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Functional and Anti-Nutritional Properties, *in-vitro* Protein Digestibility and Amino Acid Composition of Dehulled *Afzelia africana* Seeds

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Abstract. Analysis of *Afzelia africana* seed flour showed that the seeds possessed high water absorption capacity (128.31%), good oil absorption capacity (588.49%) and fairly good emulsion property (35.25%). However, it had the least gelation concentration (6.00% w/v) and foaming properties (8.00%, 3.00%). Anti-nutritional factors were very low, with the highest being phytate (13.59%) and tannin the least (0.43%). Total amino acid composition was 796.6 mg/g protein. Essential amino acids (48.5%) were in high proportion with *in-vitro* digestibility of 71.5%.

Keywords: protein digestibility, amino acids, *Afzelia africana*

Introduction

The inadequate supply of protein-food in Nigeria and other developing countries has led to protein-energy malnutrition especially in regions where diets are derived mainly from starchy roots and tuber crops (Petzke *et al*., 1997); high demand has made protein food less affordable and almost out of reach of the common man (Akanji, 2002). One of the ways to overcome this problem is the exploitation of non-conventional and cheap legume trees of forests (Becker, 1986).

Apata and Ologhobo (1994) observed that under-utilized crops contained useful amount of nutrients for body development. However, before these crops can be utilized and incorporated into any food system, it is necessary to carry out studies so as to know their nutritional potential.

Afzelia africana is a savannah crop belonging to the family Leguminosae and fruits between December and March. The seeds are ellipsoid in shape, about 18-30 mm long, glossy black in colour and arranged transversely in grooves at the middle of the pod (Keay *et al*., 1964); they contain high amount of protein. This study was undertaken to determine the functional properties, amino acid composition, anti-nutritional properties and multi-enzyme digestibility of dehulled seeds of *A. africana*.

Materials and Methods

Preparation of legume flour. Mature dry seeds of *A. africana* were collected from Ikere-Ekiti, Ekiti State, Nigeria and the seeds were further dried for two weeks and later oven-dried for 2 h at 70 °C. Dehulling was done manually immediately after the seeds were removed from the oven. The dehulled cotyledons were pulverised and stored in clean plastic containers for further analysis.

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Water absorption and oil absorption capacity of the sample were determined as described by Beuchat (1977). Emulsion capacity was determined by the method of Yatsumatsu *et al*. (1972). Modified procedure of Coffman and Garcia (1977) was used for determining gelation property, foaming capacity and stability. For the protein solubility of the sample, 200 mg of the sample were dissolved in 5 mL distilled water; pH was adjusted to the desired value with 0.1 M HCl/NaOH. Then it was centrifuged and protein content of the supernatant was determined by MicroKjeldahl method.

The method of Young and Graves (1940) was employed for phytic determination while the method of Makkar and Goodchild (1996) was used for the determination of tannin content. Oxalate content was determined using the method of Day and Underwood (1986) while cyanide was determined using the method described in AOAC (1990). For thioglucoside determination, 10 g defatted sample was weighed in a conical flask and 250 mL distilled water was added. The mixture was hydrolysed at 54 °C for 1 h, then boiled for 2 h keeping the volume constant (using a condenser) and filtered; the filtrate was retained and the residue was washed thrice with 50 mL hot water. The washings were added to the initial filtrate, the volume was made up to 600 mL, heated and excess 5% barium chloride solution was added to precipitate barium sulphate. The solution was left in the water bath for about 2 h before filtering using a suction pump. The residue retained was gently scrapped with a spatula into a pre-weighed crucible, oven-dried and ashed in a muffle furnace at 600 °C for 4 h. Thioglucoside percentage was calculated as:

$$\text{Thioglucoside (\%)} = \frac{\text{molecular weight of thioglucoside} \times \text{weight of } BaSO_4}{\text{molecular weight of } BaSO_4 \times \text{weight of sample}} \times 100$$

Determination of *in-vitro* protein digestibility was carried out using the method of Hsu *et al*. (1977). pH of 50 mL of suspension of the sample in distilled water (6.24 mg sample per mL) was adjusted to 8.0 with 0.1 M HCl and/or 0.1 M NaOH, while stirring in a water bath at 37 °C. The multi enzyme solution consisting of 1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg peptidase per mL was maintained in an ice bath and pH was adjusted to 8.0 with 0.1 M HCl and/or 0.1 M NaOH. 5 mL sample of the multi enzyme solution was added to the sample suspension with constant stirring at 37 °C. pH of the suspension was recorded after 15 min of addition of the multi enzyme solution. The *in-vitro* digestibility was calculated using the regression equation of Hsu *et al*. (1977).

$$Y = 210.46 - 18.10\,x$$

where:

Y is *in-vitro* digestibility (%) and
x is the pH of the sample suspension after 15 min digestion with the multi-enzyme solution.

The amino acid profile of the known sample was determined using methods described by Sparkman *et al*. (1958). The known sample was dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and loaded into the Technicon sequential multi-sample amino acid analyser (TSM).

Results and Discussion

Functional properties. The result of the functional properties of dehulled *A. africana* seed flour is shown in Table 1.

Water absorption capacity. Water absorption capacity (WAC) of *A. africana* seed flour is 128.31% (Table 1), which is comparatively lower than that reported by Oshodi *et al*. (1999) for benniseed (182.00%) and quinoa (147%) but higher than that of pearl millet (115%). It is also higher than that of melon seed (12%), reported by Olaofe *et al*. (1994), but comparable with the value of soy flour (Lin *et al*., 1974).

Table 1. Functional properties and *in-vitro* digestibility of *A. africana* seed flour (%) (mean, n=2)

Functional properties	%
Foaming stability	3.5
Foaming capacity	8.50
Water absorption capacity (g/g)	128.31
Oil absorption capactity (g/g)	588.49
Least gelation capacity (w/v)	6.00
Emulsion capacity	35.25
Emulsion stability	51.50
In-vitro digestibility	71.5

The comparative value of the WAC of *A. africana* and soy flour suggests that the former can find application as a functional ingredient like soy bean flour. The high WAC of *A. africana* flour makes it useful as a soup thickener just like melon seeds (*Citrullus vulgaris*).

Oil absorption capacity. The oil absorption capacity (OAC) of *A. africana* flour (588.49%) is higher than the values reported for most legumes such as melon seed (122.00%; Olaofe *et al*., 1994), pigeon pea flour (89.70%; Oshodi and Ekperigin, 1989), sun flower flour (207%; Lin *et al*., 1974) and *Cucumeropsis vulgaris* (302%; Ige *et al*., 1984). The capacity of a protein to absorb fat had been attributed to the physical entrapment of oil by numerous non-polar side groups of the protein (Kinsella, 1976). The result of OAC of *A. africana*, therefore, shows that it is an excellent flavour retainer and can find application in products where flavour retention is employed.

Least gelation concentration. The least gelation concentration (LGC) of *A. africana* seed flour (6.00%) is lower than the values reported by Oshodi and Ekperigin, (1989) for pigeon pea (12%) and Fagbemi and Oshodi (1991) for full-fat fluted pumpkin (36% w/v). The value is, however, comparable to that reported by Oshodi and Adeladun(1993) for the brown species of lima bean (8.00% w/v). The ability of protein to form gel provides a structural matrix for holding water, flavour, sugars and food ingredients and thus is useful in food application and in new product development, thereby providing an added dimension to protein functionality (Oshodi *et al*., 1997). The low LGC of *A. africana* seed flour shows that it is a poor gel-forming material.

Emulsion capacity and stability. The emulsion capacity and stability of *A. african* are 35.25% and 51.50%, respectively (Table 1). The emulsion capacity figure is lower than the values reported by Oshodi *et al*. (1999), for benniseed (63.00%), pearl millet (89.00%) and quinoa (104.0%) but higher than the values reported for soy bean flour (18%) and wheat flour (7-11%) by Lin *et al*. (1974). The emulsion stability after 5 h was 51.50%.

Foaming capacity and stability. The foaming capacity of the sample was 8.50% (Table 1). This value is lower than those reported for soy bean flour (66%) and pigeon pea (68%) by Lin *et al*. (1974) and Oshodi and Ekperigin (1989), respectively, but compares favourably with those of quinoa flour (9.0%) and dehulled full-fat seed flour of *Adenopus breviflorus* (8.03%) reported by Oshodi *et al*. (1999) and Oshodi (1992), respectively.

The foaming stability of 30 min (3.5%) was lower than that of soy flour (14.6%) and pigeon pea flour (20.0%) (Oshodi and

Ekperigin, 1989), but comparable to values reported by Fagbemi and Oshodi (1991) for full-fat fluted pumpkin seeds (5.0%) and quinoa (2.0%) reported by Oshodi *et al*. (1999).

A. africana seed flour showed minimum foaming capacity after 30 min and collapsed within 60 min.

Protein solubility. The result of the variation of solubility of *A. africana* seed flour with pH is depicted in Fig. 1. The minimum protein solubility was at pH 4.0, which may correspond to the isoelectric point of the protein (Ogungbenle, 2003). The pH observed for minimum protein solubility is lower than the value reported by Oshodi *et al*. (1999) for pearl millet and quinoa flours (pH 6.0) and benniseed (pH 5.0), but higher than the value reported for white melon (pH 3.0) by Ogungbenle (2006). It is however equal to the value for fluted pumpkin seed (pH 4.0) (Fagbemi and Oshodi, 1991). Minimum protein solubility of *A. africana* in the acid region of pH indicates its potential usefulness in the formulation of carbonated beverages (Kinsella, 1976) and low-acid foods.

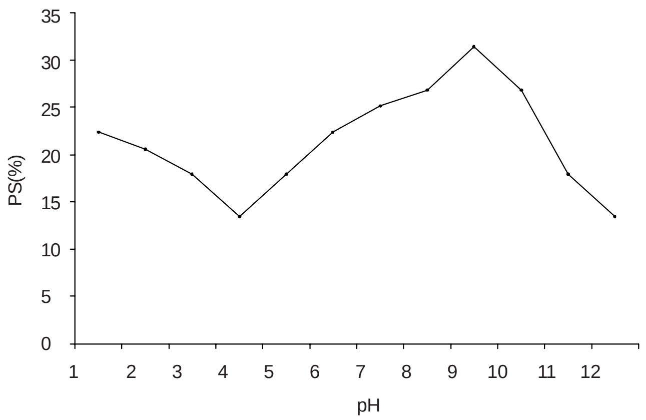

Fig. 1. Variation of protein solubility (PS) of *A. africana* flour with pH.

Anti-nutritional properties. Table 2 presents the result of the phytochemical constituents of *A. africana* seed flour. Phytate composition was the highest (13.59%) while tannin composition had the least value (0.43%); oxalate, cyanide and thioglucoside were 2.84%, 2.19% and 3.10%, respectively. The value of tannin is lower than that of cooked wall nut (2.33%) reported by Ogungbenle (2009) which indicates that it requires no supplementation during consumption.

The value of thioglucoside (3.1%) was lower than that reported for Ife Bimpe variety of cowpea (10.4%), comparable with the IT84E-124 variety (3.15%) but higher than those of Ife Brown (2.69%), K59 (2.39%) and TVX716 (0.67%) (Aletor and Aladetimi, 1989).

Table 2. Anti-nutritional constituents of *A. africana* seed flour

Component	%
Phytate	13.59
Thioglucoside	3.10
Oxalate	2.84
Cyanide	2.19
Tannin	0.43

It has been reported that heat-unstable components such as protease inhibitors and lectins or heat-stable factors, phytate and tannin, are the most important seed constituents which may modify the availability of amino acids (Phillips, 1993). The high content of such factors in *A. africana* seed will reduce its digestibility and bioavailability. Consumption of oxalates (for example, the grazing of animals on oxalate-containing plants such as greasewood) may result in kidney disease or even death due to oxalate poisoning.

Amino acid composition. Table 3 presents the amino acid composition of *A. africana* seeds in mg/g protein. Glutamic acid (141.1 mg/g protein) and aspartic acid (95.0 mg/g protein) were the most abundant amino acids in *A. africana*. Arginine was the most concentrated essential amino acid (66.5 mg/g protein) followed closely by leucine (64.0%). The methionine content (13.8 mg/g protein) of *A. africana* is higher than that reported by Yuwai *et al.* (1991) for soybean (14.0 mg/g protein).

Table 4 shows the amino acid score of the essential amino acids of *A. africana*. The limiting amino acid was threonine (0.74). Adeyeye (1997a) also reported that threonine was

Table 3. Amino acid composition of *A. africana* seed

Amino acid	Concentration (mg/g protein)	FAO/WHO reference values
Lysine	55.7	43.2
Histidine	30.0	-
Arginine	66.5	-
Aspartic acid	95.0	-
Threonine	29.5	28.8
Serine	35.1	-
Glutamic acid	141.1	-
Proline	32.7	-
Glycine	28.6	-
Alanine	34.6	-
Cystine	12.6	-
Valine	41.6	43.2
Methionine	13.8	23.0
Isoleucine	38.7	43.2
Leucine	64.0	49.0
Tyrosine	30.2	28.0
Phenylalanine	46.9	28.8

Table 4. Amino acid score of *A. africana*

Amino acid	Suggested level (mg/g crude protein)	Amino acid score
Isoleucine	40	0.97
Leucine	70	0.91
Lysine	55	1.01
TSAA	35	0.75
Phenyl+Tyr	60	1.29
Threonine	40	0.74
Valine	50	0.83
Total	350	-

Phenyl+Tyr = phenylalanine plus tyrosine.

found to be limiting in four samples of African yam bean. Histidine and Arginine which are particularly essential for children (FAO/WHO/UNU, 1985; Harper, 1984) are present in *A. africana* in fairly good quantity (Table 3). This suggests that *A. africana* can be employed in food formulation for children.

Total amino acids (TAA) were 796.6 mg/g protein (Table 5); the value is higher than that of kamilin faba bean (117 g/kg protein) reported by Salem (2009). Total essential amino acids (TEAA) in the sample with and without histidine is 48.5% and 41.6%, respectively. The implication of this is that *A. africana* may contribute significantly to the supply of EAA in diets. Total essential amino acids without histidine (386.7 mg/g protein) were lower than that of soya bean (444 mg/g protein, Yuwai *et al*., 1991) and benniseed (443.3 mg/g protein; Ogungbenle, 2006).

The percentage of total neutral, acidic and basic amino acids were 51.3%, 29.6% and 19.1%, respectively, suggesting that the protein is acidic in nature.

Table 5. Essential, non-essential, acidic, basic and neutral amino acids of *A. africana* seed

Amino acid	Proteins mg/g percent
Total amino acids	796.6
Total non-essential amino acids	409.9
Total essential amino acids with histidine	386.7
Total essential amino acids without histidine	331.0
Total acidic amino acids	236.1
Total basic amino acids	152.2
Total neutral amino acids	408.3
Total essential amino acids with histidine	48.5
Total essential amino acids without histidine	41.6
Total acidic amino acids	29.6
Total basic amino acids	19.1
Total neutral amino acids	51.3
Total non-essential amino acids	51.5

In general, the amino acid analysis of *A. africana* seeds reveals that it will meet the requirements of infants and school children for protein since the comparison between the amino acid content of *A. africana* and amino acid reference values of FAO/WHO/UNU (1985) (Table 4) show that arginine, histidine, phenylalanine, threonine and leucine are on the high side. It would also meet the amino acid requirements of adults since the amino acid composition which meets the requirement of a child would meet that of an adult (FAO/WHO/UNU, 1985).

***In-vitro* digestibility.** The *in-vitro* digestibility of the roasted *A. africana* seed flour (Table 1) (71.5%) is slightly lower than those reported for raw and heat-treated samples of pigeon pea (77% and 84%, respectively) by Oshodi and Hall (1993), and all six different colour varieties of raw, dehulled African yam bean reported by Adeyeye (1997a). It is, however, comparable to the raw, hulled samples (Adeyeye, 1997b) except the white variety (74.1%) where the difference is about 2%. The comparison shows that the *in-vitro* digestibility of roasted *A. africana* after 15 min is lower than that of the six different colour varieties of African yam bean (both hulled and dehulled samples) reported by Adeyeye, (1997a). This could be due to the phytate and tannin content of the seed. The graph of the variation in pH with time is shown in Fig. 2.

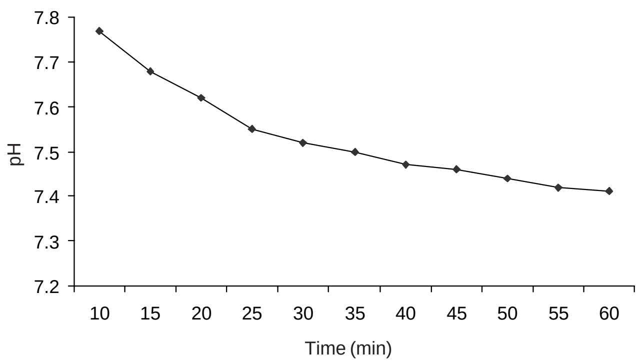

Fig. 2. The pH *vs* time curve obtained by incubating *A. africana* with the multienzyme system.

The pH drops rapidly for the first 25 min of the hydrolysis. The difference between successive pH at 5 min interval was smaller between 25 min and 60 min of the hydrolysis.

Conclusion

It can be concluded that *A. africana* has better nutritive potential and high proportion of essential amino acids useful for both children and adults and also has low levels of anti-nutritional factors.

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