Antinutrients Level in Staple Food Crops in Ebonyi-Nigeria

1C.O. Edeogu and 2C.E. Ekuma
1Department of Medical Biochemistry, College of Medicine and Health Sciences, Ebonyi State University, Abakaliki Nigeria
2Department of Industrial Physics, Faculty of Applied Natural Sciences, Ebonyi State University, Abakaliki, Nigeria

Abstract: The antinutrient levels of staple food crops were determined colorimetrically using spectrophotometer. The results indicate the presence of phytate, oxalate, lectin, alkaloid, cyanogenic glycoside, saponin, tannin and chymotrypsin inhibitor in the samples analyzed. The distribution of these antinutrients appears to have a consistent pattern for the three groups of crops (tubers, cereals and legumes) irrespective of the sample location. The study indicates that the antinutrient factors may not be due to environmental factors but are endogenous attributes of each of the staple food cultivars.

Key words: Antinutrients, spectrophotometer, staple food crops, environmental factors

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, b; Narlkey, 1980; Venneland 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 gastrointestinal 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, 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 stones (Sangkhetkit 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 (Sangkhetkit et al., 1999). Evaluating the levels of these antinutrients in staple foods is an important aspect of integrated and multi-sectorial approaches required to achieve the goals set under the National Nutrition Policy (NNP). 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, central eastern region of Nigeria. Specifically, the studied sites are Abakaliki, Afikpo North, Ohaukwu, Ohaokara and Ikwo Local Government Areas, respectively. 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.

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, linamarase 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$_2$Fe(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:

$$P_t = \frac{A_t \times S \times D_t}{100} \text{ (mg g}^{-1} \text{ tannin)}$$

where $P_t$ is the percentage of tannin, $A_t$ is the absorbance, $S$ is the slope, $D_t$ is the dilution factor.

Determination of Saponin

The saponin was 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 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. Hence, amount of saponin calculated as:

$$P_s = A_s \times S \times D_s \times 100 \text{ (mg g}^{-1} \text{saponin)}$$  \hspace{1cm} (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 phytate 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 cuvette and the absorbance measured at 420 nm using spectrophotometer. Phytate present in the sample was calculated from a graph of known weights of phytate plotted against their absorbance.

**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 milliliters of distilled water were added followed by the addition of 1 mL of concentrated H$_2$SO$_4$ and the mixture allowed to stand for 1 h. The solution was made up to 50 mL using distilled water. Five milliliters of the extract was pipetted into a test tube and 1 mL of Schiff’s 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.

**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$_2$SO$_4$. 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 spectrophotometer. The quantity of alkaloid present in each sample was obtained from the 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.

**Calculation**

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

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$_2$SO$_4$. 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.

**Determination of Chymotrypsin Inhibitor**

The status of chymotrypsin inhibitor was determined Colorimetrically as reported by AOAC (1984). The food crop samples were ground and dried in electric oven for 3 h and about 0.5 g of the ground sample was weighed into test tubes. Ten milliliters of 0.01 M of NaOH was added into each sample and they were allowed for an hour. About 2 mL of buffer solution was added; shaken properly and allowed to stand for 10 min as to attain equilibrium. Ten milliliter solution of chymotrypsin enzyme was added and allowed for 30 min. This was followed by addition of 10% of acetic acid solution. The mixture was shaken thoroughly and allowed for another 30 min. The absorbance was read at 420 nm and the optical density was recorded. The concentration in IU was obtained by multiplying absorbance by 1000.

**RESULTS AND DISCUSSION**

The levels of the antinutrient factors in the staple food crops are as presented in Fig. 1-6, while that of chymotrypsin inhibitor level is as shown in Fig. 7. Cassava has the highest value of cyanogenic glycoside followed by yam (Fig. 1 and 3). Maize had the least value of cyanogenic glycoside and the highest antinutrient factor in maize is phytate for all the study areas (Fig. 2). However Fig. 5 and 6 reveal that legumes (cowpea and African yambean) had the highest levels of phytate among the staple food crops and across the localities studied. The mean values of chymotrypsin inhibitor levels in (IU) for all the staple food crops were high with legumes (cowpea and African yambean) topping the list (Fig. 7).

Results from the study reveal the presence of the following antinutrient factors in all the samples; phytate, oxalate, lectin, alkaloid, cyanogenic glycoside, tannins and saponin. Phytate concentration was found to be highest in legumes with concentration ranges of between 153 mg g$^{-1}$ to about 400 mg g$^{-1}$ of sample, but is virtually absent in tubers. In a similar manner cyanogenic glycoside is predominant in legumes.

![Graph](image)

**Fig. 1: Concentration of antinutrients in rice**

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Fig. 2: Concentration of antinutrients in maize

Fig. 3: Concentration of antinutrients in yam

Fig. 4: Concentration of antinutrients in cassava
Fig. 5: Concentration of antinutrients in cowpea

Fig. 6: Concentration of antinutrients in African yambean

Fig. 7: Mean concentration of chymotrypsin inhibitors (IU) in staple foods
in tubers than in other samples studied (Fig. 1-6). In general, the distribution of these antinutrients appears to have a consistent pattern for the three groups of crops (i.e., tubers, cereals and legumes) irrespective of the sample location. The results of this study suggest that antinutrient factors may not be under environmental control, they are endogenous attributes of specific food crops.

Chymotrypsin inhibitor levels in the staple food crops across the localities did not show any significant difference. However, the legumes (cowpea and African yam bean) recorded the highest mean concentrations of chymotrypsin inhibitors and the trend was consistent in the five local government areas studied (Fig. 7). The result of this research agrees with the values of chymotrypsin inhibitors in legumes as reported by (McArthur et al., 1972; Miller, 1979; Onwueme, 1984).

CONCLUSION

From the foregoing discussions, it can be inferred 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 suggesting that the antinutrient factors may not be under environmental control but are endogenous attributes of specific food crops.

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