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In Vitro Starch Digestibility and Predicted Glycemic Index of Corn Tortilla, Black Beans, and Tortilla–Bean Mixture: Effect of Cold Storage

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People in the rural areas of Mexico consume corn tortillas and beans as basic components of their diet. However, little is known about the nutritionally relevant features of starch present in such combined meals. The objective of the present study was to evaluate the in vitro bioavailability of starch in tortilla−bean mixtures stored at 4 °C for different times, as compared to that of corn tortilla and boiled black beans kept separately under the same conditions. Available starch (AS), resistant starch (RS), and retrograded resistant starch (RRS) contents were measured. The in vitro starch hydrolysis indices (HI) of freshly cooked and cold-stored samples were evaluated using a chewing/dialysis digestion protocol. HIs were used to predict glycemic indices (pGI) of the samples. AS in tortilla and beans decreased between 3 and 6% after 48−72 h, whereas values in the mixture fell by 3% after 48 h, with no further change by 72 h. Only minor rises in RS contents (1.5−1.6%) were recorded for tortilla and beans after 72 h of storage, and a lower increase (0.4%) was recorded in the mixture. Judging from RRS values, an important proportion of RS is due to starch retrogradation. The HI and pGI were higher in tortilla than in bean and the mixture. Hydrolysis rate values decreased in the stored samples, a pattern that corresponded with RS and RRS changes. The slow digestion features of common beans are largely retained by the legume−tortilla combination. Data support the perceived health beneficial properties of starch in this traditional cereal−legume food.

KEYWORDS: Starch; resistant starch; glycemic index; tortilla; black bean

INTRODUCTION

In Mexico tortillas and beans are the principal staple foods in both urban and rural areas. The daily per capita consumption of tortillas is ∼325 g (*1*). Tortillas also supply 70% of the calories and 50% of the protein consumed daily (*2*). Corn proteins are considered to have low nutritional quality, because zein, the main protein fraction in corn, has low concentrations of the essential amino acids lysine and tryptophan (*3*). Nixtamalization (treatment with lime and partial cooking) improves the nutritional quality of tortillas. Many studies have been conducted on the nutritional aspects of nixtamalized maize, but very little research has been carried out on the bioavailability of its carbohydrate constituents (*4*, *5*). Carbohydrates represent 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.

The common bean (*Phaseolus vulgaris*) has an important place among the legumes of major production and consumption in Africa, India, Latin America, and Mexico (*6*−*8*). In rural areas of Mexico, consumption of beans in a normal diet represents 15% (*9*). The amino acid pattern of common bean proteins is characterized by their deficiency in the sulfur amino acids (methionine and cysteine) and tryptophan (*8*). Carbohydrates account for 55−65% of dry legumes. Of these fractions, starch and nonstarch polysaccharides (dietary fiber) are the major constituents, with smaller but significant amounts of oligosaccharides (*10*).

The digestibility of starch in foods may vary widely (*11*, *12*). 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 (*13*). Resistant starch (RS) is defined as the sum of starch plus starch degradation products not absorbed in the small

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intestine of healthy individuals (*14*). Various RS types have been characterized in common foods. According to Englyst et al. (*13*), the indigestible starch fractions may be classified according to both the nature of the starch and its environment in the food. Thus, RS1 corresponds to physically inaccessible starches, entrapped in a cellular matrix, as in legume seeds. RS2 starches are native uncooked granules of some starches, such as those in raw potatoes and green bananas, the crystallinity of which makes them scarcely susceptible to hydrolysis. RS3 starches are retrograded starches, which may be formed in cooked foods that are kept at low or room temperature. The ability of the gelled starch to recrystallize in pastes, gels, and baked foods during storage, a process often described by the term "retrogradation", greatly influences the texture and shelf life of products (*15*, *16*).

Both the rate and extent of starch digestion will affect a number of physiological functions and thus will have different effects on health, for example, caloric value reduction, influence on hypocholesterolemic action, and protective effects against colorectal cancer (*17*−*20*). Also, the rate of digestion/absorption exerts a great influence on the postprandial glycemic and insulinemic responses (*11*, *13*, *17*) and their profound metabolic consequences (*21*). Among the factors affecting the bioavailability of starch, processing, storage time, and botanical origin of the food are of major importance (*11*).

Traditionally, people in the rural areas of Mexico and Central America consume a mixture of tortilla, beans, and chili, often called "taco". It is well-known that such a mixture improves some of the nutritional characteristics of the individual items but, surprisingly, little research has been done on the nutritionally relevant features of the starch present in this composite food. Results from a recent study suggested that most of the beneficial "slow release carbohydrate" features of black beans are retained by the mixed bean−tortilla meal (*22*), an observation that may provide basis for new dietary uses of these traditional foods. Therefore, the objective of the present work was to assess the bioavailability of starch in the tortilla−bean mixture and how this starch property changes during cold storage, as compared to that of individual ingredients. For this, available and resistant starch contents, in vitro starch hydrolysis index, and the corresponding predicted glycemic index were evaluated.

MATERIALS AND METHODS

Sample Preparation. Black common bean seeds, cv. Cotaxtla, were bred through a special improvement program of INIFAP-Iguala. Fresh dry beans were cooked using a Mattson cooker type to determine cooking time (*8*). Using the selected cooking time (90 min), 100 g of beans was cooked in 200 mL of water. The mixture of cooked beans plus cooking water was divided into four subsamples, which were cooled to room temperature; three of them were stored for 24, 48, and 72 h at 4 °C, respectively, simulating cooking and storage conditions applied in Mexican households. After each storage time, the sample was equilibrated to room temperature (25 °C) for 15 min before analysis. The fourth subgroup (control sample) was analyzed without cold storage. Three replicates were run for each sample. All cooked samples were mechanically homogenized with a Polytron PT 1200 (Kinematica AG) under controlled conditions (speed level 2, 1 min), for further chemical and enzymatic analyses. Commercial corn tortillas were purchased from a "tortillería" (a small traditional tortilla factory) and immediately brought to the laboratory. After cooling, tortillas were packed into polyethylene bags (20 × 30 cm, Plásticos de México, S.A. de C.V.) and stored for 24, 48, and 72 h at 4 °C. After storage, the samples were oven-reheated for 30 s on each side, at an approximate temperature of 250 °C, cooled to 30 °C, homogenized as mentioned above, and analyzed. For the mixture, a 4:6 bean−tortilla proportion (w/w) was selected; this ratio resembles those found in common Mexican tacos.

In Vitro Digestibility Tests. Potentially available starch content was assessed following the multienzymatic protocol of Holm et al. (*23*) using Termamyl (Novo A/S, Copenhagen, Denmark) and amyloglucosidase (Boehringer, Mannheim, Germany). Resistant starch was measured according to two different protocols: (1) Retrograded resistant starch (RRS or RS3) content was measured as starch remnants in dietary fiber residues, following the so-called "Lund method" as modified by Saura-Calixto et al. (*24*). (2) The method proposed by Goñi et al. (*25*) was employed to estimate the total amount of indigestible starch (comprising RS2, RS3, and part of RS1 fractions).

Starch Hydrolysis Index of Products "As Eaten" (Chewing/Dialysis Test). The in vitro rate of starch hydrolysis was assessed with the protocol developed by Granfeldt et al. (*26*). Samples of beans or tortilla, containing 1 g of available starch, were tested. Mixtures of the two items, containing 0.6 and 0.4 g of available starch from tortilla and bean, respectively, were also analyzed. Before the digestion assay, tortillas and drained beans were warmed to 65−70 °C on a hot plate, simulating the regular household procedure for preparing the mixture. Six healthy subjects participated in the chewing phase of the experiments, which consisted of 15 chews in ∼15 s. The chewed material was carefully expectorated into a 20 mL beaker containing 0.05 M phosphate buffer adjusted to pH 1.5 with HCl, and the mixture was incubated with bovine pepsin for 30 min (37 °C), neutralized (pH 6.9), and incubated with porcine pancreatic α-amylase in a dialysis bag. The reducing amylolysis products appearing in the dialysate were measured colorimetrically and expressed as maltose equivalents. Data were plotted as degree of hydrolysis versus time curves, and the hydrolysis index (HI) was calculated as the area under the curve (0−180 min) for the test product expressed as a percentage of the corresponding area for commercial white bread, chewed by the same person.

Statistical Analysis. A completely random design with three replications was used to analyze changes during storage of the samples. Data from AS, RS, and RRS 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. The average HI was calculated from the six digestion replicates run for each sample, and means were compared by the Wilcoxon matched-pair signed-rank test, each person being his own control. The predicted glycemic index (pGI) was calculated from HI values, using the empirical formula proposed by Granfeldt (*27*): $\text{pGI} = 0.862(\text{HI}) + 8.198$, for which the correlation coefficient (r) is 0.026 ($p < 0.00001$).

Statistical analyses were run using the computer SPSS v. 6.0 software (SPSS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

AS contents recorded for fresh tortillas and cooked beans (63.4 and 36.2%, respectively, **Table 1**) were similar to those reported for canned black beans and commercial long shelf life tortillas (*22*), whereas the AS level exhibited by the taco mixture, 52.5% (**Table 1**), was in agreement with that calculated from the individual ingredients. There was a decrease in the available starch (AS) values of all three samples (taco and its ingredients), but it became significant only after 48 h of cold storage (**Table 1**). As mentioned below, such a reduction in AS with the storage time seems to be due to retrogradation, which occurs during the storage of starchy products, with marked dependence on the storage temperature (*28*): the lower the temperature, the faster the retrogradation, because nucleation and crystal growth are favored (*15*). We know of no other study looking at AS in stored tortilla−bean mixtures. RS contents (**Table 1**) were higher in beans (5.33−6.88%) than in tortillas (2.14−3.78%). Initial values remained essentially constant in the two samples for 48 h of storage, showing a 1.3−1.6% rise by the end of 72 h. Only minor changes were observed in the 48-h-stored mixture values, which showed no further rise. The tortillas used in this study underwent greater RS increase with storage than those

Table 1. Available Starch (AS), Total Resistant Starch (RS), and Retrograded Resistant Starch (RRS) in Beans, Tortilla, and Taco[a]

product/storage (h)	AS (%)	RS[b] (%)	RRS[c] (%)
beans			
0	37.13 ± 0.38a	5.33 ± 0.16a	3.47 ± 0.08a
24	36.42 ± 0.18a	5.42 ± 0.16a	3.50 ± 0.21a
48	34.40 ± 0.52b	5.58 ± 0.55a	4.38 ± 0.36b
72	34.29 ± 0.27b	6.88 ± 0.40b	4.63 ± 0.13b
tortilla			
0	65.21 ± 0.41c	2.14 ± 0.11c	1.05 ± 0.05c
24	64.41 ± 0.29c	2.61 ± 0.07c	1.52 ± 0.09d
48	60.68 ± 0.30d	2.86 ± 0.08c	1.64 ± 0.06d
72	59.72 ± 0.24d	3.78 ± 0.03d	1.80 ± 0.07d
taco			
0	52.58 ± 0.29e	3.93 ± 0.04e	3.14 ± 0.13e
24	51.84 ± 0.49ef	3.99 ± 0.21ef	3.33 ± 0.11ae
48	49.68 ± 0.23f	4.21 ± 0.15f	3.63 ± 0.10f
72	48.32 ± 0.30f	4.31 ± 0.12f	3.87 ± 0.09g

[a] The data are for foods as eaten and are given as means ± SD; $n = 3$. Values followed by the same letter in the same column are not significantly different ($P < 0.05$). [b] Using the method of Goñi et al. (*25*). [c] Using the method of Saura-Calixto et al. (*24*).

recorded by Rendón-Villalobos et al. (*4*), whereas absolute RS levels in the 72-h-stored preparations were similar in both studies. However, the RS pattern displayed following storage of tortillas prepared from nixtamalized flours (*5*) resembles the present data for traditionally made tortillas (*4*). The RS values for bean were higher than those in tortillas, and this can be explained by the crystallite structure, physical inaccessibility (*29*, *30*), and marked susceptibility to retrogradation (*31*) exhibited by starch in legume seeds, factors that result in a restricted enzymatic hydrolysis. However, lower RS values have been determined in unstored cooked beans (*32*−*34*) as compared to the control sample (unstored) of this study. Taking into account the legume−tortilla ratio in the taco, the mixture exhibited RS values closer to those of bean, suggesting its major role in the overall starch digestibility of taco. It is noteworthy that indigestible starch contents determined according to the method of Goñi et al. (*25*) (RS) were always higher than those evaluated using the Saura-Calixto et al. (*24*) protocol (RRS, **Table 1**). The differences in the results given by the two methods are due to the fact that the Goñi et al. (*25*) 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 et al. (*24*) method assesses RRS only (*12*). Similar values and patterns were found in experimental tortillas prepared with masa nixtamalized in the laboratory (*4*), the starch of which had a lower tendency to retrograde than bean starch. The dissimilar behavior can be attributed to the different starch types present in cereals and beans (*11*, *29*, *31*). Interestingly, the RRS values of the mixture are closer to those determined for RS, which, again, suggests the major importance of bean retrograded resistant fractions for the taco starch digestibility.

Metabolic responses affect and govern a number of physiological processes, and they are closely related to the rate of digestion and absorption of ingested starch, which is particularly low for beans and other pulses (*11*, *17*, *30*). The kinetic component of starch bioavailability in the various samples was estimated by means of the chewing/dialysis digestion system proposed by Granfeldt et al. (*26*). This digestion system has shown good correlation with the postprandial glycemic responses in vivo (*11*, *26*, *27*) and therefore has advantages over direct α-amylolysis assays. Starch hydrolysis percentages in the samples stored for different times are shown in **Table 2**, and, as an example, the hydrolysis curves for the different products stored for 24 h are depicted in **Figure 1**. White bread, used as reference, showed a digestion value of ∼50% after 180 min, which agrees with the values reported in the original protocol by Granfeldt et al. (*26*). Coinciding with previous observations for canned beans, ready-to-eat commercial tortillas, and the corresponding bean−tortilla taco (*22*), the course of hydrolysis differed greatly among the various samples. Hydrolysis was markedly slow for beans and faster for the bean−tortilla mixture. Tortilla and the reference bread showed an increased rate of hydrolysis over bean or bean−tortilla, regardless of storage time. Although Rendón-Villalobos et al. (*4*) and Osorio-Díaz et al. (*35*) reported greater starch hydrolysis rates for tortillas and beans, it is important to note that those studies used a simple α-amylolysis test. A slight tendency to reduced hydrolysis rates was observed in the stored samples, changes that were more evident for the taco mixture (**Table 2**). The reduction in hydrolysis rates upon cold storage agrees with the tendency recorded for RS and RRS. However, certain molecular associations in the mixed food matrix could have an additional influence on the apparent reduction in digestion rate. Such putative interactions have not been studied so far, and their investigation is therefore warranted.

Table 2. Degree of Starch Hydrolysis in a Chewing/Dialysis Digestion System over 0–180 min[a]

	degree of hydrolysis (%)					
product/storage (h)	30 min	60 min	90 min	120 min	150 min	180 min
white bread reference	10.18 ± 0.72a	18.41 ± 0.78a	26.36 ± 0.83a	32.66 ± 1.42a	38.89 ± 1.40a	47.58 ± 1.5a
beans						
0	3.37 ± 0.71b	3.85 ± 0.69bg	5.19 ± 0.66bh	6.00 ± 0.72b	7.97 ± 0.58b	11.14 ± 0.79b
24	0.72 ± 0.41c	2.02 ± 0.62b	3.96 ± 0.82b	5.97 ± 0.99b	8.17 ± 1.12b	10.50 ± 1.28b
48	1.42 ± 0.23c	1.36 ± 0.26b	3.57 ± 0.25b	6.18 ± 0.58b	8.90 ± 0.78b	10.68 ± 0.88b
72	1.15 ± 0.65c	1.73 ± 0.88b	2.96 ± 1.05b	5.36 ± 1.34b	7.48 ± 1.47b	9.87 ± 1.65b
tortilla						
0	5.45 ± 0.95d	13.31 ± 1.50c	19.98 ± 1.06c	25.59 ± 0.71c	32.08 ± 1.16c	38.86 ± 1.32c
24	4.28 ± 1.17d	10.73 ± 1.64d	17.33 ± 2.11cd	23.80 ± 2.66cd	29.51 ± 2.55cf	32.93 ± 2.93d
48	4.68 ± 0.84d	12.02 ± 0.90cd	16.91 ± 1.05cd	22.74 ± 1.11cg	27.17 ± 1.27df	32.20 ± 1.20d
72	4.18 ± 0.72d	10.76 ± 1.04d	15.92 ± 1.36cd	21.42 ± 1.71dg	25.70 ± 1.63d	30.01 ± 2.08d
taco						
0	2.12 ± 0.54ce	7.67 ± 1.20e	11.94 ± 1.36de	17.73 ± 1.53e	21.06 ± 1.72e	25.08 ± 1.90e
24	2.36 ± 0.88ce	6.24 ± 1.40f	10.20 ± 1.66eg	14.59 ± 2.16eh	13.99 ± 1.59g	18.02 ± 2.35f
48	1.76 ± 0.30ce	5.86 ± 0.73f	8.40 ± 0.65fg	11.57 ± 1.03fh	14.50 ± 1.40g	17.07 ± 1.24f
72	1.85 ± 0.58ce	5.09 ± 0.54fg	7.62 ± 0.68fh	10.56 ± 0.68f	12.84 ± 0.66g	15.35 ± 0.87f

[a] Data are means ± SEM; $n = 6$. Values in columns not sharing the same letter are significantly different ($P < 0.05$).

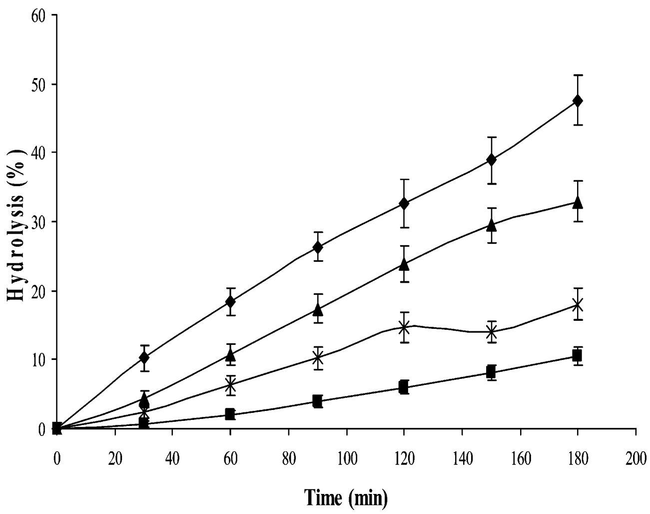

Figure 1. Rate of starch hydrolysis following chewing, incubation with pepsin, and subsequent incubation with pancreatic α-amylase, in tortilla (■), beans (×), and taco (▲) stored for 24 h; white bread reference (◆). Values are means of six chewing and digestion experiments. Areas under curves were used for calculation of HI.

Table 3. Hydrolysis Index (HI) and Predicted Glycemic Index (pGI) of Tortilla, Beans, and Taco[a]

product/storage (h)	HI[b] (%)	pGI[c] (%)
beans		
0	21.39 ± 2.10a	27
24	18.59 ± 1.39a	24
48	17.79 ± 2.47a	24
72	16.07 ± 3.50a	22
tortilla		
0	77.57 ± 1.76b	75
24	67.49 ± 5.34c	66
48	66.42 ± 2.60c	65
72	61.05 ± 3.65c	62
taco		
0	49.08 ± 4.43d	51
24	36.83 ± 4.32e	40
48	34.02 ± 2.94e	38
72	31.11 ± 1.57e	35
white bread reference	100 f	94

[a] Data are means of six chewing and dialysis replicates ± SD; $n = 6$. Means followed by different letters are significantly different ($P < 0.05$). [b] Hydrolysis index (HI) was compared with white bread (*26*). [c] Predicted glycemic index (pGI) = 0.862(HI) + 8.198 (*27*).

HI calculated from the hydrolysis curves and the corresponding pGI are presented in **Table 3**. Although the remarkably low indices recorded for the freshly cooked beans are in agreement with previous data (*22*), HI for fresh tortilla (77%) was lower than the 91% reported in the same study for commercially packed samples. The differences in hydrolysis rates favor the influence of the nixtamal/tortilla-making recipe and corn variety on the starch digestibility features of a particular product, suggested by Rendón-Villalobos et al. (*4*) and Agama-Acevedo et al. (*5*). The bean−tortilla meal exhibited intermediate HI and pGI between those of tortilla and bean alone (**Table 3**), a tendency also shown by all stored samples in this study. Interestingly, mathematical analysis of data does not give an accurate forecast of the actual experimental behavior of the mixture HI, as values recorded for all taco samples were lower than the index calculated using the ingredients' HIs and their starch ratio in the taco mixture. As an example, the HI of 72-h-stored beans was 16.07%, whereas the corresponding tortilla sample showed 61.05%. Therefore, the theoretical bean contribution should be 6.43% and that of tortilla equals 37.17%, making a sum of 43.60. However, the experimental HI value for the mixture was only 31.11%. The reduced actual hydrolysis rate indicates that the bean portion in the mixture plays a predominant role in the overall starch bioavailability. The observation is in line with the starch digestion properties reported for commercial tortilla and canned black bean taco (22), showing an HI closer to the bean rate than to the tortilla. Both HI and GI are notably influenced by food texture, particle size, and viscosity of the material subject to digestion in the dialysis tubing or in the small intestine, respectively (*17*, *26*). As matter of fact, viscous soluble dietary fibers in beans have been suggested to slow not only digestion but also diffusion of digestion products to the absorptive mucosa (*2*, *11*, *17*, *30*). Perhaps the viscosity developed in the bean−tortilla digestion mixture is high enough to retard the absorptive phase of digesta, resulting in a rather "slow" feature. Starch digestibility measurement of bean and tortilla mixed at different ratios would be of interest in order to assess the strength of this putative viscosity-governed effect of bean on the hydrolysis/absorption rate of the whole meal.

Predicted GIs (**Table 3**) suggest important "slow digestion" features for the taco mixture, retaining part of the health-beneficial characteristic of starch in beans (*29*, *36*, *37*). The predicted GI value for taco (51%) approaches the 56% glycemic index reported in normal subjects for corn tortilla served with pinto beans (*38*). The reduced index of cold-stored mixtures, which parallels the decreasing trend exhibited by the tortilla and bean ingredients alone (**Table 3**), is also noteworthy, as it may permit further reduction of metabolic responses following ingestion of this traditional Latin American meal. In addition, this type of functional-oriented handling of bean-based food items might represent a way to diversify the dietetic uses of pulses on a global scale (*39*).

In conclusion, differences in AS, RS, and RRS were found among the samples analyzed, with higher rates in tortilla than in bean. In the taco mixture the digestion rates were closer to those of bean alone, and slightly reduced digestion rates were noted upon cold storage, with lowered AS levels and greater RS contents. HI and pGI were higher in tortilla than in beans and the two-ingredient mixture. In taco, the kinetic digestion indicators decreased as a consequence of storage. Data presented here provide support for perceived health-beneficial characteristics of a traditional cereal−bean food, the popularity of which is increasing in industrialized markets.

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