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Amino and fatty acids composition of African yam bean (*Sphenostylis stenocarpa*) flour

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The amino acids and the fatty acids of African yam bean (*Sphenostylis stenocarpa*) flour were analysed. Amino acid analysis showed that the protein contained nutritionally useful quantities of most of the essential amino acids including sulphur-containing amino acids. The total essential amino acids amounted to an average of 55·0%. Both the total amino acids and the total essential amino acids showed significant difference among the six cultivars analysed. Stearic and linoleic acids were the most predominant fatty acids with average values of 34·4 and 29·4%, respectively. Capric, lauric, palmitoleic, oleic and erucic acids were present in small quantities and no significant differences were observed in the fatty acid compositions among the African yam bean cultivars.

INTRODUCTION

The African yam bean (AYB) is one of the under-utilised legumes in Nigeria (Aletor & Aladetimi, 1989). It is a source of seeds and tubers. Areas of cultivation include West Africa and parts of equatorial Africa (Tindall, 1986). AYB seeds can be brown, white, speckled or marbled with a hilum having a dark-brown border. The seeds form a valuable and prominent source of plant proteins in the diet of Nigerians and are cultivated as a pulse for human consumption.

AYB and lima bean (*Phaseolus lunatus*) have both been cited as legumes having exceptional potential for adaption to lowland tropical conditions and as potentially important food legumes (Rachie, 1972) and this has attracted research interest in recent times. The chemical composition of the AYB has been evaluated by Okigbo (1973), Aletor and Aladetimi (1989), Edem *et al.* (1990), and Kine *et al.* (1991). The characteristic problem of hard-to-cook phenomena which hinders the extensive use of AYB has been substantially reduced by precooking treatments (Njoku *et al.*, 1989). Studies aimed at providing alternative methods of utilising AYB have been reported (Njoku *et al.*, 1991; Ofuya *et al.*, 1991). Studies have been carried out on the comparative food qualities of AYB (Kine *et al.*, 1991), *in-vitro* multienzyme digestibility of protein of AYB (Oshodi & Hall, 1993) and functional properties of some varieties of AYB (Adeyeye *et al.*, 1994). Evans and Boulter (1974) reported on the amino acid composition of meals of three AYB varieties. This paper reports on the amino acid composition of six varieties (cultivars) of AYB as well as the fatty acid composition of the six cultivars on which no information is available.

MATERIALS AND METHODS

African yam bean seeds were collected from the farm located at Ayedun-Ekiti, Ondo State, Nigeria. Six different colour varieties of white (A_1), light-brown with black strips (B_1), reddish-brown (C_1), reddish-brown with black strips (D_1), light-brown (E_1) and black with light-brown strips (F_1) were identified, sorted and screened to remove the bad seeds and the remaining good seeds were dry-milled into fine flours. All chemicals used were of analytical grade.

Amino acid analysis was conducted on the milled samples. The sample was hydrolysed at 150°C for 1·5 h using a modification of the Waters 'Picotag' system (Bidlingmeyer *et al.*, 1984). The amino acid analysis was carried out as described by Bidlingmeyer *et al.* (1984) and Gardner *et al.* (1991).

The oil sample was extracted from the seed flours by Soxhlet extraction using petroleum ether of Analar grade (British Drug Houses, London), boiling range 40–60°C, for 5 h. The oil extracted was converted to the methyl ester using the method described by Hall (1982). The fatty acid methyl esters were analysed using a PYE Unicam 304 gas chromatograph (PYE

Unicam, Cambridge, UK) fitted with a flame ionisation detector and a PYE Unicam PU4810 computing integrator. Helium was used as the carrier gas. The column initial temperature was 150°C rising at 5°C/min to a final temperature of 220°C while the injection port and the detector were maintained at 220 and 250°C, respectively.

A polar (25QC3/BP1 - 0·5) capillary column (25·00 m × 0·33 mm; SGE Scientific Glass Engineering Co., UK) was used to separate the esters. The peaks were identified by comparison with standard fatty acid methyl esters obtained from Sigma Chemical Co. (St Louis, MO, USA). All the data generated were analysed statistically (Steel & Torrie, 1960).

RESULTS AND DISCUSSION

The protein content in legume seeds is governed by the genotype and the environmental conditions under which they are grown (Salunkhe *et al.*, 1985). It has been shown that legume proteins are mainly deficient in sulphur-containing amino acids and tryptophan, but are rich in lysine, in which cereals are relatively deficient (Oke *et al.*, 1975; Gopalan *et al.*, 1981; Moose & Baudet, 1983; Leung & Salunkhe, 1985). In general, legume proteins exhibit a wide range of variation in their essential amino acid composition (Salunkhe *et al.*, 1985). Environmental factors under which food legumes are grown can also influence amino acid composition (Gupta, 1982). The application of phosphorus, molybdenum and nitrogen has been shown to increase the level of methionine (Kadam *et al.*, 1977; Kapoor & Gupta, 1977; Dhage *et al.*, 1984). The application of sulphur-containing fertilisers increases the cystine content of Bengal gram proteins (Arora & Luthra, 1970).

Table 1 presents the results of the amino acid analyses of the African yam bean. The table shows that aspartic acid (Asp) and glutamic acid (Glu) together make up 193 mg/g on an average basis. The total essential amino acids in AYB amount to values ranging from 311 to 603 mg/g which is either higher or lower than that of soya bean: 444 mg/g (Altschul, 1958) depending on the cultivar under consideration.

Comparison between the amino acid content and the FAO/WHO (1985) amino acid reference values shows that lysine (Lys), Phenylalanine + tyrosine (Phe + Tyr), methionine + cystine (Met + Cys) are on the high side of the recommended range of amino acid requirement for infants and significantly higher than the values recommended for pre-school children and school children. AYB is also a good source of leucine (Leu), isoleucine (Ile) and valine (Val). This means AYB has high-quality protein and it will be a good source of essential amino acids for school children. One can also conclude that the AYB amino acids will meet the requirements of adults since the amino acid composition that meets the requirements of a child will also meet that of an adult (WHO, 1973; FAO/WHO, 1985). AYB flour may be suitable for the fortification of maize food products which are highly deficient in Lys and which are the most common weaning foods for children in most African countries (Akinrele & Edward, 1971). Oshodi *et al.* (1993) found Phe to be the most abundant essential amino acid in pigeon pea

Table 1. Amino acid composition of African yam bean (*Sphenostylis stenocarpa*) flour (whole seeds) (mg/g crude protein)

Amino acid	Sample[a]						$\bar{X}$	SD	CV(%)
	A_1	B_1	C_1	D_1	E_1	F_1			
Aspartic acid	100	92·4	74·1	82·4	96·7	96·6	90·5	10·1	11·1
Serine	50·4	44·9	37·2	36·0	40·1	42·7	41·9	5·20	12·4
Glutamic acid	121	105	87·0	91·1	105	108	103	12·0	11·7
Proline	45·8	32·5	28·9	39·7	43·5	44·4	39·1	6·79	17·4
Glycine	41·1	35·7	29·0	31·9	34·9	34·3	34·6	4·02	11·7
Alanine	38·5	33·5	28·6	31·4	35·6	37·2	34·5	3·63	10·6
Threonine[b]	45·8	32·3	25·9	29·0	31·9	32·7	32·9	6·22	18·9
Cystine	54·4	30·8	18·6	25·0	26·3	56·2	35·2	14·7	41·6
Methionine[b]	12·0	8·80	6·44	8·28	9·32	10·2	9·17	1·71	18·6
Valine[b]	48·9	34·8	29·6	37·5	43·0	44·6	39·7	6·47	16·3
Isoleucine[b]	44·7	32·8	26·5	31·4	35·1	38·9	34·9	5·79	16·6
Leucine[b]	79·0	54·8	45·1	50·9	56·8	60·5	57·7	10·3	17·9
Tyrosine	38·0	28·0	20·6	24·6	27·2	28·3	27·8	5·26	19·0
Phenylalanine[b]	61·3	40·6	32·7	38·8	40·2	42·9	42·7	8·85	20·7
Lysine[b]	88·0	58·9	45·7	57·4	64·8	66·1	63·5	12·8	20·2
Histidine[b]	55·1	34·7	27·0	30·0	32·3	30·5	34·9	9·31	26·7
Arginine[b]	63·9	42·6	32·4	39·3	43·1	42·4	44·0	9·65	22·0
Total essential amino acids	603	399	311	372	410	453	425	90·7	21·4

[a] A_1 (white); B_1 (light brown with black strips); C_1 (reddish brown); D_1 (reddish brown with black strips); E_1 (light brown); F_1 (black with light brown strips).
[b] Essential amino acids.

protein. Lys is, however, most abundant in the AYB. Phe has been earlier found to be the predominant anti-sickling agent in pigeon pea seed extract (Ekeke & Shode, 1990), AYB extract may likely be able to perform this function since it contains a reasonable amount of Phe.

Asp and Glu have their iso-electric points at pH 3·0 and 3·1, respectively. The remaining amino acids have their iso-electric points at pH values greater than 4·5 (Oshodi *et al.*, 1993). Adeyeye *et al.* (1994) reported that the minimum solubility of AYB protein occurred at about pH 4–5, which corresponds to the iso-electric point of AYB protein. The high value of Asp and Glu in the protein as observed in the present amino acid analysis may account for the occurrence of the minimum solubility of AYB protein at about pH 4–5.

The quality of dietary protein can be measured in various ways (FAO/WHO, 1991) but basically it is the ratio of available amino acids in the food of diet compared with needs expressed as a ratio (Orr & Watt, 1957; FAO, 1970; Bender, 1992). Using data from Table 1, together with the scoring pattern from Table 2, we find the value of the essential amino acids in the AYB as follows:

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

Results of the amino acid score are shown in Table 3. Table 1 shows a wide range of variation in the essential amino acids of AYB and this agrees with the report of Salunkhe *et al.* (1985). The amino acid scores in AYB also show this type of variation. Table 3 shows that Ile has the least coefficient of variation (14·3%). Met + Cys has an average of 0·20 with CV of 40·0% but both Lys and Phe + Tyr have an average 0·19 and CV of 21·1%. As a result of this variation in the amino acid scores, limiting amino acids also vary among the cultivars. The amino acid score with the least value for A_1, B_1 and C_1 is Val while the least value for Thr is recorded in F_1. However, in D_1, the least position is shared by Leu, Thr and Val but Leu and Thr share the least amino acid score in E_1. These differences in the limiting amino acids among the AYB samples are a further manifestation of the variation in the amino acid composition in the AYB.

Both histidine (His) and arginine (Arg) are particularly essential for children (FAO/WHO/UNU, 1985; Harper, 1984) and from these results AYB is a good source of both amino acids.

The data obtained for the total amino acids and those for essential amino acids score were subjected to an analysis of variance (ANOVA). In both analysis, $F_c > F_t$ ($P < 0.05$) showing that significant differences occur among the amino acid compositions in the AYB. The protein content in legume seeds is governed by the genotype and the environmental conditions under which they are grown (Salunkhe *et al.*, 1985). Since both environmental conditions and analytical conditions are similar for the various cultivars of AYB in this report, the variation in the amino acid compositions should be due to genotypical differences. Among the samples, however, sample A_1 appears the best of any in its amino acid content.

Table 4 shows the fatty acid composition of the oil from AYB. In our result, the saturated acids are caprylic (8:0), capric (10:0), lauric (12:0), myristic (14:0), palmitic (16:0) and stearic (18:0); the monounsaturated acids include palmitoleic (16:1), oleic (18:1), eicosenoic (20:1) and erucic (22:1) while the polyunsaturated components are linoleic (18:2) and linolenic acid (18:3). The total lipid content of legumes varies with variety, origin, location and climate as well as seasonal and environmental conditions, and the type of soil in which they are grown (Worthington *et al.*, 1972).

Table 4 shows that, on the average, stearic acid (18:0) has the highest (34·4%) concentration, linoleic acid is second with an average of 29·4% and palmitic acid (16:0) is in the third position with a value of 19·9%. Linoleic acid (18:2) is the most concentrated

Table 2. Scoring patterns for evaluation of proteins (mg/g N) (Pellett & Young, 1980)

Amino acid	Scoring pattern
Isoleucine	250
Leucine	440
Lysine	340
Methionine + cystine (TSAA) [a]	220
Phenylalanine + tyrosine	380
Threonine	350
Tryptophan	60
Valine	310

[a] TSAA, Total sulphur amino acids.

Table 3. Amino acid scores for the whole seeds of African yam bean flour

Amino acid	Sample score						$\bar{X}$	SD	CV(%)
	A_1	B_1	C_1	D_1	E_1	F_1			
Ile	0·18	0·13	0·11	0·13	0·14	0·16	0·14	0·02	14·3
Leu	0·18	0·12	0·10	0·12	0·13	0·14	0·13	0·03	23·1
Lys	0·26	0·17	0·13	0·17	0·19	0·19	0·19	0·04	21·1
Met + Cys	0·30	0·18	0·11	0·15	0·16	0·30	0·20	0·08	40·0
Phe + Tyr	0·26	0·18	0·14	0·17	0·18	0·19	0·19	0·04	21·1
Thr	0·18	0·13	0·10	0·12	0·13	0·13	0·13	0·03	23·1
Val	0·16	0·11	0·09	0·12	0·14	0·14	0·13	0·12	15·4

fatty acid in both pigeon pea (54·8%) (Oshodi *et al.*, 1993) and soya bean (52·0%) (Paul & Southgate, 1985). Linoleic and oleic acids are the major fatty acids in peanut, soya bean, chickpea, garden pea, broad bean and lentil. Linoleic is the major fatty acid in horse gram, red gram, field bean and lima bean. Cowpea, black-eyed pea, kidney and California small white bean have linoleic and linolenic acids as the major fatty acids. Many lipids from legume seeds contain substantial amounts of saturated fatty acids, especially palmitic acid (Lee & Mattick, 1961; Privett *et al.*, 1973; Mahadevappa & Raina, 1978). A higher proportion of either linoleic or linolenic acid is associated with legumes containing insignificant amounts of lipids (Salunkhe *et al.*, 1985). Caprylic, lauric, myristic, palmitic, palmitoleic and stearic acids are more concentrated in the AYB than the soya bean (Paul & Southgate, 1985). The value of the unknown fatty acid in the AYB is 9·25%, on the average, while it is 5·2% and 0·9% in pigeon pea (*Cajanus cajan*) and soya bean, respectively (Oshodi *et al.*, 1993). Caprylic acid is absent in the soya bean.

A major cause of death in some parts of the industrialised world is coronary heart disease (CHD) and saturated fatty acids have been implicated as an important dietary risk factor (Bender, 1992). The first stage of development of the disease is a narrowing of the coronary arteries by deposition of a complex fatty mixture on the walls — a process termed atherosclerosis. The fatal stage is the formation of a blood clot that blocks the narrowed artery — thrombosis. Even if the thrombosis is not fatal the reduced blood flow to the heart muscle deprives it of oxygen and can lead to extensive damage — myocardial infarction (Bender, 1992).

The saturated fatty acids (SFA), myristic (14:0) and palmitic (16:0), have been established as the most important of the dietary risk factors in CHD (Bender, 1992). There are three types of lipoproteins (protein–lipid complexes) in the blood; these are low-density lipoproteins (LDL) in which 46% of the molecule is cholesterol, high-density lipoproteins (HDL) which include 20% as cholesterol and very-low-density lipoproteins (VLDL) which have 8% cholesterol. High levels of total blood cholesterol are associated with the incidence of CHD and high intakes of saturated fatty acids (Bender, 1992). Myristic and palmitic acids appear to be the principal dietary factors that increase the blood cholesterol and do so by increasing LDL (Bender, 1992). The other main SFA in the AYB, stearic acid, does not have the same effect (apparently because it is converted to oleic acid which is monounsaturated); fatty acids of shorter chain length appear to have no effect (Bender, 1992).

The monounsaturated fatty acid (MUFA) of main interest is oleic acid (18:1) which is plentiful in rape

Table 4. Fatty acid composition of the African yam bean (whole seeds) flour (% total fatty acids)

Fatty acid	Sample						$\bar{X}$	SD	CV(%)
	A_1	B_1	C_1	D_1	E_1	F_1			
Caprylic (8:0)	0·68	0·68	0·82	0·42	0·33	0·76	0·62	0·18	28·9
Capric (10:0)	ND[a]	ND	0·45	ND	ND	ND	—	—	—
Lauric (12:0)	0·11	0·10	0·26	0·16	0·18	0·15	0·16	0·05	33·1
Myristic (14:0)	0·49	4·54	0·43	0·79	0·54	0·43	0·20	1·50	124
Palmitic (16:0)	20·1	18·3	21·6	20·4	20·0	18·7	19·9	1·07	5·40
Palmitoleic (16:1)	0·32	0·43	0·42	0·33	0·84	0·22	0·43	0·20	46·3
Stearic (18:0)	32·0	35·4	36·7	34·0	33·5	34·7	34·4	1·47	4·28
Oleic (18:1)	0·25	0·20	0·29	0·27	0·64	1·60	0·54	0·49	90·7
Linoleic (18:2)	35·8	30·1	25·2	27·4	28·8	29·0	29·4	3·22	11·0
Linolenic (18:3)	3·16	2·74	3·05	2·63	1·84	2·90	2·72	0·43	15·8
Eicosenoic (20:1)	1·11	0·12	0·83	0·78	1·52	0·98	0·89	0·42	47·2
Erucic (22:1)	0·41	0·54	0·81	0·96	0·60	0·72	0·67	0·18	26·8
Unknown	5·65	6·86	9·16	12·8	11·3	9·76	9·25	2·44	26·4
Unsaturation	41·0	34·1	30·6	32·4	34·3	35·5	34·4	3·25	9·44
EFA[b]	38·9	32·8	28·3	30·0	30·7	31·9	32·1	3·36	10·5

[a] ND, Not detected.
[b] Essential fatty acid.

Table 5. Fatty acids distributed according to saturation and unsaturation of components (%) (in African yam bean whole seeds flour)

Fatty acid[a]	Sample						$\bar{X}$	SD	CV(%)
	A_1	B_1	C_1	D_1	E_1	F_1			
SFA	53·4	59·1	60·2	55·8	54·5	54·8	56·3	2·49	4·43
MUFA	2·09	1·29	2·35	2·34	3·60	3·52	2·53	0·81	31·9
DUFA	35·8	30·1	25·2	27·4	28·8	29·0	29·4	3·23	11·0
TUFA	3·16	2·74	3·05	2·63	1·84	2·90	2·72	0·43	15·9
Unknown	5·65	6·86	9·16	12·8	11·3	9·76	9·25	2·44	26·4

[a]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; DUFA, diunsaturated fatty acid; TUFA, triunsaturated fatty acid.

seed and high-oleic safflower oils (Bender, 1992). The relatively high intake of olive oil and consequently the proportionately low intake of SFA are believed to be important dietary factors in the low incidence of CHD in Mediterranean countries compared with northern Europe (Bender, 1992). The polyunsaturated fatty acids (PUFA) are fatty acids with between two and six double bonds and long carbon chains of 18–22 carbon atoms. Linoleic and linolenic acids are essential in the diet and the very long-chain fatty acids are formed from them in the body. Linoleic and linolenic acids are the most important essential fatty acids required for growth, physiological functions and body maintenance (Salunkhe *et al.*, 1985).

Table 5 shows the distribution of results in Table 4 into SFA, MUFA, DUFA, TUFA and unknown. SFA is 56·3% on average with the least variation of 4·43%. 61·1% of the SFA is composed of stearic acid (18:0) while both myristic (14:0) and palmitic (16:0) acids make up 37·4% of the total SFA. The probability of AYB fatty acids contributing to CHD is very low, and particularly when the AYB is not an oil seed. The essential fatty acids (C 18:2 and C 18:3) (EFA) are reasonably comparable to EFA in rice and lower than the EFA in sorghum, millet and maize (Adeyeye & Ajewole, 1992) but generally lower than the EFA values obtained by Ajewole and Adeyeye (1993) for some Nigerian citrus seed oils. Holland *et al.* (1991) have shown that the total SFA in soya bean is 12·4%, MUFA is 18·8% and PUFA is 48·9%, while our results show that the total SFA in AYB is 56·3%, MUFA is 2·53% and PUFA is 32·1%. The best source of EFA among our samples is the white (A_1) variety.

The data obtained for the total fatty acids (Table 4) and those obtained for various levels of saturation/unsaturation (Table 5) were subjected to analysis of variance (ANOVA). In both analyses, $F_c < F_t$ ($P < 0{\cdot}05$) showing that significant difference does not occur in the fatty acid compositions in the African yam bean seeds. Although the coefficient of variation shows some unequal distribution of the fatty acids among the various cultivars, such variations are not significant.

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