

Effect of storage time on *in vitro* digestion rate and resistant starch content of tortillas elaborated from commercial corn masas

Edith Agama-Acevedo, Rodolfo Rendón-Villalobos, Juscelino Tovar, Sergio Rubén Trejo-Estrada and Luis Arturo Bello-Pérez

Centro de Desarrollo de Productos Bióticos del IPN, Yautepec, Morelos, México. Instituto de Biología Experimental, Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela. Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada del IPN, Unidad Puebla, Puebla, México

SUMMARY. Tortilla samples were elaborated by four small commercial factories in Mexico, employing masas prepared with the traditional nixtamalization process. Samples were stored at 4 °C for up to 72 hours and their chemical composition and *in vitro* starch digestibility features were evaluated. Chemical composition did not change with the storage time, but soluble carbohydrates decreased slightly during storage. A significant decrease in available starch content upon storage was observed, concomitant with increased resistant starch (RS) levels. These changes are possibly due to retrogradation. Retrograded resistant starch (RRS) values increased with storage time; in some samples, RRS represented more than 75% of total RS whereas in others it only accounted for 25%. The digestion rate (DR) in the freshly prepared tortillas was similar for the various samples, but after 72 h storage some differences among tortillas were found. Also, when a single tortilla sample was compared throughout the different storage times, lower DRs were determined in samples subjected to prolonged storage, which is related to the concomitant increase in RRS. The differences found among the various tortilla samples may be due to minor variations in the commercial processing conditions and to the use of different corn varieties.

Keywords: Com, texture, tortillas, resistant starch, starch digestibility.

RESUMEN. Efecto del tiempo de almacenamiento sobre el contenido de almidón resistente y la tasa de digestión *in vitro* de tortillas elaboradas con masas comerciales de maíz. Se elaboraron tortillas utilizando masa a partir de cuatro tortillerías, la cual fue obtenida mediante el proceso tradicional de nixtamalización. Las muestras fueron almacenadas a 4 °C hasta por 72 h y se determinó su composición química y digestibilidad del almidón *in vitro*. La composición química no cambió con el tiempo de almacenamiento, pero los carbohidratos solubles disminuyeron ligeramente durante el almacenamiento. Se observó que el contenido de almidón disponible disminuyó y el almidón resistente (AR) se incrementó con el tiempo de almacenamiento. Estos resultados se deben posiblemente a la retrogradación del almidón. Los contenidos de almidón resistente retrogradado (ARR) se incrementaron con el tiempo de almacenamiento; en algunas muestras el ARR representó más del 75 % del AR total y en otras muestras fue sólo el 25 %. La velocidad de digestión (VD) en las tortillas recién elaboradas fue similar, pero se encontraron diferencias significativas entre las tortillas almacenadas por 72 h. Al comparar las VDs de las tortillas a los diferentes tiempos, se encontró que disminuyeron significativamente con el tiempo de almacenamiento, lo cual está relacionado con el incremento en el contenido de ARR. Las diferencias determinadas en las tortillas pueden deberse a variaciones en las condiciones del proceso de nixtamalización que usa cada tortillería así como la variedad de maíz utilizada.

Palabras clave: Maíz, textura, tortillas, almidón resistente, digestibilidad del almidón.

INTRODUCTION

The nixtamalization of corn is an ancient process developed by the Mesoamerican civilizations and is still utilized in the production of "tortillas" and other corn related food products (i.e., pozole). The corn grains are cooked with alkali (i.e. lime) and steeped, in a process known as nixtamalization. After grinding and washing the "nixtamal" (i.e., alkaline-cooked corn grains) a soft dough, known as "masa", is obtained. The

"masa" is a mixture constituted by starch polymers, mixed with partially gelatinized starch granules, intact starch granules, pieces of endosperm, and lipids. All these components develop a complex heterogeneous network in a continuous water phase (1). "Masa" is used in the production of tortillas, which are the principal staple food in the Mexican diet, representing the main source of carbohydrates and calcium (2). Nowadays, table tortillas are highly popular in the United States and, to some extent, also in Canada and some

European countries (3).

The nixtamalization process produces changes that improve the nutritional quality of tortillas. Many studies have been conducted on nutritional aspects of nixtamalized corn, but very few studies have been carried out on the bioavailability of its carbohydrate constituents (4). Carbohydrates represent the main fraction of cereal grains, accounting for up to 50-70 % of the dry matter; of these, starch and non-starch polysaccharides (dietary fiber) are the main constituents. Besides, being a major plant metabolite, starch is also the dominating carbohydrate in the human diet (5,6). Current knowledge on nutritional features of starch indicates that the bioavailability of the polysaccharide in foods may vary widely (7). Hence, a nutritional classification of dietary starch has been proposed, which takes into account both the kinetic component and the completeness of its digestibility, thus comprising rapidly digestible, slowly digestible and indigestible – or resistant- fractions (8). The resistant starch (RS) is defined as the sum of starch plus the products of starch degradation not absorbed in the small intestine of healthy individuals (9). The main classification of RS has been proposed by Englyst *et al.* (8); it is based both on the nature of the starch and its environment in the food. RS1 corresponds to physically inaccessible starches, entrapped in a cellular matrix, as in legume seeds (10). RS2 are native uncooked granules of starch, such as raw potato or banana starches, whose crystallinity makes them scarcely susceptible to hydrolysis (11,12). RS3 are retrograded starches, which may be formed in cooked foods that are kept at low or room temperature (13). It has been proposed that both the rate and extent of starch digestion, and therefore the RS content of foods, will affect a number of physiological functions and thus will have different effects on health, e.g. reduction of the glycemic and insulinemic response to a food, hypocholesterolemic effects and protective action against colorectal cancer (14-17). Among the factors affecting the rate and extent of starch digestion, food processing, storage time and botanical origin of the food have a major importance. Starch in raw foods is not easily digested, exhibiting variable levels of RS2 fractions. However, during cooking, starch is gelatinized and rendered available, although a fraction of this available starch retrogrades upon cooling and becomes resistant to enzymatic digestion (RS3) (5,14,18,19). Gelatinized starch gels are thermodynamically unstable structures and, on cooling, reassociation of the starch molecules may occur. The ability of starch chains to form ordered structures in pastes, gels and baked foods during storage, a process often described by the term “retrogradation”, greatly influences the texture and shelf-life of these products (20).

In a previous study (4), experimental tortillas were prepared from laboratory-nixtamalized corn, using standardized

conditions throughout the whole process. Such materials were evaluated in terms of their *in vitro* starch digestibility. The objective of the present study was to evaluate the influence of storage on the “*in vitro*” digestibility of starch in tortilla made at the traditional commercial level in Mexico, which is more representative of the currently consumed food.

MATERIALS AND METHODS

Sample preparation: “Masas” were purchased from four small factories (A, B, C and D) called “tortillerias”, in Yautepec, Mexico, and brought immediately to the laboratory, always kept at temperatures between 28 and 32°C. These masas were obtained according to the traditional method to produce nixtamal, which shows minor differences among the different “tortillerias”; such variations relate mainly to the length of the steeping step (4). Once in the laboratory, the masa was quickly molded by pressure and extruded into thin circles to obtain 1mm-thick “tortillas”. Tortillas were baked in a home gas fired oven (Hotpoint, 6B4411LO, Leisser S.A. de C.V., San Luis Potosí, México) for 1 min per side, at an approximate temperature of 250°C. After cooling, tortilla samples were either processed for analysis (control tortillas) or packed into poly-ethylene bags (20 X 30 cm, Plásticos de México, S.A. de C.V., México) and stored for 24, 48 and 72 h at 4°C. After baking (control) or following the corresponding storage period, samples were frozen in liquid nitrogen, freeze-dried and stored at room temperature in sealed plastic containers. In the case of stored tortillas, before freeze-drying, samples were placed in an oven (30 s each side), at an approximate temperature of 250°C, cooled down to 30°C, a procedure that resembles the domestic reheating applied to cold-stored tortillas.

Chemical composition: Moisture content was determined gravimetrically ($130 \pm 2^\circ\text{C}$ for 2 h) using between 2-3 g of ground sample (sieved through 50 U.S. mesh). Ash, protein and fat were analyzed according to AACC(21) methods 08-01, 46-13, and 30-25, respectively (21). Soluble carbohydrates were determined as follows: a sample dispersion in deionized water (1% wt/wt, neutral pH) was prepared in a flask and then heated in a boiling water bath for 30 min with stirring every 5 min. The slurry was then centrifuged (5000 x g/10 min) and the supernatant volume measured. Total soluble carbohydrates were determined by colorimetry in aliquots of the supernatant (22).

***In vitro* digestibility tests:** Total starch was determined using the method of Goñi *et al.* (23). Potentially available starch content was assessed following the multienzymatic protocol of Holm *et al.* (24) using Termamyl® (Novo A/S, Copenhagen) and amyloglucosidase (Boehringer, Mannheim). Resistant starch was measured by two different protocols: 1) Retro-

graded resistant starch (RRS or RS3) content was measured as starch remnants in dietary fiber residues, according to the so called "Lund method" as modified by Saura-Calixto *et al.* (25,2) The method proposed by Goñi *et al.* (26) was employed to estimate the total amount of indigestible starch (comprising RS2, RS3 and part of RS1 fractions). The *in vitro* rate of hydrolysis was measured using hog pancreatic amylase, according to Holm *et al.* (27); each assay was run with 500 mg available starch.

Statistical analysis: A randomized complete design with three replications was used to analyze changes during tortilla storage. Data were analyzed using one-way Analysis of Variance (ANOVA) procedures. Where analysis showed significant differences ($p < 0.05$), means were compared using Tukey's tests at a level a significance of 0.05. Statistical analyzes were run using the computer SPSS V. 6.0 (software (SPSS Institute Inc., Cary NC) (28).

RESULTS

Chemical composition

The chemical composition of tortillas is presented in Table 1. In general, moisture content in the same kind of tortilla did

not change with the storage time; confirming that the polyethylene bags where the tortillas were stored restricted water losses. Protein concentration did not change significantly ($P \leq 0.05$) with storage time. The highest values were recorded in sample A and the lowest ones in sample C. Ash content in tortillas did not change with the storage time nor with the masa source. Fat content in tortillas A and D had a slight decline with storage time, but samples B and C did not show changes in fat values. Soluble carbohydrates decreased with the storage time; samples A and B showed the highest values whereas C and D exhibited the lowest ones.

Total starch

The values of total starch content (TS) are presented in Table 2. Freshly cooked sample B showed the highest values and the A tortillas the lowest ones.

Available starch

The values of available starch (AS) in the tortillas analyzed ranged between 67.04 and 76.04% (Table 2). In all samples, AS values decreased with storage time. Such a tendency was more evident than in the case of TS.

TABLE 1
Chemical composition of tortillas

Simple/Storage (h)	Moisture ¹	Protein ^{2,3}	Ash ²	Fat ²	Soluble Carbohydrates ²
A					
0	9.41 ± 0.05 ^a	9.17 ± 0.40 ^a	1.47 ± 0.05 ^{a,b,c}	3.32 ± 0.08 ^a	38.33 ± 0.70 ^a
24	9.98 ± 0.22 ^b	9.58 ± 0.14 ^a	1.45 ± 0.08 ^{a,b,c}	2.95 ± 0.05 ^b	35.69 ± 0.85 ^b
48	9.61 ± 0.37 ^b	9.56 ± 0.05 ^a	1.45 ± 0.05 ^{a,b,c}	3.00 ± 0.08 ^b	33.94 ± 0.95 ^b
72	9.69 ± 0.11 ^b	9.12 ± 0.20 ^a	1.51 ± 0.17 ^{b,c}	3.28 ± 0.27 ^c	29.23 ± 0.25 ^c
B					
0	8.33 ± 0.19 ^c	8.97 ± 0.20 ^{a,b}	1.61 ± 0.05 ^{b,c}	3.23 ± 0.16 ^{a,c}	45.45 ± 1.11 ^d
24	8.68 ± 0.15 ^d	8.61 ± 0.12 ^b	1.48 ± 0.15 ^{b,c}	3.24 ± 0.19 ^c	40.19 ± 0.45 ^e
48	8.56 ± 0.17 ^{c,d}	8.99 ± 0.22 ^{a,b}	1.63 ± 0.00 ^{b,c}	3.43 ± 0.00 ^c	39.60 ± 0.93 ^{a,c}
72	8.48 ± 0.05 ^{c,d}	9.07 ± 0.00 ^a	1.60 ± 0.00 ^{b,c}	3.40 ± 0.19 ^c	39.21 ± 0.46 ^{a,c}
C					
0	9.64 ± 0.03 ^b	8.05 ± 0.23 ^c	1.67 ± 0.00 ^{b,c}	3.97 ± 0.00 ^d	28.47 ± 0.67 ^c
24	10.38 ± 0.10 ^{e,f}	7.90 ± 0.00 ^c	1.66 ± 0.00 ^{b,c}	4.10 ± 0.05 ^c	26.55 ± 0.69 ^f
48	9.60 ± 0.11 ^b	8.64 ± 0.19 ^{a,b}	1.67 ± 0.00 ^{b,c}	4.38 ± 0.23 ^e	24.70 ± 0.54 ^g
72	10.06 ± 0.40 ^{e,f}	8.63 ± 0.12 ^{a,b}	1.71 ± 0.07 ^{b,c}	4.19 ± 0.00 ^c	23.41 ± 0.67 ^h
D					
0	10.14 ± 0.55 ^f	8.79 ± 0.23 ^{a,b}	1.56 ± 0.23 ^c	3.15 ± 0.18 ^a	30.80 ± 0.47 ⁱ
24	9.63 ± 0.57 ^b	8.77 ± 0.11 ^{a,b}	1.55 ± 0.18 ^{b,c}	2.75 ± 0.08 ^g	30.43 ± 0.35 ^{i,j}
48	9.53 ± 0.20 ^b	8.77 ± 0.11 ^{a,b}	1.48 ± 0.00 ^{a,b,c}	2.86 ± 0.08 ^{b,g}	30.04 ± 0.53 ⁱ
72	9.70 ± 0.28 ^b	9.39 ± 0.30 ^a	1.51 ± 0.15 ^{a,b,c}	2.85 ± 0.00 ^h	29.86 ± 0.28 ^j

¹ Means of three replicates ± standard error ($n = 9$ determinations).

² Means of three replicates ± standard error, dry weight basis ($n = 9$ determinations).

³ $N \times 5.85$.

Values followed by the same letter in the same column (all samples) are not significantly different ($P \leq 0.05$)

TABLE 2
Total Starch (TS), Available Starch (AS), Total Resistant Starch (RS) and Retrograded
Resistant Starch (RRS) in tortillas¹

Simple/Storage (h)	TS (%)	AS (%)	RS (%) ²	RRS (%) ³
A				
0	74.79 ± 0.54 ^a	74.42 ± 0.58 ^a	1.36 ± 0.17 ^a	1.06 ± 0.10 ^a
24	74.05 ± 0.27 ^a	73.34 ± 0.49 ^b	1.75 ± 0.17 ^b	1.21 ± 0.11 ^a
48	72.73 ± 0.21 ^b	72.81 ± 0.42 ^b	2.18 ± 0.20 ^c	1.45 ± 0.10 ^b
72	72.13 ± 0.63 ^b	67.38 ± 0.43 ^{c,e}	2.70 ± 0.04 ^d	1.74 ± 0.11 ^c
B				
0	79.73 ± 0.26 ^c	70.45 ± 0.46 ^{d,g}	2.39 ± 0.25 ^c	0.88 ± 0.10 ^a
24	78.81 ± 0.35 ^d	69.79 ± 0.64 ^{d,g}	2.47 ± 0.17 ^c	1.33 ± 0.12 ^b
48	77.04 ± 0.37 ^e	67.71 ± 0.49 ^{c,e}	3.28 ± 0.31 ^e	1.61 ± 0.10 ^{b,c}
72	76.37 ± 0.50 ^e	67.20 ± 0.71 ^e	4.18 ± 0.15 ^f	2.13 ± 0.12 ^d
C				
0	76.04 ± 0.31 ^e	76.04 ± 0.64 ^f	1.94 ± 0.19 ^{b,c}	1.18 ± 0.10 ^a
24	75.03 ± 0.45 ^{a,c}	75.13 ± 0.63 ^{a,f}	2.34 ± 0.12 ^c	1.44 ± 0.10 ^b
48	72.24 ± 0.34 ^b	72.64 ± 0.87 ^b	2.56 ± 0.16 ^d	1.65 ± 0.10 ^{b,c}
72	72.57 ± 0.46 ^b	70.35 ± 0.99 ^g	2.92 ± 0.12 ^e	2.22 ± 0.10 ^d
D				
0	76.30 ± 0.44 ^e	70.10 ± 0.39 ^{d,g}	3.05 ± 0.20 ^e	0.67 ± 0.10 ^e
24	76.29 ± 0.31 ^e	69.96 ± 0.42 ^{d,g}	3.02 ± 0.21 ^e	1.16 ± 0.10 ^a
48	76.22 ± 0.45 ^e	68.03 ± 0.44 ^{c,e}	2.97 ± 0.19 ^e	1.32 ± 0.08 ^b
72	76.02 ± 0.46 ^{a,c}	67.04 ± 0.41 ^{c,e}	3.12 ± 0.27 ^e	1.57 ± 0.08 ^{b,c}

¹ Dry basis ² Using method of Goñi *et al* (26) ³ Using method of Saura-Calixto *et al* (25)

Means of three replicates ± standard error, dry basis ($n = 9$ determinations).

Values followed by the same letter in the same column are not significantly different ($P \leq 0.05$).

Resistant starch

In general, total resistant starch (RS) values in tortilla samples A, B and C increased with storage time; this in accordance with the fact that tortillas were cold stored, thus favoring the retrogradation phenomenon (RRS). However, since RS in D tortillas did not change upon storage (Table 2); corn varietal differences may again be important for this characteristic.

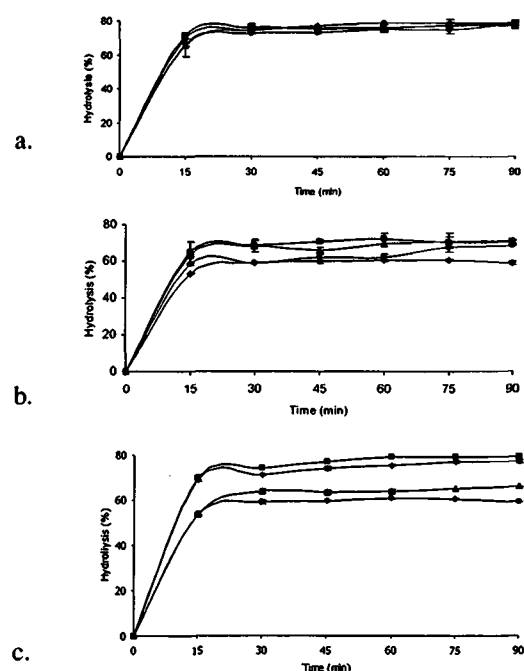
Rate of enzymatic starch hydrolysis

The *in vitro* α -amylolysis reaction of tortillas is represented in Figure 1. The control samples (0 h of storage, Fig. 1a) did not show differences at the different hydrolysis times. Starch in tortillas was rapidly hydrolyzed by pancreatic amylase, as 70% of the polysaccharide in the samples was digested in 15 minutes.

DISCUSSION

Differences were found in moisture level among the different tortilla samples, which may be due to either corn variety or, more likely, to variations in the nixtamalization process, as all tortillas were cooked under the same temperature and time conditions. Rendón-Villalobos *et al.* (4) reported moisture content (between 6.90 and 7.80%) in tortillas stored

FIGURE 1



In vitro starch hydrolysis of Tortillas (a, 0 hr, control samples ■), Tortilla A: ♦, Tortilla B: ▲, Tortilla C: ● Tortilla D. (b, 72 hr of storage): ■, Tortilla A; ♦, Tortilla B; ▲, Tortilla C; ● Tortilla D. (c) Tortilla B: (■) at 0 hr, (♦) at 24 hr, (▲) at 48 hr, (●) at 72.

during different times and freeze dried values that were lower than those found in this study. However, they found increased moisture levels at the longest storage times. The differences found in protein content among the tortillas prepared with masas from different "tortillerias" may be attributed to perceived differences in corn variety and the process for "masa" preparation used in each "tortilleria"; as a matter of fact, Méndez-Montealvo et al. (unpublished data) found protein values that ranged between 8.2 and 11.2% in raw samples of twenty corn varieties cultivated in Mexico. Similarly, Rendón-Villalobos et al. (4) reported protein values (7.35-7.84%) in tortillas; levels that did not change with storage time ($P \leq 0.05$). Ash content usually does not vary with the nixtamalization process (29,30). The ash values ranged between 1.45 and 1.71%, which were similar to those reported by Méndez-Montealvo et al. (unpublished data) in raw corn (1.1-1.7%), and by Rendón-Villalobos et al. (4) for tortillas (1.55-1.59%). These values are in agreement with the fact that lime concentration used in the nixtamalization process applied to the different samples was rather similar. The differences determined in fat values may be also attributable to the corn variety and conditions prevailing during nixtamalization process. Méndez-Montealvo et al. (unpublished data) determined fat content in raw corn varieties between 4.0 and 7.0%, which is lower than those found by Rendón-Villalobos et al. (4) (2.40-2.61%) in tortillas stored at different times. There is no clear explanation for the soluble carbohydrate pattern, although it might be due in part to the retrogradation phenomenon, as crystalline structures are formed during storage, resulting in decreased starch solubilization. However, leaching of partly hydrolyzed starch fractions during grain nixtamalization cannot be ruled out.

Variations in TS among the diverse tortilla samples may be explained by the corn variety used for the masa preparation in each "tortilleria". Regarding the storage impact, in general, TS values showed only minor decay with time; the exception was tortilla sample D, the TS level of which remained constant. To the best of the authors' knowledge, there are no published data on TS content in corn tortillas.

The reduction in AS content during storage may be explained by the formation of resistant starch due to the retrogradation phenomenon that takes place when a cooked starch product is cold-stored (8). The tortilla sample B exhibited the highest difference between TS and AS, whereas A and C presented the lowest one. Thus, when stored, the latter samples seem to contain smaller amounts of indigestible starch fractions. Differences in TS-AS values among the diverse tortillas may reflect differences in corn variety and nixtamalization process, since there is appreciable variation in AS measured in twenty Mexican corn hybrids and varieties (unpublished data). It is therefore important to make the appropriate choice of corn variety for obtaining major or minor

AS content in tortillas. According to Rendón-Villalobos et al. (4), AS values in tortillas ranged between 72.92 and 70.97, and they tended to decrease during cold storage.

The AS value variability recorded among tortillas elaborated with the different masas may also be related to corn variety or nixtamalization conditions, specially in the steeping time or nixtamal washing which, in addition to an important removal of fibrous material (31), may as well result in RS reduction. Tortillas A and B showed the highest variation in RS with storage time, as the "fresh" control samples contained 1.36% and 2.39%, respectively, but after 72 h values rose to 2.70% and 4.18%, which represents a relative increase of 98% and 75%, respectively. For tortilla C, the RS values changed from 1.94 (t=0h) % to 2.97 % (t=72h), i.e., a relative increase of 50 %. These data may have interesting implications, as tortillas bought from different "tortillerias" are likely to have different RS levels and, presumably, different structural firmness. In general, the A, C and D tortillas exhibited lower RS values than those determined in laboratory-made tortillas (3.12-3.87%) (4); corn botanical type and nixtamalization conditions may play an important role in this behavior. Such a raise in RS during cold storage is consistent with the recorded AS decrease after 72 hours (Table 2). Wet thermal treatment followed by cooling and storage produces retrograded resistant starch (RRS), as reported for corn flour (32) and for various starch gels (33,34). The formation of retrograded starch requires dehydration of the gelatinized sample (5,33), a phenomenon that is likely to take place when tortillas are baked, at approximately 250°C, and cooled. It should be mentioned that the additional heating/cooling step applied to tortillas before analyses, probably influenced present results, but this condition is also valid for the product "as eaten".

It is noteworthy that RS and RRS values in 72h-stored C tortilla are similar (Table 2); a fact that suggests most indigestible starch in this sample consists of retrograded fractions. Appreciable differences between RS and RRS levels were registered for the remaining 72h-stored samples, which indicates that RS2, and perhaps some RS1, are also present in these materials.

A RRS value of 2.5% was determined in tortillas immediately elaborated and analyzed (2); a value that was higher than those reported here. It has been mentioned that during baking, heat treatment promotes the interaction of starch with other corn components, making it less accessible to enzyme hydrolysis; the tortilla making process appears largely responsible for the recorded changes in indigestible starch levels. RRS values between 2.2% and 2.9% were determined in tortillas stored for 2, 24, 48 and 72 h, either at room or refrigeration temperature, without appreciable differences at both temperatures (35). Hence, the tortilla-making process may be considered a suitable way to increase

resistant starch levels in corn-based products.

Rendón-Villalobos *et al.* (4) determined RRS values for stored tortillas between 1.06 and 1.84%, similar to here reported ones. However, they are lower than those reported by Campas-Baypoli *et al.* (2,35). This apparent discrepancy may be consequence of the accuracy differences existing among resistant starch methods of analysis (7), although the impact of varietal or processing conditions cannot be ruled out.

The high hydrolysis rate (70% of starch was digested during the first 15 minutes) was also observed by Tovar *et al.* (36), and is an indicator of the effective gelatinizing effect of nixtamalization and tortilla making on corn starch. For the 72 h-stored materials (Fig. 1b), on the other hand, differences were found among tortillas elaborated with diverse masas; in fact, B and D tortillas did not show differences ($p < 0.05$) between 30 and 60 min hydrolysis time, with constant values (approximately 70% of hydrolysis) after 30 min. However, at 75 min the A, C and D tortillas behaved similarly. B tortilla had the lowest digestion index at 15 min and the hydrolysis values remained constant during the experiment (approximately 58%). When the behavior of this tortilla sample was compared along the different storage times (Fig. 1C), two groups were described: a) fresh tortillas and those stored for 24 h had the greatest hydrolysis rates, with values (approximately 70%) that did not change along most of the reaction time, and b) tortillas kept for 48 and 72 h displayed a similar pattern, with constant hydrolysis degrees (of approximately 60%). Hence, present results suggest that longer storage times increase RS contents (Table 2) and produce a concomitant decrease in hydrolysis rates.

CONCLUSIONS

No change was observed in moisture content in tortillas stored for different times, which is likely due to the restricted water-losses occurring in the poly-ethylene containers used. Protein, lipids and ash contents did not change with storage time, but protein and ash contents in tortillas were similar to those reported in raw corn, but lower lipid values were determined, which may be related to solubilization during nixtamalization and nixtamal washing. Decreased available starch and slightly augmented resistant starch (RS) levels were recorded upon cold-storage. However, retrograded resistant starch (RRS) content was variable in the different tortillas; in some samples RRS represents more of 70% of RS, whereas in others it only accounts for approximately 25%. α -amylolysis rates of stored samples tended to decrease at prolonged (72h) storage times. The differences found in starch bioavailability in tortillas may be mainly due to both corn variety and nixtamalization conditions used in each tortilleria for masa preparation. In general, hydrolysis rate fell as storage time

increased, this pattern relates to RRS formation during tortilla storage.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the economic support from CGPI-IPN and COSNET. Technical assistance of Tech. Juan Alcantar and Tech. Teresa Rodríguez is gratefully acknowledged.

REFERENCES

1. Gomez MH, Rooney LW and Waniska RD. Dry corn masa flours for tortilla and snack food. *Cereal Foods World* 1987;32:372-377.
2. Campas-Baypoli ON, Rosas-Burgos EC, Torres-Chávez PI, Ramírez-Wong B and Serna-Saldívar SO. Physicochemical changes of starch during maize tortilla production. *Starch/Stärke* 1999;51:173-177.
3. Yau JC, Waniska RD and Rooney LW. Effects of food additives on storage stability of corn tortillas. *Cereal Foods World* 1994;39:396-402.
4. Rendón-Villalobos JR, Bello-Pérez LA, Osorio-Díaz P, Tovar J and Paredes-López O. Effect of storage time on in vitro digestibility and resistant starch content of nixtamal, masa and tortilla. *Cereal Chem* 2002;79:340-344.
5. Björck IM, Granfeldt Y, Liljeberg H, Tovar J and Asp NG. Food properties affecting the digestion and absorption of carbohydrates. *Am J Clin Nutr* 1994; 59:699S-705S.
6. Skrabanja V, Liljeberg HM, Hedley CL, Kreft I and Björck IME. (1999). Influence of genotype and processing on the in vitro rate of starch hydrolysis and resistant starch formation in peas (*Pisum sativum* L.). *J Agric Food Chem* 1999;47:2033-2039.
7. Tovar J. Métodos para la determinación de almidón resistente en los alimentos. In: *Fibra Dietética en Iberoamérica: Tecnología y Salud. Obtención, caracterización, efecto fisiológico y aplicación en alimentos* (edited by F.M. Lajolo, F. Saura-Calixto, E. Wittig de Penna, and E.W. Menezes). Sao Paulo: Varela Ltd. Press. 2001 Pp.143-154.
8. Englyst HN, Kingman SM and Cummings JH. Classification and measurement of nutritionally important starch fractions. *Eur J Clin Nutr* 1992;46 (Suppl. 2) S33-S50.
9. Asp NG. Resistant starch. Proceedings from the second plenary meeting of EURESTA. *Eur J Clin Nutr* 1992; 46 (Suppl.2), SI.
10. Tovar J, Björck IM and Asp NG. Incomplete digestion of legume starches in rats: A study of precooked flours containing retrograded and physically inaccessible starch fractions. *J Nutr* 1992;122:1500-1507.
11. Englyst HN and Cummings JH. Digestion of polysaccharides of potato in the small intestine of man. *Am J Clin Nutr* 1987;45:423-431.
12. Faisant N, Gallant DJ, Bouchet B and Champ M. Banana starch breakdown in the human small intestine studied by electron microscopy. *Eur J Clin Nutr* 1995;49:98-104.

13. Noah L, Guillon F, Bouchet B, Buleon A, Molis C, Gratas M and Champ M. Digestion of carbohydrate from white beans (*Phaseolus vulgaris* L.) in healthy humans. *J Nutr* 1998;128:977-85.
14. Asp NG, Van Amelsvoort JMM and Hautvast JGAJ. Nutritional implications of resistant starch. *Nutr Res Rev* 1996;9:1-31.
15. Cassidy A, Bingham SA and Cummings JH. Starch intake and colorectal cancer risk: an international comparison. *Brit J Cancer* 1994;69:937-942.
16. De Deckere EAM, Kloots WJ and Van Amelsvoort JMM. Both raw and retrograded starch decrease serum triacylglycerol concentration and fat accretion in the rat. *Brit J Nutr* 1995;73:287-289.
17. Jenkins DJA, Wolever TMS, Collier GR, Ocana A, Rao AV, Buckley G, Lam Y, Mayer A and Tompson LU. Metabolic effects of a low-glycemic-index diet. *Am J Clin Nutr* 1987;46:968-975.
18. Bravo L, Siddhuraju P, and Saura-Calixto F. Effect of various processing methods on the in vitro starch digestibility and resistant starch content of indian pulses. *J Agric Food Chem* 1998;46, 4667-4674.
19. Snow P, and O'Dea K. Factors affecting the rate of hydrolysis of starch in food. *Am J Clin Nutr* 1981;34, 2721-2727.
20. Biliaderis CG. The structure and interactions of starch with food constituents. *Can J Physiol Pharmacol* 1991; 69, 60-78.
21. American Association of Cereal Chemists (A.A.C.C). Approved Methods of the AACC. St. Paul, MN. 2000
22. Dubois M, Gilles KA, Hamilton JK, Rebers PA, and Smith F. Colorimetric method for determination of sugars and related substances. *Anal Chem* 1956; 28, 350-356.
23. Goñi I, Garcia-Alonso A, and Saura-Calixto F. A starch hydrolysis procedure to estimate glycemic index. *Nutr Res* 1997;3, 423-433.
24. Holm J, Bjorck I, Drews A, and Asp NG. A rapid method for the analysis of starch. *Starch/Stärke* 1986; 38, 224-229.
25. Saura-Calixto F, Goñi I, Bravo L, and Mañas E. Resistant starch in foods: Modified method for dietary fiber residues. *J Food Sci* 1993;58, 642-645.
26. Goñi I, Garcia-Diaz L, Mañas E, and Saura-Calixto F. Analysis of resistant starch: A method for foods and food products. *Food Chem* 1996;56, 445-449.
27. Holm J, Bjorck I, Asp NG, Sjöberg LB, and Lundquist I. Starch availability in vitro and in vivo after flaking, steam-cooking and popping of wheat. *J Cereal Sci* 1985;3, 193-200.
28. SPSS para Windows. Programación y Análisis Estadístico. México: Mc-Graw Hill. 1996.
29. Saldaña G, and Brown HE. Nutritional composition of corn and flour tortillas. *J Food Sci* 1984; 49, 1202-1203, 1205.
30. Flores-Farias R, Martínez-Bustos F, Salinas-Moreno Y, and Rios E. Characterization of comercial nixtamalized flours. *Agrociencia* 2002;36, 557-567.
31. San Martín-Martínez E, Jaime-Fonseca MR, Martínez-Bustos F, and Martínez-Montes JL. Selective nixtamalization of fractions of maize grain (*Zea mays* L.) and their use in the preparation of instant tortilla flours analyzed using response surface methodology. *Cereal Chem* 2003;80, 13-19.
32. García-Alonso A, Goñi I, Jiménez-Escrig A, Martín-Carrón N, Bravo L, and Saura-Calixto F. Assessment of some parameters involved in the gelatinization and retrogradation of starch. *Food Chem* 1999;66, 181-187.
33. Fredriksson H, Björck I, Andersson R, Liljeberg H, Silverio J, Eliasson AC, and Aman P. Studies on α -amylase degradation of retrograded starch gels from waxy maize and high-amylopectin potato. *Carbohydr Polym* 2000;43, 81-87.
34. Tovar J, Melito C, Herrera E, Rascón A and Pérez E. Resistant starch formation does not parallel syneresis tendency in different starch gels. *Food Chem* 2002;76, 455-459.
35. Campas-Baypoli ON, Rosas-Burgos EC, Torres-Chávez PI, Ramirez-Wong B, and Serna-Saldívar SO. Physicochemical changes of starch in Maize tortillas during storage at room and refrigeration temperatures. *Starch/Stärke* 2002;54, 358-363.
36. Tovar J, Sáyago-Ayerdi S, Peñalver C, Paredes-López O, and Bello-Pérez, LA. In Vitro Starch Hydrolysis Index and Predicted Glycemic Index of Corn Tortilla, Black Beans and Mexican "taco". *Cereal Chem* 2003;80, 533-535.

Recibido: 25-06-2004

Aceptado: 24-01-2005