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# Antinutritional factors in protein fractions of tepary bean (*Phaseolus acutifolius*)*

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Proteins from tepary bean were extracted according to their solubility in sodium phosphate buffer (SPB), sodium chloride (NaCl), ethanol, 2-mercaptoethanol (2-ME), and sodium dodecyl sulfate (SDS) and evaluated for trypsin inhibitor activity (TIA), hemagglutinating activity (HA), and phytic acid (PA). TIA varied from 1·91 TIU/mg sample for 2-ME to 161·01 TIU/mg sample for ethanol fraction. HA was high in SPB (29 300 HU/g sample) and tepary flour (TF) (20 000 HU/g), but lower in NaCl (5000 HU/g), 2-ME (1800 HU/g), and ethanol (100 HU/g) fractions. No HA was detected in SDS fraction. PA was high in TF (4·61 mg/g sample) and SPB (6·83 mg/g) and lower in SDS (0·06 mg/g) and 2-ME (0·41 mg/g).

## INTRODUCTION

Tepary bean (*Phaseolus acutifolius*) is an indigenous legume of the semi-arid and arid areas of the southwestern United States and Mexico where it has been consumed by Indians for thousands of years. Tepary bean is tolerant to temperature stress and salinity and has been regarded as a promising potential food crop for the semi-arid and arid lands of Africa, the Middle East, and Asia. Seeds contain 15–32% protein and about 41% starch (Waines, 1978; Scheerens *et al.*, 1983; Idouraine *et al.*, 1989*a*). The antinutritional factors of these beans have been studied by several authors. Trypsin inhibitor activity levels ranged from 12 to 32 TIU/mg sample (Thorn *et al.*, 1983). Hemagglutinating activity of uncooked tepary beans has been reported high when assayed with red blood cells of rabbit and much lower when tested in cooked products, suggesting that it is relatively heat labile (Thorn *et al.*, 1983; Tinsley *et al.*, 1985). Phytic acid levels varied from 0·63 to 1·02% (Thorn *et al.*, 1983). Biological evaluation of tepary beans and their isolated water soluble protein fractions have been undertaken recently (Idouraine *et al.*, 1990). When mixed in a diet, tepary flour and its water soluble protein fraction resulted in the death of all mice in 3–4 days. Autoclaving samples for 30 min led to similar results but in 1–2 weeks. However, soaking then cooking tepary beans in water for 20 min or autoclaving tepary flour or protein concentrate for 45 min completely eliminated toxicity and resulted in appreciable protein efficiency ratio (PER) and weight gains. In view of these results, it was assumed that anti- nutritional factors might be responsible for such a toxicity.

The purpose of this study was, therefore, to determine trypsin inhibitor activity, hemagglutinating activity, and phytic acid levels in tepary flours and their isolated protein fractions.

## MATERIALS AND METHODS

### Sample preparation

Tepary beans were ground in a hammer mill to pass through a 40 mesh screen. The flour was defatted and stored in a freezer until use. Proteins from defatted tepary flour were extracted according to their solubility in sodium phosphate buffer (SPB; 0·001 M, pH 7·0), sodium chloride (NaCl; 0·5 M,) ethanol (70%), 2-mercaptoethanol (2-ME; 0·6%), sodium dodecyl sulfate (SDS; 0·5%) as described by Pan and Reeck (1988). Ethanol fraction was rinsed five times to remove all

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trace of ethanol. Protein contents (N × 6·25) of the fractions, determined by the micro-Kjeldahl method, were 65·08, 85·19, 10·17, 16·03 and 5·16%, respectively. Tepary flour (TF) and final residue had sequentially a protein content of 19·53% and 2·56%. Duplicate dry samples of each protein fraction, tepary flour, and final residue were analyzed for trypsin inhibitor activity (TIA), hemagglutinating activity (HA), and phytic acid (PA) levels.

### Trypsin inhibitor activity

Trypsin inhibitor activity was determined according to the AACC (1983) method. Duplicate fat-free dry samples were extracted with 0·001 M NaOH then incubated with trypsin and benzoyl-DL-arginine-*p*-nitroanalide hydrochloride for 10 min at 37°C in a water bath shaker. TIA expressed as trypsin inhibitor unit per mg sample (TIU/mg sample) was calculated from absorbance read against a blank in a spectrophotometer. One trypsin unit is defined as an increase of 0·01 absorbance units at 410 nm per 10 ml of reaction mix.

### Hemagglutinating activity

All samples were extracted with 0·9% NaCl (1 : 10, w/v) at room temperature for 30 min, centrifuged at 13 600*g* for 10 min at 25°C and supernatants were used for hemagglutinating activity determinations. Trypsinized rabbit red blood cells (4% suspension in 0·9% sodium chloride) were mixed gently (0·2 ml) with 0·1 ml of extract and allowed to stand at room temperature for 2–4 h. At the end of incubation, tubes were gently tapped to discern whether the erythrocytes had agglutinated. All assays were done in duplicate. One hemagglutinating unit (HU) was defined as the least amount of hemagglutinin which produced positive evidence of agglutination. A HU/g sample was calculated as described by Liener and Hill (1953). All assays were done in Bio-Rad titertubes (1 ml capacity).

### Phytic acid

Phytic acid analysis was performed according to the method of Harland and Oberleas (1977) as modified by Thorn *et al.* (1983). Duplicate fat-free dry samples weighing 2 g were extracted in 50 ml of 1·2% HCl solution for 2 h at 37°C in water bath shaker. Inorganic phosphorus was eluted with 15 ml deionized water followed by 30 ml of 0·05 M NaCl. Phytic acid (organic phosphorus) was washed out with 17 ml of 0·7 M NaCl and digested in concentrated acids. Phosphorus was quantified by the Koening and Johnson (1942) method.

## RESULTS AND DISCUSSION

Trypsin inhibitor activity (TIA) was detected in all fractions (Table 1). Ethanol extract showed the highest level of TIA suggesting that the inhibitor might be ethanol soluble. The high cysteine content found in this fraction (1·45%) when compared to SPB (trace), salt soluble (0·18%) and 2-ME (0·87%) protein extracts (Idouraine *et al.*, 1989*b*) might be related to the presence of trypsin inhibitor since this compound is generally associated with cysteine. Kunitz (1946), while isolating TIA from soybeans, noticed an increase of trypsin inhibitor crystals after washing samples with 80% ethanol. These indications might explain the high TIA level observed in the ethanol fraction. No data have been found in TIA of ethanol soluble protein fraction or prolamine for comparison. SPB extract had also a relatively high TIA when compared to salt soluble extract and other fractions. Similar results have been reported on water and salt soluble proteins of dry bean (Autunes & Sgarbieri, 1980; Sathe & Salunkhe, 1981). Little has been reported on TIA of protein fractions of legumes; more investigations are, therefore, needed.

Hemagglutinating activity (HA) was observed in all fractions except in SDS extract. The highest level of HA

**Table 1. Trypsin inhibitor activity (TIA), hemagglutinating activity (HA), and phytic acid (PA) levels of tepary bean protein fractions[a]**

| Sample | TIA[b] (TIU/mg sample) | HA[c] (HU/g sample) | PA[d] (mg/g sample) |
|---|---|---|---|
| Tepary flour | 14·05 | 20 000 | 4·61 |
| SPB extract | 90·17 | 29 300 | 6·83 |
| NaCl extract | 9·54 | 5 000 | 0·39 |
| Ethanol extract | 161·01 | 100 | 0·07 |
| 2-ME extract | 1·91 | 1 800 | 0·41 |
| SDS extract | 8·60 | 0 | 0·06 |
| Residue | 3·63 | — | 0·05 |

[a] Calculated on fat-free dry weight on duplicate samples.
[b] Expressed as trypsin inhibitor unit (TIU/mg sample).
[c] Expressed as hemagglutinating unit (HU/g sample).
[d] Expressed as mg phytate/g sample.

was found in SPB extract and TF suggesting that they might impose a considerable risk if consumed raw. Salt soluble and 2-ME extracts showed intermediate HA while ethanol extract had low levels. Little has been reported in literature on HA of different protein fractions of legumes. Autunes and Sgarbieri (1980), analyzing HA in protein fractions of dry beans (*Phaseolus vulgaris*) found that HA was concentrated mostly in albumins and to a lesser extent in globulins and water soluble extracts. Controversially, Sathe and Salunkhe (1981) noticed no HA in these fractions. Muelenaere (1965) reported a wide range of HA in legume flour. White tepary beans were found to contain 6000 HU/g sample which is lower than the value reported in the authors' study.

Phytic acid (PA) levels in TF was similar to those reported for other legumes (Reddy *et al.*, 1989) and varied among tepary protein fractions. SPB extract had more PA than TF and the remaining protein fractions. This could be due to the interaction between the strong negative charges of PA and positive charges of SPB proteins. The way this fraction has been extracted and the possibility of PA to bind to mineral–protein complex might also be involved. After dialysis, and unlike other fractions, the whole dialysate was freeze-dried, trapping all PA in the extract. Since most phytates in beans have been reported to be in soluble rather than insoluble form, SPB could retain more PA than other extracts. In other fractions, the dialysates were centrifuged and supernatants discarded, therefore contributing to removal of some PA. In salt soluble, ethanol, 2-ME, and SDS extracts PA levels appeared related to the protein content of the samples. Several authors have shown that phytates were more concentrated in protein isolates and concentrates and that the different protein fractions have tendency to bind PA differently (Naczk *et al.*, 1986; Vaintraub & Lapteva, 1988; Reddy *et al.*, 1989). Protein level, process of fractionation and protein conformation certainly affected the PA level in the isolated extracts.

## CONCLUSION

While HA and PA appeared concentrated primarily in SPB extract and secondarily in salt soluble extracts, TIA was found mainly in ethanol extracts. Ethanol might be, therefore, used for partial removal of TIA in samples where heat cannot be used. Since no data on TIA level of prolamine and other fractions have been reported, more investigations on antinutritional factors of different protein fractions are, therefore, needed.

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