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Granule size affects the acetyl substitution on amylopectin populations in potato and sweet potato starches

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Abstract

Specific enzymatic degradation in combination with chromatographic and spectrometric techniques was used to understand acetyl group distribution over the amylopectin populations of differently sized granule fractions from potato and sweet potato starches. The hydrolysates obtained after α-amylase, ß-amylase, pullulanase, and the combination of pullulanase, α-amylase and amyloglucosidase treatment were investigated by high-performance size-exclusion chromatography (HPSEC), high-performance anion-exchange chromatography (HPAEC) and Maldi-Tof-MS (Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Mass Spectrometry). The acetyl groups were found to be located near the branching point, in the external chain and in the internal chain regions. The acetyl group distributions were different for amylopectin from different granule size fractions. Higher DP (degree of polymerization) fragments were present in the digests of acetylated amylopectin populations of the small size granule starches. Our studies confirmed that acetyl groups were unevenly distributed over the amylopectin populations.

Keywords: Acetyl group; Degree of substitution; Amylopectin; Granule size; Starch

1. Introduction

Amylopectin, in general, is the major component of starch. It is a highly branched, high molecular weight polymer. The fine structure of amylopectin from various botanical sources has been intensively studied and continues to be the subject of research (Bertoft & Koch, 1999; Hizukuri, 1986; Hizukuri & Maehara, 1990; Manners, 1989; Thompson, 2000). Chemical modification at the hydroxyl groups is an efficient way to introduce different functional groups to starch in order to obtain products with desired properties for certain applications (Huber & BeMiller, 2000). Acetylation is one of the most commonly used esterification at the hydroxyl groups of starches for food applications. Substitution with acetyl groups causes changes in the properties of starch, e.g. improvement of stability against retrogradation, improvement of elasticity, and lower gelatinization temperature. The functionality of acetylated starches depends on the structure, degree of substitution (DS) and acetyl group distribution pattern over the amylose and amylopectin components (Bertoft, 2004). The acetyl substitution pattern over the amylose populations isolated from acetylated potato and sweet potato starches was investigated previously by Chen, Schols, and Voragen (2004). Acetyl groups show different distribution patterns over the amylose populations of differently size granule starches. Although the DS of amylopectin population is lower than that of the corresponding amylose population, the largest part of the total acetyl groups (in mole) is present in the amylopectin population (Chen et al., 2004). Here we report on the acetyl group distribution within the amylopectin molecule of differently sized granule fractions from potato and sweet potato starches.

2. Experimental

2.1. Materials

Acetylated potato and sweet potato (XuShu18) starches were prepared by AVEBE R&D (Foxhol, the Netherlands). The starches were modified in aqueous suspension by adding dropwise acetic anhydride in such amount that a theoretical DS (mole acetyl group per mole glucose) of 0.08 is obtained for all the modified starches.

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Alpha-amylase (product number 10069, from *Bacillus subtilis*, 393 U/mg) and beta-amylase (product number 10100, from barley, 22 U/mg) were purchased from Fluka (Switzerland). Pullulanase (P2986, from *Bacillus acidopullulyticus*, 400 U/mL) and amyloglucosidase (A9268, from *Aspergillus oryzae*, 1400 U/mL) were purchased from Sigma (USA). α-Amylase was dissolved in distilled water, ß-amylase was dissolved in sodium acetate buffer (0.01 mol/L, pH 4.8), pullulanase was diluted in sodium acetate buffer (0.01 mol/L, pH 5.0) and amyloglucosidase was diluted in sodium acetate buffer (0.01 mol/L, pH 4.5), to yield dilutions containing 0.38, 0.22, 0.22 and 0.14 U/μL, respectively.

The acetylated starches were fractionated by sieving according to Chen, Schols, and Voragen (2003). The large (>53 μm) and small (<20 μm) size granule fractions of acetylated potato starch, and the large (>20 μm) and small (<20 μm) size granule fractions of acetylated sweet potato (XuShu18) starch were used for this study.

Amylopectin populations were obtained using the aqueous leaching method as reported by Chen et al. (2004).

2.2. Saponification of acetylated amylopectin

Five milligrams of acetylated amylopectin was saponified with 150 μL, 0.02 mol/L NaOH for 2 h at room temperature and neutralized with 150 μL 0.02 mol/L citric acid.

2.3. Enzymatic digestion

Five milligrams of acetylated amylopectin (with and without saponification) was solubilized in 1 mL distilled water and incubated with 5 μL of pullulanase solution at 40 °C for 8 h. After inactivation by boiling for 5 min, half of the hydrolysate was used for high-performance size-exclusion chromatography (HPSEC) analysis. The remaining solution was further incubated with 5 μL of α-amylase at 25 °C for 6 h and then incubated with 5 μL of amyloglucosidase at 55 °C for 8 h. The reaction was stopped by boiling for 5 min. The hydrolysates were submitted to high-performance anion-exchange chromatography (HPAEC) and matrix assisted laser desorption/ionization time of flight-mass spectrometry (MALDI-TOF-MS) analyses.

Digestion of amylopectin samples with α-amylase and ß-amylase, respectively was performed as described in our previous report on the corresponding amylose populations (Chen et al., 2004).

2.4. HPSEC

High-performance size-exclusion chromatography (HPSEC) was performed on three TSK gel columns (7.8 mm ID×30 cm per column) in series (G4000PWXL, G3000 PWXL, G2500PWXL; Tosohaas, Japan), in combination with a PW_{XL}-guard column (Tosohaas, Japan). Elution was at 30 °C using 0.2 mol/L sodium nitrate at a flow rate of 0.8 mL/min. The elution was monitored using a Shodex SE-61 Refractive Index detector. Calibration was performed using pollulans (Polymer laboratories, UK).

2.5. HPAEC

For high-performance anion-exchange chromatography (HPAEC) analysis, a Thermo Quest HPLC is used which included a quaternary gradient pump and AS3000 auto-sampler completed with a Helium degassing unit and a PED detector in PAD mode (Dionex, USA). The data was processed using a Thermo Quest PC 1000 data handling system. A CarboPac PA1 column (4×250 mm) with guard column (Dionex, USA) was operated at a flow rate of 1.0 mL/min at 20 °C. The gradient was obtained by mixing solutions of 0.1 mol/L NaOH and 1 mol/L NaOAc in 0.1 mol/L NaOH. After 15 min equilibration with 0.1 mol/L NaOH, 20 μL of the sample was injected and a linear gradient to 0.50 mol/L NaOAc in 0.1 mol/L NaOH within 30 min was followed by a linear gradient in 5 min to 1 mol/L NaOAc in 0.1 mol/L NaOH. Finally, the column was washed for 5 min with 1 mol/L NaOAc in 0.1 mol/L NaOH.

2.6. MALDI-TOF-MS

A Voyager-DE RP Biospectrometry workstation (PerSeptive Biosystems Inc., Framingham, MA, USA) was used for MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Mass Spectrometry) determination according to Kabel et al. (2002).

3. Results and discussion

3.1. Structures of amylopectin populations isolated from large and small size granule potato and sweet potato starches

The cluster model has emerged as the most probable molecular structure of amylopectin (Manners, 1989). The general feature of this model is a highly branched polymer in which the side chains are arranged in clusters and that these clusters are linked by some long side chains which extend into two or more clusters (Hizukuri, 1986). Highly purified amylolytic enzymes make it possible to investigate the fine structure of amylopectin. Pullulanase, which selectively hydrolyse the α-(1, 6)-D-glucosidic inter-chain linkages in amylopectin, has been used in the exploration of the amylopectin macromolecule. The HPSEC elution pattern of pullulanase hydrolysates of amylopectin populations isolated from large and small size granule fractions of potato and sweet potato starches (Fig. 1) showed three fractions corresponding to A chains, B1 chains (short B chains, being components of the units of clusters) and B2 chains (long B chains that span over two or more clusters). The DP (degree of polymerization) of A chains was about 3–8, short B chains (B1) was about 8–25 and long B chains (B2) was about 25–68. The A:B chain weight ratios of the amylopectin populations of both large and small size granule fractions of potato starch were 0.34:1. However, The B1:B2 chain weight ratio of small size granule

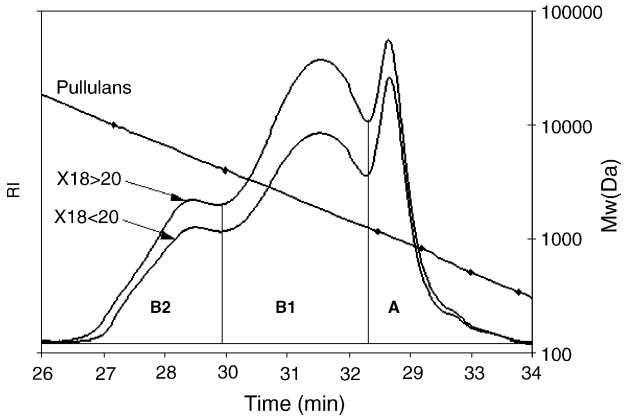

Fig. 1. HPSEC elution profiles of the pullulanase hydrolysates of the amylopectin populations isolated from large and small size granule fractions of native sweet potato starch. X18 > 20: XuShu18 sweet potato starch (granule size larger than 20 μm).

fraction (2.4:1) was higher than that of large size granule fraction (2.1:1) indicating the presence of more short B chains in small size granule fraction of potato starch. The A:B chain weight ratio of the amylopectin population isolated from small size granule fraction of sweet potato starch (0.32:1) was higher than that from large size granule fraction (0.26:1). There was no difference for the B1:B2 chain ratio between the amylopectin populations isolated from large and small size granule fractions of the sweet potato starch. For both potato and sweet potato starches there were more short chains (A chain or B1 chain) present in small granule size fractions than in large granule size fractions. The A:B chain weight ratio of potato amylopectin was in accordance with the data reported by Hizukuri (1986).

3.2. Distribution of acetyl groups over amylopectin populations

Aceylated starch is a commonly available derivative used as food additive. The acetylation is made directly on the granular starch. The substitution pattern of the starch components is different because of the granular structure. It has been reported that the degree of molar substitution (DS) of amylopectin is lower than the corresponding amylose population (Table 1). The DS of amylopectin increased with decreasing starch granule size. In addition to the DS, the distribution of acetyl groups is also of interest to predict the functional properties of the starch. For this purpose, enzymes were used as analytical tools to investigate the distribution of acetyl groups over the amylopectin populations.

Table 1
Molar substitution of amylose and amylopectin populations isolated from differently sized acetylated potato and sweet potato starches (Chen et al., 2004)

Sample	Degree of molar substitution		
	Starch	Amylose	Amylopectin
ACP > 53 μm	0.0469	0.0825	0.0275
ACP < 20 μm	0.0763	0.0598	0.0835
ACX18 > 20 μm	0.0406	0.0608	0.0335
ACX18 < 20 μm	0.0590	0.0627	0.0495

ACP > 53 μm: acetylated potato starch (granule size larger than 53 μm). ACX18 < 20 μm: acetylated XuShu18 sweet potato starch (granule size smaller than 20 μm).

3.2.1. Pullulanase digestion

Comparing the HPSEC elution profiles of the pullulanase hydrolysates of the amylopectin populations isolated from large and small size granule fractions of native and acetylated potato and sweet potato starches (Figs. 1 and 2B), it can be clearly concluded that all the acetylated samples were less degraded. This indicates that acetyl groups are located near the branch points of the amylopectin of both acetylated potato and sweet potato starches. The result confirms that the branching point regions are the areas of amylopectin which easily react with esterifying and etherifying reagents, because the regions in the vicinity of the branching points are responsible for the amorphous parts of the semi-crystalline starch granule (Biliaderis, 1982; Richardson, Nilsson, Bergquist, Gorton, & Mischnick, 2000). More acetyl groups were located near the branch points of the amylopectin isolated from the small size granule fractions (Fig. 2), since the amylopectin of the acetylated small size granule fraction appeared less accessible to pullulanase digestion. This is in agreement with our previous result that the DS is higher in amylopectin of acetylated small size granule fractions (Chen et al., 2004).

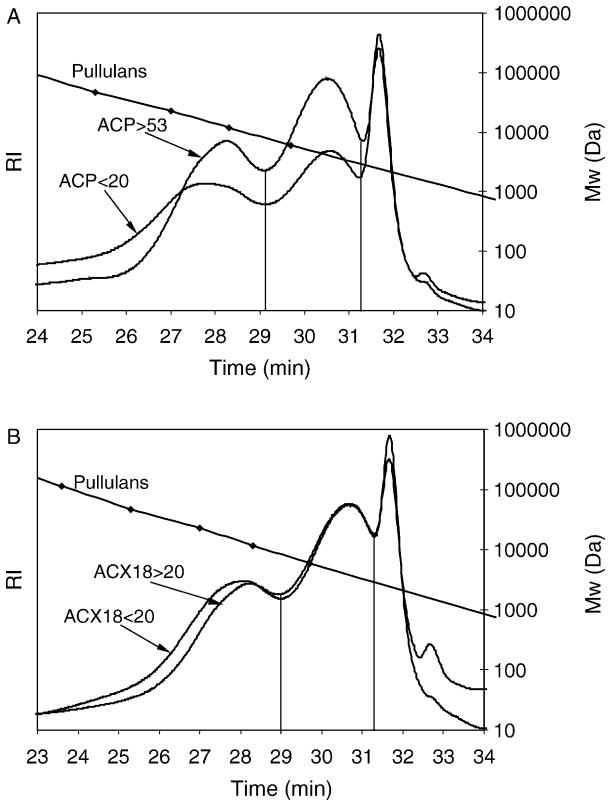

Fig. 2. HPSEC elution profiles of the pullulanase hydrolysates of the amylopectin populations isolated from large and small size granule fractions of acetylated potato (A) and sweet potato (B) starches. ACP > 53: acetylated potato starch (granule size larger than 53 μm). ACX18 < 20: acetylated XuShu18 sweet potato starch (granule size smaller than 20 μm).

Table 2
β-limit dextrin values of amylopectin populations isolated from differently sized acetylated potato and sweet potato starches and their corresponding saponified samples after β-amylase digestion

Sample	β-limit dextrin value (%)	
	Acetylated sample	Saponified sample
Potato > 53 μm	50	48
Potato < 20 μm	59	49
XuShu18 > 20 μm	54	46
XuShu18 < 20 μm	61	41

XuShu 18 > 20 μm: XuShu18 sweet potato starch (granule size larger than 20 μm).

3.2.2. ß-Amylase digestion

The ß-limit dextrin value is defined as the relative amount of ß-limit dextrin remaining after ß-amylase hydrolysis. The ß-limit dextrin value was calculated as described by Chen et al. (2004). ß-limit dextrin value, expressed as percentage of amylopectin, can be used as an indicator for the proportion of external chain (A chains and fractions of B chains which are outside the branch point). It was found that the percentage of external chains was slightly higher in amylopectin isolated from large size granule fraction of saponified potato starch than in that of its small size granule fraction (Table 2). The opposite was found for the amylopectin isolated from large size granule fraction and small size granule fraction of saponified sweet potato starch. The ß-Limit dextrin value can also be used for the estimation of substituents along the external chains. By comparing the results found for acetylated samples with data found for saponified samples, it can be concluded that all acetylated amylopectin populations are less degraded by ß-amylase. This indicates that there were only a relative small number of acetyl groups distributed over the external chains. The amylopectins isolated from small size granule fractions of both acetylated potato and sweet potato starches appeared more resistant to ß-amylase digestion. This reveals that in small size granule fractions, there were more acetyl groups distributed over the external chain or located closer to the non-reducing end of external chain of the acetylated amylopectins.

3.2.3. α-Amylase digestion

It has been reported that α-amylase is hindered by acetyl groups in degrading the glucan chains of the amylose population isolated from acetylated potato and sweet potato starches (Chen et al., 2004). The same phenomennon was found in the analysis of the corresponding amylopectin population. This was an indication of some acetyl groups distributed over the internal chains (segments of the B chains between the branches, excluding the branch point residues) of amylopectin. For both acetylated potato and sweet potato starches, amylopectin isolated from small size granule fractions were less degraded by α-amylase than that from large granules (Fig. 3). This suggests that there are more acetyl groups distributed over the internal chains of the amylopectin populations isolated from small size granule fractions. This is in accordance with the fact that there are more acetyl groups

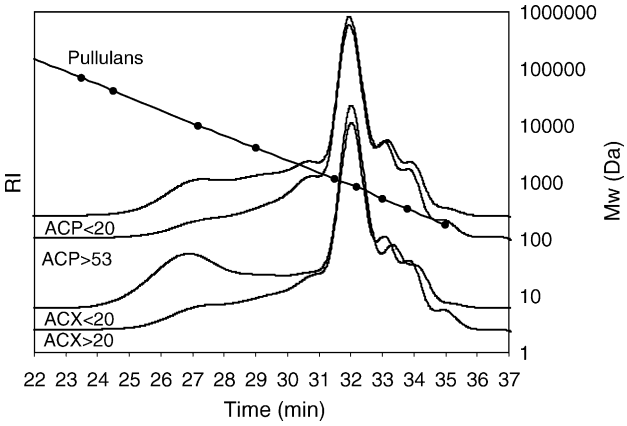

Fig. 3. HPSEC elution profiles of the α-amylase hydrolysates of the amylopectin populations isolated from large and small size granule fractions of acetylated potato and sweet potato starches.

(in mole) present in amylopectin isolated from acetylated small size granule fractions (Table 1). For small granule size starches, the hydrolysates of acetylated amylopectin isolated from sweet potato starch showed more fractions of high molecular weight, although the DS of amylopectin isolated from small potato starch granule is higher than that from small sweet potato starch. This may be due to the different distribution patterns of acetyl groups.

3.2.4. Combined digestion with pullulanase, α-amylase and amyloglucosidase

Theoretically native amylopectin can be completely digested by the combination of pullulanase, α-amylase and amyloglucosidase into glucose. HPAEC analysis (Fig. 4) showed that the saponified acetylated amylopectin isolated from sweet potato starch was indeed almost completely degraded by the combination of these three enzymes. However, for the acetylated samples, there were still quite some enzyme resistant fragments present due to the steric effect of the acetyl groups. For both acetylated potato and sweet potato starches, amylopectin isolated from small size granules showed more resistance to the combination digestion of pullulanase,

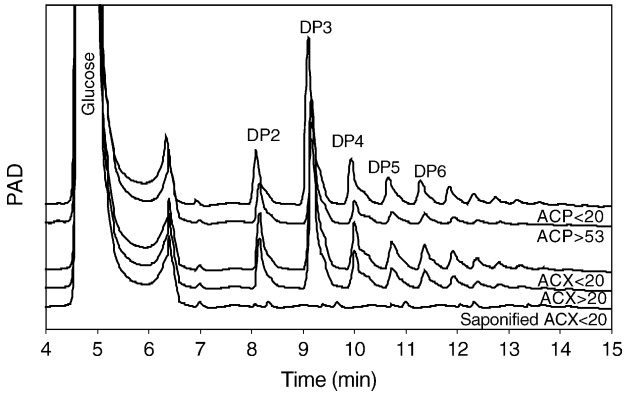

Fig. 4. HPAEC elution profiles of the pullulanase, α-amylase and amyloglucosidase hydrolysates of amylopectin populations isolated from large and small size granule fractions of acetylated potato and sweet potato starches. (DP: degree of polymerization)

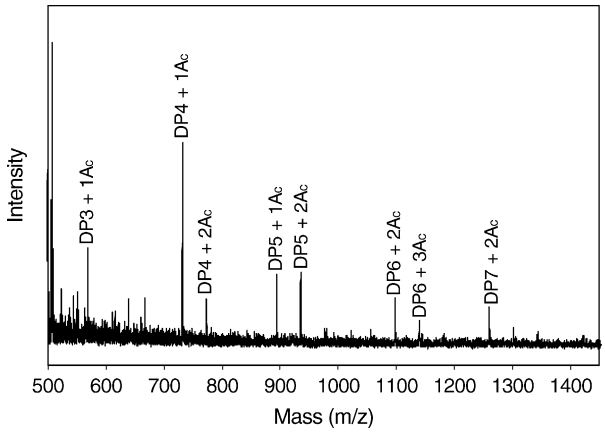

Fig. 5. MALDI-TOF mass spectrum of the enzyme (pullulanase, α-amylase and amyloglucosidase) hydrolysates of amylopectin populations isolated from small size granule fraction of acetylated XuShu18 starch (Ac: acetyl group).

α-amylase and amyloglucosidase, resulting in the presence of higher DP (degree of polymerization) enzyme resistant residues in the hydrolysates of acetylated amylopectin isolated from small size granule starches. This is due to the fact that more acetyl groups distribute over the amylopectins isolated from small size granule fractions.

MALDI-TOF mass spectrum of the enzyme hydrolysates showed that the enzyme resistant residues contained DP3 to DP5 with 1 acetyl group, and DP4 to DP7 with 2 acetyl groups in acetylated amylopectin isolated from small size granule potato starch. Enzyme resistant residues of DP6 with 3 acetyl groups were found for small size sweet potato starch sample (Fig. 5). These results confirm that the acetyl groups distribute unevenly over the amylopectin populations of the acetylated starches.

4. Conclusions

The fine structure of amylopectin is affected not only by the botanical source, but also by the granule size of starch. For large and small granule size potato starch, A:B chain weight ratios of amylopectin were constant, while for sweet potato starch, the values were different between different size granule fractions. The B1:B2 chain weight ratios showed the opposite, it was not constant for small and large granule size fraction from potato starch, while for sweet potato starch it was constant. More short chains (A chain or B1 chain) were present in small granule size fractions than in large granule size fractions for both potato and sweet potato starches.

As expected, degradations by pullulanase, α-amylase and ß-amylase were hindered by the acetyl groups present in the amylopectin. The acetyl group distributions over amylopectin were different between different granule sizes. For both potato and sweet potato starches, acetylated amylopectin isolated from small size granule fractions showed less degradability by pullulanase, ß-amylase and the combination of pullulanase, α-amylase and amyloglucosidase than that from large granules. These indicate the presence of more acetyl groups in the vicinity of branch points, near the non-reducing end of external chain regions, and in the internal chain regions over amylopectin isolated from small size granule fractions. The results are in agreement with the DS values. MALDI-TOF mass spectra confirmed that acetyl groups distributed unevenly over the amylopectin populations. Combination of results of the corresponding amylose populations, suggests that the heterogeneous distribution of acetyl groups within both starch components is due to the amorphous and crystalline areas inside the starch granules. Thus in addition to the source of starch, granule size may influence the properties of acetylated starch.

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