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# Antinutrients Evaluation of Staple Food in Ebonyi State, South-Eastern, Nigeria

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**Abstract:** Evaluating the levels of antinutrients in the staple foods is an important aspect of nutritional studies. The levels of these antinutrients were quantitatively estimated in staple foods in a major food producing rural areas of Ebonyi State, South-Eastern Nigeria using spectrophotometric method. Results indicated that phytate was not detected in tubers while legumes recorded the highest mean values of phytate (260.07 mg $g^{-1}$). The oxalate levels of legumes were significantly high compared to tubers and cereals ($p<0.000$). Tubers had the highest mean concentration of cyanogenic glycoside 15.20 mg $g^{-1}$ followed by legumes while cereals were the least. Correlation analysis shows that the cyanogenic glycoside was negatively related to all other antinutrients except tannin. Also tannin was related to all other antinutrients except cyanogenic glycoside. Interestingly, all other antinutrients were positively related to each other except cyanogenic glycoside and tannins. The consumption of staple foods rich in these antinutrients pose a health risk to livestock and poor communities that reside around the study sites, especially children.

**Key words:** Antinutrients, staple foods, correlation, rural areas, consumption, health risk

## INTRODUCTION

Many food crops used for food contain natural chemical substances known to have effects on the nutritional status of the food. Some of these naturally occurring toxicants are antinutrients such as cyanogenic glycoside, phytate, oxalate, lectin, saponins, alkaloids, pressor amines, flatulence factors, chymotrypsin inhibitors etc (Conn, 1979a and b; Nartey, 1980; Vennesland *et al*., 1982; Rosling, 1987). Chronic exposure to these natural substances through intake of staple foods rich in them may lead to the problem of toxicity. Plant products (notably cassava) if not adequately processed are toxic because of its high cyanogenic content (hydrogen cyanide-HCN). The hydrogen cyanide is readily absorbed by the gastro intestinal and respiratory tracts, the liquid and possibly the concentrated vapour are absorbed directly through the skin (Hartung, 1982; US EPA, 1984). A consequence of the absorption process prominent in the body, the cyanide is readily distributed through the blood. Short-term exposure to high concentration of HCN produces almost immediate collapse, respiratory arrest and death (Hartung, 1982; US EPA, 1985). Thyroid toxicity has been reported in humans and animals following oral and inhalation exposure to hydrogen cyanide (US EPA, 1984 and 1985). The thyroid toxicity is associated with cyanide metabolism via conversion to thiocyanate which inhibits the intra-thyroidal uptake of iodine, causing an increase in the secretion of Thyroid Stimulating Hormone (TSH) and reduction of thyroxin levels, thus it is a goitrogenic agent (Tewe, 1993).

Staple foods rich in oxalate have adverse effects when eaten because oxalate binds calcium and other minerals causing calcium deficiency and the formation of kidney stone (Sangketkit *et al*., 1999). Consumption of large doses of oxalate foods have also been implicated in corrosive gastroenteritis, shock, convulsive symptoms, less plasma calcium ions and renal damage (Sangketkit *et al*., 1999). Evaluating the levels of these antinutrients in staple foods is an important aspect of integrated and multisectorial approaches required to achieve the goals set under the National Nutrition Policy (NNP). Also, lectins, sometimes called phytohemagglutinins, are glycoproteins that bind to certain carbohydrate groups on cell surfaces, such as intestinal epithelial cells, where they cause lesions and severe disruption and abnormal development of the

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microvilli. One of the major consequences of the lectin damage to the intestinal mucosa appears to be serious impairment in the absorption of nutrients across the intestinal wall (OECD, 2003). However, available data on antinutrients level of the staple foods in many parts of Nigeria including Ebonyi is grossly inadequate. This lack of information has seriously affected the nutritional assessment in this part of the world. The aim of this research was therefore to evaluate the levels of some antinutrients in the immediate environment of a major food producing rural area in Nigeria. The health implications of the overload of these antinutrients to human are highlighted.

## MATERIALS AND METHODS

The study areas are located in Ebonyi State, in the central part of the eastern region of Nigeria. Specifically, the project sites are Abakaliki, Afikpo North, Ohaukwu, Ohaozara and Ikwo Local Government Areas, respectively and the research was conducted between the months of September to November 2006.

The samples were sorted and the damaged, discoloured and infected ones were removed. The good ones were used for the analysis. Tubers (yam, cassava) were peeled and crushed into slurry while cereals (rice samples) were dehusked and the edible portion ground. Samples that do not have husks (maize, African yambeans and cowpea) were also ground into slurry before they were used for the analysis.

The material for this study is basically the spectrophotometer.

## EXPERIMENTAL PROCEDURE

**Determination of cyanogenic glycoside:** The level of Cyanogenic glycoside was determined using the procedure as reported by Bradbury *et al.* (1999). Metal balance was used to weigh 100 mg of the slurry samples and placed on a round paper disc containing the buffer (pH 6.0) and the enzyme, Iinamarse in a flat-bottomed plastic bottle. About 0.5 mL of distilled water was added using plastic pipette. A yellow picrate paper was suspended in the flask attached to a plastic strip such that it does not touch the liquid in the bottle. The flask was covered with a screw cap lid. The capped flask was allowed to stand for 16-24 h at room temperature after which was opened and the picrate paper matched against the shades of colour of the colour chart supplied in the kit.

**Determination of tannin:** Tannin was quantitatively determined as reported in the Manual of food quality control (AOAC, 1984). The samples were ground into slurry and 0.5 g of the slurry sample was weighed into a conical flask and mixed with 10 mL of distilled water. This was shaken and allowed to stand for 1 h. About 1 mL of the extract was pipetted into another test tube. This was followed by the addition of 5 mL of distilled water. Two drops of $FeCl_3$ in 0.1 M HCl was added. It was shaken to mix properly and about 4 drops of potassium Ferro cyanide ($K_3Fe(CN)_6$) was also added. The absorbance of the portion of the mixture was read at 620 nm using spectrophotometer. The concentration of tannin was calculated as:

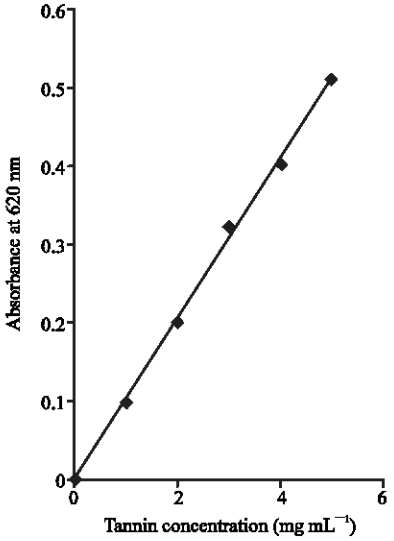


Fig. 1: Standard curve for the determination of tannin concentration

$$P_T = A_b \times S \times D_F \times 100 \text{ (mg g}^{-1} \text{ tan nin)} \quad (1)$$

where, $P_T$ is the percentage of tannin, $A_b$ is the absorbance, S is the slope from the standard curve (Fig. 1), $D_F$ is the dilution factor.

**Determination of saponin:** The saponin is determined using the colorimetric method (AOAC, 1984). The procedure involved is: The food samples were ground into slurry and 0.5 g of the sample was weighed and put into a test tube followed by the addition of 10 mL of distilled water. The mixture was shaken and allowed to stand for 1 h. The formation of stable foaming froth was observed. About 1 mL of the mixture was pipetted into another test tube with about 5 mL of distilled water added to this extract. This was followed by addition of a drop of olive oil. The test tube with its content was shaken and it became cloudy. The absorbance was measured at 620 nm using spectrophotometer. The

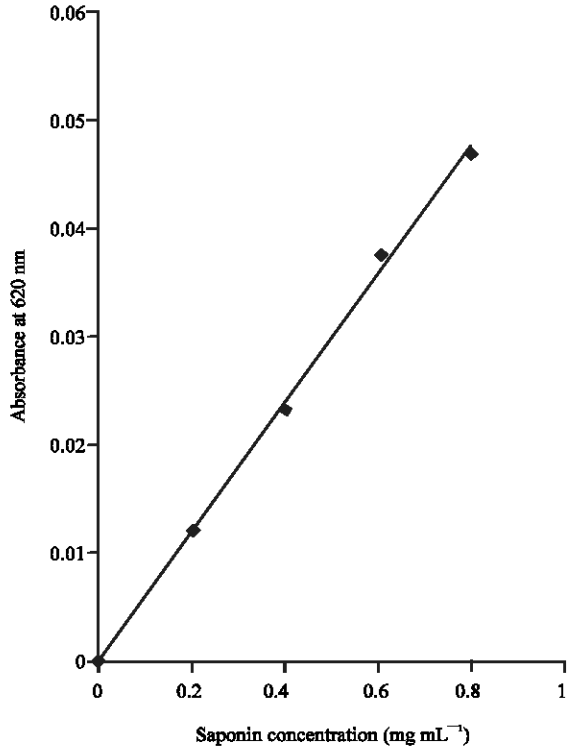


Fig. 2: Standard curve for the determination of saponin concentration

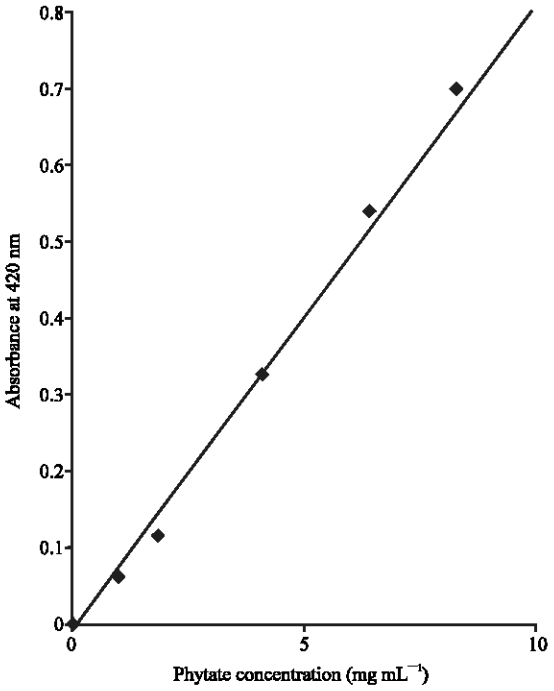


Fig. 3: Standard curve for the determination of phytate concentration

quantity of saponin contained in each sample was estimated from the standard saponin curve obtained from plotting the concentration of the standard concentration against the absorbance (Fig. 2). Hence, amount of saponin calculated as:

$$P_s = A_b \times S \times D_F \times 100 \text{ (mg g}^{-1} \text{ saponin)} \quad (2)$$

where, $P_s$ is the percentage of saponin and other symbols retain their usual meaning.

**Determination of phytate:** The method used in determining the status of phylate was as reported in the Manual of Food Quality Control of FAO (AOAC, 1984). The procedure involved is: Samples were ground into slurry and 0.5 g of the sample was weighed into a test tube. About 10 mL of distilled water was added. Approximately 2 mL of concentrated HCl was also added and the mixture was shaken and allowed to stand for 1 h. About 1 mL of the extract was pipetted into the test tube followed by the addition of 5 mL of distilled water. The mixture was shaken and portion was put into a curette and the absorbance measured at 420 nm using spectrophotometer. Phyteate present in the sample was calculated from a graph of known weights of phytate plotted against their absorbance (Fig. 3).

**Determination of lectins:** The method adopted for the determination of lectins is the Colorimetric Method as reported in the Manual of Food Quality (AOAC, 1984).

Here, the samples were ground into slurry and 1 g of the slurry sample weighed into a crucible. Ten milliliter of distilled water was added followed by the addition of 1 mL of concentrated $H_2SO_4$ and the mixture allowed to stand for 1 h. The solution was made up to 50 mL using distilled water. Five milliliter of the extract was pipetted into a test tube and 1 mL of Schiffs reagent added to the test tube. A portion of the solution in the test tube was put into cuvette and the absorbance measured at 510 nm. The value of lectin in each sample is estimated from the standard curve of the lectin (Fig. 4).

**Determination of alkaloids:** Following the colorimetric method (AOAC, 1984), the alkaloid status of the samples were determined. Samples were ground into slurry and 1 g of the slurry sample was weighed into a crucible containing about 10 mL of distilled water which was followed by the addition of 2 mL of $H_2SO_4$. The mixture was allowed to stand for 1 h after shaking. About 5 mL of the extract was pipetted into a test tube and 1 mL of trichloroacetic acid was added. A portion from the test tube was put into a cuvette and the absorbance measured at 420 nm using spectro-photometer. The quantity of alkaloid present in each sample was obtained from the

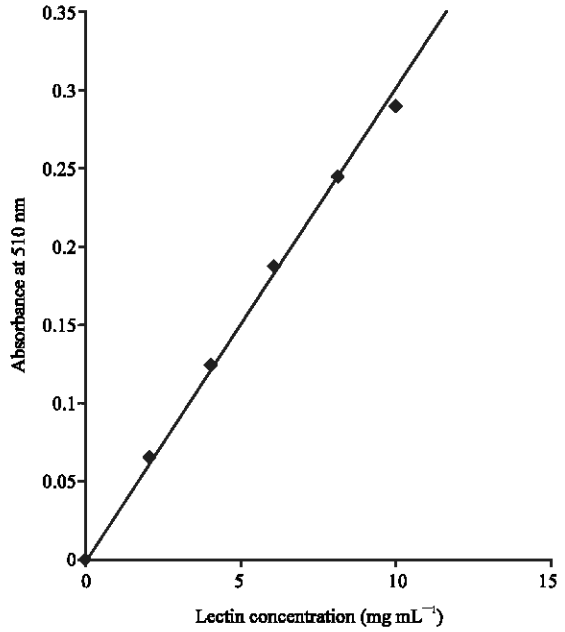


Fig. 4: Standard curve for the determination of lectin concentration

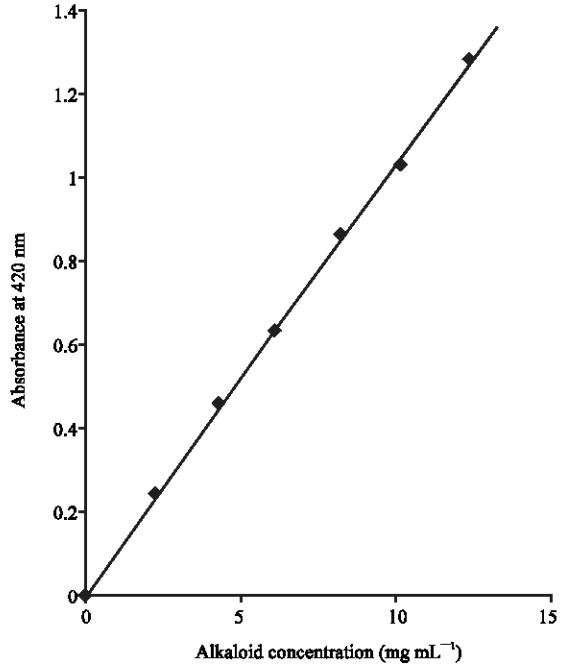


Fig. 5: Standard curve for the determination of alkaloid concentration

graph of a standard. Known weights of samples containing alkaloid were treated as the sample tests and the absorbance recorded. The results were used to obtain the standard curve (Fig. 5).

**Calculation:**

$$Q_a = A_b \times S \times D_F \times 100 \text{ (mg g}^{-1}\text{alkaloid)}$$

where, $Q_a$ is the quantity of alkaloids and other symbols retain their usual meaning.

**Determination of oxalate:** Again using the Colorimetric Method (AOAC, 1984), the oxalate level in the samples were determined. The samples were ground into slurry and 1 g of the sample was weighed into a crucible dish. About 10 mL of distilled water was added, followed by the addition of 1 mL concentrated $H_2SO_4$. This was allowed to stand for an hour. The volume was made up to 50 mL with distilled water. About 5 mL of the extract was pipetted into a conical flask and titrated against potassium permanganate in a burette. A colour change was noted which indicates the end point and the reading of the burette was taken when the red colour remained steady for some seconds. The concentration of oxalate (mg g$^{-1}$) in each of the sample was got by multiplying the burette reading by 11.5.

## RESULTS AND DISCUSSION

The results of the antinutrients concentrations (mg g$^{-1}$) of the various staple foods (expressed as plus or minus of the standard deviation) from different locations in Ebonyi State, south- Eastern Nigeria are as shown in Table 1-6.

A perusal at the various tables indicates that phytate was not detected in tubers (yam and cassava) while legumes had the highest concentrations of phytate. Cowpea has a range of 155.00-557.50 mg g$^{-1}$, cereals recorded values of 1.00-1.85 mg g$^{-1}$ and mean levels of 1.43 mg g$^{-1}$ for rice while maize had a range 188.50-141.00 mg g$^{-1}$ and mean concentration of 126.20 mg g$^{-1}$. The range and mean levels of oxalate in legumes were 8.25-21.30 mg g$^{-1}$ and mean value of 11.42 mg g$^{-1}$ while cereals recorded a range of 2.65-5.75 mg g$^{-1}$ and mean value of 4.53 mg g$^{-1}$ and tubers have 2.55-90 mg g$^{-1}$ and mean value of 2.75 mg g$^{-1}$.

The statistical analysis of the antinutrient levels of the staple food were also carried out and it shows that there are significant differences in the antinutrient content of staple foods from Ebonyi state with regards to phylate, oxalate, lectin, alkaloid, cyanogenic glycoside, tannin and saponin ($p>0.000$). However, the result show that there is no significant different in phytate, oxalate, lectin, alkaloid, cyanogenic glycoside, tannins and saponin of the staple foods got from the different local government areas of Ebonyi state ($p>0.000$).

Table 1: Concentration of antinutrients (mg $g^{-1}$) of rice

| | Study area | | | | |
|---|---|---|---|---|---|
| Antinutrients | Abakaliki | Afikpo | Ohaozara | Ikwo | Ohaukwu |
| Phytate | 1.45±0.07 | 1.52±0.12 | 1.00±0.14 | 1.35±0.35 | 1.85±0.07 |
| Oxalate | 4.25±0.50 | 7.90±1.13 | 5.55±0.64 | 2.65±0.50 | 2.80±0.28 |
| Lectin | 0.55±0.07 | 0.55±0.07 | 0.70±0.14 | 0.45±0.07 | 0.45±0.07 |
| Alkaloid | 0.65±0.07 | 0.75±0.07 | 1.55±0.21 | 1.50±0.14 | 1.50±0.14 |
| Cyanogenic glycoside | 4.20±0.14 | 2.45±0.50 | 1.45±0.07 | 1.80±0.28 | 1.05±0.07 |
| Tannins | 0.55±0.07 | 0.55±0.07 | 0.60±0.00 | 0.50±0.14 | 0.55±0.07 |
| Saponin | 0.35±0.07 | 0.30±0.14 | 0.75±0.21 | 0.15±0.07 | 0.25±0.07 |

Table 2: Concentration of antinutrients (mg $g^{-1}$) of maize

| | Study area | | | | |
|---|---|---|---|---|---|
| Antinutrients | Abakaliki | Afikpo | Ohaozara | Ikwo | Ohaukwu |
| Phytate | 131.50±2.120 | 123.00±1.41 | 108.50±0.71 | 141.00±1.41 | 127.00±1.41 |
| Oxalate | 5.15±1.20 | 5.75±0.35 | 5.50±0.71 | 2.75±0.35 | 2.95±0.07 |
| Lectin | 0.45±0.07 | 0.45±0.07 | 0.70±0.14 | 0.55±0.07 | 0.45±0.07 |
| Alkaloid | 1.50±0.14 | 0.80±0.14 | 0.70±0.14 | 1.50±0.14 | 0.65±0.07 |
| Cyanogenic glycoside | 1.90±0.14 | 5.05±0.07 | 1.70±0.14 | 2.05±0.07 | 1.50±0.14 |
| Tannins | 0.85±0.07 | 0.35±0.07 | 0.45±0.07 | 0.55±0.07 | 2.35±0.07 |
| Saponin | 0.65±0.07 | 0.15±0.07 | 0.00±0.00 | 0.45±0.07 | 0.05±0.07 |

Table 3: Concentration of antinutrients (mg $g^{-1}$) of yam

| | Study area | | | | |
|---|---|---|---|---|---|
| Antinutrients | Abakaliki | Afikpo | Ohaozara | Ikwo | Ohaukwu |
| Phytate | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| Oxalate | 2.75±0.35 | 2.80±0.28 | 2.70±0.42 | 2.90±0.14 | 2.70±0.42 |
| Lectin | 0.35±0.00 | 0.20±0.00 | 0.25±0.07 | 0.30±0.00 | 0.30±0.00 |
| Alkaloid | 0.50±0.14 | 0.25±0.07 | 0.25±0.07 | 0.30±0.14 | 0.40±0.14 |
| Cyanogenic glycoside | 5.30±0.14 | 5.15±0.07 | 5.15±0.07 | 5.25±0.07 | 4.70±0.14 |
| Tannins | 1.65±0.07 | 2.10±0.00 | 1.95±0.07 | 1.95±0.07 | 2.30±0.14 |
| Saponin | 0.35±0.07 | 0.55±0.21 | 0.25±0.00 | 0.35±0.07 | 0.35±0.07 |

Table 4: Concentration of antinutrients (mg $g^{-1}$) of cassava

| | Study area | | | | |
|---|---|---|---|---|---|
| Antinutrients | Abakaliki | Afikpo | Ohaozara | Ikwo | Ohaukwu |
| Phytate | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| Oxalate | 2.70±0.42 | 2.95±0.07 | 2.75±0.35 | 2.55±0.64 | 2.65±0.50 |
| Lectin | 0.30±0.00 | 0.35±0.07 | 0.35±0.07 | 0.35±0.07 | 0.45±0.07 |
| Alkaloid | 0.25±0.07 | 0.50±0.14 | 0.35±0.07 | 0.45±0.21 | 0.35±0.07 |
| Cyanogenic glycoside | 10.25±0.07 | 10.30±0.14 | 15.35±0.78 | 12.35±0.64 | 21.85±1.34 |
| Tannins | 1.85±0.07 | 2.35±0.07 | 1.50±0.14 | 1.95±0.21 | 1.50±0.14 |
| Saponin | 0.45±0.07 | 0.55±0.07 | 0.50±0.14 | 0.50±0.14 | 0.70±0.14 |

Table 5: Concentration of antinutrients (mg $g^{-1}$) of cowpea

| | Study area | | | | |
|---|---|---|---|---|---|
| Antinutrients | Abakaliki | Afikpo | Ohaozara | Ikwo | Ohaukwu |
| Phytate | 557.50±61.52 | 155.00±21.21 | 162.50±0.71 | 202.00±1.41 | 211.50±0.71 |
| Oxalate | 8.25±0.35 | 9.05±0.75 | 21.30±1.84 | 11.25±0.35 | 8.25±0.35 |
| Lectin | 4.20±0.28 | 2.15±0.07 | 1.75±0.21 | 2.15±0.07 | 1.80±0.28 |
| Alkaloid | 5.45±0.07 | 1.30±0.14 | 1.50±0.14 | 0.65±0.07 | 1.25±0.21 |
| Cyanogenic glycoside | 3.85±0.07 | 4.50±0.14 | 4.30±0.14 | 4.50±0.14 | 2.30±0.28 |
| Tannins | 1.20±0.14 | 2.50±0.14 | 0.60±0.14 | 0.55±0.07 | 0.95±0.07 |
| Saponin | 6.55±0.21 | 6.70±0.14 | 4.25±0.07 | 0.10±0.00 | 6.15±0.21 |

Correlation analysis shows that cyanogenic glycoside is negative related to all other antinutrients except tannin. Also, tannin is negatively related to all other antinutrents except cyanogenic glycoside. Interestingly all the other antinutrients are positively related to each other but not with cyanogenic glycoside and tannin. The t-test value for mean comparison for significant difference between rice and maize constituents, t (8, 4.005): 23.337, $p<0.000$ for phytate but not for others. There are significant differences in the antinutrient constituents of tubers with respect to lectin, t (8, 7.987): -2.263, $p<0.053$; cyanogenic glycoside t (8, 4.019): -4 107,

Table 6: Concentration of antinutrients (mg $g^{-1}$) of African yambean

| | Study area | | | | |
|---|---|---|---|---|---|
| Antinutrients | Abakaliki | Afikpo | Ohaozara | Ikwo | Ohaukwu |
| Phytate | 153.50±2.12 | 299.00±1.41 | 411.50±6.36 | 307.00±9.89 | 206.50±0.71 |
| Oxalate | 11.55±1.34 | 11.25±0.35 | 9.19±0.92 | 9.90±0.98 | 14.25±0.35 |
| Lectin | 5.95±0.21 | 2.85±0.07 | 6.30±0.57 | 4.40±0.28 | 1.25±0.07 |
| Alkaloid | 5.35±0.07 | 1.40±0.14 | 1.25±0.07 | 1.50±0.14 | 5.45±0.07 |
| Cyanogenic glycoside | 4.50±0.14 | 4.25±0.07 | 4.80±0.14 | 4.70±0.14 | 4.30±0.14 |
| Tannins | 2.20±0.14 | 0.65±0.07 | 0.65±0.07 | 2.70±0.14 | 0.55±0.07 |
| Saponin | 2.75±0.35 | 8.65±0.35 | 3.40±0.00 | 1.40±0.14 | 6.30±0.14 |

$p<0.003$ and saponin t (8, 7.868), -2.608, $p<0.031$. There are no significant differences in the antinutrient levels in legumes.

The phytate values for African yambean and cowpea in Ebonyi State were higher than values reported by Vijayakuman *et al*. (1996), but lower than values reported by Khokhars and Chauham (1986). The moderate levels of phytate in the legume staple foods from Ebonyi State and environs suggest that it will not render several minerals especially iron and zinc biological unavailable to animals and humans that feed on these staple foods in the study areas (Morris and Ellis, 1982). Also the moderate levels of the phytate are beneficial in human diets as it reduces the incidence of heart diseases and act as anticarcinogens (Saied and Shamsuddin, 1998; Zhou and Erdman, 1995).

The high levels of cyanogenic glycosides in the cassava of the areas studied are a threat to life because, if the cassava is not properly processed before using as food, it will expose the inhabitants to HCN toxicity. Short-term exposure to high levels of HCN produces almost immediate collapse, respiratory arrest and death (US EPA, 1985). Oshuntokun (1972) reported that consumption of cassava which is not properly processed over a long period, could give rise to ailments such as anoxia, neuropathy and goitre. Thyroid toxicity of hydrocyanic acids due to its metabolite-thiocyanate that inhibits the intra thyroidal uptake of iodine. This causes the stimulation of Thyroid Stimulating Hormone (TSH) and reduction in thyroxine levels which is the goitrogenic factor (Tewe, 1993).

The levels of oxalate in legumes were found to be significantly higher than in tubers and cereals. This finding is not in line with the reports of Alastair *et al*. (1999) Holmes and Kennedy (2000) that root crops (tubers) contain markedly high levels of soluble and insoluble oxalates. Consumption of staple foods rich in oxalate has adverse effects in humans because oxalates bind calcium and other minerals thereby making them unavailable to the biological system. Sangketkit (1999) reported that consumption of large doses of foods containing oxalate leads to corrosive gastroenteritis, shock, convulsive symptoms, less plasma calcium and renal damage. Ruminants that consume plants rich in oxalates develop increasing among of tolerance to oxalate because of the presence of oxalate degraders (*Oxalobacter formigenes*) in their rumen (Allison *et al*., 1995). The activity of this bacterial flora is constantly lost in humans (Sidhu *et al*., 1997). Therefore people and especially the inhabitants of the study area that consume legumes rich in oxalates are at greater risk of oxalate toxicity.

However, a major set back to this research was our inability to compare our findings with morbidity and morality information from the study area. The reason for this was due to the remote nature of the locality with the inhabitants of the study area, largely illiterates and perforating local herbs to orthodox medicine. Medical records were essential absent and no systematic assessment of the antinutrient toxicity has been conducted in the study area. Further studies to address these problems are recommended.

## CONCLUSIONS

From the proximate composition of the major staple food studied, it can be concluded that the distribution of the antinutrient factors (phytate, oxalate, lectin, alkaloid, cyanogenic glycoside, tannin and saponin) in the staple food crop cultivars in Ebonyi State have a consistent pattern for the three groups of food crops irrespective of the sample location. Hence, suggesting that the antinutrient factors may not be due to environmental factor but are endogenous attributes of specific food crops.

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