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COMPARABILITY OF THE AMINO ACID COMPOSITION OF ARIL AND SEED OF *BLIGHIA SAPIDA* FRUIT

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ABSTRACT

Akee apple fruit (*Blighia sapida* Konig), is one of the popular small-scale tropical fruits and it is an important crop. *B. sapida* may be eaten raw (without the pink raphe attaching the aril to the seed) or after cooking when it resembles scrambled eggs. Fruits like the akee apple are novelties for many people except in the savannah belt; in the localities where they grow they are eaten and relished. In view of this, an investigation into the concentrations of amino acids of the aril and seed parts of *Blighia sapida* fruit was carried out using standard methods to determine amino acid profiles; quality of dietary protein was determined using various methods like: amino acid scores determination [(in three different ways); (i) amino acid score based on the whole hen's egg, (ii) essential amino acid score based on the provisional amino acid scoring pattern, (iii) essential amino acid score based on suggested school child requirement] essential amino acid index and predicted protein efficiency ratio. For quick precipitation of protein, the isoelectric point was also determined. Glutamic acid was the most abundant amino acid (11.4-12.7 g/100 g) and while Arg was the most abundant (7.25 g/100 g) essential amino acid in the aril, it was Leu (6.58 g/100 g) in the seed. The total essential amino acid in aril was 33.7 g/100 g (50.2 %). It was 33.8 g/100 g (45.8 %) in the seed. The limiting essential amino acid (based on provisional scoring pattern) was Met + Cys (0.60) in aril and Thr (0.59) in seed. The essential amino acid index ranged from 1.08 (seed) to 1.62 (aril); the predicted protein efficiency ratio was 1.83 in aril and 2.20 in the seed whereas the isoelectric point ranged between 3.89 in aril and 4.0 in the seed. At α 0.05, significant differences existed in the samples in amino acid profiles and calculated isoelectric point (pI). The results of this study indicated that the amino acid profiles of akee apple aril and seed are similar in composition, being good sources of many of the essential amino acids. Whilst the aril is eaten fresh, the seed can be exploited for human food.

Key words: *Blighia sapida*, amino acid profiles, quality

INTRODUCTION

Akee apple fruit (*Blighia sapida*, Konig) is one of the popular small-scale tropical fruits and important crop but not as important as orange and mango fruits. The origin and the early history suggested that the fruit occurred in the wild forests of West Africa, where it is often planted. In the late 18th century, it was introduced in Jamaica [1]. *B. sapida* belongs to the family *Sapindaceae* and has been given various local names in Nigeria: Hausa (*gwanja kusa*), Fulani (*feso*), Yoruba (*ishin*) [2].

Akee is an evergreen, polymagous tree of about 7-25 m high. Seedlings begin to fruit in about five years [1]. Only the aril part of the ripe fruit is edible. The unripe fruits when eaten can cause vomiting and circulatory collapse.

B. sapida has been put into many medicinal uses [3]. The unripe fruits are pounded together and used as fish poison. The family name *Sapindaceae* took its name from soapberry tree *Sapindus saponaria* which contains saponin. It was, therefore, thought that *B. sapida* would contain saponin, as in *S. saponaria*. Phytochemical studies on the plant showed the presence of steroid saponin which could be useful in the manufacture of steroid drugs [4].

B. sapida may be eaten raw (without the pink raphe attaching the aril to the seed) or after cooking when it resembles scrambled eggs. Fruits like the akee and baobab are novelties for many people except in the savannah belt; in the localities where they grow they are eaten and relished. The information on the nutritional qualities of *B. sapida* is scanty. Adeyeye and Oyarekua [5] reported on the proximate composition and some nutritionally valuable minerals in the dehulled seeds and seed hull of *Blighia sapida*. The present investigation is, therefore, to evaluate and compare the amino acid profiles of the aril and seed of ripe and naturally opened *Blighia sapida* fruits. This type of information may improve the food composition tables of *B. sapida*.

MATERIALS AND METHODS

Sampling

About 100 naturally – opened fresh akee apple fruits were collected from the same location in Ado-Ekiti, Ekiti State, Nigeria. The samples were put in a clean sterilized polythene bag and were taken to the laboratory. The fresh fruits were examined at room temperature (28 ^{0}C) while some were stored at 4 ^{0}C for further use.

Sample treatment

In the laboratory, the aril and the seeds were separated and sun-dried until constant weight was obtained. Later, the seed testa was removed. Both aril and dehulled seeds were now milled into fine particles using the laboratory mortar and pestle; the fine particles were sieved using a 200 mm mesh sieve separately for each sample and stored in airtight containers in the refrigerator at -4 ^{0}C until used.

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Determination of amino acids

Defatting

About 2.0 g of each sample were weighed into the extraction thimble and the fat extracted with chloroform/methanol (2:1 v/v) mixture using a Soxhlet apparatus [6]. The extraction lasted, 5- 6 h.

Hydrolysis of samples

About 30 mg of the defatted sample was weighed into glass ampoules. Seven (7) milliliters of 6 M HCl were added and oxygen expelled by passing nitrogen gas into the samples. The glass ampoules were sealed with a Bunsen flame and put into an oven at 105$\pm$5 ^{0} C for 22 h. The ampoule was allowed to cool; the content was filtered to remove the humins. The filtrate was then evaporated to dryness at 40 ^{0} C under vacuum in a rotary evaporator.

Samples analysis

Amino acid analysis was by ion exchange chromatography (IEC) [7] using the Technicon Sequential Multisample (TSM) Amino Acid Analyser (Technicon Instruments Corporation, New York). The period of analysis was 76 min for each sample. The gas flow rate was 0.50 ml/min at 60 ^{0} C with reproducibility consistent within $\pm$3 %. The net height of each peak produced by the chart recorder of the TSM (each representing an amino acid) was measured and calculated. The amino acid determinations were in duplicate. Tryptophan was not determined. Norleucine was the internal standard.

Estimation of quality of dietary protein

Amino acid score

The amino acid score was calculated in three different ways.

(a) The amino acid score based on the whole hen's egg [8]. It was calculated by using the ratio of test protein to the reference protein for each amino acid.

(b) The essential amino acid score based on the provisional amino acid scoring pattern using the following formula [9]:
Amino acid score= Amount of amino acid per test protein [mg/g]/ Amount of amino acid per protein in reference protein [mg/g].

(c) The essential amino acid score based on suggested school child requirement [10].

Essential amino acid index (EAAI)

It was calculated by using the ratio of test protein to the reference protein for each of the eight essential amino acids plus histidine in the equation that follows [11]:

$$\text{Essential amino acid index} = \sqrt[9]{\frac{\text{mg lysine in 1g test protein}}{\text{mg lysine in 1g reference protein}} \times \text{for all 8 essential amino acids + His}}$$

Predicted protein efficiency ratio (P-PER)
This was determined using one of the equations developed by Alsmeyer *et al*. [12],
P-PER= -0.468+0.454 (Leu) – 0.105 (Tyr).

Determination of other quality parameters
Determination of the total essential amino acid (TEAA) to the total amino acid (TAA) (TEAA/TAA); total sulphur amino acid (TSAA); percentage cystine in TSAA (% Cys/TSAA); total aromatic amino acid (TArAA); etc., the Leu/Ile ratios were also calculated.

Estimation of isoelectric point (pI)
The pI for the mixture of the amino acids was estimated from the equation of the form [13]:

$$IPm = \sum_{i=1}^{n} IPiXi$$

where IPm is the isoelectric point of the mixture of amino acids, IPi is the isoelectric point of the i^{th} amino acid in the mixture and Xi is the mass or mole fraction of the i^{th} amino acid in the mixture.

Statistical analysis
Calculations made were the mean, standard deviation (SD), coefficient of variation in percent (CV %), linear correlation coefficient (r_{xy}), and the comparison of r-value (computed from the analytical data) with tabular value at $r_{0.05}$ with n-2 degrees of freedom [14].

RESULTS

Table 1 shows the amino acid (AA) compositions for each sample. Glutamic and aspartic acids were in the highest concentrations among their groups and are both acidic AA. Phenylalanine and tyrosine constituted the highest essential amino acid (EAA) concentration in both samples. The coefficient of variation percent (CV %) values were low with the exception of Cys with a value of 69.2 %, while the rest ranged from 0.00 %-41.7 %.

Table 2 shows the concentrations of total AA (TAA), total essential AA (TEAA), total acidic AA (TAAA), total neutral AA (TNAA), total sulphur AA (TSAA), total aromatic AA (TArAA) and their percentage levels. The CV % values were mostly low with a range of 0.25-39.

Table 3 shows the AA scores based on the whole hen's egg. While Ser (0.22) had the lowest score in the aril, it was Met (0.39) in the seed. Cys had the highest CV % (68.8) while Met showed no variation (0.00 %); other CV% range was 4.23-41.1. Table 4 shows the EAA scores based on the provisional EAA scoring pattern. Here Met + Cys (0.60) were limiting in aril but Thr (0.59) was limiting in the seed. The CV % ranged from 5.05-39.8. Table 5 shows the EAA scores based on the suggested pre-school child requirement. In aril sample, Met + Cys (0.84) were again the limiting AA and Thr (0.69) was again limiting in the seed sample. CV % range was 5.33-39.9.

Table 6 shows r_{xy} in Tables 1 and 2 (for pI), to be significantly different at probability level of $r_{0.05}$ at n-2 degrees of freedom.

DISCUSSION

The protein levels in the two samples had a variation of 22.7 %. With the higher level of protein in the aril sample, one would expect that the overall levels of the amino acids in it would be more than in the seed. However, Table 1 shows that the AA in the aril were generally lower than in the seed showing that the crude protein of the seed would contain more true protein than in the aril.

Glu, Asp and Phe+Tyr trends in the current study followed the trend as observed in *Gymnarchus niloticus* (Trunk fish) [15] and in *Clarias anguillaris*, *Oreochromis niloticus* and *Cynoglossus senegalensis* [16]. Arg (4.40-7.25 g/100 g cp) is essential for children and reasonable levels were present here particularly in the aril. The Lys contents of the samples (4.03-5.11 g/100 g cp) were close to the content of the reference egg protein (6.3 g/100 g cp), and the aril will, therefore, serve as a good source for fortification of cereal weaning foods. It is interesting to note that Met shared similar levels of 1.25 g/100 g cp in both samples (Table 1), which gave 0.00 % variation. Cys had the highest variation of 0.86-2.51 g/100 g cp or 69.2 %.

The contents of TEAA of 33.7 and 33.8 g/100 g cp without tryptophan (which was not determined) (Table 2) were close to the value for egg reference protein (56.6 g/100 g cp) [8]. The current contents of TEAA are comparable to some literature values (g/100 g cp);

33.6 in *Anacardium occidentale* [17]; 31.2 in *Parkia biglobosa* seeds [18]; 22.1 in endosperm of ripe coconut [19], 37.6 – 51.8 in six different varieties of dehulled *Sphenostylis stenocarpa* flour [20]; values from oil seeds such as 45.2 in pigeon pea [21], 53.4 (melon seed), 38.3 (pumpkin seed), and 53.6 (gourd seed) respectively [22]; and soy bean with 44.4 [23]. The contents of TSAA were generally lower than the 5.8 g/100 g cp recommended for infants [10]. The TArAA range suggested for ideal infant protein (6.8-11.8 g/100 g cp) [10] has current values close to the minimum, that is, 6.14-7.13 g/100 g cp. The ArAA are precursors of epinephrine and thyroxin [24].

The percentage ratios of TEAA to the TAA in the samples were 50.2 % (aril) and 45.8 % (seed), which were well above the 39 % considered to be adequate for ideal protein food for infants, 26 % for children and 11 % for adults [10]. The TEAA/TAA percentage contents were strongly comparable to that of egg (50 %) [25], 43.6 % reported for pigeon pea flour [23] and 43.8-44.4 % reported for beach pea protein isolate [23]. The percentages of total neutral AA (TNAA) ranged from 50.9–54.5, indicating that these formed the bulk of the AA; total acidic AA (TAAA) ranged from 29.5-29.9, which was lower than the % TNAA, while the percent range in total basic AA (TBAA) was 16.0-19.2, which made them the third largest group among the samples.

Most animal proteins are low in cystine (Cys) and hence in Cys in TSAA. For example, (Cys/TSAA) % were 35.5 % in *Archachatina marginata*, 38.8 % in *Archatina archatina* and 21.0 % in *Limicolaria* sp., respectively [26]; 29.8 % in *G. niloticus* [15]; 23.8 % in *C. anguillaris*, 28.4 % in *O. niloticus* and 30.1 in *C. senegalensis*, respectively [16]. In contrast, many vegetable proteins contain substantially more Cys than Met, for example, 62.9 % in coconut endosperm [19]; 44.4 % in *P. biglobosa* [18]; 44.3 % in *Cola acuminata*, 37.8 % in *Garcinia kola* and 50.5 % in *A. occidentale* [17]. Although FAO/WHO/UNU [10] did not give any indication of the proportion of TSAA which can be met by Cys in man, for rats, chicks and pigs, the proportion is about 50 % [10]. The current result of (Cys/TSAA) % ranged from 40.8-66.8, which were closer to plant literature results than the animals. Information on the agronomic advantages of increasing the concentration of sulphur-containing amino acids in staple foods shows that Cys has positive effects on mineral absorption, particularly zinc.

The P – PER values were higher than 1.21 (cowpea), 1.82 (pigeon pea); 1.62 (millet *ogi*) and 0.27 (sorghum *ogi)* [27] and close to 2.0 (*P. biglobosa*) [18]; reference casein with PER of 2.50 [27]; 1.89-2.22 in three different fish samples [16]; but much lower than 4.06 in modified corn *ogi* [27].

In the consumption of maize and sorghum, it has been suggested that an amino acid imbalance from excess Leu might be a factor in the development of pellagra [28].

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The current Leu/Ile ratio range was 1.78-2.26 with a difference of 2.88-3.15 g/100 g cp or 43.8-55.8 %.

Clinical, biochemical and pathological observations in human and rat experiments showed that high Leu in the diet impairs the metabolism of Try and niacin and is responsible for the niacin deficiency in sorghum eaters. High Leu is also a factor contributing to the pellagragenic properties of maize. Excess Leu could be counteracted by increasing the intake of niacin or Try and also with supplementation with Ile. These studies suggested that the Leu/Ile balance is more important than dietary excess of Leu alone in regulating the metabolism of Try and niacin and hence the disease process [28]. The present Leu/Ile ratios were low in value. Also all of the current Leu values were less than 11.0 g/100 g cp; and actually the range was 5.65-6.58 g/100 g cp, and therefore considered safe and could be beneficially exploited to prevent pellagra in endemic areas.

The essential amino acid index (EAAI) ranged between 1.08-1.62. The essential amino acid index method can be useful as a rapid tool to evaluate food formulations for protein quality. However, it does not account for differences in protein quality due to various processing methods or certain chemical reactions [29]. Essential amino acid index for defatted soy flour is 1.26 [29].

The isoelectric points (pI) as calculated for the AA were 3.89 (aril) and 4.03 (seed) (Table 2). The total neutral amino acid has pI of 5.0–6.3, the TAAA has pI of 3.0-3.1 while pI for TBAA is 7.6-10.8. Olaofe and Akintayo [13] used this method to predict pI of legume and oilseed proteins from their AA in which the overall average percentage deviation was 23.3 %. This method is, therefore, a good starting point in predicting pI for proteins in order to enhance a quick precipitation of protein isolate from a biological sample.

The amino acid scores based on whole hen's egg are shown in Table 3. Histidine (His) is a semi-essential AA particularly useful for children growth. This same characteristic also applies to Arg; both His and Arg had high scores in comparison to hen's egg. Ser had the lowest score (0.22 or 22.0 %) in aril while Met had the least score (0.39 or 39.0 %) in seed. The correction ratio for the whole AA in aril would be 100/22 x aril protein and 100/39 x seed protein or 4.55 x aril protein and 2.56 x seed protein respectively. Table 4 shows the limiting EAA (LEAA) to be Met + Cys (0.60 or 60.0 %) in aril and Thr (0.59 or 59.0 %) in seed. Corrections here would, therefore, be 100/60 x aril protein and 100/59 x seed protein or 1.67 x aril protein and 1.69 x seed protein, respectively in order to bring all the EAA to the required standards when they serve as sole sources of protein. Table 5 shows the AA scores based on the suggested pre-school child requirements. The LEAA for aril was Lys (0.69 or 69.0 %) and Thr (0.69 or 69.0 %) in seed. For correction, each would require 100/69 or 1.45 x sample protein to satisfy the requirement when each serves as the sole source of dietary protein. On the overall scoring pattern, Gly was best in Table 3 (1.19-1.28), Phe + Tyr was best in Table 4 (1.02-1.19) and Val was best in Table 5 (1.15-1.24), respectively.

Table 6 shows the summary of statistical analysis from Tables 1, 2, 3, 4 and 5. The correlation coefficient (r_{xy}) for Tables 1 and 2 values were positively high and significant at $r_{=0.05}$ and n-2 degrees of freedom since $r_{calc.} > r_{table}$. From Tables 4 and 5, the values there were negatively correlated and not significant; values of r_{xy} from Table 3 were positive but not significant.

CONCLUSION

In summary, this study indicates that the amino acid profiles of *Blighia sapida* aril and seed have close composition (see Table 7 particularly the EAA under both factors A and B means). Both are good sources of many of the essential amino acids. While the aril is eaten fresh, the seed can be exploited for human food.

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Table 1: Amino acid profile of the *Blighia sapida* fruit on dry weight (g/100 g crude protein)

Amino acid	Aril	Seed	Mean	SD	CV%
Lysine (Lys) [a]	4.03	5.11	4.57	0.76	16.7
Histidine (His) [a]	1.61	2.30	1.96	0.49	25.0
Arginine (Arg) [a]	7.25	4.40	5.83	2.02	34.6
Aspartic acid (Asp)	8.65	9.18	8.92	0.37	4.20
Threonine (Thr) [a]	3.50	2.35	2.93	0.81	27.8
Serine (Ser)	1.71	3.14	2.43	1.01	41.7
Glutamic acid (Glu)	11.4	12.7	12.1	0.92	7.63
Proline (Pro)	1.63	2.75	2.19	0.79	36.2
Glycine (Gly)	3.56	3.03	3.30	0.37	11.4
Alanine (Ala)	3.04	3.84	3.44	0.57	16.4
Methionine (Met) [a]	1.25	1.25	0.00	0.00	0.00
Cystine (Cys)	0.86	2.51	1.69	1.17	69.2
Valine (Val) [a]	4.33	4.04	4.19	0.21	4.90
Isoleucine (Ile) [a]	2.50	3.70	3.10	0.85	27.4
Leucine (Leu) [a]	5.65	6.58	6.12	0.66	10.8
Phenylalanine (Phe) [a]	3.60	4.11	3.86	0.36	9.35
Tyrosine (Tyr)	2.54	3.02	2.78	0.34	12.2
Tryptophan (Try)*	-	-	-	-	-
Protein [b]	21.3	15.4	18.4	4.17	22.7

*(-), Not determined. [a]Essential amino acid. [b]Dry and fat free.

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Table 2: Concentrations of essential, non-essential, acidic, neutral, sulphur, aromatic, (g/100 g crude protein) of *B. sapida* samples (dry weight of sample)

Amino acid	Aril	Seed	Mean	SD	CV %
Total amino acid (TAA)	67.1	74.0	70.6	4.88	6.82
Total non-essential amino acid (TNEAA)	33.4	40.1	36.8	4.74	12.9
Total essential amino acid (TEAA) – with His	33.7	33.8	33.8	0.07	0.21
- no His	32.1	31.5	31.8	0.42	1.33
% TNEAA	49.8	54.2	52.0	3.11	5.98
% TEAA, -with His	50.2	45.8	48.0	3.11	6.48
-no His	47.8	42.6	45.2	3.68	8.13
Total neutral amino acid (TNAA)	34.2	40.3	37.3	4.31	11.6
% TNAA	50.9	54.5	52.7	2.55	4.83
Total acidic amino acid (TAAA)	20.1	21.8	21.0	1.20	5.74
% TAAA	29.9	29.5	29.7	0.28	0.95
Total basic amino acid (TBAA)	12.9	11.8	12.4	0.78	6.30
% TBAA	19.2	16.0	17.6	2.26	12.9
Total sulphur amino acid (TSAA)	2.11	3.76	2.94	1.17	39.8
% TSAA	3.14	5.08	4.11	1.37	33.4
% Cys/TSAA	40.8	66.8	53.8	18.4	34.2
Total aromatic amino acid (TArAA)	6.14	7.13	6.64	0.70	10.6
% TArAA	9.15	9.64	9.40	0.35	3.69
P-PER[a]	1.83	2.20	2.02	0.26	13.0
Leu/Ile ratio	2.26	1.78	2.02	0.34	16.8
Leu/Ile ratio (difference)	3.15	2.88	3.02	0.19	6.33
% Leu-Ile (difference)	55.8	43.8	49.8	8.49	17.0
EAAI[b]	1.62	1.08	1.35	0.38	28.3
Isoelectric point (pI)	3.89	4.03	3.96	0.10	2.50

[a]Predicted protein efficiency ratio. [b]Essential amino acid index

Table 3: Amino acid scores of *B. sapida* samples based on whole hen's egg

Amino acid	Aril	Seed	Mean	SD	CV%
Lys	0.65	0.82	0.74	0.12	16.4
His	0.67	0.96	0.82	0.21	25.2
Arg	1.19	0.72	0.96	0.33	34.6
Asp	0.81	0.86	0.84	0.04	4.23
Thr	0.69	0.46	0.58	0.16	28.3
Ser	0.22	0.40	0.31	0.13	41.1
Glu	0.95	1.05	1.00	0.07	7.07
Pro	0.43	0.72	0.58	0.21	35.7
Gly	1.19	1.28	1.24	0.06	5.15
Ala	0.56	0.71	0.64	0.11	16.7
Cys	0.48	1.39	0.94	0.64	68.8
Val	0.58	0.54	0.56	0.03	5.05
Met	0.39	0.39	0.39	0.00	0.00
Ile	0.45	0.66	0.56	0.15	26.5
Leu	0.68	0.79	0.74	0.08	10.5
Tyr	0.64	0.76	0.70	0.08	12.1
Phe	0.71	0.81	0.76	0.07	9.30

Table 4: Essential amino acid scores of *B. sapida* samples based on provisional amino acid scoring pattern

Amino acid	Aril	Seed	Mean	SD	CV%
Lys	0.73	0.93	0.83	0.14	17.0
Thr	0.88	0.59	0.74	0.21	27.9
Val	0.87	0.81	0.84	0.04	5.05
Met + Cys	0.60	1.07	0.84	0.33	39.8
Ile	0.63	0.93	0.78	0.21	27.2
Leu	0.81	0.94	0.88	0.09	10.5
Phe + Tyr	1.02	1.19	1.11	0.12	10.9
Try	-	-	-	-	-
Total*	0.81	0.93	0.87	0.08	9.75

* Total is based on the total essential amino acid of FAO/WHO [9].

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Table 5: Essential amino acid scores of *B. sapida* samples based on suggested pre school child requirement

Amino acid	Aril	Seed	Mean	SD	CV%
Lys	0.69	0.88	0.79	0.13	17.1
His	0.85	1.21	1.03	0.25	24.7
Thr	1.03	0.69	0.86	0.24	28.0
Val	1.24	1.15	1.20	0.06	5.33
Met + Cys	0.84	1.50	1.17	0.47	39.9
Ile	0.89	1.32	1.11	0.30	27.5
Leu	0.86	1.00	0.93	0.10	10.6
Phe + Tyr	0.97	1.13	1.05	0.11	10.8
Try	-	-	-	-	-
Total*	0.91	1.07	0.99	0.11	11.4

* Total is based on the total essential amino acid of FAO/WHO/UNU [10].

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Table 6: Summary of statistical analysis from Tables 1, 2, 3, 4 and 5

Table	[a]r_{xy}	[b]Remark
1	0.9222	Significant
2 (pI only)	0.8219	Significant
3	0.4739	Not Significant
4	-0.0389	Not Significant
5	-0.0587	Not Significant

[a]Correlation coefficient. [b]Results significantly different at $r_{0.05}$ and n-2 degrees of freedom.

Table 7: Summary of the amino acid profiles into factors A and B

	Blighia sapida fruit(Factor A)		Factor
	Aril	Seed	B means
Amino acid composition (Factor B)			
Total essential amino acid	33.7	33.8	33.8
Total non-essential amino acid	33.4	40.1	36.8
Factor A means	33.6	37.0	35.3

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