Nutrient, Antinutrient Contents, and Solubility Profiles of Nitrogen, Phytic Acid, and Selected Minerals in Winged Bean Flour[1]

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ABSTRACT

Protein, fat, mineral, and antinutrient content of the meal of 12 cultivars of winged bean (*Psophocarpus tetragonolobus*) seeds grown in Sri Lanka were determined. Protein and oil contents in dehulled seed flour ranged from 37.4 to 46.3 and from 20.9 to 26.5% on dry weight basis, respectively. Of the antinutritional factors, phytic acid and trypsin inhibitor levels were 1.0–1.7% and 52.2–99.5 trypsin inhibitor units per milligram of flour, respectively. Tannin content in the whole meal varied between 0.2 and 0.7 mg of catechin equivalent per gram of flour. pH modification markedly affected the solubility of nitrogen, phytic acid, and minerals in seed meal slurries. Nitrogen solubility dropped from 32% at pH 2.0 to 12% at pH 4.0. Conversely, phytic acid was 25% soluble at pH 2.0 and 48.0% soluble at pH 4.0; at neutral pH, the solubility of nitrogen and phytic acid were 50 and 80%, respectively. Phytic acid solubility decreased drastically to 5.5% at pH 12.0, but nitrogen solubility remained high at 72%. The solubility of calcium, phosphorus, and zinc decreased as the pH was increased from 2.0 to 6.0 and above. Such results demonstrate that differences in solubility of nitrogen and phytic acid can be utilized to prepare a protein concentrate with low phytic acid content.

During the last few years, the overall nutrient composition, including some antinutritional factors, of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) seeds has been reported for varieties grown in experimental field plots in the United States and Puerto Rico (Harding et al 1978, Garcia and Palmer 1980, Okezie and Martin 1980, Hildebrand et al 1981a), India (Rao and Belavady 1979, Japan (Ibuki et al 1983, Yanagi et al 1983), Malaysia (Tan et al 1983), Peru (Gross 1983), Philippines (Del Rosario et al 1981), and Sri Lanka (Hettiarachchy and Sri Kantha 1982). However, literature searches (NAS 1981, Khan 1982, Sri Kantha and Erdman 1984) have not revealed information on the physical characteristics of the seeds of many varieties. Furthermore, although the pH solubility profile of nitrogen in the seeds has been reported (Gillespie and Blagrove 1978, Dench 1982, Narayana and Narasinga Rao 1982), Sathe et al 1982, little is known about the pH solubility profile of minerals and phytic acid of winged bean flour. In addition, reports on the tannin content in the seeds show conflicting values (Sri Kantha and Erdman 1984).

The present investigation is part of a continuing study on the nutritional aspects of winged bean in our laboratory. Properties of trypsin inhibitor (Hildebrand et al 1981b), production of winged bean curd (Sri Kantha et al 1983), and bioavailability of zinc and iron (Hettiarachchy and Erdman 1984) have been reported previously. In our study, 12 winged bean cultivars grown in Sri Lanka were surveyed for their nutrient and antinutrient compositions. Three of the five cultivars (Nakhon Sawan, UPS-121, and UPS-122) recommended by the First International Winged Bean Trials Report (Khan et al 1984) were included in this study. Based on the solubility profiles of nitrogen, phytic acid, and minerals, attention also focused on developing a laboratory scale procedure for preparing a low phytic acid protein concentrate.

MATERIALS AND METHODS

Winged bean seeds (cultivars SLS-1, SLS-6, SLS-11, UPS-31, UPS-45, UPS-47, UPS-61, UPS-121, Chimbu, Nakhon Sawan, TPT-2, and TPT-8) grown at the Central Agricultural Research Institute Experimental Trials, Gannoruwa, Sri Lanka, were used (supplied by H. P. M. Gunasena). Some seeds were stored at 0–4°C as long as 6 months before the preparation of defatted flour. Bonus 79 soybean, obtained from the University of Illinois Experimental Station, was used for comparative analysis.

All the chemicals used were of reagent grade and were purchased from Sigma Chemical Company (St. Louis, MO). Defatted winged bean seed flour was prepared as outlined in Figure 1 and stored at 0–4°C.

Physical Characteristics of Seed

Selected physical characteristics of the cultivars were measured as described by Williams et al (1983).

Analytical Methods

Proximate analyses of the seed flour were done according to standard AOAC (1975) methods 14.006, 14.018, and 14.026. A conversion factor of 6.25 was used for calculation of percent protein. Minerals were analyzed by atomic absorption spectrophotometry (Perkin-Elmer Model #306, Norwalk, CT) on the dry ash samples of cotyledons.

Trypsin inhibitor content was assayed by the method of Kakade et al (1974) using the synthetic substrate benzoyl-DL-arginine-*p*-

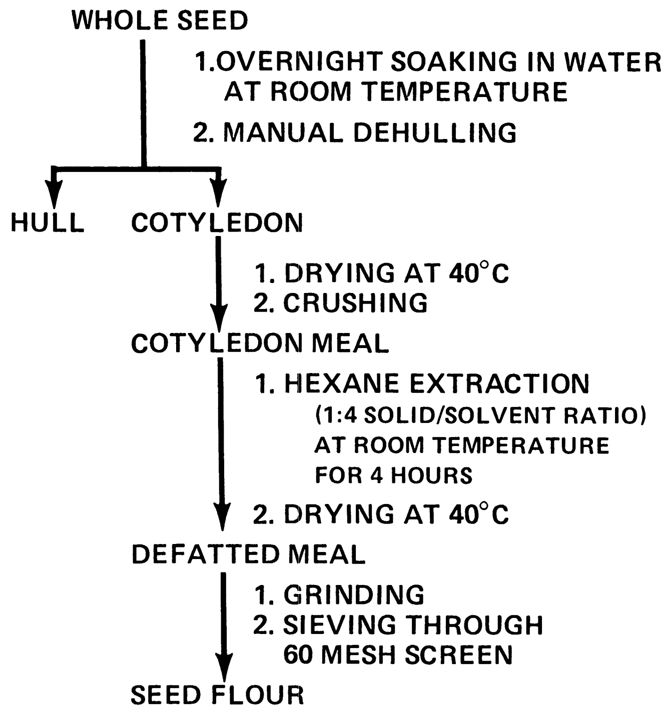

Fig. 1. Preparation of defatted flour from winged bean seed.

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nitroanilide hydrochloride (BAPNA) and expressed as trypsin inhibitor units (TIU) per milligram of flour. One gram of cotyledon flour, passed through 60-mesh screen, was homogenized in a Waring blendor with 50 ml of 0.01 N NaOH for 5 min, and the resulting slurry was diluted 1:30 and assayed.

Tannin content was determined according to the vanillin-HCl method on the methanol extracts of seed flour (Burns 1971), as modified by Price and Butler (1977) and expressed as catechin equivalents per milligram of flour. One gram of whole flour, passed through 60-mesh was extracted with 10 ml of methanol for 18 hr at room temperature, with mechanical shaking.

Phytic acid levels were determined by the supernatant difference method (Thompson and Erdman 1982) and expressed as percent of flour. A 2-g sample of cotyledon flour was extracted with 100 ml of 1.2% HCl for 2 hr on a mechanical shaker.

pH Solubility

Nitrogen, phytic acid, and mineral solubility of raw seed meal was determined in the pH range of 2–12, using 2 g of flour with a flour-water ratio of 1:20 and extraction for 60 min at room temperature (28°C). A Beckman pH meter (Model 3500, digital) was used to check the pH, and commercially prepared pH 4.00, 7.00, and 11.00 buffers were used to calibrate the pH meter. The pH of the system was adjusted by the addition of 1 N HCl or 1 N NaOH. The supernatant from the resulting slurry was separated by centrifugation at 1,500 $\times$ g for 20 min and analyzed for nitrogen, phytic acid, and minerals.

RESULTS AND DISCUSSION

The mean seed size (0.31 g per seed) of winged bean is almost twice that of the Bonus 79 soybean (0.16 g per seed). The seed size, seed volume, and density results of our study (Table I) are similar to the values reported for chick-peas (Williams et al 1983). Moisture content of the original beans ranged between 4.5 and 7.5% (mean $\pm$ S.D., 5.7 $\pm$ 1.3). Although winged bean is slightly denser than soybean, values were similar for the hydration and swelling capacities of both bean types.

The moisture content of the prepared dehulled flour ranged between 4.6 and 6.1% (mean 5.3%). The protein content ranged from 37.4 to 46.3% (mean 42.8%), and oil content ranged from 20.9 to 26.5% (mean 23.2%) on a dry weight basis (Table II). These values compare favorably with those previously published (Garcia and Palmer 1980, Okezie and Martin 1980, Hildebrand et al 1981a) and show that the winged bean closely resembles the soybean in protein and oil content.

Of the macrominerals in the dehulled seed flour, calcium (mean 0.57%) and phosphorus (mean 0.50%) contents were relatively high in winged bean (Table III) in comparison to other beans such as broad bean, chick-pea, cowpea, kidney bean, peanut, lentil, lima bean, and soybean (FAO 1982). However, winged bean dehulled seed flour is a relatively poor legume source of iron, having a mean value of 84 ppm.

The pH solubility profile of nitrogen and phytic acid for the TPT-2 seed flour is shown in Figure 2. The nitrogen solubility dropped from 32% at pH 2.0 to 12% at pH 4.0, which is the isoelectric point of this seed protein. Similar nitrogen solubility patterns have been reported previously (Gillespie and Blagrove 1978, Dench 1982, Narayana and Narasinga Rao 1982). In contrast, phytic acid was 25% soluble at pH 2.0 and 48% soluble at pH 4.0; at the neutral pH, the solubility of nitrogen and phytic acid were 50 and 80%, respectively. Phytic acid solubility decreased drastically to 5.5% at pH 12.0, whereas nitrogen solubility remained high at 72%. The pH solubility profile of nitrogen from winged bean flour appears to be similar to the pattern exhibited by soybean protein (deRham and Jost 1979), peanut meal (Fontaine et al 1946), mung bean, pea bean, and red kidney bean proteins (Hang et al 1970) but is quite different from cotton seed meal (Fontaine et al 1946). The pH solubility profile of winged bean phytic acid also showed the same pattern of that of soybean phytic acid (deRham and Jost 1979) but varied from the patterns of cotton seed and peanut seed meals (Fontaine et al 1946). Maximum solubility of phytic acid (soybean 80% and winged bean 82%) was at pH 8.0 in both soybeans and winged beans.

The pH solubility profiles of calcium, phosphorus, zinc, and iron are shown in Figure 3. The solubility of calcium and zinc decreased as the pH was increased from 4.5 to 6.5; solubility of phosphorus decreased significantly as the pH was increased from 6.5 to 12.0; there was not much fluctuation in the solubility of iron, within the pH 3.0–12.0 range.

The trypsin inhibitor content in raw winged bean seed flour ranged from 52.2 to 99.5 TIU/mg (Table IV) in comparison to 59.9 TIU/mg of raw soybean meal flour. Kakade et al (1974) reported 87.6–109.4 TIU/mg of flour for raw soymeal although the variety used was not identified. Because trypsin inhibitor content in raw winged bean seeds has been measured when using different substrates such as casein and *p*-toluenesulfonyl arginine methyl ester (deLumen and Salamat 1980, Hettiarachchy and Sri Kantha 1982), the values are difficult to compare. Our data for 12 cultivars, expressed on a protein basis, ranges between 1.2×10^5 and 2.3×10^5 TIU/g of protein, which is within the range (7.7×10^4–8.7×10^5) reported by Tan et al (1984), who used the BAPNA substrate in raw seed flour for three varieties grown in Malaysia.

Tannin content in the whole meal of the 12 cultivars we analyzed ranged from 0.2 to 0.7 mg of catechin equivalents per gram of flour (Table IV). Of the two previously published studies, Price et al (1980) reported zero values for four varieties, using the vanillin-HCl method of tannin determination. In contrast, 0.3–7.5 mg/g of flour was reported for 16 varieties by Tan et al (1983), which compares favorably with the range found in our study. The 30-min

TABLE I
Characteristics of Seven Cultivars of Winged Bean Seeds[a]

Accession	Weight (g/seed)	Volume (ml/seed)	Density (g/ml)	Hydration		Swelling	
				Capacity	Index[b]	Capacity	Index[c]
SLS-1	0.31	0.24	1.29	0.20	0.65	0.70	2.92
SLS-6	0.22	0.18	1.22	0.13	0.59	0.64	3.56
SLS-11	0.35	0.30	1.17	0.32	0.91	0.82	2.73
TPT-2	0.31	0.24	1.28	0.20	0.65	0.72	3.00
TPT-8	0.30	0.24	1.25	0.25	0.83	0.84	3.50
Chimbu	0.38	0.32	1.20	0.30	0.79	0.82	2.56
Nakhon Sawan	0.31	0.24	1.28	0.20	0.65	0.72	3.00
Mean	0.31	0.25	1.24	0.23	0.72	0.75	3.04
S.D.	0.05	0.05	0.05	0.07	0.12	0.08	0.37
Soybean (Bonus 79)	0.16	0.14	1.14	0.22	1.38	0.74	5.29

[a] Each value represents the mean of four determinations.
[b] Hydration index = hydration capacity/original seed size.
[c] Swelling index = swelling capacity/seed volume.

extraction employed by Price et al (1980) may have been inadequate to extract tannins. In addition, the TPT-2 and Chimbu varieties studied by Price et al were in the lower range of tannin content (0.2 mg/g of flour) found in the present study when 18-hr extraction was employed. Based on the values reported by Tan et al (1983) and in our study, we conclude that the tannin content in winged bean flour is considerably less than the tannin content of other legumes such as cowpeas (0–0.7%), pigeon peas (0–0.2%), and adzuki beans (0.3%) reported by Price et al (1980). No tannins were detected in soybean flour in this study. Our results generally support the assertion of Tan et al (1983) that winged bean seed does not contain sufficiently high levels of tannin to be of concern as an antinutritional factor.

Although Tan et al (1983) did not identify the seed coat color of the varieties stored, we attempted to evaluate seed coat color and tannin content but noted no association between these two factors. In fact, the three accessions with black seed coats (UPS-31, UPS-47, and Chimbu) had lower tannin levels than the nine accessions with seed coats with various shades of brown. Most of the winged bean accessions have brown and black seed coats, although cream color has also been found in one or two cultivars. According to Khan (1982), seed coat color may be greatly modified by the environment in which seed matures and by storage conditions. deLumen and Salamat (1980) reported 1.58 mg of tannic acid per gram in the Chimbu cultivar, as assayed by Folin-Denis reagent. However, this reagent determines total phenolic content of seed rather than tannin content.

Phytic acid values of cotyledon flour (Table IV) ranged from 1.0 to 1.7% for 12 cultivars. Like soybean (Weingartner et al 1979), winged bean hull contains less than 0.1% phytic acid. The 1.0–1.7% range for cotyledon flour in our study is higher than the range (0.62–0.70%) reported by Kadam and Salunkhe (1984), who investigated three cultivars. Lolas et al (1976) reported 1.00–1.47% for 15 soybean cultivars, and Thompson and Erdman (1982) found 1.54% phytic acid dehulled soybean meal in Bonus 1975. The phytic acid level in dehulled winged bean flour is higher than the levels in cereal grains such as common corn (0.89%), high lysine corn (0.99%), brown rice (0.89%), oats (0.79–1.01%), and proso millets (0.17–0.47%) (deBoland et al 1975, Lolas et al 1976, Lorenz 1983).

As noted, winged beans and soybeans are very similar in phytic acid content and have remarkably similar solubility profiles. Because soybean phytic acid decreases zinc bioavailability (Erdman 1979), winged bean phytic acid must be considered as a significant antinutritional factor. Hartman (1979) described an alkaline extraction procedure to decrease phytic acid content of soybeans. This procedure was adapted for preparation of low phytic acid winged bean protein concentrate from defatted flour (Fig. 4).

Although the yield of protein is low (Table V), a low phytic acid protein concentrate can be produced by heating and filtering

TABLE II
Proximate Composition of Dehulled Winged Bean Seed Flour[a]

Origin Accession	Protein	Oil	Ash	Carbohydrate[b] and Crude Fiber
Sri Lanka				
SLS-1	37.4	23.5	4.8	34.3
SLS-6	46.0	23.6	4.2	26.2
SLS-11	37.8	22.8	3.9	35.5
Papua New Guinea				
UPS-31	45.9	22.8	3.5	27.8
UPS-45	43.9	21.4	3.5	31.2
UPS-47	44.1	24.6	4.0	27.3
UPS-61	43.6	23.8	4.1	28.5
UPS-121	40.2	23.8	4.0	32.0
Chimbu	43.3	26.5	3.9	26.3
Nigeria				
TPT-2	43.3	21.5	4.3	30.9
TPT-8	46.3	22.6	3.9	27.2
Thailand				
Nakhon Sawan	41.8	20.9	3.8	33.5
Range	37.4–46.3	20.9–26.5	3.5–4.8	26.2–35.5
Mean	42.8	23.2	4.0	30.1
S.D.	3.0	1.5	0.4	3.3

[a]Each value is the mean of four determinations, expressed as g/100 g, dry weight basis.
[b]Calculated by difference.

TABLE III
Mineral Composition of Dehulled Winged Bean Seed Flour[a]

Accession	Ca (%)	P (%)	Mg (%)	K (%)	Na (%)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)
SLS-1	0.55	0.42	0.23	0.64	0.01	53	49	24	27
SLS-6	0.53	0.53	0.33	0.85	0.05	101	58	27	40
SLS-11	0.48	0.56	0.26	0.77	0.02	59	54	27	20
UPS-31	0.66	0.60	0.27	1.01	0.03	90	64	37	45
UPS-47	0.43	0.64	0.22	0.91	0.03	73	45	21	19
UPS-121	0.50	0.49	0.33	0.89	0.04	100	74	37	55
TPT-2	0.52	0.47	0.23	0.71	0.02	74	44	27	29
TPT-8	0.73	0.40	0.31	1.09	0.04	125	36	52	40
Chimbu	0.45	0.49	0.21	0.69	0.02	83	51	23	27
Nakhon Sawan	0.85	0.36	0.30	0.95	0.01	85	53	21	51
Mean	0.57	0.50	0.27	0.85	0.03	84	53	29	35
S.D.	0.13	0.09	0.04	0.15	0.01	21	11	10	12

[a]Each value is the mean of three determinations, dry weight basis.

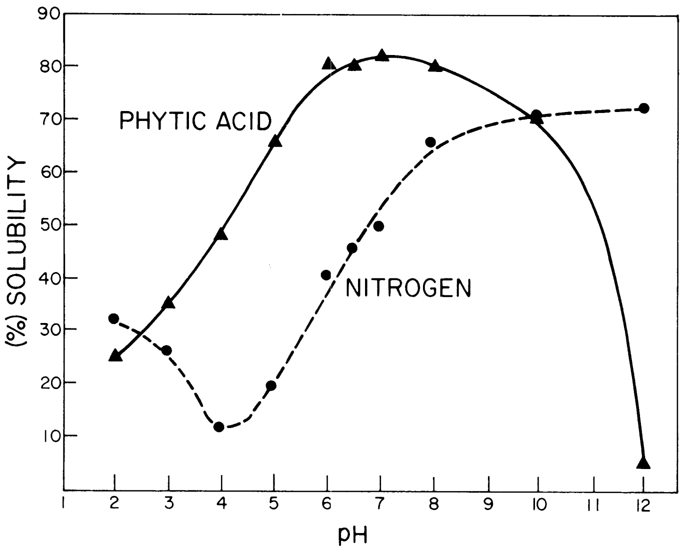

Fig. 2. pH-solubility profile of nitrogen and phytic acid in flour from winged bean cultivar TPT-2.

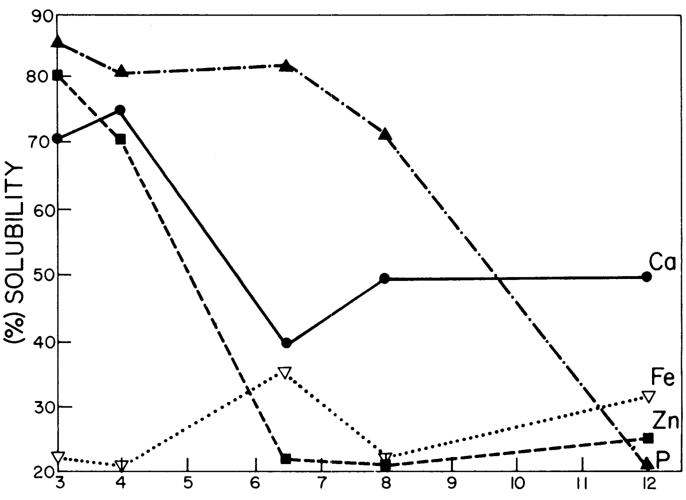

Fig. 3. pH-solubility profile of minerals Ca, Zn, Fe, and P in flour from winged bean cultivar TPT-2.

TABLE IV
Antinutrient Content in Winged Bean[a]

Accession	Seed Coat	Cotyledon: Phytic Acid (%)	Cotyledon: Trypsin Inhibitor (TIU/mg flour)	Tannin in Whole Flour[b] (mg/g)
SLS-1	light brown	1.1	66.4	0.6
SLS-6	light brown	1.5	99.5	0.4
SLS-11	light brown	1.5	99.4	0.5
UPS-31	black	1.7	67.9	0.3
UPS-45	dark brown	1.3	79.1	0.2
UPS-47	black	1.6	65.6	0.3
UPS-61	dark brown	1.6	65.7	0.2
UPS-121	dark brown	1.5	73.5	0.2
TPT-2	red brown	1.4	74.6	0.2
TPT-8	red brown	1.0	52.2	0.7
Chimbu	black	1.5	85.8	0.2
Nakhon Sawan	brown	1.1	85.1	0.2
Range		1.0–1.7	52.2–99.5	0.2–0.7
Mean		1.4	76.2	0.3
S.D.		0.2	14.3	0.2
Soybean (Bonus 79)	cream	1.4	59.0	0.0

[a] On as-is moisture basis; 60-mesh screened flour (minimum particle size 250 μm). Each value is the mean of four determinations.
[b] Catechin equivalents.

TABLE V
Protein and Phytic Acid Content of Protein Fractions[a]

Sample	Percent Recovery of Solids	Protein, %: of Sample	Protein, %: Recovery	Phytic Acid, %: of Sample	Phytic Acid, %: Recovery
Winged bean defatted seed flour	100.0	39.9	100.0	1.43	100.0
pH 12.0 residue (high phytic acid fraction)	45.0	19.8	22.3	1.40	44.1
pH 4.0 isoelectric concentrate (high protein fraction)	30.0	61.8	46.5	0.47	9.8

[a] Each value is the mean of three determinations.

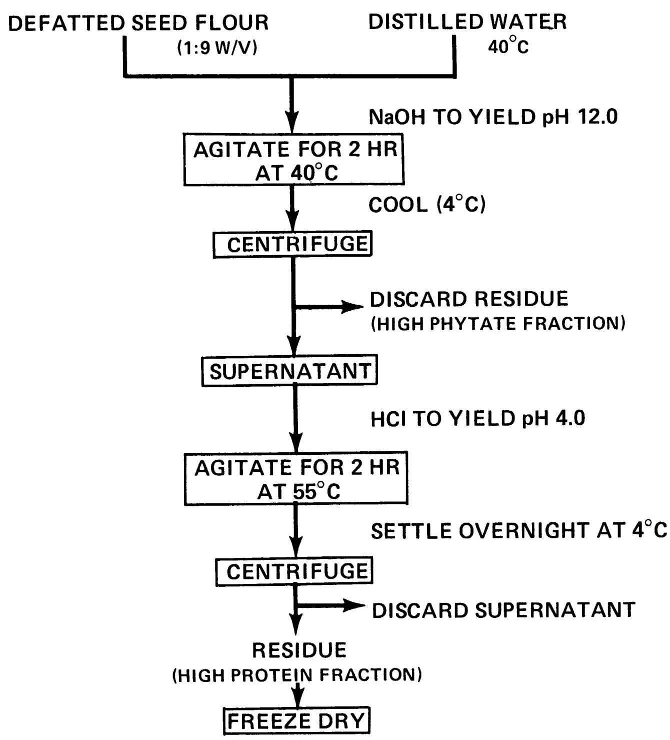

Fig. 4. Preparation of low phytic acid winged bean protein concentrate.

winged bean flour at pH 12.0 before isoelectric precipitation of protein. Thus, the differences in solubilities of nitrogen and phytic acid can be utilized to prepare a winged bean protein concentrate that is low in phytic acid. Lack of a large supply of winged bean seed has restricted further studies on improved yield and protein content of the concentrate, but the feasibility of concentrate production has been demonstrated. Further studies should investigate the reduction of antinutritional factors and the possible production of lysinoalanine at the elevated pH utilized to produce winged bean protein concentrate.

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