

SHORT COMMUNICATION

Trypsin Inhibitor and Phytohaemagglutinin Contents in the Seeds of Six Legumes commonly consumed in Sri Lanka

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Green gram (*Phaseolus aureus* Roxb.; Mung ata-S*; Paasi Payaru-T)† Black gram (*Phaseolus mungo* L.; Undu-S; Ulundu-T), Red gram (*Cajanus cajan* L. Millsp.; Parippu-S, Tuvaram Paruppu-T), Bengal gram (*Cicer arietinum* L.; Konda-kadala-S, Konda Kadalai-T), Cow pea (*Vigna unguiculata* L.; cowpea-S, Payaru-T), and Soybean (*Glycine max* L. Merr.; soya bonjee-S, soya avarai-T)³ are the six commonly consumed legumes which are grown in Sri Lanka.¹⁰

Though earlier workers^{2,5} had reported on the proximate composition of legumes popularly consumed in Sri Lanka there is hardly any information so far on the levels of two main anti-nutritional factors, trypsin inhibitor (Ti) and phytohaemagglutinin (PHA) contents.

Since efforts are currently in progress to introduce legume grain flour into substitute milk formulae for infants and into various common foods, an attempt was made in this study to assess the six legumes nutritionally with emphasis on these two anti-nutritional factors.

500g samples of the six legumes were purchased from Peradeniya market; ground with a laboratory hammer mill (model 317/0195) and passed through a sieve with mesh no. 22. For the determination of moisture, protein, lipid, ash and crude fibre, the methods described by the Association of Official Agricultural Chemists¹ were used. Protein was calculated by multiplying the nitrogen content by 6.25. Carbohydrate was determined by difference.

Trypsin inhibitor activity was assayed by the method of Kakade *et al.*⁶ as modified by Hettiarachchy *et al.*⁴ Phytohaemagglutinin activity was assayed by the method of Liener and Hill.⁸

The proximate composition of the six legumes are shown in Table 1. The protein content of five legumes (*P. aureus*, *P. mungo*, *C. arietinum*, *V. unguiculata* and *C. cajan*) range from 22.3 to 30.6 percent, while soybean with a protein content of 40.6

* S - Sinhala; † T - Tamil

percent is the richest source of this major nutrient. Similarities of the five legumes are further extended in the composition of lipid. The lipid levels range from 1.0 to 5.0 percent in these five legumes while in the variety of *G. max* tested, the lipid value recorded was 23.0 percent. Most of the values obtained in this study is comparable with the values reported by Bamber² and Joachim and Pandittesekera.⁵ Disparity among the reported values in protein and lipid is noticed only in the case of soybean. This may be due to the difference in the species of soybean analysed by the earlier workers.

TABLE 1. Proximate composition of the Six legumes (percent)

Legumes	Protein	Lipid	Carbo- hydrate*	Ash	Fibre	Moisture	Reference
<i>Phaseolus aureus</i>	28.4	1.0	52.0	3.8	2.8	12.0	Present study
<i>Phaseolus aureus</i>	21.7	1.0	57.7	4.2	3.3	12.1	Joachim and Pandittesekera (1938)
<i>Phaseolus mungo</i>	26.3	1.2	54.4	3.3	0.8	14.0	Present study
<i>Phaseolus mungo</i>	23.0	1.1	59.4	3.1	0.0	13.4	Joachim and Pandittesekera
<i>Phaseolus mungo</i>	22.7	2.2	55.8	4.8	4.4	10.1	Bamber (1921)
<i>Vigna unguiculata</i>	27.6	1.6	51.5	4.1	4.2	11.0	Present study
<i>Vigna unguiculata</i>	26.8	1.1	52.4	3.9	4.9	10.9	Joachim and Pandittesekera
<i>Vigna catieng</i>	24.1	1.3	56.8	1.8	3.5	12.5	Bamber
<i>Cicer arietinum</i>	30.6	4.6	41.1	2.6	3.1	18.0	Present study
<i>Cicer arietinum</i>	21.7	4.2	59.0	1.0	3.6	11.5	Bamber
<i>Cajanus cajan</i>	22.3	5.0	58.8	3.5	1.2	9.2	Present study
<i>Cajanus cajan</i>	21.3	1.0	63.0	2.5	1.1	11.1	Joachim and Pandittesekera
<i>Cajanus indicus</i>	22.3	2.1	60.9	1.2	3.0	10.5	Bamber
<i>Glycine max</i>	40.6	23.0	14.7	3.9	3.2	14.6	Present study
<i>Glycine hispida</i>	37.0	14.2	27.9	5.0	2.9	13.0	Joachim and Pandittesekera
<i>Glycine soja</i>	25.3	18.9	26.0	4.2	14.6	11.0	Bamber

* calculated by difference

The results obtained for trypsin inhibitor activity assay of six legumes are shown in Table 2. Trypsin inhibitor activity was present in soybean and red gram extracts to a considerable extent; i.e., 33.5 and 20.7 mg Ti/g sample respectively. While three other legumes tested (*P. aureus*, *P. mungo* and *V. unguiculata*) are devoid of Ti activity, *C. arietinum* extract showed the presence of 1.9 mg Ti/g sample.

Table 3 shows the results obtained for the PHA activity in the six legumes. Phytohaemagglutinin activity though detected in all the legume extracts tested were in low profile ranging from 100 to 800 haemagglutinating units/g sample. Soybean and cowpea exhibited the highest values recorded, 800 HU/g sample; green gram and black gram recorded the lowest values of 100 HU/g sample.

TABLE 2. Anti-tryptic activity in the raw seed extracts† of six legumes

Legume	TU*	TIU*	TIU/mg sample	TIU/mg protein	mg TI/g**
(control)	55.0	—	—	—	—
<i>P. aureus</i> (Mung ata, Paasi payaru)	55.0	—	—	—	—
<i>P. mungo</i> (Undu, Ulundu)	55.0	—	—	—	—
<i>C. arietinum</i> (Konda kadala, Kondai kadalai)	52.7	2.3	3.4	11.0	1.9
<i>V. unguiculata</i> (cow pea, payaru)	55.0	—	—	—	—
<i>G. max</i> (soya bonjee, soya avarai)	15.0	40.0	59.7	129.0	33.4
<i>C. cajan</i> (Parippu, Tuvaram paruppu)	30.0	25.0	37.3	166.7	20.8

† extracted by homogenising 670 μ g finely ground seed meal (on fresh weight basis) in 1.0 ml of 0.1M, pH 7.6 phosphate buffer for 5 minutes in a Waring blender and clarified by centrifugation.

* Trypsin units (TU) as defined by Kakade *et al.* (1969); mean of three determinations. Trypsin inhibitor units (TIU) calculated as follows: —
 $TIU = TU \text{ of control} - TU \text{ of test sample.}$

** Calculated on the basis that 1.79 TIU is equivalent of 1 μ g of TI; Kakade *et al.* (1969).

$$\text{mg TI/g sample} = \frac{\text{TIU/mg sample}}{1.79}$$

TABLE 3. Phytohaemagglutinin activity of raw seed extract* assayed by using trypsinated human erythrocytes

Legume	Haemagglutination titre (reciprocal)	HU/g sample†
<i>P. aureus</i> (Mung ata, Paasi payaru)	1	100
<i>P. mungo</i> (Undu, Ulundu)	1	100
<i>C. arietinum</i> (Konda Kadala, Kondai Kadalai)	4	400
<i>V. unguiculata</i> (cow pea, payaru)	8	800
<i>G. max</i> (soya bonjee, soya avarai)	8	800
<i>C. cajan</i> (parippu, Tuvaram paruppu)	4	400

* Extracted by homogenising one gram sample in 50 ml phosphate buffered saline (0.1M, pH 7.6) for 5 minutes in a Waring blender and clarified by centrifugation.

† Expressed as Haemagglutinating units (HU)/g sample on fresh weight basis as defined by Liener and Hill (1953).

The quantification of the trypsin inhibitor and phytohaemagglutinin levels in this study was on a fresh weight basis, in view of the fact that both these anti-nutritional factors had been reported to be thermolabile in nature. ^{7,9,11}

From the data obtained in this study, it is inferred that of the six commonly consumed legumes, soybean is the richest source for protein and lipid nutrients. In addition, trypsin inhibitors and phytohaemagglutinins are also present in considerable amounts in soybean in comparison to the other five legumes.

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