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Physicochemical characterization of flours and rheological and textural changes of masa and tortillas obtained from maize fertilized with nejayote and ovine manure

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A b s t r a c t. The agronomic management of maize (*Zea mays* L.) modifies the structure and composition of maize grain and its products like flour, masa, and tortillas. Results have shown that the protein content in flour obtained from maize grains treated with nejayote applied at 150 m^3 ha^{-1} (10.36 g × 100 g^{-1}) and nejayote applied at 75 m^3 ha^{-1} with ovine manure applied at 25 t ha^{-1} (10.17 g × 100 g^{-1}) was higher than that determined in flour treated with chemical fertilizer (10.05 g × 100 g^{-1}). The flours obtained from maize fertilized without nejayote showed the highest viscosity values and the lowest values were for chemical fertilizer (2816 mPa s) and 75 m^3 ha^{-1} of nejayote with ovine manure applied at 25 t ha^{-1} (2498 mPa s). The highest elastic and viscous moduli were obtained for masa with the following fertilization regimes: 75 m^3 ha^{-1} of nejayote with 25 t ha^{-1} of ovine manure, and 150 m^3 ha^{-1} of nejayote with 25 t ha^{-1} of ovine manure and the lowest values of these parameters were obtained for 75 m^3 ha^{-1} of nejayote with 50 t $ha^{-1}$of ovine manure. The cohesiveness of masa was the lowest for maize fertilized with nejayote applied at 75 to 150 m^3 ha^{-1}, and 50 t ha^{-1} of ovine manure. The highest concentration of 150 m^3 ha^{-1} for nejayote and the lowest level for ovine manure applied at 25 t ha^{-1} had a positive influence on the production of nixtamal and tortilla.

K e y w o r d s: ovine manure, nejayote, pasting profiles, rheological parameters, masa, tortilla

INTRODUCTION

Maize (*Zea mays* L.) is the most widely produced and consumed cereal in Mexico, and it is the raw material used to manufacture masa and tortilla. However, the maize plant requires nutrients like nitrogen, phosphorus, and potassium to produce high yields of seeds and grains. These nutrients may enter the soil through chemical fertilizers or manure and crop residues (Salazar *et al*., 2009). Pollution and the high cost of chemical fertilizers are making it necessary to use novel alternatives to fertilization. For example, the application of liquid manure to maize increases its yield and improves the balance amount of nutrients that the plant consumes and generates (Schröder *et al*., 2015). Likewise, nejayote is a by-product of the nixtamalization process that contains approximately 2% solids (pericarp, proteins, starch, calcium, germ, and others) (Valderrama-Bravo *et al*., 2012). Nejayote damages the environment because the small-scale producer disposes of it in the form of wastewater into the local drainage network. However, nejayote is an organic nutrient that contains ferulic acid, phenolic, and antioxidant compounds as well as colloids (Niño-Medina *et al*., 2009). Therefore, innovative and environmentally

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friendly research has been performed to reduce the pollutant load of nejayote. Examples of these activities include treatment by bioreactors and the use of chitosan to reduce biochemical oxygen demand (BOD) (López-Pacheco *et al*., 2019; Suarez-Meraz *et al*., 2016). Also, nejayote solids may be used as a substrate for fermentation with edible fungi, which has a positive influence on the content of free phenolic compounds and dietary fibre as well as its antioxidant activity, with potential use as a high added value food ingredient (Acosta-Estrada *et al*., 2019). Thus, nejayote treated with fermentation or composted and mixed with manure could be used as an alternative organic fertilizer. Mahmood *et al*. (2017) applied it as an organic nutrient source with urea, and showed that the growth and yield of maize were substantially improved by fertilizer application alongside organic manures and that the soil total organic content increased when inorganic fertilizers were applied in combination with organic manures. Zepeda-Bautista *et al*. (2007) applied different doses of fertilizer to maize seeds (N, P, and K); and reported that fertilization applied at 0.3 t ha^{-1} of nitrogen modifies maize structure, which consequently influences the nixtamalization process. On the other hand, Vázquez-Carrillo *et al.* (2015) mentioned that the physicochemical, rheological, and textural properties of masa as well as the quality of the tortillas depends on the maize type used and the conditions of the nixtamalization process. Valderrama-Bravo *et al*. (2017) reported that tortillas processed with hard maize (hybrid H-70) had a more rigid texture. Conversely, tortillas from soft endosperm contained more swollen starch granules, which improves softness. Osorio-Díaz *et al*. (2011) attributed these results to a higher retrogradation rate. Santiago-Ramos *et al.* (2017) reported that hard maize starch showed the highest gelatinization temperature and the lowest enthalpy due to its highly compacted endosperm and high amylose content. Tortillas of intermediate and soft grains had a higher retrogradation rate than the tortillas of hard grains. Therefore, the objective of this research was to evaluate changes in flours, masa, and tortillas obtained from maize grains fertilized organically with nejayote and ovine manure. This research is based on the assumption that fertilization is decisive in the growth and development phase of the maize plant. Different regimes of nutrient application or fertilizer type produce changes in grain quality. Therefore, it is to be expected that these differences would show in the physical and chemical properties of the flours, masa, and tortillas obtained from such maize grains.

MATERIALS AND METHODS

Field experiments were conducted in "Laguna Seca" Ranch, Ahuazotepec, Puebla, during the spring-summer agricultural cycle of 2016. The maize hybrid AS-722 (Aspros^{TM}) was sown at a density of 75 000 plants per ha. Field experiments were conducted in the "Laguna Seca" Ranch, Ahuazotepec, Puebla, during the spring-summer agricultural cycle of 2016. The maize hybrid AS-722 (Aspros^{TM}) was sown at a density of 75 000 plants per ha on April 25. The experimental plot was located at 20° 01' 51.6" N and 98° 07' 15.6" W, at an altitude of 2268 m a.s.l. The soil is characterized as medium textured Andisol. The experiment design was carried out as a factorial with two factors and three levels: nejayote (m^3 ha^{-1}): N0 = 0, N1 = 75, and N2 = 150 m and ovine manure (t ha^{-1}): A0 = 0, A1 = 25, and A2 = 50). An additional chemical fertilization treatment was established for comparison, where urea, ammonium diphosphate and potassium chloride ($CO(NH_2)_2$, $(NH_4)_2HPO_4$ and KCl) were used as sources of N, P and K, respectively (Agrogen^{TM}). Nejayote was obtained from the mills of the region and it was produced during a nixtamalization process, in which maize grains (50 kg) were cooked (2 h) and steeped (16 h) in alkaline conditions 0.36% $Ca(OH)_2$, Nixtacal 1TM, Mexico) to make nixtamal, and then tortillas. On a daily basis the cooking liquid (nejayote) was collected in 30 L plastic containers. Nejayote characterization was performed by Dominguez-Hernández *et al*. (2020). The experimental plots were set up as randomized blocks designed with three replicates per treatment. Each experimental unit consisted of six rows 10 m long and 0.8 m wide. The nejayote and ovine manure mixtures were composted in plastic containers for 20 days, stirring daily the first five days of nejayote collection time, and then stirring every other day the remaining time (15 days).

The organic and chemical fertilizers were applied manually at 20, 40, and 60 days after sowing (Fig. 1). A physicochemical analysis (pH, organic matter, nitrogen, phosphorus and potassium) of nejayote, manure and mixtures were presented in Dominguez-Hernández *et al*. (2020).

The harvest was carried out by hand when the formation of a black layer was observed, which indicated physiological maturity. The drying process was natural.

Maize grains were cleaned by removing foreign material, impurities, and broken grains. Twenty clean grains were measured (length, diameter, and thickness) and the geometric mean diameter (Dg) was calculated according to Vilche *et al*. (2003) and Valderrama-Bravo *et al*. (2017). The hectolitre weight, flotation index and weight of 100 grains were evaluated according to Abdalaa *et al*. (2018). Moisture content was evaluated according to the AACC method 44-11 (AACC, 2000).

Samples were prepared by cooking 500 g of maize in a solution of 1500 mL of water and 5g of food-grade calcium hydroxide (Fermont, Monterrey, Mexico). The maize samples were boiled at 90°C for 40-45 min and steeped for 12 h. The nejayote was separated, and the nixtamalized samples were washed with 750 mL of water and milled in a manually operated mill (Nixtamatic, Edo. Mex., México). The nixtamal obtained was dried by using a forced air oven, at 55°C during 24 h. The dried samples were milled using

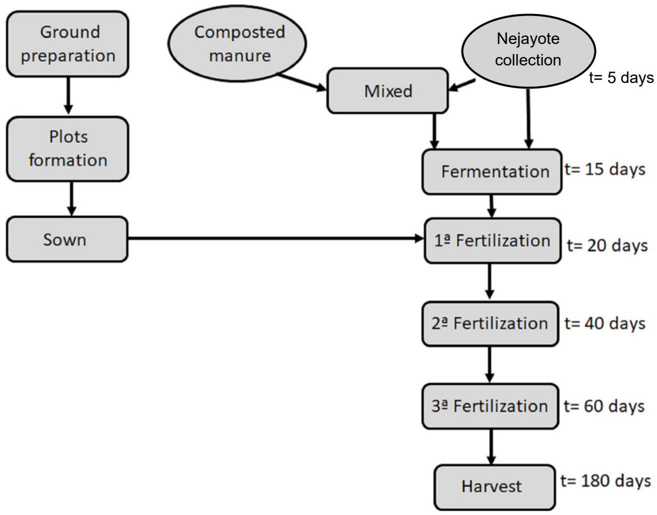

Fig. 1. Experimental diagram.

a hammer mill (Pulvex 200, Mexico) with a 0.8 mm mesh. The maize flours thus obtained were packed in airtight polyethylene bags and stored at 4°C until use.

Chemical analysis of maize flours, moisture content, ash, protein (N x 5.85), fat and total fibre were evaluated according to AACC methods 44-15, 08-01, 46-13, 30-25 and 32-05, respectively (AACC, 2000).

The water solubility index (WSI) and swelling power (SP) were determined by applying the methodology described by Ayala-Rodríguez *et al*. (2009) and modified in the laboratory. Maize flour (1 g, dry base) was weighed in a 15 mL centrifuging tube. Then 10 mL of distilled water was added. The flour suspensions were heated and shaken at 30°C for 30 min in a water bath. Then they were centrifuged at 3 000 rpm for 15 min, the supernatant was decanted, and sediments were weighed to ascertain the swelling power. The supernatant was dried at 105°C for 24 h. All measurements were conducted in triplicate. The WSI and SP were calculated by Eqs (1) and (2), as:

$$WSI = \frac{weight\ of\ dried\ supernatent\ solids}{weight\ of\ sample} 100, \quad (1)$$

$$SP = \frac{weight\ of\ sediment\ paste}{weight\ of\ sample - weight\ of\ dried\ supernatent\ soilds}. \quad (2)$$

The starch pasting profiles of nine water suspension samples were analysed using an Anton Paar MCR 102 Rheometer, equipped with a starch cell using the methodology proposed by Rincon-Londoño *et al*. (2016). The starch samples (3 g) were suspended in 18 mL of water. The suspension was heated over 5 min from 50 to 90°C; next, the suspension was maintained at a constant temperature of 90°C for 5 min and finally the samples were cooled down to 50°C for 5 min. The rotation speed of the system was 194 rpm.

Maize flours were rehydrated until they reached 54% of moisture to obtain rollable masa. The colour of the masa samples were measured using a Minolta CR-300 colorimeter (Minolta, Osaka, Japan). The colour parameters CIELAB (L*, a, and b, where L* = luminosity (100 = white, to 0 = black), the a = greenish-reddish, and b*= yellowish-bluish. The total colour difference was also calculated (ΔE) by Eq. (3). The L* = 98.75, a = 0.72 and b = 2.58 values were evaluated with a white reference:

$$\Delta E = \sqrt{(\Delta a)^2 + (\Delta b)^2 + (\Delta L*)^2}, \quad (3)$$

$\Delta a = a_{masa} - a_{reference}$, $\Delta b = b_{masa} - b_{reference}$,
$\Delta L* = L*_{masa} - L*_{reference}$.

The rheological tests of the masa were performed with the use of an Anton Paar MCR-102 rheometer equipped with a 2.5 mm rough plate. A dynamic deformation percentage sweep test was conducted to determine the linear viscoelastic region (LVR) in a range of 0.1 to 1% at 25°C. Frequency sweeps, which increased from 0.1 to 10 Hz, were evaluated to determine the storage (elastic) modulus (G´) and loss (or viscous) modulus (G´´) using the accompanying software.

Masa and tortilla analysis were performed using a Texture Analyser Brookfield Model CT3 25 K USA. Cylindrical masa (35 g) was formed with a stainless-steel mould (45 mm diameter and 15 mm high). Samples were kept in a polyethylene bag for 15 min, and then the texture profile analysis (TPA) of the masa was performed. The samples were measured to a 33% compression cycle using a TA General Probe Kit with a TA25/1000 test probe cylinder 50.8 mm diameter and 20 mm length at a speed of 1 mm s^{-1}. Two compression cycles were measured. Hence, it was possible to measure hardness, cohesiveness, adhesiveness, and elasticity. Three replications were conducted at 24 ± 1°C.

The masa was shaped into discs (diameter, 10.9 cm; thickness, 1.3 mm; and weight, 30 g) using a manual tortilla machine. The disc samples were laid on a "comal" frying pan and cooked at (270°C) for 20 s on one side, and then turned over and baked for another 30 s, finally, they were turned over again until they were inflated and bubbles were formed.

The tensile strength of the tortillas was measured using samples of 34 x 70 mm (width x length). The samples were placed in retention pincers TA-DGA dual grip, and the test was carried out at a velocity of 2 mm s^{-1} until the tortilla was fragmented. The tensile strength was expressed as the peak force (N) required to break the tortilla. Also, the extensibility was measured as the length of the masa until the breaking/cutting point. The tortilla rollability test was carried out by rolling the tortillas around an aluminium cylinder with a 2.5 cm diameter and 17.9 cm length at 1 mm s^{-1} of velocity. The force required to roll the tortilla was reported in (N).

The Minitab® Statistical software, version 15 (Minitab Inc., State College PA, USA) was used to analyse data by applying ANOVA at a probability $p \leq 0.05$, and significant differences among the means were defined by using the Tukey test. All of the measurements were conducted three times.

RESULTS AND DISCUSSION

The results of the physical characterization of the grains such as hectolitre weight and moisture showed no significant difference. The Mexican Official Standard NMX-FF-024/1-SCFI-2002 (SAGARPA, 2002), establishes a maximum moisture content of 14% for the conservation and storage of grains and a minimum hectolitre weight of 74 kg hl^{-1}. The moisture values obtained were <14%, and the hectolitre weight was >74 kg hl^{-1}, both measures are within limits established in the Mexican Official Standard. Maize treated with organic fertilizer increased the weight of the 1000 grains; however, the weight of the 1000 grains for corn treated with chemical fertilizer and without fertilizer showed the lowest values (Table 1). The flotation index showed a significant difference because according to NMX-FF-024/1-SCFI-2002 (SAGARPA, 2002) the grains are classified as hard and very hard.

Chemicals analysis of the flours obtained from the maize treated with organic fertilizer showed a significant difference ($p \leq 0.05$) between all treatments of the organically fertilized flours for protein, fat, ashes and fibre (Fig. 2). The determinations of the moisture values for the flours ranged from 6.78 to 7.31 g 100 g^{-1}, low values that may increase their shelf life during storage. The protein content of the flour obtained from maize grains N2-A0 (without manure 10.36 g 100 g^{-1}) and N1-A1 (10.17 g 100 g^{-1}) was higher than that of the flour from treatment with chemical fertilizer (10.05 g 100 g^{-1}). These results corresponded with those of Flores-Farias *et al*. (2000), who reported protein contents of maize flours of between 8.5-10.27 g 100 g^{-1}. Nonetheless, the protein contents obtained in our research were higher than the ones obtained by Bello-Pérez *et al*. (2014) for traditional flour (7.72 g 100 g^{-1}) and Ayala-Rodríguez *et al*. (2009) for commercial flour MASECA (8.98 g 100 g^{-1}). Nevertheless, all tortillas were found to contain high levels of protein (10.73-12.44 g 100 g^{-1}). These results correspond with the findings of Vázquez-Carrillo *et al*. (2012), who determined the protein content in tortillas processed from landraces maize (9.76-12.54 g 100 g^{-1}) and in maize hybrids (8.24-11.34 g 100 g^{-1}). Besides, the highest lipid content was found for N2-A1 (3.25 g 100 g^{-1}), a similar result to the ohmic heated flours produced by the batch process (Ramírez-Jiménez *et al*., 2019). Some authors have attributed this phenomenon to a saponification reaction caused

Table 1. Physical characterization of maize grains

Maize	Hectolitre weight (kg hL^{-1})	Moisture (%)	Weight of 1000 grains (g)	Flotation index (%)	Hardness
N0-A0	$76.67^{a} \pm 2.52$	$11.53^{a} \pm 0.57$	$333.23^{ab} \pm 4.36$	$15.5^{a} \pm 1.0$	Hard
N0-A1	$78.17^{a} \pm 1.26$	$13.20^{a} \pm 0.26$	$378.66^{a} \pm 8.39$	$13.5^{ab} \pm 2.5$	Hard
N0-A2	$77.83^{a} \pm 0.29$	$12.50^{a} \pm 0.44$	$371.67^{ab} \pm 7.23$	$14.0^{ab} \pm 1.0$	Hard
N1-A0	$77.67^{a} \pm 0.58$	$12.20^{a} \pm 1.31$	$365.30^{ab} \pm 8.19$	$10.0^{ab} \pm 1.0$	Very hard
N1-A1	$77.67^{a} \pm 0.58$	$11.90^{a} \pm 0.20$	$359.35^{ab} \pm 9.17$	$9.5^{ab} \pm 0.5$	Very hard
N1-A2	$77.83^{a} \pm 0.76$	$13.43^{a} \pm 0.78$	$374.33^{a} \pm 7.77$	$11.5^{ab} \pm 1.5$	Very hard
N2-A0	$77.33^{a} \pm 1.26$	$13.23^{a} \pm 0.31$	$372.33^{ab} \pm 3.06$	$7.0^{b} \pm 1.0$	Very hard
N2-A1	$77.50^{a} \pm 0.50$	$12.17^{a} \pm 0.50$	$361.67^{ab} \pm 9.50$	$12.0^{ab} \pm 1.0$	Hard
N2-A2	$77.33^{a} \pm 0.2$	$12.80^{a} \pm 0.87$	$368.25^{ab} \pm 8.72$	$14.0^{ab} \pm 2.5$	Hard
ChF	$77.50^{a} \pm 0.50$	$12.47^{a} \pm 2.04$	$352.67^{bc} \pm 3.46$	$11.5^{ab} \pm 1.5$	Very hard

Mean ± standard deviation values followed by a different letter in each row, are significantly different ($p < 0.05$).

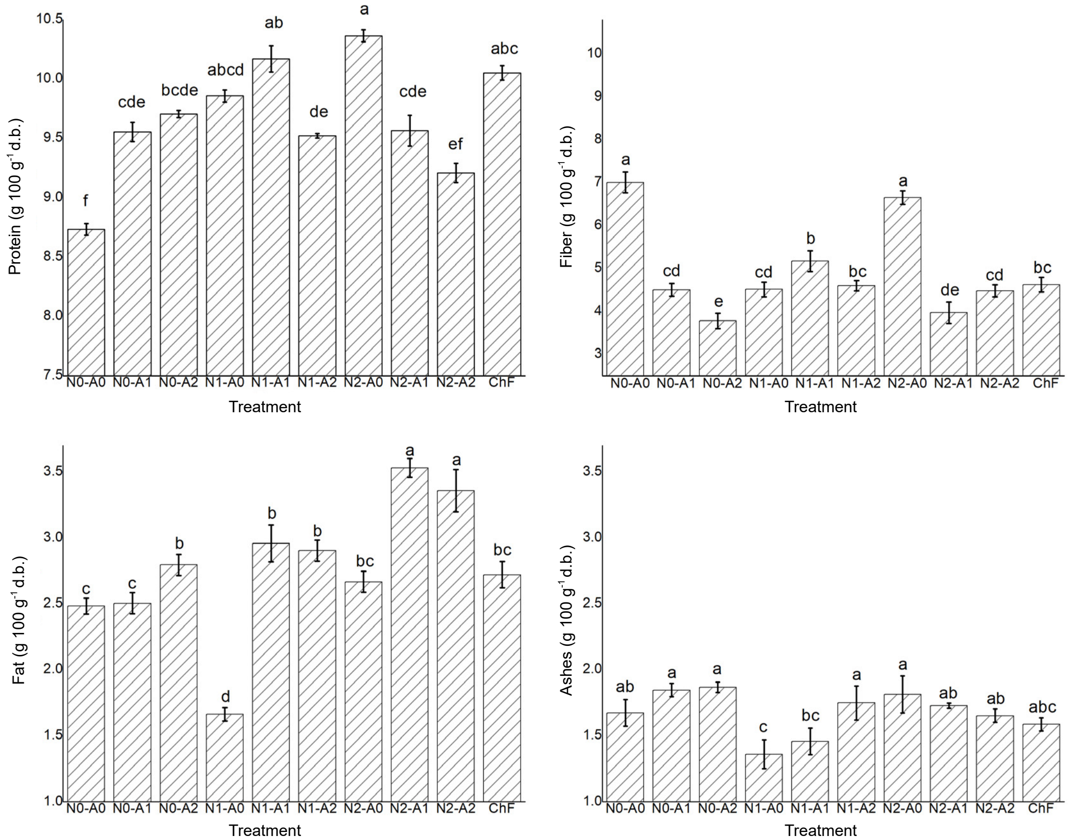

Fig. 2. Chemical analysis of different flours obtained from organically fertilized maize.

by the addition of $Ca(OH)_2$ (Martinez-Flores *et al*., 2006). Moreover, the formation of amylose-lipid complexes has been reported during the alkaline cooking of corn (Thachil *et al*., 2014), which in turn, may be affected by the extraction and the quantification of lipids. Also, the fibre content of N0-A0 and N2-A0 was significantly greater than that of N0-A2. Maize flour N2-A0 presented a high protein content, and fibre; both are components that contribute to the formation of the food matrix (Camelo-Méndez *et al*., 2017). Maize flour N2-A0 was obtained from grains that were fertilized with fermented nejayote, which is a liquid that is rich in fibre and gums. Furthermore, it has been reported that fibre content contributes to water holding capacity, to the development of viscosity, and to the formation and gelling of the food matrices, and it has a specific affinity for ions and aromatics compounds (Vitaglione *et al*., 2008).

Flours obtained from maize grains treated with organic fertilizer presented a significant difference ($p \leq 0.05$) in swelling power (SP) and water solubility index (WSI) (Fig. 3). The SP values are between 3.15-3.59 g 100 g^{-1}, these results are similar to those reported for nixtamalized maize flours (Ayala-Rodriguez *et al*., 2009; Flores-Farias *et al*., 2000). Maize flour N1-A2 was found to have a higher SP (3.59 g 100 g^{-1}) than the other flours. The SP in the nixtamalized maize flours depends on protein content, pH, enzyme-susceptible starch, and particle size and the SP is

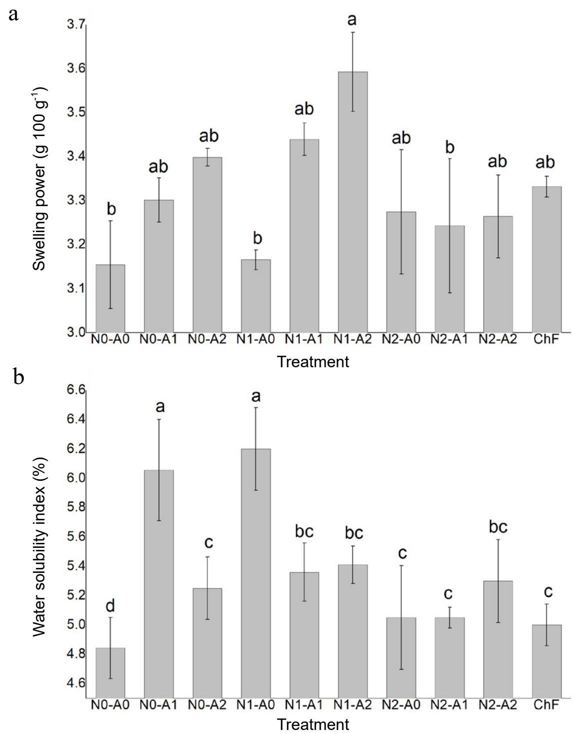

Fig. 3. Flours obtained from organically fertilized maize: a) swelling power and b) water solubility index.

related to the presence of natural gums from the hydrolysis of the pericarp or additives (Flores-Farías *et al*., 2000). Furthermore, the hydration properties are associated with the structure of the porous matrix, formed by polysaccharide chains that may contain large amounts of water due to the formation of hydrogen bonds (Kethireddipalli *et al*., 2002). Additionally, the protein within the maize flour can also hold water through weak forces such as hydrogen bonds (Shi *et al*., 2016). On the other hand, Jan *et al*. (2017) suggested that the high SP may be linked to a low amylose content, reinforcing the internal network within the starch granules, thereby restricting the swelling power. Water solubility index values (WSI) had a range of 4.84 to 6.20%. These numbers are higher than the ones previously reported by Shi *et al*. (2016) and Ayala-Rodríguez *et al*., (2009). However, these values are, according to other research, 4.4 to 7.2% (Flores-Farías *et al*., 2000). The water solubility index was determined as an approximate measure of the soluble starch and the soluble proteins in the flour samples (Wang *et al*., 2010). This fact may indicate the degree of maize cooking. However, the WSI provides evidence of the interaction magnitude between the starch chains within the amorphous and the crystalline domains. The solubility of the different flours differs due to the different treatments of organic fertilization in the maize grains. Likewise, the increase in water solubility could refer to a partial disruption of the amylopectin helices and a greater ability of the amylose to leach out from the damaged granules during the nixtamalization process (Rocha-Villareal *et al*., 2018; Mir *et al*., 2013).

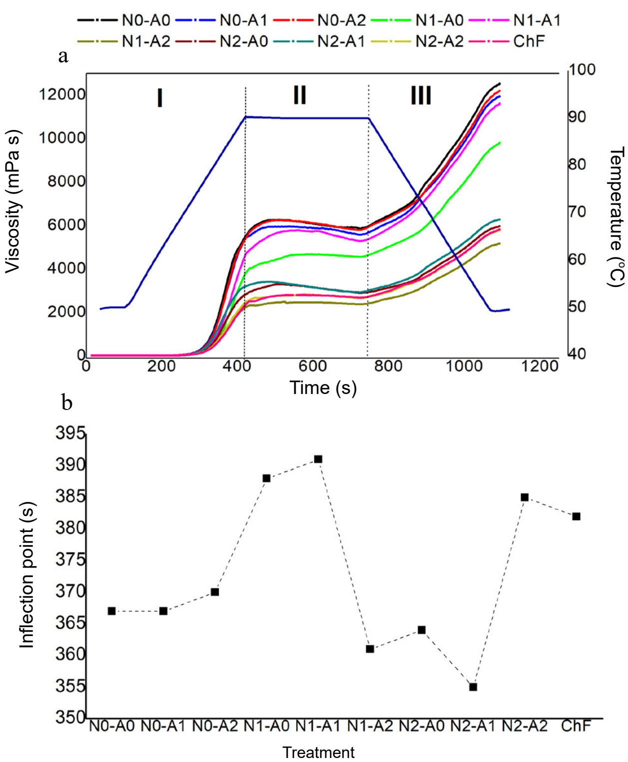

Fig. 4. Apparent viscosity of flours from fertilized maize: a) pasting profile b) inflection points.

The pasting profile (Fig. 4a) was divided into three stages as follows: stage I is the heating process of the starch slurry from 50-90°C; stage II is the isothermal process at 90°C; finally, stage III is the cooling process from 90-50°C. In all phases, changes in viscosity to the maize flours were observed. For stage I, the inflection point (Fig. 4b) shows the time in which drastic changes occur before reaching a state of maximum viscosity in the flour slurry. This point may be related to the time taken for the starch granule to become fully swollen without changing its granular form. Likewise, Rincón-Londoño *et al*. (2016) reported drastic changes in starch morphology from granules to “donuts” at the maximum peak of the pasting profile which occurs at the maximum viscosity of the flour slurry. The graphs show similar inflection points (367-370 s) and a maximum gelatinization temperature of the slurry at 87°C obtained for fertilized maize without nejayote (N0-A0, N0-A1, and N0-A2). Meanwhile, the flour slurry N1-A1 showed the highest inflection point (391 s) at a temperature of 89.6°C. During this stage, the starch granules of these flours absorb water more slowly and swell when heated continuously.

In stage II, the maximum viscosity peak was reached. The fertilized maize without nejayote N0-A0, N0-A1 and N0-A2 (6 272, 5 961, and 6 255 mPa s) showed the highest viscosity values respectively. The lowest viscosity values are correlated with the maize produced with chemical fertilizer (2 816 mPa s) and N1-A2 (2 498 mPa s). Villada *et al*. (2017) hypothesized that the viscosity peak was related to hydrogel formation (corn-lime-water) and that starch is the main reason for the increase in viscosity. Through cooling, the viscosity level reached at the end of stage III for flour N0-A0 was 2.5 times higher (12 550 mPa s) than that of N1-A2 (5 179 mPa s).

Starch is an important store of energy that is captured by plants using sunlight, water, carbon dioxide, and soil nutrients (Keeling and Myers, 2010). In this research, the flours were obtained from maize grains which were organically fertilized with nejayote, manure and mixtures of the two, 20, 40, and 60 days after sowing; during this period of plant growth possible modifications in the starch structure may occur. Starch is known as a semicrystalline material because the granules contain crystalline and amorphous regions. These crystalline regions are mainly amylopectin polymers from which the outer branches are hydrogen bonded to each other. The amorphous regions of the granules are mainly composed of amylose and amylopectin branch points (Athene, 2001). On the other hand, nejayote is constituted by $CaCO_3$, fibre, proteins, fat, ash (Valderrama-Bravo *et al*., 2013) and OH ions which are a product of alkalizing reactions during the nixtamalization process with pH 11. However, during composting, the levels of pH vary in response to the raw material used in the original composting mixture. In the first days of active composting, the period is characterized by a drop in the pH value to levels between 4 and 5; this may be the result of the formation of organic acids (USDA, 2010).

Therefore, the changes in composted nejayote could modify the crystalline and amorphous regions of the starch forming a new hydrogen bond between amylose and amylopectin. Consequently, changes in pasting properties occur in all flours. They occur because changes in starch content take place during gelatinization and this has been attributed to the differences between the amorphous and crystalline regions (Wajira and David, 2006). Also, viscosity changes may be associated with the high ash content of the flours N0-A0, N0-A1, and N0-A2 (Fig. 2). Santiago-Ramos *et al*. (2015) indicated that the additional ash might induce crosslinks or ionic interactions among the maize flour components, mainly with proteins, which increases viscosity.

The total colour difference (ΔE) and "*b*" parameters of maize masa showed significant differences ($p < 0.05$) among the masa samples. Masa N1-A0 showed the highest ΔE (32.47) and the lowest "*b*" value (10.88). Conversely, the masa N0-A1 (14.42) and N2-A2 (13.43) showed the highest "*b*" values, which indicated that the masa had a stronger shade of yellow. Ayala-Rodriguez *et al*. (2009) reported that lime quantity affects the colour of the nixtamalized maize flours and Amador-Rodríguez *et al*. (2019) revealed that the yellow colour in masa depends on calcium hydroxide concentration and on the pericarp percentage retained by the grain during the nixtamalization process. However, if maize grains were nixtamalized under equal process conditions in all experiments; then physical properties like hardness, colour, and the size of maize grains in masa and tortillas would be modified by genotypic variation and environmental growth conditions (Vázquez-Carrillo *et al*., 2012). Likewise, Zhang *et al*. (2015) indicated that ovine manure application in soils are beneficial to the buffering of soil acidification to avoid harmful effects on plant growth, which changes the chemical and physical properties of maize grains. Hence, mixtures composted with ovine manure and nejayote buffered the pH value because organic solids from ovine manure probably contain a high quantity of microorganisms like fungi, which consume the acids produced during the first stages of composting. Consequently, the masa N2-A1 obtained from organically fertilized maize grains and with chemical fertilizer, produced a yellow colour and low ΔE value.

The results of rheological parameters were within the region of linear viscoelasticity (where the moduli are independent of the strain); they showed a constant strain range between 50 and 80 Pa. As a consequence, a strain of 70 kPa was chosen to carry out all oscillatory frequency tests. The results of the sweep frequency for elastic moduli (G´) and viscous moduli (G´´) that were dependent on frequency showed significant differences ($p < 0.05$) between all of the treatments. In all masa samples, G´ values predominated over G´´ values (Fig. 5). This fact indicates that the behaviour of masa is similar to that of a weak gel (Valderrama-Bravo *et al*., 2015). Valderrama-Bravo *et al*. (2017) and Vázquez-Carrillo *et al.* (2015) reported a similar behaviour. The highest elastic and viscous moduli values were produced by masa from N1-A1, and N2-A1 while the lowest G´ and G´´ values were produced by masa from N2-A2. The results show that the application of organic fertilizer to maize during its production modifies the viscoelastic modulus G´, and G´´ of the produced masa. On the other hand, the mixtures of organic fertilizer N1-A1 (75 m^3 – 25 t ha^{-1}) and N2-A1 (150 m^3 – 25 t ha^{-1}) used for the fertilization of maize during its production generated a higher content of phosphorus, potassium, and sodium (Domínguez-Hernández *et al*., 2020).

Higher plants, like maize, synthesize and store starch in the form of granules in storage tissues such as seeds, and in a temporary form in leaves, roots, and stems (Keeling and Myers, 2010; Song *et al*., 2016). Little starch is accumulated during the 'cell division' or endosperm differentiation phase (~10 DPA), which defines the final cell number of the grain (Altenbach *et al*., 2003). Fertilization, supplied mainly by nitrogen (N) changes the composition of maize grain, by modifying the starch, protein, and oil content

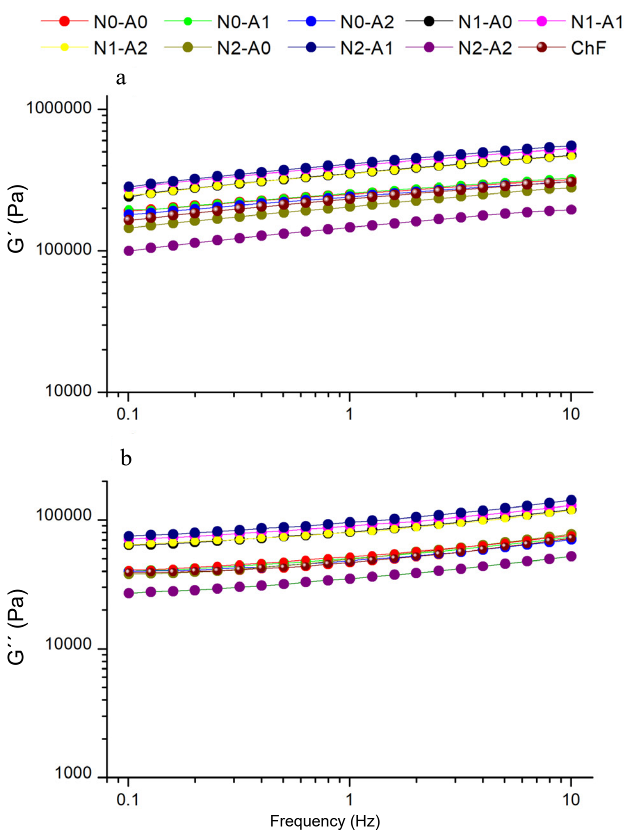

Fig. 5. Rheological parameters of masa obtained from organically fertilized maize a) elastic module (G´) and b) viscous module (G´´).

(Tofiño *et al*., 2007; Seebauer *et al*., 2010). Also, nitrogen deficiency before or during the early reproductive development stages decreased the accumulation of starch in the kernel (Thitisaksakul *et al*., 2012). The N supply from the organic fertilizers (nejayote-manure) was commenced 20 days before anthesis could change the composition of the maize kernel by modifying the starch structure. The use of the organic fertilizer meant that N2-A2 had the highest content of nitrogen in the grain (Domínguez-Hernández *et al*., 2020) and the masa obtained from the fertilized maize with this mixture showed drastic changes in G´, and G´´ (Fig. 5).

Likewise, Contreras-Jiménez *et al*. (2017) noted that the main components of nixtamalized flours that might affect the viscoelastic properties of masa are starch, non-starch polysaccharides, proteins, lipids, and minerals. In addition, nejayote contains hemicellulose, cellulose, arabinoxylans (non-cellulosic cell wall polysaccharides present in maize pericarp) and phenolic acids like ferulic acid (Niño-Medina *et al*., 2009; Daglia, 2012). During the composting process of nejayote with manure by microorganisms, the biodegradation of hemicellulose, cellulose and another polymers to short lateral chains like sugars occurs (Pérez *et al*., 2002). Similarly, Pei-Yin and His-Mei (2011) stated that the conversion of free sugars to starch resulted in the progressively-decreasing total sugar content of the developing grains. Also, the mixtures of organic fertilizer with nejayote have a higher content of sugars that are nutrients for the maize plant, which could modify the starch structure. Consequently, the viscoelastic moduli G´ and G´´ of the masa obtained from the organically fertilized maize were different.

The parameters of texture profile analysis, obtained for the prepared masa according to the experimental design (Table 2) show that the masa from fertilized maize with nejayote at 75 m^3 ha^{-1} contributed to an increase in the hardness of the masa by 50% with respect to those without nejayote and ovine manure (N0-A0). This effect was greater in the masa from maize that was fertilized with greater amounts of ovine manure (A2) during plant development. Hardness is a parameter measured by the force required to deform the masa, at optimal moisture conditions, without causing its disintegration (Gaytán-Martínez *et al*., 2011). In addition, the masa fertilized with more ovine manure had a lower elasticity, which means that they adhere less to the surface when tortillas are prepared, this coincides with those results mentioned by Flores-Farías *et al*., 2000, who made a comparative study of nixtamalized maize flours and commented that adhesiveness is associated with sticky masa and low machinability. The cohesiveness of the masa from maize fertilized with 75 or 150 m^3 ha^{-1} of nejayote and 50 t ha^{-1} of ovine manure was at its lowest level, which implies that the constituents of the masa had a lower mutual affinity. Therefore, the masa disintegrates more easily, suggesting that a more significant amount of ovine manure during the growth and development of the maize cob considerably reduces the cohesiveness properties of the masa prepared with this treated maize. Although it has been observed that the addition of nejayote does not show a significant effect on the textural parameters, it is necessary to control the amount of manure used as fertilizer, since the concentration influences the characteristics of the masa obtained. Cohesion is the force between the internal interactive bonds, the shape of the material structures (also known as "body") during compression and it is also an essential parameter in the preparation of tortillas. Therefore, the texture of the dough is crucial during tortillas production (Chel-Guerrero *et al*., 2014). Therefore it is necessary to control the addition of manure as fertilizer, and its concentration because it influences the characteristics of the masa obtained.

When considering the results obtained for the textural parameters of tortillas (Table 3), a lower resistance to stress is observed in the samples from maize produced with 150 m^3 ha^{-1} of nejayote (N2) and a more considerable

Table 2. Texture parameters of masa

Treatment	Hardness (N)	Elasticity (mm)	Adhesiveness (mJ)	Cohesiveness
N0-A0	11.60 ± 1.70 ^{ab}	3.29 ± 0.68 ^{a}	6.63 ± 1.05 ^{a}	0.3957 ± 0.06 ^{a}
N0-A1	10.51 ± 0.46 ^{b}	1.91 ± 0.21 ^{ab}	3.90 ± 1.04 ^{abc}	0.4110 ± 0.02 ^{a}
N0-A2	10.12 ± 0.30 ^{b}	2.38 ± 0.26 ^{ab}	5.27 ± 0.71 ^{abc}	0.3628 ± 0.01 ^{a}
N1-A0	14.05 ± 2.08 ^{a}	1.93 ± 0.40 ^{ab}	2.43 ± 1.02 ^{bc}	0.3321 ± 0.04 ^{ab}
N1-A1	11.23 ± 0.40 ^{ab}	1.88 ± 0.06 ^{ab}	3.50 ± 0.26 ^{abc}	0.3082 ± 0.02 ^{ab}
N1-A2	14.01 ± 0.32 ^{a}	0.86 ± 0.06 ^{b}	3.30 ± 1.02 ^{abc}	0.1486 ± 0.03 ^{c}
N2-A0	12.49 ± 1.34 ^{ab}	2.14 ± 0.29 ^{ab}	4.80 ± 0.98 ^{abc}	0.3491 ± 0.02 ^{a}
N2-A1	11.25 ± 0.46 ^{ab}	1.67 ± 0.37 ^{b}	3.10 ± 0.44 ^{bc}	0.3220 ± 0.07 ^{ab}
N2-A2	10.09 ± 0.25 ^{b}	1.04 ± 0.04 ^{b}	1.87 ± 0.15 ^{c}	0.1980 ± 0.02 ^{bc}
120N-60P-30K	6.99 ± 0.17 ^{c}	2.45 ± 0.87 ^{ab}	5.87 ± 1.01 ^{ab}	0.2978 ± 0.06 ^{ab}

Mean ± standard deviation values followed of different letter in each row, are significantly different ($p < 0.05$).

Table 3. Texture parameters of tortilla

Treatment	Tensile strength (N)	Extensibility (mm)	Rollability (N)	Rollability 24 h (N)
N0-A0	4.34 ± 0.48 [abc]	4.44 ± 0.36 [a]	0.28 ± 0.08 [c]	0.60 ± 0.01 [b]
N0-A1	4.80 ± 0.33 [a]	4.55 ± 0.28 [a]	0.41 ± 0.04 [bc]	0.85 ± 0.04 [ab]
N0-A2	3.31 ± 0.52 [bcd]	4.83 ± 0.98 [a]	0.37 ± 0.02 [c]	1.00 ± 0.11 [a]
N1-A0	3.08 ± 0.52 [cd]	2.57 ± 0.33 [cd]	0.62 ± 0.04 [a]	1.04 ± 0.02 [a]
N1-A1	4.04 ± 0.07 [abc]	2.82 ± 0.22 [bcd]	0.62 ± 0.05 [a]	0.74 ± 0.01 [ab]
N1-A2	3.56 ± 0.57 [abcd]	2.33 ± 0.33 [d]	0.53 ± 0.09 [ab]	0.84 ± 0.03 [ab]
N2-A0	3.83 ± 0.42 [abcd]	3.75 ± 0.24 [abc]	0.55 ± 0.03 [ab]	0.89 ± 0.09 [ab]
N2-A1	4.56 ± 0.79 [ab]	3.99 ± 0.27 [ab]	0.56 ± 0.02 [a]	0.87 ± 0.09 [ab]
N2-A2	2.55 ± 0.25 [d]	2.91 ± 0.68 [bcd]	0.65 ± 0.02 [a]	1.07 ± 0.07 [a]
120N-60P-30K	4.34 ± 0.64 [abc]	3.05 ± 0.17 [bcd]	0.62 ± 0.05 [a]	0.86 ± 0.11 [a]

Mean ± standard deviation values followed of different letter in each row, are significantly different ($p < 0.05$).

amount of ovine manure applied at 50 t ha^{-1}. This resistance to stress represents a 41% lower value than the samples that did not have any treatment with nejayote or ovine manure (N0-A0). It is also revealed that there was an interaction between the addition of nejayote and ovine manure. The high concentrations of nejayote applied at 150 m^3 ha^{-1} and the low levels of ovine manure applied at 25 t ha^{-1} (N2-A1) had a positive influence on maize behaviour during nixtamalization. Concerning the tensile strength of the tortillas, they showed no statistically significant difference regarding those produced without nejayote or ovine manure during plant growth. The extensibility results for the masa obtained from maize without nejayote or manure during the development of plant and maize cobs were similar to those reported by Ruiz-Gutiérrez *et al*. (2012). Masa and tortillas prepared with maize treated with N2-A1 and N0-A1 showed a statistically significant difference in extensibility. However, N0-A0 and N0-A2 presented an increase in extensibility, although their resistance to stress was lower when compared to the samples with treatment.

The rollability test showed that, in freshly prepared tortillas, both the addition of nejayote and maize treated with chemical fertilization presented an increase in the force required to roll the tortillas. Compared to the samples without nejayote and ovine manure, the addition of ovine manure has a significant effect on the rollability loss. However, the results obtained coincide with those reported by Peña-Reyes *et al*. (2017), who evaluated the impact of different nixtamalization conditions on the textural properties and reporting values between 0.22 to 0.29 N in freshly prepared tortillas. In the present study, the addition of nejayote as a fertilizer during the development and growth of maize cob is the factor with the most considerable influence on rollability. Nonetheless, the results obtained were lower than 0.8 N, which implies that they had an acceptable rollability behaviour compared to other studies (Peña-Reyes *et al*., 2017; Wu and Arntfield, 2016), in which the subjective rollability was greater than 4. Moreover, water absorption is an important parameter which depends on the characteristics of maize, and therefore, on the nutritional conditions of the plant during the development of the maize cob. However, the results obtained after 24 h of preparation showed that there was a statistically significant difference in the strength required to roll the tortillas in comparison with those without ovine manure, although they are still within the intervals reported in a study in which the capacity of subjective and objective rollability were evaluated.

CONCLUSIONS

1. Different fertilizer types produce changes in grain quality such as hectolitre weight, flotation index and hardness, and also resulted in physicochemical changes in flours and rheological and textural ones in masa and tortillas.

2. The protein content in flours obtained from maize grains treated with N2-A0 and N1-A1 were higher than those seen in flour resulting from treatment with a chemical fertilizer.

3. The inflection point obtained from the pasting profile of NI-A1 was the highest one out of all the flour slurries tested, it was obtained from fertilized maize grains with 75 m^3 ha^{-1} of nejayote and 25 t ha^{-1} of ovine manure. This fact shows that a period of water absorption of the starch granules before maximum viscosity is reached results in an improvement in the cohesiveness of the masa and the rollability of the tortilla.

4. The organic fertilizer N2-A2 had the highest content of nitrogen, and the masa obtained from the fertilized maize showed the lowest elastic and viscous moduli, but it produced the highest rollability in tortillas.

5. The texture profile analysis for masa and tortilla (tensile strength and rollability) showed that treatments with more ovine manure, considerably reduced the cohesiveness

of the masa obtained with fertilized maize. Likewise, the treatments with nejayote as a fertilizer influenced the rollability of tortilla.

6. The highest concentrations of nejayote applied at 150 m^3 ha^{-1} and the low levels of ovine manure applied at 25 t ha^{-1} (N2-A1) had a positive influence on maize behaviour during nixtamalization and tortilla production. Therefore, the application of organic fertilizer (nejayote with ovine manure) during the growth of the maize plant is a positive alternative that could replace chemical fertilizers.

7. In this research, changes to the physicochemical properties of flours (chemical analysis, pasting profile, water solubility index and swelling power) as well as the textural and rheological properties in masa and tortilla were evaluated. Future studies could continue the work and determine if the application of biofertilizers (nejayote and ovine manure) during the growth period of the plant modifies the phenolic and ferulic compounds and hence the antioxidant capacity of maize grains and tortillas.

Conflict of interest: The Authors confirm that there is no conflict of interest affecting their work as researchers.

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