_{\text{branched}}} + 4 \quad (1)$$

In this equation, $\text{mole\%}_{\text{linear}}$ and $\text{DP}_{\text{linear}}$ are the relative molar percentage and average DP of linear dextrins (DP 1–3), respectively; $\text{mole\%}_{\text{branched}}$ is the relative molar percentage of branched building blocks ($\text{DP} \geq 5$); 4 relates to the two remaining glucose stubs on average in each of the adjacent building blocks based on the work by Umeki and Yamamoto (1972, 1975).

IB-CL in clusters of NM and WM were 5.9 and 6.0, respectively (Table 3). This agreed well with a previous report on waxy maize starch (Bertoft et al., 2012a), and also fitted well with the definition of clusters in amylopectins as proposed previously (Bertoft, 2007b). Various other structural parameters for building block structure of the clusters were also estimated (Table 3) from the size-distribution analyses of the clusters and building blocks (Figs. 2D and 4A, respectively). DP of the building blocks (DP_{Bbl}), density of building blocks in clusters (DBbl) and the number of building blocks per cluster (NBbl) also generally agreed with a previous report (Bertoft et al., 2012a). The latter parameters are important for the characterization of the internal structure of amylopectin, and the result suggested that possible interference from branches in the amylose component was negligible.

Whole starches were also subjected to α-amylolysis with a high concentration of enzyme to directly produce building blocks which were further treated with β-amylase and analyzed by GPC (Fig. 4B) and HPAEC (Fig. 5). The linear fragments produced in the whole starches could be attributed to the amylose fractions, external chains from amylopectins and also inter-cluster and inter-block chain segments. From the fractions of K_{av} 0.2–0.5 (Fig. 4B), the large building blocks of groups 5 and 6 could be characterized. These large building blocks have been suggested to be critical in starch biosynthesis (Bertoft et al., 2012b) and also in the formation of amorphous regions in the granules (Zhu, Corke, Åman, & Bertoft, 2011b). The profile of groups of larger building blocks of whole starches revealed by HPAEC were also almost identical with that of clusters and may be feasibly used for fingerprinting starches from different sources (Bertoft et al., 2012b). Furthermore, using whole starch for direct building block analysis may provide structural information of branching patterns when the amount of samples is limited. Overall, the method presented here needs further work to improve its robustness by using starch from other sources and with different amylose contents. The impact of intermediate materials and its structure also needs to be studied.

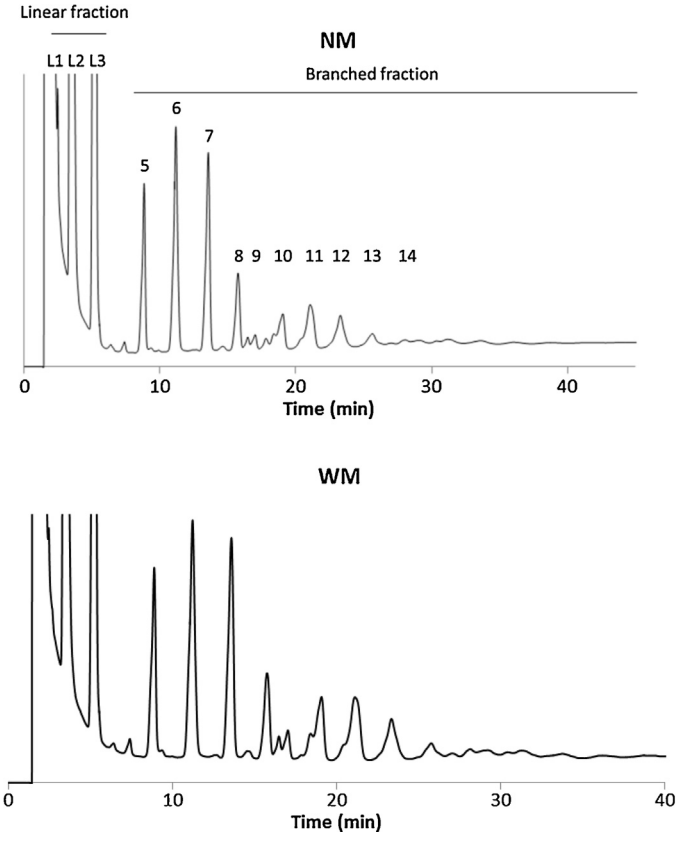

Fig. 5. HPAECs of building blocks directly from whole starches of normal (NM) and waxy maize (WM). Indication of the nature and DP of building blocks are illustrated with NM where L1, L2, and L3 represent glucose, maltose, and maltotriose, and the rest are branched dextrins with respective DP.

4. Conclusions

The internal branching pattern of whole normal maize starch was characterized to assess if meaningful information about the internal structure of amylopectin can de determined without going through the labour and time intensive process of first separating amylopectin from starches. Clusters in the form of β-LDs produced from the normal starch had somewhat more of chains compared to clusters from waxy maize starch. The clusters from normal starch were also somewhat larger. However, structural characteristics with regards to NC, CL and TICL were similar. Furthermore, the clusters had inter-block chain length (IB-CL) of ~6.0 and density of building blocks of ~7.3. Each cluster contained ~5.2 building blocks. These latter parameters have relevance to certain functional properties of starches and can be used as a fingerprint to differentiate starches from different botanical sources. It may thus be concluded that important internal structural parameters of whole amylose-containing maize starch are assessed using this protocol, even though some details of amylopectin structure may not be identical to that obtained from isolated amylopectin.

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