Biochemical Assessment of Some New Varieties of Soybeans

Anthony D. Ologhobo & Babatunde L. Fetuga

Division of Nutrition Biochemistry, Department of Animal Science, University of Ibadan, Ibadan, Nigeria

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ABSTRACT

The nutrient, fatty and amino acid compositions of six new varieties of soybean were studied by chemical analysis, gas-liquid and column chromatography techniques. All the varieties studied, namely GE 104, GE 109, TGM 767, TGX 536, TGX 342 and TGX 303, showed desirable nutrient characteristics such as low cellulose and lignin contents, high total fat content, high crude protein content, high linoleic acid content and adequate amounts of essential and non-essential amino acids. Cystine, methionine, iso-leucine and threonine were the amino acids in shortest supply in most of the varieties. Although the new improved soybean varieties were adequate in some essential nutrients, there is still need for greater attention to be paid to improving the amino acid profile, especially the sulphur amino acids, which were limiting in the varieties analysed.

INTRODUCTION

Despite the importance of legumes in the diets of people with low incomes in the developing countries, lack of availability—and consequent high price—reduce the frequency of their consumption. The nutritional contribution of legumes could be raised by increasing yields and by improving the quality and quantity of the seed protein.

Extensive breeding studies with several legumes in this country have resulted in the availability of a number of improved varieties of soybean which combine such desirable characteristics as early maturity, highharvest index and good yield potentials. Very little is, however, known

103

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about the nutritional value of these varieties and there has been no screening of genetic stocks for protein content and quality. Differences in chemical composition have been attributed to soil, climate, strain and fertiliser treatment (FAO, 1966). Reports from other investigators using different approaches for the protein evaluation found differences between species as well as between varieties of the same species.

Presently, there are several accessions and different breeding lines of cultivated soybean varieties in Nigerian model farms and research institutes. Within these materials it is possible that a soybean genotype with superior protein quality already exists.

This paper is an account of a series of investigations designed to biochemically evaluate some of the new varieties of soybeans cultivated in Nigeria for food.

MATERIALS AND METHODS

Six varieties of soybean (*Glycine max*) were employed in this study. These were accessions obtained from the Plant Breeding unit of the International Institute of Tropical Agriculture, Ibadan, Nigeria. All samples were properly air-dried, ground and stored in screw-cap bottles at 4° C until required for analysis.

Analytical procedure

All the analyses were done in duplicate and the results were expressed on a dry matter basis after correcting for residual moisture. The proximate compositions of the samples were determined according to standard methods (AOAC, 1975). Mineral contents were determined by first wetashing the samples with a mixture of nitric acid, perchloric and sulphuric acids, followed by flaming in a Perkin-Elmer atomic absorption Spectrophotometer 290, using different lamps. The phosphovanado-molybdate method was used for the estimation of phosphorus (AOAC, 1975).

Fatty acid analysis

Fat was extracted by the Soxhlet method (Pearson, 1976) with diethyl ether as the solvent. Hydrolysis of the fat and esterification of the fatty acids were effected by the method described by Metcalf & Schmidt (1960). A Packard 419 (FID) chromatograph was employed for GLC analysis of fatty acid esters, using a column containing 5% SE 30 on Chromosorb GAW, DMSC, 80/100 mesh. The temperature was programmed between 150°C and 273°C. Rate of temperature rise was $1^{\circ}C \min^{-1}$. Identification of the peaks was carried out using internal standards and the proportions calculated from ratios of the weights of peaks cut from photocopies of chromatograms.

Carbohydrate analysis

The ethanol-soluble sugars were extracted from the legume flour by repeated shaking with 80% v/v ethanol and the extracts pooled. The extractions were repeated until the final extract showed a negative test for sugars. The ethanol was separated from the pooled extracts under vacuum at 40 °C and quantitative analysis of the sugars present was accomplished by paper chromatography using ethylacetate-pyridinewater (8:2:1 v/v) as a solvent. Sugars were detected by spraying with p-anisidine hydrochloride reagent (Mukherjee & Srivastava, 1952). Oligosaccharides were separated on Whatman No. 3 paper by developing the chromatogram for 4h using propanol-ethanol-water (7:1:2) and constituent sugars were determined by the phenol-sulphuric acid method (Dubois et al., 1956). Starch was determined by the glucoamylase method (Thivend et al., 1972). Cell wall constituents (unavailable carbohydrates) were estimated by acid hydrolysis (Southgate, 1969) and quantitative investigations of the polysaccharide hydrolysates were carried out as previously described for the ethanol extracts. Sugars identified on paper chromatograms were quantified. Fructose, glucose and sucrose were determined by combined enzymatic and chemical methods (Johnson et al., 1964) and pentoses were determined by the method of Albaum & Umbreit (1947).

Amino acid analysis

Amino acid contents were determined by the method of Moore & Stein (1954) as modified by Bidmead & Ley (1958). The amino acids were separated with the column chromatographic technique, using the automated Technicon Model TSM Sequential Analyser. Cystine was determined as cysteic acid by the method of Moore (1963) while tryptophan was chemically determined by the method of Miller (1967). All the assays were performed in duplicate.

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Varieties	Protein	Fat	Available	Crude	Ash				8111	per 100	b.0			
	$(N \times 6.25)$ $(\%)$	(%)	carbohydrate (%)	fibre	(%)	Ca	Ρ	Mg	K	Na	Мn	Fe	Cu	Zn
GE 104 GE 109 TGM 767 TGX 536 TGX 336 TGX 303 Mean Mean Standard deviation	36.7 36.7 36.3 36.3 35.4 35.9 35.9 0.82	21.6 23.7 24.7 26.5 26.5 26.5 24.9 2.03	29.7 26.9 28.6 28.6 24.0 25.6 25.6 25.8 25.8 25.8	3.85 3.85 4.69 4.82 6.75 5.96 5.96 3.98 3.98 5.01	7.15 5.16 6.59 8.09 8.14 5.03 6.69	220 220 210 210 220 220 213 213 8-17	590 590 560 580 580 580 582 582 11·7	220 190 210 200 195 205.83 12.8	2 900 3 400 3 2 900 3 2 900 3 2 000 1 96	28.0 26.0 28.0 28.0 25.0 25.0 1.37	12.0 11.0 12.0 12.0 10.0 11.2 0.98	8.00 9.33 9.33 9.33 9.33 9.33 9.33 9.33 9	1:50 1:50 1:50 1:50	11-4 16-4 13-5 13-6 14-5 13-9 1-76
Per cent coefficient of variation	2.28	8.15	7.88	22.8	20-5	3-83	2.01	6·22	6-41	5.20	8-77	31.0		12.7

TABLE 1 Chemical Composition (Dry Basis) of Some Varieties of Soybean

RESULTS AND DISCUSSION

Proximate and mineral compositions

The average values for the proximate and mineral composition of the different varieties of soybean are presented in Table 1. All varieties contained uniformly high crude protein, with values ranging from $35 \cdot 4\%$ in TGX 342 to $36 \cdot 7\%$ in GE 104. This range of protein levels compares well with the values reported for several other varieties of soybean (Kakade *et al.*, 1972) but higher than the locust bean seed, green gram, pigeon pea, kidney bean (Amirshahi & Tavakoli, 1970), cowpea (Ologhobo & Fetuga, 1982*a*) and limabeans (Ologhobo & Fetuga, 1983). Total fat ranged between 21.6 and 26.6% while crude fibre was highest in TGX 536 (6.75%) and lowest in GE 104 (3.85%). Crude fibre contents did not show any varietal differences and the values obtained are comparable with levels in the cowpea, limabean (Ologhobo, 1980), water melon seed (Oyenuga & Fetuga, 1975) and groundnut seeds (Oyenuga, 1968). This indicates that the digestibility of associated nutrients by monogastric animals is likely to be efficient.

Of all the mineral constituents, potassium was the most abundant and the values obtained are higher than the levels encountered in many legumes, particularly those of the kidney bean, green gram, pigeon pea (Oyenuga, 1968), cowpea (Ologhobo & Fetuga, 1982*a*), peanut meal (Woodroof, 1969) and limabean (Ologhobo & Fetuga, 1983). Phosphorus ranged between 560 and 590 mg/100 g, calcium between 200 and 220 mg/100 g and magnesium between 190 and 220 mg/100 g. The concentration of iron was found to be highest in GE 104, TGX 342 and TGX 303 with 8.0 mg/100 g, respectively. It would appear from these results that these varieties of soybean would constitute a valuable source of the major elements and of Fe, Zn, Mn and Cu, particularly in diets for humans in which no special provision is made for the supply of these important nutrients.

Available and unavailable carbohydrates

The concentrations of the available and unavailable sugars in the soybean varieties are presented in Table 2. On chromatographic analysis, glucose, fructose and sucrose were identified. Oligosaccharides with stachyose and raffinose as constituent sugars were also identified.

	Lignin	ľ	1.20	1·56	1.50	1.35	00.1	1 10	<u></u>	1.38	0.15	10.9
	Cellulose		4-23	4-58	4-45	4.62	4.50	00.4	4.38	4-46	0.14	3.14
tter)	llulose ysate	Pentose	0.50	0.60	0.50	0.55		00-0	0.60	0-56	0.05	8.93
g Dry Ma	Hemice hydrol	Glucose	4-78	6.40	5-77	5.25		07.9	5.10	5.58	0.64	11.5
s per 100	te	Pentose	1.05	0-91	1.28	0.06	00.0	1-20	1.16	1.09	0·14	0-13
s (Gram	tter-solubl ysacchario	Glucose	1.56	1.30	1-48	20.1	C7.1	I-70	<u>.</u>	1·46	0.17	11.6
n Varietie	Wc pod	Fructose	3-02	2.93	2.88		C1.C	2.75	3.06	2.96	0.13	4.39
FABLE 2 in Soybea	Starch		16.4	15.4	16.3		10-0	16.0	15-9	16.1	0-45	2.80
T ohydrates i		Raffinose	4.04	3.86	3.38		4.10	3.95	3.95	3.88	0.26	6-70
ble Carbo	ugars	Stachyose .	8.60	7.05	VC.8	-7.0	9.50	7.88	7-90	8.18	0.32	3.91
Unavaila	l-soluble s	Sucrose	6.54	50.9	20.5	CO. /	6.83	6-60	7.12	6.85	0.24	3.50
tble and	Ethano	Fructose	0.45	01-0		0+.0	0.56	0.55	0.45	0.47	0-07	14.9
Availa		Glucose	0.07	10 0 0 0	20-0	/0.0	0.07	0.08	0-07	0.07	0.005	7.14
	Varieties		CE IM			10M /0/	TGX 536	TGX 342	TGX 303		Mean Standard deviation	Per cent coefficient of variation

Glucose and fructose were only present in traces while sucrose values ranged from 6.54% for GE 104 to 7.12% for TGX 303. Stachyose was uniformly high in all varieties with values ranging between 7.88 and 8.60% while raffinose was highest in TGX 536 (4.10%) and lowest in TGM 767 (3.38%). Results of the qualitative analysis of the water-soluble polysaccharide hydrolysates revealed the presence of fructose, glucose and pentoses. The highest concentration of this polysaccharide occurred in variety GE 104 while fructose, glucose and pentoses existed in highest concentrations in TGX 536 (3.13%), TGX 342 (1.70%) and TGM 767 (1.28%), respectively. Values for these component fractions were, however, fairly uniform in all varieties.

Glucose constituted the major sugar in the hemicellulose fraction with trace amounts of pentose sugars. Cellulose concentration was uniformly high in all varieties with values ranging between 4.23 and 4.62%. Lignin levels were comparatively low and values in all varieties did not differ to any great extent from each other.

These results obtained for the soybean present a fairly different pattern from that known for several other legume types, particularly in sucrose and starch contents. While glucose and fructose levels compare favourably, sucrose content was high compared with the range of 2.36-4.22% that has been reported for several other legumes including the mungbean, pintobean, navy bean, horse bean (Noivikull & D'appolonia, 1978) cowpeas (Longe, 1980) and limabeans (Ologhobo & Fetuga, 1982b). Oligosaccharide sugar contents were also higher than values that have been reported for other varieties of the soybean (Aspinall et al., 1967), field bean (Pritchard et al., 1973), chickpea, mung bean (Aman, 1979) and rapeseed meal (Siddiqui & Wood, 1977). These high levels are likely indications of the rôles these soybean varieties might play in flatus production. Soybean seeds have been particularly shown to produce large quantities of intestinal gases in rats (Hendin & Adachi, 1962) and human subjects (Steggerda & Dimmick, 1966), the volume of flatus produced being directly related to the quantity of beans consumed. Starch contents, on the other hand, were comparatively lower than in the cowpea, navy bean, limabean, mungbean and wrinkled peas, where contents have been as high as 47 % (Schoch & Maywald, 1968).

Like other legumes, however, cellulose and lignin contents were low and are not likely to affect the overall quality of these soybean varieties in the digestibility of associated nutrients by monogastric digestive systems.

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A STATE OF A	$C_{22:I}$	-	Propagation Processor	-	Makkalarin	- I MARANA		-	Readily on the		-
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	C.20:1	and the second se			-	-	-	-	-		***
	C 20:0	2.09	2·08	2-08	2·12	1.98	1.85	2·03	0·10		4.93
	C_{Re3}	4.86	5.13	5.20	4-96	5.05	4.98	5.03	0·12		2.4 4
	$C_{I8:2}$	43.8	55-8	45-3	50.9	48.6	48.0	48-7	4.26		8-73
	$C_{I8:I}$	27-3	23-4	25-8	26.5	23-9	26.0	25.5	1.53		6.00
	$C_{I8:0}$	3.10	2-86	2.75	3.15	2.98	2.80	2.94	0.16		5:44
	$C_{l6:1}$	0.68	0·34	0.55	0.60	0-48	0-54	0-53	0·11		20·8
	$C_{I6:0}$	17.9	11-0	11.2	10.7	9.86	11.0	10.6	0-64		6-06
	$c_{\mu i}$			-	-	-		-			
	C _{H:0}	0.10	0.10	0·10	0.50	0.10	0-69	0-27	0.26		96-3
	$C_{12:0}$		-		I		-	ł			-
	Total	21.6	26-6	23-7	24-7	26.5	26.5	24.9	2.03		8.15
	Varieties	GE 104	GE 109	TGM 767	TGX 536	TGX 342	TGX 303	Mean	Standard deviation	Per cent coefficient	of variation

 TABLE 3

 Total Fat and Fatty Acid Composition in Soybean Varieties (Per Cent Dry Matter)

Fatty acid composition

Total fat content and the fatty acid pattern in the extracted fat samples are shown in Table 3. The major fatty acids, in descending order of abundance, were essentially linoleic $(43\cdot8-55\cdot8\%)$, oleic $(23\cdot4-27\cdot3\%)$, palmitic $(9\cdot71-11\cdot2\%)$, linolenic $(4\cdot86-5\cdot20\%)$ and stearic $(2\cdot75-3\cdot15\%)$ acids. The level of saturation was low in all varieties due primarily to the low contents of palmitic, stearic and arachidonic acids and the complete absence of lauric, behenic and lignoceric acids. The highest linoleic acid content was obtained in variety GE 109 $(55\cdot8\%)$ which also had the highest total fat content.

When the above results are compared with reports on other soybean varieties (Oyenuga, 1968), no striking differences are apparent. But compared with the analysis of other tropical samples (Ologhobo, 1980) and Indian samples (Patel, 1975), the values for linoleic acid were higher, stearic lower, palmitic and linolenic in consonance with those reported by Goddard & Goodall (1958) but consistently lower than the reported Indian samples (Patel, 1975). Several varieties of soybean are known in this country but their classification into definitive groups is incomplete and the variations in the relative proportions of the fatty acids may be related to varietal differences or to differences in analytical techniques.

The presence of fairly high amounts of the essential fatty acid, linoleic suggests that the soybean is a highly nutritious oil. It is at present being used as a cooking oil in many parts of the tropics and its use should be encouraged if only to supply the much needed linoleic acid, which is also a precursor of arachidonic acid, another essential fatty acid which was very deficient in the varieties analyzed.

Amino acid composition

Table 4 shows the total amino acid composition of the soybean varieties and that of a standard protein, whole hen's egg.

Of the non-essential amino acids, these appeared, in all cases, to attain the levels found in whole egg and, in many cases, exceeded it. In some varieties where the values fell short, the glutamic and aspartic acid contents were high and these are known to be used to a large extent in the synthesis of the non-essential amino acids in the animal body. It is therefore considered unlikely that the non-essential amino acids would constitute any dietary problem.

	Narieties (g/16 g N)
TABLE 4	Amino Acid Composition of Soybear

Amino acids (g/16 g N)	GE 104	GE 109	TGM 767	TGX 536	TGX 342	TGX 303	Freeze-dried eggs
Lysine	6-23	96.9	6.50	6.35	7.15	6.85	6.98
Histidine	3.81	2.51	3.25	4.10	3-63	3.92	2.43
Arginine	6-00	6.90	7.30	6-22	5.96	6.55	6·10
Aspartic acid	11.5	9-64	11.0	9.85	12·1	10.9	9.02
Threonine	3-38	4·33	3.80	2.99	4.15	3-56	5.12
Serine	4.67	4.51	4.80	4-77	5.23	4.48	7-65
Glutamic acid	18-3	15.5	17.7	18.3	16.0	17.6	12.7
Proline	5-27	6-08	5.90	6.16	5.46	5-80	4.16
Glycine	4.28	3-71	3.90	4.22	3.65	3.70	3.31
Alanine	4.17	3.85	4·08	4-25	3.90	3.85	5-92
Cystine	0-97	1.04	<u>66</u> .0	0.92	1.02	1-06	2-43
Valine	4.86	4-49	4.88	4.50	4.16	4.53	6·85
Methionine	1.73	1.87	1.70	1.85	1.86	1.77	3.36
Iso-leucine	4.68	4.38	4-60	4.25	5.10	4-82	6.29
Leucine	5.95	5.89	6.70	7.50	6.00	5-80	8-82
Tyrosine	4-21	3.74	3.90	5.56	4.70	3.86	4.16
Phenylalanine	7-07	5.86	8·02	6-25	7.66	8-06	5-63
Tryptophan	1.65	1-60	1.28	1.64	1.55	1.62	1·62

Perhaps of more immediate importance are the values for lysine, tryptophan, methionine, cystine and threonine. With the exception of TGX 342 and perhaps GE 109, all the soybean varieties were borderline with regard to lysine: GE104, 6.23 g/16gN; GE109, 6.96 g/16gN; TGM 767, 6.50 g/16 g N; TGX 536, 6.35 g/16 g N; TGX 342, 7.15 g/16 g N; TGX 303, 6.85 g/16 g N. It is perhaps worthy to note here that even the lowest value (6.23 g/16 g N) obtained for this amino acid is higher than in most other plant sources reported to be high in lysine (cotton seed meal, 4.90 g/16 g N; African locust bean, 6.17 g/16 g N; African oil bean, 5.46 g/16g N; bambara nut, 6.08 g/16g N; field bean, 5.50 g/16g N). The importance of a cheap plant protein source high in lysine is readily understood when one views it against a background of its low content in cereals which make up about 70 to 80% of the total composition of Nigerian foods. A cheap source of lysine such as soybean meal is of primary importance to us for successful feeding of our teeming population and these varieties of soybean appear to show much promise.

More recently, threonine has come to be regarded as a possible limiting amino acid in cereal-based diets. All the soybean varieties showed fairly low threonine values compared with the whole hen's egg. It is possible, therefore, that diets in which these plant protein sources serve as the sole protein concentrate may be marginally deficient in threonine.

The most pronounced deficiencies were, however, obtained in the sulphur amino acids, methionine and cystine. The freeze-dried whole egg sample gave values of 3.36 g/16 g N for methionine and 2.43 g/16 g N for cystine while methionine values in the soybean samples ranged between 1.70 and 1.87 g/16 g N and cystine between 0.92 and 1.06 g/16 g N. These values do not differ to any extent from those reported in the literature by past workers such as Oyenuga (1968) who worked on Nigerian feeds which included several soybean varieties, and Owusu-Domfeh et al. (1970), who worked on Ghanaian feeding stuffs. Deficiency in sulphur amino acids in legumes has long been reported by these and several other authors and breeding studies should now aim at not only producing agronomically acceptable soybean varieties, but also at improving protein quality. Greater attention needs to be given to their genetic diversity to improve amino acid profiles, particularly the sulphur containing ones. The trend has been established and several new varieties of soybean with considerably improved traits are being released regularly. We are hopeful that, before long, our new soybean varieties will not only be agronomically suitable and resistant to pests and microbial infections, but will have a desirable package of nutrients adequate in all the essential amino acids.

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