Effect of Gas Flaring on the Anti-nutritional Composition of Four Green Leafy Vegetables from Eleme in Rivers State, Nigeria

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Abstract

The effect of gas flaring on the anti-nutritional composition of four green leafy vegetables, scent leaf (Ocimum gratissimum), bitter leaf (Vernonia amygdalina), water leaf (Talinum triangulare) and fluted pumpkin leaf (Telfairia occidentalis) harvested from Ilasa Eleme, an environment highly polluted by gas flaring and Igirita Ali, an environment not known for gas flaring both within the rainforest region of Rivers State were investigated. The samples were collected, processed and subjected to anti-nutrient screening using standard official methods of analysis of the Association of Analytical chemists. The result of the screening showed varying levels of anti-nutrient compositions in samples from the two localities. The concentrations of alkaloid, oxalate, saponins and cyanogenic glycosides in scent leaf and bitter leaf were significantly higher (p<0.05) in samples from non-polluted area compared to samples from polluted areas. Tannin level in fluted pumpkin and oxalate level in water leaf from polluted area were significantly higher (p<0.05) than samples from non-polluted area. Data obtained revealed that the anti-nutrient composition of vegetables studied are responsive to pollution due to gas flaring and therefore may be under environmental control.

Keywords: Gas flaring; Pollution; Anti-nutrient; Phytochemicals; Green leafy vegetables

1. Introduction

Green leafy vegetable consumed as cooked complements to the major staples like yam, maize, rice, plantains and beans constitute an indispensable constituent to human diet in West Africa and Nigeria in particular (Oguntona, 1986). They are plants parts usually herbaceous that contain edible portion such as roots, stems, leaves, fruits or a combination of these parts depending on one’s knowledge of them and their availability. They may be eaten raw or cooked depending on the type but most often are cooked. They are excellent sources of important micronutrient in diets such
as vitamins and minerals calcium, iron, magnesium, sodium, potassium and copper are among the minerals found in appreciable amount in leafy vegetables (Fox and Cameron, 1984; Ihekoronye and Ngoddy, 1985; Oguntona 1998; Onyeike, 2003). Vitamins C, E and A are also found in high quantity in many vegetable.

Nwachukwu (2002) pointed out that it is not enough to say that a particular food is rich in nutrient; such nutrient must be biologically available. This is due to the fact that most of the anti-nutritional factors chelates the essential minerals and affects their complete absorption thereby making them unavailable for their biochemical roles. In severe cases, manifest symptoms of the nutrient deficiency may result.

These vegetables are widely cultivated in the Niger Delta region of Nigeria. The Niger-Delta is replete with petroleum pollution from crude oil exploration and gas flaring. In this area of the world that lacks pipeline and other gas transportation infrastructure, vast amounts of gas are commonly flared as wastes or unstable gas. Such flaring constitutes hazard to plants well-being as it can affect soil composition and thus pose threat to plants metabolism by altering their nutrient composition (Baker, 1970). This study however, evaluates the effect of flare gases on the anti-nutrient composition of these leafy vegetables which serves as plant food in the polluted Niger-Delta environment of Nigeria.

2. Materials and Methods

2.1 Site Description

The study areas are located in Rivers State. Specifically the studied sites are Ilasa-Eleme the home to Portharcourt Refinery Company and Nigeria National Petroleum Company Department and Igirita Ali were the control samples was gotten from. The research was conducted between the months of July to November, 2014.

2.2 Sample Collection and Preparation

The samples were harvested from farms at the studied sites and scientifically identified by Taxonomist attached to National Root Crops Research Institute Umuahia, Abia State, Nigeria. The sample were sorted and the damaged, discoloured and insect infested ones were removed while the good ones were rinsed with distilled water and dried at 65 °C to constant weight before grinding with Author Thomas milling machine. The powdered form was sieved using 1mm sieve. It was then stored at room temperature and used for all the analysis.

2.3 Phytochemical Analysis

2.3.1 Determination of Tannin

Tannin was quantitatively determined as reported by AOAC (1954). Half a gram (0.5g) of powdered sample was weighed into a 250ml conical flask and mixed with 10 ml of distilled water. This was shaken and allowed to stand for 1hr. About 1ml of the extract was transferred into another test tube and diluted with 5ml of distilled water. Two drops of ferric chloride (FeCl₃) in 0.1M
hydrochloric acid (HCl) was added and then shaken to mix properly. Four drops of potassium ferrocyanide (K$_3$Fe(CN)$_6$) was introduced and the absorbance of the mixture was then read spectrophotometrically at 620nm. The concentration of Tannin contained in each sample was estimated from the standard Tannin curve obtained from plotting the concentration of the standard against the absorbance.

\[
\% T = \frac{Ab x S x Df x 100}{mg g^{-1} \text{ tannin}}
\]

where, T is tannin, Absorbance of the sample (S) is the slope of the standard curve, Df is the dilution factor.

2.3.2 Determination of Phytate

The method used in determining the concentration of phytate in the samples was as reported by AOAC (1984). Half a gram (0.5g) of the sample was weighed into a test tube. About 10ml of distilled water was added. Approximately 2ml of concentrated HCL was also added and the mixture was shaken and allowed to stand for 1hr. One ml of the extract was pipette into the test tube followed by the addition of 5ml of distilled water. The mixture was shaken and absorbance read at 420nm using spectrophotometer. Phytate present in the sample was calculated from a graph of known weights of phytate plotted against their absorbance.

2.3.3 Determination of Alkaloids

The alkaloid levels of the samples were determined colorimetrically (AOAC, 1984) by weighing 1g of the powdered samples into a crucible containing 10 ml of distilled water followed by the addition of 2 ml of H$_2$SO$_4$. The mixture was allowed to stand for 1hr after shaking. Five ml of the extract was introduced into a test tube and 1ml of trichloroacetic acid added. The reading of the sample was taken spectrophotometrically at 420nm. The quantity of alkaloid present in each sample was obtained from the graph of a standard. Known concentration of alkaloid containing samples was treated as the test samples and the absorbance recorded. The results were used to obtain the standard curve.

Calculation:

\[
\% Qs = \frac{Ab x S x Df x 100}{mg g^{-1} \text{ tannin}}
\]

where Qs is the quantity of alkaloids and other symbols remains as defined.

2.3.4 Determination of Saponins

The procedure involved in the determination of saponin according to AOAC 1984 is by colorimetric method. In this method, 0.5g of the powdered sample was weighed into a test tube and 10ml of distilled water added. The mixture was shaken and allowed to stand for 1hr. Stable foaming froth was observed. One ml of the extract was then pipette into another test tube and was made up to 5mls with distilled water. A drop of olive oil was added to the extract and was shaken till it becomes cloudy. Then the absorbance was measured at 620nm using spectrophotometer. The quantity of saponin contained in each sample was estimated from the standard saponins curve obtained from plotting the concentration of the standard concentration against the absorbance. Hence amounts of saponin were calculated thus.

\[
Ps = \frac{Ab x S x Df x 100}{mg g^{-1} \text{ saponin}}
\]
where \( Ps \) is the percentage of saponin and other symbols remain as defined.

### 2.3.5 Determination of Oxalate

Oxalate level was determined using calorimetric method of AOAC 1984. One gram of powdered samples was weighted into a crucible dish and 10ml of distilled water was introduced into the crucible followed by addition of 1ml concentrated \( \text{H}_2\text{SO}_4 \) and was allowed to stand for an hour. This volume was later made up to 50ml with distilled water. Five ml of the extract was then pipette into 250ml conical flask and titrated against potassium permanganate in a burette. A colour change was noted which indicated the end point and the reading of the burette was taken when the read 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.

### 2.3.6 Determination of cyanogenic glycoside

The level of cyanogenic glycoside was determined using the procedure as reported by Bradbury et al (1999). About 100g of powdered sample was weighed using and placed on a round paper disc containing the butter (pH 6.0) and the enzyme, linamarase in a flat-bottomed plastic bottle. Half a ml (0.5ml) 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 then covered with a screw cap lid. The capped flask was allowed to stand for 16-24hrs at room temperature after which was opened and the picrate paper matched against the shades of colour the colour chart supplied in the kit.

### 2.4 Statistical Analysis

The data obtained from the chemical analysis were subjected to descriptive statistical analysis and one way analysis of variance (ANOVA) and correlation were carried out using SPSS/PC+ package version 18 and differences between means were compared using Duncan’s (1955) multiple range test.

### 3. Results and Discussion

Studies on the anti-nutritional composition of four edible leafy vegetables from polluted and unpolluted environments provided insight on the effects of pollutants on metabolism of a whole plant as well as effects it could possibly have on those who consume them. Result obtained from this study shown that the leafy vegetables; scent leaf, bitter leaf, water leaf and fluted pumpkin leaf contains appreciable amounts of alkaloid, phytate, oxalate, saponins, tannins and cyanogenic glycosides.

The Phytochemicals in scent leaf and bitter leaf from Eleme polluted environment are shown in (Table 1). Alkaloid, oxalate, Saponins and Cyanogenic glycosides content of scent leaf and bitter leaf from Igirita Ali non polluted area were significantly higher \((p<0.05)\) than samples from Eleme polluted environment (Table 2). Phytate and tannin concentration did not vary significantly when samples from the two sites were compared. In waterleaf and fluted pumpkin leaf, alkaloid, phytate, saponin content in samples from polluted environment did not vary significantly \((p<0.05)\) while the
levels of oxalate and tannin in waterleaf and pumpkin respectively were significantly higher ($P<0.05$) than samples from unpolluted. The levels of cyanogenic glycosides in waterleaf and fluted pumpkin leaf was significantly higher ($P<0.05$) than the samples from polluted environment.

Table 1 Result of antinutrient composition of four leafy vegetables from Ilasa Eleme in Rivers State.

<table>
<thead>
<tr>
<th>Antinutrient</th>
<th>Scent leaf</th>
<th>Bitter leaf</th>
<th>Water leaf</th>
<th>Pumpkin leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>2.10±0.04b</td>
<td>3.85±0.04a</td>
<td>0.36±0.02a</td>
<td>0.50±0.04c</td>
</tr>
<tr>
<td>Phytate</td>
<td>0.49±0.01a</td>
<td>0.44±0.04b</td>
<td>0.24±0.02c</td>
<td>0.23±0.01c</td>
</tr>
<tr>
<td>Oxalate</td>
<td>0.12±0.03c</td>
<td>0.49±0.04a</td>
<td>0.26±0.03b</td>
<td>0.09±0.01c</td>
</tr>
<tr>
<td>Sapouin</td>
<td>2.11±0.04b</td>
<td>2.96±0.04a</td>
<td>0.24±0.01c</td>
<td>0.18±0.02d</td>
</tr>
<tr>
<td>Tannin</td>
<td>0.09±0.02b</td>
<td>0.012±0.04b</td>
<td>0.40±0.10a</td>
<td>0.10±0.00b</td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>13.37±0.04a</td>
<td>9.93±0.01b</td>
<td>6.14±0.02d</td>
<td>6.20±0.03c</td>
</tr>
</tbody>
</table>

Values were presented as mean±standard deviation of triplicate determination; values in the same row bearing the same superscript letters are not significantly different at 5 % level.

Table 2 Result of antinutrient Composition of Four Leafy Vegetables from Igirita Ali in Rivers State

<table>
<thead>
<tr>
<th>Antinutrient</th>
<th>Scent leaf</th>
<th>Bitter leaf</th>
<th>Water leaf</th>
<th>Pumpkin leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>3.64±0.25b</td>
<td>4.98±0.08a</td>
<td>0.37±0.03d</td>
<td>0.56±0.04c</td>
</tr>
<tr>
<td>Phytate</td>
<td>0.50±0.00a</td>
<td>0.34±0.03b</td>
<td>0.24±0.01c</td>
<td>0.22±0.01c</td>
</tr>
<tr>
<td>Oxalate</td>
<td>0.18±0.02b</td>
<td>0.85±0.09a</td>
<td>0.04±0.01c</td>
<td>0.10±0.00b,c</td>
</tr>
<tr>
<td>Sapouin</td>
<td>2.21±0.01b</td>
<td>4.85±0.02a</td>
<td>0.26±0.03c</td>
<td>0.21±0.01d</td>
</tr>
<tr>
<td>Tannin</td>
<td>0.08±0.01c</td>
<td>0.15±0.02b</td>
<td>0.40±0.01a</td>
<td>0.07±0.01c</td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>12.97±0.03a</td>
<td>10.32±0.02b</td>
<td>6.30±0.01c</td>
<td>6.27±0.03c</td>
</tr>
</tbody>
</table>

Values were presented as mean±standard deviation of triplicate determination; values in the same row bearing the same superscript letters are not significantly different at 5 % level.

These secondary metabolites are used by some plants for defense and protection (Beecher, 2003). They are beneficial chemicals and predator and parasite repelling effects. In humans and most animals, alkaloids and tannin have been observed to possess antimicrobial, antidiuretic and analgesic effects (Owoyele et al., 2002). However, they inhibits certain mammalian enzymatic actions such as those of phosphodiesterase; prolonging the actions of cyclic AMP. These antinutrients can induce actions of glucagon and thyroid stimulating hormone even when it is not needed (Okaka et al., 1992).

The concentration of phytate was non-toxic. Phytate (myo-inositol hexa phosphate) is an antinutrient that chelates mineral in the body and makes their bioavailability impossible. Phytates forms insoluble complexes with variety of minerals including calcium, zinc, iron, copper, manganese, selenium, magnesium thus reducing the availability of these nutrients (Golam et al., 2011). Phytate can also form complexes with basic residues of protein and therefore it may interfere with the activity of endogenous enzymes and digestibility of nutrients other than minerals (Golam et al., 2011). Cyanogenic glycoside in the vegetable irrespective of the sites ranged from 6.14±0.02 – 13.37±0.04. This is higher than 4.42±0.03 and 4.23±0.03 as reported by Ujowundu et al (2013) for Bambera groundnut and African bread fruit respectively. Cyanogenic glycoside is toxic and when hydrolysed releases hydrogen cyanide (HCN) which has been reported to cause marked
weight change (Aleto, 1993). They have ability of linking with metals (Fe$^{2+}$, Mn$^{2+}$ and Cu$^{2+}$) which are functional groups of many enzymes. HCN linkage with metals could inhibit processes like the reduction of oxygen in cytochrome respiratory chain electron transfer in photosynthesis and the activities of enzymes like catalase and oxidase (Cheeke, 1995, Mcmahon et al, 1995). Concentrations of anti-nutrients can be significantly reduced by boiling (Sidduraju et al, 1996). This is a major processing method of preparing vegetable.

Scent leaf and bitter leaf from non-polluted area contains appreciable levels of oxalate and saponins than those from polluted environment. Oxalate interferes with minerals availability particularly calcium. It binds with calcium and forms insoluble calcium oxalate which cannot be absorbed in the body. This may lead to death due to hypocalcimia in the renal tubules while saponin reduces the uptake of glucose and cholesterol at the gut through intra-lumenal physicochemical interactions (Price et al, 1987).

Pollution due to oil exploration and gas flaring has definitely affected the Niger Delta ecosystem. This assertion is supported by the significant changes observed in the photochemistry of plants studied. The plants may have been affected by atmospheric pollutants especially oxidants and acid soil due to acid rain which ultimately adversely affects the growth of plants. Percy (2002) reported that plants grown under enriched atmospheric CO$_2$ and volatile organic compounds (VOCs) typically showed anti-nutrient changes. It has been reported that certain anti-nutrient play important roles in antioxidant defense systems of plants (Ugochukwu and Babady, 2003). Pollution by gas flaring results in absorption of pollutions within cells or tissue of plants leading to generation of free radicals in plants growing in the surrounding environment (Baker, 1970). Thus it is expected that such plant will have low antioxidant defense compounds.

4. Conclusion

From the foregoing discussion, it can be inferred that the distribution of anti-nutrient factors (alkaloid, phytate, oxalate, Saponin, tannin and cyanogenic glycosides) in the leafy vegetables studies suggest that the anti-nutrient factors though endogenous are affected by environmental factors.

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Conflict of Interest

None
Reference


