Anti-nutritional constituents of six underutilized legumes grown in Nigeria

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Abstract

Six underutilized legume seeds grown in Nigeria namely, red and white lima beans, brown and cream pigeon pea, African yam bean and jackbean were analysed for different anti-nutritional factors. Sojasapogenol B was identified as the predominant sapogenol in lima beans and jackbeans by capillary gas chromatography. The content of total inositol phosphates and individual inositol phosphates (IP6, IP5, IP4 and IP3) were analysed by ion-pair HPLC, being in the range of other legumes. Trace quantities of lupanine were identified as the alkaloid in jackbean. α-Galactosides were present in all the legume seeds, stachyose being the predominant galactoside in lima beans, African yam bean and jackbean, and verbascose in pigeon pea. The haemagglutinating activity was estimated as a measure of the lectin content of the samples. African yam bean was found to have the highest heamagglutinating activity. Tannins were found to be in low quantities. The presence of these anti-nutrients in relation to the nutritional value of the legume is discussed.   © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Legume seeds are a major source of cheap protein in the diets consumed by a large segment of the human population in Nigeria [1]. Food legumes have twice as much protein as cereals but their biological value is limited by the presence of anti-nutritional factors.

Despite the large number of existing grain legumes, the most commonly consumed are common beans (*Phaseolus vulgaris*), soya bean (*Glycine max*) and cowpea (*Vigna unguiculata*). Accordingly, the compositional evaluation of these seeds has been carried out [2–4]. Lima beans (*Phaseolus lunatus*),

pigeon pea (*Cajanus cajan*), African yam bean (*Sphenostylis stenocarpa*) and jackbean (*Canavalia ensiformis*) are less commonly utilized. Apata and Ologhobo [5] and Oshodi et al. [6] reported the nutritional composition of lima beans, pigeon pea, jackbean and African yam bean.

Recently, considerable interest has been focused on the utilization of these relatively neglected legumes for human food and livestock feed components. However, feeding protein rich legume seeds to monogastric animals is hindered by the presence of anti-nutritional factors. Anti-nutrients commonly found in plant foods as saponins, tannins and phytate have been shown to reduce the availability of nutrients and cause growth inhibition. Some of them contribute to flatulence production in consumers.

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Others such as alkaloids and lectins can be toxic for human and animal nutrition [1].

This paper is aimed at providing a comprehensive picture of the sapogenol B, alkaloids, total inositol phosphates, total α-galactosides, lectins and tannins present in the raw seeds.

2. Experimental

Raw plant seeds were purchased from open markets in Uromi and Igueben areas of Edo State, Nigeria. The dry seeds under investigation were: (i) lima beans (*Phaseolus lunatus*); red and white variety (ii) pigeon peas (*Cajanus cajan*); brown and cream variety (iii) African yam bean (*Sphenostylis stenocarpa*); (iv) jackbean (*Canavalia ensiformis*).

Healthy bean seeds were dried to constant mass at 70°C. The seeds were cooled in a desiccator and milled to pass through a 100-mesh sieve (Tecator cyclotec 1093). The bean flour was stored in airtight plastic containers at 4°C until needed.

2.1. Analysis of anti-nutritional factors

2.1.1. Saponins
Bean flour was extracted as described by Ayet et al. [7]. The defatted flour (3 g) was extracted with methanol for 30 h and the extract purified by SiO_2–C_{18} flash chromatography. The column was sequentially eluted by distilled water (75 ml) and methanol (75 ml) under pressure. The methanol fraction was evaporated to dryness and made up to 3 ml for analysis. The saponins were hydrolysed, trimethylsilyl derivatives prepared [8], and analysed in a Perkin-Elmer (Autosystem) gas chromatograph. The quantitative determination of sapogenol was carried out with a calibration curve using soyasapogenol B obtained from hydrolysis of soyasaponin I as described by Cuadrado et al. [8]. The standard soyasaponin I was isolated from seeds of *Pisum sativum* as described in Ayet et al. [9].

Qualitative analyses by thin-layer chromatography (TLC) were done by two chromatographic systems: (a) reversed-phase C_{18} bonded to silica gel (20×20 cm, 200 μm, Whatman), methanol–water (3:2); and (b) silica gel 60F 254 (20×20 cm, 250 μm, Merck), chloroform–methanol–water (65:35:10 lower layer).

Both plates were developed at room temperature for a distance of 15 cm in a tank and air-dried plates were sprayed with a mixture of *p*-anisaldehyde–glacial acetic acid–concentrated sulfuric acid reagent (1:100:2).

Soyasaponin I was used as standard.

2.1.2. Inositol phosphates
Inositol phosphates were extracted and determined as described by Burbano et al. [10]. The extracts were passed through a strong anion-exchange (SAX) column (quaternary amine bonded silica, 500 mg; Varian, Harbor City, CA, USA). The eluent was determined by ion-pair reversed-phase high-performance liquid chromatography (HPLC) in a Beckman System Gold instrument [10]. Two extractions were made for each sample and one injection for each extract.

2.1.3. Alkaloids
Alkaloids were extracted as described by Muzquiz et al. [11]. 2.0 g of defatted bean flour were homogenised with an Ultraturrax homogeniser for 1 min in 20 ml 5% trichloroacetic acid and centrifuged at 700 *g* for 5 min. The extraction was repeated twice and the supernatants decanted while the precipitate was discarded. The alkaloid in the sample extract was extracted with dichloromethane and analysed in a Perkin-Elmer Autosystem gas chromatograph with an SPB-1 capillary column (30 m×0.25 mm I.D.; Teknokroma, Bellefonte, PA, USA), a phosphorus–nitrogen detection (PND) system and Turbochrome for instrument control and data analysis. Codeine was used as an internal standard. The injector and detector were at 240°C and 300°C, respectively. The initial oven temperature was 150°C with a temperature ramp of 5°C/min to 235°C and final hold time of 15 min at 235°C.

TLC was done by applying 20 μl of sample onto Whatman silica gel LK6DF (20 cm×20 cm, 250 μm). The plates were developed with chloroform–cyclohexane–diethylamine (6:4:1). Dragendorff's reagent was used for visualisation and when the plates were dried they were sprayed with Bouchardat's reagent.

2.1.4. Total oligosaccharides
Total oligosaccharide were extracted from bean

flour as described by Muzquiz et al. [12]. 0.5 g bean flour was homogenized in aqueous ethanol (80%, 5 ml) for 1 min at room temperature using an Ultraturrax homogeniser. The mixture was centrifuged for 5 min at 700 *g*, the supernatant decanted and the procedure repeated twice.

The sample extract was deionized using Dowex (Dow Serva Chemical) 50WX 8 (200–400 mesh) and QMA minicolumns using a vacuum system (Supelco, Bellefonte, PA, USA). The eluent was evaporated to dryness, redissolved in deionized water and determined by HPLC using a Beckman System Gold instrument [12]. Two extractions were made for each sample and one injection for each extract.

2.1.5. Lectins

The haemagglutinating activity was estimated as a measure of the lectin content of the samples using the method of Grant [13]. Two hundred mg of bean flour was homogenised with an Ultraturrax homogenizer in 1 ml of ice cold phosphate-buffered saline (PBS), pH 7.4 for 1 min. The extract was centrifuged (4300 *g*, 4°C) and the supernatant removed.

The haemagglutinating activity of the seed extracts were evaluated with native rat and rabbit erythrocytes and trypsin treated rat erythrocytes [14].

2.1.6. Tannins

Tannins were determined spectrophotometrically by the acidified vanillin method [15] using tannic acid as the tannin standard for analysis. The background colour of the sample with the vanillin reagent was measured to avoid the over estimation of tannins

[16]. The tannin was determined in duplicate with good agreement.

2.2. Statistical analysis

The data were analysed for variance using the BMDP-7D analysis of variance (ANOVA) program [17] and the mean values compared using Duncan's multiple range test.

3. Results and discussion

Although many methodologies have been introduced and applied to the study of anti-nutrient compounds in plants, relatively few of these sensitive and reliable methods have been used in the analysis of tropical legumes. Considerable interest has been aroused on the utilization of these relatively neglected legume sources for human food and as livestock feed component. Taking this into account the main purpose of this paper was to apply precise chromatographic methodologies to evaluate the anti-nutritional composition in such materials.

The saponin levels determined as soyasapogenol B are shown in Table 1. The method used in this study is accurate and precise [18] enabling us to obtain purified saponin extracts free from artifacts found with other techniques. Qualitative determination of saponins using TLC showed that lima beans and jackbean seeds contained soyasaponin I by co-chromatography with authentic soyasaponin I standard. The use of the two chromatographic systems gave a better identification of the saponins with a R_F value

Table 1
Sapogenol B, alkaloids, inositol phosphates, tannins, α-galactosides and lectins of raw seeds of lima beans, pigeon pea, African yam bean and jackbean

Samples	Sapogenol B (g/kg)[c]	Total inositol phosphate (mg/100 mg)[c]	Alkaloids (mg/100 mg)[c]	Total α-galactoside (mg/100 mg)[c]	Lectin HU (mg/100 mg)[c]	Tannins (mg/100 mg)[c]
Lima beans (red)	1.268±0.004	0.475±0.029	ND	3.372±0.002[a]	0.075±0.028[a]	0.140±0.001[a]
Lima beans (white)	1.391±0.002	0.377±0.012[a]	ND	3.628±0.085[a,b]	2.749±0.709[b]	0.080±0.001[b]
Pigeon pea (brown)	ND	0.322±0.032[a]	ND	2.346±0.232	0.085±0.021[a]	0.140±0.001[a]
Pigeon pea (cream)	ND	0.349±0.034[a]	ND	3.517±0.079[a,b]	0.085±0.021[a]	0.080±0.001[b]
African yam bean	ND	0.148±0.013[a]	ND	3.844±0.011[b]	5.556±1.389	0.140±0.001[a]
Jackbean	0.489±0.004	0.299±0.013[a]	0.041±0.002	2.830±0.034	1.701±0.340[a,b]	0.090±0.002[d]

Means of the same column followed by the same superscript are not significantly different at 5% level by Duncan test.
[c] Values are means±standard error of two determinations.

of 0.24 in chromatographic system A and 0.11 in the system B. Other substances were resolved on the TLC plates but were not identified due to lack of appropriate saponin standards. They could be mono-desmosides saponin [19] as reported in lentils. The chromatographic system A revealed widely separated saponins with a R_F value of 0.65. This could also be bidesmosidic saponin [19] as in lentils. The application of fast atom bombardment (FAB) mass spectrometry (MS) would be necessary to identify these compounds.

Gas chromatography (GC) of lima beans showed two peaks corresponding to sapogenols A and B with retention times (t_R) of 24 min and 22 min, respectively. Sapogenol B was the predominant sapogenol being minimum the amount of sapogenol A. Jackbean seeds showed only one peak corresponding to sapogenol B with a retention time of 22 min. Price et al. [19] reported the absence of saponins in jackbean (*Canavalia ensiformis*) but in the present study, TLC and GC showed that jackbean contains saponins although in low levels. The results for lima beans are lower than those reported by Osagie et al. [20] using similar methodology. This difference could be due to origin and genotype differences of lima beans.

Sodipo and Arinze [21], reported that saponin levels of beans (*Phaseolus vulgaris*), guinea corn (*Sorghum vulgare*), millet (*Pennisetum typhoideum*) and groundnut (*Arachi hypogea*) grown in Nigeria to have a considerable amount of saponin, 0.245, 0.073, 0.195 and 0.049 g/kg dry mass basis, respectively. Achinewhu [22] also reported the saponin content of some Nigerian oil seeds. Their methodology gave over estimated results for the saponin levels in these seeds.

Total and individual inositol phosphate content of the raw bean samples is shown in Table 2. The total inositol phosphate of the different varieties of bean varied from 0.148 mg/100 mg in African yam bean to 0.475 mg/100 mg in red lima bean. There were significant differences between red lima beans and the other bean samples, which were not significantly different. The lower inositol phosphates (IPs) were present in very small quantities. IP3 was absent while IP6 had the highest content in the seeds. The results obtained are lower than that reported by Harland et al. [23] in lima beans and Akpapunam and Sefa-Dedeh [24] in jackbean. The method of Burbano et al. [10] used for the analyses of bean samples has a greater sensitivity compared to the methods utilized by these authors. The additional advantage of the HPLC method is the ability to differentiate inositol hexaphosphate and inositol penta phosphate from the lower inositol phosphates. Only IP6 and IP5 appear to affect the bioavailability of minerals [25]. The occurrence of high levels of IP6 in legumes is of nutritional significance because of its ability to bind minerals and form complexes with proteins, which may inhibit peptic digestion [26]. The total inositol phosphate of the legume seeds studied was found to be in the range of other legumes [10]. Total inositol phosphate levels were lowest in African yam bean than the other legumes studied.

Alkaloids were absent from the seeds except in jackbean. The alkaloid was identified by co-chromatography with an authentic standard of lupanine on TLC plates. Capillary GC revealed lupanine as the alkaloid in jackbean with a value of 0.041 mg/100 mg. The identity of this alkaloid was unequivocally confirmed by its mass spectral degradation pattern and comparison with the literature {MS: *m/z* 248

Table 2

Individual and total inositol phosphate in the raw seeds of lima beans, pigeon pea, African yam bean and jackbean (mg/100 mg)[a]

Samples	IP3	IP4	IP5	IP6	Total
Lima beans (red)	ND	ND	0.032±0.004	0.443±0.026	0.475±0.029
Lima beans (white)	ND	ND	0.026±0.001	0.350±0.011	0.377±0.012
Pigeon pea (brown)	ND	ND	0.033±0.003	0.289±0.029	0.322±0.032
Pigeon pea (cream)	ND	0.007±0.002	0.052±0.004	0.289±0.029	0.349±0.034
African yam bean	ND	0.001±0.000	0.009±0.001	0.139±0.013	0.148±0.013
Jackbean	ND	0.005±0.001	0.053±0.004	0.242±0.012	0.299±0.013

[a] Values are means±standard error of two determinations.

$[M]^+$ (32), 136 (100), 149 (52), 98 (28), 150 (34), 110 (12), 84 (12)} [27]. This confirms the presence of this alkaloid in the seed. The possible biological activity of lupanine was reported for lupin seeds [28], affecting the use of lupins for human and animal nutrition. Since jackbean seeds showed only trace quantity of lupanine this should not negatively affect the nutritional value of jackbean.

Total α-galactosides varied from 2.346 mg/100 mg in brown pigeon pea to 3.844 mg/100 mg in African yam bean. Table 3 shows the main α-galactosides found in these seeds are raffinose, stachyose and verbascose. Stachyose was the predominant sugar in lima bean, jackbean and African yam bean as occurs in some common legumes (i.e., lupins, beans, chickpeas and lentils). However, lupins showed twice as much stachyose as found here [29]. Revilleza et al. [30] also reported stachyose as the principal oligosaccharide in lima bean and jackbean. Similarly to faba bean and peas, verbascose appeared as the main oligosaccharide in pigeon pea. Raffinose oligosaccharides in legumes produce flatulence in man and animals [31].

The lectin contents ranging from 0.075 mg/100 mg haemagglutinating units (HU) in red lima beans to 5.556 mg/100 mg HU in African yam bean are given in Table 1. These results are similar to those recorded for pigeon pea [32] and lima beans [33]. Aletor and Aladetimi [34] reported no detectable levels of haemagglutinating activity in African yam bean seeds. In this study, haemagglutinating activity was measured using different types of mammalian erythrocytes to give consistent detectable haemagglutinating activity. Akpapunam and Sefa-Dedeh [24] and Grant et al. [14] reported high haemagglutinating activity of jackbean seeds with rabbit and

trypsin treated rat erythrocytes. This study reports lower values. Kakade et al. [35] observed very significant quantitative differences when the haemagglutinating activity in extracts from different soybean cultivars were compared. Apart from the ability of lectins to cause marked alterations in certain pancreatic and hepatic enzymes [36,37], growth depression also occurs [14].

The tannin contents of the seeds studied are shown in Table 1. The darker coloured seed coats are significantly different and higher in tannin content than the lighter coloured seed coat. Varietal differences in lima bean tannins and pigeon pea was reported by Ologhobo [38] and Singh [39], respectively, showing that brown or red pigeon pea contained twice the tannin levels of white pigeon pea with values varying greatly from 0.3 to 18.3 g/kg. Our results show tannin content ranging from 0.080 mg/100 mg in white lima beans to 0.140 mg/100 mg in red lima beans. These results are in good agreement with those of Ene-Obong [40] and Osagie et al. [20] in pigeon pea and African yam bean. Tannins are reported to interact with protein and reduce its net value. The levels of tannins obtained in these legume seeds are low and are not likely to be of any nutritional significance. Chang and Fuller [41] suggested that tannin in plants does not affect the nutritional potential unless at very high levels i.e., over 10% or more of the dry mass.

The nutritional significance of these anti-nutrients in the raw grain legume is important because Nigerians depend heavily on legume protein because animal protein is expensive. The utilization of sensitive and reliable methods for the study of these anti-nutrients has helped to produce a comprehensive picture of the different anti-nutritional factors that

Table 3
Composition of α-galactosides in the raw seeds of lima beans, pigeon pea, African yam bean and jackbean (mg/100 mg)[a]

Samples	Sucrose	Raffinose	Stachyose	Verbascose	Total
Lima beans (red)	0.806±0.021	0.297±0.016	2.829±0.077	0.246±0.063	3.372±0.002
Lima beans (white)	0.770±0.018	0.277±0.016	3.157±0.112	0.194±0.011	3.628±0.085
Pigeon pea (brown)	1.186±0.149	0.423±0.057	0.857±0.065	1.067±0.110	2.346±0.232
Pigeon pea (cream)	1.666±0.012	0.620±0.003	0.335±0.006	1.562±0.070	3.517±0.079
African yam bean	1.090±0.003	0.664±0.002	2.863±0.036	0.317±0.027	3.844±0.011
Jackbean	1.443±0.260	0.284±0.012	2.298±0.038	0.248±0.016	2.830±0.034

[a] Values are means±standard error of two determinations.

could limit the beneficial utilization of the legume seeds.

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