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Amino Acid Profiles of Some Varieties of Rice, Soybean and Groundnut Grown in Ghana

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Abstract

Amino acid profiles of some Ghanaian varieties of rice, soybean and groundnut were investigated to augment existing data on their physicochemical properties and provide information to guide their application in weaning foods formulation. Five varieties of rice and four varieties each of groundnuts and soybeans were analyzed. Amino acids were separated using HPLC. Without post-column derivatization, Evaporative Light Scattering Detector (ELSD) was used to determine their concentrations against standard amino acids. Local rice varieties lacked tryptophan, valine, glycine, glutamic acid and lysine and histidine was present in only *Nerica-2* variety but *Nerica-1* variety had the highest total essential amino acid content of 36.42 g/kg. Quarshie soybean variety expressed all the amino acids under study and also had the highest total essential amino acids content of 169.14 g/kg. *Sinkarzie* and *F-Mix* groundnut varieties expressed all amino acids except tryptophan, with *Sinkarzie* having the highest total essential amino acids content of 100.62 g/kg.

Keywords: Amino acids profiles; Evaporative light Scattering Detector (ELSD); Grain legumes; HPLC

Introduction

The weaning of infants on gruels made from traditional cereal or root tuber staples like rice, maize or yam has made protein-energy malnutrition prevalent in infants in most developing countries [1,2]. Gruel made from cereals like maize, millet, sorghum or guinea corn is the major traditional weaning food in most developing countries, known as *koko* in Ghana and *Ogi* in Nigeria [3]. These gruels, although low in proteins and deficient in some essential amino acids still persist because the commercial weaning foods available for infant feeding are too expensive for low income families.

In an attempt to combat the persistent protein-energy malnutrition challenge, especially among children, in Africa, several strategies have been developed to produce healthy nutritious food, rich in proteins, for infant feeding; Rice, soybean and groundnut composites yielded diets with improved nutritional composition [3]. Malted cereals, soybeans and groundnuts composites yielded diets rich in proteins and minerals [4]. Germinated sorghum, soybean, sesame and groundnut composited diet had high protein contents. Cowpeas, common beans and green peas served as good protein supplements for sorghum and finger millet based diets [5]. Rice, soybeans and groundnuts are cheap and readily available in Ghana and have been recommended as composites for infant weaning diets.

Cereal-legume combinations could therefore yield low-cost diets with improved nutritional contents for infant weaning in Africa. However information for the right proportions of cereals and legumes for infant weaning food formulation is limited. This is because the exact nutritional profiles of traditional cereals and legumes are not known. Thus, weaning foods formulated from cereals and legume composites could still be deficient in some essential micro or macro nutrients. The study seeks to determine the amino acid profiles of the predominant varieties of rice, soybean and groundnuts grown in Ghana. This is necessary to provide information to facilitate the formulation of low-cost weaning foods with the optimum protein and amino acid profile, for infant growth and metabolism.

Materials and Methods

The rice, soybean and groundnut varieties were obtained from the Crops Research Institute, Fumesua, Kumasi and the Savannah Agriculture Research Institute, Tamale.

Rice varieties: *Sikamo, Digang, Jasmine-85, Nerica-1* and *Nerica-2*

Soybean varieties: *Salintuya, Quarshie, Anidaso* and *Jenguma*

Groundnut varieties: *Sinkarzie*, *F-mix*, *Chinese* and *Manipinta*

Sample preparation

Moisture content of all samples were between 4-5%. About 40 mg previously milled sample (using the Retchmill machine) was mixed with 5 ml of 3% Phenol in 6 N Hydrochloric acid in glass tubes. Samples were incubated at 110°C for 16 hours in a heating block (Liebisch), cooled and 1 ml of standard solution (0.656 mg/ml norleucine) was pipetted into the glass tube and mixed. The mixture was filtered using Whatman 595½ filter paper and washed repeatedly with water. The resulting supernatant was divided into 1 ml portions and dried in a Rotavap centrifuge (Thermo Electron, Asheville, NC, USA) under vacuum at 60°C for 2-3 hours to remove the excess water and HCl. After complete drying the hydrolysate was suspended in 1 ml buffer A (5 mM Heptafluorobutyric acid/0.7% Trifluoroacetic acid) and untrasonicated for 15 minutes. Subsequently the samples were filtered through 0.45 um filter for HPLC analysis. Measurements were conducted in duplicates for each variety.

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HPLC Analysis of Amino Acids in Samples

In this research, sample concentration for HPLC was 6.67 mg/ml, injected volume, 20 µl and flowrate, 1 ml/min. Chromatographic analysis was performed using the HPLC (SP thermo separation products) and ELSD (Polymer laboratories, PL-ELS2100, USA). The solvent system consisted of two buffers. Buffer A (0.653 ml 5 mM 98% heptafluorobutyric acid in 7 ml 0.7% trifluoroacetic acid solution) and buffer B (acetonitrile). Samples were injected onto the HPLC column (Grace Alltech Prevail C18 5 µm column, 4.6×250 mm). Stepwise elution was used beginning with 0% B or 100% A for 6 mins, followed by 15% B for 2 mins then 35% B for 17 min and finally held at 0% B for 5 min. The nebulisation and the evaporation temperatures, and the nitrogen flow rate were optimised at 60°C and 2.0 l/min, respectively.

Calibration curves were prepared for each amino acid standard. Area was plotted against concentration. Power trend lines were calculated from 5 dilutions. Regression coefficients were found in the range of 0.95-1.0. No correction factor was used for the internal standard because recovery was approximately 100%.

Statistical analysis

The data obtained was analyzed using ANOVA. The significant differences between factors were determined using the Tukey's HSD test at 95% confidence level (p<0.05). The statistical tool used was STATGRAPHICS Centurion Version XIV.I.

Results and Discussion

A total of sixteen (16) amino acids, eight (8) essential and seven (7) nonessential, were detected using the HPLC-ELSD. Glutamic acid and Lysine however, eluted together for soybean and groundnuts and were recorded as such. The method could not detect glutamic acid and lysine in rice. This may be due to the hydrolyses procedure which causes variation in the determined and composition of aminoacids. Asparagine and glutamine were converted to their corresponding acids during the hydrolysis procedure. Cysteine and Methionine were oxidized into cystic acid and methionsulphon in the rice samples but could not be extracted from soybean and groundnut samples.

All rice varieties investigated were deficient in the essential amino acids (EEA) tryptophan, valine and lysine as well as the nonessential amino acids (NEEA) glycine and glutamic acid. All varieties, except *Nerica-2*, were deficient in histidine while *Digang* was deficient in proline. Threonine was the most predominant essential amino acid in the rice varieties, ranging from 10.70 g/kg for *Digang* and 21.17 g/kg in *Nerica-1* while the predominant nonessential amino acid was Serine, ranging from 1.53 g/kg for *Jasmine-85* to 29.30 g/kg in *Nerica-1*. The total EEA for all five varieties ranged from 36.42 g/kg to 20.52 g/kg while total NEEA ranged from 26.16 g/kg to 54.90 g/kg with *Nerica-1* having the highest and *Digang* the lowest in both cases. The amino acid profile of the different rice varieties is shown in Table 1.

The amino acid profile of *Nerica-1* rice compared well to Thai rice having total essential amino acid content of 34.68 g/kg [6]. The rice variety, environment and/or soil type for rice cultivation as well as pre-harvest and postharvest practices account for differences in nutritional composition.

All the local soybean varieties had good amino acid profiles. All the essential and nonessential amino acids were detected in *Quarshie* soybeans, while *Salintuya* and *Anidaso* soybeans were deficient in tryptophan and *Jenguma* soybean was deficient in histidine and proline. Threonine was the most abundant essential amino acid in the soybean varieties, ranging from 54.36 g/kg to 58.80 g/kg while alanine was the predominant non essential amino acid ranging from 63.96 g/kg to 75.30 g/kg. Total EEA ranged from 154.32 g/kg to 169.14 g/kg while total NEEA ranged from 218.10 g/kg to 233.34 g/kg with *Quarshie* having the highest and *Jenguma* the lowest in both cases as shown in Table 2.

Amino Acid	***Digang***	***Nerica-1***	***Jasmine-85***	***Nerica-2***	***Sikamo***
Glutamic acid+Lysine	-	-	-	-	-
Histidine	-	-	-	0.92	-
Threonine	10.70[a]	21.17[e]	11.86[b]	17.58[d]	13.49[c]
Isoleucine	1.96[a]	3.55[e]	2.04[b]	3.51[d]	2.98[c]
Leucine	4.13[a]	6.81[d]	4.82[b]	7.27[e]	5.95[c]
Phenylalanine	3.55[a]	4.91[d]	3.80[b]	5.03[e]	4.45[c]
Tryptophan	-	-	-	-	-
Valine	-	-	-	-	-
Total EAA	20.72	36.42	22.5	33.36	26.88
Glycine	-	-	-	-	-
Serine	13.69[b]	29.30[e]	12.53[a]	17.03[d]	15.71[c]
Aspartic acid	4.72[a]	7.56[e]	5.07[b]	7.50[d]	5.86[c]
Alanine	3.46[a]	7.27[e]	4.22[b]	7.14[d]	5.32[c]
Tyrosine	1.07[a]	2.93[e]	1.50[b]	2.70[d]	2.10[c]
Proline	-	2.11[c]	1.52[a]	2.28[d]	1.68[b]
Arginine	3.17[a]	5.76[e]	3.88[b]	5.71[d]	4.44[c]
Total NEAA	26.16	54.9	28.68	42.36	35.16

*Different letters in the same row between corresponding pairs indicates significant differences (P<0.05) by Tukey's test.
Not detected

Table 1: Amino Acid Composition of Ghanaian rice varieties (g/kg).

Amino Acid	**Quarshie**	**Salintuya**	**Anidaso**	**Jenguma**
Glutamic acid+Lysine	23.70[c]	20.16[a]	24.24[d]	20.28[b]
Histidine	7.32[c]	7.02[b]	6.78[a]	-
Threonine	57.48[c]	58.80[d]	54.36[a]	54.72[b]
Isoleucine	20.16[d]	20.04[c]	19.38[b]	19.14[a]
Leucine	32.40[d]	31.44[b]	31.56[c]	30.60[a]
Phenylalanine	21.36[d]	20.64[b]	20.76[c]	19.98[a]
Tryptophan	2.46[a]	-	-	3.12[b]
Valine	4.26[b]	6.78[d]	3.90[a]	6.48[c]
Total EAA	169.14	167.94	160.98	154.32
Glycine	16.86[d]	15.18[a]	16.68[c]	15.60[b]
Serine	46.08[a]	48.30[b]	55.92[d]	52.44[c]
Aspartic acid	41.53[d]	39.36[b]	39.00[a]	39.78[c]
Alanine	69.12[c]	67.98[b]	63.96[a]	75.30[d]
Tyrosine	14.52[d]	14.10[c]	13.38[b]	13.20[a]
Proline	21.18[a]	21.48[c]	21.24[b]	-
Arginine	16.80[a]	23.58[d]	23.16[c]	21.78[b]
Total NEAA	226.09	229.98	233.34	218.1

Different letters in the same row between corresponding pairs indicates significant differences (P<0.05) by Tukey's test.
– Not detected

Table 2: Amino Acid Composition of Ghanaian soybean varieties (g/kg).

The local soybean varieties contained lower lysine, valine, histidine and tryptophan (from 2.46 g/kg to 24.24 g/kg) when compared with Brazillian and USA soybeans which ranged from 4.51 g/kg-100.58 g/kg. Threonine and alanine levels were about three times higher in the local varieties than soybeans from Brazil and USA while isoleucine, leucine and phenylalanine contents in the local soybeans were comparable to that of Brazil and USA [7].

Tryptophan was not detected in all four groundnut varieties. *F-Mix* and *Sinkarzie* expressed all the other amino acids investigated. Co-

Amino Acid	Manipinta	Sinkarzie	F-Mix	Chinese
Glutamic acid+Lysine	-	10.32[a]	10.20[b]	-
Histidine	-	4.08[a]	3.64[b]	2.10[c]
Threonine	-	41.64[a]	34.74[b]	38.58[c]
Isoleucine	12.78[a]	10.14[b]	10.32[c]	8.10[d]
Leucine	24.12[a]	18.12[b]	17.82[c]	14.28[d]
Phenylalanine	18.90[a]	13.08[b]	13.74[c]	12.06[d]
Tryptophan	-	-	-	-
Valine	11.28[a]	3.24[b]	3.72[c]	6.30[d]
Total EAA	67.08	100.62	94.2	81.42
Glycine	26.22[a]	17.40[b]	16.14[c]	-
Serine	-	31.73[a]	30.48[b]	-
Aspartic acid	14.60[a]	27.30[b]	25.26[c]	22.02[d]
Alanine	-	31.68[a]	24.18[b]	20.94[c]
Tyrosine	12.96[a]	9.90[b]	8.64[c]	7.08[d]
Proline	11.16[a]	7.30[b]	7.32[c]	6.78[d]
Arginine	5.46[a]	22.63[b]	22.62[c]	16.92[d]
Total NEAA	70.6	147.94	133.4	73.74

*Different letters in the same row between corresponding pairs indicate significant differences (P<0.05) by Tukey's test.
– Not Detected

Table 3: Composition of amino acids in four Ghanaian groundnut varieties (g/kg).

eluting Glutamic acid and lysine as well as serine were not detected in *Manipinta* and *Chinese*. Histidine and threonine were also not detected in *Manipinta* while *Chinese* was lacking in glycine. Threonine (34.74 g/kg to 41.64 g/kg) and alanine (20.94 g/kg to 31.68 g/kg) were the predominant amino acids in *Sinkarzie*, *F-Mix* and *Chinese* while leucine (2.12 g/kg) and glycine (26.22 g/kg) were predominant in *Manipinta* as shown in Table 3. *Sinkarzie* groundnuts had the highest total EEA and total NEA of 100.62 g/kg and 147.94 g/kg respectively while *Manipinta* expressed the least total EEA and total NEA of 67.08 g/kg and 70.60 g/kg respectively.

All the groundnut varieties had lower total EEA than Indian JL groundnut variety (121 g/kg) and USA peanuts (127.43 g/kg) but threonine levels of the local groundnut varieties were about four times higher than in the Indian or USA groundnuts.

Tryptophan is essential in the manufacture of serotonin, needed for balancing mood and sleep patterns but was the least expressed amino acid in all the local rice, groundnut and soybean varieties [8]. Alanine was expressed in high amounts in almost all the rice, soybean and groundnut varieties. Alanine however can be produced by the body and is thus nonessential. Excess alanine in the diet may result in flushing and tingling in the muscles of humans and must therefore be regulated in weaning foods [9].

The FAO [10] recommends weaning foods to contain not less than 42 g/kg each of lysine, leucine, isoleucine and valine, 28 g/kg each of threonine, tyrosine and phenylalnine and 14 g/kg tryptophan. With the exception of tryptophan requirements, weaning diets formulated with the local rice, soybean and groundnut varieties will not meet all other essential amino acid requirements. Thus, there is the need for fortification of the weaning diets with protein isolates.

Conclusion

While rice varieties were lacking in valine, glycine, glutamic acid and lysine and histidine, soybean and groundnut varieties expressed most amino acids investigated. *Nerica-1* rice, *Quarshie* soybean and *Sinkarzie* groundnut had the highest total EAA. The predominant amino acids in all varieties tested were threonine and serine. However, composites of rice, soybean and groundnut varieties in weaning foods will still present some deficiencies in the essential amino acid requirements of infants. Fortification of weaning diets, formulated with the local rice, soybeans and groundnut varieties investigated, with protein isolates is necessary to meet the protein energy requirement of infants.

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