INTERNATIONAL JOURNAL OF NATURAL AND APPLIED SCIENCES (IJNAS),VOL. 4, NOS.1& 2 (2009); P. 1 – 10, 4 TABLES

Determination of the effect of *Telfairia mosaic virus* on vitamins and amino acids profile of two ecotypes of *Telfairia occidentalis* (fluted pumpkin)

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ABSTRACT

The vitamins and amino acids profile of *Telfairia occidentalis* inoculated with *Telfairia mosaic virus* (TeMV) from Cross River State (Ecotype A) and Akwa Ibom State (Ecotype B) were investigated. Freshly harvested leaves from symptomatic and healthy plants were oven-dried at 65^0C to a constant weight and milled into powder. The milled samples were analyzed for the effect of the virus on vitamins and amino acids profile. The mean values obtained for healthy samples of ecotype A and B ranged from 0.74 to 15.01mg/100g and 0.83 to 18.10mg/100g respectively. Corresponding values for infected samples ranged from 0.68 to 12.4mg/100g and 0.61 to 13.00mg/100g. Ecotype B recorded higher percentage difference of 76% as against 36.8% for ecotype A. Infection of TeMV on amino acids produced significant decreases in infected samples of ecotype A and B with a range in mean value of 0.76 to 13.17g/16N and 0.79 to 12.81g/16N respectively. Associated values for healthy samples ranged from 1.50 to 10.65g/16N and 0.90 to 11.17g/16N marked increase in non-essential amino acids in infected samples was recorded for glutamic acid, aspartic acid, alanine and proline in both ecotype studied. TeMV produced marked decreases in the nutrients investigated.

INTRODUCTION

The plant, fluted pumpkin *(Telfaria occidentalis)* which belong to the family *Cucurbitacea* is a creeping or climbing vegetative shrub that spread low across the ground with large lobed leaves, and long twisting tendrils (Horsfall and Spiff, 2005). If given support, its stem can grow up to a length of 10m. It is widely cultivated as garden and farm vegetable, it is a native of West Africa, growing in humid tropical climate in well drained soils. Although the farmers grow it in farms as annual crops because of the shifting cultivation system, the plant behave like a perennial plant and can grow for many years if there is enough moisture in the soil (Enwere, 1995). The leaves of fluted pumpkin are important vegetable for the people of the South Eastern and Midwestern states of Nigeria (Oguntona, 1998). The nutritional interest in this vegetable stems from its rich contents of essential amino acids, vitamins and minerals (Fasuyi and Aletor, 2005). The leaves of the plant are important articles of trade throughout Nigeria and they are highly cherished vegetables in popular local traditional dishes such as Edikang Ikong soup of the Ibibios and Efiks. As a result, large hectares of land are under cultivation of *Telfairia occidentalis* to meet the nutritional needs of rural and urban dwellers.

The production of this vegetable is reported to be limited by a number of diseases, the most important of which are viruses (Anno-Nyako. 1988). Three viruses, inducing mosaic and mosaic-like sumptoms, have been reported on fluted pumpkin in Nigeria. These include a Y-strain of *Cucumber mosaic virus* (CMV-Y) (Atiri, 1985), a strain of *Pepper veinal mottle virus* (PVMV-TYVC) Atiri, (1986); Shoyinka and Thottappilly, (1998) and *Telfairia mosaic virus* (TeMV) (Nwauzo and Brown, 1975; Shoyinka et al; 1987). Anno-Nyako, (1988) reported the virus to be seed-brone in *T. occidentalis*.

Nwauzo and Brown (1975), Atiri (1975, 1986) and Shoyinka et al; (1987) centered their studies on the characterization and identification of TeMV causing mosaic diseases of *T. occidentalis*. Ossom, (1986), Akwaowo et al; (2000) reported on mineral and antioxidant contents in healthy leaves of fluted pumpkin. However, no information on other vitamins and amino acids exists on the effect of TeMV infection on ecotypes of *T. occidentalis*. The present study examines the effect of the virus on vitamins and amino acids profile of two ecotypes of *T. occidentalis*.

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MATERIALS AND METHODS

Collection of seeds and planting

Fruits of *T. occidentalis* from which seeds were obtained were purchased from local farmers in two localities. The two localities are;

1. Akparabong Town in Ikom Local Government Area (LGA) of Cross River State (CRS), plants from this zone represent ecotype A. This is a derived savanna zone.
2. Eket Municipality, Eket LGA of Akwa Ibom State, plants from this zone represent ecotype B. This is a rainforest zone.

The seeds of both ecotypes were removed and sorted in order to select seeds of uniform size. They were sun-dried for two days to enhance germinability and thereafter sown in steam-sterilized garden soil in 16cm diameter polyethylene bags. The seed germinated 14 days after planting and the germinated seeds (seedlings were staked to promote, adequate leaf production.

Virus sources, preparation of inoculum and virus propagation

The virus used in this study was supplied by Dr. H. J., Vetten of the Federal Biological Research Centre for Agriculture and Forestry, (BBA) Braunscheig, Germany. Infected dried lead material stored under liquid nitrogen. The virus was re-activated by triturating the leaf tissues in pre-sterilized cold pestle and mortar in sodium phosphate (Na_2HPO_4) buffer 0.03M, pH 8.0. The inoculum was applied by the conventional leaf-rub method (mechanical or sap inoculation) with cotton swab onto Nicotiana benthamiana, pre-dusted with carborundum (800mesh). The inoculated leaves were then rinsed with water and kept for symptom development. Subsequent inoculations using the sap transmission method were carried out on *T. occidentalis* in order to propagate and maintain the virus under greenhouse condition at 25 ± 3^0C.

Experimental design and inoculation of experimental plants

Test plants from the localities were arranged in two groups each containing 30 plants each which were set out in three rows or ten plants. Prior to inoculation, plants to be inoculated with the virus and those of controls in both groups were kept in a randomized complete block design in the greenhouse with average temperature of 25 ± 3^0C. The inoculation of the test plants was as described above. Within each plant groups, fifteen were inoculated with the virus and the remaining fifteen inoculated with buffer only to serve as controls. The set up was monitored for symptom expression (8-10 years post-inoculation) which included mosaic, severe leaf malformation and distortion, characteristics of TeMV infected *T. occidentalis*.

Vitamin analysis

Determination of vitamin A by spectrophotometry

Vitamin A content of the samples was determined spectrophotometrically using the hexane method. Five grams of sample was saponified with a mixture of water, 3A alcohol (methanol and absolute ethanol) at a ratio of 1:19 and 75% potassium hydroxide in amber saponification flasks. The saponified material after cooling was diluted and hexane was added and spurned on a magnetic stirrer (Type – OP 912/3, Hungary) after which it was allowed to stand for layer separation. Aliquot of the upper layer (hexane layer) was made up with isopropanol and absorbance was read at 325nm against isopropanol blank and concentration of the vitamin A content was calculated from the readings.

Determination of vitamin C content (reduced ascorbic acid)

The modified method of Bessey (1944) was used. To 2gm of dried sample was added 150ml of metaphosphoricacetic acid (w/v) (prepared by dissolving 5g metaphosphoric acid in 10ml glacial acetic acid) and made up to 450ml by the addition of distilled water and filtered. 10 to 100ml aliquot of this was titrated with standard indophenol solution (prepared by dissolving 42mg Na_2CO_3 and 52mg sodium – 2,.6 – dichlorophenol in 100ml of water and stored in the refrigerator. One hundred (100) grams of crystalline ascorbic acid was dissolved in 100ml of metaphosphoric acid solution (w/v) and titrated with the indophenol solution until a pink colour persisted for about 5 min. The strength of the indophenol solution was calculated and expressed as mg of ascorbic acid equivalent per ml of reagent. The ascorbic acid content was calculated using the formula: VxSxD.

where:

V = ml of dye used to titrate unknown

S = standardization value expressed in mg ascorbic acid per ml.

D = dilution factor.

Determination of thiamine (Vit B_1) (AOAC,1995)

Reagents used include the following: Anhydrous sodium sulphate, 15.0g of NaOH (15% (w/v sodium hydroxide), 1.0g of $K_3Fe(CN)_6$ (1.0% w/v) were dissolved in 100ml of distilled water. The reagent was stored in a brown bottle, 3ml of 1% $K_3Fe(CN)_6$ solution (Alkaline potassium fericyanide) were diluted to 100ml with 15% NaOH solution. This was diluted to 1.0 litre distilled water,8.5ml of concentrated HCl (specific gravity 1.8) (0.1N hydrochloric acid solution),2.8ml of concentrared H_2SO_4 (0.1N sulphuric acid solution), 205g of anhydrous sodium acetate NaC_2 H_3O_2. $3H_2O_2$ (2.5M solution), isobutyl alcohol (B.D.H), 6.og of diastase were suspended in 100ml of 2.5M sodium acetate buffer. The mixture was thoroughly

shaken before use, 8.5ml of KCl of concentrated HCl (Acid 25% (w/v) potassium chloride solution) were diluted to 1 litre with 25% KCl solution, 250g of KCl, activated lacalso: 200g of 14-52 mesh amberlite resin were pounded vigorously in a mortar and filtered through a 60 mesh sieve. 1oog were transferred into a Buchner funnel and 225ml of hot 3% acetic acid were added and allowed to remain in contact with Decalso for 10-15 minutes. A mild suction was then applied to remove the fluid. The washing was repeated twice with hot acetic acid solution. The remaining KCl was flushed by washing the Decalso with 50ml portions of hot water, until it was chloride free (tested with drops of 1% with $AgNO_3$ solution).

Thiamine, hydrochloride, crystals were dried over P_2O_5 in a descicator for 24 hours. 100mg of the dried thiamine were dissolved in 25% (w/v) ethanol and diluted to 1 litre with the same reagent. The solution was stored in a dark brown bottle in a refrigerator and used as stock solution. 5.0ml of the stock solution were diluted to distilled water (Intermidiate thiamine solution).

Working thiamine solution was made of 4ml of the intermediate thiamine solution and transferred into a 100ml flask containing 75ml of 0.1N H_2SO_4 and 5ml sodium acetate.The solution was finally diluted to 100ml with distilled water, 100mg of quinine sulphate were dissolved in 1 litre of 0.1N H_2SO_4 to give a final concentration of 0.3ug/litre.,95% ethanol(B.D.H.,England) and 30ml of glacial acetic acid (3% v/v) acetic acid solution) all of these were diluted to 1 litre with distilled water.

Procedure for extraction, 0.5g of sample were weighed accurately into an Erlenmeyer flask containing 75ml of 0.1N HCl. The contents were heated in a constant boiling water bath for 30 minutes. The extract was cooled to 50^0C and 5ml of freshly prepared diastase enzyme suspension were added and incubated for 12 hours at 45^0C. The diluent was cooled to room temperature and diluted with water to 100ml. The solid material which remained after enzyme digestion was separated from the extract by centrifugation.

For purification, the chromatography column was filled with water. 5.0g of activated resin was allowed to settle in the column under gravity. The water was allowed to drain until the level of water was just above the top of the resin. 25ml of the sample extract were transferred into the column and allowed to pass through the resin at the rate of 2ml/minute. The adsorption column was washed thrice with 10ml portions of hot water and all the washings were discarded. 10ml of acid KCl were poured into the column and eluate was collected in a 25ml volumetric flask. The contents of the flask were made to 25ml with the acid KCl. 25ml of the eorking thiamine solution containing 0.2ug/ml were applied to the column and the above two steps repeated.

Convention to thiochrome started with the transfer of 5ml of the eluate into a separating funnel, 3ml of alkaline ferricanide solution were added, mixed gently, then 15ml of isobutyl alcohol added. The mixture was shaken vigorously for 90 seconds. The aqueous layer (lower) was discarded. 2-3g of anhydrous Na_2SO_4 were added into the alcohol solution. The mixture was shaken for 30 seconds and allowed to stand until it was clear and colourless.

For measure of thiochrome, the clear isobutyl alcohol extracts were decanted into separate cuvetts.Using the working quinine sulphate solution, the spectrofluorimeter was adjusted to 1.00 on the digital scale and pure isobutanol was used to set the reading to zero. A fresh working standard quinine sulphate solution was used as a check in between reading.

For the blank, 5ml of acid – KCl eluate was transferred into a separating funnel and treated in the same way as the sample extract except that 15% of NaOH was added instead of the alkaline ferricyanide. The blank and sample extract were run simultaneously. Under this condition, the blank provides an assay solution which include thiochrome. This eliminates the fluorescence of non thiamine compounds.

The thiamine content of the sample was calculated as follows:

$$\frac{U-UB}{S-SB} \; x \; \frac{25}{V} \; x \; \frac{100}{wt\ of\ sample}$$

where:

U	=	reading of sample
UB	=	reading of sampl blank
S	=	reading of standard thiamine
SB	=	reading of standard thiamine blank
V	=	volume of solution applied to column.

The value was converted to ug/100ml and then to mg/100mg of sample.

Determination of riboflavin (Vit B_2) flourimetric method (A.O.A.C,1995)

Reagents used included the following : 850ml of concentrated HCl (10N HCl), 100ml of 10N HCl (1N HCl) , 100ml of 1N HCl (0.1N HCl), 400g of NaOH (10N NaOH) pellets, and 100ml of 10N NaOH (1N NaOH) were all diluted to 1 litre with distilled water.

Precipitating of interfering impurities,done by autoclaving. After this, the flask was cooled to room temperature and the pH of the extract was adjusted to 6.0, using 1N NaOH and a pH meter. The extract was stirred continuously during the addition of the alkali to prevent local high concentration of NaOH which destroy the riboflavin.After adjusting the pHto 6.0, it was quickly readjusted to pH 4.5 using 1N HCl and pH meter. The extract was then diluted to 100ml with distilled water and centrifuged. To a 50ml aliquot of the supernatant obtained by centrifugation 0.1N HCl was added dropwise

until no precipitate was formed. This was filtered through a whatman filter paper (No.12) and diluted to 100ml with distilled water.

Accidification of extract was done using 10ml of the filtrate and 1ml of water pipetted into a test tube marked A and B and mixed thoroughly. Another 10ml portion of the sample filtrate and 1ml of riboflavin working standered (0.5µg/ml) were pipetted into a second test tube marked Band mixed thoroughly. 1.0ml of glacial acetic acid was added to each of the tubes A and B.

Oxidation of interfering impurities was carried out by adding 0.5ml of 3% $KMnO_4$ to each of the test tubes A and B, mixed allowed for 2 minutes. 0.5ml of 3% H_2O_2 was added and mixed thoroughly. The dark coloration disappeared within a few seconds.

To ensure that the fluorimeter gave a linear response, the following concentrations of riboflavin were used; 0.1, 0.2, 0.3, 0.4, and 0.5/u/mg. The flourescence of the samples was measured at 440nm.The fluorescence of the extracts in tubes A and B were measured and readings noted as reading A and B respectively. About 20mg of $Na_2S_2O_4$ were added to test tube A only and the fluorescence was noted as reading C.

The riboflavin contents of the sample was calculated from the formula given below:

$$ug\ riboflavin/g = \frac{A-c}{B-C}\ x\ \frac{riboflavin\ increment}{10}\ x\ \frac{dilution\ \ factor}{sample\ wt\ (g)}$$

where:

A = Fluorescence of the extractin tube A

B = Flourescence of the extract in test tube B containing 0.5ug standard riboflavin

C = Flourescence of the extract in test tube A after addition of Na_2SO_4. Added riboflavin =0.5ug 0.5ug riboflavin were added as internal standard of test B.

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Determination of nicotinamide (Spectrophotometric method)

0.3% kH_2PO_4 solution was added in equal mL to 2mg of niacinamide. The mixture was shaken to disperse and heated 15 min in boiling water bath or in autoclave at 15lb (121) pressure. This was diluted to Ca 5µg/mL with 0.3% kH_2PO_4 solution and filtered.

Separate blank for each test sample was prepared by replacing CNBr with H_2O. To 1mL working standard solution or assay solution in spectrophotometer tube, was added 0.5mL CNBr solution, mixed, stoppered and allowed to stand for 25-30min. 10mL barbituric acid solution was also added and swirled.

The spectrophotometer was set to zero absorbance at 550nm with appropriate blank in which CNBr was replaced with H_2O. Readings of reaction product A at maximum color development were taken.

Niacinamide in original weight of samples taken:

$$\text{Weight (mg)} = \frac{A \times 5 \times dilution\ \ factor}{A' \times 1000}$$

where A and A' refer to test and standard solution, respectively and 5 = µg niacinamide / mL working standard solution.

Determination of Amino Acid Profile

The amino acid profile in *T. occidentalis* ecotypes was determined using methods described by Spackman et al (1958). *T. occidentalis* samples were dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon sequential Multi-sample Amino Acid Analyzer (TSM).

Defatting sample

A known weight of the dried sample was weighed into extraction thimbie and the fat was extracted with chloroform/methanol (2:1 mixture) using soxhlet extraction apparatus as described by AOAC (2006). The extraction lasted 15 hours.

Nitrogen Determination

A small amount (200mg) of ground sample was weighed wrapped in whatman filter paper (No.1) and put in a Kjeldhal digestion flask. Concentrated sulphuric acid 910ml) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 were added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.

The flask was then put on Kjedhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (10ml) of the diluted solution with 10ml of 45% sodium hydroxide were put into the Markhlam distillation apparatus and distilled into 10ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70ml of distilled was collected.

The distillate was then titrated with standardized 0.01N hydrocholirc acid to grey coloured end point. The percentage nitrogen in the original sample was calculated using the formula:

$$\text{Percentage nitrogen} = \frac{(a-b) \times 0.01 \times 14 \times 100}{W \times C}$$

where:

a. = Titre value of the digested sample

b = Titre value of blank sample

v = Volume after dilution (100ml)

w = Weight of dried sample (mg)

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C = Aliquot of the sample used (10ml)

14mg = Nigtrogen constant in mg

100 = Conversion factor to percentage

Hydrolysis of the sample

A known weight of the defatted sample was weighed into glass ampoule. 7ml of 6NHCL was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis e.g. methionine and cystine). The glass ampoule was then sealed with Bensen burner flame and put in an oven preset at $105^0C \pm 5^0C$ for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humans.

The filtrate was then evaporated to dryness at 40^0C under vacuum in a rotary evaporator. The residue was dissolved with 5ml to acetate buffer (pH2.0) and stored in plastic specimen bottles, which were kept in the freezer.

Loading of the hydrolysate into the TSM analyzer

The amount loaded was between 5 to 10 microlitre. This was dispense into the cartridge of the analyzer. The TSM analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of an analysis lasted for 76 minutes.

Method of calculating amino acid values from the chromagram peaks

The net height of each peak produced by the chart recorded of TSM (each representing an amino acid) was measured. The half-height of the peak on the chart was found and width of the peak on the half-height was accurately measured and recorded. Approximate area of each peak was then obtained by multiplying the height with the width at half-height.

The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

$$NE = \frac{Area\ of\ norleucine\ peak}{Area\ of\ each\ amino\ acid}$$

A constant S was calculated for each amino acid in the standard mixture:

S_{Std} = NE_{Std} x mol. Weight x μMAA_{Std}

Finally, the amount of each amino acid present in the sample was calculated in g/16N or g/100g protein using the following formula:

Concentration (g/100g protein) = $NH\ x\ W@HN/2\ x\ S_{std}\ x\ C$

$$C = \frac{Dilution \times 16}{Sample\ wt(g) \times N\%\ 10 \times vol.\ Loaded} \times NH \times W\ (nleu)$$

where: NH = Net height

W = Width @ half height

nleu = Norleucine

Data analysis

The data obtained were analyzed using the student t-test at 95% confidence limited. Values presented were means of three determinations.

RESULTS

The results of the effects of *Telfairia mosaic virus* (TeMV) on vitamins composition of *Telfairia occidentalis (T. occidentalis)* ecotype A and B are presented in table 1 and 2. Vitamin A, B_1, C and nicotinamide were significantly reduced while B_2 was marginally reduced by the virus. The mean values obtained for healthy samples of ecotype A were 1.90 ± 0.05 (iv), 3.47 ± 0.01, 15.0 ± 0.28 and 0.90 ± 0.05(mg/100g) respectively while corresponding values for infected samples were 1.20 ± 0.01 (iv), 2.75 ± 0.03, 12.45 ± 0.45 and 0.78 ± 0.013(mg/100g). Percentage difference in composition produced by the virus ranged from 8.1% for riboflavin to 36.8% for vitamin A. Ecotype B recorded higher significant reduction in vitamins engendered by the virus. All the vitamins were significantly reduced in the following order for healthy samples; Vitamin B_1, A, C, B_2 and nicotinamide. The mean values obtained were 3.78 ± 0.013, 2.10 ± 0.02 (iv), 18.10 ± 0.2, 0.,83 ± 0.02 and 0.91 ± 0.02(mg/100g) respectively with associated values for infected samples as follows 0.90 ± 0.03, 1.30 ± 0.06(iv), 13.0 ± 0.17, 0.61 ± 0.02 and 0.72 ± 0.03(mg/100g). Values for percentage difference ranged from 20.8% for nicotinamide to 76.2% for thiamine. Ecotype B was more affected by the virus than A.

Result in table 3 and 4 highlights the effect of TeMV on amino acids profile of *T. occidentalis* ecotypes. Results show that TeMV infection significantly reduced (P = 0.05, 0.01) amino acids profile in infected samples of both ecotypes compared to the healthy with exception of glutaminc acid, aspartic acid, alanine and proline which had significantly higher values in infected samples compared to the healthy. Levels of reductions produced by the virus were higher in ecotype A than compared to ecotype B. The mean values obtained for healthy samples of ecotype A ranged from the highest value 10.65 ± 0.03 for glutamic acid to lowest value of 1.05 ± 0.1g/100gN for methionine respectively with corresponding values for infected sample of 13.17 ± 0.06 for glutamic acid and 0.79 ± 0.13g/100gN. Percentage reductions in amino acids recorded ranged from 5.92% for leucine to 27.61% for cystine. The mean value for healthy samples of

ecotype B ranged from the highest value of 11.17 ± 0.1 for glutamic acid to the lowest value of 0.90 ± 0.06g/100gN for cystine. Associated value for infected sample ranged from 12.81 ± 0.06 for glutamic acid to 0.79 ± 0.1g/100gN for cystine. Values for percentage reductions ranged from 2.74% for leucine to 24.8% for methionine (Table 4).

Table 1, Effect of TeMV on vitamin composition of *Telfairia occidentalis* ecotype A

Vitamins (unit)	**Healthy**	**Infected**	**Percentage difference**
Vitamin A (iv)	1.90 ± 0.05	1.20 ± 0.01**	36.8
Thiamine Vit.B_1 (Mg/100g)	3.47 ± 0.01	2.75 ± 0.03*	20.7
Riboflavin Vit.B_2 (Mg/100g)	0.74 ± 0.06	0.86 ± 0.01	8.1
Noctinamide (Mg/100g)	0.90 ± 0.05	0.78 ± 0.013*	13.3
Ascorbic Acid Vit.C (Mg/100g)	15.0 ± 0.28	12.4 ± 0.45*	17.3

Values are Mean ± SD, n = 3 replicates

P = 0.05, 0.01

Percentage difference was obtained by expressing the difference between the value for healthy and infected samples as a percentage of the healthy

Table 2. Effect of TeMV on vitamin composition of *Telfairia occidentalis* ecotype B

Vitamins (unit)	**Healthy**	**Infected**	**Percentage difference**
Vitamin A (iv)	2.10 ± 0.02	1.30 ± 0.06**	38.0
Thiamine Vit.B_1 (Mg/100g)	3.78 ± 0.02	0.90 ± 0.03**	76.2
Riboflavin Vit.B_2 (Mg/100g)	0.83 ± 0.02	0.61 ± 0.02*	26.5
Noctinamide (Mg/100g)	0.91 ± 0.02	0.72 ± 0.03*	20.8
Ascorbic Acid Vit.C (Mg/100g)	18.10 ± 0.2	13.0 ± 0.17*	28.2

Values are Mean ± SD, n = 3 replicates P = 0.05, 0.01

Percentage difference was obtained by expressing the difference between the value for healthy and infected samples as a percentage of the healthy.

Table 3. Effect of TeMV on amino acids profile of *Telfairia occidentalis* ecotype A

Amino acids	g/100g protein or g/100gN		Percentage difference (%)
	Healthy	Infected	
Lysine	4.91 ± 0.1	4.35 ± 0.06	11.41
Histidine	2.32 ± 0.1	2.01 ± 0.3**	13.36
Arginine	5.28 ± 0.1	4.35 ± 0.06*	17.61
Aspartic acid	8.91 ± 0.13	9.67 ± 0.06*	8.53
Threonine	3.44 ± 0.06	2.80 ± 0.13**	18.60
Serine	3.15 ± 0.13	2.90 ± 0.06	7.94
Glutamic acid	10.65 ± 0.03	13.17± 0.06**	23.66
Proline	3.01 ± 0.06	3.29 ± 0.06	9.30
Glycine	4.25 ± 0.1	3.90 ± 0.06**	8.24
Alanine	3.26 ± 0.1	4.16 ± 0.1**	27.61
Cystine	1.10 ± -.06	0.80 ± 0.06	27.27
Valine	5.17 ± 0.1	4.06 ± 0.16	21.47
Methionine	1.05 ± 0.1	0.79 ± 0.13	24.76
Isoleucine	3.68 ± 0.1	3.19 ± 0.1*	13.55
Leucine	6.72 ± 0.1	7.12 ± 0.13*	5.92
Tyrosine	3.30 ± 0.03	3.04 ± 0.1*	7.88
Phenylalanine	4.40 ± 0.33	3.79 ± 0.03	6.18

Values are Mean ± SD, n = 3 determinations. P = 0.05, 0.01

Percentage difference was obtained by expressing the difference between the value for healthy and infected samples as a percentage of the healthy.

Table 4. Effect of TeMV on amino acids profile of *Telfairia occidentalis* ecotype B

Amino acids	g/100g protein or g/100gN		Percentage difference (%)
	Healthy	Infected	
Lysine	5.26 ± 0.06	4.30 ± 0.06**	18.25
Histidine	2.51 ± 0.06	2.38 ± 0.06*	5.18
Arginine	5.62 ± 0.03	5.02 ± 0.06*	10.68
Aspartic acid	9.61 ± 0.06	10.47 ± 0.06*	8.95
Threonine	4.06 ± 0.1	3.66 ± 0.06*	9.85
Serine	2.61 ± 0.03	2.48 ± 0.06*	4.98
Glutamic acid	11.17 ± 0.1	12.81 ± 0.06*	12.80
Proline	2.91 ± 0.06	3.29 ± 0.13	11.55
Glycine	4.62 ± 0.06	4.04 ± 0.06	12.55
Alanine	3.96 ± 0.1	4.86 ± 0.06**	18.52
Cystine	0.90 ± 0.06	0.79 ± 0.1*	12.2
Valine	4.51 ± 0.1	4.01 ± 0.13	11.09
Methionine	1.25 ± 0.06	0.94 ± 0.1*	24.8
Isoleucine	4.03 ± 0.2	3.66 ± 0.06	9.18
Leucine	8.02 ± 0.1	7.80 ± 0.06	2.74
Tyrosine	3.05 ± 0.13	2.90 ± 0.,06	4.92
Phenylalanine	3.96 ± 0.26	3.47 ± 0.13	12.37

Values are Mean ± SD, n = 3 determinations P = 0.05, 0.01

Percentage difference was obtained by expressing the difference between the value for healthy and infected samples as a percentage of the healthy.

DISCUSSION

Vegetables are very important food and highly beneficial for the maintenance of good health and prevention of diseases (Ayto, 1993) and good health is the one free from diseases (Schwartz, 2006). Some leaves including the leaves of *T. occidentalis* can be beneficially used in heart diseases, hypertension, hypoglycemia, diabetes and even in fatal cases of meningitis. They have been effective in lowering blood cholesterol and preventing blood clothing (Weiner, 1992). Leafy vegetables are of great importance to the health of individuals and communities.

The results obtained from the study showed high significant reductions (P = 0.05) in vitamins A, B_1, nicotinamide and C posed by TeMV in both ecotypes. This significant reductions in quantities of vitamins A and C resulting from infection of *T. occidentalis* by the virus correspond with previous work .The effect of TeMV appeared to be more in plants of ecotype B than A. The decrease in vitamin composition in infected plants obtained maybe due to their utilization by the pathogen for its metabolism during pathogenesis (Goodman et al; 1986). These micronutrients, though required in much smaller amounts, are essential for proper nutrition. Results of their deficiencies are very great (Estelle and Karen, 1999).

Infection of *T. occidentalis* by TeMV produced significant decreases (P = 0.05) in essential and non essential amino acids in both ecotypes studied. Fraser, (1987) estimated that TMV infection reduced host protein synthesis by up to 75% during the period of virus replication. A reduction in the amount of the most abundant host protein-ribulose bisphophate carboxylase-oxygenase (ribcs or rubisco) is one of the commonest effects of viruses that cause mosaic and yellowing diseases by TeMV (White and Brakke, 1983). Decrease in amino acid may be due to their utilization by the virus and enhanced enzymes activities in leaves. Marked increase in non essential amino acids was recorded for glutamic acid, aspartic acid, alanine and proline in infected plant samples of both ecotype corresponded to an increase in one or both of the amides, glutamine and asparagines (Hull, 2002). The amino acid, pipecolic acid, has been reported to occur in relatively high concentrations in several virus-infected tissues. This increase in amino acids which are protein constituents in infected samples is due to the fact that the coat protein of a virus (TeMV) can come to represent about half the total protein in diseased

leaf (Matthew, 1991). In many diseases, increase in metabolic activities is accompanied by marked increased in nucleic acid and protein synthesis. This agreed with previous work by Uritani (1971) who reported that diseased leaves have 24:13 percent higher protein content than healthy leaves. This increase may also be due to the pathogen or to newly synthesized proteins in host (Hull, 2002).

High significant reductions in vitamins, essential and non-essential amino acids produced by TeMV in ecotypes of *T. occidentalis* studied present a threat to the health of consumers. For each of these vitamins, specific deficiency symptoms occur when the vitamin composition is low or lacking in the diet. Many vitamins play roles as coenzymes in many metabolic pathways in the body, while others are directly involved in the synthesis of indispensable compounds (Estelle and Karen, 1999).

Vitamin A is involved in the formation of visual pigments, necessary for the maintenance of epithelical tissue. It deficiency causes night blindness and xerophthalmia. Thiamine ($Vit.B_1$) is involved in the metabolic breakdown of carbohydrates. Because of its central role in metabolism, the symptoms of thiamine deficiency are profound: fatigue, depression, mental confusion, cramping, burning and numbers in the legs, edema, enlarged heart, and eventually death from cardiac failure: (Beriberi). Nicotinamide is used to form the coenzyme NAD^+ and $NADP^+$ involved in oxidative-reductions in many energy yielding metabolic pathway. Without niacin, every organ of the body is severely impacted, and a severe deficiency disease, pellagra, develops. The symptoms of which are referred to as 4 DS: dermatitis (skin disorders), dementia (mental confusion), diarrhea, and eventually death if niacin is not supplied (Estelle and Karen, 1999).

The amino acids help in regulating specific functions in the human body for the well being of an individual. Thus leucine is responsible for regulating the body sugar concentration, growth and repair of muscles/tissues and wound healing, it deficiency causes dizziness, headaches, fatigue depression, irritability and hypoglyceamia in infants (Reference Guide, 1995); isoleucine helps in development of haemoglobin, repair of muscles and regulation of energy while lysine is to ensure adequate absorption of calcium and formation of antibodies, hormones and enzymes. Recent studies have shown that lysine may be effective against Herpes by improving the balance of nutrients that reduce viral growth. A deficiency may result in tiredness, inability to concentrate, irritability, bloodshot eye, retarded growth, and hair loss, anemia and reproductive problems. Alanine (non-essential amino acid) is an important source of energy for muscle tissue, the brain and central nervous system, strengthens the immune system by producing antibodies; helps in the metabolism of sugars and organic acids. These essential amino acids come from the diet and need to be replenished adequately regularly. The significant reduction in these essential nutrients is worrisome. Higher significant decreases in vitamins composition was recorded for ecotype B while ecotype A recorded higher amino acids reduction. Biotechnological research that will result in the production and deregulation of virus-resistant *T. occidentalis* through vitamins and coat-protein gene transfers between the two ecotypes is recommended. There is therefore the need to prevent TeMV infection of *T. occidentalis* in order to ensure high yield, quality and guarantee its nutritive value. Presently the use of resistant varieties is the only practicable means of controlling the viral infection (Thottappilly and Rossel, 1992).

REFERENCES

Association of Official Analytical Chemists (A.O.A.C)(1995). *Official Method of Analysis.* 16th Edition,Washington, D. C.

Association of Official Analytical Chemists(A.O.A.C) (2006). Official method of analysis of the AOAC (W. Horwitz, Ed.) 18th Edition, Washington, D. C.,

Akwaowo, E. U, Ndon, D. A. and Etuk, E. U. (2000). Minerals and antinutrients in fluted pumpkin (*Telfairia occidentalis* Hook. F.). *Food Chemistry* 70(2): 235-240.

Anno-Nyako, F. O. (1998). Seed transmission of *Telfairia mosaioc virus* in flutted pumpkin (*Telfairia occidentalis*, Hook, F.) in Nigeria. *Journal of Phytopathology* 121:85-87.

Atiri, G. I. (1985).An isolate of cucumber mosaic virus (CMV) from field-infected fluted pumpkin in Nigeria. *Phytopathologie.Zeitschrift III:*268-273.

Atiri, G. I. (1986). A disease of fluted pumpkin (*Telfairia occidentalis* Hook F.) caused by a yellow vein clearing strain of pepper veinal virus in Nigeria. *Journal of Plant Protection in the Tropics* 3:105-110.

Ayto, J. (1993). Dictionary of word origins, Arcade, NewYork,254pp.

Bessey, D. A. (1944). Determination of ascorbic acid by titrimetric method. *Journal of Association of Agricultural Chemists* 27: 537.

Enwere, N. J. (1995). Effect of tempering and drying on the functional properties of cowpea flour during akara and moi-moi preparation. M.Sc Thesis, Department of Food Science and Technology, University of Nigeria, Nsukka.

Estelle, L. and Karen, M. (1999). Plant and Society 2nd edition. McGraw- Hill, New York.

Fasuyi, A. O. and Aletor, V. A (2005). Varietal composition and functional properties of cassava (*Manohot esculenta* Grantz) leaf and leaf protein concentrates. *Pakistan Journal of Nutrition*, 4(1): 43-49.

Fraser, R. S. S. (1987). Biochemistry of virus infected plants. Research Studies Press Limited, Letchworth, Hertford, 641pp.

Goodman, R. N., Kiraly, Z. and Wood, K. R. (1987). The biochemistry and physiology of plant disease. University of Missouri Press, Columbia, 433pp.

Horsfall, M. Jr and Spiff, I. A (2005). Equilibrium sorption study of Ac3+, C02+ and Ag+ in aqueous solutions by fluted pumpkin (*Telfairia occidentalis* Hook F.) waste biomas. *Acta Chim Slov*., 52:174-181.

Hull, R. (2002). Matthew's Plants Virology. Academic Press Incorporated, London, 864pp.

Matthews, R. E. F. (1991). Plant Virology (3rd Edition). Academic Press Incorporated, New York, 835pp.

Nwauzo, E. E. and Brown W. M. (1975). *Telfairia* (Cucurbitaceae) mosia virus (TeMV) in Nigeria. *Plane Disease Reporter* 59:430-432.

Oguntona, T. (1998). Green leafy vegetables. In : Nutritional quality of plant foods (Osagie A. U. and Eka O. U. Eds.), Post Harvest Research Unit AFBIK Publics, Benine City, Nigeria 120:130.

Ossom, E. M. (1986). Influence of harvest interval on yeild crude fibre, N. P. and K contents on longevity of the fluted pumpkin, *Telfairia occidentalis* Hook. Tropical Agriculture (Trinidad and Tobago) 61(1):63-65.

Reference Guide for amino acids visitiors (1995). 671:323

Schwartz, S. (2006). Good health and vegetables. Oxford Science, Oxford, London, 485pp.

Shoyinka, S. A. and Thottapilly, G. (1998). Occurrence of *Telfairia mosaic virus* in Nigeria. *African Crop Science Journal* 6(1):69-78.

Shoyinka, S. A., Brunt, A. A., Lesemann, D., Thottapilly G. and Lastra, R. J. (1987). The occurrence, properties and affinity of *Telfairia mosaic virus* (TeMV) pot virus Prevent in *Telfairia* (Cucurbitaceae) in South West Nigeria. *Journal of Phytopathology* 119:13-17

Speakman, D. H. Stein, E. H. and Moore, S. (1958). Automatic recording apparatus for use in the chromatography of amino acids. *Analytical Chemistry* 30:1191.

Thottappilly, G. and Rossel, H. W. (1992). Virus disease cowpea in tropical Africa. *Tropical Pest Management* 38(4):337-348.

Uritani, I. (1971). Protein changes in diseased plants. Annual Review of Phytopathology 9 : 211-234.

Weiner, M. A. (1992). *Earth Medicine*. Macmillan, New York, 12pp.

White, J. L. and Brakke, M. K. (1983). Protein changes in wheat infected with w*heat streak mosaic virus and in barley stripe mosaic virus. Physiological Plant Pathology* 22: 87-100.