Impact of L-cysteine addition on nixtamalized corn dough: proteins and viscoelasticity

Impacto de la adición de L-cisteína en la masa de maíz nixtamalizado: proteínas y viscoelasticidad

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ABSTRACT

The effect of L-cysteine on the solubility, protein structure, rheological and textural characteristics of corn masa was investigated. Corn masa was prepared by adding L-cysteine at two levels (0.25, 0.50 %). Size exclusion chromatography and Fourier transform infrared spectroscopy were utilized for protein characterization. Dynamic rheology and textural profile analysis were carried out on corn masa. There was a significant difference in relative solubility between the control and masa containing L-cysteine. Treatments with L-cysteine increased the relative solubility of proteins. FT-IR spectroscopy revealed that addition of L-cysteine did not affect the secondary structure of protein. Results also showed significant differences in textural properties. Masas with L-cysteine improved masa springiness, adhesiveness and cohesiveness. This investigation revealed that L-cysteine, improves the elasticity or storage modulus, important characteristics of corn masa.

Key words: L-cysteine, corn masa, proteins solubility, FT-IR, rheological properties

RESUMEN

Se investigó el efecto de la L-cisteína en la solubilidad, estructura proteica, características reológicas y texturales de masas de maíz. La masa se preparó con L-cisteína a dos niveles (0.25, 0.50 %). La cromatografía de exclusión por tamaño y la espectroscopia infrarroja por transformada de Fourier se utilizaron para la caracterización de proteínas. Se realizaron análisis de reología dinámica y perfil de textura. Hubo una diferencia significativa en la solubilidad relativa entre el control y las masas que contienen L-cisteína. Los tratamientos con L-cisteína aumentaron la solubilidad relativa. La determinación de FT-IR reveló que la adición de la L-cisteína no tuvo efecto en la estructura secundaria de la proteína. Los datos también mostraron diferencias significativas en las propiedades de textura. Masas con L-cisteína mejoraron la elasticidad, adhesividad y cohesión. Esta investigación revela que la L-cisteína mejora la elasticidad o los módulos de almacenamiento, características importantes de la masa de maíz.

Palabras clave: L-cisteína, masa de maíz, solubilidad de proteínas, FT-IR, propiedades reológicas

INTRODUCTION

Corn tortillas are one of the most important staple foods on the daily diet for Mexico and Central America. In Mexico, the tortilla consumption *per-capita* is around 75 kg/year (Solano-Perez, 2018). Tortillas have been elaborated using fresh masa prepared from traditional nixtamalization, extruded and instant nixtamalized corn flours (Arambula *et al*., 1999). These processes promotes important structural, physicochemical, and nutritional alterations in corn components (Quintanar-Guzman *et al*., 2011). It has been reported that changes occurring in starch, are responsible for the rheological and textural properties of masa and final products (Campas-Baypoli *et al*., 1999). However, it has been recently proposed that corn prolamins could affect the rheological behavior of masa (Chaidez-Laguna *et al*., 2016).

Corn masa must have cohesiveness, adhesiveness, and machinability properties, which are necessary for proper processing; besides, they have an impact on the rheological properties of the corn masa and, therefore, on the quality of tortilla. Corn tortillas should be flexible and rollable, textural characteristics that are affected during storage, observing an increase in firmness and brittleness. In general, tortilla has a short shelf life suffering several physical and chemical changes attributed to starch retrogradation (Campas-Baypoli *et al*., 1999). To try to solve this problem, strategies have been implemented, such as addition of hydrocolloids and enzymes.

Hydrocolloids have been investigated for making quality corn extruded masa, including xanthan gum, carboxymethylcellulose, guar gum, or a gums blends (Aguirre-Cruz *et al*., 2005; Platt-Lucero *et al*., 2010). These researchers have reported that hydrocolloids can improve masa properties, providing high water absorption capacity, which is closely related to the final textural characteristics of tortilla.

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Other investigations have focused on the use of enzymes, to improve flours functionality (Gys et al., 2004) and textural properties of corn tortillas (Platt-Lucero et al., 2013). Moreno-Rivas et al. (2014) suggested xylanase as a good alternative for the production of less firm corn masa during storage. However, the role of proteins on functionality of the corn masa and tortillas was not considered on these studies.

On the other hand, deficiencies in wheat dough during processing can be overcome by the incorporation of additives and enzymes which change the functionality of the proteins (Joye et al., 2009). Food additives such as L-cysteine can have effects on the masa functionality, which is the reason of it use in the present investigation. L-cysteine, contains thiol groups, recognized as a reducing agent that stabilize free radical species generated during processing of bread or wheat tortillas (Koh *et al*., 1996; Li and Lee, 1996). Also, L-cysteine modifies the disulfide bonds proportion and rheological characteristics of bread dough (Gomez *et al*., 2005), and decreases their resistance to extension, dough hardness, and viscosity (Angioloni and Rosa, 2007).

There is no evidence in the literature about the effect of L-cysteine on corn masa, therefore, the aim of this investigation was to evaluate the addition of L-cysteine in protein solubility, viscoelastic properties, and texture of corn masa.

MATERIALS AND METHODS

Materials

Commercial white maize and commercial lime (calcium hydroxide; purity ~ 91 % cal pirámide) were acquired from a local store. L-cysteine hydrochloride monohydrate (7048-04-6), acetonitrile (75-05-8), 1-propanol (78-83-1), and trifluoroacetic acid (76-05-1) (HPLC-grade) were purchased from Sigma-Aldrich (St. Louis, MO).

Nixtamalization process

Four kg corn were cooked in 12 L of lime solution at 1 % (grain weight basis). Maize 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 masa with a final moisture content of 56.0 %, using a commercial stone grinder.

Masa preparation

Fresh masa was made according to Ramirez-Wong *et al*. (1994), with slight modifications. The fresh masa was homogenized for 30 s. L-cysteine prepared at concentrations of 0.25 and 0.5 %, was dissolved in the water before masa preparation, then gradually incorporated and mixed for 3 min at room temperature. The samples were stored in plastic bags at 40 ± 1°C for 30 min, in order to reduce the starch retrogradation.

Fourier transform infrared spectroscopy (FTIR) analysis

FTIR spectra of control masa (CM), 0 %; L-cysteine dose 1, 0.25 % and L-cysteine dose 2, 0.50 % (w/v) corn masa 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 1652−1657 cm−1 and β-sheet at 1620−1637 cm−1) from the resolved spectra.

Sample preparation before protein analysis

All samples were prepared 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 % 1-propanol. Samples were placed in a stirrer (Vortex Genie2, Scientific Industries, Bohemia, NY) 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.

Viscoelastic properties

After mixing, samples were rested in plastic bags at 40 ± 1°C for 30 min. Rheological properties of masa formulations were studied by a rheometer (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 a*l., 2010). Approximately 2.5 g of masa was compressed between two plates separated by a gap of 2.5 mm. The parallel plates were covered with petroleum jelly to avoid loss of moisture during the test.

A frequency sweep test, ranging from 0.1 to 100 rad/s, was used to study the masa rheological properties. The test was carried out using a software (RSI Orchestrator, Rheometrics Scientific). The viscoelastic behavior obtained in the frequency range used were the 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'). The tests were carried out by triplicate.

Texture profile analysis (TPA) of masas

Texture profile analysis (TPA) was performed using a texturometer (Model TA-XT2, Surrey, UK) equipped with a cylinder probe with 36 mm diameter was used in this test according to AACC standard method 74-9 (AACC, 2000). A 3 g load cell was used at the speed of 1 mm s1. The masa samples were cut in a cylinder shape. The probe was moved down in certain speeds up to 50 % of samples height, and then moved back up in the same speed, and this movement was repeated in a 10 s time interval. Indicators determined by this test include springiness, cohesiveness and adhesiveness, obtained using the texturometer (Stable Micro System TA.TXplus Texture Analyzer). The test was done by five replications.

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.

RESULTS AND DISCUSSION

Solubility study

The relative solubility of corn proteins in 50 % propanol was determined by the area under the curve in the SE-HPLC chromatograms. Figure 1 A–C shows the chromatograms of the effect of L-cysteine in proteins solubility samples extracted with 50 % propanol. Total soluble protein was significantly ($p < 0.05$) different in corn, masa treatments, and nixtamal. The amount of soluble polymeric protein increases from maize to nixtamal and is reduced in the masa, which is in accordance with previous studies (Chaidez-Laguna *et al*., 2016). However, an increase in the proportion of the soluble polymer protein (SPP) was observed in the treatments that contained L-cysteine. Table 1 summarizes the results of the effect of cysteine on the corn masa. The addition of L-cysteine into corn masa causes an increase in the SPP compared to control masa (Table I). L-cysteine, at dose 2, showed the largest increase on the solubility, which can be attributed to the L-cysteine mechanism of action, which break the disulfide bonds forming sulfhydryl groups. Therefore, the protein solubility is increased, modifying the strength and structure of the existing protein network (Bloksma 1990). The results obtained are similar to those found in wheat flour doughs added with L-cysteine (Angioloni and Rosa, 2007; Popineau *et al*., 2002).

FT-IR Analysis

The FTIR spectra were deconvoluted and are shown in Figure 2. The effect of L-cysteine on the secondary structure of the protein was analyzed through the region correspon-

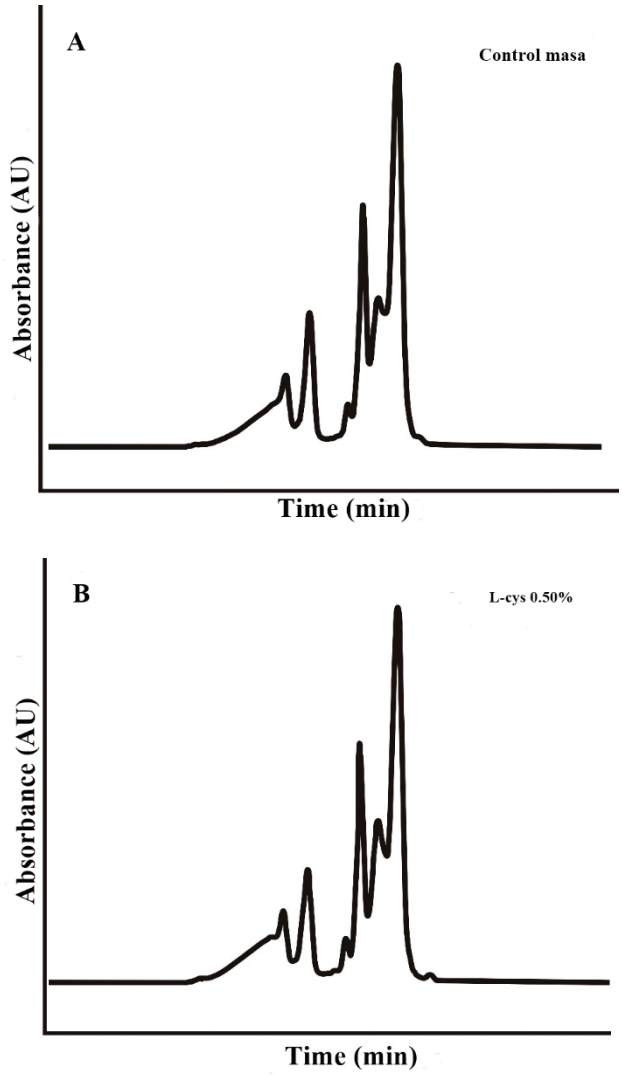

Figure 1. SE-HPLC chromatograms of control and L-cysteine dose 2 masa.
Figura 1. Cromatogramas SE-HPLC de la masa control y la masa con L-cisteína dosis 2.

Table 1. Effect of L-cysteine addition on soluble polymeric protein (SPP), total soluble protein (TSP) of corn masas nixtamalized.
Tabla1. Efecto de la adición de L-cisteína en la proteína polimérica soluble (PPS), proteína soluble total (PST) en las masas de maíz nixtamalizadas.

Treatments	SPP[1,2]	Other fractions[1]	TSP[1,3]
	(AU x 10^9)	(AU x 10^9)	(%)
Maize	9.88 b	13.18 b	23.06 b
Nixtamal	10.40 a	13.56 a	23.96 a
Control masa	7.36 e	11.22 d	18.58 d
0.25% L-cysteine	8.54 d	12.60 c	21.13 c
0.50% L-cysteine	9.49 c	13.63 ab	22.85 b

[1] Means in the same column with the same letter did not present significant differences ($p<0.05$).
[1] Medias en la misma columna con la misma letra no presentan diferencias significativas ($p<0.05$).
[2] SPP, soluble polymeric protein.
[2] SPP, proteína polimérica soluble.
[3] TSP, total soluble protein, sum of areas of peak chromatogram.
[3] TSP, proteína soluble total, suma de áreas del cromatograma.

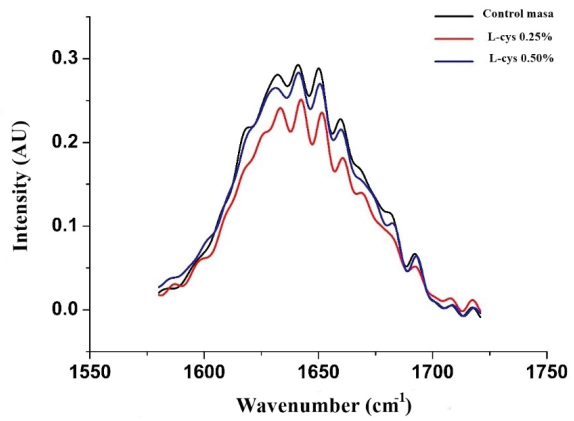

Figure 2. FT- IR spectras deconvoluted. Control masa (black line), L-cysteine masa dose 1 (red line) and L-cysteine masa dose 2 (blue line).
Figura 2. Espectros FT-IR deconvolucionados. Masa control (línea negra); masa L-cisteína dosis 1 (línea roja) y masa L-cisteína dosis 2 (línea azul).

ding to amide I (1650 cm-1). Some authors have suggested that for viscoelastic systems, changes in amide II have the disadvantage of being more sensitive to hydration and, therefore, less reliable in determining the secondary structure (Wellner et al., 1996). The amide I band is wide and contains information of the secondary structure of proteins, such as α-helix, β-sheet, turns, and random protein structure (Surewicz and Mantsch, 1988). It is between 1600 – 1500 cm-1 where we can find the associations with the N-H bending and C-N stretching modes. For control masa and L-cysteine samples, the amide I region showed five main peaks each in all cases. Amide I components centred ~ 1650 and 1660 cm-1 (Bandekar, 1992; Barth and Zscherp, 2002; Barth et al., 1984; Surewicz and Mantsch, 1988) corresponding to α-helix structures (Surewicz and Mantsch, 1988), while bands between 1615 – 1640 cm-1, and 1679 cm-1, indicate the presence of β-sheet. Bands at 1653 and 1691 cm-1 have been assigned to turns and the disordered structures are located at ~ 1639-1654 cm-1 (Barth and Zscherp, 2002).

According to the deconvoluted spectra, the content of α-helice was 46 % for control masa, 45.67 % in masa L-cys dose 1, and 46.72 % for masa L-cys dose 2. These results are in agreement with Argos et al. (1982), who demonstrated that the zeins have a content of ~ 50 % of α-helice.

On the other hand, the content of β-sheet structure was 25.71 %, 25.57 % and 25.73 % for control, L-cys dose 1 and L-cys dose 2 masa, respectively. It can be seen that the effect of cysteine on the conformation of β-sheet did not show significant changes in its content. That is, the addition of L-cysteine did not promote the disaggregation of the protein matrix, a necessary condition to functionalize these proteins. This may be due in part to the structure of the zeins. These proteins have a high degree of aggregation and are found in protein bodies. According to Argos et al. (1982) and Matsushima et al. (1997), it has been proposed that zeins have a helical wheel model where nine homologous repeat units are arranged in an anti-parallel form stabilized by hydrogen bonds, forming hexagonal structures compatible with hydrogen bonds and hydrophobic interactions. This complex structure prevents the change in the conformation of secondary structure, mainly the increase in the content of β-sheet, which constitutes a condition to functionalize these proteins.

Rheological properties

To obtain a complete description of the mechanical properties of the corn masa, a frequency scan from 0.1 to 100 rad/s was used to determine the influence of L-cysteine on the viscoelasticity of masa. The frequency profiles of the storage moduli (G ‘), loss moduli (G “) and loss tangent (Tan δ) of control masa and treatments with L-cysteine, are shown in Fig. 3 A-C, respectively. The masa added with L-cysteine showed lower G’ values, in comparison with the control masa (Figure 3 A), that is, these masa showed lower elasticity. However, increasing the concentration of cysteine, increased this value. This can be explained according to the continuous network present in the structure of the protein, where the elasticity of the masa depends of the number of disulfide bonds present on the protein network (Bloksma, 1988). These results cannot be compared with previous studies in corn, since to date there are no investigations about the effect of L-cysteine on the rheological properties of corn masa. On the other hand, a different behavior was found in wheat masa, as the moduli G’ is expected to grow with the increase of disulphide bonds in wheat gluten. These differences may be due, in part, to the different protein structure of both cereals. Both wheat and corn proteins have complex structures. However, the zeins are highly aggregated and are deposited in protein bodies, surrounded by proteins linked by disulfide bonds (Matsushima et al., 1997) which are highly compact, which limits in the reductor effect of L-cysteine.

Several authors have been suggested that addition of cysteine reduces mixing time, decreasing the elastic component of the masa and promoting its relaxation (Elkhalifa and El-Tinay, 2002; Srinivasan et al., 2000).

In both Figures it was observed high values of G’ and low values of G” for the masas, indicating a highly structured material and an elastic gel-like behavior. These results are in agreement with that reported by Quintanar-Guzman *et al*. (2011) and Santos *et al*. (2014) for masas of nixtamalized corn.

On the other hand, the corn masa began to be relatively more viscous when the concentration of L-cysteine increased from 0.25 to 0.50 %. However, it was appreciated that masa control and masa with L-cys dose 1, presented a very similar behavior. Also, the values of Tan δ of the masa with cysteine were lower compared to the control, but when increasing the dose, this value increased (Figure 3 C). In all treatments, the value of Tan δ was found between 0.18 - 0.21, that is, less than 0.5, which indicates that the elastic behavior was greater than the viscous. This has previously been mentioned according to the results of G ‘ y G”.

Finally, it is necessary to consider that in the formulations of the masa studied, there is a three-dimensional network formed by carbohydrates, proteins, L-cysteine, and

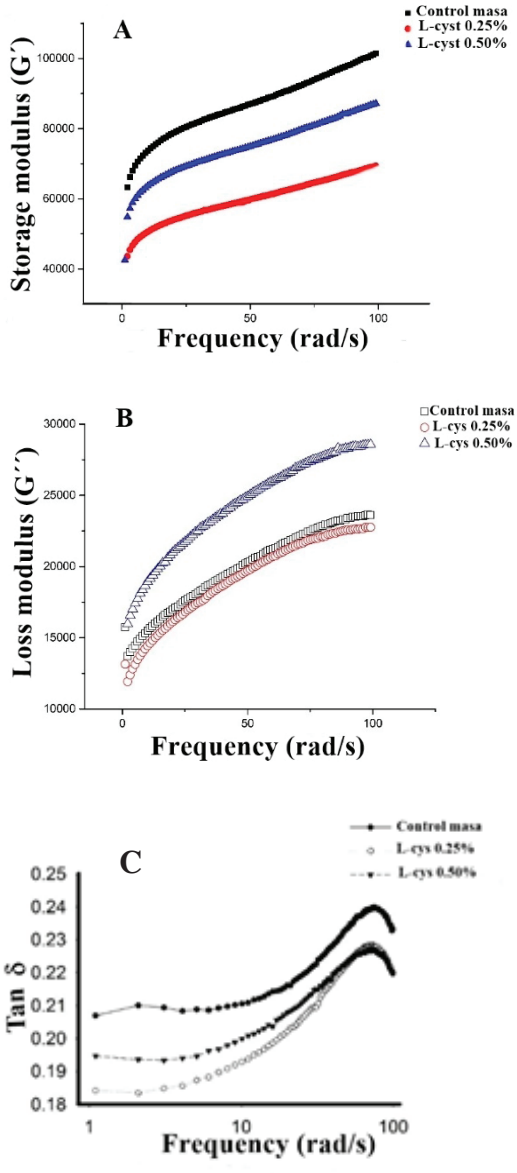

Figure 3. Effect of L-cysteine on rheological properties of masas.
A, Storage modulus (G´) control masa; L-cysteine dose 1; L-cysteine dose 2
B, Loss modulus (G´´) control masa; L-cysteine dose 1; L-cysteine dose 2
C, Tan δ, control masa; L-cysteine dose 1; L-cysteine dose 2.
Figura 3. Efecto de L-cisteína en las propiedades reológicas de las masas.
A, Storage modulus (G´) masa control; L-cisteina dosis 1; L-cysteine dosis 2
B, Loss modulus (G´´) masa control ; L-cisteina dosis 1; L-cisteina dosis 2.
C, Tan δ, masa control; L-cisteína dosis 1; L-cisteína dosis 2.

water that interact with each other and these interactions play an important role in the rheological properties of the masa.

Textural propierties

The texture profile results of masas added with L-cysteine are presented in Table 2. Significant statistical differences were observed in the samples analyzed for each of the properties. The addition of L-cysteine to the corn masas caused a decrease in adhesiveness as compared to the control masa, observing the greatest effect at the lowest

Table 2. Effects of L-cysteine on corn masa texture profile.
Tabla 2. Efectos de la L-cisteína en el perfil de textura de la masa de maíz.

Treatments	Texture profile analysis		
	Springiness[1]	Adhesiveness[1]	Cohesiveness[1]
Control masa	0.71 a	-139.45 b	0.27 a
Masa 0.25 % L-cys[2]	0.36 b	-73.34 a	0.24 b
Masa 0.50 %L-cys[3]	0.48 ab	-91.46 ab	0.21 c

[1] Each value is the average of five repetitions. Mean values within column, followed by the same letter did not present significant differences ($p < 0.05$).
[1] Cada valor es el promedio de cinco repeticiones. Valores promedio con la misma letra, dentro de la columna, no presentan diferencias significativas ($p < 0.05$).
[2] Masa, L-cysteine dose 1.
[2] Masa, dosis de L-cisteína 1.
[3] Masa, L-cysteine dose 2.
[3] Masa, dosis de L-cisteína 2.

concentration. However, the masas with cysteine did not show significant statistical differences for this characteristic between them. Regarding elasticity, it was found that the control masa obtained the highest value, but it was not statistically different from the masa with the higher dose of the amino acid. Therefore, the concentration of added L-cysteine had a determining role in promoting the change in elasticity. This result is consistent with the behavior found in the viscoelastic properties.

Cohesiveness is considered an important textural property of masa. Significant differences were found in the treatments compared to the control masa, although the effect of the amino acid promoted a decrease in the cohesiveness. It was observed that cohesiveness decreased as the L-cysteine dose was increased. Therefore, the masas to which cysteine was added resulted less adhesive. No significant differences were found in the masas added with the amino acid in the adhesiveness (Table 2), so the effect of the amino acid was not dependent on the concentration used. This can be attributed to the effect of L-cysteine in the disulfide bonds that favors its machinability (Angioloni and Rosa, 2007).

In general, these results show that the addition of L-cysteine decreases the adhesiveness and cohesiveness of the masa, but favors the increase in elasticity. It has been proposed that on the masa there should be a balance between these properties, with the purpose of obtaining a manageable masa and after baking, a tortilla that presents adequate textural characteristics during the storage period. However, this stage was not investigated in the present research, and is recommended for future work.

CONCLUSION

The addition of L-cysteine in corn masa changes the solubility and structure of proteins, as well as the rheological and textural properties of corn masa. The reducing agent, L-cysteine promoted the increase in the proportion of SPP of the masa, in addition to diminishing their elastic behavior, adhesiveness and cohesiveness. However, the determination

of the secondary structure of the protein did not reveal a change in the content of β-sheet. Corn masa with L-cysteine added, showed acceptable textural properties, and consequently, a good machinability is predicted, that may result in tortillas with good textural quality. However, it is necessary to make tortillas and to evaluate the textural changes that occur during storage, using objective and subjective methods as well sensory evaluation.

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