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Biological and Chemical Characterization of Haemagglutinins from Three Edible Varieties of Lima Beans (*Phaseolus Lunatus*, Linn.)

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ABSTRACT

Haemagglutinins from three edible varieties of lima bean were extracted, purified, lyophilized and characterized with respect to their specific haemagglutinating activities, sugar specificities, amino acids, minerals (trace and major) and available carbohydrate contents. The specific activities of the haemagglutinins were 292, 306 and 308 haemagglutinating units (HU)/mg sample for the TPL 13, TPL 237 and TPL 323 varieties, respectively. N-*Acetyl*-D-*galactosamine specifically inhibited the haemagglutination of rabbit erythrocytes. Generally, amino acid values ranged from 12·5 g/16 g N for aspartic acid to 1·23 g/16 g N for methionine. Manganese was the most abundant mineral with a value of 2·24 μg/g in TPL 323 while potassium was the least abundant (0·20 μg/g in TPL 237). Alcohol-soluble sugars (total reducing sugars (TRS), glucose and fructose) varied from 0·43 to 3·25 g/100 g DM while starch varied from 6·32 g/100 g DM in TPL 237 to 7·88/100 g DM in TPL 323. Distinct varietal differences were observed for some minerals and alcohol-soluble sugars. This was indicated by the high coefficients of variation obtained for Mg, K and Na (42·2, 56·7 and 41·2%, respectively) and for TRS and glucose (38·3 and 32·8%, respectively).*

INTRODUCTION

Lima beans (*Phaseolus lunatus*, Linn.) are among the most widely cultivated pulse crops especially in the southern part of Nigeria where the meals

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contribute a substantial amount of protein, particularly in the diets of rural dwellers. Perhaps, due to the immense contribution of grain legumes (including lima bean) to the amelioration of protein dearth, their endogenous content of a myriad of anti-nutritional factors has remained a subject of immense concern to the food chemist and nutritional biochemist. This concern derives in part from the fact that a number of culinary practices may not completely inactivate these factors.

One well known example of these toxic factors is haemagglutinin—a protein with the remarkable property of agglutinating erythrocytes. In addition to agglutinating red blood cells and precipitating polysaccharides and glycoproteins, the haemagglutinins (also called lectin from the Latin word, *legere*, to gather or select) are known to agglutinate malignant cells and induce mitosis in lymphocytes (Sharon & Lis, 1972). Evidence that part of the toxic action of raw or improperly processed legumes is related to the presence of haemagglutinin came from Liener & Palansch (1952), who indicated that a growth depression of about 75% relative to that of control was obtained in the rat by the addition of soya bean haemagglutinin at 1% of a diet containing autoclaved soya bean meal. Other studies by Jaffe & Vega-Lette (1968), Pusztai *et al.* (1979), King *et al.* (1980) have demonstrated that ingested haemagglutinins exert part of their toxic effects in mammals by interfering with the normal digestive and absorptive processes of nutrients in the gastrointestinal tract.

It is now widely believed that an in-depth elucidation of the structure and chemical constituents of the haemagglutinins would lead to a clearer understanding of the molecular basis of their sugar specificity and their mechanism of action on cells. The present account is an effort in this direction. Additionally, information on varietal differences in the chemical composition of lima bean haemagglutinins does not appear to be available even though differences in the physical characteristics such as bean shape, size and colour are well recognized.

MATERIALS AND METHODS

Materials

Three commonly consumed accessions of lima bean varieties were collected from local farms and identified at the International Institute of Tropical Agriculture (IITA), Ibadan, as TPL 13, TPL 237 and TPL 323. The dried mature seeds were milled to fine flour in the laboratory before use.

Methods

Haemagglutinin extraction and purification

Haemagglutinin was extracted and purified from the defatted flour by a modification of the four-step purification method of Huprikar & Sohonie (1965). Details of the modifications which allowed for larger scale production are reported elsewhere (Aletor & Fetuga, 1985). The haemagglutinin solutions were concentrated by vacuum dialysis for 18 h at 4°C and thereafter lyophilized, weighed and stored in vials and kept deep-frozen prior to use.

Haemagglutinating activities and sugar specificities

The relative and specific haemagglutinating activities of the raw bean, as well as the lyophilized extracts, were determined using 0·25% saline-washed trypsinized rabbit red blood cells in the twofold serial dilution technique of Kabat & Mayer (1961). The relative haemagglutinating activity (titre) was the reciprocal of the highest dilution at which agglutination was visible. One haemagglutinating unit (HU) was equal to this relative activity. The specific activities, given by

$$\frac{\text{relative activity/ml}}{\text{mg N/ml}}$$

were at each purification step used as indices of purity. The haemagglutinins were regarded as pure when their final specific activities were at least seven to eight times those of their crude saline extracts (Ikegwuonu & Bassir, 1976). The nitrogen content of the haemagglutinins was determined by the micro-Kjeldahl method as recommended by Bassir (1971).

In determining the sugar specificities of the haemagglutinins, up to 2 mg/ml of a number of saccharides (Table 1) were tested for inhibitory action on agglutination (Tomita *et al.*, 1970).

Analytical procedure

The amino acid composition of the haemagglutinins was determined by hydrolysing a 100-mg sample with 170 ml of 6 N HCl and refluxing for 24 h at 110°C. The hydrolysate was transferred to a 250-ml volumetric flask and made up to the mark. After filtration, a 10-ml aliquot of the filtrate was heated in a rotary evaporator (40°C) to remove excess acid before further examination with a Perkin-Elmer automatic amino acid analyser (Model LKB 4400). Norleucine was added as internal standard to correct for slight fluctuations in the amino acid peaks. Methionine sulphoxide was also included in the standard to detect the peak for this acid, which might be

TABLE 1
Haemagglutinating Activities (HU/mg Sample) and Sugar Specificities of Lima Bean Varietal Extracts

Varieties	*Crude saline extracts*	*Purified extract*	*Sugar specificity*[a]
TPL 13	32·0	292	*N*-acetyl-D-galactosamine
TPL 237	32·0	306	*N*-acetyl-D-galactosamine
TPL 323	32·0	308	*N*-acetyl-D-galactosamine
Mean	32·0	302	
Standard deviation	0·0	8·72	
Coefficient of variation (%)	0·0	2·89	

[a] Sugars tested for inhibitory action: D-xylose, D-arabinose, D-glucose, D-fructose, D-galactose, D-mannose, L-fucose, D-glucosamine, D-galactosamine, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine and *N*-acetyl-D-mannosamine.

produced from the oxidation of methionine. Using a molar relationship between methionine and methionine sulphoxide, a factor of 0·84 was used to convert the methionine sulphoxide values to methionine. Cystine was determined by the method of Moore (1963) after performic acid oxidation to cysteic acid, while tryptophan was determined chemically by the basic hydrolysis of proteins as described by Miller (1967).

Mineral content of the haemagglutinins was determined by wet-ashing the samples with a mixture of nitric acid, perchloric acid and sulphuric acid followed by flaming in a Perkin-Elmer Atomic Absorption Spectrophotometer 290, using different lamps.

Soluble sugars were extracted with 85% (v/v) methanol and the total reducing sugars (TRS) were estimated using arsenomolybdate reagent by the colorimetric method of Nelson (1944). Glucose was determined by the glucose oxidase method of Hugget & Nixon (1957) and fructose by the colorimetric method of Johnson *et al.* (1964). Starch was determined after extraction of the alcohol-soluble sugars by the method of Thivend *et al.* (1972). Data obtained (means for three determinations) were analysed statistically (Steel & Torrie, 1960).

RESULTS AND DISCUSSION

The specific activities and sugar specificities of both the crude saline and purified haemagglutinin extracts from the different lima bean varieties are shown in Table 1. The haemagglutinating activities of the crude saline

extracts were identical for all the varieties. Of the purified and lyophilized extracts, TPL 323 had the highest activity of 308 HU/mg while TPL 13 had the least value of 292 HU/mg. Of the sugars tested, only *N*-acetyl-D-galactosamine specifically inhibited the haemagglutination of rabbit red blood cells.

The amino acid, mineral and available carbohydrate composition of the haemagglutinins are presented in Tables 2, 3 and 4, respectively. In general, amino acid values for all the haemagglutinins ranged from 12·5 g/16 g N for aspartic acid in TPL 13 to 1·23 g/16 g N for methionine, also in TPL 13. Levels of the non-essential amino acids were generally higher than those of the essential ones. Relative to other amino acids, values for the sulphur-containing amino acids (methionine and cystine) were lowest in all the varieties. The different varieties showed low coefficients of variation (2·93–20·99%) in their amino acid content.

Manganese with a value of 2·24 μg/g in TPL 323 was the most abundant mineral while potassium was the least with a value of 0·20 μg/g in TPL 237. The minerals (Mg, K and Na) showed distinct varietal differences in their

TABLE 2
Amino Acid Profile of Haemagglutinins from Different Varieties of Lima Bean (g/16 g N)

Amino acids	*Varieties*					
	TPL 13	*TPL 237*	*TPL 323*	*Mean*	$\pm SD^a$	CV^b *(%)*
Lysine	5·32	4·11	4·66	4·79	0·61	13·0
Histidine	3·34	3·01	3·13	3·16	0·17	5·38
Arginine	5·13	4·71	3·92	4·59	0·16	13·3
Aspartic acid	12·5	12·2	11·9	12·2	0·28	2·29
Threonine	4·93	5·21	4·33	4·82	0·44	9·12
Serine	8·18	6·81	7·76	7·58	0·70	9·23
Glutamic acid	9·83	8·14	7·08	8·35	1·39	16·7
Proline	4·77	4·73	4·22	4·57	0·31	6·78
Glycine	3·35	2·61	3·11	3·02	0·38	12·5
Alanine	4·01	3·65	4·20	3·95	0·28	7·07
Cystine	2·28	2·85	3·01	2·71	0·38	14·2
Valine	4·45	4·21	3·92	4·19	0·27	6·44
Methionine	1·23	1·88	1·76	1·62	0·34	21·0
Isoleucine	4·25	4·01	4·21	4·16	0·13	3·13
Leucine	6·92	5·92	6·88	6·57	0·57	8·68
Tyrosine	3·51	3·34	3·46	3·43	0·10	2·93
Phenylalanine	4·13	4·10	3·83	4·02	0·17	4·23
Tryptophan	4·79	4·50	4·22	4·50	0·29	6·44

[a] SD = standard deviation.
[b] CV = coefficient of variation.

TABLE 3
Mineral Composition of Haemagglutinins from Different Varieties of Lima Bean (μg/g)

Minerals	*Varieties*					
	TPL 13	*TPL 237*	*TPL 323*	*Mean*	$\pm SD^a$	CV^b *(%)*
Calcium	0·80	0·86	1·12	0·93	0·17	18·3
Magnesium	0·30	0·65	0·36	0·45	0·19	42·2
Manganese	1·90	2·11	2·24	2·08	0·17	8·17
Iron	0·51	0·55	0·60	0·55	0·05	9·09
Zinc	0·90	1·00	0·82	0·91	0·90	0·89
Potassium	0·50	0·20	0·22	0·30	0·17	56·7
Copper	0·52	0·61	0·41	0·51	0·10	19·6
Sodium	1·00	0·51	0·52	0·68	0·28	41·2

[a] SD = standard deviation.
[b] CV = coefficient of variation.

levels as illustrated by the high coefficients of variation (42·2, 56·7 and 41·2%, respectively).

The TRS, glucose and fructose were present in small amounts (Table 4). Starch (amylose) values ranged from 6·32 g/100 g DM in TPL 237 to 7·88 g/100 g DM in TPL 323. Only glucose and TRS values showed appreciable varietal differences as indicated by the coefficients of variation (32·8 and 38·3%, respectively).

Results from the three varieties suggest that their haemagglutinins are glycoproteins. This corroborates earlier reports (Sharon & Lis, 1972; Liener, 1974; 1980) that most of the known haemagglutinins, with the exception of concanavalin A (and possibly also the haemagglutinin of the garden pea), are glycoproteins. However, the associated available carbohydrates (with

TABLE 4
Carbohydrate Content of Haemagglutinins from Different Varieties of Lima Beans (g/100 g DM)

Carbohydrates	*Varieties*					
	TPL 13	*TPL 237*	*TPL 323*	*Mean*	$\pm SD^a$	CV^b *(%)*
TRS	1·41	2·61	3·25	2·43	0·93	38·3
Glucose	0·80	0·43	0·51	0·58	0·19	32·8
Fructose	0·50	0·46	0·60	0·52	0·07	13·5
Starch (amylose)	7·50	6·32	7·88	7·23	0·81	11·2

[a] SD = standard deviation.
[b] CV = coefficient of variation.

the exception of starch) in this study were present at low levels. In all the varieties, the haemagglutinin proteins appeared generally richer in the non-essential amino acids than in the essential ones. This amino acid pattern compares favourably with an earlier report by Evans *et al.* (1973) for haemagglutinin from navy bean (*Phaseolus vulgaris*). In TPL 13 for example, whereas values for aspartic acid, glutamic acid and serine were 12·5, 9·83 and 8·18 g/16 g N, respectively, those for the sulphur-containing amino acids (methionine and cystine) were 1·23 and 2·28 g/16 g N, respectively. Values of 1·23 and 1·08 g/16 g N have been reported for methionine and cystine, respectively, in the whole bean by Ologhobo (1980), while Evans *et al.* (1973) have reported the methionine level to be as low as 0·30% in navy bean haemagglutinin. The relative preponderance of the metal ions, Mn^{2+} and Ca^{2+}, may have to do with their requirements for carbohydrate binding and agglutinating activities of the haemagglutinins (Yariv *et al.*, 1968; Howard *et al.*, 1971). Although there was no regression analysis, it appeared that the varieties with the higher specific haemagglutinating activity (Table 1) also had higher Mn and Ca contents (Table 3).

With the exception of some of the minerals (Mg, K and Na) and alcohol-soluble sugars (TRS and glucose), the amino acid compositions of the haemagglutinins did not show distinct varietal differences. However, since only three of the numerous varieties of lima bean have been studied, such a generalization remains tentative.

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