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Composition and Digestibility of Albumin, Globulins, and Glutelins from *Phaseolus vulgaris*

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Fifteen Brazilian varieties of *Phaseolus vulgaris* were tested for digestibility in vitro, trypsin inhibitor, and protein content. Four varieties with extreme digestibility values were assayed in rats and showed similar digestibilities in vivo. The protein from the Carioca variety fractionated for detailed studies yielded the following: albumin, 31.5% (richest in sulfur amino acids and trypsin inhibitors); globulin G_1, 38.5%; globulin G_2, 13.8%; glutelin, 22.4%. The in vitro digestibilities of the unheated globulins and glutelins were low but improved by heating. The albumins were well digested in the raw state but after heating digestibility dropped; the effect was pH dependent. The residue left after digestion of autoclaved albumin contained peptides with molecular weights of 14 000 and 20 000. Evidences on a relatively heat stable trypsin inhibitor in the albumin fraction are presented. The extent of digestion of the four fractions was tested by using either trypsin, pancreatin, or pepsin–pancreatin.

Beans (*Phaseolus vulgaris*) are an important source of protein in Brazil where they are usually consumed together with rice. However, several biochemical problems limit the optimal biological utilization of the amino acids (Kakade, 1974; Bressani and Elias, 1979).

The low digestibility of bean protein has been documented, but the reasons for it are not well understood and are probably due to a combination of factors. Improper storage at high relative humidities is known to increase cooking time and to reduce the protein digestibility and the biological utilization of bean amino acids (Molina et al., 1975; Antunes and Sgarbieri, 1979). Excessive heating to inactivate antinutritional factors is also prejudicial to the digestibility and amino acid availability; processes such as dry roasting and extrusion cooking are better for maintaining the biological value of beans (Molina et al., 1975; Yadav and Liener, 1978). Antiphysiological factors such as hemagglutinins and trypsin inhibitors are inactivated by proper heat treatment and can probably be excluded as a cause. The exception may be the heat-stable protease (enzyme) inhibitors which seem to be phenolic in nature and present in the seed coat of some colored beans (Elias et al., 1979).

Protein-complexing substances such as tannins appear to be partly implicated. They can either be extracted in the cooking water or migrate to the center of the cotyledon, thus reducing digestibility directly by reacting with the proteins or indirectly by inactivating digestive enzymes. The low biological value of the cooking broth has been attributed to the influence of these phenolic pigments (Elias et al., 1979; Mondragon and Gonzales, 1978).

Litte is known about the influence of the protein itself. Seidl et al. (1969) isolated a globulin from kidney beans which was resistant even after heating to digestion by 10 different proteolytic enzymes, and Romero and Ryan (1978) also observed low digestibility of an isolated G_1 globulin when compared to that of denaturated bovine albumin. The beneficial effect of denaturation was also recently observed by Liener and Thompson (1980), who studied the digestibility of the G_1 fraction both in vitro and with rats. Evans et al. (1974) and Sgarbieri et al. (1979) observed reduced availability of the sulfur amino acids in rats fed autoclaved beans. Evans and Bauer (1978) also indicated the existence of a dialyzable toxic compound in the soluble fraction of the cooking broth.

This paper reports research on the composition and digestibility behavior of different bean protein fractions and the effect of heat on them. Results indicating the existence of a heat-stable trypsin inhibitor are also reported.

EXPERIMENTAL SECTION

Materials. Beans (*P. vulgaris*) of different varieties were obtained from the Agronomy School of Lavras. Bovine trypsin twice crystallized (Type III; 10 000 BAEE units/mg), pepsin of hog stomach (twice crystallized; 2500 units/mg), pancreatin from hog pancreas (Grade VI), and Pronase (Type VI) were purchased from Sigma Chemical Co. All other compounds were reagent grade. Deionized

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and glass-distilled water was used.

Methods. *Extraction and Fractionations of the Proteins.* The whole beans ground to a flour in a laboratory mill to pass a 0.297-mm screen were fractionated as described below. So that the albumins could be obtained, a 20% suspension of the flour in water was extracted for 1 h under agitation at room temperature, filtered through a cheesecloth, and centrifuged at 30000*g* for 30 min. The supernatant was dialyzed for 48 h against distilled water, followed by a 24-h dialysis against deionized water at 4 °C. This last step eliminated most of the contaminating globulins.

The globulins were extracted and separated essentially as described by McLeester et al. (1973). Ten grams of the flour was extracted with 100 mL of a 0.5 N NaCl–0.25 M ascorbic acid solution (pH 2.2) for 1 h at room temperature. The supernatant obtained after centrifugation of the extract (23000*g*; 30 min) was diluted 5 times with deionized water to reduce the ionic strength to 0.08 M and to precipitate the G_1 globulin which was then collected by centrifugation at 23000*g* for 30 min at 0–4 °C. The supernatant left was again dialyzed at 0–4 °C for 24 h to precipitate the G_2 fraction which was collected in the same way.

The glutelin and prolamin were obtained from the residue left after the NaCl–ascorbic acid solution extraction. Ten grams of the residue (dry weight) was extracted with 50 mL of 70% ethanol to separate the prolamin. The residue was reextracted twice with 0.1 N NaOH for 1 h at room temperature. The extracts were centrifuged at 23000*g*, and the combined supernatants, dialyzed and freeze-dried, represented the glutelin fraction. For the preparation of the glutelin, beans were used which had the seed coat previously removed: the seeds were soaked for 8 h in water (1:1.5 beans to water ratio) to facilitate the operation and then were dried at 45 °C in a circulating air oven and ground to a flour as previously described.

Digestibility Tests. Digestibility in vitro was studied by using trypsin, pancreatin, or pepsin–pancreatin according to the experiment.

For trypsin or pancreatin hydrolysis, a dispersion of the proteins under study containing 5 mg of protein/mL in 0.05 M phosphate buffer (pH 7.0) was incubated at 37 °C with a solution of the enzyme in 10^{-3} M HCl. The enzyme to substrate ratio was maintained at 1:40. At different incubation intervals, aliquots where taken, Cl_3AcOH (5% final concentration) was added, and after standing 1 h, the undigested material that precipitated out was separated at 23000*g* for 30 min.

Split peptide links were evaluated in the supernatant by measuring the α-amino nitrogen produced by the ninhidrin reaction according to Spies (1957) and using leucine as the standard.

For the pepsin–pancreatin sequential hydrolysis, the Akeson and Stahmann (1964) technique was used with a 2 mg/mL protein concentration and an enzyme to substrate ratio of 1:40. The digestion time for the pepsin (first enzyme) was 3 h, followed by the pancreatin digestion for 3 h more. Liberation of the α-amino nitrogen was evaluated as described above. Preliminary testing indicated that more prolonged digestion time did not significantly enhance the amount hydrolyzed.

Digestibility was expressed as milligrams of leucine produced per gram of protein and was compared to Hammerstein soluble casein which was used as a control.

The screening test for digestibility of the different bean varieties (Table I) was done on beans previously heated in water (2:10 bean to water ratio) for 30 min (time needed for softening). The resulting material, homogenized with the cooking broth and properly diluted, was digested as described above.

When digestibility of unheated proteins was tested, the trypsin inhibitor was previously complexed by titration with trypsin, and after 10 min to allow complete coupling, the hydrolysis was started by adding the enzyme solution.

Digestibility in vivo was evaluated in young rats fed twice daily a diet containing 10% of the protein under study following a restriction technique used by Kakade and Evans (1966). The feces were collected each day over a period of 5 days, pooled, and analyzed for N by the micro-Kjeldahl technique.

Analytical Techniques. Protein concentration was determined by either the micro-Kjeldahl (AOAC, 1970) or Lowry (Lowry et al., 1951) procedures.

Trypsin inhibitor activity was measured according to Kakade et al. (1969b); the protein-bound sugars were measured by the phenol–sulfuric acid method of Dubois et al. (1956) after precipitation of the protein with 10% Cl_3AcOH. Electrophoreses in polyacrylamide gel were run according to Davies (1964); both acrylamide and bis(acrylamide) were recrystallized (Schuster, 1971). Monomer concentration varied from 5 to 10% according to the experiment. The runs were conducted at 3 mA/tube with Tris–glycine buffer (pH 8.3), using bromphenol blue as the front marker, and the protein bands were visualized with Coomassie brilliant blue. Molecular weight distribution of proteins before and after digestion was estimated by electrophoresis in 1% $NaDodSO_4$ (Weber and Osborn, 1969). The run was at 7 mA/tube, and cytochrome *c* (M_r = 12500), trypsin (M_r = 23300), aldolase (M_r = 40000), ovalbumin (M_r = 45000), and bovine serum albumin (M_r = 67000) were used as standards.

Amino acid analysis was done in a Beckman 120 °C analyzer with the standard resin (Moore and Stein, 1963). Sulfur amino acid was determined as cysteic acid and methionine sulfone by oxidation of the samples with performic acids before acid hydrolysis. For tryptophan determination, the Spies and Chambers (1949) technique was used.

RESULTS AND DISCUSSION

Protein Contents and Digestibility of Different Bean Varieties. Table I provides the results of the initial screening tests of 15 Brazilian varieties of *P. vulgaris* for total and soluble protein contents, in vitro digestibility by pepsin–pancreatin, and trypsin inhibitor (TI) activity. These results are in agreement with previous published data (Moraes and Angelucci, 1971; Sgarbieri et al., 1979). The Kjeldahl measurement of total protein ($N \times 6.25$) ranged from 17.4 to 27.4% while the salt-soluble extractable protein was lower, reaching in one case (Pintado variety) only 62% of the total measured protein. It is interesting to note that heating decreased the solubility of the salt-soluble fraction by different amounts, depending on the variety of bean. The use of polyvinylpyrrolidone, a phenol-complexing agent, or the previous removal of the pigmented seed coat did not change the yield of extraction or lability to heat.

The digestibility recorded varied from 17 to 40% of the maximum theoretically possible obtained after acid hydrolysis, (with the variety exhibiting the most digestion (Rosinha G-2) reaching only 50% of the value of casein (casein digestion was ~82% of the theoretical). Also worth noting is the large variation in TI content, ranging from 4.4 to 13.9 g/kg of bean.

No correlation could be established between digestibility and soluble proteins or trypsin inhibitor content. However,

Table I. Total and Salt-Soluble Protein Content, Percent Digestion by Pepsin-Pancreatin, and Trypsin Inhibitor Activity in Brazilian Varieties of *P. vulgaris*

variety	protein, % total	protein, % soluble[a] unheated	protein, % soluble[a] heated	digestibility,[b] %	TI, g/kg
Venezuela 350	21.1	15.3	13.6	28	8.6
Rico 23	27.4	19.9	12.6	31	7.4
Manteigão fosco	21.5	20.0	11.7	26	4.4
Rico pardo	20.7	16.6	10.0	25	13.9
Jalo	19.8	17.3	12.6	27	12.0
Lavras	17.8	15.9	11.0	19	12.2
Carioca	17.6	16.3	10.0	21	6.8
Pintado	20.6	12.9	8.9	17	10.0
Paraná	20.4	15.2	11.0	23	9.0
Rosinha G-2	20.3	18.8	11.0	40	7.0
Costa Rica	20.3	15.6	10.7	33	10.5
Bico de ouro	17.4	14.8	12.6		8.4
Piratã 1	21.1	18.3	9.9	24	11.3
Roxinho	21.0	17.4	12.6	28	6.3
Mulatinho precoce	20.8	15.4	11.0	26	9.0
casein	nd[c]	nd[c]	nd[c]	82	nd[c]

[a] Soluble in 0.85% NaCl solution. Heated at 121 °C for 30 min. [b] Percent of the total peptide bonds split. The total peptide bonds were measured with ninhidrin after acid hydrolysis by boiling under reflux with 6 N HCl; the hydrolysate was rotary evaporated at 40 °C to dryness, washed twice, and dissolved in 0.2 M citrate buffer, pH 2.2. [c] nd, not determined.

Table II. Digestibility of the Proteins of Four Bean Varieties for Rats[a]

variety	rat weight,[b] g initial	rat weight,[b] g final	feed consumption, g/day	digestibility, %
casein	61.9	78.3	7.8	88
Rosinha G-2	64.6	60.7	5.1	69
Carioca	63.3	63.8	5.7	71
Roxinho	60.1	60.5	5.4	70
Rico 23	61.0	61.1	5.6	72

[a] Beans heated for 30 min at 121 °C in a water to bean ratio of 10:2. [b] Period of 5 days.

the lower values were found among the red-brown beans, a fact already observed by Elias et al. (1979), who suggested that this might be caused by the higher tannin content of those varieties.

Four of the fifteen varieties tested in vitro were selected to be studied in vivo with rats. Unlike the observed differences in digestibility among the varieties in vitro, Table II demonstrates the similarities in digestibility in rats (~70%). This was probably due to the restricted feeding technique used—the rats were fed twice a day for 1-h periods. In these extreme conditions, digestibility was still lower than that for casein which was 88% absorbed.

Isolation and Partial Characterization of the Major Protein Fraction of the Carioca Variety. For identification of a specific protein component which might be responsible for the low digestibility observed in the varieties studied, five major protein fractions, albumin, globulins G_1 and G_2, prolamins, and glutelins were isolated from the Carioca bean. The NaCl–ascorbate system of McLeester et al. (1973) was used for the fractionation because it reduces the cross contamination of globulins with the albumins (only the acid-soluble albumins are coextracted) and also because it allows for easy separation of G_1 and G_2 by a simple reduction of the ionic strength, thus avoiding the need for prolonged dialysis.

Table III shows the yield obtained for each fraction, the bound carbohydrate, and the trypsin inhibitor contents, and Table IV provides data on the amino acid compositions.

The carbohydrate contents varied according to the fraction. Results presented in Table III on the G_2 globulin are comparable to those obtained by Pusztai (1966) in the vicilin of *P. vulgaris*, while the values for the albumin and total globulins are similar to values reported by Satterlee et al. (1975) in the great Northern beans. The presence of glycoproteins was confirmed in all fractions and observed in most of the bands obtained by electrophoresis by specific staining with fucsine–sulfite after periodic acid oxidation. The presence of bound carbohydrates is important as a possible barrier to enzyme action and also because reactions such as β-elimination can occur during processing. This is especially true in alkaline media (Whitaker and Feeney, 1977) which is sometimes used in the preparation of beans to soften the seed coats and shorten the cooking time.

The trypsin inhibitors were primarily extracted with the albumins (73% of the total) although the glutelins still had a significant portion (14% of the total). The seed coats of the variety used did not show a significant inhibitory action.

The first nutritional limiting factor of bean proteins is the low content of sulfur amino acids. The albumins and glutelins are the best sources of those amino acids while both globulins, G_1 and G_2, have negligible amounts (Table IV). The negligible content of cysteine in the G_1 globulin was also observed by Millerd et al. (1971) in *Vicia faba* and Padhye and Salunkhe (1979) in the black gram. The

Table III. Yield, Carbohydrate Content, and Trypsin Inhibitor Activity of Protein Fractions Isolated from Carioca Bean

	albumins	globulins G_1	globulins G_2	glutelins	prolamins
yield, %[a]	31.5	38.1	13.8	22.4	1.7
carbohydrate, %[b]	8.0	11.7	13.8	nd[c]	nd[c]
TI, %[b]	6.1	0.4	0.7	1.2	nd[c]

[a] Expressed as percent of the total protein of the bean (the bean contained 17.6% protein). [b] Expressed as percent of the fraction. [c] nd, not determined.

Table IV. Amino Acid Composition of the Albumins, Globulins (G_1 and G_2), and Glutelins[a] Isolated from Carioca Bean

amino	albumins	globulins G_1	globulins G_2	glutelins
Lys	8.8	6.8	6.4	9.5
Hys	2.8	3.0	1.8	3.8
Arg	5.9	5.7	3.5	6.3
Asp	14.1	12.8	13.4	10.7
Thr	7.2	3.2	7.2	5.2
Ser	7.1	6.4	8.2	8.0
Glu	13.1	19.0	12.2	10.0
Pro	4.8	3.8	4.2	0.3
Gly	4.3	3.5	4.3	8.5
Ala	4.7	3.3	4.4	7.5
Cys	2.9	0.0	t[b]	0.0
Val	7.7	6.6	7.7	7.1
Met	0.8	0.3	0.0	0.7
Iso	4.6	4.7	4.9	5.7
Leu	7.3	11.3	11.4	9.3
Tyr	2.8	3.2	3.3	3.9
Phe	5.7	5.7	6.7	2.9
Try	2.6	0.3	0.6	nd[c]

[a] Grams of amino acid/100 g of protein. [b] t, traces. [c] nd, not determined.

Table V. Inactivation of Trypsin Inhibitors as a Function of pH[a]

pH	residual act., %: crude extract,[a] boiled	albumins: boiled[a]	albumins: autoclaved[b]
control (unheated)	100	100	100
1.8	75 (74)	77	78
3.0	56	78	42
5.0	52	91	36
7.0	25 (21)	83	18
8.5	15	81	10

[a] 1% protein suspensions were heated for 30 min in a boiling water bath at the indicated pHs. Numbers in parentheses were obtained after 1 h of boiling. (Unheated controls were held at the same pH as heated samples.) [b] Autoclaved 30 min at 121 °C.

chemical scores (CS) for sulfur amino acids, as calculated in relation to egg protein, were 0.7 for albumin, 0.05 for G_1, 0.0 for G_2, and 0.12 for the glutelins.

The second limiting factor varied according to the fraction; it was leucine/isoleucine for albumin (CS = 0.8), threonine for G_1 (CS = 0.68), histidine for G_2 (CS = 0.8), and the aromatic amino acids for the glutelins (CS = 0.7). It should also be noted that histidine is in high excess in the glutelins (CS = 1.73). From the nutritional point of view, it appears that the best fraction is albumin, followed by glutelins, although more information is needed on the availability of the amino acids.

Inactivations of the Trypsin Inhibitors. It has already been shown (Table III) that the greater amount of trypsin inhibitor activity is associated with the albumin fraction. We also observed that the TI demonstrated great dependence of pH for inactivation and appears to be relatively heat resistant (Table V). Even after boiling crude extracts containing albumins and globulins for 1 h at pH 1.8, 75% of the original TI activity was still present. However, when the pH was near neutrality, boiling for 30 min resulted in the lowering of activity to 25%, and at the end of 1 h, the activity was measured at 21%. In the case of albumin fractions, boiling eliminated only 20–25% of the activity, depending on the pH. A heat-resistant TI that resisted boiling for 2 h was described by Pusztai (1968) in *P. vulgaris* and by Warsy and Stein (1973) in *V. faba*.

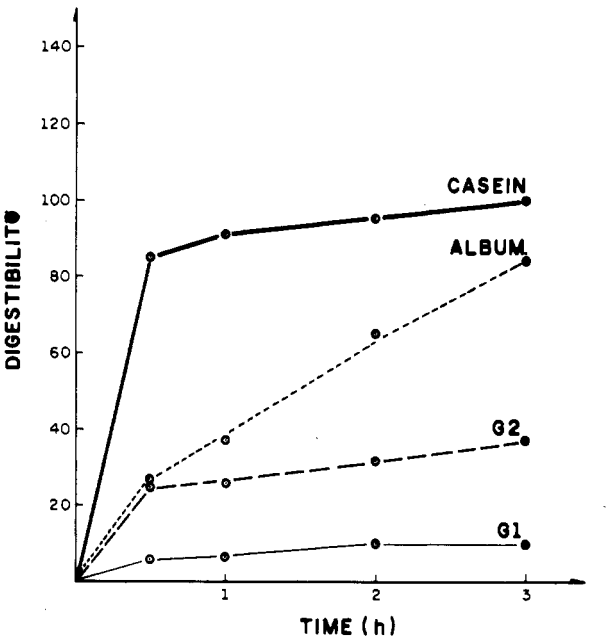

Figure 1. Digestibility (milligrams of leucine per gram of protein) by trypsin of unheated albumins, globulins, and casein. Trypsin inhibitor was previously titrated.

It was observed when preparing the controls that the simple raise of pH before any heating enhances the activity of the TI: at pH 3.0, the TI content was 200 μg/g of albumin, at pH 5.0 it was 230 μg/g, and at pH 9.0, it was 300 μg/g. This observation aids in explaining why there was a higher retention of TI activity for boiled albumin when compared to that of the crude extract; i.e., it was in part actually due to an initial activation by some unknown factor related to pH. On the other hand, heating the crude extract also helped to accelerate inactivation, probably because of the interaction between other components. Only autoclaving at 121 °C for 30 min was able to reduce the TI content of the albumin fraction to the extent of resembling that accomplished by boiling of the crude extract.

In relation to the above, it is interesting to refer the paper of Ellenrieder et al. (1980), who observed higher thermal stability of the soybean TI when aqueous suspensions were diluted; they explained the fact by the presence of a high molecular weight compound which could accelerate the thermal inactivation.

These results are significant when considering industrial or home processing techniques and the production of isolated proteins in relation to the variable content of TI in different bean varieties. Curiously, the dependence of TI inactivation on pH in beans is reversed to that observed in soybean in which the lability is higher at acidic pHs (Boonvisut and Whitaker, 1976).

Digestibility of the Separated Fractions. *Digestion with Trypsin.* The results are depicted in Figures 1 and 2 and are compared to those for casein, considered an easily digested protein.

When digested with trypsin, the raw globulins showed a reduced digestibility in relation to that of casein (12% for G_1 and 35% for G_2), while the unheated albumins were well digested, reaching 81% of the value observed for casein; only the initial velocity of hydrolysis in albumin was slower, as the shape of the curve indicates (Figure 1). Since the trypsin inhibitor had been previously titrated, the low hydrolysis in the globulins may be attributed to their structural confromations, a barrier which does not exist in albumins.

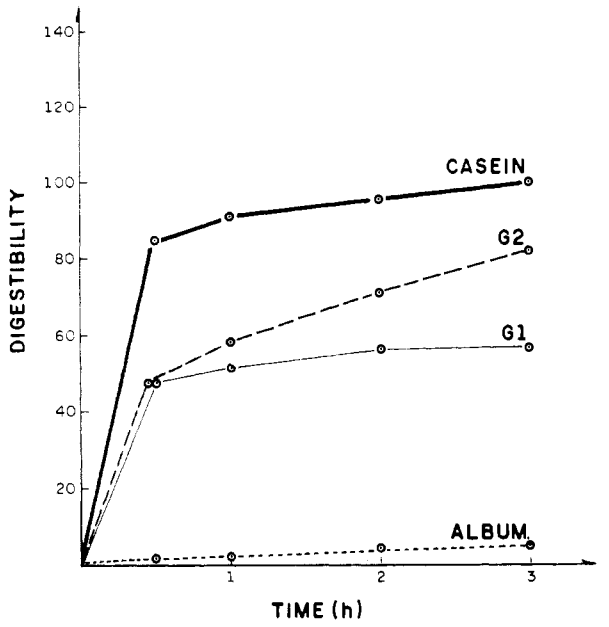

Figure 2. Digestibility (milligrams of leucine per gram of protein) by trypsin of albumin and globulins boiled for 30 min. Residual trypsin inhibitor was previously titrated.

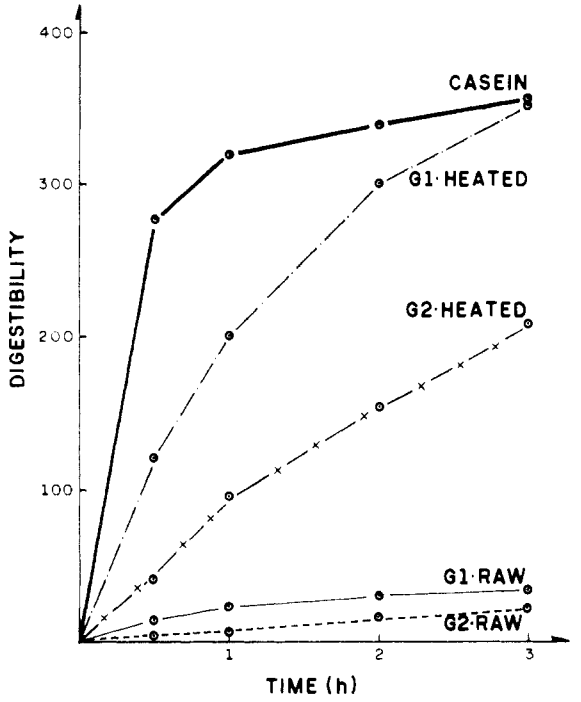

Figure 3. Digestibility (milligrams of leucine per gram of protein) of the globulins by pancreatin either boiled for 30 min or not boiled.

Heating the fractions (Figure 3) substantially increased the hydrolysis of G_1 and G_2 which reached respectively 56 and 82% of the casein value. Surprisingly, heating reduced the digestibility of albumins by trypsin from 81 to 8%. This may be explained by a sterical impediment or a blockage of amino acid residues needed for enzyme action, though the possibility of participation by some phenol or quinone, free or complexed with the protein, cannot be excluded. Similar effect of heating on the G_1 globulin was observed by Liener and Thompson (1980), either in vitro or in vivo.

Digestion by Pancreatin and Pepsin–Pancreatin. The results are presented in Figures 3 and 4. The unheated globulins showed reduced hydrolysis (10% for G_1 and 7% for G_2) in relation to casein hydrolysis which was considered as 100%. This apparent reduction in the percentage of digestibility of G_1 and G_2 when compared to the previous results obtained with trypsin (Figure 1) is actually due to an absolute increase from 100 to 360 mg of leucine/g of protein of the number of split peptide bonds in casein (Figure 3). The thermal treatment enhanced the digestibility of G_1 to a level approximating that of casein (Figure 3) and G_2 to a lesser extent (59%).

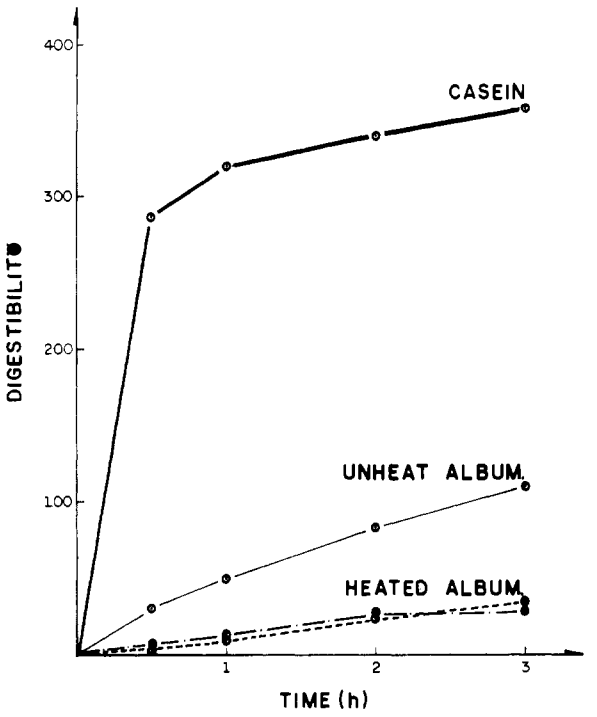

Figure 4. Digestibility of the albumins either raw or heated (boiling for 30 min) by pancreatin.

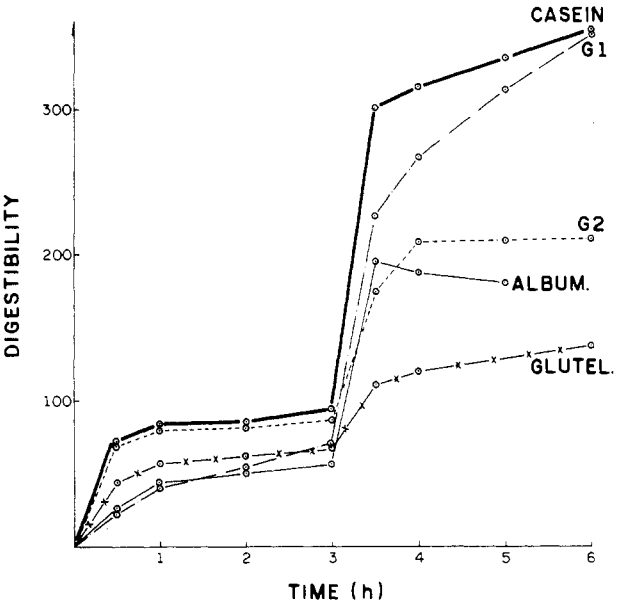

Figure 5. Digestibility of the autoclaved fractions submitted to pepsin–pancreatin.

These higher values achieved with pancreatin can be attributed to the presence of chymotrypsin and carboxypeptidase activity in the pancreatin, in addition to the presence of trypsin which was previously tested alone. The globulin G_1 fraction is a very well digested protein after denaturation. In the case of albmins, heating causes a reduction in hydrolysis which the attempted digestion by pancreatin did not alter. Pronase was also tested, but it

Table VI. Digestion of Heat-Treated Albumins as a Function of pH by Different Protease Systems

pH	digestibility, %[a] boiled (30 min), trypsin	autoclaved (121 °C/30 min) trypsin	pancreatin	pepsin–pancreatin
1.8	32	27	nd[c]	58
4.0	nd[c]	nd[c]	9	
6.0	5	18	nd[c]	nd[c]
7.0	nd[c]	28	10	52
9.0	52	52	nd[c]	nd[c]
10.0	nd[c]	46	51	82
raw albumin[a]	82	82	27	100
casein[b]	100	100	350	350

[a] Expressed as percent of the hydrolysis observed for casein as shown in the bottom of the table. [b] Hydrolysis observed for casein expressed as milligrams of leucine per gram of casein and considered as 100% for the calculation of percent digestion of the albumins. [c] nd, not determined.

did not significantly alter the digestibility of the heated albumins.

Figure 5 shows data for the four fractions, including the glutelins, autoclaved at 121 °C for 30 min and subjected to pepsin–pancreatin sequential action. The globulin G_1 was again the best digested protein, equal to casein after 6 h of incubation. Measurements at 4 and 5 h recorded decreased rates of hydrolysis relative to those of casein, but it is difficult to assess what this represents in terms of velocity of liberation and absorption in vivo. The digestibility figures for the unheated albumins (not shown in Figure 5) expressed in milligrams of leucine per gram of protein were with pepsin: 40, 60, 75, and 80, respectively, after 0.5, 1, 2, and 3 h; following the addition of pancreatin the figures were 310, 330, 340, and 340, respectively after 0.5, 1, 2, and 3 h. The albumin had the percent of digestion increased by the pepsin treatment to ~52%, but it was still much lower than that for the raw state. The total digestions, at 6 h for the other proteins relative to that for casein were 60 and 40% for G_2 and glutelin, respectively.

The pH of the protein suspension during the thermal treatment had a marked effect on the influence of heating on digestion of albumins (Table VI). At pHs between 4 and 7, the digestibility fell, indicating that the effect of heating is dependent on the ionization of amino acid residues. It was suspected that this was related to cross-links involving amino acid groups of lysine or asparagine and glutamine residues. In fact, as was observed by electrophoresis (Figure 6), heating caused the appearance of high molecular weight aggregates which did not penetrate the gel, even at low (5%) acrylamide concentration, and only at more alkaline pHs did smaller fragments appear. At higher pHs, fragmentation occurred, and the smaller aggregates produced help to explain the observed increased digestibility (see Table VI).

It should also be noted in Table VI that the pancreatin action increased the digestion of casein from 100 mg of leucine/g recorded with trypsin to 350 mg to leucine/g to protein but that albumin remained at the same level (~80 mg of leucine/g of protein) until pepsin was used in sequence with pancreatin.

Since the pepsin–pancreatin technique demonstrates a good correlation with in vivo digestibility (Saunders et al., 1973), it would be expected that rats would show similar results. In fact, calculating the percentage of each fraction digested by pepsin–pancreatin as a part of the total bean protein and in relation to casein resulted in a total digestion of 70% of the protein, which is in agreement with the rat assay (Table II) that gave a similar relative figure.

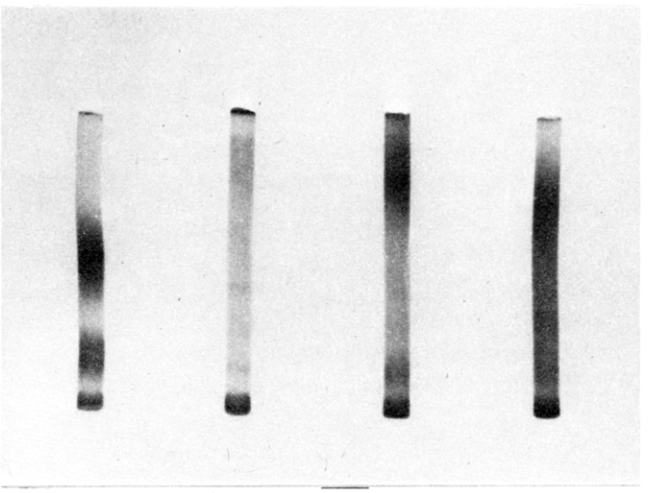

Figure 6. Electrophoretic behavior of albumins (5% polyacrylamide). From left to right: unheated albumins followed by albumin heated respectively at pH 7.0, 8.0, and 9.0 in boiling water.

Table VII. Digestibility of Each Amino Acid of the Autoclaved Albumin Fraction by Treatment with Pepsin–Pancreatin

amino acid	albumin[a] autoclaved	soluble fraction after digestion	digestibility, %
Lys	8.8	10.5	56.0
Hys	2.8	2.1	37.0
Arg	5.9	7.1	56.0
Asp	12.8	13.6	44.0
Thr	7.4	7.9	50.0
Ser	6.9	7.2	46.2
Glu	8.5	11.4	39.7
Pro	4.0	4.9	45.8
Gly	3.7	5.1	53.5
Ala	4.3	5.7	55.3
Cys	2.9	1.3	46.0
Val	6.8	5.9	35.2
Met	0.8	0.4	50.0
Iso	4.4	3.7	36.9
Leu	6.5	7.3	46.5
Tyr	2.3	2.4	50.0
Phe	5.1	5.6	50.1
Try	2.5	1.3	24.0
protein	100	46.0	46.0

[a] Expressed as grams/100 g of protein.

Amino Acids Liberated from Albumin by Digestion with Pepsin–Pancreatin. The amino acids liberated by the action of pepsin followed by pancreatin were obtained by precipitating the undigested peptides from the incubation media with Cl_3AcOH (10% final concentration). The resulting supernatant was extracted with ethyl ether until neutrality to eliminate the Cl_3AcOH. The solution, containing the digested peptides and amino acids, was dried under vacuum and resuspended in 6 N HCl for hydrolysis and amino acid analysis. After ninhydrin treatment, the ratio of the absorbance obtained for the acid hydrolysate of the undigested albumin to the absorbance for the acid hydrolysate of the supernatant (after digestion) indicated a digestibility of 46%. The ninhydrin value of the supernatant before and after acid hydrolysis indicated that its average protein peptide composition was in the form of tripeptides.

Table VII shows that some amino acids, lysine, arginine, threonine, glycine, alanine, and tyrosine, have higher digestibilities than the protein average (46% of hydrolysis),

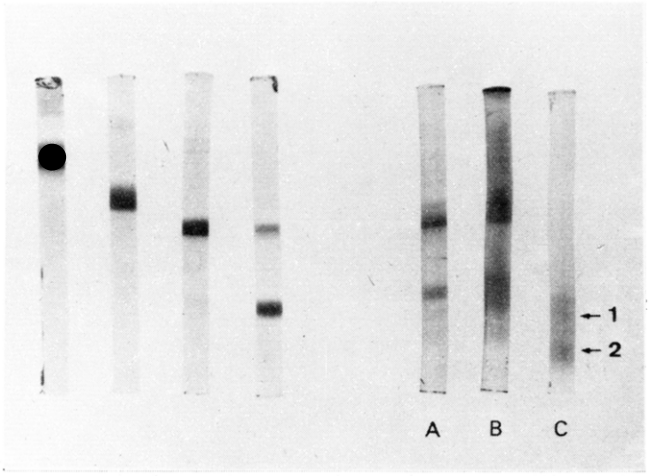

Figure 7. Electrophoretic behavior of albumins after digestion (10% polyacrylamide–1% $NaDodSO_4$). From left to right: bovine serum albumin (M_r = 67 000); ovalbumin (M_r = 45 000); aldolase (M_r = 40,000); trypsin (M_r = 23,300); unheated albumins (A); autoclaved albumins before (B) and after digestion with pepsin–pancreatin (C). The arrows show the peptides bands (1) M_r = 20 000 and (2) M_r = 14 000.

while tryptophan, glutamic acid, valine, and isoleucine demonstrated lower digestibility. However, the differences among them were small and are probably not nutritionally significant with the exception of tryptophan which is slowly liberated (50% slower than the average) and lysine with a digestibility 25% higher than the average. This was expected in view of the specificity of trypsin and the high amount of lysine in the albumin. In view of the lowering of digestibility of albumin when heat treated (Figures 1 and 4), the presence of aggregates in the heated albumin (Figure 6), and the low availability of methionine for rats (Evans and Bauer, 1978; Sgarbieri et al., 1979), it was initially supposed that some undigestible peptide containing a large proton of methionine and cysteine might have been formed. But contrary to the expected, after digestion of the albumin, the distribution of sulfurated amino acids between the supernatant and the residues was similar to the average digestibility of the whole protein. However, this does not exclude the possibility of the existence of small peptides left in the supernatant after Cl_3AcOH precipitation which are not available to enzymes because the supernatant was hydrolyzed with acid before analysis.

Methionine content was not affected by autoclaving (Table VII), which is in accordance with data provided by Hernandez-Infante et al. (1979) showing that cooking has no effect on the availability of methionine added as a nutritional enrichment to beans.

The greater portion (54%) of the amino acids were precipitated as larger peptides from the incubation media after digestion of the heated albumin. This is especially important for sulfur amino acids that are already scarce and causes a reduction in the chemical score from 0.70 to ~0.30. Electrophoresis in PAA (Figure 7) showed the undigested material resulted in two major bands composed of large peptides with molecular weights of 14 000 and 20 000 that were present even after treatment with Pronase (not shown).

These bands appear to be related to the observed heat-resistant TI. In fact, the molecular weight of TI in beans has been reported to be between 10 000 and 15 000 and with an abundance of disulfide bridges which help to stabilize the structure (Pusztai, 1968). This may explain the low availability of the sulfur amino acids in beans (Kakade et al., 1969a). On the other hand, aggregates and undigestible compounds formed during heating of the albumin are present and are most likely responsible for part of the decreased digestibility.

More research directed at this last area and at explaining the low digestibility of glutelin, an important fraction of beans, is currently under way in this laboratory.

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