THE AMINO ACIDS COMPOSITION OF SMOOTH LOOFAH (*Luffa cylindrical* L.), ROSELLE (*Hibiscus sabdariffa*) AND SESAME (*Sesamum indicum*) SEEDS

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Abstract: The amino acid composition of smooth loofah, roselle and sesame seeds were determined using standard analytical techniques. The amino acid analysis revealed that all the samples contained nutritional useful quantities of most of the essential amino acids (EAA). The total amino acid values were high at 97.2 g/100g (smooth loofah); 72.8 g/100g (roselle); 97.5 g/100g (sesame). The total essential amino acid (TEAA) ranged from 34.7 g/100g in roselle to 44.6 g/100g in sesame and 32.1 g/100g in smooth loofah and 42.0 g/100g in sesame with and without histidine, respectively. The most concentrated amino acid for the three seeds was glutamic acid wiyh values range from 15.7 g/100g protein in roselle to 31.4g/100g cp in smooth loofah. The predicted protein efficiency ratio (P-PER) range was 1.6 – 2.1. The isoelectric point (pI) ranged from 4.2 in roselle to 5.8 in sesame, showing the samples protein to be in acidic medium of the pH range. In the amino acid scores, limiting amino acids were serine (0.165) in smooth loofah, and lysine (0.435 and 0.468) in roselle and sesame, respectively in whole hen's egg; Lys in all (0.25-0.53) in provisional EAA scoring pattern; only roselle and sesame would supply virtually the required EAAs for the pre-school child as most determinations were above 100% requirement. Isoleucine had the highest score in smooth loofah 1.75 g/100g and roselle (1.15 g/100g) while Met+Cys had the highest score in sesame seed (1.34 g/100g).

Keywords: Amino acids, analysis, composition, roselle, sesame, smooth loofah

Introduction

The importance and health benefits of grain and beans consumption in the prevention of chronic diseases have been documented. Even though nutritional guidelines put grains and grain products at the base of the food grade pyramid to emphasize their importance for optimal health, little attention has been paid to grains, beans and also to seed consumption compared to fruits and vegetables. Most seeds are very cheap andnutritious. Researchers have confirmed their nutritional usefulness and many different interpretations and views have been discussed about its nutritional benefits (Salleh, 1992; Barampana & Zimard, 1993). In view of this, it is very important to study the amino acid of seeds of smooth loofah (*Luffa cylindrical* L.), roselle (*Hibiscus sabdariffa*) and sesame (*Sesamumindicum*).

Sponge guards are the fruit of *Luffa cylindrical* L. which is nicely used throughout the world. It is a plant material that comes from the fruit of the natural vegetable sponge *Luffa cylindrical* L. which is a member of the *Cucurbitaceae* family. It looks like cucumber. It is already part of beauty and skin regimens because it is often used as both sponges for their gentle exfoliating properties. Roselle (*Hibiscus sabdariffa* Linn) is a member of the plant family *Malvaceae*. It is a very versatile plant similar to the coconut tree. Roselle can be found in almost all warm countries such as India, Saudi Arabia, Malaysia and Nigeria, etc. It is known in Nigeria as *Zobo*, in Indonesia *Rosella*, in Namimbia *Omutete*, etc. The brilliant red colour and unique flavour makes it valuable food product (Tsai *et al*., 2002). Sesame (*Sesamum indicum*) is an ancient seed and one of the oldest oil seed crops cultivated in the world. This warm-season annual crop that is drought resistant is primarily adapted to areas with long growing seasons and well – drained soils and has spread from its place of origin in Iraq to many parts of the world. The objective of this work is to investigate the amino acid composition of the three seeds. This would provide useful information on their better health benefits.

Materials and Methods

Seeds of roselle (*Hibiscus sabdariffa*) were bought from Agbado Ekiti in Gbonyin Local Government Area of Ekiti State, Nigeria. Smooth loofah (*Luffa cylindrical* L.) seeds were obtained from a health tree at Aba Erifun road while sesame (*Sesamum indicum*) seeds were bought from Oja Oba market both in Ado-Ekiti, Ekiti State, Nigeria. The seeds were sun-dried, decorticated, milled, sieved and stored in polythene bag prior to use. All analyses were done in triplicate.

Amino acid analysis

Two grams (2.0 g) each of the seed samples were defatted with 40:60 chloroform/methanol mixtures. 30 mg of each defatted sample were put in glass ampoule. 7 ml of 6MHCl was added to each sample and oxygen expelled. The sealed ampoule of each sample was put in oven at 105 ± 5^{o}C for 22 h and later allowed to cool in a dessicator before the contents were filtered. The filtrates were evaporated to dryness at 40^{o}C under vacuum. Residues were dissolved with 5 ml acetate buffer (pH 2.0). The amino acid analysis was carried out by the ion-exchange Chromatography method (FAO/WHO, 1991) using the technicon sequential multisample Amino Acid Analyzer (TSM) (Technicon Instruments Corporation, New York).

Quality parameters determination

Determination of amino acid scores

Determination of the amino acidscores wasfirst based on whole hen's egg (Paul *et al*., 1976). In this method, both essential and non-essential amino acids were scored.Secondly, amino acid score was calculated using the provisional essential amino acid scoring pattern (FAO/WHO, 1973). Amino acid score = (amount of amino acid per test protein (mg/g))/(amount of amino acid per protein in reference pattern (mg/g)).

Amino acid score based on pre-school child essential amino acid requirement for ages 2-5 yrs (FAO/WHO/UNU, 1985) was also calculated.

Estimation of isoelectric point (pI)

The estimation of the isoelestric point (pI) for a mixture of amino acids was calculated using the equation of the form:

$$\text{pIm} = \sum_{i=1}^{n} pIiXi$$

Where pIm is the isoelectric point of the mixture of amino acids, pI$\boldsymbol{i}$ is the isoelectric point of the $\boldsymbol{i}$th amino acids in the mixture and x$\boldsymbol{i}$ is the mass or mole fraction of $\boldsymbol{i}$th amino acid in the mixture (Olaofe & Akintayo, 2000).

Determinations of the ratio of total essential amino acids to the total amino acids (TEAA/TAA), total sulphur amino acid (TSAA), percentage cystine in TSAA (%Cys/TSAA); total

aromatic amino acid (TArAA); total neutral amino acid (TNAA); total acidic amino acid (TAAA); total basic amino acid (TBAA) were done from the results obtained for the amino acid profile while the predicted protein efficiency ratio (P-PER) was determined using one of the equations developed by Alsmeyer *et al.* (1974), that is, P-PER = -0.468 + 0.454 (Leu) – 0.105 (Tyr). The leucine/isoleucine ratio was also calculated.

Results and Discussion

Table 1 shows the amino acid composition of smooth loofah (*Luffa cylindrical*), roselle (*Hibiscus sabdariffa*) and sesame (*Sesamum indicum*). In g/100g protein, glutamic acid (15.7 – 31.4) had the highest concentration in the three seed samples followed by arginine (6.8 – 12.6). Cystine was the least abundant amino acid in roselle (1.2) and sesame (1.7) while it was not detected in smooth loofah.

Tables 2 and 3 depict the concentrations of total amino acid (TAA), total essential amino acid (TEAA), total acidic amino acid (TAAA), total basic amino acid (TBAA), total sulphur amino acid (TSAA), total aromatic amino acid (TArAA) and their percentage values. The predicted protein efficiency ratio (P-PER), Leu/Ile ratios and Leu-Ile (difference) are also contained in Table 3. The results are summarized in (g/100g protein): TAA (72.8 – 97.5) TEAA with His (34.7 – 44.6), TEAA minus His (32.1 – 42.0), TAAA (20.9 – 38.3), TBAA (11.6 – 18.1), TNAA (40.3 – 51.8), TSAA (1.5 – 4.7), TNEAA (38.1 – 59.5), TArAA (3.3 – 8.0). The percentage values ranged from 38.8 – 45.7 (% TEAA with His), 33.0 – 44.8 (%TEAA minus His), 52.3 – 61.2 (%TNEAA), 28.3 – 39.4 (%TAAA), 15.9 – 18.6 (% TBAA), 1.5 – 4.8 (% TSAA) and 3.4 – 9.3 (% TArAA). It has shown that TAAA was higher than TBAA in all the seed samples. Smooth loofah and roselle recorded the same value (1.6) of P-PER whereas sesame had 2.1. Leu/Ile ratios ranged from 0.7 – 1.9 and isoelectric point pI had a range of 4.2 – 5.8.

Tables 4, 5 and 6 show the amino acid score based on whole hen's egg amino acid, amino acid scoring pattern and the suggested amino acid requirements for pre-school children, respectively. Lysine had the lowest score (0.25 – 0.53) based on amino acid scoring pattern in all the seed samples.

The most concentrated essential amino acid in all the samples is arginine (Arg) with values of 10.1 g/100g protein (smooth loofah), 6.8 g/100g protein (roselle) and 12.6 g/100g protein (sesame). Literature showed that presence of substantial amounts of Arg in diets enhances Ca^{2+} absorption, but under most physiological circumstances, this is of little consequence (White *et al*., 1973).

The total aromatic amino acid (6.8 g/100g) in roselle seed compared favourably with the suggested minimum (6.8 g/100g cp) for ideal infant protein (FAO/WHO/UNU, 1985) but smooth loofah (3.3 g/100g) was lower and that of sesame (8.0 g/100g cp) was comparatively higher than the standard. The predicted protein efficiency ratio (P-PER) (Table 3) of the seeds fell between 1.6 -2.1. That of sesame (2.1) was better than 1.82 of pigeon pea (Salunkhe and Kadam, 1989), 0.27 of sorghum /ogi and millet / ogi (Oyarekua and Eleyinmi, 2004).

Table 1: Amino acid composition (g/100g crude protein) of seed flour of Smooth loofah (*Luffa cylindrical* L.), Roselle (*Hibiscus sabdariffa*) and Sesame (*Sesamum indicum*)

Amino acid	Smooth loofah	Roselle	Sesame	Mean	SD	CV%
Glycine	8.9	3.5	5.8	6.07	2.71	44.6
Alanine	1.7	4.2	4.8	3.57	1.14	45.9
Serine	1.3	3.8	5.3	3.47	2.02	58.2
Proline	9.3	2.1	4.3	5.23	3.69	70.6
Valine	1.5	3.3	4.6	3.13	1.56	49.8
Threonine	2.5	3.4	4.4	3.43	0.95	27.7
Isoleucine	7.0	4.6	3.4	5.0	1.83	36.6
Leucine	4.8	5.1	6.5	5.47	0.907	16.6
Aspartic acid	6.9	5.2	8.8	6.97	1.80	25.8
Lysine	1.4	2.7	2.9	2.33	0.814	34.9
Methionine	1.5	2.3	3.0	2.27	0.751	33.1
Glutamic acid	31.4	15.7	18.8	22.0	8.32	37.8
Phenylalanine	3.3	4.4	4.6	4.10	0.70	17.1
Histidine	5.6	2.1	2.6	3.43	1.89	55.1
Arginine	10.1	6.8	12.6	9.83	2.91	29.6
Tyrosine	ND	2.4	3.4	-	-	-
Cystine	ND	1.2	1.7	-	-	-
Total	97.2	72.8	97.5	89.2	14.2	15.9

ND = not detected

Table 2: Total amino acid, total essential, non essential and neutral amino acids (g/100g) of smooth loofah, roselle and sesame seeds

Amino acid	Smooth loofah	Roselle	Sesame	Mean	SD	CV%
Total amino acid (TAA)	97.2	72.8	97.5	89.2	14.2	15.9
Total non-essential amino acid (TNEAA)	59.5	38.1	52.9	50.2	11.0	21.9
Total essential amino acid (TEAA)						
– with His	37.7	34.7	44.6	39.0	5.08	13.0
– without His	32.1	32.6	42.0	35.6	5.58	15.7
% TNEAA	61.2	52.3	54.3	55.9	4.67	8.35
% TEAA						
– with His	38.8	47.7	45.7	44.1	4.67	10.6
– without His	33.0	44.8	43.1	40.3	6.38	15.8
Total neutral amino acid TNAA	41.8	40.3	51.8	44.6	6.25	14.0
% TNAA	43.0	55.4	53.1	50.5	6.60	13.1

Table 3: Total acidic amino acid (TAAA), total basic amino acid (TBAA), total sulphur amino acid (TSAA), total aromatic amino acid (TArAA) (g/100g), P-PER, pI and Leu/Ile ratio of smooth loofah, roselle andsesame seeds

	Smooth loofah	Roselle	Sesame	Means	SD	CV%
Total acidic amino acid (TAAA)	38.3	20.9	27.6	28.9	8.78	30.4
% TAAA	39.4	28.7	28.3	32.1	6.30	19.6
Total basic amino acid (TBAA)	17.1	11.6	18.1	15.6	3.50	22.4
% TBAA	17.6	15.9	18.6	17.4	1.37	7.87
Total sulphur amino acid (TSAA)	1.5	3.5	4.7	7.73	6.32	81.8
% TSAA	1.5	4.8	4.8	3.70	1.91	51.6
% of Cys in TSAA	0.0	34.3	36.2	23.5	20.4	86.8
Total aromatic amino acid (TArAA)	3.3	6.8	8.0	6.03	2.44	40.5
% TArAA	3.4	9.3	8.2	6.97	3.14	45.1
P-PER	1.6	1.6	2.1	1.77	0.289	16.3
Leu / Ile ratio	0.7	1.1	1.9	1.23	0.611	49.7
Leu-Ile (difference)	-	0.5	3.1	-	-	-
pI	5.4	4.2	5.8	5.13	0.83	16.2

Table 4: Amino acid scores of smooth loofah, Roselle and Sesame seeds based on whole hen's egg amino acid

Amino acid	Table score	Smooth looofah	Roselle	Sesame	Mean	SD	CV%
Gly	3.0	2.97	1.17	1.93	2.02	0.904	44.8
Ala	5.4	0.315	0.778	0.889	0.661	0.304	50.0
Ser	7.9	0.165	0.481	0.671	0.439	0.256	58.3
Pro	3.8	2.45	0.553	1.13	1.38	0.972	70.4
Val	7.5	0.20	0.44	0.613	0.418	0.207	49.5
Thr	5.1	0.490	0.667	0.863	0.673	0.187	27.8
Ile	5.5	1.25	0.821	0.607	0.893	0.327	36.6
Leu	8.3	0.578	0.614	0.783	0.658	0.109	16.6
Asp	10.7	0.645	0.486	0.822	0.657	0.168	25.8
Lys	6.2	0.226	0.435	0.468	0.376	0.131	34.8
Met	3.2	0.469	0.719	0.938	0.709	0.235	33.1
Glu	12.0	2.62	1.31	1.57	1.83	0.694	37.9
Phe	5.1	0.647	0.863	0.902	0.804	0.137	17.0
His	2.4	2.33	0.875	1.08	1.43	0.788	55.1
Arg	6.1	1.66	1.11	2.07	1.61	0.482	29.9
Tyr	4.0	-	0.60	0.85	-	-	-
Cys	1.8	-	0.667	0.944	-	-	-

Table 5: Amino acid scores of smooth loofah, roselle and sesame seeds based on amino acid scoring pattern (FAO/WHO, 1973)

Amino acid	Table score	Smooth loofah	Roselle	Sesame	Mean	SD	CV%
Ile	40	1.75	1.15	0.85	1.25	0.46	37.0
Leu	70	0.69	0.73	0.93	0.73	0.13	17.8
Lys	55	0.25	0.49	0.53	0.42	0.15	35.7
Met + Cys	35	0.43	1.00	1.34	0.92	0.46	50.0
Pha + Tyr	60	0.55	1.13	1.33	1.09	0.48	44.0
Thr	40	0.63	0.85	1.10	0.86	0.24	28.0
Val	50	0.30	0.66	0.92	0.63	0.31	4

The contents of TEAA of 34.7 – 44.6 g/100g protein (without tryptophan) in the seeds were lower than the value for egg reference protein (56.6 g/100g) (Paul *et al*., 1976). The TEAA of the present report were higher than 31.2 g/100g reported for fermented locust bean (Adeyeye, 2006) but only those of smooth loofah (37.7 g//100g cp) and sesame (44.6 g/100g cp) were higher than 35.1 g/100g in*Zonocerus variegatus* (Adeyeye, 2005) and 35.5 g/100g cp in *Gymnarchus niloticus* (Adeyeye and Adamu, 2005). The EAA requirements according to FAO/WHO/UNU (1985) are (g/100g cp) (with His): infant (46.0), pre-school (2-5 y) (33), school child (10-12 y) (24.1) adult (12.7); (without His): infant (43.4), pre-school (32.0), school child (22.2) and adult (11.1). The TEAA levels in this report meet the above standards.

The % TEAA to TAA: 47.7% for roselle and 45.7% for sesame seeds were well above the 39% considered to be adequate for ideal protein food for infants, 26% for children and 11% for adults (FAO/WHO/UNU, 1985). The %TEAA in roselle and sesame seeds were comparable to that of egg (50%) (FAO/WHO, 1990); 43.6% for pigeon pea flour (Oshodi *et al.*, 1993) and 43.8 – 44.4% for beach pea protein isolate (Chavan *et al*., 2001).

Table 3 revealed that TSAA for the three seeds ranged from 1.5 – 4.8 g/100g with % Cys present in roselle and sesame were 34.3% and 36.2%, respectively. The results of TSAA in this study were lower than the 5.8 g/100g crude protein recommended for infants (FAO/WHO/UNU, 1985). Information on the agronomic advantages of increasing the concentration of sulphur – containing amino acids in staple food showed that cystine has positive effects on mineral absorption particularly zinc (Sandstrom *et al*., 1989; Mendoza, 2002). Most animal proteins are low in cystine; in contrast, many vegetable proteinscontain substantially more cystine than methionine (Adeyeye, 2006. The present values of % Cys in TSAA: 34.3% (roselle) and 36.2% (sesame) were comparatively lower than 62.9% reported forcoconut endosperm (Adeyeye, 2006), 51.2-53.1 for raw, steeped, germinated millet (Adeyeye, 2009).

The Leu /Ile ratio was 0.7 in smooth loofah, 1.1 in roselle and 1.9 in sesame. These ratios revealed that leu<Ile in smooth loofah while Leu >Ile in roselle and sesame. In the consumption of maize and sorghum, it has been suggested that amino acid imbalance from excess Leu might be a risk factor in the development of pellagra (FAO, 1995). Also, clinical biochemical and pathological observations in human and rat experiments showed that high Leu in the diet impairs the metabolism of tryptophan and niacin and is responsible for the niacin deficiency in sorghum eaters (Ghafoorunissa and Narasing Rao, 1993). Excess Leu could be countered by

increasing the intake of niacin and tryptophan or with supplementation with Ile (Belavady and Gopalan, 1969).

The calculated isoelectric point (pI) were 5.4 for smooth loofah, 4.2 for roselle and 5.8 for sesame seeds, showing that the samples were in acidic medium of the pH range. This is a good starting point in predicting the pI for proteins in order to enhance a quick precipitation of protein isolate from biological samples (Olaofe and Akintayo, 2000). In the amino acid scores based on whole hen's egg amino acid, glycine (Gly) (2.97), Proline (Pro) (2.45), isoleucine (Ile) (1.25), glutamic acid (Glu) (2.62), histidine (His) (2.33) and arginine (Arg) (1.66) (smooth loofah); Gly (1.17), Glu (1.31) and Arg (1.11) (roselle); Gly (1.93), Pro (1.13), Glu (1.57), (His) (1.08) and Arg (2.07) had scores greater than 1.00. Serine (Ser) had the least score in smooth loofah (0.165); Lys in both roselle (0.435) and sesame (0.468).

The results of the amino acid scores of theseeds based on amino acid scoring pattern showed that Lys had the lowest essential amino acid scores (EAAS) of 0.25 (25%) in smooth loofah, 0.49 (49%) in roselle and 0.53 (53%) in sesame. This showed that Lys is limiting in all the seed samples. This means, to correct for the amino acid needs from the samples, 100/25 or 4.0 times as much smooth loofah seed protein, 100/49 or 2.04 times as much roselle seed protein and 100/53 or 1.89 times as much sesame protein has to be taken when they are sole protein in the diet (Bingham, 1977). The amino acid scores based on the suggested amino acid requirements of pre-school children (2-5 years) showed that only roselle and sesame would be able to provide the required essential amino acids for the pre-school children as most of the EAA scores were above 1.00 (100%) requirements (Bingham, 1977).

Conclusion

This study indicates that the seed samples: smooth loofah, roselle and sesame seeds are good sourcesof essential amino acids. The predicted protein efficiency ratio values obtained showed that the samples proteins would be utilized physiologically. The amino acid scores indicated that both roselle and sesame would supply virtually required essential amino acids for the pre-school children as most of the parameters determined were more 100% requirement. Generally, the results from the samples are fairly close when compared on pair wise basis as it was evident in the levels of the coefficient of variation percent (CV%).

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