Biochemical Characterization and *in Vitro* Digestibility of the Major Globulin in Cashew Nut (*Anacardium occidentale*)†

Shridhar K. Sathe,*,‡ K. W. Clara Sze-Tao,‡ Walter J. Wolf,§ and Bruce R. Hamaker||

Department of Nutrition, Food and Movement Sciences, Florida State University, Tallahassee, Florida 32306-1490, National Center for Agricultural Utilization Research, 1815 North University Street, U.S. Department of Agriculture, Peoria, Illinois 61604-3999, and Department of Food Science, Smith Hall, Purdue University, West Lafayette, Indiana 47907

The major globulin (anacardein) in cashew nut (*Anacardium occidentale*) is a 13S globulin. The globulin is not a glycoprotein and is composed of at least two major types of polypeptides with estimated molecular weights in the range 18000−24000 and 30000−37000. The globulin has $A^{1\%}_{280nm}$ of 9.88, 10.56, 9.68, and 9.59 in distilled water, 0.5 M NaCl, 0.02 M sodium phosphate buffer pH 7.5, and 0.02 M Tris-HCl buffer pH 8.1, respectively. The Stokes radius of the globulin was 57 ± 3.2 Å (n = 17). The isoelectric pH (pI) of the globulin was in the pH range 6.2−7.2. Hydrophobic, uncharged polar, acidic, and basic amino acids respectively accounted for 36.4, 19.88, 25.3, and 18.4% of the total amino acids. Sulfur amino acids and threonine were respectively the first and second limiting amino acids in the purified globulin. Among the proteinases tested, pepsin was the most efficient in hydrolyzing the globulin *in vitro*.

Keywords: *Cashew; nuts; globulin; protein; in vitro digestibility*

INTRODUCTION

Tree nuts enjoy global acceptance and are valued for their sensory and nutritional attributes. Typically, tree nuts are high in lipids (45−70%, w/w) and proteins (20−25%, w/w) and are therefore energy-rich foods. Among tree nuts, cashew nuts command a premium price partly because the demand far exceeds the supply. The cashew nut supply is limited because growing cashew trees is not only expensive but also difficult. The cashew tree is native to the northeastern Brazil (Morton, 1961): the areas from southern Mexico to Peru, and the West Indies (Morton, 1993). It was introduced to the tropics of the old world, especially the west coast of India and the east coast of Mozambique, by the Portuguese and Spanish in order to halt the soil erosion (Morton, 1993). The name "cashew" is derived from the Brazilian word *acaju*, usually abbreviated as *caju*. The cashew tree belongs to the Anacardiaceae family. This family also includes some other important food species such as the mango (*Mangifera indica* L.) and pistachio (*Pistacia vera* L.) as well as some notoriously poisonous plants such as the poison ivy (*Rhus toxicodendron* L.) and poison sumac (*R. vernix* L.) (Morton, 1961).

Depending on the variety, cashew nut weight may vary considerably (range, 3−32 g) [Morton, 1993]. Cashew nuts provide 7.76 kcal/g, 21% protein, and 48% lipids and are a good source of several minerals such as the phosphorus, potassium, magnesium, and iron (Fetuga *et al*., 1974). Cashew nut chemical composition and nutritive value have been investigated (Piva *et al*., 1971; Barroso *et al*., 1973; Fetuga *et al*., 1974; Maia *et al*., 1975, 1976; Filho and Ainouz, 1977; Nagaraja and Krishnan Nampoothiri, 1986; Nagaraja, 1987a,b; Nagaraja, 1989; Toschi *et al*., 1993). On the basis of these studies, cashew nuts have high nutritional quality with respect to the fatty acid composition [C18:1 (oleic) and C18:2 (linoleic) respectively account for 73.4 and 11.9% of triglycerides] as well as protein digestibility.

Cashew nut proteins are primarily soluble in aqueous media (Damodaran and Sivaswamy, 1936; Sathe, 1994). Earlier studies have shown that a single globulin dominates the total protein composition (Damodaran and Sivaswamy, 1936; Nagaraja, 1987a; Sathe, 1994) of the cashew nut and may account for up to 50% of the total nitrogen (Damodaran and Sivaswamy, 1936). Nagaraja (1987a) did not find any varietal differences in the electrophoretic patterns of salt extractable proteins. He observed only two bands in native gels, and based on the densitometric scans (Figure 2A in his paper) of these two, only the faster moving band was the major protein. Sathe (1994) reported that this faster moving band (the major globulin) had a complex polypeptide composition depending on how it was isolated (Figures 2−5 in his paper). That this globulin is also water soluble (provided sufficient ionic strength is present), was noted by Damodaran and Sivaswamy (1936). They termed this protein anacardein and reported that the globulin could be easily purified by simple sodium chloride (10%) extraction followed by precipitation at pH 4.8 (0.05 N acetic acid was used for pH adjustment). Ventura and Filho (1964) used potassium phosphate−sodium chloride buffer [0.029 M KH_2PO_4, 0.021 M K_2HPO_4, NaCl (10% w/v)], pH 7.0, ionic strength (μ) = 1.80 to extract the globulin and then used successive precipitations at pH 4.8 followed by salting out with ammonium sulfate at pH 7.0 between 60 and 70% saturation to purify it. They obtained a 94% pure globulin based on ultracentrifugal analysis. Ventura and Filho also reported that the globulin was a 12.85S protein with intrinsic viscosity and partial specific volume (at 20 °C) of 0.027 $dL \cdot g^{-1}$ and 0.737 $mL \cdot g^{-1}$,

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* Author to whom correspondence should be addressed [fax (850) 644-0700; e-mail ssathe@mailer.fsu.edu].

‡ Florida State University.

§ U.S. Department of Agriculture.

|| Purdue University.

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respectively. They estimated the molecular weight of the globulin to be 238 000 (Archibald method), 250 000 (light-scattering method), and 260 000 (by sedimentation−intrinsic viscosity method) and reported that the protein had a frictional ratio (f/f_0) of 1.07.

As a part of long-term ongoing investigations on tree nuts, we have isolated the cashew major globulin. This paper reports cashew globulin preparation using Sephacryl S300 HR column chromatography as well as some of the biochemical properties of the globulin that have not been reported earlier.

MATERIALS AND METHODS

Materials. Unprocessed cashew nuts were purchased at a local grocery store. Electrophoresis chemical sources were the same as reported earlier (Sathe, 1993). Other chemicals were from either Sigma Chemical Co., St. Louis, MO, or Fisher Scientific Company, Norcross, GA.

Methods. *Preparation of Flour.* Defatted cashew nut flour was prepared as described earlier (Sathe, 1994).

Globulin Purification. The defatted flour was extracted with 2 M NaCl containing 0.001 M NaN_3 (flour:solvent ratio, 1:10 w/v) with constant magnetic stirring for 3 h at 4 °C and centrifuged (12000*g*, 10 min, 4 °C). The supernatant was filtered through glass wool and the residue reextracted with 10 more volumes of the same solvent for an additional 1 h at 4 °C with constant magnetic stirring. The slurry was then centrifuged (as above) and the supernatant filtered through glass wool and combined with the first supernatant. The combined supernatants were then dialyzed against distilled water at 4 °C for 36−48 h (4−6 changes of water, dialysis tubing MW cutoff 6000−8000), lyophilized, and stored at −20 °C in an air-tight container until further use. The lyophilized protein was dissolved in 0.02 M Tris [tris(hydroxymethyl)-aminomethane]-HCl buffer (pH 8.1) containing 0.1 M NaCl and 0.001 M NaN_3 in a minimum volume (typically 100 mg of protein/mL of buffer). The protein solution was centrifuged (13600*g*, 5 min, 25 °C), and the supernatant was loaded on to a Sephacryl S300 HR column (1.6 × 96.5 cm). The column equilibrium and elution buffer was 0.02 M Tris-HCl pH 8.1 containing 0.1 M NaCl and 0.001 M NaN_3. The column was equilibrated with 4 volumes of this buffer prior to loading the protein sample. The column flow rate was maintained at 20 mL/h with a peristaltic pump, and fractions were collected every 15 min. The column elution was monitored by measuring the absorbance of effluent at 280 nm as well as by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE). Fractions containing the major globulin were pooled, dialyzed against distilled water (36−48 h, 4−6 changes, dialysis MW cutoff 6000−8000), lyophilized, and stored at −20 °C in an air-tight container until further use. All protein extractions, centrifugations, column chromatography, and dialysis were done in a cold room (4 °C).

Protein Determination. Soluble protein content was determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard protein. A standard curve was prepared in an appropriate solvent for every assay.

Ultraviolet Spectrum. Ultraviolet spectra for the globulin were determined using a Perkin-Elmer λ 3 UV/vis spectrophotometer. Appropriate solvent was used as a blank in each case. The protein concentration was 0.787 mg/mL in each case.

Ultracentrifugal Analysis. Samples were analyzed in a Beckman Model E analytical ultracentrifuge at 20−25 °C with a 30 mm double-sector cell at 48 000 rpm. The solvents used were 2 M NaCl and potassium phosphate−sodium chloride buffer (0.033 M K_2HPO_4, 0.0026 M KH_2PO_4, 0.4 M NaCl) pH 7.6, $\mu = 0.5$ (also referred to as standard buffer). Compositions were estimated by enlarging the ultracentrifuge patterns and measuring the area under each peak. Areas were corrected for radial dilution, and compositions are expressed as percentages of the total area.

Electrophoresis. Nondenaturing and nondissociating (NDND)−polyacrylamide gel electrophoresis (PAGE) was done according to Andrews (1986), and the SDS−PAGE was done by the method of Fling and Gregerson (1986). Details for the NDND−PAGE and the SDS−PAGE were the same as described earlier (Sathe, 1993). To detect the presence of glycoprotein, gels were stained with periodic acid−Schiff's (PAS) stain (Dubray and Bezard, 1982). Isoelectric focusing (IEF) was done using a flat bed electrophoresis apparatus. The IEF gels were 5.4% (w/v) monomer acrylamide gels of 0.5 mm thickness and contained 8 M urea. Ampholines used for these gels were pH 4−6 plus pH 3−10 (1:1.5 v/v). The cashew globulin was dissolved in 0.01 M Tris-HCl buffer, pH 8.0, at a concentration of 1.8 mg of globulin/200 μL of buffer and 60 μL of the globulin solution was loaded on to the IEF gels.

Amino Acid Analysis. Amino acid composition was determined using the Waters Pico-Tag column amino acid analyzer (Waters Chromatography Division, Milford, MA). Typically, 0.5 mg of protein was hydrolyzed in 600 μL of 6 N HCl in the presence of nitrogen (18 h, 110 °C), and 4 μL of the hydrolyzate was injected for analysis. Tryptophan content was determined by the colorimetric method of Spies and Chambers (1948).

In Vitro Digestibility. Protein *in vitro* digestibility was determined using TPCK−trypsin (bovine pancreas, type XIII, batch number 66F-8135), TLCK−chymotrypsin (bovine pancreas, type VII, batch number 75F-8025), and pepsin (porcine stomach mucosa, batch number 88F-8010), all of which were from Sigma Chemical Co., St. Louis, MO. The substrate protein (cashew globulin) was dissolved in distilled water (10 mg/mL) to prepare the stock solution. For moist heat treatment (when required), aliquots of the globulin stock solution were heated (boiling water bath, 100 °C, 30 min) in closed plastic microcentrifuge tubes (1.5 mL capacity) and cooled (running tap water at ∼15 °C) rapidly to room temperature. The digestion buffer for TPCK−trypsin and TLCK−chymotrypsin was 0.05 M Tris-HCl pH 8.1 containing 0.01 M $CaCl_2$ and 0.05 M HCl for pepsin. Final digestion conditions were (1) substrate protein concentration, 1 mg/mL; (2) substrate protein:enzyme ratio, 100:1 (w/w); (3) digestion temperature, 37 ± 1 °C; (4) digestion time, variable (up to 30 min); and (5) digestion volume, 200 μL. At the end of the digestion period, an equal volume of SDS−PAGE sample buffer [0.05 M Tris-HCl pH 6.8, 1% SDS, 0.01% bromophenol blue, 30% glycerol, and 2% β-mercaptoethanol (β-ME)] was added to the digestion mixture (in case of pepsin, 190 μL of SDS−PAGE sample buffer + 10 μL of 10 M NaOH), and the samples were heated for 5 min in a boiling water bath, cooled immediately (ice−water mixture), and electrophoresed immediately after the last digestion in a set (30 min sample) was completed. For "0" time samples, the SDS−PAGE buffer was added prior to the enzyme addition. All digestions were initiated by enzyme addition. Appropriate protein and enzyme controls were run simultaneously.

All experiments were done at least in duplicate, and averages were reported.

RESULTS AND DISCUSSION

Globulin Purification. Typically, we obtained ∼6 g of lyophilized crude globulin from 30 g of defatted cashew flour (20% w/w of the defatted flour) which is consistent with the reported yield of 18.5% (w/w of the defatted flour) by Damodaran and Sivaswamy (1936). This crude globulin fraction is primarily composed of one major globulin [see Figure 2, lane 4, in Sathe (1994)] and several minor components (based on NDND−PAGE data). The crude globulin typically elutes off the Sephacryl S300 column in to two major and three minor peaks (Figure 1). The first major peak (tubes 13−17 in Figure 1) contains fractions that have a "milky" appearance and could be possibly due to the presence of large aggregates and large molecular species distinctly different from the major globulin (anacardein). The second major peak (tubes 19−25 in Figure 1) is primarily composed of the major globulin which on rechromatography on the same column elutes as a single peak (data not shown). The crude globulin has similar behavior

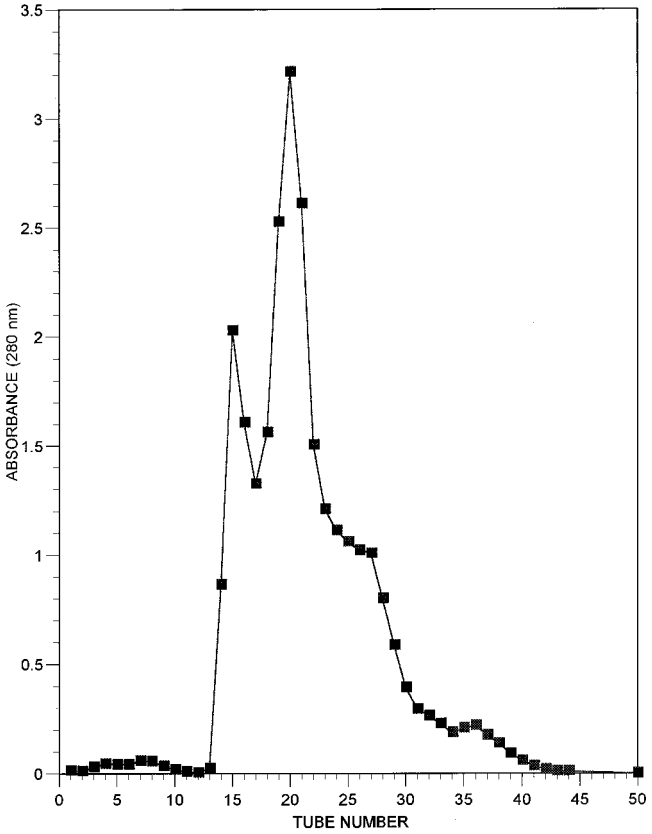

Figure 1. Elution profile of cashew nut crude globulin off Sephacryl S300 HR (1.6 × 96.5 cm) column. The equilibrium and elution buffer was 0.02 M Tris-HCl buffer pH 8.1 containing 0.1 M NaCl and 0.001 M NaN_3. Fractions were collected every 15 min, and the column flow rate was 20.6 mL/h. The crude globulin (215 mg) dissolved in 2 mL of 0.02 M Tris-HCl buffer pH 8.1 containing 0.001 M NaN_3 was loaded on to the column. Fractions containing the globulin (tube numbers 19−25) were pooled.

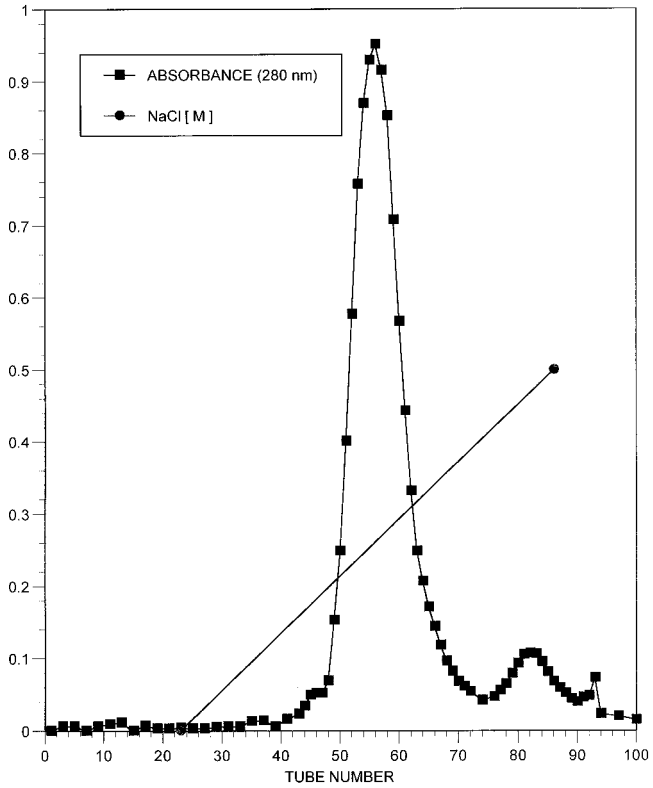

Figure 2. Elution profile of the cashew nut globulin off DEAE DE-53 anion exchange column (2.6 × 21.2 cm). The equilibrium buffer was 0.02 M Tris-HCl pH 8.1, and the elution buffer was equilibrium buffer containing 0−0.5 M NaCl linear gradient (500 mL each) followed by 2 M NaCl. Fractions were collected every 15 min, and the column flow rate was 61.9 mL/h. The globulin off the Sephacryl S300 HR column (∼200 mg) in 150 mL equilibrium buffer was loaded onto the column. Fractions containing the globulin (tubes 45−64) were pooled.

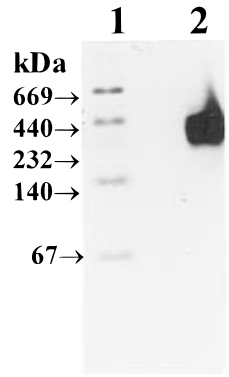

Figure 3. NDND−PAGE (3−30% linear gradient acrylamide gel, acrylamide:bis = 37:1 w/w) for the cashew globulin (lane 2, protein load was 50 μg). Gels were run at a constant current (15 mA/gel) with cooling (15 °C) provided by running tap water. Protein standards (Pharmacia HMW Kit) were as follows (from top to bottom, lane 1): thyroglobulin (669 kDa), ferritin (440 kDa), catalase (232 kDa), lactate dehydrogenase (140 kDa), and albumin (67 kDa).

on Sepharose CL-6B (5 × 44.5 cm column using 0.02 M sodium phosphate buffer pH 7.5 containing 0.1 M NaCl and 0.001 M NaN_3, data not shown). This major globulin also elutes as a single peak off an anion exchange column (DEAE DE-53; Figure 2) and travels as a single band on NDND−PAGE (Figure 3). At lower protein loads on NDND−PAGE, we have observed multiple bands with similar electrophoretic mobilities (data not shown). This observation (charge heterogeneity) is consistent with the data earlier reported by Sathe (1994). This charge heterogeneity is further confirmed by the isoelectric focusing (IEF) data (Figure 4). The IEF analysis of the major globulin suggests the presence of at least seven bands within the narrow pI range of 6.5−7.2. On the basis of three independent criteria (gel filtration and anion exchange column chromatography, and the NDND−PAGE), our major globulin preparation appeared to be homogeneous. Staining the gels (both NDND−PAGE and the SDS−PAGE) with PAS indicated that the globulin did not contain carbohydrate and was therefore not a glycoprotein (data not shown).

When this globulin preparation was subjected to the analytical ultracentrifuge analysis (Figure 5 and Table 1), the globulin was primarily composed of 66−67% 13S component with 13−18% 3S, 12−14% 6S, and 3−7% 16S. This is contrary to the findings of Ventura and Filho (1964) who reported a 94% homogeneous anacardein (12.85S component) using a series of two precipitations at pH 4.8 followed by an ammonium sulfate fractionation between 60 and 70% saturation. Our ultracentrifuge data on total extractable proteins (Table 1) in both 2 M NaCl and the standard buffer are however consistent with those of Ventura and Filho (1964) in that the total extractable proteins were primarily composed of the 13S (47−54%) and the 3S (31−37%) components, with 6S (5−9%) and 16S (7−9%) constituting minor amounts. The ultracentrifuge data in our investigations suggest that a portion of 3S fraction may associate with the major globulin and/or the major globulin undergoes dissociation in to 3S and

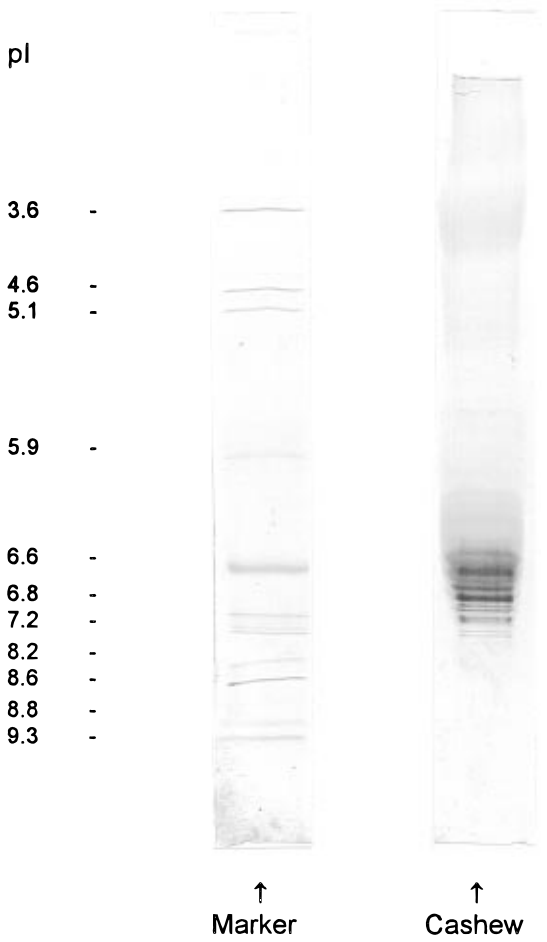

Figure 4. Isoelectric focusing of the cashew globulin in polyacrylamide gels.

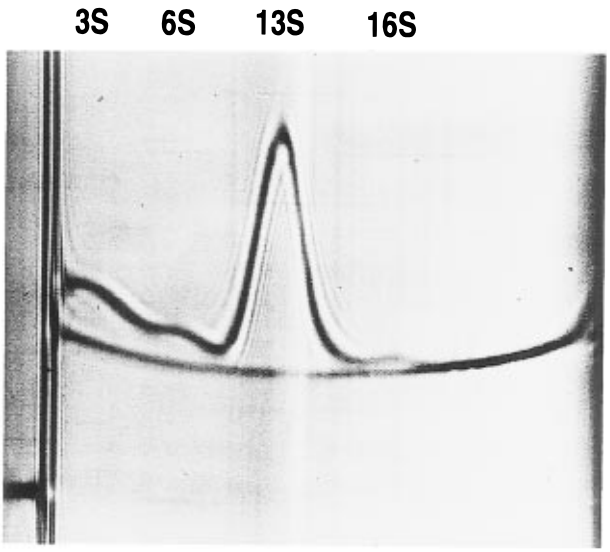

Figure 5. Ultracentrifuge pattern for the ~0.5% (w/v) solution of the cashew globulin in the standard buffer. Sedimentation is from left to right.

6S fractions. Such association of a globulin into faster moving components is consistent with earlier observations of Wolf (1993) on soybean major globulin glycinin (11S). As mentioned earlier, since gel filtration chromatography at two different pH values (pH 7.5 and at pH 8.1) essentially gave similar profiles, subtle changes in the protein structure due to change in the buffer pH appeared unlikely. We cannot however rule out the possibility of proteolysis during the globulin preparation

Table 1. Ultracentrifuge Analysis of Cashew Globulins

		composition (%)			
sample	solvent	3S	6S	13S	16S
total globulins	2 M NaCl	36.5	8.9	47.1	7.5
total globulins	standard buffer	31.7	5.3	54.2	8.8
globulin[a]	2 M NaCl	13.0	14.2	65.8	7.0
globulin[a]	standard buffer	17.9	11.7	66.9	3.4

[a] Column chromatographically purified globulin.

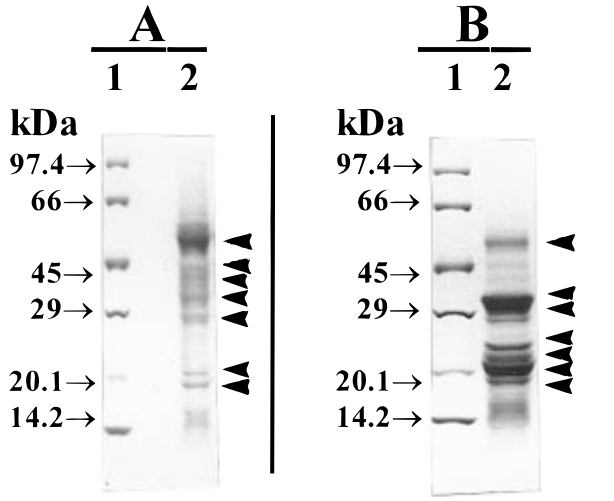

Figure 6. SDS−PAGE for purified globulin in the absence (A) and in the presence (B) of 2% (v/v) β-ME. The gels were 8−25% linear acrylamide gradient gels (1.5 mm thick). For both A and B, lane 1, low MW markers phosphorylase b (97.4 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa), soybean Kunitz trypsin inhibitor (20.1 kDa), and α-lactoglobulin (14.2 kDa); lane 2, cashew globulin (50 μg in A and 100 μg in B). Gels were run at a constant current (10 mA/gel) with cooling provided by the running tap water. Arrowheads in both A and B indicate the major polypeptides.

by neutral or alkaline proteinases. Such proteolysis of the globulin was however unlikely due to trypsin and chymotrypsin like proteinases because the presence of trypsin and chymotrypsin inhibitors in the crude globulin preparations from defatted cashew nut flour has been reported (Filho and Ainouz, 1977). These investigators also noted that the crude inhibitor preparations were heat stable (100 °C, pH 7.0, 30 min) and that pepsin treatment of the crude inhibitor (at pH 3.0, 5 h, 37 °C) was also ineffective in inactivating these "anti-trypsin and anti-chymotrypsin activities of the crude inhibitor". We used salt (0.1 M NaCl in the gel filtration buffer and 2 M NaCl in the extraction medium) and cold temperature (4 °C) during all stages of the globulin preparation to minimize possible proteolysis.

Globulin Properties. The globulin, when electrophoresed in the presence of a denaturant SDS and in the absence of a reducing agent β-ME (Figure 6A), revealed at least seven prominent polypeptides (subjectively based on the band intensity and width, indicated by arrowheads). These polypeptides (from top to bottom) had estimated MWs of 53 000, 36 950, 33 050, 31 990, 28 190, 20 960, and 18 990, respectively. We have consistently noted a large diffuse area (perhaps containing several polypeptides) that stains intensely in crude globulin fraction (MW <20 000 but >14 200). These polypeptides elute off the Sephacryl S300 HR column in the tail portion of the second peak (SDS−PAGE analysis of the column profile, data not shown). In addition to the seven major bands, we have been able

to detect as many as 18 additional minor bands (MWs in the range 11780−70880) in one-dimensional SDS−PAGE gels [with both the Coomassie Brilliant Blue R and silver staining (data not shown)]. When the globulin was subjected to SDS−PAGE in the presence of β-ME (Figure 6B), it had a complex polypeptide composition consisting of seven major polypeptides (subjectively based on the band intensity and width, indicated by arrowheads) with estimated MWs (from top to bottom) of 51 110, 33 170, 32 300, 23 180, 22 420, 20 580, and 18 860. As many as 23 additional polypeptides were visible on these gels (MWs ranging from 12 230 to 68 100) with Coomassie Brilliant Blue R staining. These data were consistent with those reported earlier for the crude globulin fraction isolated from the defatted cashew nut flour (Sathe, 1994). These data suggest that the major globulin in cashew nut is primarily composed of two types of polypeptides (the light chains in the MW range of 18000−24000 and the heavy chains in the MW range 30000−37000) that are possibly linked by disulfide bond(s) to yield a polypeptide with MW ~53 000. Six such polypeptides may constitute the native molecule which would have an estimated MW of 300000−320000, consistent with the 11S−13S protein model proposed to be the most common type of salt soluble globulin in dicots (Borroto and Dure, 1987). Ventura and Filho (1964) had estimated the MW of the cashew globulin to be in the range 230000−260000 based on three different methods. This apparent MW would give a somewhat lower estimate than would be expected from a hexameric molecule if one were to accept the hexameric nature of the molecule. When we analyzed our gel filtration data for apparent molecular mass determination, we obtained a linear regression equation of $y = -2.628918x + 5.962574$ ($r = 0.986$) for the standard proteins (data not shown). Using this equation we *estimate* the apparent molecular mass of the globulin to be 275 590 ± 45 190 (Mean ± SEM, $n = 17$). The MW reported by Ventura and Filho may be an underestimate of the true MW of the globulin. This is because, if their data for frictional ratio f/f_o (1.07), partial specific volume v (0.737 mL/g), and MW (250 000) are used to calculate the hydrodynamic radius of the cashew globulin (in the standard buffer) using the following equation (see Siegel and Monty, 1966),

$$f/f_o = \text{Stokes radius}/[3vM/4\pi N]^{1/3}$$

where N = Avogadro's number (6.023×10^{23}) one would obtain a value of 81.37 Å. This value for the Stokes radius is comparable to that of thyroglobulin (Stokes radius = 85 Å, MW 669 000, and a $f/f_o = 2.34$). The frictional ratio value of 1.07 reported by Ventura and Filho would suggest the cashew globulin to be a reasonably symmetrical molecule which would make the Stokes radius of 85 Å inconsistent with the normally expected behavior of the globular proteins on gel filtration columns. We determined the Stokes radius (mean ± SEM) of cashew major globulin to be 57 ± 3.2 Å (linear regression for the standard protein plot of $(-\log_{10} K_{av})^{1/2}$ versus Stokes radius was $y = 0.01073617x + 0.2251670$; $r = 0.989$), which is greater than that of catalase (52.2 Å, MW 232 000) and less than that of the ferritin (61 Å, MW 440 000). It is recognized that a very small change in the partial specific volume can significantly change the f/f_o and therefore other calculations based on that frictional ratio.

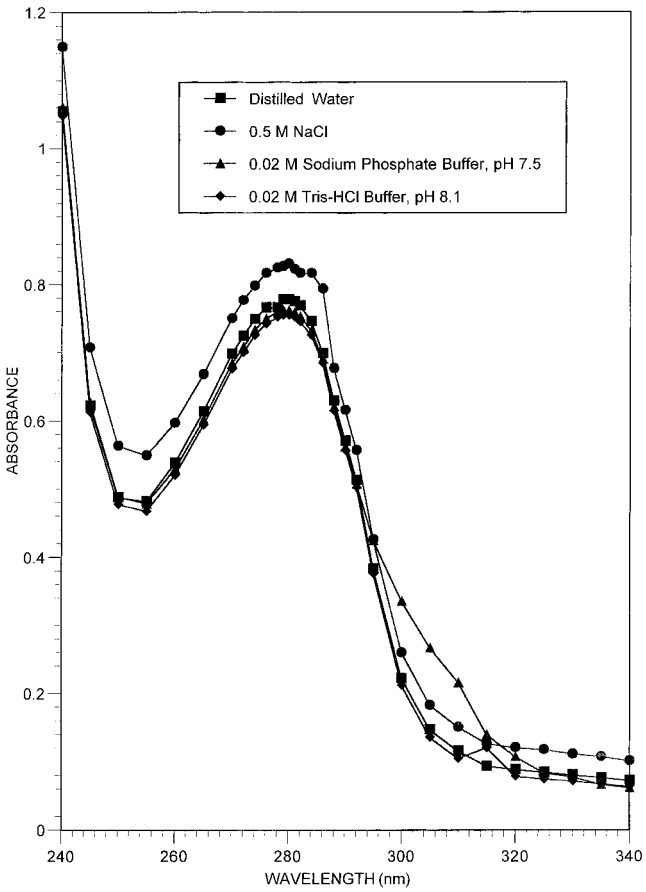

Figure 7. Ultraviolet absorption spectra of the cashew globulin. Protein concentration was 0.787 mg/mL in each case.

The globulin registered absorption maxima at 280 nm regardless of the buffer used (Figure 7). The protein concentration in all these spectra was 0.787 mg/mL (Lowry *et al.* method). The $A^{1\%}_{280nm}$ values for the globulin were 9.88, 10.56, 9.68, and 9.59 when the globulin was dissolved in distilled water, 0.5 M NaCl, 0.02 M sodium phosphate buffer pH 7.5, and 0.02 M Tris-HCl buffer pH 8.1, respectively.

The amino acid composition of the globulin (Table 2) indicated that the hydrophobic, uncharged polar, acidic, and basic amino acids respectively accounted for 36.4, 19.88, 25.3, and 18.4% of the total amino acids. The ratio of essential to total amino acids [E/T (%)] was 40.46. The sulfur amino acids (methionine + cystine/2) and threonine were, respectively, the first and second limiting amino acids in the globulin, when compared with the FAO/WHO recommended amino acid pattern for a weaned child of 2−5 years age (FAO/WHO, 1990).

***In Vitro* Digestibility.** The native globulin was easily hydrolyzed by pepsin to polypeptides with estimated MW <14 000 (Figure 8). The TPCK−trypsin and TLCK−chymotrypsin caused a significant but limited proteolysis of the globulin to smaller polypeptides (estimated MWs in the range of ~10000−32000). Heat denaturation of the globulin (moist heat, 100 °C, 30 min) however overcame this resistance to proteolysis. The polypeptide pattern of the pepsin-digested globulin essentially remained the same regardless of the heat denaturation treatment. These data indicated that the cashew globulin was effectively hydrolyzed by pepsin in both the native and the heat-denatured states to polypeptides with estimated MWs <14000. Time course digestion of the native and heat-denatured globulin with pepsin (Figure 9) indicated that this proteolysis was

Table 2. Amino Acid Composition of Cashew Globulin[a]

amino acid	g/100 g of protein	FAO/WHO[b]
Asx	7.95	
Glx	17.35	
Ser	6.19	
Gly	7.84	
His	1.48 (0.7789)	1.9
Arg	9.90	
Thr	3.01 (0.8853)	3.4
Ala	5.00	
Pro	5.29	
Tyr	2.48	
Val	6.52 (1.8629)	3.5
Met]	0.80 (0.4640)]	2.5
Cys/2]	0.36]	
Ile	4.34 (1.5500)	2.8
Leu	8.02 (1.2152)	6.6
Phe	4.13 (1.0490)[c]	6.3[c]
Lys	7.02 (1.2103)	5.8
Trp	2.30 (2.0910)	1.1
E/T (%)[d]	40.50	

[a] Data are corrected for 100% recovery. Figures in parentheses are the amino acid scores for the corresponding amino acid. [b] Recommended for children (2−5 years), although recently recommended for dietary protein quality evaluation for all age groups (except infants) by the joint FAO/WHO Expert Consultation (1990). [c] Phe + Tyr. [d] Essential/total amino acid ratio.

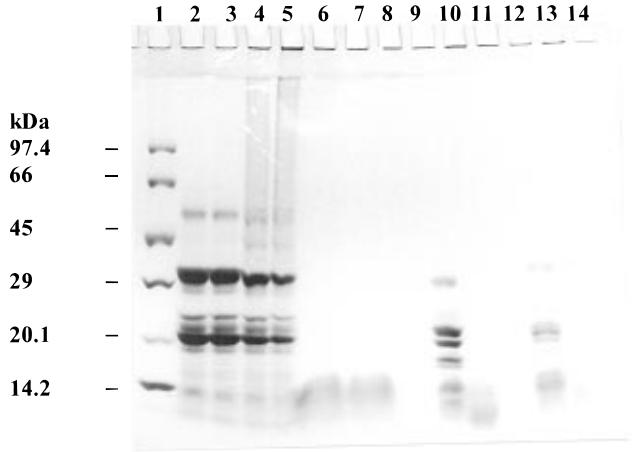

Figure 8. SDS−PAGE for cashew globulin digested with different proteinases. This is a 8−25% linear acrylamide gradient gel (1.5 mm thick). Lane 1, low MW standards (same as in Figure 6); lane 2, native globulin control; lane 3, native globulin 30 min incubation control; lane 4, heat denatured globulin, no incubation control; lane 5, heat denatured globulin 30 min incubation control; Lanes 6, 9, and 12, native globulin digested with pepsin, TPCK−trypsin, and TLCK−chymotrypsin, respectively, for 30 min; lanes 7, 10, and 13, heat-denatured globulin digested with pepsin, TPCK−trypsin, and TLCK−chymotrypsin, respectively, for 30 min; lanes 8, 11, and 14, pepsin, TPCK−trypsin, and TLCK−chymotrypsin enzyme controls, respectively. Globulin was denatured in distilled water at 100 °C for 30 min when required. Globulin to proteinase ratio was 100:1 (w/w) each, and the digestions were done at 37 °C for 30 min. Globulin load in each lane was 50 μg and that for the enzyme controls was 0.5 μg each.

rapid (≤1 min) and that the polypeptide pattern did not change to any major extent after 1 min (up to 30 min). The resistance of the native globulin to TPCK−trypsin and TLCK−chymotrypsin is similar to the *in vitro* resistance of almond proteins to these proteinases (Sathe, 1993). These data are consistent with those reported for the total cashew protein *in vitro* digestibility (15 min digestions at 37 °C) estimated by measuring the 5% trichloroacetic acid soluble peptides and amino acids (Nagaraja, 1989).

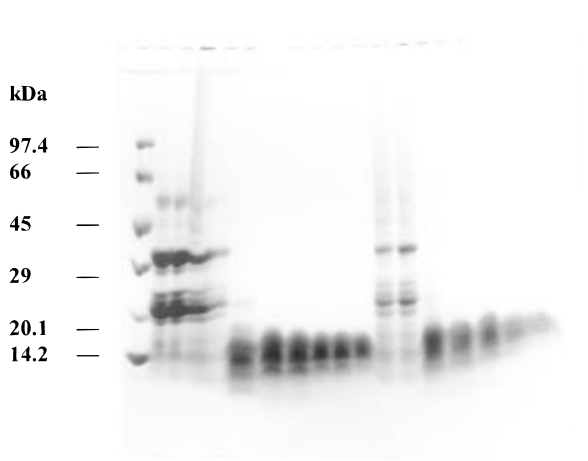

Figure 9. SDS−PAGE for time course of cashew globulin digested with pepsin. Lane 1, MW standards (same as in Figure 6); lane 2, native globulin, no incubation control; lane 3, native globulin, 30 min incubation control; lane 4, heat-denatured globulin, 30 min incubation control; lanes 5, 6, 7, 8, 9, 10, and 11, native globulin digested with pepsin for 0, 1, 3, 5, 10, 15, and 30 min, respectively; lanes 12, 13, 14, 15, 16, 17, and 18, heat-denatured globulin digested with pepsin for 0, 1, 3, 5, 10, 15, and 30 min, respectively; lane 19, pepsin enzyme control. Globulin was denatured in distilled water at 100 °C for 30 min when required. Globulin to proteinase ratio was 100:1 (w/w) each and the digestion was done at 37 °C for the time indicated. The globulin and the pepsin incubation controls were incubated for 30 min at 37 °C. Globulin load in each lane was 40 μg and the pepsin load was 0.4 μg.

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