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Differently sized granules from acetylated potato and sweet potato starches differ in the acetyl substitution pattern of their amylose populations

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Abstract

Acetylated potato and sweet potato starches were fractionated according to granule size. From the fractions obtained amylose and amylopectin were isolated and characterized with respect to degree of substitution (DS) and degradability with α-amylase, β-amylase and amyloglucosidase. The DS of the amylose populations of differently sized granule fractions was quite constant. In contrast, the DS of the amylopectin populations originating from the differently sized fractions increased with decreasing granule size. The acetylation was confirmed to occur throughout the amorphous regions, *and* only take place in the outer lamellae of crystalline regions of starch granules. The amylose populations isolated from small size granule fractions of the acetylated starches tested were less susceptible to all the enzyme digestions than the amylose originating from the large granule fractions, even though the DS was similar. The acetyl populations groups over the amylose molecules are more heterogeneously distributed and located more closely to the non-reducing ends for amylose originating from small size granule fractions when compared to amylose from lager sized granules.

Keywords: Acetyl group; Degree of substitution; Amylose; Granule size

1. Introduction

Acetylated starches with a relatively low degree of substitution (DS) are widely used in the food industries already for many years because of their typical physicochemical characteristics such as low gelatinization temperature, high solubility, and good cooking and storage stability (de Graaf, Broekroelofs, & Janssen, 1998; Liu, Ramsden, & Corke, 1999; Wang & Wang, 2002). The physicochemical properties of acetylated starches depend on their chemical structures, DS and acetyl group distributions. Until now only a few publications deal with structural features of acetylated starches (Biliaderis, 1982; Heins, Kulicke, Käuper, & Thielking, 1998; Laignel, Bliard, Massiot, & Nuzillard, 1997; Wang & Wang, 2002). Biliaderis (1982) reported that high substitution exists only in certain parts of the amylopectin fraction of acetylated starch and he assumed that acetylation of smooth pea starch occurred exclusively in the outer lamella of the granules. In highly acetylated starches, it was shown by NMR analysis that the glucose residues are equally substituted in O-2 and O-3 position, while for hydroxyethyl starches the position O-2 is highly preferred (Heins et al., 1998).

Acetylated starches are successfully applied in products such as bakery, frozen and canned foods (Jarowenko, 1986). In a previous study, we reported that acetylated potato and sweet potato starches significantly improve the quality of white salted noodle by replacing part of the wheat flour commonly used (Chen, Schols, & Voragen, 2003a). This is in contrast to replacing part of the wheat flour with non-modified or hydroxypropylated starches. Starches from different sweet potato varieties differ in both average granule size and granule size distribution. The differently sized granule fractions of potato and sweet potato starches were found to differ greatly in chemical compositions (e.g. amylose, phosphorous), gel properties, and processibility to starch noodles (Chen, Schols, & Voragen, 2003b).

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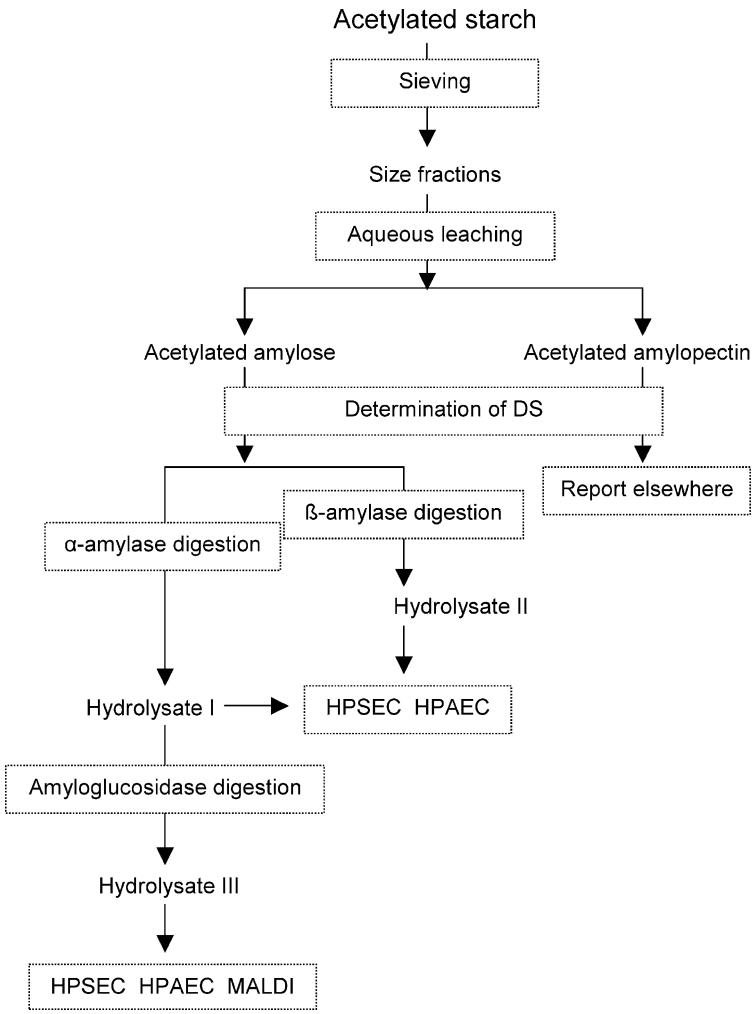

Fig. 1. Schematic overview of methodologies used to reveal the acetyl group distribution of amylose populations isolated from acetylated potato and sweet potato starches.

We assume that differently sized starch granules differ in their susceptibility to chemical reagents (e.g. acetic anhydride), which react with the hydroxyl groups of the glucose moieties of both amylose and amylopectin populations present. Since the amorphous regions are more easily accessible for chemical reagents and amylose prevails in these amorphous regions we firstly studied the extent of distribution with acetyl groups (DS) and substitution pattern of amylose populations isolated from differently sized granule fractions of acetylated potato and sweet potato starches. An outline of this study is schematically shown in Fig. 1. Similar studies on the amylopectin fractions will be reported elsewhere.

2. Experimental

2.1. Materials

Acetylated potato and sweet potato (SuShu2 and XuShu18; Chen et al., 2003b) starches were prepared by AVEBE R&D (Foxhol, the Netherlands). The starches were modified in aqueous suspension by adding drop-wise 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.

α-Amylase (product number 10069, from Bacillus subtilis, 393 U/mg) and β-amylase (product number 10100, from barley, 22 U/mg) were purchased from Fluka (Switzerland), and amyloglucosidase (A9268, from Aspergillus oryzae, 1400 U/ml) was purchased from Sigma (US). α-Amylase was dissolved in distilled water, β-amylase was dissolved in acetate buffer (0.01 M, pH 4.8) and amyloglucosidase was diluted in acetate buffer (0.01 M, pH 4.5), to yield dilutions containing 0.38, 0.22 and 0.14 U/μl, respectively.

The acetylated starches were fractionated by sieving according to Chen et al. (2003b). Acetylated potato starch was separated into four fractions: larger than 53 μm, between 36 and 53 μm, between 20 and 36 μm and smaller than 20 μm, while acetylated sweet potato starches (SuShu2 and XuShu18) were separated only into two fractions: larger than 20 μm and smaller than 20 μm.

2.2. Amylose and amylopectin separation

Amylose and amylopectin were separated using the aqueous leaching method of Shi and BeMiller (2002) with minor modification. Acetylated starch slurry (4 g starch in 100 ml distilled water) was gently stirred just below the gelatinization temperature (measured with a Kofler hot-stage polarizing microscope) overnight and then centrifuged at 10,000*g* for 20 min. The supernatant (amylose population) was separated and freeze dried. The residues was gently restirred in 100 ml distilled water at 95 °C for 2 h and then centrifuged at 10,000*g* for 20 min. The supernatant was discarded and the procedure repeated five times for the residue. The purity of the separated amylose was checked with high-performance size-exclusion chromatography (HPSEC) using the equation (purity (%) = amylose peak area/sum of all peak areas) according to Shi and BeMiller (2002), while the purity of the separated amylopectin was checked by iodine potentiometric method (Bates, French, & Rundle, 1943).

2.3. Degree of substitution assay

Five milligrams of samples were saponified with 15 μl of 0.02 M NaOH for 2 h and neutralized with 15 μl of 0.02 M citric acid. The acetate released was determined using an acetic acid assay kit (Enzytec™, Scil Diagnostics GmbH Martinsried, Germany). The DS is calculated as molar substitution (mol acetate/mol glucose).

2.4. Enzymaticdigestion

Five milligrams of acetylated amylose was solubilized in 1 ml distilled water and incubated with 5 μl of α-amylase solution at 25 °C for 8 h. After inactivation by boiling for 5 min, half of the hydrolysate was used for high-performance anion exchange chromatography (HPAEC) and HPSEC analysis. The remaining solution was further incubated with 5 μl of amyloglucosidase at 55 °C for 8 h. The reaction was stopped by boiling for 5 min. For β-amylase digestion, 5 mg of acetylated amylose was solubilized in 1 ml acetate buffer (0.01 M, pH 4.8) and incubated with 5 μl of β-amylase solution at 25 °C for 8 h and then heated by boiling for 5 min. The hydrolysates were submitted to HPSEC, HPAEC or matrix-assisted laser esorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analysis as such and after saponification.

The percentage of the released maltose of β-amylase digestion can be calculated from the HPAEC peak and the sample concentration. Therefore, β-limit dextrin can be roughly calculated as: β-limit dextrin (%) = 100 − released maltose (%).

2.5. HPSEC

HPSEC was performed on three TSK gel columns (7.8 mm ID × 30 cm per column) in series (G4000, G3000, G2500; Tosohaas), in combination with a PWXL-guard column (Tosohaas). Elution was at 30 °C using 0.2 M sodium acetate at a flow rate of 0.8 ml/min. The elute was monitored using a Shodex SE-61 Refractive Index detector. Calibration was performed using pullulans (Polymerlabs). The software was obtained from Thermo Quest.

2.6. HPAEC

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

2.7. MALDI-TOF-MS

A voyager-DE RP Biospectrometry workstation (PerSeptive Biosystems, Inc., Framingham, MA, USA was used for MALDI-TOF-MS determination according to Kabel et al. (2002).

3. Results and discussion

3.1. Degree of molar substitution of differently sized granule starches and their amylose and amylopectin populations

Differently sized granule fractions of the acetylated starches were obtained by sieving. The yields of the size fractions were in agreement with the fractions obtained after sieving of the corresponding native starches (Chen et al., 2003b).

As shown in Table 1, it was established that the amylose content decreases significantly with decreasing granule size dimension, while phosphorous content showed the opposite trend (from Chen et al. (2003b)). Protein contents are rather constant in different granule size fractions. Since acetylation would not significantly change the starch chemical compositions, the chemical compositions of the acetylated starch samples should be similar to those of their corresponding native starches.

Table 1
The yields and chemical composition of the amylose and amylopectin populations isolated from native starches (Chen et al., 2003a)

Sample	Yield (%)	Amylose (%)	Protein (%)	Phosphorus (%)
P	100	22.8 ± 0.17b	0.09 ± 0.03a	0.69 ± 0.02c
P > 53 μm	36	23.6 ± 0.15a	0.08 ± 0.04a	0.60 ± 0.03d
P36–53 μm	42	22.9 ± 0.10b	0.09 ± 0.03a	0.69 ± 0.02c
P20–36 μm	17	22.1 ± 0.25c	0.08 ± 0.04a	0.75 ± 0.03b
P < 20 μm	5	21.3 ± 0.12d	0.10 ± 0.04a	0.98 ± 0.06a
S2	100	19.4 ± 0.10ab	0.13 ± 0.03a	ND
S > 20 μm	42	19.7 ± 0.21a	0.12 ± 0.03a	ND
S < 20 μm	58	19.2 ± 0.20b	0.10 ± 0.04a	ND
X18	100	20.5 ± 0.20b	0.22 ± 0.02a	ND
X18 > 20 μm	33	22.5 ± 0.15a	0.24 ± 0.01a	ND
X18 < 20 μm	67	19.7 ± 0.15c	0.23 ± 0.02a	ND

P, potato starch; ACP > 53, potato starch fraction (granule size >53 μm); S2, SuShu2 starch; X18, XuShu18 starch; ND, not determined. All values are the mean of three measurements. Values with different letters in the same column of same variety are significantly different at $p < 0.05$. N, value was not measured.

The purity of the amylose populations isolated from differently sized granule fractions of acetylated potato and sweet potato starches were higher than 92%, while for the isolated amylopectin the purity was higher than 96% (Table 2). High purity (>90%) hydroxypropylated corn amylose was also obtained by leaching amylose in water followed by centrifugation (Shi & BeMiller, 2002). The recovery of acetyl groups after fractionation by sieving was between 88 and 96%. When the starch was further fractionated into amylose and amylopectin populations the recovery of the acetyl groups was between 76 and 96%. The loss of acetate was due to the fact that not all materials could be recovered in the pools after fractionation of amylose and amylopectin.

It was found that in general the DS of the isolated amylose population was higher than that of the amylopectin populations (Table 2). The DS of the amylose populations were 40–200, 25–54 and 27–82% higher than those of the amylopectin populations for acetylated potato, SuShu2 and XuShu18 starch fractions, respectively. The same phenomenon was observed for hydroxypropylated potato starch by Kavitha and BeMiller (1998) who found that the molar substitution of hydroxypropylated whole starch is 0.099, of amylose 0.113, and of amylopectin, 0.096. The difference in DS between amylose and amylopectin is slightly lower than that for acetylated starches in our study. This may be due to the fact that hydroxypropyl groups can randomly distribute throughout the amorphous regions of amylopectin part (Seib, 1997). Although the DS of amylose population was 25–200% higher than that of the amylopectin population, it should be realized that due to the relative large amount of amylopectin most acetyl groups were located in the amylopectin part. It was calculated that 28–49, 23–28, and 25–35% of the total acetyl groups (in molar) were distributed in the amylose populations of acetylated potato, SuShu2 and XuShu18 (small-large fraction) starches, respectively. The percentage of acetyl groups present in the amylose populations decreased with decreasing granule size. Contrarily, the percentage of acetyl groups present in the amylopectin population increased with decreasing granule size dimension indicating the increasing susceptibility to acetylation of the amylopectin populations. It can be seen that the DS of amylose is constant for differently sized granule fractions, while the DS of amylopectin, similar to the DS of the total starch fraction, increases with decreasing granule size dimension.

Table 2
Purity and molar substitution of amylose and amylopectin populations isolated from differently sized granule fractions of acetylated potato and sweet potato starches

Sample	Purity (%)		Degree of molar substitution (%)		
	Amylose	Amylopectin	Starch	Amylose	Amylopectin
ACP	93	97	0.0648c (0.0012)	0.0827a (0.0013)	0.0418b (0.0009)
ACP > 53 μm	94	96	0.0469e (0.0002)	0.0825a (0.0006)	0.0275d (0.0011)
ACP36–53 μm	92	98	0.0596d (0.0013)	0.0812a (0.0014)	0.0349c (0.0008)
ACP20–36 μm	96	96	0.0679b (0.0002)	0.0835a (0.0009)	0.0427b (0.0005)
ACP < 20 μm	93	97	0.0763a (0.0006)	0.0835a (0.0010)	0.0598a (0.0011)
ACS2	96	96	0.0578b (0.0009)	0.0641a (0.0004)	0.0431b (0.0008)
ACS > 20 μm	95	97	0.0471c (0.0003)	0.0631a (0.0004)	0.0411c (0.0009)
ACS < 20 μm	94	98	0.0598a (0.0007)	0.0635a (0.0009)	0.0509a (0.0004)
ACX18	95	97	0.0550b (0.0007)	0.0609a (0.0020)	0.0383b (0.0011)
ACX18 > 20 μm	94	96	0.0406c (0.0007)	0.0608a (0.0019)	0.0335c (0.0010)
ACX18 < 20 μm	95	97	0.0590a (0.0004)	0.0627a (0.0003)	0.0495a (0.0010)

ACP, acetylated potato starch; ACP > 53, acetylated potato starch (granule size >53 μm); ACS2, acetylated SuShu2 starch; ACX18, acetylated XuShu18 starch. All values are the mean of three measurements. Values with different letters in the same column of same variety are significantly different at $p < 0.05$. Values in the brackets are the standard deviations.

This suggests that the variation in DS of different granule size starches is due to the different DS of the corresponding amylopectin part, but not of the amylose part. This is not in agreement with the observations of Van der Burgt (2000) for methylated starches, who found no difference between substitution levels of granules of various sizes. It is known that amylose is mainly located in the amorphous regions while the branched chains of amylopectin make up the crystalline regions of starch granules. The results indicate that amylose as present in the amorphous regions are equally acetylated whereas amylopectin in the crystalline regions is selectively acetylated with the favor of decreasing granule size dimension. Since, in general, small granules have larger specific surface areas than the large ones (Morrison & Scott, 1986), the crystalline regions in the outer lamellae of small granules have more space to react with the chemical reagent (acetic anhydride) resulting in higher DS in the amylopectin present. This also indicates that the acetylation cannot take place throughout the crystalline regions of the whole granule but only occur in the outer lamellae. This may be due to the poor penetrating ability of acetic anhydride in starch granules. Our results confirm the assumption of Biliaderis (1982) who reported that the acetylation of smooth pea starch occurred exclusively in certain parts of the granule, presumable the outer lamellae, and the high-density substitution exists only in certain parts of the amylopectin. In Fig. 2, a model showing the areas of acetylation in the starch granule is proposed.

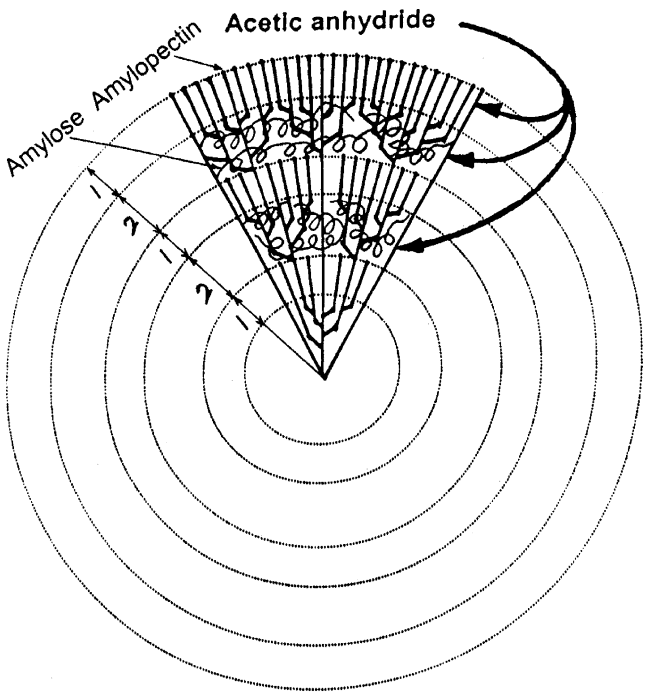

Fig. 2. Proposed model for the granular starch acetylation: (1) crystalline region; (2) amorphous region.

3.2. Distribution of acetyl groups over amylose population as revealed by enzyme digestion

The cyclosidic linkages between glucose moieties in amylose can be split by starch enzymes (e.g. α-amylase, β-amylase, amyloglucosidase) in a defined way. The introduced acetyl groups in amylose may hinder the actions of enzymes. The information obtained from these enzyme hydrolysis studies can give information over the distribution of the substituted groups over the amylose population.

3.2.1. α-Amylase digestion

α-Amylase is an endo-enzyme which hydrolyzes starches randomly at α-(1 → 4) D-glucosidic linkages to produce glucose and oligosaccharides containing two to seven glucose residues (Hizukuri, 1996). α-Amylase digestion may be hindered by acetyl substitution and studying the enzyme degradation products consequently provides information on the distribution of acetyl group over the acetylated amylose. The DS of the amylose population of differently sized granule fractions of acetylated potato, acetylated SuShu2 and acetylated XuShu18 are about 0.08, 0.06, and 0.06 corresponding to acetyl contents (w/w) of 2.1, 1.5 and 1.5%, respectively. Although only small amounts of acetyl groups were introduced, it can be seen that α-amylase digestion was hindered by the acetyl group from comparison of the HPSEC and HPAEC profiles of the acetylated amylose and their saponified samples (Figs. 3 and 4). The HPSEC elution profile showed more degradation for the saponified sample digested with α-amylase than that of the acetylated samples. The HPSEC elution profile of the acetylated amylose showed less digestion of the acetylated amylose originating from small sized granule fractions than amylose form larger sized granule fractions.

It can be clearly seen from the HPAEC elution profile that the saponified sample was digested to oligomers smaller than DP = 6 (DP, degree of polymerization),

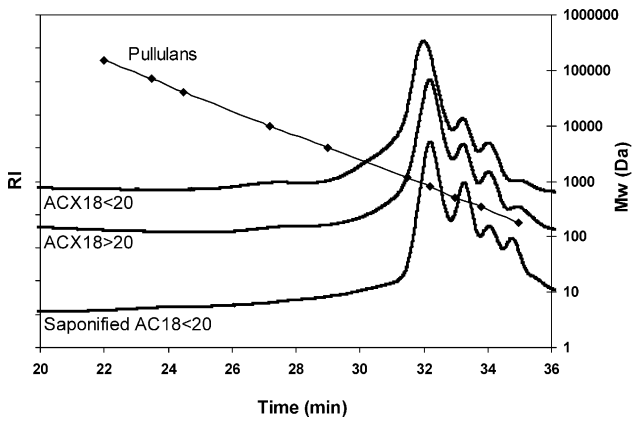

Fig. 3. HPSEC elution profiles of the α-amylase hydrolysates of the amylose populations isolated from differently sized granule fractions of acetylated XuShu18 sweet potato starch (AC18 > 20, amylose isolated form large size granule fraction (>20 μm) of acetylated XuShu18 starch).

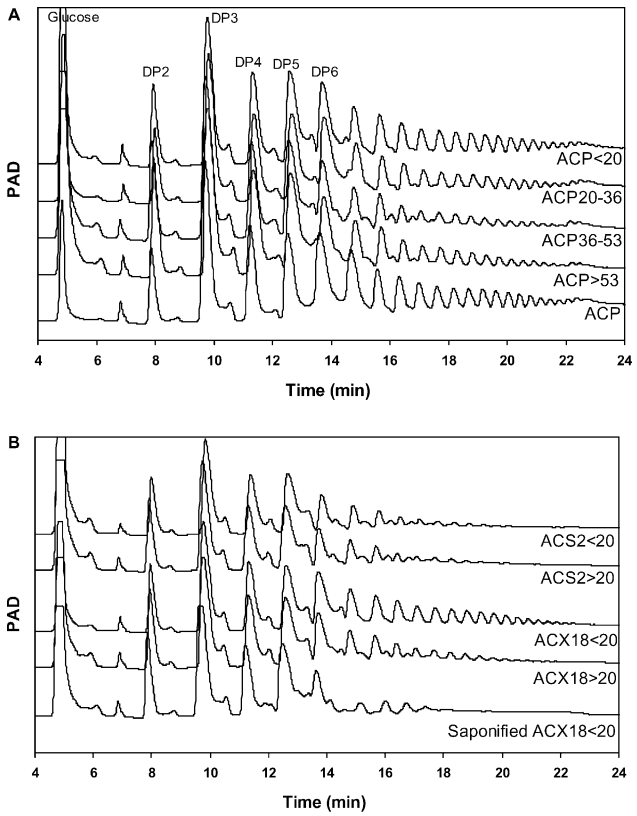

Fig. 4. HPAEC elution profiles of the α-amylase hydrolysates of the amylose populations isolated from differently sized granule fractions of acetylated potato (A) and sweet potato (B) starches (ACP, amylose isolated from acetylated potato starch; ACS2 < 20, amylose isolated from small size granule fraction (<20 μm) of acetylated SuShu2 starch; AC18 > 20, amylose isolated from large size granule fraction (>20 μm) of acetylated XuShu18 starch).

whereas oligomers higher than DP = 6 were found in the residues of all acetylated samples. This is in agreement with Biliaderis (1982) who proposed that acetyl substituents exert a similar effect as hydroxyethyl groups in restricting α-amylase attack on adjacent α-(1 → 4) glucosidic linkages. It was also found that higher DP fragments were present in the amylose isolated from small size granule fractions. Although the DS of the amylose populations of different size granule fractions was quite similar, the susceptibility of the amylose populations of both acetylated potato and sweet potato (SuShu2 and XuShu18) starches to α-amylase degradation was different. The susceptibility decreased with decreasing granule size dimension. This indicates that the acetyl group distribution patterns in the amylose populations of small size granule fractions are more restricting for α-amylase digestion suggesting a more heterogeneous distribution of acetyl groups. Moreover, it could also be observed that amylose from differently sized granule fractions of acetylated potato starch were less susceptible to α-amylase than that from acetylated sweet potato (SuShu2 and XuShu18) starches. This is partly due to the higher DS in the amylose of acetylated potato starch. However, the DS of amylose from differently sized granule fractions of acetylated SuShu2 and XuShu18 are similar, but the susceptibility to α-amylase between the amylose form large and small size granule fractions is still different for both starches. This suggests that the acetyl group distribution not only depends on granule size but also depends on other parameters like granule density, granule shape and surface.

Since all HPSEC and HPAEC elution patterns of acetylated potato, SuShu2 and XuShu18 digested with enzymes showed the same trends, we only show the elution profile of acetylated XuShu18 as a typical representative.

3.2.2. β-Amylase digestion

β-Amylase is an exo-enzyme which hydrolyses every second α-(1 → 4) linkage from the non-reducing end to release maltose, but stops on average two glucose units before a branching point (Richardson, Nilsson, Bergquist, Gorton, & Mischnick, 2000). β-Amylase hydrolysis will reveal information about the substituent location whether it is near or far away from the non-reducing end of amylose. From the released amount of maltose, the β-limit dextrin value can be calculated after digestion. By comparing β-limit dextrin values of acetylated and saponified samples, it can be seen that β-amylase was also hindered by acetyl substituents (Table 3). The β-limit dextrin values of the saponified amylose isolated from differently sized acetylated potato starch were constant indicating that the number of branches of native potato amylose were independent of granule size. Contrarily, β-limit dextrin values of the saponified amylose isolated from large size granules of acetylated sweet potato starches (XuShu18 and SuShu2) were slightly higher than those from the small size granules suggesting that native large size granule sweet potato amylose is more branched. However, for acetylated samples, both potato and sweet potato starches showed that β-limit dextrin values increased with decreasing granule size. This indicates that acetyl groups were located more close to the non-reducing ends of amylose for

Table 3
β-Limit dextrin values of amylose 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	36	22
Potato 36–53 μm	38	21
Potato 20–36 μm	47	22
Potato <20 μm	56	23
XuShu18 > 20 μm	38	20
XuShu18 < 20 μm	44	15
SuShu2 > 20 μm	38	19
SuShu2 < 20 μm	41	16

small granules than for large granules, since their DS were quite similar.

3.2.3. Amyloglucosidase digestion

The α-amylase hydrolysates were subjected to further digestion with amyloglucosidase. Amyloglucosidase is capable to hydrolyze completely both α-(1 → 4) and α-(1 → 6) linkage in polysaccharides through an exo-mechanism from the non-reducing terminal residues producing β-D-glucose (Hizukuri, 1996). Simultaneous α-amylase and amyloglucosidase digestion of normal starches will result in complete conversion into glucose (Richardson et al., 2000). The acetylated amylose digested by α-amylase and then followed by amyloglucosidase will reveal the absolute steric hindrance of the acetyl group to amyloglucosidase. After amyloglucosidase digestion, it could be observed from HPSEC profiles (Fig. 5) that some oligomers remained in the digested acetylated amylose, while the saponified samples were completely digested to glucose. This indicates that amyloglucosidase is also hindered by acetyl groups. The susceptibility of the amylose of differently sized granule fractions of acetylated potato and sweet potato (SuShu2 and XuShu18) starches to amyloglucosidase digestion showed the same trends as the α-amylase digestion. The different susceptibility to enzyme digestion is probably due to the different acetyl group distribution in the amylose population of the differently sized granules. It is clearly seen from HPAEC elution profiles (Fig. 6) that higher DP oligomers resistant to further digestion were present in the amylose population of small size granule fractions of acetylated starches. This suggests that although the DS of amyloses isolated from differently sized granule starches is not different, the acetyl groups were more heterogeneously distributed in the amylose of small size granules, since the size of the enzyme-resistant substituted fragments will be larger when the acetyl substituents are more clustered. The mass results of the enzyme digested hydrolysates determined

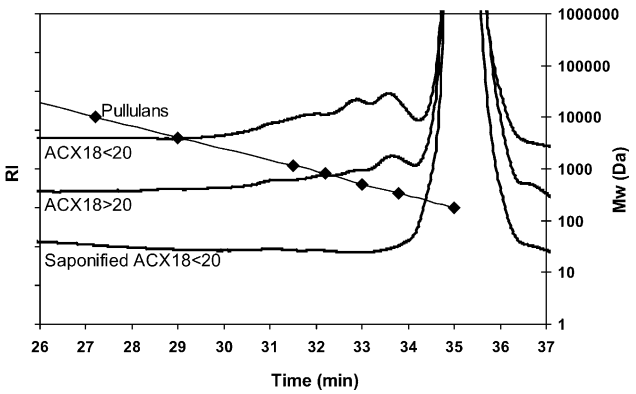

Fig. 5. HPSEC elution profiles of the α-amylase and amyloglucosidase hydrolysates of amylose populations isolated from large and small size granule fractions of acetylated XuShu18 starch (AC18 > 20, amylose isolated from large size granule fraction (>20 μm) of acetylated XuShu18 starch).

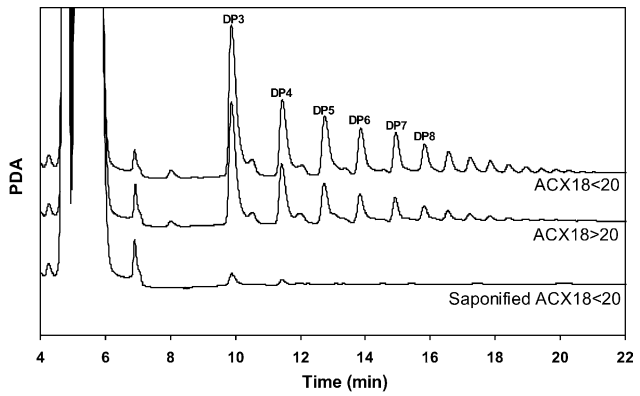

Fig. 6. HPAEC elution profiles of the α-amylase and amyloglucosidase hydrolysates of amylose populations isolated from large and small size granule fractions of acetylated XuShu18 starch (AC18 > 20, amylose isolated from large size granule fraction (>20 μm) of acetylated XuShu18 starch).

by MALDI-TOF-MS showed that the enzyme-resistant component maltotriose contains 1 acetyl group, DP4 contains 1 and 2 acetyl groups, and DP5, DP6 and DP7 contains 2 and 3 acetyl groups, respectively (Fig. 7). These results confirm that the enzyme-resistant residues contain at least 1 acetyl group and the hindrance for the enzymes (α-amylase and amyloglucosidase) digestion is caused by acetyl group. However, nothing can be said yet about the precise location of the acetyl group in the amylose molecular structure. Heins et al. (1998) indicated that the O-2 and O-3 position of the glucose residues may be equally substituted while the O-6 position clearly is less substituted in the relatively highly acetylated starches (DS = 0.42–0.81). However, the position of the substituents in the enzyme-resistant fragments carrying two acetyl groups found in the digests from the low-substituted acetylated starches used in our study need to be confirmed by further analysis such as NMR spectroscopy.

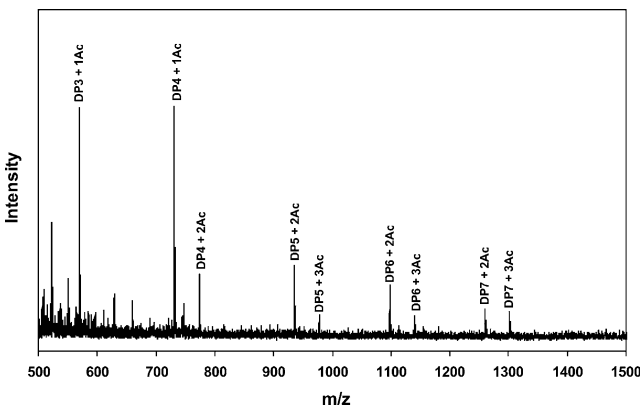

Fig. 7. MALDI-TOF mass spectra of the enzyme (α-amylase and amyloglucosidase) hydrolysates of amylose populations separated from small size granule fractions of acetylated XuShu18 starch (Ac, acetyl group).

The DS of the amylose of acetylated potato and sweet potato starches are about 0.08 and 0.06 corresponding, on average, to 1 acetyl group in every 13 and 17 glucose units, respectively. However, fragments released after enzyme digestion, showing 1–3 acetyl groups present in 3–7 glucose units, clearly indicating that the acetyl groups were unevenly distributed in amylose. It was also shown that this distribution depends on the granule size and on the starch origin as well.

4. Concluding remarks

The degree of acetylation of the starch isolated from differently sized granule fractions differed significantly: the DS increased with decreasing starch granule size dimension. The DS of the isolated amylose populations of differently sized granule fractions remains constant while the DS of the isolated amylopectin populations showed the same trends as the total acetylated starch themselves: DS increased with decreasing granule size dimension. From the fact that amylose is mainly located in amorphous region and the branched chains of amylopectin are in crystalline region, and the specific surface area of starch granule increases with decreasing starch granule dimension, we conclude that acetylation occurs in all amorphous regions and only in the outer lamella of crystalline regions. Enzymatic digestion showed that α-amylase, β-amylase and amyloglucosidase were hindered by acetyl groups. Although the amylose populations isolated from differently sized granule fractions have similar DS, they clearly showed different susceptibility to enzymatic digestion. Enzyme-resistant residues with higher DP and higher β-limit dextrin values were present in the amylose populations isolated from small size granule fractions. This indicates that the distribution of the acetyl group over the glucan backbone is more heterogeneous and also close to the non-reducing ends in the amylose populations isolated from small size granule fractions.

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