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ORIGINAL ARTICLE

Amino acid composition of *Lagenaria siceraria* seed flour and protein fractions

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Abstract Defatted seed flours of *Lagenaria siceraria* (calabash and bottle gourd) were fractionated into their major protein fractions. The amino acid composition of seed flours and their protein fractions were determined and the protein quality was evaluated. Glutamic acid (139–168 mg/g protein) was the most abundant amino acid followed by aspartic acid (89.0–116 mg/g protein) in both the seed flours and their protein fractions. The total essential amino acid ranged from 45.8 to 51.5%. The predicted protein efficiency ratio and the predicted biological value ranged from 2.4 to 2.9 and 8.7 to 44.0, respectively. Lysine and sulphur amino acids were mostly concentrated in the globulin fractions. The first and second limiting amino acids in seed flours and protein fractions were methionine and valine or threonine. The seed flours contained adequate essential amino acids required by growing school children and adults. The seed has potential as protein supplement in cereal based complementary diets or in the replacement of animal proteins in conventional foods.

Keywords *Lagenaria siceraria* · Calabash · Bottle gourd
Protein fractions · Amino acid composition

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Introduction

Studies on the utilization of vegetable proteins continue to attract attention globally due to the increasing demand for cheap and affordable dietary proteins, particularly among the low income group. Projections based on the current trends indicate a gap between human population and protein supply (Vijayakumari et al. 1997). Hence, the need to examine unconventional legumes and oilseeds as alternative protein sources for the future (Egbe and Akinyele 1990; Onweluzo et al. 1994; Chau and Cheung 1998; Fagbemi 2007). This development has stimulated research on the utilization of *Lagenaria siceraria*, an indigenous underutilized oil rich seed as alternate protein source.

Lagenaria siceraria (calabash and bottle gourd) belongs to Cucurbitaceae family. The plants are annual, herbaceous, and monoecious with creeping stems. The seeds are edible and used in the preparation of local soups, fermented food product (ogiri), fried cake (robo) and pudding (igbalo or ugbaotiri). The seeds of *Lagenaria siceraria* are rich in dietary proteins (Fokou et al. 2004).

Researchers have worked extensively on other species of Cucurbitaceae such as *Colocynthis citrullus, Citrullus vulgaris* and *Telfairia occidentalis* (Akobundu et al. 1982; Sathe et al. 1982; Ige et al. 1984; Fagbemi and Oshodi 1991; Fagbemi et al. 2005, 2006; Fagbemi 2007). Report on *Lagenaria siceraria* has been limited to its proximate composition and functional properties (Fokou et al. 2004). The knowledge of its amino acid composition and amino acid profile is necessary in food product formulations. This investigation was aimed at fractionation of *Lagenaria siceraria* seed flour into their major protein fractions and determination of their amino acid composition to further exploit the seeds potential use in food system.

Materials and methods

Two varieties of *Lagenaria siceraria* (calabash seed LS_1 and bottle gourd seed LS_2) were bought from farmers in Irele Ekiti, Ekiti State, Nigeria. The seeds were manually shelled, washed and later dried in a hot air oven at 50 °C. The seeds were pulverized using a Brabender blender, defatted by refluxing continuously for 8 h using n-hexane. Defatted meals were dried, pulverized and sieved to pass through a 500 µm sieve.

Protein fractions were extracted according to their solubility in different solvents as described by Wisal et al. (2003). Defatted *Lagenaria siceraria* seed flour (3.5 g) was extracted twice with 50 ml of distilled water for 30 min at room temperature (28±2 °C). The extract was centrifuged at 4,500 rpm for 20 min and the supernatant was used for the determination of water soluble albumin. The residue was then extracted successively in a similar manner with 1 M NaCl, or 1 M NaOH solution, extract was collected separately and used to estimate the salt soluble (globulin) or alkali soluble (glutelin) fractions.

The amino acid profiles of the seed flours and protein fractions were determined using ion exchange chromatography. The samples were defatted, hydrolyzed and evaporated in a rotatory evaporator and then injected into the Technicon sequential multisampling Amino Acid Analyzer (Technicon Instrument Co. Ltd., United Kingdom), (Adeyeye and Afolabi 2004). Tryptophan content of the seed flours was determined using the method of Concon (1975) as modified by Ogunsua (1988). The amino acids obtained were used to evaluate the protein quality of seed flour. Predicted biological value (BV) was calculated using the regression equation of Morup and Olesen (1976) as reported by Chavan et al. (2001).

$$\mathrm{BV} = 10^{2.15} \times \mathrm{q}^{0.141}{}_{\mathrm{Lys}} \times \mathrm{q}^{0.60}{}_{\mathrm{phe+tyr}} \times \mathrm{q}^{0.77}{}_{\mathrm{met+lys}} \times \mathrm{q}^{2.14}{}_{\mathrm{thr}} \times \mathrm{q}^{0.21}{}_{\mathrm{trp}}$$

Where,

$q = \frac{ai\ sample}{ai\ reference}$ for ai sample ≤ ai reference

or

$q = \frac{ai\ reference}{ai\ sample}$ for ai sample ≥ ai reference

a_i = mg of the amino acid per g of total essential amino acids.

The predicted protein efficiency ratio (PER) was calculated using one of the equations developed by Alsmeyer et al. (1974) as stated below.

$$\mathrm{PER} = -0.464 + 0.454(\mathrm{Leu}) - 0.105(\mathrm{Tyr})$$

Isoelectric point (IP) was estimated from the amino acids using the equation of the form given by Olaofe and Akintayo (2000).

$$\mathrm{IP_m} = \sum_{i=1}^{n} IPiXi$$

where, *IP* is the isoelectric point of the i^{th} amino acid in the mixture, X_i is the mass or mole fraction of the i^{th} amino acid in the mixture and IP_m is the isoelectric point of the mixture.

Determinations were carried out in triplicate, along with standard deviations. Data were subjected to analysis of variance using SPSS 15 computer programme.

Results and discussion

Variety and fractionation have significant ($p<0.05$) effect on the amino acid composition of *Lagenaria siceraria* seed flours (Table 1). Glutamic acid was the most abundant amino acid in both seed flours and all protein fractions. The values ranged from 140 to 168 mg/g protein with minimum value in LS_2 globulin fraction and maximum in LS_1 albumin fraction. The second most abundant amino acid in all the seed flours was aspartic acid ranging from 89.0 to 116 mg/g protein in LS_2 albumin and seed flour, respectively. Mora-Escobedo et al. (1990) reported similar observation for the amino acids of the albumin and globulin fractions of amaranth. Oshodi et al. (1998) reported tryptophan to be the most concentrated amino acid in legumes. The most concentrated essential amino acid in all seed flours and their protein fractions was leucine with values ranging from 60.0 to 72.1 mg/g protein (LS_2 albumin and the seed flour, respectively).

The lysine content of *Lagenaria siceraria* seed flours and their protein fractions ranged between 37.5 and 60.1 mg/g protein, this is similar to the lysine content of fluted pumpkin (37.5–66.6 mg/g cp) reported by Fagbemi (2007). The lysine content of seed flour is comparable with that of reference egg protein (63 mg/g crude protein, FAO/WHO/UNU 1985). Hence, *Lagenaria siceraria* seed flours and their protein fractions could be mixed with cereals like maize in weaning food formulation (Chavan et al. 2001). Globulins were however, richer in lysine than the water-soluble albumin or alkali soluble glutelin. This indicates that globulin fraction may be a better supplement in cereal based diet preparation. Tryptophan content of seed flours ranged from 8.1 to 13.9 mg/g protein. The calculated isoelectric point varied from 4.3 to 5.1 in LS_2 albumin and LS_1 seed flour respectively. This will serve as useful guide in quick precipitation of proteins from biological samples (Olaofe and Akintayo 2000).

Table 1 Amino acid composition of total seed flours and protein fractions of *Lagenaria siceraria* (mg/g protein) variety

Amino acid	LS_1				LS_2			
	Seed flour	Albumin	Globulin	Glutelin	Seed flour	Albumin	Globulin	Glutelin
Cystine *	12.3±0.08c	10.6±0.08d	13.9±0.08a	10.6±0.08d	12.5±0.08b	9.9±0.08e	12.6±0.08b	10.5±0.08d
Methionine*	10.4±0.16c	7.0±0.08a	11.2±0.08b	8.3±0.08f	12.5±0.08a	8.8±0.08e	12.6±0.08a	9.1±0.08d
Aspartic acid	109±0.08d	99.7±0.16f	112±0.33c	108±0.24e	116±0.16a	89.0±0.33 g	109±0.33d	113±0.33b
Threonine*	29.1±0.08e	21.6±0.08 h	25.0±0.08 g	30.1±0.08d	32.6±0.16c	25.9±0.08f	36.2±0.08b	41.1±0.08a
Serine	40.4±0.04c	31.7±0.16 h	39.1±0.08d	32.0±0.08 g	50.4±0.08a	32.8±0.08f	46.6±0.16b	38.0±0.08e
Glutamic acid	166±0.24b	168±0.24a	152±0.41d	161±0.41c	143±0.16f	149±0.16e	140±0.41 g	148±0.24e
Proline	37.5±0.08a	30.8±0.08e	34.2±0.08b	32.0±0.08d	32.5±0.08c	29.0±0.08 g	30.1±0.08f	28.0±0.08 h
Glycine	31.7±0.08 g	40.6±0.16b	40.1±0.08c	38.9±0.08d	32.4±0.04f	40.3±0.08c	35.0±0.08e	47.0±0.16a
Alanine	40.6±0.08ab	40.1±0.08b	36.3±0.08e	37.2±0.08d	32.6±0.08 g	34.7±0.08f	40.9±0.08a	37.9±0.08c
Valine*	40.6±0.08b	30.2±0.08 g	43.6±0.08a	39.2±0.16d	40.0±0.08c	31.1±0.08f	40.2±0.08c	32.5±0.08e
Isoleucine*	33.0±0.08d	35.1±0.08b	35.5±0.08a	30.4±0.08f	35.6±0.08a	28.2±0.08 g	31.4±0.08e	34.5±0.08c
Phenylalanine*	46.3±0.08b	32.9±0.08 g	41.4±0.16d	36.3±0.08f	48.8±0.08a	37.2±0.08e	45.6±0.16c	37.2±0.08e
Lysine*	56.2±0.16c	42.4±0.16 g	60.1±0.24a	57.2±0.016b	50.8±0.16e	37.5±0.08 h	52.6±0.16d	48.9±0.16f
Arginine*	58.6±0.16d	50.2±0.16 g	62.9±0.24a	60.3±0.24b	55.2±0.16e	49.4±0.08 h	54.4±0.16f	59.6±0.16c
Histidine*	26.1±0.08a	16.3±0.08 g	24.4±0.08b	22.4±0.08e	23.0±0.08e	16.9±0.08f	22.6±0.08d	24.5±0.08b
Leucine*	65.8±0.016e	65.0±0.24f	71.1±0.16b	66.2±0.16d	72.1±0.16a	60.0±0.16 h	68.3±0.08c	61.5±0.21 g
Tyrosine*	34.9±0.08b	22.5±0.08 g	35.6±0.08a	22.5±0.08 g	30.1±0.04d	27.4±0.08f	30.5±0.08c	29.0±0.08e
Tryptophan*	11.9±0.16c	13.0±0.16b	13.9±0.24a	10.6±0.08d	9.9±0.08e	8.1±0.08f	10.8±0.16d	10.7±0.16d
Cal. Isoelectric point	5.1	4.5	5.1	4.9	4.9	4.3	4.9	4.9

*Essential amino acids; values followed by different letters in the same row are significantly different ($p<0.05$); LS_1: Calabash seed flours; LS_2: Bottle gourd seed flours

The total amino acid content ranged from 715 to 851 mg/g protein (LS_2 albumin and LS_1 seed flour, Table 2). The total essential amino acid of *Lagenaria siceraria* flours ranged between 340 and 439 mg/g protein. This is lower than 566 mg/g protein reported for egg reference protein (Paul et al. 1980). It is, however comparable with values (190–503 mg/g protein) reported for some oilseeds such as *Colocynthis citrullus*, peanut meal and soybean flours (Lusas 1979; Akobundu et al. 1982; Sosulski 1983; Kuri et al. 1991). The range of percentage total essential amino acid (45.8–51.5) obtained for *Lagenaria siceraria* seed flour and their protein fractions is well above 36%, which is considered adequate for an ideal protein (FAO/WHO 1973). This suggests that seed flours and their protein fractions may find use as a food supplement. Globulin fraction had the highest percentage of total essential amino acid (51.0 and 51.5%) in all the protein fractions. The total sulphur amino acid content ranged from 17.6 to 25.2 mg/g protein with cystine ranging from 50.0 to 60.2%. The range of total neutral, acidic and basic amino acids were 49.1–53.8%, 30.4–35.4% and 14.4–17.4%, respectively, which showed that protein in seed flours and their protein fractions may be acidic in nature. Similar observation was reported by Aremu et al. (2006) for some Nigerian underutilized oilseeds.

The predicted PER of *Lagenaria siceraria* seed flours and their protein fractions ranged from 2.4 (LS_2 albumin and glutelin) to 2.9 (LS_2 seed flour). The predicted PER values were higher than seed proteins of cowpea (1.21), pigeon pea (1.82) and *L. sativus* (negative value to 0.03) (Salunkhe and Kadam 1989). These values are also higher than the ranges of 0.66–1.24 and 0.63–2.21 for fluted pumpkin seed flours and cotton seed, respectively (Fagbemi 2007). The predicted BV of *Lagenaria siceraria* protein ranged between 8.7 and 44.0. Fractionation affected BV. Globulin and glutelin fractions have higher BV than their albumin fraction. The predicted BV of *Lagenaria siceraria* compared well with the range of 36.5–40.13 reported for beach pea protein isolates (Chavan et al. 2001)

The chemical score ranged from 21.9 (LS_1 albumin) to 39.4 (LS_2 globulin) (Table 3). The first limiting amino acid in both raw seed flours and their protein fractions was methionine (7.0–12.6 mg/g protein) followed by valine (30.2–43.6 mg/g protein) except in LS_1 globulin fraction, where threonine (25.0 mg/g protein) was the second limiting amino acid. The recommended sulphur amino acid for infants, growing preschool children and growing school children are 42, 25 and 22 mg/g crude protein respectively (FAO/WHO/UNU 1985). The seed flours on this basis provide 41.9–60%; 70.4–100.8% and 80—over 100% of the recommended sulphur amino acid for infant, a growing preschool child and growing school child, respectively. Salt soluble proteins are found to be more concentrated in sulphur amino acid. In general, *Lagenaria siceraria* seed flours and their protein fractions contained adequate amounts of most of the essential amino acids required by preschool children and all amino acids essential for school children and adults.

Conclusion

Amino acid profile of *Lagenaria siceraria* seed flours and their water, salt and alkali (albumin, globulin and glutelin, respectively) soluble protein fractions are of high quality

Table 2 Summary of amino acid composition of the total seed flour and protein fractions of *Lagenaria siceraria* (mg/g protein) variety

	LS_1				LS_2			
	Seed flour	Albumin	Globulin	Glutelin	Seed flour	Albumin	Globulin	Glutelin
Total amino acids (TAA)	851	758	852	803	830	715	819	811
Total essential amino acids (TEAA)	426	347	439	394	423	340	418	399
TEAA/TAA (%)	49.9	45.8	51.5	49.1	51.0	47.6	51.0	49.2
Total non essential amino acids (TNEAA)	425	411	413	409	407	375	402	412
Total sulphur amino acids (TSAA)	22.7	17.6	25.1	18.9	25.0	18.7	25.2	19.6
Cystine (%) in TSAA	54.2	60.2	55.4	56.1	50.0	52.9	50.0	53.6
Total aromatic essential amino acids phe.+tyr. (ArEAA)	81.2	54.4	77.0	58.8	78.9	64.6	76.1	66.2
Total acidic amino acids (TAAA)% Glu. + Asp.	32.4	35.4	30.9	33.5	31.2	33.3	30.4	32.2
Total basic amino acids (TBAA)% Lys. + Arg. + His.	16.6	14.4	17.3	17.4	15.5	14.5	15.8	16.4
Total neutral amino acids (TNAA)%	50.0	50.2	51.8	49.1	53.3	52.2	53.8	51.4
Ratio of TEAA:TNEAA	1.0	0.8	1.1	1.0	1.0	0.9	1.0	1.0
Predicted protein efficiency ratio (PER)	2.5	2.7	2.8	2.7	2.9	2.4	2.7	2.4
Predicted biological value (BV)	27.1	8.7	21.0	23.4	31.4	12.3	40.2	44.0

LS_1 Calabash seed flours; *LS_2* Bottle gourd seed flours

Table 3 Amino acid scores of the total seed flour and protein fractions of *Lagenaria siceraria* variety

Essential amino acid	Reference[a]	LS_1								LS_2							
		Seed flour		Albumin		Globulin		Glutelin		Seed flour		Albumin		Globulin		Glutelin	
		EAAC	AAS(%)	EAAC	AAS(%)	EAAC	AAS(%)	EAAC	AAS(%)	EAAC	AAS(%)	EAAC	AAS(%)	EAAC	AAS(%)	EAAC	AAS(%)
Cys.	18.0	12.3	68.3	10.6	58.9	13.9	77.2	10.6	58.9	12.5	69.4	9.9	55.0	12.6	70.0	10.5	58.3
Met.	32.0	10.4	32.5	7.0	21.9	11.2	35.0	8.3	25.9	12.5	39.1	8.8	27.5	12.6	39.4	9.1	28.4
Thre.	51.0	29.1	57.1	21.6	42.4	25.0	49.0	30.1	59.0	32.6	63.9	25.9	50.8	36.2	70.9	41.1	80.6
Val.	76.0	40.6	53.4	30.2	39.7	43.6	57.4	39.2	51.6	40.0	52.6	31.1	40.9	40.2	52.9	32.5	42.8
Iso.	56.0	33.0	58.9	35.1	62.7	35.5	63.4	30.4	54.3	35.6	63.6	28.2	50.4	31.4	56.1	34.5	61.6
Phe.	51.0	46.3	90.8	32.9	64.5	41.4	81.2	36.3	71.2	48.8	95.7	37.2	72.9	45.6	89.4	37.2	72.9
Lys.	63.0	56.2	89.2	42.4	67.3	60.1	95.4	57.2	90.8	50.8	80.6	37.5	59.5	52.6	83.5	48.9	77.6
Arg.	61.0	58.6	96.1	50.2	82.3	62.9	103	60.3	98.9	55.2	90.5	49.4	80.9	54.4	89.2	59.6	97.7
His.	24.0	26.1	109	16.3	67.9	24.4	102	22.4	93.3	23.0	95.8	16.9	70.4	22.6	94.2	24.5	102
Leu.	83.0	65.8	79.3	65.0	78.3	71.1	85.7	66.2	79.8	72.1	86.9	60.0	72.3	68.3	82.3	61.5	74.1
Tyr.	40.0	34.9	87.3	22.5	56.3	35.6	89.0	22.5	56.3	30.1	75.3	27.4	68.5	30.5	76.3	29.0	72.5
Trp.	18.0	11.9	66.1	13.0	72.2	13.9	77.2	10.6	58.9	9.9	55.0	8.1	45.0	10.8	60.0	10.7	59.4
Chemical score		32.5		21.9		35.0		25.0		39.1		27.5		39.4		28.4	
1st limiting a. a		Methionine		Methionine		Methionine		Methionine		Methionine		Methionine		Methionine		Methionine	
2nd limiting a. a		Valine		Valine		Threonine		Valine		Valine		Valine		Valine		Valine	

[a] Provisional amino acid pattern egg as reference (FAO/WHO/UNU 1985). *EAAC* Essential amino acid composition (mg/g protein)

AAS Amino acid score; *a.a* amino acid; LS_1 Calabash seed flours; LS_2 Bottle gourd seed flours and very high in lysine. The percentage total essential amino acids in all flour samples were well above the recommended values. *Lagenaria siceraria* seed flours and their protein fractions may be incorporated into cereals for the formulation of a wide range of cereal based weaning foods and other complementary diets because of their high lysine content.

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