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Comparative amino acid composition and quality parameters of *Moringa oleifera* testa and cotyledon

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ABSTRACT

Introduction: *Moringa oleifera* is a drought-resistant plant, widely used in the tropical region. The leaves and stems have been extensively utilised in foods and neutraceuticals preparation, with less attention to the seeds. In this study, amino acid (AA) compositions of *M. oleifera* testa and cotyledon were examined comparatively. **Methods:** Samples were separately defatted, hydrolyed, and neutralised. The AA solution was purified by cation-exchange solid-phase extraction, derivatised and analysed by gas chromatography. **Results:** Glutamic (acidic amino acid) and phenylalanine (essential amino acid, EAA) were the most concentrated in both samples. Total EAA (g/100g crude protein, cp) was higher in cotyledon (51.0) than testa (41.9). Predicted protein efficiency ratios (P-PERs) were higher in testa (0.605-1.530) than cotyledon 0.286-1.460). EAA index ranged between 0.951-1.13 (soybean comparison) and 83.0-96.9 (egg comparison) with corresponding biological value of 78.7-93.9. The following AA had scores >1.0 in comparison to whole hen's egg, testa: glycine (Gly), glutamic acid (Glu), phenylalanine (Phe), histidine (His), and cysteine (Cys); cotyledon (Gly), proline (Pro), Glu, Phe, His, arginine (Arg) and Cys. In comparison with requirements of pre-school children, six AA (6/9 or 66.7%) had scores >1.0 in each sample. In provisional AA scoring pattern, isoleucine (Ile) (1.25) and Phe + tyrosine (Tyr) (1.68) had scores >1.0 in testa while methionine (Met) + Cys, Phe+Tyr, and tryptophan (Trp) in cotyledon. However, tryptophan and lysine were the limiting AAs in testa and cotyledon, respectively. **Conclusion:** The study showed that both anatomical parts would complement each other in terms of amino acid supply.

Keywords: amino acid scores, derivatisation, essential amino acid, hydrolysis, isoelectricpoint

INTRODUCTION

Moringa oleifera is a member of the Moringaceae family. It is a deciduous, draught-resistant, and fast growing tree with an average height of 12 metres at maturity (Olaofe, Adeyeye & Ojugbo, 2013). Moringa species has many varieties such as *Moringa ruspoliana, Moringa boziana, Moringa ovalifolia, Moringa longituba, Moringa oleifera* and *Moringa rivae.* Out of all these varieties, *Moringa oleifera* happens to be the

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most popular and widely planted in the tropical region (Jahn, 1988). *Moringa oleifera* has a lot of common names. It is fondly called drum stick tree due to the fact that the shape of its pod resembles the stick used for beating the drum (Olaofe *et al.*, 2013). Other common names are Marango, Mulangay, Sajna, Benz olive; and in Nigeria, it is popularly called Ewe Igbale (Gbile, 1984). Every part of the *Moringa oleifera* tree is used for one purpose or another; the seed is used for cooking and cosmetic oil, while the leaves, flowers and green pods are usually planted and useful as vegetable. The seeds may be eaten raw without any heat processing (Ramachandran, Peter & Gopalakrishnan, 1980). *M. oleifera* leaf is a very good source of omega-3 fatty acid (Olaofe *et al.*, 2013). The insulin-like protein observed in *M. oleifera* seed coat has antigenic epitopes similar to insulin and is known to display hypoglycemic activity on oral administration (Paula *et al.*, 2016). *Moringa oleifera* can be cultivated in tropical and subtropical areas. The temperature requirement for survival is between 25-35°C and it requires a net rainfall of between 250-3000mm.

Both the nutritional and medicinal importance of *Moringa oleifera* are well documented. *Moringa oleifera* contains a lot of nutritionally essential minerals such as calcium, iron and zinc. It was reported in the literature that *Moringa oleifera* contains more iron than spinach (Fuglie, 2005). Another report showed that *Moringa oleifera* meets the daily requirement of zinc as it contains around 25.5-31.0 mg of Zn/kg (Barminas, Charles & Emmanuel, 1998). The leaves also contain abundant essential vitamins such as vitamin A, folic acid, nicotinic acid and pyridoxine of vitamin B; with vitamins C, D and E also present (Mbikay, 2012). *Moringa oleifera* has been very popular in herbal medicine especially in countries like India and Nigeria due to the presence of phytochemicals. Research studies have revealed that aqueous extracts of *Moringa oleifera* can cure both streptozotocin-induced Type 1 diabetes and insulin-resistant Type 2 diabetes in rats (Divi, Bellamkonda & Dasireddy, 2012). Traditionally, *Moringa oleifera* has been used for the treatment of asthma, eye and ear infections, cholera, cough, headache, anaemia, killing of intestinal worms and glandular swelling in India, Puerto Rico and Malaysia (Barminas *et al.*, 1998).

The whole seed of *Moringa oleifera* consists of testa and cotyledon. The testa which develops from the integument of the ovule is the outermost covering of the seed. It protects the delicate inner part of the seed against external aggression from fungi, bacteria or insects. The cotyledon is the inner part of the seed that forms the first leaf when the plant germinates. It contains stored food that helps provide internal energy that the plant needs for the development of its embryo during germination.

There is no doubt that research studies have been published on *Moringa oleifera*, some of which include the nutritional values of *Moringa* leaves and pods (Barminas *et al.*, 1998), comparative study of proximate, amino acid and fatty acids of *Moringa oleifera* tree (Olaofe *et al.*, 2013) and synergy between *Moringa oleifera* seed powder and alum in the purification of domestic water (Dalen *et al.*, 2009). The present study is aimed at investigating the amino acid profiles and quality parameters of *Moringa oleifera* testa and cotyledon on comparative basis.

MATERIALS AND METHODS

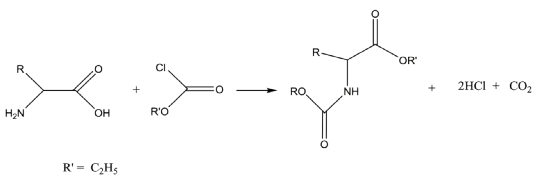
Sample collection

The *Moringa oleifera* seed samples were obtained from a farm in Odo-Ayedun Ekiti, Ikole Local Government area of

Ekiti State, Nigeria. The seeds were authenticated at the Plant Science and Biotechnology Department, Ekiti State University, Ado-Ekiti. They were sorted to remove defective ones, washed with distilled water to remove dirt and later soaked overnight. The testa of the soaked seeds was carefully separated from the cotyledon. Both testa and cotyledon were dried separately in an oven (Electric blast dry box, PEC Medical USA) set at 45°C. They were later dry-milled into fine powder using the Mallex electric blender and stored separately inside polythene containers prior to use for analysis.

Sample extraction and analysis

The extraction and amino acid analysis in this study were carried out following the procedures described by Danka *et al.* (2012). 10g of sample was accurately weighed into a 250ml conical flask. The sample was separately defatted using about 40ml petroleum ether (40-60°C) to extract fat content. The extraction was carried out three times inside a Soxhlet extractor equipped with thimble. The sample was hydrolysed three times to enhance amino acid recovery. The defatted sample was soaked with 30ml of 1M KOH (potassium hydroxide) solution and was incubated for two days at 110°C in a hermetically closed borosilicate glass vessel. The hydrolysate was later neutralised until pH was in the range of 2.5 – 5.0; the purification of the solution was achieved by cation-exchange solid-phase extraction. The amino acids in purified solution was derivatised with ethylchloroformate by the following established mechanism:



(equation 1)

After derivatisation, the reagent was removed by scavenging with nitrogen. The resulting amino acid was made up to 1ml in a vial for gas chromatography (GC) analysis. The GC conditions for the analysis were: GC: HP6890 powered with HP chemstation; injection temperature: split injection; split ratio: 20:1; carrier gas: hydrogen; flow rate: 1.0 ml/minute; oven programme: initial temperature at 110°C, first ramp at 27°C/min at 320°C, second ramp was constant for 5 mins at 320°C; inlet temp: 50°C; column type: EZ; column dimension: 10m by 0.2mm by 0.25 µm; compressed air: 35 psi; detector: PFPD; detector temperature: 320°C; hydrogen pressure: 20 psi.

Evaluation of quality parameters for the amino acids

Isoelectric point (pI)

The isoelectric point for a mixture of amino acids was determined by the equation of Olaofe & Akintayo (2000) as follows:

$$IPm = \sum_{i=1}^{n} IPiXi \qquad \textit{(equation 2)}$$

In the above equation, *I*Pm represents the isoelectric point for the mixture of amino acids, *I*Pi is the isoelectric point of the i^{th} amino acid in the mixture and Xi is the mole fraction of the i^{th} amino acid in the mixture.

Predicted protein efficiency ratio (P-PER)

Equations of Alsmeyer *et al.* (1973) were used to determine the protein efficiency ratios 1, 2, and 3 as given below:

$P–PER_1$ = -0.468 + 0.454 (Leu) - 0.105 (Tyr) *(equation 3)*

$P–PER_2$ = -0.684 + 0.456 (Leu) - 0.047 (Pro) *(equation 4)*

$P–PER_3$ = -1.816 + 0.435 (Met) - 0.78 (Leu) + 0.211 (His) - 0.944 (Tyr) *(equation 5)*

Essential amino acid index (EAAI)
The EAAI was calculated based on the ratios of essential amino acids (EAAs) in a protein relative to the respective amount in whole egg protein according to the following equation:

$$EAAI = \sqrt[n]{\frac{Lys_p}{Lys_s} x \frac{Trp_p}{Trp_s} x \ldots \frac{His_p}{His_s}} \quad \textit{(equation 6)}$$

where subscript 'p' represents food protein subscript s represents egg protein (standard) n represents the number of amino acids [Pairs like methionine (Met) + cysteine (Cys) and phenylalanine (Phe) + tyrosine (Tyr) are counted as one] (Nielsen, 2002).

The obtainable value in the above equation (equation 6) was taken to be $EAAI_1$. In another form, EAAI may be estimated from amino acid composition relative to total nitrogen in both the food and the standard. The estimation was computed logarithmically and used to calculate $EAAI_2$ (Albanese, 1959).

Biological value (BV)
Equation suggested by Albanese (1959) was used to compute the biological value. The equation is given below:
BV = 1.09 (EAAI) – 11.73 *(equation 7)*

Amino acid scores (AAS)
Computation of AAS was done using three different established procedures.
(i) Scores computed based on amino acid values of whole hen's egg (Paul, Southgate & Russel, 1976).
AAS = [g/100g of test protein (from results)]/g/100g of reference pattern

(ii) Scores based on EAA scoring pattern (FAO/WHO, 1973).
AAS = [mg/g of test protein (from results)]/mg/g of reference pattern

(iii) Scores based on EAA requirement for pre-school children (FAO/WHO/UNU, 1985).

Amino acid requirements for school boys (10-12years)
This requirement was based on the required EAA in mg/kg/day for 30kg body weight of school boys (age 10-12 years) computed using the following equation (FAO/WHO/UNU, 1985):

Amino acid requirement = EAA × 10 × protein (g/100g) *(equation 8)*

Parameters such as leucine/isoleucine ratio, (Leu/Ile), total essential amino acid (TEAA), total non-essential amino acid (TNEAA), total aromatic amino acid (TArAA), total essential aromatic amino acid (TEArAA), as well as their percentages were also calculated.

Statistical analysis
Results obtained from the amino acid composition in Table 1, AAS based on whole hen's egg amino acid (Table 3), AAS based on pre-school children standard (Table 4) as well as essential AAS based on provisional EAA standard requirements (Table 5) were subjected to both descriptive and inferential statistical analysis (Oloyo, 2011) using Microsoft Excel package in order to establish the existence of significant differences or otherwise among the samples.

RESULTS

The amino acid compositions (g/100g) of *Moringa oleifera* testa and cotyledon are depicted in Table 1. Among the amino acids investigated, glutamic acid (Glu) had the highest concentration in both samples: 17.3g/100g (testa), 18.0g/100g (cotyledon). However, tryptophan (Trp) had the least value in testa (0.283g/100g) and Met in cotyledon (1.44g/100g). Among the EAAs, Phe was the most concentrated in each sample: 7.59g/100g (testa),10.9g/100g (cotyledon). The following EAAs had

Table 1. Amino acid levels (g/100g) of *Moringa oleifera* testa and cotyledon

AA	*CID*	*Testa*	*Cotyledon*	*Mean±SD*	*CV%*	*Difference*	*%difference*
Gly	750	5.60	4.65	5.13±0.67	13.10	+0.95	+16.96
Ala	5950	3.46	3.61	3.54±0.11	2.99	-0.15	-4.34
Ser	5951	4.26	5.15	4.71±0.63	13.40	-0.89	-20.89
Pro	145742	3.69	4.49	4.09±0.57	13.80	-0.80	-21.68
Val	6287	3.94	4.29	4.12±0.25	6.00	-0.35	-8.88
Thr	6288	3.41	2.44	2.93±0.69	23.40	+0.97	+28.45
Ile	791	4.98	3.39	4.19±1.12	26.70	+1.59	+31.93
Leu	6106	4.99	5.00	5.00±0.01	0.14	-0.01	-0.20
Asp	5960	6.43	4.41	5.42±1.43	26.40	+2.02	+31.42
Lys	5962	3.51	1.99	2.75±1.07	38.90	+1.52	+43.30
Met	6137	0.85	1.44	1.15±0.42	36.30	-0.59	-69.41
Glu	33032	17.3	18.0	17.7±0.50	2.80	-0.70	-4.05
Phe	6925665	7.57	10.9	9.24±2.35	25.40	-3.33	-43.99
His	6274	2.46	2.87	2.67±0.29	10.90	-0.41	-16.67
Arg	6322	5.09	9.99	7.54±3.46	45.90	-4.90	-96.27
Tyr	6057	2.50	3.21	2.86±0.50	17.60	-0.71	-28.40
Trp	6305	0.28	1.47	0.88±0.84	95.70	-1.19	-425.00
Cys	67678	2.41	3.99	3.20±1.12	35.00	-1.58	-65.56
Total		83.20	91.30	87.30±5.73	6.56	-8.10	-9.74
CP		18.80	32.50	25.70±9.69	37.70	-13.70	-72.87

AA – amino acid, CID – chemical identification number, SD – standard deviation, CV% – coefficient of variation percent, CP – crude protein

their values greater in testa than in cotyledon: threonine (Thr) (28.4% more), isoleucine (Ile) (31.9% more), and lysine (Lys) (43.3% more). However, out of the eighteen amino acids investigated, thirteen (13) were more abundant in cotyledon than in testa.

The statistical analysis of the results in Table 1 are shown in Table 2. The values of mean, standard deviation (SD) and coefficient of variation (CV%) in testa (4.60, 3.65, and 79.3, respectively) were close to those in cotyledon (5.07, 4.09, and 80.7, respectively).

Table 2. Statistical description of amino acid composition results in Table 1 (testa/ cotyledon)

Statistics	*Testa*		*Cotyledon*
Total amino acid value	83.20		91.30
Mean value of amino acid	4.60		5.07
Standard deviation	3.65		4.09
Coefficient of variation (%)	79.30		80.70
Correlation coefficient (r_{xy})		0.91	
Variance (r_{xy}^2)		0.83	
Regression coefficient (R_{xy})		1.02	
Coefficient of alienation (C_A)		0.42	
Index of forecasting efficiency (IFE)		0.59	
Remark		*	

* - the results were significantly different at n-2=16 and $r_{=0.01}$ with critical value=0.590

The summary of amino acid compositions and quality parameters of Moringa testa and cotyledon are presented in Figures 1(a) and (b). Total essential amino acid (TEAA) levels were (g/100g crude protein, cp): testa (37.0) and cotyledon (43.8), with their corresponding percentages at 44.5% and 48.0%, respectively. This implies that both the moringa testa and cotyledon may adequately provide the required EAA for people of various age categories, including pre-school children (46.0), school children (24.1), and adults (12.7) (FAO/WHO/UNU, 1985). Total non-essential amino acids (TNEAA) were (g/100g cp): testa (46.2) and cotyledon (47.5), indicating lesser values of TEAA than TNEAA in both samples.

The AAS based on whole hen's egg amino acid, pre-school children standard, as well as the FAO/WHO (1973) standard are shown in Table 3. For whole hen's egg amino acid, glycine (Gly), glutamic acid (Glu), Phe, histamine (His), and Cys had values greater than one in testa. In the cotyledon, Gly, proline (Pro), Glu, Phe, His, arginine (Arg), and Cys had their AAS values greater than one. In the pre-school children standard, six amino acids had scores greater or equal to one in both samples. In the FAO/WHO standard, only Ile and Phe + Tyr had scores above 1.0 in testa, whereas Met + Cys, Phe + Tyr and tryptophan (Trp) had values greater than 1.0 in cotyledon.

Table 4 presents the summary of statistical analysis of the results obtained from Table 3. For egg scores (testa/ cotyledon in column 2), the correlation coefficient (r_{xy}) was high at 0.781. In pre-school children EAA scores, as well as in provisional EAA scoring pattern (FAO/WHO standard), the calculated correlation coefficient values were lower than the critical values, at 0.798 and 0.834, respectively.

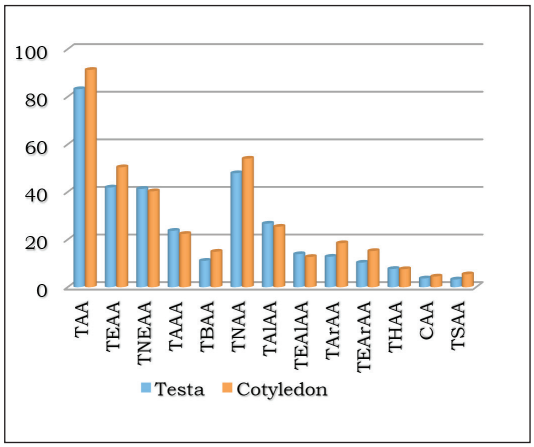


Figure 1(a). Summary of the amino acids class

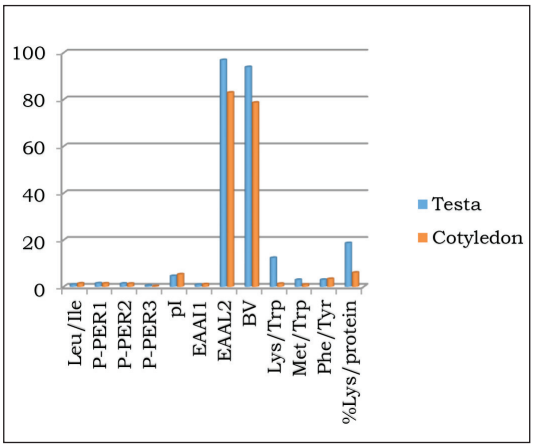


Figure 1(b). Amino acid quality parameters

TAA: Total amino acid; TEAA: total essential amino acid; TNEAA: total non-essential amino acid; TAAA: total acidic amino acid; TBAA: total basic amino acid; TNAA: total neutral amino acid; TAlAA: total aliphatic amino acid; TEAlAA: total essential aliphatic amino acid; TArAA: total aromatic amino acid; TEArAA: total essential aromatic amino acid; THAA: total hydroxylic amino acid; TCAA: cyclic amino acid; TSAA: total sulphur amino acid; P-PER1,2,3:predicted protein efficiency ratios; p*I* : isoelectric point; EAAI 1,2: essential amino acid index; BV: biological value

Table 3. Amino acid (AA) scores of *Moringa oleifera* testa and cotyledon using whole hen's egg amino acid (X), pre-school children standard (Y), and FAO/WHO (1973) standard (Z)

AA	*X*		*Y*		*Z*	
	Testa	*Cotyledon*	*Testa*	*Cotyledon*	*Testa*	*Cotyledon*
Gly	1.87	1.55				
Ala	0.64	0.67				
Ser	0.54	0.65				
Pro	0.97	1.18				
Val	0.53	0.57	1.13	1.23	0.79	0.86
Thr	0.67	0.48	1.00	0.72	0.85	0.61
Ile	0.89	0.61	1.78	1.21	1.25	0.85
Leu	0.60	0.60	0.76	0.76	0.71	0.71
Asp	0.60	0.41				
Lys	0.57	0.32	0.61	0.34	0.64	0.36
Met	0.27	0.45				
Glu	1.44	1.50				
Phe	1.48	2.14				
His	1.03	1.20	1.29	1.51		
Arg	0.83	1.64				
Tyr	0.63	0.80				
Trp	0.16	0.82	0.26	1.34	0.28	1.47
Cys	1.34	2.22				
Met+Cys			1.30	2.17	0.93	1.55
Phe+Tyr			1.60	2.24	1.68	2.35
Total	0.83	0.91	1.09	1.21	0.96	1.06

Table 4. Summary of statistical analysis of the score results in Table 3

Statistics	*Egg scores (Testa/ cotyledon)*	*Pre-school child scores (Testa/cotyledon)*	*FAO/WHO (1973) scores (Testa/cotyledon)*
r_{xy}	0.78	0.53	0.52
r_{xy}^2	0.61	0.28	0.27
R_{xy}	1.03	0.70	0.80
[a]Mean	0.86	1.08	0.89
[a]SD	0.45	0.48	0.42
[a]CV%	53.70	44.7	47.00
[b]Mean	0.99	1.28	1.10
[b]SD	0.59	0.64	0.65
[b]CV%	60.00	49.80	59.10
C_A	0.63	0.85	0.86
IFE	0.38	0.15	0.15
Remark	*	NS_1	NS_2

a- statistical results for testa, b- statistical results for cotyledon, *- results significantly different at n-2 = 16, $r_{=0.01}$ (critical value = 0.590), NS_1- results not significantly different at n-2 = 7, $r_{=0.01}$ (critical value = 0.798), NS_2- results not significantly different at n-2 = 6, $r_{=0.01}$ (critical value = 0.834)

DISCUSSION

In this research work, Glu was observed to have the highest level among all amino acids in both samples. Glu is the most abundant amino acid in most plant and animal samples, such as testa, dehulled, and whole seed of *Vigina subterranea* (Adeyeye & Olaleye, 2012), smooth loofa, Roselle, and sesame seeds flour (Adubiaro, Ogunbusola & Olaleye, 2017), as well as *Moringa oleifera* tree (Olaofe *et al.*, 2013). The difference between the total amino acid in testa and cotyledon was 8.10g/100g. In terms of percentage difference, cotyledon was just 9.74% higher than testa. This shows that both testa and cotyledon contained varied composition of amino acids, especially EAAs. Therefore, the testa could be seen as an important component of the seed, contributing immensely to its nutritive value, particularly as it contributes a higher percentage of the first limiting amino acid (Lys) to the seed protein. Although the difference in total amino acid between the testa and the cotyledon was small, the cp in cotyledon (32.5g/100g) was almost double than in testa (18.8g/100g). The implication of this is that the Moringa testa might have higher levels of solubilising protein and true protein than in the cotyledon. With the exception of Arg (45.9%) and Trp (95.7%), the coefficient of variation percent (CV%) of all amino acids were less than 40.0%, showing the closeness of the samples in all parameters.

In Table 2, there was a high level of correlation coefficient (0.910), showing significant difference of results at $r_{=0.01,}$ with a critical value of 0.590. The coefficient of alienation (C_A) was low at 0.4146, while a corresponding high index of forecasting efficiency (IFE) (0.5854) was noted. While C_A indicates the level at which there is lack of relationship between the samples, IFE is a measure of reduction in the level of error of prediction between the two samples. High IFE indicates low level of C_A. The high level of IFE in Table 2 show that there was high reduction in the error of prediction of relationship between the testa and the cotyledon. Therefore, it was easy to predict that the amino acids of the testa would be able to serve the functions of those in the colyledon and vice versa. The R_{xy} value of 1.02 showed that for each 1.00g/100g in the amino acid value of the testa, there was a corresponding increase of 1.02g/100g in the cotyledon; this attested to the fact that amino acid concentration in the testa was lower than in the cotyledon.

The TEAA (g/100g cp) values in this study as shown in Figure 1(a) were higher than those reported for leaves (35.4), stem (26.3), and root (28.4) of *Moringa oleifera* (Olaofe *et al.*, 2013). Percentage levels of total acidic amino acid (%TAAA): 28.5% (testa) and 24.5% (cotyledon) were higher than percentage levels of total basic amino acid (%TBAA): 13.3% (testa) and 16.3% (cotyledon). The following classes of amino acid were higher in testa than cotyledon (g/100g) - total acidic amino acid (TAAA): testa (23.6) and cotyledon (22.4); total aliphatic AA (TAIAA): testa (26.7) and cotyledon (25.4); total essential aliphatic AA (TEAIAA): testa (13.9) and cotyledon (12.7); total hydroxylic AA (THAA): testa (7.67) and cotyledon (7.59).

The total aromatic AA (TArAA) in this study, 12.8g/100g cp (testa) and 18.5g/100g cp (cotyledon) were higher than the recommended 6.8 - 11.8 g/100g cp for infants (FAO/WHO/UNU 1985). Total sulphur AA (TSAA) in the samples were 3.26 g/100g (testa) and 5.43 g/100g (cotyledon), and percent cysteine (Cys) in TSAA were 73.9% (testa) and 73.5% (colytedon). This shows that Cys was far more abundant than methionine in each sample. Higher Cys than Met in this study corroborates the literature reports on most proteins

of plant sources: coconut endosperm (62.9%) (Adeyeye, 2004), *Phoenix dactylifera* (56.1%) (Olaleye, 2013), and guinea corn samples (58.9 - 72.0%) (Adeyeye, 2008). Cys has been shown to have enhanced effects on Zn absorption (Mendoza, 2002).

The level of Leu/Ile ratio in testa (1.00) and cotyledon (1.47) [Figure 1(b)] showed that testa had almost equal levels of Leu and Ile, but Leu > Ile in cotyledon. Excess Leu in the diet could negatively affect Trp and niacin metabolism (Ghafoorunissa & Nasaringa Rao, 1973). The levels of predicted protein efficiency ratio (P-PER) for testa were PER_1 (1.53), PER_2 (1.42), and PER_3 (0.605); and for cotyledon: PER_1 (1.46), PER_2 (1.38), and PER_3 (0.286). In all three parameters used for calculating P-PER, values in the testa were higher than those in the cotyledon. This shows that protein in the testa would be more effectively utilised than in the cotyledon.

The isoelectric point (p*I*) levels in the samples were 4.65 for testa and 5.37 for cotyledon, showing the p*I* to be in the acidic medium of the pH. The samples may therefore be useful in preparing very low acid foods such as meat products. Olaofe *et al.* (2013) had earlier worked on *Moringa oleifera* tree parts; the p*I* results were: leaves (5.8), stem (5.5), and roots (5.4). The p*I* results in this study were close to the above literature values. The calculated isoelectric point is very useful for organic samples, whereby p*I* could be predicted without going through the rigorous process of obtaining the minimum pH value via protein solubility determination.

The levels of EAAI in the samples were $EAAI_1$: testa (0.951), cotyledon (1.13); $EAAI_2$ testa (96.9), cotyledon (83.0). $EAAI_1$ was computed based on comparison with soybean EAA index and $EAAI_2$ was computed based on comparison with whole egg protein EAA index. Biological value (BV), which was calculated from $EAAI_2$, ranged from 78.7 in cotyledon to 93.9 in testa. EAA index is important in the evaluation of food formulation for protein quality (Nielson, 2002). Also, BV is important in determining the percentage level of a given nutrient source that is utilised in the body (Mune, Mbome & Minka, 2013). High levels of EAAI and BV in this study showed that the samples' protein is of good quality and utilisation. The present study recorded the following ratios: Lys/Trp – testa (12.4), cotyledon (1.35); Met/Trp – testa (3.00), cotyledon (0.980); and Phe/Tyr - testa (3.03), cotyledon (3.40). Both the first two ratios were higher in testa than in cotyledon, whereas Phe/Tyr ratio was almost equal in both samples. The following ratio values are present in mammalian tissue patterns, Lys/Trp: plasma protein (6.2), viscera (5.3), muscle (6.3); Met/Trp: plasma (1.1), viscera (2.0), muscle (2.5) (Albanese, 1959). The optimum ratio of Phe/Tyr has been shown to be 1.5 (i.e., 60% Phe and 40% Tyr) (Pencharz, Hsu & Ball, 2007).

In the AAS based on whole hen's egg amino acid as shown in Table 3, out of the eighteen amino acids determined, five amino acids had their values greater than one in testa. This represents 5/18, with a percentage value of 27.8%. The remaining thirteen out of eighteen i.e., 13/18 (72.2%) had their scores less than 1. Also, two out of the five AAS with score values greater than 1 were EAAs - Phe (1.48) and His (1.03). In the cotyledon, 7/18 with a percentage value of 38.9% had their AASs greater than 1. Out of these, three were essential Phe (2.14), His (1.20), and Arg (1.64). Other amino acids (61.1%) had scores less than 1.0. The limiting amino acid (LAA) in testa was Trp, with a value of 0.157, and in cotyledon, it was Lys (0.321). Therefore, to make all AAs available for metabolism in the body, the LAAs have to be corrected for; 100/15.7 or 6.37 times

as much testa protein and 100/32.1 or 3.12 times as much cotyledon protein have to be eaten when they are served as the sole protein source in the diet.

For pre-school children standard, in each of the samples, six out of the nine parameters (66.7%) had scores greater or equal to one. Other amino acids Leu, Lys, and Trp in testa, and Leu, Lys, Thr in cotyledon had scores less than 1.0. The limiting essential amino acids (LEAAs) in testa and cotyledon were Trp (0.257) and Lys (0.343), respectively. The correction factor were 100/25.7 (3.89) times of testa and 100/34.3 (2.92) times of cotyledon, respectively, in order to make all EAAs available.

In the EAAS based on the FAO/ WHO (1973) standard, Trp (0.283) and Lys (0.362) were the LEAAs in testa and cotyledon, respectively. The correction factors for these two amino acids would be 100/28.3 (3.53) times of testa protein and 100/36.2 (2.76) times of cotyledon protein that must be consumed when the samples are the sole source of protein in the diet. Comparing the results of these two samples using the three scoring procedures, cotyledon was considered to have a higher number of scores greater than 100%.

In the statistical analysis of results summary obtained from Table 3, the correlation coefficient (r_{xy}) (0.781) for egg scores was higher than the critical value (0.590) at n -2 = 16 and $r_{= 0.01}$. The result of variance (r_{xy}^2) showed that 60.99% of variance in the cotyledon was associated with variance in the testa. The results were not significantly different at n -2 =7 and $r_{=0.01}$ (pre-school children), as well as n-2 = 6 and $r_{=0.01}$ (provisional). The coefficient of alienation in all the scoring patterns were high within the range of 0.62446 – 0.8552, with corresponding low levels of IFE ranging from 0.1448 – 0.3754. The high C_A implied that there was high level of lack of relationship between the two samples in terms of scores and low levels of IFE showed low reduction in the error of prediction of relationship between samples.

CONCLUSION

Findings of this study showed that *Moringa oleifera* contained appreciable amounts of amino acids, especially EAAs. The results of EAAI and BV clearly revealed that the proteins in the samples were of good quality. Though most of the AAS were close to or greater than 100% in both samples, the cotyledon was better in terms of score values. However, some amino acids, especially EAAs, were more concentrated in testa. Also, the protein efficiency ratio values showed that proteins in the testa would be more effectively utilised than those in the cotyledon. The testa should be seen as an important integral anatomical part of the seed; both the testa and the cotyledon would complement each other in terms of amino acid contribution and therefore should be consumed together in the diet. It will be an eye opener for those who are always fond of discarding the testa when preparing certain meals. This comparative study will provide new and additional information in the application of *M. oleifera* in food industry, especially in the production of animal feeds.

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Authors' contributions

EIA, the principal researcher, conceptualised and designed the research, wrote the original draft, and reviewed the manuscript; AAO, conducted the study, data analysis and interpretation, reviewed the manuscript; OTI, helped in writing the manuscript, data analysis and interpretation; HOA, conducted the study and reviewed the manuscript; KEA, conducted data analysis and interpretation, reviewed the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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