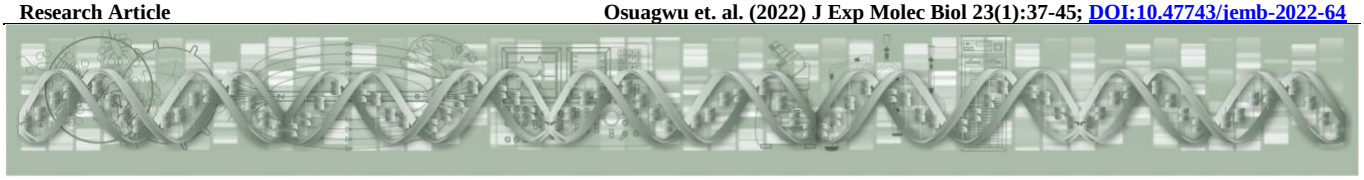

Amino acid Profile of *Trichosantes cucumerina* (L.) from four Geopolitical Zones in Nigeria.

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Abstract

The aim of the study was to investigate the amino acid profile of *T. cucumerina* (snake gourd) from four geopolitical zones in Nigeria. Fresh ripe fruits of *T. cucumerina* were collected from the research farm of Michael Okpara University of Agriculture, Umudike, Umuahia Abia State Nigeria. The pulps were extracted from the fresh ripe fruits, blended and stored in the refrigerator for further use. Amino acid content was determined through HPLC method . The study revealed that the amino acid profile of *T.cucumerina* pulp contained eighteen amino acid types. The investigation revealed that glutamate was the predominant amino acid in the pulp which ranged from 14.4643-15.23094g/100; followed by aspartate with 2.0981-12.20628g/100, leucine with 7.20846-8.9831g/100. Glycine content was the lowest with a range of 0.0435-4.2118g/100. The snake gourd amino acid profile was found highest in North-Central ecotype (Benue State) with a range of (1.115-14.46497) g/100 protein; followed by South-West ecotype (Ekiti and Osun States) with a range of (0.87481-12.20628), (0.0435-15. 23094) g/100protein respectively. South-East ecotype (Abia State*) T.cucumerina* result ranged from (0.54978-14.67031)g/100 protein. The least amino acid content was found in South-South ecotype with 0.35514-14.46243g/100 g. Variations in amino acid content in ripe fruits of *T. cucumerina* revealed that glycine had the highest coefficient variation of 140% followed by tyrosine with 54.31%, aspartate 47.56%, and lowest from valine 5.62%, isoleucine 7.05%, tryptophan 6.51 cysteine 6.82% and glutamate 2.14%. There were three clusters in the dendrogram of amino acid composition of selected snake tomato ecotypes investigated. The amino acid content analysis revealed that the highest amino acid content was found in snake gourd from the North -Central and lowest in South-South ecotype respectively.

Keywords: Amino acid, *Trichosanthes cucumerina*, Ecotypes, Snake gourd, Alanine, Glutamate, Phenylalanine, Threoonine, Argnine, Serine

Introduction

Neglected and underutilized species (NUS) have started receiving attention due to their abilities in mitigating the risk of agricultural production systems and their nutritional qualities. (Padulosi *et al*., 2013). Underutilized crops/plant species are those that many communities traditionally use for food, fiber, animal fodder, oil or medicine, but they have other undeveloped potential uses (Arora,2014). Underutilized plant species have great potential for contributing to food security, health (nutritional and/or medicinal), income generating activities and environmental services, but these have remained underexploited (Arora,2014). *Trichosanthes cucumerina* is commonly called snake gourd, viper gourd, long tomato or snake tomato (Liyanage *et al.,* 2016). *Trichosonthes cucumerina* is an annual climber with a diploid chromosome number of twenty-two (2n=22) Alam *et al.,* (2012). *Trichosanthes cucumerina* L. belongs to the *Trichosanthes* genus, which is one of the most important of the Cucurbitaceae family (Ekeke and Agogbua, 2018). The geographical distribution of the species belonging to this genus ranges from Southeast Asia from India eastward to Taiwan, the Philippines, Japan and Southeast ward to Australia, Fiji and Pacific Islands (Bharathi *et al*., 2013).

Snake gourd is cultivated in Southeast Asian countries for the immature fruit that can be cooked and served as a vegetable (ECHO, 2019). Aderibigbe *et al*., 2019 reported that in Nigeria the matured fruits of snake tomato are used for traditional gastronomy because of sweet and aromatic taste *Trichosanthes cucumerina* has diuretic properties in traditional medicine, it helps the liver increase urination, thereby speeding up the elimination of toxins from the body. The diuretic nature of snake gourd helps against dryness and dehydration and increases the function of the kidneys and bladder (Devi, 2017). Aderibigbe *et al*., 2019 showed that there was a higher content of β-carotene, vitamin C, Fe, Zn, Ca, K in snake gourd paste than in vine tomato paste. *Trichosanthes cucumerina* is a natural antibiotic, expectorant, laxative, it cures constipation and has been shown to be excellent for diabetes (Edeoga *et al,* 2010), *T. cucumerina* is used in the treatment of wounds, boils, sores, skin eruptions, such as eczema and dermatitis (Arawwawala et al, 2009).

Trichosanthes cucumerina (L) has been neglected by agriculturists in Nigeria that had stopped its cultivation and now is rarely found in home gardens, thus it is going extinct (Ugbaja *et al*., 2017). This threat (extinction) that snake gourd species face gives rise to the need for a study of amino acid profile to prove its importance in agriculture, medicine and alimentation.

Amino acids are very essential for human health and are linked to numerous biological processes. Amino acids are involved in protein synthesis, cell signaling, cellular metabolism, immune response. Amino acid (AA) evaluation has been essential and commonly used in biomedical analysis, ecology, proteomics, food science and environmental science (Mndrioli *et al*. 2013;

Obat and Fernie, 2012, Huayun *et al*. 2017). Amino acids have been classified into non-essential and essential amino acids (Kumar *et al*. 2015). The essential amino acids are produced by plants only, such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Kumar *et al*. 2015). Non-essential amino acids are alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, tyrosine and B-alanine, which are produced by animal and plant organisms (Bhardwaj *et al*., 2015).

The aim of this study was to determine the amino acid profile of T.*cucumerina* pulp from four geopolitical zones in Nigeria.

Materials and methods

Viable seeds of snake gourd (*Trichosanthes cucumerina)* ecotypes were collected from different states in Nigeria from the following locations (Table 1).

Table 1: The sources of Viable seeds of snake gourd (Trichosanthes cucumerina) ecotypes

S/N	SOURCE LOCATION	ECOTYPE	DESIGNATION
1.	Oshogbo Osun State	Rainforest	South -West
2.	Iwo, Osun State	Rainforest	South-West
3.	Ikwuano, Abia State	Rainforest	South -East
4.	Ikom, Cross River State	Rainforest	South -South
5	Makurdi, Benue State	Derived savannah	North -Central

Preparation of plant materials

The ripe snake gourd (*T.cucumerina*) fruits were collected from the research farm of Michael Okpara University of Agriculture, Umudike, Umuahia. Pulps were extracted from snake gourd and blended. The blended pulps were stored in an airtight container in the refrigerator for further use.

Amino acid procedure HPLC apparatus

The HPLC equipment consisted of a Spectra Physics (San Jose, CA) HPLC apparatus comprising an 8700 XR ternary pump, a 20-µL Rheodyne (Cotati, CA) injection loop, an SP8792 column heater, a 8440 XR UV-vis detector, and a 4290 integrator linked via Labnet to a computer running WINner 8086 software (operating system, MS.DOS version 3.2). For separation, a 250- × 4.6-mm column packed with 5-µm Spherisorb C_{18} (Sugelabor, Madrid, Spain) was used.

Standard and samples preparation

Prior to derivatization, *T.cucumerina* ripe fruit pulp proteins were hydrolyzed as follows: 0.1-g lyophilized *T.cucumerina* pulp were weighed into a 16- × 125-mm screw-cap Pyrex (Barcelona, Spain) tube, 15 mL of 6N hydrochloric acid was added, and the tube was thoroughly flushed with N_2, quickly capped, and placed in an oven at 110°C for 24 h . After hydrolysis, the tube contents were vacuum filtered (Whatman #541, Maidstone, England) to remove solids, the filtrate was made up to 25 mL with water, and an aliquot of this solution was further filtered through a 0.50-µm pore-size membrane (Millipore, Madrid, Spain). A standard solution containing 1.25 µmol/mL of each amino acid in 0.1N hydrochloric acid was created.

Derivatization procedure

The procedure used was a modification of the method of Elkin and Griffith(1985). A standard solution (5, 10, 15, or 20 µL) or 50 µL of *T. cucumerina* ripe fruit pulp solution was pipetted into a 10- × 5-mm tube and dried in vacuo at 65°C. 30 µL of methanol-water-Phenylisthiocianate (2:2:1 [v/v]) were added and then removed in vacuo at 65°C. Next, 30 µL of the derivatizing reagent methanol-water-Phenylisothiocianate (7:1:1:1 [v/v]) was added, and the tube was agitated and left to stand at room temperature for 20 min. Finally, the solvents were removed under a nitrogen stream, and the tube was sealed and stored at 4°C, pending analysis. Prior to injection, 150 µL of diluent consisting of 5mM sodium phosphate with 5% acetonitrile was added to each tube.

Chromatographic procedure

Chromatography was carried out at a constant temperature of 30°C using a gradient elutionion as follows. Eluent A was an aqueous buffer prepared by adding 0.5 mL/L Triethylamine to 0.14M sodium acetate and titrating it to pH 6.20 with glacial acetic acid; eluent B was acetonitrile-water (60:40 [v/v]). The gradient program is shown in Table 2.

Table 2. Gradient Program Employed for the Separation of PTC-Amino Acids

Time (min)	Flow rate (mL/min)	% Eluent A	% Eluent B
0	1.0	90	10
12.0	1.0	70	30
20.0	1.0	52	48
22.0	1.0	0	100
24.0	1.0	0	100
30.0	1.0	0	100
37.0	1.0	90	10

Statistical analyses

Statistical analyses were carried out including the coefficient of variation percentage (CV%) and dendrogram using Minitab 16.

Results and Discussion

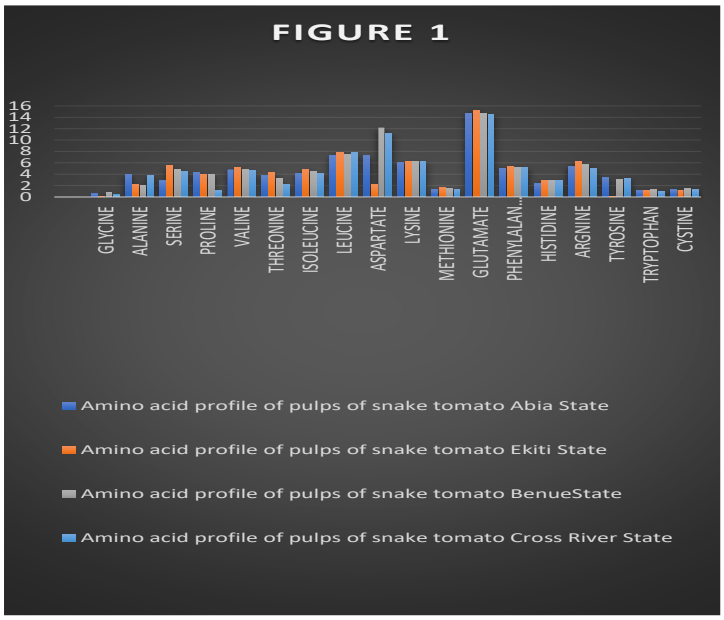
The investigation of the amino acid profile of *T. cucumerina* indicated that 18 amino acids were found in the fruit pulps. These amino acids are glycine, alanine, serine, proline, valine, threonine, isoleucine, aspartate, lysine, methionine, histidine, arginine, glutamate, phenylalanine, tyrosine, tryptophan, and cystine in various quantities (Fig 1). Generally, glutamate was the highest amount of amino acid type (14.46243 _ 15.23094g / 100g) protein and the lowest was glycine (0.0435 _ 4.21184g / 100g) protein contained in the pulps of the fruits of the snake gourds. The result of the study of the amino acid profile of *T. cucumerina* fruit pulps based on amino acid types and the various ecotypes (accessions) indicates that the amount of glutamate was highest in pulps from Benue (North central) 15.23094g/100g protein and lowest least in pulps from Osun (South West) 0.04g/ 100g protein, alanine was highest in pulps from Benue {NC)6.29368g/100gprotein and lowest in that of Ekiti(SW) 2.0667g/100g protein. The serine content of the pulps was highest from Osun (SW) 5.46763g/100g and lowest: 2.83959g/100g protein from Abia(SE). The proline content was highest in Abia(SE) 4.32329g/100g protein and lowest in those from Cross River(SS) 1.4255g/100g protein. It was also found that the highest amount of valine amino acid was from pulps from Osun(SW)5.26242g/100g protein and the lowest from Cross River(SS)4.5729/100g protein. The threonine content of *T.cucumerina* of fruit pulp evaluated indicates that the highest amount was from those pulps from Osun(SW) 4.36555g/100g protein. Comparatively, the isoleucine content of the pulps snake gourd ripe fruits was highest in Osun(SW)4.83299g of protein and lowest from 4.1004g/100g protein, while the highest leucine content was from the pulps from Benue(NC) 8.9831g/100g protein and lowest in the pulps of fruits from Abia(SE) 7.20846g/100g protein (fig 1).

The aspartate content of pulps of ripe fruits of *T.cucumerina* from Ekiti(SW) was highest 12.20638g/100g protein and the lowest was in those from Osun(SW) 2.09891g/100g protein. This study shows that the highest amount of lysine was observed from Benue(NC) 9.72016g/100g protein and the lowest lysine content was in pulps from Abia(SE) 6.11876g/100g protein. Methonine content was highest in the pulps of snake gourd fruits from Osun(SW) 1.58601g/100g protein and the lowest content was in those from Abia(SE)1.29821g protein. The amino acid profile of the pulps of *T .cucumerina* indicates that those from Osun(SW) had the highest glutamate content (15.23094g/100g protein), while the lowest was from those from Cross Rivers(S/S) 14.46243g/100g protein. The highest content of phenylalanine was obtained from pulps of snake gourd from Benue(NC) 5.3447g/100g protein, and the lowest from Abia(SE) 3.91049g/100g protein. The study also showed that amount of histidine was obtained from Abia (SE) 2.31885g/100g protein. The amino acid profile showed that the highest arginine content was obtained from pulps of fruits Benue (NC) 6.55257g/100g protein and the lowest from Cross Rivers (SS) 4.97718/100g protein.

The amino acid profile of the pulp of snake gourd fruits showed that the tyrosine content was highest in those fruits from Abia(SE) 3.3974g/100g protein, and lowest from those from Osun(SW) 0.099213g/100g protein. Tryptophan content of the pulps from Ekiti(S/W) was highest (1.43662g/100g protein) and the lowest was obtained from those from Cross River (SS) 1.04747g/100g protein. The amino acid profile that the cystine content of the pulps of the fruits of *T.cucumerina* was highest in those from Ekiti(SW)1.43662g/100g protein and the lowest from those from Osun(SW) 1.22739g/100g protein.

The result obtained from the amino acid profile of the pulp of *T.cucumerina* indicated eighteen (18) amino acid types were present. This differs from the report of Okonwu and Mounekw (2019), which showed that the seeds of *T.cucumerina* seeds contained twenty (20) amino acid types. Glutamic acid and asparagine are found in the seeds but not in the pulp. This is an indication of variation in the number of amino acid types in the various parts of the fruits. The work of Swetha and Muthukumar (2016) on the same amino acid profiling that the peels of *Luffa autangula* contained twenty-one (21) amino acid types showing variations in the number of amino acid types in different Cucurbitaceae groups. Nazary and Basumatary (2019) reported that there were twenty-five(25) amino acid types from six wild consumed vegetables while *T. cucumerina* fruit pulps had eighteen (18) amino acid types, thus this is not in agreement with this study. This work is not in agreement with the report of Aja *et al.,* (2021) that evaluated the amino acid profile of three leafy vegetables (*Solanum aethiopicum*, *Amaranthus hybridus* and *Telfaria occidentalis*) and observed that the glutamate content had the highest value with 12.59, 11.20, 11.96g/ followed by leucine with 9.81, 7.94, 9.28g/100g protein and aspartic acid with 8.99, 8.62 and 9.74g/100g protein in *S. aethiopicum*, *A. hybridus* and *T. occidentalis*. This work is not in agreement with the findings of Borokini *et al.* (2017) who determined the amino acid profile of two vegetables (*Basella alba* and *Senecio biafrae*) and reported that histidine (2.14, 2.27) isoleucine (3.01, 3.43), leucine (7.75, 8.85), lysine (2.92, 4.62) methionine (0.89, 0.99), phenylalanine (3.88, 4.22), threonine (2.84, 3.15) and non-essential amino acids valine (3.13, 3.71), arginine (3.97, 4.03), cysteine (0.79, 0.86), tyrosine (2.22, 2.70), aspartic acid (7.32, 8.28) serine 2.09, 2.22), glutamic acid (10.15, 10.83), proline (2.24, 2.65) glycine (3.07, 3.56).

Kubmarawa *et al.* (2011) reported on the amino acid profile of the leaves of *Cassia tora* and *Celtis integrifolia* and observed seventeen (17) amino acids in varying proportions.



Variation in amino acid content in fruits of *T. cucumerina* accessions investigated showed that glycine had the highest coefficient of variation of 140%, followed by tyrosine with 54.31%, aspartate 47.56%, and lowest from valine 5.62%, isoleucine 7.05%, tryptophan 6.51% and cysteine 6.82% glutamate 2.14% fig 1. Variation in amino acid content in fruits of *T. cucumerina* (Snake gourd) (fig xviii). The coefficient of variation of three leafy vegetables as reported by Aja *et al*. (2021) varied from 4.50 – 80.1%, which differed from this study, in which it ranged from 2.14 to 140%. Olubummi *et al.* (2015) reported the coefficients of variation of ten amino acid profile from indigenous leafy vegetables of south-West, Nigeria, which varied from 4.30 – 14.46% and differed from this study. in which it ranged from 2.14 to 140%.

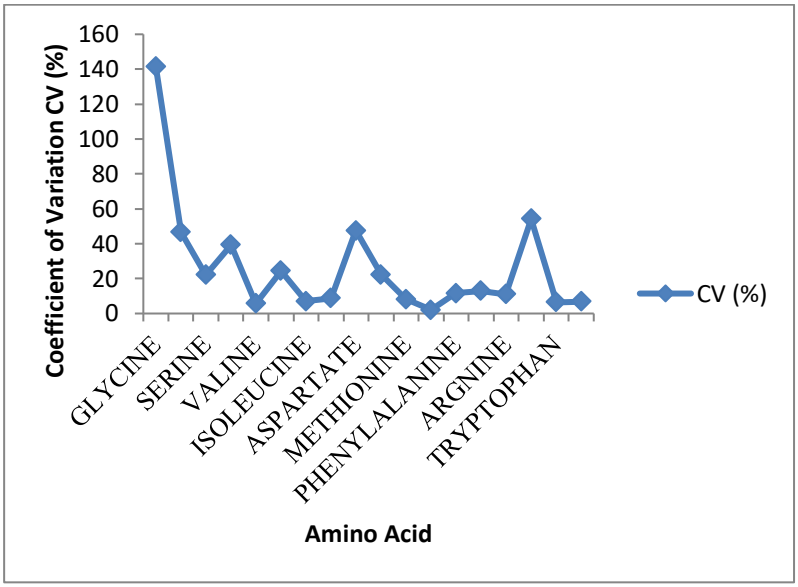


Figure 2: Variation in Amino Acid Content in Fruits of Snake Tomato

There were three clusters in this dendrogram of amino acid composition of selected snake tomato accessions. Oye-Ekiti, Ekiti and Cross River were closely related to each other with 68% similarity. Abia State accession was not closely related to Ekiti with a similarity of 45%. Benue accession was on the second cluster with a similarity of 40%. Osun accession was placed in the third group respectively not closely related to Benue with a similarity of 13.72% (Fig.III). Dendrogram of the amino acid composition of selected *T. cucumerina* snake tomato accession Fig. 2This is in agreement with the work of Nunez-Gomez *et al.* (2021) who reported that there were three clusters in the dendrogram of (*Ficus carica* L) Breba fruit illustrating the distance of morphological and chemical parameters. This is not in agreement with the work of Maro *et al*. (2011) who reported that there were five clusters in the dendrogram of *Malu domestica* Borkh cv. Annura for free amino acid profile from different Caserta districts (Campania Italy).

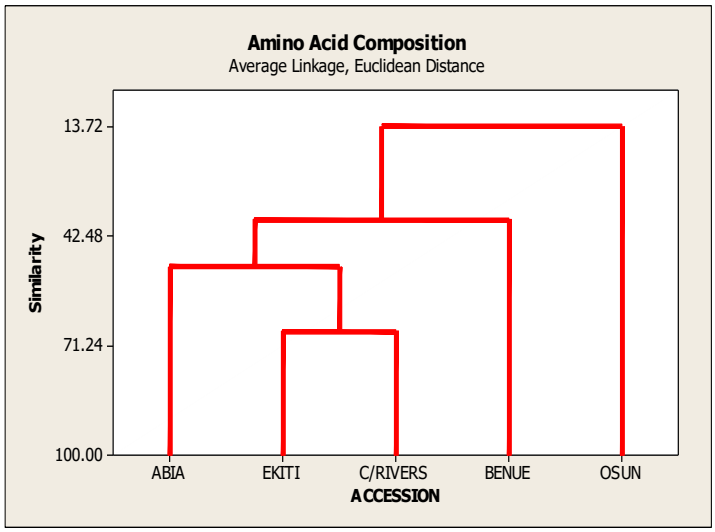


Figure3: Dendrogram Based on Amino Acid Composition of Selected Snake Tomato Accessions

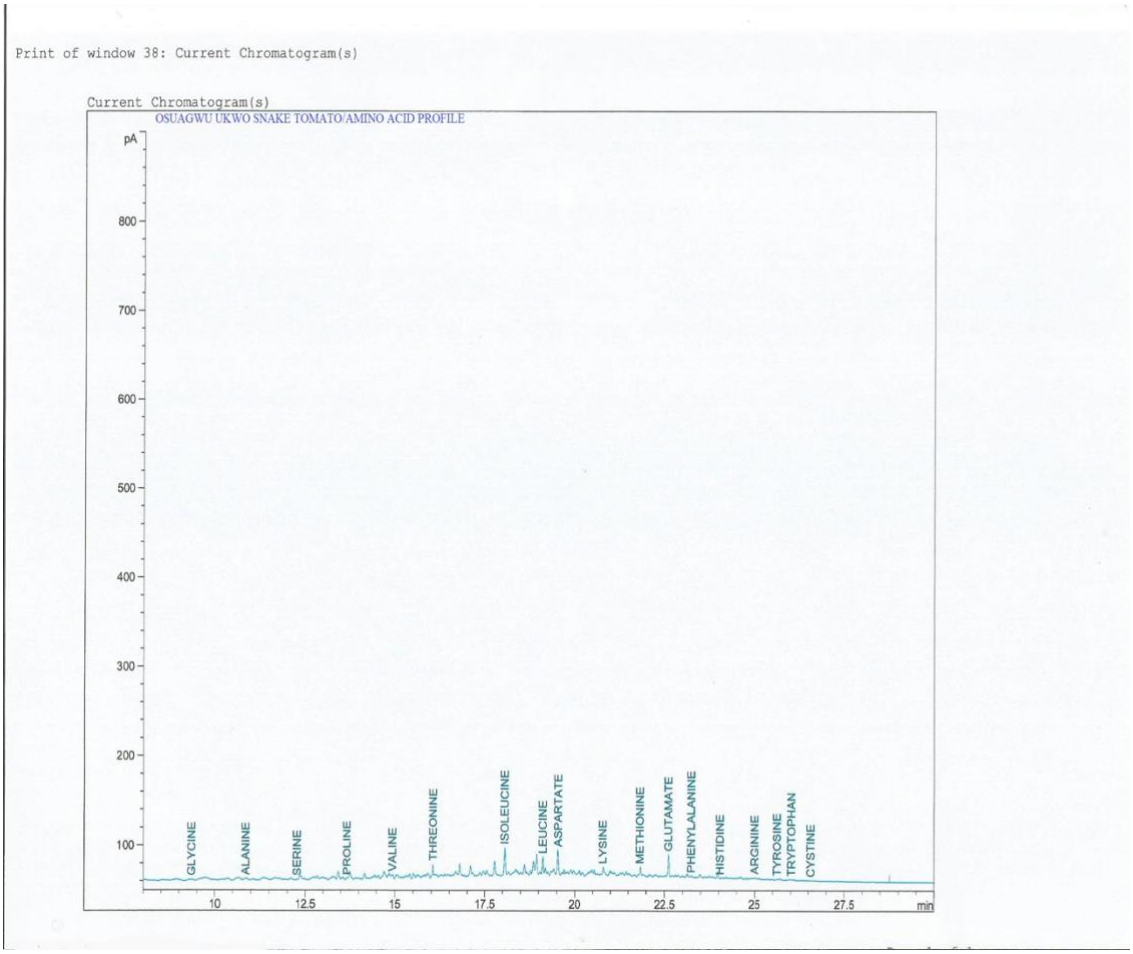


Fig 1 Amino acid profile of pulps of snake tomato Abia State South-East

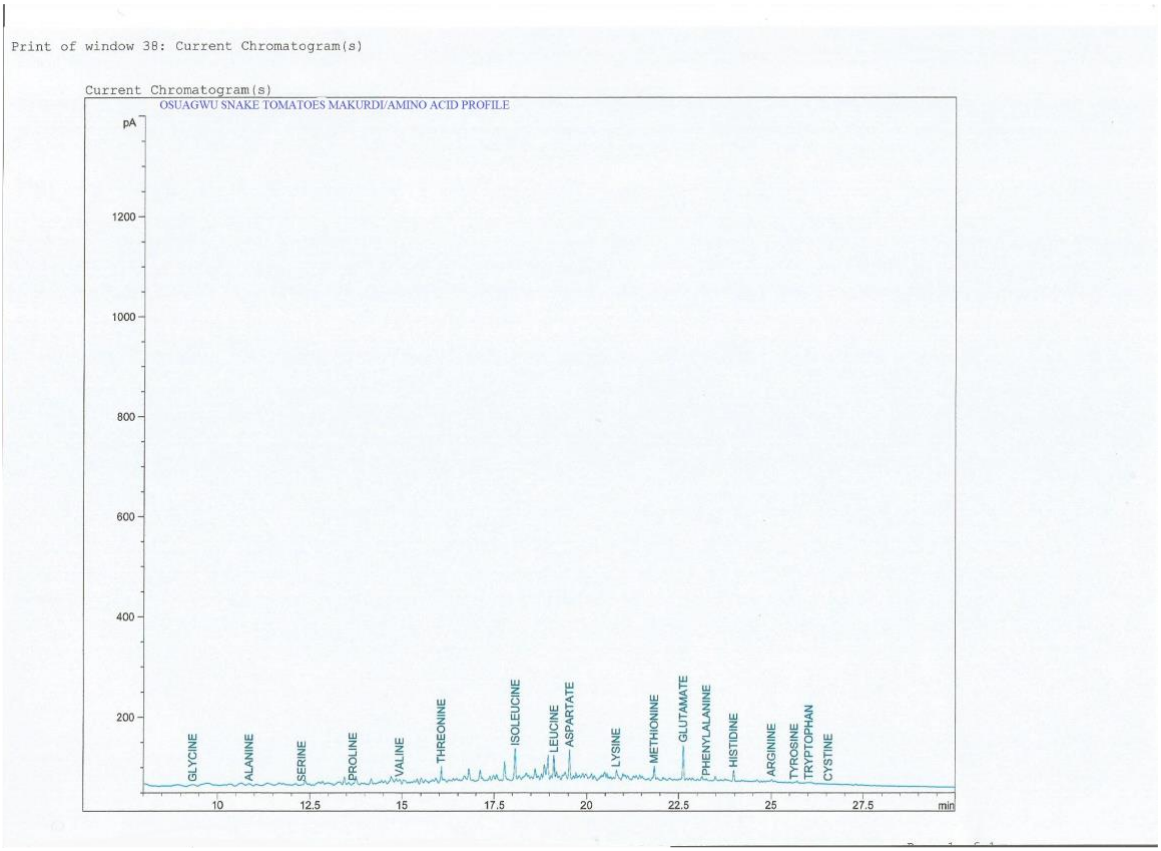


Fig II Amino acid profile of pulps of snake tomato Benue State North Central

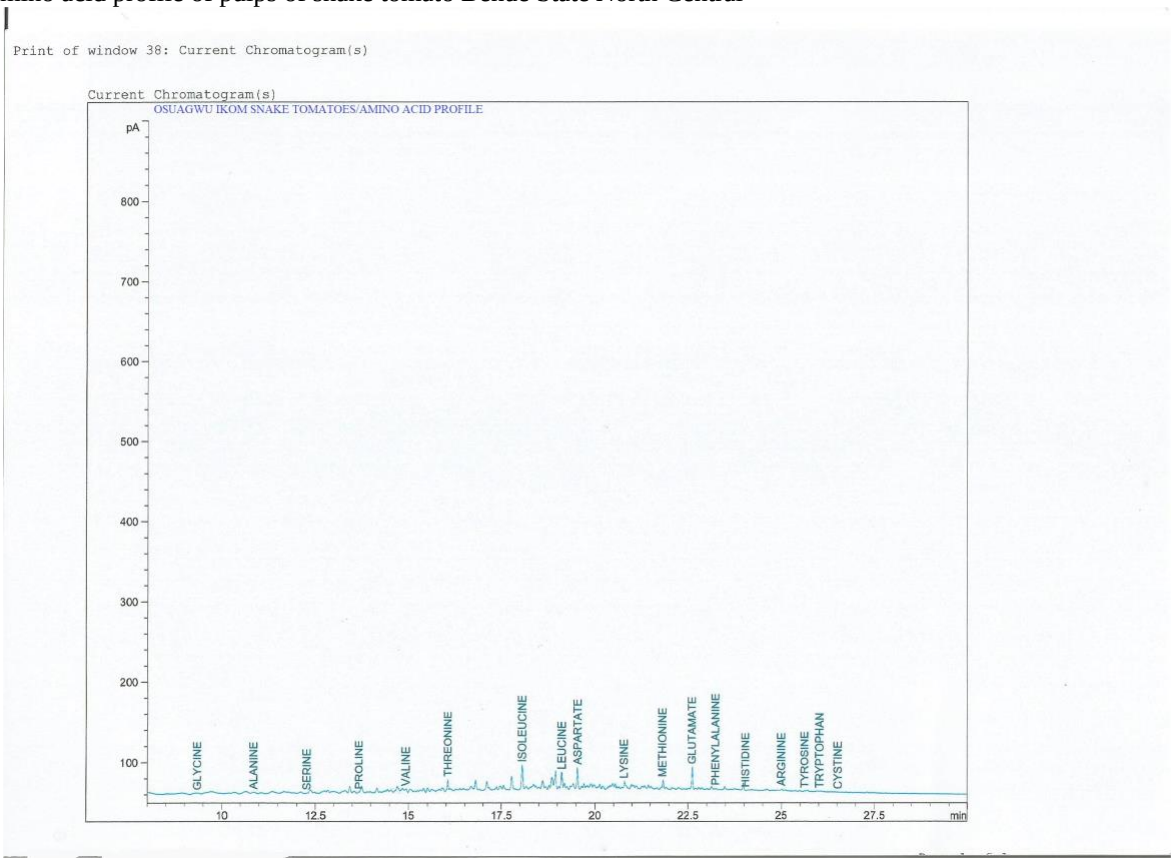


Fig III: Amino acid profile of snake tomato Cross River South-South

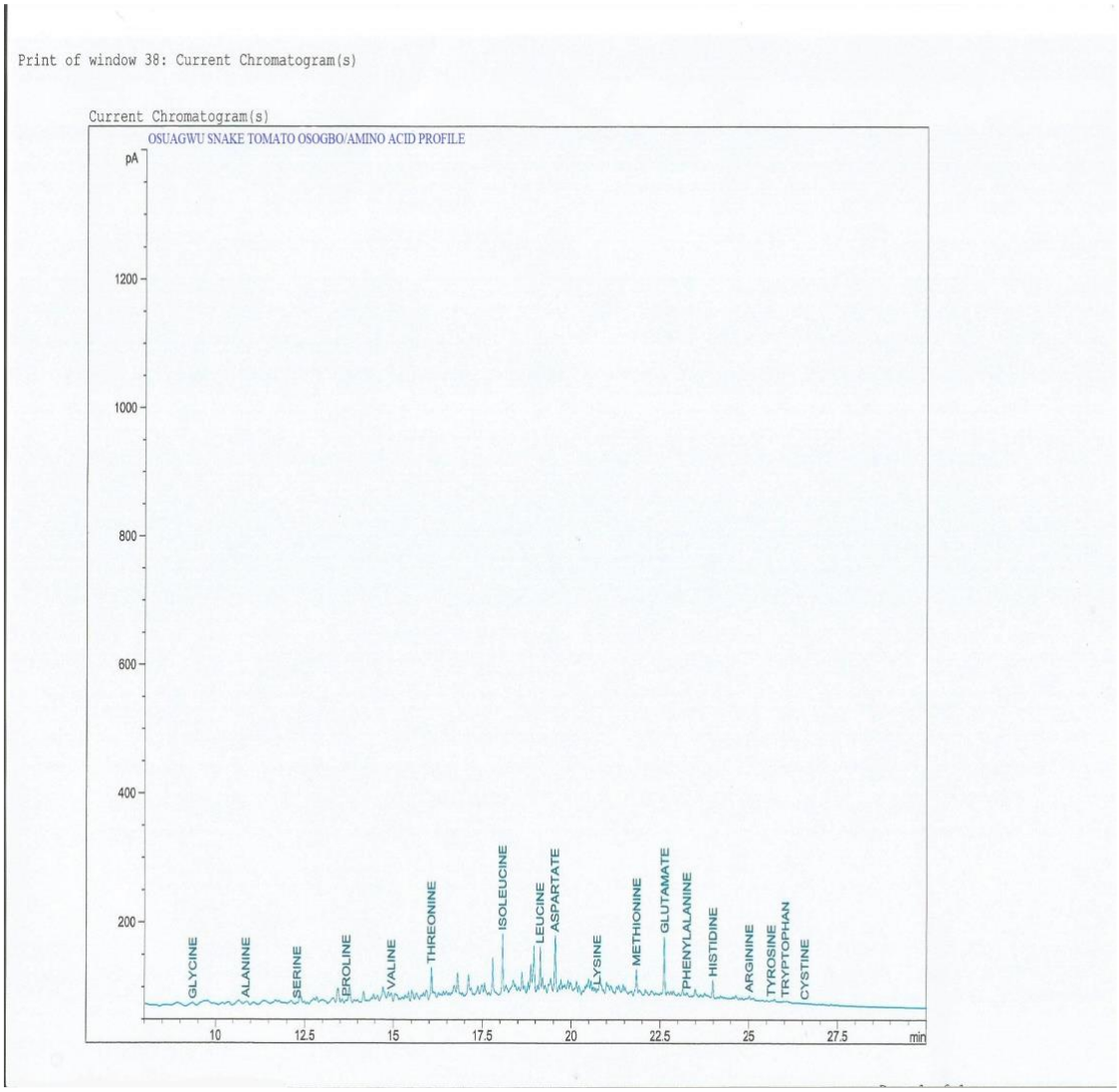


Fig IV: Amino acid profile of pulps of snake tomato Osun State South-West

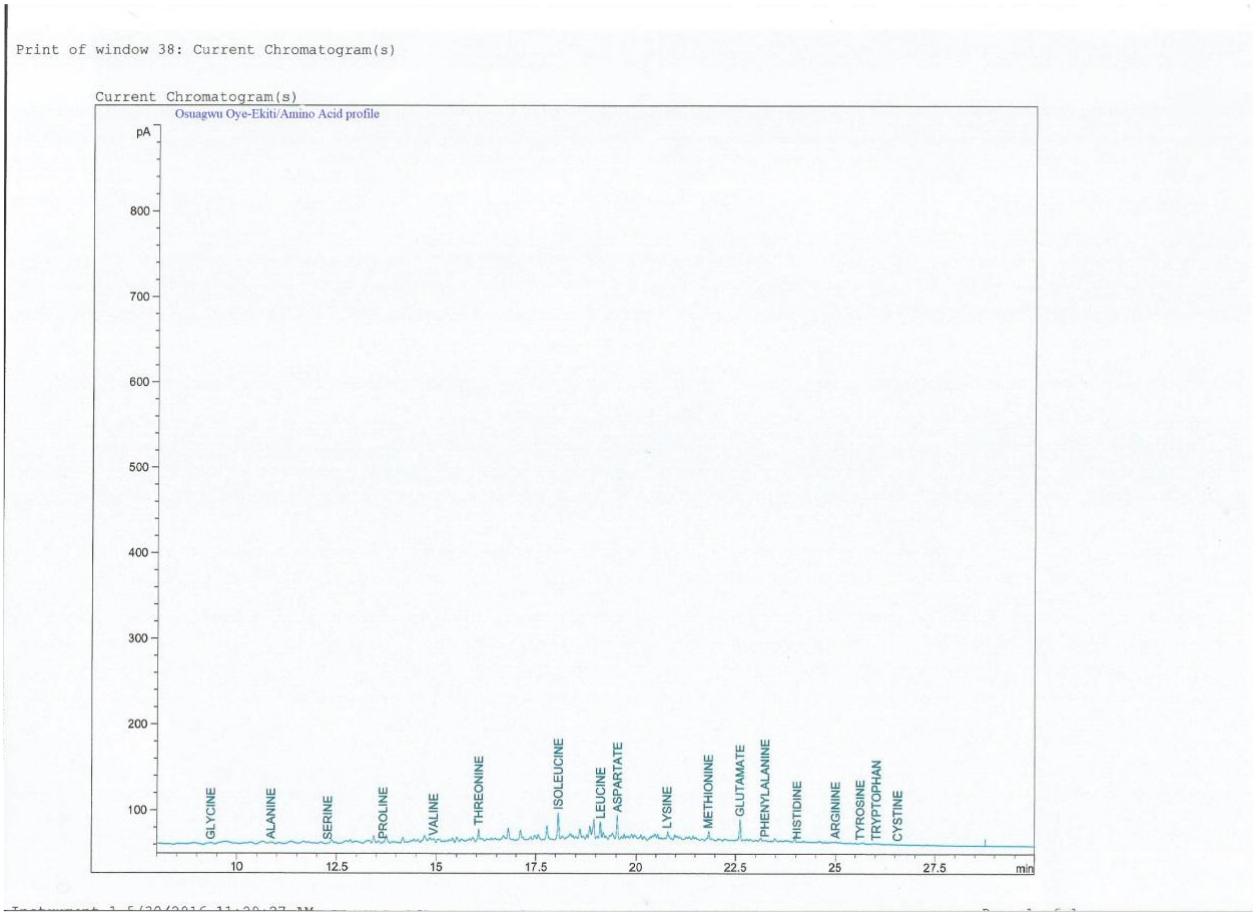


Fig V: The chromatograph of pulps of *T. cucumerina* (snake gourd) Ekiti State South West

Conclusions

The amino acid profile of pulps of the fruits of *T..cucumerina* accessions revealed the presence of eighteen (18) amino acids, both essential and non-essential. Amino acid analysis results revealed that the highest amino acid content was found in snake gourd from the North-Central and lowest in South-South ecotype respectively. There was variability in the amino acid of ripe fruit snake gourd investigated which revealed that glycine had the highest coefficient variation of 140% followed by tyrosine with 54.31%, aspartate 47.56%, and the lowest from valine 5.62%, isoleucine 7.05%, tryptophan 6.51% and cysteine 6.82% and the lowest from glutamate 2.14%. There were three clusters revealed from the dendrogram of investigated snake gourd.

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