Bull. Chem. Soc. Ethiop. **2008**, 22(1), 11-17. ISSN 1011-3924
Printed in Ethiopia

THE INTERCORRELATION OF THE AMINO ACID QUALITY BETWEEN RAW, STEEPED AND GERMINATED GUINEA CORN *(SORGHUM BICOLOR)* GRAINS

E.I. Adeyeye*

Department of Chemistry, University of Ado Ekiti, P.M.B. 5363, Ado-Ekiti, Nigeria

(Received January 31, 2007; revised May 31, 2007)

ABSTRACT. Levels of amino acids were determined in the grains of guinea corn, *Sorghum bicolor* (L.) Moench. The steeped sample was best in His, Arg, Thr, Ser, Pro, Gly, Ala, Met, Cys, Val, Phe and Tyr contents whereas germinated sample was best in Lys, Asp, Glu, Leu and Ile. The total amino acid contents were: steeped [57.71 g/100 g crude protein (c.p.)], germinated (53.37 g/100 g c.p.) and raw (37.91 g/100 g c.p.) with respective essential amino acids of 30.70 g/100 g c.p., 28.33 g/100 g c.p. and 21.48 g/100 g c.p. Percentage cystine/total sulfur amino acid (% Cys/TSAA) trend was 72.0 (steeped) > 71.1 (germinated) > 58.9 (raw). The Predicted Protein Efficiency Ratio (P-PER) levels were 0.23 steeped, 0.29 (germinated) and none (raw). The Leu/Ile ratio was 0.42 for steeped, 0.38 for germinated and 0.24 for raw grains. The limiting amino acid was Leu for all the samples with 0.30 (steeped), 0.31 (germinated) and 0.16 (raw). The two treatments enhanced the quality of the guinea corn amino acid levels. However, no significant differences occurred between raw/steeped, raw/germinated and steeped/germinated samples at $p < 0.05$.

KEY WORDS: Amino acid quality, Raw, Steeped, Germinated, Guinea corn

INTRODUCTION

Sorghum and millets have been important staples in the semi-arid tropics of Asia and Africa for centuries. These crops are still the principal sources of energy, protein, vitamins and minerals for millions of the poorest people in these regions [1]. Sorghum and millets are grown in harsh environments where other crops grow or yield poorly. They are grown with limited water resources and usually without application of any fertilizers or other inputs by a multitude of small-holder farmers in many countries. Therefore, and because they are mostly consumed by disadvantaged groups, they are often referred to as "coarse grain" or "poor people's crops".

Sorghum bicolor (L.) Moench, is known under a variety of names: great millet and guinea corn in West Africa, kafir corn in South Africa, dura in Sudan, mtama in eastern Africa, jowar in India and kaoliang in China [2]. In the United States it is usually referred to as milo or milo-maize. Sorghum belongs to the tribe Andropogonae of the grass family Poaceae. Sugar cane, *Saccharum officinarum*, is a member of this family and a close relative of sorghum. The genus *Sorghum Moench* is characterized by spikelets borne in pairs. Sorghum is treated as an annual, although it is a perennial grass and in the tropics it can be harvested many times [1].

In Nigeria *Sorghum bicolor* has been given various local names: Yoruba – *Oka baba*; Ibo – *Okili*; Hausa – *Dawa.* It is the main staple food crop of northern Nigeria but it is grown as far south as Oyo and Ibadan. Its production is concentrated in the Guinea and Sudan savannah zones. In the north, millet progressively replaces sorghum where the annual rainfall is less than 750 mm [3]. Eight groups of cultivated *Sorghum bicolor* are identified in Nigeria of which the following four are important. The *Guinea* group is adapted to the northern Guinea savannah zone where rain is more than 100 mm. Local varieties from this group are known by the Hausa name 'Fara Fara'. The *Kaura* group is more adapted to the drier part of northern Nigeria. The *Fara Fara* group is a hybrid between the previous two groups. The *Chad* group is found in the north-east region. It is the earliest group. Most of the local varieties are photosensitive as they

*Corresponding author. E-mail: eiadeyeye@yahoo.com

flower at the end of the rainy season (October-November) and grain ripens after the rains have finished. The plants are tall, ranging from 2.5-4.0 m in height and the tall stalks are desirable for a variety of building purposes and for fencing [3].

More than 95 percent of total food use of sorghum occurs in countries of Africa and Asia. In Africa, human consumption accounts for almost three–quarters of total utilization and sorghum represents a large portion of the total calorie intake in many countries [1].

This work reports on the amino acid composition of the raw, steeped and germinated grains of *Sorghum bicolor*. This will give information on the best treatment to enhance the protein quality for its various food uses.

EXPERIMENTAL

Sampling. Samples of *Guinea corn* grains were purchased from the main market, Ado-Ekiti in the southern part of Nigeria in Ekiti State. About 1.5 kg of the grains were used for the experiments. Stones, damaged grains, glumes and glumela were manually removed. Then the endosperm was extracted from kernels. The sample was divided into three equal parts for use as raw, steeped and germinated samples and labeled accordingly.

Sample treatment. The portion labelled as raw (0.5 kg) was not specially treated but only dried to constant weight (6.55 g/100 g moisture content). The portion labelled for steeping (0.5 kg) was placed in plastic container, distilled water poured to cover the grains and left in the laboratory at ambient temperature (30.9 ^{o}C) at 0.41 Im^2/ft light intensity for four days. After this, the grains were washed with distilled water, dried in the sun to constant weight (6.4 g/100 g moisture content) stored in a covered plastic container. The portion labelled for germination (0.5 kg) was treated as follows: grains were soaked in water at room temperature for 24 h; the grains were then spread on a damp fabric, protected from the direct sun for approximately 48 h, until 5.04 cm sprouts developed; germinated grains were dried in the sun for 3 days until constant weight of 7.2 g/100 g moisture content; the sprouts were manually removed and the desprouted grains were stored in a plastic container [4]. Each sample was then homogenized, sieved using a 200 mm mesh and kept in the refrigerator (-4 ^{o}C) pending analysis. Six replicates of steeped and germinated grains were used.

Amino acid analysis. *Guinea corn* flour (2.0 g) of each group was defatted with chloroform/methanol (2:1) mixture. Between 30-35 mg of the defatted sample was put in a glass test tube, 7 mL of 6 M HCL was added and oxygen expelled by flushing with nitrogen gas. The sealed test tube was put in an oven at 105 ± 5 ^{o}C for 22 h and later allowed to cool before the content was filtered. The filtrate was evaporated to dryness at 40 ^{o}C under vacuum. The residue was dissolved with 5 mL acetate buffer (pH 2.0). The method of amino acid analysis was by ion-exchange chromatography (IEC) [5], using the Technicon Sequential Multisample Amino Acid Analyzer (TSM) (Technicon Instruments Corporation, New York). Determinations were in duplicate. The period of an analysis lasted for 76 min for each sample. The column flow rate was 0.50 mL/min at 60 ^{0}C with reproducibility within ± 3 %.

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 **[6]**. This was the method used here.

Estimation of quality of dietary protein. The quality of dietary protein was measured by the method described in FAO/WHO **[7]** which is the ratio of available amino acids in the food or diet compared with the needs expressed as a ratio **[8]**. The formula below was used to calculate the essential amino acid scores.

$$\text{Amino acid score} = \frac{\text{Amount of amino acid per test protein [mg/g]}}{\text{Amount of amino acid per protein in reference pattern [mg/g]}}$$

Determination of the ratio of total essential amino acids (TEAA) to the total amino acids (TAA), i.e. (TEAA/TAA), total sulfur amino acid (TSAA), percentage cystine in TSAA (% Cys/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 obtained for the amino acids profile while the predicted protein efficiency ratio (P–PER) was determined using one of the equations developed by Alsmeyer *et al*. **[**9**]**, i.e. P-PER = – 0.468 + 0.454 (Leu) – 0.105 (Tyr). The leucine/isoleucine ratio was calculated.

Statistical analysis. All data generated were analysed statistically **[**10**]**. Calculated for were the grand mean, and standard deviation. Subjected to F-test analysis were the amino acid composition of the raw/steeped, raw/germinated, amino acid composition differences 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 $p < 0.05$. The calculations were meant to determine the level of variation among the data obtained for raw, steeped and germinated samples and to determine if significant differences existed between raw/steeped, between raw/germinated in the amino acid composition and amino acid scores.

RESULTS AND DISCUSSION

Table 1 shows the amino acid composition of the samples. In most of the results on pairwise basis, the values for steeped samples were generally better than the values in raw and germinated samples. Specifically, levels of His, Arg, Thr, Ser, Pro, Gly, Ala, Met, Cys, Val, Phe and Tyr in steeped samples were correspondingly higher than those in raw and germinated samples although germinated sample was best in Lys, Asp, Glu, Ile and Leu. The highest amino acid (AA) was Glu with values (g/100 g crude protein, c.p.) of 6.05, 8.20 and 9.12 for raw, steeped and germinated grains, respectively.

The essential amino acid (EAA) with the highest concentration was Ile with values (g/100 g c.p.) of 4.62, 5.09 and 5.60 for raw, steeped and germinated grains, respectively. The least varied AA was Phe, which ranged from 2.16 g/100 g c.p. in raw grains to 2.60 g/100 g c.p. in germinated grains. The most widely varied AA was Ala with values (g/100 g c.p.) of 1.29, 4.39 and 2.01 for raw, steeped and germinated grains. F-test results showed the $F_{calculated}$ values between raw/steeped, between raw/germinated to be 1.29 and 1.91 at $p < 0.05$, respectively which were both lower than F_{Table} (2.24) showing them not to be significantly different. Tryptophan was not determined in all the samples. The AA values in the present report were all lower than the reported values in sorghum *ogi* (a smooth, creamy, free-flowing thin porridge obtained from wet-milled, fermented sorghum) **[**11**]**. Even though Lys contents in the samples

(1.48-2.00 g/100 g c.p.) were lower than the Lys content of the reference egg protein (6.3 g/100 g c.p.), they can be enhanced by mixing with legumes, which are high in Lys. The increase in amino acid content of steeped sorghum may be due to increase in the protease activity of enzymes which breaks down the protein to release amino acids needed for the growth.

In all cases, the AA content of both the germinated and steeped samples were individually better than the raw sample. This further shows the quality improvement of the AA in both steeped and germinated over raw sorghum grains.

However, the differences in concentrations between raw/germinated were less than between raw/steeped samples. The differences observed for both raw/steeped and raw/germinated in Leu were very low. The F-test value of differences between raw/steeped and raw/germinated was not significant at $p < 0.05$.

Table 1. Amino acid (g /100 g crude protein) composition of raw, steeped and germinated guinea corn.

Amino acid	Guinea corn			Grand mean	SD[a]
	Raw	Steeped	Germinated		
Arginine (Arg)*	3.19	4.28	3.94	3.80	0.46
Histidine (His)*	1.65	2.29	1.81	1.92	0.27
Isoleucine (Ile)*	4.62	5.09	5.60	5.10	0.40
Leucine (Leu)*	1.09	2.13	2.14	1.79	0.49
Lysine (Lys)*	1.48	1.88	2.00	1.79	0.22
Methionine (Met)*	0.62	0.80	0.70	0.71	0.07
Phenylalanine (Phe)*	2.16	2.60	2.34	2.37	0.18
Threonine (Thr)*	2.24	3.23	3.02	2.83	0.43
Tryptophan (Try)*	[b] -	-	-	-	-
Valine (Val)*	1.85	3.81	3.03	2.90	0.81
Alanine (Ala)	1.29	4.39	2.01	2.56	1.32
Aspartic acid (Asp)	3.74	4.19	5.52	4.48	0.76
Cystine (Cys)	0.89	2.06	1.72	1.56	0.49
Glutamic acid (Glu)	6.05	8.20	9.12	7.79	1.29
Glycine (Gly)	1.36	2.93	2.30	2.20	0.65
Proline (Pro)	0.99	3.29	2.19	2.16	0.94
Serine (Ser)	3.00	4.01	3.90	3.64	0.45
Tyrosine (Tyr)	1.69	2.53	2.03	2.08	0.34
Protein	5.11	8.24	4.93	6.09	1.52

*Essential amino acid. [a]Standard deviation. [b]Not determined.

Table 2 shows several quality parameters of protein in the samples. The essential amino acids (EAA) ranged between 21.48 g/100 g c.p. to 30.70 g/100 g c.p. These values were far from the value of 56.6 g/100 g c.p. of the egg reference protein [12], but close to 45.3 g/100 g c.p. for peanut meal [13] and 19.0 g/100g c.p. for *Colocynthis citrilus* [14] flours. The total sulfur AA (TSAA) of the samples were 1.51, 2.86 and 2.42 g/100 g c.p. for raw, steeped and germinated, respectively, with the value for the steeped sample being close to halve the value (5.8 g/100 g c.p.) recommended for infants [15]. The aromatic amino acid (ArAA) range suggested for ideal infant protein (6.8-11.8 g/100 g c.p.) [15] is much higher than the current report (3.85-5.13 g/100 g c.p.) indicating when sorghum should be used to prepare gruel as the weaning food, it should be supplemented with ArAA rich foods particularly when raw or germinated sorghum is used. The percentage ratio of EAA to TAA in the flours ranged between 53.1-56.7. These values were well above the 39 % considered to be adequate for ideal protein food for infants, 26 % for children and 11 % for adults [15]. The percentages of EAA/TAA for

the sorghum samples could be favourably compared with that of egg (50 %) **[7],** pigeon pea flour (43.6 %) **[**16**]**, beach pea protein isolate (43.8-44.4 %) **[**17**]**. The predicted protein efficiency ratio (P-PER) as shown in Table 2 ranged between -0.15 to 0.29. The experimentally determined PER usually ranged from 0.0 for a very poor protein to a maximum possible of just over 4 **[**18**]**. From Table 1, it could be seen that the values for Leu and Tyr (from which P-PER were calculated) were similar in steeped (2.13 and 2.53 g/100 g c.p., respectively) and in germinated (2.14 and 2.03 g/100 g c.p., respectively) unlike in the raw grains where the difference was much greater (1.09 and 1.69 g/100 g c.p., respectively). On the whole, the raw sorghum sample would be poorly utilized in the body.

Table 2. Total, essential, non-essential, neutral, acidic, basic, sulphur, aromatic amino acid (g/100 g crude protein). Protein efficiency ratio (P-PER), isoelectric point (pI), Leu/Ile ratio, Leu/Ile difference of guinea corn.

Amino acid	Guinea corn			Grand mean	S.D
	Raw	Steeped	Germinated		
TAA[a]	37.91	57.71	53.37	49.66	8.50
TNEAA[b]	16.43	27.01	25.04	22.83	4.59
TEAA[c]					
- with His	21.48	30.70	28.33	26.84	3.91
- no His	19.83	28.41	26.52	24.92	3.68
TNAA[d]	21.8	36.7	30.98	29.83	6.14
TAAA[e]	9.79	12.39	14.64	12.27	1.98
TBAA[f]	6.32	8.45	7.75	7.51	0.89
TSAA[g]	1.51	2.86	2.42	2.26	0.56
TArAA[h]	3.85	5.13	4.37	4.45	0.53
P-PER	< 0.00	0.23	0.29	_	_
Leu/Ile	0.24	0.42	0.38	0.35	0.00
Leu-Ile (diff.)	-3.53	-2.96	-3.46	3.32	0.25
pI	2.1	3.3	3.0	2.8	0.6

[a]Total amino 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.

The Leu/Ile values ranged as follows: 0.24 (raw), 0.42 (steeped) and 0.38 (germinated). In all the samples (Table 1) the level of Leu was lower than half of the level of Ile in each sorghum sample. Endemic pellagra in sorghum–eating populations was first described by Gopalan and Srikantia **[**19**]** 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 **[**1**]**. High Leu in the diet impairs tryptophan and niacin metabolism and is responsible for niacin deficiency in sorghum eaters **[**20**]** and hence, the hypothesis that excess Leu in sorghum is aetiologically related to pellagra in sorghum-eating populations **[**1**]**. The study of Krishnaswamy and Gopalan **[**21**]** 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, pellagra is not endemic in all the areas where sorghum is the main staple suggesting that factors other than excess Leu and poor Leu/Ile balance in sorghum proteins are responsible for the development of the disease. Krishnaswamy *et al.* **[**22**]** 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 have shown that animals fed sorghum proteins with less than 11 g percent (110 mg/g protein) Leu did

not suffer from nicotinic acid deficiency [23]. All the current Leu values were less than 11 g/100 g c.p. and the range was 1.09 to 2.14 g/100 g c.p. and, therefore, considered safe and could be beneficially exploited to prevent pellagra in endemic areas [24].

Table 2 shows that the % Cys in TSAA ranged between 58.9-72.0. Cys can spare with Met in improving protein quality and has positive effects on mineral absorption, particularly zinc [25]. % Cys/TSAA obtained in this study were very comparable to the value of 62.9 reported for coconut endosperm [26]. And stands a good chance in carrying out its functions effectively. The calculated isoelectric point (pI) was generally low with values ranging from 2.1 to 3.3. 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 [6]. The low pI value could be a function of the TAAA (9.79-14.64 g/100 g c.p. or 21.5-27.4 %) which were much higher than the TBAA (6.32-8.45 g/100 g c.p. or 14.5-16.7 %) (Table 2). The calculated pI values were close to the experimental pI with values of 3.0-4.0 with $pI_{calc.} - pI_{expt.} = -0.3$ (-10 %) for steeped, 1.0 (25 %) for germinated and 0.9 (30 %) for raw reported under the pH effect on protein solubility; the results showed that there were close similarities between the $pI_{calc.}$ and the $pI_{expt.}$

Table 3 contains the amino acid scores of the sorghum samples, which shows that Leu is the foremost limiting AA among all the samples. Therefore, in order to fulfil the day's needs for all the EAA in *S. bicolor* sample flours, 100/16 or 6.25 times as much raw *S. bicolor* protein; 100/30 or 3.33 times as much steeped *S. bicolor* protein and 100/31 or 3.23 times as much germinated *S. bicolor* protein would have to be eaten when it is the sole protein source in the diet.

Table 3. Essential amino acid scores of the guinea corn samples.

Amino acid	Guinea corn			Grand mean	S.D.
	Raw	Steeped	Germinated		
Ile	1.16	1.27	1.40	1.28	0.10
Leu	0.16	0.30	0.31	0.26	0.07
Lys	0.27	0.34	0.36	0.32	0.04
TSAA	0.43	0.82	0.69	0.65	0.16
Phe + Tyr	0.64	0.86	0.73	0.74	0.09
Thr	0.56	0.81	0.76	0.71	0.11
Try	-	-	-	-	-
Val	0.37	0.76	0.61	0.58	0.16
Total	0.48	0.69	0.65	0.61	0.09

CONCLUSIONS

Processing techniques in the treatment of *Sorghum bicolor* grains improved its protein quality in all ramifications. Among the 17 different amino acids determined, the highest concentrations of Arg, His, Met, Phe, Thr, Val, Ala, Cys, Gly, Pro and Tyr were present in steeped sample, this formed 70.6 % of all the AA determined while highest concentrations of Ile, Leu, Lys, Asp and Glu were present in the germinated sample, this formed 29.4 % of all the AA determined. Steeped *S. bicolor* grains would have high potentials for use in the weaning foods and formulations.

REFERENCES

1. F.A.O. *Sorghum and millets in human nutrition,* FAO Food and Nutrition Series, No. 27, Food and Agriculture Organization of the United Nations: Rome; **1995**; pp. 5-96.
2. Purseglove, J.W. *Tropical Crops: Monocotyledons*, Vol. 1, Longman Group Limited: London; **1972**; pp. 1-334.
3. Phillips, T.A. *An Agricultural Notebook, with Special Reference to Nigeria*, Longman Group Limited: London; **1977**; pp. 29-34.
4. W.H.O. *Complementary Feeding of Young Children in Africa and the Middle East*, World Health Organization: Geneva; **1999**; pp. 101-119.
5. F.A.O./W.H.O. *Protein Quality Evaluation*, Report of Joint FAO/WHO Expert Consultation, FAO Food Nutrition paper 51, FAO/WHO: Rome; **1991**; pp. 4-66.
6. Olaofe, O., Akintayo, E.T. *J. Technoscience* **2000**, 4, 49-53.
7. F.A.O./W.H.O. *Protein Quality Evaluation*, Report of Joint FAO/WHO Consultation Held in Bethesda, USA, 4-8 December 1989, FAO: Rome; **1990**; pp.1-51.
8. F.A.O./W.H.O. *Energy and Protin Requirements*, Medical Report Series No. 522, FAO/WHO: Geneva; **1973**; pp. 1-118.
9. Alsmeyer, R.H.; Cunningham, A.E.; Happich, M.L. *Food Technology* **1974**, 28, 34-38.
10. Christian, G.D. *Analytical Chemistry,* 2nd ed., John Wiley and Sons: New York; **1977**; pp. 58-77.
11. Oyarekua, M.A.; Eleyinmi, A.F. *Food, Agriculture and Environment* **2004**, 2, 94-99.
12. Paul, A.A.; Southgate, D.A.T.; Russel, J. *First Supplement to Mccance and Widdow-son's The composition of foods*, HMSO: London and Elsevier: New York; **1980**.
13. Lusas, E.W. *J. Amer. Oil Chem. Soc.* **1979**, 56, 242.
14. Akobundu, E.N.T.; Cherry J.P.; Simmon, J.G. *J. Food Sci.* **1982**, 47, 828.
15. F.A.O/W.H.O./U.N.U. *Energy and Protein Requirements,* W.H.O. Technical Series No. 724, W.H.O.: Geneva; **1985**; pp. 13 – 205.
16. Oshodi; A.A.; Olaofe, O.; Hall, G.M. *Int. J. Food Sci. Nutr.* **1993**, 43, 187.
17. Chavan, U.D.; Mckenzie, D.B.; Shahidi, F. *Food Chem.* **2001**, 74, 177.
18. Muller, H. G.; Tobin, G. *Nutrition and Food Processing*, Croom Helm: London; **1980**; pp. 65-108.
19. Gopalan, C.; Srikantia, S.G. *Lancet* **1960**, 1, 954-957.
20. Belavady, B.; Srikantia, S.G.; Gopalan, C. *Biochem. J.* **1963**, 87, 652.
21. Krishnaswamy, K.; Gopalan, C. *Lancet* **1971**, 2, 1167-1169.
22. Krishnaswamy, K.; Bapu Rao, S.; Raghuram, T.C.; Srikantia, S.C. *Am. J. Clin. Nutr.* **1976**, 29, 177.
23. Belavady, B.; Udayasekhara Rao, P. *Exp. Bio.* **1979**, 17, 659-661.
24. Deosthale, Y.G. *Nutrition dimension of high yielding and hybrid crop varieties: locational and varietal differences in nutritional value*, **1980**; in *FAO Sorghum and Millets in Human Nutrition*, FAO Food and Nutrition Series, No. 27, FAO: Rome; **1995**; p. 82.
25. Mendoza, C. *Int. J. Food Sci. Technol.* **2002**, 37, 759.
26. Adeyeye, E.I. *Oriental J. Chem.* **2004**, 20, 471.