Pak. J. Sci. Ind. Res. 2009 **52**(3) 122-129

Intercorrelation of Amino Acid Quality between Raw, Steeped and Germinated Pearl Millet (*Pennisetum typhoides*) Grains

Emmanuel Ilesanmi Adeyeye*
Department of Chemistry, University of Ado-Ekiti, PMB 5363, Ado-Ekiti, Nigeria

(received November 6, 2008; revised May 27, 2009; accepted May 28, 2009)

Abstract. In the study of amino acids in the pearl millet grains, *Pennisetum typhoides*, steeped sample was best in Arg, Glu, Ser and protein contents, germinated sample was best in His, Lys, Met, Phe, Thr, Val, Ala, Asp, Cys (shared with raw sample), Pro and Tyr whereas raw sample was best in Ile, Leu and Gly. Total amino acid contents in steeped grains were 432 mg/g crude protein (c.p.), in germinated grain 464 mg/g c.p. and in raw grain 439 mg/g c.p. with respective essential amino acids of 210 mg/g c.p., 233 mg/g c.p. and 224 mg/g c.p. Percentage Cys/TSAA trend was 53.1 (raw) > 52.1 (germinated) > 51.2 (steeped). Predicted protein efficiency ratio (P-PER) levels were 1.32 (steeped), 1.66 (raw) and 1.57 (germinated). The Leu/Ile ratio levels were 2.22 (raw) and 2.46 (both steeped and germinated). Amino acid scores based on whole hen's egg had Met as the limiting amino acid for the three samples. The two treatments enhanced the quality of the pearl millet amino acid levels thereby providing high potentials for use in weaning foods and formulations. However, no significant difference was seen between raw/steeped, raw/germinated and steeped/germinated samples at $p < 0.05$.

Keywords: amino acid profile, *Pennisetum typhoides*, processed grains, pearl millet

Introduction

Millet, grown predominantly in the dry areas of the Far East and Africa, is a staple food in African countries. It is used primarily as a grain crop in Nigeria. Its grain is richer in nutritive value than guinea corn and is used for making heavy bread or porridge and alcoholic beverages, *ogi* in Nigeria and is also used in animal feed, particularly for poultry and other birds (Kochhar, 1986).

There is dearth of information on the nutritional quality of flour processed millet. This work reports on the amino acid composition of the raw, steeped and germinated grains of *Pennisetum typhoides* with a view to providing information on the best treatment for enhancing the protein quality for its various food uses.

Materials and Methods

Samples of pearl millet grains were purchased from the main market of Ado-Ekiti in the southern part of Nigeria in Ekiti State. About 1.5 kg of the grains was used for the experiments. After removing stones, damaged grains, glumes and glumela manually, the endosperm was extracted from kernels and divided into three equal parts for use as raw, steeped and germinated pearl millet samples and labelled accordingly.

Sample treatment. Raw sample (0.5 kg) was not specially treated but only dried to constant weight (6.38 g/100 g moisture content). For steeping, 0.5 kg grains were placed in plastic container, covered with distilled water and left in the laboratory at ambient temperature (30.9 °C) at 0.41 Im^2/ft light intensity. After four days, grains were washed with distilled water, dried in the sun to constant weight (6.45% moisture content) and stored in covered plastic container. For germination of samples, 0.5 kg grains were soaked in water at room temperature for 24 h; then spread on a damp fabric, protected from direct sunlight, for approximately 48 h, until 5.04 cm long sprouts developed. Germinated grains were dried in the sun for 3 days until constant weight (7.41% moisture content); the sprouts were manually removed and the desprouted grains were stored in a plastic container (WHO, 1999). Each sample was then homogenised, sieved using 200 mm mesh and kept in the refrigerator (-4 °C). Six replicates of steeped and germinated grains were used for amino acid analysis.

Instrumentation. The Technicon Sequential Multisample Amino Acid Analyzer (TSM) – an automated instrument – was used to separate, detect, and quantitate amino acids.

The column operating conditions for hydrolysate were; for acid–neutral column: flow rate (0.5 ml/min), operating temperature (60 °C), resin bed (23.0–23.5 cm) and resin type (C-3); for basic column: flow rate (0.5 ml/min) operating temperature (60 °C), resin bed (4.5-5.0 cm) and resin type (C-3).

Standard samples for both hydrolysate and physiologic systems were 0.025 μmoles of each amino acid equal to 0.010 ml (10 μl) of the Technicon 2.5 μml amino acid standard. For unknown hydrolysate: hydrolysate samples may be

*E-mail: eiadeyeye@yahoo.com

taken up in either 0.01 M HCI or the pH 2.0 buffer. The equivalent of 0.05 to 0.10 mg of protein was applied as sample. An internal standard Norleucine, approx. 0.025 µM of each hydrolysate sample and 0.025-0.050 µM with each physiologic sample was included.

Amino acid analysis. Pearl millet flour (2.0 g) of each group was defatted by extraction with some chloroform-methanol (2:1 v/v) using the Soxhlet apparatus as 61.7-65.0 °C (AOAC, 2005). Extraction of lipid continued for 5 h to ensure total lipid extraction. Approximately 30-35 mg of the defatted sample was put in a glass test tube, 7 ml of 6 M HCl was added and oxygen was expelled by flushing with nitrogen gas tube was sealed and was placed in oven at 105±5 °C for 22 h, allowed to cool and content was filtered. The filtrate was evaporated to dryness at 40 °C under vacuum in rotary evaporator. The residue was dissolved in 5 ml acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer. Approximately 5-10 ml of the sample was dispensed into the cartridge of the analyzer. The method of amino acid analysis was by ion-exchange chromatography (IEC) (Spacman *et al.*, 1958) using TSM. Determinations were performed in duplicate and run time was 76 min for each sample. The column flow rate was 0.50 ml/min at 60 °C with reproducibility within ±3% using norleucine as the internal standard.

Method of calculating amino acid values from the chromatogram peaks. The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. Half-height of the peak on the chart was found and the width of the peak on the half-height was accurately measured and recorded. Approximate area of each peak was then obtained by multiplying the height with the width at half-height.

Norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

NE = Area of norleucine peak ÷ area of each amino acid

A constant S was calculated for each amino acid in the standard mixture:

$$S_{std} = NE_{std} \times \text{mol. weight} \times \mu MAA_{std}$$

Finally the amount of each amino acid present in the sample was calculated in g/16N or g/100 g protein using the following formula:

$$\text{Concentration (g/100 g protein)} = NH \times W @ NH/2 \times S_{std} \times C$$

$$C = \frac{\text{Dilution} \times 16}{\text{Sample wt (g)} \times N\% \times 10 \text{ vol. loaded}} \div NH \times W \text{ (Nleu)}$$

Where: NH = net height
W = Width @ half-height and
Nleu = Norleucine

Estimation of isoelectric point (pI). The estimation of the isoelectric point (pI) for a mixture of amino acids was calculated using the equation below:

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

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 (Olaofe and Akintayo, 2000).

Estimation of quality of dietary protein. Total amino acid scores were calculated based on the whole hen's egg amino acid profile (Paul *et al.*, 1976) while the essential amino acid scores were calculated using the following formula (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].

Predicted protein efficiency ratio (P-PER) was determined using one of the equations developed by Alsmeyer *et al.* (1974) as follows:

P-PER = -0.468 + 0.454 (Leu) -0.105 (Tyr).

Essential amino acid index 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 (Steinke *et al.*, 1980):

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

Determination of the ratio of total essential amino acids (TEAA) to the total amino acids (TAA), i.e. (TEAA/TAA), total sulphur amino acid (TSAA), percentage cystine in TSAA, total aromatic amino acid (TArAA), total neutral amino acid (TNAA), total acidic amino acid (TAAA) and total basic amino acid (TBAA) were estimated from the results of amino acid profile. The leucine/isoleucine ratio was also calculated.

Statistical analysis. All data generated were analysed statistically (Skoog *et al.,* 2004; Dixon and Massey, 1983). Mean, standard deviation and coefficient of variation (%) were

calculated. Amino acid composition of the raw/steeped, raw/germinated, steeped/germinated samples, amino acid differences in composition between raw/steeped and between raw/germinated as well as their essential amino acid scores with respective degrees of freedom of 16 and 8 at $p<0.05$ were subjected to F-test analysis. Calculations were meant to determine the level of variation among the data obtained for raw, steeped and germinated samples.

Results and Discussion

Table 1 shows amino acid composition of the samples. On pair wise basis, most of the values for germinated samples were generally better than the values for raw and steeped samples. Specifically, levels of His, Lys, Met, Phe, Thr, Val, Ala, Asp, and Pro in germinated samples were correspondingly higher than those for raw and steeped ones, indicating that germinated sample was 60.0% best in 9 parameters (9/15) of the amino acids although steeped sample was best in Arg, Glu and Ser or in 3/15 (20.0%) while the amino acid level was best in the raw (or remained unchanged) i.e. Ile, Leu and Gly or in 3/15 (20.0%). Cystine shared similar levels of amino acid (7.6 mg/g) between raw and germinated samples and Tyr shared similar levels of amino acid (18.6 mg/g crude protein c.p.) between raw and steeped samples. The protein level of steeped sample was the highest (8.54 g/100 g) > germinated (7.54 g/ 100 g) > raw (5.11 g/100 g). The results of the pearl millet were in contrast to the observations in guinea corn where the steeped samples had the best levels of amino acids (AA) in His, Arg, Thr, Ser, Pro, Gly, Ala, Met, Cys, Val, Phe and Tyr, almost like the germinated samples of pearl millet (Adeyeye, 2008a). The highest AA was Glu with values of 67.4, 79.7 and 78.2 (mg/g c.p.) for raw, steeped and germinated grains, respectively. Tryptophan was not determined. Guinea corn samples had the highest levels of Glu (Adeyeye, 2008a). Leucine was the highest concentrated essential AA in raw (51.2 mg/g), steeped (43.7 mg/g) and germinated (49.5 mg/g) samples. Ile was the highest concentrated essential AA in guinea corn samples (Adeyeye, 2008a).

The least varied AA was Lys which ranged from 20.0 mg/g c.p. (steeped) to 21.7 mg/g c.p. (germinated) with coefficient of variation of 4.13% while the most varied AA was Gly with values of 17.9 mg/g c.p. (raw), 16.9 mg/g c.p. (steeped) and 11.6 mg/g c.p. (germinated) with CV of 21.9%. F-test results showed $F_{calculated}$ values between raw/steeped, raw/germinated and steeped/germinated to be 1.22, 1.12 and 1.09, respectively, at $p<0.05$ which were all lower than F_{Table} (2.24) which are not significantly different. Results in pearl millet were comparable in His being 15.8-18.1 mg/g c.p. with His (16.5-22.9 mg/g c.p.) in guinea corn but better in Lys (20.0-21.7 mg/g c.p.) in guinea corn (Adeyeye, 2008a). Even though Lys content in the samples (20.0-21.7 mg/g c.p.) was lower than the Lys content of the reference egg protein (62 mg/g c.p.), they can be

Table 1. Amino acid (mg/g crude protein) composition of raw, steeped and germinated millet (on dry weight)

Amino acid	Raw	Steeped	Germinated	Mean	SD	CV(%)
Arginine(Arg)*	29.4	33.6	31.0	31.3	2.12	6.77
Histidine(His)*	15.8	16.2	18.1	16.7	1.23	7.37
Isoleucine(Ile)*	23.1	17.8	20.1	20.3	2.66	13.1
Leucine(Leu)*	51.2	43.7	49.5	48.1	3.93	8.17
Lysine(Lys)*	20.6	20.0	21.7	20.8	0.86	4.13
Methionine(Met)*	6.7	5.9	7.0	6.53	0.57	8.73
Phenylalanine(Phe)*	27.7	26.0	29.4	27.7	1.70	6.14
Threonine(Thr)*	20.1	17.7	22.1	20.0	2.20	11.0
Tryptophan(Try)*	-	-	-	-	-	-
Valine(Val)*	29.6	29.0	33.9	30.8	2.67	8.67
Alanine(Ala)	18.5	21.0	23.0	20.8	2.25	10.8
Aspartic acid(Asp)	57.8	50.0	60.1	56.0	5.29	9.45
Cystine(Cys)	7.6	6.2	7.6	7.13	0.81	11.4
Glutamine(Glu)	67.4	79.7	78.2	75.1	6.71	8.93
Glycine(Gly)	17.9	16.9	11.6	15.5	3.39	21.9
Proline(Pro)	8.8	7.7	9.9	8.80	1.10	12.5
Serine(Ser)	17.8	22.0	20.0	19.9	2.10	10.6
Tyrosine(Tyr)	18.6	18.6	20.3	19.2	0.98	5.10
Protein(g/100g)	5.11	8.54	7.54	7.06	1.76	24.9

*Essential amino acid; SD = standard deviation; CV = coefficient of variation; - = not determined

enhanced by mixing with legumes, which are high in Lys. The increase in AA content of germinated pearl millet may be due to increase in the protease activity of enzymes which break down the protein to release amino acids needed for the activity.

Table 2 shows several quality parameters of proteins in the samples. The essential amino acids (EAA) ranged between 214 mg /g c.p. to 231 mg /g c.p. These values were far from the value of 566 mg/g c.p. of the egg reference protein (Paul *et al.*, 1976) but slightly close to 453 mg/g c.p. for peanut meal (Lusas, 1979) and better than 190 mg/g c.p. for *Colocynthis citrullus* (Akobundu *et al.*, 1982) flours. Total sulphur AA (TSAA)of the samples was 14.3, 12.1 and 14.6 mg/g c.p. for raw, steeped and germinated, respectively; these values were individually approx. 25% of the value (58 mg/g c.p.) recommended for infants (FAO/WHO/UNU, 1985). Aromatic amino acid (ArAA) range suggested for ideal infant protein (68-118 mg/g c.p.) (FAO/WHO/UNU, 1985) is much higher than the current report (46.3-49.7 mg/g c.p.) indicating, pearl millet when used as the weaning food, should be complemented with ArAA rich foods particularly when raw or steeped pearl millet is used. The percentage ratio of EAA to TAA in the flour ranged from 48.6-51.1. These values 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 percentages of EAA/TAA for the pearl millet samples could be favourably compared with that of egg (50%) (FAO/WHO, 1990), pigeon pea flour (43.6%) (Oshodi *et al.*, 1993) and beach pea protein isolate (43.8-44.4%) (Chavan *et al.*, 2001). The predicted protein efficiency ratio (P-PER) as shown in Table 2 ranged from 1.32 to 1.66. The experimentally determined PER usually ranged from 0.0 for very poor protein to the maximum possible of just over 4 (Muller and Tobin, 1980). In the samples (Table 1) it could be seen that the values for Leu and Tyr (from which P-PER were calculated) were almost half of each other: raw (51.2 and 18.6 mg/g c.p., respectively), steeped (43.7 and 18.6 mg/g c.p., respectively) and germinated (49.5 and 20.3 mg/g c.p., respectively). The P-PER in guinea corn were: raw (<0.00), steeped (0.23) and germinated (0.29) indicating the pearl millet samples to be much better than the guinea corn (Adeyeye, 2008a).

The Leu/Ile values were 2.22 (in raw), 2.46 (in steeped) and 2.46 (in germinated) samples. In all the samples (Table 1) the level of Leu was more than twice the level of Ile. Endemic pellagra in cereal-eating populations was first described by Gopalan Srikantia (1960) particularly in poor agricultural labourers around Hyderabad in Andhra Pradesh (India). It has been suggested that an amino acid imbalance from excess leucine might be a factor in the development of pellagra

Table 2. Total, essential, non-essential, neutral, acid, basic, sulphur, aromatic amino acid (mg/g crude protein). Protein efficiency ratio (P-PER), isoelectric point (pI), Leu/Ile ratio, Leu/Ile difference of millet (on dry weight)

Amino acid	Raw	Steeped	Germi-nated	Mean	SD	CV (%)
TAA[a]	439	432	464	445	16.8	3.78
TNEAA[b]	214	222	231	222	8.50	3.83
TEAA[c]						
-with His	224	210	233	222	11.6	5.23
-no His	219	194	215	209	13.4	6.41
%TNEAA	48.9	51.4	49.8	50.0	1.27	2.54
%TEAA						
-with His	51.1	48.6	50.2	50.0	1.27	2.54
-no His	49.9	44.8	46.3	47.0	2.62	5
TNAA[d]	248	233	254	245	10.8	4.41
%TNAA	56.5	53.8	54.9	55.1	1.36	2.47
TAAA[e]	125	130	138	131	6.56	5.01
%TAAA	28.5	30.0	29.8	29.4	0.81	2.76
TBAA[f]	65.8	69.8	70.8	68.8	2.65	3.85
%TBAA	15.0	16.2	15.3	15.5	0.62	4.00
TSAA[g]	14.3	12.1	14.6	13.7	1.37	10.0
%TSAA	3.3	2.8	3.1	3.07	0.25	8.14
%Cys in TSAA	53.1	51.2	52.1	52.1	0.95	1
TArAA[h]	46.3	44.6	49.7	46.9	2.60	5.54
%TArAA	10.6	10.3	10.7	10.5	0.21	2.00
P-PER	1.66	1.32	1.57	1.52	0.18	11.8
Leu/Ile	2.22	2.46	2.46	2.38	0.14	5.88
Leu-Ile(diff.)	28.1	25.9	29.4	27.8	1.77	6.37
%Leu- Ile(diff.)	54.9	59.3	59.4	57.9	2.57	4.44
pI(calculated)	2.4	2.4	2.6	2.47	0.12	4.86
pI(expt.)	4.0	4.0	3.0	3.67	0.58	15.8
pI difference	1.6	1.6	0.4	1.20	0.69	57.5
	(40%)	(40%)	(13.3%)			
EAAI[i]	0.67	0.61	0.70	0.66	0.05	7.58

[a] = total acid; [b] = total non-essential amino acid; [c] = total essential amino acid; [d] = total neutral amino acid; [e] = total acidic amino acid; [f] = total basic amino acid; [g] = total sulphur amino acid; [h] = total aromatic amino acid; [i] = essential amino acid index

(FAO, 1995). High Leu in the diet impairs tryptophan and niacin metabolism and is responsible for niacin deficiency in sorghum eaters (Belavady *et al.*, 1963) and hence, the hypothesis that excess Leu in sorghum is aetiologically related to pellagra in sorghum-eating populations (FAO, 1995). The study of Krishnaswamy and Gopalan (1971) had 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. However, it has also been suggested that factors other than excess Leu and poor Leu/Ile balance in cereal proteins are responsible for the development of the disease. Krishnaswamy *et al*. (1976) have shown that vitamin B_6 is involved in the metabolism of Leu as well as that of Try and niacin suggesting that regional

differences in the prevalence of pellagra might be related to the nutritional status of the population in terms of vitamin B_6. Experiments in dogs fed with sorghum have shown that animals proteins with less than 11g percent (110 mg/g c.p.) Leu did not suffer from nicotinic acid deficiency (Belavady and Udayasekhara Rao, 1979). The current report shows Leu to range from 43.7-51.2 mg/g c.p. which was far less than 110 mg/g c.p., therefore, considered safe and could be beneficially exploited to prevent pellagra in endemic areas (Deosthale, 1980).

Table 2 shows that the Cys (%) in TSAA ranged from 51.2-53.1. Cys can improve protein quality and has positive effects on mineral absorption, particularly that of zinc (Mendoza, 2002). Cys/TSAA (percentage) values obtained in this study were comparable to the value of 62.9 reported for coconut endosperm (Adeyeye, 2004). The Cys in TSAA in guinea corn ranged from 58.9-72%, and it is 50.5% in cashew nut (Adeyeye *et al*., 2007); but 40.7% in *Triticum durum* (Adeyeye, 2007), 44.4% in *Parkia biglobosa* seeds (Adeyeye, 2006), 44.3% in *Cola acuminata* and 37.8% in *Garcinia kola* (Adeyeye *et al*., 2007). Most animal proteins are low in cystine e.g. 36.3 in *Macrotermes bellicosus* (Adeyeye, 2005a); 25.6 in *Zonocerus variegatus* (Adeyeye, 2005b); 35.3 in *Archachatina marginata*, 38.8 in *Archatina archatina* and 21.0 in *Limicolaria* sp.; (Adeyeye and Afolabi, 2004); 26.5 in turkey muscle and 26.0 in turkey skin (Adeyeye and Ayejuyo, 2007); 29.8 in *Gymnarchus niloticus* (trunk fish) (Adeyeye and Adamu, 2005); 13.3-15.9 in various parts of West African fresh water male crab (Adeyeye and Kenni, 2008); 26.9 in the flesh of raffia palm tree grub (Adeyeye and Aye, 2008); 23.8-30.1 in three different fresh water fish samples (Adeyeye, 2008b). Thus for animal protein, Cys is unlikely to contribute up to 50% of the TSAA (FAO/WHO, 1991). The Cys (%)/TSAA had been set at 50% in rat, chick and pig diets (FAO/WHO, 1991). The calculated isoelectric point (pI) ranged between 2.4-2.6 whereas pI experimental (not yet reported) was 3.0-4.0. The information on pI 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). The low pI values could be a function of the TAAA (125-138 mg/g c.p. or 28.5-30.0%) which were much higher than the TBAA (65.8-70.8 mg/g c.p. or 15.0-16.2%) (Table 2). The calculated pI values were close to the experimental values with $pI_{calc.} - pI_{expt.}$ range of 0.4-1.6 or 13.3-40%. The essential amino acid index (EAAI) ranged from 0.61-0.70 which were all lower than the value of 1.26 in a defatted soya flour (Cavins *et al*., 1972). The EAAI method can be useful as a rapid tool for evaluating protein quality in food formulations (Nielsen, 2002).

Table 3 contains a summary of the differences between raw/steeped and between raw/germinated samples. The highest CV(%) was observed in Tyr with a value of 133 whereas Pro and Phe had CV(%) value of 0.00. Table 3 gave all the differences in amino acid parameters.

Table 4 shows that Met was the limiting amino acid in all the samples based on whole hen's egg (Paul *et al.*, 1976); values ranged from 0.18 (18%)-0.22 (22%). Most of the results have

Table 3. Differences in amino acid composition between raw and steeped, and between raw and germinated millet samples

Amino acid	Raw-steeped	Raw-germinated	Mean	SD	CV(%)
Arg	-4.2(14.3)**	1.6(5.40)*	2.90	1.80	62.1
His	-0.4(2.50)	2.3(14.6)	1.40	1.30	92.9
Ile	5.3(22.9)	3.0(13.0)	4.20	1.60	38.1
Leu	7.5(14.6)	1.7(3.30)	4.60	4.10	89.1
Lys	0.6(2.90)	-1.1(5.30)	0.85	0.35	41.2
Met	0.8(11.9)	-0.3(4.50)	0.60	0.40	66.7
Phe	1.7(6.10)	1.7(6.10)	1.70	0.00	0.00
Thr	2.4(11.9)	2.0(10.0)	2.20	0.30	13.6
Try	-	-	-	-	-
Val	0.6(2.00)	-4.3(14.5)	2.50	2.60	104
Ala	-2.5(13.5)	-4.5(24.3)	3.50	1.40	40.0
Asp	7.8(13.5)	-2.3(4.00)	5.10	3.90	76.5
Cys	1.4(18.4)	0.0(0.0)	0.7	1.0	14.3
Glu	-12.3(18.2)	-10.8(16.0)	11.6	1.10	9.48
Gly	1.0(5.60)	6.3(35.2)	3.70	3.70	100
Pro	1.1(12.5)	-1.1(12.5)	1.1	0.00	0.00
Ser	-4.2(23.6)	-2.2(12.4)	3.20	1.40	43.8
Tyr	0.0(0.00)	-1.7(9.10)	0.90	1.20	133
Protein	-3.43(67.1)	-2.43(47.6)	2.93	0.71	24.2

* = figures in brackets are percentage values; ** = a figure preceded by '-` means both the figure and the figure in bracket carry t

Table 4. Amino acid scores of samples based on whole hen's egg amino acid profile

Amino acid	Raw	Steeped	Germinated	Mean	SD	CV(%)
Arg	0.46	0.55	0.51	0.51	0.04	7.84
His	0.66	0.68	0.75	0.70	0.05	
Ile	0.41	0.32	0.36	0.36	0.05	13.9
Leu	0.62	0.53	0.60	0.58	0.05	8.62
Lys	0.33	0.32	0.35	0.33	0.02	6.06
Met	0.21	0.18	0.22	0.20	0.02	10.0
Phe	0.54	0.51	0.58	0.54	0.04	7.41
Thr	0.39	0.35	0.43	0.39	0.04	10.3
Try	-	-	-	-	-	-
Val	0.39	0.39	0.45	0.41	0.03	7.32
Ala	0.34	0.39	0.43	0.39	0.05	12.8
Asp	0.54	0.47	0.56	0.52	0.05	9.62
Cys	0.42	0.34	0.42	0.39	0.05	12.8
Glu	0.56	0.66	0.65	0.62	0.06	9.68
Gly	0.60	0.56	0.39	0.52	0.11	21.2
Pro	0.23	0.20	0.26	0.23	0.03	13.0
Ser	0.23	0.28	0.25	0.25	0.03	12.0
Tyr	0.47	0.47	0.51	0.48	0.02	4.17

scores less than 50% except His, Leu, Phe and Glu in all the three pearl millet samples. In order to correct for the whole amino acid profile in the samples 100/21, 100/18 and 100/22, 4.76, 5.56 and 4.55 times respectively as much raw, steeped and germinated grain flour would have to be eaten when they serve as sole protein source in the diet.

Table 5. Amino acid scores of millet samples based on provisional amino acid scoring pattern

Amino acid	Raw	Steeped	Germinated	Mean	SD	CV (%)
Ile	0.58	0.45	0.50	0.51	0.07	13.7
Leu	0.73	0.62	0.71	0.69	0.06	8.70
Lys	0.37	0.36	0.39	0.37	0.02	5.41
Met+Cys (TSAA)	0.41	0.35	0.42	0.39	0.04	10.3
Phe+Tyr	0.77	0.74	0.83	0.78	0.05	6.41
Thr	0.50	0.44	0.55	0.50	0.06	12.0
Try						
Val	0.59	0.58	0.68	0.62	0.06	9.68
Total	0.59	0.53	0.60	0.57	0.04	

Table 6. Summary of the amino acid profiles into factors A and B

	Millet samples (Factor A)			Factor B means
	Raw	Steeped	Germinated	
Amino acid composition (Factor B)				
Total essential amino acid	224	210	233	222
Total non-essential amino acid	214	222	231	222
Factor A means	219	216	232	222

Table 7. Amino acid profiles of pearl millet and guinea corn compared

Amino acid	Raw		Steeped		Germinated	
	Millet	Sorghum	Millet	Sorghum	Millet	Sorghum
Arg	29.4	31.9	33.6	42.8	31.0	39.4
His	15.8	16.5	16.2	22.9	18.1	18.1
Ile	23.1	46.2	17.8	50.9	20.1	56.0
Leu	51.2	10.9	43.7	21.3	49.5	21.4
Lys	20.6	14.8	20.0	18.8	21.7	20.0
Met	6.7	6.2	5.9	8.0	7.0	7.0
Phe	27.7	21.6	26.0	26.0	29.4	23.4
Thr	20.1	22.4	17.7	32.3	22.1	30.2
Try	-	-	-	-	-	-
Val	29.6	18.5	29.0	38.1	33.9	30.3
Ala	18.5	12.9	21.0	43.9	23.0	20.1
Asp	57.8	37.4	50.0	41.9	60.1	55.2
Cys	7.6	8.9	6.2	20.6	7.6	17.2
Glu	67.4	60.5	79.7	82.0	78.2	91.2
Gly	17.9	13.6	16.9	29.3	11.6	23.0
Pro	8.8	9.9	7.7	32.9	9.9	21.9
Ser	17.8	30.0	22.0	40.1	20.0	39.0
Tyr	18.6	16.9	18.6	25.3	20.3	20.3
Protein (g/100g)	5.11	5.11	8.54	8.24	7.54	4.93

Table 5 contains amino acid scores of the pearl millet samples based on essential amino acid scoring pattern (FAO/WHO, 1973) which shows that Lys (0.37 or 37%) was the limiting AA in raw, Met+Cys (0.35 or 35%) was the limiting AA in steeped while Lys (0.39 or 39%) was also the limiting AA in germinated samples. Therefore, in order to fulfil the day's needs for all the EAA in pearl millet sample flours, 100/37 (raw), 100/35 (steeped) and 100/39 (germinated) or 2.70 times as much raw *P. typhoides* protein; or 2.86 times as much steeped *P. typhoides* protein, or 2.56 times as much germinated *P. typhoides* protein respectively would have to be eaten when it is the only protein source in the diet.

The data obtained for the TNEAA, TEAA and EAA scores as well as the scores based on whole hen's egg were all subjected to the F-test. In raw, the TEAA/TNEAA value was F_c(1.39) but F_T(3.50), not significantly different; in steeped, the F_c (4.69) but F_T (3.73), was significantly different; in germinated the F_c (4.66) but F_T (3.73), was significantly different. For TAA/TAA scores (based on whole hen's egg), F_c raw/steeped and raw/germinated was 1.45 in each case while F_c steeped/germinated was 1.0 and F_T was 2.35 showing that all the results were not significantly different in the compared samples. For scores based on essential amino acid scoring pattern, germinated/steeped had F_c of 1.25, raw/germinated, F_c was 1.15 and raw/steeped, F_c was 1.08 whereas F_T was 4.28 showing that none of the pairs was significantly different from each other. Level of significance was at $p<0.05$.

In conclusion, it is seen (Table 6) that TEAA trend was germinated > raw > steeped whereas TNEAA trend was germinated > steeped > raw in *Pennisetum typhoides*. Mean factor A had the trend: germinated > raw > steeped whereas Factor B mean values (Table 6), which relate to all the samples for their TEAA and TNEAA, give us similar values of 222 in each case. This is an interesting revelation. The germination technique is recommended for improvement of TEAA before *P. typhoides* is processed for food consumption or formulation. However, it should be noted that while germinated pearl millet had the highest TEAA, the steeped sample had the highest TEAA in the guinea corn; this comparison is easily seen in Table 7.

References

Adeyeye, E.I. 2004. The chemical composition of liquid and solid endosperm of ripe coconut. *Oriental Journal of Chemistry* **20:** 471-476.

Adeyeye, E.I. 2008a. The intercorrelation of the amino acid quality between raw, steeped and germinated guinea corn (*Sorghum bicolor*) grains. *Bulletin of Chemical Society of Ethiopia* **22:** 1-7.

Adeyeye, E.I. 2008b. Amino acid composition of three species of Nigerian fish:*Clarias anguillaris, Oreochromis niloticus* and *Cynoglossus senegalensis. Food Chemistry* **113:** 43-46.

Adeyeye, E.I. 2007. Amino acids and sugar composition of *Triticum durum* wholemeal flour. *Journal of Applied and Environmental Sciences* **3:** 128-132.

Adeyeye, E.I. 2006. Amino acids composition of fermented African locust bean (*Parkia biglobosa*) seeds. *Journal of Applied and Enviromental Sciences* **2:** 154-158.

Adeyeye, E.I., Adamu, A.S. 2005. Chemical composition and food properties of *Gymnarcus niloticus* (Trunk fish). *Biosciences Biotechnology Research Asia* **3:** 265-272.

Adeyeye, E.I. 2005a. The composition of the winged termites, *Macrotermes bellicosus*. *Journal of Chemical Society of Nigeria* **30:** 145-149.

Adeyeye, E.I. 2005b. Amino acid composition of variegated grasshopper, *Zonocerus variegatus. Tropical Science* **45:** 141-143.

Adeyeye, E.I., Afolabi, E.O. 2004. Amino acid composition of three different types of land snails consumed in Nigeria. *Food Chemistry* **85:** 535-539.

Adeyeye, E.I., Aye, P.A. 2008. The chemical composition and functional properties of the flesh and viscera of raffia palm tree grub (*Ryncophorus phoenicis*). *Pakistan Journal of Nutrition* **7:** (in press).

Adeyeye, E.I., Ayejuyo, O.O. 2007. Proximate, amino acid and mineral composition of turkey-hen muscle and skin. *Oriental Journal of Chemistry* **23:** 879-886.

Adeyeye, E.I., Kenni, A.M. 2008. The relationship in the amino acid of the whole body, flesh and exoskeleton of common West African fresh water male crab (*Sudananautes africanus africanus*). *Pakistan Journal of Nutrition* **7:** 748-752.

Adeyeye, E.I., Asaolu, S.S., Aluko, A.O. 2007. Amino acid composition of two masticatory nuts (*Cola acuminata* and *Garcinia kola*) and a snack nut (*Anacardium occidentale). International Journal of Food Sciences and Nutrition* **58:** 241-249.

Akobundu, E.N.T., Cherry, J.P., Simmon, J.G. 1982. Chemical, functional and nutritional properties of 'Egusi' *Colocynthis citrulus* seed products. *Journal of Food Science* **47:** 828-835.

Alsmeyer, R.H.,Cunningham, A.E., Hapich, M.L. 1974. Equations to predict PER from amino acid analysis. *Food Technology* **28:** 34-38.

AOAC, 2005. *Official Methods of Analysis*, 18th edition. Association of Official Analytical Chemists, Washington DC, USA.

Belavady, B., Udayasekhara Rao, P. 1979. Leucine and isoeleucine content of jowar and its pellagragenicity. *Indian Journal of Experimental Biology* **17:** 659-661.

Belavady, B., Srikantia, S.G., Gopalan, C. 1963. The effect of oral administration of leucine on the metabolism of trytophan. *Biochemistry Journal* **87:** 652-655.

Cavins, J.F., Kwolek, D.F., Inglett, G.E., Cowen, J.C. 1972. Amino acid analysis of soybean meal:Interlaboratory study. *Journal of Association of Official Analytical Chemists* **55:** 686-694.

Chavan, U.D., Mckenzie, D.B., Shahidi, F. 2001. Functional properties of protein isolates from beach pea (*Lathyrus maritimus* L.). *Food Chemistry* **74:** 177-187.

Deosthale, Y.G. 1980. Nutrition dimension of high yielding and hybrid crop varieties: locational and varietal differences in nutritional value. In: *Sorghum and Millet in Human Nutrition,* FAO Food and Nutrition Series No. 27, FAO: Rome; 1995: p. 82.

Dixon, W.F., Massey Jr., F.J. 1983. *Introduction to Statistical Analysis,* 4th edition, McGraw-Hill, New York, USA.

FAO. 1995. *Sorghum and Millet in Human Nutrition*. FAO Food and Nutrition Series, No. 27, Food and Agriculture Organization of the United Nations, Rome Italy.

FAO/WHO. 1973. *Energy and Protein Requirements*. Technical Report Series No. 522, World Health Organization, Geneva, Switzerland.

FAO/WHO. 1990. *Protein Quality Evaluation.* Report of Joint FAO/WHO Consultation Held in Bethesda, USA:4-8 December, 1989. Food and Agriculture Organization, Rome, Italy.

FAO/WHO. 1991. *Protein Quality Evaluation*, Report of Joint FAO/WHO Expert Consultation. FAO Food and Nutrition Paper 51, Rome, Italy.

FAO/WHO/UNU. 1985. Energy and protein requirement. WHO Technical Report Series No. 724, World Health Organization, Geneva, Switzerland.

Gopalan, C., Srikantia, S.G. 1960. Leucine and pellagra. *Lancet* **1:** 954-957.

Kochhar, S.L. 1986. *Tropical Crops- A Textbook of Economic Botany*. Macmillan Publishers Limited, London, UK.

Krishnaswamy, K., Gopalan, C. 1971. Effect of isoleucine on skin and electroence-phalogram in pellagra. *Lancet* **2:** 1167-1169.

Krishnaswamy, K., Bapu Rao, S., Raghuram, T.C., Srikantia, S.C. 1976. Effect of vitamin B_6 on leucine-induced changes in human subjects. *American Journal of Clinical Nutrition* **29:** 177-181.

Lusas, E.W. 1979. Food uses of peanut protein. *Journal of the American Oil Chemists Society* **56:** 242-256.

Mendoza, C. 2002. Effect of genetically modified low phytic acid plants on mineral absorption. *International Journal*

*of Food Science and Technol*ogy **37:** 759-767.

Muller, H.G., Tobin, G. 1980. Nutrition and Food Processing. Croom Helm, London, UK.

Nielsen, S.S. 2002. *Introduction to the Chemical Analysis of Foods*. CBS Publishers and Distributors, New Delhi, India.

Olaofe, O., Akintayo, E.T. 2000. Prediction of isoelectric points of legume and oilseed proteins from their amino acid compositions. *Journal of Technosciences* **4:** 49-53.

Oshodi, A.A., Olaofe, O., Hall, G.M. 1993. Amino acid, fatty acid and mineral composition of pigeon pea (*Cajanus cajan*). *Intnational Journal of Food Sciences and Nutrition* **43:** 187-191.

Paul, A.A., Southgate, D.A.T., Russel, J. 1976. *First Supplement to McCance and Widdowson's The Composition of Foods*. HMSO, London, UK.

Phillips, T.A. 1977. *An Agricultural Notebook, with Special Reference to Nigeria*. Longman Group Limited, London, UK.

Skoog, D.A., West, D.M., Holler, F.J., Crouch, S.R. 2004. *Fundamentals of Analytical Chemistry*, 8th edition. Brooks/Cole, Bangalore, India.

Spackman, D.H., Stein, W.H., Moore, S. 1958. Chromatography of amino acids on sulphonated polystyrene resins. *Analytical Chem*istry **30:** 1190-1205.

Steinke, F.H., Prescher, E.E., Hopkins, D.T. 1980. Nutritional evaluation (PER) of isolated soybean protein and combinations of food proteins. *Journal of Food Sci*ence **45:** 323-327.

Technicon Instruments Corporation 1973. Technicon Sequential Multisample Amino Acid Analyzer (TSM).TIC, New York, USA.

WHO. 1999. *Complementary Feeding of Young Children in Africa and the Middle East*. World Health Organization, Geneva, Switzerland.