Starch Content and α -Amylolysis Rate in Precooked Legume Flours

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Dried lentils (*Lens culinaris*) and beans (*Phaseolus vulgaris*, black, brown, white, and red) were boiled until soft, freeze-dried, and milled. This procedure yielded precooked flours (PCF), rich in intact cells filled with starch granules. Starch content in the various bean PCFs was underestimated by enzymic assays. Potentially available starch in PCFs was evaluated after complete release of starch by mechanical disruption of cell wall structures. Total starch content, measured after solubilization in 2 N KOH, was higher than the potentially available starch, indicating that all PCFs contained important quantities of resistant starch, i.e., retrograded amylose (3-9%, dwb). Limited enzymic availability of starch in PCFs was also demonstrated by their low α -amylolysis rate. Pepsin preincubation and additional heat treatment increased the rate and extent of in vitro enzymic hydrolysis. Considerable differences in susceptibility to α -amylase attack were observed between the various legume flours.

INTRODUCTION

Dried legume seeds generally promote slow and moderate postprandial blood glucose increase (Jenkins et al., 1980, 1982a,b). The reasons for this feature are not yet clear, but several factors, like the rigidity of cotyledonary cell walls (Würsch et al., 1986; Tappy et al., 1986), the intrinsicially low enzyme susceptibility of legume starches (Socorro et al., 1989), dietary fiber effects (Jenkins et al., 1982b; Gee and Johnson, 1985; Morón et al., 1989), and the presence of polyphenols and other α -amylase inhibitors (Jenkins et al., 1982b; Morón et al., 1989), are thought to contribute. In spite of this "slow carbohydrate" and other beneficial properties, and their wide consumption in many regions of the world, industrial uses of legume seeds and their starches are relatively scarce (Sosulski et al., 1989). It would be of practical interest to utilize legumes, for instance, in the formulation of manufactured foods for diabetics. Dry white bean flakes have already been prepared for this purpose (Golay et al., 1986; Tappy et al., 1986).

We have recently reported a procedure for preparation of a precooked red kidney bean flour that is slowly digested by pancreatic amylase in vitro (Tovar et al., 1990a). This behavior was mainly due to the entrapment of starch granules within cell walls. Such a bean powder has the advantage that it can be incorporated in a variety of foods, thus enabling a wider use of legumes.

The limited enzymic availability of starch in the cooked red kidney bean flour also influenced the accuracy of starch determination by enzymic procedures (Tovar et al., 1990a). Besides obstructing the analytical yield, an incomplete digestion of starch is also of nutritional importance. Recently, there has been a growing interest in the total digestibility of starch, and the long-held opinion of starch as a completely digestible carbohydrate has been challenged. An important fraction of starch in ordinary food items may escape digestion and absorption in the small intestine (Anderson et al., 1981; Asp et al., 1987; Englyst and Cummings, 1985; Stephen et al., 1983). Suggested mechanisms for delivery of starch to the large bowel in healthy subjects include, among others, incomplete gelatinization and retrogradation of amylose (Asp et al., 1987).

In the present investigation, the in vitro rate and extent of starch hydrolysis were studied in processed flours from lentils and several bean varieties. Efforts were also made to distinguish various types of in vitro indigestible starch.

MATERIALS AND METHODS

Seeds. Green lentils (Lens culinaris Medik) and common beans (Phaseolus vulgaris L.) of different colors (red, white, brown, and black) were obtained from the local market. Four defined cultivars of brown beans—cv. 2578 Gotland, cv. 4012 Bonita, cv. 4150 Ebbes Mors, and cv. 5026 Brun—were kindly provided by the Nordic Genebank for Agricultural and Horticultural Plants, Alnarp, Sweden.

Processing of Legumes. Seeds were soaked for 20 min in twice their weight of water, on the basis of reports suggesting significant starch losses due to prolonged soaking (Kataria and Chauhan, 1988). The soaked seeds were then cooked by boiling in water until soft as felt between fingers, employing a seed to water ratio of 1:3 (w/v) (Tovar et al., 1990a). The cooking time, as estimated in this way, was 70 min for the lentil and red kidney bean samples, whereas 120 min was required for black, brown, and white beans.

In some experiments directed to evaluate the influence of the length of the cooking period, red kidney beans and lentils were boiled for 120 min ("overcooked seeds"), while a shortened cooking time (70 min) was applied to white beans ("undercooked white beans").

The softened seeds, along with cooking water, were freezedried and ground to pass a 1-mm screen in a Cyclotec 1093 mill (Tecator AB, Höganäs, Sweden). The cooked and freeze-dried flour was kept in a desiccator until used. This preparation is referred to as precooked flour.

For comparative microscopic examination, flours from raw legumes were prepared by using the mentioned mill.

Texture Evaluation. The texture of cooked seeds was evaluated by determining the puncture peak force, using the Instron 4301 equipment. A conical puncture probe (6.6-mm largest diameter, 1.1-mm tip diameter, 8.8-mm height) was used at a speed of 30 cm/min. Fifty single seeds were tested from each legume preparation. For comparative purposes, the hardness of 6-h-soaked and autoclaved (1.05 kg/cm² for 20 min) seeds was also evaluated.

Microscopic Observations. Suspensions of the various flours in distilled water (2.5% w/v) were used for conventional light microscopy observation.

Starch Determination. Starch was analyzed by the enzymic/colorimetric method of Holm et al. (1986). This method comprises the following main steps: incubation with Termamyl for 15 min at boiling temperature, digestion with amyloglucosidase at 60 °C (30 min), and free glucose measurement using the combined glucose oxidase/peroxidase colorimetric assay.

 Table I. Textural Properties of Variously Cooked Legume

 Seeds

legume	instron hardness,ª kg			
	boiled (70 min)	boiled (120 min)	autoclaved	
white beans brown beans black beans red kidney beans lentils	0.116 (0.074) ^a 0.081 (0.013) ^a 0.049 (0.024) ^a	0.036 (0.014) ^b 0.055 (0.035) ^a 0.057 (0.033) ^a 0.057 (0.037) ^b 0.026 (0.009) ^b	0.038 (0.013) ^b 0.063 (0.016) ^a 0.063 (0.022) ^a 0.071 (0.034) ^a 0.023 (0.008) ^b	

^a Values are the mean of peak forces from 50 single-seed tests. SD is indicated in parentheses. The means for the different treatments of each legume were statistically compared; those without common superscript letters are significantly different (p < 0.05).

The precooked flours from black, brown, and red kidney beans were also analyzed by the procedure of Salomonsson et al. (1984). In this method, Termamyl and amyloglucosidase are also employed, but the enzyme:sample ratios are different and prolonged incubation times (30 min for Termamyl and overnight for amyloglucosidase) are used.

To assess the influence of sample handling on the starch yield, various treatments were applied before the assay (Tovar et al., 1990a): (a) For homogenization, the samples (500 mg) were suspended in 20 mL of distilled water and submitted to five 1-min pulses at maximal strength with a Polytron blender (Kinematica GmbH, Luzern). (b) For solubilization with KOH, the samples (500 mg) were suspended in 10 mL of water, and an equal volume of freshly prepared 4 N KOH solution was added. The mixture was kept for 30 min at room temperature and then neutralized (pH 6.5-7) with 5 N HCl.

Starch Availability in Vitro. The rate of starch hydrolysis was evaluated as described by Holm et al. (1985), using 200 units of porcine pancreatic α -amylase (Sigma Chemical Co., St. Louis) per gram of starch. Starch content in legume samples was calculated on the basis of values obtained after the homogenization treatment. Wheat starch suspensions, which were boiled for 20 min in a water bath, were assayed as a reference.

The rate of hydrolysis by pancreatic amylase was evaluated after (a) pepsin incubation and (b) boiling. (a) One hundred milligrams of pepsin (2000 FIP-U/g; Merck, Darmstadt) was added to 500 mg of starch; incubation was performed for 1 h at 37 °C and pH 1.5. (b) A suspension of the precooked flour was boiled for 20 min in a water bath. In some cases, this treatment was followed by pepsin incubation as indicated above. Blanks of treated samples, in which the starch degrading enzymes were omitted, were run in every case.

For statistical analyses, the 15- and 30-min values were taken as indicators of the initial rate of amylolysis, whereas the 60min values gave a measure of the final degree of hydrolysis.

Statistics. Means were compared by one-way analysis of variance followed by the Duncan multiple comparison test, using the spss/pc+ program.

RESULTS

Texture of Cooked Seeds. The texture of the 70min-boiled red kidney beans and the 120-min-boiled black, brown, and white seeds was similar to that of the soakedautoclaved reference (Table I). An overcooked characteristic of the 120-min-boiled red beans and the undercooked feature of the 70-min treated white seeds were also demonstrated (Table I). Lentils were harder after 70 min of boiling than after autoclaving or 2 h of boiling. However, these two processes resulted in a large number of broken or damaged seeds.

Microscopic Appearance. All the precooked flours were rich in starch granules encapsulated by apparently intact cell walls, while free granules were mainly observed in the flours obtained from raw seeds (results not shown).

Starch Content. The apparent starch content, estimated by the method of Holm et al. (1986) in the precooked flour from common beans and lentils, is

 Table II.
 Effect of Several Treatments on the Apparent

 Starch Content of Precooked Legume Flours*

	starch content, ^b %			
legume	no treatment	homogeni- zation (A)	2 N KOH (B)	$\frac{\mathrm{RS}}{(B-A)}$
white beans brown beans black beans red kidney beans lentils	34.8 (0.4) ^{c,1} 35.2 (0.6) ^{c,1} 30.4 (0.6) ^{c,2} 33.2 (0.7) ^{c,3} 41.8 (0.8) ^{c,4}	$\begin{array}{r} 35.8 & (0.8)^{d,1} \\ 39.8 & (0.6)^{d,2} \\ 34.7 & (0.5)^{d,1} \\ 36.4 & (1.0)^{d,3} \\ 41.5 & (0.5)^{c,2} \end{array}$	39.6 (0.99) ^{e,1} 45.0 (1.6) ^{e,2} 40.8 (1.0) ^{e,3} 40.3 (1.2) ^{e,3} 44.7 (0.5) ^{d,2}	3.8 5.2 6.1 3.9 3.2

^a From 70-min-boiled lentils and red kidney beans and 120-minboiled white, brown, and black beans. ^b Dry matter basis. RS, resistant starch. Values are the mean of a minimum of four assays. SD is indicated in parentheses. Means in rows without common superscript letters are significantly different (p < 0.05). Means in columns without common superscript numbers are significantly different (p < 0.05).

Table III. Apparent Starch Content of Brown, Black, and Red Bean Precooked Flours⁴ Estimated by Two Enzymic Methods

	starch content, ^b %		
legume	method of Holm et al. (1986)	method of Salomonsson et al. (1984)	
brown beans black beans red kidney beans	35.2 (0.6) ^c 30.4 (0.6) ^d 33.2 (0.7) ^c	33.1 (0.7) ^c 29.8 (1.7) ^d 32.0 (1.0) ^{c,d}	

^a From 70-min-boiled red kidney beans and 120-min-boiled brown and black beans. ^b Dry matter basis. Values are the mean of four assays. SD is indicated in parentheses. All the means were statistically compared to each other. Means without common superscript letters are significantly different (p < 0.05).

Table IV. Apparent Starch Content of Precooked Flours from Defined Brown Bean Varieties^a after Various Treatments

	starch content, ^b %			
variety	no treatment	homogeni- zation (A)	2 N KOH (B)	$\begin{array}{c} \mathrm{RS} \\ (B-A) \end{array}$
Bonita Ebbes Mors Gotland Brun	33.0 (0.9) ^{c,1} 30.7 (1.0) ^{c,2} 31.7 (0.9) ^{c,1,2} 30.0 (1.3) ^{c,2}	40.6 (0.9) ^{d,1} 37.2 (1.8) ^{d,1} 37.7 (0.9) ^{d,1} 36.8 (1.5) ^{d,1}	46.6 (0.9) ^{e,1} 45.8 (1.6) ^{e,1,2} 43.9 (0.3) ^{e,2,3} 42.8 (0.7) ^{e,3}	6.0 8.6 6.2 6.0

^a Seeds were boiled for 120 min. ^b Dry matter basis. RS, resistant starch. Values are the mean of four assays. SD is indicated in parentheses. Means in rows without common superscript letters are significantly different (p < 0.05). Means in columns without common superscript numbers are significantly different (p < 0.05).

summarized in Table II. Similar results were obtained with the black, brown, and red bean flours when the method of Salomonsson et al. (1984) was used (Table III).

Except for the lentil preparation, the homogenization treatment resulted in a significant increase in starch yield (1-4%, dwb) (Table II). A further increase in the apparent starch content was noticed for all samples after solubilization with KOH. This effect was particularly remarkable for brown and black beans, in which a 5–6% (dwb) starch increment occurred (Table II).

The flours from the four agronomically defined brown bean cultivars (Table IV) gave a similar pattern, but the absolute increases in starch yield after homogenization were higher than that in the commercial variety, ranging between 6 and 7% (dwb). KOH treatment caused an additional 6-9% rise.

Starch Availability in Vitro. Figure 1A depicts the α -amylolysis curves of the precooked flours and the boiled wheat starch used as a reference. The red kidney bean powder was the least available, whereas that from white

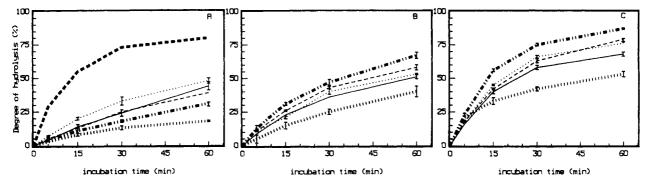


Figure 1. Hydrolysis of starch in precooked legume flours by pancreatic α -amylase. (A) Untreated samples; (B) pepsin treated; (C) boiled and pepsin treated. (--) Black bean; (--) brown bean; (|||) red bean; (--) white bean; (--) lentils; (---) boiled wheat starch. Bars indicate the standard deviation of the mean, n = 6. Cooking times were 70 min for red beans and lentils and 120 min for black, brown, and white beans.

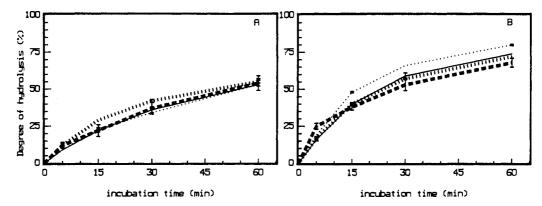


Figure 2. Hydrolysis of starch in precooked brown bean flours by pancreatic α -amylase. (A) Pepsin treated; (B) boiled and pepsin treated. (|||) cv. Bonita; (...) cv. Gotland; (...) cv. Brun; (**DD**) cv. Ebbes Mors. Bars indicate the standard deviation of the mean, n = 6. Cooking time for all samples was 120 min.

beans showed the highest rate of hydrolysis. The final degree of hydrolysis (60 min) increased in the following order: red beans < lentils \leq brown beans = black beans \leq white beans (p < 0.05).

A preincubation with pepsin led to a higher hydrolysis rate (Figure 1B). After this treatment, all bean materials were less susceptible to α -amylolysis than the lentil flour (60 min, p < 0.05). Red kidney bean powder was, however, still significantly less available than the rest of the bean samples (30 and 60 min, p < 0.05).

A further rise in the susceptibility to α -amylase was observed after boiling-pepsin treatment of the flours (Figure 1C). Red bean material showed again the lowest amylolysis rate (30 and 60 min, p < 0.05), while the lentil powder reached a pattern which was similar to that of the boiled wheat starch reference.

No significant differences in amylolytic behavior were observed between the flours prepared from the brown bean varieties studied (Figure 2A). However, the reboiled and pepsin-treated flour from cv. Gotland was more susceptible to enzymic hydrolysis than the other brown bean powders (15, 30, and 60 min, p < 0.05) Figure 2B).

Effect of Cooking Time on Starch Yield and Enzymic Availability. As indicated in Table V, 120min cooking of red kidney beans (overcooking) resulted in a flour with a lower apparent starch content than that in the 70-min-cooked seeds. Homogenization, however, ruled out differences due to cooking time. Decreasing the cooking time for white beans from 2 h to 70 min (undercooking) did not affect the starch yield. Precooked lentil flours also showed a similar starch content irrespective of the length of cooking period.

Overcooking of red kidney beans and lentils slightly affected the rate of amylolysis of the powdered material.

Table V. Influence of the Length of Cooking Period on the Apparent Starch Content of Processed Legume Flours

legume and	starch content,ª %		
cooking time	no treatment	homogenization	
red kidney beans			
120 min	27.8 (0.9) ^b	34.9 (1.3) ^{c,d}	
70 min	33.2 (0.7)°	36.4 (1.0) ^d	
white beans			
120 min	34.8 (0.5) ^b	35.8 (0.8) ^{b,c}	
70 min	34.9 (0.3) ^b	36.9 (0.4)°	
lentils			
120 min	42.7 (0.3) ^b	43.0 (0.8) ^b	
70 min	41.8 (0.8) ^b	41.5 (0.5) ^b	

^a Dry matter basis. Values are the mean of four assays. SD is indicated in parentheses. The means for different treatments of each legume were statistically compared; those without common superscript letters are significantly different (p < 0.05).

The 120-min-cooked flours were more effectively hydrolyzed than those cooked until soft (70 min) (Figure 3A,C). A similar pattern was recorded after pepsin treatment (Figure 3A,C), though higher rates and final degrees of hydrolysis were observed (60 min, p < 0.05). Additional heat treatment (boiling) of the red bean flour, in combination with pepsin incubation, resulted in a higher rate of amylolysis, the increase being more attenuated in the initially overcooked samples (Figure 3A). In contrast, the cooking time had no influence on the susceptibility to amylolysis in the case of boiled-pepsin-treated lentils (Figure 3C). The flour from undercooked white beans was more slowly hydrolyzed than that coming from softened seeds (Figure 3B), but no difference could be observed after additional boiling-pepsin treatment.

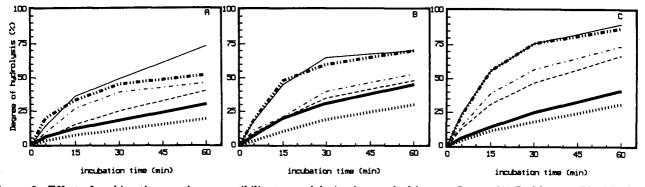


Figure 3. Effect of cooking time on the susceptibility to amylolysis of precooked legume flours. (A) Red beans; (B) white beans; (C) lentils. (||) Flour from 70-min-cooked seeds; (\longrightarrow) flour from 120-min-cooked seeds; (- -) 70-min-cooked plus pepsin incubation; (- -) 120-min-cooked plus pepsin incubation; (\blacksquare | \blacksquare) 70-min-cooked, boiled, and pepsin treated; (-) 120-min-cooked, boiled, and pepsin treated. Values are mean of four experiments.

DISCUSSION

Autoclaving is the standard heat treatment applied during industrial processing of legumes. Boiling, on the other hand, is a common way of domestic cooking of pulses in many countries (Jaffé and Flores, 1975; Kataria and Chauhan, 1988; Lowgren and Liener, 1986; Ologhobo and Fetuga, 1988; Paredes-López et al., 1989; Rodriguez and Mendoza, 1989). Therefore, conventional boiling was selected for the present study. According to the texture assays (Table I), this treatment resulted in properly softened beans, thus supporting the validity of the following observations.

The cooking pattern for lentils deserves particular consideration. After boiling for 70 min, the seeds showed a relatively hard texture as compared to the boiled for 2 h or autoclaved samples (Table I), which contained mainly disintegrated seeds. For this reason, and taking into account that short-time boiling may be considered the regular treatment for lentils (Bhatty, 1988), the 70-minboiled lentil preparation was regarded as the realistically processed material. The short cooking times required for the lentils and the various bean types are far below those characteristic of hard-to-cook seeds (Antunez and Sgarbieri, 1979; Bhatty, 1988; Paredes-López et al., 1989).

As recently reported for a flour obtained from cooked and freeze-dried red kidney beans (Tovar et al., 1990a), similarly prepared precooked flours from other legume seeds contained large amounts of apparently intact cells filled with entrapped starch granules. This is in agreement with the observations made by Golay et al. (1986) and Tappy et al. (1986) with precooked white bean flakes. The microstructural characteristics of precooked legume flours are considered elsewhere (Tovar et al., 1990b).

The present results show that the previously noticed underestimation of starch content in the red kidney bean powder occurs also in other precooked bean flours. Wet homogenization of the black, brown, and white bean flours resulted in a higher starch yield (Tables II and IV). The increase in apparent starch content when a homogenization step is included in the ordinary starch assay is likely to represent release of "entrapped" starch (Tovar et al., 1990a). Thus, physical insulation caused by cell walls seems again to be responsible for the inefficient action of the amylolytic enzymes employed in the starch assay. This problem was not overcome by the prolonged digestion and other enzyme:substrate ratio proposed by Salomonsson et al. (1984) (Table III).

In the case of lentils, the apparent starch content did not increase after mechanical disruption of cotyledonary cell surface barrier by intense homogenization (Table II). This could be explained in terms of a more labile or a less integrate cell wall structure in this seed flour than in those prepared from the various beans. Thus, "potentially available starch", which represents the amount of starch that can be hydrolyzed by amylolytic enzymes (Tovar et al., 1990a), can be directly assessed in the lentil processed flour, whereas the homogenization step is required when the various precooked bean powders are analyzed. Among the beans studied here, the cell walls of the white beans seemed to be the least resistant to heat/drying processing, since only a small increase in apparent starch content was found after homogenization (Table II). The in vivo digestibility of starch when entrapped within cell structures is not known, but some of it might be delivered to the large bowel.

A pretreatment with 2 N KOH resulted in an important rise in the apparent starch content of all the legume flours (Tables II and IV). This treatment not only solubilizes the so-called "resistant starch" (Englyst and Cummings, 1984; Siljeström et al., 1988), rendering it available to enzymic hydrolysis, but also destroys the integrity of remaining intact cell walls. Hence, for the type of samples studied, the KOH procedure provides a way to measure "total starch content" (Tovar et al., 1990a). Accordingly, the lentils and the various brown bean cultivars had a higher starch content than the other bean varieties (Tables II and IV).

Resistant starch is generated during cooling of wetcooked foodstuffs (Björck et al., 1986, 1987; Englyst and Cummings, 1984; Siljeström et al., 1988) and consists of highly crystalline retrograded amylose chains (Russel et al., 1989; Sievert and Pomeranz, 1989; Siljeström et al., 1989). The difference between starch content after solubilization with KOH and that measured after homogenization, i.e., total – potentially available starch (B - A in Tables II and IV), can be assigned to resistant starch. The relatively large amount of resistant starch found in the precooked flours (6-8%, dwb) may be a consequence of the generally high amylose content of legume starches (Eliasson, 1988).

When expressed on total starch basis, resistant starch content in the precooked legume flours was notably variable, ranging from 7 to 19% (Tables II and IV), levels that are somewhat higher than those recently found in commercially canned legume products (Siljeström and Björck, 1990). These differences could be due to the variability in the amylose:amylopectin ratio reported for starches isolated from different legumes species and varieties (Eliasson, 1988; Hoover and Sosulski, 1985). Resistant starch is known to be indigestible also in vivo (Björck et al., 1986, 1987). Hence, all the precooked flours contained an appreciable amount of starch that is likely to be delivered to the large intestine. Malabsorbed starch fractions provide additional energy for the colonic microbiota (McBurney et al., 1990). Short-chain fatty acids produced by bacterial fermentation have been implicated in various physiologically important processes (Jenkins et al., 1987; McBurney et al., 1990). On the other hand, the gas generated by colonic degradation of resistant starch may contribute to the flatulence problems of legumes, which are attributed mainly to low molecular weight indigestible sugars (Savage and Deo, 1989).

With some exceptions, the rate of starch hydrolysis in vitro is considered a good indicator of the rate and magnitude of the increase in blood glucose levels after a particular meal is eaten (Bornet et al., 1989; Jenkins et al., 1982a). All the samples studied were slowly hydrolyzed in vitro, even after pepsin digestion (Figures 1 and 2), suggesting that the procedure employed to prepare precooked red kidney bean flour (Tovar et al., 1990a) is of general applicability for legumes. The rise in availability caused by pepsin was previously observed for the red bean product (Tovar et al., 1990a) and seems to be due to proteolytic alteration of cell walls, though the release of protein-starch associations (Wong et al., 1985; Slack et al., 1979; Tovar et al., 1989) cannot be ruled out.

Because of the occurrence of pepsin in the gastric phase during in vivo digestion, the behavior of pepsin-treated flours may be considered the most relevant when predictions of glycemic response are made. As the rate of α -amylolysis of the precooked materials was considerably lower than that recorded for boiled wheat starch (Figures 1A,B and 2A) and for other "rapid" carbohydrates (Bornet et al., 1989; Socorro et al., 1989; Tovar et al., 1989), a "slow" feature may be expected. However, with regard to the studied seed batches, the red kidney bean flour seems to be the most promising material in this respect, showing a remarkably low rate of amylolysis (Figure 1B).

Previous observations indicated that the slow carbohydrate properties of the red kidney bean powder are negatively modified by reheating under wet conditions. probably due to loss of cell wall integrity (Tovar et al., 1990a). This was confirmed and verified for other processed legume flours in the present work. Results from α -amylolysis of boiled-pepsin-treated samples (Figure 1C) show that the second boiling treatment promotes a further rise in the enzymic susceptibility of starch. However, despite a similar initial rate of hydrolysis of pancreatic amylase, the hydrolysis index after 60 min differs markedly between the various legume preparations, decreasing in the following order: lentils > brown beans = white beans > black beans > red kidney beans (Figure 1C). It is noteworthy that the flour from brown beans cv. Gotland showed a higher degree of hydrolysis than the samples from other brown varieties, indicating that the final digestibility index may differ even among bean varieties of the same pigmentation (Figure 2B). Such differences could reflect unequally labile cell walls as well as possible differences in starch composition and structure, or in the content of antiamylolytic factors.

The effect of additional heating on the susceptibility to amylolysis (Figures 1C and 2B) should be kept in mind when legume flours are incorporated into foods. After the severe boiling/pepsin treatment, starch in red bean flour proved to be considerably slower than in lentils or other bean types (Figures 1C and 2B). The more heat resistant behavior together with the beneficial properties of the flour as such (Figure 1B) points out the precooked red kidney bean flour as the most suitable material for future work oriented to the formulation of diabetic-aimed food items. Reboiled lentil powder, on the other hand, is not different from boiled wheat starch (Figure 1A,C), while black, brown, and white beans rank as intermediate. However, possible differences between batches of the same legume and/or between varieties of the same species must be considered.

Another relevant parameter to be evaluated is the influence of the length of the heat treatment applied to the raw seed on the availability of the final powdered product. Except for a lower yield obtained in the flour from overcooked red kidney beans, the starch content was not affected by the cooking period (Table V). The difference between the red seed samples could be due to a larger generation of retrograded amylose after overcooking. This observation reiterates the importance of studying the influence of cooking conditions on the generation of resistant starch in legumes.

Also important is the observed dependence of the susceptibility to α -amylolytic attack on cooking time. Overcooked red bean and lentil flours were more efficiently hydrolyzed than cooked until soft seed powders (Figure 3A,C). Such differences may be an indication of a more extended cell wall damage occurring during extensive cooking. Undercooking of white beans, on the other hand, resulted in a less available starch preparation. This might be due to incomplete gelatinization, since the hydrolytic behavior of the reboiled flours was almost identical (Figure 3B). These results stress the importance of optimizing cooking time and conditions to achieve a powder that keeps the slow carbohydrate characteristic.

The present study showed that the limited enzymic availability of starch, previously observed in cooked and pulverized red kidney beans (Tovar et al., 1990a), is a general feature for precooked legume flours. Underestimation of starch content as a consequence of the presence of encapsulated starch was observed in most of the legume samples studied, whereas quantitatively important amylose retrogradation and a relatively low rate of starch hydrolysis were common to all of them. However, the rate of amylolysis was affected by physical and chemical treatments to variable extent in different precooked flours.

Apart from the beneficial consequences of their slowly digested carbohydrates, a low total digestibility of starch in legumes may have some physiological implications. In most subjects, malabsorption of a limited amount of starch is probably beneficial. However, it might have undesirable consequences for infants and adults suffering from intestinal discomfort following the consumption of pulses. Encapsulated starch might be incompletely absorbed from the intestine and thus add to the malabsorbed starch and the indigestible oligo- and polysaccharide fractions. It is therefore of interest to evaluate the in vivo digestibility of starch in these materials. Such a study is now in progress.

ACKNOWLEDGMENT

J.T. is on leave of absence from The Universidad Central de Venezuela. Financial support from The Swedish Institute (Stockholm) and The Swedish Nutrition Foundation (Gothenburg) is acknowledged. We are in debt to Dr. Stig Blix and Birgitte Lund (Nordiska Gen Banken, Alnarp) for providing the brown bean cultivars and to The Meat Research Institute (Kävlinge) for lending the texturometer.

LITERATURE CITED

Anderson, I. H.; Levine, A. S.; Levitt, M. D. Incomplete absorption of the carbohydrate in all-purpose wheat flour. N. Engl. J. Med. 1981, 304, 891-892.

- Antunez, P. L.; Sgarbieri, V. C. Influence of time and conditions of storage on technological and nutritional properties of a dry bean (Phaseolus vulgaris, L. variety Rosinha G2). J. Food Sci. 1979, 44, 1703-1706.
- Asp, N.-G.; Björck, I.; Holm, J.; Nyman, M.; Siljeström, M. Enzyme resistant starch fractions and dietary fiber. Scand. J. Gastroenterol., Suppl. 1987, 22, No. 129, 29-32.
- Bhatty, R. S. Composition and Quality of Lentil (Lens culinaris Medik): A review. Can. Inst. Food Sci. Technol. J. 1988, 21, 144-160.
- Björck, I.; Nyman, M.; Pedersen, B.; Siljeström, M.; Asp, N.-G.; Eggum, B. O. On the digestibility of starch in wheat bread -Studies in vitro and in vivo. J. Cereal Sci. 1986, 4, 1-11.
- Björck, I.; Nyman, M.; Pedersen, B.; Siljeström, M.; Asp, N.-G.; Eggum, B. O. Formation of resistant starch during autoclaving of wheat starch: Studies in vitro and in vivo. J. Cereal Sci. 1987, 6, 159-172.
- Bornet, F. R. J.; Fontvielle, A.-M.; Rizkalla, S.; Colonna, P.; Blayo, A.; Mercier, C.; Slama, G. Insulin and glycemic responses in healthy humans to native starches processed in different ways: correlation with in vitro α -amylase hydrolysis. *Am. J. Clin. Nutr.* **1989**, *50*, 315–323.
- Eliasson, A.-C. Physical and chemical characteristics of legume starches. ISI Atlas Sci.: Anim. Plant Sci. 1988, 1, 89–94.
- Englyst, H. N.; Cummings, J. H. Simplified method for the measurement of total non-starch polysaccharides by gasliquid chromatography of constituent sugars as alditol acetates. *Analyst* 1984, 109, 937–942.
- Englyst, H. N.; Cummings, J. H. Digestion of the polysaccharides of some cereals foods in the human intestine. Am. J. Clin. Nutr. 1985, 42, 778-787.
- Gee, J. M.; Johnson, I. T. Rates of starch hydrolysis and changes in viscosity in a range of common foods subjected to simulated digestion in vitro. J. Sci. Food Agric. 1985, 36, 614–620.
- Golay, A.; Coulson, A.; Hollenbeck, C. B.; Kaiser, L. L.; Würsch, P.; Reaven, G. M. Comparison of metabolic effects of white beans processed into two different physical forms. *Diabetes Care* 1986, 9, 260-266.
- Holm, J.; Björck, I.; Asp, N.-G.; Sjöberg, L.-B.; Lundquist, I. Starch availability in vitro and in vivo after flaking, steam-cooking and popping of wheat. J. Cereal Sci. 1985, 3, 193-206.
- Holm, J.; Björck, I.; Drews, A.; Asp, N.-G. A rapid method for the analysis of starch. Starch/Staerke 1986, 38, 224-226.
- Hoover, R.; Sosulski, F. Studies on the functional characteristics and digestibility of starches from Phaseolus vulgaris biotypes. Starch/Staerke 1985, 37, 181-191.
- Jaffé, W. G.; Flores, M. E. Cooking of beans. Arch. Latinoam. Nutr. 1975, 25, 79-90.
- Jenkins, D. J. A.; Wholever, T. M. S.; Taylor, R. H.; Barker, H. M.; Fielden, H. Exceptionally low blood glucose response to dried beans: comparison with other carbohydrate foods. Br. Med. J. 1980, 281, 578-580.
- Jenkins, D. J. A.; Ghafari, H.; Wholever, T. M. S.; Taylor, R. H.; Jenkins, A. L.; Barker, H. M.; Fielden, H.; Bowling, A. C. Relationship between rate of digestion of foods and postprandial glycaemia. *Diabetologia* 1982a, 22, 450-455.
- Jenkins, D. J. A.; Thorne, M. J.; Camelon, K.; Jenkins, A.; Venketeshwer Rao, A.; Taylor, R.; Thompson, L. U.; Kalmnsky, J.; Reichert, R.; Francis, T. Effect of processing on digestibility and the blood glucose response: a study of lentils. Am. J. Clin. Nutr. 1982b, 36, 1093-1101.
- Jenkins, D. J. A.; Jenkins, A. L.; Wholever, T. M. S.; Collier, G. R.; Rao, A. V.; Thompson, L. U. Starch foods and fiber: reduced rate of digestion and improved carbohydrate metabolism. *Scand. J. Gastroenterol.*, Suppl. 1987, 22, No. 129, 132-141.
- Kataria, A.; Chauhan, B. M. Contents and digestibility of carbohydrates of mung beans (Vigna radiata L.) as affected by domestic processing and cooking. *Plant Foods Hum. Nutr.* 1988, 38, 51-59.
- Lowgren, M.; Liener, I. E. The effect of slow-cooking on the trypsin inhibitor and hemaglutinating activities and in vitro digestibility of brown beans (Phaseolus vulgaris, var. Stella) and kidney beans (Phaseolus vulgaris, var. Montcalm). *Qual. Plant. Plant Foods Hum. Nutr.* **1986**, *36*, 147–154.

- McBurney, M. I.; Cuff, D. J.; Thompson, L. U. Rates of fermentation and short chain fatty acid and gas production of six starches by human faecal microbiota. J. Sci. Food Agric. 1990, 50, 79-88.
- Morón, D.; Melito, C.; Tovar, J. Effect of indigestible residue from foodstuffs on trypsin and pancreatic α -amylase activity in vitro. J. Sci. Food Agric. 1989, 47, 171–179.
- Ologhobo, A. D.; Fetuga, B. L. Effects of different processes on the carbohydrates of lima bean. Nahrung 1988, 32, 173-177.
- Paredes-López, O.; Maza-Calvino, E. C.; González-Castaneda, J. Effect of the hardening on some physicochemical properties of common bean. Food Chem. 1989, 31, 225-236.
- Rodriguez, F. M.; Mendoza, E. M. T. Physicochemical basis for hardseedness in mung bean (Vigna radiata (L.) Wilczek). J. Agric. Food Chem. 1990, 38, 29–32.
- Russell, P. L.; Berry, C. S.; Greenwell, P. Characterization of resistant starch from wheat and maize. J. Cereal Sci. 1989, 9, 1-15.
- Salomonsson, A.-C.; Theander, O.; Westerlund, E. Chemical characterization of some swedish cereal whole meal and bran fractions. Swed. J. Agric. Res. 1984, 14, 111-117.
- Savage, G. P.; Deo, S. The nutritional value of mung bean and urd (Vigna radiata var. aureus and var. mungo). Nutr. Abstr. Rev. 1989, 59, 639-662.
- Sievert, D.; Pomeranz, Y. Enzyme-resistant starch. I. Characterization and evaluation by enzymatic, thermoanalytical, and microscopic methods. *Cereal Chem.* 1989, 66, 342–347.
- Siljeström, M.; Björck, I. Digestible and undigestible carbohydrates in autoclaved legumes, potatoes and corn. Food Chem. 1990, in press.
- Siljeström, M.; Björck, I.; Eliasson, A.-C.; Lönner, C.; Nyman, M.; Asp, N.-G. Effect on polysaccharides during baking and storage of bread - in vitro and in vivo studies. *Cereal Chem.* 1988, 65, 1-8.
- Siljeström, M.; Eliasson, A.-C.; Björck, I. Characterization of resistant starch from autoclaved wheat starch. Starch/ Staerke 1989, 41, 147-151.
- Slack, P. T.; Baxter, E. D.; Wainwright, T. Inhibition by hordein of starch degradation. J. Inst. Brew. 1979, 85, 112-114.
- Socorro, M.; Levy-Benshimol, A.; Tovar, J. In vitro digestibility of cereal and legume (Phaseolus vulgaris) starches by bovine, porcine and human pancreatic α -amylases. Effect of dietary fiber. Starch/Staerke 1989, 41, 69–71.
- Sosulski, F.; Waczkowski, W.; Hoover, R. Chemical and enzymatic modifications of the pasting properties of legume starches. *Starch/Staerke* 1989, 41, 135-140.
- Stephen, A.; Haddad, A. C.; Phillips, S. F. Passage of carbohydrate into the colon - Direct measurements in humans. *Gastro*enterology 1983, 85, 589-595.
- Tappy, L.; Würsch, P.; Randin, J. P.; Felber, J. P.; Jéquier, E. Metabolic effect of pre-cooked instant preparations of bean and potato in normal and diabetic subjects. Am. J. Clin. Nutr. 1986, 43, 30-36.
- Tovar, J.; Björck, I.; Asp, N.-G. On the nutritional properties of starch and dietary fiber in cassava bread. Nutr. Rep. Int. 1989, 39, 1237–1246.
- Tovar, J.; Björck, I.; Asp, N.-G. Analytical and nutritional implications of limited enzymic availability of starch in cooked red kidney beans. J. Agric. Food Chem. 1990a, 38, 488-493.
- Tovar, J.; de Francisco, A.; Björck, I.; Asp, N.-G. Relationship between microstructure and in vitro digestibility of starch in precooked legume flours. J. Food Struct. 1990b, submitted for publication.
- Wong, S.; Traianedes, K.; O'Dea, K. Factors affecting the rate of starch hydrolysis in legumes. Am. J. Clin. Nutr. 1985, 42, 38-43.
- Würsch, P.; Del Vedovo, S.; Koellreuter, B. Cell structure and starch nature as key determinant of the digestion rate of starch in legumes. Am. J. Clin. Nutr. 1986, 43, 25-29.

Received for review December 21, 1989. Accepted April 27, 1990.

Registry No. Starch, 9005-25-8; amylose, 9005-82-7.