

Chemical Evaluation of the Effect of Pest Infestation on the Nutritive Value of Cowpeas *Vigna unguiculata*

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ABSTRACT

The effect of insect infestation of the cowpea legume Vigna unguiculata was investigated. Samples of the infested cowpeas with and without the infesting insects were analysed and compared with the uninfested cowpeas for possible variation in nutrients.

Results showed increase in ash, crude protein and crude fibre content with infestation. Values for the infested samples ($\text{mg } 100 \text{ g}^{-1}$) were: ash, 3.70 ± 0.01 ; crude protein, 27.1 ± 0.80 and crude fibre, 3.30 ± 0.64 , as against 3.40 ± 0.03 (ash), 23.6 ± 0.88 (crude protein) and 1.9 ± 0.01 (crude fibre) for the uninfested samples.

The amino acid pattern showed that the levels of aspartic acid, threonine, serine, alanine, cysteine and isoleucine were not much affected by the infestation. Marked reduction in level was observed for lysine, glutamic acid, glycine, valine, methionine and leucine. Only arginine showed an increase in level with infestation.

All the vitamins assayed showed decreases in levels with infestation. Values per 100 g sample were $14.6 \pm 0.24 \mu\text{g}$ (carotenes), $0.91 \pm 0.02 \text{ mg}$ (thiamine), $0.17 \pm 0.1 \text{ mg}$ (riboflavin) for the uninfested samples and $8.40 \pm 0.02 \mu\text{g}$ (carotenes), $0.41 \pm 0.02 \text{ mg}$ (thiamine) and $0.08 \pm 0.03 \text{ mg}$ (riboflavin) for the infested cowpeas.

Toxic substances such as phytic acid, oxalates and tannins were higher in the infested cowpeas than in the uninfested. Hydrocyanic acid decreased with infestation.

INTRODUCTION

The cowpea legume *Vigna unguiculata* is very popular in the diets of Nigerians. It constitutes a major source of concentrated and cheap vegetable proteins (Ezedinma, 1965; Oyenuga, 1968). Figures from the Federal Office of Statistics of Nigeria up to 1966 put the yearly production of cowpea in the Northern States of Nigeria at about a million tons (Anon., 1966). By the year 1967 it was estimated that about 24 000 tons representing about 2.5% were being lost yearly through pest infestation (Caswell, 1967). About five years later the total wastage in Nigeria contributed mostly by pests was estimated at 20% (Olayide *et al.*, 1972; Olayide & Olayemi, 1978). It is evident that pest infestation of cowpeas has been on the increase. This poses a serious problem to the food economy in Nigeria since a good quantity of valuable protein is being diverted by the pests for their own use.

The two major groups of pests of cowpeas in Nigeria are the lepidopterous larvae, mainly *Maruca testularis* (Usua, 1976) and *Laspeyresia ptychora* which attack the flowers and pods, and the hemiferous nymphs and adults notably *Acanthomia*, *Riptortus*, and *Anophocnemis* species which attack the pods and cause their premature shrivelling (Taylor, 1965).

The most important insect attacking cowpea seeds in store is the weevil *Callosobruchus maculatus* of the family *Brucidae* (Booker, 1965). There are two closely related weevils namely *Scatoscelides abtectus* (bean weevil) and *Bruchus pisorum* (pea weevil) still of the family of *Brucidae* which also attack the cowpea seeds. In the course of their development, the larvae of these weevils consume the central portion of the beans. Under heavy infestations as many as a dozen or more weevils may develop from a single seed (Davidson & Peairs, 1966).

There is evidence that insects divert the major nutrients in food for their own feeding. Thiamine losses in weevil infested legumes have been reported (Sowunmi, 1978). Literature is, however, scanty on the results of such studies particularly in Nigeria. The present series of investigations was carried out to estimate by chemical analysis the effect of infestation of cowpea seeds by the bruchid *Callosobruchus maculatus* on their nutritive value with special reference to proximate amino acids, mineral elements, vitamins composition as well as the content of some toxic substances such as oxalic, phytic, tannic and hydrocyanic acids.

EXPERIMENTAL

Collection of samples and treatment before analysis

Samples of cowpea seeds *Vigna unguiculata* were purchased from the local markets in Calabar and conveyed to the laboratory. The control (uninfested sample) was separated from the infested seeds by careful handpicking. The infested sample was further separated into two groups namely infested samples with the insects inside the seed and infested samples with all the insects and larvae removed.

Samples were taken out from each group for moisture determinations. The remainder were separately ground in a steel-bladed mill (National Model MK308, Japan) into very fine powder and then spread on clean, dry plastic trays and dried in the hot air circulating oven at about 60 °C for 12–24 h. The samples were stored in airtight bottles from where required quantities were taken out for the various chemical analyses.

Analysis of Samples

The methods of treatment of samples and analysis were those recommended by the Association of the Official Analytical Chemists (AOAC) (1975).

The ash was determined by incineration of known weights of the samples in a muffle furnace at 550 °C until ash was obtained. The lipid composition was determined by extracting, exhaustively, a known weight of the samples with petroleum ether, boiling point 40–60 °C, using a Soxhlet apparatus. Protein ($N \times 6.25$) was determined by the macro-Kjeldahl method. The carbohydrate content was obtained by the difference method, that is by subtracting the total crude protein and crude lipid from the organic matter. Crude fibre was determined by acid and alkaline digestion methods described by Joslyn (1970) and AOAC (1975).

The elementary composition was also determined using the methods of AOAC (1975). Sodium and potassium were determined by flame photometric methods; calcium, magnesium, zinc, iron, chromium and copper were determined using an absorption spectrophotometer; phosphorus was determined by colorimetric methods using ammonium molybdate. The amino acid contents of the samples were determined using the automatic amino acid analyser (Beckman model, Spackman *et al.*, 1958).

The vitamins were determined using the methods of the Association of Official Vitamin Chemists (AOVC) (1966). Carotene was determined colorimetrically at 455 nm. Thiamine was estimated as thiochrome by the fluorometric method; riboflavin was also estimated fluorometrically, using a Locarte fluorimeter. Vitamin C (ascorbic acid) was estimated by the *N*-bromo-succinimide method described by Evered (1960) and total ascorbic acid by the 2,4-dinitrophenylhydrazine method (Scharffert & Kingsley, 1955). Oxalate was determined by the method of Dye (1956) and as modified by Oke (1965). Hydrocyanic acid content was determined by the alkaline titration method (AOAC, 1975). Phytic acid was estimated by a photometric method adopted from the methods of McCance and Widdowson (1935). The tannin was determined by the vanillin-HCl reagent method (Joslyn, 1970; Burns, 1971).

RESULTS AND DISCUSSION

The results of the proximate composition of the cowpea samples are given in Table 1. The results showed slight increases in ash, crude protein and crude fibre content of the samples with infestation. On the other hand there were slight decreases in moisture, crude fat and carbohydrate with infestation. The increases and decreases in values of the nutrients were found to be statistically significant ($P < 0.05$).

TABLE 1
Proximate Composition and Caloric Value of Infested and Uninfested Cowpeas
Vigna unguiculata (g 100 g⁻¹) (Mean \pm Standard Deviation)

	<i>Uninfested cowpeas</i>	<i>Infested cowpeas (insects inside)</i>	<i>Infested cowpeas (without insects)</i>
Moisture	10.1 \pm 0.08	9.6 \pm 0.15	9.1 \pm 0.1
Ash	3.4 \pm 0.03	3.6 \pm 0.02	3.7 \pm 0.01
Crude protein	23.6 \pm 0.88	27.1 \pm 0.88	26.1 \pm 0.66
Crude fat	2.5 \pm 0.03	1.5 \pm 0.05	1.25 \pm 0.05
Crude fibre	1.9 \pm 0.14	2.4 \pm 0.25	3.3 \pm 0.64
Carbohydrate (excluding fibre)	58.5 \pm 0.23	55.8 \pm 0.27	56.6 \pm 0.29
Caloric value (kcal 100 g ⁻¹)	350.9 \pm 4.71	345.1 \pm 5.05	342.0 \pm 4.25

Table 2 shows the results of the mineral element composition. Increases of 20.1%, 20.6%, 15.2% and 7.6% were observed for calcium, magnesium, potassium and phosphorus in the two infested samples (with and without insects). The levels of zinc, copper, iron, chromium and sodium were not much different in the samples. The results may be explained partly by the fact that the husk which contains the greater proportion of minerals was relatively not utilized by the insects. The increases in some mineral elements could be attributed at least in part to the activity of the pests.

Table 3 shows the amino acid composition of the samples. Aspartic acid, threonine, serine, alanine, cystine, cysteine and isoleucine were not very different in these samples. Arginine was the only amino acid found to increase with infestation. Lysine, histidine, glutamic acid, valine, leucine and methionine tended to decrease with infestation. Tryptophan, tyrosine, proline and phenylalanine were not detected and this was partly attributed to the method of hydrolysis used and also partly to the fact that some of the amino acids might have been present in such low concentrations that they could not be detected. More studies using alkaline and enzyme hydrolysis are contemplated.

When compared with the FAO/WHO provisional pattern (Food and Agricultural Organisation (FAO), 1973) and also with the whole hens egg

TABLE 2
Mineral Elements of Infested and Uninfested Cowpeas *Vigna unguiculata*
(mg 100 g⁻¹) (Mean of 3 Determinations)

	<i>Uninfested cowpeas</i>	<i>Infested cowpeas (insects inside)</i>	<i>Infested cowpeas (without insects)</i>
Calcium	87	105	103
Magnesium	165	198	199
Potassium	985	1 127	1 135
Zinc	9.50	11.0	10.9
Copper	0.20	0.20	0.20
Iron	2.45	2.65	3.10
Chromium	0.80	0.60	0.60
Phosphorus	381	410	409
Sodium	0.80	0.90	1.00

TABLE 3
 Amino Acid Analysis of Infested and Uninfested Cowpeas *Vigna Unguiculata*
 (mg g^{-1} N) (Mean of 3 Determinations)

	<i>Uninfested cowpeas</i>	<i>Infested cowpeas (insects inside)</i>	<i>Infested cowpeas (without insects)</i>
Tryptophan	—	—	—
Lysine	420	370	365
Histidine	206	186	191
Arginine	431	565	558
Aspartic Acid	101	98	96
Threonine	251	242	248
Serine	335	240	339
Glutamic acid	131	81	73
Proline	—	—	—
Glycine	385	201	192
Alanine	376	379	371
Cysteine	60	64	63
Cystine	120	128	126
Valine	318	175	169
Methionine	128	78	83
Isoleucine	271	285	278
Leucine	473	247	231
Tyrosine	—	—	—
Phenylalanine	—	—	—

(—) Not detected.

standard protein (Table 4), the infested samples showed a deficiency in methionine, cysteine and valine. The sulphur-containing amino acids methionine and cysteine were the most limiting amino acids. The nutritive value of the cowpeas seeds tended to decrease with infestation on the basis of chemical scores.

Table 5 shows the content of carotenes (pro-vitamin A), vitamin B (thiamine), vitamin B₂ (riboflavin) and vitamin C (ascorbic acid) in the samples. There were decreases in all the vitamins with infestation. This further points to the adverse effect of infestation on the nutritive value of the cowpea seeds.

Table 6 shows the levels of toxic substances in the samples. There were increases in levels of oxalic acid, phytic acid and tannic acid (tannins) with

TABLE 4

Comparison of Essential Amino Acid Composition (mg g^{-1} N) of Infested and Uninfested Cowpeas with FAO/WHO Provisional Pattern (FAO, 1973) (mg g^{-1} N)

	<i>Uninfested cowpeas</i>	<i>Infested cowpeas (insects inside)</i>	<i>Infested cowpeas (without insects)</i>	<i>FAO/ WHO</i>	<i>Egg</i>
Isoleucine	271	285	278	250	413
Leucine	473	247	231	440	550
Lysine	420	370	365	340	413
Methionine + cysteine*	188	142	146	220	338
Phenylalanine + tyrosine	—	—	—	380	675
Threonine	251	242	248	250	313
Tryptophan	—	—	—	60	106
Valine	318	175	169	310	463
Protein score %	85.5	64.5	66.4		153.4

(—) Not determined.

* Limiting amino acids.

infestation but there was a decrease in hydrocyanic acid with infestation. On the whole the levels of the toxic substances were below the known toxic levels (Oke, 1966, 1969). Even though their levels were low, they are known to influence the utilisation of other nutrients even at low concentrations. For example phytic acid and oxalic acid are known to form insoluble salts with some mineral elements and therefore render

TABLE 5

Vitamin Content of Infested and Uninfested Cowpeas *Vigna unguiculata* ($\text{mg } 100 \text{ g}^{-1}$) (Mean \pm Standard Deviation)

	<i>Uninfested cowpeas</i>	<i>Infested cowpeas (insects inside)</i>	<i>Infested cowpeas (without insects)</i>
Ascorbic acid, diketogulonic acid and dehydroascorbic acid	14.2 \pm 2.30	7.70 \pm 0.85	5.4 \pm 2.30
Reduced vitamin C	0	0	0
Carotenes (pro-vitamin A)*	14.6 \pm 0.24	9.45 \pm 0.81	8.4 \pm 0.23
Thiamine	0.91 \pm 0.02	0.49 \pm 0.04	0.41 \pm 0.02
Riboflavin	0.17 \pm 0.10	0.08 \pm 0.01	0.08 \pm 0.03

* In $\mu\text{g } 100 \text{ g}^{-1}$.

TABLE 6
Toxic Substances of Infested and Uninfested Cowpeas *Vigna unguiculata*
(mg 100 g⁻¹) (Mean \pm Standard Deviation)

	<i>Uninfested cowpeas</i>	<i>Infested cowpeas (insects inside)</i>	<i>Infested cowpeas (without insects)</i>
Oxalates (soluble)	12.6 \pm 0.98	25.4 \pm 2.0	35.1 \pm 3.9
Oxalates (total)	51.4 \pm 6.3	69.1 \pm 5.7	76.7 \pm 6.0
Phytic acid phosphorus	132.1 \pm 1.3	142.0 \pm 3.6	138.0 \pm 3.9
Tannins	73.1 \pm 1.65	80.3 \pm 5.4	88.3 \pm 3.8
Hydrocyanic acid	28.4 \pm 1.7	14.8 \pm 0.47	10.4 \pm 0.43

them not available to the body of the consumer. Tannins on the other hand are known to form complexes with proteins and thus reduce their availability (Goldstein & Swain, 1965; Eka, 1977; Chakraborty & Eka, 1978).

It is evident from the findings in these studies that in most cases the nutritive value of seeds of cowpeas is adversely affected by pest infestation. There is the need for effective methods of preservation of the seeds to prevent infestation by insects and consequent reduction in nutritive value.

REFERENCES

- Anon. (1966). *Statistical Year Book*. Ministry of Economic Planning, Kaduna.
- Association of Official Analytical Chemists (1975). *Methods of Analysis*, 12th edn. Washington, DC.
- Association of Vitamin Chemists (1966). *Methods of Vitamin Assay*, 3rd edn. Interscience Publishers, London.
- Booker, R. H. (1965). Pest of cowpeas and their control in Northern Nigeria. *J. Stored Prod. Res.*, **1**, 145.
- Burns, R. E. (1971). Method of estimation of tannin in the grain sorghum. *Agron. J.*, **63**, 511.
- Caswell, G. H. (1967). The storage of cowpeas in northern states of Nigeria. *Nig. Agric. J.*, **5**, 4.
- Chakraborty, R. & Eka, O. U. (1978). Studies on hydrocyanic, oxalic and phytic acid content of foodstuffs. *W.A.J. Biol. Appl. Chem.*, **21**, 1-4.
- Davidson, R. H. & Peairs, L. M. (1966). *Insect Pests of Farms, Gardens and Orchids*, 6th edn. John Wiley & Sons, London, p. 239.

- Dye, W. B. (1956). Chemical studies on *Hologeton glomeratus*. *Weeds*, **4**, 55.
- Eka, O. U. (1977). Studies on levels of oxalic acid and phytic acid in traditional foods of northern Nigerians. *W.A.J. Biol. Appl. Chem.*, **20**, 26.
- Evered, D. F. (1960). Determination of ascorbic acid in highly coloured solutions with *N*-bromosuccinimide. *Analyst*, **85**, 515.
- Ezedinma, F. O. C. (1965). Some factors influencing the production of grain legumes in southern Nigeria. *Nig. Agric. J.*, **4**, 48.
- Food and Agricultural Organisation (1973). *Energy and protein requirements*. Report of Joint FAO/WHO ad hoc Expert Committee, WHO Tech. Rep. Series No. 522.
- Goldstein, J. L. & Swain, T. (1965). The inhibition of enzymes by tannins. *Phytochemistry*, **4**, 185.
- Joslyn, M. A. (1970). *Methods of Food Analysis*, 2nd edn. Academic Press, London.
- McCance, R. A. & Widdowson, E. M. (1935). Phytin in human nutrition. *Biochem. J.*, **29**, 2694.
- Oke, O. L. (1965). Chemical Studies on some Nigerian Foodstuffs—'Lafan'. *W.A.J. Biol. Appl. Chem.*, **8**, 53.
- Oke, O. L. (1966). Chemical studies on more commonly used leaf vegetables in Nigeria. *J.W. Afr. Sci. Assoc.*, **4**, 345.
- Oke, O. L. (1967). Studies on some Nigerian pulses. *W.A.J. Biol. Appl. Chem.*, **9**, 52.
- Oke, O. L. (1969). Role of hydrocyanic acid in nutrition. *Wld. Rev. Nutr. Dietetics*, **11**, 170.
- Olayide, S. O. & Olayemi, J. (1978). Economic aspects of agriculture and nutrition—a Nigerian study. *Fd Nutr. Bull.*, **1**, 32.
- Olayide, S. O., Olatunbosun, D., Idusogie, E. O. & Abiagom, J. D. (1972). *A Quantitative Analysis of Food Requirements, Supplies and Demands in Nigeria 1968–1985*. Federal Department of Agriculture, Lagos, Nigeria.
- Oyenuga, V. A. (1968). *Nigerian Foods and Feeding Stuffs*, 3rd edn. Ibadan University Press, Nigeria.
- Scharffert, R. R. & Kingsley, G. R. (1955). A rapid method for the determination of reduced dehydro- and total ascorbic acid in biological materials. *J. Biol. Chem.*, **212**, 59.
- Sowunmi, O. (1978). The effect of insect infestation on cowpea *Vigna unguiculata* Welw., I and II. *Nig. Stored Prod. Res. Inst.*, **4**, 43–47, 49–53.
- Spackman, D. H., Stein, W. H. & Moore, S. (1958). Automatic recording apparatus for use in chromatography of amino acids. *Anal. Chem.*, **30**, 1190.
- Taylor, T. A. (1965). An attempt at quantitative estimation of major insect damage on cowpeas. *Nig. Agric. J.*, **4**, 50.
- Usua, E. J. (1976). Description of larva and pupa of *Maruca testicularis* (Lep. pyralidae). *Nig. J. Sci.*, **10**, 179.