SOME INTRINSIC AND EXTRINSIC FACTORS OF ACETYLATED STARCHES: MORPHOLOGICAL, PHYSICOCHEMICAL AND STRUCTURAL CHARACTERISTICS

ALGUNOS FACTORES INTRÍNSECOS Y EXTRÍNSECOS DE ALMIDONES ACETILADOS: CARACTERIZACIÓN MORFOLÓGICA, FISICOQUÍMICA Y ESTRUCTURAL

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Abstract

Degree substitution (DS), morphological characteristics, X-ray diffraction (XRD), pasting properties, thermal properties and amylopectin chain length distribution were used to study the effect of botanical source (potato starch, PS; and maize starch, MS) and reagent type (acetic anhydride and vinyl acetate) on starch acetylation. The reagent type produced a different effect depending on the botanical source: for MS, the reaction with acetic anhydride produced higher DS than PS, whereas that in the reaction with vinyl acetate resulted in an inverse pattern. In general, modified starches had a Maltase cross, except for PS when acetylated with acetic anhydride. Native PS had the highest crystallinity level and the acetylated starches had a decreased amount of crystallinity (between 10% and 12 %) compared to the native starches. Acetylation produced a decrease in pasting temperature, and changes in the pasting characteristics were more evident with higher DS. Structural changes in starch components due to the acetylation reaction produced a decrease in the temperature and enthalpy of gelatinization. In general, acetylated starches had minor retrogradation. Regardless of reagent used, acetylated MS had higher retrogradation than acetylated PS. Higher DS values resulted in greater amounts of short chains in both starches.

Keywords: acetylation, physicochemical properties, retrogradation, starch structure, degree substitution.

Resumen

Se estudió el efecto de la fuente botánica (almidón de papa, PS; y almidón de maíz, MS) y el tipo de reactivo (anhídrico acético y vinil acetato) sobre el grado de sustitución (DS), las características morfológicas, la difracción de rayos X (XRD), las propiedades de formación de pastas, las propiedades térmicas y la distribución de longitud de cadena de la amilopectina durante la acetilación del almidón. El tipo de reactivo produjo diferentes efectos en la fuente botánica: para el MS, la reacción con anhídrido acético produjo mayor DS que para PS, mientras que en la reacción con vinil acetato, los resultados fueron en un patrón inverso. En general, los almidones modificados presentan la cruz de malta, a excepción del PS acetilado con anhídrido acético. El almidón nativo de papa presentó el mayor nivel de cristalinidad, mientras que los acetilados mostraron una disminución de cristalinidad (10% - 12%) comparados con los nativos. Acetilación produce una disminución de la temperatura de formación de pastas siendo estos cambios más evidentes con mayor DS. Los cambios estructurales en los componentes del almidón debido a la acetilación produjeron una disminución de la temperatura y entalpía de gelatinización. En general, los almidones acetilados tuvieron una menor retrogradación. Independientemente de reactivo utilizado, el MS acetilado mostró mayor retrogradación que el PS. Los valores más altos de DS reflejaron una mayor cantidad de cadenas cortas en ambos almidones.

Palabras clave: acetilación, propiedades físico-químicas, retrogradación, estructura del almidón, grado de sustitución.

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1 Introduction

Modified starches are widely used in diverse industries because starch does not meet the physicochemical and functional characteristics necessary for specific applications in its native state. Starch is modified chemically or physically, and dual modification is carried out in some cases to accentuate the functionality or add new characteristics to starch, such as slow digestion (Han and BeMiller, 2007). Chemical modification with various chemical reagents introduces new substituent groups replacing the hydroxyl groups of the glucosyl units. Although chemical modification of starch has been used for many years, there are many manuscripts about modification of starch and is practiced in industry, there is a lack of complete understanding of the starch granule reactivity because there is limited information about the patterns of modification within the granule and about reactions of individual starch molecules, including amylose (AM) and amylopectin (AP) (Huber and BeMiller, 2010). Granular reaction sites of modified starches have been localized using scanning electron microscopy (SEM) (Huber and BeMiller, 2001). To simplify and improve the general approach, reflectance confocal laser scanning microscopy (CLSM) has been used to localize reaction sites of modified starches (Gray and BeMiller, 2004). Additionally, the functionality of chemically modified starch depends on substituent groups for AM and AP. Chen *et al*. (2004, 2005) fractionated potato and sweet potato starches, acetylated with acetic anhydride, according granule size. They reported that acetyl groups show different patterns with AM depending on the granule size: higher granule size, lower DS. Chen and colleagues (2004, 2005) also reported that the DS is lower in AP than in the corresponding AM and that the largest number of acetyl groups is present in the AP. Results from both of their studies suggest that there is a heterogeneous distribution of acetyl groups within AM and AP and that this pattern may be due to the amorphous and crystalline areas inside the starch granules. However, these authors used native starches with large granule size and only one reagent for acetylation. Non-conventional starches as banana starch was acetylated with high and low degree substitution (Rivas-Gonzalez *et al*., 2009), and the effect of reaction time (Guerra-DellaValle *et al*., 2008), on some physicochemical and functional properties as well molecular characteristics were evaluated. Huang *et al*., (2007a) studied the effect of the reagent type (acetic anhydride and

vinyl acetate) and granule size has been tested in yellow pea starch, using the same amount of both reagents (0.088 moles of reagent per mole glucose residue starch) in the modification, and they tested two procedures of fractionation. The first procedure included fractionation in two populations (small and large granule size) followed by acetylation, and the second procedure included acetylation followed by fractionation in the same two populations. They mentioned that the distribution of acetyl groups differs not only at a molecular level but also at the granular level between the two types of reagents used for modification. Moreover, they demonstrated that the acetyl groups are more intensely distributed on and near the granule surface when acetylation is carried out with acetic anhydride compared to vinyl acetate, and they also reported that the acetyl groups are more uniform distributed throughout the granule when the modification is produced with vinyl acetate compared to acetic anhydride. In the same year (2007), these authors (2007b) reported the distribution of acetyl groups in cowpea starch acetylated with acetic anhydride and vinyl acetate followed by fractionation in small and large granule populations. They demonstrated that AM has a higher level of acetylation compared to AP regardless of reagent used (no effect of granule size was found). However, they reported that modification with vinyl acetate (the reaction is slow) produces higher DS values for AP compared to AM and that only a small difference in DS occurs for AM and AP with acetic anhydride modification (the reaction is rapid).

Acetylated starches with high DS (> 0.5) have been prepared (Shogren, 1996; Xu *et al*., 2004) with acetic anhydride under basic conditions. However, no studies have been published on the influences of selected reaction conditions on the extent of starch acetylation. High DS is useful to elucidate the acetylation pattern in starches of diverse botanical origins.

Steenekend and Woortman (2008) have reported surface effects in the acetylation of PS with acetic anhydride. They concluded that the surface effect on PS acetylation does not depend on granule size and DS if the fractions have a narrow range of granule sizes but that this may not hold true for different granule sizes within a single batch of starch granules. More studies are necessary in this sense. The acetylation of starch depends on intrinsic and extrinsic factors. The first are inherent to starch material, such as starch source, AM/AP ratio and structural characteristics, the second are related to the reaction conditions, such as reagent

type, pH, temperature and time of reaction (Huber and BeMiller, 2010).

Anatomical features, such as pores and channels, exist in starch granules of maize, sorghum and millet (Fannon *et al.*, 2004). Chemically modified potato, sorghum and waxy maize starches revealed that granular patterns of acetylation reaction are influenced by the type of starch and reagent (Fannon *et al.*, 2004). Hall and Sayre (1970) have detected internal cavities in common maize, sorghum, and waxy maize starch granules but not in barley, oat, rice or wheat starch granules. PS does not possess channels, and the reagents must pass through the external surface of PS to gain access to the granule matrix (Huber and BeMiller, 2001). The aim of this study was to evaluate the effect of botanical source and reagent type on the acetylation of starch.

2 Materials and methods

2.1 Materials

Commercial unmodified potato starch (PS) was a gift from Penford Food Ingredients Company (Centennial, Colorado). Commercial unmodified common maize starch (MS) was a gift from National Starch (Bridgewater, NJ). Acetic anhydride and vinyl acetate were reactive grade compounds.

2.2 Starch acetylation

Starch (20 g) was dispersed in distilled water (45 ml) and stirred for 1 h at 25°C. A sodium hydroxide (3%) solution was used to adjust the suspension pH to 8.0. Acetic anhydride or vinyl acetate was added drop-wise to the stirred slurry (during 1 h) while maintaining the pH in the range of 8.0 - 8.3 for acetic anhydride and pH in the range of 9.0 - 9.3 for vinyl acetate using 3% NaOH solution. This reaction was allowed to proceed for 10 min after addition of the acetylation reagent was completed. The slurry was then adjusted to pH 4.5 with 0.5 M HCl. After sedimentation, starch was washed free of acid with two washes of distilled water and one wash of 95% ethanol and was then oven-dried at 40°C. For each mole of glucose, 0.088 mol of reagent was added.

2.3 Acetyl percentage and degree of substitution

The percentage of acetylation (% acetyl) and degree of substitution (DS) were determined titrimetrically

according the method described by Sodhi and Singh (2005). DS was determined by saponification of the acetyl groups in a known amount of KOH dissolved in aqueous ethanol, and the excess of alkali was determined by back titration with aqueous HCl.

2.4 Morphological study

A polarized light microscope (Eclipse 80i, Nikon, Japan) was employed with 40x objective lenses and equipped with a digital camera (Digital Imaging Head, DC330 camera MTI, Japan). Dry native starch was sprinkled on a slide, and a cover was added to the slide. Pastes from RVA were dispersed with hot distilled water to get a final concentration of 0.4%. One drop of diluted paste was observed by light microscopy.

2.5 X-ray diffraction pattern

The XRD was obtained from a D/max 2500 X-ray diffractometer (Tokyo, Japan). A conventional X-ray tube was set to 40 kV and 200 mA. The X-ray source was Cu K radiation. Data were collected at room temperature from 2θ of 5 to 35° (θ being the angle of diffraction) with a step width of 0.02°, step time of 0.4 s, scanning speed of 8°/min, divergence slit width of 0.2 mm, scatter slit width of 0.6 mm and receiving slit width of 0.2 mm. Native and acetylated starch samples were dried at 50 °C to 10 % moisture content in a vacuum oven. The samples (50 mg) were then added into the slide for packing prior to X-ray scanning. The crystallinity percentage (%C) was determined from the diffractogram by calculating the area corresponding to the crystalline peaks (Carmona-García *et al.*, 2009):

$$\%C = \frac{Ap}{(At - N)} \qquad (1)$$

Where Ap is calculated from the difference between the area under the curve and the area of the amorphous halo; At is the total area under the curve; N is the instrumental noise (N), according to the following equation:

$$N = 149.6 \, cps * deg \qquad (2)$$

The amorphous halo was determined with the amorphous component of starch obtained with an extraction procedure previously reported (Bogracheva *et al.*, 1999), where a completely gelatinized sample on the Rapid Visco Analyzer (RVA-4) was used.

2.6 Pasting behavior

The pasting properties of starches were measured on a Rapid Visco Analyzer (RVA-4) (Newport Scientific, Warriewood, Australia) using the RVA General Pasting Method. A starch sample (4.0 g) was weighed directly in the aluminum RVA sample canister, and distiller water (25.5 g) was added to a total constant sample weight of 29.5 g. The slurry was then manually homogenized using a plastic paddle to avoid lump formation before the RVA run. The sample was heated from 50°C to 90°C at 10°C / min and was then held at 90°C for 5 min. The sample was then cooled to 50°C. The paddle speed was 160 rpm.

2.7 Thermal characteristics

Gelatinization parameters were measured by DSC using a calorimeter (DSC model 2010, TA Instruments, USA) equipped with a thermal analysis data station (Ta Instruments, Newcastle, DE) and following the procedure by Paredes-Lopez *et al.* (1999). Starch (2 mg dwb) was weighed directly in an aluminum DSC pan with a 20 μL capacity. Deionized water was added with the help of a Hamilton microsyringe to achieve a starch/water suspension containing 70% water. The pans were hermetically sealed and equilibrated for 1 h at room temperature to allow complete hydration of starch. The pan with the sample was placed in the calorimeter and heated at a rate of 10°C / min from 20 to 120°C. An empty pan was used as a reference (Paredes-Lopez *et al.*, 1999). The indium and empty aluminum pan were used as references to calibrate the DSC. The onset temperature (T_o), peak temperature (T_{peak}) and enthalpy of gelatinization (ΔH) were automatically calculated. To evaluate retrogradation, gelatinized samples were stored at 4 °C for 7 and 14 days before rescanning. After the storage, the samples were equilibrated at room temperature for 1 h. The temperature range was from 25 to 230°C, and the heating rate was 10°C / min. An empty pan was used as a reference for all measurements.

2.8 Amylopectin chain length distribution (CL)

The chain length distribution of isoamylase debranched starch was characterized by high-performance anion-exchange chromatography using a pulsed amperometric detector (HPAEC-PAD) according to the method of Kasemsuwan *et al.* (1995)

with modifications. The HPAEC-PAD (DX500, Dionex Co., Sunnyvale, CA) system consisted of a GP50 gradient pump, LC20-1 chromatography organizer, ED40 electrochemical detector, CarboPac PA1 guard column (4 × 50 mm), CarboPac PA1 analytical column (4 × 250 mm) and AS40 automated sampler. Deionized water (3.2 mL) was added to 10 mg of defatted starch and was heated in a boiling water bath and stirred for 1 h. After cooling to room temperature, 0.4 mL of a 0.1 M acetate buffer (pH 3.5) and 5 μL of isoamylase (EC 3.2.1.68) (59,000 U/mg; Hayashibara Biochemical Laboratories, Okayama, Japan) were added, and the mixture was incubated at 40°C for 4 h in a water bath shaker. Enzyme activity was arrested by neutralization with 0.21 mL of 0.2 M NaOH and heating the sample in a boiling water bath for 15 min. The sample was cooled for 5 min and filtered through a 0.45-μm syringe filter, and 0.6 mL of the filtrate was transferred into an autosampler vial for injection.

2.9 Statistical analysis

Results are presented as mean ± SEM (standard error of mean) of three separate determinations. A commercial software program (Sigma Stat, version 2.03, Jandel Corporation, San Rafael, CA) was used for one-way analysis of variance (ANOVA) to determine differences in mean values based on data collected from replications of each measurement. Statistically significant differences ($p < 0.05$) were evaluated using Tukey's multiple comparison procedure.

3 Results and discussion

3.1 Degree of substitution

The same molar amounts of reagents (acetic anhydride and vinyl acetate) were added in the derivatization. The effect of different reagents on the DS of MS and PS is shown in Table 1. PS acetylated with vinyl acetate had higher DS values than MS, but an inverse pattern was found when the acetylation was carried out with acetic anhydride. The reagent type used in the acetylation of starch produced a different effect depending on the botanical source and probably the granule size and the morphological characteristics have major effect in this pattern. This pattern of acetylation may have been related to reactivity because acetic anhydride is highly reactive, producing a side reaction with water (Huang *et al.*, 2007a). However,

there are more OH groups with a smaller granule size (as found in MS) that can react, increasing the reaction efficiency due to a larger specific surface area (Chen *et al*., 2004). Additionally, it has been reported that AM present in the amorphous regions is acetylated more than AP in the crystalline regions. However, in small granules with larger specific surface areas, the crystalline regions in the outer lamellae have more space to react with the reagent (acetic anhydride) (Chen *et al*., 2004). Diverse patterns have been reported based on the reagent type for starch acetylation.
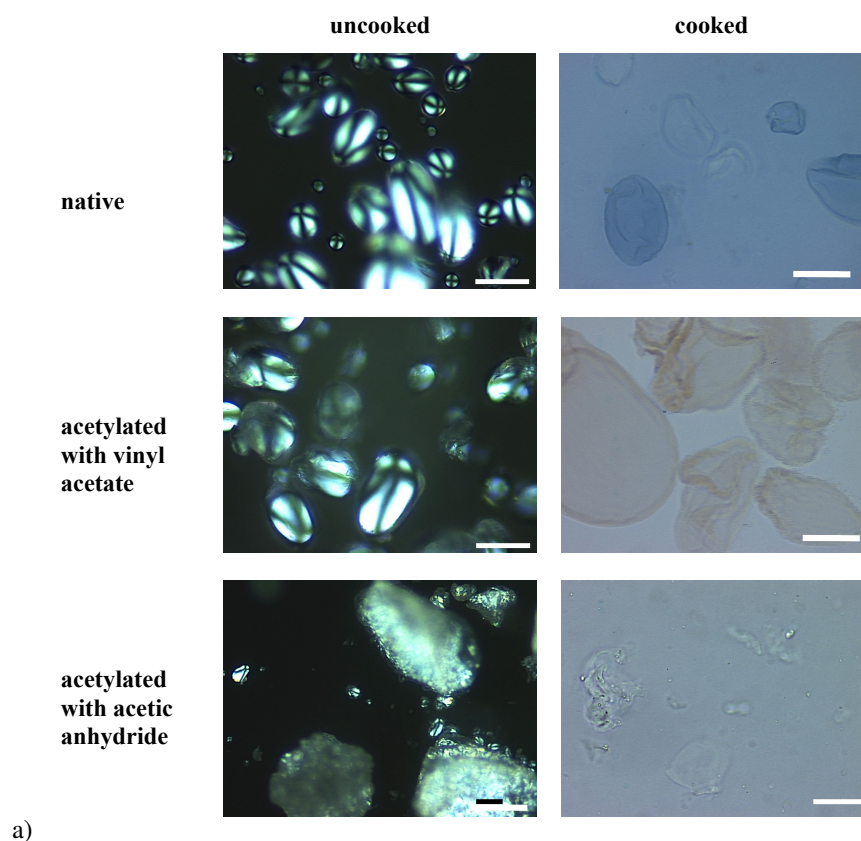
Table 1. DS of acetylated maize and potato starches using different reagents[*]

Starch	Vinyl acetate	Acetic anhydride
Maize	0.09 ± 0.00	0.16 ± 0.00
Potato	0.16 ± 0.00	0.121 ± 0.00

[*] Mean of three measurements ± standard error.

3.2 Granule morphology

Use of a polarizing microscope is one of the most effective ways to observe modified starch granules. The polarized light micrographs of uncooked native and acetylated PS are shown in Fig. 1a. Native PS showed a typical dark cross (Maltase cross), which means that the sign of birefringence is positive with respect to the spherocrystal radius, indicating an ordering of the starch components. After acetylation with vinyl acetate, the PS granules (Fig. 1a) did not show significant change in birefringence with respect to its native counterpart, which means that acetylation with this reagent had little effect on the crystalline structure. After acetylation with acetic anhydride, however, PS granules did not have birefringence, indicating a loss of order in the starch structure due to the chemical modification, and a fusion of starch granules was evident. Granule fusion after acetylation is attributed to the introduction of hydrophilic groups to the starch molecules, which results in an increase of hydrogen bonding. MS after acetylation (Fig. 1b) did not show significant change in birefringence with respect to its native counterpart, indicating that the acetylation had little effect on the crystalline structure.
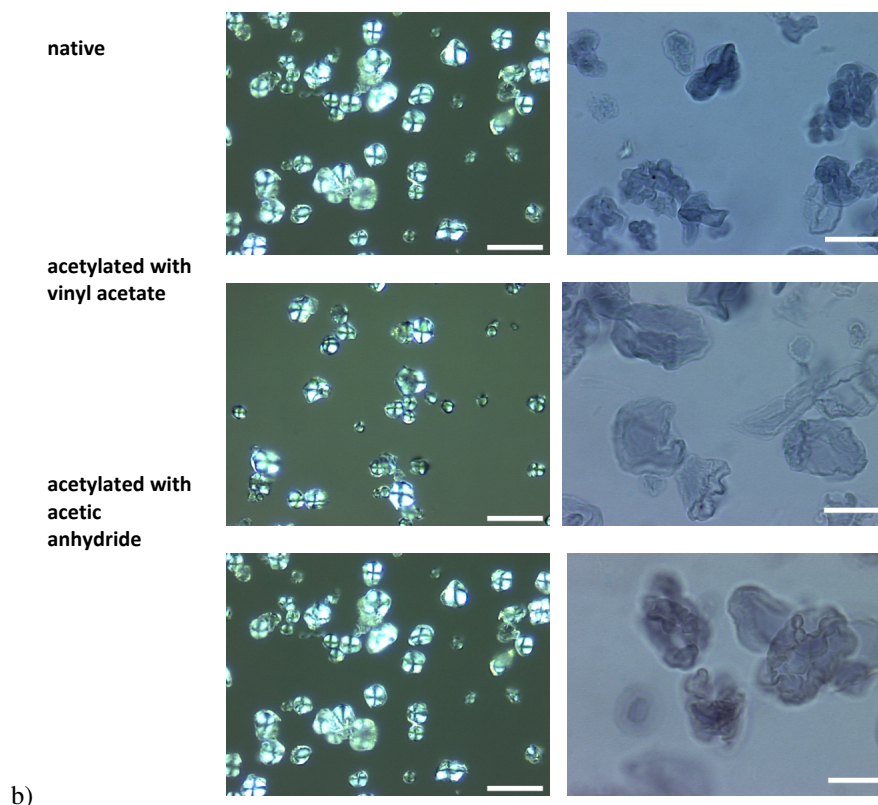
uncooked   cooked

native

acetylated with vinyl acetate

acetylated with acetic anhydride

a)

native

acetylated with
vinyl acetate

acetylated with
acetic
anhydride

b)

Fig. 1. Photograms of polarized light and light microscopy of uncooked and cooked navite and acetylated (a) potato and (b) maize starch.

When acetylated starches and their native counterparts were heated at 90ºC for 10 min, a loss of birefringence was observed (Fig. 1). In general, heating starch in the presence of excess water results in the loss of birefringence due to the melting of starch crystallites, and this phenomenon is accompanied by rapid swelling of the granule. Swollen, disrupted and undisrupted granules were found in native PS and PS acetylated with vinyl acetate. However PS acetylated with acetic anhydride did not show swollen granules and ghosts of granules were observed, indicating that these granules were not resistant to heating due to higher degree of disorganization of starch components during chemical modification. Cooking of native and acetylated MS produced swollen granules without collapse or disruption (Fig. 1b). Acetylation of MS did not change its cooking characteristics.

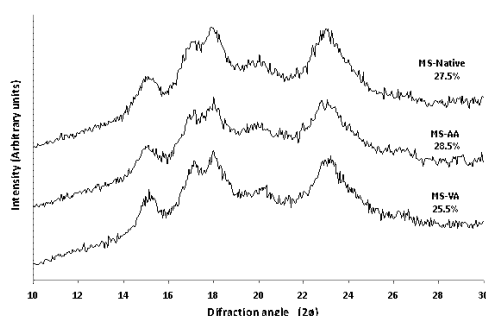### 3.3 X-ray diffraction pattern

Native PS presented a different XRD pattern and higher crystallinity level when compared to MS (Fig. 2). The arrangement of AP chains in the crystalline lamella of PS is different with longer AP chains in PS when compared to the chains in MS (Hizukuri, 1986; Yuan *et al*., 1993). Regardless of reagent used, the acetylated samples of PS had an important decrease in the crystallinity level (between 10% and 12%) when compared to native PS, and more peaks were disorganized in the chemically modified samples compared to the native samples. The effect was higher in the PS acetylated with acetic anhydride, which was a pattern that agreed with the lower DS. The effect of acetylation on the crystallinity level of MS was different. Regardless of reagent used, acetylated MS had a higher crystallinity level when compared to native MS. MS acetylated with acetic anhydride had a higher crystallinity level when compared to MS acetylated with vinyl acetate, which had a pattern that was in line with the higher DS value calculated in the MS acetylated with acetic anhydride. Therefore, a lower DS value produced lower percentages of crystallinity. These results show that the intrinsic and extrinsic factors have an important role in the acetylation of starch. Our results contrasted with those reported by several authors who demonstrated that a

change in the crystallinity of acetylated starches from diverse botanical sources was not detected with respect to their native counterparts (Wang and Wang, 2004; Jane and Chen, 1992).
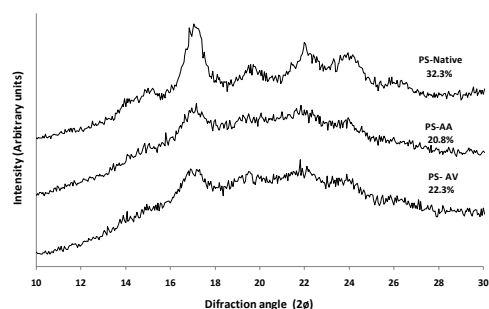
PS: Potato starch, AA: Acetic anhydride; VA: Vinyl acetate

MS: Maize starch, AA: Acetic anhydride; VA: Vinyl acetate

Fig. 2. X-ray diffraction patterns of native and acetylated maize (a) and potato (b) starches. MS: Maize starch, PS: Potato starch, AA: Acetic anhydride; VA: Vinyl acetate.

3.4 Pasting characteristics

In general, acetylation with either reagent decreased the pasting temperature. PS acetylated with acetic anhydride had the highest peak viscosity, and no difference in the peak viscosity was found between PS acetylated with vinyl acetate and its native counterpart (Table 2). Regardless of reagent used, acetylated MS had a peak viscosity similar to the peak viscosity of native MS. The acetylation of MS with either reagent did not restrict the swelling of the granules, but the acetylation of PS with acetic anhydride produced disorganization of the starch components. Disorganization of the starch components allows higher water contents to be incorporated into the granules causing the granules to swell to a higher level, and some components, including AM and long outer AP chains, are diffused out toward the continuous phase (Chen *et al.*, 2003). Although MS acetylated with acetic anhydride and PS acetylated with vinyl acetate had similar DS values, the pasting pattern was different, indicating that the DS as a number does not reflect the functionality of the acetylated starch. However, it may reflect the different distribution pattern of acetyl groups depending on the reagent (Huang *et al.*, 2007a), morphology and molecular structure of starch.

Native MS presented lower breakdown than its acetylated counterparts. However, a similar breakdown value was determined for native PS and acetylated PS. These results indicated that the MS botanical source with its specific characteristics, such as morphology, presence of pores, and channels (Huber and BeMiller, 2000) and molecular structure are important in this chemical modification. Higher breakdown values reflected susceptibility to heating and shear stress during the test, and this pattern was produced by acetylation due to partial disorganization, which was evident in MS.

Table 2. RVA parameters of acetylated maize and potato starches

Starch	Acetylation	Pasting temperature (°C)	Peak viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Reassociation (cP)
Potato	Native	66.53	8880	5810	3916	846
	Vinyl acetate	53.54	8823	5147	4803	1127
	Acetic anhydride	56.38	13592	6655	8351	1414
Maize	Native	74.60	5672	1639	5017	984
	Vinyl acetate	71.27	5659	3391	4070	1802
	Acetic anhydride	66.63	5629	2871	3992	1234

Table. 3. Gelatinization and retrogradation properties of unmodified and acetylated maize and potato starches using different reagents*.

Starch		Gelatinization			Retrogradation (7 days)			Retrogradation (14 days)		
		Native	VA	AA	Native	VA	AA	Native	VA	AA
Potato	T_o	58.18 ± 0.09	47.61 ± 0.12	49.10 ± 0.74	50.17 ± 0.30	40.80 ± 0.29	51.91 ± 0.10	45.91 ± 0.42	52.11 ± 0.97	43.30 ± 0.68
	T_p	62.42 ± 0.13	53.35 ± 0.33	55.88 ± 0.64	59.24 ± 0.32	45.40 ± 0.42	58.05 ± 0.1	58.01 ± 0.32	57.35 ± 0.80	58.47 ± 0.64
	ΔH	9.95 ± 0.06	4.23 ± 0.23	3.75 ± 0.34	2.48 ± 0.25	0.58 ± 0.01	0.68 ± 0.10	4.44 ± 0.19	0.36 ± 0.01	0.45 ± 0.04
Maize	T_o	64.82 ± 0.06	60.52 ± 0.25	57.38 ± 0.15	43.43 ± 0.56	46.30 ± 1.03	51.50 ± 0.75	41.64 ± 0.74	46.24 ± 0.14	52.44 ± 0.86
	T_p	70.71 ± 0.07	65.93 ± 0.07	63.31 ± 0.11	53.32 ± 0.15	53.21 ± 0.64	57.11 ± 0.33	51.74 ± 0.48	53.72 ± 0.81	57.7 ± 0.94
	ΔH	9.66 ± 0.07	7.09 ± 0.028	4.54 ± 0.07	6.49 ± 0.10	1.05 ± 0.33	1.91 ± 0.05	6.75 ± 0.30	1.24 ± 0.12	2.65 ± 0.17

VA = Vinyl acetate; AA = Acetic anhydride

* Mean of three measurements ± standard error.

T_o = Onset temperature (°C); T_p = Peak temperature (°C); ΔH = Gelatinization or Retrogradation enthalpy (J/g starch).

Similar to our results, others have reported that legume starches acetylated with acetic anhydride and vinyl acetate produce different profiles. Moreover, others have reported that the higher peak viscosity in starch acetylated with vinyl acetate in addition to the difference in pasting behavior between starches acetylated with either reagent is not due to a different DS value (Huang *et al*., 2007a). Yellow pea and chickpea starches acetylated with vinyl acetate have higher peak viscosities than when they are acetylated with acetic anhydride, but there is no appreciable difference in the peak viscosity when cowpea starch is acetylated with either vinyl acetate or acetic anhydride (Huang *et al*., 2007c).

3.5 Thermal properties

The gelatinization properties of native and acetylated MS and PS tested by differential scanning calorimetry (DSC) are summarized in Table 3. Native MS had a higher gelatinization temperature than native PS, but similar enthalpy values were calculated for the native starches, indicating similar ordered double helices of AP chains. In general, acetylation decreased the temperature and enthalpy of gelatinization, indicating disorganization of the starch components after acetylation (arrangement of AP double helices). MS acetylated with vinyl acetate had a higher peak temperature than MS acetylated with acetic anhydride, and an inverse pattern was found for PS. In both types of starches acetylated with acetic anhydride, the enthalpy value was less than the enthalpy value when the starches were acetylated with vinyl acetate, but the effect was higher in MS. Cowpea and chickpea starches acetylated with vinyl acetate have higher enthalpy than cowpea and chickpea starches acetylated with acetic anhydride, but there is no difference in enthalpy of the gelatinization value between yellow pea starch acetylated with vinyl acetate or acetic anhydride (Huang *et al*., 2007c). This pattern may be due to the fact that acetylation with acetic anhydride is limited at the outer lamellae of the granules (Chen *et al*., 2004) and that the modification with vinyl acetate may be located from the outer to inner lamella producing more homogenously modified granules (Huang *et al*., 2007b) and, consequently, a higher stability of the double helices.

At 7th day of storage, the native MS retrograded to a higher extent than the native PS (Table 3). However, MS retrogradation reached a plateau because the enthalpy value at 14 days of storage was similar to that at 7th day. PS continued to reorganize because the enthalpy value was higher at 14 days of storage when compared to the enthalpy value at 7 days of storage. The AP structure of PS (with longer chains) may be responsible for this pattern because the reorganization of AP takes a longer time (Ring *et al*., 1987). Acetylation decreased starch retrogradation as shown by the lower enthalpy value in acetylated starch when compared to native starch. Acetylated PS with either reagent had less retrogradation than acetylated MS, and no difference was detected between the types of reagent used in PS acetylation because a plateau was reached after 7 days of storage. The effect of the reagent during the retrogradation of MS was not evident at 7 days of storage, but MS acetylated with acetic anhydride continued to retrograde because the enthalpy increased at 14 days of storage.

3.6 Amylopectin chain length distribution

Amylopectin branch chain-length distribution for the native and acetylated starches of maize and potato are shown in Table 4. Native MS and PS starches consisted of smaller proportions of B2 and B3+ chains but greater proportions of A and B1 (with approximately 50% of the chains being B1 chains). With both reagents, acetylated MS had an increase in A and B1 chains and a decrease in B2 and B3+ chains, and this effect was more pronounced in MS acetylated with acetic anhydride. A similar pattern of chain length distribution was found for PS, but the effect of the reagent was inversed, with vinyl acetate having the higher effect. This pattern was in agreement with the DS because PS acetylated with vinyl acetate was higher than PS acetylated with acetic anhydride, and an inverse pattern was found for MS. Thus, higher DS is related with higher depolymerization, the decrease in B2 and B3+ chains resulted in a concomitant increase in A and B1 chains. The increased depolymerization of the longer chains supported the following conclusions: 1) acetylation of MS with acetic anhydride is produced in the outer lamella of the granule, but due to the arrangement of the lamella crystalline in PS, acetylation with the reagent is limited; and 2) acetylation of PS with vinyl acetate produces higher depolymerization due to the substituent being localized from the outer to inner lamellae, but acetylation of MS is limited to the outer lamella due to the crystalline arrangement of the AP chains. The distribution of acetyl groups on AM and AP depends on the reagent type, and this distribution is not affected by the granule size (Huang *et al*., 2007b). However, we also think that the crystalline

Table. 4. Chain length (CL) distribution of isoamylase-debranched native and acetylated potato and maize starches as characterized by HPAEC-PAD[a]

Starch	Acetylation	Average chain length (CL)	% Chain length distribution			
			A chain [CL 6-12]	B1 chain [CL 13-24]	B2 chain [CL 25-36]	B3+ chain [CL≥ 37]
Potato	Native	20.49±0.06	23.24±0.07	51.14±0.21	15.42±0.14	10.20±0.14
	VA	15.11±0.03	37.06±0.15	56.56±0.11	4.50±0.01	1.87±0.04
	AA	16.90±0.04	31.43±0.14	56.14±0.02	8.19±0.06	4.24±0.06
Maize	Native	20.72±0.10	23.01±0.10	49.81±0.34	17.15±0.23	10.02±0.21
	VA	17.89±0.05	30.46±0.06	52.59±0.30	11.78±0.18	5.18±0.07
	AA	16.61±0.07	33.75±0.50	54.35±0.33	8.59±0.15	3.32±0.02

VA = Vinyl Acetate; AA = Anhydride Acetic
[a] Mean of two measurements ± standard error.
CL = Chain length

arrangement (due to AP chains) is important in the acetylation of starch. The way acetylation of PS using acetic anhydride is performed depends on the granule size. It has been reported that more short chains (A and B1 chains) are present in small granule-size fractions than in large granule-size fractions for PS (Chen *et al*, 2005).

Conclusions

In summary, vinyl acetate produced a higher DS in PS when compared to MS, and an inverse pattern was found with acetic anhydride. Regardless of reagent used, acetylated PS had a lower crystallinity value when compared to acetylated MS. Acetylation with either reagent decreased the pasting temperature, and the acetylated starches with higher DS values had higher breakdown and reassociation values. MS acetylated with vinyl acetate (lower DS) had a higher gelatinization enthalpy value when compared to MS acetylated with acetic anhydride. However, there was no difference in gelatinization enthalpy values between PS acetylated with vinyl acetate or PS acetylated with acetic anhydride. Acetylation with either reagent avoided retrogradation, but acetylated MS had a higher retrogradation enthalpy value when compared to PS. Acetylation with either reagent changed the chain length distribution. In addition, more short chains were found in acetylated starches, and the effect was higher when the DS value was high. Therefore, extrinsic and intrinsic factors influenced the physicochemical and structural characteristics of acetylated starch.

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