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Acetyl substitution patterns of amylose and amylopectin populations in cowpea starch modified with acetic anhydride and vinyl acetate

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Abstract

To study the effect of reagent type on the distribution pattern of acetyl groups in acetylated cowpea starch, amylose and amylopectin populations were isolated from the starch granules after modification to a low degree of substitution (DS < 0.1) with acetic anhydride and vinyl acetate, respectively. Slowly reacting reagent vinyl acetate resulted in higher DS values for the amylopectin populations when compared to the rapidly reacting reagent acetic anhydride. The two reagents had similar effects on the acetylation level of amylose, suggesting that the amorphous regions of granules were easily accessible for both reagents. The acetyl substitution patterns were analyzed by enzymatic degradation followed by characterization of the obtained fragments using chromatographic and mass spectrometric techniques. The distributions of acetyl groups along the amylose and amylopectin chains were more clustered for modification with vinyl acetate as compared with modification with acetic anhydride. Between the two acetylation types, pronounced differences in the acetyl substitution patterns were observed for the large fragments obtained after α-amylase digestion; only slight differences were exhibited for the small fragments obtained by exhaustive enzymatic digestion of amylose and amylopectin populations.

Keywords: Cowpea starch; Amylose; Amylopectin; Acetic anhydride; Vinyl acetate

1. Introduction

It has been almost a hundred years since commercial production of starch for food and industrial applications was initiated. Two major polymeric components—amylose and amylopectin, play important roles in the structure, characteristics and properties of the different starch sources (Luallen, 2004). Amylose molecules are essentially linear and are comprised predominately of α-1,4 linked D-glucose units with a limited number of α-1,6 branching points (Seib, 1997; Bertoft, 2004). Amylose was considered to exist mainly in the amorphous region of starch granules (Luallen, 2004). Differing significantly from amylose, and having an average molecular weight about 100–1000 times that of amylose (Seib, 1997; Vermeylen, Goderis, Reynaers, & Delcour, 2004), amylopectin molecules are highly branched and are constructed of a large number of short α-1,4 linked D-glucose chains, arranged in clusters and linked by α-1,6 bonds to longer chains which transverse two or more clusters (Hizukuri, 1996; Seib, 1997; Thompson, 2000; Bertoft, 2004; Vermeylen et al., 2004). The linear arrays of double helices, formed by two neighboring chains in a cluster, alternate with clusters of branch points in the radial direction of the granule (Seib, 1997). These alternating zones of differing densities of amylopectin (Seib, 1997) make the granules both firm and flexible, which might be essential for being an energy reserve in plants. Starch modifications are

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a means of altering the structure and affecting the hydrogen bonding of amylose and amylopectin in a controllable manner to enhance and extend starch application. When low levels of alterations take place in the molecules, only slight or no change can be observed in the superficial appearance of the granule (Taggart, 2004). Following cross-linking, esterification and etherification are the second most important modifications in the starch industry (Taggart, 2004). Acetylated starches are the most typical starch ester in the market (Fleche, 1985) and they are used in many food products, such as bakery, frozen, canned foods and white salted noodles (Chen, Schols, & Voragen, 2004), to improve texture, stability and appearance. Starch acetates are also used as adhesives, and acid pH-resistant binders in the food industry, and as sizing agent in paper manufacture or textiles (Fleche, 1985).

The reagents used for preparation of starch acetate are normally acetic anhydride or vinyl acetate (Seib, 1997). Our previous research (Huang, Schols, Jin, Sulmann, & Voragen, 2006b) on the effect of reagent type on the properties of acetylated granular starches showed that the degree of substitution (DS) differed for the differently sized starch granule fractions when the starch had been acetylated by the rapidly reacting acetic anhydride. With the slowly reacting vinyl acetate, no difference in DS of the differently sized granule fractions was observed. Modification with vinyl acetate resulted in higher peak viscosity and swelling volume compared to acetic anhydride. However, the differences in DS values between the two types of acetylation were minor. Thus the substitution pattern was believed to be more important on the properties of acetylated starch. In this study, amylose and amylopectin populations were isolated from two types of acetylated cowpea starch samples, and enzymatic digestion in combination with chromatographic and mass spectrometric techniques were used to study the substitution pattern at molecular level to understand the effect of reagent type (acetic anhydride vs. vinyl acetate).

2. Materials and methods

2.1. Materials

Cowpea starch was prepared in the laboratory as reported previously (Huang et al., 2006a). Two types of acetylated cowpea starch samples were prepared by AVEBE Food Innovation Centre (Veendam, The Netherlands). Acetylated starch was prepared in granular form by reaction of starch in aqueous suspension with acetic anhydride and vinyl acetate, respectively. Both reagents were added in 0.088 moles per mole glucose residue of starch. The acetylated starches were fractionated into two granule size fractions: one larger than 20 μm and one smaller than 20 μm (Huang et al., 2006b). Since there were only slight differences in the volume mean diameters and pasting behaviours between two fractions and the amount of the large sized fractions are not sufficient for further investigations, only the smaller than 20 μm fractions with DS of 0.058 and 0.062 for modification with acetic anhydride and vinyl acetate (Huang et al., 2006b) were used for this study.

α-Amylase (EC 3.2.1.1) (product no. 10069, from *Bacillus subtilis*, 393 U/mg) and β-amylase (EC 3.2.1.2) (product no. 10100, from *barley*, 22 U/mg) were purchased from Fluka (Switzerland). Pullulanase (EC 3.2.1.41) (M2, from *Bacillus licheniformis*, 400 U/ml) was obtained from Megazyme (Ireland). Amyloglucidase (EC 3.2.1.3) (A9268, from *Aspergillus oryzae*, 1400 U/ml) was purchased from Sigma (USA).

α-Amylase was dissolved in millipore water, β-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 make solutions containing 0.38, 0.22, 0.22 and 0.14 U/μL, respectively.

2.2. Isolation of amylose and amylopectin

Amylose and amylopectin populations were isolated from acetylated cowpea starch using the aqueous leaching method according to Chen et al. (2004). The purity of isolated amylose and amylopectin was checked with high-performance size-exclusion chromatography (HPSEC) after pullulanase digestion according to Kobayashi, Schwartz, and Lineback (1985).

2.3. Determination of degree of substitution

Four milligrams of samples was saponified in 150 μL of 0.1 mol/L NaOH for 2 h at room temperature and neutralized with 150 μL of 0.1 mol/L citric acid. The amount of released acetate was determined using the EnzyPlus Acetic Acid test kit (Diffchamb, Sweden). The DS is calculated as molar substitution (mole acetate/mole glucose).

2.4. Enzymatic digestion

Five milligrams of acetylated amylose or amylopectin samples was saponified with 150 μL of 0.02 mol/L NaOH for 2 h at room temperature and neutralized with 150 μL of 0.02 mol/L acetic acid. Amylose samples were submitted to α-amylase, β-amylase, or combined α-amylase and amyloglucidase digestion according to Chen et al. (2004). Amylopectin samples were submitted to α-amylase, β-amylase, pullulanase, or combined pullulanase, α-amylase and amyloglucidase digestion according to Chen, Huang, Suurs, Schols, and Voragen (2005). The β-limit value, defined as the relative amount of maltose formed during β-amylase digestion, was calculated from HPAEC peak and the sample concentration, using pure maltose as external standard.

2.5. HPSEC, HPAEC and MALDI-TOF-MS

HPSEC (high-performance size-exclusion chromatography) was performed on a ThermoFinnigan (USA) HPLC, with three TSK gel columns (7.8 mm ID × 30 cm per

column) in series (G4000PW$_{XL}$, G3000PW$_{XL}$, G2500PW$_{XL}$; 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 pullulans (Polymer Laboratories, UK). The data were processed using ChromQuest (ThermoFinnigan, USA).

HPAEC (high-performance anion-exchange chromatography) was performed on a Dionex (USA) HPLC system. The system was equipped with a quaternary gradient pump, an autosampler completed with a helium degassing unit and an EC detector in the PAD mode. A CarboPac PA1 column (2 × 250 mm) (Dionex, USA) with a CarboPac PA1 guard column (2 × 50 mm) (Dionex, USA) was operated at a flow rate of 0.3 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. The data were processed using Chromeleon (Dionex, USA) software.

MALDI-TOF-MS (matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry) was carried out using an Ultraflex workstation (Bruker Daltonics GmbH, Germany) equipped with a nitrogen laser of 337 nm. The mass spectrometer was selected for positive ions. After a delayed extraction time of 100 ns, the ions were accelerated to a kinetic energy of 20 kV. Hereafter, the ions were detected in the reflector mode. The lowest laser power required to obtain good spectra was used. The mixture of 1 μL sample and 1 μL of matrix was dried on a sample plate. The matrix solution was prepared by dissolving 9 mg of 2,5-dihydroxybenzoic acid (DHB) in a 1 mL mixture of acetonitrile/water (300 μL:700 μL). External calibration was performed using a mixture of maltodextrins (M_w range 400–3500 Da).

3. Results and discussion

3.1. Degree of molar substitution of amylose and amylopectin populations isolated from cowpea starch modified with acetic anhydride and vinyl acetate, respectively

It is of primary importance to determine the amount of substituents because this affects the functional properties of the starch considerably. The degree of substitution (DS) is the average number of hydroxyl groups on the D-glucosyl units that have been substituted (Bertoft, 2004). Amylose and amylopectin fractions were isolated from two types of acetylated cowpea starch samples using acetic anhydride and vinyl acetate as reagents resulting in DS values of 0.058 and 0.062, respectively (Huang et al., 2006b). The purities of these fractions were checked enzymatically. After treatment with pullulanase, the elution profiles of amylose populations were similar as the un-treated counterparts. All amylopectin populations were degraded to smaller fragments by pullulanase digestion and no other peaks were detected in the HPSEC elution profiles. Therefore, the amylose and amylopectin populations isolated from two types of acetylated cowpea starch were considered to be pure and used for further studies.

For both acetylation types, the DS values of the amylose populations were much higher than those of the amylopectin populations (Table 1), indicating that the amorphous phase was more accessible for chemical reaction than the crystalline phase. This is in agreement with previous results obtained for potato and sweet potato starches modified with acetic anhydride (Chen et al., 2004). Similar findings have been reported for starch ether. The molar substitution (MS) of the amorphous domains was higher than that of the crystalline parts (van der Burgt et al., 2000a; van der Burgt et al., 1999), and a higher substitution of the amylose fraction was also observed in methylated granular starch (Steeneken & Woortman, 1994; van der Burgt et al., 2000b).

Modification with vinyl acetate resulted in higher DS than with acetic anhydride when both reagents were added in the same mole amount per glucose residue of starch (Huang et al., 2006b). Different acetylation levels were also exhibited in the amylose and amylopectin populations. The DS of amylose isolated from cowpea starch modified with acetic anhydride was higher than that of amylose isolated from starch acetylated with vinyl acetate. The opposite result was obtained for the corresponding amylopectin populations. The difference in DS between the two types of acetylated amylopectin was found to be more pronounced than that between the two types of acetylated amylose: it is the higher acetylation level in amylopectin that explains the higher DS of the parental starch modified with vinyl acetate. Since vinyl acetate reacts more slowly it can penetrate further in the starch granule and react with more hydroxyl groups of the glucosyl residues in amylopectin molecules than the rapidly reacting acetic anhydride. Higher acetylation levels in amylopectin population may contribute to the higher swelling volume and peak viscosity of the parent starch acetate modified with vinyl acetate as compared with modification with acetic anhydride (Huang et al., 2006b).

Smaller effects of reagent types on the acetylation levels were found for the amylose populations. Glucose hydroxyl groups in the amorphous domains of granules had similar relative reactivity as hydroxyl groups in dissolved starch

Table 1
Degree of molar substitution and β-limit values of amylose and amylopectin populations isolated from cowpea starch modified with acetic anhydride and vinyl acetate, respectively

Sample	Degree of molar substitution		β-Limit value (%)	
	Amylose	Amylopectin	Amylose	Amylopectin
Acetic anhydride	0.099	0.029	40	55
Vinyl acetate	0.092	0.039	37	47
Saponified	–	–	86	63

solution as has been reported for highly methylated potato starch with DS up to 0.8 (Steeneken & Woortman, 1994).

Both types of acetylation resulted in a higher substitution level in amylose than in amylopectin. However, the distribution of the amount of acetyl groups over amylose and amylopectin was not the same: for cowpea starch composed of 25.8% of amylose (Huang et al., 2006a), around 55% of the total acetyl groups was present in the amylose population when modified with acetic anhydride; while about 45% of all acetyl groups was present in the amylose population when acetylated with vinyl acetate.

3.2. Distribution of acetyl groups over amylose populations isolated from cowpea starch modified with acetic anhydride and vinyl acetate, respectively

3.2.1. α-Amylase digestion

In addition to the DS, the substitution pattern on the D-glucosyl residues within the starch components (amylose and amylopetin) is of interest. The introduced acetyl groups act as barriers to amylase attack (Chen et al., 2004), and pure and well-characterized enzymes can be used to investigate the distribution pattern of acetyl groups. α-Amylase is an *endo*-hydrolase which cleaves α-1,4-glucosidic linkages in a random fashion (Mischnick, 2001). From the HPSEC analyses of the degradation products obtained after α-amylase hydrolysis (Fig. 1A), it is obvious that the degradability of the amylose sample isolated from cowpea starch modified with acetic anhydride (AA AM) was lower than that of the vinyl acetate counterpart (VA AM). This can be partly explained by the fact that slightly more acetyl groups were present in AA AM than in VA AM.

The structural characterization of the fragments was carried out using MALDI-TOF-MS. Only fractions smaller than DP17 were observed by MALDI-TOF-MS oligomer-analysis. In Fig. 2, fractions of a certain DP are normalized to 100% according to Mischnick (2001). Although the peak intensity may not completely correlate to the concentration for oligomers with different DP, the relative ratios within one DP are only slightly distorted in MALDI-TOF-MS (Mischnick, 2001). Clear differences in the distribution of acetyl groups in the oligosaccharide fractions between AA AM and VA AM samples can be observed. The largest unsubstituted oligomer was DP8 for both AA AM and VA AM; the smallest substituted unit was DP4 with 1 acetyl group for AA AM, and DP3 with 1 acetyl group for VA AM. The highest substituted fractions were DP6 with 2 acetyl (DS 0.33), and DP4 with 2 acetyl (DS 0.5) for AA AM and VA AM, respectively. More higher substituted fragments in VA AM hydrolysates suggests that the

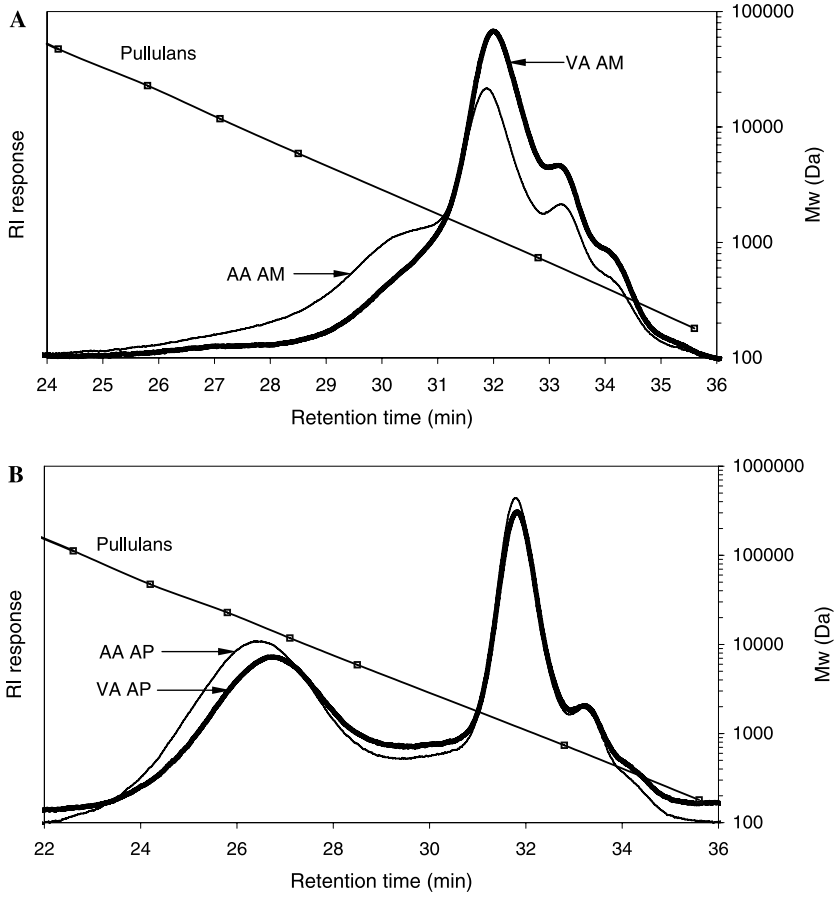

Fig. 1. HPSEC elution profiles of the α-amylase hydrolysates of (A) amylose (AM) and (B) amylopectin (AP) isolated from cowpea starch modified with acetic anhydride (AA) and vinyl acetate (VA), respectively. RI: refractive index.

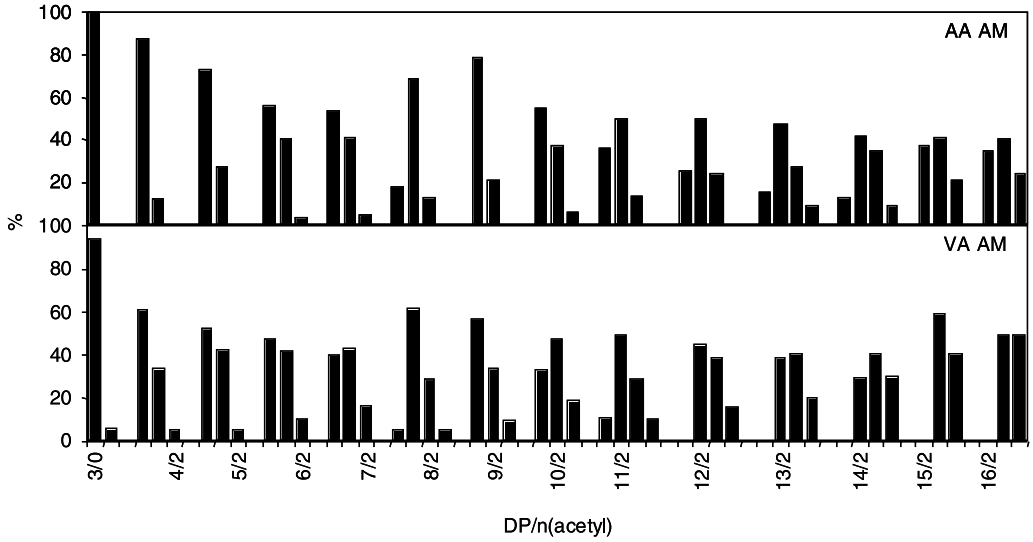

Fig. 2. Distribution of acetyl groups in the oligosaccharide fractions in the α-amylase hydrolysates of amylose (AM) isolated from cowpea starch modified with acetic anhydride (AA) and vinyl acetate (VA), respectively. The total signal intensities of the oligomers in a certain DP are normalized to 100%. DP: degree of polymerization. 3/0: unsubstituted trimer. 4/2: disubstituted tetramer.

distribution of acetyl group along the chains of VA AM was more blockwised. Besides the degree of substitution, the substitution pattern was a very important factor in the degradation by α-amylase has been reported for methylated starch by Heins, Kulicke, Käuper, and Thielking (1998).

3.2.2. β-Amylase digestion

β-Amylase splits of maltose from α-1,4-glucans starting from the non-reducing ends of the chains, but cannot attack α-1,6-linkages, neither by-pass them (Butler, van der Maarel, & Steeneken, 2004). The β-limit value, defined as the relative amount of maltose formed during β-amylase digestion (Bertoft, 2004), can be used to estimate substituents along the amylose chains. The results are summarized in Table 1. It can be seen that the saponified amylose sample was not totally converted to maltose, which might be due to the non-linear nature of the amylose population from cowpea starch, as is also known for amylose from other starches (Bertoft, 2004).

As expected, the β-limit value decreased after acetyl group has been introduced into the amylose molecules. Although the DS of VA AM was lower, the degradability by β-amylase was lower, indicating that acetyl groups were located closer to the non-reducing end of VA AM chains compared to those of AA AM.

3.2.3. Combined digestion with α-amylase and amyloglucosidase

To further investigate the acetyl distribution patterns in AA AM and VA AM, the samples were submitted to combined enzymatic digestion with α-amylase and amyloglucosidase. Amyloglucosidase is an *exo*-enzyme and release glucose from the non-reducing end of the glucan chains by attacking both α-1,4- and α-1,6-glucosidic linkages (Mischnick, 2001). From Fig. 3A, it can be seen that the saponified amylose sample was converted to a single product—glucose. The oligomers present in the digests of acetylated amylose samples after combined α-amylase and amyloglucosidase attack arose from incomplete degradation of amylose due to the presence of acetyl groups. The unsubstituted oligomers up to DP8 and substituted fractions present in the α-amylase hydrolysates could be further degraded by amyloglucosidase as revealed by MALDI-TOF-MS (Fig. 4). The fragments remaining were DP3–9 with 1–4 acetyl groups, and DP3–8 with 1–3 acetyl groups for AA AM and VA AM, respectively. The highest substituted fragment was DP3 with 2 acetyl groups (DS 0.67) for both AA AM and VA AM. The DS values of the enzymes resistant fragments were higher than the DS values of the parent amylose samples, suggesting uneven distribution of the acetyl groups along the amylose chains.

The differences in the substitution pattern between AA AM and VA AM revealed by the mass spectrum of the digest of combined enzymatic hydrolysis was not so pronounced as in the mass spectrum obtained from the sole α-amylase digest. The further degradation of the oligosaccharides by amyloglucosidase after the α-amylase attack reduced the difference of the fragments in the hydrolysates. This suggests that the effect of reagent types on the substitution pattern over the amylose chains could be better demonstrated at the level of fragments with broader chain length range obtained from α-amylase hydrolysis than at the level of the much smaller oligomers obtained from combined digestion with α-amylase and amyloglucosidase.

3.3. Distribution of acetyl groups over amylopectin populations isolated from cowpea starch modified with acetic anhydride and vinyl acetate, respectively

3.3.1. Enzymatic digestion by α-amylase, β-amylase and pullulanase

Similar approaches as discussed above were used to explore the substitution pattern of the amylopectin compo-

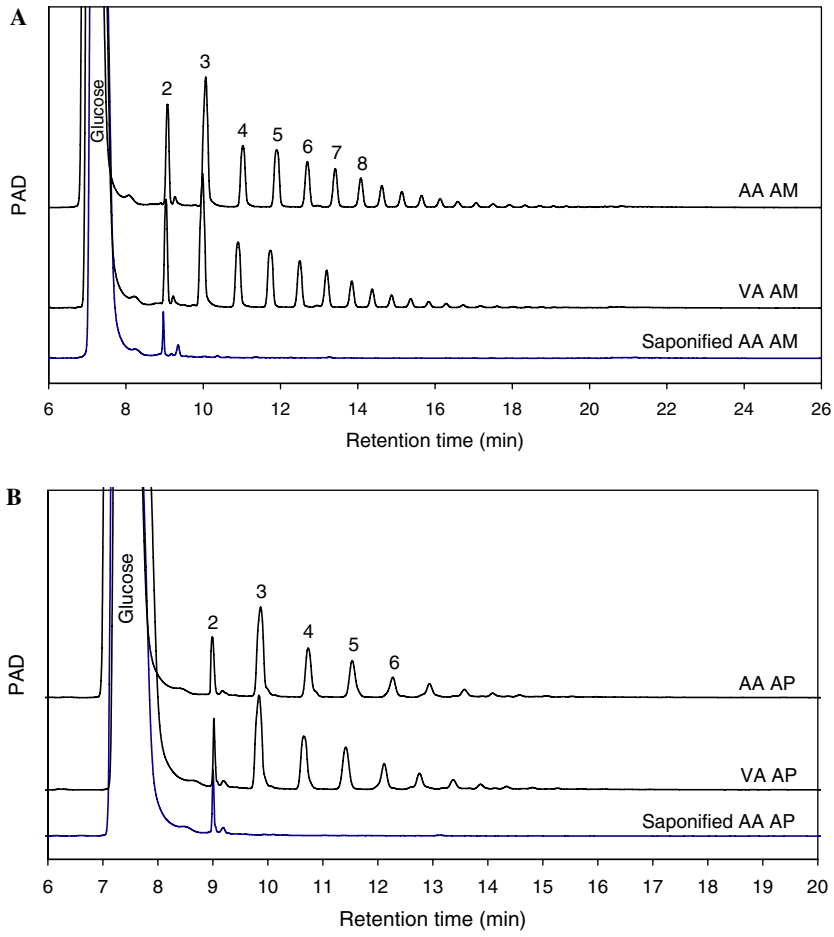

Fig. 3. HPAEC elution profile of (A) the α-amylase and amyloglucosidase hydrolysates of amylose (AM) and (B) the pullulanase, α-amylase and amyloglucosidase hydrolysates of amylopectin (AP) isolated from cowpea starch modified with acetic anhydride (AA) and vinyl acetate (VA), respectively. Numbers indicate degree of polymerization. PAD: pulsed amperometric detection.

nent. The difference in the degradability by α-amylase (Fig. 1B) between two amylopectin samples isolated from cowpea starch modified with acetic anhydride (AA AP) and the vinyl acetate counterpart (VA AP) was not so pronounced as their difference in DS. The acetyl groups in AA AP exhibited more hinder to the attack of α-amylase than those in VA AP, although the DS of AA AP was lower, suggesting a more dense distribution of acetyl groups over the internal chains of AA AP. The DP of unsubstituted oligomers in the hydrolysates was higher for amylopectin (Fig. 5) than for amylose, due to the fact that the DS of amylopectin was much lower and the fact that branch points act as barriers to α-amylase attack. For AA AP and VA AP, the smallest substituted unit was DP6 with 1 acetyl group and DP5 with 1 acetyl group, respectively. Of oligomers of DP10 and higher, fragments with 2 acetyl groups were present in VA AP, but not in AA AP, indicating that the substitution pattern along the chains of VA AP was more clustered and that more acetyl groups were located near the branch points.

As was found for the acetylated amylose samples also the acetylated amylopectin samples showed lower β-limit values than the saponified amylopectin sample due to the presence of acetyl groups (Table 1). More maltose released from AA AP is evidence for less acetyl groups being distributed along the external chains of AA AP. This result is in agreement with the observation from α-amylase digestion that more acetyl groups presented in the internal chains of AA AP.

The HPSEC elution profiles of the pullulanase hydrolysates suggest that the acetyl groups in VA AP showed more inhibition than those in AA AP (Fig. 6). This indicates more substituents in the vicinity of the branch points of VA AP. Also van der Burgt et al. (2000a) reported that methylation takes place preferably at the branched regions of amylopectin.

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

AA AP and VA AP were hydrolyzed with a combination of pullulanase, α-amylase, and amyloglucosidase. Saponified amylopectin sample produced almost exclusively glucose after combined enzymatic degradation (Fig. 3B). The amounts of enzyme resistant oligomers were much less for acetylated amylopectin samples than those of the amylose counterparts, due to the lower substitution level of amylopectin populations. The enzyme resistant fractions contained fragments of DP3–8 and

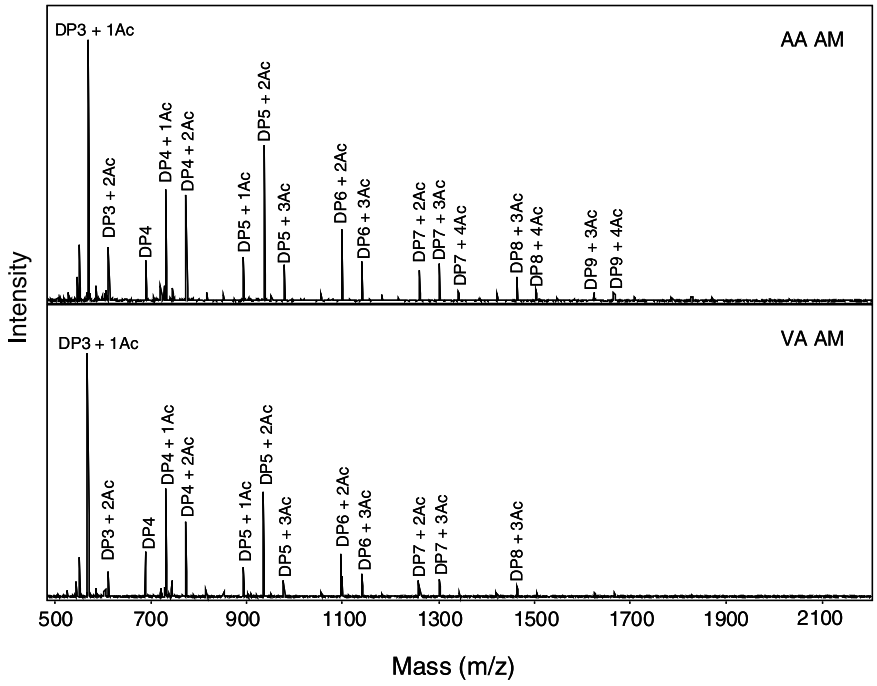

Fig. 4. MALDI-TOF mass spectrum of the α-amylase and amyloglucosidase hydrolysates of amylose (AM) isolated from cowpea starch modified with acetic anhydride (AA) and vinyl acetate (VA), respectively. DP: degree of polymerization. Ac: acetyl group.

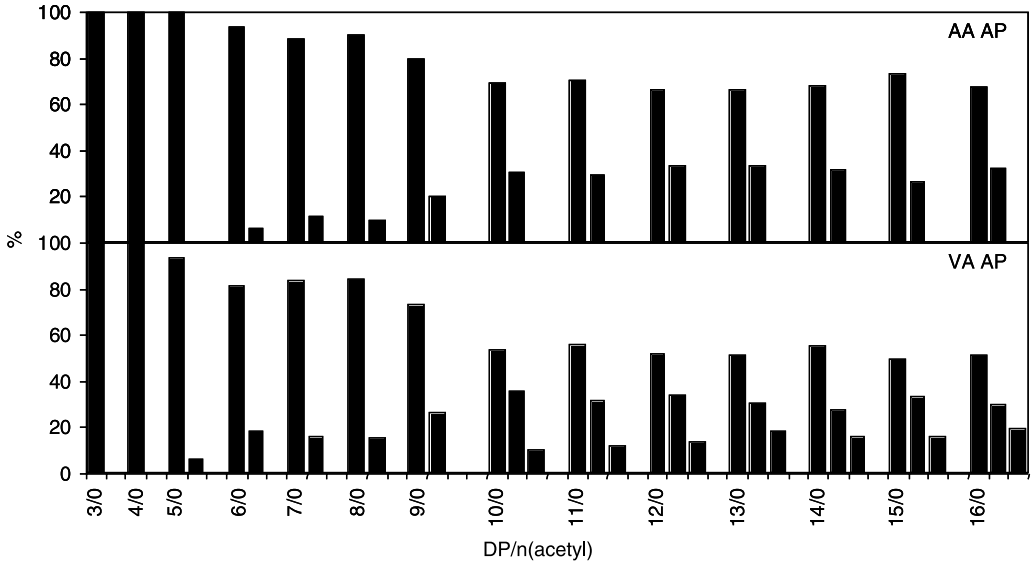

Fig. 5. Distribution of acetyl groups in the oligosaccharide fractions in the α-amylase hydrolysates of amylopectin (AP) isolated from cowpea starch modified with acetic anhydride (AA) and vinyl acetate (VA), respectively. The total signal intensities of the oligomers in a certain DP are normalized to 100%. DP: degree of polymerization. 3/0: unsubstituted trimer.

DP3–9 with 1–3 acetyl groups for AA AP and VA AP, respectively (Fig. 7). The highest DS fragment was DP5 with 3 acetyl groups (DS 0.60) for both AA AP and VA AP. It can therefore be concluded that the acetyl groups were unevenly distributed over both the amylose and amylopectin chains of the two types of acetylated cowpea starch similar as reported for potato and sweet potato starch modified with acetic anhydride (Chen et al., 2004, 2005). It is the organized nature of starch granules that obstructs the acetyl groups in being regularly distributed along the polymer chains. A similar phenomenon has been observed for methylated granular starch (Steeneken & Woortman, 1994; van der Burgt et al., 2000a).

The occurrence of unsubstituted tetramer in the combined enzymatic hydrolysates for all the acetylated samples was puzzling. It can only be stated that the presence of this fraction was due to the presence of acetyl groups in the parent amylose and amylopectin samples, since there were no oligomers presented in the hydrolysates of the corresponding saponified samples.

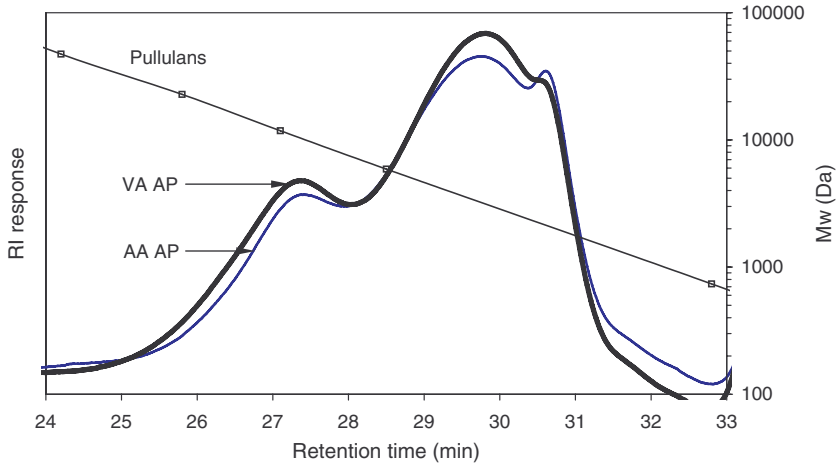

Fig. 6. HPSEC elution profile of the pullulanase hydrolysates of amylopectin (AP) isolated from cowpea starch modified with acetic anhydride (AA) and vinyl acetate (VA), respectively. RI: refractive index.

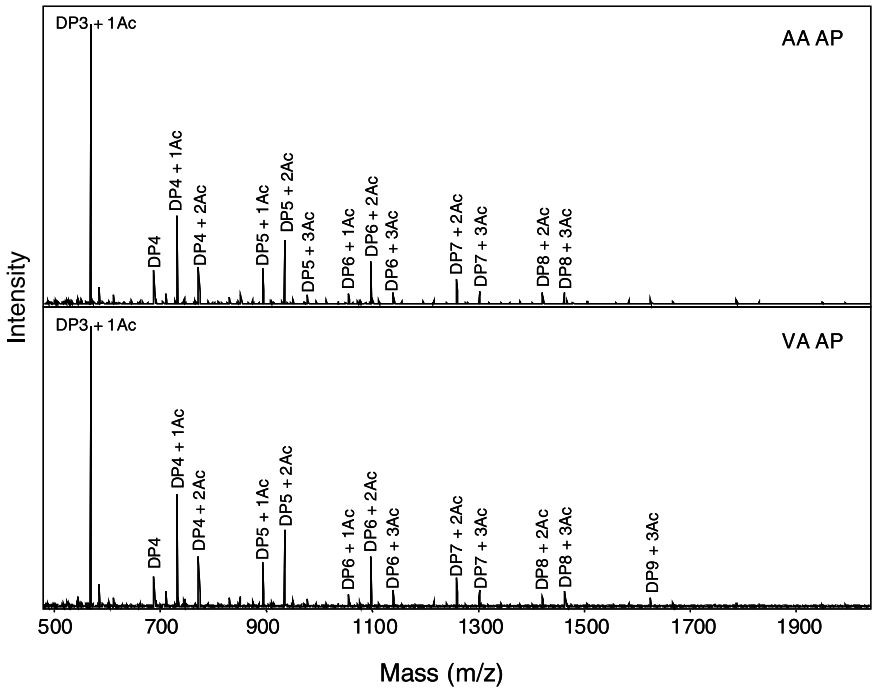

Fig. 7. MALDI-TOF mass spectrum of the pullulanase, α-amylase and amyloglucosidase hydrolysates of amylopectin (AP) isolated from cowpea starch modified with acetic anhydride (AA) and vinyl acetate (VA), respectively. DP: degree of polymerization. Ac: acetyl group.

Enzyme resistant oligomers obtained from AA AP were similar to those from VA AP as exhibited in the MALDI-TOF mass spectrum. For both amylose and amylopectin samples, combined enzymatic degradation reduced the potential to distinguish between differently substituted starch components compared to α-amylase degradation. Thus a rough estimation of the difference in acetyl substitution pattern between two types of acetylation can be made simply by analysing the fragments from α-amylase degradation.

4. Conclusions

For both acetylation types, amylose populations showed a much higher level of acetylation than amylopectin populations. Modification with slowly reacting vinyl acetate resulted in higher DS values for amylopectin and smaller DS difference between amylose and amylopectin compared to the modification with rapidly reacting acetic anhydride.

For both amylose and amylopectin, the oligomers in α-amylase hydrolysates showed clear differences between the two acetylation types as exhibited in the MALDI-TOF mass spectra. The presence of more high DS fragments in digests of vinyl acetate modified starch suggests that acetyl groups were more clustered along the polymer chains modified with vinyl acetate than of those modified with acetic anhydride.

The results of α-amylase, β-amylase and pullulanase digestion reveal that there were more acetyl groups present

in the external chains and in the vicinity of branch points of amylopectin isolated from cowpea starch after acetylation with vinyl acetate than the counterpart obtained from cowpea starch after acetylation with acetic anhydride.

Between two types of acetylation, the difference in acetyl substitution pattern can be demonstrated at the level of fragments with broader chain length range obtained from α-amylase digestion. However, such information could not be observed at the level of too small oligomers obtained from exhaustive enzymatic digestion using α-amylase and amyloglucosidase for amylose, and α-amylase, pullulanase and amyloglucosidase for amylopectin.

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