

## Viscoelastic behavior of nixtamalized maize starch gels

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### Abstract

The influence of lime and amylose–lipid complexes on the viscoelastic behavior of nixtamalized maize starch gels was studied using small amplitude oscillatory rheometry. The storage modulus ( $G'$ ) and the loss modulus ( $G''$ ) behavior showed to be lime dependent. At 0.2% w/v lime, both moduli had the highest values, and decreased with increasing lime concentration. This dependent lime behavior pattern was attributed to changes in the Ca–starch interactions, affecting swelling and solubility. Fourier transform infrared (FTIR) spectra indicated that amylose–lipid complexes are present in starch gels of maize nixtamalized with or without lime. The effect of these complexes on preventing amylose solubilization was surpassed by the stabilizing effect of the Ca–starch interactions on starch granule structure, as evidenced by scanning electron microscopy (SEM). Thus, it is suggested that the rheological properties of nixtamalized maize starch gels are influenced mostly by the effect of Ca–starch interactions.

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### 1. Introduction

The process of nixtamalization used in the elaboration of maize tortillas produces important changes in the components of the maize kernel. These changes affect the rheological, textural, and structural characteristics of masa and tortillas (Gomez, Waniska, & Rooney, 1991; Gomez, Lee, McDonough, Waniska, & Rooney, 1992; Rooney & Suhendro, 1999). A relevant role has been conferred to the structural changes of the maize starch, in the modification of the rheological properties. It was postulated that the interactions of calcium cations with starch could be responsible for the structural changes that affect the viscosity behavior of maize flour cooked in water with lime (Bryant & Hamaker, 1977). These authors obtained viscoamylograms showing decreasing peak viscosity temperatures at

low lime concentration, attributed to faster granule swelling; whereas at high lime concentration, the absence of any peak was attributed to a lack of breakdown of swollen granules. Gomez et al. (1992) reported the presence of peak viscosities in viscoamylograms of nixtamal and masa, whereas for tortillas and lime-free cooked maize the viscoamylograms showed no peak viscosity. A similar viscosity behavior was observed by Campas-Baypoli, Rosas-Burgos, Torres-Chávez, Ramírez-Wong, and Serna-Saldivar (1999), their results were attributed to the stabilization of starch granule structure by the interactions of calcium ion with starch. Data reported by Robles, Murray, and Paredes-Lopez (1988) on nixtamalized flours agreed with these previous-mentioned works, suggestion was made that hydrogen bond disruption caused by the  $\text{OH}^-$  also affect the pasting properties of the starch.

Del Valle, Santana, and Clason (1999) have pointed out that literature data on viscoamylographic behavior of raw corn, nixtamal, and tortilla are contradictory. Data reported by Tonella, Sánchez, and Salazar (1983) were found to

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be opposite to the results obtained by Campas-Baypoli et al. (1999); Gomez et al. (1992); and Robles et al. (1988). It was suggested that viscosity behavior was affected by the calcium cross-links formed between the starch chains, and that the degree of cross-linking depended on lime concentration and cooking time. Measurement of thermal diffusivity, infrared, IR, absorption coefficients, dielectric constant and electrical conductivity of corn tortillas seemed to confirm that alkali treatment induces structural changes due starch cross-linking, through the formation of calcium bridges (Rodríguez et al., 1996). Two distinct ways of calcium ion interaction with starch were proposed: at lime contents up to 0.2%, ions promote enhancement of the starch cross-linking, whereas at higher concentrations Ca ions anchor on the surface of starch granules.

Amylose–lipid complexes have been reported to be present in gels of heat-treated maize starch (Mestres, Colonna, & Buleon, 1988). Chungcharoen and Lund (1987), Galloway, Biliaderis, and Stanley (1989), and Morrison (1995) have documented that formation of amylose–lipid complexes reduced granule swelling and starch solubilization. Changes in rapid visco analyser (RVA) pasting profiles from different starches were attributed to the effects of these complexes (Becker, Hill, & Mitchell, 2001). However, little information is available concerning the effect of the amylose–lipid complexes on the viscoelastic behavior of nixtamalized maize starch gels. There is also limited information about the effect of lime on this behavior. Therefore, the present study has been undertaken to elucidate the structural starch modifications of nixtamalized maize due to lime and amylose–lipid complexes, using dynamic rheometry.

## 2. Experimental

### 2.1. Flour preparation

White dent maize (100 g) was mixed with 300 ml of lime-water solution (0%, 0.2%, 0.4%, and 0.6% w/v), cooked at boiling temperature for 30 and 60 min, and allowed to steep for 16 h. Then, the cooking solution was drained and nixtamal washed three times with distilled water. After being oven-dried at 50 °C for 36 h, germ, tip cap, and pericarp were removed by hand and the remaining endosperm ground into flour in a coffee mill with liquid nitrogen.

### 2.2. Gel preparation

Flour suspensions (5% w/v) were prepared in deionized water and heated to 90 °C for 30 min with stirring, after that, suspensions were rapidly cooled to 25 °C in an ice bath. Pastes were kept at room temperature for 10 min before performing rheological and FTIR measurements, to equilibrate the gel-like structure formed during cooling.

### 2.3. Small deformation testing

Small amplitude dynamic oscillatory testing was performed using a Carry-Med CSL<sup>2</sup> 500 (TA Instruments, Surrey, England), with a parallel plate geometry (2 cm diameter). First, the linear viscoelastic region was determined at 25 °C using stress sweeps from 1 to 150 Pa at 1 Hz. Then, frequency sweep measurements were conducted over a frequency range of 2–10 Hz. The dynamic rheological parameters determined were the storage modulus ( $G'$ ), the loss modulus ( $G''$ ), and  $\tan \delta$  ( $G''/G'$ ). The measurements were made twice for the flours obtained. The relative errors were all within the range  $\pm 8\%$ .

### 2.4. Scanning electron microscopy

Scanning electron microscopy (SEM) was performed on endosperm flour specimens. The specimens were attached to stubs with silver conducting paint and coated with a layer of gold–palladium approximately 30 nm thick. The coated specimens were scanned with a Phillips XL 30 C scanning electron microscope at an accelerating voltage of 10 kV.

### 2.5. Fourier transform infrared spectroscopy

Infrared spectra were recorded between 600 and 2000  $\text{cm}^{-1}$ , using a Perkin Elmer Spectrum One infrared spectrometer (Perkin Elmer, USA) operating at 4  $\text{cm}^{-1}$  resolution. Freshly prepared gels were poured into an ATR cell with a Zn Se crystal, and transmittance spectra were obtained by accumulation of 32 scans.

## 3. Results and discussion

### 3.1. Viscoelasticity of nixtamalized flour gels

The gel storage modulus ( $G'$ ) of nixtamalized corn flour cooked for 30 min exhibited the highest values at 0.2% lime concentration as shown in Fig. 1. On further addition of lime, the  $G'$  tended to decreased back to the values of the non-lime treated flour. Also, the  $G'$  values were higher than  $G''$  values (Fig. 2), showing that the elastic characteristic has a principal role in the gel structure.

It has been shown that lime concentration affects the thermal and rheological properties of calcium-treated starch, nixtamal, nixtamalized flour, and tortillas (Bryant & Hamaker, 1997; Fernández-Muñoz et al., 2002; Gomez et al., 1992; Nurul Islam & Mohd, 1997; Rodríguez et al., 1996; San Martín-Martínez et al., 2003, 2003). It has been suggested that at lime concentrations  $<0.2\%$ , the calcium inorganic cations form cross-links between two starch chains of the granule matrix by coordination of two ionized hydroxyl groups produced by the alkaline conditions; while at higher lime concentrations the Ca ions are more likely to anchor on the surface of the starch granules (Bryant & Hamaker, 1997; Rodríguez et al., 1996). The increase in the  $G'$  of our

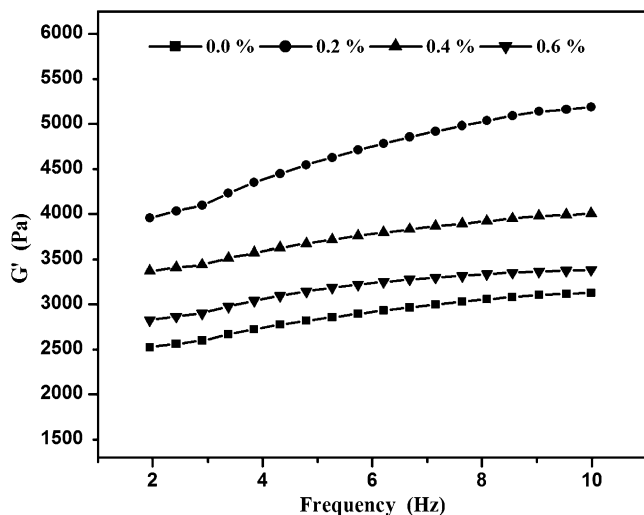


Fig. 1. Effect of lime concentration on the storage modulus ( $G'$ ) of flour gels from nixtamalized maize cooked for 30 min.

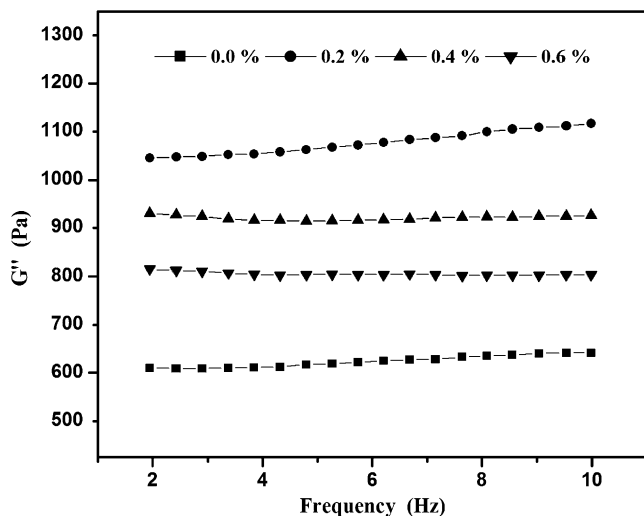


Fig. 2. Effect of lime concentration on the loss modulus ( $G''$ ) of flour gels from nixtamalized maize cooked for 30 min.

samples at low lime concentration could be taken as an indicator that these cross-links strengthened the swollen granules embedded in the solubilized amylose, resulting in stronger gel networks with higher elastic or storage modulus. The decrease in the  $G'$  at lime concentrations  $>0.2\%$  could be attributed to softer granules with uncross-linked starch chains, which originally had the Ca ions interacting on their surface. It has been suggested that these anchored ions increase the rigidity of the granules (Bryant & Hamaker, 1997; Oosten, 1982). Our results agreed with this suggestion, since samples prepared with lime exhibited higher  $G'$  values than samples prepared without lime, indicating swollen granules with increased rigidity. The highest  $G'$  values at low lime concentration seemed to indicate that the effect of Ca cross-links formed during nixtamalization on gel strength is greater than the effect of calcium ions anchored on surface starch. The lower  $G'$  values at lime

concentrations  $>0.2\%$  could also be attributed to Ca ions interrupting the crystallization of starch molecules during gel cooling, thus leading to weakened gel networks. Stabilization of gel network is related to the development of local crystalline structures due to re-association of amylose molecules (Zobel, 1988).

The effect of calcium–starch interactions on starch gel elasticity has been formerly described; however, since not only the granular structure influences the elasticity of the starch gels but also the amount of soluble component (Tsai, Li, & Lii, 1997) and both of these parameters are greatly affected by amylose–lipid complexes, their effect must also be considered. These complexes can be formed when freshly prepared starch gels of cereal starches are cooled (Mestres et al., 1988; Zobel, 1988) and they inhibit amylose leaching and starch granule swelling (Morrison, 1995). The formation of amylose–lipid complexes might decrease the gel consistency, since amylose in the complexes is not longer available for the development of the gel network, interrupting inter-chain association and decreasing the  $G'$  (Biliaderis & Zawistowski, 1990). The decreasing behavior of  $G'$  seen in Fig. 1 at lime concentrations  $>0.2\%$ , therefore, could also be attributed to the amylose–lipid complexes and not only to the Ca–starch interactions. The increased values of  $G'$  with decreasing lime content suggested the predominance of the cross-linking formation effect at low lime concentration.

The viscous response of the nixtamalized flour gels, as indicated by  $G''$  (Fig. 2), followed the same pattern as the elastic response (Fig. 1), with lime concentration changes. Other workers have shown the dependence of  $G''$  on amylose content and swollen granule structure (Lii, Shao, & Tseng, 1995). Our  $G''$  results then could be attributed to differences in the amount of amylose chains leached out of the granules during the maize cooking and to the increased rigidity of the swollen starch granules from maize cooked with lime, the characteristics of which were discussed previously. The presence of amylose–lipid complexes might diminish vibrations of dangling chains, thus contributing to decreasing of  $G''$ , as suggested for the cross-linked waxy starch (Tsai et al., 1997).

The  $G'$  and  $G''$  patterns of nixtamalized flour cooked for 60 min (Figs. 3 and 4) were similar to the patterns of flour cooked for 30 min (Figs. 1 and 2), with increasing lime concentration. However, the  $G'$  values were higher for samples cooked for 60 min (Figs. 1 and 3). Longer cooking time leads to a larger amount of gelatinized starch in nixtamalized flour and thus to larger amount of released amylose. The nixtamalization is not enough for producing complete starch gelatinization, but when the cooking time is longer more gelatinization is carried out, producing a masa with more cohesive texture. Other workers have reported that the  $G'$  tended to increase with increasing leached out amylose (Biliaderis & Zawistowski, 1990). The  $G''$  of the flour cooked with 0% and 0.2% lime was also higher at longer cooking time than at shorter cooking time, while the samples cooked

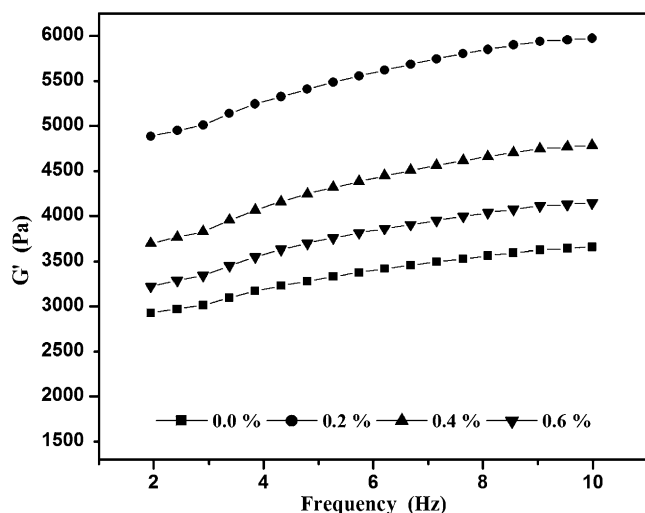


Fig. 3. Effect of lime concentration on the storage modulus ( $G'$ ) of flour gels from nixtamalized maize cooked for 60 min.

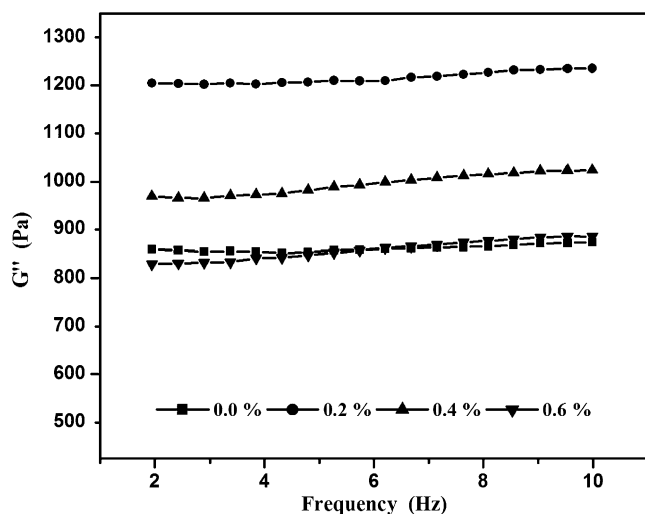


Fig. 4. Effect of lime concentration on the loss modulus ( $G''$ ) of flour gels from nixtamalized maize cooked for 60 min.

with 0.4% and 0.6% lime tended to keep the same values at both cooking times (Figs. 2 and 4). This could be due to differences not only in the solubilized amylose content but also in the volume and mobility of the swollen granules. Several investigators have established the influence of these parameters on the viscosity of pastes and gels (Fannon & BeMiller, 1992). The major amount of cross-linked swollen granules at low lime concentration will present decreased mobility increasing the viscosity of the gel. The softer granules of the samples without lime will be swollen to a greater extent resulting also in increased viscosity. The effect of the amylose–lipid complexes also contributes to this behavior as discussed previously. The granule characteristics of flour prepared with intermediate lime concentrations seem to be unaffected by the cooking time, since the  $G''$  are quite similar at both cooking times.

### 3.2. Scanning electron microscopy of flours

Figs. 5 and 6 display scanning electron micrographs of flours obtained from ground endosperm of nixtamalized maize cooked for 60 min. The size of the starch granules varied with the addition of lime to cooking water. Greater and smoother granules were observed in the nixtamalized flour without lime (Fig. 5A), compared to the granules of the nixtamalized flours with lime (Figs. 5B, 6A and B). It has been suggested that starch gelatinization – and consequently granule swelling – is inhibited by calcium–starch interactions (Robles et al., 1988), our micrographs agreed with this hypothesis. At 0.2% lime concentration, the starch granules were the less deformed and the more densely grouped of them all (Fig. 5B). On further addition of lime, granules tended to be more deformed and more loosely grouped (Figs. 6A and B). It was also apparent that the starch granules of flours from maize cooked with lime presented rougher surfaces, which could be attributed to the formations of small cracks. The cracks tended to growth with increasing lime content, as clearly shown in the flour micrograph of the corn cooked with 0.6% lime concentration (Fig. 6B). Robles et al. (1988) had suggested that during nixtamalization, the  $\text{OH}^-$  ions tend to disrupt granule structure; the observed cracks may be a consequence of this effect. The starch granule micrographs of these flours

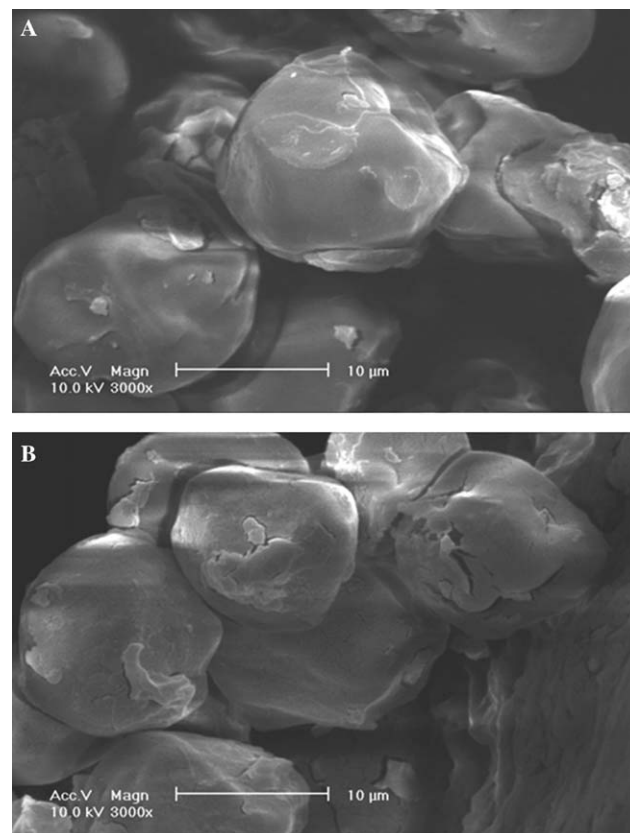


Fig. 5. Scanning electron micrographs of flours from nixtamalized maize cooked for 60 min as a function of lime concentrations (w/v, %): 0.0 (A), 0.2 (B).



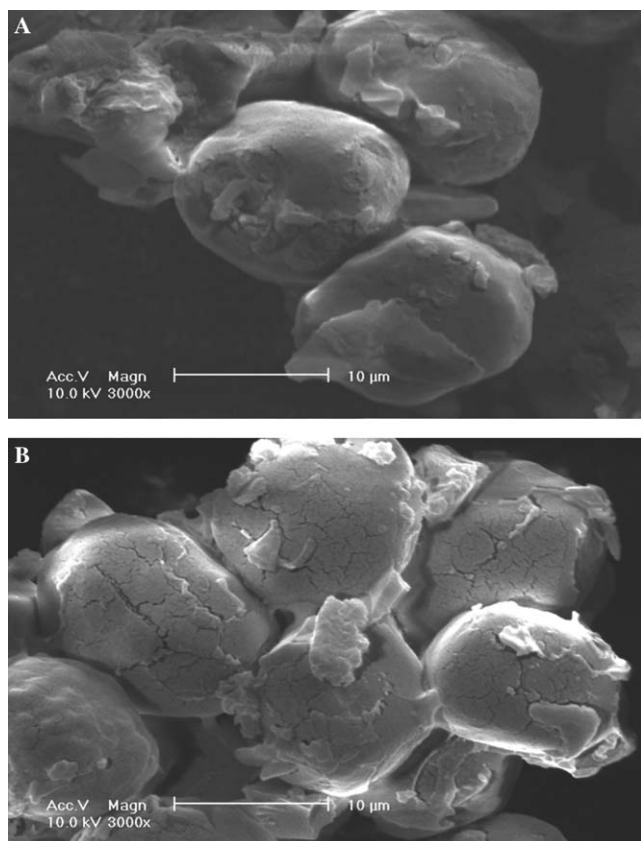


Fig. 6. Scanning electron micrographs of flours from nixtamalized maize cooked for 60 min as a function of lime concentrations (w/v, %): 0.4 (A), 0.6 (B).

agreed with the discussion made on the viscoelastic properties of their corresponding gels. The flour with the more compacted and less deformed granules (Fig. 5B), the one prepared with 0.2% lime concentration, led to gels which presented the highest  $G'$  and  $G''$  values (Figs. 3 and 4). Both gel moduli tended to decrease as the starch granule size of the flours increased with increasing lime concentration and the surface presented more pronounced cracks (Figs. 6A and B). Finally, the minor gel storage modulus  $G'$  was shown by the gels of the flour with the more swelled and deformed granules (Fig. 5A), which corresponded to the flour from maize cooked with no lime. Differences in the starch granules of nixtamalized flours cooked for 30 min were not apparent (not shown).

### 3.3. FTIR spectra of gels

The IR gel spectra of nixtamalized flours cooked for 30 and 60 min are shown in Figs. 7 and 8, respectively. The bands at 1458 and 1413  $\text{cm}^{-1}$  correspond to  $\text{CH}_2$  and CH bendings, while the bands at 1161 and 1130–1000  $\text{cm}^{-1}$  correspond to C–O stretchings; these bands are shown in the FTIR spectrum of gelatinized corn starch (Kim, Na, & Park, 2003; Rodríguez et al., 1996). Our spectra also showed a broad band at 1750–1615  $\text{cm}^{-1}$ , with a maximum intensity at 1710  $\text{cm}^{-1}$ , a

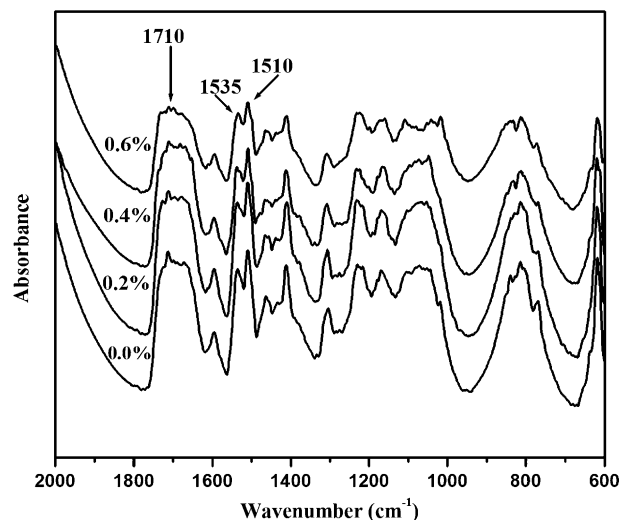


Fig. 7. FTIR spectra of flour gels from nixtamalized maize cooked for 30 min as a function of lime concentration (w/v, %).

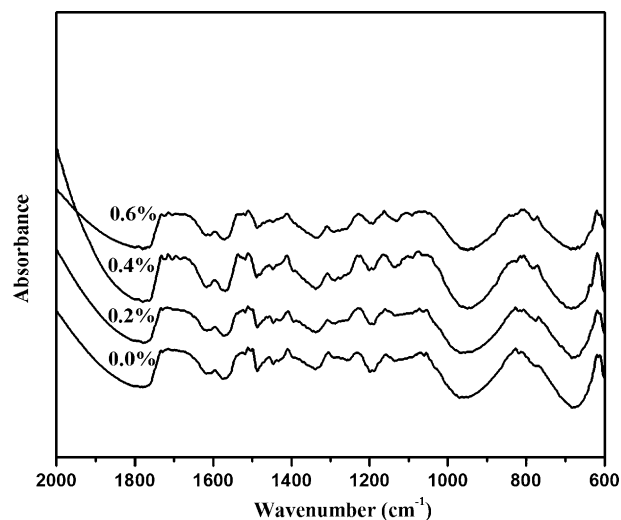


Fig. 8. FTIR spectra of flour gels from nixtamalized maize cooked for 60 min as a function of lime concentration (w/v, %).

band at 1600  $\text{cm}^{-1}$ , and a doublet at 1560–1490  $\text{cm}^{-1}$ . FTIR studies of amylose–fatty acid inclusion complexes formed during hydrothermal treatment reported a band at 1725–1700  $\text{cm}^{-1}$  which was attributed to the stretching of the fatty acid carbonyl C=O, and a doublet at 1610–1550  $\text{cm}^{-1}$  attributed to  $\text{CO}_2^-$  stretching; this doublet was only present for fatty acids with more than nine carbon atoms in the aliphatic chain (Oceguera-Hernández, 1999). Salt formation was not considered in this work since the author prepared the complexes using only hot de-ionized water, thus the vibrational modes of  $\text{CO}_2^-$  were attributed to hydrogen bonds between the O of the carboxylic OH and the glucosyl OH–C(6), resulting in ion-pair electrostatic interactions. Thus, we consider the presence of the bands in the 1710–1490  $\text{cm}^{-1}$  region, as an indicator of the presence of amylose–lipid complexes in our prepared gels. The band position did not

change for gels with or without lime, suggesting that calcium salts were not formed in samples prepared with lime. Same bands appeared in the gels of samples cooked by 60 min, but the overall intensities were diminished (Fig. 8), indicating the structural loss suffered during gelatinization.

X-ray diffraction studies have shown the presence of V-type crystalline structures, corresponding to amylose–lipid complexes, in solubilized starch of nixtamalized maize flour (Mondragón et al., 2004). These complexes affect the viscoelastic behavior of starch, depending on the type of starch and length of the carbon chain of the lipids as well as on their concentration (Singh, Singh, & Saxena, 2002). The presence of amylose–lipid complexes and of Ca–starch interactions restrain amylose leaching and starch granule swelling as pointed out earlier. Micrographs in Fig. 5A showed a great swelling degree for starch granules of maize flour cooked without lime, even when amylose–lipid complexes are present in the gels prepared from this flour as indicated by the FTIR spectra (Fig. 8). While the starch granules of maize cooked with lime showed smaller swelling degree indicating that the restricting effects of the Ca–starch interactions were stronger than the effect produced by the complexes. The final effect seems to be conditioned to lime concentration (as seen in SEM micrographs shown in Figs. 5 and 6). Therefore, we postulate that viscoelastic behavior of the starch gels, as indicated by  $G'$  and  $G''$ , may be attributed mainly to the influence of Ca–starch interactions.

#### 4. Conclusions

Viscoelastic behavior of nixtamalized starch gels was predominantly affected by the calcium interactions with starch. These interactions seemed to depend on concentration: (a) at 0.2% lime concentration, calcium may form bridges between starch chains producing very resistant granules and reducing significantly the swelling; (b) while at higher lime concentrations, the Ca ions are more likely to anchor on the surface of the starch granules and also may increase their rigidity and reduce the solubility. The storage modulus ( $G'$ ) and the loss modulus ( $G''$ ) behavior attained maximum values at 0.2% lime, this may be due to the calcium cross-links which strengthened starch structure and reduced solubility. Both moduli tended to decrease at higher lime concentration, but to higher values compared to the gels without lime, indicating that the effect of the anchored calcium on the granule rigidity and solubility was less pronounced than the effect of calcium cross-link. Amylose–lipid complexes were also present in all gels, but although when they produced the same effects as Ca–starch interactions and may affect the rheological properties of starch gels without lime, the viscoelastic behavior of starch gels with lime seemed to be finally determined by the effect of the Ca–starch interactions.

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