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A Comparative Study on the Chemical and Amino Acid Composition of Some Nigerian Under-Utilized Legume Flours

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Abstract: A comparative study was carried out on chemical and amino acid composition of six Nigerian under-utilized legume (two varieties of bambara groundnut, kersting's groundnut, two varieties of rear cowpea and cranberry beans) flours. The crude protein values ranged from 7.5g/100g in moderate brown coat cowpea to 51.5g/100g in cranberry beans. The ether extract, crude fibre, ash, moisture and carbohydrate value ranged form 3.1 – 6.9, 2.4 – 4.4, 3.2 – 4.6, 0.4 – 3.0 and 31.5 – 82.9g/100g respectively. All the studied legume flours were found to be good sources of essential minerals such as K, Mg, Ca, P, Na, Fe but Cu, Mn and Zn contents were low while Ni, Co, Pb, Cr and Hg were not detected. The amino acid analysis revealed that the legume were superior with respect to Arg, Leu and Phe when comparing the essential amino acids in the studied samples with the recommended FAO/WHO provisional pattern however supplementation may be required in Ile, Lys and Val.

Key words: comparative study, chemical, amino acid, under-utilized legumes

Introduction

Bambara groundnut (vigna subterranean), kersting's groundnut (kerstingiella geocarpa), cowpea (vigna unguiculata) and cranberry bean (phaseolus coccineus) are four under-utilized legumes grown in the Middle belt and Northern part of Nigeria. Bambara groundnut was once said to be the third most important grain legume after groundnut and cowpea in sub-Saharan Africa. It appears to have evolved in the savannas probably in West African and its compact habit seems to be an adoption to growing in hot, windy environment (Doku and Karikari, 1971; Duke 1981), Different varieties of bambara groundnut are grown in these parts of the country. Cowpea varieties that were studied and later identified are very rear. Kersting's groundnut is an annual herb, which grows on sandy loam soils in Savannah areas of West Africa from Senegal to Nigeria. It produces fruits which is a pod containing 1-3 brownish seeds with a helium and a relatively thick testa similar to that of groundnut (Tindall, 1986). The cranberry bean gets the name from the mottled cranberry red and ivory pinto markings and is related to the tongues of fire beans (Kaplan and Kaplan, 1988). Like other members of the Fabaceae, it possesses climbing stems. Its pods are gathered and left to dry in the sun before being beaten and the seeds are stored in sacks. They are cultivated by peasant farmers for home consumption in the Middle belt of Nigeria. The aesthetic value of the seeds has enabled them to be used in recreation activities.

Legumes are important major sources of plant protein and fats in tropical countries. They are good sources of essential amino acids and fats. The industrial application of them depends on the knowledge of nutritional importance and functional properties. Many workers (Oshodi and Ekperigin, 1989; Olaofe *et al.*, 1993; Ige *et al.*, 1984; Aletor and Aladetimi, 1989; Onwuliri and Obu, 2002; Fagbemi and Oshodi, 1991; Adeyeye *et al.*, 1999; Akintayo *et al.*, 1998; Siddhraju *et al.*, 2001; Adebowale and Lawal, 2004) have reported the compositional evaluation, functional properties, amino acids and protein solubility of legume flours. The present study aims at drawing attention to the nutritive value of these under-utilized legume flours and taking comparative study on them with a view to providing useful information towards effective utilization of these legumes in various food application.

Materials and Methods

Collection and preparation of samples: Bambara groundnut seeds, kersting's groundnut, cowpea and cranberry beans were collected from the farms located at Keffi and Lafia in Nasarawa State, Nigeria. Two different colour varieties of cream coat (BBG^1) and dark red coat (BBG^2) of bambara groundnut, also two different colour varieties of moderate brown coat ($cowpea^1$) and small white coat ($cowpea^2$) of cowpea, brown coat kersting's groundnut (KSG) and cranberry beans (CBB) were all identified, sorted and screamed to remove the bad seeds. Their corresponding dehulled samples were then processed for analysis. The dehulled seeds were dry-milled into fine flours. The removal of the hull and the preparation of the dehulled samples were done according to the method of Oshodi and Ekperigin, (1989).

Table 1: Proximate Composition (g/100g) of the Legume Flours

PARAMETER	BBG[1]	BBG[2]	KSG	COWPEA[1]	COWPEA[2]	CBB
Moisture	2.1 (0.01)	3.0 (0.02)	1.7 (0.12)	0.4 (0.10)	1.8 (0.25)	1.7 (0.51)
Ash	4.3 (0.13)	3.9 (0.43)	3.2 (0.05)	3.7 (0.23)	3.6 (0.02)	4.6 (0.32)
Etherextract	6.7 (0.30)	4.1 (0.10)	5.9 (0.30)	3.1 (0.04)	2.1 (0.03)	6.9 (0.35)
Crude fibre	2.1 (0.05)	4.1 (0.01)	3.1 (0.10)	2.4 (0.20)	3.2 (0.10)	4.4 (0.07)
Crude protein	11.6 (0.24)	11.1 (0.01)	12.9 (0.43)	7.5 (0.23)	12.1 (0.08)	51.1 (0.02)
Cabohydrate (by difference)	73.3 (0.20)	73.9 (0.15)	73.3 (0.12)	82.9 (0.16)	77.2 (0.12)	31.5 (0.30)
Fatty acids[a]	5.36	3.28	4.71	2.47	1.66	5.53
Energy[b] (KJ/100g)	1691.26	1595.34	1692.85	1651.93	1595.72	1659.65

Numbers in parentheses are standard deviations of triplicate determination. [a]Calculated fatty acids. [b]Calculated metabolisable energy (KJ100g) (protein x 17+fat x37+Cabohydrate x 17). BB[1] cream coat bambara groundnut. BBG[2] dark red coat bambara groundnut.
KSG Kersting groundnut. Cowpea[1] moderate brown coat cowpea. Cowpea[2] small white coat cowpea. CBB Cranberry beans

Proximate analysis: The proximate analyses of the samples for moisture, total ash and crude fibre were carried out in triplicate using the methods described in AOAC, (1990). The nitrogen was determined by the micro kjedahl method described by Pearson, (1976) and the nitrogen content was converted to protein by multiplying by 6.25. Carbohydrate was determined by difference. All the proximate values were reported in g/100g sample.

Mineral analysis: Sodium and potassium were determined using a flame photometer (Corning, UK model 403) using NaCl and KCl to prepare the standards. Phosphorus was determined colorimetrically using spectronic 20 (Gallenkamp, UK) as described by Pearson, (1976) with KH_2PO_4 as the standard. All other minerals were determined using atomic absorption spectrophotometer (Perkin – Elmer model 403, walk CT, USA). All determinations were done in duplicate. All chemicals used were of analytical grade (BDH, London). All the minerals were reported in mg/100g sample.

Amino acid analysis: Amino acid profiles were determined using the ion exchange chromatography (IEC). The samples were defatted, hydrolysed and evaporated in a rotatory evaporator and then injected into the technicon sequential multi-sample Amino Acid Analyzer. The full experimental details have been reported by Adeyeye and Afolabi (2004).

Results and Discussion

Table 1 presents the proximate composition of the legume flours. The moisture content which ranged between 0.4 – 3.0g/100g sample was generally low but is within the range expected of most legumes (Olaofe and Sanni, 1988 and Oyenuga, 1968). The low moisture content will afford a long shelf-life for the legume flours. The crude fat (ether extract) was generally low ranging from 2.1 to 6.9g/100g sample. None of these legume is qualified as oil-rich legume when compared with soya bean (22.8 23.5%) as reported by Salunkhe *et al.* (1985); and Paul and Southgate, (1980), pumpkin seed (49.2 and 47.0%) by Aisegbu (1987) and Fagbemi and Oshodi (1991) and C. Vulgaris (47.9 – 51.1%) by Ige *et al.* (1984). Cranberry beans had an exceptional high crude protein content of 51.g/100g sample among the studied legumes. This value is high compared with crude protein-rich foods such as soyabeans, groundnut and pigean peas (Olaofe *et al.*, 1993, Conkerten and Ory, 1976 and Oshodi and Ekperigin, 1989). The observed low values in BBG varieties, KSG and cowpea varieties in this study may be due to the genotype and the environmental conditions under which they were grown (Salunkhe *et al.*, 1985).

The legume flours had ash content (3.2 – 4.6g/100g), crude fibre (2.1- 4.4g/100g) and carbohydrate (31.5 – 82.9g/100), these values are comparable with some reported works in the literature (Poulter, 1981, Kay, 1979, Mtanga and Sugiyama, 1974). The calculated metabolisable energy values which ranged between 1595.34 – 1692.85 KJ/100g showed that all the studied legumes have energy concentrations favourable comparable to cereals.

The mineral composition in mg/100g of the legume flours are presented in Table 2. The abundant minerals in the studied samples were magnesium and calcium with values ranging from 54.6 – 67.4mg/100g and 59.4 – 66.5mg/100g respectively. These followed by potassium (33.5 – 43.5mg/100g) and phosporus (13.0 – 45.0mg/100g). It has been reported that calcium in conjunction with phosporus, magnesium, manganese, vitamin A, C and D, chlorine and protein are all involved in bone formation (Fleck, 1976). Sodium ranged between 6.5mg/100g in cowpea[2] to 13.4mg/100g in KSG. Phosphorus content was too high in CBB compared with other legumes among the studied samples. Manganese, zinc and copper content were generally low while nickel, cobalt, lead, chromium and

Table 2: Amino acid composition (mg/100g) of the legume floors

Mineral	BBG[1]	BBG[2]	KSG	COWPEA[1]	COWPEA[2]	CBB
Iron (Fe)	5.9	4.7	5.5	5.5	6.7	10.3
Copper (Cu)	0.1	0.1	0.2	0.3	0.3	0.3
Nickel (Ni)	ND	ND	ND	ND	ND	ND
Manganese (Mn)	1.8	2.3	1.3	1.2	1.2	1.0
Zinc (Zn)	5.3	3.9	6.5	5.7	6.0	7.3
Cobalt (Co)	ND	ND	ND	ND	ND	ND
Sodium (Na)	7.4	10.6	13.4	6.9	6.5	8.4
Potassium (K)	33.5	42.7	43.5	35.7	40.4	37.7
Magnesium (Mg)	57.3	58.0	62.4	67.7	54.6	64.4
Lead (Pb)	ND	ND	ND	ND	ND	ND
Calcium (Ca)	60.2	63.8	66.5	59.4	66.4	59.5
Phosphorus (P)	32.5	26.5	34.0	8.3	13.0	95.0
Chromium (Cr)	ND	ND	ND	ND	ND	ND
Mercury (Hg)	ND	ND	ND	ND	ND	ND
Na/K	0.22	0.25	0.31	0.19	0.16	0.22
Ca/P	1.85	2.41	1.96	7.16	5.11	0.63

ND = Not Detected. Na/K = Sodium to Potassium ratio. Ca/P = Calcium to Phosphorus

Table 3: Amino Acid Composition (g/100g Crude Protein) of the Legume Flours

Amino acid	BBG[1]	BBG[2]	KSG	COWPEA[1]	COWPEA[2]	CBB
Lysine (Lys)[a]	3.0	2.9	3.0	2.8	2.9	3.1
Hisidine (His)[a]	2.2	2.0	2.3	2.0	2.1	2.0
Arginine (Arg)[a]	4.0	3.6	4.1	3.5	3.8	3.9
Aspartic acid (Asp)	5.0	4.9	5.0	5.1	4.8	4.8
Threonine (Thre)[a]	2.5	2.4	2.5	2.3	2.3	3.2
Serine (Ser)	3.2	2.7	3.2	2.4	2.7	3.1
Glutamic acid (Glu)	16.5	18.5	19.5	15.8	14.2	13.3
Proline (Pro)	3.2	4.0	3.1	3.7	3.9	3.0
Glycine (Gly)	3.3	3.1	3.3	3.0	3.2	2.9
Alanine (Ala)	3.5	3.1	3.5	2.9	3.2	3.4
Cystine (Cys)	0.5	0.3	0.6	0.4	0.4	0.3
Valine (Val)[a]	3.8	3.2	3.9	3.1	3.1	3.3
Methionine (Met)	2.0	1.8	2.0	1.7	1.7	1.9
Isoleucine (Ile)[a]	3.8	3.1	3.8	3.1	3.0	3.8
Leucine (Leu)[a]	6.8	5.9	6.8	5.9	5.7	6.6
Tyrosine (Tyr)	3.2	3.2	3.3	2.6	3.0	3.3
Phenylalanine (Phe)[a]	4.3	3.9	4.2	3.7	3.9	4.0
Calculated Isoelectric Point (pl)	3.95	3.74	4.06	5.49	5.60	5.68

[a]Essential amino acid

mercury were not detected in any of the samples. The observable similar pattern of mineral distribution in all the legume flours is in agreement with reports that mineral content of agricultural products vary with geographical location (Olaofe *et al.*, 1987 and John, 1992).

The ratios of sodium to potassium (Na/K) and calcium to phosphorus (Ca/P) are also shown in Table 2. The Na/K ratio in the body is of great concern for prevention of high blood pressure. Na/K ratio less than one is recommended. Hence, most of these samples would probably reduce high blood pressure disease because they had Na/K less than one. Modern diets which are rich in animal proteins and phosphorus may promote the loss of calcium in the urine (Shills and Young, 1988). This has led to the concept of the Ca/P ratio. If the Ca/P ratio is low (low calcium, high phosphorus intake) more than the normal amount of calcium may be loss in the urine, decreasing the calcium level in bones. Food is considered "good" if the ratio is above one and "poor" if the ratio is less than 0.5 (Nieman et al, 1992). The Ca/P ratio in the present study ranged between 0.63 in CBB to 7.16 in cowpea[1] indicating they would serve as good sources of minerals for bone formation.

Table 3 depicts the amino acid composition of the legume flours. It is observed that glutamic and aspartic acids are the most abundant amino acids in all the food samples and make up to values which ranged from

Table 4: Analysis of Essential and Non-essential Amino acids (g/100 crude protein) of the Legume Flours

Analysis of amino acid	BBG[1]	BBG[2]	KSG	COWPEA[1]	COWPEA[2]	CBB
Total Amino Acid (TAA)	70.8	68.5	74.2	64.0	63.9	65.9
Total Non-Essential Amino Acid (TNAA)	38.5	40.1	41.4	36.1	35.4	34.1
% TNAA	54.2	58.5	55.8	55.5	55.5	51.7
Total EssentialAmino Acid With His (TEAA)	32.5	28.9	32.7	28.1	28.4	31.8
Without His	30.2	26.9	30.5	26.1	26.4	29.8
%TEAA with His	45.9	42.2	44.1	43.8	44.6	48.3
Without His	42.7	39.3	41.1	40.7	41.3	45.3
Essential alphatic Amino Acid (EAAA)	17.0	14.6	17.0	14.4	14.2	16.9
Essential Aromatic Amino Acid (EArAA)	4.3	3.9	4.4	3.7	3.9	4.0
Total Neutral Amino Acid (TNAA)	42.3	38.7	42.6	36.9	38.8	40.9
%TNAA	59.7	56.5	57.4	57.6	60.8	62.0
Total Acid Amino Acid (TAAA)	21.5	23.3	24.5	21.0	19.1	18.1
%TAAA	30.4	34.0	33.0	32.7	29.7	27.4
Total Basic Amino Acid (TBAA)	9.2	8.5	9.4	8.3	8.7	9.0
%TBAA	13.0	12.4	12.7	12.9	13.7	13.6
Total Sulphur Amino Acid (TSAA)	2.5	12.1	2.6	2.1	2.1	2.3
% Cys in TSAA	20.0	14.3	23.1	19.0	18.9	14.4

Table 5: Recommended FAO/WHO Essential Amino Acids Provisional Pattern

EAA	FAO*	BBG[1]	BBG[2]	KSG	COWPEA[1]	COWPEA[2]	CBB
Arg	2.0	4.0	3.6	4.1	3.5	3.8	3.9
His	2.4	2.2	2.0	2.3	2.0	2.1	2.0
Ile	4.2	3.8	3.1	3.8	3.1	3.0	3.8
Leu	4.8	6.8	5.9	6.8	5.9	5.7	6.6
Lys	4.2	3.0	2.9	3.0	2.8	2.9	3.1
Met	2.2	2.0	1.8	2.0	1.7	1.7	1.9
Phe	2.8	4.3	3.9	4.2	3.7	3.9	4.0
Thre	2.6	2.5	2.4	2.5	2.3	2.3	3.2
Try	1.4	nd	nd	nd	nd	nd	nd
Val	4.2	3.8	3.2	39	3.1	3.1	3.3

EAA = Essential amino acid. nd = not determined. *Source: Belschant *et at*. (1975)

18.1/100g protein in CBB to 24.5g/100g protein in KSG. Similar observation has been reported by Olaofe and Akintayo (2000); and Adeyeye (2004). Leucine was the most concentrated essential amino acid (9.6%) which was found in BBG[1]. Also BBG[1] had the highest amount of methionine (2.84%) which is even greater than that of soybeans (Temple and Aliyu, 1994). Tryptophan was not determined. The predicted isoelectric point (pI) ranged from 3.74 to 5.68. This 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).

Amino acid further analyses are also presented in Table 4. The total essential amino acid (TEAA) ranged from 42.1% in BBG[2] to 48.3% in CBB and 39.3% in BBG[2] to 45.3% in CBB with and without histidine respectively. These are comparable with values obtained from selected oil seeds (melon, pumpkin and gourd seeds) which ranged between 33.3% and 53.6% (Olaofe et al, 1993 and Ige *et al*., 1984). Essential alphatic amino acid (EAAA) was highly concentrated in BBG[1] and KSG with equal value of 17.0g/100g protein while the highest value of essential aromatic amino acid (EArAA) was also found in BBG[1] (4.3g/100g protein). The range of percent total neutral, acidic and basic amino acids were 56.5-62.0%, 27.4-34.0% and 12.4-13.7 respectively, indicating that the protein is probably acidic in nature. The amino acid profiles of the studied legume suggest that their protein have moderate nutritive value.

When comparing the essential amino acids in these legume flours with the recommended FAO/WHO provisional pattern, the legumes were superior with respect to arginine, leucine and phenylalanine while they were adequate in histidine, methionine and threonine. It was only for isoleucine, lysine and valine that supplementation may be required (Table 5).

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References

Adebowale and Lawal, 2004. Comparative study of the functional properties of bambara groundnut, jack bean and mucuna bean flours, Food Res. Int., 27: 355- 365.

Adeyeye, E.I., 2004. The Chemical composition of liquid and solid endosperm of ripe coconut. Oriental J. Chem., 20: 471-478.

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

Adeyeye, E.I., A.A. Oshodi and K.O. Ipinmoroti, 1999. Fatty acid composition of six varieties of dehulled African yam bean flour. Int. J. Food Sci. Nutr., 50: 357-365.

Aisegbu, J.E., 1987. Some biochemical evaluation of fluted pumpkin seed. J. Sci. Food Agri., 40: 151-155.

Akintayo, E.T., K.O. Esuoso and A.A. Oshodi, 1998: Emulsifying properties of some legume proteins. Int. J. Food Sci. Tech., 33: 239-246.

Aletor, V.A. and O.O. Aladetimi, 1989: Compositional evaluation of some cowpea varieties and some under-utilised edible legumes in Nigeria, Nahrung, 33: 999- 1007.

AOAC, 1990. Official methods of analysis, 15th eds. Asso. Of Official Anal. Chemists. Washington D. C.

Belschant, A.A., C.K. Lyon and G.O. Kohler, 1975. In "Food protein sources" (N. W. Pirie ed.) Cambridge University press. Cambridge, UK.

Conkerten, E.J. and R.L. Ory, 1976. Peanut proteins as food supplements: A compositional study of selected Virginia and Spanish peanuts. J. Am. Oil Chem. Sec., 54: 754-6.

Doku, E.V. and S.K. Karikari, 1971. Bambara groundnut. Economic Botany, 25: 3.

Duke, J.A., 1981. Hand book of legumes of World Economic importance, Plenum Press, New York.

Fagbemi, T.N. and A.A. Oshodi, 1991. Chemical composition and functional properties of full fat fluted pumpkin seed flour. Nig. Food J., 9: 26-32.

Fleck, H., 1976. Introduction to Nutritions. PP. 207 – 219, 3rd edition, Macmillan, New York, USA.

Ige, M.M., A.O. Ogunsua and O.L. Oke, 1984: Functional properties of the proteins of some Nigeria oil seeds casophor seeds and three varieties of melon seeds. J. Agri. Food Chem.

John, M.K., 1992. Up take of soil applied. Cadmium and its distribution in Redisties, Can. J. Plant Sci., 52: 715-719.

Kaplan, L. and L. Kaplan, 1988. Phaseolus in archaelogy in P. Gept ed. Genetic resources of Phaseolus beans P. 125-142. Dordrecht. The Netherlands Kluwer Academic.

Kay, D.E., 1979. Food legumes. TPI. Crop Digest No. 3, Trop. Products Institute, London.

Mtanga, L.A. and T.A. Sugiyama, 1974: Note of the amino acid compositions of some legume seeds grown in Tanzania, East Africa. Agri and For. J., 39: 307-310.

Nieman, D.C., D.E. Butterworth and C.N. Nieman, 1992. Nutrition, pp: 237-312, WmC. Brown Publishers. Dubugye, USA.

Olaofe, O. and E.T. Akintayo, 2000. Production of isoelectric points of legume and oil seed proteins from their amino acid composition. The J. Technoscience, 4: 49-53.

Olaofe, O. and C.O. Sanni, 1988. Mineral content of Agricultural products. Food Chem., 30: 73-77.

Olaofe, O., E.O. Oladeji and O.J. Ayodeji, 1987. Metal contents of some cocoa beans produced in Ondo State, Nigeria, J. Sci. Food and Agri., 41: 241-244.

Olaofe, O., Y.O. Umar and G.O. Adediran, 1993. The effect of nematicides on the nutritive value and functional properties of cowpea seeds. Food Chem. 46, 337-342.

Onwuliri, V.A. and J.A. Obu, 2002. Lipids and other constituents of vigna unguiculata and phaseolus vulgaris grown in Northern Nigeria. Food Chem., 78: 1-7.

Oshodi, A.A. and M.M. Ekperigin, 1989. Functional properties of pigeon pea (cajanus cajan) flour. Food Chem., 34: 1-5.

Oyenuga, V.A., 1968. Nigeria's foods and feeding stuff, 3rd edition, Ibadan University press.

Paul, A.A. and D.A.T. Southgate, 1980. The composition of foods. McCance and Widdowson's London. HMSD. Royal Society of Chemistry.

Pearson, D., 1976. Chemical analysis of foods (7th edu.) London: Church Hill Livingstone.

Poulter, N.H., 1981. Properties of some protein fractions from Bambara groundnut. J. Sci. Food Agri., 32: 44-50.

Salunkhe, D.K., S.S. Kadon and J.K. Charan, 1985. CRC Postharvest Biotechnology of food legumes. Bocaranton, F. L. CRC press.

Shills, M.E.G. and V.R. Young, 1988. Modern nutrition in health and disease. In Nutrition, D. C. Neiman, D. E. Buthepodorth and C. N. Nieman (eds), PP: 276-282. WmC. Brown Publishers Dubugue, USA.

Siddhraju, P., K. Berker and H.P.S. Makkar, 2001. Chemical composition, protein fraction, essential amino acid potential and antimetabolic constituents of an uncommon legume, Gila bean seed kernel, 82.

Temple, V.J. and R. Aliyu, 1994. Proximate composition of the cream coloured decorticated seeds of bambara groundnut (Ibandzeia subterranean). Biosci. Res. Comm., 6: 51-54.

Tindall, H.D., 1986. Vegetables in the tropics. Macmillan Edu. Ltd. Hampshire, 267-278.