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Changes on the structure, consistency, physicochemical and viscoelastic properties of corn (*Zea mays* sp.) under different nixtamalization conditions

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ABSTRACT

Aqueous corn (*Zea* mays sp.) dispersions with 1% (w/w) of calcium hydroxide/weight of corn were cooked at 90 °C (nixtamalization) for different times (10, 30, 50, 70 and 90 min), making five treatments. A control lot (nine treatments), was cooked for 10, 30, 50, 60, 70, 90, 120, 150 and 180 min. Then, processed corn samples were washed and milled to obtain masa (dough). Scanning electron microscopy, calorimetry, viscoamilography and rheological analysis were used to characterize the corn samples. The corn micrographs showed that the nixtamalization modified the shape of the starch granules and the protein bodies. Starch granules from nixtamalized samples, were round shaped, while control samples, showed polygonal shape. Proteins from nixtamalized samples usually exhibited two transition endotherms, while in control samples, only one transition was seen, suggesting some relationship with gelatinized starch. The nixtamalization shortened the corn cooking time to develop a proper texture in masa to obtain good quality tortillas, as seen in their consistencies. All corn masa samples showed weak gel-like viscoelastic behavior with the elastic modulus (G′) higher than the loss modulus (G″), over all strain and frequency domains.

1. Introduction

In Mexico and several countries of Central America, corn is consumed mainly as tortillas, which are prepared from the masa (dough) or flour obtained by cooking corn kernels with lime, and then soaking them in alkaline solution; a process known as nixtamalization, which is used in this region since pre-Hispanic times. During the nixtamalization process, the calcium content of corn kernels increases notably depending on the time of soaking the corn (Fernández-Muñoz et al., 2004; Trejo-González, Feria-Morales, & Wild-Altamirano, 1982).

Simultaneous processes of water and calcium diffusion occur during the nixtamalization process, affecting the final product's physicochemical characteristics. The thermal alkaline treatment, changes drastically the appearance of the protein bodies in some areas of the kernel. Differences in the size of both nixtamalized and non-nixtamalized starch granules in micrographs from scanning electron microscopy (SEM) have been observed (Mondragón, Mendoza-Martínez, Bello-Pérez, & Peña, 2006). Starch granules from non-nixtamalized samples; usually appear bigger and smoother compared to nixtamalized ones. Studies using SEM, have confirmed that the starch granules compaction is directly related to the kernels' hardness and their protein content, and inversely to their moisture content (Narváez-González et al., 2006).

Proteins are dynamic and interactive molecules, prone to numerous conformational and chemical changes during nixtamalization; however, these changes are not well understood. Zein, the prolamin fraction of corn proteins comprises about 50% of the starch granule associated proteins, which are composed of two different classes, the above mentioned, surface localized zeins, with molecular weights of about 10–27 kDa and the granule intrinsic proteins of 32 kDa or higher, which remain refractory to proteolysis (Duodu et al., 2002).

The texture of masa is crucial during the production of tortillas. Both, in the industrial and the domestic process, the masa should be readily cohesive to allow the formation of a sheet and thus, favor its cutting and shaping as round disks or another shape. Changes in consistency measured using the rapid visco analyzer (RVA) viscoamilograph of various starches have been attributed to changes in structure. Other studies using the RVA, have correlated the functionality of nixtamalized corn flour with the consistency of the masa produced. Also, the sizes of the particles and

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polymers have been correlated with the texture of the masa (Sahai, Buendía, & Jackson, 2001).

Several works about the rheological behavior of starch from different sources have been published (Kaur, Singh, & Sodhi, 2002; Singh, Singh, Kaur, Sodhi, & Gill, 2003), however, few studies about the rheological characteristics of starch from nixtamalized corn are found in the literature. It has been postulated that the interactions of calcium cations with starch, could be in great extent, responsible of the structural changes that affect the viscoelastic behavior of nixtamalized corn flour (Mondragón et al., 2006). These authors studied the influence of lime and the amylose–lipid complexes, on the viscoelastic behavior of gels from nixtamalized corn starch using oscillatory or low strain tests. They found that both the storage (G′) and loss (G″) moduli were somehow dependent on the concentration of lime; they concluded that the corn proteins, which lie around the starch granule in an amorphous matrix as protein bodies; modify during the nixtamalization process and also during the milling of nixtamalized corn, in such a way that the amylose, the amylopectin and the hydrated proteins form a system that notably alters the masa viscoelastic properties.

The objective of this research was to study the effect of different nixtamalization conditions on the structure, consistency, physicochemical and viscoelastic properties of corn (*Zea mays* sp.).

2. Materials and methods

2.1. Materials

The commercial corn variety named *Pioneer 30G54*, coming from "Valle de Santiago" region, was used. This was harvested in the provinces of México, Guanajuato and Michoacán. The alkaline treatment was done with commercial lime (calcium hydroxide, (cal piramide)), commonly used in the tortilla industry.

2.2. Corn cooking conditions

A traditional industrial procedure of nixtamalization was followed (Ibarra-Mendívil, Gallardo-Navarro, Torres, & Ramírez Wong, 2008). Lots of 300 kg of corn in 900 kg of water were nixtamalized, using 1% (w/w) of calcium hydroxide (lime)/weight of corn. To assess the efficiency of the corn cooking, the masa consistency profile was measured in a rapid visco analyser (RVA) (Newport Scientific PTY, Ltd., model 3D, Sydney, Australia), which is a common practice in some industries that produce tortillas. The RVA readings are given in arbitrary units called RVU, normally expressed for a given rotational speed, that can then be converted into shear viscosity at a specific shear rate and can hence be compared directly from data from other shear instruments (1 RVU is about 10 cP) (Aerts & Verspaille, 2001; Chen, Lu, & Lii, 1999). The RVU range considered to define a good corn cooking goes from 820–850 RVU, which according to the equipment dealer corresponds to about 9840–10,200 cP. All corn kernel samples were cooked at 90 °C at atmospheric pressure. Different lots of samples were cooked for different times, with lime (nixtamalization), making a total of five treatments, with processing times of 10, 30, 50, 70 and 90 min. Another series (a total of nine treatments) of non-nixtamalized samples (controls) were cooked at 90 °C for 10, 30, 50, 60, 70, 90, 120, 150 and 180 min. All lots of cooked corn were then let to rest for 14 h, which is the traditional procedure. Then, the samples were washed thoroughly with tap water until the rinsing water's pH = 7. All washed corn samples were milled in a Cyclone mill (UDY Corporation, Fort Collins, CO.), a common step in the traditional nixtamalization process, until a masa (dough) adequate to be rolled and cooked to obtain tortillas was obtained (Almeida-Dominguez, Suhendro, & Rooney, 1997). The temperature range selected, was similar to the one commonly used in the tortilla industry (personal communication, Elizabeth Quintana, R&D Fritolay Mexico, Mexico city), where corn is milled and transformed in masa, and where presumably its actual viscoelastic properties are developing.

2.3. Microscopy of corn kernels

Lyophilized corn samples were ionized with gold using the deionizer equipment (DESK II, Denton vacuum) and observed at 1000×, using a scanning electron microscope JEOL model JSM-5900LV (Duodu et al., 2002). SEM micrographs were obtained from nixtamalized and control corn kernels cooked at 90 °C for different cooking times, by using an JEOL equipment. Samples were treated with a bath of gold at 15 kV, micrographs were obtained at 1000×.

2.4. Thermal analysis of corn

Transition temperatures (T_p) and enthalpies (ΔH), were measured using a differential scanning calorimeter (DSC-7, Perkin–Elmer Corp., Norwalk, CT) following the method described by Ruales and Nair (1994). The calorimeter was calibrated with indium and the data was analyzed using the Pyris software. Samples of about 5 mg each, were adjusted with de-ionized water to 30% moisture content and then placed in pre-weighed aluminum pans. The pans were sealed and kept at rest for one hour to reach equilibrium. The scanning range was 30–120 °C at a rate of 10 °C/min. Any transitions such as starch gelatinization and protein denaturation were recorded, finding in the thermograms the initial (T_i), peak (T_p) and final (T_f) temperatures, as well as the enthalpy (ΔH).

2.5. Cooked corn consistency

The steeped and washed corn samples were milled until a particle size of 1 mm was obtained, using a Cyclone mill, to avoid starch gelatinization because of friction or starch damage. The above mentioned RVA was used to measure changes in viscosity upon heating and cooling. The sample was added to the RVA vessel at 25 °C, to make a 15% suspension in 28 g of de-ionized water. Then, it was heated at a rate of 5 °C/min to reach 50 °C in 5 min. After incubating for 2 min at 50 °C, the sample was heated at 2.4 °C/min to 95 °C, maintained at this temperature for 4 min, then, cooled down at 5 °C/min to 50 °C, and held for 5 min at this last temperature. The RVA parameters gelatinization onset temperature, pasting peak viscosity and temperatures of breakdown and set-back, were obtained from the viscogram data. All measurements were done in triplicate.

2.6. Rheology of masa

The study of the viscoelastic properties of the masas obtained using corn subjected to different cooking times (Section 2.2), was undertaken with a strain controlled TA Instruments (AR-2000) Rheometer, using a parallel plate system with a diameter of 20 mm and a sample gap of 1 mm. Once the corn samples were washed and milled, water was added until the samples reached 50% moisture to obtain a masa (dough). These masas were kept in rest for 24 h at room temperature for their stabilization. Strain amplitude sweeps were carried out within the range from 0.1% to 2%, at 1 Hz. Then, frequency sweeps were run from 0.1 to 10 Hz, at a constant strain of 0.3%. All measurements were done in triplicate at 25, and 60 °C. A temperature ramp was also run, following a heating program that started at 25 °C with a heating rate of 7 °C/min, till reaching a final temperature of 60 °C in about 5 min. To avoid moisture losses during the tests, the edges of the plates were covered with silicon. The storage or elastic modulus (G′) and the loss or viscous modulus (G″) and

the loss tangent or Tan δ (G''/G') were evaluated using the software from the equipment.

2.7. Statistical analysis

The Minitab statistical software, version-Minitab 15 (Minitab Inc., State College PA) was used to analyze data by applying one way analysis of variance at a significance level of 5% ($p = 0.05$), and significant differences among means were defined by using the Student-Newman-Keul's test (Steel & Torrie, 1980).

3. Results and discussion

3.1. Scanning electron microscopy (SEM)

Nixtamalized corn samples cooked 10 min (Fig. 1a1) show round shaped starch granules. Irregular shaped protein bodies adhered to the granules are also distinguished. Starch granules from control samples cooked for 10 min (Fig. 1b1), exhibit polygonal shape and most of them are still intact inside the cells. However, a small number of round shaped protein bodies are observed; suggesting they may have undergone some hydration. Most of the starch granules from nixtamalized samples cooked for 30 min (Fig. 1a2) have been separated from the cells and still show protein bodies adhered to them. Many starch granules inside the cells from control samples may also be seen (Fig. 1b2), those granules show polygonal shape, which indicate they have not probably undergone the characteristic swelling of starch granules. As cooking time increased up to 50 min, nixtamalized samples (Fig. 1a3) showed changes in the shape of protein bodies from irregular to round. This change indicates they have perhaps undergone some alterations because of their hydration. Cell walls seem to be slightly thicker than those from less cooked samples under the same conditions; which may be explained for the prolonged cooking time under

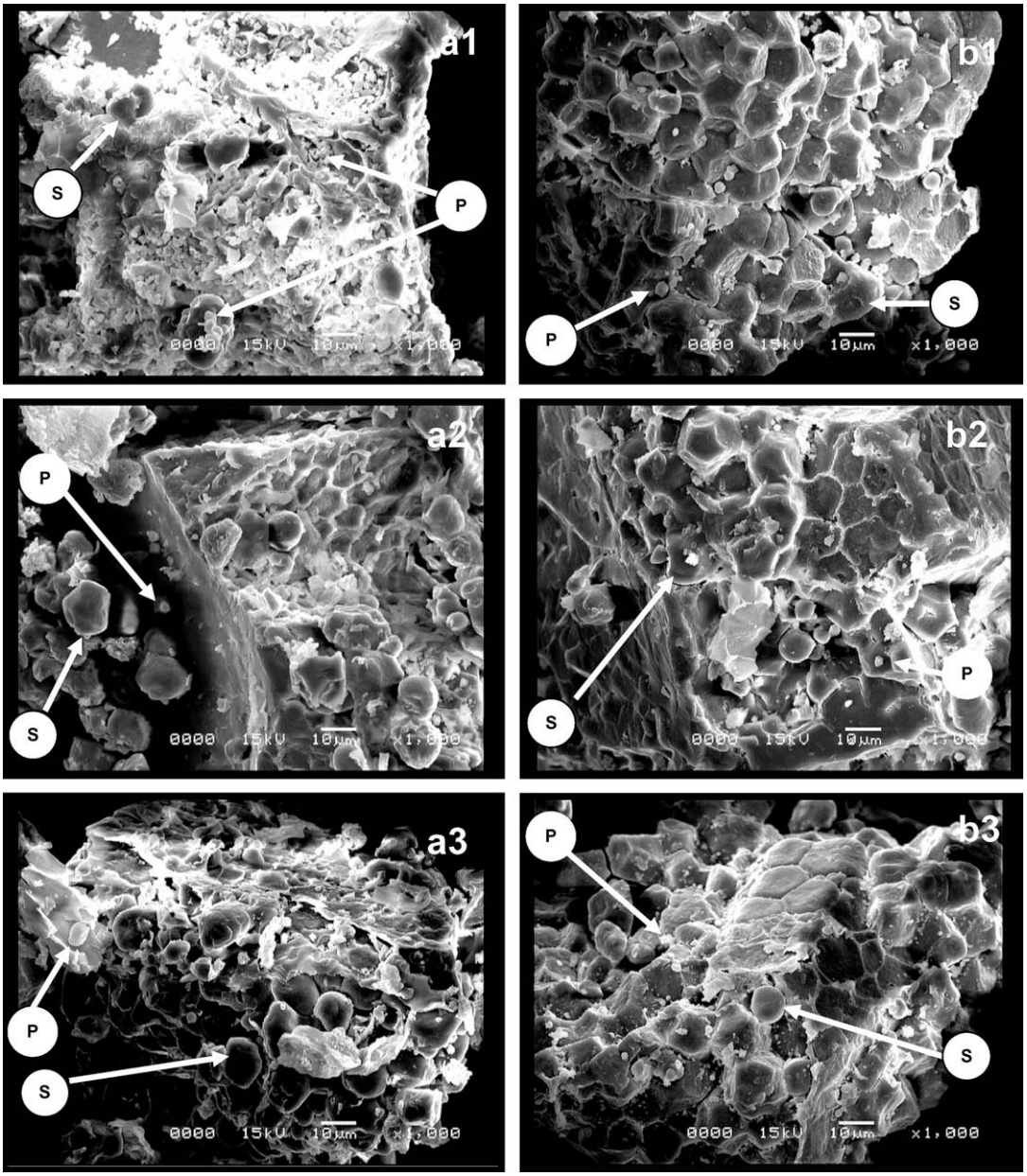

Fig. 1. Effect of nixtamalization and processing time on the structure of corn cooked at 90 °C. (a) nixtamalized and (b) control, cooked for 1 = 10 min, 2 = 30 min, 3 = 50 min. S = starch granules, P = protein bodies.

alkaline conditions, that could cause some macromolecules hydrolysis. In contrast, in control samples cooked during the same time (Fig. 1b3), although various starch granules exhibit round shape, many are still attached to the cell walls, showing a polygonal shape; which indicates they have not probably undergone complete swelling yet. As cooking time increased up to 70 min, nixtamalized samples (Fig. 2a4) exhibited a small number of starch granules and a few round shaped protein bodies, possibly because of their hydration; the surface of most of the cellular cavities are empty and exhibit an irregular appearance. Some light color structures are visible, which may be the remains of the protein matrix covering the starch granules, and might be related to the "ghost structures" reported by Han and Hamaker (2002). In the control samples (Fig. 2b4), there are still many starch granules inside the cells. Although most of them show a round shape, some still exhibit a polygonal one; characteristic of those that have not been swollen yet. It is observed that some starch granules are still covered by a protein matrix, with several round protein bodies stuck to some of them.

At 90 min of cooking, the nixtamalized samples (Fig. 2a5) practically have no visible starch granules on the endosperm tissue; suggesting their disintegration by the effect of a long cooking time. Different to samples cooked for 70 min, which had an irregular shape; in samples cooked during 90 min., it changed from rough to a smooth one, may be caused by their hydration; suggesting that these rough forms possibly correspond to protein bodies adhered to the cellular walls. In control samples cooked 90 min (Fig. 2b5), there are still many round starch granules adhered to the cellular cavities, most of them covered by a protein matrix.

Control samples cooked for 120 and 150 min (Fig. 3b6 and b7), exhibit a small number of starch granules attached to the cellular cavities, and protein bodies are still visible. Despite the extended cooking time, the granules still present a polygonal shape. Conversely, in samples cooked for 180 min (Fig. 3b8), starch granules are no longer seen and only some protein bodies are mildly joined to the cell walls. These results are in agreement with those reported by Ezeogu, Duodu, Emmambux, and Taylor (2008). They studied the influence of cooking conditions on the protein matrix of "honeycomb-like" structures, on starch hydrolysis of flours obtained from vitreous endosperm matrices of sorghum and maize by three-dimensional fluorescence microscopy, finding that cooking caused the collapse and a rough appearance of the internal cellular surface. They interpreted these effects as a consequence of expansion of the starch granules through water uptake during gelatinization, which is consistent with the results of this work.

3.2. Changes on physicochemical properties

The transition temperature and enthalpy of raw corn flour were 71.2 °C and 8.1 J/g, respectively, which might be considered typical of normal starches (Table 1). However, nixtamalized and control samples of flour cooked from none to 70 min, did not show any early transition. Most of the extracted proteins from nixtamalized corn at all cooking times, exhibited two endothermal transitions (thermograms not shown). The first phase transition lay in a range of temperatures from 56.5 to 58.4 °C with enthalpies from 0.7 to 2.6 J/g, which corresponds to protein denaturation. No early endothermal events were seen in proteins extracted from nixtamalized samples cooked for up 10 min; however, at 30 min and even until 90 min of cooking, all samples showed thermal transitions. Those results are consistent with the fact that the proteins from control samples showed an endothermal transition, solely in the corn sam-

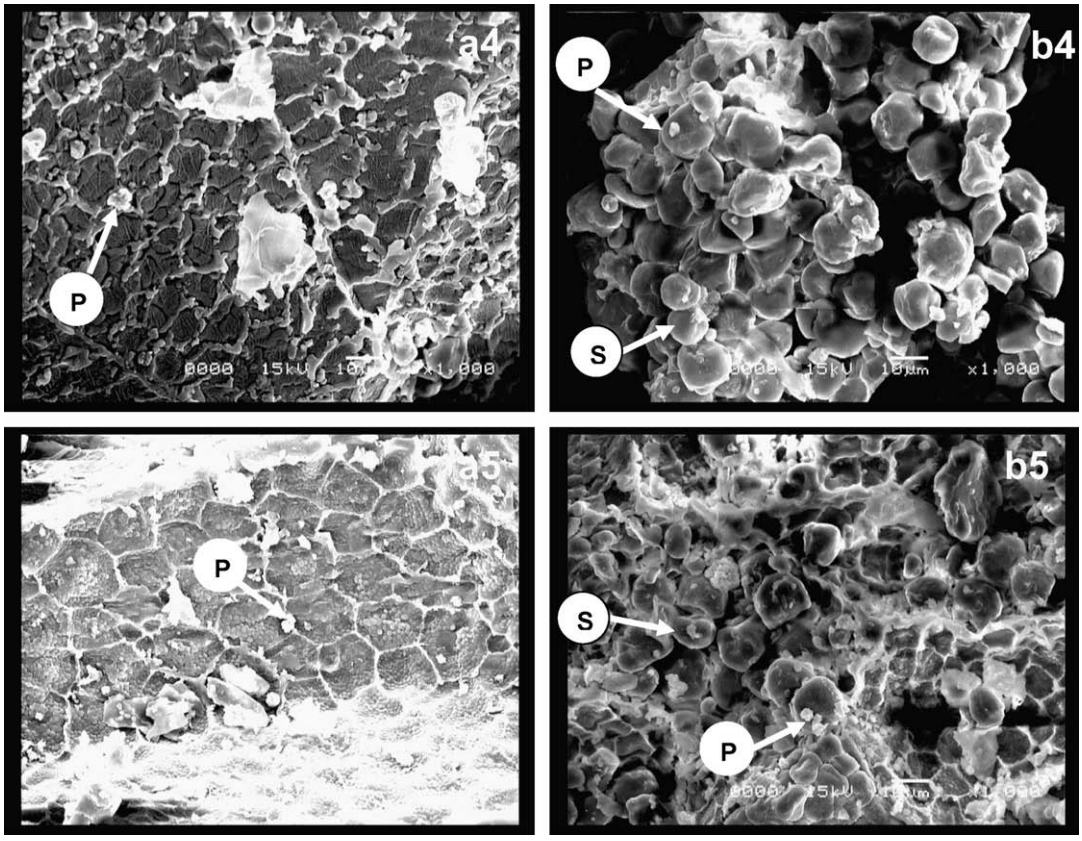

Fig. 2. Effect of nixtamalization and processing time on the structure of corn cooked at 90 °C. (a) nixtamalized and (b) control, cooked for 4 = 70 min, 5 = 90 min. S = starch grains, P = protein bodies.

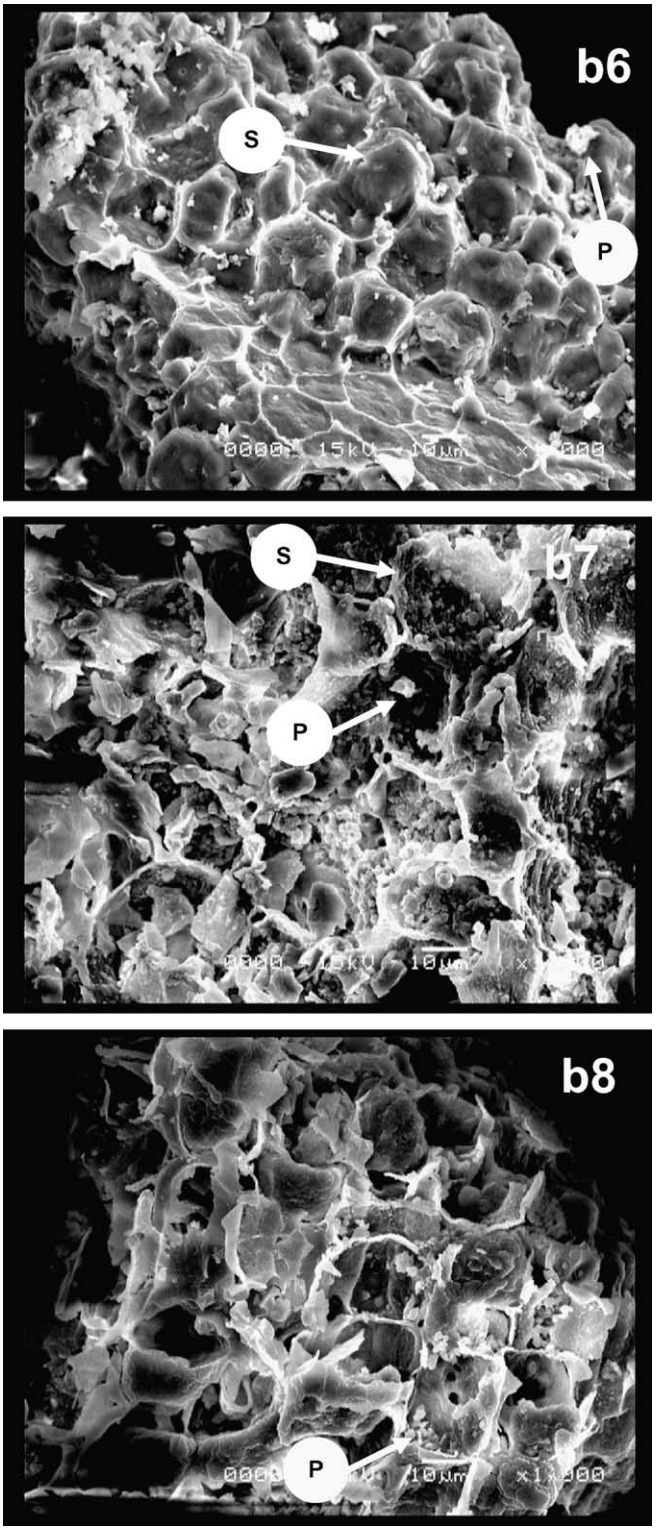

Fig. 3. Effect of processing time on the structure of control corn cooked at 90 °C, for 6 = 120 min, 7 = 150 min, 8 = 180 min. S = starch grains and P = protein bodies.

ple cooked for 120 min., suggesting that the protein has changed its secondary structure to a more ordered one, which could also be associated with its denaturation. This allowed the link among aminoacid chains by hydrophobic interactions, which in turn were lost above 90 °C, as in this case, solely after a long cooking time (120 min). The flour from nixtamalized sample cooked for 90 min, showed a higher first thermal transition than the control sample, but the enthalpies showed the opposite behavior (Table 1). In the case of extracted proteins from control samples, no endothermal phase transitions were observed, but samples cooked for 120 min showed a transition temperature (denaturation) at about 59 °C with enthalpy of 2.4 J/g. These results are in agreement with previous reports (Vivas, Serna-Saldivar, Waniska, & Rooney, 1990; Vivas, Waniska, & Rooney, 1987; Duodu et al., 2002; Cabra, Arreguin, Vazquez-Duhalt, & Farres, 2006) which indicate that nixtamalization alters the solubility and molecular weight of albumins, globulins and prolamins, by affecting their primary, secondary and tertiary structures and promoting the formation of disulphide crosslinks among neighbors polypeptides, resulting in a more ordered structure.

This first phase transition of the extracted proteins from nixtamalized corn, suggests that the protein changed its structural levels, losing its native structure as an effect of lime treatment.

A second endothermal transition was shown by all nixtamalized and control samples from flour and their extracted proteins. In control samples, this transition corresponds probably to the gelatinization. However, nixtamalized samples showed slightly higher gelatinization temperatures than those of the controls at the same cooking times.

It is known that transition (gelatinization) temperature (T_p) of starch, may be associated with the degree of starch's crystallinity, among other factors (Singh et al., 2003). A high transition temperature is the result of a high crystallinity degree, which confers structural stability to starch granules, and makes them more resistant to gelatinization. However, even though the samples studied were composed mainly of starch, but with some proteins and lipids, it might well be that the level of starch crystallinity, was not the sole factor that determined its phase transition (gelatinization temperature and enthalpy). This result also suggests that the high gelatinization temperatures could be associated with interactions among the corn kernel components (mainly starch–lipid, starch–protein, starch–calcium and protein–calcium), which conferred resistance to the gelatinization phenomenon. Overall, the longer the cooking time, the higher the gelatinization temperature; which suggests that as cooking time increased, a more ordered structure was formed.

Samples of proteins extracted from both nixtamalized and control corns, also showed a second endothermal transition, which corresponds to protein denaturation, because those samples did not contain starch. This result suggests that those proteins might also be related to the gelatinized starch. However, nixtamalized corn samples showed slightly higher transition temperatures, indicating that the nixtamalization is possibly favoring a more ordered structure due to protein–starch interactions taking place during the treatment, as seen previously in the microscopy results.

3.3. Consistency (RVU) of the corn samples

By taking the RVU range defined above (820–850 U) and fitting the data on the plots of Fig. 4 using polynomial regression; the optimum cooking time for each group of samples was obtained by interpolating viscosity values on the plots. Thus, for nixtamalized corn samples, it was necessary to have a cooking time between 16.7 and 26.8 min, while for the controls, a cooking time between 124.2 and 150.2 min was needed. This suggests that control corn samples cooked between these two latter periods of time, may present similar characteristics to those nixtamalized, cooked within the former mentioned cooking time range, because of the correspondence in optimum processing time. However, the nixtamalized corn samples produced a masa that showed an adequate texture, which allowed a cohesive but not adhesive sheet to be formed during shaping, meaning it was appropriate to make good

Table 1
Thermal properties of nixtamalized and control corns and their proteins (mean ± SD, $n = 3$).

Cooking time (min)	First endothermal phase transition						Second endothermal phase transition					
	T_p (°C)				ΔH (J/g, d.b.)		T_p (°C)				ΔH (J/g, d.b.)	
	Flour		Extracted proteins		Flour	Extracted proteins	Flour		Extracted proteins		Flour	Extracted proteins
	N	*C*	*N*	*C*	*N*	*C*	*N*	*C*	*N*	*C*	*N*	*C*	*N*	*C*
0	–	–	–	–	–	–	74.6 ± 0.1[a]	71.2 ± 0.2[a]	–	73.6 ± 0.1[a]	6.7 ± 0.1[a]	8.1 ± 0.1[a]	–	–
10	–	–	–	–	–	–	76.7 ± 0.2[b]	73.4 ± 0.2[b]	73.9 ± 0.2[a]	73.1 ± 0.1[a]	6.9 ± 0.1[a]	8.6 ± 0.1[a]	6.5 ± 0.4[b]	7.1 ± 0.1[b]
30	–	–	56.5 ± 0.3[a]	–	–	0.7 ± 0.1[a]	79.0 ± 0.6[c]	73.5 ± 0.1[b]	78.3 ± 0.8[b]	75.0 ± 1.6[b]	3.6 ± 0.2[b]	8.6 ± 0.4[a]	5.4 ± 1.4[b]	5.7 ± 1.6[a]
50	–	–	57.8 ± 1.0[a]	–	–	1.4 ± 0.1[b]	79.3 ± 0.1[c]	78.0 ± 0.2[c]	79.3 ± 0.6[b]	76.7 ± 1.6[b]	3.7 ± 0.1[b]	3.7 ± 0.3[c]	7.0 ± 0.1[c]	5.0 ± 0.4[a]
70	–	–	58.4 ± 0.6[b]	–	–	1.4 ± 0.1[b]	81.7 ± 0.1[d]	79.1 ± 1.1[c]	79.7 ± 0.1[b]	78.9 ± 0.3[c]	2.3 ± 0.1[c]	3.6 ± 0.9[c]	4.2 ± 0.2[d]	4.5 ± 1.0[a]
90	64.2 ± 0.1	59.7 ± 0.5	57.6 ± 0.2[a]	–	0.7 ± 0.1	1.2 ± 0.4	2.6 ± 0.1[c]	–	–	79.8 ± 0.2[c]	–	–	–	4.7 ± 0.6[a]
120	–	–	–	58.6 ± 0.8	–	–	–	2.4 ± 0.4	–	81.6 ± 0.1[d]	–	–	–	2.3 ± 0.2[c]
180	–	–	–	–	–	–	–	–	–					

Note: a–d Same letters after entries within each column mean that there are no significant differences ($p > 0.05$). T_p = peak temperature, ΔH = transition enthalpy, *N* = nixtamalized, *C* = control, d.b. = dry basis.

quality tortillas. Control corn samples cooked between 124.2 and 150.2 min produced in turn a highly adhesive masa. Overall, from the viscoamilographic study (RVA), nixtamalized samples cooked for 30 min corresponded to the most adequate cooking conditions to produce good quality tortillas. It can be observed that in general, there is a reduction on the maximum viscosity as a function of corn cooking time, and for nixtamalized samples, even an abrupt decrease in the cooking time is seen after 90 min. The slopes of both plots differed significantly ($p < 0.05$), being the slope of the nixtamalized sample higher than that of the control. This was as expected, since according to previous works (Fernández-Muñoz et al., 2004; González, Reguera, Mendoza, Figueroa, & Sánchez-Sinencio, 2004; Gutiérrez et al., 2007; Hurtado-Castañeda et al., 2005; Laria, Meza, Mondragón, Silva, & Peña, 2005; Laria, Meza, & Peña, 2007; Paredes-López & Sarharopulos, 1982; Trejo-González et al., 1982), one of the roles of calcium during nixtamalization is to shorten the corn cooking time, by degrading and solubilizing the cell wall components, allowing the pericarp removal, softening the endosperm structure and favoring water and ions diffusion towards the starch granules.

3.4. Viscoelastic properties

To define the linear viscoelastic region (LVR), or domain where the moduli are independent of the strain (Singh et al., 2003), preliminary tests were undertaken (at 25 and 60 °C) to masa samples from control and nixtamalized corn, cooked at different times. It was found that a frequency value of 1 Hz, an intermediate value within the LVR; was adequate to do all amplitude strain tests. The same way, a value of 0.3% strain was chosen for all oscillatory frequency tests. The strain and frequency profiles of both the storage or elastic modulus (G′) and the loss or viscous modulus (G″) of masa samples from nixtamalized corn cooked at 90 °C for 10 and 90 min, and controls cooked during 10–180 min, are shown in Figs. 5 and 6, respectively. It is seen in Fig. 5a, that irrespective of the measurement temperature, all nixtamalized samples had G′ values predominating over all of the applied strain range, conforming the LVR within the domain involving up to 1% deformation. Beyond this point, especially the plots of G″ manifest a shift in its slope, which is an indication of the beginning of a strain-dependence of the modulus (Ferry, 1980), suggesting that the masa has become sensitive to deformation, but with no real breakage of its structure even within the remaining strain range applied. Besides, samples cooked for 10 min show higher moduli (G′, G″) values than those cooked for 90 min.

Fig. 5b shows the frequency profiles of the masa samples from nixtamalized corn, where the G′ values are higher than those of G″, and the two moduli showed a tendency to be frequency-independent over the frequency domain applied. This confirms the behavior of weak gels and is also evidence of an ordered structure (Steefe, 1992). The plots are overall, essentially flat over the frequency range tested. Comparing the profiles of the moduli (G′, G″) for the nixtamalized masas from corn cooked for 10 min, they are significantly higher ($p < 0.05$) than those of the masas from corn cooked for 90 min. This behavior could be because during the first 10 min of cooking, the starch was partially gelatinized and the starch granules swelled and hydrated, presenting the elastic character a higher contribution in the masa viscoelastic profile, showing in consequence certain "stiffness" (rigidity). However, after 90 min of cooking, the already fully gelatinized starch granules had possibly lost their structure integrity, the starch components (amylose, amylopectin) had leached out from the granules and so, their masas were less rigid (Han & Hamaker, 2002). The range of Tan δ (G″/G′) values for samples cooked for 90 min (0.33–0.47) were slightly higher than those of samples cooked for 10 min (0.29–0.35), but overall, they were lower than unity,

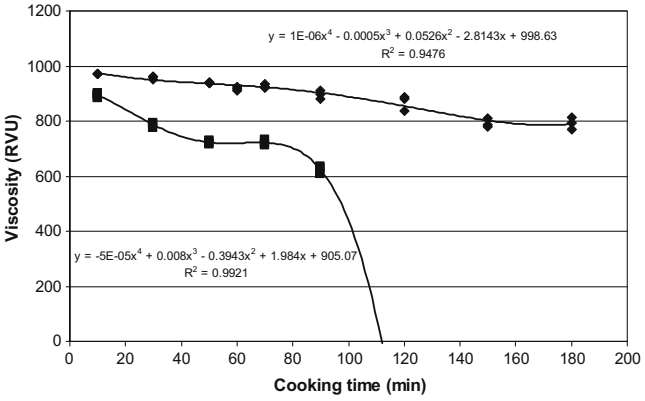

Fig. 4. Changes in the maximum viscosity (RVU) of nixtamalized (■) and control (♦) corn samples processed at different cooking times.

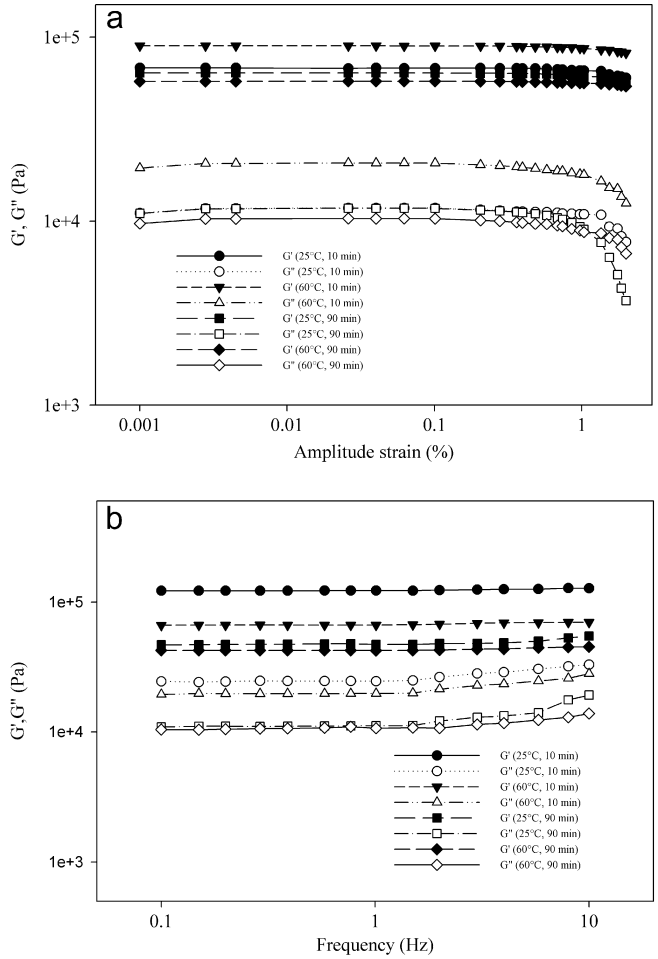

Fig. 5. (a) Amplitude strain sweep (at 1 Hz) and (b) Frequency sweep (at 0.3% strain) of masa samples from nixtamalized corn cooked at 90 °C for 10 and 90 min. The tests were measured at 25 and 60 °C. Filled symbols G′; void symbols G″.

and thus, behaving as weak viscoelastic gels with amorphous structure (Ferry, 1980).

The measurement temperature affected the mentioned profiles, because at 60 °C, a similar trend to the profiles at 10 °C was observed, but with lower moduli values, suggesting that the gels became "flaccid" at the highest measurement temperature.

In Fig. 6a (amplitude sweep), it is seen that the strain profiles of G′ from control masa samples, are flat over the applied strain

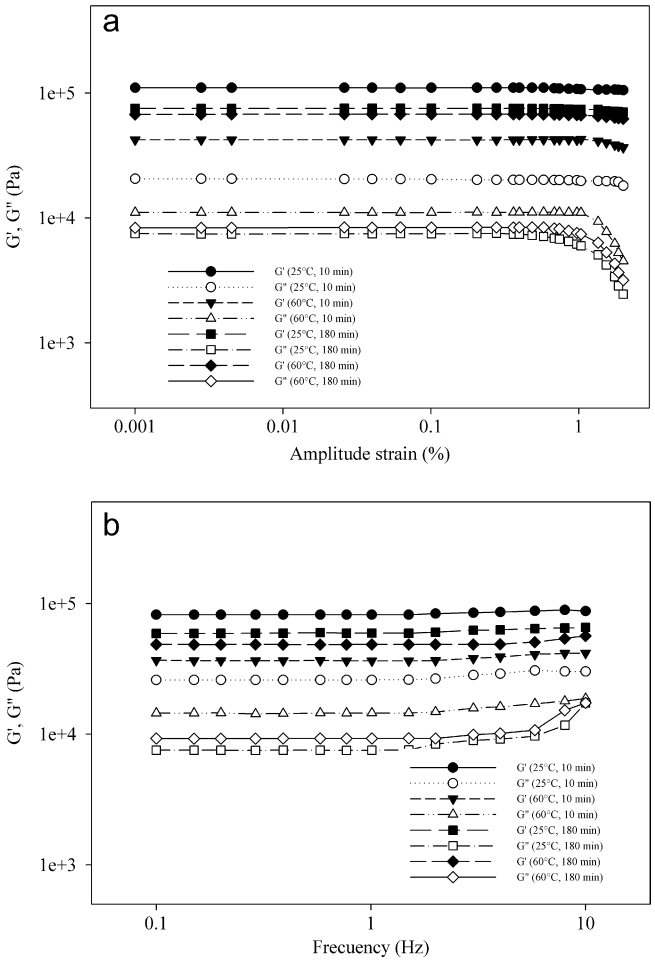

Fig. 6. (a) Amplitude strain sweep (at 1 Hz) and (b) Frequency sweep (at 0.3% strain) of masa samples from control corn cooked at 90 °C for 10 and 180 min. The tests were measured at 25 and 60 °C. Filled symbols are for G′; void symbols are for G″.

range, but the plots of G″ are overall amplitude-independent solely between 0.1% and 0.75% strain, and thus, the above strain range may be considered within the linear viscoelastic region (LVR) for the controls, here again, the values of G′ > G″. From 0.75% strain onwards, except for the sample cooked for 10 min (measured at 25 °C); all G″ strain profiles show non-linear behavior, suggesting that this might well be the "critical point" not to be surpassed, i.e., the strain where the sample structure starts breaking. Considering the profiles of G″ of the masa from controls corn cooked for 10 min; in all cases, they were significantly higher ($p < 0.05$) than those coming from corn cooked for 180 min.

The frequency profiles of the control samples are shown in Fig. 6b. All plots of G′ are flat in the frequency range tested. But in the case of the G″ profiles, those cooked for 180 min, irrespective of the measurement temperature, start presenting frequency-dependence at about 6 Hz. However, trends already seen in masas from nixtamalized corn; as the values of G′ > G″ and the overall higher moduli values in samples with lower processing times, being affected by the test temperature, are also common in these frequency profiles, suggesting that except for the lime effect (calcium–corn components interactions) in treated samples; the similar above mentioned mechanism might still be working. In this case, the range of Tan δ values for samples cooked for 10 min (0.41–0.44), were higher than those samples cooked for 180 min (0.22–0.3), but still being consistent with an amorphous structure as a result of the heat processing.

Comparing the frequency profiles of nixtamalized and control masas (Figs. 5b and 6b), it is observed that overall, at the same cooking temperatures, the moduli (G′, G″) values of the nixtamalized samples are predominating, possibly as an effect of the lime treatment.

In Fig. 7, the moduli (G′ G″) profiles of the temperature ramp are shown, most of the plots are not precisely linear or somewhat sloping, but with no indication of a real breakage of its structure over the temperature range. It is seen that irrespective of the sample (control, nixtamalized), the G′ values are higher than those of G″, over the temperature range studied (25–60 °C). It is also observed that the results are overall, consistent with those shown previously in Figs. 5 and 6 (higher moduli values at 25 than at 60 °C sharp, for treated samples cooked for 10 and 90 min and control samples cooked for 10 and 90 min and for 10 and 180 min).

For nixtamalized samples, the values of the moduli (G′, G″) increased up to a maximum at 50 min cooking. Then, as the cooking time increased, the G′ values decreased (Fig. 7a). The lowest values belonged to samples cooked for 10 min (overall G′ values for 50 min > 30 min > 70–90 min > 10 min cooking), thus among the involved treatments, 50 and 30 min are perhaps the most consistent with the RVA results previously shown and might be close to the optimum cooking time. This behavior suggests that in a way, as the cooking time increases, the masas "stiffen" reaching its maximum in 50 min cooking. At this stage, the masas became rigid (increase in G′), possibly due to starch gelatinizing in a larger extent than in the case of shorter cooking times, and thus, the swelling of granules had increased, as previously reported for starch gels (Singh et al., 2003). The viscous modulus followed a slightly different pattern (G″ values of 50 min > 30 min > 70 - min > 10 min > 90 min), but keeping the maximum values in samples cooked for 50 min. These results suggest that the moduli values depend not only on the sample story and measurement temperature, but also on the time the sample has been at that temperature (see Figs. 5 and 6).

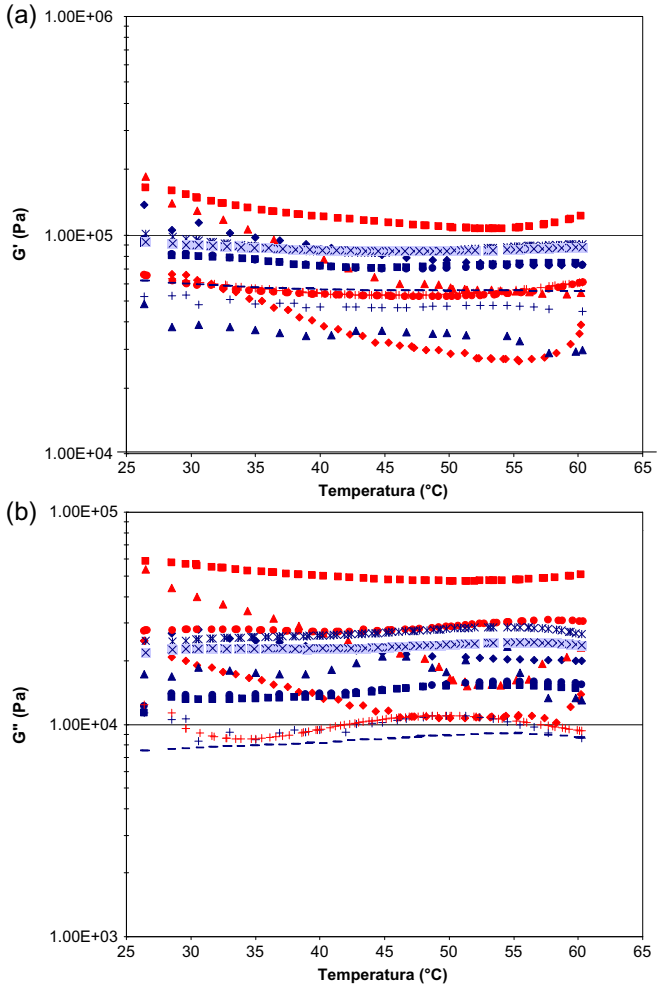

Fig. 7. Effect of the cooking time, temperature and the presence of lime on the rheological moduli of masa. (a) elastic modulus (G′). (b) Viscous modulus (G″). Cooking time (min) ♦ = 10, ▲ = 30, ■ = 50, ● = 70, + = 90, ∗ = 120, *x* = 150, – = 180. Blue symbols are for control samples; red symbols are for nixtamalized samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

In all control samples (Fig. 7), also the elastic character prevailed (G′ > G″). The maximum G′ values correspond to samples cooked for 10 min. Then, lower values were seen along the temperature range studied (Ǵ values of 10 min > 120 min > 150 min > 50–70 min > 180 min > 90 min > 30 min) (Fig. 7a). The viscous modulus, behaved in a different way as can be seen in Fig. 7b. (G″ values 120 min > 150 min > 10 min > 30 min > 50–70 min > 90 min > 180 min). According to Steefe (1992), those unusual profiles of both moduli (most of them sloping) are commonly seen in weak gels subjected to temperature ramps, due to probable inhomogeneities of the sample, manifested as the temperature changes.

The properly nixtamalized corn grains are constituted by swelled and gelatinized starch granules and a protein matrix, conforming the masa. The amylose, amylopectin and protein, form a "glue-like" system, which links the non-gelatinized starch and the endosperm intact cells in a cohesive masa. That is why the structural changes occurring during nixtamalization, affect the rheological and chemical behavior of the masa and its byproducts (e.g. tortillas) (Singh et al., 2003).

If the change of the viscoelastic behavior of the masa as affected by the cooking time was due solely to the changes occurred in starch, it would be possible to find some equivalence in the elastic and the viscous moduli between the nixtamalized and control samples. However, in this work, such a relationship was not detected. In consequence, the differences in G′ and G″ profiles, might also be attributed to probable interactions between the starch and another components found in nixtamalized samples; such as proteins and lipids, that could cause the moduli profiles of the nixtamalized specimens, to be apparently higher than those of the controls at 10 and 90 min cooking, with certain effect of the measurement temperature. That way; it is probable that the interactions which potentially occur between the starch and the other corn components, cause some "stiffeness" on the masas and eventually make them cohesive.

It was also seen over this temperature ramp, that the combined effect of the changes in the elastic and viscous moduli was as expected, evident when the loss tangent or Tan δ values were analyzed. In nixtamalized samples, overall, the range of values were between 0.2 and 0.6. Within this range, the loss tangent values were decreasing as the cooking time increased. However, the highest values were those of samples cooked for 50 min, going from 0.5–0.6 within the frequency range applied, still typical of weak viscoelastic gels with an amorphous structure (Ferry, 1980), being consistent with the lowest G′ values observed in those samples.

In the case of control samples, it was notorious that except for those cooked for 70 min, with Tan δ values between 0.45 and 0.5, no other sample showed similar values. In the remaining control samples, the Tan δ values decreased as the cooking time increased, going from 0.4 to 0.15, which are still those of weak gels, with probably mainly amorphous structure conferred by the cooking time. The Tan δ results could be indicative of the changes occurring in the components of the masa, which favors the distinctive viscoelastic properties when using a proper cooking time. These changes may involve not solely loss in cristallinity with change

to an amorphous structure, because of starch gelatinization which is the major component, but also the interactions starch–protein and starch–lipid.

4. Conclusion

From this study, as seen by SEM, it can be concluded that lime treatment (nixtamalization) during cooking for more than 30 min of corn kernels, modifies the shape from round to polygonal of both the starch granules and the protein bodies attached to them. The flours obtained from nixtamalized and control corns, over all cooking times, did not show any earlier endothermal transition, but the extracted proteins from nixtamalized samples, usually exhibited two transition endotherms, differing from control sample proteins, where solely one endothermal phase transition was observed. Within the temperatures domain involved, starch gelatinization and protein denaturation temperature increased with the cooking time; suggesting structure stabilization. Protein samples from nixtamalized corn, showed slightly higher transition temperatures, suggesting that the lime is promoting a more ordered structure, due to enhanced protein–starch interactions. The nixtamalization process, shortened substantially the corn cooking time to develop a proper texture in masa to obtain good quality tortillas, as seen in the consistency (RVU) results.

All masa samples showed a rheological behavior which is typical of "weak" gel-like viscoelastic materials, with the storage or elastic modulus (G′) predominating over the loss or viscous modulus (G″) over the applied strain and frequency ranges, with loss tangent or Tan δ values less than unity. The values of both moduli (G′, G″) were affected by the nixtamalization process and the cooking time. The overall results of this study, suggests that nixtamalization affected the starch and proteins structures of corn and also enhanced the protein–starch interactions.

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References

Aerts, L., & Verspaille, M. (2001). Absolute rheometry in the bravender viscograph by mixer viscometry calibration. *Starch/Stärke, 53*, 59–63.

Almeida-Dominguez, H. D., Suhendro, E. L., & Rooney, L. W. (1997). Corn alkaline cooking properties related to grain characteristics and viscosity (RVA). *Journal of Food Science, 62*(3), 516–523.

Cabra, V., Arreguin, R., Vazquez-Duhalt, R., & Farres, A. (2006). Effect of temperature and pH on the secondary structure and processes of oligomerization of 19 kDa alpha-zein. *Biochimica et Biophysica Acta, 1764*, 1110–1118.

Chen, J. J., Lu, S., & Lii, Y. C. (1999). Effects of milling on the physicochemical characteristics of waxy rice in Taiwan. *Cereal Chemistry, 76*(5), 796–799.

Duodu, K. G., Nunes, A., Delgadillo, I., Parker, M. L., Mills, E. N. C., Belton, P. S., et al. (2002). Effect of grain structure and cooking on sorghum and maize in vitro protein digestibility. *Journal of Cereal Science, 35*, 161–174.

Ezeogu, L. I., Duodu, K. G., Emmambux, M. N., & Taylor, J. R. N. (2008). Influence of cooking conditions on the protein matrix of sorghum and maize endosperm flours. *Cereal Chemistry, 85*(3), 397–402.

Fernández-Muñoz, J. L., Rojas-Molina, I., González-Dávalos, M. L., Leal, M., Valtierra, M. E., San Martín-Martínez, E., et al. (2004). Study of calcium ion diffusion in components of corn kernels during traditional nixtamalization process. *Cereal Chemistry, 81*, 65–69.

Ferry, J. D. (1980). *Viscoleastic properties of polymers* (pp. 20–86). New York, NY: Wiley.

González, R., Reguera, E., Mendoza, L., Figueroa, J. M., & Sánchez-Sinencio, F. (2004). Physicochemical changes in the hull of corn grains during their alkaline cooking. *Food Chemistry, 52*, 3831–3837.

Gutiérrez, E., Rojas-Molina, I., Pons-Hernández, J. L., Guzmán, H., Aguas-Angel, B., Arenas, J., et al. (2007). Study of calcium ion diffusion in nixtamalized quality protein corn as a function of cooking temperature. *Cereal Chemistry, 84*(2), 186–194.

Han, X. Z., & Hamaker, B. R. (2002). Association of starch granule proteins with ghosts and remnants revealed by confocal laser scanning microscopy. *Cereal Chemistry, 79*(6), 892–896.

Hurtado-Castañeda, D., Fernández, J., Gutiérrez, E., Rojas-Molina, I., Fernández-Muñoz, J. L., & Rodríguez, M. E. (2005). The diffusion of calcium ion into the organic layers studied by a differential photoacoustic system. *Journal of Physics, 125*, 833–836.

Ibarra-Mendívil, M. H., Gallardo-Navarro, Y. T., Torres, P. I., & Ramírez Wong, B. (2008). Effect of processing conditions on instrumental evaluation of nixtamal hardness of corn. *Journal of Texture Studies, 39*, 252–266.

Kaur, L., Singh, N., & Sodhi, N. S. (2002). Some properties of potatoes and their starches II. Morphological, thermal and rheological properties of starches. *Food Chemistry, 79*, 183–192.

Laria, J., Meza, E., Mondragón, M., Silva, R., & Peña, J. L. (2005). Comparison of overall water uptake by con kernel with and without dissolved calcium hydroxide at room temperature. *Journal of Food Engineering, 67*, 451–456.

Laria, J., Meza, E., & Peña, J. L. (2007). Water and calcium uptake by corn kernel during alkaline treatment with different temperature profiles. *Journal of Food Engineering, 78*, 288–295.

Mondragón, M., Mendoza-Martínez, A. M., Bello-Pérez, L. A., & Peña, J. L. (2006). Viscoelastic behavior of nixtamalized corn starch gels. *Carbohydrate Polymers, 65*, 314–320.

Narváez-González, E. D., Figueroa-Cárdenas, J. D., Taba, S., Castaño-Tostado, E., Martínez-Peniche, R. A., & Rincón-Sánchez, F. (2006). Relationships between the microstructure, physical features and chemical composition of different corn accessions from Latin America. *Cereal Chemistry, 83*(6), 595–604.

Paredes-López, O., & Sarharopulos, M. E. (1982). Scanning electron microscopy study of limed corn kernels for tortilla making. *Journal of Food Technology, 17*, 687–693.

Ruales, J., & Nair, B. M. (1994). Effect of processing on in vivo digestibility of protein and starch in quinoa seeds. *International Journal of Food Science and Technology, 29*(4), 449–456.

Sahai, D., Buendía, M. O., & Jackson, D. S. (2001). Analytical techniques for understanding nixtamalized corn flour. Particle size and functionality relationships in masa flour sample. *Cereal Chemistry, 78*, 14–18.

Singh, N., Singh, J., Kaur, L., Sodhi, S. N., & Gill, B. S. (2003). Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chemistry, 81*, 219–231.

Steefe, J. F. (1992). *Rheological methods in food process engineering* pp. 57–121. East Lansing, MI: Freeman Press.

Steel, R. G., & Torrie, J. H. (1980). *Principles and procedures of statistics a biometrical approach* pp. 43–98. New York: McGraw-Hill.

Trejo-González, A., Feria-Morales, A., & Wild-Altamirano, C. (1982). The role of lime in the alkaline treatment of corn for tortilla production. Modifications of proteins: Food, nutritional, and pharmacological aspects. In R. E. Freeney & J. R. Whitaker (Eds.), *Advances in chemistry* (pp. 245–263). Washington, DC: ACS.

Vivas, N. E., Serna-Saldivar, S., Waniska, R. D., & Rooney, L. W. (1990). Effect of tortilla chip preparation on the protein fractions of quality protein maize, regular maize and sorghum. *Journal of Cereal Science, 12*, 289–296.

Vivas, N. E., Waniska, R. D., & Rooney, L. W. (1987). Effect of tortilla production on protein in sorghum and maize. *Cereal Chemistry, 64*(6), 384–389.