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Mixing effect on prolamins solubility and rheological properties of corn dough during processing for tortilla production

Efecto del mezclado en la solubilidad de las prolaminas de maíz y en las propiedades reológicas de sus masas durante el procesamiento para la producción de tortilla

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ABSTRACT

The study aims to investigate the effects of mixing on the protein solubility and secondary structure in corn dough during processing for tortilla production. To evaluate how mixing affects dough rheology, the storage moduli (G´), loss moduli (G´´) and tangent of the phase angle (Tan δ) dependent on frequency and texture profile analysis (TPA) were determined. HPLC-SEC showed an increased proportion of soluble polymeric proteins (soluble high molecular weight species). FT-IR results revealed that mixing promotes an increase of the β-sheet structure and a reduction of α-helix proportion, which suggests protein aggregation. Rheological data showed that the elastic prevailed over the viscous behavior (G´ > G´´) in the corn dough, with a soft solid material and an ordered and stable structure. Mixing, an intermediate step for dough production had important effects in protein structure and dough rheological properties. The increased Tan δ (G´´/ G´) is consistent with an increased viscous character and polymers aggregation that was demonstrated in this case for proteins.

Keywords: Mixing, dough, corn protein, rheological properties

RESUMEN

El estudio tiene como objetivo investigar los efectos del mezclado sobre la solubilidad de las proteínas del maiz y su estructura secundaria, en masas de maíz durante el procesamiento para la producción de tortillas. Para evaluar cómo el mezclado afecta la reología de la masa, se determinaron los módulos de almacenamiento (G´), los módulos de pérdida (G´´) y el ángulo de fase (Tan δ) dependientes de la frecuencia y el perfil de textura (TPA). Los análisis cromatográficos (HPLC-SEC) mostraron una mayor proporción de proteínas poliméricas solubles (especies solubles de peso molecular alto). Los resultados de FT-IR revelaron que la mezcla promueve el aumento de la estructura de la hoja β y la reducción de la proporción de la hélice α, lo que sugiere la agregación de las proteínas. Los datos reológicos mostraron que en las masas de maíz prevaleció el comportamiento elástico sobre el viscoso (G´ > G´´), un material sólido, blando con una estructura ordenada y estable. El mezclado, un paso intermedio para la producción de masa, tuvo efectos importantes en la estructura de la proteína y las propiedades reológicas de la masa, el aumento de Tan δ es consistente con un mayor carácter viscoso y agregación de polímeros que se demostró en este caso para las proteínas.

Palabras clave: Mezclado, masa, proteínas, propiedades reológicas.

INTRODUCTION

Nixtamalization consists of cooking whole corn in water and lime, followed by soaking, removal of the cooking liquor, washing the kernel, grinding the nixtamal, mixing the dough, and tortilla baking. The nixtamalized corn is milled with water to produce the dough. The corn dough moisture is an essential factor in tortillas production and must be approximately 50 – 58 g/100 g (Arámbula-Villa *et al*., 2001). Each step is important, however mixing has relevant functions: blends the nixtamal and water, forming the dough with desirable textural characteristics, mainly cohesiveness and adhesiveness (Quintanar-Guzmán *et al*., 2011) and also promotes the interaction and addition of components, including proteins (Chaidez-Laguna *et al*., 2016). According to Zheng *et al*. (2000), mixing helps change conformational arrangements of the polymers in the system.

Dough is a complex system composed of starch polymers, endosperm parts, lipids, and proteins (Gomez *et al*., 1987). Complex reactions and chemical interactions happen during dough preparation. The dough behavior relates on microstructure, spatial arrangements of the components, and types of bonds, which directly affects the rheological properties (Létang *et al*., 1999).

Several studies on the nixtamalization process are available, some confirming that starch affects the rheological properties of products made from dough (Pflugfelder *et al*., 1988; Campas-Baypoli *et al*., 1999).

Zeins, prolamins of corn, compactly packed in the protein bodies, help form weak fibrous network during mixing in a gluten free dough. Nevertheless, it has been proposed that zeins, above its glass transition temperature (Tg) are capable of forming a viscoelastic dough, in a model system (Lawton, 1992; Schober *et al*., 2008).

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In this context, an accurate understanding of corn prolamins behaviour during mixing is considered of great importance. The available scientific data is limited to the effects of corn prolamin during dough mixing in the nixtamalization process, and the contribution to the rheological properties and texture.

Dynamic rheology is a widely used tool to characterize the structure and polymer rheology (Ferry, 1980), including that of food properties, such as dough. It is used to measure food viscoelasticity, where basic descriptive components of the sample are the storage modulus (G'), an indicator of materials elastic component; the loss modulus (G''), an indicator of materials viscous component; and the tan δ (G''/G'), the ratio of the viscous and elastic moduli of a material.

The rheological properties of corn dough was previously examined by Quintanar-Guzmán *et al*. (2011). They claim that the corn dough had a weak gel like viscoelastic behavior and that the storage modulus (G´) was higher than the loss modulus (G´´). On the other hand, FT-IR spectroscopy is a suitable tool that can be used to make the structural analysis of liquid, semisolid, and solid proteins (Allain *et al*., 1999).

The objective of this work was to investigate the effect of mixing in the corn protein solubility and its secondary structure, and to determine its contribution to dough rheology and textural properties.

MATERIALS AND METHODS

Materials

Commercial white corn and commercial lime (calcium hydroxide; purity ~ 91 % (cal pirámide) were acquired from local store. Acetonitrile, 1-propanol, trifluoroacetic acid (HPLC-grade) were purchased from Sigma-Aldrich (St. Louis, MO).

Nixtamalization

Corn (4 kg) was cooked in 12 L of lime solution at 1 % (grain weight basis). Corn was cooked for 20 min at boiling temperature and steeped in the same cooking vessel for 14 h. The cooking solution or "nejayote" was discarded and the resulting nixtamal was washed two times with water, to remove brain and excess lime. Nixtamal was ground into dough with a final moisture content of 56.0 %, using a commercial stone grinder.

Dough preparation

Fresh dough was made according to Ramírez-Wong *et al*. (1994), with slight modifications. The fresh dough was homogenized for 30 s, deionized water (125 mL/kg) was then incorporated and mixed (3 or 6 min) in a Hobart mixer, at room temperature. The samples were stored in plastic bags at 40±1°C for 30 min, in order to reduce starch retrogradation.

Sample preparation before protein analysis

All samples were defatted according to Lending *et al*. (1988).

Protein extraction and size exclusion high-resolution liquid chromatography (SE-HPLC)

The defatted samples were analyzed according to Bean et al. (1998), with several modifications. Flours (250 mg) were mixed with 1 mL of 50 % propanol. Samples were placed in a stirrer (Vortex Genie2, Scientific Industries, Bohemia, N. Y.) and vortexed continuously for 15 min. Samples were then centrifuged (Eppendorf AG, 5415 Hamburg) at 8000 x g for 5 min, and the supernatant was recovered. The supernatant was centrifuged at 14000 x g for 15 min and analyzed by size exclusion high-performance liquid chromatography (SEC-HPLC). The HPLC system consisted of an Agilent quaternary pump and a diode array detector (Model 1260, Agilent Technologies, Pittsburgh, PA, USA) with a Biosep-SEC-S 4000 column (Phenomenex, Torrence, CA). The mobile phase was acetonitrile-water (50:50 v/v) containing 0.1 % trifluoroacetic acid at a constant flow rate of 0.8 mL min-1. The chromatographic profile was analyzed using Open Lab Software (Agilent Technologies, Palo Alto, CA). SE-HPLC measurements were performed in triplicate.

Fourier transform infrared spectroscopy (FTIR) analysis

Spectra of fresh, 3 and 6 mixing time were recorded on a Nicolet FT-IR spectrometer (Thermo Scientific Nicolet iS50-FTIR) equipped with a diamond attenuated total reflectance (ATR) cell with a 45° aperture angle, a liquid nitrogen-cooled MCTA detector, and OMNIC software. Samples signals were obtained at 25 °C in transmission mode from 600 to 4000 cm-1 at 4 cm-1 resolution. Curve deconvolution, fitting, and peak assignment were done with PeakFit software (v4.11 Systat Software Inc., Point Richmond, CA) to quantify protein secondary structure (α-helix at 1652–1660 cm−1 and β-sheet at 1630–1679 cm−1) (Barth and Zscherp, 2002) from the resolved spectra.

Dough rheological properties

After mixing, samples were rested in plastic bags at 40±1°C for 30 min. The rheological properties of dough were studied with a dynamic mechanical spectrometer (Rheometrics Scientific, model RSF III. Piscataway, NJ, USA) equipped with parallel plates of 25 mm diameter and a chamber for temperature control (Platt-Lucero *et al*., 2010). Approximately 2.5 g of dough was compressed between two plates separated by a gap of 2.5 mm. The parallel plates were covered with petroleum jelly to avoid moisture loss during the test.

The frequency sweep test was carried out using a software (RSI Orchestrator, Rheometrics Scientific). Storage modulus (*G'*), the amount of energy that is stored, the loss modulus (G''), the amount of energy dissipated in the material after deformation and tangent of the phase angle tan δ *(G''/G')* were quantified over the frequency range from 1.1 to 100 rad/s. The tests were carried out in triplicate.

Dough texture profile analysis (TPA)

Texture profile analysis (TPA) was performed using a texturometer (Model TA-XT2, Surrey, UK) equipped with a 36 mm diameter cylinder probe according to AACC standard method 74-9 (AACC, 2000). A 3 g load cell was used at the speed of 1 mm s^{-1}. The dough samples were cut in cylinder. The probe was moved down to 50 % of samples height, and then moved back up at the same speed, and this movement was repeated in a 10 s time interval. Parameters determined by this test include hardness, cohesiveness, adhesiveness, springiness, and chewiness were obtained using the texturometer software. The test was done with five replicates.

Statistical analysis

A completely randomized design was used. Data were statistically analyzed by a one-way ANOVA test with a significance level of 5 % ($p<0.05$). Significant differences among specific treatment means were defined using Tukey's test. All statistical analyses were performed using XLSTAT (Addinsoft, 2015).

RESULTS

Solubility studies

Representative size exclusion chromatogram (SEC) of corn dough after the three mixing times is shown in Figure 1, with profiles similar to those reported by Chaidez-Laguna *et al*. (2016) for nixtamalized samples, and the area under the curve assessed the corn proteins relative solubility in 50 % propanol. Table 1 shows percentages of first peak (SPP) and soluble polymeric protein, and the area under the chromatographic curve (total area, all picks) of samples. The different soluble protein proportion of samples indicated solubility changes during the nixtamalization process. Statistical analyses showed significant differences ($p < 0.05$) in soluble

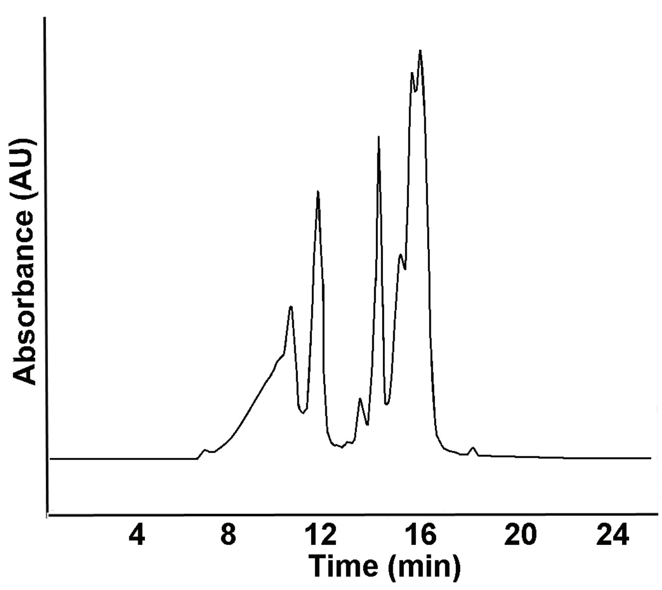

Figure 1. Representative size exclusion HPLC chromatogram of 50% 1-propanol soluble corn dough after 3 min of mixing time. The first peak, with a retention time of 11.6 min, corresponds to soluble polymeric proteins.
Figura 1. Cromatograma representativo de exclusion molecular (HPLC-SEC) de proteínas de maíz solubles en 50% propanol, después de tres minutos de mezclado. El primer pico, con un tiempo de retención de 11.6 min, corresponde a la proteína polimérica soluble (PPS).

Table 1. Soluble polymeric protein (SPP) and total soluble protein (TSP), assessed by the area under the HPLC-SEC chromatogram curve, of nixtamalized samples with different mixing times.
Tabla1. Proteína polimérica soluble (SPP) y proteína soluble total (TSP), estimados por el área bajo la curva de los cromatogramas de muestras nixtamalizadas.

Samples	SPP (%)	TSP2 (AU x 109)
Maize	42.92 c	29.94 a
Nixtamal for 3 min	43.40 c	23.96 c
Nixtamal for 6 min	43.76 c	23.92 c
Fresh dough for 3 min	44.72 bc	24.96 c
Fresh dough for 6 min	44.47 bc	24.93 c
Dough 3 min mixing	48.97 a	26.40 b
Dough 6 min mixing	45.92 a	27.17 b
Tortilla for 3 min	32.03 d	12.30 e
Tortilla for 6 min	32.12 d	17.37 d

1 Means in the same columns with the same letter did not present significant differences ($P<0.05$). SPP, soluble polymeric protein, percent of soluble protein, area of the first peak.
2 TSP, Total soluble protein, sum of areas of all peaks of the chromatogram.

protein with the exception of corn and nixtamal. Results indicated that the higher percentage of soluble polymeric protein was in dough with 3 min of mixing time, but it was not significantly different to that in dough with 6 min of mixing.

In general, the mixing increased the soluble protein proportion, and the proportion of soluble polymeric fractions. On the other hand, tortilla baking reduced the solubility of corn proteins. These results indicate that dough mixing induced the break of noncovalent and covalent bonds, due the physical strain (MacRitchie, 1975).

FT-IR spectras

The deconvoluted FTIR spectras of the corn dough with 3 and 6 min of mixing time are shown in Figure 2. In order to establish a comparison between the structures, the selected spectra region was from 1700- 1600 cm^{-1}, since this range relates to C- O interactions that constitutes amide I mode (Englander and Wand, 1987), while the amide I region is between 1600 – 1500 cm^{-1}, associated with the N-H bending and C-N stretching modes (Curley *et al.,* 1998). For the purpose of the present study, only the region of the amide I was considered for the secondary structure analysis, since changes in amide II are less reliable due to overlapped region with amino acid side chain vibrations (Chirgadze and Nevskaya, 1976); in addition, it is considered more sensitive to dough hydration (Wellner *et al*., 1996).

Structural analyzes show that the initial presence of α-helix in fresh dough was 34 %, observing a decrease after the mixing, to 28 % for 3 and 6 minutes. The content of the α-helix thus obtained is lower than those reported by Argos *et al*. (1982) and Forato *et al*. (2004), who propose that zeins

Table 2. Evaluation of texture properties (TPA), of corn dough after of 3 and 6 min mixing time.
Table 2. Evaluación de las propiedades de textura (TPA) de masas de maíz después de 3 y 6 min de mezclado.

Texture properties	Dough 3 min	Dough 6 min
Hardness (N)	1749.02 a	1410.34 b
Springiness	0.25 a	0.49 b
Adhesiveness	-28.89 a	-86.44 b
Cohesiveness	0.15 b	0.24 a
Chewiness	73.82 a	188.07 a

Each value indicates the average of five repetitions. Mean values followed by different letters for each attribute are significantly different (P < 0.05).

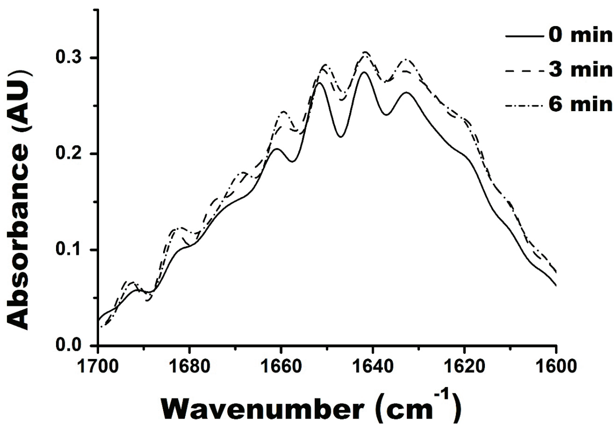

Figure 2. Deconvoluted spectra of fresh dough, and doughs with 3 min and 6 min of mixing time.
Figura 2. Espectro deconvolucionado de masa fresca, masas con 3 min y 6 min de mezclado.

contain ~ 40 – 60 % α-helix.

On the other hand, with respect to the fresh dough, it presented ~ 40.9 % of beta sheet structure, measured at 1633 y 16854 cm^{-1} (Barth and Zscherp, 2002). It was evident that the mixing step increased the β-sheet structure content by 4.0 %, which suggests that the mixing favors its formation. The number of amide groups in the strands of the sheet affects the position of these bands, but it also depends on the number of strands (Barth and Zscherp, 2002). This is desirable because several authors propose that gluten-free dough functionality is largely dependent on the formation of β-sheet type secondary structure (Mejía *et al*., 2007). However, it is necessary to control other factors such as the glass transition temperature (Tg) of zeins, because these structural changes are unstable (Mejía *et al*., 2007).

Structures found in the region of 1672 cm^{-1}, denominated like turns structures (Barth and Zscherp, 2002), showed an increase of about 4.0 % after mixing, compared to fresh dough. Finally, the unordered structure measured at the band around 1654 cm^{-1} (Barth and Zscherp, 2002) was stable after the mixing stage. A content of 17.15 % was found in fresh dough; and after 3 and 6 min of mixing, these proportions changed to 17.33 and 15.83 %, respectively, suggesting that mixing did not greatly affect these structures.

Overall, the mixing of the dough promotes structural transition decreasing α-helix and in turn, increasing the β-sheet structure. This has been previously reported by Mejía *et al.* (2007) who proposed that the mixture of zeins with water at a Tg of approximately 35 °C and at adequate levels of moisture, increases the β-sheet structure and the decrease of α-helix. Now, it is necessary to consider that these β-sheet structure changes are reflected in the dough viscoelastic properties, existing several proposed theories to explain the behavior of zein dough. One of them, establishes that the viscoelastic properties of the dough of zeins are related to the formation of a β-sheet rich secondary structure, which has also been related to the elasticy characteristic of the gluten dough (Belton, 2005).

Dough rheological properties

The rheological properties of corn dough with 3 or 6 min mixing time are presented in Figure 3 (A – C). Figures 3A and 3B show that storage (G´) and loss moduli (G´´) in dough with 3 or 6 min of mixing increased with frequency, respectively. In addition, at high frequencies in both doughs, the distribution of the data was homogeneous. This effect on dough behavior might be attributed to the temperature control (41 °C) during analyses, which reduced starch retrogradation of corn dough samples. It is widely recognized that G´ and G´´ are the most important determinations in rheological analysis, which are associated with the solid and liquid behavior in semi-solid dough (Ferry, 1980).

Both, G´ and G´´ decreased in dough when the mixing time increased from 3 to 6 min (Figure 3, A and B). Furthermore, G´ values were higher than G´´ values, indicating that, in all formed dough, predominating the elastic behavior. Platt-Lucero *et al*. (2010), Quintanar-Guzmán *et al*. (2011), and Santos *et al*. (2014) also reported similar results. These researchers proposed that corn dough with different treatments had a weak gel, and the viscoelastic behavior was that storage modulus (G´) had higher values than those of the loss modulus (G´´). Nixtamalization produces a stabilization of the structure (Quintanar-Guzmán *et al.*, 2009), and it occurs because of the interaction of starch and proteins during the nixtamalization process. Results of rheological measurements of the present work suggest also an ordered and stable structure.

On the other hand, tangent of the phase angle (Tan δ) values of corn dough, were also higher at long than at shorth mixing time (Figure 3 C). Tan δ increased steadily, as the frequency increased, which indicates that the dough structure became stronger, besides of presenting a solid type behavior (Ferry, 1980).

The rheological properties of corn dough show that mixing has important effect on elastic and viscous behavior. Additionally, this study showed the interaction of corn protein on viscoelastic properties of dough. The corn proteins were polymerized through lime with protein-starch network, thus proving that the mixing plays a primordial role in the proteins characteristics and their relationship to viscoelastic properties (Santos *et al*., 2015). Nevertheless, it has been reported that the rheological changes in corn dough are due to gelatinization reactions during traditional and extrusion nixtamalization (Enríquez-Castro *et al*., 2020; Topete-Betancourt *et al*., 2020.).

Figure 3 C shows the phase angle (Tan δ) values of corn dough at 3 and 6 min of mixing. It is observed that Tan δ values were higher with 6 min than 3 min of mixing. At both mixing times, Tan δ increased steadily with frequency, which indicates that the dough structure became stronger and presented a solid type behavior (Ferry, 1980). Values of Tan δ were in the range of 0.1 to 0.3, which indicate that the elastic behaviour predominate over the viscous one.

The rheological properties of corn dough show that mixing time has an important effect on elastic and viscous behavior. In addition, our study suggested the effect of the interaction of corn proteins on viscoelastic properties of the dough.

Dough texture profile analysis (TPA)

Table 3 summarizes the textural properties of the corn dough. The hardness is determined by the amount of force required by the teeth to compress the dough and, in a force-time curve, is designated as the maximum force necessary to achieve a deformation during the first compression cycle (Bourne *et al*., 1978). On the other hand, adhesiveness is the force necessary to overcome the forces of attraction between the product surface and the material surface with which the product comes into contact. Both properties are among the main textural properties considered of the dough. The dough with different mixing time had significant statistical differences ($p < 0.05$) for the different textural properties. The three-minute mixed dough, had higher hardness and elasticity values than those of the dough with six min, while this last showing higher adhesiveness values.

With respect to cohesiveness, defined as the strength of the internal bonds that make up the body of the product, we found that the dough with 6 min of mixing, obtained the highest cohesiveness values. In general, the dough with 3 minutes of mixing presented greater hardness and elasticity, but less cohesiveness. On the other hand, both doughs had no significant differences in the chewability characteristic. Research on corn dough with different cooking time and lime concentration, suggests that both variables have an effect on the adhesiveness of the dough, which increases directly proportional to the calcium content (Gracia-Amaya and Silva-Espinoza, 1992).

These differences in texture properties depend on the macrostructural behavior of the dough (Letang *et al*., 1999); but they can also be attributed to the processing conditions. It is for this reason that the measurement of texture of the dough is an important analysis.

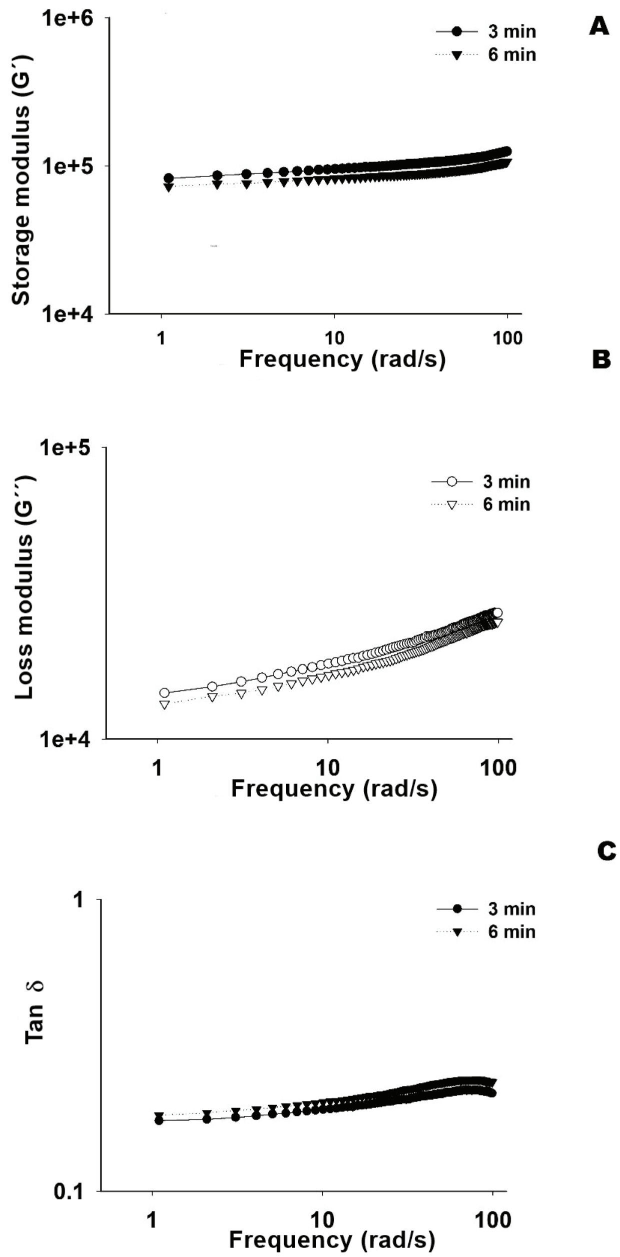

Figure 3. Effect of mixing time on viscoelasticity of the corn dough. A, storage moduli, G´; B, loss moduli G´´; C, phase angle Tan δ as a function of frequency.
Figura 3. Efecto del tiempo de mezclado en la viscoelasticidad de las masas. A., módulo de almacenamiento G´; B, módulo de pérdida, G´´; C, ángulo de fase, Tan δ, como una función de la frecuencia.

The tortilla texture depends on a number of factors, including the characteristics of the raw material as well as the baking conditions; besides, texture of the dough is critical during the process of making corn tortillas. When the dough has the proper texture, its adhesivenes and cohesivenes made dough to behave properly in the roll of the forming tortilla machine (Ramírez-Wong *et al*., 1993).

CONCLUSIONS

Mixing is a critical intermediate step during the nixtamalization process, in which, besides incorporating the nixtamal with water to obtain a homogeneous and maquinable dough to produce tortillas, also promotes significant changes. Among these, an increase in the proportion of the soluble polymer protein, as well as an increase in the secondary structure β-sheet. On the other hand, the mixing time affected the rheological and textural properties of the dough. The viscoelastic properties of corn dough showed that mixing has an important effect on elastic and viscous behavior. The dough were viscoelastic weak gel-like systems, with the elastic behavior prevailing over the viscous one.

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