



## EFFECT OF CHEMICAL MODIFICATION TYPE ON PHYSICOCHEMICAL AND RHEOLOGICAL CHARACTERISTICS OF BANANA STARCH

## EFFECTO DEL TIPO DE MODIFICACIÓN EN LAS CARACTERÍSTICAS FÍSICOQUÍMICAS Y REOLÓGICAS DEL ALMIDÓN DE PLÁTANO

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### Abstract

Isolation of non-conventional starches has increased in the last decade; chemical modification of these non-conventional starches may produce starches with improved physicochemical and functional properties that are not available from commercial starches. Banana starch was acetylated and oxidized and the thermal, pasting and rheological characteristics were evaluated. The low carbonyl and carboxyl groups might be due to the starch source. The acetylated banana starch obtained had a low degree of substitution (0.04). The acetylated banana starch had lower temperature and enthalpy of gelatinization than oxidized and native banana starches, had higher peak viscosity in the viscoamylographic profile than its native counterpart. The oxidized starch produced the peak viscosity during the cooling step and the three starches had higher viscosity during the cooling step, showing a gel conformation. The native, acetylated and oxidized starches showed a non-Newtonian behavior of the shear-thinning type. The rotational test showed that oxidized banana starch had a firmer gel than acetylated starch, which agrees with the viscoamylographic results.

*Keywords:* chemical modification, banana starch, thermal properties, physicochemical properties, rheological test.

### Resumen

En la última década ha incrementado el aislamiento de almidones de fuentes vegetales no convencionales; la modificación química de estos almidones de fuentes no convencionales puede producir almidones con propiedades funcionales y físicoquímicas mejores que no presentan los almidones comerciales. Se acetiló y oxidó almidón de plátano y se evaluaron sus propiedades térmicas, de formación de pastas y reológicas. El bajo contenido de grupos carbonilo y carboxilo puede ser debido a la fuente botánica de almidón. El almidón acetilado de plátano presentó un bajo grado de sustitución (0.04). El almidón acetilado presentó una menor temperatura y entalpía de gelatinización que los almidones oxidado y nativo, y presentó un mayor pico de viscosidad en el perfil viscoamilográfico en comparación con el almidón nativo. El almidón oxidado presentó el pico de viscosidad durante la etapa de enfriamiento y los tres almidones presentaron incrementos de viscosidad durante la etapa de enfriamiento, mostrando una conformación de gel. Los almidones nativo, acetilado y oxidado presentaron un comportamiento no Newtoniano del tipo reofludizante. La prueba rotacional mostró que el almidón oxidado presentó un gel más firme que el almidón acetilado, lo cual coincide con los resultados viscoamilográficos.

*Palabras clave:* modificación química, almidón de plátano, propiedades térmicas, propiedades físicoquímicas, prueba reológica.

### 1. Introduction

Starch is perhaps the most important polymeric carbohydrate in terms of its functionality that impacts to products in diverse industries such as: pharmaceutical, foods, adhesives, etc. Nowadays, the developments of new products in those industries are searching starches with different or better

physicochemical and functional properties such as viscosity, solubility, low retrogradation and syneresis tendency. In recent years, substantial progresses have been made in obtaining starches from non-conventional botanical sources and studying their functional and physicochemical properties (Zhao y Whistler, 1994; Pérez *et al.*, 1997; Hoover, 2001; Hernández-Lauzardo *et al.*,

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2004; Nuñez-Santiago *et al.*, 2004). Starch is the principal component of unripe banana and some studies have been carried out on its isolation and partial characterization (Bello-Pérez *et al.*, 1999; Bello-Pérez *et al.*, 2000a); also, some modifications in banana starch were reported by Bello-Pérez *et al.* (2000b, c); Guerra-Della Valle *et al.* (2008); Sánchez-Rivera y Bello-Pérez (2008).

Use of native starches has showed some drawbacks because the process conditions (e.g., temperature, pH, pressure, shear stress, etc.) limit their use in industrial applications. These shortcomings may be overcome by modification using chemical, physical and enzymatic methods (Fleche, 1990). The most common methods to modified starch properties are the chemical procedures, treating native starch with specific chemicals reagent. This definition includes acetylated, oxidized, lintnerized, pyrodextrinized, hydroxypropylated and cross-linked starches (Van Der Bij, 1976).

The acetylated starch is produced by the esterification of native starch with acetyl groups. The efficiency of the reaction is affected by factors such as reagent concentration, reaction time, pH, presence of catalyst, starch source, amylose/amylopectin ratio and amylopectin fine structure. The functional properties of starch acetates will depend of the number of acetyl group incorporated to the glucose units of starch molecule (Betancur-Ancona *et al.*, 1997).

Oxidized starch is obtained by reacting starch with a specific amount of oxidizing agent under controlled temperature and pH (Wang y Wang, 2003). Sodium hypochlorite is the oldest and the most popular commercial oxidant. Hydroxyl groups of starch molecules are first oxidized to carbonyl groups and then to carboxyl groups. Therefore, the numbers of carboxyl and carbonyl groups in oxidized starch indicate the level of oxidation, which takes place primarily at the hydroxyl groups of C-2, C-3, and C-6 position on a D-glucopyranosyl unit (Kuakpetoon y Wang, 2001; Sánchez-Rivera *et al.*, 2005; Sánchez-Rivera y Bello-Pérez, 2008).

The objective of this study was to evaluate the effect of chemical modification type on some physicochemical and rheological properties of banana starch, in order to known possible applications of both modified banana starches.

## 2. Materials and methods

### 2.1. Banana starch isolation

Unripe bananas (*Musa paradisiaca*) were purchased in the local market of Cuautla, Morelos, México. Banana starch was isolated using a pilot scale procedure (Flores-Gorosquera *et al.*, 2004). The dry powder (10.5 % moisture content) was ground to pass a US No. 100 sieve and stored at room temperature (25 °C) in a sealed plastic container. The

protein and fat content were analyzed according to AACC methods 46-13 and 30-25, respectively (AACC, 2000).

### 2.2. Preparation of oxidized starch

The procedure reported by Sánchez-Rivera *et al.*, (2005) was tested. In brief, starch slurry was prepared by adding distilled water to 200 g starch (dry basis) to final weight of 500 g in a 1-L reaction vessel. The starch slurry was maintained at 35 °C and the pH was adjusted to 9.5 with 2 N NaOH. Sodium hypochlorite, 40 g (2.0 g active chlorine/200 g starch, 1.0 % w/w) was slowly added into the starch slurry over 30 min while maintaining the pH at 9.5 with 1 N H<sub>2</sub>SO<sub>4</sub>. After the addition of NaOCl solution, the pH of the slurry was maintained at 9.5 with 1 N NaOH for an additional 50 min. The pH of the slurry was adjusted to 7.0 with 1 N H<sub>2</sub>SO<sub>4</sub>, transferred to 1-L flask to precipitate and decant the slurry, washed with deionized water and dried in a convection oven at 50 °C for 48 h. The carbonyl and carboxyl content was assessed with the procedure of Sanchez-Rivera *et al.*, (2005).

### 2.3. Preparation of acetylated starch

Acetylated starch was obtained using the method reported by Bello-Perez *et al.* (2000a). In brief, 162 g of banana starch (dry basis) was weighed in a Griffin beaker of 400 mL of capacity and 220 mL of distilled water (25 °C) was added. The starch dispersion was stirred and the pH was adjusted to 8.0 by adding 3 % sodium hydroxide solution drop wise to the suspension while keeping it under agitation. Acetic anhydride (10.2 g = 0.1 mol/100 g starch, dry basis) was slowly added drop wise to starch suspension (in agitation) while simultaneously adding 3 % sodium hydroxide sufficient to maintain the pH of the suspension at 8.0-8.4. Thereafter, the pH was adjusted to 4.5 with 1 N hydrochloric acid and then the slurry was filtered on a Buchner funnel containing Whatman No. 4 filter paper. The filter cake was air-dried to equilibrium moisture content. The acetyl content (%) and the degree of substitution were obtained with the method of Wurzburg (1964).

### 2.4. Thermal analysis

The thermal properties of starches including temperature and enthalpy of gelatinization were assessed by a differential scanning calorimetry (DSC, TA Instrument, model 2010, New Castle, DE) previously calibrated with indium. Two mg sample (dry basis) was weighed on an aluminum pan, adding 7 µL of deionized water. The pan was sealed tightly and it was allowed to stand for 1 h prior to the analysis. An empty aluminum pan was used as reference. The sample was subjected to a heating program over a range of temperature from 10 to 120

°C and a heat rate of 10 °C min<sup>-1</sup>. The peak of gelatinization temperature ( $T_p$ ) and the enthalpy ( $\Delta H$ ) were obtained directly from the software TA Instruments OS/2 version 2.1 (Paredes *et al.*, 1994).

### 2.5 Micro ViscoAmyloGraphy

The viscosity profile (Brabender Units, BU) of native, acetylated and oxidized starches was obtained using the technique proposed by the AACC (61-02, AACC, 2000). Starch dispersions with 10 % (w/v) of total solids were prepared; 100 mL of starch dispersion (sample) were transferred to the bowl of the microviscoamylograph (Brabender OHG, Duisburg, Germany). The machine was programmed to run a heating-cooking-cooling cycle that began at 30 °C and it was increased to 95 °C, holding at this temperature for 10 min, then cooling down to 40 °C and it was maintained at this temperature for 10 min. A heating and cooling rate of 2.5 °C min<sup>-1</sup> was used along the whole cycle with a speed of agitation of 125 rpm.

### 2.6 Rotational test: Flow curves

Starch dispersions with 10 % (w/v) of total solids were prepared using distilled water. Their flow properties were measured running rotational tests in a Rheometer AR 2000 TA Instruments, (New Castle, DE) using a cone system of 1° with a diameter of 40 mm, and either a heating or cooling rate of 2.5 °C min<sup>-1</sup>. The rheometer was programmed for running time sweeps with a cycle of heating from 25 to 90 °C, holding at 90 °C for 10 min, and then cooling from 90 °C to 10 °C. At the final temperature of 10 °C, it was made two cycles up-down from 0.06 to 600 s<sup>-1</sup> and third cycle down from 600 to 0.06 s<sup>-1</sup>. The power law equation was applied to analyze the data.

### 2.7 Dynamic viscoelastic method

Starch dispersions with 10 % (w/v) of total solids were prepared using distilled water. The viscoelastic properties were measured using oscillatory tests in the same system as in the rotational test. To determine the linear viscoelastic region (LVR), strain amplitude sweeps were run from 0.01 to 5 % in the 1st stage (90 °C) and 2nd stage (25 °C) of a heating (25 to 90 °C) – holding (90 °C, 10 min, stage 1)–cooling (90 °C to 25 °C, stage 2) kinetics. All tests were run at a frequency of 1 Hz. Once the LVR was determined, the rheometer was programmed for running frequency sweeps (0.1 to 10 Hz) for which a constant strain value of 0.3 % was used in the 1st and 2nd stage of the kinetics. The storage modulus ( $G'$ ), loss modulus ( $G''$ ) and loss tangent ( $\tan \delta$ ) were evaluated from each test.

### 2.8 Statistical analysis

One way analysis of variance (ANOVA) at the significance level of 5 % ( $\alpha = 0.05$ ) was applied using the statistical program Sigma-stat, version 2.1 (Fox y Urlich, 1995) and when statistical differences were found, the test of multiple comparisons of Tukey was applied.

## 3. Results and discussion

### 3.1. Degree modification of starches

The carbonyl group value in oxidized banana starch was 0.019 %; this value is likely due to the high protein (1.6 %), lipid content (2.4 %), or pigments content (Sanchez-Rivera *et al.*, 2005) in the isolated banana starch. Sanchez-Rivera *et al.* (2005) reported 0.048 % for the same starch type with similar amount of sodium hypochlorite and pH used. These results showed the variability of the oxidation reaction in starches, because the carbonyl group content in oxidized normal and waxy corn starches were higher than those reported in banana starch at the same level of sodium hypochlorite. It was reported previously (Sanchez-Rivera *et al.*, 2005) that oxidized banana starch with 2.0 % chlorine concentration had higher amount of carbonyl groups than normal corn starch and similar to waxy corn starch (Wang y Wang, 2003). The low carbonyl groups in oxidized banana starch might be related with phenolic compounds present in the fruit and in the isolated starch, where its OH groups reacted with the chlorine before that the OH groups of glucose units. Additionally, it was reported that starch with high amylose content (37 % of amylose in banana starch, González-Soto *et al.*, 2007) had a low carbonyl groups content probably due to that sodium hypochlorite was consumed first to depolymerize the amylose present in the amorphous lamella of the outer layers of the starch granules before the formation of carbonyl groups (Kuakpetoon y Wang, 2001). For this reason oxidized banana starch was whiter than its native counterpart (Rutenberg y Solarek, 1984). The oxidation degree in a modified starch is determined by the concentration of carboxyl groups. During oxidation of starches, the carbonyl groups are transformed to carboxyl groups because the OH groups of glucose are initially oxidized to carbonyl groups and thereafter to carboxyl groups (Wang y Wang, 2003). The acetyl content of banana starch was 1.15 % with a degree of substitution of 0.04. This value classified the acetylated banana starch as of low degree substitution and is lower than the maximum (2.5 %) of acetyl group allowed in food by the U.S. Federal Drug Administration (FDA), Code of Federal Registration 172.892. Both values are similar to those reported in acetylated banana starch by Bello-Pérez *et al.*, 2000b, and indicating better repeatability of this reaction.

### 3.2. Thermal properties

The gelatinization temperature ( $T_p$ ) of native banana starch was significant higher than oxidized and acetylated samples (Table 1), showing that some disorganization of starch components was carried out during both modifications. The native banana starch presented the highest enthalpy value and the acetylated starch the lowest one. This pattern is in agreement with the temperature of gelatinization assessed for native and modified banana starches. The modification type showed different changes in the structure of starch because this phase transition (gelatinization) is influenced by the organization inside starch granular structure. It was reported that oxidation produces depolymerization of starch components (principally amylopectin), decreasing the crystallinity, but this depolymerization depends of the extent of oxidation of the polysaccharide. The results obtained for the acetylated banana starch are attributed to the introduction of hydrofobic groups with the concomitant decreasing of interactions starch-starch that produce lower gelatinization temperature (Singh-Sodhi y Singh, 2005). Other hypothesis is that the introduction of acetyl groups interrupts the order structure of native starch, decreasing the gelatinization temperature (Wang y Wang, 2002). Acetylated normal potato and normal maize starch presented lower gelatinization temperatures assessed by DSC than its native counterpart (Singh *et al.*, 2007). The enthalpy value of acetylated banana starch decreased ( $6.29 \text{ J g}^{-1}$ ) compared with its native counterpart ( $13.15 \text{ J g}^{-1}$ ). This value was indicative of structural changes produced during the chemical modification; similar pattern of enthalpy values was detected in yellow pea and chickpea starches acetylated with acetic anhydride (Huang *et al.*, 2007).

Table 1. Differential scanning calorimeter of unmodified and modified banana starches.

Sample	Gelatinization temperature (°C)	Gelatinization enthalpy ( $\text{J g}^{-1}$ )
Native	$70.25 \pm 0.28^a$	$13.15 \pm 0.49^a$
Acetylated	$64.56 \pm 0.14^c$	$6.29 \pm 1.38^c$
Oxidized	$69.05 \pm 0.30^b$	$9.00 \pm 1.69^b$

Mean values of three measurements  $\pm$  standard error. Values with the same letters inside each column are not different ( $\alpha = 0.05$ ).

### 3.3. Microviscoamylography

Chemical modification produces considerable changes in the pasting and rheological properties of starches. Fig. 1 shows the pasting properties of chemically modified banana starches and its native counterpart. Native and acetylated banana starches

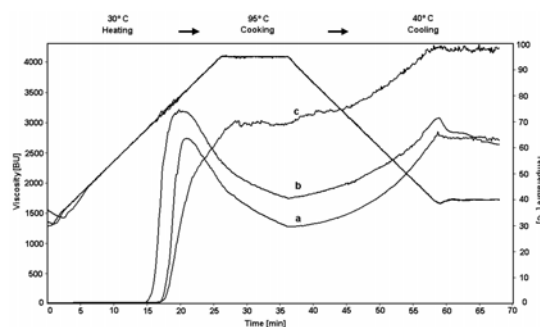


Fig. 1. Brabender profile of banana starch pastes: (a) native (b) acetylated and (c) oxidized.

had similar pasting behaviour, although the paste formation for native and oxidized banana starch started at higher temperatures than the acetylated one as demonstrated in DSC study.

The maximum viscosity values for oxidized and acetylated starches (Table 2) were similar and both were higher than the native one. These higher values of peak viscosity could be explained by the increase in the swelling power and solubility of the chemically modified starch (Singh-Sodhi y Singh, 2005). In the case of oxidized starch, this pattern is due to the carbonyl and carboxyl groups introduced to the starch molecule promoted swelling of the granules (Kuakpetoon y Wang, 2001). During the acetylation process, the starch-starch interactions in the granule are weakened by the introduction of acetyl groups (Taggart, 2004; Adebowale *et al.*, 2006; González-Soto *et al.*, 2007; Singh *et al.*, 2007), but there was no differences with the native sample during the holding step.

During the cooling period, it was observed an increase in the viscosity (Table 2) due to starch reorganization of linear chains (principally amylose) solubilized during the heating and holding steps. However, differences were shown between the two modified starches, because oxidized starch had higher viscosity than acetylated and native samples. The introduction of acetyl groups in starch chains were expected to prevent close parallel alignment of amylose chains and provoke the same setback viscosity than its native one.

Table 2. Pasting properties of unmodified and modified banana starches.

Sample	Peak viscosity (BU)	Break Down (BU)	Set Back (BU)
Native	$2645.7 \pm 80.2^b$	$1291.7 \pm 138.1^a$	$1359.0 \pm 49.3^a$
Acetylated	$3056.3 \pm 138.0^a$	$1272.7 \pm 211.0^a$	$1238.0 \pm 148.4^{ab}$
Oxidized	$3065.0 \pm 28.2^a$	$98.0 \pm 32.5^b$	$1182.3 \pm 53.7^b$

Mean values of three measurements  $\pm$  standard error. Values with the same letters inside each column are not different ( $\alpha = 0.05$ ). BU = Brabender units

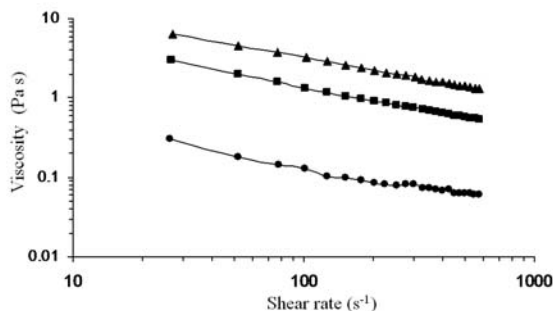


Fig. 2. Variation of apparent viscosity with shear rate of starch pastes: native (■), acetylated (▲) and oxidized (●) at 10°C.

Other characteristic of the pasting profile of oxidized banana starch is that during the cooling step had higher viscosity value than during the heating step.

### 3.4. Rotational test: Flow curves

All starches (native and modified) showed a non-Newtonian behaviour, where the viscosity decreased when shear rate rose (Fig. 2); this pattern is defined as shear thinning and is produced when the stress disorganized the arrangement of the macromolecules inside of the matrix. In our study, the Ostwald de Waele's model (power law) was used (Table 3) to fit the experimental data ( $R > 0.99$ ). All samples presented a flow behaviour index ( $n$ ) smaller than 1, although native starch showed the lowest value and more dependent of shear rate than the modified starches, the oxidized one showed the least dependency. In the case of consistency index ( $k$ ), acetylated starch showed the highest whilst oxidized the lowest value. These values agree with the fact that depending of chemical modification different chemical groups are incorporated and could produced softer system, like in the case of oxidation sample, and for the acetylated sample the gel was stronger. Wilhelm *et al.* (1998) postulated that alkaline media damaged starch structure and reduced paste viscosity.

During oxidation, there is a depolymerization which resulted in lower dispersion viscosity whilst acetylation retarded retrogradation, this is going to affect the stabilization of the starch sol. The tendency of aqueous starch dispersion to increase viscosity during cooling and finally to form a paste is related to the association of amylose molecules. Adebowale *et al.* (2006) mentioned that is probably that oxidation and acetylation is preferentially carried out on the amorphous regions of the starch granule, and depending of the modification level, a rupture of the granules is produced in minor or higher proportion, affecting the rheological properties of the modified starch.

Rutenberg y Solarek (1984) reported that the introduction of acetyl groups during the acetylation reduces the bond strength between starch molecules

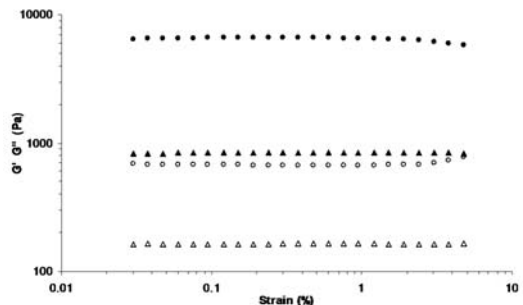


Fig. 3. Strain sweep of modified starches pastes [acetylated (▲) and oxidized (●)] in a gelatinization kinetic at 25 °C (second stage).

(amylose and amylopectin) and thereby increases the swelling power and solubility of the starch granules. This facilitates the access of water to amorphous areas, enhancing the water holding capacity of the starch matrix and developing a more organized structure, leading to a higher resistance to deformation and achieving a higher peak viscosity, this effect is more notorious at low temperatures.

Table. 3. Parameters of the Ostwald-de Waele (Power law) model of unmodified and modified banana starch pastes.

Sample	10 °C		
	R	n	k (Pa.s <sup>n</sup> )
Native	0.999	0.465	15.87
Acetylated	0.999	0.479	35.44
Oxidized	0.986	0.566	0.9213

### 3.5 Dynamic viscoelastic method

When starch approaches gelatinization is produced, an increase of dynamic moduli appears (storage module  $G'$  and loss module  $G''$ ) due to: 1) granules swelling and 2) amylose liberation from granules. When the gelatinized starch suspension was cooled down a gel was formed (Biliaderis, 1991) and then rheological dynamic tests were carried out in order to evaluate starch gelation.

During the strain sweeps at 90 °C similar values of  $G'$  and  $G''$  were obtained, being  $G'$  higher in the range of the strain studied (Figure not shown). Fig. 3 presents the strain amplitude sweep for the second stage (25 °C), where the gel conformation was determined. With these strain amplitude sweeps, the linear viscoelastic region of the studied samples was fixed. The samples analyzed had similar response in the range of the strain used, and it was determined that 0.3 % of strain was possible to carry out the studies of frequency sweep in all samples (native and modified banana starches).

The results of frequency sweeps showed that  $G'$  was higher than  $G''$ . In the first step (90 °C, Figure not shown) the differences between both moduli were of one logarithm cycle. For  $G''$  a slight

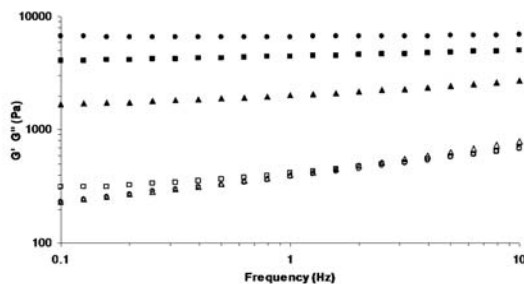


Fig. 4. Frequency sweep of modified and unmodified starches pastes [native (■), acetylated (▲) and oxidized (●)] in the last step of the gelatinization kinetic (cooling) at 25 °C (second stage).

decrease was found when the frequency decreased, and  $G''$  showed a slight increase with the frequency, which is due to the relaxation mechanism involved in the gel structure at low frequencies and might indicate the structure of a rigid biopolymer in solution. The same pattern for all starches was obtained and no differences were shown.

At low temperature (Fig. 4) the differences between both moduli also was one logarithm cycle,  $G'$  values were constant in the range of frequency applied, and  $G''$  showed a slight dependence with the frequency. This confirmed the pattern that is characteristic of the gel network. This might be due to the maximum swelling obtained at that cooling temperature (25 °C) and the elastic characteristic had the major contribution in the starch gel structure. Differences between the modified starches were presented, which may be explained to be due to the type of chemical modification used.

The variations in mechanical properties of starch gels may be attributed to differences in the molecular characteristics of amylose matrix. Acetylated starches showed weaker gels than native and oxidized starches. The variations in enthalpy indicate structural and molecular differences (amylose and amylopectin fine structure, distribution pattern of substitutes groups), and composition (amylose-amylopectin ratio). The weaker gels in the acetylated starch may be attributed to the inhibition of amylose chain interactions, reducing the formation of junction zones and leading to the formation of a softer system. Lower values of  $G'$  and  $G''$  as compared with its native starch gel confirm the less tendency of both modified banana starches to retrogradation. During oxidation, there is introduction of carboxyl and carbonyl groups, which retards recrystallization giving a gel with higher response of storage module.

## Conclusions

The chemical modification produced a decrease in the enthalpy of gelatinization and the effect was more notable in the acetylated starch. Similar pasting temperatures were exhibited by the native and

acetylated starches; however, the acetylated sample had higher peak viscosity. The oxidized starch had the maximum peak viscosity during the holding step. In the rotational test, the three starches had a non-Newtonian pattern of the shear thinning type. The oxidized banana starch showed  $G'$  values higher than the acetylated sample, this produced a stronger gel.

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