Interciencia
Asociación Interciencia
interciencia@ivic.ve
ISSN (Versión impresa): 0378-1844
VENEZUELA

2004
Perla Osorio Díaz / Edith Agama Acevedo / Roselis Carmona García / Juscelino Tovar / Octavio Paredes López / Luis A. Bello Pérez
RESISTANT STARCH AND IN VITRO STARCH DIGESTIBILITY OF COOKED "AYOCOTE" BEAN (PHASEOLUS COCCINEOUS)
Interciencia, septiembre, año/vol. 29, número 009
Asociación Interciencia
Caracas, Venezuela
pp. 510-514

RESISTANT STARCH AND IN VITRO STARCH DIGESTIBILITY OF COOKED "AYOCOTE" BEAN (*Phaseolus coccineous*)

Perla Osorio-Díaz, Edith Agama-Acevedo, Roselis Carmona-García, Juscelino Tovar, Octavio Paredes-López and Luis A. Bello-Pérez.

SUMMARY

*"Ayocote" beans (*Phaseolus coccineous*) were cooked and studied regarding their chemical composition and* in vitro *starch digestibility. Protein and ash contents were 20.46 and 1.39%, respectively, which are among the lowest levels for seeds of the* Phaseolus *genus. On the contrary, lipid content was relatively high (3.31%). Available starch (AS) values decreased with storage at 4ºC, changing from 37.93 (freshly cooked "control" seeds) to 32.18% (seeds stored for 96h). An inverse pattern was found for resistant starch (RS) content. RS ranged between 2.24 and 3.49% for the control and 96h-stored samples, which represents a 56% increase in the RS content. Resistant starch associated to dietary fiber (RSAF) had a similar behavior, as its values increased with the storage time. The α-amylolysis rate decreased with storage, i.e. long-stored (96h) cooked samples exhibited slower starch digestion features. The predicted glycemic index ranged between 62.9 and 59.7%, suggesting slow glucose-release features for starch in "ayocote" beans.*

Introduction

The nutritional value of legumes as sources of protein and carbohydrates in the diet is undeniable. Beans are a rich and inexpensive source of proteins (20-25%) and carbohydrates (50-60%) for a large part of the world's population, mainly in developing countries (Rehman *et al*., 2001), being considered as poor man's meat. In addition, they are generally good sources of slow release carbohydrates (Tharanathan and Mahadevamma, 2003). México is accepted as the center of origin of beans, since 47 of the 52 species classified in the *Phaseolus* genus were identified in Mexico; besides, Mexico possesses the wild type of the 5 cultivated species of this genus, i.e. *P. vulgaris, P. acutifolius, P. lunatus, P. coccineous and P. polyanthus* (Sousa-Sánchez and Delgado-Salinas, 1993). Castellanos *et al.* (1997) estimated an annual per capita bean consumption in Mexico of 22kg and indicated that 74% of the population eats beans 5 days a week.

Legumes are beneficial for health, with a low glycemic index (Foster-Powell and Brand-Miller, 1995). Although carbohydrates are the major component of legumes, relatively little work has been carried out on this fraction (Bravo *et al*., 1998). Of them, starch and non-starch polysaccharides (dietary fiber) are the major constituents, with a small but significant amount of oligosaccharides (Bravo *et al*., 1998; Guillon and Champ, 2002). The largest part of the great number of species and varieties of beans available in Mexico, is cultivated only for farmers' selfconsumption. These pulses may have different caloric value, depending on their starch bioavailability.

Also, being a major plant metabolite, starch is also the dominating carbohydrate in the human diet (Björck *et al*., 1994; Skrabanja *et al*., 1999). A couple of decades ago, starch was considered an available carbohydrate that was completely digested and absorbed in the small intestine. However, it is now known that there exists a starch fraction that is resistant to enzyme digestion, passing through the small intestine and reaching the large bowel, where it may be fermented by the 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). Recently there has been considerable interest in the possibility of improving diabetic patients by altering the glycemic impact of the ingested carbohydrates. For this purpose, a number of strategies have been used, such as the change or combination of different processing/storage conditions and the study of lesser explored edible plant species that present higher levels of RS or slow starch digestion properties. A tool for ranking foods with respect to their blood glucose raising potential is the glycemic index (GI) concept (Jenkins *et al*., 1981). A nutritional variable frequently linked to low GI properties is RS. The types of RS identified in foods are (Englyst *et al*., 1992): physically entrapped starch within whole or partly milled grains or seeds (RS1),

KEYWORDS / Beans / Digestibility / Legumes / *Phaseolus coccineous* / Resistant Starch / Starch /

Received: 02/25/2004. Modified: 08/03/2004. Accepted: 08/11/2004.

Perla Osorio-Díaz. Nutritionist, Instituto Politécnico Nacional (IPN), Mexico. M.Sc. in Food Technology, IPN, Mexico. Doctoral Student, Universidad Autónoma de Querétaro (UAQ), Mexico. Professor, IPN, Mexico.

Edith Agama-Acevedo. Biochemical Engineer, Instituto Tecnológico de Acapulco (ITA), Mexico. M.Sc. in Bioproduct Development, IPN. Doctoral Student, UAQ, Mexico. Professor, IPN, Mexico.

Roselis Carmona-García. Biochemical Engineer, ITA, Mexico. Graduate Student, IPN, Mexico.

Juscelino Tovar. Biologist, Universidad Central de Venezuela (UCV). Ph.D. in Applied Nutrition, Lund University, Sweden. Professor, Experimental Biology Institute, School of Sciences, UCV, Venezuela.

Octavio Paredes-López. Biochemical Engineer and M.Sc. in Food Technology, IPN, Mexico. Master in Engineering, Czech Academy of Sciences. Ph.D., University of Manitoba, Canada. Professor, IPN, Irapuato, Mexico.

Luis A. Bello-Pérez. Biochemical Engineer, ITA, Mexico. M.Sc. in Bioengineering and Ph.D. in Plant Biotechnology, CINVESTAV-IPN, Mexico. Professor, IPN. Address: Centro de Desarrollo de Productos Bióticos del IPN. Km 8.5 Carr. Yautepec-Jojutla, Colonia San Isidro, Apartado Postal 24, 62731 Yautepec, Morelos, México. e-mail: labellop@ipn.mx

RESUMEN

Se estudió la composición química y digestibilidad in vitro *del frijol "ayocote" cocido (*Phaseolus coccineous*). El contenido de proteína y cenizas fue de 20,46 y 1,39%, respectivamente, los cuales se encuentran entre los más bajos reportados para el género* Phaseolus*. En el caso de los lípidos su contenido fue relativamente alto (3,31%). Los valores de almidón disponible (AD) disminuyeron con el tiempo de almacenamiento, variando desde 37,93% (muestra recién cocinada) hasta 32,18% (muestra almacenada por 96h). El comportamiento inverso se encontró para el contenido de almidón resistente (AR). Los valores estuvieron entre 2,24% (muestra control) y 3,49% (muestra almacenada por 96h), lo cual representa un incremento del 56% en el contenido de AR. El almidón resistente asociado a la fibra dietética (ARAF) presentó un comportamiento similar, ya que los valores se incrementaron con el tiempo de almacenamiento. La velocidad de α-amilólisis disminuyó con el tiempo de almacenamiento; por ejemplo, las muestras almacenadas por 96h mostraron una velocidad de digestión lenta. El índice glucémico estimado estuvo entre 62,9 y 59,7%, sugiriendo que el almidón del frijol "ayocote" libera muy lentamente la glucosa cuando es hidrolizado por las enzimas digestivas.*

RESUMO

Estudou-se a composição química e digestibilidade in vitro *do feijão "ayocote" cozido (*Phaseolus coccineous*). O conteúdo de proteína e cinzas foi de 20,46 e 1,39%, respectivamente, os quais se encontram entre os mais baixos reportados para o gênero* Phaseolus*. No caso dos lípidos seu conteúdo foi relativamente alto (3,31%). Os valores de amido disponível (AD) diminuíram com o tempo de armazenamento, variando desde 37,93% (amostra recém cozida) até 32,18% (amostra armazenada por 96h). O comportamento inverso encontrou-se para o conteúdo de amido resistente (AR). Os valores estiveram entre 2,24% (amostra controle) e 3,49% (amostra armazenada por 96h), o qual representa um incremento de 56% no conteúdo de AR. O amido resistente associado à fibra dietética (ARAF) apresentou um comportamento similar, já que os valores se incrementaram com o tempo de armazenamento. A velocidade de α-amilólisis diminuiu com o tempo de armazenamento; por exemplo, as amostras armazenadas por 96h mostraram uma velocidade de digestão lenta. O índice glicêmico estimado esteve entre 62,9 e 59,7%, sugerindo que o amido do feijão "ayocote" libera muito lentamente a glicose quando é hidrolisada pelas enzimas digestivas.*

native (ungelatinized) granules of B-type starches (RS2), and retrograded starch (RS3).

Raw and processed legumes contain significant amounts of RS in comparison with other products such as cereals, tubers and unripe fruits (Jenkins *et al*., 1982; Tovar *et al*., 1992b; Tovar and Melito, 1996; Velasco *et al*., 1997; Bravo *et al*., 1998, 1999). For this reason, the starch digestion rate and, therefore, the release of glucose into the blood stream are slower after the ingestion of legumes, resulting in a reduced glycemic and insulinemic postprandial responses in comparison with cereal grains or potatoes (Jenkins *et al*., 1982; Tovar *et al*., 1992a). In addition to starch, legumes contain high amounts of dietary fiber in a form that gives cell walls a high resistance toward disintegration during cooking (Würsch *et al*., 1986; Tovar *et al*., 1992a; Melito and Tovar, 1995). These factors, along with the presence of certain antinutrients, may account for the low starch digestibility in pulses (Guillon and Champ, 2002).

Most studies on starch digestibility in beans in México (Osorio-Díaz *et al*., 2002, 2003; Vargas-Torres *et al*., 2004a, b), Venezuela (Tovar and Melito, 1996; Velasco *et al*., 1997), Spain (García-Alonso *et al*., 1998) and Pakistan (Rehman *et al*., 2001) were carried out in common beans (*Phaseolus vulgaris* L.). However, to our knowledge, no research has been conducted on starch digestibility of "ayocote" beans (*Phaseolus coccineous*), a species widely consumed in central and southern Mexico.

The object of the present investigation was to evaluate the *in vitro* starch bioavailability of cooked "ayocote", also known as "runner" beans, and to examine the influence of cold storage on their available and resistant starch content and *in vitro* rate of starch digestion.

Materials and Methods

Sample preparation

"Ayocote" bean seeds were purchased from a local market in Yautepec, Morelos, México. The beans were cooked using a Mattson cooker, and cooking time was measured (Reyes-Moreno and Paredes-López, 1993). The sample, cooked seeds plus cooking water, were cooled down at room temperature and stored during 24, 48, 72 and 96h at 4ºC, simulating cooking and storage conditions applied in Mexican households. After each storage period, the samples were equilibrated at room temperature (25ºC) during 15min before analysis.

Chemical analysis

Moisture content was determined by gravimetric heating (130 ±2ºC for 2h) using 2-3g of a ground sample. Ash, protein (Nx6.25) and fat were analyzed according to AACC methods 08-01, 46-13, and 30-25, respectively (AACC, 2000).

Digestibility tests

Potentially available starch content was assessed following the multienzymatic protocol of Holm *et al*. (1986) using Termamyl® (Novo A/S, Copenhagen) and amyloglucosidase (102857; Roche Diagnostics, Indianapolis, IN, USA). The method proposed by Goñi *et al*. (1996) was employed to estimate the amount of indigestible starch (comprising part of RS1 plus RS2 and RS3 fractions). Resistant starch associated to dietary fiber (RSAF) content, which is an estimate of highly resistant retrograded starch fractions (Tovar *et al*., 2002), was measured as starch remnants in dietary fiber residues, according to the so called Lund method as modified by Saura-Calixto *et al*. (1993). The *in vitro* rate of hydrolysis was measured using hog pancreatic amylase according to Holm *et al*. (1985); each assay was run with 500mg available starch. In all these enzymatic tests, a portion of cooked beans was weighed into a test tube or a beaker, and homogenized with the corresponding solution (depending on the assay) under controlled conditions: first step (speed level 2, 1min) and second step (speed level 2.5, 1min), using a homogenizer (Polytron PT 1200; Kinematica AG, Switzerland). *In vitro* estimated glycemic index was calculated from the percentage of available starch hydrolyzed within 90min (H_{90}) in the α-amylolysis assay, applying the

equation GI= 39.21 + 0.803 (H_{90}), r= 0.91 P≤0.05 (Goñi *et al*., 1997).

Statistics

Results were expressed by mean values ±standard error of the three separate determinations. Comparison of means was performed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests using the SPSS statistical program (v. 2.03, SPSS, Chicago, IL, USA).

Results and Discussion

Chemical composition

Protein content in the species studied was of 20.46 ±0.40%, a value that is in the range reported in common black beans (between 18.87 and 24.20%; Vargas-Torres *et al*., 2004a), and slightly lower than those reported by Reyes-Moreno and Paredes-López (1993), who found protein values between 20.3 and 29.0% (using the same conversion factor of 5.85) for different bean varieties harvested in Mexico. The relatively high protein values found in "ayocote" beans should be kept in mind due to the possibility of starch-protein complex formation on cooking, a well known phenomenon in bread (Preston, 1998), where staling could be inhibited by complex formation of the starch polymers with lipid and proteins during subsequent cooking. The potential impact of these complexes on starch digestibility has not been investigated in detail.

The lipid content in the "ayocote" sample was of 3.31 ±0.06%, which is higher than data recorded previously in seeds from other *Phaseolus* species, ranging between 0.90 and 2.80% (Bravo *et al*., 1999; Vargas-Torres *et al*., 2004a). This value may be of importance in the formation of amylose-lipid complexes, which may reduce the starch digestibility (Björck *et al*., 1994). In relation to ash content, "ayocote" had a value of 1.39 ±0.04%, being lower than those reported in an Indian *P. vulgaris* cultivar (Bravo *et al*., 1999) and four other black bean cultivars (*P. vulgaris* L.) from México (between 3.62 and 5.15%; Vargas-Torres *et al*., 2004a). Variety may be playing an important role in the mineral storage levels in the seed.

Total starch (TS) in "ayocote" bean was 42.1%. This is higher than those reported in *P. vulgaris* cultivars. Tovar and Melito (1996) reported TS contents in two raw bean varieties of 39.3 and 39.9%, and a similar value was recorded in raw white beans (García-Alonso *et al*., 1998). However, Bravo *et al*. (1999) reported a TS value of 34.9% for haricot beans, and Vargas-Torres *et al*. (2004a) between 33.56 and 36.69% for four cultivars of black beans. The present results confirm the relevance of the biological species on the nutritional characteristics of *Phaseolus* beans.

It should be noted that the sum of constituents herein reported does not reach 100%. Legume seeds contain other components, principally dietary fiber, whose presence may explain this apparent lack of analytical completeness. Total fiber levels in common beans range between 11.2 and 27.5% (Kozlowska, 2001; Tovar, 1994), which adds to oligosaccharides (raffinose, stachyose and verbascose) representing between 5 and 6% (Agustin and Klein, 1989), and to polyphenols (0.49-3.98%; Bravo *et al*., 1999), substances that were not evaluated in this investigation.

Available starch (AS)

When potentially AS was determined at different cold-storage times (Table I), the level decreased from 37.93% in freshly cooked "ayocote" seeds to 32.18% in the sample stored for 96h. These values represented 90 and 76% of TS, respectively, differences that may be due to the presence of resistant starch (RS). A previous study on cooked black beans from different cultivars, reported AS contents in cooked control samples (without storage) ranging between 32.1 and 21.7%; these values decreased with the storage time, reaching levels between 21.8 and 13.5% (Vargas-Torres *et al*., 2004b). In the case of "ayocote" bean, the reduction in AS was of 5.75%, resembling those found in three of the cultivars in the above-mentioned study, in which the difference between AS in the control group and in the 96h-stored sample was 6-8% (Vargas-Torres *et al*., 2004b). Such changes probably reflect the development of retrograded enzyme resistant fractions which, as it will be discussed later, is a general feature for legumes, as a consequence of the relatively high amylose/amylopectin ratio of their starches (Björck *et al*., 1994; Tovar *et al*., 2002). Nonetheless, some beans show lower AS contents and higher retrogradation tendencies than others; in the case of "ayocote" bean, the decrease of AS values in the stored samples appears relatively low. Rosin *et al*. (2002) reported a digestible starch content in non-stored cooked beans of 37.57%, but it only decreased to 35.33% in the sample stored for 30 days at -20ºC. Tovar and Melito (1996) reported lower AS contents (32.7 and 36.5%) in two varieties of common beans cooked with two different protocols and immediately analyzed. Similarly, Bravo *et al*. (1999) reported lower AS value (26.6%) in raw haricot bean (*P. vulgaris*).

TABLE I
AVAILABLE STARCH (AS), RESISTANT STARCH (RS) AND RESISTANT STARCH ASSOCIATED TO DIETARY FIBER (RSAF) CONTENT OF COOKED "AYOCOTE" BEAN

Storage time (h)	AS	RS	RSAF
0	37.93 a ±1.30	2.24 a ±0.15	1.49 a ±0.12
24	35.29 b ±0.73	2.79 b ±0.08	1.72 b ±0.16
48	34.58bc ±0.94	2.88bc ±0.17	1.89 b ±0.21
72	32.93 d ±1.04	3.08 d ±0.12	1.95 b ±0.18
96	32.18de ±0.89	3.49 e ±0.09	2.53 c ±0.22

Values are mean of three replicates ±standard error, dry matter basis.
Means inside each column with a different letter are significantly different (α=0.05)

Resistant starch (RS)

The RS values increased with the storage time (Table I). Levels ranged between 2.24% for the control sample and 3.49% for the 96h-stored preparation, representing a 56% increase in RS content with the storage. Ample variation in RS content values has been previously observed for freshly cooked common beans (*P. vulgaris*). Indeed, most cultivars exhibited greater levels than those recorded here for "ayocote" seeds (Tovar and Melito, 1996; Bravo *et al*., 1999; Vargas-Torres *et al*., 2004b). Furthermore, RS in "ayocote" beans exhibited only a moderate rise after cold-storage. Thus, this legume seems to have a modest proclivity to develop retrograded resistant starch fractions upon cooling, which is in contrast with the generally recognized behavior for starch in pulses (Tovar and Melito, 1996; Rosin *et al*., 2002; Tovar *et al*., 2002; Vargas-Torres *et al*., 2004b).

Resistant starch associated to dietary fiber (RSAF)

The value of RASF (Table I) in the control sample was of 1.49%. It increased with the storage time, reaching 2.53% after 96h. This in

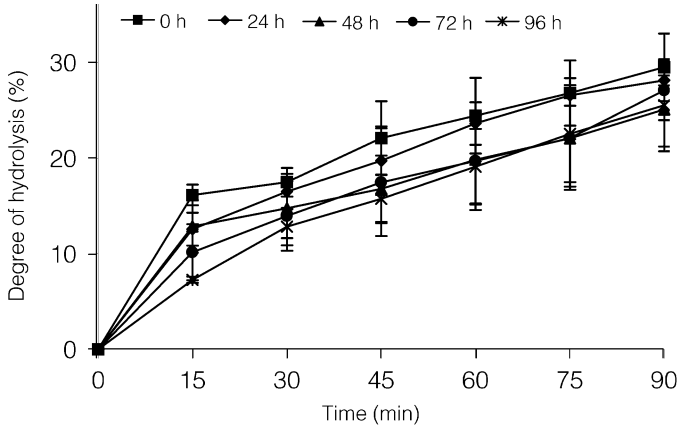

Figure 1. In vitro enzymatic (α-amylase) hydrolysis of starch in "ayocote" cooked bean.

agreement with the increased values observed for total RS upon storage. The RASF contents at 24, 48 and 72h were not different (α=0.05), but at 96h the value increased significantly; perhaps, only after this time starch retrogradation becomes quantitatively important in this system, as it was suggested for the recrystallization process in corn starch gels (Farhat *et al*., 2000). Some black bean cultivars (Vargas-Torres *et al*., 2004b) presented RASF contents that increased within the 0-72h storage period, becoming constant thereafter. Again, present data suggest that "ayocote" bean had a relatively low retrogradation rate. This is noteworthy since, among common occidental foods, legumes exhibit noticeable proneness to form indigestible starch by retrogradation (Velasco *et al*., 1997; Bravo *et al*., 1998; Tovar *et al*., 2002), a tendency that appears only moderate for ayocote beans. Also, these results may be indicative of potential nutritional uses for "ayocote" that may differ from those given to *P. vulgaris* seeds.

Hydrolysis rate

The *in vitro* α-amylolysis reaction for "ayocote" bean is represented in Figure 1. The degree of hydrolysis along the 90min digestion period appeared lower as the storage time increased, i.e. samples stored for longer times showed lower starch digestibility. However, in general, final hydrolysis scores (90min) were similar for all samples. Values between 5 and 10% were obtained at 15min reaction time, reaching hydrolysis values between 23 and 28% after 90min. The low hydrolysis rate recorded for "ayocote" bean resembles that exhibited by legumes in general; for instance, amylolysis values for a number of black bean cultivars ranged between 7 and 30% after a 90min digestion (Vargas-Torres *et al*., 2004b). However, some of these varieties exhibited hydrolysis values even lower than reported herein for "ayocote" seeds. This was the case of the Tacana cultivar, which only reached 17% of hydrolysis at 90min (Vargas-Torres *et al*., 2004b). Also, hydrolysis values between 10 and 30% were recorded after 90min digestion for various cooked Indian legumes (Bravo *et al*., 1998). Several factors are involved in the reduced bioavailability of legume starches (Tovar, 1994; Guillon and Champ, 2002). Some of them are of structural nature, such as the presence of intact tissue or cell structures enclosing starch granules, which hinders the swelling and solubilization of starch resulting in reduced *in vitro* digestion rate (Tovar *et al*., 1990). Other factors are related to endogenous dietary fiber. For instance, permanence of starch granules trapped within rather intact walled-cells in precooked legume flours was observed even after extensive homogenization and pepsin treatment (Tovar *et al*., 1991). Furthermore, the viscous fiber constituents are frequently proposed as important determinants in the "slow" characteristic of common legume seeds (Tovar *et al*., 1992b; Skrabanja *et al*., 1999). Estimated glycemic index (GI) calculated from the *in vitro* amylolysis results, ranged between 62.9% and 59.7% for 0h and 96h-stored seeds, respectively. These values were higher than that reported for common beans (50.6%), using the same empiric predictive equation (García-Alonso *et al*., 1998), although these values were calculated on a total starch basis, whereas in the present study the amylolysis assays were performed on an available starch basis. However, the calculated GI for stored beans in this study are similar to those mentioned by García-Alonso *et al*. (1998) as referential for legume GI values (60%). Hence, present results confirm that "ayocote" beans are a potential "slow carbohydrate" source.

Conclusions

"Ayocote" beans contain lower protein and ash, and higher lipid and total starch levels, than other *Phaseolus* seeds. Available starch in this bean is higher than in seeds of other species, but it tends to decrease upon cold storage. Both total resistant starch and resistant starch associated to fiber contents increased with storage time, as a consequence of starch retrogradation. Similarly, amylolysis rate decreased in samples submitted to prolonged storage, resulting in estimated GI values between 62.9 and 59.7%, which shows that starch in this type of bean is slowly digested, particularly in stored samples. Storage time, in addition to the botanical species or variety, may influence digestibility of starch pulses; suggesting that some species might be preferred for specific dietetic uses.

ACKNOWLEDGEMENTS

The authors acknowledge the economic support from CGPI-IPN, IPICS, LANFOOD, CONACYT-México and COFAA-IPN.

REFERENCES

AACC (2000) *Approved methods*. 10th ed. American Association of Cereal Chemists. St. Paul, MN, USA.

Agustin J, Klein BP (1989) Nutrient composition of raw, cooked, canned and sprouted legumes. In Matthews RH (Ed.) *Legumes: chemistry, technology, and human nutrition*. Mercer Dekker. New York, NY, USA. pp. 187-196.

Asp NG (1992) Resistant starch. Proceedings from the second plenary meeting of EURESTA. *Eur. J. Clin. Nutr. 46*: SI.

Björck IM, Granfeldt Y, Liljeberg H, Tovar J, Asp NG (1994) Food properties affecting the digestion and absorption of carbohydrates. *Am. J. Clin. Nutr. 59*: 699S-705S.

Bravo L, Siddhuraju P, Saura-Calixto F (1998) Effect of various processing methods on the *in vitro* starch digestibility and resistant starch content of indian pulses. *J. Agric. Food Chem. 46*: 4667-4674.

Bravo L, Siddhuraju P, Saura-Calixto F (1999) Composition of underexploited indian pulses. Comparison with common legumes. *Food Chem. 64*: 185-192.

Castellanos JZ, Guzmán-Maldonado SH, Jiménez A, Mejía C, Muñoz-Ramos JJ, Acosta-Gallegos JA, Hoyos G, López-Salinas E, González-Eguiarte, D, Salinas-Pérez R, Gonzélez-Acuña J, Muñoz-Villalobos JA, Fernández-Hernández P, Cázares B (1997) Preferential habits of Mexican consumers of common beans (*Phaseolus vulgaris* L.). *Arch. Latinoam. Nutr. 27*: 163-167.

Englyst HN, Kingman SM, Cummings JH (1992) Classification and measurement of nutritionally important starch fractions. *Eur. J. Clin. Nutr. 46*: S33-S50.

Farhat IA, Blanshard JMV, Mitchell JR (2000) The retrogradation of waxy maize starch extru-

dates: Effects of storage temperature and water content. *Biopolymers 53*: 411-422.

Foster-Powell K, Brand-Miller J (1995) International tables of glycemic index. *Am. J. Clin. Nutr 62*: 871S-893S.

García-Alonso A, Goñi I, Saura-Calixto F (1998) Resistant starch formation and potential glycemic index of raw and cooked legumes (lentils, chickpeas and beans). *Z. Lebensm Unter Forsch 206*: 284-287.

Goñi I, García-Alonso A, Saura-Calixto F (1997) A starch hydrolysis procedure to estimate glycemic index. *Nutr. Res. 17*: 427-437.

Goñi I, García-Díaz L, Mañas E, Saura-Calixto F (1996) Analysis of resistant starch: a method for foods and food products. *Food Chem. 56*: 445-449.

Guillon F, Champ MJ (2002) Carbohydrate fractions of legumes: uses in human nutrition and potential for health. *Br. J. Nutr. 88* (Supl. 3): S293-S306.

Holm J, Björck I, Asp NG, Sjöberg LB (1985) Starch availability *in vitro* and *in vivo* after flaking, steam-cooking and popping of wheat. *J. Cereal Sci. 3*: 193-206.

Holm J, Björck I, Drews A, Asp NG (1986) A rapid method for the analysis of starch. *Starch/ Stärke 38*: 224-229.

Jenkins DJA, Thorne MJ, Camelon K, Jenkins AL, Rao AV, Taylor RH, Thompson LU, Kalmusky J, Reichert R, Francis T (1982) Effect of processing in digestibility and the blood glucose response: study of lentils. *Am. J. Clin. Nutr 36*: 1093-1101.

Jenkins DJA, Wolever TMS, Taylor RH, Barker H, Fielder H, Baldwin JM, Bowling AC, Newman HC, Jenkins AL, Goff DV (1981) Glycemic index of foods: a Physiological basis for carbohydrates exchange. *Am. J. Clin. Nutr 34*: 362-366.

Kozlowska H (2001) Nutrition. In Hedley CL (Ed.) *Carbohydrates in grain legumes seeds*. CABI. New York, NY, USA. pp. 61-69.

Melito C, Tovar, J (1995) Cell walls limit *in vitro* protein digestibility in processed legumes. *Food Chem. 53*: 305-307.

Osorio-Díaz P, Bello-Pérez LA, Agama-Acevedo E, Vargas-Torres A, Tovar J, Paredes-López O (2002) *In vitro* digestibility and resistant starch content of some industrialized commercial beans (*Phaseolus vulgaris* L.). *Food Chem. 78*: 333-337.

Osorio-Díaz P, Bello-Pérez LA, Sáyago-Ayerdi SG, Reyes-Benítez MP, Tovar J, Paredes-López O (2003) Effect of processing and storage time on *in vitro* digestibility and resistant starch content of two bean (*Phaseolus vulgaris* L.). *J. Sci Food Agric. 83*: 1283-1288.

Preston KR (1998) Protein carbohydrate interaction. In Hamer RJ, Hoseney RC (Eds.). *Interaction: the keys to cereal quality*. American Association of Cereal Chemists. St Paul, MN, USA. pp. 81-93.

Rehman Z, Salariya AM, Zafar SI (2001) Effect of processing on available carbohydrate content and starch digestibility of kidney beans (*Phaseolus vulgaris* L.). *Food Chem. 73*: 351-355.

Reyes-Moreno C, Paredes-López O (1993) Hard-to-cook phenomenon in common beans- A review. *Crit. Rev. Food Sci. Nutr. 33*: 227-286.

Rosin MP, Lajolo MF, Menezes WE (2002) Measurement and characterization of dietary starches. *J. Food Comp. Anal. 15*: 367-377.

Saura-Calixto F, Goñi I, Bravo L, Mañas E (1993) Resistant starch in foods: modified method for dietary fiber residues. *J. Food Sci. 58*: 642-643.

Sousa-Sánchez M, Delgado-Salinas A (1993) Mexican leguminoseae: Phytogeographic endemism and origins. In Ramamoorthy TP, Bye R, Lot A, Fa J (Eds.) *Biological diversity in México: origins and distribution*. Oxford University Press. New York, NY, USA. pp. 459-511.

Skrabanja V, Liljeberg HGM, Hedley CL, Kreft I, 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. 47*: 2033-2039.

Tharanathan RN, Mahadevamma S (2003) Grain legumes- a boom to human nutrition. *Trends Food Sci. Tech. 14*: 507-518.

Tovar J (1994) Bioavailability of carbohydrates in legumes. Digestible and indigestible fractions. *Arch. Latinoam. Nutr. 44*: 36S-40S.

Tovar J, de Francisco A, Björck I, Asp NG (1991) Relationship between microstructure and *in vitro* digestibility of starch in precooked leguminous seed flours. *Food Struct. 10*: 19-26.

Tovar J, Björck I, Asp NG (1990) Analytical and nutritional implications of limited enzymic availability of starch in cooked red kidney beans. *J. Agric. Food Chem. 38*: 488-93.

Tovar J, Björck IM, Asp NG (1992a) Incomplete digestion of legume starches in rats: A study of precooked flours containing retrograded and physically inaccessible starch fractions. *J. Nutr. 122*: 1500-1507.

Tovar J, Granfeldt Y, Björck IM (1992b) Effect of processing on blood glucose and insulin responses to starch in legumes. *J. Agric. Food Chem. 40*: 1846-1851.

Tovar J, Melito C (1996) Steam-cooking and dry heating produce resistant starch in legumes. *J. Agric. Food Chem. 44*: 2642-2645.

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

Vargas-Torres A, Osorio-Díaz P, Tovar J, Paredes-López O, Ruales J, Bello-Pérez LA (2004a) Chemical composition, starch bioavailability and indigestible fraction of common beans (*Phaseolus vulgaris* L). *Starch/Starkë 56*: 74-78.

Vargas-Torres A, Osorio-Díaz P, Islas-Hernández JJ, Tovar J, Paredes-López O, Bello-Pérez LA (2004b) Starch digestibility of five cooked black beans (*Phaseolus vulgaris* L.) varieties. *J. Food Comp. Anal. 17*: 605-612.

Velasco ZI, Rascón A, Tovar J (1997) Enzymic availability of starch in cooked black beans (*Phaseolus vulgaris* L) and cowpeas (*Vigna* spp.). *J. Agric. Food Chem. 45*: 1548-1551.

Würsch P, Del Vedovo S, Koellreuter B (1986) Cell structure and starch nature as key determinants of the digestion rate of starch in legumes. *Am. J. Clin. Nutr. 43*: 25-29.