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Quantitative Evaluation of Anti-nutritional Factors in Mango *(Magniferaindica)* Fruit

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***Abstract* – This work quantitatively evaluated the anti-nutritional factors in mango (*Magniferaindica*) fruit. The results of the analysis showed that mango fruit contained phytate(0.50%), alkaloid(0.40%), oxalate (0.0594mg/100g), saponin(0.20%), Tannin(1.75%), trypsin inhibitor(1.80%). These anti-nutrients amounts are lower than the toxic levels so mango fruit is not toxic to humans and hence its consumption is encouraged.**

***Keywords* – Alkaloids, Oxalate, Saponin, Tannin.**

I. INTRODUCTION

The present uncertainty in the world food supply and the expected increase infood demand warrant the search for alternative sources of food which will be readily available and affordable for all and sundry by government planners and scientists. One of such important sources of food is the mango (*Magnifieraindica*) fruit. It is important to state here that most people in South Eastern Nigeria and indeed the world at large consume Mango fruit directly or indirectly because of its taste and nutritional potentials. Scientists such as Fetuga et al., (1973), Aletor and Aladetimi, (1989) and Fowomola et al., (2007) had studied and described the anti-nutritional factors of plant fruits and seeds.

Anti-nutritional factors are those substances or chemical compounds found in fruits and food substances in general which are poisonous to humans or in some ways limit the nutrient availability to the body.

Anti-nutritional factors are present in different food substances in varying amounts depending on the kind of food, mode of its propagation, chemicals used in growing the crop as well as those chemicals used in storage and preservation of the food substances.These anti-nutritional factors are known to interfere with metabolic processes such that growth and bioavailability of nutrients are negatively influenced (Abara, 2003; Binta and Khetarpau, 1997). Examples of anti-nutritionals factors present in mango fruits are alkaloids, tannins, phytate, trypsin inhibitor, cyanide, saponins, oxalate e t c. Phytate and oxalates have the ability to form chelates with di- and tri-valentmetallic ions such as Cd, Mg, Zn, and Fe to form poorly soluble compounds that are not readily absorbed from the gastrointestinal tract thus reducing their bioavailability (Ademoroti, 1996).

Mango*(Magniferaindica)* belong to the genius *Angifera*, consisting of numerous species of tropical fruiting trees in the flowering plant family, *Anacardiaceae*. The mango is indigenous to the Indian sub-continent and South-East Asia, cultivated in many tropical regions and distributed widely in the world. It is one of the most extensively exploited fruits for food, juice, flavor, fragrance and colour and a common ingredient in new functional fruits often called super fruits. Its leaves are ritually used as a floral decoration at weddings and religious ceremonies (Fowomola, 2009). In Nigeria, people generally regard the nutritional values of the mango fruit and ignore the presence of harmful or anti- nutritional factors because of scarcity of information about these poisonous substances.This present study is aimed at providing this information.

II. PROCEDURE FOR ANALYSIS

The fresh mango fruits were collected from Nkpor community in Anambra State, Nigeria. They were quickly taken to the laboratory for digestion and analysis. The fruits were washed using distilled water, thenthe bark was peeled off. The fleshy part or mesocarp (which will be studied) was cut and pounded using a clean mortar. Varying masses of the sample were placed in different beakers or volumetric flasks (as required by the method for the test of the different anti-nutrients) and the analysis proper was commenced as outlined below.

A. Oxalate Determination by Titration Method: This determination involved three major steps: digestion, oxalate precipitation and permanganate titration.

Digestion

i) 2g of sample was suspended in 190ml of distilled water in a 250ml volumetric flask.
ii) 10ml of 6MHCl was added and the suspension digested at 100^0c for 1 hour.
iii) The solution was cooled, and then made up to 250ml mark before filtration.

OxalatePrecipitation

Duplicate Portions of the filtrate were measured into breakers and four drops of methyl red indicator added. Then NH_4OH solution was added (drop wise) until the test solution changed from pink to faints yellow colour (PH4.0-4.5). Each portion was then heated to 90^0C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 90^0C and 10ml of 5% $CaCl_2$solution was added while being stirred constantly. The solution was then heated and left overnight at 25^0C, it was then centrifuged at 2500rpm for 5minutes. The supernatant was decanted and the precipitate completely dissolved in 10ml of 20% (v/v) H_2S0_4 solution.

Permanganate Titration

At this point, the total filtrate resulting from digestion of 2g of sample was made up to 300ml. Aliquots of 125ml of the filtrate was heated until near boiling and then titrated against 0.05M standardized $KMnO_4$ solution to a faint

pink colour which persisted for 30 seconds. The calcium oxalate content was calculated using the formula:

$$\frac{T \times (Vme)\ (Df)}{(ME) \times M_F} \times (Mg/100g) \quad (1)$$

Where T is titre of $KMnO_4$ (ml),
Vme is the volume-mass equivalent
Df is the Dilution factor= Vt/A
Where Vt is the total volume of fitrate (300ml) and A is the aliquot used i.e. 250ml,
ME is the molar equivalent of $KMnO_4$ in oxalate and
Mf is the mass of sample used

B. Alkaloid determination

5g of the sample was weighed into a 250ml beaker and 200ml of 20% acetic acid in ethanol was added and covered and allowed to stand for 4hours at 25^0C. It was then filtered and the filtrate was concentrated using water-bath (memmert) to one –quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4oH solution. It was then filtered using a pre-weighed filter paper. The residue on the filter paper is the alkaloid and was dried in the precision oven at 80^0C. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analyzed, thus:

% Weight of alkaloid =

$$\frac{\text{Weight of filer paper with residue} - \text{wt of filter paper}}{\text{Weight of sample analyzed}} \times 100 \quad (2)$$

C. Determination of Saponin

5g of the sample was put into 20% acetic acid in ethanol and allowed to stand in weterbath at 50^0C for 24hours. This was filtered and the extract was concentrated using a waterbath to one-quarter of the original volume. Concentrated NH_4OH was added drop-wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed. The saponin content was weighed and calculated in percentage of sample analyzed.

% Saponin content =

$$\frac{(\text{Weight of filter paper + residue}) - (\text{wt of filter paper})}{\text{Weight of sample analyzed}} \times 100 \quad (3)$$

D. Tannin Determination by Titration

The Follins Dennis titrating method as described by Pearson (1974) was used. To 20g of the crushed sample in a conical flask was added 100mls of petroleum ether and covered for 24hours. The sample was then filtered and allowed to stand for 15 minutes so that the petroleum ether evaporated. It was re-extracted by soaking in 100ml of 10% acetic acid in ethanol for 4 hours. The sample was then filtered and the filtrate collected. 25ml of NH_4OH were added to the filtrate to precipitate the alkaloids. The alkaloids were heated with electric hot plate to remove some NH_4OH still in solution. The remaining volume was measured. 5ml of this volume was taken and 20ml of ethanol was added to it. It was titrated with O.IM NaOH using phenolphthalein as indicator until pink end point is reached. Tannin content was then calculated in percentage ($C_1V_1 = C_2V_2$) molarity of the sample analysed.

Calculation:

$C_1V_1 = C_2V_2$

$$C_1 = \frac{C_2V_2}{V_1}$$

$$\% \text{ of tannic acid content} = \frac{C_1 \times 100}{\text{Weight of sample analyze}} \quad (4)$$

Where
C_1 = Conc. Of Tannic acid
C_2 = Conc. Of Base
V_1 = volume of Tannic acid
V_2 = volume of Base used.

E. Phytate Determination

The method used was the Young and Greaves method (1940) as adopted by Lucas Markakes (1975). 0.2g of the sample was weighed into 250ml conical flask. It was soaked in 100ml of 20% concentrated HCl for 3 hours, the sample was then filtered 50ml of the filtrate was placed in a 250ml beaker and 100ml distilled water added to the sample. Then 10ml of 0.3% ammonium thiocyanate solution was added as indicator and titrated with standard iron (III) chloride solution which contained 0.00195g iron per 1 ml.

Calculation:

$$\text{Phytic acid} = \frac{\text{Titre value} \times 0.001\ 95 \times 1.19 \times 100}{2} \quad (5)$$

F. Determination Of Trypsin Inhibitor

0.5g of sample was extracted with 50ml of distilled water for 30minutes with mechanical shaking at a speed of 200rpm. 10ml of the sample suspension was then destabilized by adding an equal volume of assay buffer and vigorously shaking for 2-3 minutes before filtering through a What No.2 filter paper. The filtrate was then further diluted with water to the point where 1ml gave 30-79% trypsin inhibitor. This was done to keep the relative standard deviation (RSD) of trypsin inhibitor activity (TIA) measured within $\pm$ 3.5%. A suitable final concentration of the sample was around 0.1mg of the sample per ml (0.1mg sample/ml diluted extract), and for heated sample, it is 0.5-1.5mg/ml.

Procedure:

The reaction was run at 37^oC. Exactly 10minutes after adding the trypsin solution, the reaction was stopped by injecting 0.5ml of 30% acetic acid solution with 1-ml syringe. The absorbance at A^S_{410} (Sample reading), was a measure of the trypsin activity in the presence of the sample inhibitors. The reaction was also run in the absence of inhibitors by replacing the sample with 1ml of water. The corresponding absorbance was symbolized as A^r_{410} (reference reading). Distilled water was then used as a blank.

Calculation:

Defining a trypsin unit as an A_{410} increase of 0.01 under the conditions of the assay, the trypsin inhibitory activity is expressed in Trypsin units inhibited (TUI) Per milligram of the sample and calculated as follows:

$$\text{TUI/mg sample} = \frac{(A_{410}^{r} - A_{410}^{S}) \times 100/\text{ml diluted extract}}{(\text{mg sample/ml diluted extract})} \quad (6)$$

This Trypsin Inhibitory Activity expressed in terms of trypsin units inhibited (TUI) is a measure of the amount of trypsin inhibitor present in any given sample.

III. Results and Discussion

Table 1: The results of anti-nutritional analysis of mango fruits

Anti-nutrient	Amount
Phytate	0.50%
Alkaloid	0.40%
Oxalate	0.0594mg/100g
Saponin	0.20%
Tannin	1.75%
Trypsin inhibitor	1.80%

Table1 indicates that mango contains phytate (0.50%), alkaloid (0.40%) oxalate(0.0594mg/100g), Saponin (0.20%), Tannin (1.75%), and Trypsin inhibitor (1.80%). This shows that the anti-nutrient in the highest amount is trypsin inhibitor (1.80%) followed closely by Tannin (1.75%), then phytate, alkaloid, Saponin and the anti nutrient present in the lowest amount is oxalate.

Tannins are aromatic compounds containing phenolic groups. They interact with salivary proteins and glycoproteins in the mouth and render the tissues astringent to taste (Howes,1953). Astringency gives tannin the medicinal value in preventing diarrhea and dysentery and for controlling hemorrhage (Sollman, 1957). They also protect plants against dehydration and rotting. When tannins polymerize, they form a protective barrier against microbial attack (Stumpf and Conn, 1981).

Phytate depresses the growth of chicks fed with phytate-casein diet by forming complex with zinc making the latex unavailable (Savage et al, 1964). Phytate form complex with protein by actions of cations e.g. Zn, Ca or Mg thereby reducing the bio-availability such minerals (Omosaiye and Cheryan, 1979).

Alkaloids cause gastro-intestinal upset and neurological disorders especially when taken in excess (Osagie, 1988). Trypsin inhibitors form complexes with trypsin so reduce its proteolytic activity which in turn reduce availability of amino acid and reduce growth (Liener, 1989).In effect, the low levels of anti-nutrients observed in mango fruit suggest that it is less toxic and can be incorporated into our daily food and also in live-stock feed without any adverse effect.

IV. Conclusion

Based on the results of this research work and the subsequent discussion, it has been revealed that the mango fruit actually contains anti-nutritional factors but the amounts present are below toxic levels. Hence mango fruit is good for human consumption and its anti-nutrients levels do not have any adverse effect on the human health.

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