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Evaluation and comparison of the Amino acid composition of three varieties of *Lagerena siceraria* melon seed flours

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ABSTRACT
Amino acids of three varieties of *Lageneria siceraria* seed flours were determined using standard methods of analysis. The three varieties are rich in essential amino acids needed for human health and growth, with a mean of 53.15%. Sulphur amino acid and aromatic amino acid of the seeds ranged from 17.06 mg/g protein to 24.10 mg/g protein and from 80.22mg/g protein to 89.00 mg/g protein respectively. The varieties of these melon seed flours are closely related; however, there are significant differences (P≥0.05) in Cystine, showing that the varieties have some genetic differences.
Key Words: *Lageneria siceraria* seed flours, Essential amino acid, Genetics, Aginine, Cystine.

1. INTRODUCTION

Protein quality and availability are functions of the amino acid present in food, and the amino acid composition determines the nutritive value of plant protein products (Johnson & Lay,1974; Alejandro & Sheikh, 1994). *Lageneria siceraria* melon seed belong to the family Cucumeropsis otherwise called Cucurbitaceae which are versatile and include hundreds of species of vine bearing coiled climbing tendrils and some of the most unusual fruits in the world (Wayne, 1994). They are generally called gourds seeds and are cultivated mostly for utility purposes rather than for consumption. The seeds are melons and are consumed by rural dwellers in the Southern part of Nigeria among the Yorubas as soup thickeners (Ogundele, & Oshodi, 2010). Melons are a major food crop in many tropical and subtropical parts of the world, especially in the sub-Saharan Africa (Mabaleha et al., 2006). The nutritional value of melon seeds compare favourably with those of soybean, sunflower seed and groundnut (Wicken et al., 1984). *Lageneria siceraria* melon seeds are underutilized and are scarcely planted for consumption probably because of the difficulty of shelling and the ignorance of the nutritive potentials of the seeds. However, researchers have recently been attracted to explore the potentials of the seeds. Analysis of the proximate and functional properties of bottle gourd *Lageneria siceraria* melon seed was reported on (Fokou, et al.2004), while the amino acid and protein fraction of the bottle gourd variety of *Lageneria siceraria* had been worked on by Ogunbusola et al., 2010. Report on the proximate and functional properties of three varieties of *Lageneria siceraria* melon seed flours by Ogundele, & Oshodi, 2010 showed that the three varieties of *Lageneria siceraria* melon seeds are very rich in protein and fat. Hence it is therefore essential to determine the different types and quantities of the amino acid and three varieties of *Lageneria siceraria* seeds. This paper therefore reports on the similarities and genetic differences in the amino acid compositions of three varieties of *Lageneria siceraria* melon seeds and their possible nutritive importance and applications.

2. MATERIALS AND METHODS

2.1 Sample Preparation

The three varieties of *Lageneria siceraria* melon seeds analysed in this research work are *Lageneria siceraria* African Winekettle Gourd(AWK) melon seed, *Lageneria siceraria* Basket Ball Gourd (BBG) melon seed and *Lageneria siceraria* Bushel Giant Gourd (BGG) melon seed as seen in figures 1-3. The seeds were purchased from Ilora in Oyo town, Nigeria and were identified by Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria and backed up by the following authors: (Brands, 1992; Omotoye, 1984). The seeds were dehusked, dried and grinded into fine flour.

2.1.1 Amino Acid Analysis

The flour samples were used for amino acid analysis. The samples were defatted, for at least 8hours with chloroform/methanol (2:1 mixture) using soxhlet extractor apparatus, hydrolyzed with 6N HCl and evaporated in a rotatory evaporator for 22 hours at 104-110^{o}C. The HCl was removed after hydrolysis in vacuum and the residue then injected into and analysed in the Amino Acid Analyzer (Technicon Instrument Co. Ltd., United

Kingdom), (Adeyeye & Afolabi 2004). Amino acid scoring was done according to (Oshodi et al., 1994). Evaluations of the amino acid profile were carried out are as follows:

$$\text{Amino acid score} = \frac{\text{mg of amino acid per g N in test protein}}{\text{mg of amino acid per g of N in reference}}$$

Total Sulphur Amino Acid (TSAA) = Methionine + Cystine
Total Aromatic Amino Acids (TArAA) = Phenylalaline + Tyrosin
Protein Efficiency Ratio (PER) = 0.498 + 0.454 (Leucine) – 0.105 (Tyrosine)
(Alsmeyer et al., 1974).

2.1.2 Statistical analysis

Statistical analysis was carried out on replicate analysis using SPSS 15 one way ANOVA with a significance level of $P \leq 0.05$. The results are expressed as mean±standard deviation.

3. RESULT AND DISCUSSION

3.1 Protein and fat contents of varieties of Lageneria siceraria melon seed

The proximate analysis of the three varieties of *Lageneria siceraria* melon seed flours reported by Ogundele and Oshodi, 2010 are seen in table 1. Protein and fat contents of the seeds are quite appreciable with values (g/100g) ranging from 27.71±0.41 (*Lageneria siceraria* AWK) to 34.64±0.08 (*Lageneria siceraria* BBG) and 46.03 ±1.14 (*Lageneria siceraria* BBG) to 53.35±0.24 (*Lageneria siceraria* AWK) respectively. The average values (g/100g) of the protein and fat contents of the three varieties of *Lageneria siceraria* melon seed flours are therefore 31.68±2.92 and 50.10±3.04 respectively.

3.2 Amino acid contents of varieties of Lageneria siceraria melon seeds

Tables 2 to 4 show the results of the amino acid content, amino acid scoring and the summary of the amino acid composition of the three varieties of *Lageneria siceraria* melon seed flours. The result of the amino acid content of the three varieties of *Lageneria siceraria* melon seeds in table 2 shows that the most abundant amino acid in *Lageneria siceraria* melon seeds is glutamic acid with an average value of 162.12 mg/g protein, followed by aspartic acid (100.28) mg/g protein mean value. Aspartic acid is needed for stamina, brain and neural health and assists the liver by removing excess ammonia and other toxins from the bloodstream. It is also very important in the functioning of RNA, DNA, as well as the production of immunoglobulin and antibody synthesis (Sallamander , 2012). Aspartic acid and glutamic acid have their iso-electric points at pH 3.0 and 3.1 respectively. The remaining amino acids have their iso-electric points at pH values greater than 4.5 (Oshodi et al., 1994). The high values of aspartic acid and glutamic acid in the protein of these varieties of *Lageneria siceraria* as observed, accounts for the iso-electric points of *Lageneria siceraria* melon seeds varieties occurring at pH 3-5. This is consistent with the pH 3 to pH5 minimum solubility points reported for the three varieties of *Lageneria siceraria* melon seeds Ogundele and Oshodi, 2010. The least abundant amino acid on the other hand is cystine followed by methionine with average values of 9.55 mg/g protein and 12.38 mg/g protein respectively. The values of cystine in the three varieties are significantly different with coefficient of variation (0.59). *Lageneria siceraria* melon seeds varieties have been reported to be good sources of protein (Ogundele and Oshodi, 2010). The total amino acid (TAA) mg/g protein of the three varieties of *Lageneria siceraria* melon seeds is equally high due to the high protein content of the seeds and they ranges from 792.80 mg/g protein (*Lageneria siceraria* BBG) to 859.00 mg/g protein (*Lageneria siceraria* AWK). These values are higher than the TAA (6758 mg/g protein) for *Luffa cylindrica* (Olaofe et al., 2008) and compete favorably with the total amino acid for some plant foods which range between 393mg/g – 765mg/g protein as reported by some researchers (Akobundu et al., 1982; FAO/WHO/UNU, 1985; Olaofe et al., 1994 and Ogungbenle, 2006).

The total essential amino acids (TEAA) of *Lageneria siceraria* melon seeds are *Lageneria siceraria AWK* (455.97) mg/g protein, *Lageneria siceraria BBG* (401.06) mg/g protein and *Lageneria siceraria BGG* (443.93) mg/g protein with an average of 433.65 mg/g protein which is equivalent to 53.15% of the whole amino acids. These results compete favorably with the total essential amino acid of soy bean 444 mg/g, whole and defatted *Adenopus breviflorus benth* seed (53.70%) and (51.40%) respectively (Oshodi, 1996). Amino acid reference values were used to compare the amino acid contents of *Lageneria siceraria* melon seeds as amino acid scoring in table 3 (FAO/WHO/UNU, 1985). The amino acid scoring got from this shows that the first limiting amino acids are the sulphur amino acids cystine + methionine for *Lageneria siceraria* (AWK) seeds while threonine is the limiting amino acid in the other two varieties of *Lageneria siceraria* melon seeds. The total sulphur amino acid TSAA (cystine + methionine) in Table 4 ranges from 17.06 mg/g protein to 24.90 mg/g protein with a mean value of 21.95 mg/g protein. The percentage of cystine in the sulphur amino acid on the average is 44.20%. The

TSAA of *Lageneria siceraria* melon seed are comparable with the TSAA of (2.50g/100g) for *Luffa cylindrica* (Olaofe et al., 2008) and lower than the 5.8g/100g recommended for infants (Salunkhe & Kadam, 1998). Total Aromatic essential amino acid (tyr.+ phen.) is however higher, with an average value of 83.72 mg/g protein. The total acidic and total basic amino acid averaged 29.33mg/g protein and 15.93 mg/g protein respectively. This indicates that the proteins of *Lageneria siceraria* melon seeds are slightly acidic in solution.

The predicted protein efficiency ratio (PER) is one of the quality parameters used for protein evaluation (FAO/WHO, 1991). The Protein efficiency ratio of the three varieties of *Lageneria siceraria* ranges from 2.13 to 2.98 with a mean value of 2.65. These are higher than the PER recorded for some legumes and concentrates like *Lathyrus sativus* (1.03) (Aremu et al., 2007), *Luffa cylindrical* (1.49) (Oshodi et al., 1993); *Phaseolus coccineus* (1.91) (Robinson, 1987) and comparable with that of *Prosopis africana* (2.3) (Aremu et al., 2008).

In conclusion, the amino acid contents of the three varieties of *Lageneria siceraria* are closely related in values but there are noticeable differences among the values of some amino acids. This is especially more pronounced in cystine contents which are significantly different with $p \geq 0.05$. However, there are also significant differences in some other amino acids in the varieties of *Lageneria siceraria* such as phenylalanine, leucine, methionine, valine, alanine, glycine, proline, threonine and arginie with coefficients of variations (16.76, 12.70, 27.14, 17.50, 14.17, 27.34, 29.65, 19.26 and 14.31)% respectively. All these account for the noticeable genetic differences in the shapes of the seeds and fruits of this specie. The high protein, amino acid as well as essential amino acid contents of the three varieties of *Lageneria siceraria* makes them good sources of protein that can compete favorably with soy protein, suitable to supply essential amino acids for proper growth of children and to also serve as a substitute for animal proteins.

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Table 1: Proximate composition (g/100g) of varieties of *Lagenaria siceraria* seed flours

Composition	*L. sceraria*(AWK)	*L. siceraria* (BBG)	*L. siceraria* (BGG)	Mean
Moisture	5.67±0.05	5.13±0.04	5.67±0.09	5.49±0.22
Fat content	46.03±1.14	53.35±0.24	50.91±1.28	50.10±3.04
Crude Protein	34.64±0.08	27.71±0.41	32.70±0.03	31.68±2.92
Crude Fibre	1.61±0.25	0.75±0.15	1.10±0.12	1.15±0.69
T. Ash	3.75±0.14	4.07±0.22	4.50±0.18	4.11±0.31
Carbohydrate	8.29±1.04	8.99±0.49	5.12±1.36	7.47±1.68

Ogundele J.O. and Oshodi A.A., 2010

Table 2: Amino Acid Profile of varieties of *Lageneria siceraria* seed flour (mg/g protein)

Amino acid	Sample			$\bar{X}$	SD	CV (%)
	LS(AWK)	LS(BBG)	LS(BGG)			
*Lysine	50.35±0.92[b]	41.95±1.76[a]	40.90±0.14[a]	44.40	4.72	10.63
*Histidine	31.55±0.92[b]	27.00±1.13[a]	25.20 ±0.42[a]	27.92	3.00	10.75
*Arginie	51.85±1.48[a]	55.90±0.99[b]	69.75±1.77[c]	59.17	8.47	14.31
Aspartic	102.85±1.48[b]	100.07 ±0.99[b]	97.30±0.99[a]	100.28	2.66	2.65
*Threonine	35.25±1.20[c]	22.80±0.99[a]	31.20 ±0.42[b]	29.75	5.73	19.26
Serine	45.85±1.20[b]	41.90±1.27[b]	37.30±0.99[a]	40.68	2.86	7.03
Glutamic	170.20±2.40[b]	163.00±4.24[b]	153.15±5.16[a]	162.12	8.29	5.11
Proline	18.90±2.26[a]	33.70±1.27[b]	20.95±0.64[a]	24.52	7.27	29.65
Glycine	23.85±0.49[a]	22.15±0.78[a]	37.05±2.42[b]	27.68	7.40	27.34
Alanine	42.10±0.99[b]	30.85 ±0.92[a]	35.20±0.72[**b**]	36.05	5.11	14.17
*Cystine	8.58±2.86[a]	10.40 ±4.96[b]	9.68±4.13[ab]	9.55	3.26	59.27
*Valine	60.35±1.63[c]	45.80±0.99[b]	41.95±1.20[a]	49.95	8.74	17.50
*Methionine	8.48±2.44[a]	14.50 ±2.05[b]	14.20±0.85[b]	12.38	3.36	27.14
*Isoleucine	1.80±0.42[b]	36.45±0.92[a]	40.55±0.49[b]	39.60	2.55	6.44
*Leucine	86.80±0.42[c]	65.60±0.85[a]	81.70±1.27[b]	78.03	9.92	12.70
*Tyrosine	46.65±1.34[b]	37.85±2.62[a]	37.75±0.49[a]	40.75	4.76	11.68
*Phenylalanine	35.30±0.71[a]	42.35 ±1.77[b]	51.25±0.35[c]	42.97	7.20	16.76

Values with different superscriptions on the same row are significantly different $p \leq 0.05$

*Essential Amino acid; X= mean; SD=standard deviation; CV= coefficient of variation

Table 3: Amino Acid score of varieties of *Lageneria siceraria* seed flour

Amino Acid	Sample				$\bar{X}$	SD	CV(%)
	LS(AWK)	LS(BBG)	LS(BGG)	*Reference			
Lysine	0.15	0.12	0.12	340.00	0.30	0.02	6.67
Threonine	0.10	0.07	0.09	350.00	0.87	0.02	2.30
Cys.+Meth.	0.08	0.11	0.11	220.00	0.10	0.02	20.00
Valine	0.19	0.15	0.14	310.00	0.16	0.03	18.75
Isoleucine	0.17	0.15	0.16	250.00	0.16	0.01	6.25
Leucine	0.20	0.15	0.19	440.00	0.18	0.26	144.44
Phe.+Tyrosine	0.22	0.21	0.23	380.00	0.22	0.01	4.55
1st LAAA	C+M	Threonine	Thre.				
2nd LAAA	Thre.	C+M	C+M				

*Reference, is scoring reference according to Pellet & Young, 1980 (Oshodi et.al., 1994).

X= mean; SD=standard deviation; CV= coefficient of variation

Table 4: Summary of amino acid composition of *Lagenaria siceraria* seed flours

	Sample			$\bar{X}$	SD	CV%
Amino acid	LS (AWK)	LS(BBG)	LS (BGG)			
TAA	859.72	792.28	825.13	825.63	33.10	4.01
TEAA	455.97	401.06	443.93	433.65	28.86	6.66
TEAA/TAA (%)	53.04	50.62	53.79	53.15	2.59	4.82
TNEAA	403.75	391.22	381.20	392.06	11.30	2.88
TNEAA/TAA (%)	46.96	49.40	46.20	47.52	1.67	3.51
TSAA	17.06	24.90	23.88	21.95	4.26	19.41
%Cystine in TSAA	50.29	41.77	40.54	44.20	5.31	12.01
TArEAA	81.95	80.20	89.00	83.72	4.66	5.57
%TAAA	24.43	33.20	30.35	29.33	4.47	15.24
TBAA	15.56	15.76	16.46	15.93	0.47	2.95
TNAA	60.01	51.04	53.19	54.77	4.68	8.54
TEAA/TNEAA	1.13	1.03	1.16	1.11	0.07	6.31
PER	2.98	2.13	2.84	2.65	0.46	17.36

TAA=Total amino acids; TEAA=Total essential amino acids; TNEAA=Total non essential amino acids; TSAA= Total sulphur amino acid= Cys+Meth; TArEAA= Total essential aromatic acid=phe.+tyr; TAAA=Total acidic =Glu. + Asp. TBAA=Total basic amino acid=Lys +Arg.+His;TNAA= Total neutral amino acids TNAA; PER= Predicted protein efficiency ratio; X= mean; SD=standard deviation; CV= coefficient of variation

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