Full Length Research Paper

Technological properties of maize tortillas produced by microwave nixtamalization with variable alkalinity

Méndez-Albores, A.[1]*, Martínez-Morquecho, R. A.[1], Moreno-Martínez, E.[1] and Vázquez-Durán, A.[2]

[1]UNAM-FESC. Campus 4. UNIGRAS. Unidad de Investigación Multidisciplinaria. Cuautitlán Izcalli, C. P. 54714, México.
[2]UANL-FA, Campus de Ciencias Agropecuarias. Francisco Villa s/n, Escobedo, Nuevo León, México.

This research was conducted to determine the quality, physicochemical, textural, compositional, nutritional, viscoamylographic and sensory properties of maize tortillas produced with a Modified tortilla-making process (MTMP) of variable alkalinity (0.125, 0.25 and 0.5% $Ca(OH)_2$ w/w) and compared to the commercial brand MASECA®. In general, tortillas from MTMP showed higher pH, total color difference (ΔE), tensile strength/cutting force, protein, lipids, crude fibre, lysine, tryptophan, *in vitro* protein digestibility and lower Hunter L value, loss of weight during cooking and moisture content than MASECA® tortillas. No significant differences were found in the sensory analysis of 22 descriptors of tortillas made from MASECA® and MTMP with $Ca(OH)_2$ concentrations of 0.125 and 0.25% (w/w). However, panelist identified principal effects on changes in four attributes (aroma, appearance, flavor, and after taste flavor) and seven descriptors in tortillas from MTMP prepared with the maximum lime concentration (0.5% w/w). Microwave nixtamalization produce tortillas with acceptable physicochemical, textural, quality, compositional/nutritional and pasting properties.

Key words: Maize, modified nixtamalization, tortillas, technological properties.

INTRODUCTION

In Mexico, maize is primarily consumed as tortillas, with a *per capita* consumption of about 120 kg/year (Plasencia, 2004) and traditionally made utilizing the ancestral alkaline-cooking process called nixtamalization. The Traditional nixtamalization process (TNP) consists of the cooking of the grain in abundant water (2 to 3 L of water/kg of maize processed), with 1 to 3 g/100 g $Ca(OH)_2$ at temperatures near boiling, for 35 to 70 min, with a steeping period of 8 to 16 h. After the steeping, the lime cooking solution (nejayote) is decanted, and the grain is thoroughly washed to leave the grain (nixtamal) ready for milling to obtain the masa (maize dough) for making the tortillas.

The TNP, though centuries old, is still used without modification. While this process when implemented on a small scale does not present serious problems, it has become the cause of ecological problems on a grand scale due to the generation of large quantities of washing water containing maize solids, calcium ions and hull. The estimated amount of nejayote generated in Mexico is about 14 million m^3 year^{-1} (Pérez-Flores et al., 2011). The tortilla is mostly elaborated by small processors discarding the nejayote into the sewage, whereas dry masa factories almost always treat the nejayote aerobically/anaerobically to reduce the Biochemical oxygen demand (BOD) and the Chemical oxygen demand (COD). Thus, nejayote is one of the most difficult waste water to treat because it has a high pH (10 to 14), contains organic soluble and insoluble solids (~5%), leftover lime (80% of the lime originally used during the nixtamalization process), thus requiring a BOD and COD of about 2.7 to 8.1 g O_2 L^{-1} and 10 to 30 g L^{-1}, respectively, for its degradation (Jackson et al., 1988; Pedroza-Islas and Durán de Bazúa, 1990; Velasco-

---
*Corresponding author. E-mail: albores@unam.mx. Tel: (+52) 5556231999. Fax: (+52) 5556239402.

Table 1. Compositional and nutritional information of commercial nixtamalized maize flour MASECA® and maize grain.

Property	MASECA	Maize (AS-900)
Proximate composition (% d.b.)		
Protein	9.01±0.10	9.38±0.02
Lipids	4.79±0.07	5.82±0.03
Ash	1.62±0.03	1.79±0.04
Crude fibre	1.44±0.03	2.23±0.03
Carbohydrates*	83.14±0.16	80.78±0.11
Nutritional		
Lysine content (g kg^{-1}protein)	23.76±0.22	33.89±0.36
Tryptophan content (g kg^{-1} protein)	5.09±0.09	7.28±0.12
In vitro protein digestibility (%)	83.53±0.34	76.18±1.05

Result is presented as mean of three replicates ± standard error. *Determined by difference.

Martinez et al., 1997; Salmerón-Alcocer et al., 2003).

The search continues for new technologies with advantages over TNP, including: process acceleration, reduction of water use, the elimination of polluting effluents, the reduction of operation costs (energy/time), and the achievement of good product quality, among others. Recently, a modified tortilla-making process (MTMP) has been developed, in which maize grits are mixed with water and lime, cooked in a microwave oven, steeped, and then milled to obtain fresh masa for the tortilla making (Pérez-Flores et al., 2011). MTMP was proven for its effectiveness in reducing the aflatoxin content in contaminated maize destined for tortilla production, being effective with low levels of aflatoxin contamination (up to 22.5 ng/g). However, technological properties of tortillas produced with this modified process have not been evaluated.

It is well known that the TNP enhances the nutritional value of maize by increasing the availability of lysine, calcium, and niacin; as well as reducing phytic acid levels (Figueroa-Cárdenas et al., 2001). Unfortunately, TNP also leads to losses of some nutrients including fat, protein, dietary fibre, vitamins and minerals (Martínez-Bustos et al., 1996). Due to this fact, the tortilla industry undergoes a compromise in quality with low protein levels and deficiencies of others nutrients in the product, due to the large variability in the lime concentration used during small-scale nixtamalization. Consequently, it is considered crucial to evaluate the effect that the MTMP could have on the technological properties of maize tortillas when using lime at relatively low and relatively high levels (since this process substantially differs from the TNP), and to compare to those properties of tortillas produced from commercial nixtamalized maize flour brand MASECA®.

MATERIALS AND METHODS

The study was conducted in the Grain and Seed Research Laboratory of the National Autonomous University of Mexico.

Maize grain and commercial nixtamalized maize flour

Regular maize of the commercial hybrid AS-900 (Aspros, Mexico) grown and harvested in 2011 at Celaya-Guanajuato, Mexico, with 10.8% moisture content (MC) was utilized. This material has a thousand-kernel weight and test weight of 283.40 ± 3.64 and 71.59 ± 1.23 kg hL^{-1}, respectively. MC was determined by drying replicate portions of 5 to 10 g each of whole grain at 103°C for 72 h, with percentages calculated on a wet-weight basis. Fresh commercial nixtamalized maize flour MASECA® lot I33213 (GRUMA, San Pedro Garza, Monterrey, NL, Mexico) was used as a control. The compositional and nutritional information of both materials are shown in Table 1.

Modified tortilla-making process (MTMP)

Nixtamalization was done following the procedure described by Pérez-Flores et al. (2011). Experimental units (1000 g) of maize were milled in a hammer mill (Glen Mills Inc. sieve 5 mm Clifton, NJ). The tap water/maize input ratio was 1:1 (v/w) and three different Ca(OH)$_2$ (JT Baker, purity ≥ 95%) contents were evaluated (0.125, 0.25, and 0.5% w/w). The maize was cooked in a microwave-resistant plastic container, and the cooking stage was carried out in a domestic commercial microwave oven (LG Electronics Inc, model MS047GR, Korea), with an average cooking power of 100% during 4 min. The power output of the magnetron specified by the manufacturer was 1650 W and the operating frequency was 2450 MHz. The cooked grain (nixtamal) was steeped 3.5 h at room temperature (22°C) before milling (FUMASA, Model MN-400, Mexico) to provide a masa with a MC of about 54%.

Tortilla preparation

Masa was compressed into thin disks of approximately 12.5 cm diameter, 1.2 mm thickness, and 28 g weight, using a commercial tortilla roll machine (Model TM-G, Casa Gonzalez. Monterrey, NL, Mexico). Tortillas were baked 17 s on one side (first side), 55 s on the other side, and again 17 s on the first side on a griddle at 270°C. The temperature was measured with a non-contact portable infrared thermometer Fluke-572 (Fluke, Melrose, MA, USA). Finally, masa (500 g), and tortillas from each treatment (n = 30) were oven

dried at 40°C for 48 h, milled and stored at 4°C in polyethylene bags for further analysis. For control tortillas, the commercial nixtamalized maize flour MASECA® (1 kg) was mixed with 1.5 L of tap water to obtain the masa, tortillas were prepared as described.

Physicochemical analysis

pH

The pH was determined according to the 943.02 AOAC method (AOAC, 2000). Ten grams (10 g) of sample were suspended in 100 ml of recently boiled distilled water. The suspension was shaken (1500 rpm, 25°C, 30 min) using an orbital shaker (Cole Parmer Model 21704-10, Vernon Hills, IL, USA). After 10 min, the supernatant liquid was decanted and the pH value was immediately determined using a pH meter, Model PC45 (Conductronic S.A., Puebla, Mexico).

Color

Tortillas were subjected to surface-color analysis with a MiniScan XE model 45/0-L colorimeter (Hunter Associates Laboratory, Reston, VA, USA). The colorimeter was calibrated with a white porcelain plaque (L = 97.02, a = 0.13, b = 1.77). Readings were made in triplicate at four positions at 90°C with respect to each other. The total color difference (ΔE) was computed from the L, a, and b readings, as:

$$\Delta E= [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

Textural properties

The texture of the tortilla (tensile strength and cutting force) was evaluated using the Texture Analyzer TA-XT2 (Texture Technologies Corp., Scarsdale, NY, USA). Texture was evaluated immediately after preparation. To avoid loss of water and temperature, tortillas were kept at 40°C inside polyethylene bags. For tensile strength evaluation, five pieces of tortilla in the form of a test strip (8.7 × 3.75 cm at each end, and 1.5 cm wide at the thinnest part) of the central part of the tortilla was cut and placed in retention clamps TA-96. The test was carried out at 2 mm/s, and pincers were opened 15 mm until the tortilla piece was broken. The pieces of tortilla that were not used for tensile strength were used to determine the cutting force using the blade TA-90 at 2 mm/s and a cutting depth of 15 mm (Cuevas-Martínez et al., 2010). Texture values were expressed as the peak force in Newtons (N) required to break and cut the tortilla strip.

Quality properties of tortillas

Puffing degree

Tortilla puffing was evaluated subjectively by using scores of 1 to 3, where: 1 = little or no puffing (0 to 25%), 2 = medium puffing (25 to 75%), and 3 = complete puffing (75 to 100%), as recommended by Cuevas-Martínez et al. (2010).

Rollability

Rollability was evaluated by rolling a tortilla over a 1 cm diameter tube, quantifying the extent of breaking using a scale of 1 to 3, where: 1 = tortillas with no breaking; 2 = a partial breaking at the center and edges of the tortilla; and 3 = completely flattened tortillas (Cuevas-Martínez et al., 2010).

Weight loss of tortilla during cooking

The weight loss (WL) of tortilla was determined by weighing the tortilla before and after cooking, value was reported as percentage (w/w) and computed as:

$$WL = [(W_a - W_b) / W_a] \times 100$$

where, W_a, W_b is the weight of tortilla before and after cooking, respectively.

Compositional analysis

The following analyses were performed in tortillas: MC (drying at 105°C for 24 h); ash (incineration at 550°C); crude protein (micro-Kjeldahl, N × 6.25); crude fat (defatting in a Soxhlet equipment with hexane); and crude fibre (acid and alkaline hydrolysis), following AOAC official methods 925.10, 923.03, 960.52, 920.39C, and 962.09E, respectively (AOAC, 2000).

Nutritional properties

Lysine

Lysine analysis was performed using the method described by López-Cervantes et al. (2006) using high performance liquid chromatography (Alliance® HPLC, Waters Associates, Milford, MA, USA), equipped with a Waters nova-pak C18 reverse phase column (4 µm, 3.9 mm × 150 mm) maintained at 38°C. Samples (50 mg) were hydrolyzed at 110°C with 10 mL of 6 M HCl for 24 h. The hydrolyzed sample was filtered and the extract was diluted 200 times with milliQ water. A 300 µl aliquot of the extract was dried and derivatized with the same amount of 9-fluorenylmethyl-chloroformate (FMOC). Standard as well as sample amounts (20 µl), were injected into a HPLC and eluted with a mobile phase of 30 mM ammonium phosphate (pH 6.5) in 15:85 (v/v) methanol/water; 15:85 (v/v) methanol/water; and 90:10 (v/v) acetonitrile/water, at a flow rate of 1.2 mL min^{-1}. The gradient program employed was as reported by López-Cervantes et al. (2006). Lysine was fluorometrically detected and identified using a fluorescence detector (Waters model 2475); the excitation and emission wavelengths were 270 and 316 nm, respectively.

Tryptophan

Tryptophan analysis was performed using the colorimetric method described by Nurit et al. (2009). Flour samples were defatted with hexane in a Soxhlet-type continuous extractor for 6 h. After hexane evaporation, 80 mg of powder was digested using 3 mL of 4 mg mL^{-1} papain solution in 0.165 M sodium acetate. The tubes were incubated at 65°C for 16 h, allowed to cool to room temperature, and centrifuged at 3600 g for 10 min. Subsequently, one milliliter of the supernatant was carefully transferred to a clean tube, and 3 mL of a colorimetric reagent (0.1 M glyoxylic acid in 7 N H_2SO_4 + 1.8 mM $FeCl_3 \cdot 6H_2O$ + 30 N H_2SO_4) was added. Samples were vortexed and then incubated at 65°C for 30 min. Samples were allowed to cool to room temperature before reading their optical density at 560 nm in a Beckman-Coulter DU-530 UV-visible spectrophotometer. Calibration curves were constructed using standard lysine and tryptophan (Sigma, St. Louis, MO, USA).

In vitro protein digestibility

In vitro protein digestion (PD) was performed using the 982.30G AOAC (2000) method. A multi-enzyme system, consisting of a mixture of porcine pancreatic trypsin type IX, porcine intestinal peptidase grade I, bovine pancreatic α-chymotrypsin type II, and bacterial protease (Sigma, St. Louis, MO, USA), was used. Sodium caseinate was used as a control protein (10 g suspended in 200 mL distilled water and adjusted to pH 8 with NaOH). Flour samples and distilled water were used to prepare 10 mL of an aqueous protein suspension (10 mg N) with pH adjusted to 8.0 ± 0.03, while stirring in a water bath at 37°C during 1 h. The multi-enzyme solution was maintained in an ice bath and adjusted to pH 8.0. While stirring, 1 mL of the multi-enzyme solution was added to the protein suspension and 10 min after addition, 1 mL of bacterial protease was added and then transferred to 55°C bath for 9 min. Exactly 19 min after reaction, vials were transferred back to 37°C bath. The rapid pH drop was recorded automatically over a 20 min period using a pH meter, Model PC45 (Conductronic S.A., Puebla, Mexico). PD was calculated using the equation:

PD (%) = 234.84 - 22.56 (pH value)

Viscoamylographic properties

Relative viscosity of water suspensions of ground material was determined in a Rapid Visco Analyser RVA-4 (Newport Scientific, Sydney, Australia). Tortillas were dried in a vacuum oven at 40°C during 48 h, then milled and sieved to provide ground material with a particle size of < 250 µm (60 mesh). A sample of 3.5 g at 14% MC was placed in a can and suspended in 25.5 g distilled water. A plastic stirring paddle was placed in the sample can, which was then fixed into the RVA, and the heating cycle was activated through a split copper block. The analyzer used a time-temperature program as follows: initiating at 50°C (1 min), increasing the temperature to 92°C at a rate of 5.6°C min^{-1} (7.5 min), remaining 5 min at that temperature, and later decreasing the temperature to 50°C at the same rate used during the heating, and remaining at that temperature for 2 min, with a total test time of 23 min. The rotating speed of the paddle was 860 rpm for the first 10 s, then 160 rpm for the remainder of analysis. From the pasting curves, the values of viscosity peak and setback (difference between the viscosity at the end and the beginning of the cooling period) were registered.

Sensory evaluation

A panel of one-hundred untrained judges was selected from students and professors of the Food Engineering Faculty of the National Autonomous University of Mexico (both sexes, 22 to 50 years old). The selection criteria for the panelists were based on the participant interest, taste/odor and texture perception, besides that they all declared to enjoy eating tortillas and to consume them in a regular basis. For sensory tests, tortillas were made from either MASECA® or from MTMP. Tortillas were kept in thermo isolated plastic containers and within 5 min of preparation, warm tortillas (65°C) were offered to candidates in individual booths under sensory controlled laboratory conditions (22°C, 50 to 60% RH). Reference tortillas were always offered before tortillas obtained through the MTMP. Participants receiving a separate set of samples within each sensory attribute were evaluated: aroma, appearance, manual and oral texture, flavor and aftertaste flavor.

For aroma evaluation, tortillas were placed in plastic bags and smelled twice. For flavor, tortilla triangles were offered on plastic plates, samples were not swallowed and purified water was offered for oral rinsing; however, samples of tortilla were consumed for

aftertaste analysis and the results were recorded after 30 s of consumption. Tortilla appearance was judged under 250 W incandescent white bulbs installed in each individual booth. During the session, participants evaluated the following attributes and their descriptors: aroma (like maize, lime, nixtamal), appearance (evenness, opacity, yellowness, puffed), manual texture (softness, roughness, fragility, bending, rollability), flavor (like maize, bitter, astringent, nixtamal), oral texture (softness, doughness, adhesiveness, tooth packing), and aftertaste flavor (bitter, astringent, nixtamal). Samples produced from MTMP with lime concentrations of 0.125, 0.25, and 0.5% (w/w), and control tortillas (MASECA®) were compared in terms of degree of deviation from the control using a non-numerical 10 cm graphic scale, where 0 = less than the control, 5 = same as the control and 10 = more than the control. Sensory tests were repeated three times on different dates. A complete balanced block design was used (Pedrero and Pangborn, 1989).

Experimental design and statistical analysis

The experiment was conducted as a completely randomized design. Treatments consisted of microwave nixtamalization with three different calcium hydroxide concentrations (0.125, 0.25, and 0.5% w/w). Data were assessed by analysis of variance (ANOVA) and means comparisons were performed according to Dunnet test using the Statistical Analysis System (SAS, 1998). A significance value of α = 0.05 was used to distinguish significant differences.

RESULTS AND DISCUSSION

Physicochemical properties

Table 2 shows some physicochemical properties of tortillas obtained through the MTMP and commercial nixtamalized maize flour MASECA®. Regarding the MC, no statistical differences were observed in tortillas from MTMP prepared with the three different Ca(OH)$_2$ concentrations; the average MC was 44.84%. However, tortillas from MASECA® had the higher MC (53.67%). Cuevas-Martínez et al. (2010) reported similar MC values for maize tortillas produced with the traditional nixtamalization process. In the case of tortillas from MASECA®, the higher MC is probable due to the incorporation of gums into the nixtamalized flour. Flores-Farías et al. (2002) reported the presence of guar and xhantan gums in commercial nixtamalized maize flours in concentrations of 0.38 and 0.18%, respectively. In general, gums are added to nixtamalized flours to improve masa and tortilla texture as well as to retain MC.

In relation to pH, tortillas from MASECA® and from MTMP with lime concentration of 0.125% (w/w) presented similar average pH values (6.50). However, as the calcium concentration increased, higher pH values were registered in tortillas from MTMP (Table 2). Thus, tortillas produced with lime concentrations of 0.25 and 0.50% (w/w), presented average pH values of 6.96 and 7.97, respectively. Ayala-Rodríguez et al. (2009) reported a pH value of 6.78 for MASECA® tortillas and 7.05 for tortillas produced from nixtamalized transgenic maize (genetically modified with the cDNA of amarantin, line

Table 2. Technological properties of tortillas from MASECA® and MTMP prepared with different $Ca(OH)_2$ concentrations.

Properties		MASECA®	MTMP (lime content % w/w)		
			0.125%	0.25%	0.5%
Physicochemical					
Moisture content (%)		53.67±0.33[a]	44.48±0.21[b]	44.60±0.29[b]	45.43±0.13[b]
pH		6.48±0.019[a]	6.52±0.006[a]	6.96±0.008[b]	7.97±0.041[b]
Color	L value	75.34±0.80[a]	70.62±0.31[b]	66.59±0.29[c]	65.03±0.39[d]
	ΔE*	25.81±0.58[a]	29.88±0.09[b]	33.44±0.02[c]	38.22±0.84[d]
Textural					
Tensile strength (N)		0.77±0.01[a]	1.09±0.03[b]	1.11±0.07[b]	1.11±0.05[b]
Cutting force (N)		6.12±0.09[a]	7.50±0.11[b]	7.47±0.10[b]	7.55±0.13[b]
Quality					
Puffing		1[a]	1[a]	1[a]	1[a]
Rollability		1[a]	1[a]	1[a]	1[a]
Loss of weight (%)		17.95±0.62[a]	21.60±0.19[b]	21.25±0.19[b]	18.64±0.70[a]
Proximate composition (% db)					
Protein		8.91±0.04[a]	9.25±0.06[b]	9.27±0.01[b]	9.23±0.02[b]
Lipids		5.10±0.09[a]	5.56±0.09[b]	5.54±0.08[b]	5.51±0.04[b]
Ash		1.63±0.08[a]	1.78±0.03[b]	1.76±0.03[b]	1.76±0.07[b]
Crude fibre		1.50±0.06[a]	2.18±0.02[b]	2.19±0.05[b]	2.18±0.08[b]
Carbohydrates[†]		82.86±0.02[a]	81.23±0.09[a]	81.24±0.07[a]	81.30±0.03[a]
Nutritional					
Lysine content (g kg^{-1} protein)		22.45±0.31[a]	32.45±0.29[b]	32.12±0.36[b]	32.02±0.40[b]
Tryptophan content (g kg^{-1} protein)		5.02±0.11[a]	7.20±0.13[b]	7.17±0.09[b]	7.07±0.11[b]
In vitro protein digestibility (%)		84.00±0.26[a]	83.55±0.45[a]	83.68±0.23[a]	84.13±0.22[a]

Result is presented as mean of three replicates ± standard error. Means with the same letter in the same row are not significantly different (Dunnet > 0.05). *ΔE = total color difference; [†]determined by difference.

(1041/1.7k) flour using a lime solution of 5.4 g $Ca(OH)_2$ L^{-1} of distilled water. Martínez-Flores et al. (2004) produced tortillas from extruded fresh masa with pH values of 6.98. Méndez-Albores et al. (2004) reported a pH value of 8.33 for maize tortillas produced with the Mexican traditional nixtamalization process using 3% (w/w) lime. These results are in close agreement with those obtained in this research.

In nixtamalized products, the lime concentration represents an important factor in color, odor, flavor, shelf life and texture characteristics; when the lime content is not sufficient to give the characteristic alkaline flavor, the tortillas are rejected by consumers. Likewise, if this compound is in excess, tortillas become astringent and are also rejected. In this research, tortillas from MTMP prepared with the maximum lime concentration (0.5% w/w) presented the highest pH value (7.97). Bedolla and Rooney (1984) reported that tortillas with pH between 7.1 to 7.2 retain better the characteristic flavor with acceptable shelf life.

Regarding surface color analysis, tortillas from MASECA® had a higher whiteness than those from MTMP, as indicated by their higher Hunter L value (75.34) and lower ΔE (25.81). In the case of tortillas produced from MTMP, as the $Ca(OH)_2$ increases, lower Hunter L values were registered (Table 2). Moreover, in these tortillas ΔE was increasing, reaching values up to 38.22. In general, tortilla color was affected by the addition of the different $Ca(OH)_2$ concentrations and consequently tortillas presented a yellowish color, as shown by the ΔE values. Ayala-Rodríguez et al. (2009) reported Hunter L and ΔE values of 80.77 and 21.43 for tortillas elaborated with MASECA® (using a white standard tile as a reference). Those differences could be related to the origin of MASECA®, MC, or time/temperature of tortilla baking. Serna-Saldívar (1996) reported that some commercial nixtamalized maize flours are added with bleaching agents, which may explain the slighter higher whiteness of MASECA® in comparison with MTMP tortillas prepared with 0.125% lime.

Changes in the color of tortillas are directly attributed to the amount of lime retained during the cooking of the nixtamal. Lime content affects the tortilla color even when tortillas are produced from white maize grains, and the color intensity is closely related to carotenoid pigments, flavonoids, and pH. However, the development of color during the alkaline-cooking process is more complex, considering that the $Ca(OH)_2$ reacts with the different

pigments found in the grain and interferes with browning reactions such as caramelization and Maillard reactions (Gómez et al., 1987).

Textural and quality properties

Related to textural properties, tortillas from MASECA® presented tensile strength and cutting force values of 0.77 and 6.12 N, respectively (Table 2). These values were slightly lower than those recorded in tortillas from MTMP. In general, the increment in the calcium content used during the MTMP had no effect on these textural properties; thus, the average tensile strength and cutting force values were 1.10 and 7.51 N, respectively (Table 2). These texture values are similar to those reported in tortillas produced with high moisture content in stored maize grain using the traditional nixtamalization process (Méndez-Albores et al., 2003). Consequently, tortillas prepared from MTMP were slightly more stretchable (to lengthen, widen or distend), elastic, and resistant to rupture and cracking than MASECA® tortillas. Table 2 also shows some quality properties of tortillas. Tortillas made from MASECA® and MTMP showed similar puffing values; all tortillas presented a value of 1, which indicates complete puffing (75 to 100%).

A good puffing is obtained when two layers are formed in the tortilla; these layers, produced during the cooking process, are impermeable, retaining the steam that gives rise to the puffing during heating. Moreover, all tortillas evaluated presented a good rollability, with a value close to 1 (Table 2), defined as no breaking; therefore, tortillas were also considered within the acceptable margins of quality, presenting a soft texture and rolled without breaking. Table 2 shows the loss of weight during tortilla baking. Tortillas from MASECA® and MTMP with $Ca(OH)_2$ concentration of 0.5% had a similar loss of weight average value (18.29% w/w). On the contrary, highest values (21.43% w/w) were registered in tortillas from MTMP prepared with lime concentrations of 0.125 and 0.25% (w/w), respectively. This phenomenon is interesting, due to the fact that tortillas produced with the highest lime concentration retained slightly more MC. Figueroa-Cárdenas et al. (2001) reported loss of weight values around 23% for tortillas prepared from nixtamal, and Arámbula-Villa et al. (2001) reported values up to 23.52% of loss of weight for tortillas from extruded instant maize flour supplemented with various types of lipids. These results are consistent with the values found in this research. The lower the loss of weight the better tortilla quality, due to the fact that MC plays an important role on tortilla yield and texture (Arámbula et al., 1999).

Proximate and nutritional analysis

Table 1 shows the proximate composition and nutritional

properties of MASECA® and maize flours. Results are quite similar to those reported previously by other researchers (Aguilar-Miranda et al., 2002; Ayala-Rodríguez et al., 2009). On the other hand, Table 2 shows the proximate composition of tortillas from MASECA® and MTMP. Tortillas produced from MASECA® presented an average protein content of 8.91%; this result is consistent with the data published by Ayala-Rodríguez et al. (2009) who reported protein content of about 8.93% for MASECA® tortillas. Moreover, protein content was slightly reduced after the MTMP process. Protein content in raw maize was 9.38% db (Table 1), and when nixtamalized, protein was reduced to 9.25% db (Table 2). This reduction (1.4%) is consistent with previous reports, which indicate that protein is lost during tortilla elaboration, possibly due to solubilization of some protein fractions (Bello-Pérez et al., 2003; Milán-Carrillo et al., 2004; Cuevas-Martínez et al., 2010).

In general, tortillas produced with MTMP presented higher crude protein values in comparison with MASECA® tortillas, inherent to the maize variety and the nixtamalization process used in this research; and no statistical differences were observed at the three different alkali concentrations tested (Table 2). The same trend was observed in the case of the rest of the components (lipids, ash, and crude fibre). Gómez et al. (1987) mentioned that the compositional analysis of nixtamalized maize flour is similar to that of raw maize. However, in this research, tortillas from MTMP presented higher values in lipids (5.54 versus 5.10% db), ash (1.77 versus 1.63% db), and crude fibre (2.18 versus 1.50% db), than MASECA® tortillas (Table 2). In the case of carbohydrates, statistical differences were not observed in tortillas from all treatments. In resume, the higher values in those components are principally due to the use of integral maize during the MTMP.

Regarding the nutritional properties, lysine and tryptophan were reduced in MASECA® tortillas in 5.5 and 1.4%, respectively. The same phenomenon was observed in tortillas prepared from MTNP, lysine and tryptophan were reduced in 4.99 and 1.84%, respectively (Tables 1 and 2). Mora-Avilés et al. (2007) reported that lysine and tryptophan content in quality protein maize (QPM) were reduced in 8% after lime treatment (traditional nixtamalization) while in regular maize they were reduced 25 and 15%, respectively. Rojas-Molina et al. (2008) reported reductions of 36 and 32% in the total lysine and reactive lysine, as well as 38.7% for tryptophan content during traditional nixtamalization of QPM (H-368C). These differences in amino acid reductions are attributable to the conditions used during the nixtamalization process, such as lime concentration, temperature, cooking and steeping time. Also, the protein solubility distribution and the essential amino acids location could partially explain the protein quality changes observed during nixtamalization.

Tryptophan, an amino acid highly sensitive to the

thermal-alkaline treatment, can be considered the first limiting amino acid of MTMP tortillas (7.15 g kg^{-1} protein), the second limiting amino acid being lysine (32.19 g kg^{-1} protein), covering 74 and 59% of the FAO/WHO requirements (FAO/WHO/UNU, 1985). Table 1 also shows the *in vitro* protein digestibility (PD) values of flours; as expected, MASECA® flour had the highest PD value (80.53%) in comparison with maize flour (76.18%). In this context, Ayala-Rodríguez et al. (2009) reported PD values of 73.65 and 74.98% for MASECA® flour and nixtamalized transgenic maize flour (NTMF), respectively.

In this research, this parameter increased when the flour was processed into tortillas (up to 84.13%), and no statistical differences were observed in tortillas from MASECA® and from MTMP with the three different Ca(OH)$_2$ concentrations tested (Table 2). The authors before mentioned also reported PD values of 76.80 and 77.36% for tortillas from MASECA® and NTMF, respectively. Wolzak et al. (1981) reported that the response of different types of proteins to the multi-enzyme assay is different. They found highly significant correlation between *in vivo* and *vitro* studies for unprocessed vegetable samples, but this was not the case when samples were thermally processed.

Viscoamylographic properties

When starch granules are heated in water excess, granules irreversibly swell and lose their native birefringence and crystalline order and form a paste. In contrast, pasting is the phenomenon following gelatinization, involving granular swelling, exudation of molecular components and eventually, total disruption of the granule (Atwell et al., 1988). Figure 1 (profile A) shows the typical viscoamylographic curves of MASECA® and maize flours. MASECA® flour presented the highest peak of viscosity (898 cP) in comparison with maize flour (765 cP). On the contrary, Figure 1 (profile B) shows the viscoamylographic curves of tortillas. In the case of viscosity peak, the same trend was observed; highest viscosity was registered in tortillas from MASECA®, reaching values up to 239 cP. The presence of gums and the maize variety in the commercial flour probably were factors that contributed to the higher viscosity of the tortillas, which was significantly different from those of the MTMP. In the case of tortillas from MTMP, as the lime concentration increases, lower values in viscosity were observed. Thus, tortillas produced with Ca(OH)$_2$ concentrations of 0.125, 0.25 and 0.5% (w/w) presented viscosity values of 100, 94, and 67 cP, respectively.

When cooled, pastes will form a viscoelastic gel, the molecular reassociation that occurs during the cooling and storage of gelatinized starch molecules to form an ordered structure are defined as starch setback or "retrogradation". Regarding the setback, statistical differences were not observed in the case of flours; samples presented an average value of 409 cP (Figure 1, profile A). Moreover, there were no statistical differences in setback between tortillas produced from MASECA® and from MTMP with Ca(OH)$_2$ concentration of 0.5% (w/w); the average setback value in these tortillas was 52 cP. The same phenomenon was observed in the case of tortillas produced from MTMP with lime concentrations of 0.125 and 0.25%; however, setback was slightly higher, presenting an average value of 72 cP (Figure 1, profile B). Starch setback is influenced by the fine structure of amylopectin and the amylose/amylopectin ratio (Ward et al., 1994).

During tortilla production, there is enough time and moisture content to gelatinize starch granules, to disperse some of the starch, and to have much of the amylose become insoluble (retrograde) by the time the product has cooled to room temperature. Retrogradation of amylopectin is believed to involve association of its outer branches and requires a longer time and a lower temperature than amylose to retrograde. Thus, retrogradation of amylopectin occurs with time after the product has cooled (Keleckci et al., 2002). It is most likely that extreme pH values are also a contributing factor to starch degradation, resulting in lower viscosity, as in the case of tortillas from MTMP prepared with higher lime concentrations.

In this work, it appears that viscosity peak and setback in tortillas from MTMP were more influenced by the different Ca(OH)$_2$ concentrations evaluated.

Sensory evaluation

In general, tortillas made from MASECA® and MTMP with calcium hydroxide concentrations of 0.125 and 0.25% (w/w) had similar acceptability during sensory evaluation (Table 3). The panelist appreciated that tortillas from these treatments had similar aroma (descriptors: like maize, lime, nixtamal), appearance (descriptors: evenness, opacity, yellowness, puffed), flavor (descriptors: like maize, bitter, astringent, nixtamal), manual and oral texture (descriptors: softness, roughness, fragility, bending, rollable, doughness, adhesiveness, tooth packing), and after taste (descriptors: bitter, astringent, nixtamal) attributes. On the contrary, when tortillas were produced from MTMP with the maximum lime concentration (0.5% w/w), panelist identified principal effects on changes in four attributes and seven descriptors: aroma (lime), appearance (opacity and yellowness), flavor (bitter and astringent), and after taste flavor (bitter and astringent). In general, good quality tortillas were obtained using the MTMP produced with lime concentrations of 0.125 and 0.25% (w/w), respectively. This statement was taken into account since sensory properties were statistically similar to those obtained for the control tortillas (MASECA®).
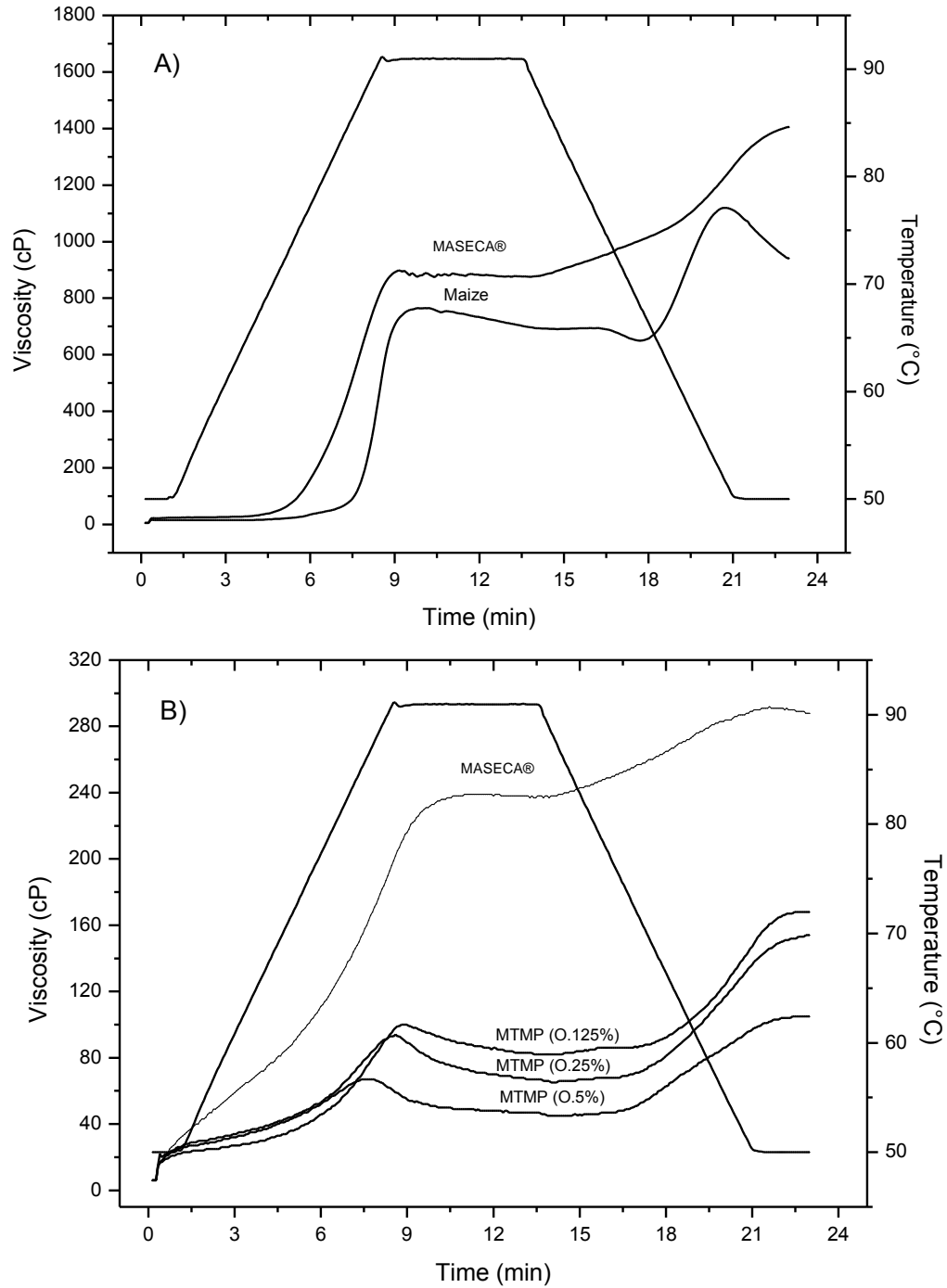
Figure 1. Viscoamylographic profiles of flours (profile A) and tortillas (profile B) measured by rapid visco analyzer.

Conclusion

The fact that tortillas from MTMP with calcium hydroxide concentrations of 0.125 and 0.25% (w/w) has similar sensory properties to those from commercial flour MASECA® is important to take advantage of the process, since microwave nixtamalization does not produce wastewater and produce tortillas with acceptable physicochemical, textural, quality, compositional/nutritional and pasting properties. A carefully designed process tunnel in which maize is properly exposed to the microwave field could provide

Table 3. Sensory properties of tortillas from MASECA® and MTMP prepared with different $Ca(OH)_2$ concentrations.

Attribute	Descriptor	MASECA®	MTMP (lime content % w/w)		
			0.125%	0.25%	0.5%
Aroma	Like maize	4.12±0.46[a]	4.57±0.31[a]	4.23±0.17[a]	4.43±0.15[a]
	Lime	4.28±0.23[a]	3.97±0.15[a]	4.43±0.21[a]	7.43±0.20[b]
	Nixtamal	4.41±0.49[a]	4.57±0.37[a]	4.77±0.15[a]	4.83±0.06[a]
Appearance	Evenness	4.37±0.42[a]	4.18±0.22[a]	4.31±0.45[a]	4.09±0.33[a]
	Opacity	5.13±0.32[a]	5.09±0.36[a]	5.12±0.34[a]	7.00±0.28[b]
	Yellowness	4.00±0.46[a]	4.19±0.38[a]	5.27±0.33[b]	7.29±0.11[c]
	Puffed	4.37±0.31[a]	4.12±0.86[a]	4.22±0.45[a]	4.18±0.74[a]
Manual texture	Softness	4.63±0.25[a]	4.31±0.17[a]	4.26±0.38[a]	4.33±0.56[a]
	Roughness	4.77±0.35[a]	4.82±0.24[a]	4.79±0.62[a]	4.90±0.73[a]
	Fragility	4.53±0.30[a]	4.12±0.34[a]	4.27±0.44[a]	4.19±0.71[a]
	Bending	4.20±0.10[a]	4.16±0.37[a]	4.35±0.38[a]	4.29±0.55[a]
	Rollable	4.51±0.23[a]	4.27±0.42[a]	4.19±0.21[a]	4.44±0.49[a]
Flavor	Like maize	4.43±0.41[a]	4.69±0.55[a]	4.72±0.48[a]	4.57±0.53[a]
	Bitter	4.33±0.15[a]	4.16±0.91[a]	4.51±0.49[a]	6.75±0.77[b]
	Astringent	4.27±0.33[a]	4.30±0.54[a]	4.90±0.71[a]	6.13±0.31[b]
	Nixtamal	4.93±0.46[a]	5.01±0.22[a]	4.97±0.44[a]	5.22±0.38[a]
Oral texture	Softness	5.53±0.32[a]	5.54±0.75[a]	5.48±0.16[a]	5.21±0.33[a]
	Doughness	4.50±0.27[a]	5.00±0.81[a]	5.11±0.67[a]	4.98±0.89[a]
	Adhesiveness	5.50±0.30[a]	5.11±0.34[a]	5.19±0.45[a]	5.20±0.36[a]
	Tooth packing	4.87±0.15[a]	4.89±0.55[a]	4.85±0.91[a]	4.90±0.16[a]
After taste	Bitter	4.53±0.29[a]	4.55±0.33[a]	4.61±0.82[a]	6.87±0.88[b]
	Astringent	4.81±0.14[a]	4.92±0.16[a]	4.89±0.58[a]	7.12±0.99[b]
	Nixtamal	4.55±0.18[a]	5.00±0.11[a]	5.12±0.34[a]	5.36±0.67[a]

Means with the same letter in the same row are not significantly different (Dunnet > 0.05). Mean of three replicates ± standard error.

utilization to industrial scale without the size restriction usually imposed by the domestic microwave heating systems. More research, however, pertaining to the shelf-life of nixtamalized flours and tortillas produced with this modified process, needs to be conducted.

ACKNOWLEDGEMENTS

REFERENCES

Aguilar-Miranda ED, López GM, Escamilla-Santana C, Barba de la Rosa AP (2002). Characteristics of maize flour tortilla supplemented with ground *Tenebrio molitor* larvae. J. Agric. Food Chem. 50:192-195.

AOAC (2000). Official Methods of Analysis of the AOAC. (Methods 920.39C, 923.03, 925.10, 943.02, 960.52, 962.09E, 982.30G).17th Ed., Washington, DC, USA. Association of Official Analytical Chemists.

Arámbula VG, Mauricio SRA, Figueroa CJD, González-Hernández J,

Ordorica FCA (1999). Corn masa and tortillas from extruded instant corn flour containing hydrocolloids and lime. J. Food Sci. 64:120-124.

Arámbula-Villa G, González-Hernández J, Ordorica-Falomir CA (2001). Physicochemical, structural, and textural properties of tortillas from extruded instant corn flour supplemented with various types of corn lipids. J. Cereal Sci. 33:245-252.

Atwell WA, Hood LF, Lineback DR, Varriano-Marston E, Zobel HF (1988). The terminology and methodology associated with basic starch phenomena. Cereal Foods World 33:306-311.

Ayala-Rodríguez AE, Gutiérrez-Dorado R, Milán-Carrillo J, Mora-Rochín S, López-Valenzuela JA, Valdez-Ortiz A, Paredes-López O, Reyes-Moreno C (2009). Nixtamalised flour and tortillas from transgenic maize (*Zea mays* L.) expressing amarantin: Technological and nutritional properties. Food Chem. 114:50-56.

Bedolla S, Rooney LW (1984). Characteristics of US and Mexican instant maize flours for tortilla and snack preparation. Cereal Foods World 29:732-735.

Bello-Pérez LA, Osorio-Díaz P, Agama-Acevedo E, Solorza-Feria J, Toro-Vázquez JF, Paredes-López O (2003). Chemical and physicochemical properties of dried wet masa and dry masa flour. J. Sci. Food Agric. 83:408-412.

Cuevas-Martínez D, Moreno-Ramos C, Martínez-Manrique E, Moreno-Martínez E, Méndez-Albores A (2010). Nutrition and texture evaluation of maize-white common bean nixtamalized tortillas. Interciencia 35:828-832.

FAO/WHO/UNU (1985). Energy and Protein Requirements. Report of a Joint FAO/WHO/UNU Expert Consultation. WHO Technical Report Series. Geneva, Switzerland. p. 724.

Figueroa-Cárdenas JD, Acero-Godínez MG, Vasco-Méndez NL, Lozano-Guzmán A, Flores-Acosta LM, González-Hernández J

(2001). Fortificación y evaluación de tortillas de nixtamal. Arch. Latinoam. Nutr. 51:293-302.

Flores-Farías R, Martínez-Bustos F, Salinas-Moreno Y, Ríos E (2002). Caracterización de harinas comerciales de maíz nixtamalizado. Agrociencia 36:557-567.

Gómez MH, Rooney LW, Waniska RD, Pflugfelder RL (1987). Dry maize masa flours for tortilla and snack production. Cereal Foods World 32:372.

Jackson DS, Rooney LW, Kunze OR, Waniska RD (1988). Alkaline processing properties of stress-cracked and broken corn (*Zea mays* L.). Cereal Chem. 65:133-137.

Keleckci NN, Pascut S, Waniska RD (2002). The effects of storage temperature on staling of wheat flour tortillas. J Cereal Sci. 37:377-380.

López-Cervantes J, Sánchez-Machado DI, Rosas-Rodríguez JA (2006). Analysis of free amino acids in fermented shrimp waste by high-performance liquid chromatography. J. Chromatogr. A. 1105:106-110.

Martínez-Bustos F, Figueroa JDC, Sánchez-Sinencio F, González-Hernández J, Martínez JDL, Ruiz MT (1996). Extrusion apparatus for the preparation of instant fresh corn dough or masa. US patent number 5,558,886.

Martínez-Flores HE, Gaytán-Martínez M, Figueroa-Cárdenas JD, Martínez-Bustos F, Reyes-Vega ML, Rodríguez-Vidal A (2004). Effect of some preservatives on shelf-life of corn tortillas obtained from extruded masa. Agrociencia 38:285-292.

Méndez-Albores JA, Arámbula VG, Vázquez BME, Mendoza EM, Preciado ORE, Moreno-Martínez E (2003). Effect of high moisture maize storage on tortilla quality. J. Food Sci. 68:1878-1881.

Méndez-Albores JA, Villa GA, Del Río-García JC, Martínez EM (2004). Aflatoxin-detoxification achieved with Mexican traditional nixtamalization process (MTNP) is reversible. J. Sci. Food Agric. 84:1611-1614.

Milán-Carrillo J, Gutiérrez-Dorado R, Cuevas-Rodríguez EO, Garzón-Tiznado JA, Reyes-Moreno C (2004). Nixtamalized flour from quality protein maize (*Zea mays* L.). Optimization of alkaline processing. Plant Foods Hum. Nutr. 59:35-44.

Mora-Avilés A, Lemus-Flores B, Miranda-López R, Hernández-López D, Pons-Hernández JL, Acosta-Gallegos JA, Guzmán-Maldonado SH (2007). Effects of common bean enrichment on nutritional quality of tortillas produced from nixtamalized regular and quality protein maize flours. J. Sci. Food Agric. 87:880-886.

Nurit E, Tiessen A, Pixley KV, Palacios-Rojas N (2009). Reliable and inexpensive colorimetric method for determining protein-bound tryptophan in maize kernels. J. Agric. Food Chem. 57:7233-7238.

Pedrero DL, Pangborn RM (1989). Evaluación Sensorial de los Alimentos. Métodos Analíticos. México, DF., Alambra Mexicana.

Pedroza-Islas R, Durán de Bazúa C (1990). Aerobic treatment of maize-processing wastewater in a 50-liter rotating biological reactor. Biol. Wastes 32:17-27.

Pérez-Flores GC, Moreno-Martínez E, Méndez-Albores A (2011). Effect of microwave heating during alkaline-cooking of aflatoxin contaminated maize. J. Food Sci. 76:48-52.

Plasencia J (2004). Aflatoxins in maize: a Mexican perspective. J. Toxicol. 23:155-177.

Rojas-Molina I, Gutiérrez E, Cortés-Acevedo ME, Falcón A, Bressani R, Rojas A, Ibarra C, Pons-Hernández JL, Guzmán-Maldonado H, Cornejo-Villegas A, Rodríguez ME (2008). Analysis of quality protein changes in nixtamalized QPM flours as a function of the steeping time. Cereal Chem. 85:409-416.

Salmerón-Alcocer A, Rodríguez-Mendoza N, Pineda-Santiago V, Cristiani-Urbina E, Juárez-Ramírez C, Ruíz-Ordaz N, Galíndez-Mayer J (2003). Aerobic treatment of maize-processing wastewater (nejayote) in a single-stream multi-stage reactor. J. Environ. Eng. Sci. 2:401-406.

SAS (1998). Introductory guide for personal computers. 6.12 ed. Cary, N.C.: SAS Institute.

Serna-Saldívar SO (1996). Química, almacenamiento e industrialización de los cereales. México, DF. A.G.T. Editor, S.A. p. 519.

Velasco-Martinez M, Angulo O, Vázquez-Couturier DL, Arroyo-Lara A, Monroy-Rivera JA (1997). Effect of dried solids of nejayote on broiler growth. Poult. Sci. 76:1531-1534.

Ward KEJ, Hoseney RC, Seib PA (1994). Retrogradation of amylopectin from maize and wheat starches. Cereal Chem. 71:150-155.

Wolzak A, Bressani R, Gómez-Brenes R (1981). A comparison of *in vivo* and *in vitro* estimates of protein digestibility of native and thermally processed vegetable proteins. Plant Foods Hum. Nutr. 31:31-43.