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BIOCHEMICAL COMPOSITION AND NUTRITIONAL POTENTIAL OF *UKPA*: A VARIETY OF TROPICAL LIMA BEANS (*PHASEOLUS LUNATUS*) FROM NIGERIA – A SHORT REPORT

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Limitations of protein supplies make the hunt for new protein sources and their quality evaluation necessary. *Ukpa* seeds, a variety of lima beans (*Phaseolus lunatus*), was biochemically evaluated. Analysis on fresh weight bases gave crude protein content of 23.17 g/100 g, true protein value of 19.57 g/100 g and appreciable amounts of total carbohydrates (71.14 g/100 g) and minerals comparable to commonly consumed staples. Crude fat content was 0.21 g/100 g while those of total sugar and starch were 4.48 and 67.72 g/100 g respectively. Levels of trypsin inhibitor, phytic acid and oxalate were not striking but comparable with other conventional food sources. However, polyphenols were not detected in the seeds, which could be a nutritional advantage. While marginally limiting in S-containing amino acids (Cyst + Meth) (score 90.4), other indispensable amino acids (IAA) were at higher than adequate concentrations and easily satisfied the recommended requirements. It was concluded that *ukpa* seeds represent a potential source of protein and energy that could alleviate shortages, and efforts towards the domestication, multiplication and conservation of this indigenous variety should be pursued.

INTRODUCTION

As common with most developing nations of the world, the state of nutrition in Nigeria is still characterised by inadequate calorie and protein supplies [FAO, 2004]. The growth rate of food production continues to lag behind population growth rate with adverse consequences for food security. The increasing pressure of population and predicted food shortages are creating a demand for new food sources either: for direct use as a food or animal feed ingredient or as a raw material for seed protein and oil extraction.

Protein foods and particularly animal protein have continued to be in short supply. Most experts consider protein deficiency as the commonest form of malnutrition in the developing countries, especially in regions where diets are mainly based on roots and tuber crops [Pelletier *et al.,* 1995]. The production of protein-rich foods (leguminous seeds and particularly animal products) has been much less efficient. The solution to the food problem has to be sought through a combination of all available sources. Therefore, in an attempt to widen the narrow food base, food and agricultural scientists are screening lesser known and under-exploited native plants for possible potential sources of food [Ezeagu & Ologhobo, 1995; Vietemeyer & Janick, 1996; Murray *et al*., 2001]. Recent reports have revealed that quite a large number of lesser-known native crop species are high in nutrients and many could possibly become advanced crops in the future if given priority and research resources [Nordeide *et al*., 1996; Lalas & Tsaknis, 2002]. One such crop identified in this context is *ukpa*, a variety of tropical lima beans (*Phaseolus lunatus)* found in Nigeria. Lima bean, though a minor crop, has been an important source of plant protein to millions of Nigerian. The *ukpa* variety is characterised by large rhomboid-shaped flattened seeds. It has white cotyledons and the white seed coat bears black streaks radiating from the dark hilum giving it a zebra-skin coloration.

In view of the urgent need for new or unusual sources of food, especially high quality protein, efforts at popularization of *ukpa* are therefore justified. But prior to utilization of such novel resources data indicating the nutrient composition, antinutritional factors and toxicants and the methods of processing that will enhance their utility as food or feed ingredient are all necessary in order to achieve optimal utilization. This study therefore seeks to elucidate further the nutrient and antinutrient composition and nutritive quality of this variety of lima beans and to compare them to those of commonly consumed food grains.

MATERIALS AND METHODS

About 1 kg of the *ukpa* seeds were collected from Igbo--Ukwu village market in Anambra State of Nigeria. A soy bean sample (TGX 1660-15F), obtained from IITA, Ibadan, was included for comparison. The seeds were ground to flour using a Wiley Mill with the 0.5 mm mesh sieve and stored at –4°C until analysis. Moisture content was determined by drying at 110°C using an oven (T 6030, Heraeus Instruments, Hanau, Germany) until reaching weight constancy (at least

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for 24 h). Total nitrogen (N) was determined by the standard micro-Kjeldahl method [AOAC, 1990] using a digestion apparatus (Kjeldatherm System KT 40, Gerhardt Laboratory Instruments, Bonn, Germany) and a titration system (T110-TR160-TA-TM120, Schott-Geräte GmbH, Hofheim, Germany). Crude proteins content was calculated by multiplying percentage N by factor 6.25. Crude lipid content was assayed by extraction with petroleum ether (b.p. 40-60°C) in a Soxhlet extractor [AOAC, 1990]. Carbohydrate content was calculated by difference. Gross energy was determined by the use of adiabatic bomb calorimeter (IKA-Calorimeter C4000, Janke & Kunkel, IKA Analysentechnick, Heitersheim, Germany).

Total soluble sugars and starch were determined by the combined methods of Duboise *et al.* [1956] and Kalenga *et al.* [1981]. Dietary fibre was determined by the enzymatic-gravimetric method as outlined by AOAC [1990]. Trypsin inhibitor activity was determined using benzoyl-DL-arginine-p-nitroanilide as a substrate [Kakade *et al*., 1974], and total polyphenols were determined colorimetrically by the Folin-Denis method [AOAC, 1990]. The presence of alkaloids was qualitatively detected by the combined methods of Seaforth [1964] and Hultin & Torssell [1965]. To this end, 0.5 g of sample was extracted in 20 mL of 1.0% aqueous HCl, and 5 mL of the extract were tested with Mayer's reagent [Seaforth, 1964]. The strength of the precipitate obtained was visually compared to that resulting from strychnine solution (0.1 mg/100 mL). Ash, phytate and oxalates were estimated by the methods of AOAC [1990], Davies & Reid [1979] and Baker [1952], respectively. Minerals were determined by wet digestion and using an atomic absorption spectrophotometer (BUCK Scientific, 200A).

Amino acid analyses were carried out according to the recommendations in the Report of the Joint FAO/WHO Expert Consultation [FAO/WHO, 1991] as previously described [Petzke *et al*., 1997]. Using the method of Carpenter [1960] as modified by Booth [1971], available lysine was estimated as fluorodinitrobenzene-reactive lysine. The *in vitro* protein digestibility (IVPD) was assayed by the multi-enzyme technique of Hsu *et al*. [1977]. Dietary fibre was determined by the enzymatic-gravimetric method as outlined by AOAC [1990].

For fatty acid analysis, 10 mg of aliquot of the lipid extract were transmethylated with trimethylsulfonium hydroxide (TMSH) according to Schulte & Weber [1989]. The fatty acid methyl esters (FAME) were analysed in a gas-liquid chromatograph (Hewlett-Packard GmbH, Waldbronn, Germany) equipped with a flame-ionization detector as previously described [Ezeagu *et al*., 1998].

TABLE 1. Biochemical composition of *ukpa* seed meal as compared to soybean (f.w.b)[1].

Specification	*Ukpa*	*G. max*
Proximate composition (g/100 g)		
Moisture	2.70	6.61
Total N	3.71	6.19
Amino acid N (%)	84.42	91.9
Crude protein	23.17	38.7
Crude protein corrected[2]	19.57	35.50
Crude fat	0.21	20.1
Ash	2.78	4.60
Carbohydrates	71.14	22.12
Total dietary fibre	18.40	22.80
Total sugars	4.48	8.36
Starch	67.72	18.44
Available lysine (g/100 g protein)	5.47	5.3
Available lysine as% total lysine	85.26	78
In vitro protein digestibility (%)	73.79	75.05
Gross energy, kJ (kcal)/100 g	1665 (396.43)	2256 (537.14)
Minerals (mg /100 g)		
Sodium	182.4	10.0
Potassium	817.4	192.0
Calcium	95.3	260.0
Magnesium	111.5	320.0
Phosphorus	nd	750.0
Iron	3.20	7.0
Copper	1.03	nd
Zinc	3.29	nd
Manganese	3.26	nd

f.w.b.: Fresh Weight Bases; [1]Mean of two independent determinations; [2]Using correction factor (Cf) based on the amino acid N content according to the formula: (N x 6.25)/(Cf), where Cf = 100/(percentage N determined as amino acid N); nd: Not determined.

RESULTS AND DISCUSSION

The biochemical composition (fresh weight bases) of *ukpa* seeds are presented in Table 1. The crude protein content (23.17 g/100 g) though lower than that of soybean (38.7 g/100 g), but appreciably higher than those of cowpea, maize and some commonly cultivated staples [Kadwe *et al*., 1974; Nwokolo, 1987; FAO, 1982]. Similar crude protein levels of 20.4–30.3 g/100 g have been reported for some other varieties of lima beans [Apata & Ologhobo, 1994; Offei *et al*., 2003; Granito *et al*., 2007; Bello-Perez *et al*., 2007]. Crude protein, fat and total carbohydrate levels were also similar to and within the ranges of 17.75–23.99 and 0.66–1.27 reported for a wide variety of beans [Barampama & Simard, 1993].

It must be noted, however, that crude protein analysis conventionally based on multiplying the total nitrogen (N) (Kjeldalh N) by a factor of 6.25 has been suspect. This factor leads to overestimation due to the fact that a good percentage of the total N content is of non-protein origin [Ezeagu *et al*., 2002]. Some secondary compounds, such as alkaloids, chlorophyll, and certain glycosides contain nitrogen. Various other non-protein nitrogenous compounds such as nitrates, alkaloids, B-vitamins, nitrogenous lipids, ammonium salts, glycosides and nucleic acids could add up to the level of non-protein N [McDonald *et al*., 1981; Smith, 1987]. Also amino acids of non-protein origin such as α- β-diamino-propionic acid or canavanine and 3,4-diphenylhydroxlamine (L-DOPA) were found in significant amounts in several seeds of leguminous family [Evans & Bandemer, 1967; Mohan & Janardhana, 1994; Ezeagu *et al*., 2003]. Therefore, based on amino acid analysis of *ukpa* seed protein, only 84.42% of the total N is of amino acid or protein origin. A correction factor was therefore derived for the crude protein to obtain the true protein content of *ukpa* seed value of 19.57 g/100 g.

Crude fat content of 0.21 g/100 g was not comparable to soyabean and lower than the range of 1.3-2.3 g/100 g reported for some lima bean varieties [Bello-Perez *et al*., 2007; Granito *et al.,* 2007; Apata & Ologhobo, 1994] and a range of 0.66-1.27 g/100 g reported for several other food grains [Barampama & Simard, 1993]. Ash content of 2.78 g/100 g suggests a mineral level that could be suitable for animal feed. Higher ash contents of 4.14-4.4 g/100 g occurred in some lima beans [Bello-Perez *et al*., 2007]. Mineral analysis revealed potassium (817.4 g/100 g) to be the most abundant mineral and appears higher than levels in other cultivated staples [Apata & Ologhobo, 1994; FAO, 1982]. Total sugar (4.48g/100 g) was lower but the starch content (67.72 g/100 g) was much higher than the 18.44g/100 g value recorded for soybean and a range of 31.8–38.0 g/100 g reported for some lima bean varieties [Bentancur-Ancona *et al*., 2004; Bello-Perez *et al*., 2007, Granito *et al*., 2007]. The high level of starch in the *ukpa* seed qualifies it as a good source of industrial starch, and possibly, slow-release carbohydrates with potential benefits for human health [Bello-Perez *et al*., 2007]. Also the total carbohydrate and gross energy contents of 71.14 g/100 g and 1665 kJ/100 g, respectively, shows that *ukpa* seeds could be a good source of energy and a useful supplement for scarce cereals in food and animal feeding. *In vitro* protein digestibility (IVPD) of *ukpa* seeds (73.79%) was on the same level with that of soybean (75.05%). While similar IVPD of 72.4% was reported by Bentancur-Ancona *et al*. [2004], Egbe & Akinyele [1990] reported a lower value of 43% for lima bean; indicating that digestibility may be dependent on varieties. Total dietary fibre was 18.40 g/100 g and similar value of 19.3 g/100 g was reported by Granito *et al*. [2007] for lima bean.

Levels of antinutritional factors, trypsin inhibitors, oxalates and phytic acid, were not striking (Table 2) but at a similar level with those reported for commonly consumed food grains [Siddhuraju *et al*., 1996; Apata & Ologhobo, 1994]. Polyphenols were not detected in the seed which could be of nutritional advantage. Polyphenols (more especially tannins) are known to inhibit the activities of digestive enzymes and are present in most lima bean varieties [Offei *et al*., 2006; Granito *et al*., 2007]. Content of alkaloid, qualitatively compared to a standard strychnine solution (0.1 g/100 mL), showed occurrence of alkaloid (Table 3) to be strong but, however, lower than the level in the soybean sample. Antinutritive and toxic factors widely contained in plant-derived foodstuffs could lower digestibility and biological value of proteins [Krupa, 2008]. However, several processing techniques, such as cooking, soaking and germination are employed to reduce or eliminate antinutritional factors and toxicants in legumes resulting in improved digestibility and nutritive value of seed proteins [Badifu, 2001; Ihimire *et al*., 2004; Ezeagu, 2006].

The results of amino acid profile (Table 4) revealed a balanced complement of indispensable amino acids (IAAs), limiting marginally only in total S-amino acids (cysteine+methionine) (score of 90.4). Methionine seems to be the widely reported limiting amino acid in lima beans [Meredith & Thomas, 1982]. Other IAAs are higher than adequate concentrations when compared with the recommended pre-school age (2-5 years) reference protein and adult requirements [FAO/WHO, 1991]. The total IAAs in *ukpa* seed protein was at the same level with that of *G. max* and

TABLE 2. Contents of some antinutritional components in *ukpa* and *G. max* seeds (g/kg)[1].

Antinutritional components	*Ukpa*	*G. max*
Polyphenols	nd	2.20
Phytic acid	0.077	0.099
Total oxalate	0.69	0.66
Soluble oxalate	0.51	0.41
Soluble as% of total oxalate	74	62.1
Trypsin inhibitor (TIU/mg)	29.7	26.35

[1]Mean of two independent determinations; nd: not detected.

TABLE 3. Standard alkaloid test.

	Strychnine soln (0.1 g/100 mL)	*Ukpa*	*G. max*
Strength of precipitate:	+	+++	++++

Explanations: +: weak; +++: very strong; ++++: exceedingly strong.

TABLE 4. Amino acid profile of *ukpa* seed (g /100 g protein)[1].

Specification	*Ukpa*	*G. max*	FAO/WHO [1991]	
			Child (2-5 yrs)	Adult
Lysine	6.42	6.75	5.80	1.60
Methionine	1.01	1.10		
Cysteine	1.25	2.30		
Total S-amino acids	**2.26**	**3.40**	**2.50**	**1.70**
Isoleucine	5.06	4.69	2.80	1.30
Leucine	8.30	8.11	6.60	1.60
Phenylanine	6.05	5.34		
Tyrosine	4.24	4.24		
Total aromatic amino acids	**10.29**	**9.58**	**6.30**	**1.90**
Threonine	4.30	4.10	3.40	0.90
Tryptophan	1.38	1.60	1.10	0.5
Valine	5.58	5.07	3.50	1.30
Histidine	2.74	2.72	1.90	1.60
Total IAAs	**46.33**	**46.02**	**33.90**	**12.70**
Proline	4.90	6.82		
Alanine	4.34	4.43		
Serine	6.56	5.38		
Glutamic acid	14.54	19.98		
Aspartic acid	12.51	12.45		
Arginine	6.42	7.98		
Glycine	4.14	4.69		

[1] Mean of two independent determinations.

higher than those of the reference proteins. As common with most seed proteins, glutamic and aspartic acids (14.54 and 12.51 g/100 g, respectively) were most abundant dispensable amino acids. The fairly high concentration and wide spectrum of amino acids and the high level of IAAs and available lysine (5.47 g/100 g protein) present in *ukpa* seed protein qualify it as a potential supplement for the bulk of low protein starchy tubers and cereals consumed locally to yield diets of good nutritional value. *Ukpa* seed proteins, with high S-amino acid, could also enrich local diets containing conventional pulses which are limiting in methionine+cysteine. With 78% of the total lysine available and high tryptophan level, *ukpa* seed could also be suitable for protein extraction and for supplementation of cereal-based weaning diets [Jansen, 1977].

Data on the fatty acid composition of crude oil, as shown in Table 5, indicate linoleic (7.13) and oleic (6.87 g/100 g oil) acids as the most abundant fatty acids, making up 23.42 and 22.56% respectively of total fatty acids. On the other hand, palmitic acid (5.57 g/100 g oil) is the predominant saturated acid. High proportion of linolenic acid, associated with high iodine value, has been reported in some varieties [Korytnyk & Metzler, 2006]. However, in view of the very low seed oil content, these fatty acids are not likely to be of any nutritional significance in *ukpa* diets.

Taking account of the overall nutrient quality, *ukpa,* could be promoted for wider diffusion and adoption as an alternative cheap source of protein and could improve a balanced diet. This can alleviate food shortages and provide extra income for the rural populations where it is grown. Moreover, this variety is likely to be better adapted to the local and marginal soil environment, requiring less agricultural inputs than most exotic grain legumes. Efforts towards the domestication, multiplication and conservation of this indigenous lima beans variety should therefore be pursued.

TABLE 5. Fatty acid composition of *ukpa* seed oil (g/100 g oil)[1].

Fatty acids	*Ukpa*	*G.max*
Myristic $C_{14:0}$	0.58	nd
Palmitic $C_{16:0}$	5.57	10.49
Palmitoleic $C_{16:1n-7}$	3.98	nd
Hexadecadienic $C_{16:2n-4}$	nd	1.96
Stearic $C_{18:0}$	1.62	3.25
Oleic $C_{18:1n-9}$	6.87	22.32
Oleic (isomer) $C_{18:1n-7}$	1.16	1.57
Linoleic $C_{18:2n-6}$	7.13	44.32
Linolenic $C18:_{3n-6}$ γ	nd	0.46
Linolenic $C_{18:3n-3}$ α	2.39	5.66
Arachidic $C_{20:0}$	0.20	0.33
Gadoleic $C_{20:1n-9}$	nd	0.24
Docosahexaenoic $C_{22::6n-3}$	nd	nd
Behenic $C_{22:0}$	0.48	0.41
Lignoceric $C_{24:0}$	0.47	nd
Total[2]	30.45	90.98
Sat[3]	8.92	14.48
U/S ratio[4]	2.41	5.28

[1]Mean of two independent determinations; [2]Sum of fatty acids; [3]Sum of all saturated fatty acids; [4]Sum of unsaturated / Sum of saturated; nd: not detected.

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