Effect of Various Processing Methods on the in Vitro Starch Digestibility and Resistant Starch Content of Indian Pulses

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The effect of different processing treatments on the in vitro starch digestion rate and resistant starch (RS) formation in two little-known Indian legumes (moth bean and horse gram) as compared with a pulse widely consumed in Asian countries (black gram) was studied. Samples were cooked until edible without previous treatment, after overnight soaking in water or in a 0.02% sodium bicarbonate solution, and after sprouting. Cooked samples were analyzed for their RS content immediately after cooking or after storing for 24 h at 4 °C. The in vitro starch digestion rate was also measured in the freshly cooked (FC) legumes, and the hydrolysis index (HI) and glycemic index (GI) were calculated. RS in processed samples varied between 2.7 and 7.9 g kg^{-1} of dry matter, the highest values corresponding to the stored legumes. Sprouting and direct cooking resulted in the lowest RS content in FC and stored legumes, respectively. Soaking significantly improved in vitro starch digestibility in the little-known pulses but not in black gram. Sprouting yielded the highest HI and GI.

Keywords: *Little-known legumes; processing; resistant starch; starch digestibility*

INTRODUCTION

Grain legumes are a rich and inexpensive source of dietary protein [15−30% of dry matter (dm)] and contribute substantially to the protein and energy intake of a large part of the world's population, mainly in developing countries. Carbohydrates constitute the main fraction of grain legumes, accounting for up to 55−65% of the dm. Of these, starch and nonstarch polysaccharides (dietary fiber) are the major constituents, with smaller but significant amounts of oligosaccharides.

The nutritional significance of pulses has recently promoted the search for new, underexploited legume species as potential sources of energy and nutrients. In this respect, most work in the literature deals with the study of the protein and antinutritional factors of such little-known legumes (Carnovale et al., 1991; Siddhuraju et al., 1995a,b, 1996; Sudha et al., 1995), as well as on the effects of processing on protein digestibility and antinutrient activity (Adewusi and Falade, 1996; Barimalaa and Anoghalu, 1997; Veena et al., 1995; Vijayakumari et al., 1993, 1995, 1996). However, little attention has been paid to the influence of processing on the digestibility of starch, the main carbohydrate fraction of these little-known legumes.

Until recently, starch had been 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 is 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). RS has been classified into three different types: RS_1 represents physically inaccessible starch such as that occurring in partly milled seeds and grains or in densely packed structures such as pasta; RS_2 is composed of the native starch granules found in uncooked foods; RS_3, or retrograded starch, corresponds to the indigestible starch fraction formed after starch-containing foods are heated in the presence of water followed by cooling and may be present in cooked, cooled potatoes, canned legumes, etc. (Englyst et al., 1992).

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, protective effects against colorectal cancer) (Asp et al., 1996; Cassidy et al., 1994; De Deckere et al., 1995; Jenkins et al., 1987). Among the factors affecting the rate and extent of starch digestion, food processing has a major importance: starch in raw foods is barely digestible, corresponding with RS type 2. However, during cooking starch is gelatinized and rendered available, although a fraction of this available starch is retrograded upon cooling and made resistant to enzymatic digestion (RS_3). Also, depending on the botanic origin of the food, the starch digestion rate varies (Asp et al., 1996; Björck et al., 1994; Snow and O'Dea, 1981).

Processed legumes have been shown to contain significant amounts of RS in comparison with other food products such as cereals and potatoes, irrespective of the processing treatment (Björck et al., 1994; Jenkins et al., 1982; Tovar et al., 1992a; Tovar and Melito, 1996; Velasco et al., 1997). In consequence, 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

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potatoes (Jenkins et al., 1982, 1988; Tovar et al., 1992b). In addition to starch, legumes contain high amounts of dietary fiber, which gives cells a high resistance toward disintegration in cooking (Tovar et al., 1992a; Würsch et al., 1986). This, along with the presence of certain antinutrients, may account for the low digestibility of legume starch.

Nevertheless, most studies on the effect of processing on the starch digestibility of grain legumes are limited to common pulses. However, to gain a better understanding of the nutritional properties of underexploited legumes in order to select those species suitable for human consumption, it is necessary to determine the digestibility of their carbohydrate fraction.

Moth bean [*Vigna aconitifolia* Jacq. (Marechal)] and horse gram [*Macrotyloma uniflorum* Lam. (Verdc.), previously *Dolichos biflorus*] are two legume species that grow in dry zones of southern India. These pulses are consumed by rural communities and the urban poor, as well as by the Malayali and Kurumba tribes in these regions. The National Academy of Sciences recognized these little-known pulses as potential food resources in its report on tropical legumes (National Academy of Sciences, 1979). Previous studies showed that these little-known legumes are a good source of protein, carbohydrates, and energy, with a composition comparable to those of other widely consumed pulses such as black gram or chickpeas (Bravo et al., 1998).

The objective of the present work was to study the effect of different traditional domestic treatments on the in vitro starch digestibility and RS formation in moth bean and horse gram. These pulses were compared with another legume widely consumed mostly in Asian countries, black gram (*Vigna mungo* L.).

MATERIALS AND METHODS

Samples. Dried moth bean and horse gram were obtained from a local market in Salem (Tamil Nadu, India). Black gram was purchased from a local market in Madrid (Spain). Grains were thoroughly cleaned and freed from broken seeds, dust, and other foreign materials. Moth beans were kept at 45 °C overnight to kill infesting pests, which was not necessary with the other seeds.

Processing Methods. All samples were cooked until edible either directly or after different domestic treatments commonly applied in Indian households. These treatments were as follows: soaking in distilled water (12 h, 30 °C); soaking in a 0.02% sodium bicarbonate solution (12 h, 30 °C); and sprouting [samples were soaked overnight in distilled water (12 h, 30 °C), drained, and placed onto sterile Petri dishes lined with wet filter paper; seeds were germinated during 32 h at 30 °C in the dark with frequent watering to maintain moisturized conditions].

Soaking liquid was drained and sprouts were rinsed prior to cooking in distilled water. The weight/volume ratios used for soaking and cooking as well as the cooking times applied to each legume are shown in Table 1. These cooking volumes and times were adjusted to each species and treatment to ensure edible conditions prior to the analysis.

To prevent possible retrogradation of the starch, samples were analyzed for their in vitro starch digestibility rate and RS content immediately after cooking. Additionally, a batch of cooked samples was stored during 24 h at 4 °C to assess the impact of postcooking storage on RS formation. In both cases, three to five seeds (~100 mg) were soaked and cooked in triplicate in separate centrifuge tubes and the cooking liquid was discarded.

Another set of samples was cooked in greater amounts, maintaining the weight/volume ratios and cooking times. These samples were freeze-dried after the cooking liquid had been drained. Freeze-dried pulses were milled to a particle size of <1 mm in a Cyclone sample mill (Tecator, Höganäs, Sweden). These milled samples were used for the determination of total starch (TS). TS and RS were also analyzed in the corresponding raw, milled grains.

Table 1. Cooking Times and Weight/Volume Ratios Used for Soaking and Cooking Legumes

	times (min) and w/v ratios	cooked	water soaked and cooked	$NaHCO_3$ soaked and cooked	sprouted and cooked
moth bean	time	35	15	15	45
	soaking		1:5	1:5	1:5
	cooking	1:10	1:5	1:5	1:9
horse gram	time	65	45	45	45
	soaking		1:5	1:5	1:5
	cooking	1:11	1:7	1:7	1:9
black gram	time	50	20	20	60
	soaking		1:5	1:5	1:5
	cooking	1:10	1:5	1:5	1:10

Analytical Procedures. *Total starch (TS)* content was determined according to the Goñi et al. (1997) method. Raw and cooked freeze-dried samples (100 mg) were dispersed in 2 M KOH. Solubilized starch was then hydrolyzed with amyloglucosidase (EC 3.2.1.3; catalog no. 102857, Boehringer-Mannheim, Germany) in a water bath at 60 °C. Liberated glucose was quantified using the peridochrom oxidase/peroxidase (GOD-PAP) reagent (catalog no. 676543, Boehringer-Mannheim), and TS was calculated as glucose × 0.9. No correction of free glucose was required in the cooked samples because these were previously drained of the cooking liquid.

Free Glucose and O-Linked Glucose. The glucose content of raw legumes, both free glucose and the glucose moiety of sucrose, was determined to correct the TS values obtained as described previously. Samples dispersed in 2 M KOH were treated with invertase (EC 3.1.1.26; catalog no. 390203D, BDH, Madrid, Spain) during 30 min at 37 °C. After centrifugation, a 1 mL aliquot was precipitated with 2 mL of 96% ethanol and centrifuged again and glucose analyzed in the supernatants using the peridochrom oxidase/peroxidase reagent (Bravo and Saura-Calixto, 1998).

RS. Cooked legumes, both freshly cooked and stored, were homogenized (Polytron PCU, Kinematica, GmBH, Switzerland) prior to analysis. Both raw and cooked samples were treated with 20 mg of pepsin (1 g of pepsin/10 mL of HCl−KCl buffer; Merck, catalog no. 7190) and 1 mL of α-amylase (40 mg/mL Tris maleate buffer; EC 3.2.1.1; A-3176, Sigma Chemical Co., Madrid, Spain) to remove protein and digestible starch. After centrifugation, residues were dispersed in 2 M KOH and hydrolyzed with amyloglucosidase (EC 3.2.1.3; catalog no. 102857, Boehringer-Mannheim) and the liberated glucose was quantified, all as described above for TS. RS was calculated as glucose × 0.9 (Goñi et al., 1996). This method measures total RS: RS_1 is measured because samples are either finely milled (particle size <1 mm in raw samples) or homogenized (cooked samples) to ensure enzyme accessibility to starch. Alkaline treatment ensures dispersion of native starch granules in raw samples and retrograded starch in the processed legumes.

Digestible starch (DS) was calculated as the difference between TS and RS.

In Vitro Starch Digestion Rate. Freshly cooked samples were used to assess the kinetics of starch digestion. Cooked samples containing equivalent starch amounts were drained of the cooking water and homogenized in HCl−KCl buffer of pH 1.5 at constant speed during 1 min. Protein was removed as indicated above. The pH was then adjusted to 6.9 after the addition of Tris maleate buffer, and 5 mL of a solution of α-amylase (containing 2.6 IU of α-amylase in Tris maleate buffer) was added. Samples were then incubated at 37 °C in a shaking water bath. At 30 min intervals, portions of 1 mL of the supernatants were taken and placed into tubes that were shaken vigorously at 100 °C during 5 min to inactivate the enzyme. Digested starch in these portions was fully hydro-

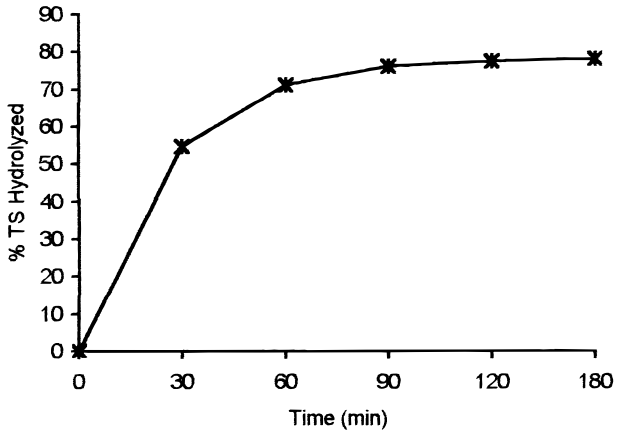

Figure 1. In vitro starch hydrolysis rate of fresh white bread (reference material).

lyzed with amyloglucosidase, and glucose was determined as described above by calculating digested starch as glucose × 0.9 (Goñi et al., 1997).

The rate of starch digestion was expressed as the percentage of TS hydrolyzed at different times. The hydrolysis curves follow a first-order equation as described in a previous paper (Goñi et al., 1997). The area under the hydrolysis curve (AUC) was calculated using the equation

$$\mathrm{AUC} = C_\infty(t_f - t_0) - (C_\infty/k)[1 - \exp[-k(t_f - t_0)]] \quad (1)$$

where C_∞ corresponds to the concentration at equilibrium (t_{180}), t_f is the final time (180 min), t_0 is the initial time (0 min), and k is the kinetic constant.

Once the area under the starch hydrolysis curve of the test samples was established, a hydrolysis index (HI) was calculated by comparison with the AUC of a reference food (fresh white bread loaves; hydrolysis curve shown in Figure 1) as described before (Goñi et al., 1997). This HI was shown to be a good predictor of the glycemic response to a food and was highly correlated with the glycemic index (GI) in vivo. Therefore, from the HI obtained in vitro we estimated the predicted GI using the equation established in the mentioned study (Goñi et al., 1997):

$$\mathrm{GI} = 39.71 + (0.549 \times \mathrm{HI}) \quad (2)$$

Statistics. Results were expressed as mean values ± standard deviations of three separate determinations. A one-way analysis of variance (ANOVA) followed by Duncan's multiple-comparison test was performed to assess the effect of the processing methods and the variation according to the legume species (significance level: $p \leq 0.05$). Statistical analyses were performed with a Statgraphics Plus program version 2.1 (Statistical Graphics Corp.).

RESULTS

Starch Fractions of Raw and Processed Legumes. Effect of Processing. Table 2 shows the TS, RS, and DS contents of the raw and processed legumes. Raw samples had similar TS contents and very high amounts of RS (mainly RS_2), ranging from 30% to up to 70% of the TS, resulting in low apparent starch digestibilities and low DS contents. Horse gram seemed to be the legume with the poorest starch digestibility, whereas the comparatively low RS content of moth bean points to a potentially higher digestibility of this little-known legume.

Processing resulted in an apparent increase of TS in the grain legumes, the TS content of the cooked pulses being significantly higher than in the raw ones. Of all the treatments assayed, soaking in sodium bicarbonate and sprouting followed by cooking resulted in the lowest TS values among the cooked legumes. TS in these processed samples was closer to the TS values of the raw pulses than the legumes cooked directly or after soaking in distilled water. Quantitatively, the smallest differences in the TS content between raw and processed legumes were observed in black gram, whereas these differences were bigger in the little-known pulses, their starch content apparently enriched after processing.

DS, calculated as the difference between TS and RS, was higher in the processed legumes than in the raw ones (Table 2). About 90% of the starch in all of the processed seeds was digestible as compared with 69% in raw moth bean, 48% in black gram, and only 27% in horse gram. The DS content of stored samples was slightly lower than in the freshly cooked legumes due to their higher RS content.

Cooked pulses contained significant amounts of RS (mainly RS_3), although processing greatly reduced RS in all samples (Table 2), because native granules, responsible for the high RS content of raw samples, are gelatinized during cooking. Among the processed pulses, horse gram was the legume with the highest RS concentration, whereas there was no significant difference in the RS contents of moth bean and black gram, irrespective of the processing method.

Only minor differences in the RS content were observed after the application of the various treatments to each legume. In general, cooking the seed legumes directly or after soaking in water seemed to be the treatments leading to the highest RS formation in freshly cooked legumes (Table 2). Although the RS values of the water-soaked legumes were consistently higher than in the samples soaked in the sodium bicarbonate solution, there were no statistically significant differences among these treatments either in freshly cooked or in the stored samples. The only exception was observed in the stored horse gram, the RS content being significantly higher in the water-soaked seeds than in the grains soaked in the sodium bicarbonate solution.

The lowest RS formation occurred in the germinated legumes analyzed immediately after cooking. However, when these samples were stored at 4 °C during 24 h, their RS concentration increased significantly. Moreover, in stored horse gram and black gram, germination was the treatment leading to the highest RS formation.

Storing the processed legumes resulted in a significant increase of the RS content (Table 2). The highest increase was observed in sprouted and cooked legumes, whereas the lowest occurred in samples cooked without previous soaking. Contrary to what happened to the freshly cooked samples where direct cooking yielded comparatively high RS values, storing these cooked samples resulted in the lowest RS contents of the stored legumes.

In Vitro Starch Digestion Rate. The effect of the assayed processing treatments on the rate of in vitro starch digestion of the three freshly cooked legumes is shown in Figure 2, where the rate of starch digestion is shown as a percentage of the TS hydrolyzed at different times. Table 3 shows the actual hydrolysis values and statistical significance.

The highest rate and extent of in vitro starch digestion in moth bean were achieved in the sprouted seed (Figure 2A). Cooking without previous soaking resulted in a reduced digestion rate in this pulse, whereas there

Table 2. Total Starch (TS), Resistant Starch (RS), and Digestible Starch (DS) of Raw and Processed Indian Legumes (Grams per Kilogram of Dry Matter)[a]

	moth bean			horse gram			black gram		
	TS	DS	RS	TS	DS	RS	TS	DS	RS
raw	$39.54 \pm 1.7^{a\alpha}$	27.34	$12.20 \pm 0.77^{a\alpha}$	$36.03 \pm 1.17^{a\alpha}$	9.61	$26.42 \pm 0.20^{b\alpha}$	$37.87 \pm 1.05^{a\alpha}$	18.21	$19.66 \pm 1.67^{c\alpha}$
freshly cooked									
cooked	$46.24 \pm 0.66^{a\beta}$	42.34	$3.90 \pm 0.06^{a\beta}$	$47.32 \pm 2.58^{a\beta}$	42.12	$5.21 \pm 0.64^{b\beta\gamma}$	$40.73 \pm 0.23^{b\beta\gamma}$	37.33	$3.40 \pm 0.06^{a\beta\gamma}$
WSC	$47.88 \pm 0.40^{a\gamma}$	44.15	$3.72 \pm 0.41^{a\beta\gamma}$	$46.29 \pm 0.45^{a\beta\gamma}$	40.60	$5.69 \pm 0.24^{b\beta}$	$41.02 \pm 0.14^{c\beta}$	37.35	$3.68 \pm 0.25^{a\gamma}$
SBSC	$44.49 \pm 0.89^{a\delta}$	41.26	$3.23 \pm 0.28^{a\gamma\delta}$	$43.15 \pm 1.30^{a\gamma}$	38.07	$5.08 \pm 0.06^{b\beta\gamma}$	$39.74 \pm 0.22^{b\alpha\gamma}$	36.09	$3.64 \pm 0.24^{a\gamma}$
SpC	$44.75 \pm 0.37^{a\delta}$	42.08	$2.67 \pm 0.22^{a\delta}$	$44.66 \pm 0.30^{a\beta\gamma}$	40.22	$4.44 \pm 0.71^{b\gamma}$	$38.99 \pm 0.83^{c\alpha}$	35.96	$3.03 \pm 0.30^{a\beta}$
cooked, stored at 4 °C for 24 h									
cooked		41.45	$4.79 \pm 0.70^{ab\epsilon}$		41.57	$5.75 \pm 0.17^{b\beta\delta}$		36.74	$3.99 \pm 0.01^{a\delta}$
WSC		41.91	$5.97 \pm 0.46^{a\kappa}$		38.64	$7.65 \pm 0.42^{b\epsilon}$		35.73	$5.29 \pm 0.56^{a\epsilon}$
SBSC		39.22	$5.27 \pm 0.44^{a\epsilon\kappa}$		36.83	$6.32 \pm 0.79^{b\delta}$		34.90	$4.84 \pm 0.39^{a\delta\epsilon}$
SpC		40.23	$4.52 \pm 0.34^{a\epsilon}$		36.77	$7.89 \pm 0.68^{b\epsilon}$		33.50	$5.49 \pm 0.80^{a\epsilon}$

[a] WSC, water soaked and cooked; SBSC, sodium bicarbonate soaked and cooked; SpC, sprouted and cooked. Different superscript Latin characters denote statistically significant differences among legume species; different superscript Greek characters denote statistically significant differences among processing treatments ($p \leq 0.05$).

were no significant differences between samples soaked in water or in sodium bicarbonate (Table 3).

Although there were no statistically significant differences in the starch hydrolyzed at different times between the horse gram seeds soaked in the sodium bicarbonate solution and those sprouted, the percentage of hydrolysis tended to be higher in the former (Figure 2B; Table 3). Cooking without soaking also resulted in the lowest rate of starch digestion in this legume, although it was statistically comparable to the starch digestion of water-soaked seeds.

As in moth bean, sprouting also proved to be the best treatment to increase the in vitro amylolysis rate in black gram, with no statistically significant differences among the other three processing methods (Figure 2C; Table 3).

Table 4 shows the experimental HI and the estimated GI of the three studied legumes after processing. In general, the highest HI were observed in black gram. Except for sprouting, the different processing methods had little influence on the availability of starch in this sample. On the contrary, the type of processing markedly affected the starch digestibility of the little-known legumes. In both moth bean and horse gram the lowest HI and GI were obtained when the seeds were cooked without previous processing (Table 4). The highest starch digestibility and in consequence the highest estimated GI of the studied little-known pulses were achieved when moth bean and horse gram were sprouted and soaked in sodium bicarbonate prior to cooking, respectively.

DISCUSSION

It is now known that the culinary and technological processings of foods are important factors determining the availability of dietary starch (Asp et al., 1996; Björck et al., 1994). Because two of the parameters considered in the evaluation of new potential food resources are the quality and bioavailability of their constituent nutrients, it is important to study the starch content and digestibility of such new foods as affected by traditional cooking procedures.

The apparent increase of the TS content observed in the processed legumes (Table 2) may be due to the partial loss of soluble materials during soaking and cooking. Soluble sugars, oligosaccharides, soluble polyphenols, or soluble dietary fiber components are some of the constituents that can be lost during processing. Periago et al. (1996, 1997) also reported an increase of the TS content of cooked peas and chickpeas in comparison with the raw materials. On the other hand, starch in legume seeds is known to be hydrolyzed to oligosaccharides and ultimately to monosaccharides during germination; monosaccharides are then utilized to produce the energy required for various metabolic processes taking place during germination (Kataria and Chauhan, 1988; Kumar and Venkataraman, 1976). This amylolysis, catalyzed by phosphorylase and amylases, may be responsible for the decreased TS content of sprouted legumes in comparison with the other processed samples.

Among the factors known to influence starch digestibility in foods, the presence of certain antinutrients such as α-amylase inhibitors can greatly determine the extent of starch hydrolysis. However, during cooking and also during germination, these heat-labile inhibitors are inactivated (Mulumani et al., 1994) and the starch digestibility thus also improved. Therefore, the presence of α-amylase inhibitors could partly account for the low starch digestibility of raw legumes; however, no amylase inhibitor activity was detected in uncooked moth bean and horse gram according to Subbulakshmi et al. (1976). Moreover, during starch analysis samples are incubated with concentrated alkali and proteolytic enzymes that would inactivate amylase inhibitors. Therefore, the reduced digestibility of starch in the raw pulses should be attributed to factors different from the presence of α-amylase inhibitors.

Starch in raw foods is contained within granules that are poorly affected by hydrolytic enzymes and it is, therefore, mostly indigestible (Colonna et al., 1992). This accounts for the high RS content of raw legumes (RS_2). The difference in the starch digestibility among the studied samples might be due to differences in the degree of crystallinity or amylose/amylopectin ratio of the starch granules, which are factors also known to affect starch digestibility (Englyst et al., 1992; Saura-Calixto and Abia, 1991).

During cooking, starch granules are gelatinized and partly solubilized, becoming available to digestive enzymes. This explains the great improvement of starch digestibility attained after cooking, with a significant decrease in the RS values. However, there is still a fraction of starch not digested by the amylolytic enzymes in the processed seeds. Because these samples were analyzed immediately after cooking, avoiding drops in temperature, it should be assumed that no retrograded starch (RS_3) was formed. Legumes are characterized by their poor starch digestibility, with appreciable

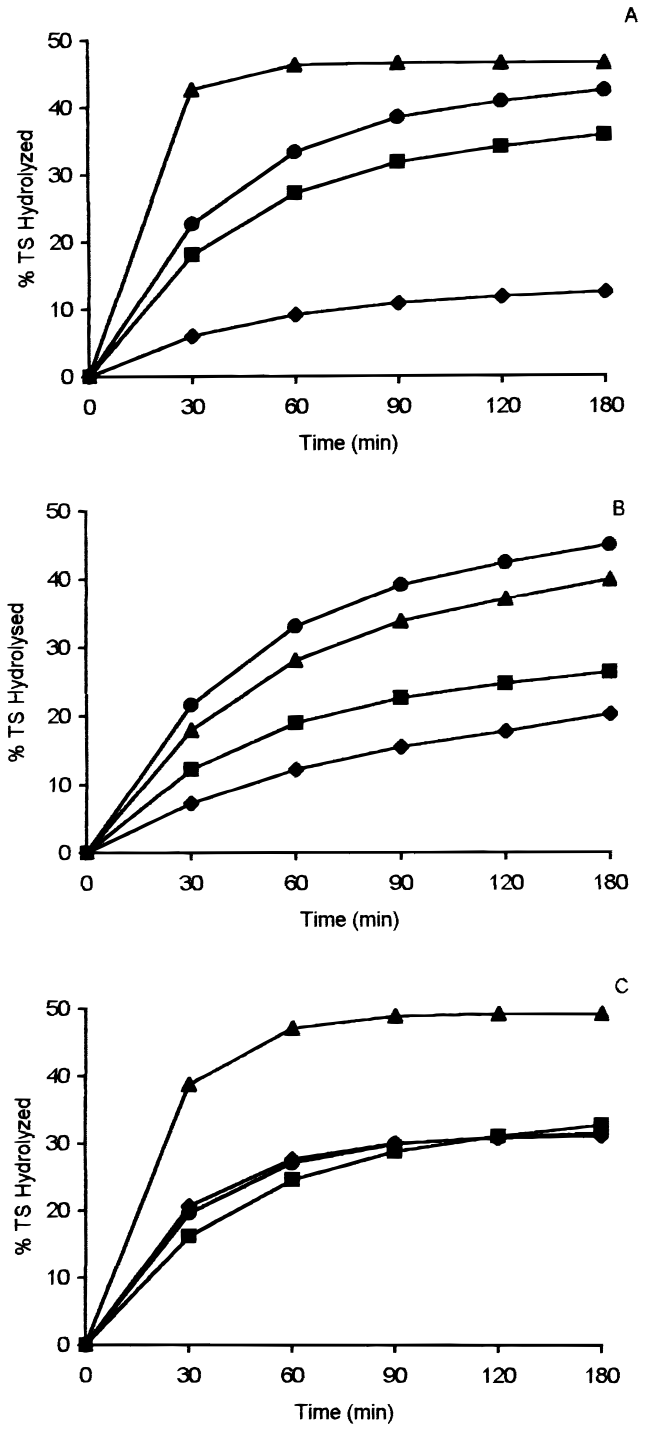

Figure 2. In vitro starch hydrolysis rate of processed legumes: (A) moth bean; (B) horse gram; (C) black gram. Treatments: direct cooking (◆); water soaking and cooking (■); sodium bicarbonate soaking and cooking (●); sprouting and cooking (▲).

amounts of RS in cooked samples (Periago et al., 1996, 1997; Tovar et al., 1990a,b). Several factors are involved in the reduced bioavailability of legume starches. The presence of intact tissue/cell structures enclosing starch granules hinders the swelling and solubilization of starch, resulting in a reduced digestion rate in vitro (Tovar et al., 1990b; Würsch et al., 1986) and incomplete digestion in vivo (Tovar et al., 1992a; Schweizer et al., 1990). Permanence of intact starch granules trapped within cells in precooked legume flours was observed by Tovar et al. (1991), even after extensive homogenization and pepsin treatment. This could account for the RS found in freshly cooked samples (Table 2). Other factors affecting legume starch digestibility are the high contents of viscous, soluble dietary fiber components as well as the relatively high amylose/amylopectin ratios of legume starches and the presence of antinutrients such as polyphenols (Longstaff and McNab, 1991; Thompson and Yoon, 1984; Wong et al., 1985). As a consequence of this poor starch digestibility, pulses promote slow and moderate postprandial glucose and insulin responses (Jenkins et al., 1980, 1982). This "lente carbohydrate" property of legumes suggests the importance from a nutritional viewpoint of increasing legume consumption (Björck et al., 1994). Such lente foods have been suggested to have beneficial effects in the management of diabetes and hyperlipidemia (Jenkins et al., 1988, 1994).

Sprouting seemed to be the best processing method to facilitate starch gelatinization during cooking because RS contents in freshly cooked samples were always lower in sprouted pulses than in any other processed sample (Table 2). Also DS, as a percentage of TS, was higher in sprouted legumes. This higher starch digestibility may result from a partial starch hydrolysis during germination due to the activation of amylases and phosphorylase, which can initiate amylolysis (Kumar and Venkatarman, 1976). Also, it has been suggested that cooking germinated grain legumes may promote metabolic and structural changes with modifications of the nature of interaction between legume starch and fiber/protein, rendering the former more readily digestible (El Faki et al., 1984). Increased starch digestibilities have also been reported by other authors in germinated legumes (Kaur and Kapoor, 1990; Subbulakshmi et al., 1976; Urooj and Puttaraj, 1994; Veena et al., 1995). As to the other treatments, there is certain variability depending on the studied sample as to which processing method is best to reduce RS values.

When cooked pulses were kept at 4 °C during 24 h, the RS contents significantly increased in all samples due to partial retrogradation of gelatinized starch (RS_3) (Colonna et al., 1992). Similar reduced starch availability in cooked and stored pulses was observed by Velasco et al. (1997) in black beans and cowpeas. In our experiment, the samples that showed the lowest RS content when analyzed immediately after cooking also showed the highest relative increase in RS formation upon storing (Table 2). The lower RS content in freshly cooked samples reflects better amylose and amylopectin availabilities. Upon cooling and storing, those amylose and amylopectin chains may undergo retrogradation, resulting in high RS (RS_3) contents.

In moth bean, there was an inverse relationship between the RS content of freshly cooked samples and the in vitro starch digestion rate. The processing method leading to the lowest RS content—sprouting and cooking (Table 2)—also resulted in the highest starch digestion rate (Table 3; Figure 2A). This processing method also rendered the lowest RS content and highest in vitro rate of starch digestion in black gram. As mentioned above, activation of amylolytic enzymes takes place during germination, which could account for the higher digestibility of sprouted legumes.

Concerning horse gram, sprouting also proved to be a good treatment to achieve a high starch digestion rate in vitro (Figure 2B). It also resulted in the lowest RS content of the freshly cooked seeds as compared with

Table 3. Kinetics of in Vitro Starch Digestion of Processed Indian Legumes (Percent Total Starch Hydrolyzed at Different Times)[a]

treatment	sample	30 min	60 min	90 min	120 min	180 min
cooked	moth bean	$8.54 \pm 1.51^{a\alpha}$	$8.74 \pm 1.70^{a\alpha}$	$8.85 \pm 1.62^{a\alpha}$	$9.65 \pm 1.62^{a\alpha}$	$15.18 \pm 3.54^{a\alpha}$
	horse gram	$9.53 \pm 0.11^{a\alpha}$	$11.94 \pm 0.85^{a\alpha}$	$13.99 \pm 1.33^{a\alpha}$	$16.48 \pm 0.76^{a\alpha}$	$21.51 \pm 1.88^{a\alpha}$
	black gram	$22.96 \pm 5.11^{b\alpha}$	$25.78 \pm 5.60^{b\alpha}$	$26.98 \pm 4.78^{b\alpha}$	$31.85 \pm 5.67^{b\alpha}$	$33.64 \pm 4.35^{b\alpha}$
WSC	moth bean	$19.55 \pm 2.19^{a\beta}$	$28.67 \pm 3.47^{a\beta}$	$29.24 \pm 4.18^{a\beta}$	$32.79 \pm 3.86^{a\beta}$	$38.18 \pm 3.43^{a\beta}$
	horse gram	$14.28 \pm 3.39^{a\alpha}$	$17.26 \pm 3.73^{a\alpha\beta}$	$21.86 \pm 4.00^{a\alpha}$	$24.54 \pm 5.98^{a\alpha}$	$27.15 \pm 6.69^{a\alpha}$
	black gram	$18.90 \pm 2.22^{a\alpha}$	$23.55 \pm 2.27^{a\alpha}$	$27.26 \pm 2.26^{a\alpha}$	$29.28 \pm 2.56^{a\alpha}$	$35.42 \pm 3.09^{a\alpha}$
SBSC	moth bean	$24.39 \pm 5.24^{a\beta}$	$32.84 \pm 6.09^{a\beta}$	$37.36 \pm 8.41^{ab\gamma}$	$38.91 \pm 7.37^{a\gamma}$	$44.80 \pm 5.75^{a\beta\gamma}$
	horse gram	$24.84 \pm 6.51^{a\beta}$	$31.66 \pm 7.56^{ab\gamma}$	$37.96 \pm 9.50^{b\beta}$	$41.46 \pm 7.32^{a\beta}$	$47.16 \pm 9.13^{a\beta}$
	black gram	$22.47 \pm 3.98^{a\alpha\beta}$	$24.97 \pm 2.89^{b\alpha}$	$27.03 \pm 3.54^{a\alpha}$	$29.27 \pm 2.61^{b\alpha}$	$36.01 \pm 5.98^{b\alpha}$
SpC	moth bean	$43.09 \pm 3.44^{a\gamma}$	$43.89 \pm 3.73^{a\gamma}$	$46.39 \pm 1.01^{a\delta}$	$47.24 \pm 1.72^{a\delta}$	$48.02 \pm 2.63^{a\gamma}$
	horse gram	$22.46 \pm 4.19^{b\beta}$	$25.82 \pm 4.77^{b\beta\gamma}$	$30.43 \pm 3.56^{b\beta}$	$36.95 \pm 4.52^{b\beta}$	$42.01 \pm 6.22^{a\beta}$
	black gram	$40.80 \pm 8.75^{a\beta}$	$43.06 \pm 9.26^{a\beta}$	$47.80 \pm 4.54^{a\beta}$	$48.39 \pm 6.75^{a\beta}$	$54.00 \pm 5.44^{a\beta}$

[a] WSC, water soaked and cooked; SBSC, sodium bicarbonate soaked and cooked; SpC, sprouted and cooked. Different superscript Latin characters denote statistically significant differences among legume species; different superscript Greek characters denote statistically significant differences among processing treatments ($p \leq 0.05$).

Table 4. Hydrolysis Index (HI) and Predicted Glycemic Index (GI) of Processed Legumes[a]

sample	treatment	HI	GI
moth bean	cooked	14.50	47.67
	WSC	42.82	63.22
	SBSC	51.44	67.95
	SpC	66.67	76.31
horse gram	cooked	21.01	51.24
	WSC	30.26	56.23
	SBSC	52.75	68.57
	SpC	45.24	64.55
black gram	cooked	40.70	62.05
	WSC	38.66	60.93
	SBSC	40.20	61.78
	SpC	68.10	77.10

[a] WSC, water soaked and cooked; SBSC, sodium bicarbonate soaked and cooked; SpC, sprouted and cooked.

the other studied treatments (Table 2). However, the in vitro starch digestion rate tended to be higher when this pulse was soaked in a sodium bicarbonate solution than after sprouting, although the difference was not statistically significant (Table 3). The fact that this sample required longer cooking times to render it edible might suggest that a longer sprouting time could have also improved the starch digestion rate as has been reported in soybean (Boralkar and Reddy, 1985).

Except for black gram, cooking without previous treatment resulted in higher RS values and slower starch digestion rates in the little-known pulses as compared with the processed ones. Soaking in water or in a sodium bicarbonate solution may reduce the levels of phytate, polyphenols, and other antinutritional factors, which are known to reduce the rate of starch digestion and the glycemic response (Björck and Nyman, 1987; Thompson and Yoon, 1984), thus improving starch digestibility (Boralkar and Reddy, 1985; Khokar and Chauhan, 1986). Also, structural changes, derived from an increased swelling of starch granules during soaking, may play a role in the observed enhanced starch digestibility. Surprisingly, soaking black gram seeds prior to cooking did not affect their starch digestibility; this only improved after sprouting (Figure 2C).

A useful tool, from a nutritional point of view, to compare the starch digestibility of different food samples as affected by processing is the HI. This index expresses the digestibility of the starch in any foodstuff in relation to the digestibility of starch in a reference material, namely white bread. White bread is also used as reference of 100% starch digestibility to calculate the GI. This is defined as the incremental postprandial blood glucose area after ingestion of the test product as percentage of the corresponding area after ingestion of an equicarbohydrate portion of the reference product (Jenkins et al., 1983). Similarly, the HI is also defined as the area under the starch hydrolysis curve of a test material expressed as percentage of the corresponding hydrolysis area of the reference product (Goñi et al., 1997).

In a previous paper we showed the excellent correlation between the GI measured in vivo and the HI analyzed (Goñi et al., 1997), recommending the use of the latter to predict the glycemic responses to foods due to the difficulty of assaying in vivo the GI of every single foodstuff. Thus, from the measurement of the in vitro starch digestion rate and the calculation of the corresponding area under the hydrolysis curve (using eq 1), the HI is calculated and used to estimate the corresponding predicted GI using eq 2.

As could be expected, the samples with the highest in vitro starch digestion rate were also those that would elicit the highest glycemic response in vivo as suggested by their GI (Table 4). From these results it can be seen that little-known legumes, horse gram and moth bean, maintain the lente carbohydrate quality common to all pulses, with predicted glycemic responses similar to or even lower than those of a commonly consumed legume such as black gram.

CONCLUSIONS

Both little-known Indian pulses, moth bean and horse gram, were comparable to a widely consumed one such as black gram in terms of their starch digestibility. Nevertheless, depending on the legume species and the type of processing applied prior to cooking, the RS content of the cooked pulses and the glycemic response derived from their consumption will vary.

Sprouting or soaking in a 0.02% sodium bicarbonate solution prior to cooking were the best of the studied processing methods to achieve the highest rate of in vitro starch digestion and the lowest RS in *Vigna* spp. (moth bean and black gram) and in horse gram, respectively. These traditional culinary treatments proved to be adequate to improve starch digestibility. However, these legumes thus cooked would elicit higher postpran-

dial blood glucose and insulin responses than when cooked following some of the other studied procedures. Also, storing cooked samples at 4 °C during 24 h significantly increased the RS content of these pulses. Therefore, depending on the use of these foods, an appropriate processing method could be recommended among the ones studied here. If these legumes were consumed in rural areas of India, where malnutrition can be a major problem, processing methods leading to the highest starch digestibility should be used. In turn, diabetic patients should consume legumes eliciting a low postprandial glycemic response with the appropriate processing applied in consequence.

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