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Protein Solubility of Raw and Cooked Beans (*Phaseolus vulgaris*): Role of the Basic Residues

Marina Carbonaro,† Paola Vecchini,‡ and Emilia Carnovale*,†

National Institute of Nutrition, via Ardeatina 546, 00178 Rome, Italy, and Department of Biochemical Sciences, University of Rome "La Sapienza", 00185 Rome, Italy

The protein solubility of raw and cooked beans (*Phaseolus vulgaris*) was tested in the pH range 1.0–13.0. The protein solubility of the raw beans in water increased, up to 90–100%, on both sides of pH 4.0, while in 0.5 M NaCl it was higher than in water in the pH range 2.0–7.0. A reduction to 15–20% in protein extraction was observed, both in water and in NaCl, after bean cooking up to pH 10.0, where solubilization sharply occurred. Protein solubilization of cooked beans in water, as analyzed over 24 h at increasing pH (3.3–12.0), showed that the higher the pH, the higher the rate of protein solubilization, but a marked increase at pH 12.0, and in 8 M urea, was detected. Protein solubilization of the raw beans in water increased by lysine acetylation and succinylation or by arginine modification with phenylglyoxal. Only succinylation had a positive effect on the protein solubilization of cooked beans. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and ultracentrifugation of the protein water-extracted at pH 6.5 showed a higher protein dissociation in cooked than in raw beans and further dissociation in cooked bean proteins at pH 12.0. The involvement of the basic residues in the association–dissociation of raw and cooked bean proteins is suggested.

INTRODUCTION

Alterations in legume protein structure that occur upon heating are reflected by changes in nutritional, physicochemical, and functional properties (Bressani and Elias, 1974; Kinsella et al., 1985). Among these, nitrogen solubility is decreased after heat treatment (Lo et al., 1968; McWatters and Holmes, 1979b; Dench, 1982), possibly as the consequence of protein denaturation and subsequent aggregation. Legume proteins are characterized by a high content in polar amino acids with opposite charge (aspartic and glutamic acid on one side and lysine and arginine on the other side) (Duke, 1981). Therefore, it is reasonable to suppose that these amino acids are involved in the association–dissociation phenomena of protein subunits that are at the basis of the solubility properties of legume oligomeric proteins.

In the attempt to clarify the mechanism of the interaction of legume protein components, chemical modification of specific residues, both in native and in processed proteins, appears to be a powerful technique. However, attention to chemical modifications has been focused as a tool for improving functional properties of food proteins. Among chemical modifications, acylation of lysine residues (acetylation or succinylation) appears by far the most widely used. Actually, lysine acylation has been reported to increase nitrogen solubility and surface properties of legume proteins (Franzen and Kinsella, 1976; Beuchat, 1977; Narayana and Narasinga Rao, 1984). Subsequently, the improvement in the functional properties has been correlated with modifications in the physicochemical properties, which often reflect drastic structural changes of the proteins, especially when high levels of lysine residues are chemically blocked (Kim and Rhee, 1989; Narayana and Narasinga Rao, 1991). Conformational changes in the structure of legume storage proteins, which occur during acylation, typically involve a loss of the

* Author to whom correspondence should be addressed.
† National Institute of Nutrition.
‡ University of Rome "La Sapienza".

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quaternary structure through a step-by-step dissociation of the oligomeric protein into subunits (Schwenke et al., 1986, 1990). Similar changes in the association state of legume proteins can be induced when the pH is raised from the acidic to the basic range (Sun et al., 1974; Yanagi, 1983).

The association–dissociation properties of legume proteins are expected to be changed by heat processing, resulting in a decrease in protein solubility. However, in spite of the marked reduction in the solubility of several legume proteins observed upon heating (Lo et al., 1968; McWatters and Holmes, 1979b; Narayana and Narasinga Rao, 1982), most of the available data on the mechanism of the thermal dissociation and association of protein subunits, responsible for the solubility alterations, comes from studies carried out on soybean (German et al., 1982; Sathe et al., 1989). Moreover, we are not aware of any reports on the effect of chemical modifications on the solubility behavior of heat-processed legume proteins, which could prove to be useful for clarifying the mechanism of thermal aggregation.

Therefore, the aim of the present work was to analyze the solubility properties of proteins extracted from two varieties of raw and cooked beans (*Phaseolus vulgaris*) in different conditions of pH and ionic strength. The effect of chemical modifications of the basic protein residues, lysine (by acetylation and succinylation) or arginine (with phenylglyoxal), on the protein solubility behavior was examined. Preliminary characterization of the protein fraction, water-extracted from raw and cooked beans, was carried out by SDS–PAGE and ultracentrifugation.

MATERIALS AND METHODS

Dry common beans (*P. vulgaris*) with white (var. Cannellini) or brown (var. Borlotti) seed coats were obtained from the local market. Cooked beans were prepared after soaking in water (1:3 w/v) at room temperature for 2 h. The beans, with the soaking water, were autoclaved (20 min, 120 °C) and then freeze-dried. Raw and cooked beans were ground in a Cyclotec 1093 Tecator (50 μm). Protein content (N × 6.25) was determined according to the Kjeldahl method (AOAC, 1984).

Protein Solubility. Protein solubility of raw and cooked white/brown beans was detected in the pH range 1.0–13.0 on a 3% (w/v) flour suspension either in water or in 0.1 and 0.5 M NaCl. Adjustments of pH were carried out by the addition of small amounts of 0.5 N HCl or 0.5 N NaOH. The suspension was left shaking for 1 h and centrifuged (15 min, 4000 *g*). Protein solubility was estimated in the supernatant according to the method of Lowry et al. (1951) and expressed as percentage of the total protein content (N × 6.25) of the flour.

Protein solubilization of cooked beans in water at different pH values (3.3, 7.5, 8.5, 10.2, and 12.0) was tested over 24 h. The suspension (3% w/v), at each pH value, was incubated for various time intervals with stirring. Aliquots of the incubation mixture were centrifuged and assayed for protein content according to the method of Lowry et al. (1951). Protein solubilization of raw beans in water was followed at pH 7.5 and 8.5.

Lysine Modification. Lysine residues of raw and cooked bean proteins of both varieties were modified by acylation with acetic and succinic anhydride. A 3% (w/v) aqueous flour suspension was adjusted to pH 7.5 with 2 N NaOH. Acetic or succinic anhydride (0.6 g/g of protein) was added to the suspension over 30 min, and the pH was maintained between 7.0 and 8.0 by addition of 2 N NaOH. When the pH attained a constant value of 7.5, the suspension was incubated for 24 h with stirring. The effect of lysine modification on the protein solubilization of raw and cooked beans was analyzed over 24 h, as described above. The time courses obtained were compared with those detected for the corresponding untreated proteins at pH 7.5. Acylated flours were then dialyzed overnight against deionized water to remove the excess of anhydride and freeze-dried. The extent of acylation was quantified by the decrease in the "available lysine" content with respect to the untreated flour proteins after reaction with 1-fluoro-2,4-dinitrobenzene (FDNB) (Carpenter, 1960).

Arginine Modification. Phenylglyoxal was used to modify arginine residues of raw and cooked beans of both varieties. Phenylglyoxal (0.6 g/g of protein) was dissolved in ethanol and added to a 3% (w/v) aqueous flour suspension at pH 8.5. The pH was assessed at 1 h and readjusted to the initial value with 2 N NaOH. The suspension was incubated for 24 h, with stirring. The effect of arginine modification on the protein solubilization of raw and cooked beans was tested over 24 h as described above and compared with the solubilization for the corresponding untreated proteins at pH 8.5. The suspension was then dialyzed against deionized water and freeze-dried. The degree of chemical modification of arginine residues was estimated by amino acid analysis after a 20-h hydrolysis in 6 N HCl, in vacuo, at 110 °C. Hydrolysates were analyzed with a Beckman 118BL amino acid analyzer, after reaction with ninhydrin (Moore et al., 1958). The extent of arginine modification was measured by the decrease in the arginine content with respect to the unmodified proteins. Comparison of amino acid compositions between native and modified proteins revealed no significant differences in the levels of amino acids different from arginine.

All of the experiments were carried out at room temperature in duplicate or triplicate and the means of determinations are reported (variability coefficients <8.5%). The statistical significance of the differences between mean values was determined by the analysis of variance.

SDS–PAGE. SDS–PAGE was performed according to the method of Laemmli (1970) on a slab gel of 13% acrylamide. Phosphorylase *b* (97 400), bovine serum albumin (66 200), ovalbumin (42 700), carbonic anhydrase (31 000), soybean trypsin inhibitor (21 500), and lysozyme (14 400) (Bio-Rad) were used as standard proteins. The gel was stained with Coomassie brilliant blue R-250.

Ultracentrifugal Analysis. Sedimentation velocity experiments were carried out on a Spinco Model E analytical ultracentrifuge equipped with a temperature control unit (RTIC). Sedimentation coefficients were determined from the maximum ordinate of the schlieren peaks and were reduced to $s_{20,w}$ by standard procedures (Schachman, 1959). The runs were performed at 196500 *g* (t = 8.25 °C) at a protein concentration of 7 mg/mL. Before the run, the proteins water-extracted at pH 6.5 and 12.0 were dialyzed against 0.1 M phosphate buffer, pH 6.5, and 0.1 M borax–NaOH, pH 10.8, respectively.

RESULTS AND DISCUSSION

Protein Solubility. The protein solubility of the raw and cooked white/brown beans at different pH values in water and NaCl solutions is shown in Figure 1. Protein content (N × 6.25), on dry basis, was 26.6% for the raw white bean, 28.0% for the cooked white bean, 23.0% for the raw brown bean, and 23.2% for the cooked brown bean. The percentage of protein extracted from the two bean varieties in the different conditions was very similar, in line with our previous data showing close analogy between the proteins of these different seed-colored beans (Marletta et al., 1992). The protein solubility of the raw beans at various pHs in water (Figure 1A) resulted in profiles similar to those previously reported for several legume proteins, such as common and winged bean (Hang et al., 1970b; Sathe and Salunkhe, 1981; Dench, 1982; Narayana and Narasinga Rao, 1982), soybean (McWatters and Holmes, 1979a), and faba bean (Carnovale et al., 1988). All profiles were characterized by a minimum solubility around pH 4.0. On both sides of this pH value the protein solubility increased up to a maximum (90–100%), detected at the extreme values of the pH range. The protein solubility of the raw beans was significantly higher ($p < 0.05$) in 0.1 M NaCl than in water at pH 4.0 (Figure 1B) and in 0.5 M NaCl than in 0.1 M NaCl in the pH range 2.0–7.0 (Figure 1C). NaCl has already been shown to

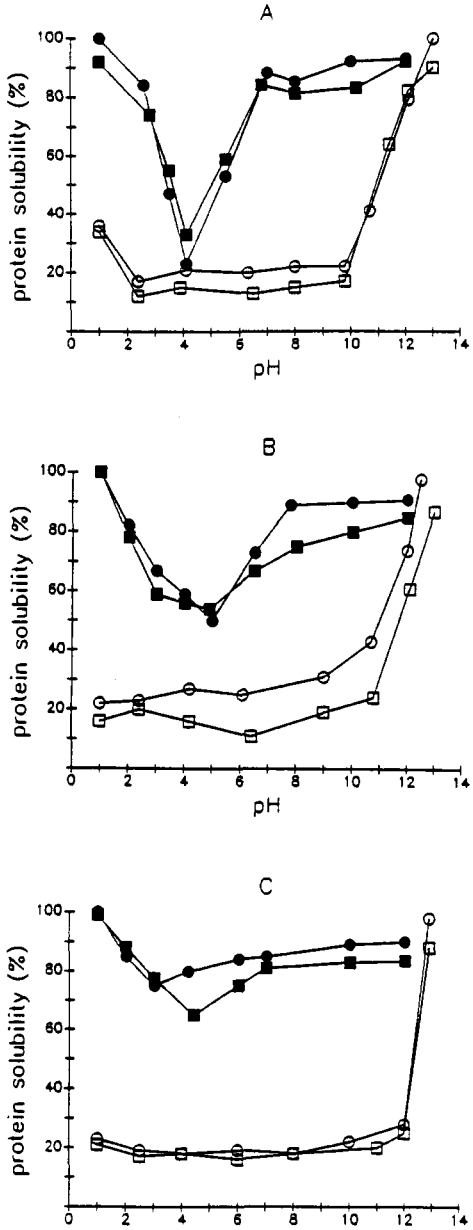

Figure 1. Protein solubility of the raw and cooked beans at different pH values in (A) water, (B) 0.1 M NaCl, and (C) 0.5 M NaCl: (■) raw white bean; (●) raw brown bean; (□) cooked white bean; (○) cooked brown bean.

improve the extractability of legume proteins, and similar profiles for the percentages of proteins extracted from different legumes by increasing concentrations of NaCl have been reported (Hang et al., 1970a,b; McWatters and Holmes, 1979a; Dench, 1982). This trend possibly reflects the ionic strength effect in decreasing intermolecular electrostatic interactions between oppositely charged groups. The amount of protein extracted from the white and brown beans after cooking was reduced both in water and in the NaCl solutions up to pH 10.0–12.0, where more solubilization occurred (Figure 1A–C). Neutralization of charged groups by NaCl did not markedly increase the protein solubility of cooked beans and, especially at the highest concentration used, even caused a broadening of the pH range where low protein solubility was observed (Figure 1B,C). It appears that electrostatic interactions between external charged residues are not the main force responsible for the low protein solubility of cooked beans. Other molecular forces (hydrogen bonds, hydrophobic interactions) may be involved in the aggregation of bean

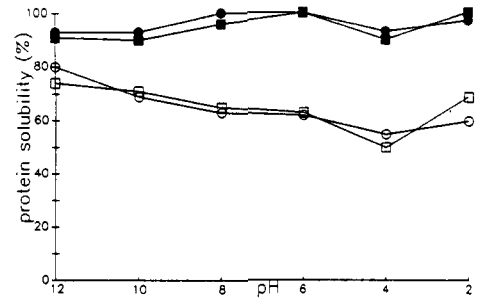

Figure 2. Reversibility of the raw and cooked bean protein solubilization: (■) raw white bean; (●) raw brown bean; (□) cooked white bean; (○) cooked brown bean. Protein solubility in water was measured in the pH range 12.0–2.0 after 1 h of extraction at each pH value.

protein components, as expected when thermal denaturation (unfolding) takes place.

The overall levels of protein extracted from our cultivars of common bean (*P. vulgaris*) after cooking were lower than those reported for winged bean (*Psophocarpus tetragonolobus*) (Dench, 1982; Narayana and Narasinga Rao, 1982). Some differences in the heat-processing conditions might account for the reduced protein solubility of common bean with respect to winged bean. However, it should be stressed that *Phaseolus* and *Psophocarpus* native proteins have already been shown to present dissimilar physicochemical properties: proteins extracted from the common bean showed a higher trend to make up associated (fast sedimentating) forms, at low pH values, than other storage-related proteins, such as those of winged bean and soybean (Yanagi, 1983). Low levels of protein (albumin and globulin) solubility (ca. 20% at neutral pH) have also been reported to follow high-temperature extrusion of other varieties of *P. vulgaris* (Gujska and Khan, 1990, 1991). Moreover, extractablity of proteins from red kidney beans (*P. vulgaris*), at pH 4.0, has been found to be only 20% when temperatures from 35 to 55 °C were used for extraction (Hang et al., 1970b).

The reversibility of the raw and cooked bean protein solubilization in water was tested, and the results are reported in Figure 2. When the pH of the white or brown bean suspension was gradually changed from the basic (pH 12.0) toward the acidic (pH 2.0) side of the range, the trend of the protein solubility of both raw and cooked beans appeared markedly different. The percentage of protein extracted, below pH 10.0 and 12.0 for raw and cooked beans, respectively, was much higher than that previously obtained at the corresponding pH values (Figure 1A). In addition, a significant ($p < 0.05$) minimum solubility at pH 4.0 was always observed, probably indicating some protein precipitation through electrostatic interactions around the isoelectric pH. This solubility behavior might indicate that some conformational changes have occurred at high pH values. This could involve the transition from an associated form (soluble for raw beans but insoluble for cooked beans) to a dissociated form (soluble for both raw and cooked beans) of the protein. The higher percentage of protein solubility of raw beans and the gradual decrease of protein solubility of cooked beans between pH 12.0 and 2.0 might reflect a higher stability of "native" with respect to "denatured" bean protein components. pH-dependent association–dissociation phenomena of globulins from several legume species have been widely described. The dissociation to low S-value components of purified globulins of *P. vulgaris*, which occurs above pH 10.0, has been found to be partially reversible (Sun et al., 1974; Yanagi, 1983).

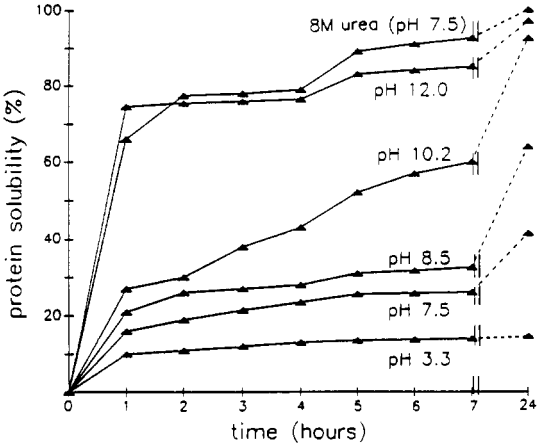

Figure 3. Protein solubilization of the cooked white beans at different pH values in water and at pH 7.5 in 8 M urea.

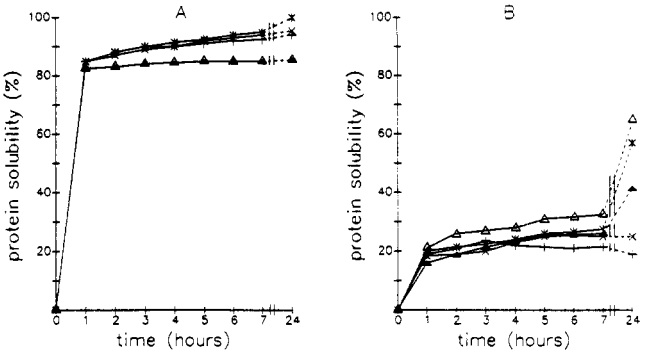

Figure 4. Effect of lysine and arginine chemical modifications on protein solubilization of the raw (A) and cooked (B) white beans in water: (*) succinylation; (×) acetylation; (+) modification with phenylglyoxal. Protein solubilization of the raw (A) and cooked (B) beans in water at pH 7.5 (▲) and 8.5 (△) is also shown.

Protein solubilization of cooked beans in water at different pH values (3.3–12.0) and in the presence of 8 M urea, pH 7.5, is shown in Figure 3. Similar results were obtained with the cooked white and brown bean varieties. It is readily evident that the higher the pH of the solution, the higher the rate of cooked bean protein solubilization. At acidic pH values protein solubilization was slowest and was not substantially improved by extraction time longer than 1 h. However at pH 7.5, 8.5, and 10.2 high levels of protein were solubilized by increasing extraction time up to 24 h. Finally, at pH 12.0, a marked increase in the rate of protein solubilization was observed and most proteins were extracted after 1 h (Figure 3). The trend obtained at the highest pH was similar to that of cooked bean proteins extracted at pH 7.5, in 8 M urea. Since basic protein residues undergo loss of positive charge through deprotonation at high pH values (the pK_a values of lysine and arginine being 10.0 and 12.5, respectively), the possibility existed that these amino acids were involved in the pH-dependent association–dissociation of protein subunits that may be the basis of raw and cooked bean protein solubilization. However, the additional involvement of hydrogen bonds and hydrophobic interactions in the aggregation of cooked bean components was suggested by the positive effect of 8 M urea on protein solubilization at neutral pH (Figure 3) (Privalov, 1979). The same molecular forces have been reported to be responsible for the heat formation of gels from soybean 7S globulins (Utsumi and Kinsella, 1985).

Effect of Chemical Modification of the Basic Residues on the Protein Solubility Behavior. The effect of lysine and arginine chemical modification on the protein solubilization of raw and cooked beans was therefore analyzed (Figure 4). No differences were noted in the extent of modification between the white and brown bean varieties: 80% and 75% of lysine residues were acetylated, while 70% and 60% were succinylated in raw and cooked bean proteins, respectively. The extent of arginine modification was slightly lower, being 55% in raw beans and 40% in cooked beans of both varieties. The degree of acetylation and succinylation of the raw (white and brown) bean proteins was similar to that reported for winged bean proteins when modified by the same anhydride concentration (Narayana and Narasinga Rao, 1984). The lower percentage of lysine and arginine that undergoes chemical modification in cooked with respect to raw bean proteins may be related to the high level of protein insolubility found after cooking. Aggregation of protein components, subsequent to denaturation, would make a number of the basic protein residues unavailable to the modifying agent.

The degree of protein solubilization of the raw beans was significantly ($p < 0.05$) increased by chemical modification of the basic residues, at incubation time exceeding 5 h (Figure 4A). This result is in line with previous reports on the positive effect of lysine acylation on the functional properties of several food proteins (Franzen and Kinsella, 1976; Beuchat, 1977; Narayana and Narasinga Rao, 1984). Dissociation of acetylated and succinylated proteins into lower molecular weight components (Schwenke et al., 1986; Narayana and Narasinga Rao, 1991) could explain the increase in the protein solubility. On the other hand, succinylation had a positive effect ($p < 0.025$) only on the protein solubilization of cooked beans obtained after 24 h. A significant inhibition of the long-term (24 h) protein solubilization, with respect to the untreated samples at the same pH values, was observed for both lysine acetylation ($p < 0.01$) and arginine modification ($p < 0.005$) (Figure 4B). Irrespective of the percentage of protein solubilized, the highest protein solubilization was achieved for both raw and cooked beans after succinylation (introduction of hydrophylic groups), followed by acetylation and by arginine modification with phenylglyoxal (introduction of hydrophobic groups) (Figure 4).

Our findings on the protein solubility properties of raw and cooked beans together with the results on the effect of chemical modification of the basic residues on the protein solubility behavior suggest that lysine and arginine have some role in the association–dissociation phenomena of bean protein components. The positive effect of neutralization of charge on lysine and arginine side chains on the protein solubilization of raw beans (Figure 4A) indicates that the association of native protein components is enhanced by interactions of oppositely charged residues, i.e., lysine/arginine and aspartic/glutamic acid. This is also consistent with the salting-in effect of NaCl reported previously (Figure 1). The high content of these amino acids in the bean proteins (Marletta et al., 1992) would represent the condition for this mechanism of interaction to occur effectively. Such a mechanism might be common among legume proteins, as all of these are characterized by a very high amount of both acid and basic amino acids (Duke, 1981).

After cooking, the solubility properties are expected to change due to protein denaturation. Actually, not only the protein solubility profile (Figure 1) but also the effect of the chemical modifications on the bean protein solubilization (Figures 3 and 4), appeared to be very different. Indeed, only the high increase in the net negative charge

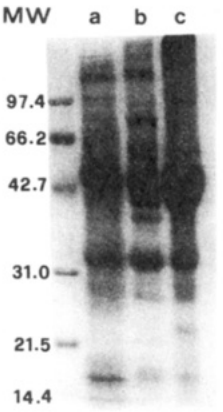

Figure 5. SDS–PAGE of the protein fraction water-extracted from raw white beans at pH 6.5 (a) and from cooked white beans at pH 6.5 (b) and 12.0 (c). Three hundred micrograms of protein was loaded on each lane.

of the proteins by succinylation improved the long-term protein solubilization. In this case aggregation may have been overcome by repulsive interactions between charged carboxyl groups. On the contrary, neutralization of the positive charge of the basic residues by introduction of hydrophobic groups led to a stabilization of the insoluble form of the protein, as reflected by the lack of the long-term protein solubilization (Figure 4B). A decrease in stability at high levels of acetylation has also been described for different native proteins, including chymotrypsinogen (Cupo et al., 1982), soy glycinin (Kim and Rhee, 1989), and cottonseed and winged bean proteins (Rahma and Narasinga Rao, 1983; Narayana and Narasinga Rao, 1984). As far as the molecular forces responsible for cooked bean protein aggregation are concerned, it seems likely that hydrophobic interactions (and perhaps hydrogen bonds) are involved, since a rapid solubilization was observed in 8 M urea (Figure 3). Loss of solubilization occurred with the introduction of hydrophobic groups into the proteins (Figure 4B). Nevertheless, the aggregation forces appear to be completely suppressed at high pH values, where deprotonation of basic protein residues typically takes place (Figure 1).

Preliminary Characterization of the Protein Extracted from Raw and Cooked Beans. *SDS–PAGE.* Figure 5 shows the SDS–PAGE pattern of the water-soluble fraction obtained from raw white beans at pH 6.5 (a) and from cooked white beans at pH 6.5 and 12.0 (b and c, respectively) after 1 h of extraction. The electrophoretic profile of the water-soluble fraction of the raw and cooked bean proteins extracted at pH 6.5 appeared to be characterized by a series of bands in the molecular weight range 14 400–110 000 (Figure 5a,b). Among these, the band pattern MW 43 000–51 000, generally ascribed to phaseolin subunits (Bradbear and Boulter, 1984; Deshpande and Nielsen, 1987a), was evident. The overall electrophoretic pattern was similar to that previously reported for the water-soluble protein fraction of other varieties of *P. vulgaris* (Deshpande and Nielsen, 1987b). Raw bean proteins (Figure 5a) contained a higher number of high molecular weight components (above 43 000) than cooked bean proteins (Figure 5b). Thus, it was supposed that some of the high molecular weight components of raw beans were dissociated by heating. This was also supported by an increase in the intensity of some bands below 43 000 MW in the cooked beans (Figure 5b). Dissociation of high molecular weight components during extrusion at high temperature has also been described for proteins (globulin and albumin) from navy and pinto beans (*P. vulgaris*)

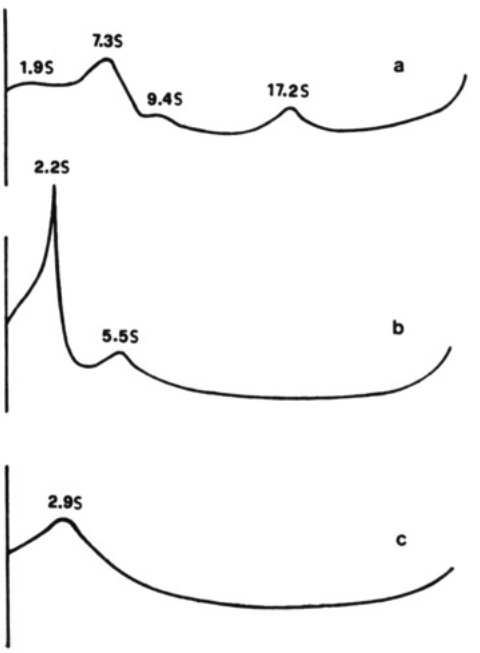

Figure 6. Ultracentrifuge pattern of the protein fraction water-extracted from raw white beans at pH 6.5 (a) and from cooked white beans at pH 6.5 (b) and 12.0 (c). Protein concentration was 7 mg/mL.

(Gujska and Khan, 1991). The protein fraction extracted from cooked beans at pH 12.0 (Figure 5c) showed the loss of most of the components with MW >43 000, concomitantly to the increase in the intensity of the 43 000–51 000 bands. It seems that, at pH 12.0, cooked bean protein solubilization is achieved by further dissociation of bean protein components. However, the trend of these subunits to aggregate might be monitored by the appearance of very high molecular weight components, which remained at the top of the running gel (Figure 5c). It may be that some of the protein components, dissociated at pH 12.0, underwent reaggregation under the prevailing pH conditions of the stacking gel (pH 6.8). Partial changes in the heat-induced interactions of soybean protein subunits have already been reported to occur during electrophoresis (Utsumi et al., 1984).

Ultracentrifugal Analysis. The sedimentation–velocity pattern of the water-soluble fraction extracted from the raw white bean at pH 6.5 (Figure 6a) showed a main component with a sedimentation coefficient of 7.3 S and three minor 1.9-, 9.4-, and 17.2-S components. Our results are in agreement with those available from characterization data of proteins similarly extracted from other varieties of *P. vulgaris* (Yanagi, 1983). After cooking, changes in the sedimentation profile of the water-soluble fraction extracted at pH 6.5 were observed (Figure 6b). These consisted of the disappearence of the 17.2- and 9.4-S components, while the 7.3-S protein was shifted to a lower S value (5.5 S) and decreased in concentration. The main species in the sedimentation pattern was represented by a very slow component, with a sedimentation coefficient of 2.2 S. Thus, the bean proteins appeared to be more dissociated after cooking, in agreement with the results of the SDS–PAGE (Figure 5a,b). At pH 12.0 (Figure 6c) the water-extracted protein fraction showed a single slow species, with $s_{20,w} = 2.9$ S, indicating that all bean proteins dissociated into low molecular weight components at high pH values. It was possible that the 2.9-S component consisted of a mixture of many proteins, since several bands (with high electrophoretic mobility) were seen in the SDS–PAGE pattern of cooked bean proteins extracted at pH

12.0 (Figure 5c). A similar behavior has been described for the 2.5-S native protein fraction of winged bean (Yanagi et al., 1983).

These results suggest that extensive aggregation of *P. vulgaris* proteins, which results in the loss of solubility, occurs upon cooking after dissociation (and denaturation) of protein subunits, following a mechanism similar to that already described for the basic subunits of soybean 11S protein (German et al., 1982). Insoluble protein aggregates are held together by hydrophobic interaction (and hydrogen bonds) but are again resolved into soluble components above pH 10.0. The mechanism of the rapid cooked bean protein solubilization observed at high pH values is probably quite complex. It can be reproduced in urea, a strong dissociating agent, but not when charge neutralization of lysine and arginine is achieved by chemical modification. It is possible that a rapid dissociation of cooked bean proteins requires the loss of charge of most basic residues, a condition that is realized only at high pH values. This explanation would be also consistent with the observation that our modified insoluble proteins still maintain the capacity to undergo high-pH solubilization, although at a low rate (data not shown). This would also imply a role for internal basic residues in further stabilizing protein aggregates through electrostatic interactions, which would be enhanced in the nonpolar environment. In this situation the loss of charge of internal lysine and arginine, allowing the formation of hydrogen bonds between the ϵ-amino and guanidino groups of aggregated protein components and water molecules, might represent the first step for protein solubilization to be achieved. The presence of some of the basic residues in the interior of the protein molecule might also explain the failure in total blocking of lysine ϵ-amino groups, even at high excess of the modifying agent, often reported for native legume proteins (Schwenke et al., 1990). Moreover, it should be noted that the compact structure of legume storage proteins, such as phaseolin or vicilin, has been found not to be completely unfolded after heating (Deshpande and Damodaran, 1989). It is proposed from the results of this study that the basic protein residues play a role in stabilizing bean protein structure, both in native and in heat-induced conformations.

Aggregation mediated by basic residues subsequent to cooking may have an effect on the rate and extent of digestive degradation of bean proteins, especially in the intestinal tract, where trypsin and chymotrypsin are present. In addition, essential nutrients, such as amino acids and minerals, might be trapped in protein aggregates as a biounavailable form. Actually, iron has been reported to be included within large protein and peptide aggregates (Schnepf and Satterlee, 1986; Yoshida, 1989).

Further studies are in progress to further clarify the role of lysine and arginine in the interaction of native and heat-processed bean protein subunits, as well as the consequence of such a mechanism of aggregation on bean protein quality and digestibility and mineral availability.

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