Analytical and Nutritional Implications of Limited Enzymic Availability of Starch in Cooked Red Kidney Beans

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Measured with an enzymic method, the starch content of a raw red kidney bean (*Phaseolus vulgaris*) flour (RBF) was higher than that of a cooked and blended (CBB) and of a cooked, freeze-dried, and milled (CBF) preparation of the seeds. Wet homogenization as well as pepsin pretreatment of CBF increased the starch yield, indicating that starch in the cooked samples is not completely available to enzymic degradation unless cell wall entrapped granules are released by mechanical or enzymatic disruption of the fibrous walls. Solubilization of resistant starch in CBF with 2 N KOH resulted in a further increase in measured starch, which reached the RBF value. Influence of encapsulated and resistant starch fractions on dietary fiber values was also noticed. CBF showed remarkably low values of in vitro amylolysis rate and starch digestibility index in a digestion/dialysis system, features that seemed to depend also on the integrity of cell walls.

Legume seeds are an important component of the human diet in many countries (Jaffé, 1970). Their protein content and nutritional value have been profusely investigated (Liener, 1979; Nielsen, 1988). Studies on legume starches are, however, less abundant. It is well-known that the glycemic response to pulses is generally lower than to other starchy foods (Jenkins et al., 1980; Shaheen and Fleming, 1987). Because of this, during the last years legumes have received great attention concerning both availability, i.e. rate of hydrolysis, and total digestibility of their starch (Fleming and Vose, 1979; Jenkins et al., 1982a; Würsch et al., 1986; Fleming et al., 1988; Socorro et al., 1989). However, the results from these research efforts, coming from different laboratories, have led to a number of inconsistencies. As an example of this, Jenkins et al. (1982b) reported that the in vitro rate of starch hydrolysis in processed legumes shows good correlation with the glycemic response, whereas O'Dea and Wong (1983) did not find such a relationship.

In the present investigation, after a freeze-dried powder was prepared from cooked red kidney beans, a remarkably lower starch content was noticed as compared to that measured in the raw material. Such an observation prompted us to assess the possibility of obtaining inaccurate values for starch and dietary fiber content in cooked bean samples by employing common enzymic methods. This could partially explain some of the controversial data reported in the literature dealing with the rate and extent of legume starch digestibility. In addition, interesting in vitro digestibility features of the precooked bean powder were also recorded.

MATERIALS AND METHODS

Processing of Beans. Red kidney beans (*Phaseolus vulgaris* L.) obtained from the local market were soaked for 20 min in twice their weight of water. The soaked seeds were then cooked by boiling in water until soft as felt between fingers (60–70 min), employing a seed to water ratio of 1:3 (w/v). The relatively short cooking time required ruled out the possible occurrence of the "hard-to-cook" phenomenon (Antunes and Sgarbieri, 1979), which is frequently observed in aged beans. The softened seeds along with cooking water were freeze-dried and ground to pass a 1-mm screen in a Cyclotec 1093 mill (Tecator AB, Höganäs). The cooked–freeze-dried flour was kept in a desiccator until use. This preparation is referred to as cooked and freeze-dried flour (CBF).

A freshly cooked bean preparation was also studied, after the seeds and cooking water were blended in a mortar with pestle (cooked and blended beans) (CBB).

Table I. Treatments Applied to Red Kidney Beans before Measuring Starch Content

	additional treatment			
init treatment	homogenizn[a]	homogenizn + boiling[b]	pepsin[c]	KOH[d]
dry-milling	×	×	×	×
cooking[e] and blending	×			
cooking,[e] freeze-drying, and milling	×	×	×	×

[a] Wet homogenization (two, five, or ten 1-min pulses). [b] Wet homogenization (five 1-min pulses) and boiling for 20 min. [c] 200 mg of enzyme/g of sample, 1 h, 37 °C, pH 1.5. [d] 2 N, 30 min, room temperature. [e] Boiled for 60–70 min.

For comparative purposes, a bean flour was prepared by dry-milling of raw seeds in the above-mentioned equipment (raw flour) (RBF).

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

To assess the influence of sample handling on the starch yield, various treatments were applied before the analysis (see Table I):

(*a*) *Homogenization.* The samples were suspended in water and submitted to several 1-min pulses at maximal strength with a Polytron blender (Kinematica GmbH, Luzern).

(*b*) *Pepsin Incubation.* A 100-mg portion of pepsin (2000 FIP-U/g; Merck, Darmstadt) was added to 500 mg of sample; incubation was performed for 1 h at 37 °C and pH 1.5.

(*c*) *Solubilization with KOH.* A 500-mg sample was 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 neutralized (pH 6.5–7) with 5 N HCl.

Blanks of treated samples, in which the starch-degrading enzymes were omitted, were run in every case.

Dietary Fiber Determination. Total dietary fiber was analyzed gravimetrically after enzymic digestion of the sample, according to Asp et al. (1983). Fiber values were calculated after correction for ash and protein (N × 6.25) content of the residues.

Resistant and Residual Starch Evaluation. The determination was performed as described by Siljeström et al. (1988). The dietary fiber residues were solubilized for 30 min at room

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Table II. Treatments Applied to Red Kidney Beans Prior to the Determination of the Rate of Starch Hydrolysis in Vitro

	additional treatment		
init treatment	homogenizn[a]	heating[b]	pepsin[c]
cooking,[d] freeze-drying, and milling	×	×	×

[a] Wet homogenization (five 1-min pulses). [b] 20 min at 40 °C, 60 °C, or boiling. [c] See Table I. [d] See Table I.

temperature in 2 N KOH, neutralized, and analyzed for total starch with amyloglucosidase. Residual starch was measured after the fiber residues were suspended in distilled water followed by incubation with amyloglucosidase. Resistant starch was then calculated as total starch less residual starch and expressed as polysaccharide (glucose × 0.9) on sample dry weight basis.

The precision of these analyses was evaluated according to

$$s = \left[\frac{1}{2k}\sum_{i=1}^{k}(X_{i1} - X_{i2})^2\right]^{1/2}$$

where s = standard deviation, k = number of samples (two analyses per sample), and X_{i1} and X_{i2} = duplicate values for sample i.

s values obtained for a large number of samples analyzed in this laboratory were 0.025 for residual starch and 0.067 for resistant starch. This means that 0.1% (i.e., 2s) residual starch or higher and 0.2% resistant starch (dry weight basis) or higher are significantly different from zero.

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. In some experiments, a preincubation with pepsin was performed as described under Starch Determination. The effect of wet homogenization for 5 min and of heating (40 °C, 60 °C, or boiling) a suspension of the samples for 20 min before the amylolysis experiment was also investigated (see Table II). Starch content calculations for cooked bean samples were done on the basis of values obtained after the homogenization treatment. Boiled samples of wheat starch were assayed as a reference.

Dialysis Experiments. The rate of reducing sugars release from starch in a digestion/dialysis system was evaluated in the cooked–freeze-dried bean powder and in a boiled wheat starch reference, essentially after Tovar et al. (1989). A 10-mL portion of a 2.5% (w/v, starch basis) suspension of the sample was gently mixed with 280 μL (65 μg/mL) of pancreatic α-amylase solution, and 5 mL of the mixture was transferred into a dialysis tube (250-7U, cutoff 12 000, Sigma). The tube was placed in a bottle containing 130 mL of 0.05 M sodium/potassium phosphate buffer at 37 °C (pH 6.9) and magnetically stirred. The dialysis buffer was assayed for reducing power by the 3,5-dinitrosalicylic acid method (Hostettler et al., 1951) after 1, 2, and 3 h of incubation, and the degree of starch hydrolysis was calculated as the percentage (maltose equivalents) of the starch appearing in the dialysate. Wet homogenization, heating, and pepsin effects were studied after the sample was treated in the same way as indicated above (see Table II).

Statistics. Means were compared by the Student's t-test.

RESULTS

Gross and Microscopic Appearance. Cooking and freeze-drying of red kidney beans resulted in a very soft material that could be easily pulverized by hand-pressing. The powder obtained by milling this sample was observed under light microscope, showing starch granules mainly encapsulated by cell walls. This picture contrasts to the one obtained from the raw flour, which was rich in free and birefringent starch granules. Wet homogenization of the cooked–freeze-dried flour liberated starch granules from the cell structures, whereas boiling did not produce any obvious difference (results not shown).

Table III. Effect of Several Treatments on the Apparent Starch Content of Red Kidney Bean Samples

treatment	starch content,[a] %
Raw Flour	
none	38.7 (0.8)[d,e]
homogenization	38.9 (0.5)[d,e]
homogenization and boiling	38.3 (0.4)[d]
pepsin[b]	38.8 (0.9)[d,e]
2 N KOH[c]	39.7 (0.5)[e]
Cooked and Freeze-Dried	
none	33.2 (0.7)[f]
homogenization	36.4 (1.0)[g]
homogenization and boiling	36.2 (1.1)[g]
pepsin[b]	36.0 (0.8)[g]
2 N KOH[c]	40.3 (1.2)[e]
Cooked and Blended	
none	15.4 (1.4)[h]
homogenization	34.9 (1.5)[g]

[a] Dry matter basis. [b] 100 mg of enzyme, 1 h at 37 °C. [c] 30 min at room temperature. (d–g) Values are the means of a minimum of six assays. SD is indicated in parentheses. All the treatments were statistically compared to each other. Means without common superscript letters are significantly different ($p < 0.05$).

Table IV. Total Dietary Fiber Content of Red Kidney Bean Samples

	dietary fiber,[a,b] %	resid starch,[a] %	resistant starch,[a] %
raw flour	18.9 (0.8)[c]	0.2	0.6
cooked and freeze-dried	22.8 (1.2)[d]	0.7	2.8
cooked and blended	24.6 (0.9)[d]	0.9	3.3

[a] Values are referred to the original sample on a dry matter basis. [b] Corrected for protein and ash. (c, d) Numbers in parentheses indicate the standard deviation of the mean; $n = 6$. Dietary fiber values were statistically compared. Means without common superscript letters are significantly different ($p < 0.01$).

Starch Content. The impact of various treatments on the starch content of raw and cooked red kidney beans is summarized in Table III. A value of 39% was estimated for the untreated raw flour. Similar yields were obtained after homogenization, pepsin preincubation, or KOH solubilization of the sample. The cooked and freeze-dried material had a considerable lower content (33%). The yield was increased to 36% by homogenization, either by itself or in combination with boiling, as well as by pepsin preincubation. A further increase in the starch yield was observed after 2 N KOH treatment of the sample, reaching the same value as that in the alkali-treated raw flour (40%). Neither higher alkali concentrations nor longer incubation periods changed the estimated starch content. An extremely low starch value was found for cooked–blended beans (15%). Homogenization of this sample resulted in a starch yield similar to that measured in the freeze-dried flour after homogenization.

Dietary Fiber Content. As indicated in Table IV, both the blended and freeze-dried cooked bean samples showed higher total (soluble plus insoluble) dietary fiber content than the raw ones. This difference can be explained by the important quantities of starch present in the fiber residues obtained from cooked beans. Most of the remaining starch (2.8–3.3% dwb; dwb = dry weight basis) was "resistant starch", that is, starch unavailable to amylases unless solubilized in alkali. A small amount of resistant starch (0.6% of the flour dry weight) was found also in the raw material.

Homogenization of the cooked–freeze-dried sample did not change its dietary fiber content (Table V). Strong alkali preincubation, however, resulted in a lower fiber value, which was similar to that obtained in the raw flour.

Table V. Effect of Homogenization and Alkali Treatment on the Total Dietary Fiber Content of Cooked–Freeze-Dried Beans

treatment	dietary fiber,[a,b] %	resid starch,[a] %	resistant starch,[a] %
none	22.8 (1.2)[d]	0.7	2.8
homogenization	22.4 (0.4)[d]	0.7	2.3
homogenization plus 2 N KOH[c]	18.3 (0.8)[e]	0.3	0.1

[a] Values are referred to the original sample on a dry matter basis. [b] Corrected for protein and ash. [c] 30 min at room temperature. (d, e) Numbers in parentheses indicate the standard deviation of the mean; $n = 6$. Dietary fiber values were statistically compared. Means without common superscript letters are significantly different ($p < 0.01$).

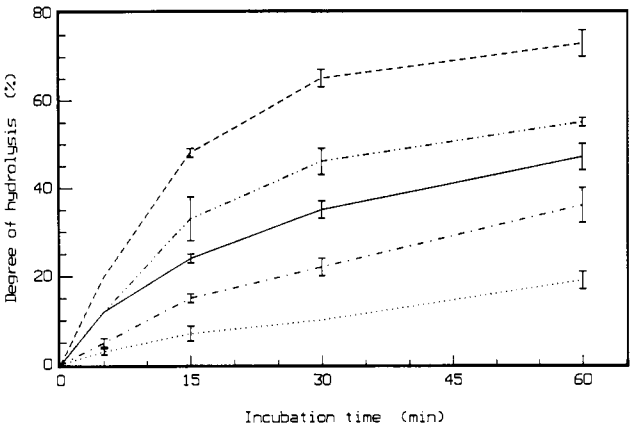

Figure 1. Starch hydrolysis of cooked–freeze-dried bean flour by pancreatic α-amylase: ···, control; – · –, pepsin treated; —, boiled; – ·· –, boiled and pepsin treated; - - -, boiled wheat starch. Bars indicate the standard deviation of the mean; $n = 6$.

Only a small amount of remaining resistant starch was detected in the KOH-treated material.

Starch Availability in Vitro. Figure 1 shows the results from the evaluation of the rate of amylolysis of the cooked–freeze-dried bean samples with various additional treatments, as compared to a boiled wheat starch reference. All the cooked–freeze-dried materials were digested at a significantly lower rate than the boiled wheat starch reference. The most slowly digested sample was the untreated freeze-dried flour. Pepsin treatment enhanced the susceptibility to amylolytic attack. Boiling and boiling plus pepsin treatments resulted in a further increase in the rate of hydrolysis.

The effect of wet homogenization and heating on the starch availability of the cooked–freeze-dried flour is summarized in Table VI. Homogenization produced a marked increase in the degree of starch hydrolysis. A 5-min treatment resulted in a higher value than that of the boiled sample and similar to that observed after boiling plus pepsin treatment. Heating at 60 °C for 20 min promoted a 1.6-fold increase of the rate of hydrolysis, even at a high (6%) starch concentration.

The index of hydrolysis of the cooked–freeze-dried beans was also low in a digestion–dialysis system (Figure 2), showing a degree of hydrolysis of 22% after 3 h as compared to 57% with boiled wheat starch. Under these experimental conditions, all treatments tested resulted in an equivalent (1.8-fold) increase in the rate of appearance of reducing sugars in the dialysis buffer.

DISCUSSION

Enzymic methods for evaluation of the starch content of foods have gained in popularity during the last years

Table VI. Effect of Various Treatments on the Availability of Starch in Cooked–Freeze-Dried Beans

treatment	deg of hydrolysis[a]
none	20
homogenization (2 min)	40
homogenization (5 min)	54
homogenization (10 min)	54
heating at 40 °C (20 min, 3% w/v[b])	22
heating at 60 °C (20 min, 3% w/v[b])	34
heating at 60 °C (20 min, 6% w/v[b])	32
boiling (20 min, 3% w/v[b])	44

[a] 100 × milligrams of starch digested (maltose equivalents × 0.95)/milligrams of starch incubated with pancreatic amylase for 1 h at 37 °C. [b] Analyzed starch, weight basis. Values are the means of four determinations.

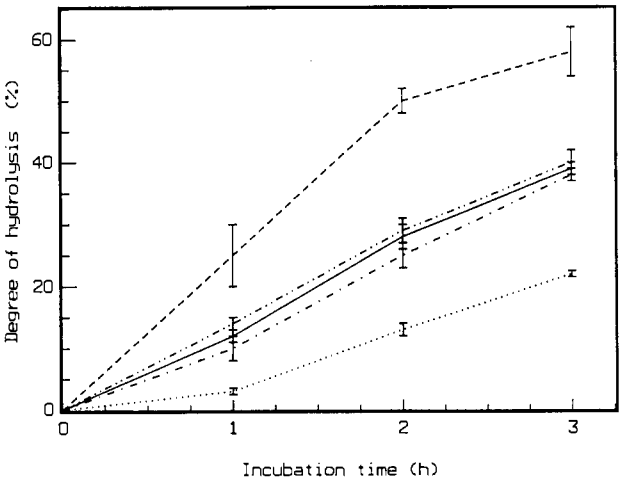

Figure 2. Starch digestibility of cooked–freeze-dried bean flour in a dialysis system: ···, control; – · –, pepsin treated; —, boiled; – ·· –, boiled and pepsin treated; - - -, boiled wheat starch. Bars indicate the standard deviation of the mean; $n = 6$.

(AOAC, 1984 (Method 14.073); Holm et al., 1986; Theander and Westerlund, 1986). The results reported here indicate that these methods require certain modifications when true starch content is measured in cooked beans. Hence, depending on sample handling and/or analytical modifications, the starch yield ranged from 15 to 40% (dwb). Mechanical disruption as well as proteolytic or alkali treatment of the samples prior to the analytical procedure had a considerable impact on starch recovery (Table III). However, these treatments did not modify the starch yield in the raw flour. Therefore, no modification of common enzymic assays seems to be necessary for the analysis of true starch content in raw beans.

A prominent increase in starch yield was observed following homogenization of the cooked–freeze-dried and the cooked–blended seeds (Table III). In both cases, homogenization resulted in a starch value that came closer to that of the raw flour. Microscopy studies have shown that after cooked *Phaseolus vulgaris* seeds were gently blended, most of the starch appeared gelatinized but remained encapsulated within rather intact cell wall structures (Würsch et al., 1986; Fleming et al., 1988). This finding was confirmed in the present work. Furthermore, milling of the freeze-dried samples resulted in a flour that also retained intact cell structures. This contrasts with the effect of dry-milling of raw beans, a procedure that liberates starch granules (Golay et al., 1986; Fleming et al., 1988). Obviously, the starch in cooked–blended and cooked–freeze-dried beans is not com-

pletely available to the amylolytic enzymes used in the starch assay, unless the cell wall structures are previously disrupted, for instance by a strong homogenization step.

A pepsin preincubation of the freeze-dried flour also increased the starch yield (Table III). Thus, it seems that some alterations of the protein/fiber associations occurring in the cell walls are required and a complete disruption of the cell structures is not necessary. Considering that in vivo ingested materials are always exposed to pepsin digestion, this observation may also indicate that, even if underestimated by the routine enzymic starch analysis, the cooked beans have a potentially available starch content of about 36%.

Another interesting finding was the increase in starch yield observed after incubation of the cooked–freeze-dried material with 2 N KOH (Table III). It is important to mention that the alkali incubation itself was not able to liberate glucose, suggesting that the treatment rendered available some previously indigestible starch. It is well documented that heat treatment of starch and starchy foods may generate resistant starch, i.e., starch fractions that are not available to amylolytic enzymes unless a KOH or dimethyl sulfoxide (DMSO) solubilization step is included (Englyst and Cummings, 1984; Siljeström et al., 1988; Russel et al., 1989). This type of starch, which has been mainly studied in cereals, is believed to consist of retrograded amylose (Russel et al., 1989; Siljeström et al., 1989). Legume starches have a relatively high amylose/amylopectin ratio (Hoover and Sosulski, 1985; Eliasson, 1988). Hence, the 4% difference observed between the homogenized and the KOH-treated cooked–freeze-dried flour seems to account for the resistant starch generated during processing.

This 4% resistant starch content in cooked beans is considerably higher than that formed during baking of wheat bread, which is about 1% (Björck et al., 1986). Freeze-drying has only a marginal effect on formation of resistant starch, as judged from studies with wheat products (Björck et al., 1987). Similarly, the presence of resistant starch in cooked–freeze-dried beans should not be attributed to the postcooking manipulation of the sample, since the cooked–blended material had a similar content of resistant starch (Table IV). It can be concluded that the total starch content, including resistant starch, in both the raw and cooked beans is 40%.

Resistant starch has been shown to be physiologically indigestible, thus behaving similarly to dietary fiber components (Björck et al., 1986, 1987). Because of this, it is important to discriminate between total and available starch content of foods. The methods discussed above provide the possibility of obtaining separate figures for potentially available starch and indigestible starch in cooked beans by simple enzymic analyses. KOH treatment appears to solubilize encapsulated as well as resistant (retrograded) starch, whereas homogenization or pepsin treatment mainly affects recovery of encapsulated starch.

Whether the impact of sample handling on the starch yield of red kidney bean is generalizable to other legumes remains to be elucidated. However, it is possible to anticipate discrepancies in bean starch values as reported by different laboratories, since no universal procedure for the treatment of cooked samples has been settled. Comparisons between raw and processed bean composition could also be influenced by these phenomena. For instance, both cell wall encapsulated starch and the appearance of resistant starch may partially explain the decrease in analyzed starch observed after cooking of mung beans (Kataria and Chauhan, 1988) and lima beans (Ologhobo and Fetuga, 1988). Measurement of the degree of starch gelatinization in this type of materials by enzymatic procedures (Chiang and Johnson, 1977) may also lead to inaccurate figures.

Underestimation of starch content may also have consequences regarding evaluation of nutritional properties. Thus, it leads to a concomitant overestimation of the degree of starch hydrolysis, an important parameter frequently considered in starch digestibility investigation. Similarly, errors in analyzed starch content may invalidate conclusions regarding postprandial glucose and insulin responses to leguminous food items.

An efficient starch removal is a critical step during dietary fiber analysis (Asp and Johanson, 1984). Consequently, the presence of encapsulated and resistant starch influenced the results during the estimation of dietary fiber content in cooked beans. Both the blended and the freeze-dried flour showed significantly higher total fiber values than the raw beans (Table IV).

As observed with black beans and other legume seeds (Schinagel and Tovar, 1987), residual starch in the raw bean fiber residue is very low (Table IV), indicating that the combined action of Termamyl and pancreatic amylase (pancreatin) removed most of the available starch in these materials. However, the resistant starch content of this raw flour is relatively high (0.6%) and comparable to that observed in wheat bread crust (Björck et al., 1986). The true nature of the resistant starch detected in this sample should be investigated. It is possible that a minor starch fraction, different from retrograded amylose, may require alkali treatment for complete solubilization. Such a feature might be displayed, for instance, by highly crystalline regions in the starch granules.

The cooked samples show a 3–4 times higher content of residual starch and, as expected, more important quantities of resistant starch (2.8–3.3%) (Table IV). Correction of fiber values for the fiber-associated starch (residual plus resistant) results in only a small difference between raw and cooked beans. The contribution of resistant starch to the high fiber level obtained with cooked samples is stressed by comparing the freeze-dried flour to the KOH-pretreated one (Table V). After alkali solubilization, 97% of the resistant starch fraction disappeared and the fiber value approached that reported for the raw flour.

It is noteworthy that even if the starch content of the cooked–freeze-dried flour is lower than that observed after the homogenization treatment (Table III), the total fiber values do not differ (Table V). Since the dietary fiber procedure includes pepsin, encapsulated starch will probably be released, thus resulting in similar dietary fiber values.

The cooked–freeze-dried flour described here retains a nutritionally important feature of whole beans, that is a low in vitro rate of digestion by pancreatic amylase (Table VI; Figure 1). Cooked–blended white beans and drum-dried flakes prepared from the same seeds have also been shown to be slowly digested in vitro (Würsch et al., 1986) and to produce a moderate glycaemic response in human patients (Golay et al., 1986; Tappy et al., 1986; Würsch et al., 1988). One important reason for these properties is the entrapment of bean starch in fibrous thick-walled cells (Würsch et al., 1986). It was observed during the present work that the digestibility of the cooked–dried powder is importantly increased by prolonged homogenization (Table VI). Therefore, the presence of "entrapped" rather than nongelatinized starch seems to

be one of the mechanisms controlling the low digestion rate also in this sample.

It would be of interest to include the precooked bean flour in the preparation of special foods to improve the dietary management of diabetics. Some experiments were therefore run in order to assess the stability of the "slow" feature. Boiling of the freeze-dried flour resulted in a higher rate of amylolysis, as reflected by a 2.2-fold increase in the percentage of hydrolysis after 1 h of digestion (Figure 1; Table VI). Such an observation suggests that additional heat processing may affect the integrity of the cell wall layers, although no evident difference could be detected by observation of the boiled material under the microscope. The digestibility begins to increase after 20 min of incubation at 50–60 °C, and it was not possible to prevent these changes by manipulation of the starch to water proportion (Table VI). As expected from the starch measurements results, preincubation of the sample with pepsin also increased the susceptibility to α-amylolytic attack (Figure 1).

Nevertheless, even if increased by homogenization, heating, or pepsin incubation, the digestibility rate of the cooked bean powder was always significantly lower than that observed for boiled wheat starch. This may be explained by the intrinsically low susceptibility to digestion by pancreatic amylase exhibited by *P. vulgaris* starch (Socorro et al., 1989) as well as by the interference of polyphenols (Singh, 1984; Björck and Nyman, 1987) and fiber (Morón et al., 1989) with α-amylase activity in vitro.

Results from the amylolysis rate in the dialysis model deserve special attention. Contrasting with the observations made with the regular amylolysis assay, boiling and/or pepsin predigestion produced the same increase in the rate of appearance of reducing sugars in the external medium (Figure 2). This indicates that the increase in rate of digestion does not run in parallel with the "absorptive rate", simulated here by diffusion through the dialysis membrane. Considering that the average increase under such conditions (1.8-fold) is lower than that observed in the common procedure, the diffusion rate appears to be the limiting step for the release of starch digestion products (maltodextrins) from the dialysis tubing. Viscous fiber components present in these pulses may be responsible for the decrease in the diffusion rate, as reported for other dietary fibers in this type of system (Chonchol and Tovar, 1988; Tovar et al., 1989). This behavior of the cooked bean powder is noteworthy since results obtained with the dialysis model have shown good correlation with in vivo glycemic responses (Jenkins et al., 1984).

In conclusion, the present study shows that enzymic methods for starch analysis in cooked red kidney beans may lead to significant underestimations of the content unless an appropriate treatment of the sample is done prior to the assay. Efficient wet homogenization or preincubation with pepsin seems to be enough for the quantification of potentially available starch. However, solubilization under strongly alkaline conditions is necessary for determination of total starch content. It was also established that, due mainly to the presence of resistant starch, the dietary fiber content in cooked beans is higher than in raw seeds. The validity of these findings, which are of profound analytical importance, should be evaluated also in other legume seeds.

In addition, present data suggest that starch in the precooked red kidney bean flour is hydrolyzed at a significantly lower rate than gelatinized wheat starch. Furthermore, the gelling properties of its dietary fiber components appear to reduce the diffusion rate of maltodextrins in vitro. These nutritionally interesting properties of the processed flour suggest possibilities for its use in the formulation of foods for diabetics or patients with hyperlipidemia. The assessment of these possibilities as well as a detailed study of the cotyledonary cell wall changes associated with bean processing are now in progress. The possible influence of sample handling on total starch digestibility in vivo will be also evaluated.

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A *Heliothis zea* Antifeedant from the Abundant Birchbark Triterpene Betulin

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Four triterpenoids bearing α,β-unsaturated A-ring functionality have been prepared from betulin, an abundant and readily isolated triterpene found in the bark of birch (*Betula spp.*). These compounds were evaluated as antifeedants in laboratory leaf-disk choice assays against bollworm larvae, *Heliothis zea*. One triterpenoid, 19β,28-epoxy-2-(β-D-glucopyranosyloxy)-18α-olean-1-en-3-one (VIII), was found to display high antifeedant activity.

The presence of defensive phytochemicals confers upon many species of plants a degree of protection against insect herbivores. These allelochemicals may act as insecticides, repellents, growth regulators, or antifeedants. There is thus considerable interest in application of these compounds, or related synthetic models, as components of integrated pest management (IPM) programs for protection of agricultural crops (Cutler, 1988; Whitehead and Bowers, 1983). Antifeedants, or feeding deterrents, are often highly specific in their action, an advantage in IPM

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