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Acetylation of banana (*Musa paradisiaca* L.) and corn (*Zea mays* L.) starches using a microwave heating procedure and iodine as catalyst: II. Rheological and structural studies

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ABSTRACT

The effect of iodine concentration on the acetylation of starches with low and moderate degree of substitution (DS < 0.5) and its impact on the physicochemical feature and structural features was evaluated. The acetylated starches were prepared with 0.03 mol anhydroglucose unit, 0.12 mol of anhydride acetic, and 0.6, 0.9 or 1.4 mM of molecular iodine as catalyst in a sealed Teflon vessel using microwave heating (600 W/2 min). Pasting profile and rheological properties were obtained under steady flow; dynamic oscillatory test was used. Structural features were obtained by HPSEC-RI. In acetylated starches, DS and acetyl groups increased when the iodine concentration increased, corn starch showed higher values than banana starch. The viscosity of acetylated starches decreased relative to unmodified starches while, acetylated corn starch had lower value than acetylated banana starch. In the flow curves, a non-Newtonian pattern (*shear-thinning*) was shown in the pastes of native and modified starches. Storage modulus (G') and loss modulus (G") showed low dependence on frequency ($G' \alpha \omega^{0.1}$; $G'' \alpha \omega^{0.2}$) on frequency sweep test, which is characteristic of a viscoelastic gel. Debranched native banana and corn starches presented trimodal chain-length distribution. The pattern was maintained in the acetylated starches, but with different level of short and long chains. The structural differences in native and acetylated samples explain the rheological characteristics in both starches.

1. Introduction

Acetylated starch exhibits increased stability in food applications compared to its native counterpart; therefore, it has been used to increase the stability and resistance of food products to retrogradation (Singh, Chawla, & Singh, 2004). Chemical modification of native starches is often required to improve their properties, as well as to overcome the undesirable changes in product texture and appearance caused by retrogradation or breakdown of starch during storage and processing (Hung & Morita, 2005; Miladinov & Hanna, 2000). Starch acetates are prepared commercially with a low (<0.1) degree of substitution (DS) through the reaction of an aqueous suspension of starch granules with acetic anhydride and sodium hydroxide over several hours (Biswas et al., 2008; Shogren, 2003; Shogren & Biswas, 2006).

The potential of microwave-assisted reactions for the modification of bio-materials, including native starches, has recently been discussed (Biswas et al., 2008; Sánchez-Rivera et al., 2010), particularly for esterification. The esterification of starch can be accelerated by heating the reaction mixture in a pressurized vessel using a microwave oven since high temperatures can be reached rapidly through microwave heating. The esterification reaction rate can be increased with the addition of iodine (I_2) as a catalyst because this activates the carbonyl carbon of acetic anhydride, making the latter more reactive (Diopa, Li, Xie, & Shi, 2011). The advantage of a microwave-assisted reaction is that it provides fast and uniform heating (Shogren & Biswas, 2006) and is economical and environmentally friendly due to minimizing the energy consumption, level of solvent toxicity, amount of catalyst needed, and by-products (Biswas et al., 2008). Certainly, microwave heating of a polar solvent is similar to conventional heating.

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It is possible to achieve production of starch acetate on an industrial scale as well as to use a tubular reactor maintaining the heat by using microwave energy. However, success on a large scale may be limited and problematic. In addition, localized superheating during (or as a result) microwave heating, which is a problem that needs to be considered depending on the microwave instrumentation employed.

Acetylated banana and corn starches were acetylated with acetic anhydride and iodine as the catalyst at different concentrations. The acetylated starches had a moderate degree of substitution (0.5), and FT-IR at 1740 cm^{-1} showed a difference in the intensity signal when the iodine concentration increased. The crystallinity level was reduced with an increase in the iodine concentration. Corn modified granules showed more exo-corrosion and fusion than banana granules, as revealed by SEM (Sánchez-Rivera et al., 2010). In the present study, the reaction of dry starch with acetic anhydride in the presence of molecular iodine as a catalyst at a high temperature (100 °C) and pressure (0.1–50 bar) was studied in order to complement previously published results (Sánchez-Rivera et al., 2010). The objective of this study was to evaluate the rheological and structural properties of banana and corn starches acetylated by microwave heating in the presence of iodine as a catalyst.

2. Materials and methods

2.1. Materials

Unripe bananas (*Musa paradisiaca* L.) were purchased in a local market at Cuautla, Morelos, México and starch was isolated (Flores-Gorosquera et al., 2004). Commercial corn starch was purchased from Sigma (St. Louis, MO, USA). Reagents analytical grade were purchased from Fermont, S.A. de C.V. (D.F. México).

2.2. Preparation of acetylated starches

The acetylated starches were prepared according to the method reported by Sánchez-Rivera et al. (2010). Starch (4.86 g, 0.03 mol anhydroglucose unit), acetic anhydride (12.25 g, 0.12 mol) and molecular iodine (0.6–1.4 mmol) were added into a 50 mL Teflon vessel containing a magnetic stir bar. The vessel was covered with parafilm and the mixture was stirred for 5 min with magnetic bar; thereafter, placed in a polycarbonate microwave bomb (mod. 4782, Parr Instrument Co., Moline, IL, USA). The microwave bomb was heated for 2 min at 600 W, using a domestic microwave oven (900 W) (Panasonic de Mexico, S. A. de C. V.). The starting temperature was 23 °C and after 1 min reached to around 220 °C (Bello-Perez et al., 1998). The bomb was removed from the microwave oven and cooled down in ice bath for 40 min. A saturated sodium thiosulphate solution (2 mL) was added to the reaction mixture then transferred into an Erlenmeyer flask (250 mL) containing 50 mL of ethanol (96°) and stirred for 30 min. Excess alcohol was removed by filtering through a Buchner funnel containing a filter (Whatman No. 4). The solids were washed with water and alcohol then dried in an oven at 40–45 °C for 24 h.

2.3. Degree of substitution

The DS was determined titrimetrically following the method reported by Sánchez-Rivera et al. (2010). Acetylated starch (1.0 g) and 50 mL of 75% aqueous ethanol were placed in a 250 mL flask. The loosely stopper flask was agitated, warmed to 50 °C for 30 min, cooled and 40 mL of 0.5 M KOH were added. The excess alkali was back-titrated with 0.5 M HCl using phenolphthalein as an indicator. The solution was left to stand for 2 h, and then the alkali leached from the sample was titrated. A blank, using the original unmodified starch, was also used. Initially, the acetyl (%) was calculated as:

$$\text{Acetyl}\,(\%) = \frac{(\text{Vol Blank} - \text{Vol Sample}) \times \text{Molarity of HCl} \times 0.043 \times 100}{\text{Sample wieght}} \qquad (1)$$

and the DS was calculated as:

$$\text{DS} = \frac{162 \times \text{Acetyl}\,(\%)}{4300 - 42 \times \text{Acetyl}\,(\%)} \qquad (2)$$

2.4. Starch pasting and rheological measurements

Acetylated starches were cooked “in situ” in a Stress Control Rheometer AR-1000N (TA Instruments, New Castle, DE, USA) using parallel plate fixture (sandblasted plate) (diameter = 60 mm, gap = 1000 μm) under constant shear rate (50 s^{-1}). Starch dispersions (7.5%, w/w) were prepared by suspending 0.3 g (dry weight) of starch in 3.7 mL of distilled water at room temperature. The parallel plates were covered with mineral oil to avoid water evaporation during the test. The change in viscosity was measured under the following in-sequence steps: (a) heating (2.5 °C/min) from 25 °C to 90 °C; (b) holding at 90 °C during 10 min; (c) cooling (2.5 °C/min) to 60 °C. Once at 60 °C, steady shear rate sweeps were carried out. Two consecutive up and down sweeps from 0.06 to 300 s^{-1} were chained to a final logarithmic ascendant from 1 to 300 s^{-1}.

2.5. Viscoelastic properties

Viscoelastic properties were carried out at 25 °C under low amplitude oscillatory shear in a Stress Controlled Rheometer (AR-1000N, TA Instruments, Newcastle, DE, USA) using a parallel plates fixture (sandblasted plate, diameter = 60 m; GAP = 1000 μm). The starch dispersions (7.5%) were cooked “in situ” following in-sequence steps: (a) heating (2.5 °C/min) from 25 °C to 90 °C; (b) holding at 90 °C during 10 min (1st stage); (c) cooling (2.5 °C/min) to 25 °C; (d) holding at 25 °C during 10 min (2nd stage). Linear viscoelastic region was determined for each sample by strain amplitude sweep tests at the oscillation mode. The oscillatory strain sweeps were made at a constant frequency of 1 Hz over with 5 steps per log cycle and the amplitude was increased from 0.1 to 5% to determine the linear viscoelastic region (LVR). Measurements of frequency sweeps were made over a frequency range of 0.1–10 Hz and a constant strain at 0.7% (1st stage) and 0.9% (2nd stage).

2.6. HPSEC-RI analyses

Defatted native and acetylated starches (10 mg) were solubilized in 3.2 mL Millipore Waters by heating in a boiling water bath and agitation for 30 min. The starch solution was cooled at room temperature (25 °C) and debranching was undertaken according to the method described by Kasemsuwan, Jane, Schnable, Stinard, and Robertson (1995) with some modifications. Acetate buffer 0.1 M (pH 3.5) (0.4 mL) and 5 μL of isoamylase enzyme (59,000 U/mL, HBL, Japan) were added to solubilized starch; the reaction blend was held at 45 °C with stirring for 2 h, and subsequently neutralized with 0.2 M Sodium hydroxide. The enzymatic reaction was stopped by immersion the vial in a boiling water bath for 15 min. After cooling for 5 min, 1.5 mL of the debranched solutions were mixed for 1 min in a screw cap test tube with 0.2 g of bed exchange resin anionic/cationic (IONAC NM-60, J.T. Baker, Phillipsburg, NJ, USA) to eliminate the interference from the buffer prior to injection into High-Performance Size-Exclusion Chromatography (HPSEC) system, consisting of a 515 HPLC pump (Waters Corporation, Milford, MA, USA) with an injector of 100 μL sample loop, an in-line degasser, a 2410 Refractive Index Detector (IR) (Waters Corporation) maintained at 40 °C, and a series of Shodex OHpak columns, including a guard column (Shodex OHpak SB-G) and KB-804 (Shoko

Co., Japan) column maintained at 55 °C with a column heater. The mobile phase was an aqueous solution of 0.1 M $NaNO_3$ containing 0.02% NaN_3 (vacuum-filtered twice through a 0.1 μm membrane filter) and the flow rate was 0.3 mL/min. The starch components profiles were analyzed from retention time and integrated peaks area in the normalized chromatograms.

3. Results and discussion

3.1. Degree of substitution (DS)

The DS values, as in a prior publication (Sánchez-Rivera et al., 2010), increased with the increasing iodine concentration for banana starch 0.06, 0.09, and 0.26 for 0.6, 0.9, and 1.4 mmol iodine, respectively, for corn starch 0.08, 0.13, and 0.47. An important increase in DS was determined for both starches at the highest iodine concentration; for instance, DS increased about three to five times. The effect was major for corn starch because higher DS values were obtained. This pattern is in agreement with the patterns reported in the acetylation of banana and corn starches using acetic anhydride, where banana starch showed a higher resistance to this chemical modification, and diverse factors such as amylose content, granule size distribution, amylopectin structure, as well as the presence of other components in the starch, might be affecting the introduction of acetyl groups in the starch components.

3.2. Pasting profiles

In a previous paper, Sánchez-Rivera et al. (2010) showed the pasting profiles in an RVA machine with 3% of acetylated starch and volumetric fraction, $\phi < 1.0$. In this paper, the viscosity changes were evaluated in pastes at 7.5% of acetylated starch ($\phi > 1.0$) with a rheometer. The results were similar to those obtained in the RVA machine. In this case, the volume fraction of the starch granules (>1.0) indicated that the amount of water was not enough to hydrate the starch granules completely, so the rheological behavior was controlled by partially swollen granules, and the viscosity values were higher than those previously reported by Sánchez-Rivera et al. (2010) due to the higher level of starch in the test. On the other hand, the results obtained during cooking of the starch *in situ* showed a similar pattern to the results obtained in the RVA machine.

The pasting profiles of unmodified and acetylated banana and corn starches are presented in Fig. 1. The viscosities of acetylated starches were found to be lower than for unmodified starches because reduction of peak viscosity (PV, arrow in Fig. 1) was observed in all acetylated starches, with different concentrations of iodine as the catalyst. The introduction of acetyl groups in the glucose monomers of starch chains restricted the swelling of the granules due to the hydrophobic character of the acetyl groups. This characteristic of acetylated starches can be important for specific applications (Saartrat, Puttanklek, Rungsardthong, & Uttapap, 2005). When starch is heated in the presence of water, granules swell while some components including amylose and long (external) amylopectin chains diffuse out to the continuous phase, resulting in swollen granules inside the network of lineal chains (Gunaratne & Corke, 2007; Núñez-Santiago, Bello-Pérez, & Tecante, 2004).

When the native starches and those acetylated with the same amount of iodine of both sources are compared (Fig. 1a and b), a higher PV was shown in the banana starch, indicating that higher acetylation was produced in the corn starch due to minor restrictions of the swelling that was present in the banana starch. The amylopectin structure, which was different in both starches (Espinosa-Solis, Jane, & Bello-Pérez, 2009; Espinosa-Solis, Sánchez-Ambríz, Hamaker, & Bello-Pérez, 2011) played an important role in the acetylation and in the PV viscosity, as shown later. The DS of acetylated starches had an influence on the breakdown and setback values due to the fact that the corn starch with the highest DS did not show both parameters, indicating that a weak network was produced. This pattern is related to the depolymerization of starch chains during acetylation or the higher hydrophobic character that increased with the DS, preventing realignment to form a network among linear chains from amylose or external long chains of amylopectin (Gunaratne & Corke, 2007).

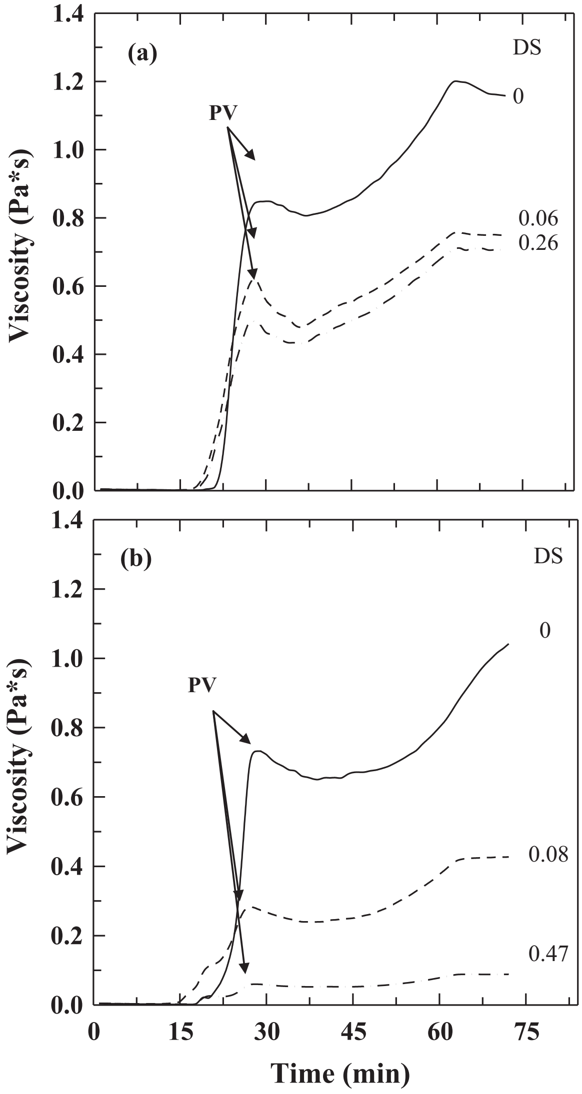

Fig. 1. Effect of DS on the profile of acetylated (a) banana and (b) corn starches. The continuous line is for starches without acetylation.

3.3. Flow behavior

Fig. 2 shows the flow behavior at 60 °C of acetylated banana and corn starches as a function of DS. At this temperature, starch pastes are a mixture of swollen and partially disrupted granules suspended in a macromolecular solution (Núñez-Santiago et al., 2004). The shear stress *vs* shear rate curve shows the non-Newtonian shear-thinning character of the pastes. The native starches showed higher shear stress in the banana starch at the same shear rate than that of the corn starch. This pattern indicates a higher apparent viscosity in banana starch than corn starch due to the higher

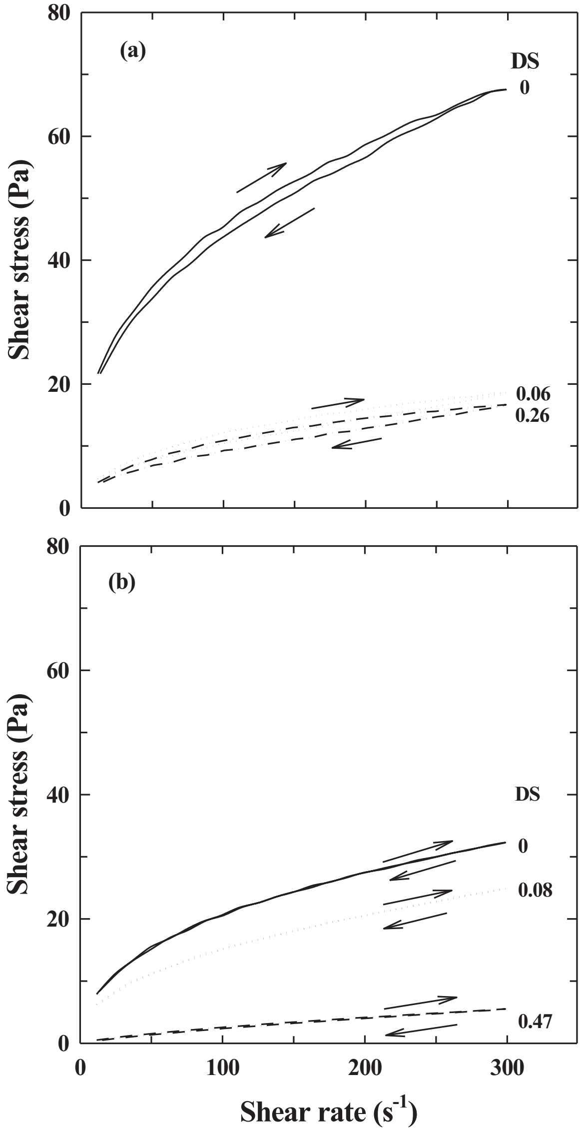

Fig. 2. Flow curves of (a) banana and (b) maize starches acetylated at 60 °C.

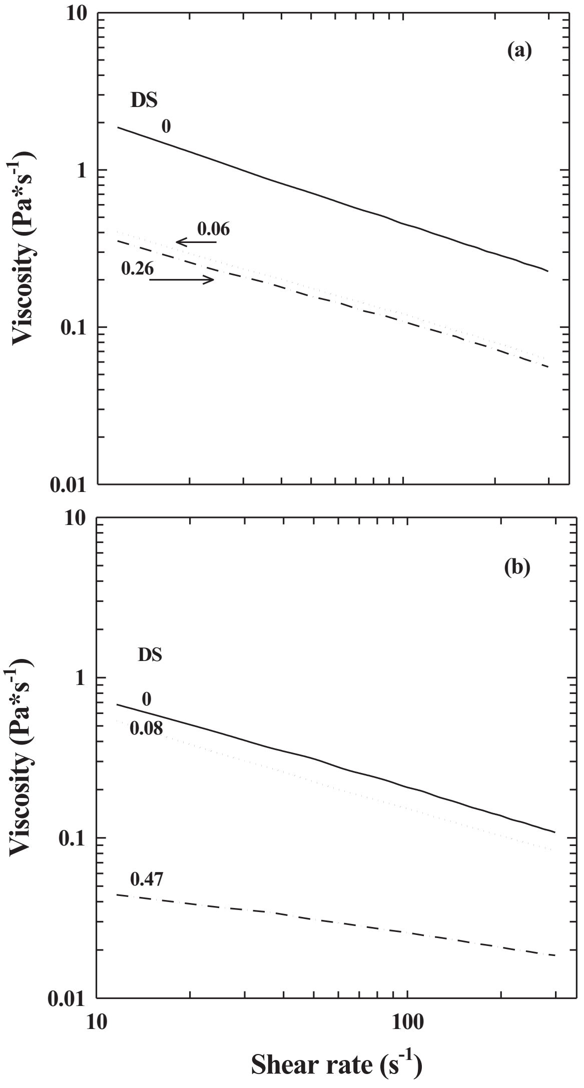

Fig. 3. Variation of apparent viscosity with shear rate of starch pastes at 60 °C. (a) Banana and (b) maize starch.

resistance of the structure breaking with the shear stress. The acetylation produced a decrease in shear stress due to the weakness of the structure; consequently, pastes with higher flow ability are obtained. The effect was higher in corn starch, indicating that its structure is more susceptible to acetylation and produces paste with low viscosity. The profiles of acetylated banana starches with low and high DS were similar, while those of acetylated corn starches were different. The dependence of shear stress on DS was observed in corn starch, where the lowest values in shear stress were produced with the highest DS; the structural differences between banana and corn starch were responsible for the degree of modification, and, consequently, in the viscosity profiles.

Fig. 3 shows the results from 1 to 300 s^{-1}. A Power law model was used to correlate viscosity with shear rate:

$$\eta = K \cdot \gamma^{n-1}$$

where η is the viscosity, K is the consistence index (Pa s^n), and n is the flux index. Table 1 shows K and "n" values for banana and corn acetylated starch pastes. Despite the different DS values in banana starch, the value of "n" showed a slight increase even at the highest DS. However, a decrease in K value in acetylated starches even at the lowest DS was evident. Corn starch showed a similar pattern to banana starch in "n" values, but the difference between the sample with the lowest and the highest DS was about double. K values for corn starch were lower than for banana starch and showed a decrease when the DS value increased. An increase in "n" value indicates that the fluid showed a trend toward Newtonian behavior due to the arrangement of the macromolecules, which were weak, as was observed by the decrease of the K value with

Table 1
Power law model ($\eta = K \cdot \gamma^{n-1}$) for pastes of banana and corn starches acetylated (7.5%, w/w) by microwave heating, using iodine as catalyst, at 60 °C.

DS	n	K (Pa s^n)	R^2
Banana			
0	0.431	8.91	0.999
0.06	0.435	1.67	0.999
0.09	0.478	1.46	0.999
0.26	0.445	1.44	0.999
Corn starch			
0	0.427	2.86	0.998
0.08	0.432	2.09	0.998
0.13	0.479	1.10	0.998
0.47	0.721	0.09	0.997

DS, degree of substitution; n, flux index; K, consistence index.

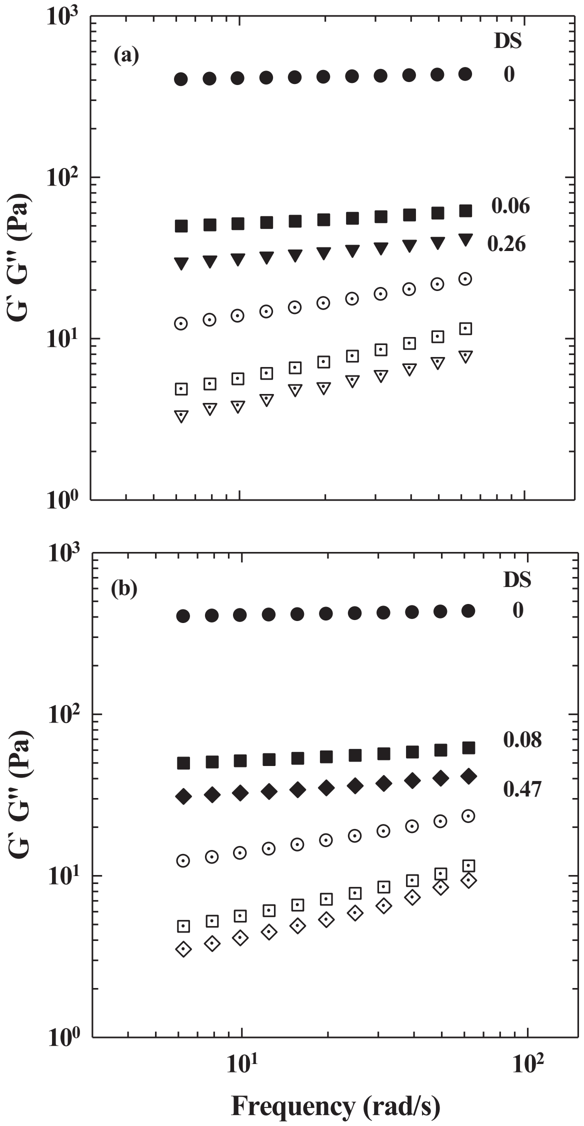

Fig. 4. Effect of DS in the dynamic properties of (a) banana and (b) maize starches aceylated by microwave heating. G' (filled symbols) and G'' (empty symbols).

the corresponding low viscosity of the paste. The "*n*" and *K* values were in agreement with the pasting profiles and flow curves.

3.4. Dynamic properties

Fig. 4 shows the viscoelastic behavior of the gels formed at 25 °C. In general, all starches showed a characteristic behavior of a gel, where G' > G", with both modules slightly dependent on the frequency ($G' \alpha\, \omega^{0.15}$, $G'' \alpha\, \omega^{0.25}$) and tan $\delta < 0.1$ (data not shown). The gels of both native starches showed higher stiffness than their acetylated samples, and this parameter decreased when DS increased. This pattern is related to the chain length of the starch granules (amylose and some long external chains of amylopectin) which increased when higher acetylation of the starch was produced. Those chains leached from the starch granules contributing to the development of a network that was more ordered when the leached chain level increased. In gels of both starches, at the highest DS value, G' showed lower values than acetylated starches with lower DS, indicating more weakening of associative forces in the amorphous areas of the granules and high resistance to reorganization (Saartrat et al., 2005). These results were consistent with the profiles of the pastes and flow behavior reviewed previously.

Table 2
Normalized HPSEC of isoamylase-debranched fractions (Fr. I, Fr. II and Fr. III) of native and acetylated starches with different degree of substitution (DS).

Sample	Fr. I	Fr. II	Fr. III	Fr. III/Fr II
Banana starch				
Native	21.74	25.03	53.24	2.13
DS = 0.06	19.52	29.53	50.95	1.73
DS = 0.09	20.93	29.35	49.76	1.70
DS = 0.26	19.18	29.74	51.08	1.72
Corn starch				
Native	23.57	15.04	61.39	4.08
DS = 0.08	20.47	23.74	56.05	2.39
DS = 0.13	23.22	23.36	53.42	2.29
DS = 0.47	23.18	20.86	55.95	2.68

HPSEC, high-performance size-exclusion chromatography.

3.5. Amylose and amylopectin content by HPSEC-RI

Table 2 shows the normalized high-performance size-exclusion chromatograms of native and acetylated starches after debranching with isoamylase. A three-modal (Fr. I, II and III) distribution was observed in unmodified and acetylated banana and corn starches. In the banana starches, Fr. I corresponded to amylose chains after acetylation. The peak moved for a longer time due to the decrease in the chain length. Amylose was mainly located in the periphery of the starch granules and during the chemical modification, depolymerization was produced. Native and acetylated banana starches presented a shoulder indicated by the Fr. II (data not shown), which corresponds to long chains of amylopectin. No appreciable change in this fraction was observed after acetylation. The main fraction is Fr. III, which corresponds to short chains of amylopectin, and a slight increase in this fraction was observed after acetylation compared with its native counterpart.

Native and acetylated corn starches presented a three-modal distribution too (Fr. I, II and III). The HPSEC patterns of both native starches indicated a structural difference, with a higher amount of Fr. I (amylose) in corn starch than banana starch (Espinosa-Solis et al., 2009). The Fr. I in the acetylated samples presented an increase in the elution time (data not shown), indicating a decrease in the molecular weight of amylose due to depolymerization. Structural differences between both starches before acetylation played an important role in the level of the modification, and the structural changes observed in the acetylated starches explain the rheological characteristics.

4. Conclusions

Structural differences existed in both starches and also based on degree of substitution (DS) of the acetylated samples. Banana starch was more resistant to acetylation, so lower DS values were calculated. The pasting profile of acetylated starches presented a lower peak viscosity, indicating that acetylation restricted the swelling of starch granules, with the effect being higher in corn starch at similar DS. The pastes of acetylated starches presented a high flow ability, with this characteristic being higher in corn starch. Rheological properties revealed that all starches presented the storage modulus (G') > loss modulus (G") values. The flow behavior was influenced by DS. The structural study revealed differences in acetylated corn and banana starches, which influenced the rheological characteristics. It is possible to produce acetylated starches with specific rheological characteristics, which to a large extent is a function of the structural features of the native starch.

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