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Fractionation and partial characterization of tepary bean (*Phaseolus acutifolius*) proteins

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Tepary bean (*Phaseolus acutifolius*) proteins were fractionated sequentially according to solubility in sodium phosphate buffer (SPB), sodium chloride (salt), ethanol, 2-mercaptoethanol (2-ME), and sodium dodecyl sulfate (SDS) solutions, and characterized. The SPB protein fraction was significantly ($P < 0.05$) the highest (83.2% of the recovered protein) followed by salt protein fraction (13.7%), 2-ME (1.5%), ethanol (0.8%), and SDS (0.8%) protein fractions. The amino acid compositions of SPB, salt, ethanol, and 2-ME protein fractions were not significantly different. Methionine and cysteine concentrations were low in all fractions. The ethanol protein fraction had a significantly ($P < 0.05$) higher cysteine content (1.5%) than 2-ME (0.95%), salt (0.2%) or SPB (trace) protein fractions. SDS-PAGE of SPB and salt protein fractions contained 37 and 27 polypeptides, respectively, with major bands at 29, 45, and 49 kDa. Ethanol and SDS protein fractions had only a limited number of small polypeptides.

INTRODUCTION

Tepary bean (*Phaseolus acutifolius*) is an indigenous legume of the arid and semi-arid areas of the southwestern United States and Mexico where it is traditionally consumed by American Indians (Nabhan & Felger, 1978). The tepary plant produces well with limited moisture, is tolerant to temperature stress and salinity, and is regarded as a potential food crop for the arid lands of Africa, the Middle East, South America and Asia (Marsh & Davis, 1985). Tepary beans contain 15–32% protein and about 41% starch (Scheerens *et al.*, 1983; Abbas & Berry, 1986). Like other legumes, they are low in vitamins A and C (Idouraine *et al.*, 1989). Tepary beans can be consumed fresh, germinated, boiled, steamed or fried. They are acceptable when substituted for cowpeas or other local legumes traditionally used in African and Middle Eastern recipes (Tinsley *et al.*, 1985; Idouraine *et al.*, 1989).

In the last decade, chemical studies on tepary beans were limited to proximate and amino acid compositions (Tinsley *et al.*, 1985; Idouraine *et al.*, 1989), mineral content (Taggart *et al.*, 1983) and anti-nutritional factors (Thorn *et al.*, 1983). A few studies reported physico-chemical and rheological properties of tepary starch and its potential use in food products (Abbas & Berry, 1986; Abbas *et al.*, 1986). More recently, functional properties of tepary flour, and water- and salt-soluble tepary proteins, were studied and biologically evaluated. Water- and salt-soluble proteins were found to be highly soluble at acid and alkaline pH values and had good emulsion and coagulability properties (Idouraine *et al.*, 1991). When fed to mice, tepary flour and the water-soluble protein extract had a protein efficiency ratio similar to that of other *Phaseolus* species (Idouraine *et al.*, 1992*a*). These studies showed that tepary flour and tepary protein fractions have a promising potential use in food. There is, however, no information on the characterization and nutritional value of the different tepary protein fractions. Such information is needed for comparative studies with other legume proteins. Several techniques such as electrophoresis, chromatography, and ultracentrifugation have been used to characterize protein. Because of its simplicity and rapidity, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is routinely used in many laboratories to identify and determine the molecular weight of the different polypeptides in legume protein fractions. Recently, amino acid composition and SDS-PAGE patterns of faba bean, chickpea, urdbean, and other legumes have been reported (Hamza *et al.*, 1988; Mahajan *et al.*, 1988).

The purpose of this study is: (1) to isolate tepary bean proteins sequentially according to solubility in sodium phosphate buffer, sodium chloride, ethanol, 2-mercaptoethanol, and sodium dodecyl sulfate solutions; (2) to determine the amino acid composition, and (3)

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to examine the SDS-PAGE patterns of the isolated protein fractions.

MATERIALS AND METHODS

Preparation of tepary bean samples

White tepary beans (Phaseolus acutifolius) from the 1986 harvest were obtained from Mexico. They were placed in plastic jars and stored at 3–4°C and 40% relative humidity until needed. Beans were cleaned manually and ground in a hammer mill to pass through a 30 mesh-screen size. The flour (100 g), was defatted with 400 ml of hexane for 12 h in a Soxhlet apparatus. The defatted meal was freeze-dried and stored at −18°C until used.

Protein fractionation

Fractionation of proteins from the defatted flour was carried out in duplicate as described by Pan and Reeck (1988). Tepary bean proteins were extracted sequentially according to their solubility in 0.001 M sodium phosphate buffer, pH 7.0 (SPB), 0.5 M sodium chloride (salt), 70% aqueous ethanol, 0.6% 2-mercaptoethanol (2-ME) in 0.1 M sodium carbonate buffer, pH 10.0, and 0.5% sodium dodecyl sulfate (SDS) in 0.1 M sodium carbonate buffer, pH 10.0. Protein fractions were extracted at 6°C, except the SDS protein fraction which was extracted at room temperature. Protein fractions were prepared by extracting 100 g of flour for 2 h with 600 ml of appropriate solvent with mechanical stirring. Each protein fraction was centrifuged at 13 000 × g for 30 min. The residues were rinsed twice, for 1 h each, with corresponding solvent and centrifuged as before. The supernatants were combined and filtered through glass wool to remove any particles. Supernatants, excluding the SDS protein fraction which was dialyzed at room temperature, were dialyzed against deionized water at 6°C with Spectrapor membrane tubing (MW cutoff, 6000–8000 Da). Fractions were dialyzed for 72 h with five water changes. Dialyzates, excluding that of the SPB protein fraction, were centrifuged at 13 000 × g for 30 min. The supernatants were discarded while the pellets were freeze-dried and stored in a freezer until used. For SPB, the dialyzate was freeze-dried and stored until needed.

Analyses

Crude protein (N × 6.25) of defatted tepary flour and protein fractions isolated using SPB, salt, ethanol, 2-ME, and SDS solutions were determined in duplicate with a micro-Kjeldahl method (AACC, 1983). Solubilized protein in recovered pellets and defatted flour was determined by the bicinchoninic acid (BCA) method as described by Smith *et al.* (1985). Quadruplicate samples of 2–30 mg proteins were diluted in 2 ml 5% SDS and centrifuged for 20 min at 4000 × g to sediment insoluble proteins. Supernatants were incubated for 30 min at 37°C and absorbance measured at 562 nm in a Sequoia-Turner Model 340 spectrophotometer. The protein content of each supernatant was calculated with a standard curve prepared with known concentrations of bovine serum albumin (Pierce Chemical Company, Rockford, IL). Amino acid composition was determined with the Spackman *et al.* (1958) method. Duplicate protein fraction samples were hydrolyzed by transferring about 50 mg of precisely weighed protein fraction into a 15-ml ampoule, adding 5 ml 6 N HCl, sealing the vial under vacuum and digesting at 110°C for 18 h. Cysteine concentration was determined by adding 4 ml performic acid to the sample and hydrolyzing with 5 ml 6 N HCl in an autoclave for 18 h at 121°C. Amino acid analysis was performed with a Spectra Physics SP 8000A amino acid analyzer using a Beckman fluorescence detector.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis

SDS-PAGE was determined following the method described by Sathe *et al.* (1987). Duplicate 20-mg protein fractions or defatted tepary flour were separately extracted with 200 μl of SDS-PAGE buffer for 2 min in a boiling-water bath and centrifuged at room temperature in a Beckman table-top centrifuge for 5 min at 13 600 × g. The supernatant of each protein fraction was mixed with 200 μl of additional SDS-PAGE buffer and analyzed. The SDS-PAGE buffer was 0.05 M tris-HCl (pH 6.8) containing 1% SDS, 30% glycerol, 0.01% bromophenol blue as tracking dye and 2% β-mercaptoethanol. Duplicate protein fractions of SPB, salt, ethanol, 2-ME, and SDS were prepared at 5 mg per ml of SDS-PAGE buffer. Each protein fraction was heated for 10 min and 20 μl each was loaded in Fling and Gregerson (1986) type gels (8–25% linear acrylamide gradient). The composition, dimension and running conditions of the gels were similar to the condition described by Sathe *et al.* (1987). The gels were stained with 50% methanol containing 10% acetic acid and 0.25% Coomassie brilliant blue for 16 h, and destained for 2 h with a solution of 50% methanol containing 10% acetic acid followed by another solution of 5% methanol with 7.5% acetic acid. The molecular weights (MW) of the different protein fractions were estimated using a Sigma molecular weight standard. MW standards included phosphorylase b (97.4 kDA), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa), soybean trypsin inhibitor (20.1 kDa), myoglobin backbone polypeptide (16.95 kDa), α-lactalbumin (14.2 kDa), myoglobin fragment I + II (14.4 kDa), myoglobin fragment I (8.16 kDa), and myoglobin fragment II (6.21 kDa).

Statistical analysis

Data, expressed as means ± SD, were statistically analyzed using the one-way analysis of variance and

t-test with significance set at $P < 0.05$ (Steel & Torrie, 1960).

RESULTS AND DISCUSSION

Isolation of tepary protein fractions

Data on isolated protein fractions are reported in Table 1. The SPB protein fraction was significantly higher than all other protein fractions together and accounted for 55.8% of the total protein. The salt-soluble protein fraction was significantly higher than the ethanol, 2-ME and SDS protein fractions. The SPB and salt protein fractions represented 83.2% and 13.7% of the recovered protein, respectively. No significant differences were found among the ethanol, 2-ME and SDS protein fractions. Protein recovered plus residual proteins totalled 75.3%. The unaccounted for portions (24.7%) could be related to the non-protein nitrogen which might have increased the initial amount of protein analyzed and/or to a small portion of water-soluble protein lost during dialysis or discarded with the supernatant after centrifugation. Depending on the method and solvent used to extract protein, various yield values have been reported in the literature. The values of the present study agree with those reported by Sathe and Salunkhe (1981), Rahma (1988), and Tjahjadi *et al.* (1988) for Great Northern bean, faba bean, and adzuki bean, respectively. Protein contents determined both by micro-Kjeldahl and bicinchoninic acid methods were similar, except that of the SDS fraction and final residue. The lower level of solubilized protein in the SDS extract and final residue might be related to a high amount of insoluble proteins. Tepary proteins are mostly water-extractable, which is an advantage since water-extractable proteins generally have a wider range of use in the food industry. Though numerous studies on the fractionation of legume proteins are available, there is no report on the fractions of tepary bean. Estimates of the proportions and protein recovery of different fractions vary widely depending on bean species, types of solvent used to isolate protein fractions, conditions of extraction and methods of analyses. Depending on these factors, data on the proportions of different protein fractions in legumes vary considerably.

Tjahjadi *et al.* (1988) reported that albumin and globulin fractions compose 15.8% and 2.3% of the whole adzuki bean (*Vigna angularis* cv. Takara) proteins or 73.3 and 10.4% of the total extractable protein, respectively. Rahma (1988) indicated that faba bean (*Vicia faba*) proteins contain 53.1% albumin, 20.4% globulin, 6.6% prolamine, and 8.1% glutelin. Abd El-Aal and Rahma (1986) fractionated fenugreek (*Trigonella foehum graecum* L.) proteins in water, 1.0 M NaCl, 70% ethanol and 0.2 M NaOH, and reported 43.8% albumin, 27.2% globulin, 17.2% glutelin, and 7.4% prolamine. Mahajan *et al.* (1988) fractionated urdbean (*Vigna mungo*) proteins and reported 62.8% globulins, 20.9% glutelins, 12.1% albumins and 1.0% prolamines. The final residue contained 1.8% of the total protein. Gopinathan *et al.* (1987) extracted and isolated proteins of different progenies of *Vigna minima*. Seed proteins were made up of 10.8–19.2% albumins, 38.4–54.4% globulins, 1.1–4.1% prolamines, and 20.2–30.2% gluteins. The proteins of chickpea (*Cicer arietinum*) contain 12.6% albumin, 56.6% globulin, 2.8% prolamines, and 18.1% glutelins (Singh & Jambunathan, 1982). Common bean (*Phaseolus vulgaris*) proteins contain 11.1–19.8% albumins, 39.5–56.2% globulins, 19.6–29.5% glutelins, and 2.2–3.7% prolamines. The amount of protein in the final residue ranged from 1.9 to 3.9% (Ma & Bliss, 1978; Marquez & Lajolo, 1981).

Amino acid composition of the protein fractions

Amino acid composition of the defatted tepary flour proteins and isolated protein fractions is presented in Table 2. Defatted tepary flour protein, SPB, salt, ethanol, and 2-ME protein fractions showed similar

Table 1. Protein content of fractions obtained by sequentially extracting tepary bean flour with sodium phosphate buffer (SPB), sodium (Salt), ethanol, 2-mercaptoethanol (2-ME), and sodium dodecyl sulfate (SDS)[a]

Fraction	Protein recovered (g/100 g tepary flour)	Protein recovered[b] (g/100g total protein)	BCA protein[c] (g/100g total protein)
SPB	10·9 ± 0·44[d]	55·8 ± 2·28[d]	55·6 ± 2·26[d]
Salt	1·8 ± 0·16[e]	9·2 ± 0·80[e]	8·4 ± 0·71[e]
Ethanol	0·1 ± 0·01[f]	0·5 ± 0·07[f]	0·3 ± 0·03[f]
2-ME	0·2 ± 0·07[f]	1·1 ± 0·04[f]	0·9 ± 0·03[f]
SDS	0·1 ± 0·01[f]	0·5 ± 0·07[f]	0·2 ± 0·02[f]
Total recovered	13·1 ± 1·28	67·1 ± 3·26	65·4 ± 3·05
Residue	1·6 ± 0·34	8·2 ± 1·74	ND
Total protein	19·5 ± 0·05	—	18·7 ± 1·67

ND = not detected.
[a] Determined in duplicate fat-free dry sample (means ± SD).
[b] Determined by micro-Kjeldahl method ($N \times 6.25$).
[c] Determined by bicinchoninic acid (BCA) method of Smith *et al.* (1985).
[d,e,f] Mean values with the same superscript within the column are not significantly different ($P < 0.05$).

Table 2. Amino acid composition of defatted tepary flour and sequentially isolated protein fractions[1]

Amino acids	Defatted sample (%)	SPB fraction (%)	Salt fraction (%)	Ethanol fraction (%)	2-ME fraction (%)	SDS fraction (%)	Final residue (%)
Aspartic acid	10.9 ± 0.48^{abc}	11.9 ± 0.57^{ab}	12.0 ± 0.09^{ab}	9.6 ± 3.13^{bcd}	13.6 ± 1.70^{a}	7.2 ± 0.07^{d}	8.0 ± 1.26^{cd}
Glutamic acid	15.9 ± 0.17^{a}	14.8 ± 0.02^{a}	15.4 ± 0.63^{a}	10.7 ± 2.42^{b}	12.2 ± 1.45^{b}	6.4 ± 0.24^{c}	6.7 ± 0.04^{c}
Serine	4.9 ± 0.10^{b}	6.2 ± 0.13^{a}	6.0 ± 0.52^{a}	5.5 ± 0.27^{ab}	6.1 ± 0.68^{a}	3.1 ± 0.13^{c}	2.9 ± 0.03^{c}
Histidine	2.3 ± 0.11^{b}	2.9 ± 0.06^{a}	3.0 ± 0.25^{a}	2.1 ± 0.23^{b}	2.9 ± 0.35^{a}	1.1 ± 0.13^{c}	1.0 ± 0.16^{c}
Glycine	4.5 ± 0.15^{bc}	5.3 ± 0.12^{a}	4.9 ± 0.39^{ab}	4.1 ± 0.39^{c}	4.5 ± 0.59^{bc}	2.6 ± 0.07^{d}	3.1 ± 0.07^{d}
Threonine	3.1 ± 0.01^{b}	4.6 ± 0.05^{a}	2.8 ± 0.35^{bc}	4.0 ± 0.29^{a}	4.6 ± 0.61^{a}	2.1 ± 0.44^{cd}	1.9 ± 0.06^{d}
Arginine	5.8 ± 0.15^{b}	7.8 ± 0.25^{a}	8.0 ± 0.46^{a}	5.6 ± 0.43^{b}	6.5 ± 0.76^{b}	2.9 ± 0.49^{c}	2.8 ± 0.08^{c}
Alanine	4.4 ± 0.10^{b}	5.5 ± 0.24^{a}	4.7 ± 0.02^{b}	4.4 ± 0.54^{b}	4.7 ± 0.61^{b}	2.9 ± 0.15^{c}	2.6 ± 0.08^{c}
Tyrosine	3.0 ± 0.18^{c}	3.7 ± 0.09^{ab}	4.1 ± 0.14^{a}	3.5 ± 0.20^{bc}	3.0 ± 0.28^{c}	2.0 ± 0.52^{d}	1.9 ± 0.01^{d}
Methionine	1.0 ± 0.08^{a}	1.0 ± 0.02^{a}	0.8 ± 0.11^{ab}	0.9 ± 0.15^{a}	0.8 ± 0.15^{ab}	0.3 ± 0.06^{c}	0.6 ± 0.02^{b}
Valine	5.2 ± 0.06^{b}	5.7 ± 0.06^{ab}	5.1 ± 0.18^{b}	5.9 ± 0.78^{ab}	6.4 ± 0.82^{a}	3.4 ± 0.33^{c}	3.2 ± 0.03^{c}
Phenylalanine	5.1 ± 0.08^{b}	5.9 ± 0.14^{b}	5.7 ± 0.19^{b}	5.9 ± 0.59^{b}	7.1 ± 0.80^{a}	3.1 ± 0.42^{c}	2.7 ± 0.06^{c}
Isoleucine	4.3 ± 0.05^{a}	4.7 ± 0.07^{a}	4.7 ± 0.11^{a}	4.7 ± 0.60^{a}	4.6 ± 0.59^{a}	2.8 ± 0.22^{b}	2.4 ± 0.02^{b}
Lysine	6.2 ± 0.12^{b}	8.3 ± 0.16^{a}	6.9 ± 0.26^{b}	6.5 ± 1.10^{b}	7.4 ± 0.96^{ab}	4.2 ± 0.23^{c}	4.3 ± 0.03^{c}
Leucine	7.4 ± 0.15^{a}	8.4 ± 0.23^{a}	8.7 ± 0.33^{a}	7.9 ± 1.21^{a}	7.7 ± 0.92^{a}	4.9 ± 0.27^{b}	4.3 ± 0.03^{b}
Cysteine	0.5 ± 0.03^{c}	0.0 ± 0.00^{d}	0.2 ± 0.01^{d}	1.5 ± 0.25^{a}	0.9 ± 0.03^{b}	1.0 ± 0.06^{b}	1.4 ± 0.03^{a}
Recovery	84.5	96.7	93.0	82.8	93.0	49.9	50.7

[1] Determined in duplicate fat-free dry samples (mean ± SD), expressed as a percentage of protein ($N \times 6.25$). Mean values with the same superscript within the row are not significantly different ($P < 0.05$).

amino acid composition. No significant difference among most amino acids was found within the major protein fractions. SPB and salt protein fractions, when compared to other protein fractions, had a significantly lower cysteine content. Contrary to the present study, this amino acid has previously been reported to be concentrated mainly in albumin and globulin fractions, respectively, in *Vigna minima*, chickpeas, and common beans (Gopinathan *et al.*, 1987; Marquez & Lajolo, 1981; Singh & Jambunathan, 1982). Cysteine was significantly high in the ethanol protein fraction and may be associated with the high trypsin inhibitor activity concentration (1583 TIU/mg protein) reported in this fraction (Idouraine *et al.*, 1992*b*). The SDS protein fraction and the protein of the final residue showed significantly lower amino acid concentrations than the protein fractions cited above. The cysteine content in the final residue was, however, not significantly different from that of the ethanol protein fraction and might be associated with glycoproteins. Similar to other legume species, defatted tepary flour protein and the isolated protein fractions contain high amounts of glutamic and aspartic acids and low levels of methionine and cysteine. Quantitatively and qualitatively the SPB protein fraction appears the most interesting protein in tepary beans.

SDS-PAGE of defatted flour protein and isolated protein fractions

SDS-PAGE of tepary flour protein and isolated protein fractions are presented in Fig. 1. Tepary flour protein showed at least 44 polypeptides of varying molecular weights (MW of 6.5 to 116 kDa). The proteins of defatted tepary flour have three major bands with an apparent MW of 29, 45, and 49 kDa, respectively, and six intermediate ones with a MW ranging from 17 kDa to 98 kDa. SPB protein fractions showed 37 polypeptides with one major band at 29 kDa and three medium ones at 29, 45 and 49 kDa. The salt protein fraction had 27 polypeptides with two major bands at 45 and 49 kDa and six medium ones in the 8–64 kDa range. The ethanol protein fraction showed six small polypeptides in the 8–29 kDa range. This fraction may be composed of low MW polypeptides which were eliminated during dialysis. The 2-ME protein fraction

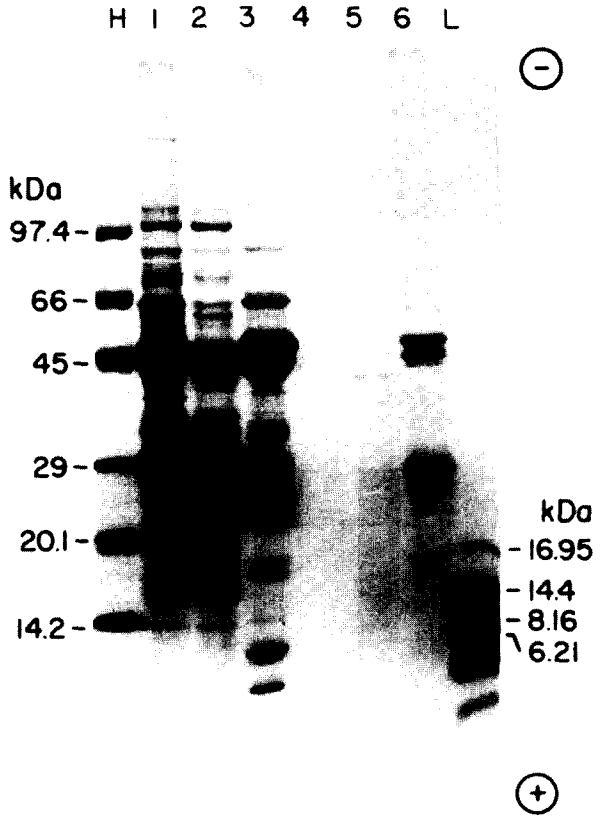

Fig. 1. SDS-PAGE patterns of tepary proteins: (H) high MW marker, (1) defatted tepary protein, (2) SPB, (3) salt, (4) ethanol, (5) SDS, (6) 2-ME extracts and (L) low MW marker.

contained three medium-size polypeptides at 28, 45, and 49 kDa and smaller ones in the 14.6–97.0 kDa range. The SDS protein fraction showed several small polypeptides at 35–75 kDa. The SDS-PAGE patterns of the defatted tepary flour protein and isolated protein fractions in the present study were close to those reported in the literature in legume proteins and their isolated protein fractions. Mahajan *et al.* (1988) found that the electrophoretic profile of urdbean protein exhibited 21 different bands with MW ranging from 8.9 to 117.4 kDa, while the albumin, globulin, prolamine and glutelin fractions isolated from these beans had 8–13 polypeptides with MW varying from 10.2 to 118 kDa. Hamza *et al.* (1988) found that the water-extracted protein fraction of faba bean, chickpea, lentils, lupin and fenugreek had major bands between 20 and 50 kDa. The albumin fraction of mungbeans, lentils, faba beans, vetch, dry beans, peas, chickpeas and lathyrus was composed of 19–25 polypeptides with MW ranging from 10 to 100 kDa (Kawakishi & Namiki, 1982). Sathe and Salunkhe (1981) reported that bean flour protein, and albumin and globulin fractions of the Great Northern bean (*Phaseolus vulgaris* L.) were made up of 22, 14 and 10 polypeptides, respectively. Their MW ranged from 14.7 to 550.0 kDa, 14.0 to 554.0 kDa and 19.3 to 450 kDa, respectively. The number of polypeptides and the MW of the protein fractions may vary depending on the method used and legume species. In the present study the major bands in isolated protein fractions were located at 29, 45, and 49 KDa.

CONCLUSION

Tepary bean proteins were mostly water-extractable, which is an advantage since proteins soluble in water generally have a wider range of use in the food industry. SPB proteins are quantitatively and nutritionally the most important fractions in tepary beans. When assessing the nutritional values of the tepary protein fractions, emphasis should be given to this fraction. Ethanol, 2-ME and SDS protein fractions are not significantly different and represent only a small portion of the recovered proteins. The amino acid compositions of SPB, salt, ethanol, and 2-ME protein fractions were generally similar. Methionine and cysteine contents were low in all isolated protein fractions. The highest cysteine content was found in the ethanol protein fraction and final residue. SDS-PAGE of defatted tepary protein contained at least 44 polypeptides with varying MW (6.5–116 kDa). The SPB and salt protein fractions had 37 and 27 polypeptides, respectively, with major bands at 29, 45, and 49 kDa.

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