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Comparative Study of Amino Acid and Proximate Composition of *Citurllus colocynthis* and *Citrullus vulgaris* Seeds

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Abstract: *Citrullus colocynthis and Citrullus vulgaris* both look alike and belong to the genus *Citrullus* except that *Citrullus colocynthis* has thick black ring round its circumference. However, they are among the underutilized seeds of the World. *Citrullus colocynthis and Citrullus vulgaris* seed flours were analyzed for their amino acid and proximate compositions which was further subjected to statistical analysis to determine the correlation between the proximate compositions of the two species. The seeds show significant difference in fat and protein contents, but they are both good sources of fats and protein with values (56.00 and 49.59) % fat and (24.37 and 32.96)% protein contents for *Citrullus colocynthis* and *Citrullus vulgaris* respectively. However, they are closely related in fibre (1.91 and 2.00)%, moisture (3.08 and 2.75)% and ash contents (3.15 and 3.53)% respectively. The percentage essential amino acids of the seeds are also closely related: *Citrullus colocynthis* (52.73) and *Citrullus vulgaris* (50.86). The biological values of *Citrullus colocynthis and Citrullus vulgaris* are significantly different (12.83 and 26.35) with Protein efficiency ratios that are closely related (2.71 and 2.58) respectively.

Key words: Amino acid, chemical composition, protein, fat and diet

INTRODUCTION

Citrullus colocynthis and *Citrullus vulgaris* belong to the family Cucubitaceae and the same genus *Citrullus* (Zipcode Zoo, 2008). They are generally called egusi in Yoruba land and *Citrullus colocynthis* is specifically called egusi Bara by Omotoye (1984). Bara, also known as papa, has large brown seeds with thick black edges thickened towards the apex, about 16 x 9.5 mm and is common in the northern and western parts of Nigeria (Denton and Adeiran, 1988). Plant proteins are an abundant and relatively inexpensive source of protein that is widely recognized due to their nutritional values and excellent physicochemical properties (Issoufou *et al*., 2010). It is therefore important to determine the available protein content of these two *Citrullus* species and the similarities in their proximate compositions.

Cysteine (or sulphur-containing amino acids), tyrosine (or aromatic amino acids) and arginine are required by infants and growing children (Imura and Okada, 1998; FAO/WHO/UNU, 2007). Attempts have been made to express the "quality" or "value" of various kinds of protein in measures such as the biological value, net protein utilization, protein digestibility-corrected amino acid score. These concepts are also important in the livestock industry, because the relative lack of one or more of the essential amino acids in animal feeds would have a limiting effect on growth and thus on feed conversion ratio (Wikipedia, 2010). Although proteins from plant sources tend to have a relatively low biological value, in comparison to protein from eggs or milk, they are nevertheless "complete" in that they contain at least trace amounts of all of the amino acids that are essential in human nutrition (McDougall, 2002). Discovery of the nutritional and amino acid component of *Citrullus colocynthis* and *Citrullus vulgaris* seeds will enhance their potential use as food supplements and in food compounding as to fortify the protein and fat contents of food substances for local consumption and industrial application. The knowledge of their similarities and differences will also go a long way to indicate the possibility of substituting one for the other in food compounding and processing.

MATERIALS AND METHODS

Citrullus colocynthis and *Citrullus vulgaris* seeds were purchased from Akure central market. The seeds were dehulled, dried and milled into fine flour. Proximate analyses and sample treatments of the seeds flour were carried out according to the standard methods of analysis as given by AOAC (1990) and Crude fibre was determined according Pearson (1981).

The amino acid analysis was carried out on the milled flour samples using the method described by (Karen *et al*., 1996) with some modifications. The following evaluations were further carried out on the results of the amino acids:

Evaluation of protein quality: The amino acid results were used for protein evaluation of the seed flours as stated below. The parameters determined are:

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1. Proportion of The Essential Amino Acid (TEAA) to the Total Amino Acid (TAA) i.e. (TEAA/TAA)
2. Amino acid score

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

or

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

The most complete sets of amino acid data on food express the values as mg/g Nitrogen.

3. Total Sulphur Amino Acid (TSAA) = Methionine + Cystine
4. Total Aromatic Amino Acids (TArAA) = Phenylalaline + Tyrosin
5. Predicted Biological value

$$BV = 10^{2.15} \times q^{0.41}_{Lys} \times q^{0.60}_{Phen+Try} \times q^{0.77}_{Met+Cys} \times q^{2.41}_{Thr} \times q^{0.21}_{Trp}$$

Where

$$q = \frac{\text{ai sample}}{\text{ai reference}} \text{ for ai sample} \leq \text{ai reference}$$

$$q = \frac{\text{ai reference}}{\text{ai sample}} \text{ for ai sample} \geq \text{ai reference}$$

ai = mg of the amino acid per gram of total essential amino acids

(Chavan *et al*., 2001).

6. Predicted Protein Efficiency Ratio (P-PER)

P-PER = -0.468+0.454 (Leucine)-0.105 (Tyrosine) (Alsmeyer *et al*., 1979).

Statistical analysis: Analyses in triplicates are expressed as mean±standard deviation (SAS/STAT, 1988).

RESULTS AND DISCUSSION

Table 1 shows the proximate composition of *Citrullus colocynthis* and *Citrullus vulgaris*. The protein content of *Citrullus vulgaris* (32.96%) is significantly higher than that of *Citrullus colocynthis* (24.37%); while the average of their protein content is (28.67%). However, the two *Citrullus* species are good sources of protein and these values are consistent with protein values reported for some other melons like three varieties of *Lagenaria siceraria* with protein contents (27.71, 32.70 and 34.64)% (Ogundele and Oshodi, 2010). The consumption of *Citrullus colocynthis* and *Citrullus vulgaris* 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 feeds that are low in protein content. The fat contents of these two melons are equally high. *Citrullus colocynthis* has higher value (56.61%) than *Citrullus vulgaris* (49.59%). These values make them both good sources of fat like is reported for *Cucumeropsis manni* (42.29%), *Cucumeropsis edulis* (40.26%) *Cucumis sativus* (45.21%) (Abiodun and Adeleke, 2010) and the three varieties of *Lagenaria siceraria* (46.03, 50.91 and 53.35)% (Ogundele and Oshodi, 2010). However, the values of the moisture (3.08 and 2.75)%, Ash (3.15 and 3.53)% and Fiber (1.91 and 2.00)% are in smaller quantities and with little of no significant differences $p>0.05$. Carbohydrate of *Citrullus colocynthis* (10.88)% is significantly higher than that of *Citrullus vulgaris* (9.17%) with an average value of (10.03%) for the two species. This is however higher than the average value reported for three varities of *Lagenaria siceraria* (7.47) (Ogundele and Oshodi, 2010).

The values of all the amino acid content and scoring of *Citrullus colocyynthis* and *Citrullus vulgaris* with the exception of Tryptophan that was not determined can be seen in Table 2. The amino acid contents of the two melons are significantly different. The most concentrated amino acid for the samples (mg/g) is Glutamic acid with the values (142.45±1.06) for *Citrullus colocyynthis* and (151.40±0.99) for *Citrullis vulgaris*. The first least concentrated amino acid in the samples is Cystine, with (9.30±3.68) for *Citrullus colocyynthis* and (10.01±4.51) for *Citrullus vulgaris*. Methionine is the second least concentrated amino acid with values (12.30±2.12) for *Citrullus colocyynthis* and (14.40±2.12) for *Citrullus vulgaris*. The Total Amino Acid (TAA) mg/g of the seeds are (738.25) *Citrullus colocyynthis* and *Citrullus vulgaris* (785.61). These values are higher than the TAA (6758 mg/g) for *Luffa cylindrica* (Olaofe *et al*., 2008) and comparable with the TAA for some plant foods which range between 393-765 mg/g as reported by some researchers (Olaofe *et al*., 1994; Ogungbenle, 2006; FAO/WHO/UNU, 1985 and Akobundu *et al*., 1982).

Table 1: Proximate composition of *Citrullus colocynthis* and *Citrullus vulgaris* (g/100 g)

Parameter	*Citrullus colocynthis*	*Citrullus vulgaris*	Mean	SD[a]	CV%[b]
Protein	24.37±2.13	32.96±2.53	28.67	4.30	15.00
Fat	56.61±0.10	49.59±1.40	53.10	3.51	6.61
Moisture	3.08±0.80	2.75±0.27	2.91	0.17	5.84
Ash	3.15±0.30	3.53±0.32	3.34	0.19	5.69
Fiber	1.91±1.00	2.00 ±1.00	1.96	0.05	2.55
Carbohydrate	10.88±3.03	9.17±2.88	10.03	1.08	10.77

[a]Standard deviation; [b]Coefficient of variation

Table 2: Amino Acid profile and scoring of *Citrullus colocynthis* and *Citrullus vulgaris* seeds

	Amino acid contents (Mg/g protein)		Reference	Amino acid scoring	
Amino acid	*Citrulus colocynthis*	*Citrulus vulgaris*	Soya bean	*Citrulus colocynthis*	*Citrulus vulgaris*
*Lysine	36.95±0.35	34.60±0.71	60.80	60.77	56.91
*Histidine	23.05±0.07	24.10±1.13	25.00	92.20	96.40
*Arginie	66.10±0.57	63.55±0.78	61.00	108.36	104.18
Aspartic acid	91.05±0.64	95.15±0.92	113.00	80.58	84.20
Threonine	23.40±1.27	30.00±1.14	51.00	45.88	58.82
Serine	32.45±1.34	35.80±1.56	56.70	57.23	63.14
Glutamic acid	142.45±1.06	151.40±0.99	169.00	84.29	89.59
Proline	30.30±1.13	29.65±1.20	48.60	62.35	61.01
Glycine	29.50±2.12	36.45±0.78	40.10	73.57	90.90
Alanine	23.20±0.59	37.05±1.20	42.30	54.85	87.59
*Cystine	9.30±3.68	10.01±4.51	17.00	54.70	58.88
*Valine	37.05±1.20	39.60±1.13	45.90	80.71	86.27
*Methionine	12.30±2.12	14.40±2.12	12.20	100.00	118.03
*Isoleucine	32.75±1.06	31.10±0.85	46.20	70.89	67.31
*Leucine	76.95±1.20	74.60±1.71	77.20	99.68	96.63
*Tyrosine	29.75±0.35	32.25±0.78	33.90	87.76	95.13
*Phenylalanine	41.70±2.75	45.90±0.59	48.40	86.16	94.83
1st Limiting EAA				Cystine	Lysine
2nd Limiting EAA				Lysine	Cystine

Amino acids values are mean of duplicate analysis ± standard deviation

The scoring of the amino acids in Table 2 shows that the amino acids of *Citrullus colocynthis* and *Citrullus* vulgaris can compete favourably with those of Soya bean. The Total Essential Amino Acid (TEAA) (mg/g) for *Citrullus colocynthis* (389.30) and *Citrullus vulgaris* (399.56) with corresponding percentages of (52.73%) for *Citrullus colocyynthis* and *Citrullus vulgaris* (50.86%). These values compete favorably with those of *Adenopus breviflorus benth* seed (53.70%) for the whole seed and (51.40%) for the dehulled seeds (Oshodi, 1996) and Soya beans (50.31%). The Total Sulphur Amino Acid (TSAA) mg/g for the melons are (21.60) *Citrullus colocyynthis* and (24.41) *Citrullus vulgaris*, these are comparable with the TSAA of (2.50 g/100 g) for *Luffa cylindrica* (Olaofe *et al*., 2008) and lower than the 5.8 g/100 g recommended for infants (Salunkhe and Kadam, 1989). The Total Essential Aromatic Amino Acid (EarAA) mg/g are (71.45) *Citrullus colocyynthis* and (78.15) *Citrullus* vulgaris and are higher than the Essential Aromatic Amino Acid (EArAA) (3.25 g/100 g) for *Luffa cylindrica* (Olaofe *et al*., 2008). However, these values fall within the range suggested for ideal infant protein (6.8-11.8 g/100 g) (Salunkhe *et al*., 1985). The first limiting essential amino acids are Cystine (54.70) and Lysine (56.91) while the order is reversed for the second limiting essential amino acids which are Lysine (60.77) and Cystine (58.88) for *Citrullus colocynthis* and *Citrullus vulgaris* respectively. On the other hand, the most concentrated essential amino acid (mg/g) for the two melon samples is Leucine (76.95±1.20) *Citrullus colocyynthis* and (74.60±1.71) *Citrullus vulgaris*, followed by Arginine for *Citrullus collosynthis* and *Citrullus vulgaris* with values (66.10±0.57 mg/g) and (63.55±0.78) respectively. Arginine is an essential amino acid for children's growth (NRC, 1989; Olaofe *et al*., 2008), hence *Citrullus colocyynthis* and *Citrullus vulgaris* are good sources of Arginine which will enhance proper growth of children.

The P-BV of *Citrullus colocyynthis* (12.83) is lower than that of *Citrullus vulgaris* (26.37) (Table 3). However, they are both lower than that of beach pea protein isolates (36.5-40.13) (Chavan *et al*., 2001). These values are however lower than the suggested Biological value for plant based proteins (45) (Huge, 2011). The Predicted Protein Efficiency Ratio (P-PER) is one of the quality parameters used for protein evaluation (FAO/WHO, 1991). The P-PER for the two Citrullus spiecies are closely related with values (2.71) for *Citrullus colocyynthis* and (2.58) for *Citrullus vulgaris*. 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) (Olaofe *et al*., 2008); *Phaseolus coccineus* (1.91) (Robinson, 1987) and comparable with that of *Prosopis africana* (2.3) (Aremu *at al*., 2008).

The recommended daily intake of amino acid by FAO/WHO/UNU (2007) is used to calculate the available amino acids in the samples. The percentage protein content of *Citrullus colocyynthis* (24.37) and *Citrullus vulgaris* (32.97), when used in calculating Isoleucine available in 100 g of the samples gave (798.12 and 1022.07) mg/100 g of *Citrullus colocynthis* and *Citrullus vulgaris* respectively. These quantities are actually more than the daily requirement of Isoleucine for a child of about 10-15 years old with an assumed weight of 35 g which is 700 mg. In the same vein, 100 g of the melon samples also contain (902.91 and 1305.61) mg Valine/100 g samples respectively which will adequately

Table 3: Summary of Amino acid composition of *Citrullus colocynthis* and *Citrullus vulgaris* seeds

	Citrullus colocynthis	*Citrullus vulgaris*
Total Amino acid (TAA)	738.25	785.61
Total Essential Amino acid (TEAA)	389.30	399.56
%TEAA	52.75	50.86
Total Sulphur Amino Acids (Methionine + Cystine) (TSAA)	21.60	24.41
%Cystine in TSAA	43.06	41.00
Total Aromatic Amino Acid: (Phenylalanine + Tyrosine)	71.45	78.15
%Total Aromatic Amino Acid	10.07	9.95
Total Basic Amino Acid (Lysine + Argine + Histiden)	126.10	132.25
%TBAA	17.08	15.56
%Total acidic AA (TAAA) Glu. + Asp	31.63	31.42
%Total Neutral AA	51.29	53.02
Total Non-Essential Amino Acid (TNEAA)	348.95	386.05
%Total Non-Essential Amino Acid	47.27	49.14
TEAA/TNEAA	1.12	1.04
Predicted Biological Value (PBV)	12.83	26.35
Protein Efficiency Ratio (PER)	2.71	2.58
%PER	108.40	103.20

meet the daily Valine requirement for growing children. The same thing is applicable to the other amino acid contents of the melon seeds; hence *Citrullus colocyynthis* and *Citrullus vulgaris* seeds are good sources of essential and non essential amino acids and can be used to supplement food substances which are deficient in any of these amino acids for proper growth of children and good health of adults.

Conclusion: *Citrullus colocyynthis* and *Citrullus vulgaris* seeds are significantly different p>0.05 in protein, fat and biological values show their genetic differences as different species. However, they are good sources of protein, fat and essential amino acids which will enhance proper growth of children and meet the daily amino acid requirements of adults at calculated quantities. They can therefore be used to supplement food substances that are deficient in protein, fat or any of these amino acids (FAO/WHO/UNU, 2007; Salunkhe and Kadam, 1998).

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