In Vitro Digestibility of Albumin Proteins from *Phaseolus vulgaris* L. Effect of Chemical Modification

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The *in vitro* digestibility of albumins from *Phaseolus vulgaris* was studied. The extent of hydrolysis of native albumins was low (32%), and when they were heated (99 °C for 30 min), it was reduced even more, to 20%. Chemical deglycosylation increased proteolysis of both native (44.4%) and heated (30.7%) albumins. Reversible blockage of the ϵ-amino groups of lysine residues during heating resulted in an extent of hydrolysis (29.8%) similar to that of native albumins. Heating (99 °C for 30 min) caused formation of new disulfide bonds and incomplete inactivation of trypsin inhibitors, which was attained only by autoclaving for a prolonged time (2 h). A digestibility similar to that of casein was only achieved during a 27 h prolonged pepsin−pancreatin hydrolysis of chemically denatured and deglycosylated albumins. Primary structure, native conformation, heat-stable trypsin inhibitors, steric hindrance imposed by carbohydrates, and chemical reactions induced by heating seem to be major factors responsible for the low digestibility of albumins.

Keywords: *Phaseolus vulgaris; albumin proteins; digestibility; methionine bioavailability; chemical modification*

INTRODUCTION

Dry bean (*Phaseolus vulgaris*) proteins have a low nutritional value due to reduced digestibility and low methionine content and bioavailability (Bressani, 1993). Phaseolin, the major seed storage globulin, is very poorly digested when in its native state but is effectively hydrolyzed after heat treatment, both *in vitro* and *in vivo* (Liener and Thompson, 1980; Deshpande and Nielsen, 1987a). Contrarily, as we observed in previous papers, the albumin fraction from dry beans has a low digestibility that is reduced by heat treatment (Marquez and Lajolo, 1981; Deshpande and Nielsen, 1987b). Since albumin proteins represent a high percentage of total bean proteins and the ratio between albumins and globulins varies from 0.44 to 1.15 according to the variety (Deshpande and Nielsen, 1987c), investigating the problem is important for the development of new bean varieties (Lumen, 1990) and processing technologies.

The purpose of this project was to further investigate the causes of low albumin digestibility and the bioavailability of methionine, studying reactions occurring during heating and the effect of chemical modifications upon it.

MATERIALS AND METHODS

Dry seeds of *P. vulgaris* L. cv. Carioca were obtained from the Instituto Agronômico de Campinas, Campinas, Brazil. After they were dehulled, the seeds were ground in a mill, and the resultant flour was passed through a 0.25 mm sieve. Pepsin (P-7012, 2690 units/mg) and pancreatin (P-1750, activity equivalent to 4 × USP specifications) were purchased from Sigma Chemical Co. All other chemicals used were of analytical grade.

Albumin Extraction. A 5% suspension of the flour in water was extracted for 2 h under agitation at room temperature. After centrifugation (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. The precipitated globulins were separated by centrifugation, and the supernatant (albumin fraction) was freeze-dried.

Phaseolin Isolation. Phaseolin was isolated by the method of Hall et al. (1977). The flour was extracted under acidic conditions (0.5 M NaCl in 0.025 M HCl; 20 mL/g of flour); after centrifugation, the supernatant was diluted with 5 volumes of distilled water (4 °C). Phaseolin was precipitated two more times and finally dialyzed against water and freeze-dried. Purity, shown by electrophoresis, was more than 95%.

Oxidation with H_2O_2. Samples of albumins, phaseolin, and Hammarsten casein (10 mg/mL distilled water) were heated at 99 °C for 30 min and, after cooling, oxidized with 4% H_2O_2 at 60 °C for 2 h, dialyzed, and freeze-dried. Samples of albumins also were oxidized in the same way without previous heat treatment.

Albumin Deglycosylation. Chemical deglycosylation was done according to the method of Edge et al. (1981). Thirty milligrams of albumins was dissolved in anisole−trifluoromethanesulfonic acid (1:2 v/v), mixed in an ice−water bath for 2 h, and then diluted with a 2-fold excess of diethyl ether at −40 °C. After removal of the ether phase, the albumins were dialyzed against distilled water and freeze-dried. Protein recovery was about 66%, and 84−85% of the carbohydrates were removed, as determined by the method of Dubois et al. (1956).

Albumin Citraconylation. Citraconylation of albumin proteins was performed according to the method of Atassi and Habeeb (1972). Briefly, albumins were dissolved in 0.1 M sodium phosphate buffer (pH 8.2), and a total of 500 μL of citraconic anhydride (C-2395, Sigma Chemical Co.) was added, in aliquots of 100 μL, to the magnetically stirred solution, at room temperature. The pH of 8.2 was maintained by the addition of 1 N NaOH with a pH-stat. A total of 100% citraconylation was obtained, as checked by the method of Fields (1972) for free amino groups. After heat treatment, removal of blocking groups was carried out at pH 3 for 3 h/40 °C. Following dialysis against distilled water, decitraconylated albumins were freeze-dried. About 94% of the amino groups were regenerated.

Heat Treatment. Aqueous solutions of albumins (10 mg/mL), chemically modified or not, were heated in a water bath at 99 °C for 30 min, or as indicated, and then freeze-dried.

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Alternatively, samples were autoclaved at 121 °C (15 psi) for 15 min, 30 min, 1 h, or 2 h. Native albumins were also heated (99 °C for 30 min) in the presence of 20 mM *N*-ethylmaleimide (NEM) or 10 mM β-mercaptoethanol (ME) to observe the effect of sulfhydryl−disulfide interchange reactions on the *in vitro* digestibility. After dialysis against distilled water, samples were freeze-dried.

Albumins' Dissociation. Freeze-dried albumins were denatured and the disulfide bonds reduced in 50 mM Tris-HCl buffer (pH 8) containing 8 M urea and 2% β-mercaptoethanol (β-ME). After the solution was allowed to stand at room temperature for 1 h under nitrogen, sulfhydryl (SH) groups were blocked with an excess of NEM to prevent subsequent disulfide formation. After dialysis against distilled water, samples were freeze-dried.

***In Vitro* Digestibility.** Enzymatic hydrolysis was carried out according to the method of Mauron (1973), with slight modifications. Protein solutions (10 mg/mL KCl-HCl buffer, pH 2) were first digested with pepsin (enzyme to substrate ratio of 1:66.7) at 37 °C for 3 h. Then, the samples were diluted with the same volume of 0.2 M sodium phosphate buffer (pH 8), and after addition of pancreatin (1:25), digestion was allowed to proceed for 4 h at 37 °C. Samples were taken at 1 h intervals, and the digestion was interrupted with 1.8 M $HClO_4$ (0.2 mL/mL of hydrolysate). After centrifugation (10 000*g* for 10 min), the supernatants were assayed for an increase in free amino groups to determine the extent of hydrolysis. The precipitates (hydrolysis residues) were freeze-dried after dialysis against distilled water. Two blanks, one containing only the enzymes and the other only the proteins, were run simultaneously.

Extent of Hydrolysis. The extent of hydrolysis was determined, on the basis of the measurement of free amino groups released with picryl sulfonic acid (TNBS), according to Fields (1972). The absorbances at 420 nm of the controls were subtracted from those of the digested samples, and the percentage of peptide bond hydrolysis was the ratio of the number of new amino groups released (calculated using 2.2×10^4 M^{-1} cm^{-1} as the molar extinction coefficient for TNP-α amino groups) to the total number of peptide bonds in the protein (calculated by dividing the *g* of the substrate by 113, the average molecular weight of amino acid residues in the protein).

Trypsin Inhibitory Activity. Trypsin inhibitory activity was measured according to Kakade et al. (1969a) using BAPA (benzoyl-DL-arginine *p*-nitroanilide) as the substrate. One trypsin unit (TU) was arbitrarily defined as an increase of 0.01 absorbance unit at 410 nm. Results were expressed as the number of trypsin units inhibited (TUI) per milligram of protein.

Sulfhydryl and Disulfide Contents. Sulfhydryl groups were determined by a modification of the method of Beveridge et al. (1974). Freeze-dried albumins, or their hydrolysis products, were resuspended in 0.086 M Tris−0.09 M glycine−0.006 M EDTA (pH 8.0) buffer containing 8 M urea. To 3 mL of these solutions was added 30 μL of DTNB (4 mg/mL Tris−glycine buffer, pH 8.0), and the absorbance at 412 nm was measured 10 min later. Determination of disulfide bonds was carried out according to Li-Chan (1983); 0.01 g of albumins or their hydrolysis products was solubilized in 10 mL of Tris−glycine−EDTA (pH 8) buffer containing 10 M urea and 2% β-mercaptoethanol. After 1 h of incubation at room temperature, 10 mL of 24% trichloroacetic acid (TCA) was added and the samples were centrifuged. The pellets were twice resuspended in 10 mL of 12% TCA and finally dissolved in Tris−glycine−EDTA (pH 8) buffer with 8 M urea. To 3 mL of this solution was added 50 μL of DTNB, and $A_{412\ nm}$ was read after 10 min. Half the value obtained after subtracting the sulfhydryl (SH) content from the total SH was defined as a measure of the disulfide (SS) content. Sample and reagent blanks were included for each determination, and the analyses were made in triplicate.

Bioavailable Methionine. Methionine bioavailability of bean proteins was determined according to Genovese and Lajolo (1993), corresponding to the unoxidized methionine released during *in vitro* hydrolysis by the successive pepsin−pancreatin method. Unoxidized methionine was assayed by the modification proposed by Horn et al. (1946) of the sodium nitroprusside colorimetric reaction initially developed by McCarthy and Sullivan (1941). A protein blank, for which methionine was previously oxidized (see Oxidation with H_2O_2), was used to determine possible color formation of histidine or other interferents.

Methionine Content. Methionine content of albumins and the respective pepsin−pancreatin hydrolysis residue was determined after 6 N HCl hydrolysis (110 °C for 22 h) with a Dionex DX 300 aminoanalizer.

SDS−Polyacrylamide Gel Electrophoresis (SDS−PAGE). Slab SDS−PAGE was carried out according to the procedure of Laemmli (1970) at gel concentrations of 12 or 15% and a constant current. Gel slabs were fixed and stained with 0.2% Coomassie Blue R in methanol−acetic acid−water (10:7:83).

RESULTS AND DISCUSSION

***In Vitro* Digestibility of Bean Proteins**. *In vitro* digestibility of phaseolin (the major reserve protein from *Phaseolus*) and albumins, before and after heat treatment, was determined and compared to that of heated casein (Figure 1A). Denatured phaseolin was hydrolyzed at almost the same rate and degree as denatured casein (89% of hydrolysis) by the successive pepsin−pancreatin treatment. On the contrary, denatured albumins showed a very low extent of hydrolysis (20%), smaller than that of native albumins (32%) and similar to that of native phaseolin (23%). Marquez and Lajolo (1981) and Deshpande and Nielsen (1987b) also reported a decrease in the digestibility of albumins upon heating, while the increased susceptibility of heated phaseolin, leading to an extent of proteolysis similar to that of casein, has been reported by several authors (Romero and Ryan, 1978; Liener and Thompson, 1980; Bradbear and Boulter, 1984; Deshpande and Nielsen, 1987a; Ahn et al., 1991).

Effect of pH and Heating Time. To determine the pH dependence of the digestibility decrease of albumins upon heating, protein suspensions were heated (99 °C for 30 min) at pH 2, 4, 6, 8, and 10 (Figure 1B). In all cases with heated albumins, there was a digestibility drop compared to native albumins, although at higher pHs, the effect was less severe (extent of hydrolysis at pH 10 of 26%), showing that ionic interactions are not involved in albumin modifications provoked by heating. Albumins also were heated for prolonged times (1, 2, and 3 h) to verify whether the effect was cumulative, intensifying the extent of albumin modification with time of heating, or whether it could be reversed, by a greater unfolding of proteins leading to a facilitated protease access (Figure 1C). There was no subsequent reduction in digestibility with time of heating up to 3 h, and 15 min of heating was enough to decrease the extent of hydrolysis to the values observed. Prolonged heating at autoclaving temperatures was not able to reverse the deleterious heat effects either, as would be expected if the heat treatment at 99 °C had not been sufficient for the complete denaturation of albumins (data not shown). With autoclaving periods of 15 min to 2 h (121 °C), not more than 14−16% hydrolysis of albumins was achieved.

SDS−PAGE Profiles. SDS−PAGE of native (Figure 2) and heated (Figure 3) albumins subjected to pepsin−pancreatin digestion showed that susceptibility of proteins to hydrolysis changed upon heating. In heated samples, the hydrolysis product showed a predominance

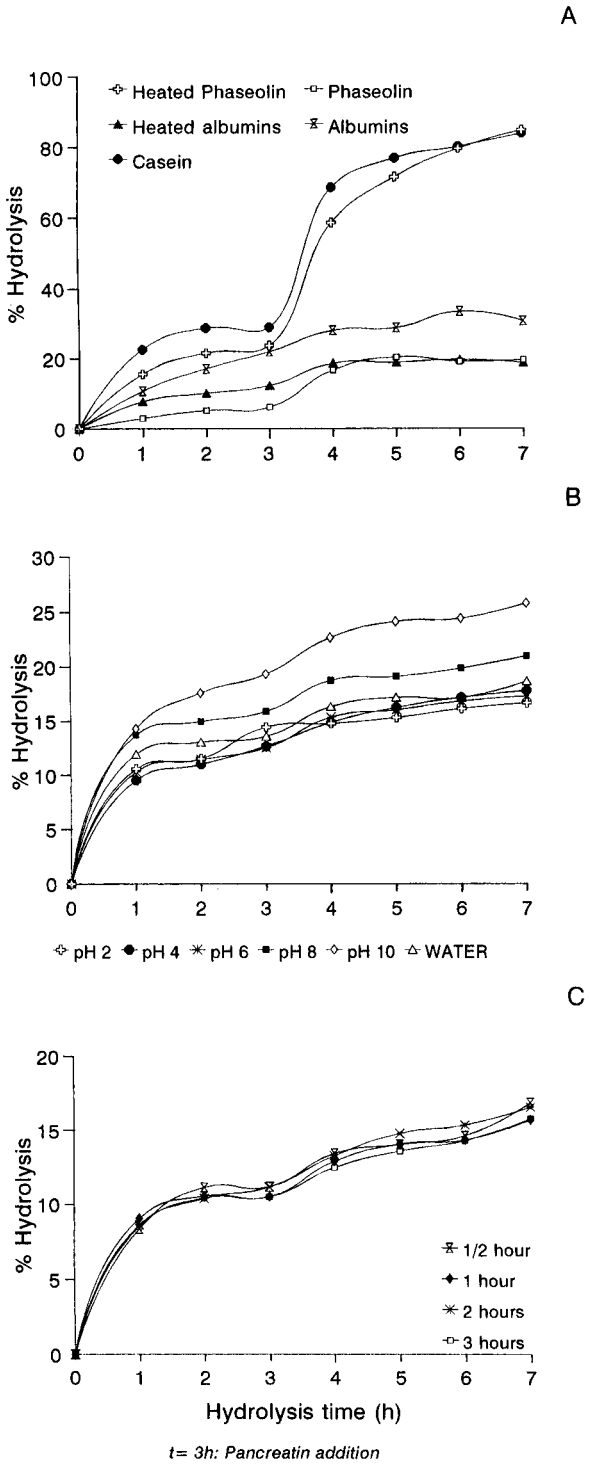

Figure 1. Pepsin−pancreatin digestibility of native and heated (99 °C for 30 min) phaseolin and albumins (A), albumins heated (99 °C for 30 min) at different pHs (B), and albumins heated (99 °C) for different time intervals (C).

of lower-molecular mass peptides (<20 kDa) when compared to native samples. Peptides in the 32−34 kDa region (probably lectins), resistant to hydrolysis in the native fraction, were completely digested after heating. Also, heating was shown to promote aggregation, observed by the presence of proteins that were not able to enter the stacking gel (Figure 3, compare to Figure 2). These aggregates were not dissociated by SDS and β-mercaptoethanol but completely disappeared after 1 h of pepsin hydrolysis (Figure 3), suggesting that they would not impair digestibility. When samples were treated in SDS−PAGE buffer in the absence of β-mercaptoethanol, hydrolysis products of heated albumins demonstrate the presence of high-molecular mass aggregates, unable to enter the stacking or resolving gel (Figure 4), not observed for native albumin's hydrolysis products (not shown, as the profile was identical to that in Figure 2). These results suggest that formation of high-molecular mass protein aggregates through disulfide bonds may be one of the main reasons for the low hydrolysis susceptibility of heated albumins.

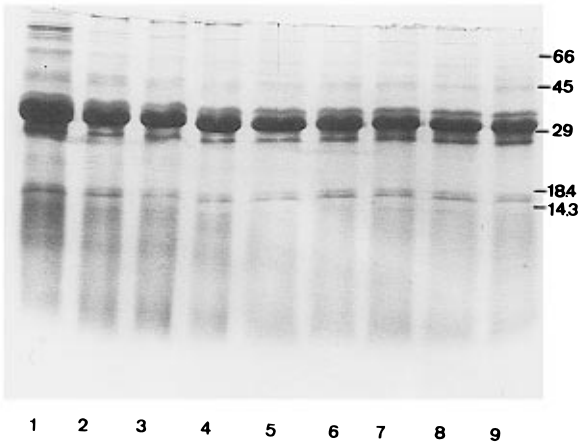

Figure 2. SDS−PAGE (15%) profile of native albumins sequentially digested with pepsin−pancreatin: lane 1, native albumins; lane 2, after 1 h of pepsin hydrolysis; lane 3, after 2 h of pepsin hydrolysis; lane 4, after 3 h of pepsin hydrolysis; lane 5, after 1 h of pancreatin hydrolysis; lane 6, after 2 h of pancreatin hydrolysis; lane 7, after 3 h of pancreatin hydrolysis; lane 8, after 4 h of pancreatin hydrolysis; lane 9, hydrolysis residue. Numbers on the right indicate the position of molecular mass markers.

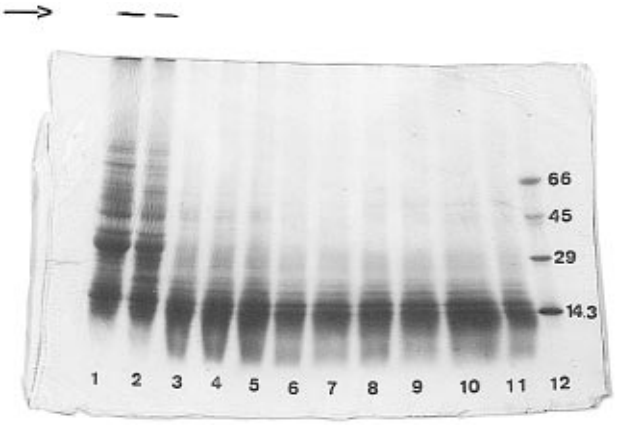

Figure 3. SDS−PAGE (5−20%) profile of heated albumins sequentially digested with pepsin−pancreatin: lane 1, heated albumins (99 °C for 30 min); lane 2, after 2 min of pepsin hydrolysis; lane 3, after 1 h of pepsin hydrolysis; lane 4, after 2 h-pepsin hydrolysis; lane 5, after 3 h of pepsin hydrolysis; lane 6, after 2 min of pancreatin hydrolysis; lane 7, after 1 h of pancreatin hydrolysis; lane 8, after 2 h of pancreatin hydrolysis; lane 9, after 3 h of pancreatin hydrolysis; lane 10, after 4 h of pancreatin hydrolysis; lane 11, hydrolysis residue; and lane 12, molecular mass markers. The arrow indicates the position of aggregates that did not enter the stacking gel.

Trypsin Inhibitory Activity. The presence of protease inhibitory activity in the albumin fraction might be partially related to its low digestibility, but not to the decrease caused by heating. A much greater amount of trypsin inhibitory activity has been reported to be associated with the albumins compared to that associated with phaseolin (Marquez and Lajolo, 1981). As reported in Table 1, native albumins showed a high trypsin inhibitory activity, which is only partially

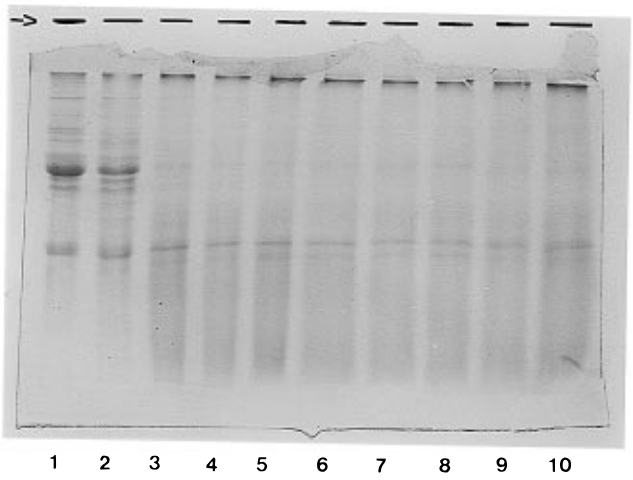

Figure 4. SDS−PAGE (15%) profile of heated albumins sequentially digested with pepsin−pancreatin. Samples were in SDS−PAGE buffer without β-mercaptoethanol: lane 1, heated albumins (99 °C for 30min); lane 2, after 2 min of pepsin hydrolysis; lane 3, after 1 h of pepsin hydrolysis; lane 4, after 2 h of pepsin hydrolysis; lane 5, after 3 h of pepsin hydrolysis; lane 6, after 1 h of pancreatin hydrolysis; lane 7, after 2 h of pancreatin hydrolysis; lane 8, after 3 h of pancreatin hydrolysis; lane 9, after 4 h of pancreatin hydrolysis; lane 10, hydrolysis residue. The arrow indicates the position of aggregates that did not enter the stacking gel.

Table 1. Trypsin Inhibitory Activity of Native and Heated Albumins

	TUI/mg	residual activity (%)
native phaseolin	traces	
native albumins	170.4 ± 2.6	100
heated albumins (99 °C)		
30 min	117.9 ± 2.8	69.2
3 h	106.1 ± 1.3	62.3
autoclaved albumins (121 °C)		
15 min	94.3 ± 3.9	55.3
1 h	30.2 ± 1.4	17.7
2 h	0	0.0

[a] TUI = trypsin units inhibited.

eliminated (31%) upon heating for 30 min at 99 °C. Sgarbieri and Whitaker (1982) reported that Brazilian pink bean inhibitors retained 100% of their activity after heating for 15 min at 97 °C. Deshpande and Nielsen (1987b) also observed the presence of heat-stable trypsin inhibitors in bean albumins. In our case, heating at 99 °C for as long as 3 h was not enough to eliminate more than 38% of the initial activity. Similarly, Weder and Link (1993) reported that extracts of pink bean (Rosinha G2) heated at 95 °C for 2 h maintained high residual activities against both bovine and human trypsin and chymotrypsin. Autoclaving aqueous suspensions of albumins was more effective in inactivating trypsin inhibitors, although total inactivation was only achieved after a 2 h treatment (Table 1). These results show that protease inhibitors can be held partially responsible for the low digestibility of native albumins, but not for the decrease caused by heating. When albumins autoclaved for total elimination of trypsin inhibitor activity were subjected to pepsin−pancreatin hydrolysis, only 14.3% hydrolysis was achieved, indicating that, even after total elimination of trypsin inhibitory activity, the albumins' digestibility is very low and heating has a deleterious effect upon it. Trypsin inhibitors, on the other hand, could be implicated in the formation of high-molecular mass aggregates during heating, due to their high content of disulfide bonds (Belitz and Weder, 1990).

Effect of Carbohydrates. Albumins have a high content of carbohydrates (11.6%) compared to that of phaseolin (4.8%), as determined by the phenol−H_2SO_4 method (Dubois et al., 1956). Therefore, the glyco groups may cause a decreased digestibility of this fraction. Gu et al. (1989) reported an easier degradation of deglycosylated hen ovomucin by trypsin, when compared to that of the native ovomucin. Semino et al. (1985) also observed an increase in the action of trypsin on lupin seed glycoproteins related to the amount of glyco groups removed. To investigate this, albumins were chemically deglycosylated, and after heat treatment, their extent of hydrolysis was determined. Deglycosylation resulted in a greater susceptibility to proteolysis of heated albumins (30.7%), similar to that of native albumins (Figure 5A). The results could be explained by their much lower content of carbohydrates after deglycosylation, the presence of which may impair the action of proteases by steric hindrance. However, deglycosylated albumins that were not subjected to heat treatment showed a much higher extent of hydrolysis (44.4%) than native ones, indicating that heating also has a deleterious effect on susceptibility to proteolysis of deglycosylated albumins. This effect might be the same observed for albumins that were not modified, involving formation of disulfide bonds, or the result of hydrophobic aggregation of albumins caused by the removal of glyco groups, or even both of them.

Effect of Lysine Blockage during Heating. As shown in Figure 3, heating provoked formation of protein aggregates that were not dissociated by SDS and β-mercaptoethanol, suggesting that cross-links, as those involving the ϵ-amino group of lysine, could have been formed in albumin proteins. In order to verify the involvement of lysine residues in the digestibility decrease upon heating, ϵ-amino groups were reversibly blocked by reaction with citraconic anhydride and the modified albumins were subjected to heat treatment. After removal of the blocking groups, the *in vitro* digestibility was determined (Figure 5A) and an extent of hydrolysis (29.8%) similar to that of native and heated deglycosylated albumins was observed. Also, protein aggregates were not formed as observed through SDS−PAGE (not shown), suggesting a possible involvement of lysine residues in the decreased digestibility of heated albumins.

Sulfhydryl and Disulfide Contents. Sulfhydryl and disulfide contents of native and heated albumins and their hydrolysis residues are in Table 2. Albumins showed a high content of both sulfhydryl and disulfide groups. However, heat treatment of albumins caused the formation of new disulfide bonds, which could be related to their lower digestibility, as a more compact structure would impair protease access to labile peptide bonds. A high content of disulfide bonds also was found in the hydrolysis residues, but the level was lower for the native albumin residue. If we consider the amount of total SH (free SH + two SS), we again observe a higher value for the hydrolysis residue of heated albumins (64.2) as compared to that of native albumins (50.6). This seems to corroborate the role of disulfide bonds on the higher hydrolysis resistance of heated albumins. The decrease in sorghum protein digestibility observed after cooking has also been attributed to disulfide bond formation (Hamaker et al., 1987; Oria et al., 1995).

Effect of Sulfhydryl Blockage and Disulfide Cleavage. As it was shown in Table 2, disulfide bonds

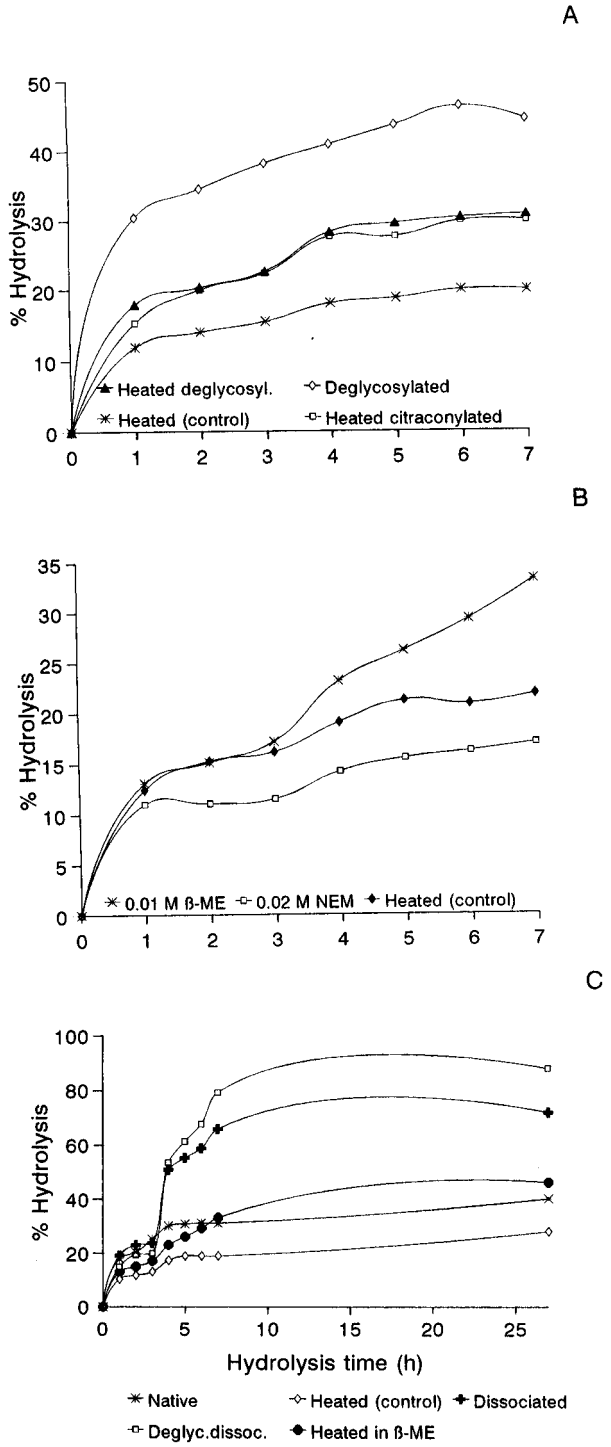

Figure 5. Effect of deglycosylation and citraconylation (A), sulfhydryl blockage (NEM) and disulfide cleavage (β-ME) during heating (99 °C for 30min) (B), and chemical dissociation (C) on the *in vitro* pepsin−pancreatin digestibility of albumins.

were formed upon heating the albumins. *N*-Ethylmaleimide (NEM) added prior to heating blocked free sulfhydryl groups in the protein, preventing the occurrence of sulfhydryl−disulfide interchange reactions, and also blocked thiol groups possibly liberated from breakage of disulfide bonds, preventing disulfide-linked polymer formation. When albumins were heated in the presence of NEM, all free sulfhydryls were blocked and a disulfide content of 14.9 ± 0.2 μmol/g was found (data not shown), meaning that seven bonds were broken during heating and immediately blocked by the excess of NEM in the solution (compare with Table 2). However, when *in vitro* digestibility of this fraction was determined, a lower value (17%) than that of heated albumins (21%) was found (Figure 5B). This result suggests that NEM might have bound in great quantity to proteins, impairing protease access. In the case of β-mercaptoethanol (β-ME) addition, apart from blocking SH−SS interchange reaction, it can reduce inter- and intramolecular disulfide bonds in the protein and thus cause a greater unfolding during the thermal treatment. As can be seen in Figure 5B, heating in the presence of β-ME led to a higher hydrolysis (33.3%), which could still be enhanced with time (see curve inclination). Sulfhydryl and disulfide content in this fraction were 39.0 ± 0.2 and 9.2 ± 0.3 mmol/g, respectively. When sorghum was cooked in the presence of β-ME or other reducing agents, protein digestibility also increased significantly (Hamaker et al., 1987), confirming the importance of disulfide bonds in the stability to hydrolysis. β-ME also caused a great decrease in trypsin inhibitory activity of albumins (22.6 ± 1.1 TUI/mg), probably due to reduction of SS bonds, which decreased the heat stability of inhibitors.

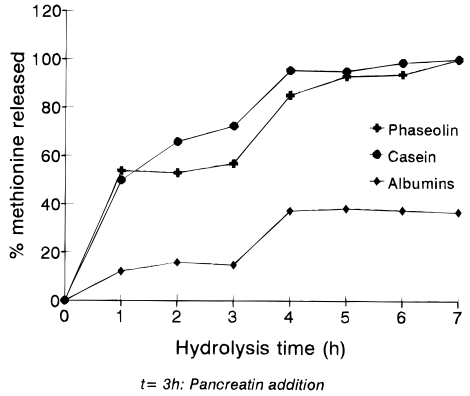

Figure 6. Methionine release during *in vitro* pepsin−pancreatin hydrolysis of heated casein, phaseolin, and albumins.

Table 2. Sulfhydryl (SH) and Disulfide (SS) Contents of Native and Heated Albumins and Their Respective Hydrolysis Residues

	SH (μmol/g)	SS (μmol/g)
native albumins	13.9 ± 0.2	22.1 ± 0.1
heated albumins	5.6 ± 0.1	26.0 ± 0.3
hydrolysis residue from native albumins	0.4 ± 0.0	25.1 ± 0.3
hydrolysis residue from heated albumins	4.2 ± 0.1	30.0 ± 0.3

Effect of Chemical Dissociation and Prolonged Digestion Time. The effect of chemical dissociation of native and deglycosylated albumins, by urea, β-ME, and NEM, on *in vitro* digestibility can be seen in Figure 5C. After 7 h of hydrolysis, an extent of hydrolysis of 66 and 79.3% was observed, respectively, for dissociated and deglycosylated dissociated albumins. However, with prolonged hydrolysis (27 h), deglycosylated dissociated albumins reached an extent of hydrolysis similar to that of casein (88%), while for dissociated albumins, it was only 72%. Prolonged digestion time caused a slight increase in hydrolysis of native albumins (40.3%) and albumins heated in β-ME (47%). No trypsin inhibitory activity was detected after chemical dissociation. Albumins seem to have a very compact structure, maintained mostly by hydrophobic interactions and disulfide bonds, which, along with attached carbohydrates, impair digestibility of this fraction. As expected, the changes in protein conformation brought about by urea and β-mercaptoethanol increased the number of

accessible peptide bonds and thus increased the rate of proteolysis. The results also showed that, even with complete inactivation of trypsin inhibitors and without the deleterious effects of heating, albumins have a lower rate of hydrolysis, compared to phaseolin, and carbohydrates display a significant role on proteolysis susceptibility.

Methionine Bioavailability. Unoxidized methionine released during *in vitro* pepsin−pancreatin hydrolysis of heated phaseolin and albumins was taken as a measure of its bioavailability. All the methionine was released from heated phaseolin and casein after 7 h of hydrolysis (0.97 and 2.58 g of Met per 100 g of protein, respectively) (Figure 4). On the contrary, the amount of methionine released from heated albumins was only 0.42 g per 100 g, corresponding to 37.2% of the total (1.13 g per 100 g, determined by ion-exchange chromatography). The low methionine bioavailability of this fraction could be related to the low values that have been reported for the beans as a whole (Evans and Bauer, 1978; Sgarbieri et al., 1979; Rayas-Duarte et al., 1988) and to its low digestibility. Methionine content of the hydrolysis residue of heated albumins was 1.33 g per 100 g (determined by ion-exchange chromatography), and the residue represented 54.7% of the total N in albumins, meaning that 0.73 g of methionine remained in the hydrolysis residue for each 100 g of albumins, confirming the results obtained for the methionine released. This fact is significant due to the low methionine content of bean protein (1%).

Effect of Oxidation with H_2O_2. During determination of methionine bioavailability, we used a protein blank, previously heated and oxidized with H_2O_2, to see the interference of other amino acids released during *in vitro* hydrolysis on the colorimetric reaction for methionine. Although H_2O_2-treated casein and phaseolin showed a digestibility similar to that of unoxidized samples (around 88%), H_2O_2-treated albumins had a much higher digestibility (48%) than that of the unoxidized and the native samples (Figure 7A). H_2O_2 treatment of native albumins also was able to increase the extent of hydrolysis to 44.1%. H_2O_2 is known to react with methionine ($-SCH_3 + H_2O_2 \rightarrow -SOCH_3 + H_2O$) and cysteine ($-SH + 3H_2O_2 \rightarrow -SO_3H + 3H_2O$) (Feeney et al., 1982). The results obtained here indicated that H_2O_2 possibly was also responsible for disulfide cleavage, which could be related to the increased digestibility observed. Confirming this, we did not find free sulfhydryls in native and heated albumins treated with H_2O_2, and disulfide content was 8.2 ± 0.2 (native) and 9.0 ± 0.2 μmol/g (heated), showing that 14 and 17 disulfide bonds were broken, respectively, by H_2O_2-treatment (compare with Table 2). This corroborates the evidences that disulfide bonds have an important role in the hydrolysis stability of albumins. Treatment with H_2O_2 of both native and heated albumins also caused a significant reduction of trypsin inhibitory activity (to 8.4 ± 0.6 and 7.8 ± 0.4 TUI/mg, respectively) (data not shown). H_2O_2 treatment of deglycosylated albumins (previously heated) was able to provoke a higher increase of its digestibility, from 30.7 to 52%. However, oxidation of deglycosylated albumins that were not previously heated caused a decrease in the susceptibility to pepsin, from 38.1 to 27.4%, but the extent of hydrolysis reached after the successive pepsin−pancreatin treatment was a little higher (50.1 compared to 44.4%) (Figure 7B). These could have been due to conformational modifications provoked by the conditions used in the oxidation process (heating at 60 °C during 2 h).

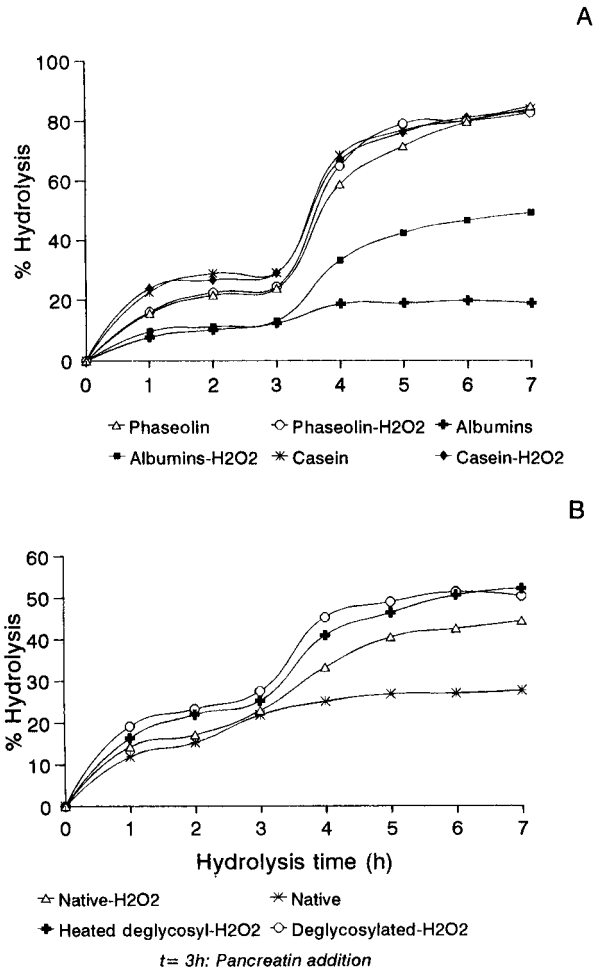

Figure 7. Effect of H_2O_2 oxidation of heated casein, phaseolin, and albumins (A) and of native, deglycosylated, and heated deglycosylated albumins (B) on pepsin−pancreatin digestibility.

Boonvisut and Whitaker (1976) also observed that performic acid oxidation of disulfide bonds had a marked effect on the pepsin−trypsin digestibility of soybean proteins.

As disulfide cleavage is more effective than heating in decreasing the resistance of albumins to proteolysis and trypsin inhibitory activity, bean processes using specific additives (e.g. cysteine or sodium bisulfite) could be used as a means of improving nutritional quality. However, heating would also be necessary in these processes to denature phaseolin so it is digestible.

Conclusions. The low digestibility of albumin proteins from beans seems to be multicausal. In the native state, steric hindrance due to glycosylation, compact structure involving disulfide bonds, and protease inhibitors are the main causes of low proteolysis. When heated, as happens during the processing of beans, albumins have, contrary to what was expected, a reduction of their digestibility, caused by a mechanism involving chemical reactions, such as disulfide bond formation, resulting in high-molecular mass aggregates resistant to proteolysis. Chemical dissociation of albumins, which eliminates trypsin inhibitor activity, reduces disulfide bonds, and unfolds the proteins, was not able to produce a digestion of more than 66%, demonstrating that attached carbohydrates also play a significant role in proteolysis susceptibility.

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