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Pasting properties and (chemical) fine structure of acetylated yellow pea starch is affected by acetylation reagent type and granule size

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Abstract

Yellow pea starch was fractionated into small and large size granule fractions and then modified with acetic anhydride and vinyl acetate (acetylation after sieving) or first acetylated in the same way and then fractionated into small and large size fractions (acetylation before sieving). Acetylation with different procedures (acetylation *before* or *after* sieving) resulted in different degrees of substitution for small size granule fractions when acetic anhydride was used as reagent. Modification with vinyl acetate gave products with much higher peak viscosities than modification with acetic anhydride for the same granule size and same degree of substitution (DS). The location and distribution of acetyl groups was investigated by analyzing the α-amylase hydrolysates of isolated amylose and amylopectin with chromatography and mass spectrometry. Mass spectra showed that the substituent distribution mainly depended on the type of reagent and was not affected by the granule size. Both amylose and amylopectin were modified in an heterogeneous way and the reactions took place in different regions of the granule. It is postulated that acetylation occurs more homogeneously throughout the granule when vinyl acetate is used as reagent, while the reaction with acetic anhydride to a large extent takes place in the outer lamellae of granule.

Keywords: Yellow pea starch; Amylose; Amylopectin; Acetic anhydride; Vinyl acetate

1. Introduction

Starches from various sources exhibit different granule morphologly (size, shape), molecular structure (amylose and amylopectin fine structures) and composition (amylose content, non-starch components) (Huber & BeMiller, 2001). Granules from a given starch type also consist of a range of sizes. Chemically substituted granular starches play important roles in many industrial applications. The granular structure, preserved throughout the course of derivatization reactions, influences greatly the substitution pattern of the starch components (Bertoft, 2004). Stapley and BeMiller (2003) separated maize, wheat and potato starches into granule subpopulations by sedimentation *after* hydroxypropylation with propylene oxide and found similar degrees of molar substitution for the different size fractions within each starch. In contrast, granule size has been reported to affect the degree of substitution values for acetylated potato and sweet potato starches using acetic anhydride as reagent (Chen, Schols, & Voragen, 2004): potato and sweet potato starches were fractionated by sieving into different size granule fractions after modification with acetic anhydride and it was found that small size granule fractions showed higher DS values than the large size granule fractions. In previous work (Huang, Schols, Jin, Sulmann, & Voragen, 2007), we established the effect of

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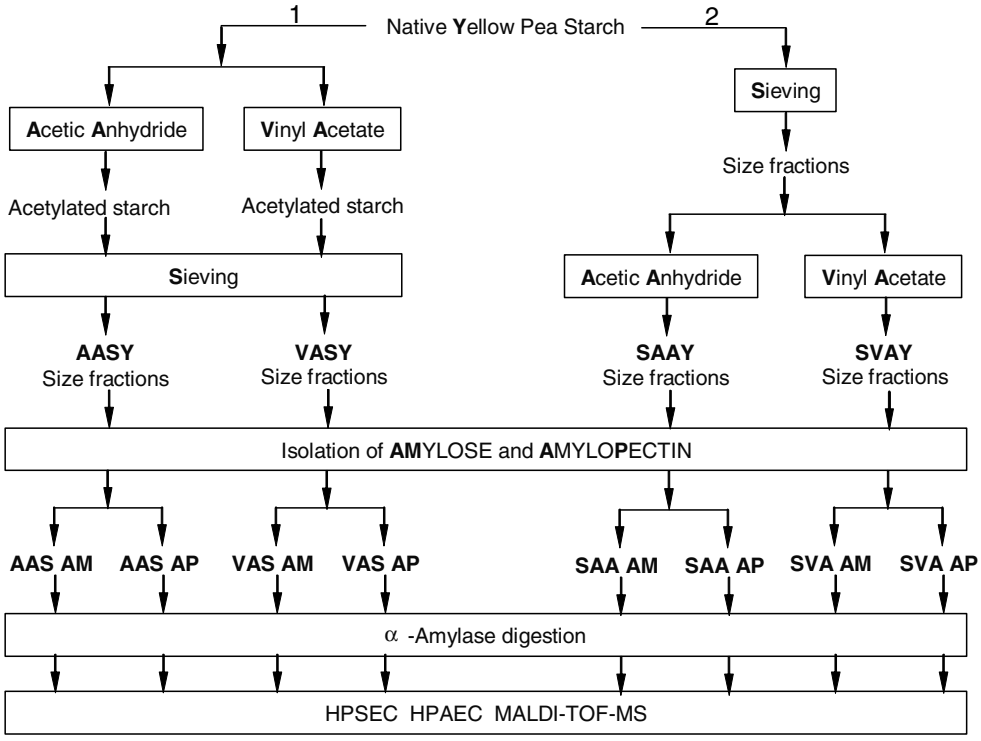

Fig. 1. Schematic overview of the approach followed to reveal the impact of reagent type and granule size on the fine structure of acetylated yellow pea starch.

reagent type on acetylated starches by sieving the starch after the modification process for yellow pea, cowpea and chickpea starches. The DS values differed for the differently sized starch granules acetylated by the rapidly-reacting acetic anhydride, which confirmed the findings of Chen et al. (2004) for potato and sweet potato starches. It was for the first time reported that differently sized granule fractions showed similar DS values when slowly-reacting vinyl acetate was used. In all these cases, the starches were acetylated before fractionation by size. Furthermore, there is no report on the substitution pattern of size fractions for acetylated starch modified with vinyl acetate. In this study two acetylation procedures were employed to determine how reagent type (acetic anhydride vs. vinyl acetate) and differently size granule fractions affect properties and fine structures of acetylated yellow pea starch: the first procedure included fractionation of the starch into small and large size granule fractions and then acetylation of these fractions (acetylation after sieving), and the second procedure included acetylation of the starch and then fractionation into small and large size fractions (acetylation before sieving) (Fig. 1).

2. Materials and methods

2.1. Materials

Yellow pea starch was a gift from COSUCRA (Warcoing, Belgium). Since the purities of both this starting material and its >32 μm granule size fraction were rather low (92.3% and 72.5%, respectively; Huang, Schols, Jin et al., 2007), the starch was further purified in the laboratory by sieving through a series of test sieves (0.250 mm, 125 μm and 71 μm) on a Retsch AS200 digit shaker (Retsch GmbH & Co., Haan, Germany) with deionized water and then dried at 40 °C. The starch contents of the purified starch samples were determined by using the enzymatic Roche starch test kit (Boehringer Mannheim, Darmstadt, Germany). α-Amylase (EC 3.2.1.1) (product number 10069, from *Bacillus subtilis*, 393 U/mg), purchased from Fluka (Switzerland), was dissolved in millipore water to make a solution containing 0.38 U/μL of enzyme.

2.2. Acetylation and separation of differently sized granule fractions

Two procedures were used to prepare acetylated yellow pea starch (Fig. 1). One procedure included acetylation *after* sieving, while in the other procedure acetylation was performed *before* sieving. Yellow pea starch samples were separated by sieving into three fractions: smaller than 20 μm, 20–32 μm and larger than 32 μm. The small (<20 μm) and large (>32 μm) size granule fractions were used in this study. Acetylated yellow pea starch samples were prepared by AVEBE Food Innovation Centre (Veendam, The Netherlands). Acetylation reaction was performed by treating aqueous starch suspensions (38–40%) at pH 7.5–9.0 for acetic anhydride and pH 9–10 for vinyl

acetate at 20–25 °C for about 1–2 h. Sodium carbonate is used as catalyst and buffer in reactions with vinyl acetate. For all the starches, per mole glucose 0.088 moles of reagent were added.

2.3. Particle size distribution and pasting behavior

Particle size distribution was measured in water using a laser diffraction system (H1140, Sympatec Inc., USA). The pasting behaviors of the starches were measured using a Rapid Visco Analyzer (RVA-4, Newport Scientific Pty. Ltd., Australia). These analyses were carried out as described by Huang, Schols, van Soest et al. (2007).

2.4. Isolation of amylose and amylopectin

Amylose and amylopectin populations were isolated from acetylated yellow pea 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).

2.5. Determination of degree of substitution and α-amylase digestion

The degrees of molar substitution (DS) of whole starch samples were determined using the titration method according to Huang, Schols, Jin et al. (2007). The DS values of amylose and amylopectin samples were determined using the EnzyPlus Acetic Acid test kit (Diffchamb, Sweden) according to Huang, Schols, Klaver, Jin, and Voragen (2007). Five milligrams of acetylated amylose or amylopectin samples were submitted to α-amylase digestion according to Chen et al. (2004).

2.6. 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}, G3000 PW_{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).

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, a helium degassing unit and an electrochemical 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 of HPSEC and HPAEC were processed using Chromeleon software (Dionex, USA).

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 in a 1 mL mixture of acetonitrile:water (300 μL:700 μL). External calibration was performed using a mixture of maltodextrins (Mw range 400–3500 Da).

3. Results and discussion

3.1. Characterization of small and large granule fractions of acetylated yellow pea starch samples

3.1.1. Starting materials

Since the purity of >32 μm granule fraction separated from yellow pea starch as studied before was quite low (72.5%; Huang, Schols, Jin et al., 2007), the commercial starch as well as this fraction were further purified in the laboratory by sieving to a purity of 94.8% and 92.5%, respectively. The purity of another starting material, <20 μm granule fraction of purified yellow pea starch, was 95.9%. Eight samples were obtained from two procedures (Fig. 1): SAAY <20; SAAY >32; AASY <20; AASY >32; SVAY <20; SVAY >32; VASY <20; and VASY >32.

3.1.2. Particle size distribution and degree of substitution

The particle size distributions of the eight acetylated yellow pea starch samples are presented in Fig. 2. The four <20 μm granule fractions all showed similar size distribution patterns and their volume mean diameters (VMD) measured in water were all in the range of 19.1–20.8 μm. The granule size distributions of SAAY >32 and SVAY >32 were unimodal with VMD of 30.1 and 31.2 μm, while for AASY >32 and VASY >32 there was a slight shoulder at high granule diameters with higher VMD values (46.9 and 45.0 μm). The shoulder may be due to the presence of fiber-like impurities as observed by microscopy. Due to the purification, the >32 μm granule fraction did not show a much broader peak than <20 μm granule fraction

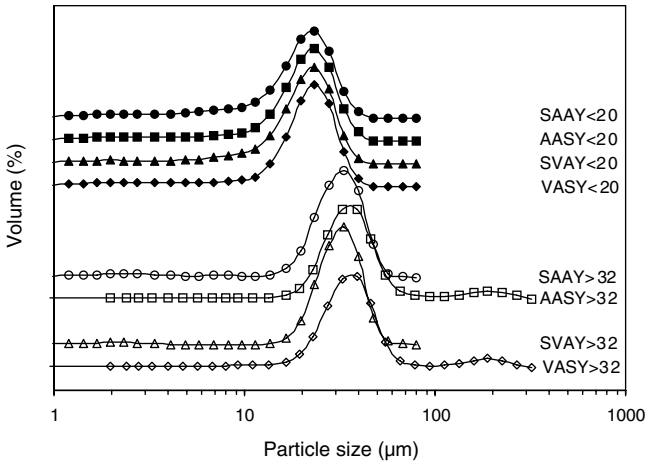

Fig. 2. Particle size distributions of small (<20 μm) and large (>32 μm) granule size fractions of yellow pea starch (Y) modified with acetic anhydride (AA) and vinyl acetate (VA) before and after sieving (S), respectively.

as found in our previous work (Huang, Schols, Jin et al., 2007).

For chemically substituted starches, the degree of substitution (DS) is a basic parameter to get information about reaction efficiency and the amount of substituents introduced. Acetylation with acetic anhydride using different procedures (acetylation *before* and *after* sieving) resulted in different degrees of substitution for the <20 μm granule fractions (Table 1). In the case of acetylation using vinyl acetate, similar acetylation levels were found for all size fractions obtained either before or after sieving. In our previous work, the DS values were also found to differ for differently sized granule fractions acetylated by acetic anhydride but not for the size fractions acetylated by vinyl acetate (Huang, Schols, Jin et al., 2007). It should be noted that, for modification with acetic anhydride, the <20 μm and >32 μm granule fractions that were obtained from the first procedure (acetylation *after* sieving) showed less difference in DS levels compared to the <20 μm and >32 μm fractions obtained from the second procedure (acetylation *before* sieving).

When samples were fractionated after modification, rather than fractionated before modification, small and larger size granule fractions compete for the rapidly reacting reagent as mentioned by Stapley and BeMiller (2003) for hydroxypropylated starches. It was not surprising that the extent of difference in reaction efficiency between the two size fractions was larger when there was a competition for acetic anhydride than when there was no competition. The lower reaction efficiency of the large (>32 μm) granule fraction than that of the small (<20 μm) fraction even when they reacted separately with acetic anhydride (acetylation after sieving) could be explained by the difference in specific surface area of these two fractions (Chen et al., 2004). Assuming that the granule is a sphere and that the densities of small and large granules are uniform, it can be approximated that the diameter of a >32 μm granule is about 1.5 times that of a <20 μm granule, which means that the specific surface area of the <20 μm fraction is about 1.5 times that of the >32 μm fraction. When reacting with same amount of acetic anhydride, the distribution of the reagent over the granule surface is different between these two fractions: a thin layer around small granules and thicker layer around large granules. The thin layer of acetic anhydride around small granules may increase the chance of reaction with starch and thereby reduce the chance of its side reaction with water and consequently result in the high reaction efficiency.

When vinyl acetate was used, which had time to penetrate deeply into granule before reacting, the reaction efficiency was independently from the specific surface area of the granules. Thus, especially for acetylation with acetic anhydride, sieving prior to reaction resulted in greater level of difference in the acetylation level of the differently sized granule fractions which may consequently have impact on the physical properties.

Table 1
Degree of substitution of amylose and amylopectin isolated from small (<20 μm) and large (>32 μm) size granule fractions of yellow pea starch (Y) modified with acetic anhydride (AA) and vinyl acetate (VA) before and after sieving (S), respectively

	Starch (μm)	Degree of substitution[a]		
		Starch[b]	Amylose	Amylopectin
Procedure 1	SAAY <20	0.067c	0.092b	0.042c
Acetylation	SAAY >32	0.061d	0.082d	0.038d
after	SVAY <20	0.067c	0.093b	0.043c
Sieving	SVAY >32	0.066c	0.089c	0.042c
Procedure 2	AASY <20	0.079a	0.097a	0.057a
Acetylation	AASY >32	0.064cd	0.082d	0.041cd
before	VASY <20	0.068cb	0.089c	0.046bc
Sieving	VASY >32	0.071b	0.088c	0.045bc

Values with different letters in the same column are significant different at $p < 0.05$.
[a] Values are means of triplicate.
[b] Values are based on dry matter.

3.1.3. Pasting behavior

The pasting behaviors of acetylated yellow pea starch samples were determined by RVA (Rapid Visco Analyzer). The small size granule fractions exhibited the same pasting temperature, while producing a higher pasting viscosity compared to the corresponding large size granule fractions (Fig. 3). Small and large size granule fractions modified with vinyl acetate exhibited greater peak viscosities than the corresponding size fractions modified with acetic anhydride. Therefore, a higher pasting viscosity of acetylated starch could be obtained by using vinyl acetate instead of acetic anhydride as the reagent and/or choosing smaller size granule fractions.

The DS value of SVAY <20 μm was the same as that of SAAY <20 μm. While SVAY <20 μm showed higher peak viscosity than SAAY <20 μm. These findings confirm that the difference in pasting behavior between two acetylation types is not due to a different DS value (Huang, Schols,

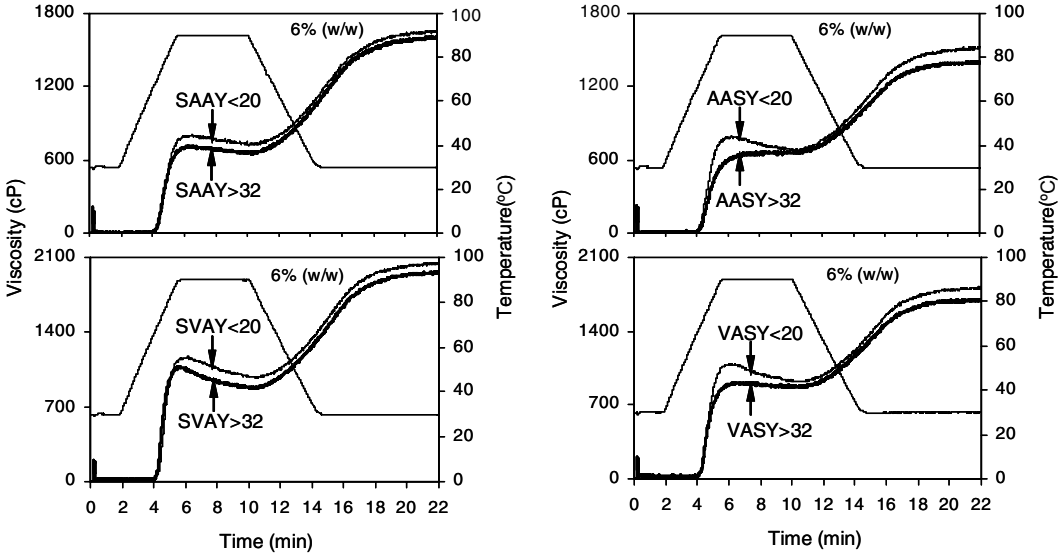

Fig. 3. RVA pasting curves of the small (<20 μm) and large (>32 μm) granule size fractions of yellow pea starch (Y) modified with acetic anhydride (AA) and vinyl acetate (VA) before and after sieving (S), respectively. RVA, rapid visco analyzer.

Jin et al., 2007). One possible explanation is that acetylation with acetic anhydride was limited to the outer lamellae of the granule (Chen et al., 2004), thus a large proportion of the inner granule remained unmodified which contributed to the apparent pasting behavior of the starch to such an extent that it was similar to that of native yellow pea starch (Huang, Schols, Jin et al., 2007). When vinyl acetate was used, reactions may have been located throughout the granule (from outer to inner lamellae), the more homogeneously modified granules showed significantly different pasting viscosity than did native granules (Huang, Schols, Jin et al., 2007). Biliaderis (1982) already postulated this explanation based on enzymatic debranching studies of two modified starches: an acetylated (DS 0.06) smooth pea starch obtained after reaction with acetic anhydride and a commercial hydroxypropyl (DS 0.09) waxy maize starch. The size exclusion patterns showed that the chain length profile of the acetylated derivative nearly matched that of the unmodified pea starch, whereas the profile of the hydroxypropyl waxy maize starch did not match that of its blank. Those results led Biliaderis (1982) to conclude that the acetylation occurred exclusively in certain part of the granule, whereas hydroxypropylation was more uniform throughout the starch granule.

For modification with acetic anhydride, the peak viscosities of AASY <20 μm and SAAY <20 μm were similar in spite of the fact that the acetylation level of AASY <20 μm was much higher than that of SAAY <20 μm. The results suggest that although different modification procedures may induce differences in the acetylation level for the same size fractions, the influence on the pasting behavior was only minor. The peak viscosity of the >32 μm fraction obtained from the first procedure (acetylation *after* sieving) was higher than that of the >32 μm fraction obtained from the second procedure (acetylation *before* sieving) within one acetylating type. This might be due to the lower volume mean diameter values obtained from the first procedure than from the second procedure of the >32 μm fractions.

It may be concluded that for the low acetylation level (DS < 0.1) used in our study the DS value obtained for modification with acetic anhydride was not so important for the pasting behavior of the starch as was the case for modification with vinyl acetate. Thus, it is essential to mention the reagent used when discussing properties of acetylated starch.

3.2. Characterization of amylose and amylopectin isolated from acetylated yellow pea starch samples

3.2.1. Degree of substitution of amylose and amylopectin populations

The purities of isolated amylose and amylopectin were checked by treating them with pullulanase. The elution profiles of acetylated amylose samples were similar to those of their untreated counterparts. The acetylated amylopectin samples exhibited smaller fragments and no high molecular weight material was found to be present. Therefore, the isolated amylose and amylopectin samples were considered to be pure and used for further studies.

For all acetylated yellow pea starch samples, the DS of amylose was much higher than that of amylopectin (Table 1). Similar observations have also been reported for potato and sweet potato starches modified with acetic anhydride (Chen et al., 2004), methylated potato starch (Steeneken & Woortman, 1994; van der Burgt et al., 2000), and hydroxypropylated potato starch (Kavitha & BeMiller, 1998). This suggests a difference in reactivity of amorphous and crystalline regions. It is likely that granule derivatization starts in the more amorphous regions and proceeds

to the more crystalline regions of the granule (Gray & BeMiller, 2004).

The acetylation level of amylose was 1.7–2.2 times that of amylopectin, but it should be realized that, due to the higher weight fraction of amylopectin (Huang, Schols, Jin et al., 2007), 52–58% of the total acetyl groups were present in the amylopectin population of the yellow pea starch.

For modification with acetic anhydride, amylose and amylopectin that were obtained from small size granule fractions showed higher DS than obtained from large size fractions. The DS ratios of <20:>32 μm fractions for amylose were 1.1 and 1.2 for acetylation after and before sieving, respectively. The DS ratios of <20:>32 μm fractions for amylopectin were 1.1 (acetylation after sieving) and 1.4 (acetylation before sieving). The reactivity of amylopectin increased to a larger extent than that of amylose in small size fraction when there was a competition for acetic anhydride between two size fractions (acetylation before sieving) compared to when there was no competition (acetylation after sieving). The clusters of amylopectin chains arrange in the radial direction of the granule (Seib, 1997). More amylopectin chain ends may therefore locate on the surface of small size granule fraction than on the surface of large size fraction.

When yellow pea starch samples were modified with vinyl acetate, there were only small differences between two size fractions and two procedures. The DS of amylose from the <20 μm fraction was slightly higher than that of amylose from the >32 μm fractions for acetylation after sieving. Similarity in DS values between the two size fractions were found for amylose obtained from acetylation before sieving and for amylopectin from both procedures. The observations were in accordance with those of whole starch samples.

Chen et al. (2004) separated potato and sweet potato starch size fractions after modification with acetic anhydride. DS values determined for isolated amylose and amylopectin showed that the acetylation level of amylose was constant for differently sized granule fractions, while that of amylopectin was increased with decreasing granule size. This may be due to the different architecture of granules from different sources.

3.2.2. Distribution of acetyl groups over amylose populations

Investigation of the substitution patterns of amylose and amylopectin isolated from cowpea starch samples modified with acetic anhydride and vinyl acetate has revealed that the difference in acetyl substitution pattern between two types of acetylation can be demonstrated better by α-amylase degradation than by combined enzymatic (α-amylase and amyloglucosidase for amylose samples, α-amylase, pullulanase and amyloglucosidase for amylopectin samples) degradation (Huang, Schols, Klaver et al., 2007). In this study, the distribution and location of acetyl groups on amylose and amylopectin were determined by analyzing the degradation products after α-amylase hydrolysis.

α-Amylase is an endo acting enzyme which hydrolyzes α-(1,4)-D-glucosidic linkages in starch in a random fashion. Its action might be hindered by acetyl substitution (Biliaderis, 1982; Chen et al., 2004). Acetic anhydride modified amylose that was isolated from small size fractions exhibited apparent differences in susceptibility to α-amylase degradation between two modification procedures (acetylation before and after sieving). There were more high molecular weight fragments in the digests of AAS AM <20 than in the digests of SAA AM <20 as revealed by HPSEC elution profiles (Fig. 4). HPAEC analysis showed that much less glucose was produced from AAS AM <20 than from SAA AM <20 (results not shown). Although this might be partly explained by the fact that more acetyl groups were present in AAS AM <20 than in SAA AM <20, the DS difference (0.097 vs. 0.092) was only minor when compared to the difference in the degradation profiles obtained after α-amylase treatment of these two samples. The DS values of SAA AM <20 and AAS AM <20 and were 1.1 and 1.2 times those of SAA AM >32 and AAS AM >32, respectively. The susceptibility to α-amylase degradation was similar for SAA AM <20 and SAA AM >32, but obviously different for AAS AM <20 and AAS AM >32 as shown in the HPSEC elution profiles.

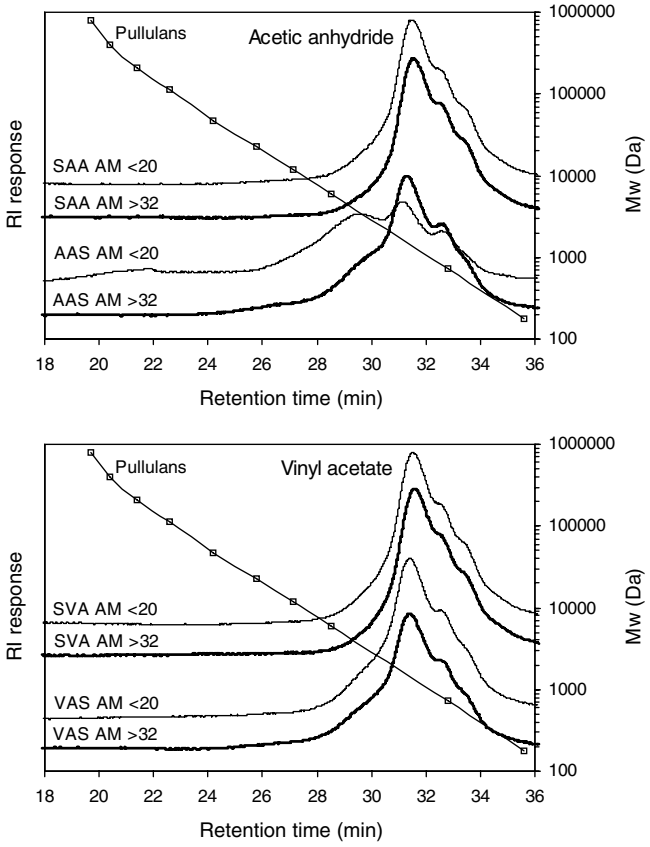

Fig. 4. HPSEC elution profiles of the α-amylase hydrolysates of amylose (AM) isolated from small (<20 μm) and large (>32 μm) granule size fractions of yellow pea starch modified with acetic anhydride (AA) and vinyl acetate (VA) before and after sieving (S), respectively. RI, refractive index.

Similar phenomena have been found by Chen et al. (2004) for both acetylated potato and sweet potato starches, although the DS of amylose samples obtained from different size granule fractions were quite similar. Therefore, it is the substitution pattern rather than the DS that induces the different degradability by α-amylase of acetic anhydride acetylated amylose populations obtained by the two procedures. For modification with vinyl acetate, the four amylose samples showed similarity in the HPSEC and HPAEC (not shown) elution profiles, which was consistent with their DS results.

The location of acetyl groups on amylose molecules was determined by using MALDI-TOF-MS after α-amylase digestion. When modified with acetic anhydride, large size granule fractions (SAA AM >32 and AAS AM >32) showed similar spectra as the corresponding small size fractions (SAA AM <20 and AAS AM <20), although the DS values of small size fractions were higher. The comparison of SAA AM <20 and AAS AM <20 is given as an example to show differences between the two procedures (Fig. 5). Fragments not larger than DP14 were observed by MALDI-TOF-MS oligomer-analysis. Fragments of DP5–6, DP8–10 and DP11–13 with 2, 3 and 4 acetyl groups, respectively, were present in SAA AM <20, but not in AAS AM <20. On the other hand, the fragments of unsubstituted DP8–9 and of DP11–13 with 1 acetyl group were present in AAS AM <20, but not in SAA AM <20. The highest substituted fragments were DP5 with 2 acetyl (DS 0.40), and DP7 with 2 acetyl (DS 0.29) for SAA AM <20 and AAS AM <20, respectively. The observation of lower amount of higher substituted fragments in AAS AM <20 hydrolysates suggests that the acetyl groups were distributed in a more uniform manner along the polymer chain of AAS AM <20 compared to SAA AM <20. In addition, the acetylation level of AAS AM <20 was higher, therefore, acetyl groups seemed to be present on more chains of amylose, which resulted in much less glucose released from AAS AM <20 than from SAA AM <20. There was more glucose present in the α-amylase hydrolysates of SAA AM >32 as revealed by HPAEC analysis (not shown) since its DS was lower than that of SAA AM <20.

For modification with vinyl acetate, there was little difference in the distribution pattern between large size granule fractions (SVA AM >32 and VAS AM >32) and the corresponding small size fractions (SVA AM <20 and VAS AM <20) (spectra not shown). The largest unsubstituted oligomer was DP7, and the smallest substituted unit was DP4 with 1 acetyl group for all four samples, similar to the results for cowpea amylose (DS 0.092), the corresponding oligomers of DP8 and DP3 with 1 acetyl group were found to be present in the α-amylase hydrolysates (Huang, Schols, Klaver et al., 2007). Only slight differences were found between the two procedures, for example, DP6 with 3 acetyl groups and DP10 with 4 acetyl groups were present in SVA AM <20 and SVA AM >32, but not in VAS AM <20 and VAS AM >32. α-Amylase degradation revealed that amylose was not homogeneously modified as indicated by the presence of unsubstituted oligomers and oligomers

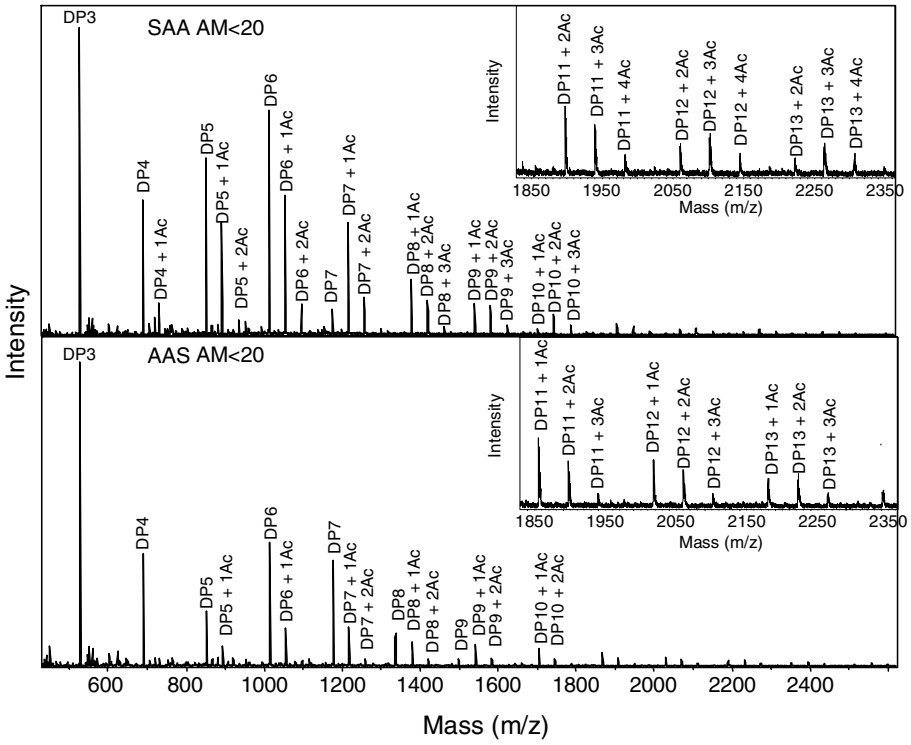

Fig. 5. MALDI-TOF mass spectra of the α-amylase hydrolysates of amylose (AM) isolated from small (<20 μm) granule size fractions of yellow pea starch modified with acetic anhydride (AA) before and after sieving (S), respectively. DP, degree of polymerization; Ac, acetyl group. A zoom of DP11–13 is inserted.

with different substitution levels in the digests of acetylated amylose.

3.2.3. Distribution of acetyl groups over amylopectin populations

Amylopectin samples isolated from small size fractions showed different degradation pattern by α-amylase for the two modification procedures when modified with acetic anhydride. HPSEC elution profiles suggested that α-amylase was hindered to a larger extent by the presence of acetyl groups in AAS AP <20 than by those in SAA AP <20 (Fig. 6). Less maltose was released from AAS AP <20 than from SAA AP <20 as determined by HPAEC (results not shown). This is in accordance with the higher acetylation level of AAS AP <20 (0.057) compared to the DS of SAA AP <20 (0.042). However the distribution pattern on AAS AP <20 was similar to that on SAA AP <20 as shown in the MALDI-TOF mass spectra (Fig. 7). For the first modification procedure, SAA AP <20 showed similar degradability by α-amylase as SAA AP >32 and a similar substitution pattern despite the slight DS difference (0.042 vs. 0.038). For the second procedure, AAS AP <20 was less degraded by α-amylase than AAS AP >32, confirming the results found for acetylated potato and sweet potato starches (Chen, Huang, Suurs, Schols, & Voragen, 2005). In all four amylopectin samples modified with acetic anhydride, unsubstituted fragments of DP3 to 14, and substituted fragments of DP5 to 14 with 1 acetyl group were observed, which was similar to the results for amylopectin of acetylated cowpea starch (Huang, Schols, Klaver et al., 2007).

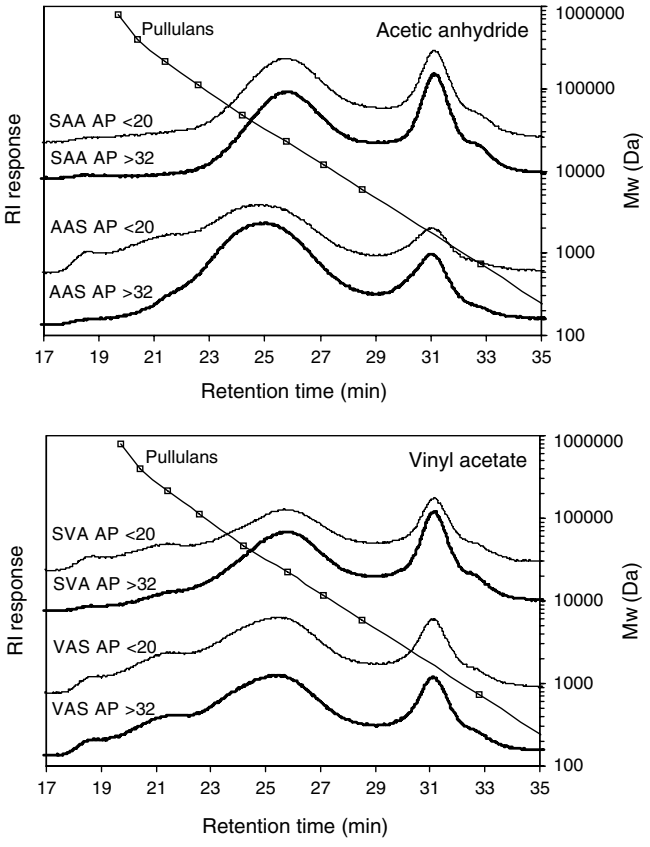

Fig. 6. HPSEC elution profiles of the α-amylase hydrolysates of amylopectin (AP) isolated from small (<20 μm) and large (>32 μm) granule size fractions of yellow pea starch modified with acetic anhydride (AA) and vinyl acetate (VA) before and after sieving (S), respectively. RI, refractive index.

The DP of unsubsitituted oligomers in the hydrolysates was higher in the amylopectin than in the amylose fraction (Fig. 7 vs. Fig. 5), and more high molecular weight fragments were present in the digests of amylopectin than in that of amylose (Fig. 6 vs. Fig. 4). This was due to the fact that the DS of amylopectin was lower and the fact that branch points act as barriers to α-amylase attack (Huang, Schols, Klaver et al., 2007).

With low reagent levels, hydroxyl groups in granular starch selectively react in the amorphous region and on the surface of crystals (Seib, 1997). Our findings may well agree with those of Biliaderis (1982), stating that, at the *granular* level, the acetylation predominantly occurred in certain regions of granules. In addition, our observations also suggest that, at the *molecular* level, the acetylation reaction was limited to hydroxyl groups in certain parts of amylopectin molecules (like branch points).

For modification with vinyl acetate, there was little, if any, difference between small size granule fractions (SVA AP <20 and VAS AP <20) and large size fractions (SVA AP >32 and VAS AP >32) with respect to degradability by α-amylase and substitution pattern. Fragments of DP9 and higher with 2 acetyl groups were present in the hydrolysates of amylopectin obtained from modification with vinyl acetate but not in that of the counterparts obtained after modification with acetic anhydride. For both amylose and amylopectin of yellow pea starch, modification with vinyl acetate resulted in a more blockwise distribution of acetyl groups compared to modification with acetic anhydride, which is in agreement with the observation for cowpea starch (Huang, Schols, Klaver et al., 2007).

These findings, combined with our previously results (Huang, Schols, Klaver et al., 2007), led us to believe that the distribution of acetyl groups differed not only at a molecular level but also at the granular level between modification with acetic anhydride and with vinyl acetate. It is proposed that, for acetylated granular yellow pea starch, acetyl groups are more intensely distributed on and near the granule surfaces when modified with acetic anhydride, while they are more uniformly distributed throughout the granule when modified with vinyl acetate (Fig. 8). Reaction sites within starch granules have been located by utilizing microscopy (Huber & BeMiller, 2001; Gray & BeMiller, 2004). Investigations on phosphorylated potato starch and hydroxypropyl waxy maize starch revealed that phosphoryl chloride (highly reactive) appeared to react most prominently on peripheral surfaces of potato granules, while reaction with propylene oxide analog (less reactive) appeared to occur throughout the granule matrix of waxy maize starch in a more uniform manner. Findings from this

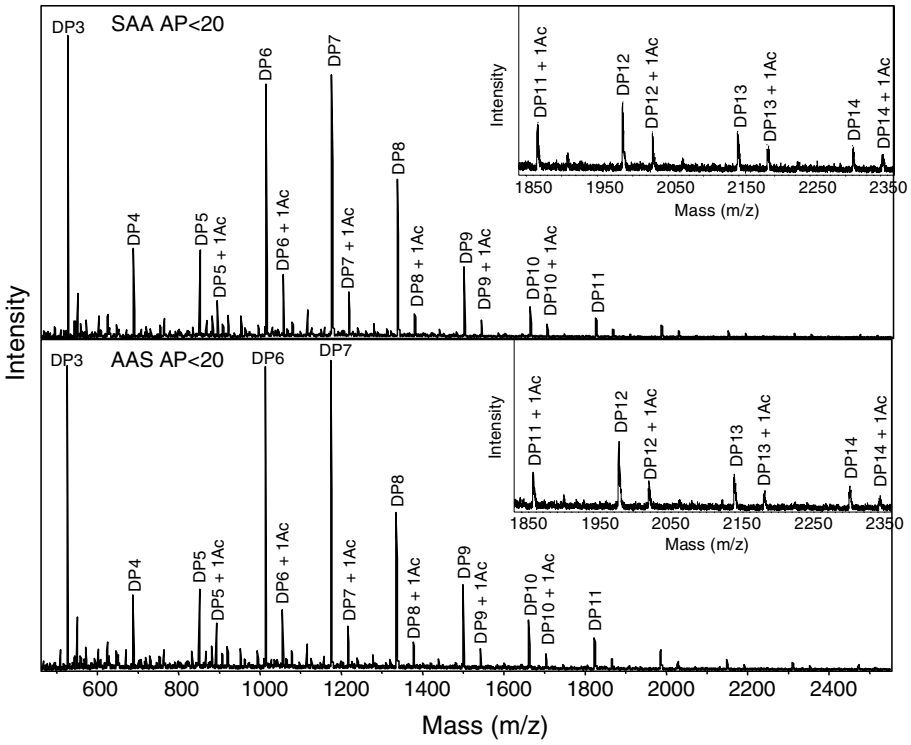

Fig. 7. MALDI-TOF mass spectra of the α-amylase hydrolysates of amylopectin (AP) isolated from small (<20 μm) granule size fractions of yellow pea starch modified with acetic anhydride (AA) before and after sieving (S), respectively. DP, degree of polymerization; Ac, acetyl group. A zoom of DP11–14 is inserted.

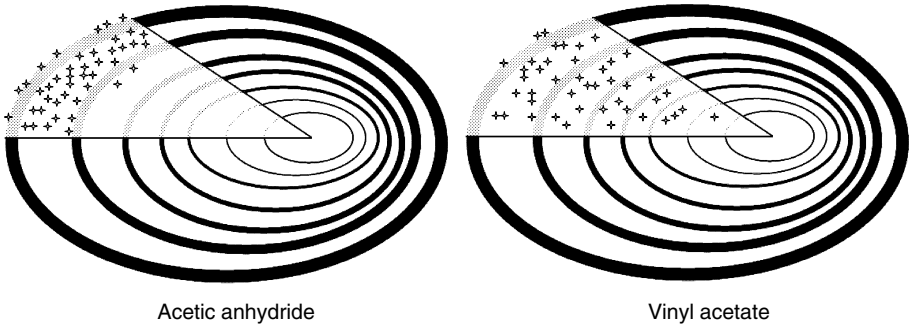

Fig. 8. Proposed model structures for acetylated granular yellow pea starch modified with acetic anhydride and vinyl acetate, respectively. The alternating crystalline (dark) and amorphous (light) lamellae are shown.

study suggest that when acetyl groups distributed only in certain regions of granule, the amount of acetyl groups introduced has little impact on the pasting properties and that the reagent type is an important fact for the functional properties of acetylated starch.

4. Conclusions

When modified with the rapidly reacting acetic anhydride, acetylation *before* and *after* sieving resulted in rather different DS values but similar pasting behaviors for small size granule fractions. This suggests that when the distribution of acetyl groups was limited to certain (outer) regions of the granule, the amount of acetyl groups introduced had little impact on the functional properties of starch. When the slowly reacting vinyl acetate was used, the acetylation level and the pasting behavior of the same size fractions were similar for the two modification procedures. A higher pasting viscosity of acetylated starch could be obtained by using vinyl acetate instead of acetic anhydride as the reagent and/or choosing smaller size granule fractions. Mass spectrometry analyses revealed that the acetyl distribution on amylose and amylopectin molecules depends mainly on the type of reagent and is not affected by the granule size. The importance of these factors for the properties of acetylated starch maybe in the order of: reagent type > granule size > acetylation level. It is essential to mention the reagent used when discussing properties of

acetylated starch. The distribution of acetyl groups differed not only at molecular level but also at granular level between modification with acetic anhydride and vinyl acetate. It is proposed that with acetic anhydride only hydroxyl groups in certain parts of the granule, preferably those in the outer lamellae will react, while acetylation occurs throughout the whole granule when the starch reacts with vinyl acetate.

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