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Nutritional and Antinutritional Composition of Adenopus breviflorus Benth Seed Protein Isolate

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Abstract: *The nutritional and anti nutritional composition of Adenopus breviflorus benth seed flour and protein isolate were studied. The protein isolated was found to have 95.13% protein while the seed flour has 30.44% protein, the percentage fat, fibre, ash in the seed flour are 52.63%, 3.33% and 3.11% respectively. Fat was not detected in the isolate while the ash 1.09%, fibre 1.03% are lower for the isolate than the value obtained for the full fat flour. The mineral composition ranged from 0.39 to 164.94mg/100g for the full fat flour with phosphorus being the most concentrated while the mineral composition of the isolate range from 0.07 to 95.15% with potassium being the most concentrated. The Na/K ratio (0.74) of the isolate was lower than the 1.0 recommended. The amino acids values for the flour and protein isolate range between 10.17-196.30mg/100g and 10.50-143.87mg/100g respectively. The most concentrated amino acid in the flour is leucine (78.47mg/100g) and arginine (111.11mg/100g) the most concentrated amino acid in the isolate. The percentage TEAA and % TNEAA for the flour and isolate (50.63), (49.38) and (50.37 and 49.63) respectively. The P-PER and P-PV for the flour are 2.7 and 33.05 respectively while for the isolate are 2.67 and 23.44. The limiting amino acid for the flour and the isolate are meth+cystine and valine based on the egg amino acid scoring pattern. The anti nutritional factor studied; phytate, tannin, saponin, alkaloid, flavanoid and cyanide in the isolate were very low and constitute no nutritional significance. These studies have shown that Adenopus breviflorus flour is a good source of protein and the protein is of a very high quality. It also revealed that the isolate can be used in supplementing cereal based diet.*

Keywords: *Adenopus breviflorus benth seed, protein isolates, nutritional composition, anti-nutritional composition*

I. Introduction

Plant proteins play a significant role in human nutrition, particularly in developing countries where average protein intake is less than that required. Because of inadequate supplies of food proteins, there has been a constant search for unconventional legumes as new protein sources for uses in both functional food ingredients and nutritional supplements [22]. Bridging the gap between increasing food consumption and production is among the most challenging task round the globe especially in developing countries[16].

Generally, there are two main sources of protein, that is, from animal and plant. Provision of adequate animal protein is difficult due to high cost and changing consumer's attitude towards animal based protein.

Consumers are more conscious in their food selection which is due to growing awareness about nutritional dependent ailment. An alternative for improving protein intake of the people is to supplement the diet with plant protein. For that reason, consumption of plant protein isolate with special reference to legumes is beneficial[16;14]. The use of plant protein isolate as functional ingredients to improve the nutritional quality of the product or for economic reason is very extended. Nevertheless, their application in the food trade are almost limited to protein from soybeans seeds, whereas, other vegetable proteins are less used[28]

Amongst these are those from Adenopus Breviflorus Benth seed which is underutilized.

Adenopus breviflorus benth seed is an oil seed commonly found in the savanna forest region of southern Nigeria. Adenopus breviflorus belongs to the family of cucurbitaceaea. The family is moderately large and it consists of 110 genera and 640 species[8;23] reported the nutritional and functional properties of the protein concentrates. This paper aims at reporting the nutritional and antinutritional composition of Adenopus Breviflorus Benth seed protein isolate.

II. Materials And Methods

Source of materials: denopus breviflorus benth seed was obtained from the local market in Edo state, Nigeria.

The seeds were sorted manually to remove stones, damages and immature seeds after which it was milled and the fat was extracted with hexane in a soxhlet extractor for nine hours at room temperature to remove the fat content.

2.1 Preparation of Protein Isolate
The Chart Showing The Preparation Of Protein Isolates

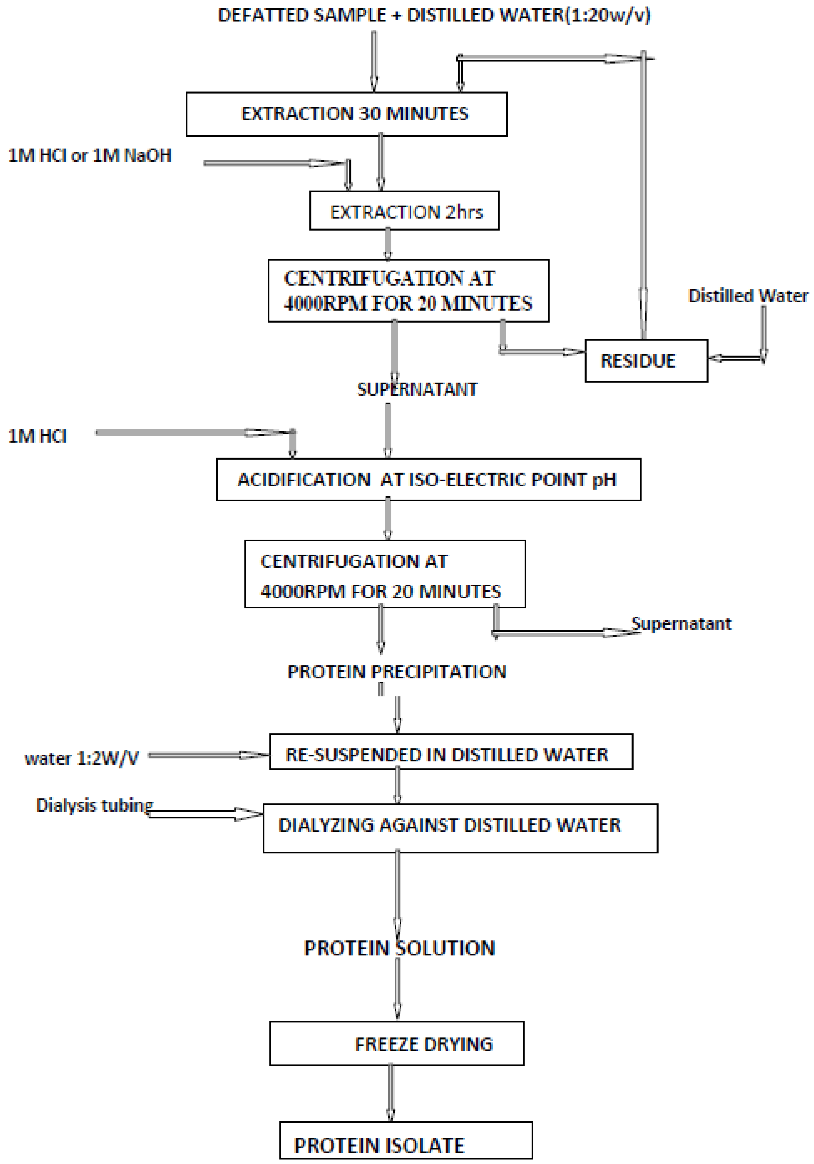

Fig 1. Chemical analyses- the following analyses were carried out on both the flour and isolate.

2.2 Proximate analysis: The flour and isolate were analysed for moisture, ash, crude protein, crude fat, and fibre according to[3]. The carbohydrate was determined by difference.

2.3 Mineral element

The mineral element of flour and isolate were carried out using the dry ashing procedure. The total ash was digested in 10%HClv/v which was followed by analysis using atomic absorption spectrophometer. Sodium and potassium was determined using flame photometer.Amino acid profile was determined using gas chromatography. The sample extractor was dried by the modified method of [3].The amino acid obtained was used to evaluate the protein quality of the seed flour and the isolate. Predicted biological value (BV) was calculated using regression equation of Morup and Olesen,1976 as reported by [7].

2.4 Anti-nutritional Composition

Phytic acid was determined using the method of Young and Greeves 1940. Tannin content by modifying the procedure of [15]. Oxalate content was determined by Day and Underand (1986), total cyanide was carried out using standard method of [3]. Determination of saponin was done by the method described by Obadoni and Ochuko (2001), flavanoid by Boham and Kocipal-Abyazan (1994) and alkaloid by Harborne (1993). In-vitro multi enzymes protein digestibility of the flour and isolate were determined following the procedure of [13]. Statistical analysis: all determinations are carried out in triplicates, errors recorded as standard deviations from the mean. Data were subjected to analysis of variance using spss15 computer programme, while means were separated using new Duncan multiple range test . significance was accepted at 5% level of probability.

III. Results And Discussion

Table 1: Proximate Composition of the Full-fat seed flour and Protein Isolate. (%)

Parameters	Full fat	Isolate
Moisture content	$4.05^{b}±0.09$	$2.58^{a}±0.04$
Fat	52.63±0.33	ND
Crude protein	$30.44^{a}±0.19$	$95.31^{b}±0.02$
Ash	$3.33^{b}±0.11$	$1.08^{a}±0.11$
Crude fibre	$3.15^{b}±0.03$	$1.03^{a}±0.06$
Carbohydrate	6.40±0.31	ND

Values with different superscript on the same row are significant ($p \leq 0.05$)

The result of the proximate composition of the flour and protein isolate for A. breviflorus is presented in table one. The value of moisture content of both the flour (4.05±0.01) and isolate (2.58±0.04) are lower than the 10% recommended for the stability of flour and this indicate good shelf -life characteristic. The high protein content of the flour (30.44±0.19%) showed that it is a good source of protein. This result compares favourably well with the report of Ogundele and Oshodi, 2010 on three varieties of Lagenaria siceraria with protein content of (27.71, 32.72 and 34.64)%. These also indicate that A. breviflorus could contribute significantly to alleviating the problem of protein malnutrition in the third world and developing countries. The high fat content (52.6±0.33) suggest that it is an oil seed. The fat content is higher than those obtained from Cucumeropsis Manni (42.29%,), Cucumeropsis Edulis (40..26%), Cucumis Sativa (45.21%) as reported by Abiodun and Adeleke, 2010. The ash content (3.3%) and crude fibre (3.15%) are in smaller quantities. On isolation, the protein content increased significantly (95.31±0.02)%. No fat and carbohydrate were determined. The high protein content of the isolate showed that they can find use in both food and feed formulations.

Table 2: Mineral Analysis of the Full fat flour and The Protein Isolate (mg/100g)

MINERALS	FULL FAT	ISOLATE
IRON	12.32^{b} ± 0.81	1.46^{a} ± 0.04
CHROMIUM	ND	ND
NICKEL	ND	ND
COPPER	0.39^{a} ± 0.54	0.28^{a} ± 0.02
COBALT	ND	ND
MANGANESE	0.40^{b} ±0.30	0.07^{a} ± 0.02
ZINC	21.32^{b} ± 0.30	3.63^{a} ± 0.01
LEAD	ND	ND
CALCIUM	120.53^{b} ± 0.62	25.16^{a} ± 0.08
MAGNESIUM	125.06^{b} ± 0.11	18.51^{a} ± 0.02
POTASSIUM	129.62^{a} ± 0.77	116.60^{a} ± 0.09
SODIUM	151.26^{b} ± 0.31	86.43^{d} ± 0.04
PHOSPHORUS	164.94^{b} ± 0.05	95.13^{a} ± 0.03
CADMIUM	ND	ND
Na/K	1.17	O.74
Ca/K	0.73	0.26
Ca/Mg	0.96	1.36

Values with different superscript on the same row are significant ($p \leq 0.05$)

The mineral composition of the flour and the isolate are presented in TABLE 2. The result revealed potassium to be the most abundant mineral in the protein isolate and phosphorus in the seed flour. Iron, zinc and copper were of low values while Cr, Ni, Co, Pb and Cd were not detected. Though isolation reduced the values of these mineral elements, the values of K, P and Na are still relatively high. The ratio of Na/K of less than one recorded for the isolate suggest that they will be very suitable for hypertensive patient.

Table 3: Amino Acid Profile of the Full fat seed flour and the Protein Isolate. (mg/g)

Amino acids	Full fat	Isolate
GLYCINE	57.33[a] ± 0.16	68.18[b] ± 0.08
ALANINE	59.70[b] ± 0.10	44.30[a] ± 0.05
SERINE	41.73[a] ± 0.15	43.80[b] ± 0.05
PROLINE	33.60[a] ± 0.10	46.60[b] ± 0.05
VALINE	28.40[a] ± 0.20	36.42[b] ± 0.08
THREONINE	36.01[b] ± 0.01	31.73[a] ± 0.08
ISOLEUCINE	39.10[b] ± 0.10	38.39[a] ± 0.03
LEUCINE	78.47[b] ± 0.31	65.30[a] ± 0.05
ASPARTIC ACID	76.53[a] ± 0.12	106.82[b] ± 0.08
LYSINE	54.33[b] ± 0.15	52.40[a] ± 0.02
METHIONINE	10.17[a] ± 0.06	12.01[b] ± 0.02
GLUTAMIC ACID	196.30[b] ± 0.10	143.87[a] ± 0.04
PHENYLALANINE	64.50[b] ± 0.10	50.50[a] ± 0.02
HISTIDINE	23.17[a] ± 0.06	28.40[b] ± 0.02
LEUCINE	78.47[a] ± 0.31	111.11[b] ± 0.01
ARGININE	68.43[b] ± 0.15	23.59[a] ± 0.04
TYROSINE	59.10[b] ± 0.10	10.57[a] ± 0.04
CYSTINE	35.18[a] ± 0.08	111.11[b] ± 0.01

Values with different superscript on the same row are significant ($p \leq 0.05$)

Table 4: Amino Acid Summary of the Full fat Flour and the Protein Isolate.

Amino acid	Full fat	Isolate
TOTAL A.A.(T.A.A.)	942.09[b] ± 1.51	913.99[a] ± 0.30
T. ESSENTIAL A.A. (T.E.A.A)	476.86[b] ± 0.92	460.41[a] ± 0.19
T.E.A.A./T.A.A(%)	50.63[b] ± 0.01	50.37[a] ± 0.01
TOTAL NON-ESS A.A. (TNEAA)	465.23[b] ± 0.59	453.58[a] ± 0.12
TN.E.A.A / T.A.A. (%)	49.38[b] ± 3.03	49.63[a] ± 0.01
T. SURPHUR A.A. (CYST + METH)	25.35[b]	22.58[a]

	± 0.09	± 0.04
% CYSTINE T.S.A.A.	59.88[b] ± 0.08	46.79[a] ± 0.11
T. AROMATIC E.A.A. (PHE+TYR)	123.60[b] ± 0.10	74.09[a] **± 0.06**
TOTAL ACIDIC A.A. (GLU + ASP)	272.83[b] ± 0.06	250.69[a] ± 0.12
T. BASIC A.A (LYS + HIS)	77.50[a] ± 0.17	80.80[b] ± 0.01
T. NEUTRAL A.A. (TNAA)	591.76[b] ± 1.28	582.50[a] ± 0.19
RATIO T.E.A.A: T.N.E.A.A.	1.02[a] ± 0.01	0.99[a] ± 0.01
% T.A. A.A.	28.96[b] ± 0.04	27.43[a] ± 0.06
% B.A.A.	8.23[a] ± 0.01	8.84[b] ± 0.01
T. AROMATIC E.A.A. (PHE+TYR)	123.60[b] ± 0.10	8.84[a] ± 0.01
TOTAL ACIDIC A.A. (GLU + ASP)	272.83[b] ± 0.06	250.69[a] **± 0.01**
T. BASIC A.A (LYS + HIS)	77.50[b] ± 0.17	0.78[a] ± 0.01
% T.N.A.A.	62.81[a] ± 0.03	63.73[b] **± 0.01**
T.E.A.A./ T.N.A.A.	0.81[a] ± 0.01	0.78[a] ± 0.01

Values with different superscript on the same row are significant ($p \leq 0.05$)

The amino acid profile, amino acid score and summary of the amino acids of the seed flour and the protein isolate are shown in TABLES 3, 4 and 5.

The major abundant major abundant amino acids in both the flour and protein isolate is glutamic acid and aspartic acid while the least is methionine and cystine, the observation is consistent with the majority of other plant protein. Glutamic acid was the highest in the oil seed [18] as well as in six varieties of dehulled African yam bean flour [2]. The most concentrated amino acid in both flour and isolate are Leucine and Arginine respectively. Arginine is an essential amino acid for children growth [17;18] the total essential amino acid for the flour (476.86mg/g) and isolate (460.42mg/g) are lesser than the value reported for soya flour (503mg/g) protein and whole egg protein (506mg/g)(Paul et al, 1980). But the percentages ratio of TEAA/TAA of (50.63) flour and (50.37) for the isolate compete favourably those values obtained for dehulled seed [23]. However, the values are higher than those of leaf protein A. Hybridus (39.5mg/g) reported by [2]. The % ratio of the flour and isolate were all above 39% which were considered adequate for an ideal protein for infant, 16% for children and 11% for adult [10]. The total sulphur for the flour and isolate are (25.35mg/g and 22.58mg/g) respectively. These values are comparable with the values obtained for citrullus colocynthis (21.60mg/g) and citrullus vulgaris (24.41mg/g) as reported by Ogundele 2012 but lower than (5.8g/100g) recommended for infant [27]. The total essential aromatic amino acid are 123.60mg/g for the flour and (74.09mg/g) for the isolate, these values fell within the range suggested for ideal infant protein [9] the first limiting essential amino acid for the flour is valine (40.57) and the second limiting essential amino acid is methionine+cystine (50.70) while the reverse was the order for the isolate methionine+cystine (45.16) and valine (52.03). The amino score obtained compared favorably with suggested reference standard. In most cases, the values obtained from the present study almost doubled the suggested levels. The relatively low level of methionine and cystine has been reported by other investigators [4]. The high lysine content of A. breviflorus protein is a very important attribute and because it makes the legume a significant supplementary potein with cereal based diet which is known to be deficient in lysine. The P-BV of the flour (33.09) is lower than the isolate 23.44. these values are lower than that of beach pea protein isolate (36.50-40.13) [7] and also lower than the suggested biological value for plant based protein (45) [12].

P-Per is one of the quality parameters used for protein evaluation [9]. In general, PER below 1.5 implies a protein of low or poor quality, while PER between 1.5 and 2.0 indicate an intermediate protein quality and then PER above 2.0 indicate protein of high quality (Friedman, 1996). The value of P-PER obtained for the flour is 2.7 and protein isolate (2.67) are higher than the P-PER recorded for some legumes and concentrate like

Lathyris Sativus (1.03) Aremu et al, 2007, Luffa cylindrical (1.49) Olaofe et al, 2008, Phaseolus Coccireus (1.91),robinson,1987.

Table 5: Result of the Invitro Multi Enzymes Protein Digestibility of the Full fat Flour and the Protein Isolate.

Samples	Full Fat	Protein Isolate
A. Breviflorus	**80.14[a] ± 0.01**	**95.87[b] ± 0.56**

Values with different superscript on the same row are significant ($p \leq 0.05$)

The In-vitro multi enzymes digestibility is as shown in TABLE 7

The IVPD of 80.14 for the flour is lower than the value reported for cucumeropsis manii seed flour (84.00±0.30) by Ogunbusola et al. 2013. The protein isolate (95.87%) shows higher digestibility when compared with the flour. This may be due to the presence of trypsin and chymotrypsin inhibitors and also to the globular structure of protein in the seed flour. On isolation, the protease inhibitors are removed and protein is being partially denatured which makes them more accessible to digestive enzymes[26].

Table 6: The Antinutritional Composition of the Full fat Flour and the Protein Isolate

Parameters	Full Fat Flour	Protein Isolate
Phytate mg/g	**11.74[b] ± 0.02**	**4.67[a] ± 0.02**
Tannin mg/100g	**0.14[a] ± 0.02**	**0.13[a] ± 0.01**
Saponin %	**1.32[a] ± 0.02**	**0.37[a] ± 0.03**
Oxalate mg/g	**1.33[a] ± 0.01**	**1.24[a] ± 0.02**
Cyanide mg/kg	**2.34[b] ± 0.02**	**0.66[a] ± 0.02**
Alkaloid (%)	**0.46[b] ± 0.02**	**0.14[a] ± 0.02**
Flavanoid (%)	**1.31[b] ± 0.01**	**0.26[a] ± 0.01**

Values with different superscript on the same row are significant ($p \leq 0.05$)

The anti nutritional composition of the flour and the protein isolate are presented in TABLE 6.

Generally, the anti nutrients were higher in the flour but, on isolation, they were all significantly reduced. This may be due to the processes involved in the isolation. The nutritional effect of these anti nutrients are related to the interaction with protein and minerals. Poly phenols and tannin also reduces the absorption of vitamin B12, tannin-protein complex are insoluble in water and thus decreases protein digestibility. The low level for these anti-nutrients as reported for the isolate are desirable from the functional and nutritional view point and in the preparation of high quality food product. However, the value of alkaloid (0.46±0.02 for flour, 0.14±0.02 for isolate), flavanoid (1.31±0.01 for flour, 0.26±0.01 for isolate) and saponin (1.32±0.02 for flour, 0.37±0.00 for isolate) are low and constitute no nutritional significance.

IV. Conclusion

A.Breviflorus flour and isolate are good sources of protein and the amino acids are of high qualities particularly the essential amino acids. This can enhance the proper growth of children and also meet the daily requirement of adults. The isolate can also be used as supplement in cereal based products.

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