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EFFECT OF MOISTURE CONTENT AND TEMPERATURE, ON THE RHEOLOGICAL, MICROSTRUCTURAL AND THERMAL PROPERTIES OF MASA (DOUGH) FROM A HYBRID CORN (*Zea mays* sp.) VARIETY

EFECTO DEL CONTENIDO DE HUMEDAD Y DE LA TEMPERATURA, EN LAS PROPIEDADES REOLÓGICAS, MICROESTRUCTURALES Y TÉRMICAS DE MASA DE UNA VARIEDAD DE MAIZ (*Zea mays* sp.) HÍBRIDO

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Abstract

The effect of the moisture content (55, 60 and 65% (*w/w*)) and temperature (30, 40 and 50 °C) on the X-ray diffraction (XRD), microstructural, pasting, thermal and rheological properties of nixtamalized dough (masa) from a corn hybrid variety (*Zea mays* sp.) is reported. A complete set of rheological tests including temperature and frequency sweeps, steady shear and transient shear flow was performed in order to get detailed information on the food processing issues. The nixtamalization process affected significantly ($P < 0.05$) the microstructural and thermal properties of masa. Polarized Light microscopy and XRD showed that crystallinity in starch granules decreased for the masa; whereas, Scanning Electron Microscopy (SEM), showed swollen granules dispersed into a plasticized surface. Moreover, Differential Scanning Calorimetry (DSC) showed significant ($P < 0.05$) differences on the gelatinization enthalpy (ΔH_{gel}) of masa, where it increased with increasing the moisture content from 6.7 ± 0.84 J/g for 55% (*w/w*) to 10.2 ± 0.4 J/g for 60% (*w/w*). Frequency sweeps showed a predominant elastic behavior where the storage modulus (G´) was higher than the loss modulus (G´´) and they were significantly ($P < 0.05$) affected by the moisture content and temperature into a small range. The steady shear data exhibited a shear-thinning flow behavior and followed a power law equation, where the power law index (n) decreased when the temperature and the moisture content increased. The effect of temperature on the shear-viscosity (η) was well described by the Arrhenius equation, exhibiting energy activation energy (E_a) values in the range from 25.52 to 59.35 KJ/mol. For transient shear test, all masas presented a stress overshoot at high shear rate before reaching a steady state. It was found that the amplitude of this overshoot depends on the shear rate. On the other hand, the stress relaxation test, which gives the main relaxation time (τ), showed fast relaxation decay at short times, whereas at long times, a slow relaxation was observed. The τ values ranged from 10.46 to 0.43 s, which decreased with increasing shear rate. The rheological behavior of masa was related to a weak gel-like structure formation, composed by dispersed swollen starch granules into a cross-linked starch network, similar to a biocomposite material.

Keywords: nixtamalized corn masa (dough), starch, linear and non-linear rheology, microstructure, calorimetry, pasting curves.

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Resumen

Se reporta el efecto del contenido de humedad (55, 60 y 65% en peso) y la temperatura (30, 40 y 50 °C) en la difracción de rayos X (DRX), curvas de formación de pasta y las propiedades microestructurales, térmicas y reológicas de masa de una variedad híbrida de maíz (*Zea mays* sp.). Se realizó una serie completa de pruebas reológicas que incluyeron barridos de temperatura y frecuencia y barridos de velocidad de corte en estado estacionario y transitorio, con el fin de obtener información detallada sobre variables de procesamiento de alimentos. El proceso de nixtamalización afectó significativamente ($P < 0.05$) a las propiedades microestructurales y térmicas de la masa. Por medio de Microscopía de luz polarizada y DRX se mostró que la cristalinidad de los gránulos de almidón de la masa disminuyó; mientras que por medio de Microscopía Electrónica de Barrido (SEM), se observaron gránulos hinchados y dispersos sobre una superficie plastificada de almidón. Por otra parte, por medio de calorimetría diferencial de barrido (DSC), la entalpía de gelatinización (ΔHgel) de la masa mostró diferencias significativa ($P < 0.05$), donde esta aumentó con el contenido de humedad de 6.7 ± 0.84 J/g para 55% en peso a 10.2 ± 0.4 J/g para 60% en peso. Los barridos de frecuencia mostraron un comportamiento predominantemente elástico, donde el módulo de almacenamiento (G´) fue mayor al módulo de pérdida (G´´), y fueron afectados ($P < 0.05$) significativamente en un intervalo pequeño de contenido de humedad y la temperatura. Los barridos de velocidad de corte en estado estacionario exhibieron adelgazamiento al corte y siguieron la ley de la potencia, donde el índice de comportamiento de flujo (n), disminuyó cuando la temperatura y el contenido de humedad aumentaron. El efecto de la temperatura sobre la viscosidad de corte (η) fue bien descrito por la ecuación de Arrhenius, exhibiendo valores de energía de activación de energía (E_a) en el intervalo de 25.52 a 59.35 kJ/mol. En las mediciones transitorias de esfuerzo, todas las masas presentaron un aumento abrupto a altas velocidades de corte antes de alcanzar un estado estacionario. Se encontró que la amplitud de este sobre-aumento depende de la velocidad de corte. Por otro lado, la prueba de relajación de esfuerzo, de donde se obtuvo el tiempo de relajación principal (τ), mostró una relajación rápida a tiempos cortos, mientras que a tiempos largos se observó una relajación lenta. Los valores de τ variaron entre 10.46 to 0.43 s, y disminuyeron con la velocidad de corte. El comportamiento reológico de la masa se atribuyó a la formación de una estructura similar a la de un gel-débil, compuesto por gránulos de almidón hinchados y dispersos en una red reticulada, similar a un material biocomposito.

Palabras clave: masa de maiz nixtamalizado, almidón, reología lineal y no-lineal, microestructura, calorimetría, curvas de formación de pasta.

1 Introduction

Corn or maize (*Zea mays* sp.) has an ancient tradition, dating from the pre-Hispanic era in America, and until today, it constitutes an important part of the daily diet of people in Mexico, and several countries in Central America. The nixtamalized dough or masa (as is called in Mexico and Central American countries), obtained from this grain cereal through nixtamalization, is used to produce different foodstuffs. However, tortillas are still the most popular staple food among consumers (Caballero-Briones *et al*., 2000). The consumption of tortillas not only is addressed to the local market, but also to international markets. In countries like the US, this food product has also become very popular. Nixtamalization is considered an alkaline process, where the dried corn kernels are cooked with slaked lime (calcium hydroxide), until the corn is soft (nixtamal) enough to be ground using stone ground mills, to obtain a soft and moist masa (Serna-Saldivar *et al*., 2008). During nixtamalization, there are some processing factors that affect the final characteristics and quality of masa and hence of tortillas, such as lime concentration and water content. Water is very important because it acts like a plasticizer, affecting the gelatinization of starch granules during grinding and cooking (Sudha and Leelavathi, 2011). Low moisture content could produce compact and solid masa, whereas high moisture content could produce watery and sticky masa. Hence, water controls important rheological characteristics such as hardness, cohesiveness and adhesiveness, which are of considerable importance in masa processing, as it influences the quality of the final product (Quintanar-Guzmán *et al*., 2009).

Masa can be considered as a biocomposite material, consisting of amylose and amylopectin (starch biopolymers) molecules, dispersed in partially gelatinized starch and intact granules, granule fragments (endosperm) and lipids. All these components forms a continuous complex biopolymer matrix, which during processing, specific interactions at molecular level could modify its microstructure, for example: (1) breakdown of covalent bonds; (2) hydrogen bonding; (3) formation of complexes between starch and lipids (Mondragon *et al*., 2006), and between starch and proteins (Quintanar-Guzmán *et al*. (2010); (4) enzyme-substrate coupling and (5) hydrophobic interactions. To understand the microstructure of complex foods at a micromolecular

level (1-100 nm), various techniques have been used, such as microscopy, light scattering, laser and X-ray diffraction. However, by using techniques to measure properties at a macromolecular level (2 to 1×10^4 μm) such as rheology, they are directly affected by the changes and properties at the microscopic level (Rao, 2007). During food processing, it is very important the evaluation of rheological properties such as viscosity, which are subjected to varying temperatures, because this factor could affect masa viscosity during storage and consumption (Rao, 1999). Thus, rheological properties that include linear and non-linear ones, have a direct relationship with their macrostructure and must be understood, especially the non-linear ones, which have a repercussion on the quality of the finished product. Some published researches address the linear rheology to study the effect of the cooking time (Quintanar-Guzmán *et al*., 2009), the maize proteins content (Quintanar-Guzmán *et al*., 2010; 2011) and starch content (Mondragón *et al*., 2006) of masa on their viscoelastic behavior. Moreover, some authors have reported the rheological properties of nixtamalized corn flours (Bello-Perez *et al*., 2002; Flores-Farías *et al*., 2000); meanwhile, some others have forwarded their researches to study the rheological properties of starch gels from maize (Mondragon *et al*., 2006; Casas-Alencáster and Parfo-García, 2005; Aparicio-Saguilán *et al*., 2006). On the other hand, pasting is another important physicochemical property, useful in the food industry from the product quality viewpoint. An "over-gelatinized" starch (due to overcooking) produces sticky masa, making it difficult to handle; meanwhile, undercooking generates a non-cohesive masa, which do not process adequately on the appropriate masa processing equipments and do not yield a satisfactory product. To our knowledge, and despite the large amount of researches about masa, very few studies have been reported on rheological measurements that include transient non-linear analysis.

The objective of this study was to assess the linear and non-linear rheological behavior of masa of a hybrid variety, as a function of moisture content and temperature, in order to relate its rheological properties to its microstructure. A complete set of rheological parameters with application in food processing techniques were selected and performed at a small-amplitude oscillatory (SAO) and low shear flow rates, i.e., food molding process. The steady state and transient non-linear responses were fitted to a power law and an exponential model, respectively; where the latter allowed it to obtain the relaxation time (τ) of the masa. In order to relate masa rheological properties to its microstructural properties, the microstructural changes of the raw corn and masa by polarized light microscopy, scanning electron microscopy (SEM), and X-ray diffraction (XRD) are reported.

Moreover, thermal properties of the masa were studied by determining its starch gelatinization, by using differential scanning calorimetry (DSC) and rheometry as a function of moisture content.

2 Materials and methods

2.1 Materials

The corn (*Zea mays*) used in this work, was a commercial white dent hybrid corn, named Pegaso, which was kindly donated by Unisem S.A. de C.V. Company (Mexican corn seeds producer), and harvested in the region of Atotonilco el Alto, province of Jalisco (located in the western-pacific area of Mexico). Commercial lime (calcium hydroxide (cal piramide)) was purchased at the local market. Bidistilled water was obtained from a Millipore purification system (Milli-Q, Merck Millipore, MA, USA).

2.2 Masa (dough) preparation

The nixtamalized dough (masa) was prepared by following a traditional industrial procedure of nixtamalization (Quintanar-Guzmán *et al*., 2009). About 1000 g of corn in 3000 g of water were nixtamalized, using 1% (w/w) of calcium hydroxide (lime)/weight of corn. All corn kernel samples were cooked at 90 °C for 90 minutes at atmospheric pressure (627.3 mm Hg). Then, the nixtamal samples were allowed to stand for 13 h in the cooking liquor named as nejayote, which was similar to the traditional procedure reported elsewhere (Quintanar-Guzmán *et al*., 2011). The samples were washed thoroughly with tap water until reaching a pH value of 7. The different lots of cooked grains were ground separately, using a Victoria grain mill (Maquinas Gonzalez, NL, MEXICO), a common step used in the traditional nixtamalization process (Quintanar-Guzmán *et al*., 2009) to obtain the masa. Then, the masa samples were dried in a vacuum oven (Precision) (GCA Co., IL, USA) at 50 °C and 15 kg/cm2, until constant weight. To obtain masa flour, the dried samples were sieved using a testing sieve with a 50 U.S mesh (Daigger, IL, USA), with a mesh size of 297 μm in

diameter, by using a Ro-TAP equipment to obtain the granulometric analysis. The mesh size aperture was chosen according to a previous report (Palacios-Fonseca *et al*., 2009). The sieving procedure was performed according to the ASAE Standards for 100 g of flour during 10 min of sieving (S319.2, ASAE, 1995). The flour particles with size smaller than the sieve-opening pass through were used. Then, all flour samples were stored in hermetic plastic bags at 4 °C, until further use.

The masa was prepared by weighing 100 g of dry flour, with just enough bidistilled water, in order to adjust the moisture content to 55, 60 and 65% (w/w). The resultant masa was homogenized manually, and allowed to stand for 60 min into a hermetic plastic bag, to avoid water evaporation before each measurement. The range of moisture content in the masa, was selected to be similar to previous studies reported elsewhere (Quintanar-Guzmán *et al*., 2009).

2.3 Microstructural characterization

Polarized light microscopy, was used to identify the starch granule crystalline regions in the raw corn kernel and nixtamalized corn flour. Samples were examined with an Olympus BX51 optical microscope (Tokyo, Japan) using an objective of 20x, equipped with polarizers and a digital camera QICAM Fast 1394 (Q Imaging, Burnaby, BC, Canada), and image analysis software Linksys 32 (Linkam Scientific Instruments Ltd, Surrey, UK). The dried samples were sprinkled in a glass microscope slide with a drop of bidistilled water, where the tests were run at 25 °C. Starch granules were selected at random in order to observe the presence of Maltese crosses.

SEM was used to determine changes in the microstructural characteristics of the starch granules of the corn kernels, caused by the nixtamalization and grinding processes, and the effect of the moisture content in masa. A JEOL-JSM-5900LV scanning electron microscope at an accelerating voltage of 20 kV (JEOL LTD. Tokyo, Japan) was used for this study. Samples were prepared as follows: approximately 5 g of masa were placed into a hermetic plastic bag and frozen at -20 °C during 12 h in a low temperature freezer model EF21326D (Daigger, IL, USA). Then, 1 cm^2 of frozen sample was placed in a brass sample holder, and SEM micrographs of the internal surfaces were taken at the magnification of x600 under vacuum.

2.4 X-ray diffraction

X-ray diffraction (XRD) analysis was performed in a Theta-theta diffractometer system Stadip (STOE & Cie GmbH, Darmstadt, Germany) equipped with a copper source, operated at 30 kV, 15 mA, with radiation of ($K\alpha$ =1.5406 Å) and over a scattering angle (2θ) range of 5°-70°. Dried samples were ground to a fine powder and then were fixed over a glass slide with Vaseline, which did not interfere with the sample measure. The sample crystallinity was analyzed qualitatively in this study, with the observation of diffraction peaks in the XRD patterns at 2θ = 15°, 17.5°, and 23.5°.

2.5 Thermal properties

DSC measurements were conducted to determine gelatinization temperatures and enthalpies of raw corn flour, and the masa at different moisture contents. Thermograms were obtained with a DSC 6 differential scanning calorimeter (Perkin Elmer, Inc., MA, USA), that was previously calibrated with indium, water and n-octane standards. All scans were performed at heating rate of 5 °C/min. Hermetical aluminum pans (Perkin Elmer, Inc., MA, USA) were used to minimize losses by evaporation. Samples in the sealed pans were weighed before and after each test. Results from samples that lost weight were discarded. The enthalpic changes were determined by measuring the area under the curve of the thermograms, using the Pyris Instrument Managing Software, Version 10.1 (Perkin Elmer, Inc., MA, USA).

2.6 Rheological properties

Temperature ramps, oscillatory, steady simple-shear and transient shear tests were performed in an ARG2 rotational stress-controlled rheometer (TA Instruments Co., DE, USA) on its strain mode, with a cross-hatched parallel-plate geometry with 25 mm of diameter and a gap between the fixed and mobile plates of 2000 μm. A humidification chamber was set around the geometry to minimize water evaporation from samples. Temperature was controlled within ± 0.1 °C during measurements, with a Peltier plate system. For each test, about 0.5 g of well-mixed and homogeneous sample was carefully transferred to the rheometer plate, a pre-shearing of 0.5 mNm to stabilize the sample was applied for about 20 s, then to minimize any possible destruction of the structure, the sample was let to stand for about two

minutes, before running any test. Measurements were carried out at 30, 40 and 50 °C. All rheological measurements were performed at least three times, reporting average values. Strain amplitude sweeps at constant frequency (ω=5 rad/s) were performed to determine the maximum deformation attainable by the samples in the linear range from 0.1 to 100% and the linear viscoelastic region (LVR), defined as the strain domain where the storage (G´) and the loss (G´´) moduli are strain independent. The frequency sweeps were run at an oscillatory deformation of 0.2%, which was within the LVR, and from 1 to 100 rad/s. Simple shear rate sweeps were performed under steady state conditions and at low shear rates from 0.01 to 1 s^{-1}. Temperature ramp measurements from 25 to 95 °C, were conducted to study the pasting profiles of masas. All measurements were fixed at a constant frequency of 5 rad/s, a constant deformation of 0.2% and a ramp-heating rate of 1 °C/min. The shear stress growth and stress relaxation after cessation of steady shear flow of masa, was obtained at the shear rates of: 0.01, 0.05, 0.1, 0.5 and 1.0 s^{-1}. In order to examine the no-slip phenomena, shear tests with different gaps (1500-2500 μm) were performed; it was found that wall slip not happen within the conditions used.

The steady simple-shear viscosity was fitted using the power law equation, applied to the shear-thinning region (Rao, 1999):

$$\sigma = K\dot{\gamma}^n \tag{1}$$

Where σ is the shear stress (Pa), K is the consistency index (Pa s^n), $\dot{\gamma}$ is the shear rate (s^{-1}) and n is the power law index.

The shear stress relaxation was fitted using a simple exponential decay expression (Ferry, 1980):

$$\sigma = \sigma_{ss} e^{-t/\tau} \tag{2}$$

Where σ is the shear stress (Pa), σ_{SS} is steady shear stress (Pa), t is the measurement time (s) and τ is the longest relaxation time of the system (s) related to the material.

2.7 Statistical analysis

The rheological and calorimetric experimental data were statistically analyzed using the Statgraphics Centurion XVI software, Version 16.1.15 (Statpoint Technologies, Warrenton, VA, USA). Comparisons between sample measurements were made with analysis of variance (ANOVA) and the least significant difference (LSD) test was used with a probability $P < 0.05$.

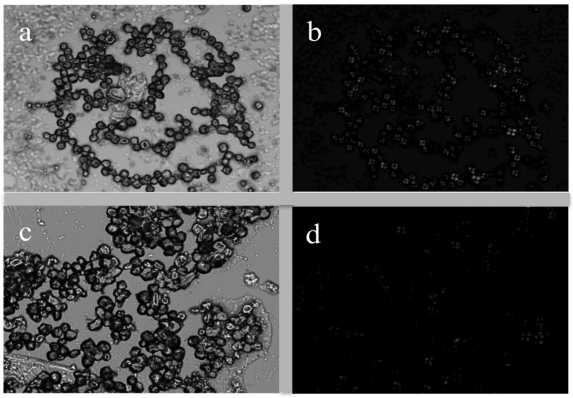

Fig. 1 Photographs taken by light microscopy of (a) raw corn kernel and (c) nixtamalized corn flour, and polarized light microscopy of (b) raw corn kernel and (d) nixtamalized corn flour.

3 Results and discussion

3.1 Morphological characterization

3.1.1. Polarized light microscopy

Polarized and non-polarized light microscopy images of raw corn kernels and masa are shown in Fig. 1. The non-polarized light microscopy image of raw corn, showed starch granules with a roughly spherical shape and with the presence of pinholes in the center of the granule (Fig. 1a). Meanwhile, for the nixtamalized corn flour, swollen angular shaped starch granules and some damage in their structure, are observed (Fig. 1c).

The Maltese cross was observed in both samples (birefringence) (Figs. 1b and 1d), indicating the presence of a radial order arrangement, with a crystalline nature of the amylose and amylopectin molecules within the starch granule. However, for the masa (Fig. 1d), only a few starch granules showed Maltese cross, due to the fact that the majority of the granules altered their molecular structure, because of starch gelatinization. Notice, that birefringence confers a high degree of molecular orientation within the granule, without any reference to any crystalline form. Similar observations were reported in native maize starch (Núñez-Santiago *et al*., 2011).

3.1.2. Scanning electron microscopy (SEM)

SEM images of raw, nixtamalized corn kernel and masa prepared with different moisture content are depicted in Fig. 2. Raw corn (Fig. 2a) shows irregular shape starch granules, with no damage,

and an average size of 8-12 μm in length and 6-10 μm in width, which is common in corn starch granules (Quintanar-Guzmán *et al*., 2011). It was observed some strand-like structures, which might be those of proteins, which are not uncommon to see accompanying starch granules. Meanwhile, the nixtamalized corn kernel (Fig. 2b) reveals damaged swollen starch granules with irregular shape and a size increase due to swelling (10-18 μm in length and 10-14 μm in width). Strand-like structures of possibly proteins are also observed but in lower amounts than the above-mentioned raw corn, probably because of the effect of nixtamalization.

Figures 2c and 2d show the masa with moisture contents of 55 and 65% (*w/w*), respectively. Fig. 2c shows a smoothed gelatinized starch paste, indicating that the highest degree of gelatinization was due to the friction at grinding, leading to an increase of disruption of granules, and a dispersion of them into the matrix, which acts as a glue that join the masa. The masa surface observed was inhomogeneous, with the presence of furrows and pores, where angular shaped and partially damaged starch granules are still observed over the surface. Moreover, an irregular dispersion of the starch granules is also observed, with grooved regions without starch granules. As will be discussed later, the masa microstructure resulted from the process where the leached amylose chains from the starch granules re-associate by hydrogen bonding to form a gelatinized paste.

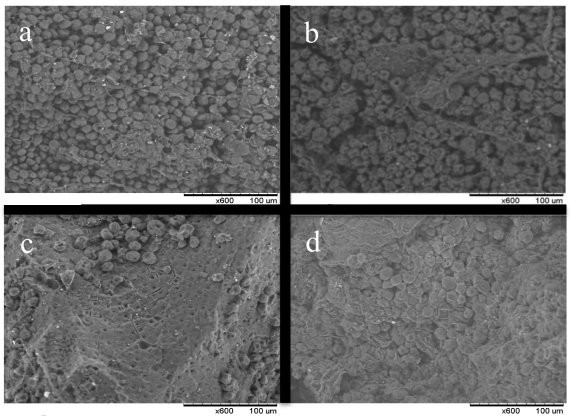

Fig. 2 Photographs taken by scanning electron microscopy (SEM) of (a) raw corn kernel, (b) nixtamalized corn kernel, (c) masa with 55% (*w/w*) of moisture content and (d) masa with 65% (*w/w*) of moisture content.

On the other hand, when increasing the moisture content to 65% (*w*/*w*) (Fig. 2d), a more homogeneous plasticized surfaced is produced, where it can be observed smoothed spherical shaped starch granules, partially embedded into the paste, with good dispersion and no grooved regions. The differences observed in the gelatinized surface of masa (Figs. 2c and 2d), were attributed to the increase of moisture content.

3.2 X-ray diffraction

Fig. 3 shows the XRD of raw corn and nixtamalized corn flour. It is seen that overall, the nixtamalization process did not alter the diffraction pattern of the native starch. Most of the peaks are typical from those seen in cereal starches, however, there are also thin differences believed to be because of nixtamalization. A-type crystallinity indices were determined for the two specimens. Thus, A-type crystalline patterns were displayed, indicated by a broad and small peak detected at about 2θ = 15°, and more prominent than another characteristic peak, at 2θ = 17.5°, and even the peak at 2θ = 23.5° is broader in the raw corn flour diffractogram. The XRD from nixtamalized flour shows an amorphous-crystalline pattern with broad peaks, but lower crystallinity than those of the original material (raw corn), possibly as a result of starch granules melting during heat processing. Nonetheless, crystallinity persisted, since location of peaks was almost the same, although the intensities became smaller (Thomas and Atwell, 1999). These results may be the residual cristallinity that persisted after processing. This XRD pattern is similar to native maize starch reported elsewhere (Núñez-Santiago *et al*., 2011).

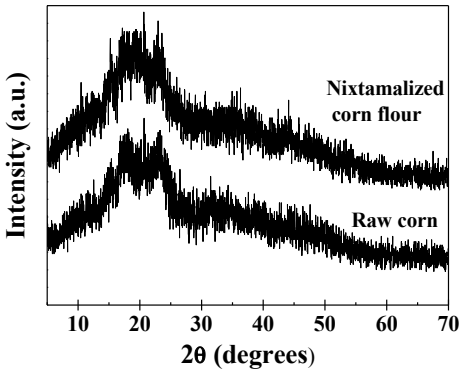

Fig. 3 X-ray diffraction patterns of raw and nixtamalized corn flour.

Table 1. Gelatinization parameters raw corn flour with 55% (*w/w*) of moisture and masa prepared with different moisture content*.

Moisture (% (*w/w*))	T_0^a °C	T_P^b °C	T_c^c °C	ΔH^d J/g	$(T_c - T_0)$ °C
Raw corn flour	43.2±0.70$^\alpha$	5.2±0.71$^\alpha$	67.9±0.85$^\alpha$	12.4±0.07$^\alpha$	24.7$^\alpha$
M‡ 55	53.0±0.53	59.5±0.8	69.0±0.96	6.7±0.84$^\alpha$	16.0
M‡ 60	54.1±0.63	61.2±0.56	68.7±1.45	10.2±0.4α	14.6
M‡ 65	54.3±0.61	61.7±0.9	67.6±1.12	11.2±0.21$^\alpha$	13.3

*Mean value of three measurements ± standard deviation (n = 3)

‡M = masa

[a] Onset temperature

[b] Peak or gelatinization temperature

[c] Final temperature

[d]Enthalpy of gelatinization (based on starch weight).

[α]The statistical analysis applied was the analysis of variance (ANOVA) and the least significant difference (LSD) test, which indicated significance with a probability of ($P < 0.05$).

3.3 Thermal properties

DSC thermograms of raw corn at 55% (*w/w*) of moisture content and masa with 55, 60 and 65% (*w/w*) of moisture content are shown in Fig. 4. As can be noticed, a single endothermic transition, caused by gelatinization of starch, can be appreciated in each curve. The shape of the thermograms of raw corn flour and masas prepared with nixtamalized corn at different moisture content are similar, with some differences, i.e., the gelatinization temperature (T_p) increases from 55.2±0.71 °C for the raw corn flour to 59.5 ± 0.8 °C for the masa with 55% (*w/w*) moisture content. Gelatinization temperatures and enthalpies are reported in Table 1. The ANOVA analysis applied separately to Tp, onset temperature (T_0), final temperature (T_c) and $\Delta T = (T_c - T_0)$, showed significant ($P < 0.05$) differences between the raw corn flour and the masa; meanwhile, the moisture content in the masa did not show significant ($P > 0.05$) differences. This suggests that the formation of a more ordered structure in masa compared with the raw corn, possibly due to a starch network formation. Moreover, since the T_p values are usually related to the amylose content, the degree of crystallinity has an important impact on the thermal properties of starch (Chel-Guerrero *et al*., 2011). As was shown in Fig. 3, masa presented crystallinity caused by two macromolecules; amylopectin which is a highly branched and large macromolecule whose molecular weight that can go from 10^7 to 10^9 g/mol (Daltons), depending on its source, and amylose; that is mainly a linear macromolecule with a degree of polymerization which usually lies between 300 to 104 g/mol (Daltons), depending on its origin (Thomas and Atwell, 1999). The structure of starch granules consists of concentric shells of amorphous rings of mainly amylose and crystalline rings of amylopectin. Upon heating, the starch granules in an aqueous environment swell, and amylose and water diffuse out of the granules. At higher temperatures, amylopectin melting begins, enabling the release of certain amount of amylose that was trapped between amylopectin layers. This phenomenon is called gelatinization and is closely related to physicochemical starch properties (Biliaderis *et al*., 1986).

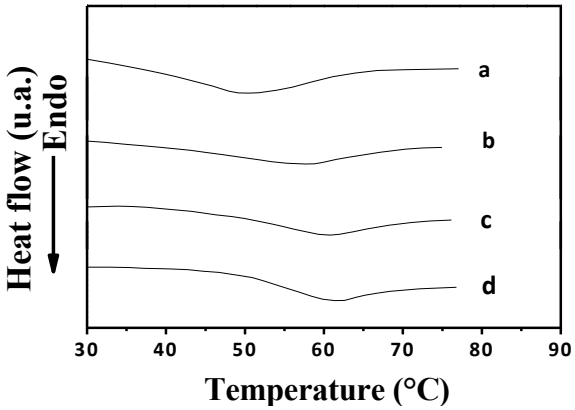

Fig. 4 Thermograms of (a) raw corn flour with 55% (*w/w*) of moisture; (b) masa with 55% (*w/w*) of moisture; (c) masa with 60% (*w/w*) of moisture and (d) masa with 65% (*w/w*) of moisture content.

On the other hand, the gelatinization enthalpy (ΔH_{gel}) or enthalpy of gelatinization of the raw corn (flour) (12.7 J/g), was higher than those enthalpies of

the masas prepared at all the moisture contents, which were within the range of 6.7-11-2 J/g. The ΔH_{gel} provides information of the energy changes during the melting of recrystallized amylopectin. Since the nixtamalization process requires heating, after cooling down the nixtamalized corn flour, starch retrodegradation occurs leading to a re-association process (hydrogen bonding between starch chains) that causes crystallization. Therefore, the reduction on ΔH_{gel} of masa (containing retrograded starches) could be attributed to a weaker starch crystallinity formation (Sasaki *et al*., 2000). Moreover, the gelatinized starch fraction increased for the masa, requiring then less energy to complete the sample (starch) thermal transition or gelatinization (ΔH_{gel}). The ANOVA analysis applied to ΔH_{gel} showed significant ($P < 0.05$) differences between the raw corn flour and the masas prepared at different moisture contents. Moreover, increasing the moisture content of the masa, the ΔH_{gel} increases from 6.7 ± 0.84 J/g for 55% (w/w) to 10.2 ± 0.4 J/g for 60% (w/w), which where significantly different ($P < 0.05$) (Table 1). Whereas, no significant differences where found for the masa samples with 60 and 65% (w/w) of moisture content. These results, suggest that the gelatinization process is controlled by the high amount of moisture, where at moisture contents higher than 60% (w/w), ΔH_{gel} remained unchanged, which indicates that the transition approaches to the equilibrium conditions (Ratnayake and Jackson, 2007). Moreover, the water excess presented in the masa, which during gelatinization, it modifies the thermodynamic values, i.e., ΔT, in as much as, water acts as a plasticizer (decrease of intermolecular forces, and increase in the mobility of polar polymeric chains). This in turn, reduced the glass-rubber transition temperature (T_g) of the amorphous regions of the granules, thus facilitating melting or reorganization of starch crystallites, which is similar to that observed in semicrystalline polymers (Biliaderis *et al*., 1986). Some authors have reported for nixtamalized corn flours, values of T_p and ΔH_{gel} within the ranges of 70.7-73.6 °C and 2.3-3.9 J/g, respectively (Bello-Perez *et al*., 2002). Meanwhile, other researchers have obtained for corn varieties, values of T_p and ΔH_{gel} within the ranges of 72.59-75.46 °C and 14.15-13.68 J/g, respectively (Aparicio-Sanguilán *et al*., 2006). The differences in T_p and ΔH_{gel} between this work and those reported in the literature, may be attributed among other factors, to differences in shape and size of starch granules, amylose content, internal molecular arrangement of starch fractions within the granule as well as different botanical starch sources and linked proteins (Yañez-Farias *et al*., 1997). It is known that for samples with the same moisture content, T_p increases proportionally with the amylose content, meanwhile ΔH_{gel} decreases; this has been attributed to the variation in amylose/amylopectin ratio, because amylopectin have a crucial role in starch granule crystallinity, and amylose has to do more with the starch amorphous lamella structure conformation, and less with crystalline regions (Li and Yeh, 2001). But, also other factors could be attributed to those variations, such as polydispersity, total molecular dimension, internal mobility and the proportion of the length in the amylopectin structure (Aparicio-Sanguilán *et al*., 2006).

3.4 Pasting properties

The pasting properties of masa were determined by using oscillatory temperature ramp test as a function of moisture content. Dynamic shear rheology is a sensible technique, which has been used to study the changes produced by the processing and storage of food products, since the small-amplitude of the equipment does not alter the arrangement of the macromolecules inside the food matrix. In these experiments, the storage modulus (G´) measures the energy stored in the material per cycle of deformation, meanwhile the loss modulus (G´´), measures the energy lost per cycle of deformation (Steffe, 1996). The ratio of the loss modulus to the storage modulus for each cycle of deformation is defined as loss tangent or tan delta (tan δ) =G´´/G´, which reflects the phase changes taking place in the material, being another rheological parameter used to characterize the viscoelastic properties of samples. A tan $\delta < 1$ indicates a predominantly elastic behavior, meanwhile a tan $\delta > 1$, indicates a predominantly viscous behavior. Fig. 5 shows the effect of temperature on tan δ, for the masa at different moisture contents. It is seen that the overall tan δ values of the masas or doughs, ranged from 0.13 to 0.25, which are still those of weak gels, with mainly amorphous structure as was shown by SEM (Fig. 2), probably conferred initially by the nixtamalization process and enhanced by the testing temperature. The tan δ progressively increases with temperature to a maximum peak $(\tan \delta)_{max}$, and then, drops with continued heating until reaching a minimum peak $(\tan \delta)_{min}$. The initial increase could be attributed to the degree of granular swelling of starch, where swollen starch granules and water occupy the available volume of the system (Eliasson,

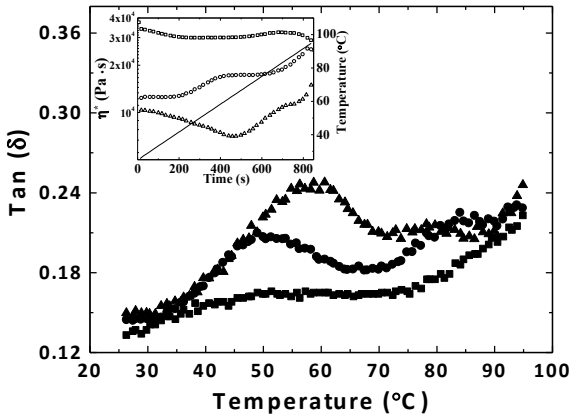

Fig. 5 Temperature ramp test for tan δ (G´´/G´) of masa with different percentages of moisture content (% (*w*/*w*)): (■) 55; (●) 60; (▲) 65. Inset: complex viscosity ($|\eta^*|$) versus time of masa with different moisture contents (% (*w*/*w*)): (■) 55; (●) 60; (▲) 65. The solid line is the applied temperature ramp.

1986). The temperature at $(\tan \delta)_{max}$ was considered the temperature at the maximum starch swelling. Increasing the temperature, the intergranule contact increases, which increases their friction, and creates a three-dimensional network, formed with those swollen granules and linear chains of amylose that were previously leached out in the nixtamalization process (Sankarakutty *et al*., 2010). Hence, the masa stability improved and consequently the viscosity increased (inset in Fig. 5). Thus, tan δ decreased to reach the $(\tan \delta)_{min}$ peak, which was attributed to the gelatinization temperature of the masa. With further increase in temperature, tan δ increased, indicating that the network was destroyed. This destruction was attributed to rupture and disintegration of the granules, due to the increase in temperature, resulting in a gradual decrease in the volume fraction of the granules in the masa. The rupture of granules also results in a release of amylose, which contributes to the viscosity reduction of the masa (Rao, 1999).

On the other hand, tan δ increased with the moisture content, indicating an increase of G´´ and consequently, of the complex viscosity ($|\eta*|$) (inset of Fig. 5). Moreover, $(\tan \delta)_{max}$ peak shifted to a higher temperature with the moisture content, i.e., $(\tan \delta)_{max}$= 50.6 °C for the masa with 55% (*w*/*w*) of moisture content and $(\tan \delta)_{max}$= 58.2 °C for the masa with 65% (*w*/*w*) of moisture content.

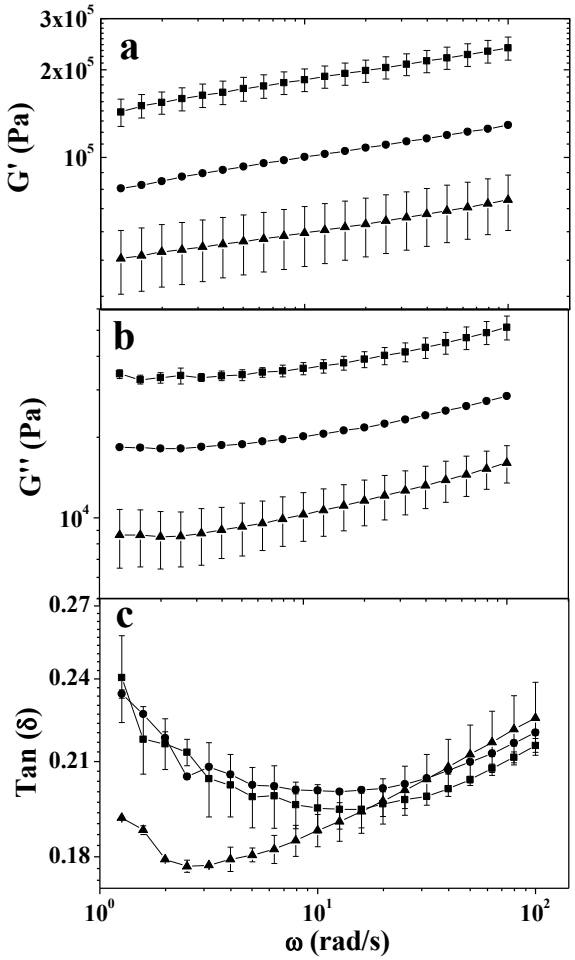

Fig. 6 Frequency sweep test for (a) storage modulus (G´), (b) loss modulus (G´´) and (c) tan δ (G´´/G´), for masa with different moisture content (% (*w*/*w*)) measured at 30 °C: (■) 55; (●) 60; (▲) 65. The solid lines are aide to the eye. Error bars indicate standard deviation for triplicate measurements.

3.5 Dynamic shear

Fig. 6 shows frequency sweep profiles of masa measured at 30 °C as a function of the moisture content (% *w*/*w*). All masas showed a predominant elastic behavior, i.e., G´ was higher than G´´ around an order of magnitude (Figs. 6a and 6b). The ANOVA analysis applied separately to G´ and G´´ data, showed a significant ($P < 0.05$) effect due to the increase of the moisture content. Also, tan δ was significantly ($P < 0.05$) affected by the moisture content when it was evaluated as a single factor, i.e., not considering its interaction with frequency. Meanwhile, taking into account its dual interaction with both, moisture content and frequency, as well as, for the plots at 55

and 60% (w/w), of moisture content (cf. Fig. 6c), tan δ was not significantly affected, meaning that overall, the involved rheological systems were essentially the same (Steffe, 1996). Both moduli showed overall flat profiles with some frequency dependence (ω), with no cross-over points between the moduli over the frequency range studied, suggesting the existence of relaxation processes occurring at short time scales. A crossover of G´ and G´´ at a characteristic frequency (ω_c) would correspond to the main relaxation (or disentanglement) time (τ) of the material. It was not possible to analyze its viscoelastic response in the terminal zone (lower frequencies) due to evaporation of the sample. However, for flow measurements at very low shear rates, were possible to analyze its viscoelastic behavior by measuring the stress relaxation (section 3.7). Overall, the results reported in Fig. 6 indicate that tan δ was smaller than unity, meaning a predominant elastic behavior, indicating that masa had a behavior of weak gel-like materials (Steffe, 1996; Rao, 1999). Hence, these results suggest that its structure was conformed by a starch matrix, composed by dispersed swollen starch granules and probably granular starch fragments. As expected, increasing the moisture content in masa, i.e., from 55 to 65% (w/w), resulted in a reduction of both G´ and G´´. This behavior could be produced by the presence of a cross-linked biopolymer matrix embedded in a softer matrix, where the water excess caused this effect. The biopolymer chains in the continuous phase, were subjected to an increase of mobility and stretching, which reduced the moduli (Fig. 6) and viscosity (Fig. 8). Thus, in agreement with the morphological results (Fig. 2), the excess of moisture content plasticized the masa and changed its structure. Water, or other plasticizers (such as oil), enables the starch to obtain homogeneous smooth surfaces, which considerably reduces the flow properties (Sudha and Leelavathi, 2011). Some authors have attributed the formation of the elastic network to the crystallinity domains derived from the complexation reaction between amylose and lipids (Della Valle *et al*., 1998). Others, to the formation of complexes between starch and proteins, that causes some "stiffenes" of masas (Quintanar-Guzmán *et al*., 2009). In fact, Quintanar-Guzmán *et al*. (2009) reported G´ values around an order of magnitude lower than those masas prepared with similar moisture content (55% (w/w)) reported in this work (Fig. 6a). Factors that are attributed to the formation of complexes protein/starch are i.e., pH, ionic strength, temperature, the protein/starch ratio, as well as, their molecular characteristics (Tolstoguzov, 1991). Besides to possible complexes formation, differences in the elastic response found in this study compared with other published works, could be related to several factors, i.e., alkaline cooking, steeping time and drying step, as well as lime concentration (Flores-Farías *et al*., 2000). However, although the formation of some type of complexes between the starch and another molecules during the nixtamalization process was not evaluated in this work, the elastic response was attributed to a composite-like structure, with starch granules dispersed in a continuous phase (Figs. 2c and 2d).

Fig. 7 shows frequency sweep profiles as a function of temperature for nixtamalized masas prepared with 55% (w/w) of moisture content. Similar to Fig. 6, a predominant elastic response was observed (G´>G´´), with moduli frequency dependence, and without cross-over of G´ and G´´ over the frequency range studied. The ANOVA analysis applied to G´, G´´ and tan δ, showed that they were significantly ($P < 0.05$) affected by the temperature and frequency; however, no significant differences at 30 and 40 °C were observed. The overall viscoelastic behavior of masa when the temperature was increased, from 30 °C to 50 °C, was because the network structure was weakened by the temperature increase, evidenced by the G´ drop (Fig. 7a). Similar explanation exposed for the pasting profiles (Fig. 5) are applicable here, since the gelatinization temperatures were around 50 °C. On the other hand, the viscoelastic response of masa was found to be time dependent ($t = \omega^{-1}$) (Fig.7b and 7c), in as much as, at lower frequencies ($\omega < 10$ rad/s) (long times), the viscous character predominated for the masa at 50 °C than that at 30°C ($\tan \delta_{(50^oC)} > \tan \delta_{(30^oC)}$); whereas, at higher frequencies ($\omega > 10$ rad/s) (short times), an opposite behavior was seen, where ($\tan \delta_{(50^oC)} < \tan \delta_{(30^oC)}$). Although the overall behavior of masa was predominantly elastic in all the ω range studied (Fig. 7a), these results are attributed to a thermal dependence of the relaxation times of the system, where the viscoelastic response is dominated by the dissociation/attraction between the biopolymer chains.

The dynamic viscosity ($|\eta^*|$)(Fig. 8) of masa as a function of moisture content also showed a similar trend as G´ and G´´; i.e., significant differences ($P < 0.05$) due to the increase of the moisture content for the masa at 30 °C (Fig. 8a). Besides, no significant differences were observed for the masa prepared with 60 and 65% (w/w) of moisture content measured at 40

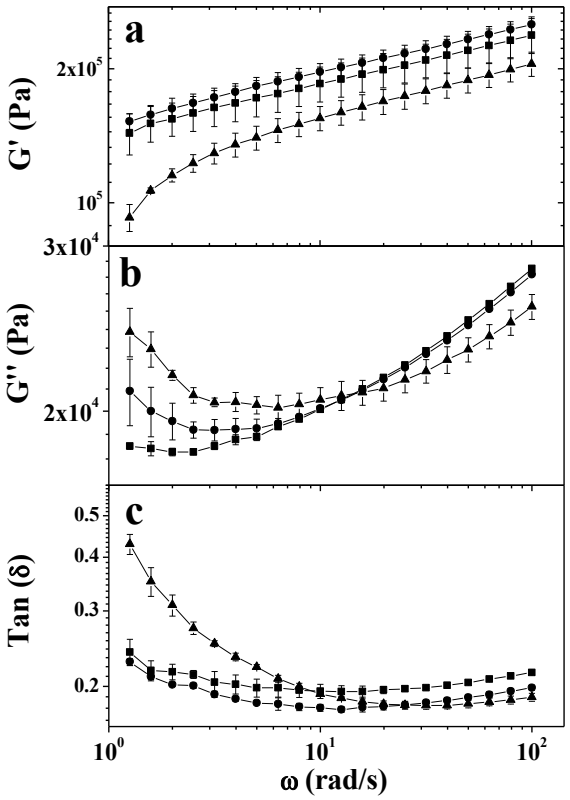

Fig. 7 Frequency sweep test for storage modulus (G´), (b) loss modulus (G´´) and (c) tan δ (G´´/G´), for masa with 55% (*w/w*) of moisture content and measured at different temperatures (°C): (■) 30; (●) 40; (▲) 50. The solid lines are aide to the eye. Error bars indicate standard deviation for triplicate measurements.

and 50 °C (Figs. 8b and 8c). For all masa samples, $|\eta^*|$ decreased with frequency, indicating a shear thinning behavior, and no zero complex viscosity ($|\eta_0^*|$) was observed within the range of frequencies studied. Shear-thinning behavior is characterized by a viscosity decrease with increasing frequency, due to a molecular motion, under small-amplitude oscillatory (SAO) measurements [$|\eta^*| = |G^*|/\omega$], being $|G^*|$ the complex modulus (Ferry, 1980). When the masa is subjected to an oscillatory shear at low frequencies, within the range where the sample is not deformed permanently (linear region), the viscosity reduction may be explained in terms of a freer molecule-to-molecule interaction. When increasing frequency, the molecules have less time to undergo translation, and consequently the viscosity decreases, but the elastic behavior prevails. Moreover, as it was mentioned above, the decrease of viscosity with moisture content was due to the reduction of intermolecular forces within

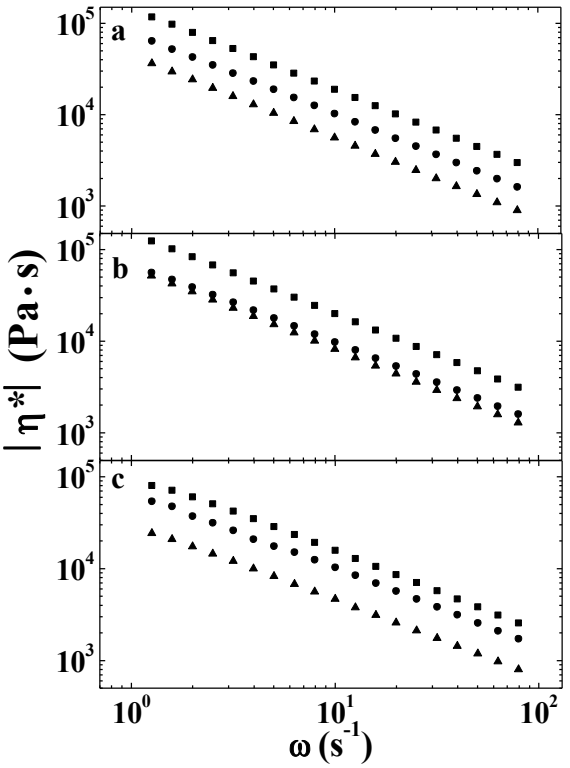

Fig. 8 Complex viscosity ($|\eta^*|$) for masa measured at (a) 30, (b) 40 and (c) 50 °C for masa with different moisture content (% (*w/w*)): (■) 55; (●) 60; (▲) 65.

the masa, and increased biopolymer chain mobility, causing the observed decrease in viscosity. This plasticizing effect on masa is similar to that reported for starch pastes (Sankarakutty *et al*., 2010).

The dynamic moduli were subjected to linear regression in order to assess the magnitudes of the slope (*m*); the values of which are disclosed in Table 2. The slope values were small and positive, within the ranges from 0.105 to 0.159 for G´ and 0.09 to 0.185 for G´´. The trend of the slopes values was the increase of G´ values with temperature, while those of G´´ decreased. The slope values of G´ reached a maximum at 60% (*w*/*w*) of moisture content, while G´´ values decreased. This is again consistent with the behavior of a weak viscoelastic gel-like system (Aparicio-Sanguilán *et al*., 2006; Steffe, 1996).

3.6 Steady shear

Steady shear viscosity (η) *versus* shear rate ($\dot{\gamma}$) as a function of temperature for nixtamalized masas with 55, 60 and 65% (*w*/*w*) of moisture content

are presented in Figs. 9a, 9b and 9c, respectively.

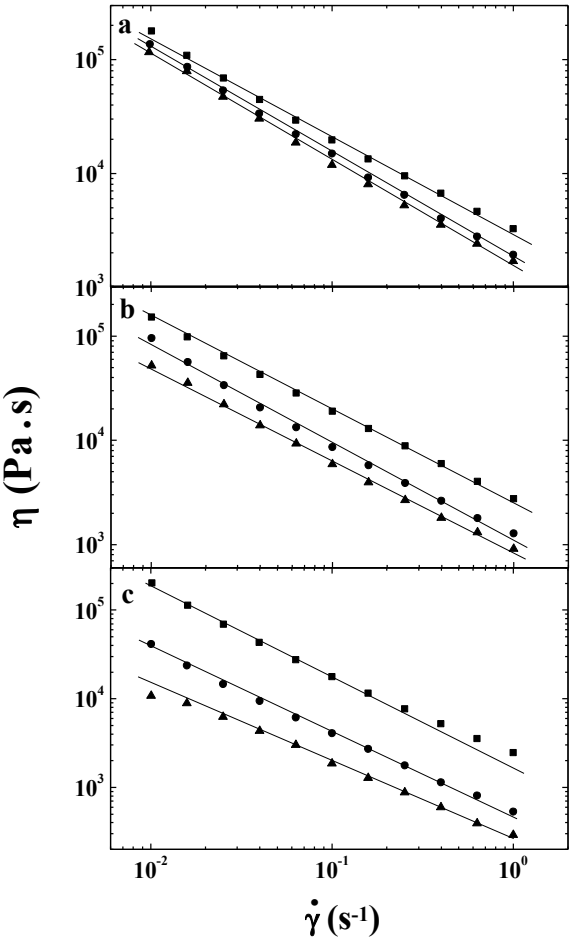

Fig. 9. Shear viscosity (η) for masa with (a) 55, (b) 60 and (c) 65% (*w/w*) of moisture content as a function of temperature (°C): (■) 30; (●) 40; (▲) 50. The solid lines are the best fit with the Eq. (1).

All samples showed a shear-thinning (pseudoplastic) behavior, at all shear rates studied. Under flow measurements, shear-thinning behavior is characterized by a viscosity decrease with increasing shear rate, due to a structure alignment or destruction, and is presented by most of the non-Newtonian foods (Rao, 1999). During food processing, pseudoplastic foods became less viscous when its mixing or transporting rate increased (Bello-Perez *et al*., 2002). Solid lines in Fig. 9 are the best fit of the power law (Eq. (1)), which is applied at the shear-thinning region, the values of which are disclosed in Table 3. The power law is an important model for understanding the flow behavior of many types of foods, which indicates the extent of shear-thinning behavior as n deviates from unity. It was found that the flow behavior index value was less than unity ($n < 1$), indicating that the masa behaves like a shear-thinning fluid (Rao, 1999). The masa exhibited a decrease in the power law index (n), as the temperature and the moisture content increased. Bello-Perez *et al*. (2002) studied the flow properties of nixtamalized corn flours. They found a range of n values for nixtamalized corn flours with 15% (*w/w*) of solids at 25 °C of between 0.51 and 0.78. Other authors have found an n value for waxy cornstarch pastes at 25 °C of about 0.51, and for mixtures of waxy cornstarch with guar and xanthan gums of 0.52 and 0.36, respectively (Achayuthakan and Suphantharika, 2008).

Comparing $|\eta^*|$ in Fig. 8 with η in Fig. 9, the data did not follow the Cox-merz rule, $|\eta^*| = \eta$, which is an indication of the elastic character of the masa starch network. As was shown in Fig. 2, masa was formed with interspersed swollen starch granules that conferred it a network structure. Deviations from Cox-Merz rule, are attributed to structure destruction in the case of steady shear, and also reflect the weak gel-like nature of the gelatinized network. A similar result has been reported for different starch based systems (Chel-Guerrero *et al*., 2011).

Fig. 10 depicts the average of the steady shear viscosity (η) measured at a fixed shear rate ($\dot{\gamma} = 0.1 s^{-1}$), showing a linear dependence with temperature, under steady shear rate measurements for masa with different moisture contents. When applying ANOVA to η, the samples presented significant differences ($P < 0.05$), where η decreased with temperature and moisture content for all samples, except for the samples measured at 30 °C, where no significant differences were found. During heating, the intermolecular forces of the masa are broken, provoking in the samples less opposition to be sheared.

The inset in Fig. 10 shows a η *versus* inverse temperature plot obtained from the masa samples. The temperature effect on masa viscosity fitted an Arrhenius type equation, which indicates a thermal activated process that follows the equation:

$$\eta(T) = \eta(T_0) \exp\left(\frac{Ea}{RT}\right) \tag{3}$$

where R is the gas constant, E_a is the activation energy and $\eta(T_0)$, is a coefficient depending on the nature of the material. E_a is the energy barrier that must be overcome before the elementary flow process can occur (Chun and Yoo, 2004).

Table 2. Slope parameters of log G´ and log G´´ versus log ω for masa*

	Content of moisture (% (*w/w*))											
T	55				60				65			
(°C)	m´†	R^2	m´´‡	R^2	m´†	R^2	m´´‡	R^2	m´†	R^2	m´´‡	R^2
30	0.109±0.01	0.998	0.151±0.02	0.983	0.111±0.01	0.998	0.136±0.01	0.98	0.105±0.01	0.996	0.185±0.02	0.995
40	0.110±0.01	0.998	0.146±0.01	0.983	0.128±0.01	0.999	0.141±0.01	0.985	0.107±0.01	0.998	0.148±0.01	0.99
50	0.125±0.01	0.998	0.119±0.01	0.951	0.142±0.01	0.997	0.09±0.01	0.935	0.159±0.02	0.998	0.152±0.01	0.992

*Mean value of three measurements ± standard deviation (n=3)

† m´, Slope of G´

‡ m´´, Slope of G´´

Table 3. Power Law parameters for masa†

	Content of moisture (% (*w/w*))								
T (°C)	55			60			65		
	K*	n**	R^2	K*	n**	R^2	K*	n**	R^2
30	2717±496	0.119±0.01	0.981	1461±415	0.087±0.02	0.999	852±266	0.01±0.0.003	0.981
40	1763±353	0.063±0.01	0.998	909±214	0.049±0.01	0.985	466±114	0.058±0.01	0.978
50	1459±227	0.052±0.01	0.997	705±140	0.062±0.01	0.999	314±62	0.199±0.03	0.99

†Mean value of three measurements ± standard deviation (n=3)

* K, Consistency index (in Pa s^n)

** n, Power law index

Table 4. Activation energy values for masa

Moisture content (% (*w/w*))	$\eta_{(T_0)}$	$E_a^{\ddagger}$ (KJ/mol)	R^2
55	6.6304	25.52	0.95
60	2.62×10^{-5}	30.04	0.90
65	3.44×10^{-7}	59.35	0.99

‡E_a, Activation energy

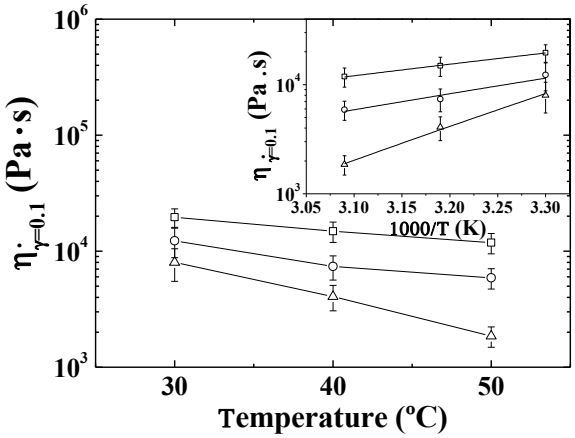

Fig. 10. Shear viscosity (η) measured at $\dot{\gamma}$=0.1 s^{-1} versus temperature for masa with different percentages of moisture content (% (*w/w*)): (□) 55; (o) 60; (△) 65. The solid lines are aide to the eye. Error bars indicate standard deviation for triplicate measurements. Inset: Arrhenius plot of the shear viscosity (η) measured at $\dot{\gamma}$=0.1 s^{-1} for masa with different percentages of moisture content (% (*w/w*)): (□) 55; (o) 60; (△) 65. The solid lines are the best fit with the Eq. (3). Error bars indicate standard deviation for triplicate measurements.

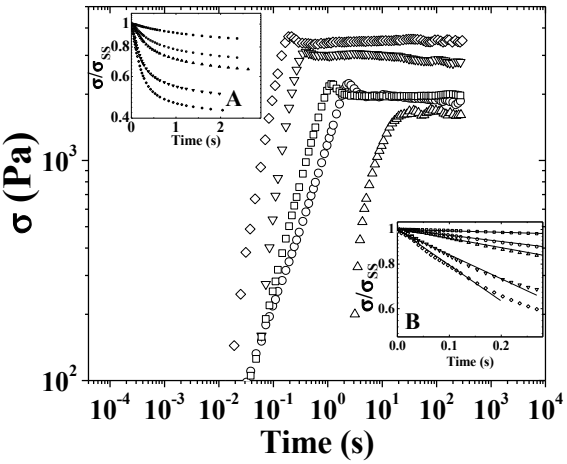

Fig. 11 Stress growth versus time for masa with 55% (*w/w*) of moisture content measured at 30 °C and different applied shear rates (s^{-1}): (■) 0.01; (●) 0.05; (▲) 0.1; (▼) 0.5; (♦) 1.0. Insets: (A) Normalized stress relaxation after cessation of steady shear flow at long times for masa with 55% (*w/w*) of moisture content measured at 30 °C at different applied shear rates. (B) Normalized stress relaxation after cessation of steady shear flow at short times for masa with 55% (*w/w*) of moisture content measured at 30 °C at different applied shear rates. The solid lines are the prediction with Eq. (2).

The values of the activation energy, calculated with Eq. (3) and from the inset in Fig. 9 are reported in Table 4 as a function of moisture content. A good fitness (R^2) with linearity was found. The activation energy of masa was dependent on their moisture content. Higher E_a means higher viscosity dependence on temperature changes (Bello-Perez *et al*., 2002). Kim and Wang (1999) reported an E_a value of 57.1 KJ/mol for waxy cornstarch pastes, similar to that with 60% (*w/w*) moisture of this study.

3.7 Transient shear

Fig. 11 depicts the stress growth after inception of shear flow for masa with 55% (*w/w*) of moisture content, measured at 30 °C with different shear rates applied. At lower shear rates ($\dot{\gamma} < 0.05 s^{-1}$), the stress growth is monotonic, and reached the steady state with the highest relaxation time of the sample (Table 5). An increase in the shear rate ($\dot{\gamma}$), promoted an increase in the stress and the steady state was reached faster developing an overshoot (Ferry, 1980). An important parameter in transient shear test, which is very informative to the materials structure, is the presence or absence of an overshoot. In a shear flow test, the stress can go through a maximum before reaching a steady state. This maximum (overshoot) is related to changes in the morphological structure of composite materials. Fig. 11 reveals that, an overshoot was formed as shear rate was increased. This overshoot is believed to be the result of alignments in the biopolymer chains, rearrangement of particles (starch granules) and the breakdown of the network. Furthermore, since the starch granules are not spherical (Fig. 2), the stresses could induce them to an orientation under the flow. Thus, the structural reorganization and breakup of the network would explain the increased stress observed.

Inset A in Fig. 11 depicts the normalized stress relaxation σ/σ_{ss}, being σ_{ss} the steady shear stress prior to cessation of flow) versus time after cessation of steady shear flow as a function of the applied shear rate for masa with 55 % (*w/w*) of moisture content and measured at 30 °C. The ANOVA analysis applied to σ/σ_{ss} showed significant differences ($P < 0.05$) with $\dot{\gamma}$. For values lower than ca. 0.05 s^{-1}, σ/σ_{ss}, the relaxation is single exponential. For larger values, non-linear behavior is observed, with the relaxation being faster, and a sudden decrease of the stress with time, followed by a more gradual decrease for

longer times. Note that two main relaxation times are discernible with a transition region between them. Therefore, two different slopes are observed, with different mechanism; one relaxation slope at times lower than ca. 0.2 s, which is very fast and its duration depends on the previous shear rate, whereas, the second relaxation slopes at a higher time than ca. 0.2 s. The evidence of two relaxation mechanisms suggests the existence of two different structures that coexists in masa and relaxes at different times. This also suggests that masa structure is composed by one structure, embedded into another structure (similar to a composite material), supporting the hypothesis that it was formed by a biopolymer matrix with dispersed starch granules. Thus, the fast relaxation mechanism (short times) would correspond to the biopolymer matrix, whereas, the slow relaxation process (long times) is probably related to the starch granules, which are more elastic. Noteworthy facts are that: (1) the slopes of the fast mechanism increase with the applied shear rate, (2) the relaxation time decreases as the shear rate increases in the fast process, and (3) at long times, the curves are nearly parallel, i.e., they present similar slopes. This suggests that the faster process is due to the relaxation of segments of amylose between entanglement points of hydrogen bonds being related to the average lifetime of the associations (Ferry, 1980). On the other hand, the second relaxation process indicates that the structure relaxes similarly, being independent of the shear rate, and meaning that the starch granules are highly elastic (Fig. 2). The relaxation time (τ) of masa was estimated by using a monoexponential decay expression (Eq. (2)), applied in the fast relaxation step, which is related to the structure of masa (see inset B in Fig. 11).

Table 5. Stress relaxation parameters for masa with 55% (*w/w*) moisture content, measured at 30 °C and different shear rates ($\dot{\gamma}^{\ddagger}$)

$\dot{\gamma}^{\ddagger}$ (s^{-1})	τ^{*} (s)	σ_{SS}^{**} (Pa)	R^2
0.01	10.46±0.05	1638±51	0.99
0.05	2.46 ±0.01	1820±64	0.99
0.1	1.65 ±0.01	1956±48	0.99
0.5	0.64±0.01	2823±60	0.99
1	0.43±0.01	3512±83	0.99

?Mean value of three measurements ± standard deviation (n=3)

$^{\ddagger}\dot{\gamma}$, Shear rate

$^{*}\tau$, Relaxation time

$^{**}\sigma_{SS}$, Steady shear stress

The solid lines in the inset B in Fig. 11 are the best fit of Eq. (2), the values of which are disclosed in Table 5.

Conclusions

In this work, a complete study of the linear and non-linear rheological properties of nixtamalized dough (masa) as a function of their moisture content and temperature was presented. The microstructural, pasting and thermal properties of masa were analyzed in order to relate the changes caused by the nixtamalization process and processing conditions (moisture content and temperature) with its rheological behavior. Birefringence and XDR patterns, presented the characteristic Maltese cross and amorphous-crystalline patterns in masa, respectively; indicating that certain crystallinity degree of the starch granule remained after the nixtamalization process. This crystallinity diminished the gelatinization enthalpy (ΔH_{gel}) in masa, compared with the raw corn, since it is a measure of the energy changes that take place during the melting of recrystallized amylopectin. Moreover, (ΔH_{gel}) was affected with the moisture content in masa, indicating that the excess of water controls the gelatinization of masa. On the other hand, SEM micrographs indicated that the moisture content induced in masa a surface plasticization, where disperse starch granules into an amorphous masa surface were observed. For small amplitude oscillatory shear test, the moduli (G´ and G´´) showed dependence with frequency and, G´ was higher than G´´ in all cases. These data indicate that masa has a weak gel-like behavior, which was related to the formation of a starch-granule network as a result of the nixtamalization process, where hydrogen bonding between the starch granules and starch (amylose) chains were formed. The steady state shear test showed shear-thinning flow behavior, where shear-thinning flow developed, and η values were reduced with the increase of both moisture content and temperature. Furthermore, rheological data fitted an Arrhenius type equation, where the activation energy (E_a) of masas was found to be dependent on their moisture content; also, higher E_a meant higher viscosity dependence on temperature changes. For transient test (step rate), the data were also consistent with the assumption of a starch-granule network formation. At low shear rates, the stress curves monotonically increased and did not show any overshoot; while at higher shear rates, the masas

presented an overshoot before reaching a steady state. Moreover, the overshoot was also observed only for the masa with 55% (w/w) of moisture content. The stress overshoot was related to changes in structure orientation under flow, as well as breakdown of the starch network at high temperature. The stress relaxation data depict two-relaxation process; a faster relaxation mechanism at short times, and a slower relaxation at long times, where the latter was associated to the main relaxation time of the system. These measurements indicate that two phases formed the masa structure, similar to a composite material, which is composed by starch granules disperse into a biopolymer matrix.

In general, oscillatory shear data of masa, within the range where the sample is not deformed permanently (into the linear region), a little dependence with moisture content and temperature was observed; above the critical moisture content of 60% (w/w) and the temperature of 40 °C, the oscillatory shear data were similar, indicating that the starch network almost did not change. On the other hand, under shear flow measurements, masa behaved like a pseudoplastic material and a strong effect with the moisture content and temperature was observed, where a rupture of the network was caused by the higher water content and temperature, respectively. These results were in agreement with the Cox-Merz rule, which was not followed for this material. Finally, the rheological results were found to be very helpful in understanding the structure-property relationship of masas with important processing variables such as the moisture content and temperature. However, although a general scheme was presented in this study, further research is needed, to better understand and quantify the interactions of masa structure at a molecular level.

Nomenclature

T_o	onset temperature
T_p	peak temperature
T_c	final temperature
ΔT	temperature increase
ΔH_{gel}	gelatinization enthalpy
G´	storage modulus
G´´	loss modulus
ω	frequency
ω_c	characteristic frequency
(tan δ)	loss tangent
(tan $\delta)_{max}$	maximum loss tangent peak
(tan $\delta)_{min}$	minimum loss tangent peak
$\lvert\eta^*\rvert$	complex viscosity
$\lvert\eta_0^*\rvert$	zero-shear viscosity
$\lvert G^*\rvert$	complex modulus
σ	shear stress
σ_{ss}	steady shear stress
$\dot{\gamma}$	shear rate
η	shear viscosity
K	consistency index
$\eta(T_0)$	coefficient depending of nature of the material
R	gas constant
τ	main relaxation time
n	power law index
E_a	activation energy

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