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Amino acids profile of loofah gourd *Luffa cylindrica* (M J Roem) seeds subjected to different heat processing methods

[1,*]Onigemo, M. A., [2]Dairo, F. A. S. and Oso, Y. A. A.[3]
[1]*Department of Animal Production Technology, Lagos State Polytechnic, Ikorodu, Nigeria*
[2]*Department of Animal Science, Ekiti State University, Ado-Ekiti, Nigeria*
[3]*Department of Agricultural Technology, Lagos State Polytechnic, Ikorodu, Nigeria*
Corresponding author: *maonigemo@gmail.com;+2348022770999*

Abstract

The major limiting factor to the utilisation of loofah gourd seeds (LGS) as food is its high content of anti-nutrient. Heat processing is one of the process of reducing the concentration of anti-nutrients in food resources which may adversely influence the amino acids contents of such resources. Raw seeds of loofah gourds (RLGS) were toasted (TLGS), boiled (BLGS) and cooked (CGLS) and analysed for the amino acids content. The amino acids score, the essential amino acids score and the predicted (P-PER) of the raw and heat treated RLGS were calculated using standard equations. Results revealed that heat treatment significantly reduced (P<0.05) the essential amino acids, total sulphur containing amino acids and total basic amino acids while significantly (P<0.05) increasing the total amino acids, and total non-essential amino acids. cysteine is the most reduced amino acids and toasting had the highest reduction effect on the amino acids. P-PER of LGS were significantly enhanced by heat processing. Glutamic acid and aspartic acid were the most abundant amino acids while cysteine and methionine were the major limiting amino acids in the raw and processed LGS. In conclusion, heat processing enhances the nutritional potentials of LGS and wet heat processing boiling and cooking are the most preferred processing methods for loofah gourd seed.

Keywords: loofah gourd seeds, toasting, boiling, cooking, amino acids.

Introduction

Loofah (*Luffa cylindrica* L.) is a plant that originated from India which produces berry like fruit whose colour at tender stage is green with cucumber-like interior when immature, and yellow at puberty with a network of fibre surrounding at least 30 or more flat black seeds (Sofowora, 1982; Newton, 2006; Oyetayo and Ojo, 2012; Silva *et al.,* 2012). The fruit is harvested before puberty and eaten as vegetables in some part of Asia and Africa and serves as the source of the sponge (Newton, 2006). It is an excellent fruit in nature containing all the essential constituents required for good health of humans (Rahman, 2003). Its kernel contains between 45 – 51% oil which composed of mainly oleic and linoleic acids; the seeds have laxative properties due to their high oil content and contains a wide range of secondary metabolites with distinct biological activities that could be useful in the treatment of asthma, sinusitis and fever (Nagao *et al.,* 1991). It is reported to possess antiviral, anti-tumor, antioxidant, anti-inflammatory and immunomodulatory activities (Tannin-Spitz *et al.,* 2007).

In Nigeria, the plant grows in the wild, abandoned building structures, fences and walls in towns and villages, while in some parts of the country, loofah plants are grown for its sponge which is used to wash dish, human bathing sponge and in traditional medical practice (Dairo *et al.,* 2007; Onigemo *et al.,* 2015). The seeds which are often discarded as waste are leguminous in nature, potentially rich in energy and protein and could be used as a source of vegetable protein and energy in human and animal diets (Lee and Yoo, 2006; Dairo *et al.,* 2007; Abitogun and Ashogon, 2010). One main criterion in the use of any material as feed ingredients is its nutrient composition and there is dearth of information on the amino acids content of loofah seeds. Also, the types of processing procedure feedstuff are subjected to also influence their nutrient profile and availability (Ajala, 2009). This study was therefore designed to determine the amino acids composition of loofah gourd seed subjected to three heat processing methods so as to confirm its potentials as a source of vegetable protein in livestock diets.

Materials and methods

Luffa cylindrica (M J Roem) was harvested during the dry season in the south west, and north central geographical regions of Nigeria. The gourds were harvested when the gourds coat had dried and turned leathery brown. Seeds were removed from the cucumber shaped fruits by breaking the leather colour coating on the gourd to expose the sponge in it. The black hard coated seeds were removed by vigorously agitating. The seeds obtained from the sponge were soaked in fairly hot water at 60°C for 12 hours and thereafter dehulled by removing the black seed coat that had been softened by the soaking process. The dehulled seed was then sundried. Foreign materials were removed from the sundried dehulled seeds by winnowing and the thoroughly cleaned seeds were divided into four parts for dry and wet heat processing namely cooking, boiling and toasting. The first part of the clean dried seeds was poured into already boiling water and left for 30 minutes to cook. The water was allowed to cover the seeds and the ratio of water to seeds was 5:1 by volume. The cooked seedswere poured into a sieve and allowed to drain properly. The cooked drained seed was then sun dried for three days and labelled cooked loofah gourd seed (CLGS). The second part of the clean dried seed was poured in a jute bag and immersed in hot boiling water and heated for five minutes when the water begins to boil again. The boiled seed was then sun dried for three days and labelled boiled loofah gourd seed (BLGS). The third part of the clean dried seed was roasted for 30 minutes at 100 - 110°C in a gari fryer along with clean fine sand to prevent the seed from getting burnt and to ensure uniform distribution of heat. The seeds and sand mixture were continuously stirred throughout the toasting process using a wooden spatula. The toasted seeds were sieved out of the fine sand and allowed to cool. This was labelled toasted loofah gourd seed (TLGS). The forth part was not exposed to any form of heat treatment and was labelled raw loofah gourd seed (RLGS). The three differently processed seeds as well as the raw seeds were grinded using plate mill and packed in polythene sachets. The amino acids content of loofah gourd seeds were analyzed using Ingos® Automatic Amino Acids Analyzer AAA, 400 PIKRON sro®. The amino acid score was determined based on the whole hen's egg as described by Paul *et al.,* (1976). It was calculated by using the ratio of amino acid in the test protein to those in the reference protein for each amino acid. The essential amino acid score was based on the provisional amino acid scoring pattern using the FAO/WHO, (1973) formula: *Amino acid score = Amount of amino acid per test protein [mg/g]/ Amount of amino acid per protein in reference protein [mg/g].* Predicted protein efficiency ratio (P-PER) was determined using the equations developed by Alsmeyer *et al.,* (1974): *P-PER= -0.468+0.454 (Leu) –*

0.105 (Tyr). Data were analyzed using analysis of variance. The means were separated using Duncan Multiple Range Test. All statistical analysis was done using the Assistat-Statistical Assistance 7.7 beta software developed by Silva and Azevedo (2016).

Results and discussion

The amino acids value of the heat processed LGSM were significantly ($P< 0.05$) affected by heat treatment except aspartic acid, isoleucine, methionine, alanine, glutamic acid and proline (Table 1). Cysteine and methionine were the most limiting amino acid. Cysteine ranged between 5.67 - 10.14 mg/g. RLGSM recorded the highest value of 10.14 mg/g while TLGSM had the lowest (5.67 mg/g) and similar to those obtained for BLGSM and CLGSM. The values for methionine ranged from 12.25 – 12.50mg/g. Glutamic acid and aspartic acid were the most abundant amino acids in the LGSM. Heat processing significantly reduced cysteine, lysine, glycine and arginine content of LGSM with cysteine, arginine and lysine having the trend of RLGSM > BLGSM > CLGSM > TLGSM.

Table 1: Amino acids profile of loofah gourd seeds subjected to different heat processing methods

Amino acids	Methods of processing				SEM
	Raw	Toasting	Boiling	Cooking	
Lysine	41.18^{a}	39.12^{b}	39.53^{b}	39.14^{b}	0.49
Histidine	28.38	28.66	28.52	28.63	0.06
Arginine	62.88^{a}	59.78^{c}	61.64^{b}	60.77^{b}	0.66
Aspartic acid	95.76	94.99	95.35	95.67	0.17
Threonine	32.13^{ab}	31.95^{b}	32.45^{a}	32.06^{b}	0.11
Serine	33.34^{b}	33.94^{a}	33.66^{ab}	33.34^{b}	0.14
Glutamic acid	97.31	97.31	97.31	97.31	0.00
Proline	30.98	30.98	30.98	30.98	0.00
Glycine	27.98^{a}	27.54^{c}	27.78^{bc}	27.93^{ab}	0.10
Alanine	27.45	27.41	27.69	27.41	0.30
Cysteine	10.14^{a}	5.67^{b}	6.59^{b}	6.29^{b}	1.01
Valine	36.88^{b}	37.62^{a}	37.09^{b}	37.24^{ab}	0.16
Methionine	12.25	12.5	12.35	12.43	0.05
Isoleucine	32.55	32.15	31.59	32.45	0.22
Leucine	70.88^{c}	73.06^{a}	72.23^{b}	72.65^{ab}	0.47
Tyrosine	34.66^{a}	34.89^{a}	34.60^{a}	33.69^{b}	0.26
Phenylalanine	43.87^{b}	43.44^{b}	44.31^{a}	44.52^{a}	0.24
PER	28.07^{b}	29.04^{a}	28.69^{a}	28.98^{a}	0.17

abc→ **Means on the same row with different superscript differ significantly ($P< 0.05$)**

Heat processing significantly ($P< 0.05$) increased the valine and leucine value of treated LGSM above that of RLGSM. The valine content of TLGSM and CLGSM were similar ($P> 0.05$) but higher than the value for RLGSM. Histidine and methionine content of LGSM increased with heat application. Histidine ranged between 28.38 mg/g (RLGSM) to 28.66 mg/g (TLGSM). Glutamic acid and proline appeared not to be affected by the heat treatment as their values remain unchanged post processing and are the same for the treated LGSM and RLGSM. Alanine values were also not influenced by heat treatment and ranges from 27.69 mg/g for BLGSM to 27.41 mg/g for TLGSM and CLGSM. Phenylalanine was highest in CLGSM (44.52 mg/g) and lowest in TLGSM (43.44 mg/g). Serine had the highest value of 33.94 mg/g in TLGSM followed by BLGSM (33.66 mg/g) then CLGSM and RLGSM with the same value of 33.34 mg/g. Tyrosine value was highest in TLGSM (34.89 mg/g) and lowest (33.69 mg/g) in CLGSM and while the highest value of Threonine was recorded in BLGSM (32.45 mg/g) and the lowest in TLGSM (31.95 mg/g). The result of this study showed that loofah gourd seed was rich in amino acids. Also, different heat processing methods significantly affected the amino acid profile of the loofah gourd seed except histidine, aspartic acid, glutamic acid, proline, alanine, methionine and isoleucine. The values of the amino acids observed in this study are comparable to those reported in literature (Aremu *et al.,* 2006; Olaofe *et al.,* 2008; Aremu *et al.,* 2010; Aremu *et al.,* 2011). The most concentrated amino acids were glutamic acid, aspartic acid, leucine and arginine which support the findings of Ogunji *et al.* (2003); Mubarak (2005); Olaofe *et al. (*2008) and Dairo *et al. (*2013) Methionine and cysteine were slightly deficient in LGSM as indicated in the result of the study which conform with the report of other workers (FAO/WHO, 1991; Olaofe *et al.,* 2008). Methionine and cysteine appeared to be generally deficient in legumes and most of the oil-bearing seeds as documented for mung bean (Mubarak, 2005); *Mucuna pruriens* (Kala and Mohan, 2010) and Mosquito bean (Kathrivel and Kumudha, 2011). Cysteine was significantly decreased by treatment effect. The RLGSM had higher cysteine value than any of the treated LGSM which were similar in values. This might be due to the effect of heat on the amino acids that may have depleted it in the seed as reported by previous workers (Mubarak, 2005; Dairo *et al.,* 2013). The lower cysteine value of the processed seeds might be attributed to the action of heat on the amino acids. Van Barneveld *et al.,* (1994 a and b) reported that heating results in the formation of a hydroalanyl residue from cysteine, which is capable of binding the e-amino group of lysine to form lysinoalanine, hence the significant reduction in the lysine value of the treated seed when compared to RLGSM. The limiting methionine and cysteine content of raw and treated loofah gourd seed would require the use of other quality vegetable protein sources such as soybean or groundnut supplementation in order to correct for this deficiency when used in livestock feed. Dairo *et al.* (2008) has reported a 5% tolerable inclusion level or raw loofah gourd seed meal in rabbit diet supplemented with other protein source so as to reduce the high cost of protein source. Histidine, serine and valine content increased by the heat treatment with TLGSM having the highest value. This observation agrees with the report of Mubarak (2005) but at variance with that of Dairo *et al.* (2013) who reported a decreased value due to boiling and cooking. Lysine, glycine, isoleucine and phenylalanine were significantly reduced by heat treatment as compared with RLGSM which agreed with the report of previous workers on mung bean and loofah seeds (Mubarak, 2005; Dairo *et al.,* 2013). Table 2 shows the amino acid score of loofah gourd seeds (LGSM) subjected to different heat processing methods. The treatment significantly ($P < 0.05$) affected the amino acid score of loofah gourd seeds (LGSM) except serine and glutamic acid. The amino acid score of cysteine, lysine, arginine and aspartic acid were significantly ($P < 0.05$) reduced below that of RLGSM. The TLGSM value for cysteine

(25.77) was significantly (P< 0.05) lowered than those of RLGSM, BLGSM and CLGSM with values of 46.09, 29.95 and 28.59 respectively. Arginine value was significantly (P< 0.05) different among LGSM exposed to different heat processing methods. Arginine value was significantly (P< 0.05) lowest in TLGSM (89.22) and followed by CLGSM (90.70), BLGSM (92.00) and RLGSM (93.85) in ascending order. Aspartic value of the LGSM varied significantly (P< 0.05), the values of RLGSM and CLGSM were similar (P> 0.05) and higher than others. In addition, aspartic acid value of CLGSM did not differ significantly (P>0.05) from BLGSM. Glycine value of RLGSM (75.62), CLGSM (75.49) and BLGSM (75.08) were similar (P> 0.05) but significantly (P< 0.05) higher than TLGSM (74.43). Isoleucine values of RLGSM (46.50), CLGSM (46.36) and TLGSM (45.93) were similar but different significantly (P< 0.05) from what was obtained in BLGSM (45.13).

Table 2: Amino acid score of loofah gourd seeds subjected to different heat processing methods

Amino acids	Methods of processing				SEM
	Raw	Toasting	Boiling	Cooking	
Lysine	60.56^{a}	57.53^{b}	58.13^{b}	57.56^{b}	0.72
Histidine	105.11^{b}	106.15^{a}	105.63^{ab}	106.04^{a}	0.24
Arginine	93.85^{a}	89.22^{d}	92.00^{b}	90.70^{c}	0.98
Aspartic Acid	165.10^{a}	163.78^{c}	164.40^{bc}	164.95^{ab}	0.30
Threonine	58.42^{b}	58.09^{b}	59.00^{a}	58.29^{b}	0.20
Serine	43.30	44.08	43.71	43.30	0.19
Glutamic Acid	79.11	79.11	79.11	79.11	0.00
Glycine	75.62^{a}	74.43^{b}	75.08^{a}	75.49^{a}	0.27
Cysteine	46.09^{a}	25.77^{c}	29.95^{b}	28.59^{b}	4.58
Valine	44.98^{b}	45.88^{a}	45.23^{b}	45.41^{ab}	0.19
Methionine	37.12^{b}	37.88^{a}	37.42^{ab}	37.67^{a}	0.16
Isoleucine	46.50^{a}	45.93^{a}	45.13^{b}	46.36^{a}	0.31
Leucine	83.39^{c}	85.95^{a}	84.98^{b}	85.47^{ab}	0.56
Tyrosine	75.35^{a}	75.85^{a}	75.22^{a}	73.24^{b}	0.57
Phenylalanine	81.24^{b}	80.44^{c}	82.06^{a}	82.44^{a}	0.45

^{abc} → Means on the same row with different superscript differ significantly (P< 0.05).

Histidine score values in RLGSM (105.11) and BLGSM (105.63) were similar (P> 0.05) but significantly (P< 0.05) lower than those of TLGSM (106.15) and CLGSM (106.04) which were also similar (P> 0.05). The highest valine was recorded in TLGSM (45.88) which was similar to that CLGSM (45.41), but differ significantly (P< 0.05) from those of BLGSM (45.23) and RLGSM (44.98) which were also similar (P> 0.05). The score of methionine was significantly (P< 0.05) highest in TLGSM (37.88) and lowest in RLGSM. The result showed that TLGSM significantly (P< 0.05) recorded the highest value for methionine (37.88), leucine (85.95) and Tyrosine (75.85) and the lowest values were recorded in RLGSM for both methionine (37.12) and leucine (83.39) while CLGSM had the lowest tyrosine value. BLGSM had significantly (P< 0.05) higher threonine score those others which were similar in their score. Phenylalanine scores were similar in CLGSM (82.44) and BLGSM (82.06) but are significantly (P< 0.05) different from others while TLGSM (80.44) had the lowest score. The amino acid score of raw and heat-treated loofah gourd seeds significantly differ ($P < 0.05$) from one another except with respect to serine and glutamic acid. Methionine scores were similar in treated LGSM and lowest in RLGSM. However, cysteine score was found to be greatly reduced by heat treatment hence, could be regarded as one limiting amino acid in treated LGSM. This result is in line with the findings of earlier workers that cysteine and methionine are the limiting amino acids in seeds of tropical plants (Kala and Mohan, 2010; Kathirvel and Kumudha, 2011; Dairo*, et al.,* 2013; Galili and Amir, 2013). However, Olaofe *et al.* (2008) reported lysine as the limiting amino acid in a LGSM kernel. Lysine score was reduced by the heat treatment and this agrees with the findings of Dairo *et al.* (2013) who reported a similar result in dehulled LGSM exposed to cooking and boiling. Therefore, the use of heat-treated loofah gourd seed will require other quality protein sources to supplement the deficiencies. However, valine and leucine scored higher for heat treated loofah gourd seed when compared to RLGSM. Dairo *et al.* (2013) reported reduction in valine and leucine score as a result of boiling and cooking. Aspartic acid scored the highest among the amino acid found in loofah gourd seed followed by histidine. Heat treatment significantly reduced the aspartic acid score whereas, histidine scores of the loofah gourds seed was significantly increased. Dairo *et al.* (2013) also reported increased histidine score as a result of boiling. Heat treatment significantly reduced arginine and glycine score of treated loofah gourd seed with toasting having the lowest values. Table 3 shows the essential amino acid pattern of loofah gourd seeds (LGSM) subjected to different heat application methods. Heat application significantly ($P < 0.05$) influenced the amino acids pattern in LGSM. The RLGSM recorded the highest values for the sulphur containing amino acids, essential amino acids (EEA) with or without histidine, total acidic amino acids (TAA), total basic amino acids (TBA), and percentage EEA with or without histidine. The EEA values with or without histidine were similar ($P > 0.05$) in the treated LGSM. However, the predicted PER was lower in RLGSM (28.07) than the treated LGSM.

Table 3: Essential amino acid pattern of loofah gourd seeds subjected to different heat processing methods

Variables	Methods of processing				
	Raw	Toasting	Boiling	Cooking	SEM
Total aromatic amino acids	78.53^{ab}	78.33^{b}	78.91^{a}	78.21^{b}	0.15
Total sulphur containing	22.39^{a}	18.17^{b}	18.94^{b}	18.72^{b}	0.96
Total essential amino acids with histidine	342.92^{a}	339.06^{b}	339.26^{b}	339.10^{b}	0.95
Total essential amino acids	314.54^{a}	310.40^{b}	310.74^{b}	310.47^{b}	1.00
Total non-essential amino acids	404.08^{b}	464.22^{a}	465.63^{a}	464.66^{a}	15.19
Total amino acid	718.62^{b}	774.62^{b}	776.37^{a}	775.13^{a}	14.19
Total aliphatic amino acids	226.72^{b}	228.76^{a}	227.36^{b}	228.66^{a}	0.50
Total acidic amino acids	193.07^{a}	192.3^{b}	192.66^{ab}	192.98^{ab}	0.17
Total basic amino acids	132.44^{a}	127.56^{c}	129.69^{b}	128.54^{bc}	1.05
%Total essential amino acid	43.77^{a}	40.07^{b}	40.02^{b}	40.05^{b}	0.93
%Total essential amino acid with histidine	47.72^{a}	43.77^{b}	43.70^{b}	43.75^{b}	1.00

^{abc} → Means on the same row with different superscript differ significantly ($P < 0.05$)

Heat processing significantly reduced the essential amino acid in loofah gourd seed when compared with the RLGSM. Deshpande *et al.* (1982) stated that nutritive value of a protein depends primarily on the capacity to satisfy the needs for nitrogen and essential amino acids. Though the values obtained are lower than what has been reported for other legumes like soybean (Kuri *et al.,* 1991), pigeon pea (Nwokolo, 1987) and *Cajanus cajan* (Olaofe *et al.,* 1993) it however compared well with what was reported for pumpkin seed, *Leganaria sciceraria*, and *Luffa cylindrica*. (Aisegbu, 1987; Olaofe *et al.,* 2009; Dairo *et al.,* 2013). Therefore, the nutritive value of the processed seeds is still capable of providing the needed essential amino acids as the values obtained in this study are above the recommended values for leucine (19-44), valine (13-25), threonine (9-28), phenylalanine and tyrosine (19-22) for adult and children (10-12 years), respectively (FAO/WHO, 1991).

Conclusion

Heat processing enhanced the predicted protein efficiency ratio but reduced the value and score of essential amino acids. Toasting had the highest reduction effect on total sulphur containing amino acids, total essential amino acids with histidine, total essential amino acids, total aromatic amino acid and total basic amino acids and the lowest reduction effect on total aliphatic amino acids. Consequently, boiling and cooking may be preferred for processing loofah gourd seed for a better nutrient content.

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