ELSEVIER

Food Chemistry 78 (2002) 333–337

Food Chemistry

www.elsevier.com/locate/foodchem

In vitro digestibility and resistant starch content of some industrialized commercial beans (*Phaseolus vulgaris* L.)

P. Osorio-Díaz[a], L.A. Bello-Pérez[a,*], E. Agama-Acevedo[a], A. Vargas-Torres[a], J. Tovar[b], O. Paredes-López[c]

[a]*Centro de Desarrollo de Productos Bióticos del IPN. Km 8.5 carr. Yautepec-Jojutla, colonia San Isidro, apartado postal 24, 62731 Yautepec, Morelos, Mexico*
[b]*Instituto de Biología Experimental, Facultad de Ciencias, Universidad Central de Venezuela, Apartado Postal 47069, Caracas 1041-A, Venezuela*
[c]*Centro de Investigación y Estudios Avanzados del IPN, Unidad Irapuato, Apartado Postal 629, 36500, Irapuato, Guanajuato, Mexico*

Received 13 February 2001; received in revised form 3 January 2002; accepted 3 January 2002

Abstract

Commercial bean products were studied in terms of chemical composition and starch digestibility. In general, commercial cooked flours did not show differences in protein and ash contents. Canned beans also did not show statistical differences ($\alpha = 0.05$) in protein, but they were different in ash, perhaps due to botanical variety. Lipid content varied in the different flours, due to the formulation used in their preparation, whereas the canned bean samples had similar lipid values. Canned samples had the highest available starch (AS) values. These results suggest that the drying of samples decreases AS. Canned beans had the lowest total resistant starch (RS) values, and the flours obtained from canned seeds had the highest. These results agree with AS content in the samples. Retrograded resistant starch (RS type 3) showed the same pattern as RS (type 2 + type 3), but with lower absolute values. The *in vitro* α-amylolysis rate for canned beans and commercial flours was lower than for samples dried in the laboratory. Thus, the additional drying step increased the hydrolysis rate of the samples. Therefore, depending on the specific dietetic use of beans, appropriate processing methods and formulations are needed. © 2002 Published by Elsevier Science Ltd.

Keywords: Resistant starch; Beans; Starch hydrolysis; Legumes; Chemical composition

1. Introduction

Dry legumes are a rich and inexpensive source of protein and calories for a large part of the world's population, mainly in developing countries. The dry bean (*Phaseolus vulgaris*) has an important place among the legumes of major production and consumption in Africa, India, Latin America and Mexico (Bourges, 1987; Reyes-Moreno & Paredes-López, 1993; Sathe, Rangnekar, Deshpande, & Salunkhe, 1982).

Carbohydrates constitute the main fraction of grain legumes, accounting for up to 55–65% of the dm. Of these, starch and nonstarch polysaccharides (dietary fibre) are the major constituents, with smaller but significant amounts of oligosaccharides (Bravo, Siddhuraju, & Saura-Calixto, 1998). Besides, being a major plant metabolite, starch is also the dominating dietary carbohydrate in the human diet (Bjorck, Granfeldt, Liljerberg, Tovar, & Asp, 1994; Skrabanja, Liljeberg, Hedley, Kreft, & Bjorck, 1999). Until recently, starch had been considered to be 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). The main classification of RS has been proposed by Englyst, Kingman, and Cummings, (1992); it is based on 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, Bjorck, & Asp 1992a). RS2 are native granules of starch, whose crystallinity makes them less susceptible to hydrolysis, e.g. raw potato or banana starches (Englyst & Cummings, 1987; Faisant, Gallant, Bouchet, & Champ, 1995). RS3 are retrograded starch

* Corresponding author. Fax: +52-73941896.
E-mail address: labellop@ipn.mx (L.A. Bello-Pérez).

0308-8146/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0308-8146(02)00117-6

fractions, which may be formed in cooked foods that are kept at low or room temperature (Noah et al., 1998).

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 effects against colorectal cancer; Asp, Van Amelsvoort, & Hautvast, 1996; Cassidy, Bingham, & Cummings, 1994; De Deckere, Kloots, & Van Amelsvoort, 1995; Jenkins, Wolever, Collier, Ocana, Rao, Buckley, Lam, Mayer, & Tompson, 1987). Among the factors affecting the rate and extent of starch digestion, food processing, storage time and botanic origin of the food are of major importance. Starch in raw foods is barely digestible, corresponding with RS2. However, during cooking starch is gelatinized and rendered available, although a fraction of this available starch is retrograded upon cooling and thus made resistant to enzymatic digestion (RS3; Asp et al., 1996; Björck et al., 1994; Bravo et al., 1998; Snow & O'Dea, 1981).

Processed legumes have been shown to contain significant amounts of RS in comparison with other products such as cereals, tubers and unripe fruits, irrespective of the processing treatment (Bjorck et al., 1994; Bravo et al., 1998; Jenkins, Thorne, Camelon, Jenkins, Rao, Taylor, Thompson, Kalmusky, Reichert, & Francis, 1982; Tovar, Granfeldt, & Bjorck, 1992b; Tovar & Melito, 1996, Velasco, Rascon, & Tovar, 1997). 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 reduced glycemic and insulinemic postprandial responses in comparison with cereal grains or potatoes (Jenkins et al., 1982, 1987; Tovar et al., 1992b). In addition to starch, legumes contain high amounts of dietary fibre in a form that gives cell walls high resistance toward disintegration during cooking (Tovar et al., 1992a; Würsch, Del Vedovo, & Koellreuter, 1986). This, along with the presence of certain antinutrients, may account for the low digestibility of starch in pulses.

The objective of the present study was to investigate the effect of the traditional domestic treatment in comparison with some industrial processing ways relevant in Latin America on the in vitro rate of starch digestion and RS levels in beans. The influence of post-cooking storage and variety were also evaluated.

2. Materials and methods

2.1. Sample preparation

Three commercial bean flours (F1–F3) and three processed canned beans (C1–C3) were purchased in a local supermarket in Acapulco. The flours and canned beans were used directly. Canned beans were studied either directly or after drying (55 °C, 24 h) in a convection oven and milling (50 Mesh) to obtain a flour (CF1–CF3).

2.2. Chemical composition

Moisture content was determined by gravimetric heating (130±2 °C for 2 h) using 2–3 g of sample. Ash, protein ($N\times5.85$) and fat were analyzed according to AACC methods 08-01, 46-13, and 30-25, respectively (AACC, 1983).

2.3. Digestibility tests

Potentially-available starch content was assessed following the multienzymatic protocol of Holm, Bjorck, Drews, and Asp (1986) using Termamyl® (Novo A/S, Copenhagen) and amyloglucosidase (Boehringer, Mannheim). Resistant starch was assessed by two different protocols: (1) retrograded resistant starch content was measured as starch remnants in dietary fibre 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 amount of indigestible starch (comprising part of the RS1 plus RS2 and RS3 fractions; Tovar (2001). The in vitro rate of hydrolysis was measured using hog pancreatic amylase according to Holm, Bjorck, Asp, Sjoberg, and Lundquist (1985); each assay was run with 500 mg available starch.

3. Results and discussion

3.1. Chemical composition

The chemical composition of the commercial bean flours and flours prepared from canned beans is given in Table 1. Moisture content in the samples analyzed ranged between 4.44 and 7.12%, suggesting that the industrial drying process and that used in the laboratory were similar, as F1 and CF1, and F2 and CF3 did not show significantly different values ($\alpha=0.05$). Protein contents (between 17.0 and 19.9%) were slightly lower than those reported by Reyes-Moreno and Paredes-Löpez (1993), who reported protein values between 20.3 and 29.0% (using the same factor, 5.85) for different varieties harvested in Mexico. It should be stressed, however, that protein content in edible legumes may vary markedly among cultivars of a single species (Bourgues, 1987). The relatively high values of protein in these beans samples could be important due to the possibility of starch-protein complex formation, a well known phenomenon in bread (Preston, 1998) where staling could be inhibited by complex formation of the starch polymers

Table 1
Chemical composition of beans

Sample	Moisture[a]	Protein[b,c]	Lipids[b]	Ash[b]	Carbohydrates (by difference)
F1	4.62a	19.9c	12.38d	7.09b	56.0a
F2	6.86c	19.8c	2.05a	6.99b	64.3c
F3	5.82b	17.4a	13.39e	7.11b	56.3a
CF1	4.44a	17.3a	3.05b	9.14c	66.1d
CF2	7.12d	19.1a,b,c	3.64c	10.06d	60.1b
CF3	6.57**c**	17.0a,b	3.16b	8.63a	64.7c

Means in columns not sharing the same letter are significantly different ($P<0.05$). F, Commercial flour; CF, Flour obtained from canned beans
[a] Means of three replicates
[b] Means of three replicates, dry basis.
[c] N × 5.85

with lipid and proteins. Little is known about the possible effect of these complexes on starch digestibility. The influence of other food constituents on resistant starch formation has been studied using calcium and potassium ions and catechin (Escarpa, González, Morales, & Saura-Calixto, 1997) but not protein. In general, lipid content in the CFs was lower than in F samples which might reflect the fact that some industrialized flours (F1 and F3) are mixed with other ingredients, such as edible oil. The high lipid content in F1 and F3 samples can increase the amount of lipid-starch complex, decrease the retrogradation phenomenon and the formation of RRS which, as will be discussed later, seems to be the case in this study. The CF1 and CF3 samples did not present differences in lipid content, and CF3 was slightly higher than in the former samples. The differences found between CF and F2 samples were possibly due to agronomic and genotype characteristics. In the case of ash, the F samples were not significantly different ($\alpha=0.05$), although higher values, between 8.63 and 10.06, were determined in CF samples. These differences could be due to the presumably different genotypes used when preparing the commercial products. These ash values found in beans are due to the high levels of potassium, iron and some vitamins present in these grains (Reyes-Moreno & Paredes-López, 1993). The carbohydrate values (obtained by difference) of samples CF and F2 were higher than in F1 and F3 samples, this may be due to the lower lipid content of the former.

3.2. Starch contents

Relatively ample variation among samples was recorded for the AS content. The values ranged between 27.9 and 39.2% (Table 2).Tovar and Melito (1996) reported AS content in two varieties of beans cooked with three different methods ranging from 32.7–36.5%; a similar AS value (33.4%) was found in cooked white beans by García-Alonso, Goñi, and Saura-Calixto (1998). However, Velasco et al. (1997) reported as AS value in boiled black beans of 27.8%. These results suggest that the cooking method, the post-cooking handling, and perhaps the bean variety play an important role in AS content.

Table 2
Available starch (AS), total resistant starch (RS) and retrograded resistant starch (RRS) in commercial bean flours (F), canned beans (C) and canned bean flour (CF)[a]

Sample	AS (%)	RS (%)[b]	RRS (%)[c]
F1	34.0b	5.42d	1.89c
F2	27.9a	4.44c	2.33e
F3	38.3c	6.14e	2.15d
CF1	28.4a	6.44e	2.62f
CF2	36.2b,c,d	5.98e,f	2.76g
CF3	32.3b	5.72f	2.64f
C1	35.7b,c,d	2.72a	1.20b
C2	39.2c	2.43a	1.0a
C3	38.5c,d	2.78a,b	0.93a

Means in columns not sharing the same letter are significantly different ($P<0.05$)
[a] Values are mean of three replicates, dry matter basis
[b] Using method of Goñi et al. (1996)
[c] Using method of Saura-Calixto et al. (1993)

3.3. Digestibility

The impact of post-cooking manipulation becomes clear when the resistant starch content of the variously treated beans is considered. Canned beans showed significantly lower RS values than the corresponding flour (CF) and the commercial powdered meal (F) (Table 2). Diminished RS contents in F and CF samples are consistent with the reduction in physically inaccessible starch fractions (RS1) that follows any treatment promoting disintegration and/or microstructural damage of seeds (Englyst et al. 1992; Tovar, de Francisco, Bjorck, & Asp, 1991). On the other hand, the higher RRS contents recorded for the powdered materials (F and CF), as compared to the whole cooked seeds (C), must be the consequence of a more extensive recrystallization of their starch fractions, which is expected to occur after

the additional heating/cooling treatment applied to these samples during the drying phase (Tovar, Bjorck, & Asp, 1990), thus leading to greater RS3 levels. However, the possible influence of other phenomena, such as transglycosidation reactions (Tovar & Melito, 1996) and annealing, cannot be ruled out.

It is also noteworthy that RS contents determined after Goñi et al. (1996) were always higher than those evaluated using the Saura-Calixto et al. (1993) protocol (Table 2). These findings are due to the fact that Goni et al. (1996) report the sum of a part of the physically inaccessible (type 1) plus the ungelatinized (type 2) and retrograded (type 3) resistant fractions (Tovar, 2001). Hence, all preparations studied contained significant amounts of RS1 and RS2 in addition to the RS3 fractions, all in accordance with previous studies on starch digestibility in legumes (Bravo et al., 1998; Garcia-Alonso et al., 1998; Tovar et al., 1990, 1991, 1992a).

The in vitro α-amylolysis reaction for canned beans is represented in Fig. 1. Homogeneized canned samples (C1–C3) presented slightly lower susceptibility to enzymatic attack than their dried/milled counterparts (CF1–CF3). After 5 min C samples showed approximately 10% of hydrolysis and the degree of hydrolysis reached only 20% by 60 min. A similar pattern was reported by García-Alonso et al. (1998) for cooked beans, showing approximately 17% of hydrolysis after 60 min. Bravo et al. (1998) reported degrees of hydrolysis between 10 and 20% for various processed Indian legumes analyzed as eaten but, when the cooked samples were dried, the degree of hydrolysis increased, reaching values between 40 and 50% after 5 min, increasing until 60–80% at 90 min. The increase recorded for the dried samples may be explained by the disruption during grinding of starch containing cotyledon tissue and cell structures, since it has been reported that any process that disrupts tissue/cell structures will increase the rate of digestion of starch in legumes (García-Alonso et al., 1998; Tovar et al., 1991, 1992b). Furthermore, drying of the samples could be responsible for a greater gelatinization degree, which would increase the accessibility of starch polymers to amylolytic enzymes. CF1 and CF2 samples did not show differences ($\alpha = 0.05$) in degree of hydrolysis, but sample CF3 exhibited the highest rate. These differences might be explained by the potentially different genotypes.

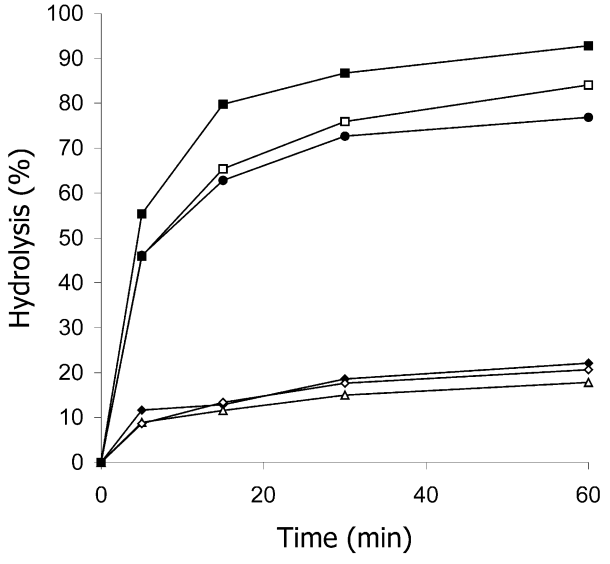

Fig. 1. In vitro starch hydrolysis of canned (C) and flour from canned (CF) beans. ◆, C1; ◇, C2; Δ, C3; □, CF1; ●, CF2; ■, CF3.

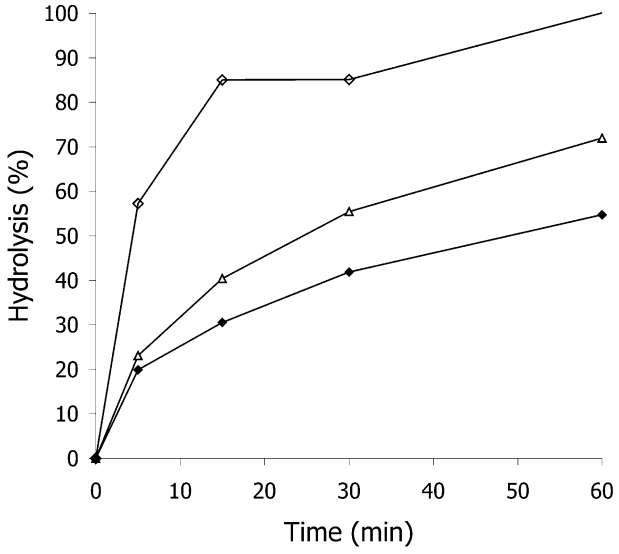

Fig. 2. In vitro starch hydrolysis of commercial bean flours (F). ◆, F1; ◇, F2; Δ, F3.

Commercial flours presented lower degree of hydrolysis (Fig. 2) than flours obtained in the laboratory from canned seeds. Differences among commercial flours were noticeable. F1 had the lowest degree of hydrolysis, F2 the highest and F3 was slightly lower than F2. The high amount of lipids present in the F1 and F3 samples could explain this behaviour due to possible formation of lipid–starch complexes during cooking, a phenomenon that decreases the polymer's susceptibility to enzymatic degradation (Bjorck et al., 1994). However, differences in the in vitro α-amylolysis reaction among commercial flours could also be influenced by the use of different genotypes in their preparation.

4. Conclusions

Processing conditions, chemical composition and agronomic variety may each influence starch digestibility in common beans. Therefore, specfic processing methods and formulations are needed for specific dietetic uses. For instance, in rural Mexican areas where beans and tortillas are the principal food, a processing method leading to the highest starch digestibility—such as the

flour preparation—should be used, whereas diabetic and hyperlipidemic subjects may be advised to consume whole cooked beans, with decreased starch bioavailability.

Acknowledgements

We appreciate the economic support from OMNI-LIFE-CONACYT, CGPI-IPN and from COSNET.

References

AACC. (1983). *Approved methods*. St. Paul, MN: American Association of Cereal Chemists.

Asp, N. G. (1992). Resistant starch. Proceedings from the second plenary meeting of EURESTA. *European Journal of Clinical Nutrition*, *46*(2), SI.

Asp, N. G., Van Amelsvoort, J. M. M., & Hautvast, J. G. A. J. (1996). Nutritional implications of resistant starch. *Nutritional Research Review*, *9*, 1–31.

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

Bourges, R. H. (1987). Las Leguminosas en la alimentación. II Parte. Publicación del Instituto Nacional de la Nutrición, CONASUPO y sus Empresas Industriales. *Cuadernos de Nutrición*, *10*, 22–30.

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. *Journal of Agricultural and Food Chemistry*, *46*, 4667–4674.

Cassidy, A., Bingham, S. A., & Cummings, J. H. (1994). Starch intake and colorectal cancer risk: and international comparison. *British Journal of Cancer*, *69*, 937–942.

De Deckere, E. A. M., Kloots, W. J., & Van Amelsvoort, J. M. M. (1995). Both raw and retrograded starch decrease serum triacylglicerol concentration and fat accretion in the rat. *British Journal of Nutrition*, *73*, 287–289.

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*(2), S33–S50.

Escarpa, A., González, M. C., Morales, M. D., & Saura-Calixto, F. (1997). An approach to the influence of nutrients and other constituents on resistant starch formation. *Food Chemistry*, *60*, 527–532.

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.

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

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

Jenkins, D. J. A., Thorne, M. J., Camelon, K., Jenkins, A. L., Rao, A. V., Taylor, R. H., Thompson, L. U., Kalmusky, J., Reichert, R., & Francis, T. (1982). Effect of processing in digestibility and the blood glucose response: study of lentils. *American Journal of Clinical Nutrition*, *36*, 1093–1101.

Jenkins, D. J. A., Wolever, T. M. S., Collier, G. R., Ocana, A., Rao, A. V., Buckley, G., Lam, Y., Mayer, A., & Tompson, L. U. (1987). Metabolic effects of a low-glycemic-index diet. *American Journal of Clinical Nutrition*, *46*, 968–975.

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

Preston, K. R. (1998). Protein carbohydrate interaction. In R. J. Hamer, & R. C. Hoseney (Eds.), *Interactions: the keys to cereal quality* (pp. 81–93). St Paul MN: American Association of Cereal Chemists.

Reyes-Moreno, C., & Paredes-López, O. (1993). Hard-to-cook phenomenon in common beans—a review. *Critical Review of Food Science Nutrition*, *33*, 227–286.

Sathe, S. R., Rangnekar, P. D., Deshpande, S. S., & Salunkhe, D. K. (1982). Isolation and partial characterization of black gram (*Phaseolus mungo* L.) starch. *Journal of Food Science*, *47*, 1524–1538.

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.

Skrabanja, V., Liljeberg, H. G. M., Hedley, C. L., Kreft, I., & Bjorck, M. E. (1999). Influence of genotype and processing on the invitro rate of starch hydrolysis and resistant starch formation in peas (*Pisum sativum* L.). *Journal of Agricultural Food Chemistry*, *47*, 2033–2039.

Snow, P., & O'Dea, K. (1981). Factors affecting the rate of hydrolisis of starch in food. *Americam Journal of Clinical Nutrition*, *34*, 2721–2727.

Tovar, J. (2001). Metodos para la determinacion de almidon resistente en los alimentos. In F. M. Lajolo, F. Sáura-Calixto, E. Wittig de Penńa, & T. Menezes (Eds.), *Fibra Dietetica en Iberoamerica: Tecnologia y Salud. Obtencion, caracterizacion, efecto fisiologico y aplicación en alimentos* (pp. 143–154). Sao Paula: Varela Press.

Tovar, J., & Melito, C. (1996). Steam-cooking and dry heating produce resistant starch in legumes. *Journal of Agricultural and Food Chemistry*, *44*, 2642–2645.

Tovar, J., Björck, I. M., & Asp, N.-G. (1990). Starch content and α-amylolysis rate in precooked legume flours. *Journal of Agricultural and Food Chemistry*, *38*, 1818–1823.

Tovar, J., de Francisco, A., Bjorck, I., & Asp, N-G. (1991). Relationship between microstructure and in vitro digestibility of starch in precooked leguminous seed flours. *Food Structure*, *10*, 19–26.

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

Tovar, J., Granfeldt, Y., & Bjorck, I. M. (1992b). Effect of processing on blood glucose and insulin responses to starch in legumes. *Journal of Agricultural and Food Chemistry*, *40*, 1846–1851.

Velasco, Z. I., Rascon, A., & Tovar, J. (1997). Enzymic availability of starch in cooked black beans (*Phaseolus vulgaris* L.) and cowpeas (Vigna spp.). *Journal of Agricultural and Food Chemistry*, *45*, 1548–1551.

Wursch, P., Del Vedovo, S., & Koellreuter, B. (1986). Cell structure and starch nature as key determinants of the digestion rate of starch in legumes. *American Journal of Clinical Nutrition*, *43*, 25–29.