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Comparative Evaluation of Amino Acid Composition and Volatile Organic Compounds of Selected Nigerian Cucurbit Seeds

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Abstract: Amino acid composition and volatile organic compounds (VOCS) of five Nigerian cucurbits namely: *Luffa aegyptiaca* (Mill.), *Citrullus lanatus* (Thunb. Matsum), *Cucurbita maxima* (Duchesne, ex Lam), *Cucumis metuliferus* (E.Mey. ex Naudin) and *Momordica balsamina* (L.) were investigated using Amino acid Analyzer and Gas Chromatography-Mass Spectrometry (GC-MS). The proportion of essential amino acids ranged from 22.75- 30.23 g/100 g protein with the highest content in *M. balsamina* with 30.23 g/100 g protein and the least in *C. lanatus* with 22.75 g/100 g protein. High content of leucine was found in *C. maxima* and *M. balsamina* with 7.04 and 6.11 g /100 g protein respectively. The GC-MS analysis revealed the presence of thirty-one compounds, the most abundant classes of organic compounds in *L. aegyptiaca* comprised of esters with 65.17% and Fatty Acids (FA) had 32.62% while hydrocarbons amounted to 29%. In *C. lanatus*, seven compounds were identified dominated by FA and hydrocarbons with 91.5 and 7.25% respectively. In *C. maxima*, ten compounds were identified dominated by FA (52.24%) and hydrocarbons (43.46%). In *C. metuliferus*, FA had 30.74% while aromatic compounds and alcohol had 9.18 and 0.68%, respectively. Among the five seeds studied, the most abundant compounds identified were linoleic acid methyl ester in *L. aegyptiaca* with 63.72%. Cis-cis linoleic acid is most abundant in *M. balsamina* (62.03%) totaling the fatty acid portion to 86.41%. These results suggest the potential of the seeds as a source of amino acids and fatty acids that could be useful in food and feed fortification strategies. The unique VOCS could be useful as biomarkers for delimitation of the studied species.

Key words: Cucurbits, amino acid, volatile compounds, fatty acids, biomarkers

INTRODUCTION

From time immemorial, man has depended on plants either directly or indirectly for existence and the resource will continue to serve as primary providers of human needs. In order to have a healthy population that can promote development, the relationship between food, nutrition and health must be reinforced (Obi *et al*., 2004). This becomes imperative since large segments of the population especially in developing countries suffer from protein malnutrition and projections based on current trends indicate a widening gap between human population and protein supply (Abdelatief, 2011). The significance of wild plants in nutrition of human populations of the Sahel is increasing due to the periodic drought and weather-related calamities that reduce yields of traditional grain staples (Robert *et al*., 1997).

The family Cucurbitaceae consists of members commonly known as melons and gourds that include cucumbers, squashes, luffas and melons. Cucurbits are among the economically most important vegetable crops (Loukou *et al*., 2007). The family is distributed around the tropics, where those with edible fruits were among the earliest cultivated plants in both the old and new worlds. It is a large family with about 125 extant genera and 960 species (Jeffrey, 1978). The family is represented by 21 genera and 41 species in Nigeria (Hutchinson, 1954). Melon, pumpkin and gourd seeds are reported to be rich in protein and could be useful in fortification of food products (Abiodun and Adeleke, 2010). Cucurbits are recognized as rich sources of secondary metabolites such as cucurbitacins, tetra cyclic triterpenoids and alkaloids that impart a bitter flavour to many cucurbits (Timothy, 1993).

Many plant proteins in the form of protein extracts or seed flours are being investigated and tested for new products (Lawhom and Cater, 1971; Lin *et al*., 1974; McWalters *et al*., 1976; Amaefule and Obioha, 1998). Seeds have been reported to have nutritive and calorific values (Farinu, 1986; Godwin, 2008; Lewu and Mavengahama, 2010; Dangoggo *et al*., 2011). Conventional cereal and vegetable protein sources being used in animal feeds are under pressure of competition through their use in human diets especially in Nigeria and most developing nations of the world (Dairo, 2008). Similarly, the ban on the use of animal by-products in feeding poultry by the European Union increases the demand for these protein sources making

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them very expensive (Farrell, 2005). This has brought challenges in the area of nutrition research in plants of lesser importance to man that may serve as a veritable source of vegetable protein due to the increased costs of high quality conventional sources (Amaefule *et al*., 2004). In order to explore the numerous economic potential of cucurbits, there is a need for thorough chemical analyses of their seeds that could be vital towards understanding their fuller potential. This paper reports on the amino acid compositions and volatile organic compounds of selected Nigerian cucurbits seeds.

MATERIALS AND METHODS

Ripe fruits of *C. lanatus* and *C. maxima* were obtained from Sokoto old market while; those of *M. balsamina, L. aegyptiaca* and *C. metuliferus* were collected from Ruggar Liman Fadama in the outskirts of Sokoto town. The plant materials were authenticated at the Department of Biological Sciences Herbarium, Usmanu Danfodiyo University, Sokoto where voucher specimens were prepared and deposited. Seeds were removed from the fruits by cutting individual fruit longitudinally and scrapping out the seeds using a clean knife and seeds were screened to remove bad ones. The seeds were dried to a constant weight in an oven at 70°C, ground using mechanical blender, placed in airtight containers and stored in desiccators for further analyses.

Amino acid profile of the samples was determined using methods (Spackman *et al*., 1958). 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). The samples were defatted by weighing two grams of each of the dried samples into extraction thimble and the fat was extracted with chloroform (2:1 mixture) using soxhlet extraction apparatus for 15 hours as described (AOAC, 1990).

Nitrogen content was determined by Kjeldhal method after samples digestion with concentrated H_2SO_4, sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) as catalyst. Percentage nitrogen in the original samples was calculated and recorded. Hydrolysis of defatted samples was carried out by addition of seven milliliters of 6 NHCl and oxygen was expelled by passing nitrogen into the ampoule (this was to avoid the possible oxidation of some amino acids during hydrolysis (e.g., methionine and cysteine). The glass ampoule was sealed with Bunsen burner flame and put in an oven preset at 105°C for 22 hours and the content was filtered to remove the humins. The filtrate was evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residue was dissolved with 5 ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles kept in the refrigerator until required. The hydrolysate was loaded into the TSM analyzer designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate for 76 minutes. Amino acid content was calculated from the chromatogram peaks. The net height of each peak produced by the chart recorder 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 half-height was accurately measured and recorded. Approximate area of each peak was then obtained by multiplying the height with width at half-height. The Non-leucine Equivalent (NE) for each amino acid in the standard mixture was calculated.

Volatile compounds were extracted by direct solvent extraction method (Parliament, 1997 and Ibrahim *et al*., 2011). Five gram each of the samples was saturated with 20 ml of diethyl ether. The mixtures were allowed to stand at room temperature for 24 hours, filtered using Whatman No. 1 filter Paper and the filtrates were collected in labelled sterile bottles, closed tightly before the analysis.

GC-MS analysis was performed using GC-MS-QP2010-Plus (Shimadzu, Japan) equipped with Flame Ionization Detector (FID). The injection was a split- less mode at 250°C for 3 min by using an inlet of 0.75 mm i.d to minimize peak broadening. Chromatographic separations were performed using DB-WAX analytical column 30 m 0.25 mm, 0.25 mm (J and W Scientific, Folsom C.A.) with helium as carrier gas at a constant flow rate of 0.8 ml/Min. The oven temperature was programmed at 60°C for 5 min, followed by an increase (held for 5 min) and finally at 10°C/min to 280°C (held for 10 min) and the temperature of the FID was set at 250°C. MS operating conditions (electron impact ionization mode) were an ion source temperature of 200°C, ionization voltage of 70 eV and mass scan range of m/z 23- 450 at 2.76 scans/s.

Chromatographic peak identification was carried out according to the method (Wanakhachornkrai and Lertsiri, 2003) by comparing their mass spectra with those of the bibliography data of unknown compounds from the NIST library mass spectra database on the basis of the criterion similarity (SI)>800 (the highest value is 1,000). Approximate quantification of volatile compounds was estimated by the integration of peaks on the total ion chromatogram using X calibur software (Vienna, VA). The result was presented as the peak area normalized (%).

RESULTS AND DISCUSSION

Amino acid profile of the seeds of five selected cucurbit studied is presented in Table 1. Appreciable quantities of essential amino acids were observed with the highest contents of leucine in *C. maxima* and *M. balsamina* with 7.04 and 6.11 g/ g protein respectively. The concentration of phenylalanine was high in *M. balsamina* with 4.28 g/ g protein and *L.*

Table 1: Amino acid profile of five species in Cucurbitaceae (g/ 100 g protein)

Amino acid	*L. aegyptiaca*	*C. lanatus*	*C. maxima*	*C. metuliferus*	*M. balsamina*
Lysine	4.16	2.02	1.80	3.50	3.94
Histidine	2.02	1.80	2.14	2.30	3.00
Arginine	7.80	6.30	7.33	7.08	6.30
Aspartic acid	9.89	8.10	9.92	11.23	9.50
Threonine	3.30	2.10	3.10	2.60	3.01
Serine	2.76	3.00	3.70	3.15	2.66
Glutamic acid	13.63	11.74	3.60	13.10	15.15
Proline	2.65	2.75	4.50	2.84	3.05
Glycine	5.04	3.80	4.50	3.95	4.01
Alanine	3.60	4.00	4.50	4.06	3.60
Cysteine	1.65	1.50	1.85	1.45	1.70
Valine	3.69	1.54	2.30	3.60	4.00
Methionine	1.90	1.54	2.30	1.46	1.50
Isoleucine	3.80	2.30	3.50	3.08	3.50
Leucine	5.65	5.18	7.04	5.80	6.11
Tyrosine	1.60	2.38	2.54	2.40	2.70
Phenylalanine	4.11	3.17	3.94	4.00	4.28
Total EAA	30.23	22.03	28.66	28.74	32.04
Total NEAA	47.02	41.19	36.17	46.86	45.97

aegyptiaca had 4.11 g/g protein. Lysine had the highest content of 4.16 g/100 g protein in *L. aegyptiaca* while *M. balsamina* and *C. lanatus* had 3.94 and 2.02 g/g protein, respectively. The contents of isoleucine was highest (3.80 g /100 g protein) in *L. aegyptiaca*, while *C. maxima* and *M. balsamina* had 3.50 g /g protein each. Similarly, the content of valine was high in *M. balsamina* and *L. aegyptiaca* with 4.00 and 3.69 g/ g protein respectively. Out of the twenty amino acids known, only seventeen were recorded in the five species studied, the reason could be attributed to the possible conversion of the amides of glutamine and asparagine into their corresponding amino acids (Salo-Vaananen and Koivistoinen, 1996) and complete destruction of tryptophan during acid hydrolysis (Wathelet, 1999). Presence of high contents of arginine in the studied seeds is an indication of possession of medicinal properties (El-Adawy and Taha, 2011). Relative to the WHO standards on essential amino acid needs of the preschool child, amounts of leucine (7.04 g/ 100 g protein) identified in *C. maxima* was greater than the recommended leucine requirement. Similarly, the combined PHE+TYR and MET+CYST contents identified in this study were greater than the recommended essential amino acid requirements by the WHO. Similarly, the amount of isoleucine in *L. aegyptiaca* was comparatively higher than that of pumpkin seeds (Muhammad, 2004). The amount of histidine (2.99 g) reported on *Largenaria siceraria* by Olaofe *et al.* (2009) is in close range to 3.00 g identified in *M. balsamina* but comparatively lower than values obtained in *C. lanatus, L. aegyptiaca, C. maxima* and *C. metuliferus*). Similarly, methionine content of 1.38 g reported is in close range to the value of 1.46 g identified in *C. metuliferus* but higher than 1.50 and 1.54 g/100 g protein found in *M. balsamina* and *C. lanatus*, respectively. The reported value for threonine (3.39 g/ 100 g protein) was higher than those obtained in this study for *L. aegyptiaca, C. lanatus, C. maxima, C. metuliferus* and *M. balsamina*. The results indicate that arginine; glutamic acid and aspartic acid were the most dominant amino acids identified which corroborate with the dominance of arginine, glutamic acid and aspartic acid contents in *C. Lanatus* (Evangelos, 1986). *C. maxima* however, had glutamic acid content of 16. 9 g/100 g protein was higher than 3.60 g obtained in this study. The valine content reported on Chinese bottle gourd is comparatively lower than values obtained in this study (Yazzie *et al.*, 1994). The differences observed could be attributed to geographical distribution in addition to intraspecific among the species (Glew *et al.*, 1997). The result of this study indicates that, the studied seeds contain substantial amounts of essential amino acids that could be exploited in feed and food fortification strategies.

GC-MS analysis revealed the presence of thirty-one volatile compounds in the studied seeds and the result is presented in Table 2. The compounds identified, their retention time and peak area normalized is summarized (Table 2). *C. metuliferus* had the highest number (20) of compounds dominated by 6-octadecenoic acid (22.2%) and 1, 6-methano (10) annulene (14.58%). Seven compounds were identified in *C. lanatus* with stearic acid and palmitic acid as the most dominant with 51.94 and 23.03% respectively. In *C. maxima*, squaline had the highest proportion of 29.70%, palmitic acid and 6-octadecenoic acid had 28.98 and 13.95%, respectively. Nine compounds were identified in *M. balsamina* with cis-linoleic acid as the most prominent compound with 62.03%. Other prominent compounds identified in *M. balsamina* were 9-octadecenoic acid with 51.94% while palmitic acid and stearic acid. *L. aegyptiaca* had the least number (six) of compounds with palmitic and 9-octadecenoic acid as the most abundant compounds. The amount of linoleic

Table 2: Volatile compounds from the seeds of five selected species of Cucurbitaceae presented as peak area normalized (%)

RT[-1]	Compounds	*L. aegyptiaca*	*C. lanatus*	*C. maxima*	*C. metuliferus*	*M. balsamina*
3.84	N-octane	-	-	-	7.83	-
6.37	N-nonane	-	-	3.35	9.00	-
8.48	5-Oxazolidinone, 3-benzoyl-2-(1, 1-dimethyl)-4-phenyl methyl	-	-	-	2.36	-
8.70	3-methyl nonane	-	-	-	2.11	-
8.9 8	Isoprophylbenzene	-	-	1.52	4.45	-
9.67	N-decane	-	-	4.08	9.37	-
12.34	Octahydro4, 7-methano indene	-	-	-	1.98	-
12.97	N-undecane	-	-	-	2.44	-
14.21	Cyclobutylbenzene	-	-	-	1.51	-
14.77	4-Phenylbut-3-ene-1-yne	-	-	-	1.91	-
18.07	1, 6-methano (10) annulene	0.72	3.61	6.53	14.31	-
18.49	1-ethylidene	0.57	-	-	-	-
21.43	1-phenyl-3-methyl penta-1, 2, 4-triene	-	-	-	0.91	-
23.25	phenol -4, 6-di (1, 1-dimethyl ethyl)-2-methyl	-	-	-	0.86	-
24.62	3, 7-dimethyl decane	-	-	-	1.10	-
26.50	Heptane, 3, 3-methyl	-	-	-	0.71	-
27.36	Palmitic acid	15.69	23.03	13.95	8.49	3.23
27.62	N-hexadecanoicacid	-	3.41	-	-	14.08
28.60	Linoleic acid methyl ester	63.72	-	-	-	9.79
28.71	Stearic acid	15.01	13.12	8.77	-	-
28.75	Oleic acid	1.92	51.94	28.98	22.25	-
28.98	Cis-cis linoleic acid	-	-	-	-	62.03
29.57	Alpha-linolenic acid	-	-	-	-	4.43
30.00	9-octadecenamide	0.92	1.25	2.78	0.61	1.51
30.59	N-1-Benzyl-N-2 (benzylidenyl-benzyl amino) benzamidin	-	-	-	-	0.75
30.64	Decamethyl enediol	-	-	-	0.68	-
31.93	Linoleic acid chloride	-	-	0.54	-	-
31.98	2-hydroxy-1-(hydroxymethyl) ethyl ester	1.45	-	-	-	-
32.00	Glycerol-1-monolinolate	-	-	-	-	2.64
33.34	Squaline/spinacene	-	3.64	29.50	7.12	1.52

[1]Retention time (RT) on DB-WBX column in GC-MS

acid in *L. aegyptiaca* (63.72%) was in close agreement with the values reported in 64.61 % melon seed (Sena *et al*., 1998) but higher than that of *C. pepo*. The content of Oleic acid content of 37.8 in melon seeds was lower than 51.94% obtained in *C. lanatus* but higher than 1.94, 28.98 and 22.25%, respectively in *L. aegyptiaca*, *C. maxima* and *C. metuliferus*. The amounts of the two fatty acids that are essential for humans, namely linoleic acid and a-linolenic acid found in *L. aegyptiaca* and *M. balsamina* were comparatively higher than those reported in western Sahel (Cook *et al*., 2000). The major fatty acids identified in this study were linoleic acid, oleic acid, palmitic acid and fatty acids and their derivatives have been described as multifunctional molecules that have a wide range of applications (Bodalo *et al*., 2005). From the results obtained in this study, the wild species studied could play a significant role in the quest for essential amino acids and fatty acids from wild plant materials that could be very promising for incorporation into the food items with the aim of ameliorating the problem of malnutrition especially among the developing nations like Nigeria. Some of the volatile compounds identified could also be useful in pharmaceutical and chemical industries.

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