



Effect of maize processing on amylose-lipid complex in pozole, a traditional Mexican dish

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ABSTRACT

One of the most traditional foods in Mexico is pozole (a soup of maize grains with pork). Maize starch interacts with fatty acids during its preparation, generating amylose-lipid complexes. This study aimed to determine the effect of lard concentration on the formation of amylose-lipid complexes and their effect on the physicochemical properties of the starch after pozole preparation. Three maize varieties were nixtamalized and cooked, using 0, 6, 8, and 10 % lard. Grain physical parameters, viscosity, resistant starch, and detailed characterization of starch granules were analyzed. X-ray diffraction patterns showed structural damage in the crystalline starch structure. Two of the maize varieties showed an amylose-lipid endotherm with the addition of lard. The viscosity profile was affected by grain hardness and heat treatment, whereas lard addition decreased the viscosity of samples. The addition of higher percentages of lard (8 and 10%) favors the formation of amylose-lipid complexes, as shown in thermal properties and resistant starch.

Introduction

Maize is the primary dietary cereal in the Mexican population, with an average per capita intake of 319 g per day (FAOSTAST, 2021). Maize is used to prepare a wide variety of foods, is the most common one the nixtamalized flatbread or tortilla. Pozole is a traditional soup prepared with maize and meat and served with vegetables (Figueroa et al., 2013). The preparation includes thermal-alkaline cooking or nixtamalization of maize kernels, followed by manual removal of kernels tip and subsequent cooking with meat (generally, pork or chicken) (Vázquez-Carrillo et al., 2013; Santiago-Ramos et al., 2018). Starch is the main component of maize and consists mainly of amylopectin and amylose, both composed of D-glucose units linked by α -(1→4) bonds. Amylopectin is the branched part of starch due to α -(1→6) bonds with every 20 to 22 D-glucose units (Escalante-Aburto et al., 2020). On the other hand, amylose is considered linear due to its low percentage of α -(1→6) bonds, and it has a turn every 6 to 8 glucose molecules forming helices.

Due to its structural arrangement, amylose can interact with different hydrophobic molecules such as lipids.

During nixtamalization, several physicochemical changes in the grain could occur as the partial removal of the pericarp, softening of the grains, high water absorption, gelatinization, and annealing of the starch granules. It also provides a characteristic flavor and smell. During the nixtamalization process, calcium content increases, and resistant starch (RS) is formed, enhancing the nutritional benefits of the kernels (Figueroa et al., 2013; Escalante-Aburto et al., 2020; Palacios-Rojas et al., 2020). RS is not enzymatically hydrolyzed after 120 min, and the gut microbiota ferments it in the colon (Dupuis et al., 2014; Figueroa-Cárdenas et al., 2016). RS can be divided into five classes: RS1 is the physically unavailable starch, RS2 is the native starch resistant to the enzymatic attack, RS3 refers to the retrograded starch, RS4 comprises starches that have been chemically modified, and RS5 consists of amylose-lipid complexes (Dupuis et al., 2014). During food processing, especially after gelatinization, amylose-lipid complexes can be formed due to the interaction between the amylose released during gelatinization and the lipids present in the food (Tan & Hong, 2019). According

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to their order level, the amylose-lipid complexes are divided into Type I and Type II. The first has a melting temperature <100 °C, and the complexes are formed when rapid nucleation occurs, resulting in a random distribution in their structure. On the other hand, amylose-lipid complexes Type II have a melting temperature >100 °C and are subdivided into Type IIa and IIb. The second shows the highest order and stability (Tufvesson et al., 2003). It has been reported that physical and chemical modifications on the starch took place due to the formation of amylose-lipid complexes, altering their pasting properties, water absorption capacity, solubility, swelling capacity, and viscosity (Cervantes-Ramírez et al., 2020). Several studies have reported this complex type in different starch sources when adding fatty acids (Tufvesson et al., 2003; Wang et al., 2018).

In Mexico, landraces like Cacahuacintle, Elotes Occidentales, Tabloncillo, and Maíz Ancho are commonly used to prepare pozole. Kernels of those landraces are big and soft, with a large proportion of floury endosperm (Santiago-Ramos et al., 2018) Figueroa et al. (2013). reported that traditional nixtamalization improves the stability of starch to collapse due to the annealing undergone. The authors also observed a V-type X-ray diffraction pattern in grains cooked in water, associated with the formation of amylose-lipid complexes.

As far as we know, the impact of the lard concentrations and the maize source on the amylose-lipid complex formation remains unknown. Likewise, the impact of the repeated cooking process during the pozole making on the resistant starch is an unexplored field. Therefore, this study evaluated the formation of amylose-lipid complexes during maize processing in a Mexican dish “pozole.” It was evaluated three maize landraces cooked with different lard concentrations (0, 6, 8, and 10%), evaluating the physicochemical, thermal, rheological, and morphological properties.

Materials and methods

Materials

Three commonly used maize landraces were used to prepare pozole: Cacahuacintle landrace kernels (CH) (purchased at a local market in Queretaro, Mexico), Pozolero 1 (P1), and Pozolero 2 (P2) (improved varieties of “Elotes Occidentales” landrace). Both, P1 and P2 varieties were provided by Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). The raw maize was manually cleaned, removing all impurities, and stored in sealed bags at 27 °C until processing.

Food grade calcium hydroxide $\text{Ca}(\text{OH})_2$ was obtained from Calhidra S.A. de C.V., and commercial lard was purchased from a local market (Querétaro, Qro., México).

Nixtamalization process and pozole preparation

One kilogram of each maize was cooked in 2 L of purified water and $\text{Ca}(\text{OH})_2$ [10 g $\text{Ca}(\text{OH})_2$ /kg of maize]. Maize was boiled at 94 °C for 25–30 min (the time was defined according to the flotation index, very soft grains (CH) were boiled 25 min, and soft grains (P1, P2) were boiled 30 min.). The cooked grains were steeped for 16 h. The steeping water (nejayote) was removed. The nixtamalized maize was washed using 1 L of water to remove the excess of $\text{Ca}(\text{OH})_2$.

Manual removal of kernel tip was performed after the nejayote was washed. Then, each nixtamalized sample was placed in aluminum pans, mixed with purified water (ratio 1:3, maize: water), and four different concentrations of lard (0, 6, 8, and 10%). The mixture was boiled for two h. The cooking water was discarded, the nixtamalized and cooked kernels were dried in an Excalibur Dehydrator (Sacramento, CA, USA) at 50 °C for 12 h. A sample of nixtamalized and cooked grains were kept from each treatment to evaluate the microstructure. The rest were ground (Krupps grinder, model 8000033105, Texas-USA), passed through a No. 60 US mesh (250 μm), and stored at room temperature (27 °C) in polyethylene bags for further analysis. Two replicates per treatment

were made. The samples were identified as raw, nixtamalized, and cooked (with 0, 6, 8, and 10% pork lard).

Scanning electron microscopy (SEM)

The starch granule morphology study was carried out in an electron scanning microscope (SEM Phenom-World BV, The Netherlands), adapted to work in a low vacuum with a beam of 5 kV. Each grain sample was prepared by cutting the kernel at the middle longitudinal section and deposited on a sample holder with double-sided tape. The images were taken at a magnification of 1250X.

X-ray diffraction

Raw, nixtamalized, and cooked (0, 6, 8, and 10% of pork lard) flours were conditioned with 7% moisture were placed on a glass surface. Samples were scanned from 5° to 50° on the 2θ scale using a diffractometer (Rigaku, model Ultima IV, Texas-USA) with a detector D/tex ultra, operated at 35 kV and 15 mA, with $\text{CuK}\alpha$ radiation ($\lambda=1.5405$ Å) (Tan and Kong, 2019).

Infrared spectroscopy (FT-IR)

The infrared spectra of the raw, nixtamalized, and cooked flours were obtained in a Perkin Elmer IR spectrometer (model Spectrum Two, Waltham-USA) with ATR (Attenuated Total Reflectance, using a Diamond/ZnSe crystal). The IR spectra were obtained in the range of 600–4000 cm^{-1} .

Thermal properties

Thermal properties were obtained by differential scanning calorimeter (DSC) (DSC Mettler Toledo, model 821, Greifensee-Switzerland). Using a modified method from Cervantes-Ramírez et al. (2020). Approximately 100 g of 250 μm -screened samples were weighed in an airtight bag, adding the required water to reach 60% moisture. The sample was held for 30 min at room temperature (27 °C). Then, 0.05 g of sample was placed into an aluminum pan (40 μL) and sealed with a Mettler Toledo press (ME-Q0119410). The samples were heated from 30 to 160 °C at a rate of 10 °C/min. Four endotherms were obtained: gelatinization and three for amylose-lipid complexes (type I, type IIa, and IIb). From each endotherm, the following features were obtained: initial temperature (To), peak temperature (Tp), final temperature (Tf), and enthalpy (ΔH).

Viscosity profile

The method report by Ménera-Lopez et al. (2013) was used with slight modifications. Using an Anton Paar rheometer model MCR 101 (Anton Paar, St Albans, United Kingdom), with a starch pasting accessory (ST24-2D/2V/2V-30-SN186530), it was determined the viscosity profile of the raw, nixtamalized, and cooked flours. Approximately 3.0 g of each flour sample (sieved through a US 60 mesh) were suspended in 18 mL of distilled water. The mix was heated from 50 °C to 92 °C (5.8 °C/min), maintained at 92 °C for 5 min, and cooled to 50 °C at a rate of 5.8 °C/min. The results were expressed as the paste temperature, maximum viscosity, minimum viscosity, and final viscosity. Each sample was measured in duplicate.

Resistant starch content

Resistant starch of the raw, nixtamalized, and cooked flours were analyzed by K-RSTAR assay kit (Megazyme International, Bray, Ireland) based on the AACC International Approved Methods of Analysis (32-40.01) (2002.02, 2005, AOAC).

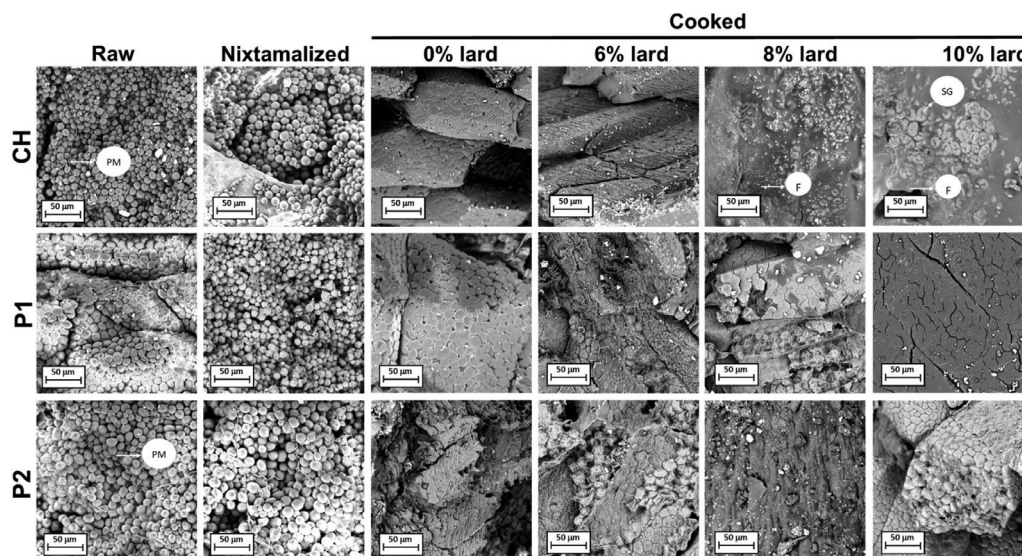


Fig. 1. SEM images of raw maize, nixtamal, and pozoles with different concentrations of lard (magnification of 1250x). SG: starch granule; PM: protein matrix; F: surface lard.

Statistical analysis

The data represent the mean of four independent measurements. A one-way analysis of variance (ANOVA) was performed at a significance level of 95% ($\alpha = 0.05$), with means comparison by the Tukey's test. The statistical analyses were conducted using the Minitab 16® statistical software.

Results and discussions

Flotation index

The flotation index is an indirect determination of the grain hardness, typically used to define the cooking time during nixtamalization. P1 had the lowest flotation index (FI), followed by P2 and CH. (Supplementary Table S1). Thus, CH was classified as a very soft grain and cooked for 25 min, while P1 and P2 were classified as soft grains and cooked for 30 min.

Starch morphology

The effect of processing on the microstructure of raw maize, nixtamal, and grain cooked with lard is illustrated in Fig. 1. The raw CH and P2 varieties samples showed spherical starch granules surrounded by a protein matrix in the floury endosperm. The P1 variety had polygonal starch granules, with fewer intragranular spaces due to higher compaction within the protein matrix, compared to CH and P2 varieties. The differences in the microstructure of maize are related to the grain hardness, where a highly packed matrix tends to show greater hardness (Figueroa et al., 2013; Narváez-González et al., 2006). The compaction degree directly affects the water diffusion and absorption into the starch granule, impacting its pasting and thermal properties (Figueroa et al., 2013).

Regardless of the variety, no apparent damages were observed in the starch granules or the internal layers of the endosperm for the nixtamalized samples. However, a partial loss of the protein matrix was observed, attributed to the alkaline treatment. Starch granules in the outer layers of the endosperm tend to swell more than starch granules from the inner endosperm. Therefore, those granules undergo more gelatinization, reducing the surrounding protein matrix (Santiago-Ramos et al., 2018).

Changes in the microstructure of the maize kernel cooked without lard were observed. P2 showed damage in the protein matrix due to the

heat treatment, while CH and P1 did not display apparent damage; they were more compact than the raw samples, attributed to the swelling of the starch granules during the treatment (Figueroa et al., 2013). reported that native starch granules without annealing are more susceptible to collapse. In contrast, pozoles starch granules do not easily collapse due to their progressive swelling during the process, which could explain the observed results.

Cervantes-Ramírez et al. (2020) showed that saturated fatty acids, such as stearic acid, tend to form a surface layer in starch granules, protecting against damage from thermal effects. In maize samples cooked with lard (mainly monounsaturated and saturated fatty acids), a thin layer of lard covered the starch granules in the treatments with the highest lard concentrations (8–10%). Spherical starch granules without apparent damage were observed, suggesting a protective effect of lard during the heating process, and preventing the starch granule from swelling.

X-ray diffraction

The X-ray patterns of the three maize varieties were identified as characteristics of A-Type starch, which indicated the presence of orthorhombic nanocrystals (Rodríguez-García et al., 2021), with the following angles/planes positions: $15.140^\circ/(200)$, $17.251^\circ/(031)$, $18.009^\circ/(211)$, and $23.029^\circ/(231)$ (2θ), typically found in cereals (Cervantes-Ramírez et al., 2020; Rodríguez-García et al., 2021).

After the nixtamalization process, a decrease in the relative intensity of the peaks corresponding to the hkl plane was observed: (020), (101), (200), and (031), corresponding to the angles 10.052 , 11.223 , 15.140 , and 17.251° (2θ) respectively, for the three varieties of maize studied (Fig. 2, red lines). However, there were no changes in the orthorhombic structure of the maize due to the nixtamalization process. The above indicates that a complete gelatinization of the starch granules was not occurring as observed in SEM images (Fig. 1) (Rojas-Molina et al. (2007). showed that during cooking, the nixtamalized grains suffer changes in the external layers of the endosperm (10 to 20%). In comparison, the remaining 80% does not exhibit significant changes in the starch integrity.

In samples from maize cooked with and without lard, orthorhombic patterns were observed (Fig. 2, colored lines). A slight increase in the intensity of the peaks at angles 7.5 and 13° (2θ) was observed. These peaks are not indexed in the orthorhombic structure proposed by Rodríguez-García et al. (2021). However, it has been associated with amylose V-type (Cervantes-Ramírez et al., 2020). The intensity of the peak located

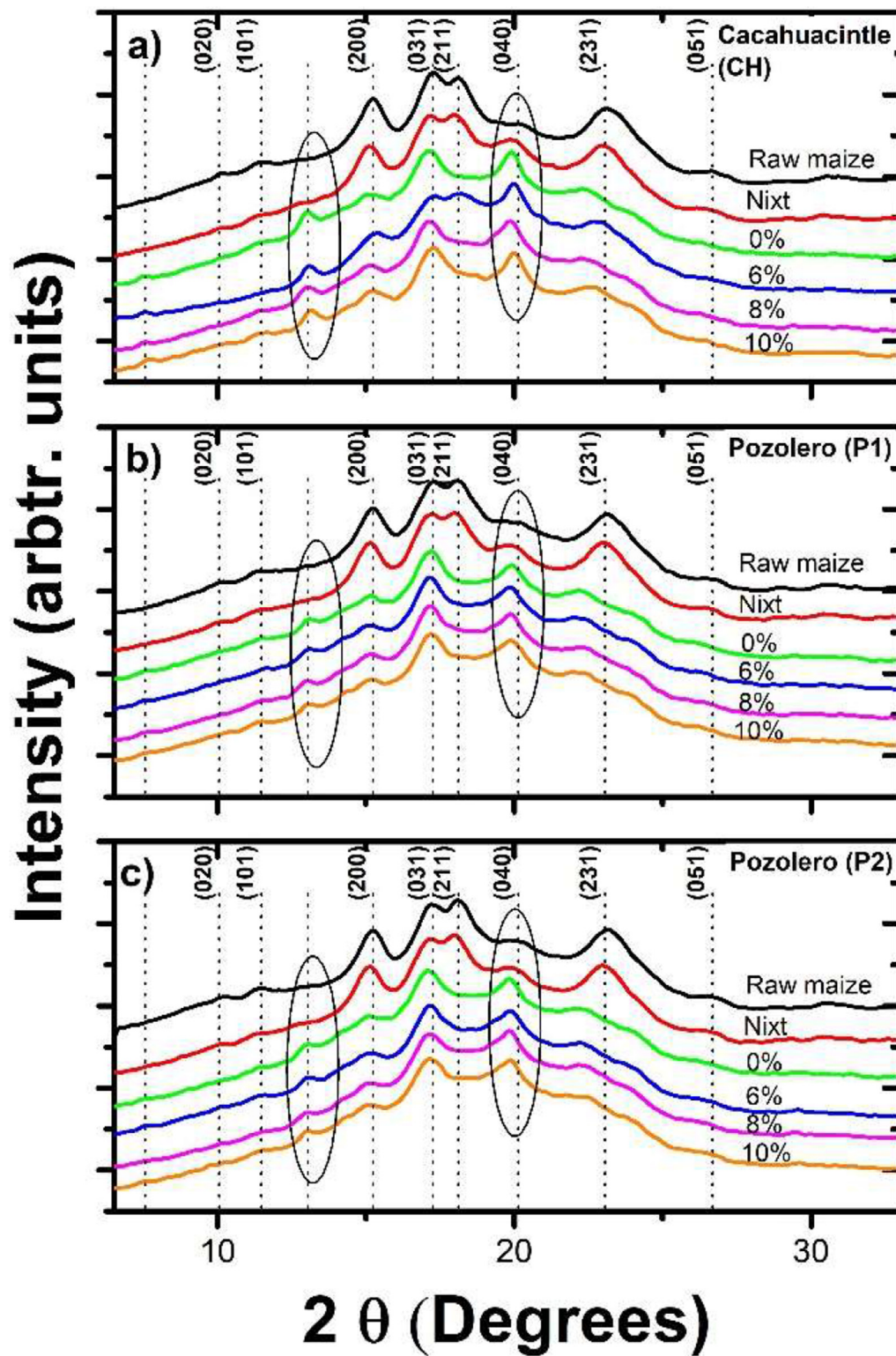


Fig. 2. X-ray diffraction of raw maize, nixtamalized maize, and pozole added with different concentrations of lard. Nixt: nixtamalized a: Cacahuacintle (CH); b: Pozolero 1 (P1); c: Pozolero 2 (P2).

in the plane (200) corresponding to angle 15.140° (2θ) decreased when the maize kernel was cooked for two hours.

In addition, an increase in the peak intensity was observed in the plane (040), corresponding to the angle 20.183° (2θ). This increase was in the nixtamalized samples (Fig. 2, red line), rising when the samples were subjected to prolonged heat treatment during the pozole cooking

(Fig. 2, colored lines). The increment in the relative intensity of the peak at 20.183° (040) could be associated with the heat treatment instead of being associated with the addition of lard. Interestingly, two revealed peaks were observed at 13.08 and 20.01° (2θ scale), positions determined using the second derivative criteria of each pattern. These peaks have been observed in popped and retrograded rice starch, associated

with a V-type crystalline structure (Murugesan and Bhattacharya, 1989). These peaks have been referred to as a new crystalline structure in the literature. However, the V-type structure has never been identified in crystallography. More importantly, two peaks that do not include the three Miller indexes cannot identify any crystalline structure from a crystallographic viewpoint. However, considering the indexing process carried out by Rodríguez-García et al. (2021) and using the Laue law " $n\lambda = 2d\sin(\theta)$ ", where $\lambda = 1.5406 \text{ \AA}$, d is the interplanar distance, and θ is the half of the measured 2θ angle for each peak, the direct calculation of the d spacing for both diffracted peaks resulted from 6.783 to 3.39 \AA , respectively. The first value for d does not correspond to any diffracted peak of the orthorhombic structure. However, the half value of 3.395 corresponds to the (051) direction. The experimental deviation concerning the reported value (Rodríguez-García et al., 2021) is only 1.39% . The second case, $d = 2.356$, corresponds to the (040) peak and an experimental deviation of 0.09% . Such results mean that it is possible to have a lamellar formation in the first case due to the water, heat, and fat during the cooking process.

The above could be attributed to the starch granules' partial and localized gelatinization process. In that sense, during the nixtamalization process, a partial gelatinization of the starch granules is promoted (Santiago-Ramos et al., 2018; Rojas-Molina et al., 2007). As can be seen, the nixtamalization process in the presence of lard produces damage in the starch integrity and damage in the orthorhombic crystalline structure (as shown in Fig. 2, for all studied grains).

Infrared spectroscopy

Independently of the treatment and maize variety, all samples presented the characteristic bands of maize starch (Fig. 3). The band at 3290 cm^{-1} was identified as symmetric stretching vibration of the O-H and C-H groups. Meanwhile, the band at 2926 cm^{-1} corresponds to an antisymmetric stretching of the functional group $-\text{CH}_2$ of the lipids, naturally present in maize (Preciado-Ortiz et al., 2018). The bands at 998 cm^{-1} and 928 cm^{-1} correspond respectively to the bending of the C-O-H bond and the skeletal model of the α -glycosidic C-O-C bond (Deeyai et al., 2012).

Cooked samples with lard (Fig. 3, solid-colored lines) present the bands associated with maize starch and the characteristic bands of cooked lard (Fig. 3, gray dashed lines). The band at 3008 cm^{-1} [v] observed in P2 added with 8% lard was associated with the stretching vibration of the C=CH and C-H groups, typical of the lipid chain Cervantes-Ramírez et al. (2020). attributed this band to oleic acid (C18:1), which, according to Ripoche and Guillard (2001), is the main component of the lard.

An increase in the intensity of the band at 2926 cm^{-1} [t], corresponding to oleic acid, was observed. This band was observed in raw samples. The increase was attributed to lard concentration. On the other hand, the band at 2854 cm^{-1} [s] represented the symmetric stretch of C-H (Wang et al., 2018). The band at 1742 cm^{-1} [r] corresponds to C=O stretching in the methyl-ester of oleic acid (Niu et al., 2017). The bands observed at 1456 cm^{-1} [o] and 1368 cm^{-1} [n] were identified as the asymmetric bending of the $-\text{CH}_3$ bond and the symmetric bending of $-\text{CH}_2$, respectively. The bands at 1242 cm^{-1} [i] and 1098 cm^{-1} [e] represented the asymmetrical stretching vibrations of C-O-C in oleic acid and the bending of C-O-H, respectively. The 1400 – 1200 cm^{-1} region has been associated with vibrations of the aliphatic groups $-\text{CH}_2$ and $-\text{CH}_3$.

Several authors associate the 1022 cm^{-1} band with amorphous structures present in starch, increasing when the starch gelatinizes and decreasing when the starch is retrograded. In contrast, the band at 1047 cm^{-1} shows an opposite behavior and has been associated with ordered structures (Cervantes-Ramírez et al., 2020; Preciado-Ortiz et al., 2018).

Finally, a band at 1715 cm^{-1} was observed in samples P1, and P2 with a higher percentage of lard added (8–10%) (Fig. 3, arrow). This band has been associated with the interaction of oleic acid molecules

and amylose (Marinopoulou et al., 2016). However, it could correspond to the vibration of the C=O group from the free fatty acid.

Thermal properties

Table 1 shows the thermal properties of the three varieties of maize studied. From the thermograms obtained, the onset temperature (T_o), peak temperature (T_p), endset temperature (T_e), and enthalpy (ΔH) for the gelatinization transitions as well as the melting transitions of amylose-lipid complexes (type I, IIa, and IIb) were reported.

In the raw samples, the gelatinization endotherm of the starch granules was observed between 60 and $81 \text{ }^\circ\text{C}$. The CH variety showed a lower temperature range (60.4 ± 0.4 – $78.4 \pm 0.5 \text{ }^\circ\text{C}$) than P1 and P2 varieties, which showed a temperature range between 68 and $82 \text{ }^\circ\text{C}$. On the other hand, the CH variety presented a gelatinization enthalpy (ΔH) of $6.28 \pm 0.04 \text{ J/g}$, followed by P2 with $7.29 \pm 0.05 \text{ J/g}$; whereas the P1 variety showed the highest gelatinization enthalpy with $7.54 \pm 0.05 \text{ J/g}$. Therefore, these results indicate that the CH variety gelatinizes faster than the P1 and P2. The above could be explained due to the hardness of the grains, where soft grains (CH) will have easier water absorption and a lower temperature of gelatinization. In contrast, hard grains (P1) will have higher compaction in starch granules (Fig. 1), requiring more energy to gelatinize them (Figueroa et al., 2013; Santiago-Ramos et al., 2017).

Nixtamal samples showed a slight increase in peak (T_p) and endset (T_e) temperatures corresponding to the gelatinization endotherm, whereas the ΔH was decreased. The most significant increase in the gelatinization temperatures was observed in the CH variety. The nixtamalized sample rose $7 \text{ }^\circ\text{C}$ in onset temperature (T_o), corresponding to the gelatinization endotherm, $6 \text{ }^\circ\text{C}$ in T_p , and $14 \text{ }^\circ\text{C}$ in T_e . On the other hand, P1 and P2 varieties slightly increased T_o , T_p , and T_e (10 – $13 \text{ }^\circ\text{C}$). These results agree with those reported in the literature, where an alteration in the starch structure can cause an increase in thermal properties due to the annealing of the starch granules during the thermal-alkaline treatment (Santiago-Ramos et al., 2018; Mariscal-Moreno et al., 2015). In contrast, a decrease in the gelatinization enthalpy of nixtamalized maize compared to raw was attributed to the partial gelatinization of the starch granules from external layers of the endosperm during nixtamalization.

The gelatinization temperature of cooked maize was lower than the raw samples. This decrease in gelatinization temperature is attributed to the fact that the endotherm corresponds to the fusion of retrograded amylopectin chains (Santiago-Ramos et al., 2017). An increase of gelatinization temperature was observed between cooked samples when lard was added, this increase of temperature could be attributed to the formation of thermally stable material.

In the treatments with lard, a second endotherm was observed in a temperature range of 83 – $98 \text{ }^\circ\text{C}$. This endotherm is attributed to Type I dissociation of the amylose-lipid complexes, which has been reported to have a melting temperature $< 100 \text{ }^\circ\text{C}$ (Mariscal-Moreno et al., 2017) Tufvesson et al. (2003). indicated that these complexes are formed when rapid nucleation occurs, resulting in a random distribution of the complexes. Similar results were reported by Figueroa et al. (2016) during the preparation of tamales.

Regarding the dissociation enthalpy of amylose-lipid complexes I, variety CH showed a range of 0.14 – 0.39 J/g , P1 exhibited a constant enthalpy (0.14 J/g), and P2 displayed a decrease in enthalpy as the lard content increased. The dissociation enthalpy of amylose-lipid complexes has been associated with the number of complexes formed in the sample (Tufvesson et al., 2003). Therefore, it can be assumed that in variety P2, the formation of Type I amylose-lipid complexes is not favored due to the decrease in their enthalpy as the lard content increases (Table 1). On the other hand, the formation of complexes, and their dissociation enthalpy, are related to the hardness of the maize grains, where soft maize grains present a higher enthalpy than hard grains (Santiago-Ramos et al., 2018). The variety with less hardness (CH) showed higher values of

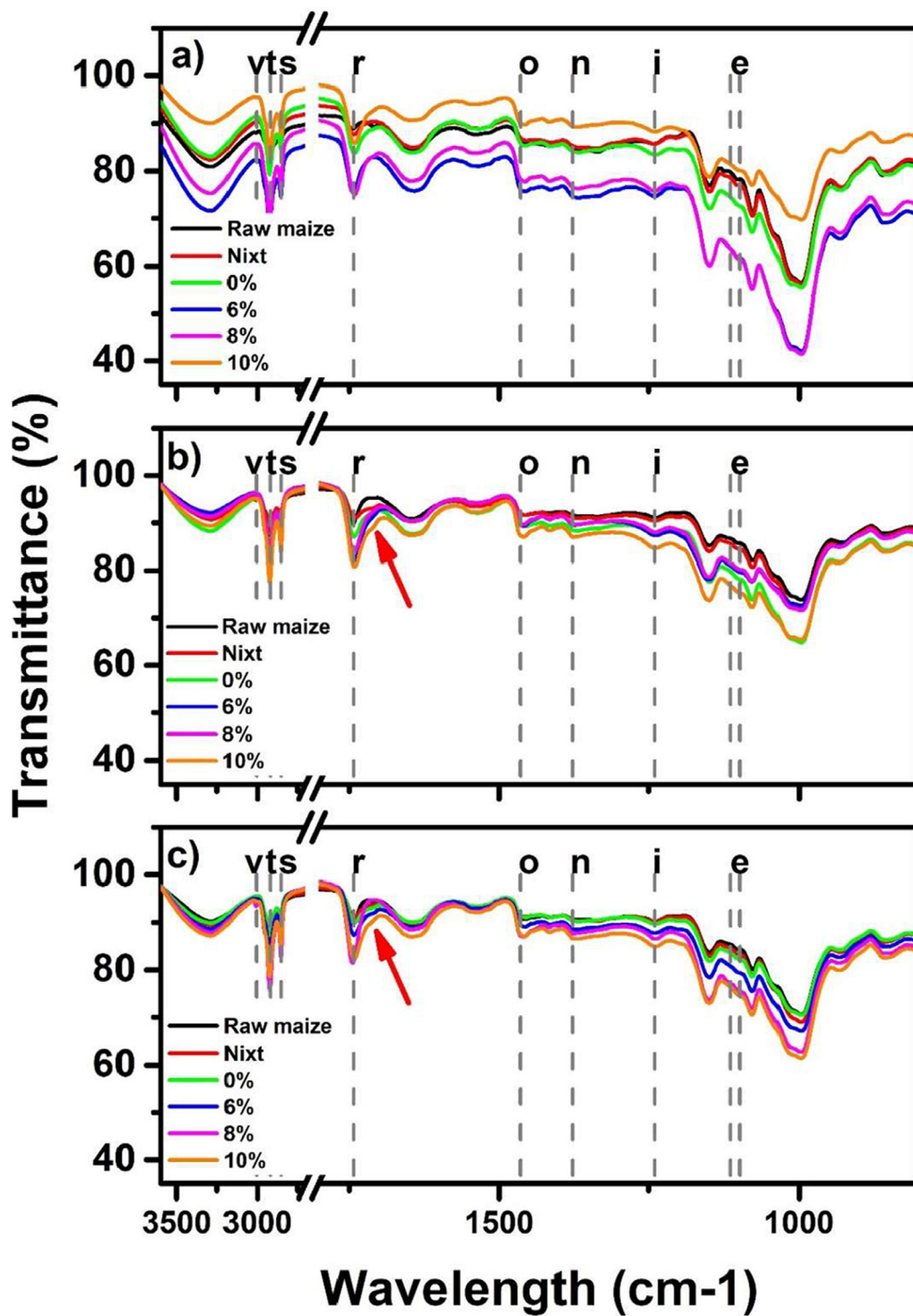


Fig. 3. Infrared spectra of raw maize, nixtamal, and pozole with different concentrations of lard. Nixt: nixtamalized; a: Cacahuacintle; b: Pozolero 1; c: Pozolero 2.

dissociation enthalpy of Type I complexes, so it could be inferred that a more significant formation of Type I amylose-lipid complexes took place.

According to their order level, the amylose-lipid complexes are divided into Type IIa and IIb, the second being the highest order and stability (Obiro et al., 2012). The Type IIa endotherm was observed independently of the variety in samples with 6 and 10% added lard. The temperature range was 107–141 °C, and enthalpy values were between 0.27 and 0.53 J/g. The results agree with Mariscal-Moreno et al. (2017) in

tamales, where endotherms for these complexes were observed between 110 and 124 °C with an average enthalpy of 0.27 J/g.

On the other hand, the Type IIb endotherm was observed in the variety CH (6 and 10%) and P2 (8 and 10%) between 122 and 155 °C. Whereas P1 showed the Type I and IIa amylose-lipid complexes; it is essential to mention that P1 has the highest hardness values, which could affect its thermal and pasting properties (Santiago-Ramos et al., 2017) as well as the formation of amylose-lipid complexes. Furthermore, increased enthalpy value in CH and P2 corresponding to the amylose-lipid

Table 1
Thermal properties of raw, nixtamal, and pozole flours added with different concentrations of lard.

Treatment	To (°C)	Tp (°C)	Te (°C)	ΔH (J/g)	Treatment	To (°C)	Tp (°C)	Te (°C)	ΔH (J/g)
<i>Starch gelatinization</i>					<i>Amylose-lipid complex I</i>				
CH: R	60.4 ± 0.4 ^e	69.6 ± 0.4 ^d	78.4 ± 0.5 ^{def}	6.28 ± 0.04 ^c	CH: 6	85.9 ± 0.5 ^a	95.3 ± 0.6 ^a	99.3 ± 0.6 ^a	0.14 ± 0.001 ^d
CH: N	67.5 ± 0.4 ^c	75.5 ± 0.5 ^{ab}	92.9 ± 0.6 ^b	1.08 ± 0.006 ^{ij}	CH: 8	85.0 ± 0.5 ^a	88.5 ± 0.6 ^b	98.7 ± 0.6 ^a	0.39 ± 0.002 ^b
CH: 0	46.1 ± 0.3 ⁱ	63.1 ± 0.4 ^h	72.9 ± 0.5 ^g	0.35 ± 0.002 ^m	CH: 10	86.0 ± 0.5 ^a	88.4 ± 0.6 ^b	93.3 ± 0.6 ^{cd}	0.21 ± 0.001 ^c
CH: 6	54.0 ± 0.3 ^g	57.4 ± 0.4 ⁱ	60.5 ± 0.4 ^j	1.04 ± 0.006 ^j	P1: 6	84.2 ± 0.5 ^a	86.9 ± 0.5 ^b	94.9 ± 0.6 ^{bc}	0.14 ± 0.003 ^d
CH: 8	75.6 ± 0.5 ^a	76.1 ± 0.5 ^a	78.3 ± 0.5 ^{def}	0.13 ± 0.001 ⁿ	P1: 8	85.2 ± 0.5 ^a	87.5 ± 0.5 ^b	91.6 ± 0.6 ^{de}	0.14 ± 0.001 ^d
CH: 10	ND	ND	ND	ND	P1: 10	84.9 ± 0.5 ^a	87.3 ± 0.5 ^b	92 ± 0.6 ^{de}	0.14 ± 0.003 ^d
P1: R	68.1 ± 0.4 ^c	73.8 ± 0.5 ^c	81.7 ± 0.5 ^c	7.54 ± 0.05 ^a	P2: 6	84.9 ± 0.5 ^a	88.5 ± 0.6 ^b	97 ± 0.6 ^{ab}	0.40 ± 0.002 ^a
P1: N	68.9 ± 0.4 ^{bc}	75.4 ± 0.5 ^{abc}	95.6 ± 0.6 ^a	0.93 ± 0.005 ^k	P2: 8	85.4 ± 0.5 ^a	88.5 ± 0.6 ^b	92.6 ± 0.6 ^{ede}	0.12 ± 0.001 ^e
P1: 0	48.8 ± 0.3 ^h	62.1 ± 0.4 ^h	65.8 ± 0.4 ⁱ	0.19 ± 0.001 ⁿ	P2: 10	84.7 ± 0.5 ^a	87.3 ± 0.5 ^b	90.6 ± 0.6 ^e	0.01 ± 0.001 ^f
P1: 6	57.7 ± 0.4 ^f	66.1 ± 0.4 ^g	74 ± 0.5 ^g	1.61 ± 0.01 ^g	<i>Amylose-lipid complex IIa</i>				
P1: 8	59.4 ± 0.4 ^e	69 ± 0.4 ^{de}	79.6 ± 0.5 ^d	2.7 ± 0.02 ^f	CH: 6	107.2 ± 0.7 ^d	109.8 ± 0.7 ^d	129.3 ± 0.8 ^{cd}	0.44 ± 0.002 ^b
P1: 10	54.4 ± 0.3 ^g	65.6 ± 0.4 ^g	74.6 ± 0.5 ^g	2.7 ± 0.02 ^f	CH: 10	136.0 ± 0.9 ^a	137.0 ± 0.9 ^a	140.5 ± 0.9 ^a	0.10 ± 0.001 ^e
P2: R	68.8 ± 0.4 ^{bc}	74.4 ± 0.5 ^{bc}	82.2 ± 0.5 ^c	7.29 ± 0.05 ^b	P1: 6	112.5 ± 0.7 ^{bc}	123.2 ± 0.8 ^{bc}	131.4 ± 0.8 ^{bc}	0.33 ± 0.002 ^c
P2: N	69.9 ± 0.4 ^b	75.3 ± 0.5 ^{abc}	92.0 ± 0.6 ^b	1.34 ± 0.008 ^h	P2: 6	111.0 ± 0.7 ^c	120.3 ± 0.8 ^c	128.0 ± 0.8 ^d	0.27 ± 0.001 ^d
P2: 0	45.1 ± 0.3 ⁱ	58.7 ± 0.4 ⁱ	70.2 ± 0.4 ^h	0.51 ± 0.003 ^l	P2: 10	114.9 ± 0.7 ^b	125.5 ± 0.8 ^b	133.3 ± 0.8 ^b	0.53 ± 0.003 ^a
P2: 6	55.4 ± 0.3 ^g	65.1 ± 0.4 ^g	76.8 ± 0.5 ^f	3.67 ± 0.02 ^e	<i>Amylose-lipid complex IIb</i>				
P2: 8	54.9 ± 0.3 ^g	67.6 ± 0.4 ^{ef}	79.3 ± 0.5 ^{de}	5.14 ± 0.03 ^d	CH: 6	131.8 ± 0.8 ^c	133.1 ± 0.8 ^c	137.1 ± 0.9 ^c	0.03 ± 0.001 ^b
P2: 10	62.8 ± 0.4 ^d	68.9 ± 0.4 ^{de}	77.4 ± 0.5 ^{ef}	1.14 ± 0.007 ⁱ	CH: 10	147.7 ± 0.9 ^a	150.0 ± 0.9 ^a	155.0 ± 0.9 ^a	0.56 ± 0.003 ^a
					P2: 8	122.5 ± 0.8 ^d	123.4 ± 0.8 ^d	125.0 ± 0.8 ^d	0.01 ± 0.002 ^c
					P2: 10	142.3 ± 0.9 ^b	145.6 ± 0.9 ^b	147.1 ± 0.9 ^b	0.01 ± 0.001 ^c

The mean ± SD of three independent experiments is shown. Different letters between treatments indicate statistical differences in the same parameter at every transition found (starch gelatinization, amylose-lipid complex I, amylose-lipid complex IIa, and amylose-lipid complex IIb). CH: cacahuacintle; P1: pozolero 1; P2: pozolero 2; T: treatment; R: raw; N: nixtamalized; ND: non-detected; To: Onset temperature; Tp: Peak temperature; Te: Endset temperature; ΔH: Enthalpy; CH: Cacahuacintle; P1: Pozolero 1; P2: Pozolero 2; Nixt: nixtamalized.

complex IIb was observed with increasing lard content in the samples, indicating that increases in the lard contents lead to Type IIb amylose-lipid complexes. According to Tufvesson et al. (2003), the complex formation will depend on the type of lipid added and the heat treatment. Moreover, at rising heat treatment time, Type II complexes formation with long-chain fatty acids will be favored. This process could be related to lard composition, where long-chain fatty acids predominate (C16:0, C18:0, C18:1). When samples were cooked for two hours, Type II complexes formed more than Type I, which could be verified with the enthalpies of dissociation, where Type II complexes presented a higher enthalpy than Type I.

Pasting properties

The variety of maize and the treatment applied had a significant effect ($p < 0.05$) on the pasting properties of the grains (Fig. 4).

In raw maize flour, CH had the lowest pasting temperature (73.53 ± 0.94 °C) (Fig. 4a, black line), followed by P1 (73.68 ± 0.31 °C) (Fig. 4c, black line) and P2 (75.92 ± 0.41 °C) (Fig. 4e, black line). No significant differences ($p < 0.05$) were found between the varieties of maize. An increase in pasting temperatures between 2 and 9 °C was observed in the nixtamalized samples (Fig. 4a, c, and e, red line). The increase in pasting temperature is associated with an increase in the stability of the starch granule to swell and collapse, indicating that a higher temperature is required for starch gelatinization (Figuroa et al., 2013). These results agree with thermal results (Table 1). The increase in gelatinization temperature is associated with an alteration in the starch structure caused by annealing.

In cooked samples with lard added (Fig. 4b, d, and f), the pasting temperature increased as the lard percentage increased. This suggests that a higher temperature is needed for starch gelatinization in the presence of lard. The above agrees with the microstructure of cooked samples, where the structure of the starch granules is preserved despite the heat treatment (Fig. 1).

In addition, the peak viscosities varied among samples (raw, nixtamalized, and cooked kernels). CH presented a higher maximum (4759 ± 750 cP) and final viscosity (5056 ± 210 cP), followed by variety P2, with 4531.85 ± 525 cP and 779.23 ± 75.92 cP for maximum

and final viscosity, respectively. Finally, variety P1 was the one with the lowest values at maximum (3532 ± 5.35 cP) and final viscosity (3687 ± 1.41 cP) (Fig. 4, black line) Figuroa et al. (2013), reported a direct relationship between the compaction degree of starch granules in the endosperm and the maximum viscosity of different maize varieties, where a more compact microstructure can restrict water absorption. CH maize was classified as a very soft grain for the three studied varieties, presenting a microstructure with a lower compaction degree than P1 and P2 (soft grain). This difference in compaction affected the maximum and final viscosity, allowing better water absorption in CH. Therefore, the hardness of the maize grains and the compaction of the starch granules significantly affected their pasting properties. The nixtamalized samples presented lower maximum and final viscosity than the raw maize samples ($p < 0.05$). It has been associated with decreased viscosity values during nixtamalization due to the interactions between amylose-amylose or amylose-amylopectin chains. The formation of amylose-lipid complexes reduces the ability of starch granules to absorb and retain water (Santiago-Ramos et al., 2018).

The addition of lard to the nixtamalized kernels had a significant effect ($p < 0.05$) on the maximum and final viscosity peaks. Cooked samples with lard had a lower viscosity than their counterpart cooked without lard (Fig. 4b, d, and f, green line). A decrease in maximum and final viscosity was observed as the percentage of lard increased. The difference can be attributed to the lard's action that prevents or limits the water diffusion into the starch granules. The above is consistent with the SEM results, where a layer of lard on the surface of the starch granules was observed (Fig. 1). Since amylose-lipid complexes reduce the ability of starch granules to absorb water, it explains the decrease of viscosity in cooked samples with lard, presumably attributed to the formation of amylose-lipid complexes. These results are consistent with the thermal properties, in which lard added samples showed enthalpies corresponding to the amylose-lipid complexes (Table 1).

Resistant starch

The RS in raw samples was statistically equal ($p > 0.05$) among maize varieties, having $0.57 \pm 0.09\%$, $1.47 \pm 0.41\%$, and $1.52 \pm 0.23\%$ for

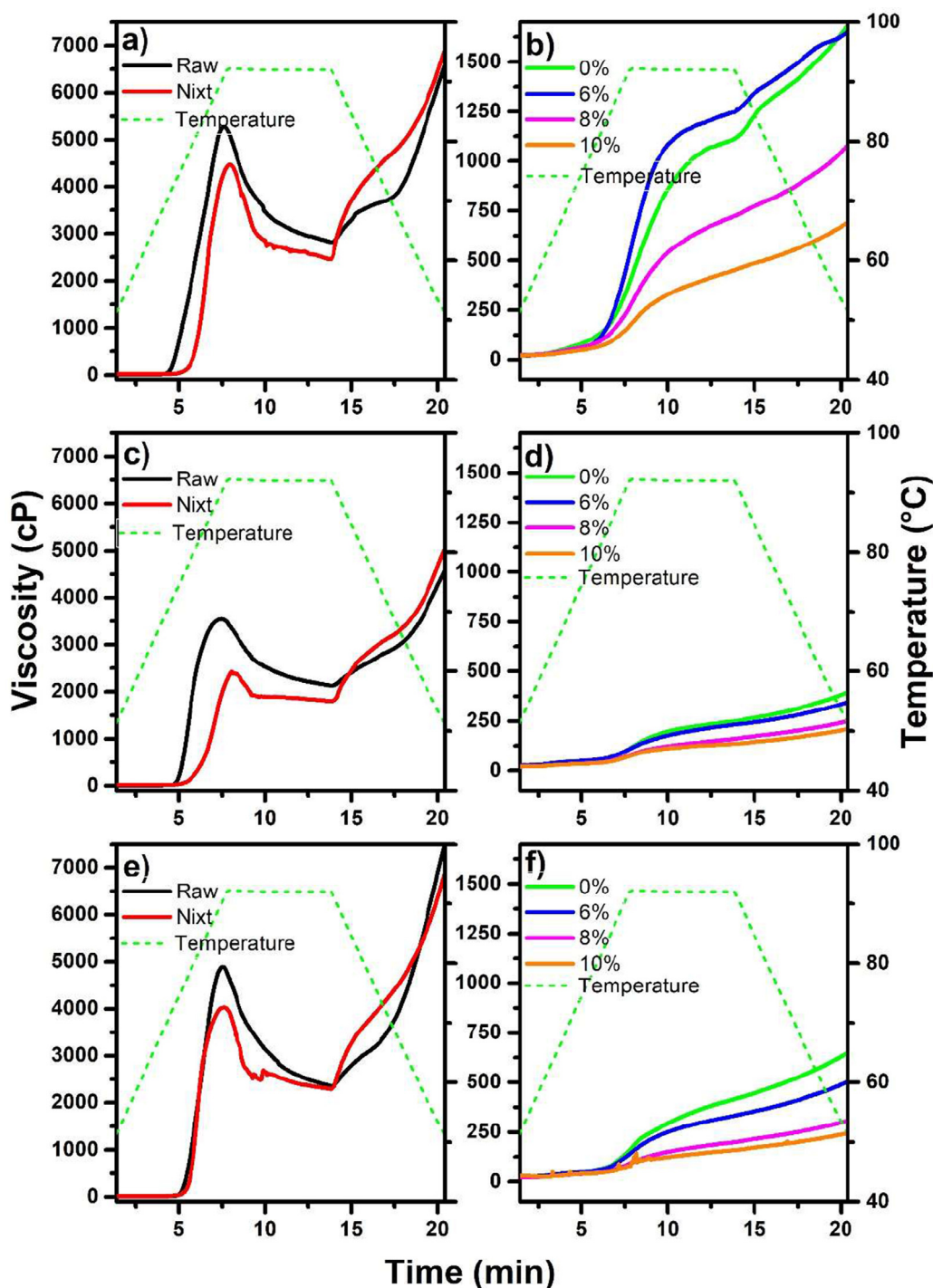


Fig. 4. Viscosity profile of pozole with different concentrations of lard. a: CH raw and nixtamalized. b: CH cooked with 0, 6, 8, and 10 % of lard. c: P1 raw and nixtamalized. d: P1 cooked with 0, 6, 8, and 10 % of lard. e: P2 raw and nixtamalized. f: P2 cooked with 0, 6, 8, and 10 % of lard.

CH, P1 and P2, respectively. In raw samples, resistant starch is mainly associated with resistant starch type 1 (physically unavailable starch) and type 2 (native starch) (Santiago-Ramos et al., 2015). Moreover, RS content observed in these samples are similar to previously reported in maize landraces (Vázquez-Carrillo et al., 2016).

A significant increase in RS was observed in all varieties ($p < 0.05$) for the nixtamalized samples (Fig. 5). CH showed the highest increase compared to raw, reaching $1.01 \pm 0.12\%$ RS. Meanwhile, P2 had $2.47 \pm 0.56\%$ RS, and P1 showed the lowest increase with $1.82 \pm 0.30\%$ RS. The increase in RS in nixtamalized samples is attributed mainly

to the formation of RS3 (retrograded starch) and to a lesser extent to RS4 due to the addition of calcium hydroxide during nixtamalization and RS5 for the amylose-lipids complexes (Santiago-Ramos et al., 2015; Figuero-Cárdenas et al., 2016; Jayakody and Hoover, 2008).

Cooked samples without added lard increased RS, mainly in CH and P1 varieties (Fig. 5). This increase is associated with the formation of retrograded resistant starch (RS3). These results are related to the thermal properties (Table 1) since the samples of cooked maize without lard did not present the characteristic endotherm of the amylose-lipid complexes.

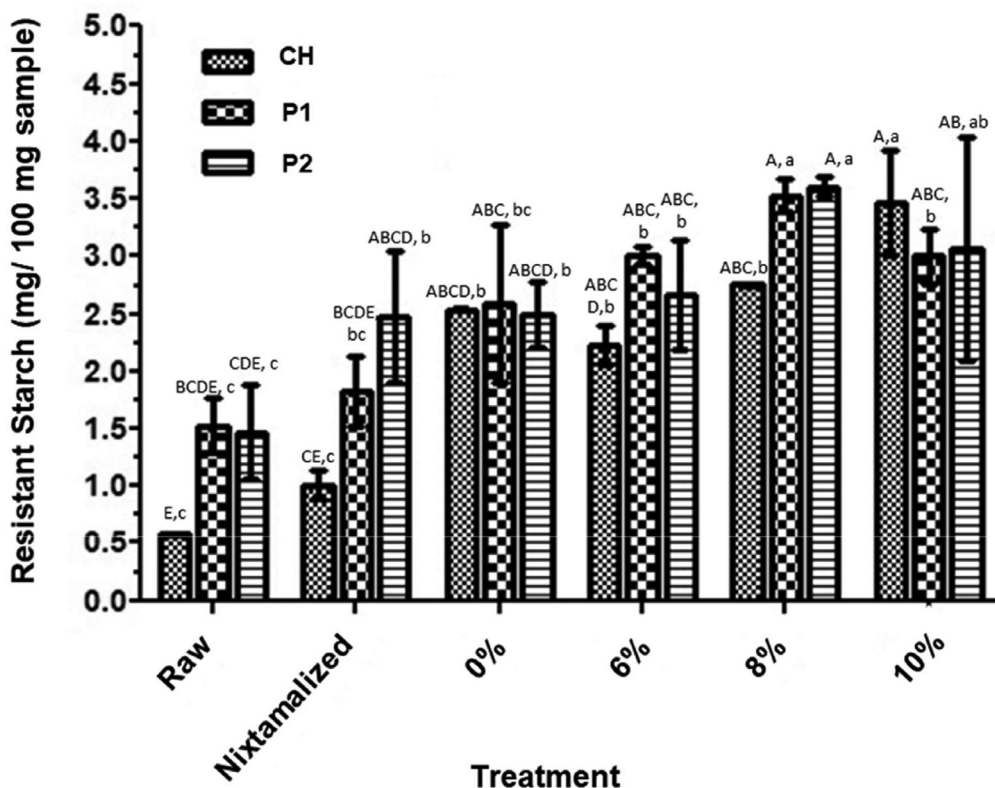


Fig. 5. Resistant starch content in raw maize, nixtamal, and pozole with lard. CH: Cacahuacintle; P1: Pozolero 1; P2: Pozolero 2. Means \pm SD followed by the same letter do not show significant differences. Uppercase letters indicate differences between varieties, and lowercase letters indicate differences between treatments.

RS increased as the lard content rose, regardless of the maize variety, regarding the cooked samples with lard. The CH variety obtained a maximum RS of $3.46 \pm 0.45\%$ after being cooked with 10% lard. Meanwhile, P1 and P2 showed a maximum RS of $3.52 \pm 0.13\%$ and $3.59 \pm 0.08\%$ when adding 8% lard. These differences could be related to the grain hardness as CH was considered a very soft grain and was the variety that presents a lower gelatinization temperature range compared with P1 and P2. To our knowledge, this is the first study on the effect of lard added on the content of resistant starch in maize destined to produce pozole. Wherein samples with a higher content of lard (8–10%) have a higher RS content, attributed to the formation of amylose-lipid complexes. Due to its structural characteristics, amylose chains can interact with other molecules, forming complexes (Wang et al., 2018). Therefore, it would be expected that the amylose interacts with the fatty acids released from the lard after being heated due to lipolysis.

According to Obiro et al. (2012), the formation of amylose-lipid complexes is affected by the ligand type, the process applied, and the amylose-amylopectin ratio, among other factors. According to Hernández-Galeno et al. (2014), the amylose content in Cacahuacintle variety ranges from 8.9 to 25.3%. In contrast, in pigmented varieties, such as Elotes Occidentales, the amylose content is between 20 and 22%. The highest percentage of RS in CH could be associated with the amylose content Tachil et al. (2014), reported that amylose-rich corn flour (45% amylose) significantly increases the formation of amylose-lipid complexes. On the other hand, the ligand used in the study was lard, which its composition predominates monounsaturated fatty acids (oleic acid [C18:1]), saturated fatty acids (palmitic acid [C16:0] and stearic acid [C18:0]), and polyunsaturated fatty acids (linoleic acid [C18:2]) (Ripoche and Guillard, 2001). In that sense, it has been reported that the interaction of amylose with palm oil (mainly composed of C16:0, C18:1, C18:2) takes place when the mixture is heated (Tufvesson et al., 2003). According to Cervantes-Ramírez et al. (2020), interactions between amylose and fatty acids such as oleic acid and stearic acid are

possible in maize subjected to thermal procedures such as extrusion. Based on the above, it could be expected that the amylose-lipid complexation may occur during the heating of the nixtamalized grain during the final step of pozole preparation. These results are consistent with the thermal properties observed due to the endotherms corresponding to the dissociation of amylose-lipid complexes type II found in samples with a higher lard content. Furthermore, the increase in RS can be related to the pasting properties since the decrease in viscosity of the pozole samples with lard can be attributed to the formation of RS during the pozole production process (Fig. 4b, d, and f).

Conclusions

This work evaluated the effect of the addition of lard on the formation of amylose-lipid complexes during de maize cooking in pozole, a Mexican dish. In that sense, the orthorhombic structure of starch (A-type) was maintained after nixtamalization and cooking, but structural damage in the crystalline structure was observed. On the other hand, a decrease in the gelatinization endotherm and a subsequent increase in their temperatures (T_0 , T_p) was observed, suggesting a more thermally stable material formation. Furthermore, the amylose-lipid complexes' endotherms fusion was observed as the lard concentration rose, indicating a formation of amylose lipid complexes type I and II. The maximum viscosity of the samples decreased as the lard concentration increased, associated with the physical impediment of water diffusion into the starch granule due to the lard and the formation of amylose-lipid complexes. Finally, the RS content increased significantly to the increase in lard, reaching a maximum RS when using 10% lard in varieties CH and 8% for P1 and P2 landraces. In that sense, lard concentrations between 8–10% could be used for further investigations. These results are relevant since, in recent years, lard has been cataloged as harmful to the organism; however, the data suggest that lard can interact with starch, providing fiber to the organism. The above can hint

at a lower starch hydrolysis degree and, subsequently, lowering their glycemic index. However, more detailed work is required to investigate this effect and the possible health effects of pozole consumption.

Declaration of Competing Interest

The authors declare no conflict of interest. The funders had no role in the study's design, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.afres.2022.100078](https://doi.org/10.1016/j.afres.2022.100078).

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