Biological Evaluation of Flour and Protein Extract of Tepary Bean (*Phaseolus acutifolius*)

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Raw, cooked, or autoclaved tepary flour (TF) and protein extract (PE) were tested for their nutritive value using mice and analyzed for their trypsin inhibitor activity (TIA), hemagglutinating activity (HA), and phytic acid (PA). Both raw and 20-min-autoclaved TF or PE, when included in the diets, were deadly to mice. Raw TF and PE had high TIA, HA, and PA contents. Soaking and cooking tepary beans for 20 min improved PER (0.97) and protein digestibility (83.54%). Autoclaving samples for 40 min showed lower digestibility (41–59%) but weight gain and PER not significantly different ($P < 0.05$) from those of cooked beans. Sixty-minute autoclaving caused a significant depressing effect on feed intake, weight gain, and PER. TIA and HA seemed not to be the only toxic factors in tepary beans. Processing caused no significant change in PA. Soaking and cooking tepary seeds appeared to be more efficient in eliminating toxicity and leading to better growth performance than autoclaving.

INTRODUCTION

Legumes are an important source of protein, carbohydrates, minerals, and dietary fiber consumed worldwide. They are generally well adapted to a wide range of climates and environmental conditions. Although many species have been extensively studied and commercially promoted, several potential legumes growing in arid and semiarid areas are still little known. Because desertification is increasing in many parts of the world, interest in such plants is growing.

Tepary bean (*Phaseolus acutifolius*) is an indigenous legume of the arid and semiarid areas of the southwestern United States and Mexico. Although consumed by Native Americans as early as 5000 years ago (Nabhan and Felger, 1978), the study of this legume is still limited. As other legumes, tepary beans are deficient in sulfur amino acids and contain antinutritional factors such as trypsin and hemagglutinating activities and phytates, which may limit their utilization (Idouraine et al., 1989, 1992a). When consumed raw, teparies have been reported to cause death in rats in 3–4 days (Gonzáles-Garza et al., 1982; Grant et al., 1983). Intragastric administration of a solution of raw tepary flour to rats has been reported to cause widespread destruction of microvilli, while that of cooked flour did not (Sotelo et al., 1983). This has been attributed to lectin activity. Studies conducted in our laboratory showed that inclusion of 10-min-autoclaved tepary flour into the diet of rats led to the death of all animals within a week (Weber, 1981, unpublished observations). Soaking and cooking tepary bean for 140 min has been reported, however, to support the growth of rats and to have a protein efficiency ratio similar to that of some common legumes (Gonzáles de Mejia et al., 1988). Several other legumes have also been shown to cause high mortality when consumed raw. Autunes and Sgarbieri (1980) noticed that the addition of raw flour or protein fractions obtained from dry beans (*Phaseolus vulgaris* var. Rosinha G2) into rat diets caused death of the animals in 3–23 days. Pusztai et al. (1977) showed that the albumin fraction was more toxic than the globulin fraction and whole bean flour. They attributed this toxicity to the higher concentration of phytohemagglutinins in the albumin fraction. Our preliminary studies revealed, however, that trypsin inhibitor activity, rather than hemagglutinating activity, might be the major cause of toxicity in tepary beans. The method of processing also seems to be an important factor in eliminating toxicity in tepary beans. Legume species, method of processing (soaking and cooking, autoclaving), and length of heat treatment have been reported to affect variably the nutritional values and toxic components of beans (Kantha and Erdman, 1988). Since only limited and contradictory data were available on nutritional evaluation of tepary beans and no study was found on the biological evaluation of tepary protein fractions, it appeared necessary to conduct a biological study on these products to assess their potential use.

The objectives of this experiment were, therefore, (1) to study the nutritive value of tepary bean and isolated protein extract when incorporated into the diets of mice as the sole source of protein, (2) to evaluate the effect of soaking and cooking and autoclaving alone on mouse growth, and (3) to determine the trypsin inhibitor activity, hemagglutinating activity, and phytic acid levels in raw and heat-treated products.

MATERIALS AND METHODS

Preparation of Tepary Flour and Protein Extract. Tepary flour (TF) was obtained by grinding tepary beans in a hammer mill to pass through a 40-mesh screen. Protein extract (PE) was prepared using defatted TF. A sample (100 g) was extracted three times for 2 h using 600 mL of sodium phosphate buffer (0.001 M, pH 7.0). The mixture was centrifugated for 30 min at 13000*g*. The supernatant (PE) was filtered through glass wool, dialyzed against deionized water for 72 h, and freeze-dried. Samples were stored in a freezer until use. Raw TF and PE were autoclaved for 20, 40, and 60 min at 121 °C under 15 lb/in^2. Cooked flour was prepared by soaking tepary seeds overnight in tap water (1:3 w/v ratio of tepary beans to water), draining off their soaking water, and cooking them in water for 20 min at 121 °C under 15 lb/in^2 using a pressure cooker. The cooked beans were freeze-dried and ground in a hammer mill to pass through a 40-mesh screen. Flour obtained from these cooked seeds was stored in a freezer until use.

Diet Formulation. Diets were formulated to contain about 8% protein as follows: (1) whole egg (control), (2) raw TF, (3) raw TF plus 0.05% methionine (Met), (4) raw PE, (5) raw PE

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Table I. Composition of Experimental Diets (Grams/100 g)[a]

ingredients	whole egg	protein extract	tepary flour
whole egg (46%)	17.40		
protein extract (66%)		12.12	
tepary flour (16%)			50.00
cerelose	60.45	74.06	39.05
corn oil	3.00	3.00	3.00
cellulose	3.00	3.00	3.00
AIN vitamin mix	1.00	1.00	1.00
AIN mineral mix	3.50	3.50	3.50
chromium oxide, mg	0.20	0.20	0.20
choline chloride	0.20	0.20	0.20
BHT	0.002	0.002	0.002
DL-methionine (if added)	0.05	0.05	0.05
bentonite	11.20	2.87	
total	100.00	100.00	100.00

[a] Protein = 8.0%; bean (ME) = 3.08 kcal/g; egg (ME) = 3.08 kcal/g.

plus 0.05% Met, (6) 20-min-autoclaved TF, (7) 20-min-autoclaved TF plus 0.05% Met, (8) 20-min-autoclaved PE, (9) 20-min-autoclaved PE plus 0.05% Met, (10) soaked then 20-min-cooked TF, (11) 40-min-autoclaved TF, (12) 40-min-autoclaved TF plus 0.05% Met, (13) 40-min-autoclaved PE plus 0.05% Met, (14) 60-min-autoclaved TF, (15) 60-min-autoclaved TF plus 0.05% Met, and (16) 60-min-autoclaved PE plus 0.05% Met. The composition of the diet is indicated in Table I.

Animal Bioassays. Three-week-old male weanling mice of Charles River CD-1 strain weighing 8–10 g were used. Each assay was composed of 10 mice which were housed individually in stainless steel cages in a controlled-temperature and -light room. Feed and deionized water were provided ad libitum for 3 weeks. Feed consumption and body weight were recorded twice weekly. Feces were collected twice a week, screened to remove feed residues, and stored in a cold room. At the end of the experiment, they were dried in an oven at 70 °C and ground to pass through a 40-mesh screen. Portions of the samples within each experiment were used to determine protein content (N × 6.25) to calculate the apparent digestibility. Protein efficiency ratio (PER) was determined according to the AOAC (1990) method. Apparent digestibility was estimated in triplicate dry samples using the chromic oxide method as reported by Schürch et al. (1950).

Analyses. Protein (N × 6.25) in the diets and feces was estimated in triplicate dry samples following the standard micro-Kjeldahl method (AOAC, 1990). Trypsin inhibitor activity (TIA) was determined according to the AACC (1983) method. Duplicate fat-free dry samples of TF or PE were extracted with 0.001 M NaOH and then incubated with trypsin and benzoyl-DL-arginine *p*-nitroanilide hydrochloride for 10 min at 37 °C in a water bath shaker. TIA expressed as trypsin inhibitor unit per milligram of sample (TIU/mg of sample) was calculated from absorbance read against a blank in a spectrophotometer. One trypsin unit is defined as an increase of 0.01 absorbance unit at 410 nm per 10 mL of reaction mix. Hemagglutinating activity (HA) was determined by extracting samples with 0.9% NaCl (1:10 w/v) at room temperature for 30 min, centrifuging at 13600*g* for 10 min at 25 °C, and using supernatants for HA 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 that produced positive evidence of agglutination. HU per gram of sample was calculated as described by Liener and Hill (1953). All assays were done in Bio-Rad titertubes (1-mL capacity). Phytic acid (PA) analysis was performed according to the AOAC (1990) method. Duplicate fat-free dry samples weighing 2 g were vigorously extracted in 40 mL of 2.4% HCl solution for 3 h at room temperature using a shaker. An aliquot (1 mL) of the sample was diluted to 25 mL with deionized water and eluted through a column containing an anion-exchange resin (AG1-X4, 100–200 mesh, Bio-Rad Laboratories, Richmond, CA) with 15 mL of deionized water and 0.1 M sodium chloride solution, respectively. PA was washed out with 15 mL of 0.7 M NaCl and digested in a mixture of concentrated sulfuric and nitric acids. PA concentration calculated as milligrams per gram of sample was determined colorimetrically at 640 nm using a Sequoia-Turner spectrophotometer.

Statistical Analysis. The data were statistically analyzed using the one analysis of variance with means separated and a least significance difference (LSD) at $P < 0.05$ (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Mice fed whole egg as a source of protein (control) gained significantly more weight ($P < 0.05$) and had a significantly higher feed intake and protein efficiency ratio (PER) than those fed heat-treated tepary flour (TF) or tepary protein extract (PE) (Table II). No mice died during the 3-week experimental period. Values were similar to those reported by Yensen and Weber (1987) and Cossack and Weber (1983).

Diets containing raw TF or raw PE were toxic to mice (Table II). Supplementing these diets with 0.05% methionine (Met) led to similar results. All animals died in 3–4 days. The high level of trypsin inhibitor activity (TIA) and hemagglutinating (HA) activity (Table III) as well as other antinutrient factors might have caused toxicity. Similar results were observed in mice and rats fed diets containing 8–10% crude protein from raw or 10-min-autoclaved TF (C. W. Weber, 1981, unpublished observations; Gonzáles-Garza et al., 1982). Autunes and Sgarbieri (1980) also noticed that when raw flour or protein fractions from dry beans (*P. vulgaris*) were included alone or with Met into the diets of rats, all animals died in 4–10 days. They attributed this mortality to HA.

Autoclaving TF or PE for 20 min at 121 °C under a pressure of 15 lb/in^2 also did not support mouse growth (Table II). Addition of 0.05% Met to autoclaved samples showed only a little improvement. Although mice did survive slightly longer after this heat treatment and the addition of Met, all lost weight and died in 4–10 days. Since HA was completely destroyed and TIA considerably reduced (Table III), we might assume that other unidentified heat-resistant toxic factors might be present in tepary bean. This finding contradicts those of Gonzáles-Garza et al. (1982) and Grant et al. (1983), who suggested that HA was responsible for the high mortality observed in rats fed raw TF. Although TIA was reduced by 92% and 52% in TF and PE, respectively (Table III), this was still not enough to eliminate toxicity. This finding might suggest that HA and TIA might not be the major toxic factors in tepary seeds. Other heat-resistant lethal substances might have been involved in the mortality of mice.

Soaking tepary seeds in water overnight and cooking them in water for 20 min using a pressure cooker eliminated toxicity completely and led to perceptible weight gain and increased PER (Table II). Feed intake, weight gain, and PER were significantly lower than the control diet and in agreement with the values reported in tepary beans cooked in water for 140 min (Gonzáles de Mejia et al., 1988) and in soaked and autoclaved dry beans (*P. vulgaris*) (Durigan et al., 1987). Since soaking water was discarded before cooking, some TIA and other soluble toxic factors might have been partially eliminated. The digestibility of soaked and cooked sample (Table II) was similar to that of control and much higher than that of autoclaved samples. The complete elimination of HA and the higher reduction of TIA when compared to other heat-treated samples as well as the removal of some other antinutrient factors during the soaking and cooking phases of tepary seeds might

Table II. Nutritional Evaluation of Tepary Flour (TF) and Protein Extract (PE) after Different Treatments

source of protein	feed intake, g/day	protein intake, g/day	wt change, g/day	PER	digestibility, %	mortality/ 10
whole egg	4.68^{A}	0.40^{A}	0.92^{A}	2.33^{A}	85.92	0
raw TF or PE[a]	ND	ND	ND	ND	ND	10
TF or PE (autoclaved, 20 min)[b]	ND	ND	ND	ND	ND	10
TF (cooked, 20 min)	3.73^{B}	0.31^{B}	0.30^{B}	0.97^{B}	83.54	0
TF (autoclaved, 40 min)	3.29^{C}	0.28^{C}	0.23^{BC}	0.82^{BC}	53.37	0
TF (autoclaved, 40 min) + 0.05% Met	3.37^{C}	0.29^{BC}	0.29^{B}	1.01^{B}	58.03	0
PE (autoclaved, 40 min) + 0.05% Met	3.19^{C}	0.27^{C}	0.19^{C}	0.69^{C}	62.48	0
TF (autoclaved, 60 min)	1.99^{D}	0.16^{D}	−0.01^{D}	−0.42^{D}	41.36	6
TF (autoclaved, 60 min) + 0.05% Met	2.04^{D}	0.17^{D}	−0.06^{D}	−0.40^{D}	58.91	5
PE (autoclaved, 60 min) + 0.05% Met	1.84^{D}	0.15^{D}	−0.06^{D}	−0.38^{D}	57.59	0

[a] TF or PE with or without 0.05% Met; mice died in 3–4 days. [b] TF or PE with or without 0.05% Met; mice died in 4–10 days. Mean values having the same superscript within columns are not significantly different ($P < 0.05$). ND, not determined.

Table III. Trypsin Inhibitor Activity (TIA), Hemagglutinating Activity (HA), and Phytic Acid (PA) Levels of Raw and Heat-Treated Tepary Flour (TF) and Protein Extract (PE)[a]

sample	TIA,[b] TIU/mg	HA,[c] HU/g	PA, mg/g
TF (raw)	14.05	20 000	6.22^{B}
TF (beans cooked, 20 min)	0.75 (95)	0	5.88^{B}
TF (autoclaved, 20 min)	1.13 (92)	0	6.16^{B}
TF (autoclaved, 40 min)	0.82 (94)	0	7.04^{B}
TF (autoclaved, 60 min)	1.07 (92)	0	7.05^{B}
PE (raw)	90.17	29 000	31.32^{A}
PE (autoclaved, 20 min)	43.00 (52)	0	32.89^{A}
PE (autoclaved, 40 min)	0.95 (99)	0	33.36^{A}
PE (autoclaved, 60 min)	7.74 (91)	0	31.38^{A}

[a] Data are means of duplicate determinations on fat-free dry sample. [b] Expressed as trypsin inhibitor unit (TIU/mg of sample); numbers in parentheses indicate percentage reduction of TIA after heat treatment. [c] Calculated as hemagglutinating unit (HU/g of sample). Mean values with the same superscript are not significantly different ($P < 0.05$).

explain these differences. Lower digestibility values (75–78%) have been reported in cooked tepary beans (Gonzáles de Mejia et al., 1988).

Autoclaving TF for 40 min supported mouse growth (Table II). Weight gain and PER were significantly lower ($P < 0.05$) than control but not significantly different from those of soaked and cooked samples. Addition of Met led to a slight increase in weight gain (26%) and PER (23%), but these values were not significantly different from those of cooked or 40-min-autoclaved TF samples. The PER values of our study were in good agreement with those reported for rats fed autoclaved dry beans (Autunes and Sgarbieri, 1980). Digestibility was lower than that of cooked samples (Table II). TIA decreased by about 94% (Table III). Autoclaving PE for 40 min and then adding Met led to weight gain and PER significantly lower than those of cooked or 40-min-autoclaved TF. The lower sulfur amino acid contents reported in this extract when compared to that of TF could explain this difference (Idouraine et al., 1992b).

Autoclaving TF or PE for 60 min depressed mouse growth and led to a high mortality (Table II). Feed intake and PER were significantly lower than those of cooked or 40-min-autoclaved samples. Addition of Met had no significant effects on feed intake and PER. The detrimental effects might be due to the combination of protein denaturation which could lead to a proteolysis resistance and loss of some amino acids such as lysine, cysteine, and cystine as well as to the formation of new components which might act as appetite-reducing agents or even be toxic to mice. This could also be explained by the low feed intake due to a poor palatability of diets leading to death of animals by starvation. Prolonged autoclaving has been reported to reduce significantly most of the essential amino acids and lead to the formation of undigestible compounds which were blamed for the nonutilization of legume proteins (Evans and Butts, 1940; Lanfer Marquez and Lajolo, 1990). Maillard-type reactions which might lead to the formation of new compounds not easily digested in the gastrointestinal tract could also affect mouse growth performance (Liener and Thomson, 1980). TIA levels were found to be slightly higher than those of the 40-min-autoclaved samples (Table III). This might be explained either by the destruction of the trypsin structure, leading to a higher number of smaller peptides which exhibited higher activity when compared to the 40-min-autoclaved samples, or by the formation of new products which might inhibit trypsin. Similar trends have been reported by some authors. Elias et al. (1976) showed that autoclaving black bean (*P. vulgaris*), cowpea (*Vigna sinensis*), and pigeon pea (*Cajanus cajan*) flours for 15 30, and 40 min drastically decreased TIA when compared to the raw samples. Although the authors gave no explanation, the 40-min-autoclaved flour exhibited a higher TIA level when compared to that of the 30-min-autoclaved samples. Prolonged autoclaving appeared, therefore, to cause negative effects on mouse growth performance. Similar results were reported by Ekpenyong and Borchers (1981) and Kim and Barbeau (1991).

Some authors have reported that soaking beans in water prior to autoclaving was necessary to eliminate the depressive effects caused in animals (Honavar et al., 1962). Others have found that autoclaving alone was sufficient (Hintz et al., 1967). In this study, both soaking and pressure cooking or autoclaving alone appeared adequate for the elimination of growth depressive effects in mice. The optimal time was found to be 20 min for soaked and pressure-cooked tepary seeds and 40 min for autoclaved TF or PE. The long autoclaving time period required to eliminate the deleterious effects caused by TF or PE might be related to bean varieties and animal species. Mice have been reported to be more strongly affected by inhibitors than rats (Westfall and Hauge, 1948).

Phytic acid (PA) contents were not significantly affected either by soaking and pressure cooking tepary seeds or by autoclaving TF or PE for different time periods (Table III). Results of our study were in agreement with those reported by several authors (Lease, 1966; Kadam et al., 1987). PA might form complexes with proteins and minerals leading to a decrease in the bioavailability of these nutrients. PA contents in teparies might range from 0.38 to 9.38 mg/g of sample (Idouraine et al., 1991).

CONCLUSION

The results indicate that consumption of raw or 20-min-autoclaved TF and PE caused the mortality of mice within 10 days. Since HA was completely eliminated and TIA considerably reduced after heating, other toxic factors seemed to be present in tepary beans. Inclusion of soaked and 20-min-cooked tepary beans or 40-min-autoclaved TF and PE in the diets of mice eliminated toxicity and led to a perceptible weight gain and increased PER. Digestibility of cooked samples was similar to that of the control diet, while that of autoclaved samples was lower. Autoclaving for 60 min had a significant depressing effect on feed intake, weight gain, and PER. Protein denaturation, some amino acid unavailability, and likely newly formed undigestible or toxic compounds might be responsible for this depression. Soaking and cooking tepary seeds appeared to be the best method of eliminating completely toxicity, leading to the best performance. Autoclaving TF or PE for 40 min appeared to be optimal for mouse growth performance. Further studies are needed to determine which other antinutrients cause toxicity and what type of interactions might limit the digestion of TF or PE.

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