Abstract

The effect of reagent type on the properties of acetylated starches was studied for yellow pea, cowpea and chickpea starches after modification with acetic anhydride and vinyl acetate. Of the same starch, samples modified with vinyl acetate showed higher peak viscosities than those acetylated with acetic anhydride. In addition, the reagents reacted differently towards granules having different sizes as present in un-fractionated starch. After sieving of the acetylated starches, the Degree of Substitution (DS) differed for the differently sized starch granules acetylated by the rapidly reacting acetic anhydride but not for the size fractions obtained from the starches acetylated by the slowly reacting vinyl acetate.

The effect of the reagents on the distribution pattern of acetyl groups was investigated for acetylated cowpea starch. Modification with vinyl acetate resulted in higher DS values for the isolated amylopectin populations when compared to modification with acetic anhydride. The two reagents had similar effects on the acetylation level of the isolated amyllose populations, suggesting that the amorphous regions of granules were easily accessible for both reagents. The acetyl substitution patterns were analyzed by α-amylase degradation followed by characterization of the obtained fragments using mass spectrometric techniques. The distributions of acetyl groups along the amyllose and amylopectin chains were more clustered for starches modified with vinyl acetate compared to modification with acetic anhydride.

Keywords: Acetylated starch, Amylose, Amylopectin, Acetic anhydride, Vinyl acetate

1. Introduction

Modified starch is defined as starch whose hydroxyl group has been altered by a chemical reaction or by enzymatic modification. Low degree of substitution (DS < 0.1) starch acetate has been applied in both food industry and industrial areas for many years (Fleche, 1985). Acetic anhydride is usually used as acetylating agent, while an alternative reagent like vinyl acetate can also be used to prepare starch acetate. Acetic anhydride and vinyl acetate are reagents of different nature and little information is available about the effect of reagent type on the properties of acetylated starch. The acetyl substitution pattern on the starch components (amylose and amylopectin) has been reported only on modification with acetic anhydride (Chen, Schols, & Voragen, 2004; Chen, Huang, Suurs, Schols, & Voragen, 2005). Investigations on yellow pea (Pisum sativum), cowpea (Vigna unguiculata) and chickpea (Cicer arietinum) starches were undertaken to obtain information on the effects of reagent type (acetic anhydride vs. vinyl acetate) on the properties of acetylated starches. 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

Yellow pea starch was a gift from COSUCRA (Warcoing, Belgium). Cowpea and chickpea starches were prepared in the laboratory as reported previously (Huang, Schols, van Soest, Jin, Sulmann, & Voragen, 2006). Two types of acetylated yellow pea, cowpea and chickpea starches were
Acetylated starch samples were fractionated by sieving through test sieves (32 µm and 20 µm) on a Retsch AS200 digit shaker (Retsch GmbH & Co., Haan, Germany) as described in Huang et al. (2006). 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, & Lineback (1985).

2.2. Analytical methods

Moisture content was determined according to Chen, Schols, & Voragen (2003). The analyses of particle size distribution, and pasting behaviour were carried out as described in (Huang et al., 2006a). The determination of degree of molar substitution (DS) and α-amylase digestion of amylose samples were carried out according to Chen et al. (2004). The α-amylase digestion of amylopectin samples was carried out according to Chen et al. (2005). HPSEC (high-performance size-exclusion chromatography) was performed as described in Chen et al. (2005), and MALDI-TOF-MS (Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Mass Spectrometry) were performed as described in Huang, Schols, Klaver, Jin, & Voragen (2006).

3. Results and Discussion

3.1. Characterization of pea starches after modification with acetic anhydride and vinyl acetate

3.1.1 Characterization of starch samples modified with acetic anhydride and vinyl acetate, respectively

The same moles of acetic anhydride and vinyl acetate were added in the derivatisation of yellow pea, cowpea and chickpea starches. However, a higher degree of substitution (DS) was obtained from modification with vinyl acetate than with acetic anhydride for all three starches (Table 1). The peak viscosity was increased after modification with vinyl acetate for all three starches (Fig. 1). With regard to acetylation with acetic anhydride, peak viscosity remained similar for yellow pea, decreased for cowpea and chickpea starches as compared with those of their native counterparts. Acetylation with vinyl acetate resulted in a higher peak viscosity than modification with acetic anhydride. This suggests that the change in viscosity after acetylation depends on the starch source and the nature of acetylated reagent.

3.1.2. Characterization of different size granule fractions of acetylated starch samples

Yellow pea, cowpea and chickpea starches were fractionated by size after reaction with acetic anhydride and vinyl acetate. When modified with acetic anhydride, small size granule fractions of yellow pea, cowpea and chickpea starches had a higher DS as compared with large size granule fractions (Table 1). This could be explained by the fact that smaller size granule fractions have a larger specific surface area (Chen et al., 2004). For acetylated starches prepared with vinyl acetate, small size granule fractions of yellow pea, cowpea and chickpea starches were acetylated at the same level as large size granule fractions. Granule size had a greater effect on DS values when modified with acetic anhydride than with vinyl acetate. Stapley & BeMiller (2003) pointed out that highly reactive reagents like phosphoryl chloride react to a large extent at granule surfaces compared to slowly reacting reagents like propylene oxide. When using the slowly reacting reagents, having time to migrate deeper into the granule matrix, the specific surface areas have less effect on the modification. These observations suggest that the difference in starch properties might be due to the different distribution pattern of acetyl groups rather than the DS.

3.2. Acetyl substitution patterns of amylose and amylopectin populations in cowpea starch modified with acetic anhydride and vinyl acetate
3.2.1. Degree of molar substitution of amylose and amylopectin populations isolated from cowpea starch modified with acetic anhydride and vinyl acetate, respectively

Amylose and amylopectin fractions were isolated from cowpea starch after acetylation, and the purities of these fractions were checked enzymatically.

The amylose (AM) and amylopectin (AP) samples isolated from cowpea starch modified with acetic anhydride (AA) or vinyl acetate (VA) are abbreviated as AA AM, VA AM, AA AP and VA AP. The DS values of AA AM, VA AM, AA AP and VA AP were 0.099, 0.092, 0.029 and 0.039, respectively, indicating that the amorphous phase of starch granules 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).

The difference in DS between the two types of acetylated amylopectin was more pronounced than the difference between the two types of acetylated amylose. The higher acetylation level in amylopectin seems to explain 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 peak viscosity of the parent starch acetate modified with vinyl acetate as compared with modification with acetic anhydride.

Table 1  Characterization of acetylated yellow pea, cowpea and chickpea starches and their different size granule fractions (from Huang et al., 2006b)

<table>
<thead>
<tr>
<th>Starch</th>
<th>Yield(%)</th>
<th>Moisture(%)</th>
<th>Volume mean diameter (µm)</th>
<th>Degree of molar substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow pea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAY</td>
<td>-</td>
<td>12.8</td>
<td>26.9</td>
<td>0.066</td>
</tr>
<tr>
<td>AAY &lt;20 µm</td>
<td>23</td>
<td>10.7</td>
<td>20.2</td>
<td>0.074</td>
</tr>
<tr>
<td>AAY 20-32 µm</td>
<td>63</td>
<td>11.9</td>
<td>26.5</td>
<td>0.058</td>
</tr>
<tr>
<td>VAY</td>
<td>-</td>
<td>12.7</td>
<td>25.4</td>
<td>0.071</td>
</tr>
<tr>
<td>VAY &lt;20 µm</td>
<td>24</td>
<td>11.5</td>
<td>20.0</td>
<td>0.068</td>
</tr>
<tr>
<td>VAY 20-32 µm</td>
<td>60</td>
<td>8.5</td>
<td>26.5</td>
<td>0.067</td>
</tr>
<tr>
<td>Cowpea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAC</td>
<td>-</td>
<td>12.7</td>
<td>15.6</td>
<td>0.059</td>
</tr>
<tr>
<td>AAC &lt;20 µm</td>
<td>89</td>
<td>8.7</td>
<td>15.3</td>
<td>0.058</td>
</tr>
<tr>
<td>AAC &gt;20 µm</td>
<td>11</td>
<td>9.8</td>
<td>15.9</td>
<td>0.053</td>
</tr>
<tr>
<td>VAC</td>
<td>-</td>
<td>12.4</td>
<td>15.8</td>
<td>0.064</td>
</tr>
<tr>
<td>VAC &lt;20 µm</td>
<td>93</td>
<td>12.8</td>
<td>15.8</td>
<td>0.062</td>
</tr>
<tr>
<td>VAC &gt;20 µm</td>
<td>7</td>
<td>9.8</td>
<td>17.5</td>
<td>0.063</td>
</tr>
<tr>
<td>Chickpea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AACH</td>
<td>-</td>
<td>12.9</td>
<td>18.0</td>
<td>0.057</td>
</tr>
<tr>
<td>AACH &lt;20 µm</td>
<td>68</td>
<td>10.5</td>
<td>17.4</td>
<td>0.057</td>
</tr>
<tr>
<td>AACH &gt;20 µm</td>
<td>32</td>
<td>10.2</td>
<td>19.8</td>
<td>0.053</td>
</tr>
<tr>
<td>VACH</td>
<td>-</td>
<td>12.7</td>
<td>18.7</td>
<td>0.068</td>
</tr>
<tr>
<td>VACH &lt;20 µm</td>
<td>78</td>
<td>8.5</td>
<td>17.7</td>
<td>0.066</td>
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<tr>
<td>VACH &gt;20 µm</td>
<td>22</td>
<td>9.1</td>
<td>20.7</td>
<td>0.067</td>
</tr>
</tbody>
</table>

a Yellow pea starch modified with acetic anhydride (< 20µm granule fraction).
b Yellow pea starch modified with vinyl acetate (20-32µm granule fraction).
c Values are measured in dry state.
Fig. 1. RVA pasting curves of native and acetylated yellow pea, cowpea and chickpea starches modified with acetic anhydride and vinyl acetate, respectively. RVA: rapid visco analyzer (from Huang et al., 2006b)
3.2.2. Distribution of acetyl groups over amylose populations isolated from cowpea starch modified with acetic anhydride and vinyl acetate

In addition to the DS, the substitution pattern on the D-glucosyl residues within the starch components (amylose and amylopectin) is of interest. The introduced acetyl groups act as barriers to amylase attack (Chen et al., 2004), and pure enzymes can be used to investigate the distribution pattern of acetyl groups. \(\alpha\)-Amylase is an \textit{endo}-hydrolase which cleaves \(\alpha\)-1,4-glucosidic linkages in a random fashion (Mischnick, 2001). The structural characterization of the fragments was carried out using MALDI-TOF-MS. Only fractions smaller than DP 17 were observed by MALDI-TOF-MS oligomer-analysis. Clear differences in the distribution of acetyl groups in the oligosaccharide fractions between AA AM and VA AM samples can be observed (results not shown). The largest unsubstituted oligomer was DP 8 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. More higher substituted fragments in VA AM hydrolysates suggests that the distribution of acetyl group along the chains of VA AM was more blockwised.

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

Similar approaches as discussed above were used to explore the substitution pattern of the amylopectin component. The DP of unsubstituted oligomers in the hydrolysates was higher for amylopectin (Fig. 2) than for amylose, due to the fact that the DS of amylopectin was much lower.

Fig. 2. MALDI-TOF mass spectrum of the \(\alpha\)-amylase hydrolysates of amylopectin (AP) isolated from cowpea starch modified with acetic anhydride (AA) and vinyl acetate (VA), respectively. Numbers indicate degree of polymerization (DP). Superscript numbers denote the number of acetyl group. A zoom of DP9-10 is inserted. + Na and + K indicate sodium and potassium adducts, respectively.
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 fractions 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 slightly more clustered and that more acetyl groups were located near the branch points.

4. Conclusions

For yellow pea, cowpea and chickpea starches, the smaller the granule size fraction, the higher the DS obtained when modified with acetic anhydride. While for reaction with vinyl acetate, different size granule fractions showed the same level of acetylation. Granule size had a greater effect on reactivity when modified with rapidly reacting acetic anhydride than with slowly reacting vinyl acetate. For all three starches, modification with vinyl acetate resulted in a higher peak viscosity than acetic anhydride. However, the differences in DS values between the two types of acetylation were minor.

For both acetylated cowpea starch samples, amylose populations showed much higher level of acetylation than amylopectin populations. Modification with vinyl acetate resulted in higher DS values for amylopectin compared to the modification with acetic anhydride. For both amylose and amylopectin isolated from cowpea starch, the oligomers in α-amylase hydrolysates showed clear differences between the two acetylation types as exhibited in the MALDI-TOF mass spectrum. 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.

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