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Investigations on the Trypsin Inhibitor, Hemagglutinin, Phytic and Tannic Acid Contents of Cowpea *Vigna unguiculata*

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ABSTRACT

Ten cultivated varieties of mature dry beans Vigna unguiculata *were analysed for trypsin inhibitor (TI) and hemagglutinating activities, phytic acid, phytic acid–phosphorus and tannic acid. The respective concentrations were: 19·6–28·2 TUI mg^{-1} protein,* 33·5–98·9 HU mg^{-1} protein,† 280–331 mg 100 g^{-1}, 131–200 mg 100 g^{-1} and 0·42–0·78 g 100 g^{-1} dry weight. Phytic acid–phosphorus as a percentage of total phosphorus was highest in Farv-13 (49·9) and lowest in Samaru local (29·8). Considerable variability in hemagglutinating activity was evident among the different varieties as indicated by the high percentage coefficient of variation. Some of these differences may be genetic and may provide an opportunity for the genetic development of cowpea strains with superior protein quality, low in hemagglutinin content.*

INTRODUCTION

Beans form a very important group of foodstuffs in Nigeria. They represent a cheap source of dietary protein and are now being successfully used in child-feeding programmes.

Of the different species of bean consumed in the country, cowpeas in

* Trypsin units inhibited (TUI) per mg protein, as defined by Kakade *et al.* (1969).
† Hemagglutinating units (HU) per mg protein, as defined by Liener (1955).

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particular have attracted attention as possible home grown sources of protein and successive selections have made it possible to introduce several high yielding varieties with desirable packages of nutrients and with resistance to pest and microbial infections. However, one major setback in these breeding efforts has been the omission of several inherent constituents of legumes into breeding objectives. Most breeding studies involving legumes have been directed towards changes in oil and protein contents and amino acid composition with little attention being paid to antinutritional components.

Although heat treatment will effectively eliminate most of these undesirable substances, careful control of processing conditions is essential to prevent both functional as well as nutritional damage to the protein. On the other hand, the breeding of varieties or strains of cowpea with low levels of one or more of the antinutritional factors offers a much more satisfactory long-term solution to this problem.

In the present study, ten different varieties of cowpea, which have shown promising agronomical characteristics, were analysed for their trypsin and hemagglutinating activities, phytic acid, phytic acid–phosphorus and tannic acid contents.

EXPERIMENTAL

Materials

The varieties of cowpea were obtained from the National Cereals Research Institute, Moor Plantation, Nigeria. The seeds were ground in a laboratory mill to a particle size of 0·5 mm and stored in screw-cap bottles at 4°C until required for analysis.

Methods

TI activity was evaluated in terms of the extent to which a portion of an aqueous extract of the cowpea meal inhibited the action of trypsin on benzoyl-D,L-arginine-*p*-nitroanilide (Kakade *et al.*, 1969). Hemagglutinating activity was determined by the photometric technique of Liener (1955) which measures the ability of hemagglutinin extracts to agglutinate rabbit erythrocytes.

For the determination of phytic acid, a combination of two methods

was used. The extraction and precipitation of phytic acid were performed according to the method of Wheeler & Ferrel (1971), while the iron of the precipitate was measured by Makower's method (Makower, 1970). An Fe/P atomic ratio of 4:6 was used to calculate phytic acid and phytic acid–phosphorus contents. Total phosphorus was determined colorimetrically after digestion of the sample with perchloric acid according to Allen's method (Allen, 1940) while tannic acid was estimated as described by Eggum & Christensen (1975). All analyses were carried out on triplicate samples.

RESULTS AND DISCUSSION

The results of the analyses are shown in Table 1. TI activity ranged between 19·6 and 28·2 TUI mg^{-1} protein with an average value of 23·7 and percentage coefficient of variation of 10·8. The values for this activity are lower than in the soybean (Kakade *et al.*, 1972), limabean (Ologhobo & Fetuga, 1983) and *Vicia faba* (Rafik El-Mahdy *et al.*, 1980). Varietal

TABLE 1
Antinutritional Components of Cowpea *Vigna unguiculata*

Variety	*Trypsin inhibitor activity, TUI mg^{-1} protein*	*Hemagglutinating activity, HU mg^{-1} protein*	*Protein, % dry matter*	*Tannic acid, g 100 g^{-1} dry matter*
Igbira	19·6	43·8	26·68	0·60
Samaru local	25·4	80·4	24·35	0·46
Kano 1696	22·8	33·5	25·93	0·58
Blackie	25·0	93·1	25·25	0·78
Adzuki	24·3	94·8	26·44	0·48
Farv-13	28·2	45·7	27·49	0·63
Westbreed	25·7	36·6	25·18	0·55
Ife brown	20·5	38·0	24·33	0·42
Prima	22·3	98·9	24·38	0·66
Nigeria B_7	23·2	49·6	25·06	0·46
Mean	23·7	61·44	25·51	0·56
Standard deviation	2·57	26·92	1·09	0·11
% Coefficient of variation	10·8	43·8	4·27	19·6

differences were not evident and apart from Igbira, Ife brown and Farv-13, all the other varieties showed close similarities in TI activity. This suggests little possibility of lowering TI levels through selection from among these varieties.

Hemagglutinating activity gave a range of 33·5–98·9 HU mg^{-1} protein with Prima, Adzuki, Blackie and Samaru local exhibiting higher hemagglutinating activities than those of Kano 1696, Westbreed, Ife brown, Igbira, Farv-13 and Nigeria B_7. It is possible from this result to arrange the different cowpea varieties, on the basis of their hemagglutinating activity, into low (33·5–49·6 HU mg^{-1} protein) and medium (80·4–98·9 HU mg^{-1} protein) hemagglutinating cultivars. This would be consistent with the observation with respect to soybean where high, medium and low hemagglutinating cultivars have been identified (Kakade *et al.*, 1972). The lower limits of the low hemagglutinating cultivars (33·5 HU mg^{-1} protein) and the upper limits of the medium (98·9 HU mg^{-1} protein), reveal about a threefold increase in activity. Such variations may be of importance in selection efforts to obtain varieties with low hemagglutinating activity considering the noxious properties of phytohemagglutinins in human and animal nutrition.

Percentage tannic acid in the ten varieties of cowpea analysed also appear in Table 1. Values ranged between 0·42 % for Westbreed and 0·78 for Blackie with mean of 0·56 and a coefficient of variation of 19·64 %. The levels of tannic acid obtained in the cowpea varieties are low and are not likely to be of any nutritional significance. Chang & Fuller (1964) had suggested that tannic acid in plants does not affect their nutritional potentials unless at very high levels, often 10 % or more of the dry weight. The highest tannic acid levels obtained in this study were in Blackie and Prima both of which contained less than 1 % of the bean dry weight.

Values for phytic acid and phytic acid–phosphorus expressed in mg 100 g^{-1} dry matter appear in Table 2. The phytic acid values ranged between 280 and 331 mg 100 g^{-1} with a mean of 310 mg 100 g^{-1} and a percentage coefficient of variation of 5·49. Phytic acid–phosphorus ranged between 131 and 200 mg 100 g^{-1} and represented between 29·8 and 49·9 total phosphorus, with an average of 39 %. These represent a significant percentage of the total phosphorus. These results are in agreement with a report by Lolas & Markakis (1975) which showed that phytic acid is a characteristic and abundant constituent of legume seeds. This observation has considerable nutritional significance in that phytic acid is able to chelate, not only phosphorus and several other minerals including Ca,

TABLE 2
Phytic Acid and Other Phosphorus Compounds of Cowpea *Vigna unguiculata* (expressed in mg $100\,g^{-1}$ dry matter)

Variety	*Phytic acid*	*Phytic acid–phosphorus*	*Total phosphorus*	*Phytic acid–phosphorus as % total phosphorus*
Igbira	314	154	480	32·0
Samaru local	300	131	441	29·8
Kano 1696	290	183	481	38·0
Blackie	331	195	481	34·8
Adzuki	316	153	440	40·5
Farv-13	280	200	401	49·9
Westbreed	301	179	400	44·6
Ife brown	326	192	481	40·0
Prima	330	197	471	41·8
Nigeria B_7	313	161	420	38·4
Mean	310	174	450	39·0
Standard deviation	17·0	23·2	33·6	5·90
% Coefficient of variation	5·49	12·3	7·47	15·1

Mg, Fe, Zn and Mo thereby reducing their availability in the intestinal tract (Oberleas, 1973), but also reacts with proteins, forming complex products, with inhibitory effects on peptic digestion (Barre, 1956). Because most Nigerians subsist on legumes and derive most of their nutrients from them, phytic acid toxicity could be a problem in cowpea based diets. There is also the possibility of increased requirement for the essential amino acids which may become unavailable in such situations, due to the inhibitory effect of the protein–phytate complex on the digestive enzymes.

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