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Determination and Comparison of the Amino Acid Composition of Seventeen *Lagenaria siceraria* Varieties and One Variety of *Citrullus colocynthis* Seed Flours

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Abstract: Seeds from 17 varieties of *Laganaria siceraria* and 1 variety of *Citrullus colocynthis* were analyzed for their proximate amino acids composition. These seeds were produced on the Abdou Moumouni University, Faculty of Sciences and Technology, experimental field. The seeds showed significant difference in their protein content: *Lagenaria siceraria* (29.45 g/100 g), *Citrullus colocynthis* (12.40 g/100 g) but were closely related in their amino acids profile. Glutamic, aspartic acids and arginine were the most abundant amino acids while Total Sulphur Amino Acids (TSAA = methionine + cystine) were the limiting amino acids. Leucine was the most concentrated essential amino acid. The percentages of essential amino acids of the seeds were also closely related: *Lagenaria siceraria* (42.85%) and *Citrullus colocynthis* (41.51%). Despite a similar amino acids profile, the chemical concentrations of each amino acid were significantly different between varieties ($p<0.001$) exception of leucine ($p = 0.06$), showing that the varieties have some genetic differences. In *L. siceraria* seeds, TSAA ranged from 3.26% to 4.35% and Total Aromatic Amino Acids (TAAA) ranged from 27.14 to 30.55%. In *C. colocynthis* seeds the concentrations were 4.5 and 27.99%, respectively. When comparing the amino acids in these varieties with the recommended FAO/WHO provisional pattern, the seeds were adequate in histidine, TAAA, threonine and TSAA but supplementation was required for lysine in all varieties and for isoleucine, leucine and valine in some varieties. Predicted Protein Efficiency Ratios (PER) was 1.98 in *C. colocynthis* and ranged from 1.81 to 2.33 in *L. siceraria*.

Key words: *Lagenaria siceraria* seed, *Citrullus colocynthis* seed, essential amino acid, Niger, lysine

INTRODUCTION

Studies on the use of plant proteins continue to globally cause a particular attention, with the growing demand of vulnerable groups. The projections based on conventional sources show a gap between demographic growth and the needs in proteins. It's therefore necessary to examine non conventional oleaginous seeds as alternative sources of protein for the future (Olaofe and Adeyeye, 2009). Niger Republic, a Sahel country, is facing such a serious situation. Protein-energy malnutrition, vitamin and mineral deficiencies constitute a major public health problem (Wuehler and Biga, 2011). Indeed, the various Demographic and Health Multiple Indicator Surveys showed that chronic undernutrition constitutes a public health problem. Women of childbearing age, Children and young people in growth are the main victims (Wuehler and Biga, 2011; Attama *et al*., 1999).

Niger has therefore set up a national nutrition policy whose overall objective was to ensure that every Niger citizen and especially the vulnerable populations, a nutritional status compatible with good health, optimal productive life and able to reduce maternal, infant and child morbidity and mortality. In rural areas where more than 80% of the populations live, diet is mainly cereals. Animal protein consumption is exceptional or casual because of their cost.

Indeed, according to UNDP (2012), 60% of Niger citizens live on less than a dollar a day, which does not allow access to most animal protein, yet deemed to be of good nutritional value. It is therefore urgent to investigate potential sources of protein of good nutritional value accessible to these populations and to achieve this goal we were interested in the seeds of Cucurbitaceae. Though several studies have investigated the pharmacological potential (Habibur, 2003; Prajapati *et al*., 2010; Mayakrishnan *et al*., 2013) and the different levels of crude nutrients (Nmila and Al, 2002; Ponka *et al*., 2005; Sabo *et al*., 2005a, 2005b; Sadou *et al*., 2007; Ullah *et al*., 2012) rare were those who are interested

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in the fine amino acid composition including essential amino acids. Such a study is, however, a prerequisite for their use against the protein-energy malnutrition and rebalancing the diets of undernourished populations.
We focused our interest on the seeds of 17 varieties of Lagenaria siceraria (Molin.) Standl and 1 variety of Citrullus Colocynthis (linn.) Schrad belonging to the cucurbitaceea family commonly consumed in Niger. In our previous studies we were interested in the physicochemical characteristics and the total chemical composition of the seeds of several species of this family (Sabo *et al*., 2005a, 2005b; Sadou *et al*., 2007). The present study is focused on the amino acid contents as well as the nutritional values of seeds proteins of the 18 studied varieties.

MATERIALS AND METHODS

Plant material: It was composed of 18 varieties of which 17 varieties (LS1 to LS17) of *Lagenaria siceraria* and one variety (CC) of *Citrullus colocynthis*. They are very varied. Berries can be large or small, of varied shapes (calabash, gourd, ladle.) and smooth or rough appearance. Seeds analyzed in this study were produced at the experimental site of the Faculty of Science and Technology of Abdou Moumouni University of Niamey (13°30' N; 2°08'E; 216 a.s.l).

Seeds preparations: The fruits were cut in half then the seeds embedded in the pulp were collected. *Lagenaria siceraria* varieties seeds were then crushed, husks removed and kernels recovered. *C. colocynthis* seeds were small, difficult to handle and the body wall firmly bound in the kernel. These seeds were therefore used whole. Finally, kernels and seeds were crushed for subsequent analyzes.

Amino acid analysis: The flour samples were used for amino acid analysis. The samples were defatted, for at least 8 h with chloroform/methanol (2:1 mixture) using soxhlet extractor apparatus, hydrolyzed with 6N HCl and evaporated in a rotatory evaporator for 22 h at 104-110°C. The HCl was removed after hydrolysis in vacuum and the residue then injected into and analyzed in the Amino Acid Analyzer (Technicon Instrument Co. Ltd., United Kingdom), (Adeyeye and Afolabi, 2004; Ogundele *et al*., 2013).
Biological values of defatted *Lagenaria Siceraria* and *Citrullus colocynthis* seed flours were determined on the basis of the amino acid profiles. Amino acid score was calculated for each essential amino acid in a given test protein using the FAO/WHO/UNU (2002) reference pattern:

$$\text{Amino acid score} = \frac{\text{mg of amino acid in 1 g of test protein}}{\text{mg of amino acid in 1 g reference}}$$

Predicted Protein Efficiency Ratio (PER) was estimated according to the regression equation proposed by Alsmeyer *et al*. (1974):

P-PER = -0.468+0.454 (Leucine)-0.105 (Tyrosine)

1: Total Sulphur Amino Acid (TSAA) = Methionine + Cystine
2: Total Aromatic Amino Acid (TAAAromatic) = Phenylalaline + Tyrosin
3: Total Acid Amino Acids (TAAA) = Aspartate + Glutamate
4: Total Basic Amino Acide (TBAA) = Lysine + Arginine + Histidine
5: Total Aliphatic Amino Acide (TAA Aliphatic) = Glycine + Valine + Leucine + Isoleucine + Alanine
6: Total Alcool Amino Acids (TAA Alcool) = Threonine + Serine
7: Total Sulfur Amino Acids (TSAA) = Cysteine + Methionine

Statistical analysis: An analysis of variance using the Statitix software has been used for all the parameters. In case of significant difference between the varieties, averages comparison tests (Tukey HSD test) have been carried out for the concerned compounds in order to determine the groups of homogeneous varieties.

RESULTS AND DISCUSSION

The crude protein content of each of the 18 studied varieties is expressed in Table 1. The seeds crude protein content of the 17 varieties of *Lagenaria siceraria* ranged from 21.64 to 34.71 g/100 g of seeds. Recently it was reported a crude protein content ranging from 27.71±0.41 to 34.64±0.08 g/100 g in the seeds of three varieties of *Lagenaria siceraria* (Ogundele *et al*., 2013). This indicated that Lagenaria siceraria seeds are good sources of protein. The consumption of *Lagenaria siceraria* will therefore go a long way to meet the daily protein need of man especially of children and can also be used in supplementing animal foods that are low in protein content. Indeed the crude protein value compared favorably with high protein seeds and legumes like soybeans (35%) and cowpea (22.7%);

Table 1: Proximate protein composition (g/100 g) of *Lagenaria siceraia* varieties kernels and the whole seed of *Citrullus colocynthis*

Variety	Protein	Variety	Protein
LS1	30.45±2.29[ab]	LS10	26.73±3.36[ab]
LS2	30.33±4.04[ab]	LS11	21.29±0.69[bc]
LS3	25.20±2.29[abc]	LS12	29.83±1.82[ab]
LS4	21.64±0.71[abc]	LS13	29.15±1.50[ab]
LS5	34.71±2.33[ab]	LS14	35.21±0.21[a]
LS6	31.13±1.39[ab]	LS15	30.57±2.21[ab]
LS7	29.02±2.02[ab]	LS16	28.44±0.50[ab]
LS8	34.33±17.19[ab]	LS17	31.48±0.52[ab]
LS9	31.06±0.35[ab]	Mean	29.45±3.97
		CC	12.40±1.75[c]

LS: *Lagenaria siceraria*
CC: *Citrullus Colocynthis*
There is no significant difference between
Means that have the same letters in each column

Table 2: Analysis of essential and non essential amino acids (g/100 g crude protein) of *Lagenaria siceraria* and *Citrullus colocynthis* varieties seed flours

Varieties	TAA	TNEAA (%)	TEAA (%)	TAAA	TAA Alcool	TAA Aliphatic	TAA Aromatic	TBAA	TSAA
LS1	74.19	57.70	42.30	28.98	9.25	29.05	8.87	16.28	3.84
LS2	77.04	56.63	43.37	28.84	8.58	29.36	9.55	15.91	4.06
LS3	73.90	57.47	42.53	28.43	9.34	28.76	9.32	16.39	4.03
LS4	81.03	57.56	42.44	27.48	9.55	29.36	8.64	17.24	3.96
LS5	79.41	56.97	43.03	27.41	9.52	29.42	9.00	17.18	3.82
LS6	70.71	56.87	43.13	28.07	9.02	29.60	8.53	16.43	3.93
LS7	69.67	56.12	43.88	28.29	9.39	29.09	9.04	16.02	4.35
LS8	75.87	56.93	43.07	28.00	9.15	29.43	9.56	16.50	3.85
LS9	72.32	57.73	42.27	30.16	8.79	29.09	9.25	15.58	3.58
LS10	82.73	56.61	43.39	27.14	9.17	29.48	8.97	17.31	4.23
LS11	73.21	57.46	42.54	29.34	9.14	29.97	8.88	15.33	3.82
LS12	76.46	57.44	42.56	27.43	9.51	30.41	8.02	16.95	4.05
LS13	69.63	58.37	41.63	30.55	9.58	27.52	9.48	15.54	3.69
LS14	70.62	56.33	43.67	28.33	10.01	28.59	8.57	16.45	4.28
LS15	71.63	57.03	42.97	29.46	9.16	29.82	8.64	15.73	3.77
LS16	71.75	56.61	43.39	29.49	9.06	29.44	9.35	15.54	3.61
LS17	72.09	57.41	42.59	30.00	8.39	28.99	9.47	16.49	3.26
CC	77.53	58.49	41.51	27.99	9.73	28.96	8.14	17.39	4.05

LS: *Lagenaria siceraria* CC: *Citrullus Colocynthis*

however, it is higher than others such as lima beans (19.8%) and chickpeas (19%) (Oshodi *et al*., 1993). The protein content of *Citrullus colocynthis* seeds observed was 12%. This value ranged between the 8.5% and 24.37% as previously reported (Gurudeeban *et al*., 2010; Ogundele *et al*., 2012). It is important to note that, *Citrullus colocynthis* seeds contained 2 to 3 times less protein than that of *Lagenaria siceraria* seeds.

In order to assess the nutritional potential of proteins, their amino acid contents were given. The variance analysis presented in Table 2 shows that the amino acid contents of the studied varieties were significantly different ($p<0.001$) except for leucine ($p = 0.06$). All the varieties being cultivated on the same ground and under the same conditions this variability of the amino acid contents could be of genetic origin.

In Table 2, amino acids were gathered according to the chemical nature of their side. Total aromatic amino acids (TAAA) and Total aliphatic amino acids (TAA aliphatics) representing about 58% of total amino acids. Total basic amino acids (TBAA), total aromatic amino acids (TAAA) and total alcool amino acides (TAA alcool) representing about 34% of total amino acids with 16, 9 and 9%, respectively. Total sulphur amino acids (TSAA) and the proline represented 4% each.

Table 2 also revealed that, the total amino acids (TAA) contents of the seeds of the 17 varieties of *Lagenaria siceraria* ranged from 69.65 (LS1) to 82.72 g/100 g protein with an average of 74.25 g/100 g protein. These TAA contents are high taking into account the high protein contents of these seeds. However, these values are relatively lower than those previously reported from 3 varieties of *Lagenaria siceraraia* in Nigeria (Ogundele *et al*., 2013b) but compete favorably with the TAA for some plant food which ranged from 39.3 g/100g to 76.5 g/100 g protein (Akobundu *et al*., 1982; Ogungbenle, 2006; Olaofe *et al*., 2008). The TAA of *Citrullus colocynthis* seeds was also higher (77.53 g/100 g protein) and compete favorably with the variety of Nigeria (73.38 g/100 g protein). However contents in crude protein are two times less high (Ogundele, 2012). This high TAA content explains probably why these seeds are actively harvested during the periods of starvation. In all cases they could be potential sources of amino acids for stripped populations or used in the foods fortification.

The total essential amino acids (TEAA) without arginine was 32.18 g/100 g protein in *Citrullus colocynthis* seeds and ranged from 29 g/100 g (LS13) to 35.90 g/100 g (LS10) with an average of 31.82 g/100 g protein in *Lagenaria siceraria* seeds. The contents of TEAA are also high and deserve a detailed attention. Percentage ratios of TEAA acids to TAA (E/T%) for *Lagenaria siceraria* and for *Citrullus colocynthis* seeds were respectively 42.85 and 41.51% without arginine and 51.53 and 51.15% with arginine. These values were above 36% which is considered adequate for an ideal protein (FAO/WHO, 1973) and similar to that reported for *Citrullus colocynthis* (52.73%) and *L. siceraria* (53.15%) (Ogundele *et al*., 2012; 2013a). They are also comparable with selected oil seeds (melon, pumpkin and gourd seeds) which ranged from 33.3 to 53.6% (Ige *et al*., 1984; Olaofe *et al*., 1993, 1994). The Total Sulphur Amino Acids (TSAA) and Total Aromatic Amino Acids (TAAA) were higher than TSAA of 2.50 g/100 g and the TEAA of 3.25 g/100 g for *Luffa cylindrica* (Olaofe *et al*., 2008).

Tables 3a and 3b present the proximate amino acids composition of the seeds protein. Glutamic, aspartic acids and arginine were the most plentiful amino acids while cystine, methionine, histidine and threonine were the most limiting amino acids in all studied varieties. Glutamic, aspartic acids and arginine content was 29.47 g/100 g protein in *Citrullus colocynthis* seeds and ranged from 25.37 (LS7) to 30.11 g/100 g protein with an average of 27.70 g/100 g protein in *Lagenaria siceraria* varieties seeds which represented, respectively 38%

Table 3a: Non essential amino acids profile of the seeds kernels of 17 varieties of *Lagenaria siceraria* (LS) and the seeds of 1 variety of *Citrullus colocynthis* (CC) (g/100 g protein)

	Alanine	Arginine	Aspartate	Glutamate	Glycine	Histidine*	Proline	Serine
LS1	3.85±0.12^{defg}	6.6±0.177^{de}	8.94±0.13^{a}	12.56±0.11^{e}	4.45±0.13^{ef}	2.15±0.14^{abc}	2.77±0.10^{cd}	3.64±0.09^{defg}
LS2	3.91±0.12^{cdefg}	6.44±0.7^{ef}	8.65±0.08^{abcd}	13.57±0.12^{a}	4.54±0.14^{def}	2.26±0.08^{abc}	2.84±0.07^{abcd}	3.68±0.10^{a}
LS3	3.66±0.08^{fg}	6.64±0.08^{de}	8.35±0.11^{ef}	12.66±0.08^{e}	4.66±0.09^{cde}	2.12±0.12^{abc}	2.76±0.12^{abcd}	3.74±0.08^{cde}
LS4	4.14±0.09^{ab}	7.84±0.07^{a}	8.84±0.06^{abc}	13.43±0.08^{ab}	5.23±0.1^{a}	2.22±0.11^{abc}	3.05±0.07^{ab}	4.11±0.12^{ef}
LS5	4.1±0.08^{bcde}	7.56±0.09^{b}	8.63±0.1^{bcde}	13.14±0.08^{bc}	4.95±0.08^{abc}	2.32±0.15^{ab}	2.9±0.08^{abc}	3.96±0.08^{cde}
LS6	4.04±0.07^{bcde}	5.62±0.09^{j}	7.93±0.08^{i}	11.92±0.07^{fg}	4.32±0.11^{fg}	2.26±0.1^{abc}	3.12±0.11^{a}	3.26±0.11^{f}
LS7	3.76±0.12^{efg}	5.66±0.10^{j}	8.05±0.07^{ghi}	11.66±0.10^{g}	3.65±0.08^{j}	2.06±0.07^{abc}	2.66±0.10^{bcd}	3.66±0.11^{f}
LS8	3.84±0.10^{defg}	6.87±0.09^{cd}	8.47±0.11^{def}	12.77±0.11^{de}	4.87±0.1^{bcd}	2.21±0.12^{abc}	2.67±0.11^{bcd}	3.7±0.12^{def}
LS9	3.94±0.05^{cdef}	6.07±0.07^{gh}	8.24±0.11^{fgh}	13.57±0.11^{a}	4±0.08^{gh}i	2.04±0.08^{bc}	2.56±0.11^{cd}	3.37±0.09^{cde}
LS10	4.38±0.18^{ab}	7.85±0.11^{a}	8.88±0.09^{ab}	13.57±0.16^{a}	5.06±0.07^{ab}	2.34±0.12^{ab}	3.06±0.06^{ab}	4.03±0.09^{cde}
LS11	4.54±0.23^{a}	5.83±0.05^{hij}	8.34±0.12^{efg}	13.14±0.08^{bc}	4.3±0.24^{fgh}	1.95±0.07^{c}	2.58±0.16^{cd}	3.34±0.13^{bcd}
LS12	4.04±0.07^{bcde}	7.15±0.01^{c}	7.84±0.08i	13.13±0.12^{bc}	5.07±0.09^{ab}	2.24±0.13^{abc}	2.78±0.08^{abcd}	3.91±0.04^{def}
LS13	3.57±0.10^{gh}	5.65±0.09^{j}	8.57±0.09^{cde}	12.7±0.07^{e}	3.97±0.06^{hi}j	1.94±0.16^{c}	2.54±0.11^{cd}	3.64±0.09^{cdef}
LS14	3.27±0.17^{h}	6.25±0.08^{fg}	7.96±0.09^{hi}	12.05±0.08^{f}	3.96±0.14^{ij}	2.25±0.07^{abc}	2.66±0.10^{bcd}	3.63±0.08^{def}
LS15	4.22±0.12^{abc}	5.76±0.07^{ij}	7.98±0.11^{hi}	13.12±0.12^{bc}	3.87±0.11^{ij}	2.25±0.09^{ab}c	2.45±0.10^{d}	3.45±0.10^{ef}
LS16	3.98±0.04^{cdef}	5.76±0.09^{ij}	8.04±0.07^{hi}	13.12±0.12^{bc}	3.93±0.05^{ij}	2.12±0.10^{abc}	2.52±0.07^{cd}	3.27±0.17^{bc}
LS17	3.67±0.11^{fg}	5.97±0.11^{ghi}	8.56±0.12^{cde}	13.07±0.08^{cd}	4.54±0.08^{ef}	2.36±0.09^{a}	2.44±0.01^{d}	3.14±0.08^{ab}
Mean±SD	3.94±0.31	6.44±0.77	8.37±0.36	12.89±0.58	4.43±0.49	2.18±0.13	2.73±0.21	3.62±0.28
CC	4.25±0.08^{abc}	7.48±0.09^{h}	8.57±0.1^{cde}	13.13±0.12^{bc}	4.93±0.06^{abc}	2.25±0.08^{abc}	2.91±0.06^{abc}	4.08±0.05^{ef}

LS: *Lagenaria siceraria* CC: *Citrullus Colocynthis* There is no significant difference between the means that have the same letters in each column

*Essential amino acids *Essential amino acid

Tableau 3b: Essential amino acids profile of the seed kernels of 17 varieties of *Lagenaria siceraria* (LS) and the seeds of 1 variety of *Citrullus colocynthis* (CC) (g/100 g protein)

Variety	Cystine*	Methionine*	Isoleucine*	Leucine*	Lysine*	Phenylalanine*	Tyrosine*	Threonine*	Valine*
LS1	1.39±0.14^{cde}	1.46±0.10^{abcd}	3.37±0.11^{bcd}	5.55±1.79^{a}	3.33±0.14^{def}	4.32±0.12^{cde}	2.26±0.09^{def}	3.22±0.10^{abcd}	4.33±0.10^{ab}
LS2	1.65±0.13^{abc}	1.48±0.16^{abcd}	3.37±0.09^{bcd}	6.7±0.13^{a}	3.56±0.10^{cde}	4.38±0.17^{cde}	2.98±0.13^{a}	2.93±0.12^{e}	4.13±0.11^{bcd}
LS3	1.52±0.17^{abcde}	1.46±0.10^{abcd}	2.83±0.07^{ef}	6.23±0.13^{a}	3.35±0.09^{def}	4.45±0.1^{bc}	2.44±0.12^{cde}	3.16±0.07^{abcde}	3.87±0.09^{def}
LS4	1.76±0.10^{ab}	1.45±0.09^{abcd}	3.65±0.08^{ab}	6.55±0.08^{a}	3.91±0.06^{ab}	4.85±0.07^{a}	2.15±0.06^{ef}	3.63±0.08^{a}	4.22±0.11^{abc}
LS5	1.61±0.14^{abcd}	1.42±0.13^{abcd}	3.61±0.16^{ab}	6.47±0.11^{a}	3.76±0.10^{bc}	4.71±0.13^{ab}	2.44±0.12^{cde}	3.6±0.15^{ab}	4.23±0.12^{abc}
LS6	1.55±0.09^{abcd}	1.23±0.10^{abcd}	2.74±0.10^{ef}	5.95±0.08^{a}	3.74±0.10^{cdef}	3.98±0.22^{cdef}	2.05±0.08^{f}	3.12±0.12^{de}	3.88±0.08^{bcd}
LS7	1.56±0.1^{abcd}	1.47±0.21^{abc}	2.87±0.09^{abc}	5.86±0.10^{a}	3.44±0.13^{bc}	4.24±0.09^{cdefg}	2.06±0.08^{f}	2.88±0.10^{abc}	4.13±0.13^{def}
LS8	1.57±0.11^{abcd}	1.35±0.09^{abcd}	3.22±0.11^{cd}	6.52±0.16^{a}	3.44±0.11^{cdef}	4.93±0.08^{a}	2.32±0.09^{def}	3.24±0.11^{abcd}	3.88±0.10^{def}
LS9	1.35±0.09^{cde}	1.24±0.11^{cd}	3.13±0.11^{de}	6.24±0.10^{a}	3.16±0.08^{f}	4.23±0.12^{cdef}	2.46±0.09^{cde}	2.99±0.12^{cde}	3.73±0.08^{efg}
LS10	1.82±0.07^{a}	1.68±0.10^{a}	3.75±0.09^{a}	6.73±0.11^{a}	4.13±0.12^{a}	4.98±0.05^{a}	2.44±0.11^{cde}	3.56±0.08^{ab}	4.47±0.10^{a}
LS11	1.45±0.13^{bcde}	1.35±0.09^{abcd}	3.36±0.08^{bcd}	5.98±0.08^{a}	3.44±0.14^{cdef}	4.00±0.07^{efg}	2.5±0.16^{bcd}	3.35±0.13^{abcd}	3.76±0.06^{ef}
LS12	1.45±0.07^{bcde}	1.65±0.14^{ab}	3.46±0.06^{abc}	6.55±0.10^{a}	3.57±0.09^{cd}	3.86±0.07^{g}	2.27±0.17^{def}	3.36±0.08^{abcd}	4.13±0.11^{bcd}
LS13	1.35±0.11^{cde}	1.22±0.08^{d}	2.44±0.08^{g}	5.73±0.17^{a}	3.23±0.11^{ef}	4.25±0.09^{cdef}	2.35±0.09^{cdef}	3.03±0.09^{cde}	3.45±0.09^{g}
LS14	1.57±0.11^{abcd}	1.45±0.10^{abcd}	3.21±0.10^{cd}	6.12±0.11^{a}	3.12±0.11^{f}	3.82±0.12^{g}	2.23±0.1^{def}	3.44±0.09^{abc}	3.63±0.08^{fg}
LS15	1.45±0.11^{bcde}	1.25±0.07^{cd}	3.35±0.10^{bcd}	5.96±0.09^{a}	3.26±0.07^{def}	4.05±0.08^{defg}	2.14±0.1^{ef}	3.11±0.11^{bcde}	3.96±0.08^{cde}
LS16	1.27±0.17^{de}	1.32±0.11^{bcd}	3.24±0.12^{cd}	6.33±0.13^{a}	3.27±0.17^{def}	4.06±0.06^{defg}	2.65±0.08^{bc}	3.23±0.11^{abcd}	3.64±0.08^{fg}
LS17	1.18±0.08^{e}	1.17±0.08^{d}	2.85±0.12^{ef}	6.11±0.09^{a}	3.56±0.07^{cd}	4.02±0.11^{efg}	2.81±0.11^{ab}	2.91±0.0^{de}	3.73±0.09^{efg}
Mean±SD	1.5±0.17	1.39±0.14	3.2±0.35	6.21±0.34	3.49±0.27	4.3±0.37	2.38±0.25	3.22±0.24	3.95±0.28
CC	1.56±0.09^{abcd}	1.58±0.15^{cd}	3.5±0.16^{fg}	5.9±0.10^{a}	3.75±0.08^{bc}	4.15±0.07^{g}	2.16±0.08^{ef}	3.46±0.10^{abcde}	3.87±0.10^{def}
FAO		2.6**	3.1	6.3	5.2	4.6***		2.7	4.2

LS: *Lagenaria siceraria* CC: *Citrullus Colocynthis* There is no significant difference between the means that have the same letters in each column

*Essential amino acids FAO: FAO/WHO/UNU (2002) pre-school child reference pattern (g/100 g protein) ** Cysine + Methionine *** Phenylalanine + Tyrosine

Table 4: Protein nutritional quality of *Lagenaria siceraria* seed kernels and *Citrullus colocynthis* seeds

			Limiting amino acids			
Varieties	Chemical score (%)	Amino acids score*	First	Second	Third	PER
LS1	64	6/8	Lysine	Leucine	-	1.81
LS2	68	6/8	Lysine	Valine	-	2.25
LS3	64	4/8	Lysine	Isoleucine	Valine	2.10
LS4	75	7/8	Lysine	-	-	2.28
LS5	72	7/8	Lysine	-	-	2.21
LS6	72	4/8	Lysine	Isoleucine	Valine	2.02
LS7	66	4/8	Lysine	Isoleucine	Valine	1.98
LS8	66	6/8	Lysine	Valine	-	2.25
LS9	61	5/8	Lysine	Valine	Leucine	2.11
LS10	79	7/8	Lysine	-	-	2.33
LS11	66	5/8	Lysine	Valine	-	1.98
LS12	69	6/8	Lysine	Valine	-	2.27
LS13	62	3/8	Lysine	Isoleucine	Valine	1.89
LS14	60	5/8	Lysine	Valine	Leucine	2.08
LS15	63	5/8	Lysine	Valine	Leucine	2.01
LS16	63	6/8	Lysine	Valine	-	2.13
LS17	68	3/8	Lysine	Valine	AAS	2.01
CC	72	5/8	Lysine	Valine	Leucine	1.98

*No. of amino acids with score >1 on a total of eight amino acids

and 37.31% of TAA. In *Lagenaria siceraria* seeds, TSAA ranged from 2.35 (LS17) to 3.50 g/100 g protein with an average of 2.89 g/100 g protein (LS10) which represented 3.89% of TAA. TSAA in *Citrullus colocynthis* seeds was 3.14 g/100 g protein that is 4.05% of TAA. The proximate percentages of methionine in the TSAA are 48.08 and 50.32%, respectively in *Lagenaria siceraria* seeds and *Citrullus colocynthis* seeds. After the sulphur amino acids the lysine isoleucine and valine are the least abundant essential amino acids.

The proximate composition in amino acids revealed that seems to be a general characteristic of cucurbitaceous seed flours proteins. Indeed similar observations were previously reported for various cucurbitaceous seeds flour proteins. However the relative proportions of these amino acids vary according to the specie and the variety (Olaofe and Akintoyo 2000; El-Adawy and Taha, 2001; Alfawaz, 2004; Aremu *et al*., 2006; Ogundele *et al*., 2012). Thus in three varieties of *Lagenaria siceraria*, the average contents of glutamic, aspartic acids and arginine represented approximately 32.16 g/100 g proteins that are 40.71% of the TAA whereas these three acids contribute for 27.70 g/100 g that is 37.23% of the total amino acids in the present study. However methionine and cystine contents were similar (Ogundele, 2013). The high content in glutamic and aspartic acids is consistent with the pH 3 to pH 5 minimum solubility points reported for the three varieties of *Lagenaria siceraria* seeds protein (Ogundele *et al*., 2013).

Leucine is the major essential amino acid (EAA) with an average content of 6.21 g/100 g protein in the seeds of *L. siceraria* and 5.90 g/100 g in the seeds of *C colocynthis* that is respectively 8.36 and 7.95% of the total amino acids. Its content is very homogeneous between varieties (p = 0.06). Tryptophan is not determined. It was destroyed during the acid hydrolysis. Leucine was also found to be the most concentrated essential amino acid (Ogundele *et al*., 2012, 2013b).

The protein nutritional quality of *Lagenaria Siceraria* and *Citrullus colocynthis* seeds is presented in Table 4. Essential amino acids reference values were used to compare the amino acids contents of the seeds as amino acid scoring (FAO *et al*., 2002). Amino acid scoring got from this ranged from 3/8 to 7/8. Three varieties of *L. siceraria* have an amino acid scoring of 7/8. In all studied varieties, lysine was the first limiting amino acid with a chemical index of 72% in *Citrullus colocynthis* and 60 to 79% in the *Lagenaria siceraria* varieties. In a country where cereals constitute the essential of energy contribution of the risk groups, it is therefore important to be vigilant when these proteins are used for fortification of foods. Isoleucine, leucine and valine could be in some varieties second or third limiting amino acid. The present observation is similar to other legumes (Akobundu *et al*., 1982; Sathe *et al*., 1982). It is important to note that, leucine is the major essential amino acid of proteins of all studied seeds however it constitutes the secondary or tertiary amino acid limiting in some varieties comparing to the recommended FAO *et al*. (2002) provisional pattern. On the contrary, whereas TSAA have the weakest chemical concentrations in all the studied varieties, they do not however constitute a limiting factor only in LS17 variety and still as third limiting amino acid.

The predicted-PER (P-PER) for *Citrullus colocynthis* was 1.98 and ranged from 1.81 to 2.33 for *Lagenaria siceraria* varieties. These values are however higher than the P-PER recorded for some legumes and concentrates like *Lathyrus sativus* (1.03) (Aremu *et al*., 2007), *Luffa cylindrica* (1.49) (Robinson, 1987; Olaofe *et al*., 2008) and comparable with that of *Phaseolus coccineus* (1.91) and *Prosopis africana* (2.3) (Aremu *et al*., 2008).

Conclusion: Seeds of *Lagenaria siceraria* varieties were found to contain high protein and compete favorably with traditional oilseeds as sesame, groundnut or soy bean. The proteins of all studied varieties were also rich in amino acids as well as in essential amino acids. However there were significant differences in their amino acid concentrations except for leucine. The high protein, of the seeds and the fairly high concentration and distribution of the amino acids, make them suitable for fortification of foods or as a substitute for animal protein. However, in a country where cereals constitute the main source of foodstuffs, lysine supplementation must be considered whatever the variety. Moreover, supplementation may be required for isoleucine, leucine, threonine or valine.

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