Amino Acid Scores and Ratios as Quality Index for Some Non- Conventional Tropical Seed Proteins

[1]Ezeagu, I. E., [2]Petzke, K. J., [3]Akinsoyinu A.O. and [1]Ogbunude, P. O. J.
[1]Nutrition Unit, Department of Medical Biochemistry, University of Nigeria, Enugu Campus, Enugu, Nigeria.
[2]Department of Physiology and Biochemistry of Nutrition, German Institute of Human Nutrition, D-14558, Nuthetal, Germany.
[3]Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

Corresponding author: Ezeagu, I. E. Nutrition Unit, Department of Medical Biochemistry, University of Nigeria, Enugu Campus, Enugu, Nigeria. Email: ikezeagu@yahoo.co.uk

Abstract

The amino acid profile of 19 non-conventional tropical plant seeds showed a fair complement of indispensable amino acids (IAAs). Individual amino acid scores indicates lysine as the most limiting in most of the seeds. Lysine levels comparable to soybean (6.75 g/16gN) occurred in Lonchocarpus sericeus (6.00 g/16gN), Prosopis africana (6.03), Pericopsis eleta (6.3 g/16gN) and Senna sieberiana (6.2 g/16gN). P. africana contains particularly high levels of leucine, and sulphur-amino acids (of 101 and 666% scores respectively). Total nitrogen (TN) ranged from 0.87 in Diospyros mespiliformis to 6.96 g/100g in Gliricidia sepium. IAA/TN ratio varied between 1.22 in Albizia zygia and 2.98 in P. africana. Low IAA/TN ratios in A. zygia, P. osun and G. sepium, indicate high content of non-proteinogenic nitrogen in the seeds. In conclusion, 12 of the plant seeds, with high amino acid scores, are likely to support growth and development in animal feeding.

Keywords: Amino acid scores, Ratios, Non-conventional, Tropical, Seed, Proteins

Introduction

As food shortages persist (McMurray, 1996; FAO, 2002) and every measure is being taken to boost food production by adopting conventional agricultural methods, considerable interest is being focused on the possibilities of exploiting the vast numbers of less familiar and neglected plant resources existing in the wild and natural bushes and forests across the nation. Several reports indicate that quite a number of nonconventional and lesser-known plant resources are high in nutrients and may represent potential food sources that could relieve critical food shortages if given adequate promotion and research attention. (Balogun and Fetuga 1986; Ezeagu *et al.*, 2004). Traditional sources take up a large part of the family budget in third World countries, whether it is for human or livestock feeding.

Development of inexpensive alternative sources of protein for farm animals could measurably reduce malnutrition in reducing the cost of livestock products. This will spare more conventional food resources for human use and thus eliminate the existing competition between man and his livestock for available resources. As a result, screening efforts for possible new crops from the wild have focused more on potential sources of concentrated proteins to supplement conventional supplies. Accurate estimation of the protein quality is necessary so as to narrow down the search to species with the highest potentials as protein sources for further research and development studies (Young and Pellet, 1994).

A prime prerequisite for the nutritional adequacy of a dietary protein is presence of sufficient amounts of all the essential or indispensable amino acids (IAAs) and also by its nutritive value. The latter is affected by factors like digestibility and absorbability of food protein, the presence of toxic substances and inhibitors, damage by heat during cooking or processing, acidity and other factors. High quality proteins are more complete and richer in IAAs than low quality proteins. Also, incomplete mixtures of amino acid are less effective even at high concentration of protein. A protein that provides amino acids in roughly the proportions in which they are required by the body is termed a balanced protein and has a high biological value (BV), while a protein that is low in one or more of the IAAs is termed an unbalanced protein and has a low BV.

The Food and Agricultural Organization (FAO) and National Research Council (NRC) have over the years set a reference pattern of the minimum quantities of most of the IAAs that a protein should contain to meet dietary requirements (FAO/WHO, 1957, 1973; NAS/NRC, 1966, 1980). References used as standard for computing amino acid score of protein have included the amino acid composition of whole egg protein and/or human milk. However, it has been observed that high levels of IAA in egg proteins give relatively low amino acid scores for many food proteins when employed as the reference standard protein (Rao *et al.*, 1964). Consequently the human amino acid requirement values have now been adopted internationally as the bases for several amino acid scoring systems (FAO/WHO, 1973, FAO/WHO/UNU, 1985), with different scoring patterns adopted for separate age groups. The most recent recommended pattern is the amino acid requirement for 2-5 years pre-school age child, which also has been adopted for all adults and children excepting infants (FAO/WHO, 1991). The amino acid composition of the human milk is adopted as the reference pattern for proteins in foods for infants under one year of age.

The objective of this study therefore, was to carry out preliminary evaluations of seed proteins of some minor crop seeds from Nigeria, which will provide a framework for their optimum utilization in food or animal feed formulation.

Materials and Methods

Total nitrogen and amino acid analysis: Mature seed samples were harvested in south-west region of Nigeria and treated as previously described (Proll et al. 1996). Total nitrogen was determined by the standard micro-Kjeldahl method and amino acids were determined by triple hydrolysis of equivalents of 2mg protein of samples as previously described (Proll et al. 1996). The different amino acids recorded were presented as g/16g N protein and compared with soybean meal and the FAO/WHO/UNU (1985) preschool age (2-5 years) reference protein.

Calculation of amino acid score: The quantity of each IAA contained in a seed protein was expressed individually as percentage of the content of the corresponding amino acid in the reference protein amino acid pattern. The pre-school age (2-5yr) pattern is used as reference (FAO/WHO/ UNU 1985). The amino acid that shows the lowest percentage is called the first limiting amino acid. The amino acid score is thus defined as:

$$\frac{\text{g of amino acid per 16g N in seed protein}}{\text{of amino acid per 16g N in reference pattern}} \times \frac{100\text{g}}{1}$$

Calculation of amino acid ratios: The ratio of the sum of the IAAs, threonine, valine, isoleucine, leucine, tyrosine, phenylalanine, lysine, cysteine, methionine and tryptophan to total nitrogen (TN) was calculated. Also the ratios of the structurally similar and antagonistic amino acids, leucine and isoleucine, leucine and lysine, arginine and lysine, were calculated as quality index.

Results and Discussion

Amino acid composition of the seed meals including the FAO/WHO/UNU (1985) pre-school (2-5 yrs) age requirement as reference, are presented in Table 1.Wide variation in amino acids levels occurring in the seed proteins and the legumes did not show any characteristic pattern in terms of lysine, tryptophan or the sulphur-amino acids, methionine and cysteine contents (Millard, 1975). Glutamic and aspartic acids, both of which are dispensable amino acids (DAAs), were the most abundant amino acids and constitute 23.2 - 34.3 % of the total amino acid content of the seed proteins. This evidence correlates the earlier observation that high levels of these two amino acids seem to be a general occurrence in all seed proteins (Olaofe *et al.*, 1994; Vijayakumaria *et al.*, 1994). The contents of DAA in diets are not as important as IAA in the evaluation of protein quality since these amino acids could be synthesized *de novo* (Borman *et al.*, 1946). Total IAAs levels, ranging from 19.5 g/16gN in *Albizia zygia* to 47.72 g/16gN in *Prosopis african,* suggest that these seeds could contribute significantly to the supply of IAAs in diets. *P. africana, Daniellia ogea, Senna sieberiana and Dalbergia latifolia* compare favorably with soybean, cowpea, pigeon pea and pumpkin seeds in total IAA contents (Olaofe *et al.*, 1994; Oshodi *et al.*, 1993).

Table 2. shows the amino acid scores of the IAAs including histidine. Lysine was found to be the first limiting amino acid in *Afzelia bella, Millettia thonningii, Pterocarpus osun, Sesbania pachycarpa, Adansonia digitata* and *Diospyros mespiliformis.* However, it was only in *P. osun* (50 % score) that the deficiency seemed to be critical, assuming 60% to be a minimum score. Therefore, seed meals of *D. ogea, E. cyclocarpum, Daniellia oliverri, S. sieberiana, D. latifolia, Pericopsis eleta, Khaya senegalensis* and *Leucaena. leucocephala,* with lysine scores ranging between 94 and 109 %, could be good sources of lysine, and if all the lysine were available, would make good supplements for the bulk of low protein cereals and tubers consumed in the local diets.

Levels of cysteine in *P. africana* (7.84 g/16gN) and *E. angolensis* (9.12 g/16gN) were higher than that of soybean (2.3 g/16gN). Since methionine can be partly replaced by cysteine, these two sulphur-amino acids (S-aas) are frequently considered together in assessing the animal requirement or for scoring purposes. *P. africana* recorded the highest S-aa score (666 %) while the lowest score (35 %) occurred in *A. zygia.* By virtue of high contents of lysine and sulphur-amino acids, *P. africana, D. latifolia, L. leucocephala* , *K. senegalensis* and *A. bella* seed proteins could be good supplement to starchy tubers and cereals to yield diets of good nutritional value. Such seed proteins, rich in sulphur-amino acid, could also enrich local diets containing conventional pulses, which are limiting in methionine+cysteine. Sulphate is reported to be a limiting nutrient in soils where legumes are grown (Goldsworthy and Heathcote, 1964). Since legumes grown in sulphate deficient soils have lowered levels of cysteine, methionine and tryptophan (Blagrove *et al.*, 1976), it is possible that the low levels of these amino acids observed in most of these seeds may be environmental factor rather the genetic status of the plants.

Except in *A. zygia*, all the plant seeds recorded fairly adequate scores (71 - 168 %) for leucine and isoleucine. Adequacy of both amino acids seems to be a general occurrence in most cereals and pulses and tubers as well (Oyenuga, 1968; Apata and Ologhobo, 1994). However, in most of the seeds leucine levels are higher than that of isoleucine, giving a simple ratio of approximately 2:1, the significance of which is discussed below.

The levels of the aromatic amino acids, phenylalanine and tyrosine, ranged from 2.19 - 4.91 and 2.10 - 4.90 g/16gN respectively. Since tyrosine can partly replace phenylalanine, both acids are usually considered together for scoring purposes. The pre-school age requirement for the combined amino acids is satisfied in all the seeds, except in *A. zygia* in which it is the second limiting amino acid with a marginal score of 68%.

Table 1: Amino acid score[1] of indispensable amino acids including histidine (%)

Species	Thr	Val	Ileu	Leu	Tyr+Phe	Lys	Cys+Met	Try	His
Leguminosaea:									
Afzelia bella	117	147	159	114	142	87	136	135	116
Albizia zygia	51	68	75	59	68	60	35	67	72
Daniellia ogea	125	136	152	102	128	94	211	119	90
Enterolobium cyclocarpum	96	121	133	118	119	96	54	95	123
Gliricidia sepium	65	94	104	87	94	68	84	96	109
Lonchocarpus sericeus	95	108	116	107	130	103	82	85	98
Millettia thonningii	104	122	125	113	131	91	104	125	99
Pterocarpus osun	88	92	90	71	101	50	98	104	68
Prosopis africana	63	209	86	101	89	104	666	80	91
Sesbania pachycarpa	80	98	111	82	108	72	124	276	120
Dalbergia latifolia	120	137	175	137	165	103	128	127	253
Daniellia oliverri	129	126	171	108	98	95	120	127	242
Leucena leucocephala	118	117	143	118	156	100	152	100	247
Pericopsis eleta	135	126	157	123	162	109	140	100	268
Senna sieberiana	132	143	161	135	165	107	96	136	263
Non-leguminosaea:									
Adonsonia digitata	86	143	126	99	115	64	127	125	104
Khaya senegalense	124	114	164	107	152	103	136	118	263
Diospyros mespiliformis	109	131	130	97	110	83	169	134	106
Entandrophragma angolensis	52	123	154	93	101	78	390	109	138
Glycine max	121	145	168	123	152	116	136	145	143

[1]Compared to 2-5 yr pre-school age requirements;Thr, Threonine; Val, Valine; Ile, Isoleucine; Leu, Leucine; Tyr, Tyrosine; Phy, Phenylalanine; Lys, Lysine; Cys, Cysteine; Meth, Methionine; Try, Tryptophan . His, Histidine.; Underlined figures represent the most limiting amino acid/amino acid score

Table 2: Amino acid[1] ratios as quality index for seed proteins

Species:	Total Nitrogen[2]	Leu/Ileu	Leu/Lys	Arg/Lys	IAA/TN[3] (g IAA/gN)
Leguminosaea:					
Afzelia bella	2.09	1.69	1.49	1.39	2.50
Albizia zygia	5.29	1.83	1.11	0.79	1.22
Daniellia ogea	2.16	1.58	1.23	0.83	2.50
Enterolobium cyclocarpum	3.53	2.11	1.41	1.10	2.16
Gliricidia sepium	6.96	1.97	1.45	2.03	1.70
Lonchocrpus sericeus	4.48	2.16	1.18	0.90	2.16
Millettia thonningii	3.60	2.13	1.42	1.91	2.27
Pterocarpus osun	4.56	1.85	1.16	1.40	1.64
Prosopis africana	3.60	2.76	1.11	1.64	2.98
Sesbania pachycarpa	5.27	1.75	1.30	1.79	1.99
Dalbergia latifolia	3.37	1.32	1.17	2.66	1.61
Daniellia oliverri	1.80	1.35	1.02	2.26	1.35
Leucena. leucocephala	4.06	1.78	0.93	2.48	1.78
Pericopsis eleta	4.06	1.17	1.10	2.66	1.68
Senna sieberiana	2.15	1.31	1.16	2.67	1.80
Non-leguminosae:					
Adansonia digitata	2.80	1.85	1.75	2.52	2.10
Khaya senegalense	2.08	1.39	1.17	2.47	1.39
Diospyros mespiliformis	0.87	1.75	1.32	1.66	2.24
Etandrophragma angolensis	1.97	1.43	1.36	1.88	2.40
Glycine max	6.19	1.73	1.20	1.18	2.71

[1]Leu, Leucine; Ileu, Isoleucine; Lys, Lysine; Arg, Arginine; [2]g/100g fresh weight bases; [3]Ratio of sum of indispensable amino acids (IAAs) / total nitrogen (TN)

Even though valine scores in *A. zygia, P. osun, G. sepium and S. pachycarpa* were less than 100% but satisfactory. Adequate valine level is reported common in most plant seed proteins (Nordeide *et al.*, 1994; VanEtten *et al.* 1967).

Seeds of *A. bella*, *D. ogea*, *M. thonningii* and *D. mespiliformis* recorded maximum threonine scores which are comparable to soybean (121 %). Threonine was the first limiting amino acid in *G. sepium*, *P. africana* and *E. angolensis* seed proteins, with marginal scores of 65, 63 and 52 % respectively, and also the second limiting amino acid in *A. zygia* (51 %). Threonine has been reported to be slightly limiting in some leguminous seed proteins (Mohan and Janardhanan, 1994; Evans and Bandemer, 1967). Except *A. zygia*, all the seed proteins appear to be quite adequate in tryptophan. Tryptophan levels in most of the plant seeds are in excess of the recommended level. Therefore, these minor plant seeds especially *A. bella* and *D. mespiliformis*, may serve as supplement to cereal-based diets in which tryptophan is generally the second limiting amino acid (Jansen, 1977).

All the plant seeds seemed to be adequate in histidine and were similar in histidine levels to those reported by Evans and Bandemer (1967) and the standard (2.4 g/16gN) given by Rose (1937) and also the rat requirement of 2.5g/16gN (FAO/WHO/UNU, 1985). Histidine is observed to be inexplicably too high in some seeds. It must be noted that histidine is not an IAA in adult man but it is indispensable in the early period of growth (FAO/WHO/UNU, 1985).

Table 3 shows the total N, IAA/TN ratios and certain antagonistic amino acid ratios. The total N was highest in *G. sepium* (6.96 g/100g) and lowest in *D. mespiliformis* (0.87 g/100g). IAA/TN ranged between 1.22 in *A. zygia* and 2.98 in *P. africana*. The IAA/TN ratios in *P. africana*, *S. sieberiana* (2.67), *D. latifolia* (2.66) are comparable to that of *G. max* (2.71). Low IAA/TN ratio in some of the seeds is an indication of high non-proteinogenic nitrogen contents of the seeds and may indicate IAA deficiency. A minimum ratio of 2.25 has been recommended by FAO/WHO (1973). IAA/TN ratios for *A. bella*, *D. ogea*, *M. thonningii, P. africana, S. sieberiana, D. latifolia, P. eleta, K. senegalensis, L. leucocephela, D. oliverri* and *Etandraphragma angolensis* were higher than or equal to 2.25. The main nitrogenous reserves of seeds are proteins and in certain species, various non-protein amino acids. Amino acids of non-protein origin such as α- β-diamino-propionic acid or canavanine and 3,4-diphenylhydroxlamine (L-DOPA) were found in significant amounts in several seeds of leguminous family (VanEtten *et al.*, 1967; Mohan and Janardhanan 1994, Ezeagu *et al.*, 2003). Other nitrogenous compounds such as nitrates, purines, pyrimidines, alkaloids, B-vitamins and nucleic acids, nitrogenous lipids and glycosides could add up to the level of non-protein N. (McDonald *et al.* 1981).

The Leu/Ile, Leu/Lys and Arg/Lys ratios illustrate the extent of amino acid imbalance and/or antagonism, which could affect the utilization of seed proteins. Excess of one amino acid usually depresses the utilization of a structurally similar amino acid. Leu/Ile ratio varied between 1.35 in *D. oliverri* and 2.76 *in P. africana*. Excess of dietary leucine was found to retard growth in rats fed low protein diets deficient in isoleucine (Harper et al., 1955). Thus high Leu/Ile ratios as found in *P. africana, M. thonningii* (2.13), *L. sericeus* (2.16) and *E. cyclocarpum* (2.11) may affect the quality of the seed proteins. On the other hand, when leucine is limiting, isoleucine and valine were shown to inhibit growth and vice versa (Brickson *et al.*, 1948). Arg/Lys ratio ranged between 0.79 in *A. zygia* and 2.52 in *A. digitata*. Ganapathy and Chitre (1970) have reported that excessive arginine in relation to lysine is a factor contributing to the poor utilization of certain plant protein foodstuffs like millet (with Arg/Lys ratio 2.0). Arg/Lys ratios for *G.* sepium (2.03), *M. thonningii* (1.91), *S. pachycarpa* (1.79), *E. angolensis* (1.88) and A. digitata (2.52) compared closely to that reported for millet (2.0), rice (2.7), wheat (1.2) and corn (1.7) (Evans and Bandermer, 1967) but, however, are lower than that of *G. max* (1.18). Seed proteins of *A. digitata, G. sepium, M. thonningii, S. pachycarpa*, *P. africana, E. angolensis and D. mespiliformis,* with Arg/Lys ratios higher than 1.8 may suffer poor protein digestibility.

Food intake and growth of rats consuming a diet in which there is an imbalance of amino acids are depressed below values for controls fed a diet with balanced pattern of amino acids (Leung and Rogers, 1975). It is also thought that Arg/Lys ratio in seed proteins is relevant to the plasma cholesterol lowering effect of plant proteins (Nagata *et al.*, 1980). Balogun *et al.* (1982) observed that rats fed diets with low Arg/Lys ratios tend to be less hypocholesterolemic than diets having higher ratios. It may therefore be possible to rank the possible hypocholestrolemic effects of these seed protein sources based on their Arg/Lys ratios.

Ranking of the seed proteins according to chemical scores, as underlined in Table 2, shows those of *D. ogea, P. africana, A. bella, M. thonningii, S.* pachycarpa, *D. mespiliformis, S. sieberiana, D. latifolia, P. eleta, K. senegalensis, L. leucocephela* and *D. oliverri* to be the most promising in terms of amino acid profile, since a score of 70% or more will be assumed to be satisfactory for growth and maintenance (FAO/WHO, 1957). This study therefore suggests that these minor seeds are promising potential sources of low cost protein for possible use in cattle or poultry feeds as supplements to scarce conventional sources.

It must, however, be noted that the calculation of chemical scores was based only on the first limiting amino acids. All other limiting amino acids and factors of digestibility, availability of amino acids, presence of antinutritional factors were not considered in this preliminary evaluation. Chemical scoring systems as a means of predicting protein quality have been criticized on these grounds. Consequently FAO/WHO (1989) have proposed, for scoring purposes, the so-called protein digestibility-corrected amino acid score (PDCAAS) (Sarwar and McDonough, 1990). It will therefore be necessary to investigate further issues of protein digestibility and amino acid availability before nutritional recommendation could be made on these minor crop seeds. It may well be that these sources of protein will provide large quantities of animal feed in the future.

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