Available online at www.sciencedirect.com
SCIENCE DIRECT®

Food Chemistry 97 (2006) 631–636

Food Chemistry

www.elsevier.com/locate/foodchem

In vitro starch bioavailability of corn tortillas with hydrocolloids

R. Rendón-Villalobos, E. Agama-Acevedo, J.J. Islas-Hernández, J. Sánchez-Muñoz, L.A. Bello-Pérez *

Centro de Desarrollo de Productos Bióticos del IPN, Km 8.5 Carr, Yautepec-Jojutla, Colonia San Isidro, Apartado Postal 24, 62731 Yautepec, Morelos, Mexico

Received 8 March 2004; received in revised form 18 April 2005; accepted 18 April 2005

Abstract

Tortillas were prepared using commercial hydrocolloids, stored for 7 and 14 days and their available, resistant and retrograded resistant starches, were evaluated alongside their in vitro starch digestibility. Available starch (AS) decreased with storage time and tortillas with hydrocolloids had lower values than the control sample. Tortillas elaborated with TC-20 gum did not present substantial differences in AS. Control tortilla had resistant starch (RS) content that increased with storage time but, in general, tortillas with hydrocolloids did not show any change in RS values with storage time, except tortillas with TC-1 gum that presented a slight increase after 7 storage days. Approximately 50% of RS is due to the retrogradation phenomenon as it was shown by the amount of retrograded resistant starch (RRS). Tortillas with added hydrocolloids had lower hydrolysis percentage and the hydrolysis was slower than in the control. In general, tortillas prepared with hydrocolloids had a lower tendency for retrogradation than control tortillas; it is important to consider this to obtain tortillas with better texture and lower RS content.

Keywords: Tortilla; Starch digestibility; Hydrocolloids; Maize; Resistant starch

1. Introduction

The nixtamalization of maize is an ancient process developed by the Mesoamerican civilizations and is still utilized in the production of "tortillas". The maize 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 maize grains) a soft dough, known as "masa", is obtained. The masa is a mélange 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 (Gomez, Rooney, & Waniska, 1987). 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 (Campus-Baypoli, Rosas-Burgos, Torres-Chávez, Ramírez-Wong, & Serna-Saldívar, 1999). The nixtamalization process produces changes that improve the nutritional quality of tortillas. Many studies have been conducted on nutritional aspects of nixtamalized maize, but very few studies have been carried out on the bioavailability of its carbohydrate constituents (Rendón-Villalobos, Bello-Pérez, Osorio-Diaz, Tovar-Rodríguez, & Paredes-López, 2002). 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 major constituents. When tortillas are cooked starch gelatinization occurs; the gelatinized starch gels are thermodynamically unstable structures and, on cooling, reassociation of the starch molecules may occur. The ability of starch

* Corresponding author. Tel.: +52 735 394 2020; fax: +52 735 394 1896.
E-mail address: labellop@ipn.mx (L.A. Bello-Pérez).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.04.026

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 (Biliaderis, 1991). Retrogradation in tortillas is a problem, because after preparation staling occurs increasing rigidity which affects palatability. On the other hand, starch retrogradation increases enzymatic resistance to starch digestion due to the formation of resistant starch which is associated with low glycaemic and insulinemic responses, and is very important for prevention of some diseases such as colon cancer and cardiovascular disease. The main classification of RS has been proposed by Englyst, Kingman, and Cummings (1992); 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 (Tovar, Björck, & Asp, 1992). RS2 are native uncooked granules of starch, such as raw potato or banana starches, whose crystallinity makes them scarcely susceptible to hydrolysis (Englyst & Cummings, 1987; Faisant, Gallant, Bouchet, & Champ, 1995). RS3 are retrograded starches, which may be formed in cooked foods that are kept at low or room temperature (Noah et al., 1998). RS4 that is due to chemical starch modification or thermal treatment at high temperatures (Tovar, Melito, Herrera, Rascón, & Pérez, 2002). It has been reported that adding hydrocolloids in tortillas retards retrogradation; however, interactions between starch and hydrocolloids may occurs in tortillas containing these additives which might decrease starch digestibility. Good quality corn tortillas are soft and can be rolled into "taco" form without damage. The textural characteristics of tortillas are related to the binding forms and the amount of water contained. The fresh masa is highly susceptible to moisture loss which makes its texture hard and therefore difficult to shape into a round flat form (Arámbula-Villa, Mauricio, Figueroa, González-Hernández, & Ordorica, 1999). A dehydrated corn masa produces hard and breakable tortillas. Thus, retention of water in masa and tortilla is important since excessive water loss makes an unacceptable product. The nixtamalization process produces changes that improve the nutritional quality of tortillas. Many studies have been conducted on nutritional aspects of nixtamalized maize in relation to protein, minerals and lipids, but very few studies have been carried out on the digestibility of its carbohydrate constituents (Campas-Baypoli, Rosas-Burgos, Torres-Chávez, Ramirez-Wong, & Serna-Saldívar, 2002; Rendón-Villalobos et al., 2002), and there are no studies on starch digestibility in tortillas mixed with hydrocolloids and the mechanisms involved in these perceived phenomenon. The objective of the present study was to evaluate the influence of the type of commercial hydrocolloid and storage on the in vitro digestibility of starch in tortillas.

2. Materials and methods

2.1. Sample preparation

The traditional 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") were cooked in 15 l of lime solution. Lime is added at 1% (grain weight basis). Maize was cooked for 1 h at boiling temperature and then steeped in the same cooking vessel during 16 h. The cooking solution -or "nejayote" – was discarded and the resulting nixtamal washed three or four times with tap water for bran and excess lime removal. Nixtamal was ground into a "masa" using a commercial stone grinder. Masa was mixed by hand with each one of commercial hydrocolloids TC-20 (2% in base of weight of masa), TC-1 (0.5%) (Gum Technology Corporation, Tucson, AZ) and WHIP (0.25%) (Colloides Naturels International, Rouen, France) until a good mixture was obtained in which the hydrocolloid was uniformly distributed in the masa. The levels of hydrocolloids used were those suggested by the producers. TC-20 is a mixture of modified food starch, guar, xanthan and mono and diglycerides, designed to enhance moisture retention in tortillas and is useful in frozen and fresh tortillas. TC-1 is a mixture of cellulose and guar gum, as designed to enhance moisture retention and therefore reduce flaking and cracking in baked goods, particularly tortilla type products. WHIP gum is a mixture of hydrolyzed wheat proteins and arabic gum, that is recommended for tortilla formulation due to its interaction with Ca ions present in masa.

The masa was molded by pressure and extruded into thin circles to obtain "tortillas" of 1 mm 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, Plasticos de México, S.A. de C.V., México) and stored for 2, 4, 7 and 14 days at 4 °C. A sample immediately baked and cooled was also analyzed (0 storage time). After each time the samples were frozen in liquid nitrogen and freeze dried. In the case of stored tortillas, the samples were reheated in a home gas fired oven for 30 s on each side, at an approximate temperature of 250 °C, cooled down to 30 °C, frozen in liquid nitrogen and freeze dried; such a variation was introduced in order to replicate the same conditions used when this product is eaten. All samples were stored at room temperature in sealed plastic containers.

2.2. In vitro digestibility tests

Potentially available starch content was assessed following the multienzymatic protocol of Holm, Björck, Drews, and Asp (1986). The sample (300 mg db) was suspended in 20 ml of distilled water and incubated with α-amylase (Termamyl® Novo A/S, Copenhagen) in a boiling water bath for 20 min. This mixture was then diluted to 100 ml with distilled water. To 0.5 ml of this suspension, amyloglucosidase (Boehringer, Mannheim, Germnay) and 0.1 mol/l Na acetate buffer, pH 4.75 (1.0 ml) were added. The mixture was incubated for 30 min at 60 °C, diluted to 10 ml with distilled water and analyzed for glucose using glucose oxidase peroxidase assay (SERA-PAK® Plus, Bayer de México, S.A. de C.V., Edo. de México). Resistant starch was measured by two different protocols: (1) Retrograded 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, Goñi, Bravo, and Mañas (1993). (2) The method proposed by Goñi, Garcia-Diaz, Mañas, and Saura-Calixto (1996) was employed to estimate the total amount of indigestible starch (comprising RS2, RS3 and part of RS1 fractions). In brief, removal of protein with pepsin P-7012 (Sigma Chemical Co., St. Louis, MO) at 40 °C, 1 h, pH 1.5, incubation with α-amylase A-3176 (Sigma Chemical Co., St. Louis, MO) at 37 °C for 16 h to hydrolyze digestible starch, treatment of the precipitate with 2 M KOH, incubation with amyloglucosidase A-7255 (Sigma Chemical Co., St. Louis, MO) at 60 °C, 45 min, pH 4.75, and determination of glucose using glucose oxidase peroxidase assay (SERA-PAK® Plus, Bayer de México, S.A. de C.V., Edo. De México). The in vitro rate of hydrolysis was measured according to Holm, Björck, Asp, Sjoberg, and Lundquist (1985); each assay was run with 500 mg available starch. Starch was hydrolyzed at different incubation times with α-amylase and maltose liberated was tested with DNS acid reaction.

2.3. 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 test at a level a significance of 0.05. Statistical analyses were run using the computer SPSS V. 6.0 software (SPSS Institute Inc., Gary NC).

3. Results and discussion

3.1. Available starch

The values for available starch (AS) are presented in Table 1. For all analyzed samples, AS, decreased when storage time increased, this pattern being due to the retrogradation phenomenon as was reported in tortillas elaborated with masa prepared in the laboratory (Rendón-Villalobos et al., 2002). When AD of tortillas without storage (0 h) and stored for 14 days were compared, tortillas control, tortillas with WHIP and TC-1 presented the same difference, indicating that these gums did not affect the AS content in tortillas. However, the lowest difference between these two values of AS was

Table 1
Available starch (AS), resistant starch (RS) and retrograded resistant starch (RRS) in corn tortillas with hydrocolloids

Sample/storage (days)	AS (%)	RS (%)[1]	RRS (%)[2]
TC			
0	70.03 ± 1.24^{a}	2.74 ± 0.07^{a}	1.66 ± 0.08^{a}
7	$68.38 \pm 0.56^{a,b}$	5.04 ± 0.11^{b}	2.83 ± 0.05^{b}
14	66.91 ± 0.72^{b}	5.23 ± 0.06^{b}	3.05 ± 0.11^{b}
T_WG			
0	$67.42 \pm 0.81^{a,b}$	2.49 ± 0.05^{a}	1.71 ± 0.08^{a}
7	64.17 ± 0.93^{c}	$3.55 \pm 0.63^{a,c}$	1.23 ± 0.13^{c}
14	62.55 ± 0.60^{c}	$3.08 \pm 0.11^{a,c}$	$1.54 \pm 0.15^{a,c}$
T_TC-1			
0	$68.00 \pm 0.67^{a,b}$	3.01 ± 0.05^{a}	1.69 ± 0.09^{a}
7	$66.44 \pm 0.70^{b,c}$	3.18 ± 0.07^{c}	1.71 ± 0.16^{c}
14	64.77 ± 0.89^{c}	3.38 ± 0.06^{c}	1.76 ± 0.13^{a}
T_TC-20			
0	65.69 ± 0.57^{c}	$3.01 \pm 0.11^{a,c}$	$1.46 \pm 0.09^{a,c}$
7	64.22 ± 0.61^{c}	3.33 ± 0.05^{c}	$1.37 \pm 0.18^{a,c}$
14	63.20 ± 067^{c}	3.27 ± 0.07^{c}	$1.48 \pm 0.11^{a,c}$

TC, control corn tortilla; T_WG, corn tortilla with hydrocolloid WHIP gum; T_TC1, corn tortilla with hydrocolloid TC-1; T_TC20, corn tortilla with hydrocolloid TC-20.

Mean values of eighteen replicates, dry matter basis. Values followed by the same letter in the same column are not significantly different ($p > 0.05$).

[1] Using method of Goñi et al. (1996).

[2] Using method of Saura-Calixto et al. (1993).

shown in tortillas with TC-20, demonstrating that this gum decreased starch reorganization in higher proportion and AS is not altered significantly. This pattern can be related with the gum formulation, because TC-20 is a mixture of modified food starch, guar, xanthan and mono and diglycerides. These components can be responsible for the decrease in starch retrogradation, as has been reported in bread (Krog, Olesen, Toernaes, & Joensson, 1989; Russell, 1983). AS values of 72.92% and 70.97% were reported in tortillas without storage and with 72 h of storage, respectively (Rendón-Villalobos et al., 2002); values that were higher to those found in this study. Similar values (between 64.52% and 76.04%) were determined in tortillas stored until 72 h (Agama-Acevedo et al., 2004).

3.2. Resistant starch

Total resistant starch (RS) values in control tortillas increased approximately 50% after storage for 7 days (Table 1). However, tortillas with added gums did not show appreciable differences with storage time; only tortillas with TC-1 gum had RS values that changed after 7 storage days. In all cases tortillas with added hydrocolloids had low retrogradation tendency, because these gums impede interactions between starch chains solubilized during gelatinization. In laboratory-made tortillas without storage was determined a RS content of 3.12%, and when this sample was stored for 3 days, the RS value increased to 3.87% (Rendón-Villalobos et al., 2002). This last RS content is comparable with that obtained in tortillas with added gums and stored for 7 days. These results indicate that hydrocolloids can retard the retrogradation phenomenon in tortillas. Other studies reported in tortillas prepared with nixtamalized maize flour, showed RS values for samples stored for 72 h between 2.52% and 3.29% (Agama-Acevedo et al., 2004), and for RS values ranged between 2.70% and 4.18% (Rendón-Villalobos et al., 2002).

Retrograded resistant starch in the tortillas studied was lower than total RS; this indicates that there are other resistant fractions (principally RS1 or RS4) that contribute to total RS. Perhaps some portions of the hydrocolloids interact with starch at high temperatures and diminish starch susceptibility, because in other studies of tortillas without hydrocolloids, the difference between RS and RRS is lower than that shown in this study (Agama-Acevedo et al., 2004; Rendón-Villalobos et al., 2002). More studies are necessary to resolve this question. Wet thermal treatment followed by cooling and storage produces retrograded resistant starch (RRS), as reported for corn flour (García-Alonso, Jiménez-Escrig, Martín-Carrón, Bravo, & Saura-Calixto, 1999) and in various starch gels (Fredriksson et al., 2000; Tovar et al., 2002). The formation of retrograded starch requires dehydration of the gelatinized sample (Björck, Granfeldt, Liljerberg, Tovar, & Asp, 1994; Fredriksson et al., 2000), a phenomenon that is likely to take place when tortillas are baked, at approximately 250 °C, and cooled. However, hydrocolloid interaction with water molecules impedes water elimination during the baking and storage steps of tortillas.

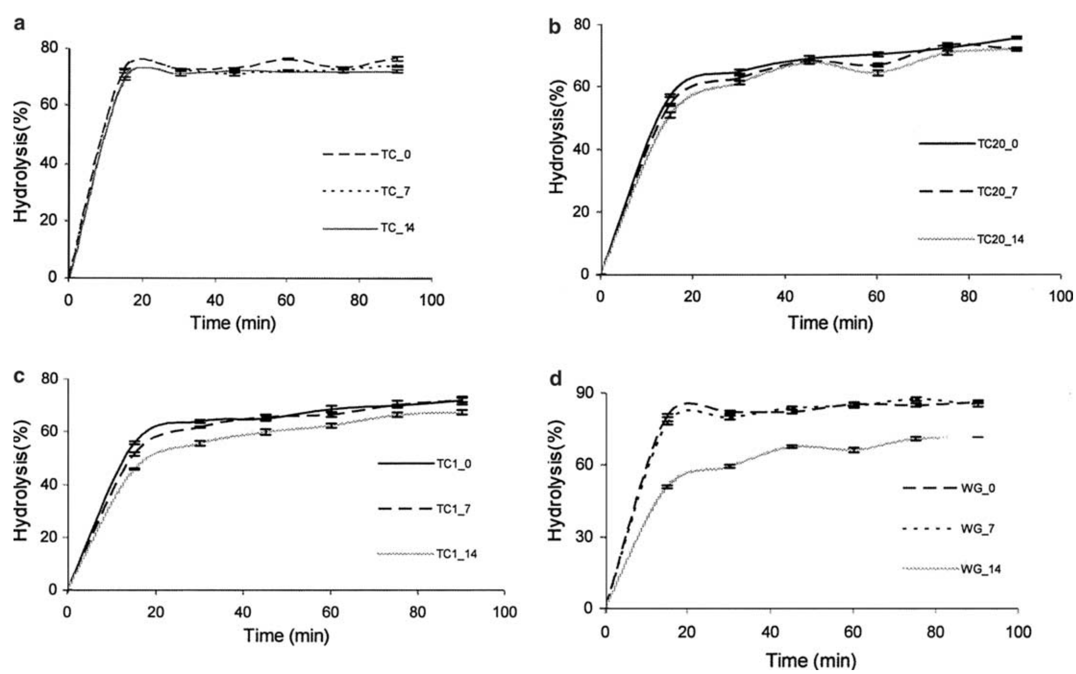

Fig. 1. In vitro starch hydrolysis: (a) control corn tortilla = TC; (b) control corn tortilla with hydrocolloid TC-20 = T_TC20; (c) corn tortilla with hydrocolloid TC-1 = T_TC1; (d) corn tortilla with hydrocolloid WHIP = WG (d).

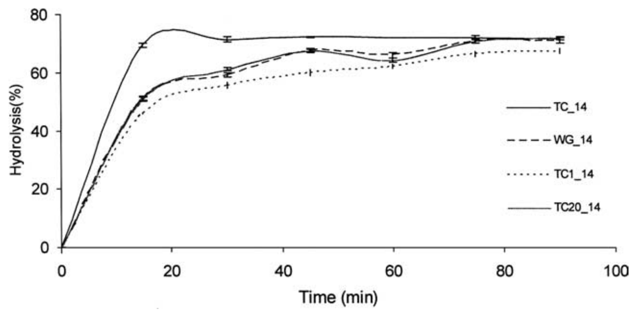

Fig. 2. In Vitro starch hydrolysis of corn tortilla without hydrocolloids (TC), with hydrocolloids TC-20, TC-1 and WHIP (WG). All samples were stored by 14 days.

3.3. Rate of enzymatic starch hydrolysis

When the behavior of tortillas were compared at the different storage times, control (Fig. 1(a)) and tortillas with TC-20 hydrocolloid (Fig. 1(b)) did not show statistical differences ($p < 0.05$). However, for tortillas with TC-1 gum (Fig. 1(c)) the samples at 0 and 7 days of storage had differences after only 30 min of the test. In this same sense, the sample stored for 14 days showed lower hydrolysis percentage. A similar pattern was evident for tortillas with added WHIP gum (Fig. 1(d)), because the samples at 0 h and 7 days storage did not show differences during the test, but the sample stored for 14 days had the lowest hydrolysis percentage (approximately 60%). The differences showed in hydrolysis rate for the tortillas with added gums studied can be related with the chemical characteristics of the hydrocolloid used. The in vitro α-amylolysis reaction of tortillas stored for 14 days is presented in Fig. 2. The control sample showed a high hydrolysis rate until 20 min (approximately 75%) and thereafter the values were constant during the test. Tortillas with added hydrocolloids had lower hydrolysis percentage and the hydrolysis was slower than the control. Tortillas with WHIP and TC-20 gums did not show statistical differences in hydrolysis percentage with time. However, tortillas with added TC-1 presented the lowest hydrolysis percentage; because these tortillas are slowly hydrolysed by digestive enzymes.

4. Conclusions

The present study shows that hydrocolloids decrease resistant starch formation in tortillas. The hydrocolloids can be used for obtaining tortillas with soft texture during storage, but with the concomitant lost of resistant starch formation and some benefits for consumers due to the lower resistant starch ingested. However, the slow hydrolysis rate in tortillas with hydrocolloids liberates glucose toward the blood more slowly and also increases the time elapsed between meals.

Acknowledgements

The authors wish to acknowledge the economic support from CGPI-IPN, COFAA-IPN, EDI-IPN and COSNET.

References

Agama-Acevedo, E., Rendón-Villalobos, R., Tovar, J., Paredes-López, O., Islas-Hernández, J. J., & Bello-Pérez, L. A. (2004). In vitro starch digestibility changes during storage of maize flour tortillas. *Nahrung/Food, 48*, 31–40.

Arámbula-Villa, V. G., Mauricio, S. R. A., Figueroa, C. J. D., González-Hernández, J., & Ordorica, F. C. A. (1999). Corn masa and tortillas from extruded instant corn flour containing hydrocolloids and lime. *Journal of Food Science, 64*, 120–124.

Biliaderis, C. G. (1991). The structure and interactions of starch with food constituents. *Canadian Journal of Physiology and Pharmacology, 69*, 60–78.

Björck, I. M., Granfeldt, Y., Liljerberg, H., Tovar, J., & Asp, N. G. (1994). Food properties affecting the digestion and absorption of carbohydrates. *American Journal of Clinical Nutrition, 59*, 699S–705S.

Campas-Baypoli, O. N., Rosas-Burgos, E. C., Torres-Chávez, P. I., Ramirez-Wong, B., & Serna-Saldívar, S. O. (2002). Physicochemical changes of starch in Maize tortillas during storage at room and refrigeration temperatures. *Starch/Stärke, 54*, 358–363.

Campus-Baypoli, O. N., Rosas-Burgos, E. C., Torres-Chávez, P. I., Ramírez-Wong, B., & Serna-Saldívar, S. O. (1999). Physicochemical changes of starch during maize tortilla production. *Starch/Stärke, 51*, 173–177.

Englyst, H. N., & Cummings, J. H. (1987). Digestion of polyssacharides of potato in the samll intestine of man. *American Journal of Clinical Nutrition, 45*, 423–431.

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition, 46*(Suppl. 2), S33–S50.

Faisant, N., Gallant, D. J., Bouchet, B., & Champ, M. (1995). Banana starch breakdown in the human small intestine studied by electron microscopy. *European Journal of Clinical Nutrition, 49*, 98–104.

Fredriksson, H., Björck, I., Andersson, R., Liljeberg, H., Silverio, J., Eliasson, A.-C., et al. (2000). Studies on α-amylase degradation of retrograded starch gels from waxy maize and high-amylose potato. *Carbohydrate Polymers, 43*, 81–87.

García-Alonso, A., Jiménez-Escrig, A., Martín-Carrón, N., Bravo, L., & Saura-Calixto, F. (1999). Assessment of some parameters involved in gelatinization and retrogration of starch. *Food Chemistry, 66*, 181–187.

Gomez, M. H., Rooney, L. W., & Waniska, R. D. (1987). Dry corn masa flours for tortilla and snack food. *Cereal Foods World, 32*, 372–377.

Goñi, I., Garcia-Diaz, L., Mañas, E., & Saura-Calixto, F. (1996). Analysis of resistant starch: a method for foods and food products. *Food Chemistry, 56*, 445–449.

Holm, J., Björck, I., Asp, N. G., Sjoberg, L. B., & Lundquist, I. (1985). Starch availability in vitro and in vivo after flaking, steam-cooking and popping of wheat. *Journal of Cereal Science, 3*, 193–200.

Holm, J., Björck, I., Drews, A., & Asp, N. G. (1986). A rapid method for the analysis of starch. *Starch/Starke, 38*, 224–229.

Krog, N., Olesen, S. K., Toernaes, H., & Joensson, T. (1989). Retrogradation of the starch fraction in wheat bread. *Cereal Foods World, 34*, 281–285.

Noah, L., Guillon, F., Bouchet, B., Buleon, A., Molis, C., Gratas, M., et al. (1998). Digestion of carbohydrate from white beans (*Phaseolus vulgaris* L.) in healthy humans. *Journal of Nutrition, 128*, 977–985.

Rendón-Villalobos, J. R., Bello-Pérez, L. A., Osorio-Diaz, P., Tovar-Rodríguez, J., & Paredes-López, O. (2002). Effect of storage time on in vitro digestibility and resistant starch content of nixtamal, masa and tortilla. *Cereal Chemistry, 79*, 340–344.

Russell, P. L. (1983). A kinetic study of bread staling study by differential scanning calorimetry and compressibility measurements. The effect of added monoglyceride. *Journal of Cereal Science, 1*, 297–303.

Saura-Calixto, F., Goñi, I., Bravo, L., & Mañas, E. (1993). Resistant starch in foods: modified method for dietary fiber residues. *Journal of Food Science, 58*, 642–645.

Tovar, J., Björck, I. M., & Asp, N. G. (1992). Incomplete digestion of legume starches in rats: A study of precookeed flours containing retrograded and physically inaccessible starch fractions. *Journal of Nutrition, 122*, 1500–1507.

Tovar, J., Melito, C., Herrera, E., Rascón, A., & Pérez, E. (2002). Resistant starch formation does not parallel syneresis tendency in different starch gels. *Food Chemistry, 76*, 455–459.