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Research note

# In vitro digestion rate and resistant starch content of tortillas stored at two different temperatures

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**Abstract**

In vitro indicators of starch bioavailability were evaluated in freshly prepared maize tortillas and compared to those exhibited by 24, 48 or 72 h-stored samples. Storage took place either at room temperature (approx. 25 °C) or under refrigeration (4 °C). Potentially available starch (AS) content decreased from 670 g $kg^{-1}$ in the control tortilla to 583 g $kg^{-1}$ in 72 h-stored preparations. Concomitant increases in total resistant starch (RS) and retrograded resistant starch (RRS) were recorded upon storage. RRS content in 72 h-stored samples (35–39 g $kg^{-1}$) doubled that of freshly prepared tortillas. Changes in AS, RS and RRS were not affected by storage temperature. Both initial rate and final point of starch hydrolysis by pancreatic amylase were reduced in samples kept for 48 and 72 h, without influence of storage temperature. Storage length is suggested as a major determinant of the bioavailability of starch in tortillas.


*Keywords:* Tortilla; Storage; Resistant starch; Digestion rate; Digestibility

## 1. Introduction

The nixtamalization is a traditional Mexican process developed by the Mesoamerican civilizations and is still utilized in the production of "tortillas" and other maize-based food products. Tortillas are the principal staple food in the Mexican diet, representing the main source of carbohydrates and calcium (Campus-Baypoli, Rosas-Burgos, Torres-Chávez, Ramírez-Wong, & Serna-Saldívar, 1999). Nowadays, table tortillas are highly popular in United States and, to some extent, also in Canada and several European countries (Yau, Waniska, & Rooney, 1994).

Many studies have been conducted on nutritional aspects of nixtamalized maize, but only limited research has been carried out on the bioavailability of its carbohydrate constituents (Agama-Acevedo et al., 2004; Rendón-Villalobos, Bello-Pérez, Osorio-Díaz, Tovar, & Parédez-López, 2002). Carbohydrates represent the main fraction of cereal grains, accounting for up to 500–700 g $kg^{-1}$ of the dry matter; of these, starch and nonstarch polysaccharides (dietary fiber) are the major constituents. Starch owes much of its functionality to two major high-molecular-weight carbohydrate components, amylose and amylopectin, as well as to the physical organization of these macromolecules into the granular structure (French, 1984). When starch is cooked in excess water, the granules swell and, at the same time, part of the components solubilize, giving rise

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to a suspension of swollen particles dispersed in a macromolecular continuous phase (Thebaudin, Lefebvre, & Doublier, 1998). These events are referred to as starch gelatinization. When starch pastes are stored for minutes, or days, retrogradation occurs and it is believed to be responsible for the textural and starch digestibility changes that take place during storage of starch-based products (Farhat, Blanshard, & Mitchell, 2000). Starch retrogradation is a process where gelatinized starch returns from a solvated, dispersed or amorphous state to an insoluble, aggregated or crystalline condition (Thygesen, Blennow, & Engelsen, 2003). This phenomenon is understood as a nonequilibrium, thermo-reversible, recrystallization process, which takes place in three consecutive steps: nucleation, propagation and maturation.

Current knowledge on nutritional features of starch indicates that the digestibility of this polysaccharide in foods may vary widely (Björck, Granfeldt, Liljeberg, Tovar, & Asp, 1994; Tovar, 2001). 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 (Englyst, Kingman, & Cummings, 1992). Resistant starch (RS) is defined as the sum of starch plus starch degradation products not absorbed in the small intestine of healthy individuals (Asp, 1992). The classification of RS has been proposed by Englyst et al. (1992); it considers both the chemical features of the starch and its environment in the food. Since maize tortillas are often prepared in large batches and stored for consumption during subsequent days, the present study was undertaken to evaluate the influence of temperature and storage time on the in vitro digestibility of starch in tortilla.

## 2. Materials and methods

### 2.1. Sample preparation

The traditional Mexican method to produce nixtamal, masa and tortillas was used. Lots of 5 kg maize (commercial grain distributed for "Industriales de la Masa y Tortilla de México", variety "criollo Acatlan") were cooked in 15 l of lime solution. Lime was added at 10 g $kg^{-1}$ (grain weight basis). Maize was cooked for 1 h at boiling temperature (95 °C) and then steeped in the same cooking vessel during 16 h. The cooking solution ,or "nejayote", was discarded and the resulting "nixtamal" washed 3–4 times with tap water for bran and excess lime removal. Nixtamal was ground into a "masa" using a commercial stone grinder. Masa was molded by pressure and extruded into thin circles to obtain "tortillas" of 1 mm of thickness. 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, tortillas were packed into poly ethylene bags (20 × 30 cm, Plásticos de México, S.A. de C.V., México) and stored for 24, 48 and 72 h, either at 4 °C or room temperature (25 °C). After storage, the samples were reheated in a home gas fired oven during 30 s each side, at an approximate temperature of 250 °C and cooled down to 30 °C; such a variation was introduced in order to replicate the conditions used when this product is eaten at household level.

### 2.2. In vitro digestibility tests

Potentially available starch (AS) content was assessed following the multienzymatic protocol of Holm, Björck, Drews, and Asp (1986), using Termamyl® (Novo A/S, Copenhagen) and amyloglucosidase (102 857 Roche Diagnostics, Indianapolis, IN, USA). The method proposed by Goñi, García-Díaz, Mañas, and Saura-Calixto (1996), was employed to estimate the amount of indigestible starch (comprising part of RS1 plus RS2 and RS3 fractions) (Tovar, 2001). Retrograded resistant starch (RRS) (RS3) content was measured as starch remnants in dietary fiber residues, according to the so-called Lund method as modified by Saura-Calixto, Goñi, Bravo, and Mañas (1993). The in vitro rate of hydrolysis was measured using hog pancreatic amylase according to Holm, Björck, Asp, Sjöberg, and Lundquist (1985); each assay was run with 500 mg AS. In all these measurements the sample was weighed into a test tube or a beaker and homogenized with the appropriate solution for each technique, under controlled conditions: first step (speed level 2, 1 min) and second step (speed level 2.5, 1 min) using a Polytron homogenizer (Polytron PT 1200 Kinematica AG, Switzerland).

### 2.3. Statistical analysis

A randomized complete block design with three replications was used to analyse changes during tortilla storage. Data were analysed 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 (Ferrán, 1996).

## 3. Results and discussion

### 3.1. Available starch

The values of potentially AS in the tortillas analysed decreased with the storage time. Values for the stored