

# Effect of Storage Time on In Vitro Digestibility and Resistant Starch Content of Nixtamal, Masa, and Tortilla

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## ABSTRACT

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Nixtamal, masa, and tortilla samples were stored for 24–96 hr and their chemical composition, retrogradation, and in vitro starch digestibility features were evaluated. Ash and fat contents in the three products were smaller than in the original corn sample, but protein levels were higher, all in accordance with previous studies. In general, a minor decrease in available starch (AS) content was observed with storage time. Masa showed the greatest AS values, followed by tortilla and nixtamal. Tortilla presented slightly higher retrograded resistant starch (RS3) values (1.1–1.8%, dmb) than masa (0.7–0.9%) and nixtamal (0.7–0.8%) and only minor increases were observed after 24 hr of storage, suggesting that retrogradation phenomenon in these samples takes place very rapidly and is more pronounced in the final product (tortilla). The development of

RS3 explains the observed decrease in AS. Higher total resistant starch values were found in all samples at a range of 2.1–2.6% for nixtamal and masa, and a range of 3.1–3.9% in tortilla. This indicates that, apart from retrograded resistant starch, some ungelatinized fractions appear to contribute to the indigestible content of these products. The  $\alpha$ -amylolysis rate of the three materials decreased with storage. Tortilla showed the greatest hydrolysis indices. Differential scanning calorimetry (DSC) analysis showed that the nixtamal, masa, and tortilla did not show differences in amylopectin crystal melting temperature with storage time, but tortilla exhibited higher enthalpy values after 72 hr of storage, in accordance with the greater total RS contents recorded after prolonged storage.

The nixtamalization of maize is an ancient process developed by the Aztecs and still utilized in the production of high-quality tortillas and other maize-related food products such as *pozole*. The Aztecs ground the maize grains after an alkaline heating treatment known as nixtamalization. Still today, several Latin American countries manufacture maize food products using nixtamalization. These products represent an important source of calories, protein, dietary fiber, and calcium (Campus-Baypoli et al 1999). Currently, as part of the ethnic food trend, table corn tortillas are highly popular in industrialized countries and are often consumed as snacks or during the main meal (Yau et al 1994). But masa production at the industrial level does not quite follow the traditional nixtamalization conditions, resulting in tortillas that are of lower quality (texture and stability during storage) when compared with tortillas made traditionally.

Carbohydrates constitute the main fraction of cereal grains, accounting for up to 50–70% of the dry matter. Of these, starch and nonstarch polysaccharides (dietary fiber) are the major constituents. In addition to being a major plant metabolite, starch is also the predominant carbohydrate in the human diet (Skrabanja et al 1999). Until recently, starch had been considered an available carbohydrate that was completely digested and absorbed in the small intestine. However, now we know there is a starch fraction that is resistant to enzyme digestion; it passes through the small intestine and reaches the large bowel where it is fermented by colonic microflora. This fraction is called resistant starch (RS) and is defined as the sum of starch and the products of starch degradation not absorbed in the small intestine of healthy individuals (Asp 1992). The main classification of RS was proposed by Englyst et al (1992) based on the nature of the starch and its environment in food. RS1 corresponds to physically inaccessible starches entrapped in a cellular matrix, as in legume seeds (Tovar et al 1992a). RS2 are native uncooked starch granules in foods such as raw potato or banana. Crystallinity makes them less susceptible to hydrolysis (Englyst and Cummings 1987;

Faisant et al 1995). RS3 are retrograded starches formed in cooked foods that are kept at low or room temperature (Noah et al 1998).

The rate and extent of starch digestion, and therefore the RS content of foods, will affect a number of physiological functions and have different effects on health including reduction of the glycaemic and insulinemic response to food; hypocholesterolemic effects; and protective effects against colorectal cancer (Jenkins et al 1987; Cassidy et al 1994; De Deckere et al 1995; Asp et al 1996). Among the factors affecting the rate and extent of starch digestion, the most important are food processing, storage time, and botanic origin of the food. RS2 starch in raw foods is barely digestible. However, during cooking, starch is gelatinized and made available, although a fraction of this available starch (RS3) is retrograded on cooling and becomes resistant to enzymatic digestion (Snow and O'Dea 1981; Björck et al 1994; Asp et al 1996; Bravo et al 1998). Gelatinized starch gels are thermodynamically unstable structures. Reassociation of the starch molecules may occur on cooling. Retrogradation, the ability of starch chains to form ordered structures in pastes, gels, and baked foods during storage, greatly influences the texture and shelf life of these products (Biliaderis 1991). In starch-rich products such as bread, amylose retrogradation is a rapid process taking only a few hours. Amylopectin retrogradation, on the other hand, which is involved in bread staling, continues over a period of several days or weeks (Colonna et al 1992).

The objective of the present study was to evaluate how the different steps in tortilla manufacturing and length of storage affect in vitro digestibility of maize starch. Also of interest was the effect of accelerated staling on RS formation and its technological and nutritional implications.

## MATERIALS AND METHODS

### Sample Preparation

The traditional method to produce nixtamal, masa, and tortillas was used. Sample lots of 5 kg of maize (commercial grain, Industriales de la Masa y Tortilla de México) were cooked in 15 L of lime solution. Lime was added at 1% (grain weight basis). Maize was cooked for 1 hr at boiling temperature, steeped in the same cooking vessel for 16 hr, and then the cooking solution (*nejayote*) was discarded. The resulting nixtamal was washed three or four times with tap water to remove bran and excess lime. Nixtamal was ground into a masa using a commercial stone grinder. Masa was pressure-molded and extruded into thin circles to make tortillas 1 mm thick. Tortillas were baked in a gas-fired domestic oven (Hotpoint,

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6B4411LO, Leisser S.A. de C.V., San Luis Potosí, México) for 1 min per side at  $\approx 250^{\circ}\text{C}$ . After cooling, nixtamal, masa, and tortillas were packed into polyethylene bags ( $20 \times 30$  cm, Plásticos de México, S.A. de C.V., México) and stored for 24, 48, 72, and 96 hr at  $4^{\circ}\text{C}$ , after which the samples were freeze-dried in liquid nitrogen. Stored tortilla samples were reheated in the gas oven for 30 sec on each side at  $\approx 250^{\circ}\text{C}$ , cooled to  $30^{\circ}\text{C}$ , then freeze-dried in liquid nitrogen. This variation was introduced to replicate the consumer preparation of this product. All samples were stored at room temperature in sealed plastic containers.

### Chemical Composition

Moisture content was determined gravimetrically ( $130 \pm 2^{\circ}\text{C}$  for 2 hr) using 2–3 g of ground sample. Ash, protein, and fat were analyzed according to Approved Methods 08-01, 46-13, and 30-25, respectively (AACC 2000). Soluble carbohydrates were determined by preparing a dispersion of sample and water (1%, w/w) in a flask and heating in a boiling water bath for 30 min stirring every 5 min. The slurry was then centrifuged ( $5,000 \times g$  for 10 min) and the supernatant volume was measured. Total soluble carbohydrates were determined by colorimetry in aliquots of supernatant (Dubois et al 1956).

### In Vitro Digestibility Tests

Potentially available starch content was assessed by following the multienzymatic protocol of Holm et al (1986) using  $\alpha$ -amylase (Termamyl, Novo A/S, Copenhagen) and amyloglucosidase (Boehringer, Mannheim). Resistant starch was assessed by two different protocols. Retrograded resistant starch (RRS or RS3) content was measured as starch remnants in dietary fiber residues, according to the Lund method as modified by Saura-Calixto et al (1993). The method proposed by Goñi et al (1996) was employed to estimate the total amount of indigestible starch (RS2, RS3, and part of RS1 fractions). The in vitro rate of hydrolysis was measured using hog pancreatic amylase according to Holm et al (1985). Each assay was run with 500 mg of available starch.

### Crystallinity Degree of Polymer Systems

Differential scanning calorimetry measurements were made using a calorimeter (model 2010, TA Instruments, New Castle, DE) previously calibrated with indium. The samples (2 mg) were weighed directly into DSC aluminum pans and deionized water was added with a microsyringe to make a 65–75% (w/w, dmb) suspension. After sealing, the pans were left to equilibrate (15 min) at room temperature and then heated from 30 to  $120^{\circ}\text{C}$  at  $10^{\circ}\text{C}/\text{min}$ . An empty pan was used as reference for all measurements.

### Statistical Analysis

Analysis of variance determined differences in mean values from replicates using statistical software (v. 6.0, SPSS, Chicago, IL).

## RESULTS AND DISCUSSION

### Chemical Composition

The chemical composition of raw maize, nixtamal, masa, and tortilla is given in Table I. The raw sample showed the highest moisture content because grains were ground to prepare a flour that retained most of the original sample humidity. The moisture level was 6.3–7.8% in nixtamal and masa, and slightly higher in tortilla. The similarity of these values is due to the drying process.

Ash and fat contents in the products were lower than in raw maize, which suggests that, during the nixtamalization process, part of these components was eliminated in the nejayote. Ash content values were 1.43–1.68%, which compares favorably with ash values of 1.3–1.7% in another study in this laboratory (Bello-Pérez et al, unpublished data) with masa and masa flours where the lime concentration used in each nixtamalization process was similar. Fat values for nixtamal, masa, and tortilla stored for different times were not significantly different ( $\alpha = 0.05$ ). On the other hand, protein concentration among the samples investigated varied at 7.10–8.82%. Those values were higher than values recorded for the raw maize sample. This behavior is typical because nixtamalization increases protein levels and its digestibility in maize (Cravioto et al 1945). Almeida-Dominguez et al (1996) reported protein values of 6.7–11.6% for dry masa flours, and Bello-Pérez et al (unpublished data) found protein values of 7.5–8.9% for masas and dry masa flours.

### Starch Content

Appreciable variation (70.20–79.64%) was recorded among samples for available starch (AS) content (Table II). AS values were slightly greater in the fresh nixtamal and masa than in the raw grain which may be a consequence of partial removal of nonstarch constituents during processing. Interestingly, grinding of freshly prepared nixtamal to obtain masa increased AS by 4%. This could reflect a extensive starch release from cellular structures, thus allowing complete enzymatic hydrolysis to glucose. However, resistant starch data do not fully support this idea.

Generally, AS content decreased with storage length. This might be related to the development of retrograded indigestible fractions on cold storage. Tortilla had the smallest variation of AS values with time. This may be due to the reheating of these samples before the freeze-drying step.

TABLE I  
Chemical Composition of Corn, Nixtamal, Masa, and Tortilla

Sample/Storage (hr)	Moisture <sup>a</sup>	Ash <sup>b</sup>	Fat <sup>b</sup>	Protein <sup>b,c</sup>
Corn	12.80 $\pm$ 0.40a <sup>d</sup>	2.37 $\pm$ 0.10a	6.61 $\pm$ 1.08a	6.59 $\pm$ 0.06a
Nixtamal				
0	6.40 $\pm$ 0.30b,c	1.46 $\pm$ 0.03b,c	2.95 $\pm$ 0.20b	7.16 $\pm$ 0.17b,c
24	6.30 $\pm$ 0.10b	1.43 $\pm$ 0.005b	2.97 $\pm$ 0.12b	7.10 $\pm$ 0.25b
48	6.80 $\pm$ 0.20c,d	1.52 $\pm$ 0.01c	2.97 $\pm$ 0.01b	7.39 $\pm$ 0.03b,c
72	6.60 $\pm$ 0.30c	1.47 $\pm$ 0.01b,c	2.68 $\pm$ 0.50b	7.39 $\pm$ 0.43b,c
Masa				
0	6.90 $\pm$ 0.20c,d	1.66 $\pm$ 0.02e	3.13 $\pm$ 0.73b	8.69 $\pm$ 0.15e
24	7.30 $\pm$ 0.05d,e	1.68 $\pm$ 0.09e	3.10 $\pm$ 0.11b	8.82 $\pm$ 0.14e
48	6.90 $\pm$ 0.10c,d	1.52 $\pm$ 0.01c	3.38 $\pm$ 0.38b	8.09 $\pm$ 0.13d
72	7.20 $\pm$ 0.03d,e	1.50 $\pm$ 0.01c	2.95 $\pm$ 0.29b	8.18 $\pm$ 0.29d
Tortilla				
0	6.90 $\pm$ 0.20c,d	1.59 $\pm$ 0.02d	2.46 $\pm$ 0.26b	7.53 $\pm$ 0.17c
24	7.00 $\pm$ 0.10d	1.59 $\pm$ 0.01d	2.32 $\pm$ 0.20b	7.84 $\pm$ 0.08c,d
48	7.40 $\pm$ 0.04e	1.55 $\pm$ 0.01c,d	2.40 $\pm$ 0.26b	7.72 $\pm$ 0.04c,d
72	7.80 $\pm$ 0.05f	1.55 $\pm$ 0.00c,d	2.61 $\pm$ 0.25b	7.35 $\pm$ 0.03b,c

<sup>a</sup> Means of three replicates  $\pm$  standard error.

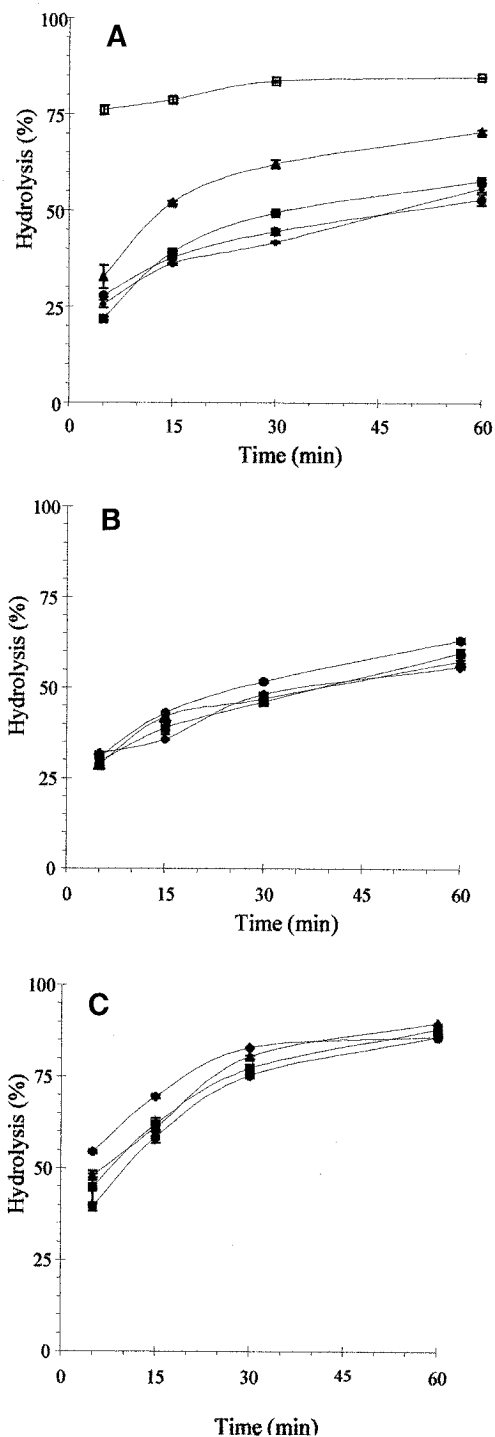
<sup>b</sup> Means of three replicates  $\pm$  standard error, dry basis.

<sup>c</sup>  $N \times 5.85$ .

<sup>d</sup> Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

## Resistant Starch

The total resistant starch (RS) value in the raw corn sample was similar to that reported by Garcia-Alonso et al (1999) in corn flour ( $1.99 \pm 0.20\%$ ) (Table II). Tortilla showed significantly higher RS values than original corn. This is in accordance with the fact that tortilla was the only material in which RRS levels rose as consequence of cold storage, with a concomitant decrease in AS content after 72 hr. Thus, starch retrogradation seems to increase after the second heat treatment and drying involved in tortilla making. Thermal treatment followed by cooling and storage produces RRS, as reported in corn flour (Garcia-Alonso et al 1999) and in waxy corn starch gels



**Fig. 1.** In vitro starch hydrolysis of corn (A) nixtamal ( $\square$ ) at 0 hr ( $\blacktriangle$ ), 24 hr ( $\blacksquare$ ), 48 hr ( $\bullet$ ), and 72 hr ( $\blacklozenge$ ); (B) masa at 0 hr ( $\blacklozenge$ ), 24 hr ( $\blacksquare$ ), 48 hr ( $\blacktriangle$ ), and 72 hr ( $\bullet$ ); (C) tortilla at 0 hr ( $\blacklozenge$ ), 24 hr ( $\blacktriangle$ ), 48 hr ( $\blacksquare$ ), and 72 hr ( $\bullet$ ).

(Fredriksson et al 2000). The formation of retrograded starch requires dehydration of the gelatinized sample (Bjorck et al 1994; Fredriksson et al 2000), a phenomenon that is likely to take place when tortillas are baked at  $\approx 250^\circ\text{C}$  and cooled.

Additional heating and cooling of tortillas before analyses may have influenced the present results. An earlier study established a similar pattern for RS in nixtamal, masa, and tortilla (Campus-Baypoli et al 1999). Thus, the tortilla-making process appears mainly responsible for the changes in indigestible starch levels. Furthermore, the process may be considered as a suitable method to increase resistant starch levels in corn-based products.

Indigestible starch contents determined according to Goñi et al (1996) were always higher than those evaluated using the Saura-Calixto et al (1993) protocol (Table II). The Goñi method reports the sum of the ungelatinized (type 2) and retrograded (type 3) resistant fractions, plus some of the physically inaccessible starch (type 1), whereas the Saura-Calixto method assess RRS only (Tovar 2001). Since grinding of nixtamal did not decrease total RS content in masa (Table II), it may be concluded that these products contain only minor levels of physically inaccessible starch, but perhaps significant amounts of RS2 in addition to RS3. In contrast, cooked legumes that complement maize-based items in traditional Latin American diets reportedly contain higher levels of RS1, RS2, and RS3 fractions (Tovar et al 1990, 1991, 1992a; Bravo et al 1998; Garcia-Alonso et al 1998).

**TABLE II**  
Available Starch (AS), Total Resistant Starch (RS), and Retrograded Resistant Starch (RRS) in Corn, Nixtamal, Masa, and Tortilla

Sample/Storage (hr)	AS (%)	RS (%) <sup>a</sup>	RRS (%) <sup>b</sup>
Corn	$73.85 \pm 1.38^c$	$1.99 \pm 0.20^a$	nd <sup>d</sup>
Nixtamal			
0	$75.89 \pm 0.14^d$	$2.18 \pm 0.01^{a,b}$	$0.85 \pm 0.02^{a,b}$
24	$71.31 \pm 0.85^{a,b}$	$2.47 \pm 0.04^{b,c}$	$0.82 \pm 0.35^{a,b}$
48	$70.42 \pm 0.13^a$	$2.58 \pm 0.01^{b,c}$	$0.81 \pm 0.23^{a,b}$
72	$70.20 \pm 0.60^a$	$2.60 \pm 0.00^{b,c}$	$0.72 \pm 0.02^a$
Masa			
0	$79.64 \pm 0.11^d$	$2.05 \pm 0.05^a$	$0.65 \pm 0.03^a$
24	$78.93 \pm 0.22^d$	$2.14 \pm 0.22^a$	$0.72 \pm 0.07^a$
48	$76.99 \pm 0.71^e$	$2.25 \pm 0.12^{a,b}$	$0.85 \pm 0.02^{a,b}$
72	$75.57 \pm 0.28^e$	$2.27 \pm 0.08^{a,b}$	$0.86 \pm 0.07^{a,b}$
Tortilla			
0	$72.92 \pm 0.44^{b,c}$	$3.12 \pm 0.01^d$	$1.06 \pm 0.00^b$
24	$72.83 \pm 0.68^{b,c}$	$3.27 \pm 0.08^d$	$1.79 \pm 0.01^c$
48	$73.13 \pm 0.10^c$	$3.45 \pm 0.54^{d,e}$	$1.82 \pm 0.00^c$
72	$70.97 \pm 0.87^a$	$3.87 \pm 0.03^e$	$1.84 \pm 0.02^c$

<sup>a</sup> Using method of Goñi et al (1996).

<sup>b</sup> Using method of Saura-Calixto et al (1993).

<sup>c</sup> Mean values of three replicates, dry matter basis. Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

<sup>d</sup> Not determined.

**TABLE III**  
Differential Scanning Calorimetry Data of Fresh and Cold Stored Nixtamal, Masa, and Tortilla

Sample	Storage Time (hr) <sup>a</sup>			
	0	24	48	72
Nixtamal				
$T_p$ ( $^\circ\text{C}$ ) <sup>b</sup>	$81.8 \pm 0.1^a$	$81.8 \pm 0.2^a$	$81.3 \pm 0.1^a$	$81.8 \pm 0.1^a$
$\Delta H$ (J/g) <sup>c</sup>	$5.6 \pm 0.8^a$	$6.0 \pm 0.3^a$	$5.6 \pm 0.3^a$	$5.8 \pm 0.3^a$
Masa				
$T_p$ ( $^\circ\text{C}$ ) <sup>a</sup>	$79.8 \pm 0.1^a$	$80.8 \pm 0.4^b$	$80.7 \pm 0.3^b$	$80.9 \pm 1.0^{a,b}$
$\Delta H$ (J/g) <sup>b</sup>	$10.4 \pm 2.3^a$	$13.1 \pm 2.2^{a,b}$	$16.5 \pm 1.0^{a,b}$	$17.0 \pm 2.1^{a,b}$
Tortilla				
$T_p$ ( $^\circ\text{C}$ ) <sup>a</sup>	$89.9 \pm 3.3^a$	$89.5 \pm 1.8^a$	$86.9 \pm 2.4^a$	$87.2 \pm 2.5^a$
$\Delta H$ (J/g) <sup>b</sup>	$4.7 \pm 0.2^a$	$5.9 \pm 0.6^b$	$7.6 \pm 1.0^c$	$7.8 \pm 0.5^c$

<sup>a</sup> Mean values of three replicates  $\pm$  standard error; storage temperature  $4^\circ\text{C}$ . Values followed by the same letter are not significantly different ( $P < 0.05$ ).

<sup>b</sup> Melting temperature.

<sup>c</sup> Melting enthalpy.

## Rate of Enzymatic Starch Hydrolysis

The *in vitro*  $\alpha$ -amylolysis reaction of nixtamal, masa, and tortilla is represented in Fig. 1. Corn flour without nixtamalization treatment showed a 76% hydrolysis after 5 min and 84.7% after 60 min. These values were higher than for nixtamalized corn and masa, although the digestion degree after 60 min of reaction was similar to those of tortillas. In general, the three materials analyzed presented hydrolysis indices that decreased with storage time. In this study, the lowest hydrolysis values were  $\approx$ 50% after 60 min, which are markedly higher than those reported for cooked beans and some Indian legumes (Velasco et al 1997; Bravo et al 1998; Garcia-Alonso et al 1998), all in accordance with the generally greater hydrolysis rates exhibited by cereals in comparison to pulses (Tovar et al 1992b; Björck et al 1994; Velasco et al 1997). Since low amylolysis rates generally correlate with moderate glycemic responses (Björck et al 1994), the controlled cold storage of tortillas may represent a way to reduce their glycemic indices.

## Crystallinity Degree of Polymer Systems

The results of the DSC analysis of the samples are shown in Table III. The melting temperature values ( $T_p$ ) correspond to retrograded amylopectin crystallites. The structures in tortilla appear to be more stable than in nixtamal and masa, as  $T_p$  values were markedly higher. This may be due to the more intense heat treatment involved in tortilla making, which may result in a more extensive reorganization of starch components.

None of the samples exhibited differences in melting temperature with storage time, therefore the crystalline structures formed at each stage of processing may be considered to disorganize at the same temperatures. Nixtamal did not show changes in melting enthalpy with storage time, but enthalpy values in masa and tortilla increased with storage time, suggesting that the retrogradation phenomenon was greater in these two samples. This apparently increased a tendency of tortilla starch to retrograde and seems to be in accordance with its relatively high RRS values (Table II), a parallelism that could not be established for masa.

This discrepancy might be explained by the possible occurrence, in masa, of significant retrogradation without a concomitant rise in RRS, since *in vitro* RRS assessment reports retrograded amylose fractions but does not account for recrystallized amylopectin (Saura-Calixto et al 1993; Tovar 2001). This phenomenon was stressed in a recent study of heat-treated high-amylopectin starches (Fredriksson et al 2000).

Campus-Baypoli et al (1999) found melting temperatures of 83.0 and 80.1°C in nixtamal and masa, respectively, but only 61.5°C was registered for tortillas, which is remarkably lower than the one recorded here. This inconsistency is indicative of an ample variability in tortilla thermal analysis parameters, a subject that deserves further evaluation.

In summary, the present study shows that AS content in the evaluated samples decreased with storage. Tortilla showed the greatest RS values and this content increased slightly with storage, a phenomenon that may have a potential in the formulation and production of reduced calories and diets promoting gut health, but could also limit the gross nutritive value of these Latin American staple food items. In addition, decreased  $\alpha$ -amylolysis rates were observed after storage, which might have consequences for the glycemic response to nixtamalized corn products. These features may be also be beneficial for consumers of Mexican ethnic food in developed countries.

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