Solubility−Digestibility Relationship of Legume Proteins

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Protein solubility of raw and cooked faba bean, lentil, chickpea, and dry bean was tested in water and in NaCl in the pH range 1.0−13.0. The solubility of all legume proteins in water typically increased on both sides of pH 4.0. In NaCl, only solubility of raw dry bean proteins was improved. A marked reduction in protein solubility was observed after cooking of all legumes up to pH 10.0, where solubilization occurred, suggesting that it was dependent on deprotonation of lysine and arginine. Amino acid analysis showed that the protein fraction that retained solubility in water (pH 6.5) after cooking had a high amount of arginine and glutamic acid, low levels of hydrophobic amino acids, and, therefore, a much higher charge density than proteins in the whole flour. The SE-HPLC profiles indicated that water-soluble raw faba bean and lentil had main protein peaks of a higher molecular weight than those of dry bean or chickpea, thus suggesting a higher trend toward association. In vitro protein digestibility of faba bean and lentil, unlike that of chickpea and dry bean, was not improved upon cooking. The results indicate that, in addition to hydrophobic forces, basic residues are involved in the stabilization of heat-induced aggregates of legume proteins, possibly contributing to their low digestibility.

INTRODUCTION

Low nutritional value of legume proteins has long been ascribed to both the presence of a limiting amount of essential amino acids, methionine and cysteine, and the poor digestibility of proteins (Evans and Bauer, 1978; Sarwar and Peace, 1986), the latter a consequence of the effect of antinutritional seed compounds (trypsin inhibitors, lectins, phytate, tannins, and dietary fiber) (Jaffé, 1968). Because heat liability of most of the protease inhibitors has been demonstrated and evidences for only a limited effect of the heat-stable antinutritional factors, such as tannins and phytic acid, have recently been provided, intrinsic structural factors of legume seed proteins have been proposed as a major cause of the low digestibility (Semino et al., 1985; Deshpande and Nielsen, 1987; Carnovale et al., 1988; Deshpande and Damodaran, 1989; Liener, 1989; Carbonaro et al., 1992; Van der Poel et al., 1992). However, it is not yet clear whether the structural constraints that exist in the native protein can be completely overcome by heating (Chang and Satterlee, 1981; Deshpande and Damodaran, 1989).

Heating is responsible for protein denaturation, eventually followed by aggregation of the unfolded molecules, which results in loss of solubility. The mechanism of thermal aggregation of the oligomeric storage proteins, the main components of legume seeds, has been investigated by several techniques in model systems with isolated 11S and/or 7S globulins. Thermal denaturation involves an initial stepwise dissociation of subunits and a subsequent reassociation of only partially unfolded molecules with formation of either soluble or insoluble complexes (Kinsella et al., 1985). Studies on isolated soy glycinin (11S) and β-conglycinin (7S) have established that a preferential interaction between different globulin subunits imparts thermal stability: insoluble aggregates that selectively consisted of basic subunits are formed upon heating a solution of glycinin, but the

aggregation is inhibited in the presence of β-conglycinin because of the association between dissociated 7S and 11S subunits (German et al., 1982; Utsumi et al., 1984). Thermal behavior of other legume proteins has not been as thoroughly investigated. In addition, a close comparison of the association−dissociation behavior of legume proteins is often made difficult by differences in the experimental conditions.

In our previous study (Carbonaro et al., 1993) we aimed at clarifying the mechanism of interaction of protein components of dry bean (*Phaseolus vulgaris* L.) before and after cooking. We suggested the involvement of electrostatic interactions between oppositely charged residues (lysine/arginine and aspartic acid/glutamic acid) in the stabilization of bean protein complexes that are built up either before or after heating. In the present study, to gain a better knowledge of the association−dissociation properties of legume proteins and of the possible consequence of heat-induced aggregation on the protein quality, we compared raw and cooked dry bean, faba bean, lentil, and chickpea for protein solubility behavior under different conditions of pH and ionic strength, protein and amino acid composition, and in vitro protein digestibility.

MATERIALS AND METHODS

Dry seeds of faba bean (*Vicia faba* L.), lentil (*Lens culinaris* Medikus), chickpea (*Cicer arietinum* L.), and dry white bean (*Phaseolus vulgaris* L.) were obtained from the local market. Legumes were cooked after soaking in water at room temperature for 2 h (1:5, w/v, for faba bean and 1:4, w/v, for the other legume species). Legumes, with the soaking water, were autoclaved for 20 min at 120 °C (1 atm) and then freeze-dried. Raw and cooked legumes were ground in a Cyclotec 1093 Tecator (50 μm). Protein content was determined by the Kjeldahl method (AOAC, 1990).

Protein Solubility. Protein solubility of raw and cooked legumes was determined in the pH range 1.0−13.0 on a 3% (w/v) flour suspension either in water or in 0.5 N NaCl. The pH was adjusted by the addition of small amounts of 0.5 N HCl or 0.5 N NaOH. The suspension was shaken for 1 h at room temperature and centrifuged (15 min, 4000*g*). Protein

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content in the supernatant was measured by the method of Lowry et al. (1951). Protein solubility is expressed as percentage of the total protein content (N × 6.25) of the flour.

Amino Acid Analysis. Amino acid composition of the legume proteins was determined after hydrolysis under vacuum with 6 N HCl at 110 °C for 24 and 72 h. Amino acids were analyzed with a Beckman 118BL amino acid analyzer (Beckman Instruments, Fullerton, CA) and quantified after reaction with ninhydrin (Moore et al., 1958). Cysteine and methionine were determined as cysteic acid and methionine sulfone, respectively, after oxidation with performic acid (Schram et al., 1954). The charge density of proteins (CHGS) was calculated according to Kinsella et al. (1985).

Size-Exclusion High-Performance Liquid Chromatography (SE-HPLC). The SE-HPLC of legume proteins was performed on a Waters (Milford, MA) protein pak 300SW column of 7.5 × 300 mm. The column was fitted to a Waters M510 HPLC apparatus, equipped with a 510 pump model. Proteins were run in 50 mM sodium phosphate, pH 6.5, 0.1 M NaCl, at a flow rate of 0.5 mL/min. A standard curve was obtained by using molecular weight protein markers including blue dextran (2 000 000), thyroglobulin (669 000), apoferritin (443 000), β-amylase (200 000), alcohol dehydrogenase (150 000), albumin (66 000), carbonic anhydrase (29 000), and tryptophan (204). Legume proteins were extracted in water at a concentration of 5 mg/mL. The suspensions (pH 6.5) were stirred for 1 h at room temperature and centrifuged at 4000g for 15 min. Supernatants were diluted in column buffer at a final concentration of 2.5 mg/mL and filtered on a 0.45 μm Whatman filter (Millipore Corp., Bedford, BA) prior to injection. The eluate was monitored at 210 nm with an ultraviolet spectrophotometric detector (PAD Waters Model 996), and the chromatograms were analyzed quantitatively through appropriate software.

In Vitro Protein Digestibility. In vitro protein digestibility was obtained by the multienzyme method of Bodwell et al. (1980). Porcine pancreatic trypsin (type IX, 15 310 units/ mg of protein), bovine pancreatic chymotrypsin (type II, 48 units/mg of solid), porcine intestinal peptidase (P-7500, 115 units/g of solid), and bacterial protease (type XIV, 4.4 units/ mg of solid) (Sigma Chemical Co., St. Louis, MO) were used for the enzymatic digestion. For each sample, 63.8 mg of protein in 10 mL of distilled water was equilibrated at 37 °C and the pH was adjusted to 8.0 with 1 N NaOH; 1 mL of a three-enzyme solution in water (1.58 mg of trypsin, 3.65 mg of chymotrypsin, and 0.45 mg of peptidase) was added to the sample, and digestion was allowed to proceed for 10 min at 37 °C. After addition of 1 mL (1.48 mg) of protease solution, the digestion was continued for 9 min at 55 °C. The pH value was measured after a further 1 min at 37 °C and used to estimate the in vitro protein digestibility in the equation $Y = 234.84 - 22.56X$, where Y is the in vitro protein digestibility (%) and X is the pH of the suspension after 20 min digestion (Bodwell et al., 1980).

Statistical Analysis. Data were subjected to analysis of variance. The significance of the differences between means was obtained by Student's t-test ($p < 0.05$).

RESULTS AND DISCUSSION

Protein Solubility. Protein solubility of raw faba bean, lentil, and chickpea in water and in 0.5 N NaCl at different pH values is presented in Figure 1. For comparison, the same figure also shows the protein solubility profile previously obtained for dry bean (*P. vulgaris* L.) proteins (Carbonaro et al., 1993). A sharp minimum solubility of proteins from dry bean and chickpea (35% and 20%, respectively) was observed in water at pH 4.0, while faba bean and lentil proteins showed a broad range of minimum solubility (15−25%) between pH 3.5 and 5.0 (Figure 1A). On either side of these pH values solubility increased significantly ($p < 0.05$). Maximum values (over 80%) were observed for all legume proteins above pH 7.0 and below pH 2.0. The
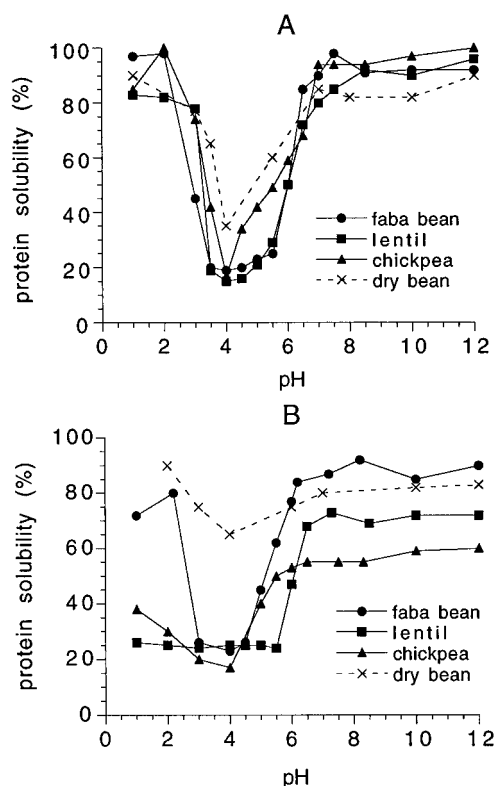

Figure 1. Protein solubility of raw legume species at different pH values in (A) water and (B) 0.5 N NaCl. The values are the mean of three determinations (variability coefficient < 6%).

profiles obtained closely resemble those already reported for several legume proteins (McWatters and Holmes, 1979; Ganesh Kumar and Venkataraman, 1980; Dench, 1982; Carnovale et al., 1988). The 0.5 N NaCl increased solubility of proteins from dry bean in the pH range from 2.0 to 7.0 (Figure 1B). On the contrary, it did not improve that of lentil and chickpea that was even significantly reduced ($p < 0.05$) at pHs far from the isoelectric point (from 1.0 to 3.0 and above 7.5). Proteins from faba bean had decreased solubility in NaCl at the acidic side of the pH range (1.0−3.0) but increased solubility from pH 5.0 to 6.0 ($p < 0.05$).

The protein solubility behavior observed in the presence of NaCl suggested that insolubilization of proteins occur through a different mechanism for dry bean compared to faba bean, lentil, and chickpea. In the dry bean, it is likely that electrostatic interactions involving ionizable amino acids with opposite charge are responsible for low protein solubility in water around the isoelectric pH. Shielding of charged groups by NaCl results in increased electrostatic repulsive force which will reduce protein aggregation and therefore improve solubility. On the other hand, the lower solubility in NaCl than in water observed for faba bean, lentil, and chickpea proteins suggests that hydrophobic forces rather than electrostatic interactions are the driving force for protein−protein association that leads to insolubilization.

A high degree of protein insolubilization either in water or in 0.5 N NaCl was determined after cooking for all legume species over most of the pH range tested (Figure 2). Above pH 10.0 protein solubility of faba bean, lentil, and chickpea in water was gradually recovered, and nearly total solubilization was obtained at pH 13.0 (Figure 2A). On the other hand, only a slight increase was obtained above pH 10.0 in the presence of NaCl (Figure 2B). Protein solubility of cooked dry bean
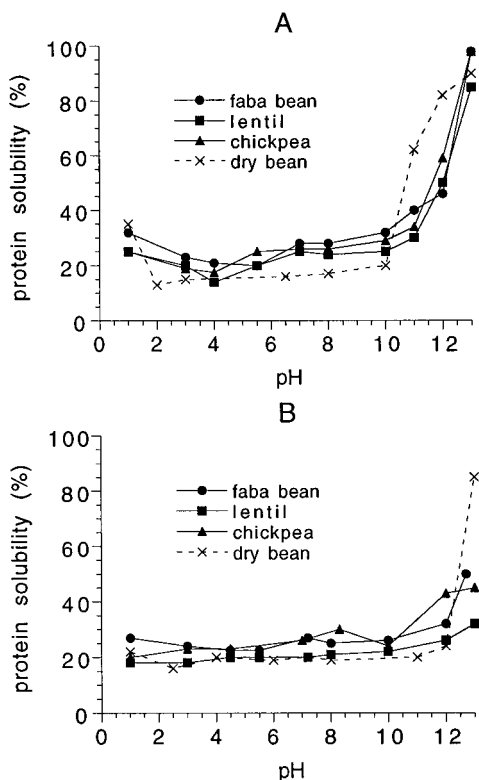
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Figure 2. Protein solubility of cooked legume species at different pH values in (A) water and (B) 0.5 N NaCl. The values are the mean of three determinations (variability coefficient < 8%).

increased sharply above pH 10.0 and 12.0 in water and in 0.5 N NaCl, respectively. We have previously provided evidence (Carbonaro et al., 1993) that solubilization of cooked dry bean proteins is dependent on loss of charge of lysine and arginine through deprotonation of the ϵ-amino and guanidino groups ($pK_a = 10.0$ and 12.5, respectively). Our present results suggest that a mechanism of association–dissociation mediated by basic residues is common among legume proteins. However, the solubilization in water for cooked faba bean, lentil, and chickpea proteins (Figure 2A) requires higher pH values than that for cooked dry bean proteins. At pH 12.0, only 45–60% protein from faba bean, lentil, and chickpea, while more than 80% protein from dry bean, were extracted ($p < 0.05$). This might suggest that deprotonation of arginine ($pK_a = 12.5$) plays a critical role in the solubilization of proteins from faba bean, lentil, and chickpea.

Amino Acid Composition. The legume proteins have similar amino acid profiles (Table 1). All of them contained low amounts of sulfur amino acids, methionine and cysteine, the first limiting amino acids in legumes. Methionine was present in a significantly ($p < 0.05$) higher amount in chickpea and dry bean than the other legume proteins, as already reported (Chavan et al., 1986; Marletta et al., 1992). Chickpea proteins also contained the highest amount of cysteine. This feature might in part explain the better protein quality of chickpea with respect to that of other legume proteins as it resulted from a comparison of several parameters of in vitro and in vivo protein quality (Chavan et al., 1986). All legume species were rich in aspartic acid/ asparagine and glutamic acid/glutamine that impart acidic character to legume proteins (Derbyshire et al., 1976). Proteins from faba bean, lentil, and chickpea contained a higher ($p < 0.05$) amount of arginine than

dry bean proteins, while the latter contained more histidine. No significant differences in the other polar charged amino acids (lysine, aspartic acid, and glutamic acid) between dry bean and the other legumes were detected (Table 1). Charged residues are mostly located on the surface of the protein molecule. Thus, the high levels of arginine in faba bean, lentil, and chickpea proteins may support the hypothesis of a specific role of this amino acid in the association–dissociation phenomenon of subunits of the oligomeric storage proteins.

Since protein solubility was found markedly decreased upon cooking for all the legume species tested (Figure 2), the percentage of acidic, basic, hydrophobic, and uncharged polar amino acids of the soluble protein fraction in water (pH 6.5) after cooking (about 15–25% of total legume proteins) was compared with that of the whole cooked flour (Table 2). Major changes in the amino acid composition (not shown) consisted in arginine and glutamic acid content that was increased ($p < 0.05$) in the soluble fraction of all legume species. Concomitantly, valine, isoleucine, leucine, and phenylalanine were present in much lower amounts in the soluble fraction than in the whole legume flour ($p < 0.05$). Complexively (Table 2), the percentage of acidic and basic amino acids was increased and that of hydrophobic amino acids was decreased in the water-soluble fraction of faba bean, lentil, chickpea, and dry bean while uncharged polar amino acids were unchanged. As a consequence, the proportion of charged groups at pH 6.5 (CHGS) of the soluble fraction was increased compared to that of the whole flour up to a value of 0.55–0.57 (Table 2), indicating a very high relative charge density of the protein fraction that retained solubility upon cooking.

SE-HPLC Analysis. The calibration curve for the SE-HPLC column is reported in Figure 3. The SE-HPLC separation of proteins in the water-soluble fraction of raw and cooked legumes (Figures 4–7) revealed a heterogeneous pattern for all the species examined and pointed out differences either among the species or within the same species before and after cooking. The chromatogram of proteins from raw dry bean (Figure 4) showed a main peak ($t_R = 14.0$ min) of MW 170 000 that accounted for 40% of the total peak area and likely corresponded to the trimeric form of phaseolin, the 7S storage protein of dry bean. Other minor components with retention times either lower or higher than 14 min were resolved. The first peak in the chromatogram ($t_R = 9.4$ min) was eluted at the void volume, indicating the presence of soluble protein aggregates of very high molecular weight.

The results of the SE-HPLC analysis (Figure 4) were in agreement with those we obtained previously by ultracentrifugal analysis of the water-soluble fraction of raw dry bean, where species with sedimentation coefficients of 1.9, 7.3, 9.4, and 17.2 S were observed (Carbonaro et al., 1993). However, the higher number of components resolved by the HPLC separation technique than by analytical ultracentrifugation indicated that some of the components that appeared as a single peak in the sedimentation velocity pattern consisted of a mixture of proteins, as already suggested (Yanagi et al., 1983; Carbonaro et al., 1993).

The HPLC elution profile of the protein fraction extracted from raw faba bean (Figure 5) and lentil (Figure 6) showed two peaks of MW 533 000 ($t_R = 11.9$ min) and MW 435 000 ($t_R = 12.4$ min) for faba bean and lentil, respectively, and a peak of MW 150 000 ($t_R = 14.4$

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Table 1. Amino Acid Composition of Faba Bean, Lentil, Chickpea, and Dry Bean Proteins (g/16 g of N)[a]

amino acid	faba bean	lentil	chickpea	dry bean
lysine	6.78 ± 0.13	6.84 ± 0.06	6.77 ± 0.18	6.93 ± 0.14
histidine	2.68 ± 0.05	2.56 ± 0.07	2.65 ± 0.06	3.03 ± 0.25
ammonia	1.96 ± 0.39	1.88 ± 0.32	1.75 ± 0.30	1.94 ± 0.36
arginine	9.24 ± 0.34	10.12 ± 0.12	9.20 ± 0.42	6.52 ± 0.33
aspartic acid	11.69 ± 0.16	13.24 ± 0.18	12.29 ± 0.18	12.03 ± 0.48
threonine	4.00 ± 0.21	4.03 ± 0.17	3.72 ± 0.09	4.13 ± 0.38
serine	5.28 ± 0.19	5.19 ± 0.17	5.30 ± 0.25	6.13 ± 0.41
glutamic acid	17.86 ± 0.30	16.70 ± 0.16	17.43 ± 0.24	16.88 ± 0.22
proline	4.15 ± 0.23	4.46 ± 0.16	4.11 ± 0.12	4.10 ± 0.27
glycine	4.38 ± 0.20	4.01 ± 0.28	4.13 ± 0.26	4.20 ± 0.20
alanine	4.46 ± 0.19	4.52 ± 0.20	4.21 ± 0.16	4.30 ± 0.24
half-cystine[b]	1.14 ± 0.16	1.04 ± 0.05	1.35 ± 0.12	1.07 ± 0.06
valine	5.12 ± 0.14	5.29 ± 0.02	4.79 ± 0.04	5.68 ± 0.13
methionine[b]	0.72 ± 0.03	0.83 ± 0.06	1.19 ± 0.05	1.28 ± 0.08
isoleucine	4.44 ± 0.19	4.48 ± 0.11	4.54 ± 0.13	4.76 ± 0.06
leucine	8.25 ± 0.24	7.79 ± 0.24	8.10 ± 0.11	8.41 ± 0.24
tyrosine	3.55 ± 0.11	3.06 ± 0.13	3.07 ± 0.48	3.65 ± 0.14
phenylalanine	4.43 ± 0.19	4.43 ± 0.19	5.87 ± 0.24	5.64 ± 0.28
tryptophan	ND[c]	ND	ND	ND

[a] Means and standard deviations of four replicates. [b] Corrected for 5% loss. [c] Not determined.

Table 2. Percentage of Amino Acids with Different Character and Proportion of Charged Residues (CHGS) of Proteins in Whole Cooked Flour and in the Water-Soluble Fraction (pH 6.5) of Faba Bean, Lentil, Chickpea, and Dry Bean[a]

amino acid	faba bean		lentil		chickpea		dry bean	
	whole	soluble	whole	soluble	whole	soluble	whole	soluble
acidic[b]	27.73	30.00	28.62	32.10	28.11	32.70	27.46	34.53
basic[c]	18.13	25.15	18.95	22.70	18.15	22.25	14.59	22.04
hydrophobic[d]	31.19	20.11	31.74	21.73	33.04	23.62	31.84	20.42
uncharged polar[e]	18.60	17.22	17.44	17.63	17.95	17.87	17.68	16.53
CHGS[f]	0.46	0.55	0.48	0.55	0.46	0.55	0.42	0.57

[a] Calculated from the mean of three determinations (SD < 0.8). [b] Aspartic acid, glutamic acid. [c] Lysine, arginine, histidine. [d] Alanine, isoleucine, leucine, methionine, phenylalanine, proline, valine. [e] Glycine, serine, threonine, tyrosine, cysteine. [f] Sum of acidic and basic amino acids minus ammonia/100.
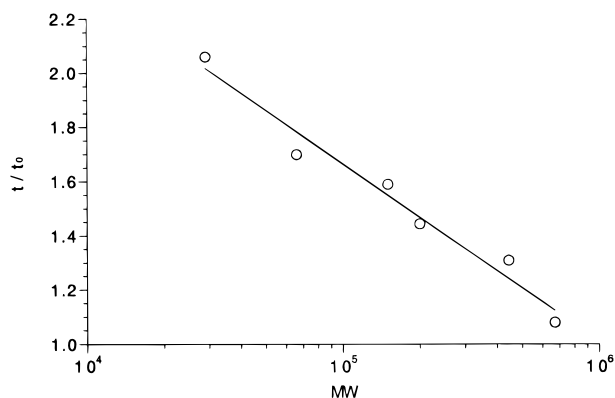

Figure 3. Calibration curve for the SE-HPLC column. t/t_0 is the ratio between the retention time of molecular weight protein markers and that of blue dextran.

min) for either species. The slow-eluting species (15–20% of the total area) (Figures 5 and 6) had a molecular weight corresponding to that indicated for the 7S protein (Derbyshire et al., 1976). However, the fast-eluting species (30% of the total area), especially for faba bean, had a molecular weight much higher than that reported for the 11S protein (about 350 000 and 375 000 for faba bean and lentil, respectively) (Derbyshire et al., 1976; Neves and Lourenco, 1995). The molecular weight of the main species resolved in the HPLC chromatogram of faba bean and lentil might arise from a higher association state of protein subunits than that described for the 11S protein.

The HPLC separation of proteins from chickpea (Figure 7) showed that the main protein fractions had MWs of 59 000 (t_R = 16.3 min), 18 000 (t_R = 18.58 min), and 3000 (t_R = 22.2 min) (each peak accounting for

about 20% of the total area) and, therefore, much lower compared to that calculated for the main peaks resolved in the chromatogram of the other legume species. In addition, a lower amount of aggregated material ($V_e = V_0$) was found.

After cooking, legume protein components were shifted to higher retention times and major peaks appeared at t_R > 15 min (MW < 83 000) (Figures 4–7). The peaks eluted at the void volume were increased in the chromatogram of the soluble fraction extracted after cooking of the dry bean (Figure 4) and chickpea (Figure 7), while they were decreased in that of cooked faba bean (Figure 5) and lentil (Figure 6). This difference may suggest a high solubility of macrocomplexes that are formed upon heating of proteins from dry bean and chickpea, unlike faba bean and lentil.

The results indicated that the fraction that retained solubility after cooking of legumes is mainly composed of protein components with a molecular weight lower than that of the species extracted from raw legumes, in line with the indications provided previously by ultracentrifugal analysis of dry bean proteins (Carbonaro et al., 1993). The protein fraction that is extracted in water (pH 6.5) from cooked legumes has been shown by amino acid analysis (Table 2) to have a very high charge density. On the basis of available data on the amino acid composition of subunits 11S and 7S storage proteins (Derbyshire et al., 1976; Kinsella et al., 1985), either acidic subunits of the 11S globulin or subunits of the 7S globulin could be present in the soluble fraction. Among components of soy proteins, basic subunits of the 11S globulin have been shown to have the highest trend to make up insoluble aggregates following heat-induced dissociation (German et al., 1982).
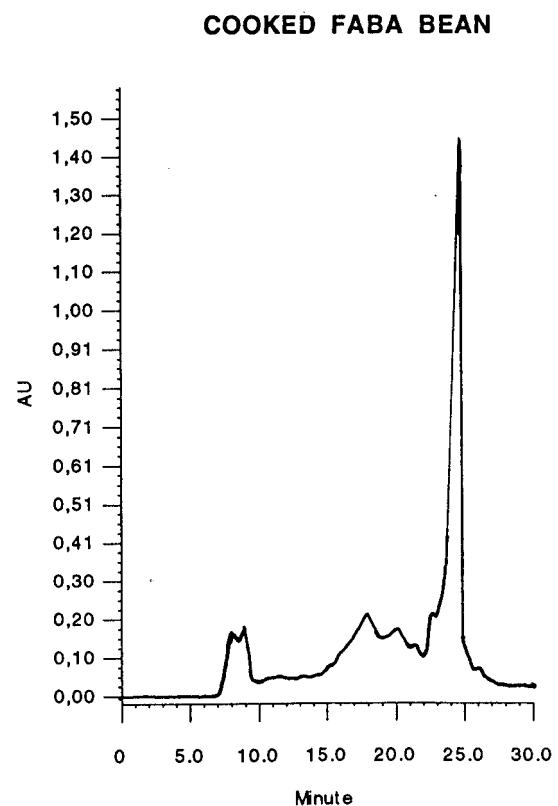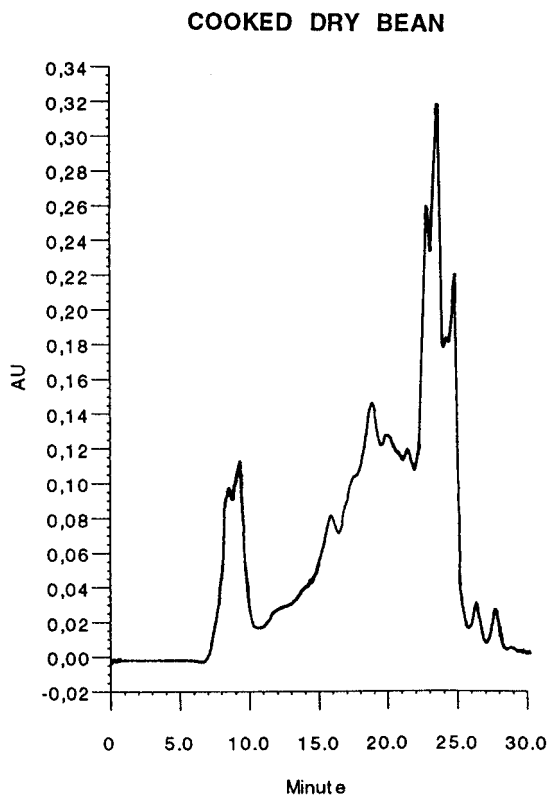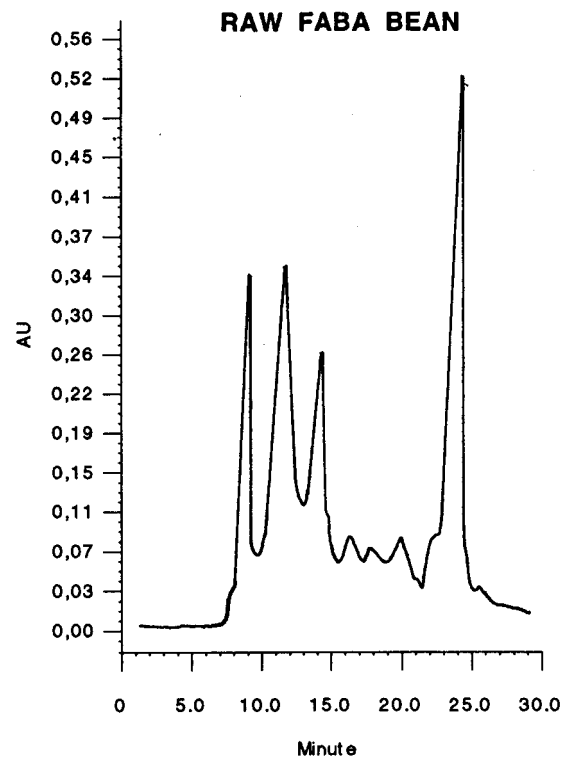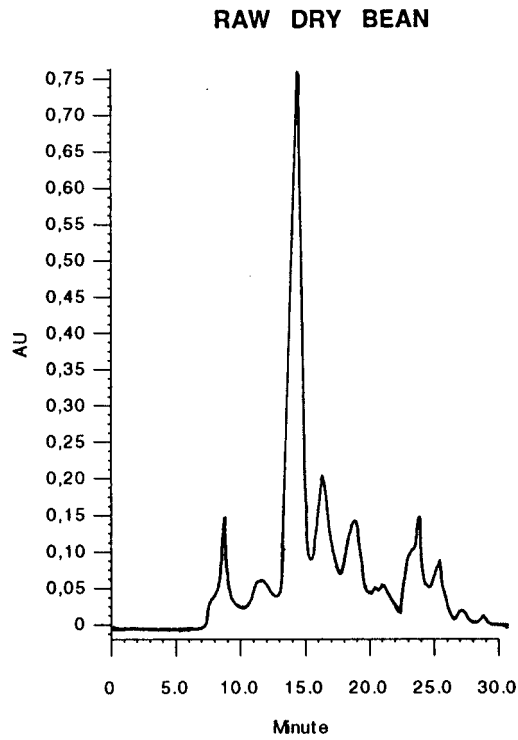
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Figure 4. SE-HPLC of the protein fractions extracted with water from raw and cooked dry bean.

Protein Digestibility. In vitro protein digestibility of raw and cooked legume species is presented in Table 3. The lowest value (73.8%) was observed for the raw dry bean, in agreement with the high content in protease inhibitors of *P. vulgaris* seeds (Marquez and Lajolo, 1981; Sgarbieri and Whitaker, 1981). Only digestibility of proteins from chickpea and dry bean was improved upon heating. On the contrary, protein digestibility of lentils was unchanged and that of faba bean was even impaired by the cooking treatment.

Figure 5. SE-HPLC of the protein fractions extracted with water from raw and cooked faba bean.

Values of cooked samples were in the range established on the basis of in vivo determinations (Sarwar et al., 1989; Friedman, 1996). Chickpea proteins presented the highest digestibility (Table 3), as already found (Chavan et al., 1986).

The increase in protein digestibility observed after cooking has generally been attributed to protein denaturation and inactivation of protease inhibitors (Carnovale et al., 1988; Friedman, 1996). However, unlike
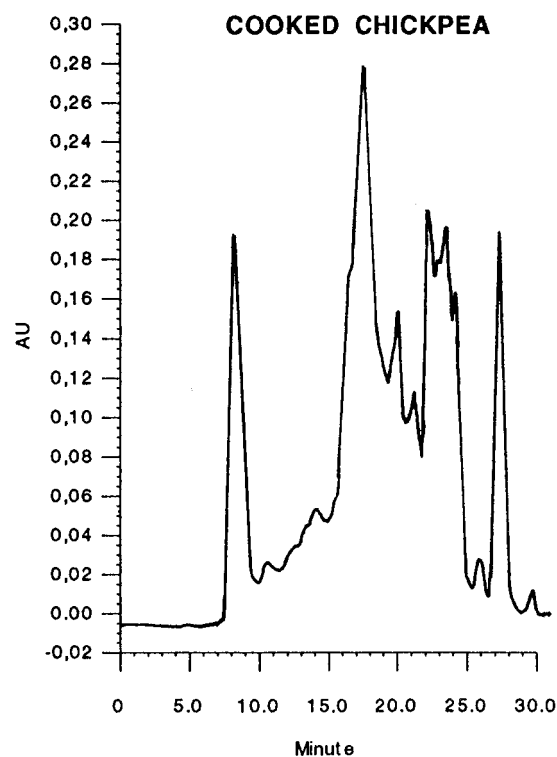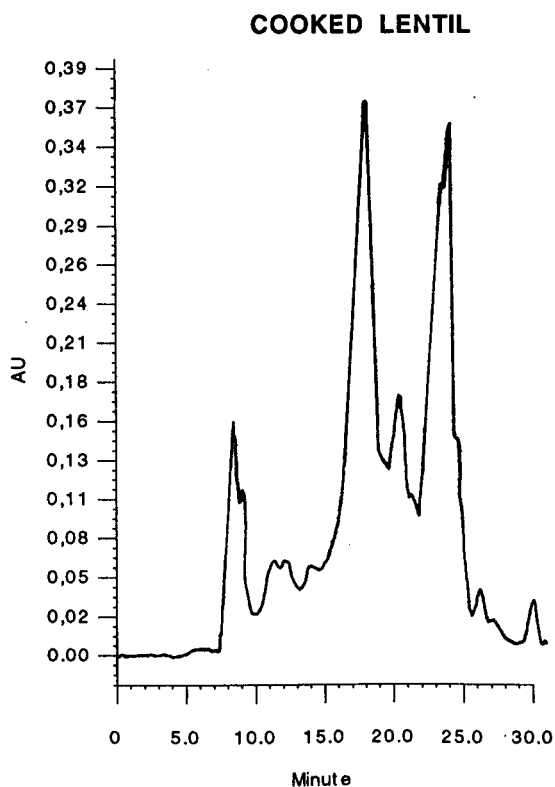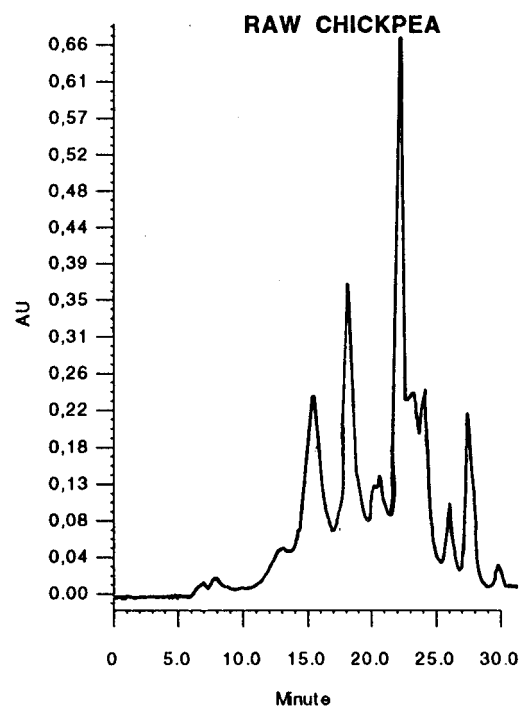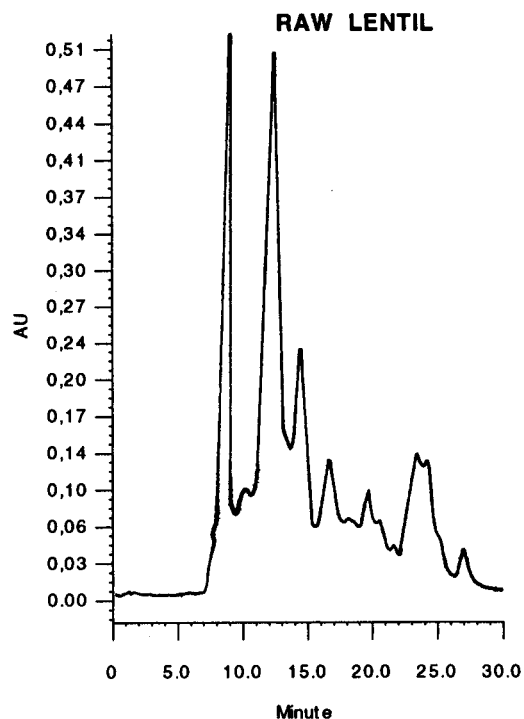
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Figure 6. SE-HPLC of the protein fractions extracted with water from raw and cooked lentil.

dry bean, faba bean, and lentil, chickpea seeds have been found to contain low levels of protease inhibitors as well as other antinutritional factors (Chavan et al., 1986; Carnovale et al., 1988). Therefore, the improvement in digestibility observed can be mainly ascribed to heat-induced denaturation of chickpea proteins, and it might indicate that enhanced accessibility of susceptible sites to proteolysis (that is typical of denatured proteins) is not compromised by protein aggregation. On the other hand, inactivation of protease inhibitors has occurred upon cooking for faba bean, lentil, and dry bean

Figure 7. SE-HPLC of the protein fractions extracted with water from raw and cooked chickpea.

Table 3. In Vitro Protein Digestibility of Legume Species before and after Cooking[a]

legume species	raw	cooked
faba bean	83.07 ± 1.82	79.57 ± 1.92*
lentil	82.50 ± 0.91	81.66 ± 1.84
chickpea	78.28 ± 1.43	83.16 ± 1.72*
dry bean	73.76 ± 0.90	80.53 ± 1.20*

[a] Means and standard deviations of three replicates. Raw versus cooked: significantly different *$p < 0.05$.

(Carnovale et al., 1988; Marletta et al., 1992). Therefore, the lack of improvement in digestibility of faba bean and lentil may be related, at least in part, to

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protein aggregation that is consequent to the thermal treatment. This would imply a different localization of amino acid residues specific for protease action in the different legume species. In this respect, accessibility of lysine, arginine, phenylalanine, tryptophan, and tyrosine would be of primary importance because of the restricted specificity of trypsin and chymotrypsin.

In a comparative in vitro study on digestibility of native and heated legume storage proteins by various proteases (Nielsen et al., 1988), remarkable differences among the degradation protein patterns have been pointed out. Phaseolin, vicilin, glycinin, and β-conglycinin were found to be resistant to complete proteolysis by trypsin in the native state, with phaseolin showing the slowest degradation rate. Although all proteins were more readily digested by trypsin after heating, complete degradation was not achieved even after 60 min incubation. Moreover, unlike phaseolin, the other legume proteins were found to be less completely digested by a variety of proteases in the denatured state than in the native state. This is in agreement with the finding of Deshpande and Damodaran (1989) that some conformational changes induced by heating on pea vicilin, but not phaseolin, confer to it a resistance to proteolysis. In the same study it has also been demonstrated that a complete randomization of the protein structure is not achieved after either thermal or chemical denaturation, indicating a high degree of protein stability.

The hypothesis that the chemical structure of legume proteins could be responsible for an adverse effect on growth and nitrogen metabolism by affecting the rate of release of lysine and arginine has been suggested by the results of in vivo studies. Rubio et al. (1995) compared growth and nitrogen metabolism of rats fed diets in which isolated legume globulins or lactalbumin was incorporated as the only source of protein. The plasma urea levels were found to be higher in the group of rats fed legume proteins than in the lactalbumin-fed group, indicating increased protein degradation. Comparison of plasma concentration of lysine and arginine after 9 h of feeding between the group fed a lupin diet and the control group revealed that only in the latter group were both amino acids increased, but in the other group, arginine was increased and lysine was decreased.

We showed (Table 3) that the low digestibility that is typical of legume proteins is not completely overcome (as for bean and chickpea) or is even decreased (as for faba bean) by heat processing, possibly as a consequence of protein aggregation. This might result in a slower rate of absorption of amino acids (or peptides) than required for an efficient protein synthesis, thus determining an amino acid imbalance in the tissues. Our analysis of the protein solubility behavior of different legume species suggests a general role of lysine and arginine in the association−dissociation properties of legume proteins before and after cooking.

Involvement of charged protein residues in the stabilization of the oligomeric structure of 7S *P. vulgaris* protein has recently been demonstrated unequivocally by X-ray crystallographic analysis (Lawrence et al., 1994). Although crystallographic information is still lacking for 11S legume globulin, alignment of phaseolin (7S) and soybean glycinin (11S) sequences, performed on the basis of structural data, allowed to establish that 11S and 7S globulins have a very similar tertiary structure. Many of the conserved residues in the 7S and 11S sequences have been found to correspond to those forming part of the intermonomer packing in the 7S protein (Lawrence et al., 1994). In spite of the high degree of homology detected at a level of primary structure between 7S and 11S proteins, the presence of "variable regions" has also been demonstrated, especially in the 11S globulin sequence. These regions contain a number of charged residues and are, therefore, supposed to be located at the surface of the protein (Wright, 1987). Consequently, some of the differences observed in the physicochemical properties of legume proteins could depend on the existence of these variable domains. The differences observed in the pH-dependent protein solubilization in water and in the presence of NaCl between raw/cooked dry bean and the other legume species (Figures 1 and 2) may reflect specific association−dissociation properties of the prevailing seed globulin, 7S vicilin-type or 11S legumin-type, respectively.

Although HPLC analysis (Figures 4−7) revealed differences in protein patterns of the components extracted from the various legume species before and after cooking, thus suggesting a specific association−dissociation behavior, the protein fraction that retained solubility after cooking of legumes is characterized by a very similar amino acid composition, typical of proteins with an unusually high charge density (Table 2). The relative high digestibility of arginine and glutamic acid from in vivo studies (Sarwar et al., 1989) might be partially explained by the high recovery of these amino acids in the soluble fraction of cooked legume flour.

Solubility of the complexes that are built up upon heating of legume proteins has been found to be governed by rather specific interactions between the dissociated protein subunits. The absence of aggregation of glycinin (11S) in whole soy protein is due to a preferential association, exerted through electrostatic interactions, between conglycinin (7S) and dissociated glycinin basic subunits. Such an interaction gives rise to a soluble complex of very high molecular weight (more than 1 million) (Utsumi et al., 1984). However, it is not known whether digestibility of heated soy proteins is dependent on the extent of aggregation.

The results of this study suggest that the aggregation mediated by basic residues of legume proteins that occurs upon heating can be one reason for the low digestibility of cooked legume proteins. Other in vitro and in vivo research is currently being performed to further confirm this hypothesis. Progress in the understanding of the relationship between association−dissociation properties of legume proteins before and after heating and protein digestibility may provide important suggestions for improving protein quality through breeding or molecular biology techniques.

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