

Effect of the acetylation process on native starches of yam (*Dioscorea* spp.)

Efecto del proceso de acetilación sobre almidones nativos de ñame (*Dioscorea* spp.)

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ABSTRACT

Key words:

Amylose
Esterification
Degree of substitution
Viscoamylogram
Yam

In Colombia, it is necessary to produce native and modified starches for the use of amylaceous raw materials of major socioeconomic importance. In this study, the effects of the acetylation process on structural, morphological and functional properties of native starches yam, *Dioscorea* spp. (*D. alata* and *D. rotundata*) were evaluated. Chemical modification by esterification with acetic anhydride was performed at different reaction times, and morphological and structural changes were assessed using the following techniques: infrared spectroscopy (FTIR), X-ray diffraction and scanning electron microscopy (SEM). Acetylation produced slight changes in the granule morphology, and a decreased degree of crystallinity (DC) associated with a slight increase in the amylose content was observed. The introduction of acetyl groups into the starch structure caused a decrease in the gelatinization temperature and an increased retro gradation tendency. The acetylated starches had low degrees of substitution (DS<0.2), meaning they can be used in the food industry, considering that they showed greater stability, greater water absorption capacity and better solubility than native starches.

RESUMEN

Palabras claves:

Amilosa
Esterificación
Grado de sustitución
Viscoamilograma
Ñame

En Colombia es necesaria la producción de almidones nativos y modificados para el aprovechamiento de materias primas amiláceas de gran importancia socioeconómica. En esta investigación se evaluó el efecto del proceso de acetilación en las propiedades estructurales, morfológicas y funcionales de almidones nativos de ñame *Dioscorea* spp. (*D. alata* y *D. rotundata*). La modificación química por esterificación con anhídrido acético, se realizó a diferentes tiempos de reacción, evaluando cambios morfológicos y estructurales, a través de espectroscopia de infrarrojo (FTIR), difracción de rayos X y microscopia electrónica de barrido (SEM). La acetilación produjo cambios leves en la morfología del gránulo, y se observó una disminución del grado de cristalinidad (GC) asociado a un ligero aumento en el contenido de amilosa. La introducción de grupos acetilos en la estructura del almidón, provocó una disminución de la temperatura de gelatinización y mayor tendencia a la retrogradación. Los almidones acetilados presentaron bajos grados de sustitución (GS<0,2) que posibilitan su uso en la industria alimentaria, y atendiendo que muestran: una mayor estabilidad, mayor capacidad de absorción de agua y mejor solubilidad.

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Starch is an important reserve polysaccharide in tubers from the *Dioscorea* species, with the content in dry base varying between 40 and 60% (Wang *et al.*, 2008). The granule consists of amylose and amylopectin macromolecules that differ in their chemical composition and molecular structure depending of the species of origin, displaying unique physicochemical and functional properties. Numerous studies have focused on evaluating the properties of native starches from the *Dioscorea* spp. species, due to their ready availability and their widespread use in food and non-food applications; additionally, they are characterized by presenting thermally stability, good water retention capacity and a high gelatinization temperature (Wang *et al.*, 2006a, 2006b).

However, the native structure of starch can be less efficient, since its functional properties are less stable to process conditions such as high temperature, shear stress and exposure to acidic media; this decreases its use in industrial applications. These disadvantages can be rectified by modified starches designed to respond to industrial demand (Falade and Ayetigbo, 2015). One type of modification consists of the acetylation process, based on the introduction of acetyl groups ($\text{CH}_3\text{C}=\text{O}$) that interfere with the structural order of native starch, as well as with the reassociation of amylose and amylopectin after the gelatinization process, causing a decrease in the gelatinization temperature, and an increase or decrease in the swelling power and solubility, along with the storage stability. The number of acetyl groups incorporated in the molecule depends on the source of the starch, the reagent concentration, the reaction time, the amylose/amylopectin ratio, the pH and the presence of a catalyzer (Huang *et al.*, 2007; Wang *et al.*, 2008). In food applications, the United States Food and Drug Administration (FDA) only permits the use of starches with a low degree of substitution (<0.2) (Colussi *et al.*, 2015).

Previous studies regarding the effect of the acetylation process on native yam starches have been reported. Harvey *et al.* (2012) studied the susceptibility of yam starch to the acetylation process for pharmaceutical use. Other authors evaluated the changes in the physicochemical properties, including the morphology, crystalline structure and thermal properties of acetylated starches with different degrees of substitution (Wang *et al.*, 2008; Bolade and

Oni, 2015). However, few bibliographical reports exist about acetylated yam starches for food applications. For this reason, the present study was developed in order to produce acetylated starches from purple yam (*D. alata*) and white yam (*D. rotundata*), a yam with a low degree of substitution (DS), and to evaluate their structural and functional properties in order to assess their possible use in the food industry.

MATERIALS AND METHODS

Starch modification process

The native starch from the purple yam (*D. alata*) and white yam (*D. rotundata*) species was obtained from the Unitary Operations Plant of the Universidad de Sucre (Sincelejo, Colombia), using the methods described by Salcedo *et al.* (2014). The acetylation process was carried out in accordance with the instructions of Rincón *et al.* (2007) and Adebowale *et al.* (2004), with slight modifications. A 42.5% starch suspension was subjected to agitation for 60 min at 30 °C and the pH was adjusted to 8.0–8.2 with a solution of 3.0% NaOH w/v. Afterwards, 3 mL of acetic anhydride was added drop by drop, maintaining a pH of 8.0–8.4. After the reaction time was complete, the suspension was adjusted to a pH of 4.5 with 0.4 N HCl. The starch obtained was washed, dried (dryer Model TD-S/EV, Veneta Electronic-Italia), ground and sieved.

Determination of amylose content

The amylose content was determined by applying the standardized colorimetric iodine method, following the method proposed by Chen *et al.* (2003), with slight modifications using a 100 mg starch sample in dry base (d.b). Potato amylose was used (Sigma Aldrich, St. Louis, USA) as a standard for the calibration curve. The measurements were performed in triplicate with a UV-visible spectrophotometer (Thermo Scientific, Evolution 60S, USA) at an absorbance of 620 nm.

Determination of acetyl groups (AG) and degree of substitution (DS)

The percentage of acetyl groups and the degree of substitution in acetylated and native (white) starch samples were calculated by titration with 0.8 N HCl, according to the method proposed by Diop *et al.* (2011). The AG percentage was determined based on the volume of acid used during neutralization (Equation 1), where 0.043 are the meq_s of the acetyl group.

$$\text{Acetyls(\%)} = \frac{(\text{mL White} - \text{mL sample}) * (\text{NHCL} * 0.043 * 100)}{\text{g sample (db)}} \quad (1)$$

The DS corresponded to the average number of acetyl groups introduced per unit of anhydrous glucose whose molecular weight was equal to 162 units (Equation 2):

$$\text{DS} = \frac{(162) * \text{Acetyls(\%)}}{4300 - [42 * \text{Acetyls(\%)}] } \quad (2)$$

Fourier transform infrared spectroscopy (FTIR)

The spectra were obtained with an FTIR spectrometer (Thermo Scientific, IS50, USA) in the 500–4000 cm^{-1} range, from samples of starch mixed with KBr salt at a ratio of 1:5, respectively. Thirty-two readings were taken at a resolution of 4 cm^{-1} (Colussi *et al.*, 2015).

Diffraction pattern and degree of crystallinity (DC)

The diffraction patterns of the materials were obtained with an X-ray diffractometer (Panalytical, X'Pert Pro-MPD, Italy), using a detection angle of 4–35°. The machine was operated at 1.8 kW, with a current of 40 mA. The samples were previously macerated and attached to an aluminum sample holder. The DC was determined based on the ratio of the area of the absorption peaks from the crystalline regions (A_C) to the amorphous area of the sample (A_A) (Equation 3), through integral calculation using Origin Lab 8.0 software (OriginLab Corporation, USA), in accordance with Wang *et al.* (2008).

$$\text{DC} = \left(\frac{A_C}{A_A + A_C} \right) \quad (3)$$

Granule morphology

The samples were analyzed with a scanning electron microscope (JEOL, JSU-5600 LV, Japan) using the method described by Guerra *et al.* (2008). The samples were attached to a sample holder with electro-conductive carbon tape, covered with a platinum gold alloy. The observation conditions were set at 15 kV and 30 mA, and the images were captured at a magnification of 1000X.

Water absorption index (WAI), water solubility index (WSI), and swelling power (SP)

The WAI, WSI and SP were determined using the methods described by Colussi *et al.* (2015). 1 g (d.b) of

starch was deposited in a centrifuge tube, to which 25 mL of distilled water was added at 60 °C. The suspension was heated in a 60 °C bath for 30 min and agitated 10 min after the process was initiated. It was then subjected to centrifugation at 1065 g for 15 min. The supernatant was extracted (soluble starch) and the total volume (V) was determined. A 10 mL aliquot of supernatant was placed in a Petri dish that was previously weighed and oven-dried at 70 °C for 16 h. The weights of the Petri dish with the soluble matter and of the tube containing the gel (insoluble starch) were recorded. The WAI, WSI, and SP values were estimated using the following expressions (Equations 4-5-6).

$$\text{WAI} = \frac{\text{Gel weight(g)}}{\text{Sample weight(g) db}} \quad (4)$$

$$\text{WSI} = \frac{\text{Soluble weight(g)} \times (V/10)}{\text{Sample weight(g) db}} \quad (5)$$

$$\text{SP} = \frac{\text{Gel weight(g)}}{\text{Sample weight(g) - Soluble weight (g)}} \quad (6)$$

Behavior of the paste in cold and heat

Suspensions of starch at 4% (w/v) were evaluated in a rheometer (Anton Paar, MCR 302, Austria) using the method described by Rivas (2012). The starch suspensions were initially subjected to a temperature of 50 °C for 1 min; the temperature was then raised to 95 °C over the course of 7.5 min, maintained at 95 °C for 5 min, cooled to 50 °C over the course of 7.5 min, and finally, maintained at 50 °C for 2 min. The spindle speed (Anton Paar, ST24-2D/2V, Austria) was adjusted to 960 rpm for the first 20 s in order to homogenize the suspensions, and was then reduced to 160 rpm for the rest of the experiment. The results obtained from the experiments were processed with RheoCompass software, version 1.12 (Anton Paar, Austria).

Statistical analysis

For the statistical analysis of the results, a categorical multifactorial design was used with two factors: yam species (purple, white) and reaction time (10 and 240 min). The results were statistically examined using analysis of variance and Tukey's comparison of means test, at a significance level of 5%, with the statistical software Rv. 3.0

RESULTS AND DISCUSSION

Acetyl group (AG) percentage and degree of substitution (DS)

The AG percentage and DS increased significantly ($P < 0.05$) with the reaction time (Table 1). This same behavior was found in acetylated starches from various yam species, indicating that a higher quantity of acetyl groups are introduced into starch molecules as the reaction time increases (Harvey *et al.*, 2012). The AG and DS values were also similar to those reported by Muluaem (2013) in yam starches treated with acetic anhydride for two hours. On the other hand, significant

differences in the DS were found between the yam species ($P < 0.05$); analogous results were found by Harvey *et al.* (2012) in five species of *Dioscorea* spp. The variation in DS between species was likely due to the amylose content (Table 1), the form of packaging in the amorphous regions and the configuration of amylose/amylopectin chains, which could affect the substitution reaction in the glucose units within the starch macromolecules (Huang *et al.*, 2007). Muluaem (2013) also found that the degree of acetylation appeared to be affected by the reaction conditions, such as reaction time, pH, reagent concentration and starch source.

Table 1. Percentage of acetylation, degree of substitution, amylose content and degree of crystallinity for acetylated yam starches.

Species	Reaction time (min)	Amylose content (%)	Acetyl groups (%)	Degree of Substitution	Degree Crystallinity (%)
Native Purple	-	25.01 ± 0.03 ac	-	-	48 ± 0.31 ac
	10	25.47 ± 0.19 bc*	0.90 ± 0.08 ac	0.034 ± 0.009 ac	47 ± 0.46 bc
	240	25.42 ± 0.12 bc	1.34 ± 0.14 bc	0.051 ± 0.012 bc	47 ± 0.22 bc
Native White	-	23.37 ± 0.10 ad	-	-	53 ± 1.21 ad*
	10	23.65 ± 0.14 bd	0.94 ± 0.11 ad	0.036 ± 0.003 ad	50 ± 0.61 bd
	240	23.75 ± 0.04 bd	1.13 ± 0.03 bd*	0.043 ± 0.008 bd	49 ± 0.87 bd

Arithmetic mean of three replicates ± standard error. Means in the same column with different letters are significantly different ($P < 0.05$) according to Tukey's HSD test at 5%. The same first letter in each column indicates $P > 0.05$ with respect to the reaction time. The same second letter in each column indicates $P > 0.05$ with respect to the species.

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of native and acetylated yam starches are shown in figure 1. In the IR spectrum of native starches, discernable peaks can be observed at 1079 cm^{-1} and 1243 cm^{-1} . These can be attributed to the stretching of C-O bonds. Another absorption peak can be observed around 1650 cm^{-1} , corresponding to strong bonds between water (H_2O) and the starch granule (Diop *et al.*, 2011), and a very wide band can be observed around 3340 cm^{-1} and 2945 cm^{-1} , resulting from the vibration of hydroxyl (O-H) and C-H groups, respectively (Rivas, 2012). After the acetylation process, increase absorption bands between 1251 and 1255 cm^{-1} , as a typical sign of the stretching of C-O bonds by acetyl groups (Harvey *et al.*, 2012). In the region between 1650 and 1750 cm^{-1} , an increase in the absorbance signal can be observed due to the folding of the OH group, as a result of changes in the starch structure (Muluaem, 2013). Variations in the signal can be observed in the wavelength ranges 3000-3900 cm^{-1} ,

corresponding to the OH group, and 2000-2850 cm^{-1} , corresponding to the CH tension of the anhydrous glucose unit of the starch molecules, possibly associated with the introduction of acetyl groups in the carbon 6 (Xu *et al.*, 2004). These new absorption bands suggest that ester carbonyl groups were formed during the starch esterification process, when the acetylated starches were synthesized (Colussi *et al.*, 2015).

Diffraction pattern and degree of crystallinity

The native yam starches presented B-type diffraction patterns (Figure 2A, B), with peaks of greater intensity near the angles (2θ) 5.5; 14.7; 17; 22; and 24 (Wang *et al.*, 2006a). This type of pattern is characteristic of starches from tubers with high degrees of crystallinity, since the double helices from the amylopectin in their structures are packaged in a hexagonal shape and consist of 36 water molecules, forming a significantly organized structure (Rivas, 2012). After the reaction, the granules

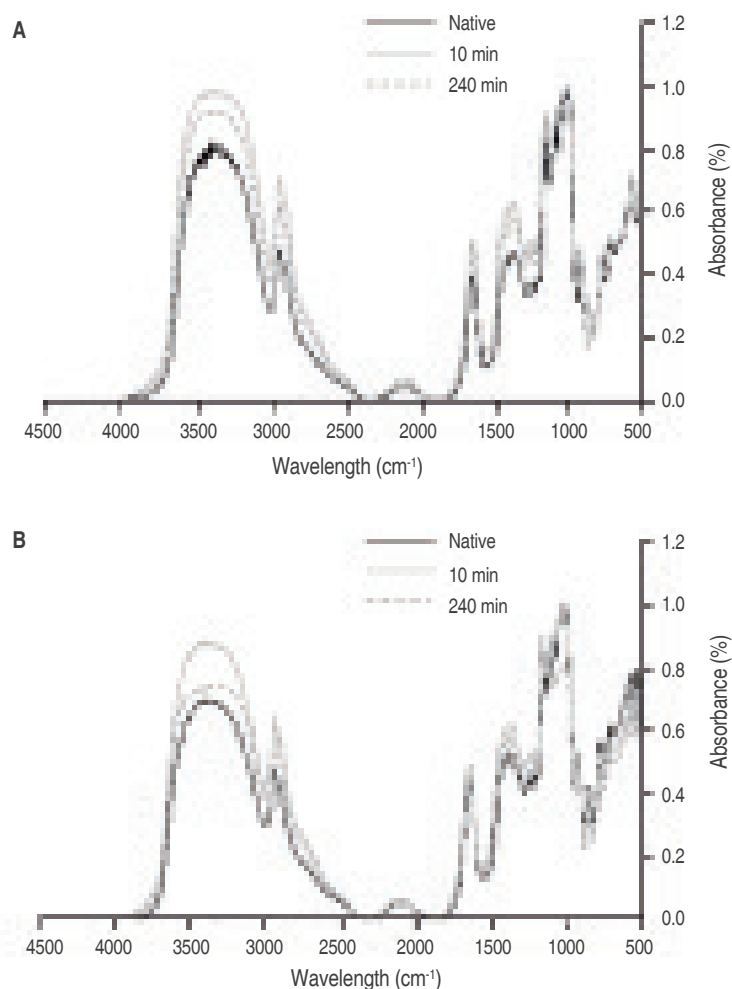


Figure 1. Infrared (FTIR) spectra in native and acetylated yam starches: (A) Purple, (B) White.

conserved the diffraction pattern characteristic of the native starches; similar results were reported in acetylated starches from corn hybrids, indicating that the most of the acetyl groups mainly enter the amorphous regions of the granule (Lawal, 2004).

The DC decreased significantly with the acetylation process (Table 1), likely due to the breaking of intermolecular bonds caused by the introduction of acetyl groups, which affected the crystalline structure of the granule in its native state (Diop *et al.*, 2011). The white yam starch presented a higher DC than the purple yam starch; this was related to a lower amylose content and reduction of the amorphous region. Additionally, in the diffraction patterns of starches acetylated for 240 min, a crystallinity profile with more pronounced peaks was observed as a result of

modifications in the structural arrangement of the starch, caused by the esterification reaction. According to Jandura *et al.* (2000), wider peaks can result from the formation of small crystals, and from structural and morphological changes caused by the acetylation process (Figures 2-3). Similar effects have been reported in acetylated starches from plantain (Rendón *et al.*, 2011).

Scanning electron microscopy (SEM)

The native purple yam starch granules presented a homogeneous morphology, of essentially oval or ellipsoidal shapes (Figure 3A), analogous to those established by Odeku and Picker (2007); meanwhile, the white yam granules (Figure 3D) exhibited oval, spherical and irregular shapes similar to those reported by Otegbayo *et al.* (2011).

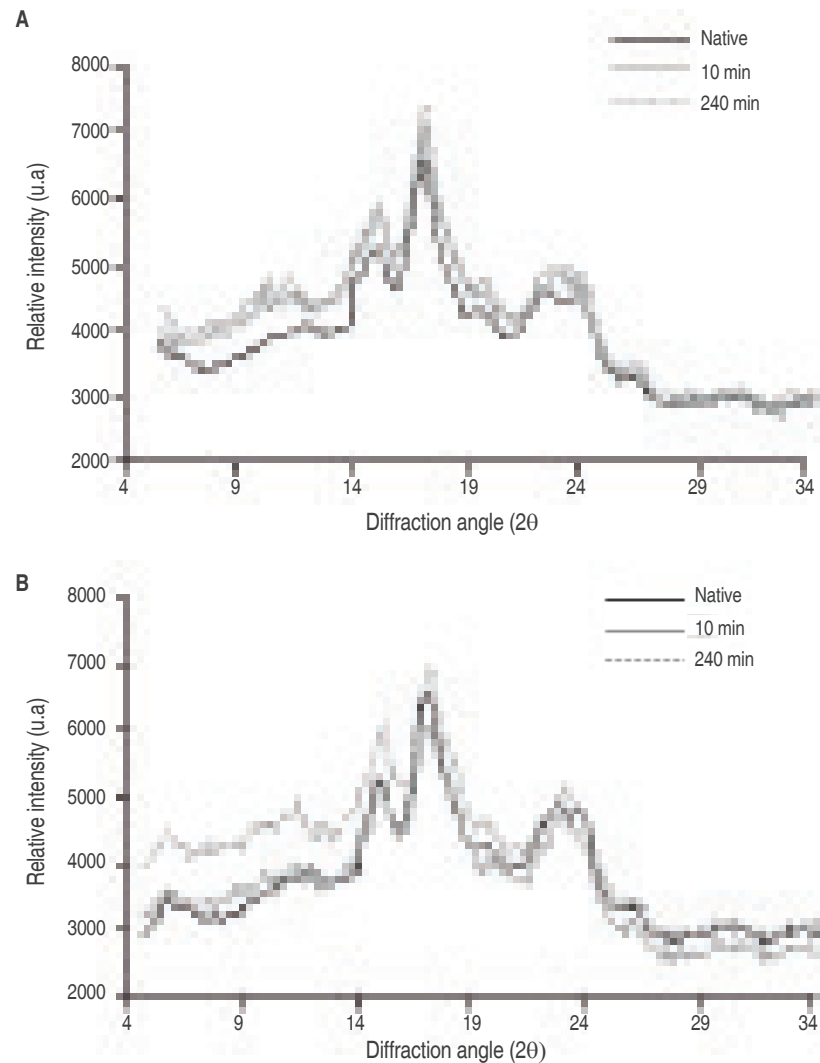


Figure 2. X-ray diffraction patterns in native and acetylated yam starches: (A) Purple, (B) White.

After the acetylation process, slight changes were observed in the granule surfaces (Figure 3B, C); however, the acetylated starches conserved their morphology. This could be because the reaction was produced preferentially in the amorphous region of the granule composed of amylose and because the absence of pores or channels on the granule surface limited penetration of acetic anhydride, controlling the action of severe changes in the surface of the granule (Chung and Solarek, 2009). Similar results were described in acetylated plantain starches, where the granules presented a heterogeneous morphology with certain imperfections (Guerra *et al.*, 2008). Additionally, Sha *et al.* (2012) reported that the change of the surfaces of acetylated rice starch granules were mild compared with

native starch, possibly due to the role of the crystalline regions in controlling a certain loss of granular structure during the reaction. It is noteworthy that the changes in the surface of acetylated white yam starches became slightly evident as the DS increased (Figure 3E, F); a similar behavior was reported by Wang *et al.* (2008) in starches of Chinese yam (*D. opposita*).

Water absorption index (WAI), water solubility index (WSI), and swelling power (SP)

The WAI, WSI and SP values are detailed in Table 2. The WAI and SP, there were significant changes in acetylated starches ($P < 0.05$) in accordance with the reaction time and the yam species. Similar results were

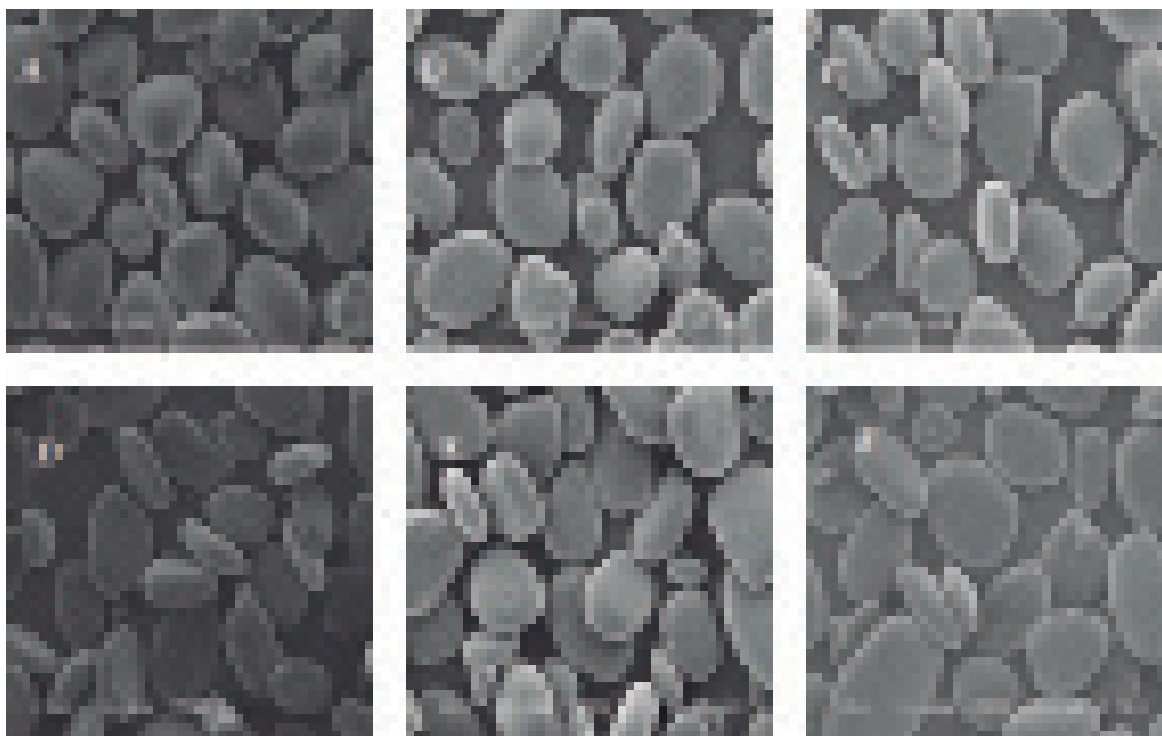


Figure 3. Appearance of native and acetylated yam granules under a scanning electron microscope (SEM). (A) Native purple, (B) Acetylated purple 10 min, (C) Acetylated purple 240 min, (D) Native white, (E) Acetylated white 10 min, (F) Acetylated white 240 min.

obtained in acetylated starches of sweet potato (Lee and Yoo, 2009) and corn (Diop *et al.* 2011). This increase in the WAI in acetylated starches could be associated with the introduction of acetyl groups that impeded intermolecular chain associations, causing a structural disorganization that facilitated water access in the amorphous region, thus leading to an increase in the hydration and swelling capacity of the granule (Xu *et al.*, 2005). Meanwhile, Chen

et al. (2003) established that differences in the WAI and SP among species could be explained by the intragranular arrangement of the chemical structure, amylose content, gelatinization temperature, shape and size of the granule.

The WSI increased significantly as a function of the reaction time ($P < 0.05$). Analogous behaviors were recorded for acetylated starches of potato, Cassava and

Table 2. Water absorption index (WAI), water solubility index (WSI) and swelling power (SP) of native and acetylated yam starches.

Species	Time (min)	WAI	WSI	SP
Native	-	1.77 ± 0.035 ac	0.001 ± 0.0004 ad	1.77 ± 0.09 ac
Purple	10	1.86 ± 0.015 bc	0.010 ± 0.0005 bd	1.86 ± 0.02 bc
	240	1.77 ± 0.005 ac	0.012 ± 0.0009 cd	1.77 ± 0.01 ac
Native	-	1.82 ± 0.008 ad	0.001 ± 0.0004 ad	1.79 ± 0.01 ad
White	10	1.82 ± 0.060 ad	0.015 ± 0.0006 bd	1.80 ± 0.03 ad
	240	1.91 ± 0.001 bd	0.008 ± 0.0007 cd	1.97 ± 0.01 bd

Arithmetic mean of three replicates ± standard error. Means in the same column with a different letter are significantly different ($P < 0.05$) according to Tukey's HSD test at 5%. The same first letter in each column indicates $P > 0.05$ with regard to the reaction time. The same second letter in each column indicates $P > 0.05$ with regard to the species.

rice (Mbougoung *et al.*, 2012; Colussi *et al.*, 2015). The change in the WSI could be attributed to the decreased interactions between the starch chains and the hydrophilic increase produced by the introduction of acetyl groups, permitting the amylose to solubilize and move to the outside of the swollen starch granule (Rincón *et al.*, 2007).

Behavior of the paste in cold and heat

Figure 4 and table 3 show the behavior of the viscosity in suspensions of native and acetylated yam starches during heating-cooling cycles. A decrease in the initial gelatinization temperature can be observed in the acetylated yam starches compared to their counterparts in a native state, possibly due to the weakening of the

intermolecular forces caused by the introduction of acetyl groups into the starch structure (Rincón *et al.*, 2007). This same behavior was reported by Bolade and Oni (2015) in acetylated starches from purple yam and sweet potato treated with acetic anhydride for five minutes. During heating, the viscosity peak of the paste in acetylated purple yam starches increased with the acetylation process, perhaps due to the increase in the hydrophilic capacity and to the development of more organized structures (Ancona *et al.*, 1997). This pattern was also observed by Rincón *et al.* (2007) in acetylated starches of *Artocarpus altilis*. However, in acetylated starches of white yam, a decrease in the viscosity peak was observed as the reaction time increased; this was possibly associated with the amylose

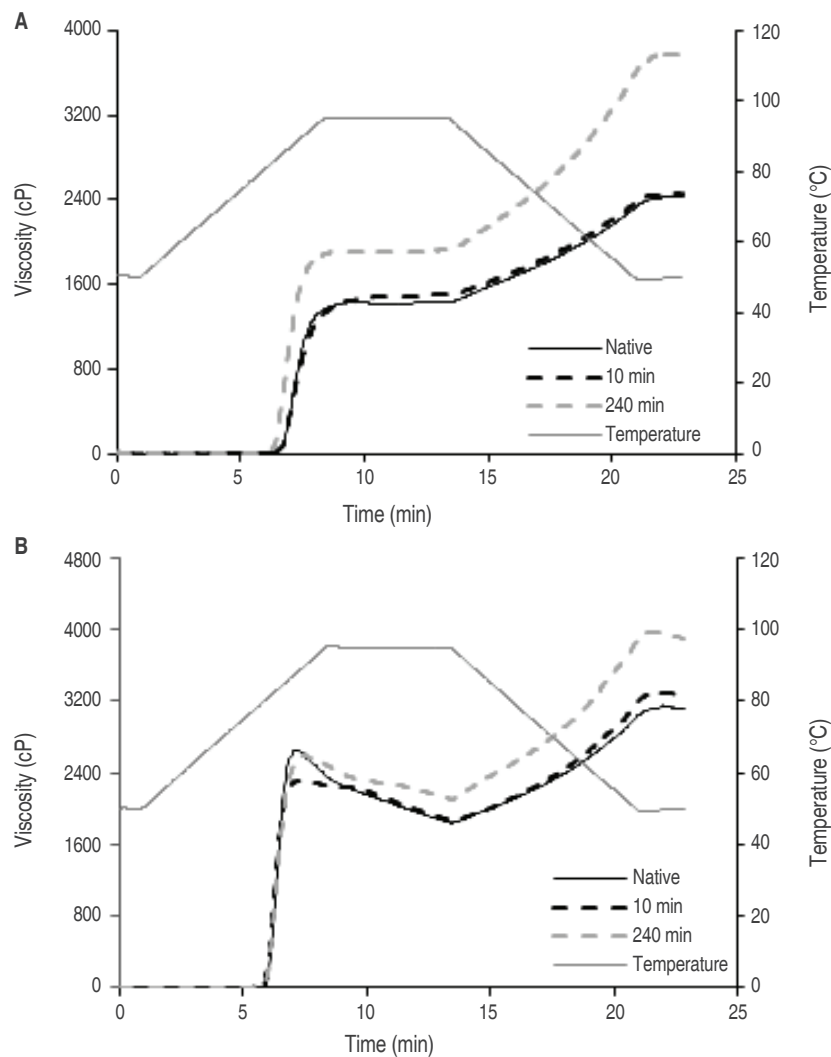


Figure 4. Viscoamylogram of native and acetylated yam starches: (A) Purple, (B) White.

Table 3. Behavior of viscosity in yam starch suspensions during heating-cooling cycles.

Species	Reaction time (min)	A	B	C	D	E	F
Native	-	82.4	1489	1454	2625	35	1021
	10	81.2	1504	1507	2452	-3	1032
Purple	240	80.1	1929	1931	3773	-2	1695
	-	79.6	2621	1843	3110	818	1201
White	10	78.6	2317	1870	3254	447	1337
	240	77.2	2605	2111	3899	494	1777

A: Initial temperature of the paste (°C); B: Maximum viscosity (cP); C: Viscosity at 95°C, 5min (cP); D: Final viscosity (cP); E: Stability (breakdown); F: Settling (setback).

content, morphology or molecular arrangement of the granule, which condition the capacity to absorb and retain water during the gelatinization process (Bou *et al.*, 2006; Huang *et al.*, 2007). A similar result was reported by Lawal (2004) in acetylated starches of corn hybrids.

On the other hand, the acetylated yam starches presented better stability, or “breakdown,” during heating than the native starches. This could be due to the acetylated starches having a more organized structure due to the introduction of acetyl groups, thus providing a greater resistance to thermal or mechanical forces. Bolade and Oni (2015) reported a constant-viscosity period during the heating phase, associated with excellent gel stability in acetylated starches of purple yam. With regard to the settling or “setback” values, the modified starches presented a greater retrogradation tendency. This can be explained as a function of the reorganization of linear chains during cooling, primarily those of amylose solubilized during the heating stages, causing a greater number of intersected bonds in the gel formation process (Moorthy, 2002; Mali *et al.*, 2003). Moreover, when yam starches present B-type diffraction patterns (Figure 2), associated with the distribution of long amylopectin chains in their structure, they acquire a more crystalline structure, increasing the retrogradation tendency of the granule (Moorthy, 2002).

CONCLUSIONS

The acetylated yam starches presented low degrees of substitution ($DS < 0.2$), which indicates their viability for use in the food industry. Using infrared analysis (FTIR), it was possible to confirm the introduction of acetyl groups by the widening or generation of new absorption bands, specifically in the region from 2700 to 3500 cm^{-1} . The acetylation process caused slight morphological

and structural changes that were manifested as slight lacerations in the granule surface, increased amylose content and a reduced degree of crystallinity.

The acetylated yam starches presented greater stability, with a resulting decrease in the “breakdown” values associated with an improved hydrophilic capacity and water absorption capacity. Based on this, it is possible to recommend their use in products that require a thickening power during processing, such as instant soups, broths, and sauces, among others. Moreover, the acetylated starches presented an increase in their final viscosity during the cooling period, which indicates a greater retrogradation tendency and, since they showed a greater solubility and water absorption capacity, suggests that they could be used in the development of confectionary products such as hard candies.

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