

# Characterization of differently sized granule fractions of yellow pea, cowpea and chickpea starches after modification with acetic anhydride and vinyl acetate

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## 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. Samples modified with vinyl acetate showed higher swelling volume and peak viscosity than those acetylated with acetic anhydride for the same starch. 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. Smaller size granule fractions exhibited larger swelling volume and higher peak viscosity as compared with the corresponding larger size fractions. The reagent type and granule size are important factors for pasting and swelling behaviours of acetylated granular starches.

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## 1. Introduction

Irrespective of the source, all starches occur in nature as minute granules. The shape of the starch granule depends on the botanical source, and many different forms are found. Also the size varies, from the tiny granules in rice and oat to the large ones in potato and banana starches. Particle size ranges 3–8  $\mu\text{m}$ , 3–24  $\mu\text{m}$ , 2–35  $\mu\text{m}$ , 5–70  $\mu\text{m}$  and 5–100  $\mu\text{m}$  have been reported for rice, amylomaize, wheat, arrowroot and potato starches, respectively (Swinkels, 1985). Because of the broad range of particle size distribution in starch, the effect of granule size on the properties and applications

of starch have received attention. Other factors affecting starch functionality are composition (amylose-to-amylopectin ratio, contents of non-starch components), crystallinity and amylopectin structure. Waxy maize starch granules with a smaller diameter showed greater enzymatic hydrolysis than larger ones, but there was no difference in the fine structure of starch residues obtained from enzymatic hydrolysis between the two size fractions (Franco, Rreto, Ciacco, & Tavares, 1998). Investigation of large, medium and small size granule fractions from barley starch revealed that small granules and large granules exhibited differences in enthalpy, swelling power, relative crystallinity, susceptibility to enzymes, retrogradation, the number-average degree of polymerisation ( $\text{DP}_n$ ) and chain-length distribution of amylopectin (Tang, Ando, Watanabe, Takeda, & Mitsunaga, 2000, 2001a, 2001b; Tang, Mitsunaga, & Kawamura,

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2004; Tang, Watanabe, & Mitsunaga, 2002). The content of phosphorus, pasting properties, and digestibility by amylase, varied greatly according to the granule size of potato starch (Noda et al., 2005). Chen, Schols, and Voragen (2003b) reported that starch noodles made from small size granule fractions of potato and sweet potato starches had better processibility and better quality than those made from large size granule fractions.

Modified starch is defined as starch whose hydroxyl group has been altered by a chemical reaction (esterification, etherification, or oxidation) or by enzymatic modification, as in the case of dextrans (Fleche, 1985). The reaction(s) chosen for starch modification usually exert a major change on the desirable physical properties. Nevertheless, low levels of modification already dramatically alter the physical properties of starch, such as paste viscosity, gelling, syneresis, clarity and adhesion properties. The most typical starch ester is acetylated starch, which is actively marketed due to the specific properties arising from the substitution groups.

Low degree of substitution (DS <0.1) starch acetate has been applied in both food industry and industrial areas (Fleche, 1985) for many years. 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. This research was designed to make a direct comparison on the properties of starch modified with acetic anhydride and vinyl acetate, respectively. Acetylated starches are prepared most often under reaction conditions that preserve granular structure. In previous work, we observed increased degree of substitution (DS) with decreasing granule size for small and large size granule fractions of potato and sweet potato starches modified with acetic anhydride (Chen, Schols, & Voragen, 2004). In this study, we investigated the effect of granule size on modification with vinyl acetate and acetic anhydride as acetylating reagents.

Yellow pea (*Pisum sativum*), cowpea (*Vigna unguiculata*) and chickpea (*Cicer arietinum*) starches, with typical legume starch properties (Huang et al., 2006), are of industrial interest to be utilized in their acetylated form as well. Hence, investigation on these three starches was undertaken to obtain information on the effects of reagent type (acetic anhydride vs. vinyl acetate) and granule size on the properties of acetylated starches.

## 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 et al., 2006). Two types of acetylated yellow pea, cowpea and chickpea starches were prepared by AVEBE Food Innovation Centre (Veendam, The Netherlands). Acetylated starch was pre-

pared 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 mol of reagent were added.

### 2.2. Separation of different size granule fractions

Native and 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) with deionized water, and then air-dried at 40 °C. Native and acetylated yellow pea starch was separated into three fractions: larger than 32 µm, 20–32 µm, smaller than 20 µm; native and acetylated cowpea and chickpea starches were separated into two fractions: larger than 20 µm and smaller than 20 µm, respectively.

### 2.3. Analytical methods

Moisture content was determined according to Chen, Schols, and Voragen (2003a). Amylose content was tested using the enzymatic amylose/amylopectin assay kit (Megazyme, Ireland). Particle size distribution was measured both in dry state and in water with a laser diffraction system (H1140, Sympatec Inc., USA). The pasting behaviours of the starches were measured using a rapid visco analyzer (RVA-4, Newport Scientific Pty. Ltd, Australia). Crystal-line structure was analyzed using a Philips diffractometer (PW 1830, the Netherlands). Gelatinization temperature was measured using a Differential Scanning Calorimeter (Perkin-Elmer DSC-7, USA). These analyses and the determination of swelling volume were carried out as described in our previous paper (Huang et al., 2006). The content of acetyl group was determined using the titration method according to Miladinov and Hanna (2001) except that the sample was saponified at room temperature for 2–3 h. The degree of substitution (DS) was expressed as moles of substituent per mole of D-glucose residue.

### 2.4. Statistical analysis

SPSS10.0 for Windows was used for statistical analysis. Differences between samples of different size fractions and different modification types were tested using the general linear model univariate test. A significance level of  $p < 0.05$  was used throughout the study.

## 3. Results and discussion

### 3.1. Characterization of different size granule fractions of native starch samples

#### 3.1.1. Particle size distribution

Starches from yellow pea, cowpea and chickpea as obtained and characterized before (Huang et al., 2006)

were further fractionated according to their granule size by sieving. The particle size distributions of different size granule fractions separated from native yellow pea starch are presented in Fig. 1. The granule size range of  $>32\ \mu\text{m}$  fraction was broader than that of  $20\text{--}32\ \mu\text{m}$  and  $<20\ \mu\text{m}$  fractions and an overlap between the fractions could be seen. According to the software used, in each fraction, 50–60% of all granules represented the given size. Similar results were obtained for cowpea and chickpea starches with respect to overlap between large and small size granule fractions. The extent of variation in VMD (volume mean diameter) between granule size fractions was in the order yellow pea  $>$  chickpea  $>$  cowpea (Table 1). The  $>32\ \mu\text{m}$  fraction of yellow pea starch when measured in water had a greater shift toward larger granule sizes when compared to the  $20\text{--}32\ \mu\text{m}$  and  $<20\ \mu\text{m}$  fractions, indicating a greater swelling of the granules. In particle size distribution measurement, the reproducibility of the  $>32\ \mu\text{m}$  fraction

was relatively bad, may be due to the presence of fiber-like impurities as observed by microscopy. This fraction also showed some agglomeration (hard to obtain a homogeneous powder), a bit brownish colour and lower purity. Therefore, the  $>32\ \mu\text{m}$  fraction is not included in the following discussion about behaviour of different size granule fractions. Nevertheless, its properties are presented for reference.

### 3.1.2. Amylose content and swelling volume

For yellow pea starch,  $<20\ \mu\text{m}$  fraction showed similar amylose level as that of  $20\text{--}32\ \mu\text{m}$  fraction. For cowpea starch, a higher amylose content was found for  $<20\ \mu\text{m}$  fraction as compared with  $>20\ \mu\text{m}$  fraction, while the opposite result was obtained for chickpea starch. No clear relationship between the amylose content and granule size was also observed for potato starch. Chen et al. (2003b) found a lower amylose level in the smaller-size potato

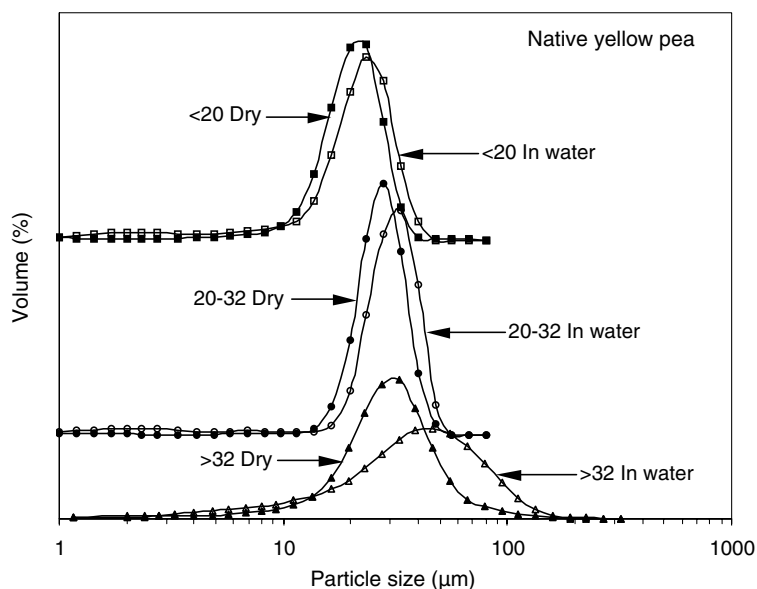


Fig. 1. Particle size distributions of different size granule fractions separated from native yellow pea starch.  $<20$  dry:  $<20\ \mu\text{m}$  granule fraction, measured in dry state.  $20\text{--}32$  in water:  $20\text{--}32\ \mu\text{m}$  granule fraction, measured in water.

Table 1

Characterization of different size granule fractions separated from native yellow pea, cowpea and chickpea starches

Starch	Yield <sup>a</sup> (%)	Moisture (%)	VMD <sup>b</sup> ( $\mu\text{m}$ )		Starch <sup>a</sup> (%)	Amylose <sup>a</sup> (%)	Swelling volume <sup>a</sup> (ml/g)
			Dry	In water			
Yellow pea $<20\ \mu\text{m}$	25	11.6	20.2	21.0	96.5a	28.4a	22.0a
Yellow pea $20\text{--}32\ \mu\text{m}$	61	12.3	27.3	29.2	94.0b	27.9a	19.5b
Yellow pea $>32\ \mu\text{m}$	14	9.6	32.1	43.8	72.5c	25.8b	20.4ba
Cowpea $<20\ \mu\text{m}$	91	12.9	15.7	16.0	94.0	25.0	33.9
Cowpea $>20\ \mu\text{m}$	9	10.4	16.1	16.9	92.5	22.5	25.3
Chickpea $<20\ \mu\text{m}$	73	10.1	17.2	18.2	94.4	25.6	28.6
Chickpea $>20\ \mu\text{m}$	27	8.8	20.4	21.2	93.0	30.2	24.7

Values with different letters in the same column of the same variety are significant different at  $p < 0.05$ .

<sup>a</sup> Values are based on dry matter and means of triplicate.

<sup>b</sup> Volume mean diameter. Values are means of duplicate.

starch granule fraction, while Noda et al. (2005) reported that there was no difference in amylose content among the differently sized potato starch granule fractions. Thus, it is hard to predict the amylose content of different size fractions from the result of un-fractionated starch.

The extent of swelling, which provides evidence of non-covalent bonding between starch molecules (Moorthy, 2004), was determined at 90 °C and expressed as swelling volume. For yellow pea, chickpea and cowpea starches, higher swelling volume values were observed for smaller size granule fractions as also reported for waxy and normal barley, potato and sweet potato starches (Chen et al., 2003b; Singh & Kaur, 2004; Tang et al., 2004, 2002). Other factors like chain-length and molecular weight distribution, degree/length of branching and conformation of amylopectin as well as the amylose content also contribute to the swelling behaviour of starches (Moorthy, 2004).

### 3.1.3. Pasting behavior

Besides some cases in which starch is used in its natural granular shape – starches used for molding in confectionery, powdering of surgical gloves, dilution of insecticides or herbicides – most of the time starch must be subjected to hydrothermal dispersion (Fleche, 1985). For the application of starch in a process, the pasting behaviour during different temperatures is of interest. It is apparent that there was little difference in pasting temperature among the granule size fractions separated from yellow pea starch (Fig. 2). Similar results were observed for chickpea and cowpea starches (results not shown). This is in agreement with the findings for sweet potato and potato starches (Chen et al., 2003b; Noda et al., 2005).

The smaller the granule size, the higher the peak viscosity was found for yellow pea and chickpea starches. For cowpea starch, the lack of substantial difference in peak viscosity between <20 µm and >20 µm fractions may be explained by the small differences in their VMD. This is an indirect evidence for the statement that the peak viscosity varies according to the granule size. However, in literature, findings on potato starch were not in agreement with each other. Chen et al. (2003b) reported similar peak viscosity of the different size granule fractions. In contrast, Noda et al. (2005) found decreased peak viscosity as the granule size decreasing.

## 3.2. Characterization of starch samples modified with acetic anhydride and vinyl acetate, respectively

### 3.2.1. Degree of molar substitution and swelling volume

The reaction level, determined by the analysis of the introduced chemical group, is usually indicated by the term “degree of substitution (DS)”. The same moles of acetic anhydride and vinyl acetate were added in the derivatisation of yellow pea, cowpea and chickpea starches. However, a higher DS was obtained from modification with vinyl acetate than with acetic anhydride for all three starches (Table 2), indicating a higher reaction efficiency for the reaction

with vinyl acetate. This may be due to the fact that acetic anhydride is a highly reactive reagent, and its side reaction with water may result in a lesser reaction efficiency.

The particle size distributions of acetylated cowpea and chickpea starches showed similar results as their corresponding native starches. The acetylated yellow pea starch showed a unimodal distribution of particle size, and a lower VMD compared to the native form. This might be due to the washing step during preparation of the acetylated starch, which can remove some fiber-like impurity that is responsible for the shoulder peak at high granule diameters exhibited in native yellow pea starch (Huang et al., 2006).

For yellow pea and cowpea starches, there were no significant differences in amylose content between the native and acetylated samples. After acetylation a higher amylose level was exhibited for chickpea starch. Reduced amylose levels for cocoyam, jack bean, maize and rice starches (González & Pérez, 2002; Lawal, 2004a, 2004b; Lawal & Adebawale, 2005), and increased values for jack bean starch (Betancur, Chel, & Canizares, 1997) after modification with acetic anhydride have been reported.

For yellow pea, cowpea and chickpea starches, the swelling volume values all increased after acetylation with vinyl acetate. After modification with acetic anhydride, the values were similar for yellow pea and chickpea, and lower for cowpea starch as compared with those of the native starches. It is obvious that modification with vinyl acetate resulted in higher swelling volumes than modification with acetic anhydride. This could be explained by the fact that vinyl acetate is a slowly reacting agent, and can diffuse deep into the granule matrix, which makes acetyl groups present on the surface of crystals in both interior and exterior lamella of the granules. However, the reaction with acetic anhydride occurs in the outer lamella of crystalline regions (Chen et al., 2004).

### 3.2.2. Thermal properties and crystal structure

Differential scanning calorimetry (DSC) was used to study the thermal characteristics of un-fractionated native and acetylated starch samples. The onset temperatures and the enthalpy of thermal transition are presented in Table 2. Yellow pea, cowpea and chickpea starches all showed lower gelatinization temperatures after acetylation as also observed for maize, cocoyam, jack bean, potato and rice starches modified with acetic anhydride or vinyl acetate (Lawal, 2004a, 2004b; Lawal & Adebawale, 2005; Liu, Ramsden, & Corke, 1997; Liu, Ramsden, & Corke, 1999; Singh, Kaur, & Singh, 2004; Wang & Wang, 2002). The introduction of acetyl groups interrupts the ordered structure of native starch, leading to decreased gelatinization temperature (Wang & Wang, 2002). There was little difference in gelatinization temperature between the two types of acetylation within each starch.

After modification with acetic anhydride, the enthalpy was reduced for yellow pea and chickpea starches, and remained similar for cowpea starch. In the case of vinyl acetate, the value decreased for yellow pea, remained

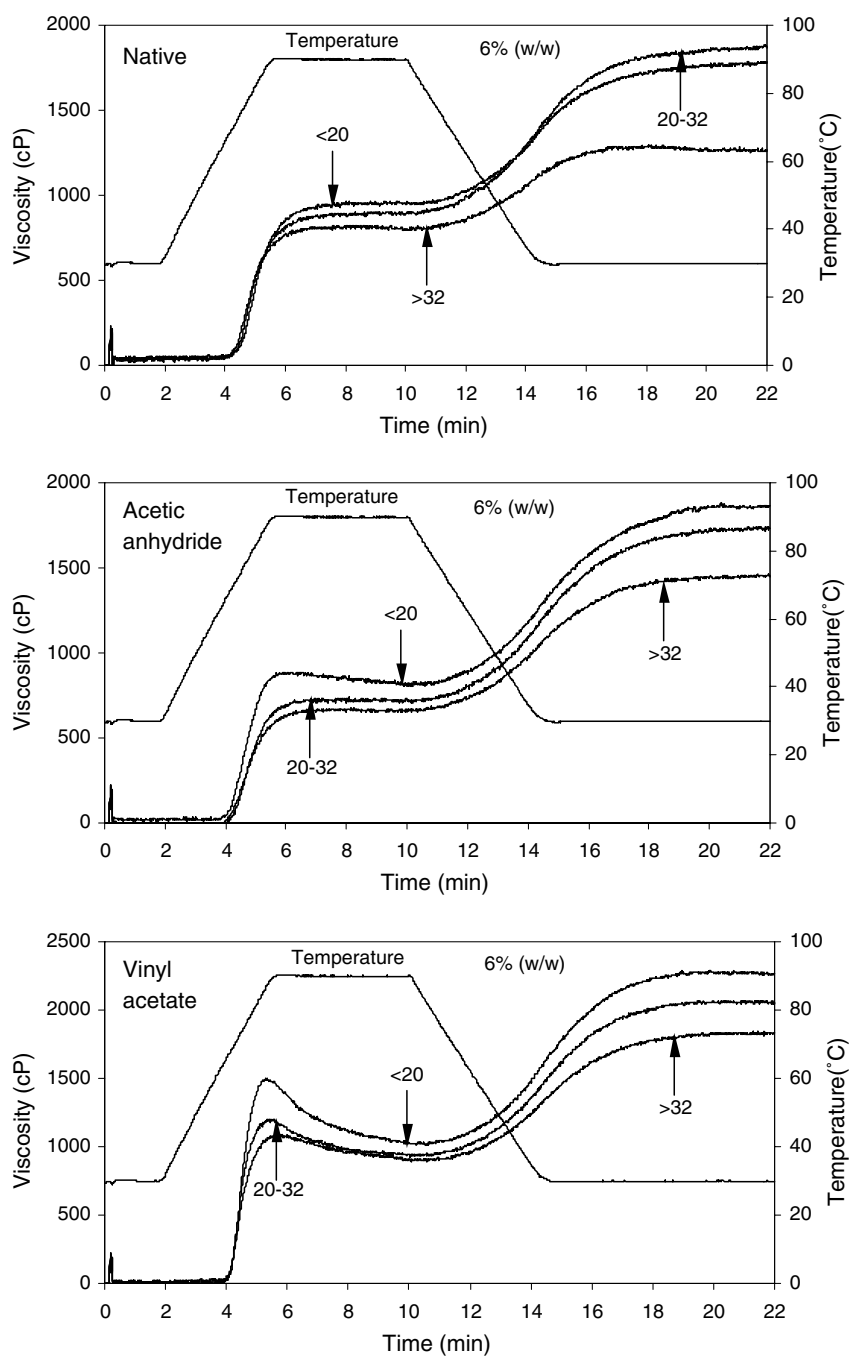


Fig. 2. RVA pasting curves of different size granule fractions separated from native and acetylated yellow pea starch modified with acetic anhydride and vinyl acetate, respectively. <20: <20  $\mu\text{m}$  granule fraction. 20–32: 20–32  $\mu\text{m}$  granule fraction. RVA: rapid visco analyzer.

similar for chickpea and increased for cowpea starch. Similarity in enthalpy was found between two types of acetylation for yellow pea starch, while acetylation with vinyl acetate resulted in higher enthalpy than modification with acetic anhydride for cowpea and chickpea starches. Decreased enthalpy after modification with acetic anhydride or vinyl acetate was reported for maize, potato, jack bean, and cocoyam starches (Lawal, 2004a, 2004b; Lawal and Adebawale, 2005; Liu et al., 1997; Singh et al., 2004). Since enthalpy is the latent heat absorbed by melting of crystallites in the granules, it depends on a number of

factors like crystallinity, intermolecular bonding, rate of heating of the starch suspension, presence of other chemicals, etc. (Moorthy, 2004). Variations in enthalpy of acetylated starches were indications of structural divergence: molecular structure (amylose and amylopectin fine structures, distribution pattern of acetyl groups) and composition (amylose-to-amylopectin ratio, DS).

X-ray diffraction patterns and the relative crystallinity did not show substantial changes after reaction with acetic anhydride and vinyl acetate for yellow pea (Fig. 3), cowpea and chickpea starches. This information confirms the



Table 2  
Properties of native and acetylated yellow pea, cowpea and chickpea starches modified with acetic anhydride and vinyl acetate, respectively

Starch	Moisture (%)	VMD <sup>b,g</sup> (μm)		DS <sup>c,d,e</sup>	Amylose <sup>d,e</sup> (%)	Swelling volume <sup>d,e</sup> (ml/g)	T <sub>o</sub> <sup>f,g</sup> (°C)	Enthalpy <sup>d,g</sup> (J/g)
		Dry	In water					
<i>Yellow pea</i>								
Native <sup>a</sup>	11.3	33.8	34.0	–	31.2a	19.8b	58.2	16.1
Acetic anhydride	12.8	26.9	30.8	0.066	30.3a	21.5b	54.2	13.7
Vinyl acetate	12.7	25.4	31.3	0.071	29.3a	29.4a	55.3	14.2
<i>Cowpea</i>								
Native <sup>a</sup>	11.5	15.5	16.5	–	25.8a	31.4b	70.5	15.2
Acetic anhydride	12.7	15.6	16.4	0.059	25.2a	26.0c	65.5	15.6
Vinyl acetate	12.4	15.8	16.4	0.064	26.1a	40.9a	64.8	20.0
<i>Chickpea</i>								
Native <sup>a</sup>	11.9	17.9	19.8	–	27.2b	26.8b	57.9	17.6
Acetic anhydride	12.9	18.0	19.5	0.057	33.3a	25.3b	55.2	15.8
Vinyl acetate	12.7	18.7	19.6	0.068	33.6a	40.0a	54.7	17.2

Values with different letters in the same column of the same variety are significant different at  $p < 0.05$ .

<sup>a</sup> From Huang et al. (2006).

<sup>b</sup> Volume mean diameter.

<sup>c</sup> Degree of molar substitution.

<sup>d</sup> Values are based on dry matter.

<sup>e</sup> Values are means of triplicate.

<sup>f</sup> Onset gelatinization temperature.

<sup>g</sup> Values are means of duplicate.

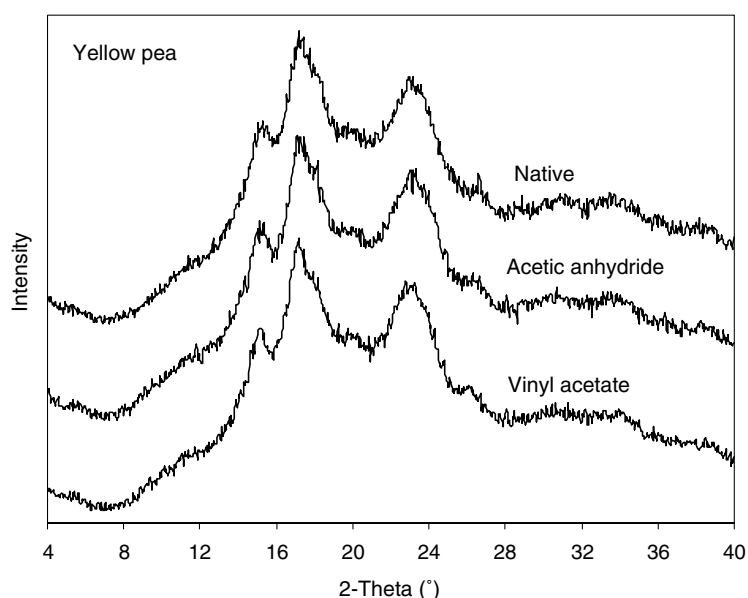


Fig. 3. X-ray diffractogram of native and acetylated yellow pea starch modified with acetic anhydride and vinyl acetate, respectively.

findings of others (Chen et al., 2004; Lawal, 2004a; Wang & Wang, 2002) for waxy maize, sweet potato, potato and cocoyam starches that at low level of acetylation, hydroxyl groups react in the amorphous region and on the surface of crystals (Seib, 1997), no substantial change occurred in crystal structures of granular starch.

### 3.2.3. Pasting behavior

The pasting viscosity during heating starch in excess water was recorded using a Rapid Visco Analyzer

(RVA). Yellow pea, cowpea and chickpea starches all showed lower pasting temperatures after acetylation, consistent with the gelatinization temperature results determined by DSC. The starch–starch interactions in the granules are weakened by the introduction of acetyl groups (Taggart, 2004). For yellow pea and cowpea starches, pasting temperatures were similar for both types of acetylation. However, for chickpea starch, modification with acetic anhydride resulted in a higher pasting temperature than vinyl acetate treatment. The reason is not clear.

As described previously (Huang et al., 2006), native yellow pea and chickpea starches showed type C pasting profiles, and cowpea starch showed a type B viscosity according to the classification of Schoch and Maywald (1968). The pasting profile of yellow pea and chickpea starches remained type C after acetylation with acetic anhydride, but changed to type B after modification with vinyl acetate (Fig. 4). For cowpea starch, both acetylated samples exhibited the same pasting profiles as the native starch. The peak viscosity was increased after modification with vinyl acetate for all three starches. 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. Increases in peak viscosity were observed for waxy maize, dent maize, jack bean, rice, smooth pea and sweet potato starches after acetylation with acetic anhydride (Betancur et al., 1997; Biliaderis, 1982; Chen, Schols, & Voragen, 2003c; González & Pérez, 2002; Liu et al., 1997; Wilkins et al., 2003a, 2003b), and for waxy rice starch after acetylation with vinyl acetate (Liu et al., 1999). Contradicting results were

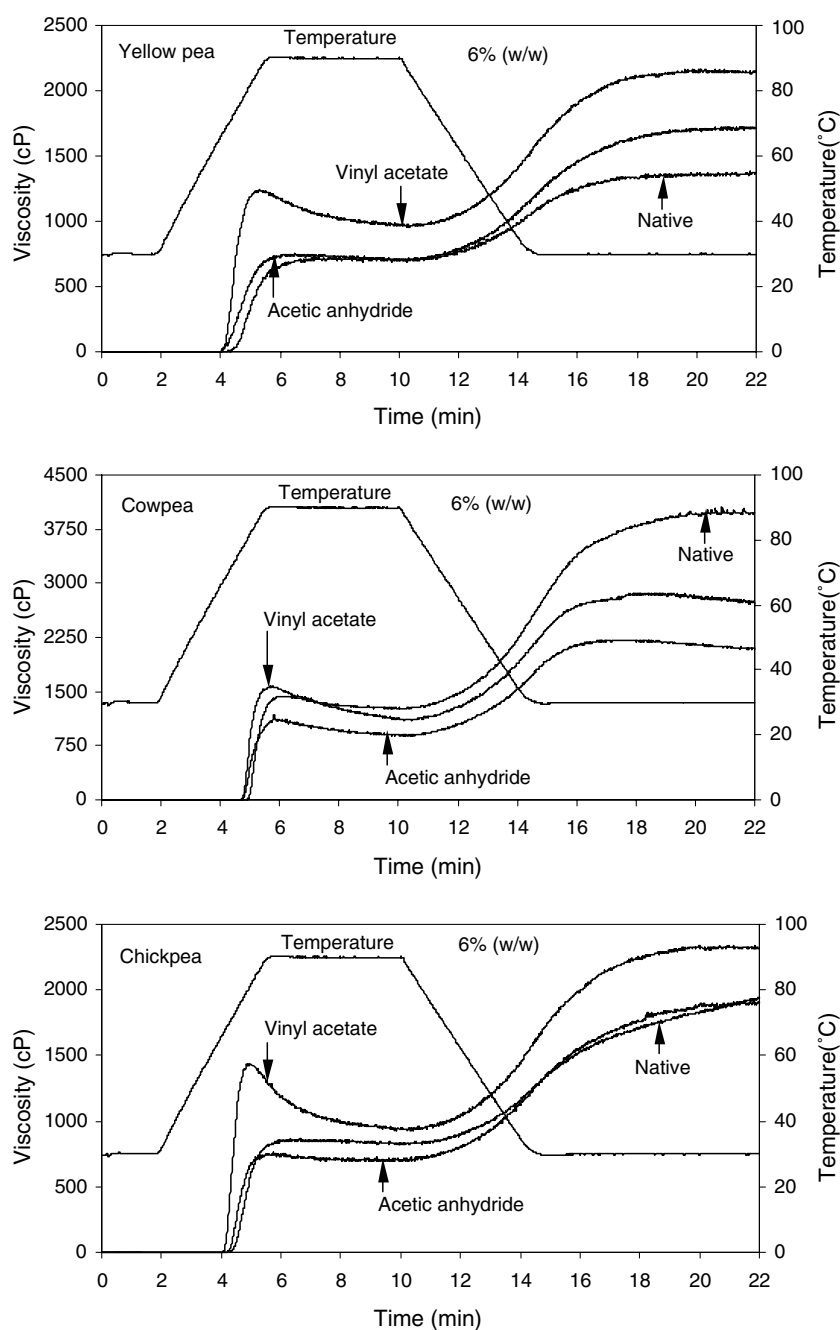


Fig. 4. RVA pasting curves of native and acetylated yellow pea, cowpea and chickpea starches modified with acetic anhydride and vinyl acetate, respectively. RVA: rapid visco analyzer.

mentioned for maize, canna, cocoyam and potato starches which showed a lower peak viscosity after acetylation with acetic anhydride (Chen et al., 2003c; Lawal, 2004a, 2004b; Saartrat, Puttanlek, Rungsardthong, & Uttapap, 2005).

A noticeable relationship between swelling volume and peak viscosity was observed. Acetylation with vinyl acetate resulted in both a larger degree of swelling and a higher peak viscosity than modification with acetic anhydride. The setback (= final viscosity – peak viscosity) values of yellow pea starch following modification with acetic anhydride and vinyl acetate were 960cP and 889cP, respectively, both higher than that of native starch (643cP, Huang et al., 2006). For cowpea starch, the setback values were lower after both types of acetylation, while reaction with acetic anhydride and vinyl acetate induced only slight change in setback value for chickpea starch. Following modification with acetic anhydride, different starch samples were found to exhibit variability in setback value. Reductions were observed for rice, cocoyam, maize and canna starches (González & Pérez, 2002; Lawal, 2004a, 2004b; Saartrat et al., 2005); and increases for waxy and high-amylose maize, and dent maize starches (Liu et al., 1997; Wilkins et al., 2003b); similarities for normal maize and waxy maize starches (Liu et al., 1997; Wilkins et al., 2003a). Our findings and those reported by others suggest that the change in viscosity after acetylation strongly depends on the starch source and the nature of acetylating reagent.

### 3.3. Characterization of different size granule fractions of acetylated starch samples

#### 3.3.1. Degree of molar substitution

Yellow pea, cowpea and chickpea starches were fractionated by size *after* reaction with acetic anhydride and vinyl acetate. The VMD of granule size fractions separated from acetylated starches (Table 3) were identical with those of their native counterparts.

When modified with acetic anhydride, smaller size (<20 µm) granule fractions of yellow pea, cowpea and chickpea starches had a higher DS as compared with larger size granule fractions (yellow pea 20–32 µm; cowpea and chickpea >20 µm). This could be explained by the fact that smaller size granule fractions have larger specific surface area (Chen et al., 2004). For acetylated starches prepared with vinyl acetate, smaller size (<20 µm) granule fractions of yellow pea, cowpea and chickpea starches were acetylated at the same level as larger size granule fractions (yellow pea 20–32 µm; cowpea and chickpea >20 µm). This phenomenon and the DS results of un-fractionated starch samples revealed that differences between reagent types lead to variations in the levels of acetylation. Granule size had a greater effect on DS values when modified with the rapidly reacting agent acetic anhydride than with the slowly reacting reagent vinyl acetate. Stapley and 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.

Table 3  
Characterization of different size granule fractions separated from yellow pea, cowpea and chickpea starches modified with acetic anhydride and vinyl acetate, respectively

Starch	Yield <sup>c</sup> (%)	Moisture (%)	VMD <sup>d</sup> (μm)		DS <sup>e,c</sup>	Amylose <sup>c</sup> (%)	Swelling volume <sup>c</sup> (ml/g)
			Dry	In water			
<i>Yellow pea</i>							
AAV <20 μm <sup>a</sup>	23	10.7	20.2	22.2	0.074b	30.2b	25.1c
AAV 20–32 μm	63	11.9	26.5	28.7	0.058d	28.9b	19.7e
AAV >32 μm	14	11.2	30.3	40.6	0.081a	31.9a	19.8e
VAV <20 μm	24	11.5	20.0	20.8	0.068cb	29.3b	30.6a
VAV 20–32 μm <sup>b</sup>	60	8.5	26.5	29.6	0.067c	29.4b	27.7b
VAV >32 μm	16	12.8	29.0	46.7	0.084a	30.2b	21.1d
<i>Cowpea</i>							
AAC <20 μm	89	8.7	15.3	16.3	0.058b	25.2ba	28.7c
AAC >20 μm	11	9.8	15.9	17.2	0.053c	24.0ba	23.7d
VAC <20 μm	93	12.8	15.8	16.1	0.062ab	27.0a	42.2a
VAC >20 μm	7	9.8	17.5	18.1	0.063a	23.6b	36.1b
<i>Chickpea</i>							
AACH <20 μm	68	10.5	17.4	18.3	0.057b	32.5a	25.3c
AACH >20 μm	32	10.2	19.8	21.3	0.053c	29.4b	22.7d
VACH <20 μm	78	8.5	17.7	18.9	0.066a	31.6a	40.6a
VACH >20 μm	22	9.1	20.7	22.8	0.067a	29.5b	36.3b

Values with different letters in the same column of the same variety are significant different at  $p < 0.05$ .

<sup>a</sup> Yellow pea starch modified with acetic anhydride (<20 µm granule fraction).

<sup>b</sup> Yellow pea starch modified with vinyl acetate (20–32 µm granule fraction).

<sup>c</sup> Values are based on dry matter and means of triplicate.

<sup>d</sup> Volume mean diameter. Values are means of duplicate.

<sup>e</sup> Degree of molar substitution.



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. The  $>32\ \mu\text{m}$  fraction of yellow pea starch was an exception, showing the highest level of derivation in both acetylation types. The most likely explanation was the difference in granule swelling. This fraction showed the largest degree of granule swelling among all native and acetylated starch samples as revealed by particle size distribution measurements. The extent of granule reaction is a function of the magnitude of granule swelling (Huber & BeMiller, 2001).

### 3.3.2. Pasting behavior

Different size granule fractions separated from acetylated yellow pea starch showed similar pasting temperatures (Fig. 2). The same results were obtained with cowpea and chickpea starches (viscoamylograph not given). The smaller the granule size, the higher peak viscosity values were found for yellow pea and chickpea starches of both native and acetylated samples, while cowpea starch exhibited little difference in peak viscosity between large and small size granule fractions. All granule size fraction samples modified with vinyl acetate exhibited higher swelling volume values and peak viscosities than did their counterparts produced with acetic anhydride. This is in accordance with the findings for un-fractionated starch samples. The  $<20\ \mu\text{m}$  fraction separated from yellow pea starch acetylated with vinyl acetate showed lower DS but higher peak viscosity and swelling volume than the corresponding fraction obtained from reaction with acetic anhydride. These observations confirm that acetylation with vinyl acetate results in higher peak viscosity and swelling volume than obtained with acetic anhydride and suggests that the difference in starch properties might be due to the different distribution pattern of acetyl groups rather than the DS.

## 4. Conclusions

The fact that acetylation did not affect the X-ray diffraction patterns is an indication that neither acetic anhydride nor vinyl acetate reacted in the crystalline region of the granules when the starches were weakly substituted (DS  $<0.1$ ). The smaller the granule size fraction, the higher the DS obtained when modified with acetic anhydride, which is due to the larger specific surface area of the smaller size granule fractions. While for reaction with vinyl acetate, different size granule fractions showed the same level of acetylation. Thus, granule size had a greater effect on reactivity when modified with acetic anhydride (a rapidly reacting reagent) than with vinyl acetate (a slowly reacting reagent).

For yellow pea, cowpea and chickpea starches and their granule size fractions, modification with vinyl acetate resulted in a larger degree of granule swelling and higher peak viscosity than acetic anhydride. However, the differences in DS values between the two types of acetylation were minor. These results suggest that the distribution pat-

tern of acetyl groups rather than the DS determines the variation in physical properties of acetylated starch.

Acetylated cowpea starch showed higher gelatinization temperature and peak viscosity than yellow pea and chickpea starches, which was in accordance with the behaviour found for the native starches in our previous work. Therefore, besides the origin of starch, factors that influence the properties of acetylated granular starches are reagent type and granule size. Further investigation is needed to understand differences between two types of acetylated starches at a molecular level by assessment of the substituent distribution on starch components (amylose and amylopectin).

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