

## CHARACTERIZATION OF COWPEA CULTIVARS FOR VARIATIONS IN SEED CONTENTS OF SOME ANTINUTRITIONAL FACTORS (ANFs)

<sup>1</sup>Afiukwa Celestine A., <sup>2</sup>Ogah Onwuchekwa, <sup>3</sup>Ibiam Udu A., <sup>3</sup>Edeogu Chuks O. and <sup>3</sup>Aja Patrick M.

<sup>1</sup>Department of Biotechnology, <sup>2</sup>Biotechnology Research and Development Centre, <sup>3</sup>Department of Biochemistry, Faculty of Biological Sciences, Ebonyi State University, P.M.B. 053, Abakaliki, Nigeria.

### ABSTRACT

Varietal variability in seed contents of 6 antinutritional factors (ANFs) of importance in cowpea was studied using 104 accessions. The aim of the study was to help broaden the selection guide for cowpea lines for consumption especially for individuals who depend largely on cowpea for protein and for selection of cultivars with intrinsic potentials to resist insect pest infestation for cultivation by farmers. The Phytic acid content varied from 2.58 – 3.91 with a mean value of  $3.09 \pm 0.24$  mg/g; Oxalate ranged from 0.57 – 0.99 with a mean of  $0.78 \pm 0.08$  mg/g; Trypsin inhibitor ranged from 14.98 - 27.93 with an average of  $21.39 \pm 2.41$  TIU/mg; Hemagglutinin varied very widely from 5.10 - 83.00 with a mean of  $63.92 \pm 10.72$  HU/mg; Tannin varied from 2.14 - 4.21 with a mean of  $3.08 \pm 0.54$  mg/g; whereas Cyanogenic glycoside ranged from 370.0 - 402.0 with an average value of  $388.63 \pm 8.48$  mg/kg. We report here a very high degree of variability in all the ANFs among the accessions ( $p < 0.0001$ ), suggesting ample opportunities - to select cultivars of choice for consumption, for genetic improvement of the crop, and for possible presence of accessions with natural ability to resist pest attack.

**KEYWORDS:** Cowpea seeds, antinutrients variability, selection choice

### INTRODUCTION

Cowpea, generally called *beans* in Nigeria, belongs to the family *Leguminosae*, subfamily *Faboideae*, tribe *Phaseoleae*, genus *Vigna*, subgenus *Ceratropis* and species *unguiculata* (Mahalakshmi *et al.*, 2007). It is a very important legume food for both humans and animals, because of its high protein, vitamins and minerals contents (Nielsen *et al.*, 1997; Singh *et al.*, 1997). The crop is consumed in many forms: the young leaves, green pods and green seeds are eaten as vegetables whereas dry seeds are used in a variety of food preparations (Nielsen *et al.*, 1997). The seeds contain about 25% protein and serve as a major source of protein for the poor in Africa, while the haulms are also very nutritious, containing about 15 to 17 % highly digestible protein and useful as fodder for livestock (Li *et al.*, 2001; Nielsen *et al.*, 1997; Singh, 2007; Tarawali *et al.*, 1997a and Tarawali *et al.*, 1997b).

It is known that cowpea protein is rich in the amino acids, lysine and tryptophan, relative to cereal grains, but deficient in the sulphur containing amino acids (SAAs), methionine and cysteine, when compared to animal protein. Its seeds are therefore useful as nutritional supplement to cereal and extender of animal protein (Steele, 1985). However, the relative low SAA content of beans may provide an advantage in calcium retention. As noted by (Chan, 1974; Remer and Manz, 1994), SAA metabolism may be partly responsible for the reported hypercalciuric effect of protein. The authors opined that the hydrogen ions ( $H^+$ ) released from metabolism of SAA in the body cause demineralization of bones and a consequent excretion of calcium in the urine. (Kerstetter and Allen, 1989) reported an estimated loss of one milligram of calcium for every one gram of animal protein consumed. Therefore, cowpea protein, being low in SAA content, may produce minimal loss of bone calcium relative to animal and non-legume derived proteins. Studies carried out by Anderson *et al.* (1987) and Breslau *et al.* (1998) on the effects of whey protein and a mixture of animal proteins respectively on urinary calcium excretion already support the above hypothesis.

Cowpea is also valued for its useful ability to fix atmospheric nitrogen through its root nodules, and grow well in poor soils (Davis, *et al.*, 1991; Duke, 1981; Yost and Evans, 1988 and Singh, 2003).

However as a legume crop, the presence of antinutritional factors is a major limiting factor to the optimal food use of cowpea (Liener, 1975). Anti-nutritional factors (ANF) or antinutrients are plant's secondary metabolites which act to reduce food nutrient utilization (Soetan, 2008; Welch and Graham, 2004). However, Akande *et al.*, 2010 noted that being an antinutrient is not an intrinsic property of a compound but depends on the metabolic processes of the ingesting animal. For instance, Trypsin inhibitors, which are ANFs for monogastric animals, do not exert any antinutrient effects in ruminants because they are degraded in the rumen. It has been suggested that the reason for formation of ANFs in plants may be a way of storing excess nutrients or a means of defence from destruction by insect pests and grazing animals (Harborne, 1989; Welch and Graham, 2004). The major ANFs in legumes include: protease inhibitors, lectins (phytohaemagglutinins), gossypol, goitrogens, antivitamin, phytates, saponins, estrogens, flatulence factors, chlorogenic acid, amylase inhibitors, allergens and lysinoalanine (Akande *et al.* 2010; Liener, 1981). Luckily, most ANFs are heat-labile. However, heat-stable ANFs (e.g., phytate and polyphenols) are not eliminated by simple soaking and heating, but through germination or fermentation (Sridharand Seena, 2006).

The objective of this study was to estimate the level of variations in the seed contents of some ANFs among cowpea varieties to provide a selection guide for safe cultivars for consumption especially for individuals who depend largely on cowpea for protein and for selection of cowpea lines with intrinsic potentials to resist insect pest infestation for cultivation by farmers.

## MATERIALS AND METHODS

### Sample Collection and Preparation

A total of 101 cowpea genotypes, comprising of 97 Nigerian landraces and four exotic lines, were used. Two of the exotic cultivars have their origin in Sudan, one was from Cote d'Ivoire, while one was from Mozambique. All the seeds were obtained from the Genetic Resources Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Before the analyses, the seeds were washed clean to devoid it of dirt, the seed coats were removed and the endosperms were ground into powder using a blender (Model, SMB – 2898).

### Determination of Seed Antinutrients Content

Phytic acid was determined by a combination of two methods. The extraction and precipitation of phytic acid were performed according to the method described by Wheeler and Ferrel (1971). Iron in the precipitate was measured according to the method of Makover (1970). A 4:6 Fe/P ratio was used to calculate the phytic acid content. The titration method described by Day and Underwood (1986) was used to determine the oxalic acid content of the powdered seed samples. The trypsin inhibitor activity of the samples was determined by the method of Kakade *et al.* (1969) using casein as substrate, while hemagglutinating activity was determined by the serial dilution method using trypsinized rabbit erythrocytes and expressed as hemagglutinating unit (HU)/mg sample as described by Liener and Hill, (1953). Tannins were determined as tannic acid equivalent by the colorimetric Folin-Denis method (Joslyn, 1970), whereas cyanogenic glycoside was estimated by measuring the amount of hydrogen cyanide (HCN) released on hydrolysis using HCN-Autoanalyser (Technicon - Pulse Instrumentation Ltd, Canada) according to the method of Rao and Hahn (1984).

## DATA ANALYSIS

The data were analyzed for significant variation by the ANOVA procedure. Differences were declared statistically significant at  $P < 0.05$  and the means were separated by the Tukey's Studentized Range (HSD). Correlations among the variables were determined using the Pearson simple correlation procedure. All the analyses were performed at 95% confidence level using SAS Software version 9.1 (SAS Institute, 1998). Means and standard deviation values were computed using Excel Software.

## RESULTS

The cowpea cultivars showed very high degree of genotypic variations in their seed contents of all the antinutritional factors analysed ( $p < 0.0001$ ). The Phytic acid content of the 104 accessions varied from 2.58 – 3.91 with a mean value of  $3.09 \pm 0.24$  mg/g; Oxalate values ranged from 0.57 – 0.99 with a mean of  $0.78 \pm 0.08$  mg/g; Trypsin inhibitor levels ranged from 14.98-27.93 with an average of  $21.39 \pm 2.41$  TIU/mg; Hemagglutinin varied very widely from 5.10-83.00 with a mean of  $63.92 \pm 10.72$  HU/mg; Tannin varied from 2.14-4.21 with a mean of  $3.08 \pm 0.54$  mg/g; while Cyanogenic glycoside ranged from 370.0-402.0 with an average value of  $388.63 \pm 8.48$  mg/kg or ppm. The above results are summarized in Table 1.

Table 1. Mean Values of some Antinutrients Contents of Cowpea cultivars.

S/No.	Accession No.	Phytate (mg/g)	Oxalate (mg/g)	Trypsin Inhibitor (TIU/mg)	Hemagglutinin, (HU/mg)	Tannin (mg/g)	Cyanogenic glycoside, (mg/kg)
1	TVu-46	3.20±0.11	0.72±0.01	19.16±0.26	65.87±0.45	3.48±0.07	395.00±1.00
2	TVu-160	3.05±0.05	0.80±0.01	23.54±0.01	70.39±0.05	3.09±0.07	399.00±1.00
3	TVu-331	3.05±0.05	0.68±0.02	20.78±0.10	57.28±0.16	3.45±0.01	388.00±1.00
4	TVu-442	2.58±0.03	0.86±0.01	21.26±0.21	80.55±0.55	2.58±0.08	380.50±1.50
5	TVu-461	2.90±0.02	0.76±0.01	23.92±0.14	77.60±0.61	3.00±0.04	401.50±0.50
6	TVu-561	3.07±0.08	0.58±0.01	22.10±0.01	34.04±2.98	3.01±0.05	396.00±1.00
7	TVu-702	3.23±0.04	0.89±0.01	23.21±0.11	59.01±0.32	2.63±0.03	383.50±1.50
8	TVu-729	3.25±0.11	0.77±0.01	22.79±0.11	63.42±0.02	3.15±0.03	399.00±1.00
9	TVu-764	3.16±0.08	0.73±0.01	21.54±0.01	56.72±1.72	2.65±0.03	386.00±1.00
10	TVu-839	3.20±0.06	0.71±0.01	21.12±0.01	60.81±0.22	4.00±0.09	380.00±1.00
11	TVu-848	3.04±0.08	0.66±0.02	20.93±0.10	57.50±0.53	3.45±0.00	399.00±1.00
12	TVu-867	3.33±0.05	0.66±0.02	23.69±0.01	72.51±0.71	3.09±0.07	398.50±0.50
13	TVu-930	2.90±0.05	0.75±0.02	23.16±0.16	52.44±0.56	3.62±0.12	386.50±1.50
14	TVu-939	3.32±0.02	0.76±0.02	23.79±0.16	66.66±0.69	3.07±0.04	392.00±2.00
15	TVu-1138	3.05±0.05	0.69±0.01	19.14±0.26	81.89±1.11	2.69±0.03	378.00±2.00
16	TVu-1197	3.41±0.01	0.61±0.02	23.59±0.10	77.89±0.32	2.68±0.05	400.50±0.50
17	TVu-1260	2.94±0.10	0.80±0.00	20.77±0.10	52.07±1.07	2.40±0.01	395.00±1.00
18	TVu-1262	3.11±0.00	0.68±0.01	21.23±0.21	49.42±0.58	2.93±0.02	385.50±1.50
19	TVu-1263	3.08±0.03	0.83±0.03	19.44±0.16	75.61±0.72	4.03±0.13	393.50±1.50
20	TVu-1455	3.27±0.02	0.74±0.01	20.85±0.16	53.98±0.02	2.74±0.01	376.00±2.00
21	TVu-3910	3.31±0.01	0.74±0.04	20.22±0.16	58.93±1.07	3.10±0.04	381.50±0.50
22	TVu-3919	3.02±0.03	0.84±0.02	24.40±0.16	72.18±1.19	3.53±0.04	384.00±0.00
23	TVu-3933	2.82±0.03	0.65±0.03	23.78±0.16	58.94±0.36	2.80±0.05	377.50±1.50
24	TVu-3960	2.76±0.03	0.88±0.02	23.15±0.16	66.46±1.58	3.57±0.03	383.50±1.50
25	TVu-4007	2.97±0.02	0.78±0.02	22.52±0.16	62.45±0.66	3.44±0.02	393.00±1.00
26	TVu-4009	3.00±0.03	0.74±0.02	21.89±0.16	54.55±0.56	2.65±0.05	398.00±1.00
27	TVu-4015	3.10±0.02	0.78±0.01	21.27±0.16	80.50±0.64	3.72±0.05	399.00±1.00
28	TVu-4028	2.89±0.02	0.85±0.01	20.64±0.16	51.68±0.68	3.13±0.02	397.00±1.00
29	TVu-4034	2.85±0.02	0.79±0.03	20.01±0.16	52.30±0.71	4.13±0.08	385.00±0.00
30	TVu-4044	3.23±0.01	0.76±0.03	19.39±0.16	68.11±1.22	2.24±0.04	385.50±1.50
31	TVu-	2.85±0.03	0.73±0.03	18.76±0.16	57.73±0.40	3.47±0.04	393.50±1.50

	4045						
	TVu-						
32	4046	2.92±0.03	0.84±0.03	18.13±0.16	77.44±0.58	3.59±0.04	377.00±2.00
	TVu-						
33	4047	3.11±0.05	0.92±0.03	17.51±0.16	72.46±0.88	2.85±0.07	384.00±2.00
	TVu-						
34	4049	2.76±0.01	0.85±0.03	19.33±0.39	74.45±0.56	2.26±0.02	393.00±1.00
	TVu-						
35	4068	2.94±0.02	0.85±0.04	19.75±0.34	66.38±0.63	2.28±0.03	386.00±1.00
	TVu-						
36	4083	2.92±0.12	0.72±0.01	15.62±0.16	55.50±0.74	2.87±0.02	397.50±1.50
	TVu-						
37	4089	3.03±0.07	0.65±0.03	19.02±0.13	74.84±0.16	3.50±0.06	398.50±1.50
	TVu-						
38	4095	3.15±0.06	0.76±0.01	18.27±0.31	67.11±1.15	2.42±0.02	401.00±1.00
	TVu-						
39	4100	3.12±0.04	0.76±0.03	16.79±0.16	58.14±0.88	2.65±0.02	399.50±0.50
	TVu-						
40	4260	3.24±0.06	0.76±0.04	18.66±0.66	54.63±0.74	2.26±0.04	376.50±0.50
	TVu-						
41	4408	3.23±0.09	0.90±0.02	15.55±0.16	65.64±0.78	4.01±0.06	393.50±1.50
	TVu-						
42	4415	3.43±0.02	0.78±0.01	17.79±0.00	73.50±1.50	2.73±0.03	384.50±1.50
	TVu-						
43	6318	2.76±0.00	0.74±0.01	20.76±0.05	65.95±0.38	2.65±0.01	377.50±1.50
	TVu-						
44	6320	2.84±0.05	0.71±0.00	21.53±0.16	71.84±0.38	2.52±0.02	396.00±1.00
	TVu-						
45	6325	3.00±0.04	0.78±0.02	20.78±0.00	67.52±1.82	2.55±0.01	373.00±1.00
	TVu-						
46	6674	3.27±0.04	0.74±0.02	16.37±0.26	69.45±0.55	2.64±0.00	395.00±1.00
	TVu-						
47	6778	3.48±0.01	0.73±0.03	15.24±0.26	53.48±0.38	3.89±0.02	401.00±1.00
	TVu-						
48	6804	2.96±0.03	0.85±0.03	15.75±0.42	62.54±0.68	2.86±0.02	375.00±3.00
	TVu-						
49	6815	3.17±0.03	0.78±0.03	23.10±0.31	75.17±0.10	2.60±0.05	392.00±2.00
	TVu-						
50	6819	3.36±0.02	0.66±0.01	22.91±0.01	76.61±0.60	4.00±0.05	387.50±7.50
	TVu-						
51	6822	3.14±0.04	0.70±0.02	18.77±0.32	49.33±0.34	3.51±0.08	393.00±1.00
	TVu-						
52	6830	3.35±0.02	0.71±0.01	25.07±0.32	52.40±0.61	3.40±0.01	399.00±1.00
	TVu-						
53	6833	3.40±0.05	0.67±0.01	21.45±0.31	78.52±0.64	3.66±0.06	387.00±1.00
	TVu-						
54	6835	3.45±0.06	0.71±0.02	20.19±0.31	77.11±1.11	3.35±0.19	380.50±0.50
	TVu-						
55	6847	3.49±0.04	0.69±0.03	22.15±0.26	49.11±1.25	3.64±0.41	386.50±2.25
	TVu-						
56	6932	3.32±0.01	0.77±0.01	21.24±0.37	64.92±0.45	3.43±0.03	393.00±1.00
	TVu-						
57	7083	3.03±0.03	0.85±0.01	21.44±0.21	69.43±0.05	3.52±0.02	397.00±1.00
	TVu-						
58	7097	2.73±0.02	0.73±0.01	24.04±0.21	56.32±0.16	2.97±0.05	386.00±1.00
	TVu-						
59	7109	2.76±0.03	0.91±0.01	21.94±0.21	79.59±0.55	2.49±0.01	378.50±1.50
	TVu-						
60	7110	3.47±0.03	0.81±0.02	24.88±0.31	76.64±0.61	2.96±0.05	399.50±0.50

61	TVu-7112	3.26±0.03	0.63±0.01	24.15±0.42	33.08±2.98	3.90±0.00	394.00±1.00
62	TVu-7117	3.87±0.03	0.94±0.01	24.51±0.05	58.05±0.32	2.87±0.07	381.50±1.50
63	TVu-7488	3.07±0.05	0.82±0.01	23.36±0.05	62.46±0.02	3.73±0.06	397.00±1.00
64	TVu-7491	2.99±0.04	0.78±0.02	22.83±0.05	55.77±1.72	2.30±0.07	384.00±1.00
65	TVu-7531	3.34±0.03	0.76±0.01	24.30±0.58	59.85±0.22	3.38±0.07	378.00±1.00
66	TVu-7815	2.87±0.08	0.71±0.02	22.72±0.00	56.54±0.53	2.59±0.03	397.00±1.00
67	TVu-7833	3.26±0.01	0.71±0.02	19.15±0.26	71.55±0.71	3.66±0.06	396.50±0.50
68	TVu-7838	2.62±0.01	0.80±0.02	18.98±0.00	51.48±0.56	3.02±0.06	384.50±1.50
69	TVu-7846	2.67±0.02	0.81±0.02	18.98±0.00	65.70±0.69	2.61±0.03	390.00±2.00
70	TVu-7848	2.95±0.00	0.74±0.01	18.98±0.00	80.93±1.11	2.31±0.06	376.00±2.00
71	TVu-7853	2.83±0.02	0.66±0.02	22.11±0.26	76.93±0.32	3.24±0.01	398.50±0.50
72	TVu-7870	3.32±0.03	0.85±0.00	23.21±0.42	51.11±1.07	2.92±0.09	393.00±1.00
73	TVu-7898	3.37±0.02	0.73±0.01	21.95±0.42	48.47±0.58	2.50±0.09	383.50±1.50
74	TVu-7920	2.63±0.04	0.88±0.03	23.10±0.31	74.65±0.72	2.45±0.09	391.50±1.50
75	TVu-7962	3.09±0.02	0.79±0.01	24.25±0.10	53.03±0.02	3.04±0.15	374.00±2.00
76	TVu-7983	2.92±0.03	0.79±0.04	25.93±0.42	57.97±1.07	3.21±0.02	379.50±0.50
77	TVu-7995	3.10±0.03	0.89±0.02	25.14±0.26	71.22±1.19	2.98±0.14	382.00±0.00
78	TVu-8042	3.45±0.05	0.70±0.03	23.57±0.05	57.98±0.36	2.77±0.05	375.50±1.50
79	TVu-8164	3.31±0.02	0.93±0.02	21.12±0.42	65.50±1.58	2.58±0.05	381.50±1.50
80	TVu-8387	2.93±0.04	0.83±0.02	21.64±0.10	61.49±0.66	4.10±0.06	391.00±1.00
81	TVu-8541	3.48±0.03	0.79±0.02	23.78±0.16	53.59±0.56	2.40±0.03	396.00±1.00
82	TVu-8546	3.38±0.04	0.83±0.01	21.00±0.21	79.54±0.64	3.60±0.05	397.00±1.00
83	TVu-8580	2.95±0.01	0.90±0.01	21.84±0.21	50.72±0.68	3.58±0.03	395.00±1.00
84	TVu-8586	3.20±0.03	0.84±0.03	18.87±0.05	51.34±0.71	3.39±0.04	383.00±1.00
85	TVu-9036	2.95±0.03	0.81±0.03	21.86±0.16	67.15±1.22	3.49±0.07	383.50±1.50
86	TVu-9167	2.90±0.02	0.78±0.03	21.24±0.16	56.77±0.40	3.67±0.07	391.50±1.50
87	TVu-9176	3.40±0.03	0.89±0.03	20.61±0.16	76.48±0.58	3.49±0.03	375.00±2.00
88	TVu-9185	3.43±0.03	0.97±0.03	23.28±0.16	71.50±0.89	2.73±0.03	382.00±2.00
89	TVu-9769	2.89±0.01	0.90±0.03	20.34±0.26	73.50±0.56	3.91±0.10	391.00±1.00
90	TVu-9772	3.15±0.03	0.90±0.04	23.19±0.52	65.43±0.63	2.71±0.08	384.00±1.00

91	TVu- 9773	2.92±0.03	0.77±0.01	24.45±0.16	54.54±0.74	3.11±0.04	395.50±1.50
92	TVu- 9774	3.12±0.02	0.70±0.03	24.30±0.16	73.89±0.16	2.98±0.03	396.50±1.50
93	TVu- 9776	3.15±0.04	0.81±0.01	24.79±0.16	66.16±1.15	2.73±0.09	399.00±1.00
94	TVu- 9779	3.18±0.01	0.81±0.03	23.24±0.10	57.18±0.88	2.15±0.01	397.50±0.50
95	TVu- 9780	3.06±0.07	0.81±0.04	23.12±0.21	53.67±0.74	2.24±0.01	374.50±0.50
96	TVu- 9784	2.98±0.02	0.95±0.02	22.28±0.21	64.68±0.78	3.62±0.06	391.50±1.50
97	TVu- 9787	3.32±0.04	0.83±0.01	21.44±0.21	72.54±1.50	2.28±0.04	382.50±1.50
98	TVu- 9788	2.99±0.01	0.79±0.01	20.59±0.21	64.99±0.38	2.40±0.05	375.50±1.50
99	TVu- 9790	3.10±0.01	0.76±0.00	27.40±0.52	70.88±0.38	3.56±0.13	394.00±1.00
100	TVu- 10112	2.99±0.01	0.83±0.02	21.01±0.26	66.56±1.82	2.79±0.05	371.00±1.00
101	TVu- 9357	2.83±0.02	0.79±0.02	19.96±0.26	68.49±0.55	2.45±0.03	393.00±1.00
102	TVu- 11979	2.65±0.04	0.78±0.03	19.19±0.05	52.52±0.38	3.12±0.04	399.00±1.00
103	TVu- 11986	2.72±0.00	0.90±0.03	21.39±0.05	61.58±0.68	3.88±0.02	373.00±3.00
104	TVu- 12348	2.98±0.03	0.83±0.03	19.60±0.05	74.21±0.10	4.05±0.02	390.00±2.00
Grand Average		3.09±0.24	0.78±0.08	21.39±2.41	63.92±10.72	3.08±0.54	388.63±8.48
Range		2.58-3.91	0.57-0.99	14.98-27.93	5.10-83.00	2.14-4.21	370.0-402.0
N		3	3	3	3	3	3
P <sub>0.05</sub>		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LSD <sub>0.05</sub>		0.15	0.07	0.84	14.22	5.43	0.25
CV (%)		1.37	2.48	1.10	6.19	2.31	0.39

LSD = Minimum Significant difference among mean values determined by Tukey's Test at 0.05 probability level and CV = Coefficient of Variation.

## DISCUSSION

The presence of antinutritional factors in legumes including cowpea has been recognized as a major factor limiting their wider food use (Grant, 1989; Liener, 1980; Oke, 1969). For instance, phytic acid and Oxalic acid reduce mineral bioavailability that may lead to some mineral deficiency diseases e.g. anaemia (Guthrie and Picciano, 1995), or form deleterious complexes with metal ions e.g. calcium-oxalate that leads to renal damage (Oke, 1969; Shukkur *et al.*, 2006). Notably, the presence of oligosaccharides in beans cause flatulence or gas production in the intestine with its associated discomfort and embarrassment that force some people to avoid eating beans entirely. It is because of the absence of the enzyme,  $\alpha$ -galactosidase in the human intestinal mucosa to cleave the  $\alpha$ -(1-6) galactose linkage in galactoside containing oligosaccharides (e.g. raffinose and stachyose), that allows these oligosaccharides to pass into the large intestine where bacteria ferment them forming large amounts of CO<sub>2</sub>, hydrogen gas and sometimes methane that are responsible for flatulence (Life Sciences Research Office, 1995). However, digestive aids are now available commercially to allow people eat beans without flatulence (Life Sciences Research Office, 1995) and changing water one or more times during cooking can markedly reduce oligosaccharide levels in beans (Anderson *et al.*, 1979). Nevertheless, there are beneficial effects of these antinutrients. Some ANFs such as phytic acid, lectins, phenolic compounds, amylase inhibitors and saponins have been shown to reduce blood glucose, plasma cholesterol and triglycerides levels. Phytic acid, phenolics, saponins and protease inhibitors have also been associated with reduced cancer risks (Thompson, 1993; Sridhar and Seena, 2006; Graf and Eaton, 1990; Harland and Morris, 1995; Vucenik *et al.*, 1997). Because of the health benefits of some antinutrients, Thompson (1993) suggested a change in their name.

This study determined the variability of phytate, oxalate, trypsin inhibitors, hemagglutinin, tannin and cyanogenic glycosides among cowpea cultivars. The results revealed significant variations in all the ANFs among the cultivars. This agrees with earlier reports by Oluwatosin (1999) and Barampama and Simard (1993), which attributed the variations to variety and location differences. Oluwatosin (1999) opined that the variability in the levels of ANFs in cowpea seeds depends largely on the environment where they are grown and by implication that a cowpea genotype grown and consumed safely in one environment can be poisonous when grown and consumed in another environment. The author reported that environmental effect was the major source of variation for tannins, haemagglutinin and phytic acid contents, whereas genotype was strongest in controlling trypsin inhibitor content. Mean separation analysis of the data showed that both protein and the antinutrients contents varied continuously among the cultivars with hemagglutinin having the highest variability (CV = 6.19 %). This study is important because, cultivars high in the ANFs including phytic acid, tannin and especially trypsin inhibitors such as TVu-9790 ( $27.40 \pm 0.52$ ) and TVu-7983 ( $25.93 \pm 0.42$ ) TIU/mg may be naturally more resistant to pest infestation than those with low antinutrient contents (Srinivasan and Durairaj, 2007; Oliveira, 1997; Udedibie and Carlini, 1998a; Udedibie and Nwaiwu, 1988; Oliveira *et al.*, 1999).

## CONCLUSION

This study showed high degree of variability in the seed contents of all the ANFs with trypsin inhibitor and hemagglutinins showing greater degree of variations among the cowpea accessions. This finding could form a base for selecting cultivars of choice for consumption and genetic improvement of the crop. It also indicated the possible presence of accessions with natural ability to resist insect pest attack.

## REFERENCES

- Akande, K. E., Doma, U. D., Agu, H. O., and Adamu, H. M. (2010). Major antinutrients found in plant protein sources: their effect on nutrition. *Pakistan Journal of Nutrition* 9 (8): 827-832
- Anderson, J.J.B., Thomsen, K. and Christiansen, C. (1987). High protein meals, insular hormones and urinary calcium excretion in human subjects. *In: Christiansen, C., Johansen J.S. and Rils, B.J. (eds), Osteoporosis Viborg, Denmark, Norhaven A/S, 240-245.*
- Anderson, R.L., Rackis, J.J. and Tailent, W.H. (1979). Biologically active substances in soy products. *In: Wilcke, H.L., Hopkins, D.T. and Waggle, D.H. (eds) Soy protein and human nutrition. New York Academic Press.*
- Barampama, Z. and Simard, R.E. (1993). Nutrient composition, protein quality and antinutritional factors of some varieties of dry bean (*Phaseolus vulgaris*) grown in Burundi. *Food Chemistry* 47(2):159-167.

- Breslau, N.A., Brinkley, L., Hill, K.D. and Pack, C.Y.C. (1998). Relationship of animal protein-rich diet to kidney stone formation and calcium metabolism. *Journal of Clinical Endocrinology and Metabolism* 66:140-146.
- Chan G.C.M. (1974). The influence of dietary intake on endogenous acid production. *Nutrition and Metabolism* 16:1-9.
- Davis, D.W., Oelke, E.A., Oplinger, E.S., Doli, J.D., Hanson, C.V. and Putnam, D.H. (1991). Alternative field crops manual. Center for Alternative Plant and Animal Products, Minnesota Extension Service, University of Wisconsin - Madison, 1- 6.
- Day, R.A. (Jnr) and Underwood, A.L. (1986). Quantitative analysis. 5th ed. Prentice -Hall publication.
- Duke, J.A. (1981). Handbook of legumes of world economic importance. Plenum Press, New York, 52-57.
- Graf, E. and Eaton, J.W. (1990). Antioxidant functions of phytic acid. *Free Radical Biology and Medicine* 8:61-69.
- Grant, G. (1989). Antinutritional factors of soybean: a review. *Progress in Food and Nutrition Science* 13:317-348.
- Guthrie, D.G. and Picciano, M.F. (1995). Human nutrition. 1<sup>st</sup> edn. WCB/ McGraw -Hill, New York. 43-44.
- Harborne, J.B. (1989). Biosynthesis and function of antinutritional factors in plants. *Aspects of Applied Biology* 19: 21-28.
- Harland, B.F. and Morris, E.R. (1995). Phytate: a good or bad food component? *Nutrition Research* 15:733-754.
- Joslyn, M.A. (1970). Tannins and related phenolics. *In: Methods in food analysis*. 701-725.
- Kakade, M.L., Rackis, J.J., McGhee, J.E. and Puski, G. (1974). Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. *Cereal Chemistry* 51: 376 - 382.
- Kerstetter, J.E. and Allen, L.H. (1989). Dietary Protein increases urinary calcium. *Journal of Nutrition* 120:134-136.
- Li, C.; Fatokun, C.A.; Ubi, B. Singh, B.B. and Scoles, G.J. (2001) Determining Genetic similarities and relationships by microsatellite markers. *Crop Science* 41:18, 117.
- Liener, I. E. (1981). Factors affecting the nutritional quality of soy products. *Journal of American Oil and Chemical Society* 58:406-415.
- Liener, I.E. and Hill, E.G. (1953). The effect of heat treatment on the nutritive value and haemagglutinating activity of soybean oil meal. *Journal of Nutrition* 49:609-619.
- Liener, I.E., (1975). Antitryptic and other anti-nutritional factors in legumes. Nutritional improvement of food legumes by breeding, 5<sup>th</sup> Edition. Wiley Interscience Publication, John Wiley and Sons, New York, 239-258.
- Liener, L.E. and Kakade, M.L. (1980). Protease inhibitors. *In: The lectins: Properties, functions, and applications in biology and medicine*. Academic Press, New York. 527- 552.
- Life Sciences Research Office (1995). Federation of American Societies for Experimental Biology. Third report on nutrition monitoring in the United States Vol.1. Washington DC, US Government Printing Press.
- Mahalakshmi, V., Ng, Q., Lawson, M. and Ortiz, R. (2007). Cowpea [*Vigna unguiculata* (L.) Walp.] core collection defined by geographical, agronomical and botanical descriptors *Plant Genetic Resources: Characterization and Utilization* 5(3); 113-119.
- Makover, R. U. (1970). Extraction and determination of phytic acid in beans. *Cereal McGraw-Hill*, New York. 43-44.



- Nielsen, S.S., Ohler, T.A. and Mitchell, C.A. (1997). Cowpea leaves for human consumption: production, utilization and nutrient composition. *In*: B.B. Singh, D.R. Moham Raj, K.E. Dashiell, L.E.N. Jackai (eds) *Advances in Cowpea Research*. Co-publication of International Institute of Tropical Agriculture (IITA) and Japan International Research Centre for Agricultural Science (JIRCAS), IITA, Ibadan, Nigeria, 326-332.
- Oke, O.L. (1969). Oxalic acid in plant and in nutrition. *World Review of Nutrition and Dietetics* 10: 267-303.
- Oliveira, A. E. A. (1997). Defence proteins of legume seed testa are homologous to vicilin storage protein. Abstract volume of 26<sup>th</sup> Annual Conference of Brazilian Society of Biochemistry and Molecular Biology, Caxambu, Brazil, May 3-6.
- Oliveira, A. E. A., Sales, M. P., Machado, O. L. T., Fernandes, K. V. S. and Xavier, F. J. (1999). The toxicity of Jack bean (*Canavalia ensiformis*) cotyledon and seed coat proteins to the cowpea weevil (*Callosobruchus maculatus*). *Entomologia-Experimentalis-et-Applicata* 92: 249-255.
- Oluwatosin, O.B. (1999). Genotype x environment influence on cowpea (*Vigna unguiculata* (L) Walp) antinutritional factors: Trypsin inhibitors, tannins, phytic acid and haemagglutinin. [\*Journal of the Science of Food and Agriculture\* 79\(2\):265-272.](#)
- Rao, P.V. and Hahn, S.K. (1984). An automated enzymatic assay for determining the cyanide content of cassava products. *Journal of Science, Food and Agriculture* 35:426-436.
- Remer, T. and Manz, F. (1994). Estimation of the renal net acid secretion by adults consuming diets containing variable amounts of protein. *American Journal of Clinical Nutrition* 59:1356-1361.
- SAS Institute Inc. (1988). SAS Statistical Software Version 9.1, Cary, NC, USA.
- Shukkur, M. F., Abdul, S. E., Karthik, H. S., Ramasamy, S., Nachiappa, G. R. and Palaninathan, V. (2006). Oxalate mediated nephronal impairment and its inhibition by C-phycoerythrin: A study on urolithic rats. *Molecular and Cellular Biochemistry* 284: 95-101.
- Singh, B. B., Ajeigbe, H. A., Tarawali, S. A., Fernandez-Rivera, S. and Abubakar, M. (2003). Improving the production and utilization of cowpea as food and fodder. *Field Crops Research* 84(1-2):169-177.
- Singh, B.B. (2007). Recent Progress in Cowpea Genetics and Breeding, Proceedings of I<sup>st</sup> International Conference on Indigenous Vegetables and Legumes. M.L. Chadha (ed), 69-75.
- Singh, S.P., Nodari, R. and Gepts, P. (1991). Genetic diversity in cultivated common bean: Allozymes. *Crop Science* 31:19-23.
- Soetan, K. O. (2008). Pharmacological and other beneficial effects of antinutritional factors in plants - A review. *African Journal of Biotechnology* 7 (25): 4713 -4721.
- Sridhar, K.R. and Seena, S. (2006). Nutritional and antinutritional significance of four unconventional legumes of the genus *Canavalia* – A comparative study. *Food Chemistry* 99:267-288.
- Srinivasan, T. and Durairaj, C. (2007). Biochemical basis of resistance in rice bean, *Vigna umbellata* Thunb (Ohwi and Ohanshi) against *Callosobruchus maculatus* F. *Journal of Entomology* 4(5):371-378.
- Steel, W.M., Allen, D.J. and Summerfield, R.J. (1985). Cowpea (*Vigna unguiculata* L. Walp). *Journal of Food Chemistry* 18(8):520-538.
- Tarawali, S.A., Singh, B.B., Fernandez-Rivera, S., Peters, M., Smith, J.W., Schutze -Kraft, R. and Ajeigbe, H.A. (1997a). Optimizing the contribution of cowpea to food and fodder production in crop-livestock systems in West Africa. Proceedings of the International Grassland Congress, Canada, 53-54.

Tarawali, S.A., Singh, B.B., Peters M. and Blade, S.F. (1997b). Cowpea haulms as fodder. *In*: B.B. Singh, D.R. Mohan Raj, K. Dashiell and L.E.N. Jackai (eds) *Advances in Cowpea Research*. Co-publication of International Institute of Tropical Agricultural Sciences and the JIRCAS, IITA, Ibadan, Nigeria, 313 -325.

Thompson, L. U. (1993). Potential health benefits and problems associated with antinutrients in foods. *Food Research International* 26(2):131-149.

Udedibie, A. B. I. and Carlini, C. R. (1998a). Questions and answers to edibility problem of the *Canavalia ensiformis* seeds – a review. *Animal Feed Science and Technology* 74:95–106.

Udedibie, A. B. I., and Nwaiwu, J. (1988). The potential of jack bean (*Canavalia ensiformis*) as animal feed. *Nigerian Agricultural Journal* 23:130–143.

Vucenik, I., yang, G.Y. and Shansuddin, A.M. (1997). Comparison of pure inositol hexaphosphate and high-bran diet in the prevention of DMBA-induced rat mammary carcinogenesis. *Nutrition and Cancer* 28:7-13.

Welch, R. M. and Graham, R.D. (2004). Breeding for micronutrients in staple wheat and wheat fractions. *Cereal Chemistry* 47:288-296.

Wheeler, E.L. and Ferrel, R.E (1971). A method for phytic acid determination in heat and wheat fractions. *Cereal Chemistry* 47:288-296

Yost, R. and Evans, D. (1988). *Green Manure and Legume Covers in the Tropics*. HITAHR-College of Tropical Agriculture and Human Resources, University of Hawaii. Research Series 055, 12 – 22.

Received for Publication: 24/02/2012

Accepted for Publication: 14/04/2012

Corresponding author

Afiukwa Celestine A.,

Department of Biotechnology, Faculty of Biological Sciences, Ebonyi State University, P.M.B. 053, Abakaliki, Nigeria.

Email: [afiukwa@yahoo.com](mailto:afiukwa@yahoo.com)