Original Research Article

Evaluation of the Proximate and Functional Properties of Flours from Brown Variety of African Yam Bean (*Sphenostylis stenocarpa*) Seeds

ABSTRACT

Aim: To create variety of flours through food product development by checking the proximate and functional properties of flours from brown variety of African yam bean (*Sphenostylis stenocarpa*) seeds
Study Design: This study was made to fit into a one way Analysis of Variance.
Place and Duration of Study: The research was carried out at the Department of Food Science and Technology laboratory, Federal University of Technology, Owerri, Nigeria, between July 2017 and September 2018.
Methodology: Brown coloured variety of African yam bean seeds were sorted, soaked, dehulled and milled to obtain full fat flour. The full fat flour was further processed to obtain defatted flour, protein isolate and protein concentrate. The different flours was the analysed to determine their proximate and functional properties
Results: The result of the proximate composition showed that the protein isolate had a higher value of 89.18±0.23% protein composition compared to the full fat, defatted and protein concentrates which has 21.83±0.16%, 23.10±0.06% and 1.46±0.21% composition respectively. There were no significant difference ($p<0.05$) between the protein concentrate, protein isolate, full fat flour and defatted flour. The functional properties revealed high bulk density of (0.50± 0.01) for the defatted flour more than the full fat flour (0.35± 0.10) while the emulsion capacity of the protein concentrate and protein isolate flour was found to be (30.7±0.19%) and (35.32± 0.16%) respectively.
Conclusion: The proximate and functional results obtained indicate that the starches from African yam bean will have useful technological properties for many applications both in food processing and non-food applications such as in paper and textile industries. It can also be said that African yam bean represents a source of alternative protein supplement and its protein isolates possess certain characteristics which show that it could be used for protein enrichment in some food products.

Keywords: African yam bean; proximate; functional; concentrates; isolates; defatted flour.

INTRODUCTION

Legumes ranked as 3rd largest family of flowering plants having more than 19500 species and over 750 genera [1] The high protein content of varieties of legumes make them important source of protein in the diet of population groups of many countries and also a very important source of dietary protein in many West African countries including Nigeria [2]. African yam bean(AYB) is a herbaceous leguminous plant occurring throughout tropical Africa, known and called different names by different tribes in Nigeria; ijiriji or uzaaku in Igbo, Girigiri in Hausa and akpaka in Delta State. The African yam bean is highly nutritious with high protein, mineral and fibre content similar to that of some major and commonly consumed legumes. It has high metabolic energy, low true protein digestibility (62.9%) and moderate mineral content. The amino and fatty acid contents are comparable to those of most edible pulses and its economic potential has been recognized especially in reducing malnutrition among Africans [3].However,

African yam bean is underutilized and faces the danger of extinction in Nigeria due to its beany flavor, long cooling time and anti nutritional factors which affect the nutrients [4] but these limitations can be overcome by processing techniques like fermentation, soaking, roasting among others [5].The main objective of this work is to determine the proximate and functional properties of flour samples of African yam bean which include full fat flour, Defatted flour, protein isolate flour and protein concentrate flour which would be a form of dietary diversification leading to food security and sustainability in Nigeria.

2. MATERIALS AND METHODS

2.1 Source of materials

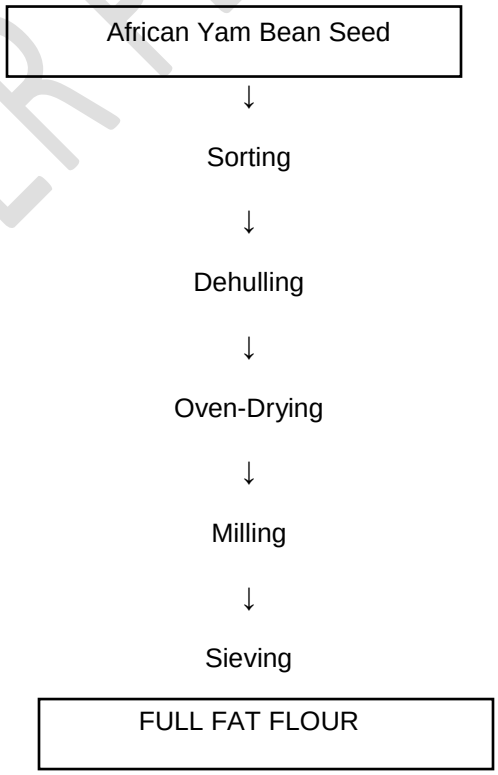
The raw mature brown (speckle) coloured variety seed of African yam bean (*Sphenostylisstenocarpa*) for this study were obtained from Mrs Onwuchikwa Glory's farm in Abia State. The equipments and chemicals used were available at Federal University of Technology (FUTO) Owerri, Imo State. All chemicals used were of analytical grade.

2.2 Sample preparation

The African yam beans were sorted manually to remove extraneous materials like dirt and spoiled seeds to obtain healthy ones.

2.2.1 Production of full fat AYB and defatted AYB flour

African yam bean seeds were soaked overnight (24hours) in water at 1:5 (W/v) ratio. The seeds were manually dehulled to separate the seed coats from the cotyledon, then dried in the oven at temperature of 30^0C for 48 hours, ground with a labouratory mill and sieved through 60mm sieve to obtain flour sample. The full fat flour was soaked in ethanol at 1:5(w/v) ratio and allowed to stand overnight at room temperature. The mixture was filtered with filteration apparatus, the fiterate which is the defatted flour was air dried for 8 hours and pulverized in a motor.



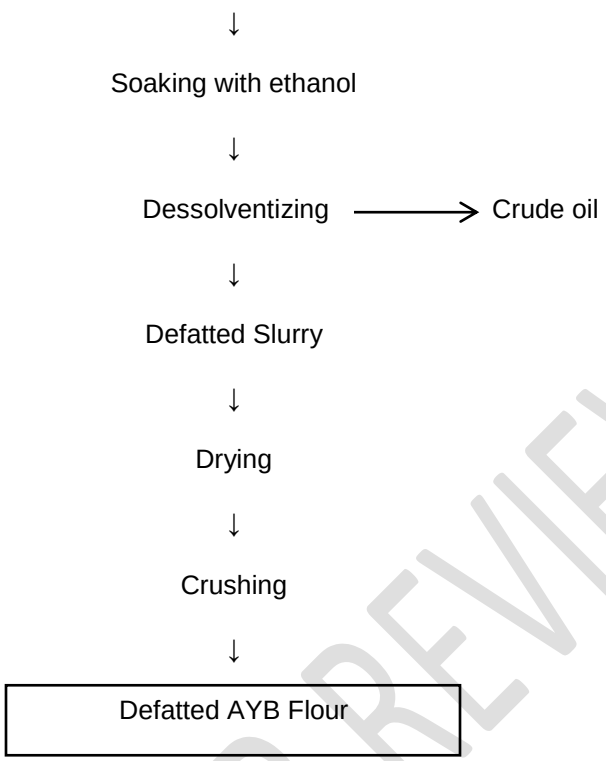
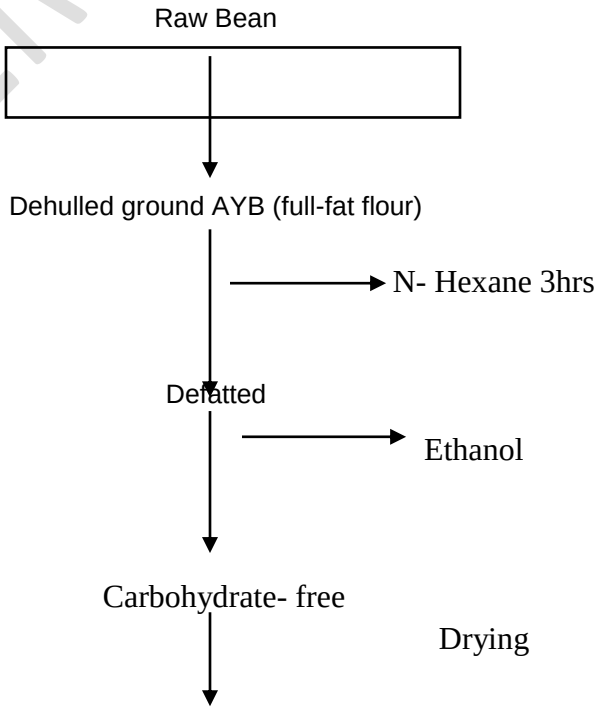


Fig 1: Flow diagram for the production of full fat AYB flour and defatted AYB flour

2.2.2 Production of AYB Concentrate

The method of [6] was employed. The method involved defatting the flour with normal hexane (soaked for 3hours and dried after sieving). The carbohydrate in the defatted flour (mainly sugars) was removed by extraction with ethanol for 30minutes. The resulting defatted, carbohydrate free concentrate was dried in the oven at 45^0C and used as the protein concentrate.



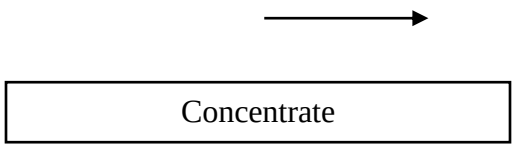
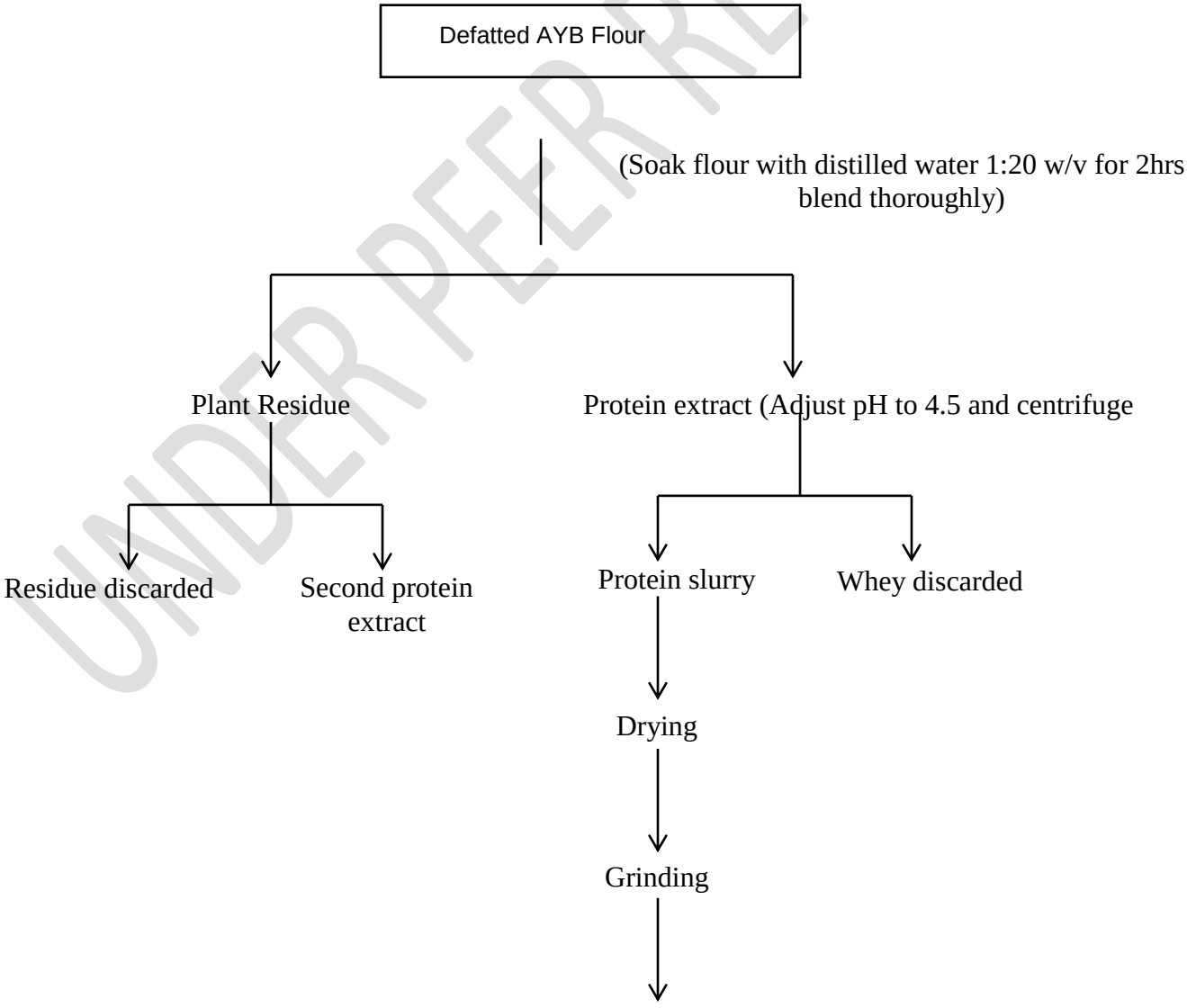


Fig 2: flow diagram for the production of protein concentrate from defatted African

2.2.3 Production of AYB Isolate

Seventy (70g) of defatted flour was added to 1400ml of water to form a 1:20 (W/v) ratio of slurry. The solution was allowed to settle for 3 hours at a pH of 6.37. The spent residue was separated from the dissolved protein extract by decanting after which centrifugation took place. The pH of the extracted protein was adjusted with HCL to its iso-electric point between 4.0 -4.3. The precipitate formed was subsequently removed by centrifugation at room temperature by removing the whey which contains soluble sugars, residual protein, peptides, salt and minor constituents. The resulted curd (protein isolate) was then dried under air using desiccators before grinding and sieving.



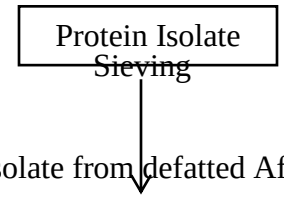


Fig 3: Flow diagram for the production of protein isolate from defatted African yam bean

2.3 PROXIMATE ANALYSIS

The proximate analysis was carried out according to the methods outlined by the Association of Official Analytical Chemists [7].

2.3.1 Moisture content

Two grams of the dried ground sample were weighed into a crucible and placed in an oven at a controlled temperature of 105^0C. The sample was allowed to dry in the oven to a constant weight.
The percentage moisture content was then expressed as the percentage of the original weight of the sample. The experiment was carried out in triplicates the percentage moisture was thus calculated:

$$\text{Percentage moisture} = \frac{(W3-W1)}{(W2-W1)} \times 100\%$$

Where W1 = weight of dried crucible
W2 = weight of dry crucible + Sample before drying
W3 = Weight of dry crucible + Sample after drying

2.3.2 Ash content

Five grams of the dried sample was measured into a crucible and placed in the muffle furnace at 550^0C until it was burnt to ash. The crucible and content were then allowed to cool in a desiccator and weighed. This was done repeatedly until a constant weight of the ash was obtained.
The percentage ash content was then expressed as percentage of the original weight of the sample on dry basis. Percentage ash content was thus calculated:

$$\%\ \text{Ash} = \left[\frac{(W3-W2)}{(w1\ \text{of sample})} \times 100\%\right]$$

WhereW1 = Weight of sample analyzed
W2 = Weight of empty crucible
W3 = Weight of crucible + Ash

2.3.3 Crude fat content

Ten (10) grams of the dried ground sample was weighed and wrapped with a clean filter paper and placed into the thimble in a soxhlet extractor. A round bottom flask was cleaned, weighed and 200mls of food grade hexane added. The flask was connected to the sample holder of the soxhlet extractor and heated slowly on a mantle for 6 hours. Refluxed hexane was recovered and the flask containing the lipid was dried in the moisture extractor in the oven at 600C for few minutes to remove any residual solvent. After drying, the flask containing the oil was cooled in a desiccator and reweighed.
By difference, the mass was determined and expressed as the percentage of the fat thus:

$$\text{Percentage (\%) Crude fat} = \left[\frac{(\text{Weight of fat})}{(\text{weight of sample})} \times 100\%\right]$$

2.3.4 Crude fibre content

Two grams (2g) of the defatted dried sample was transferred into a 100ml flask, followed by addition of 200ml of 1.25% sulphuric acid. The flask was then placed in a digest apparatus on a pre-adjusted hot plate and boiled for 30 minutes with rotation of the flask periodically to prevent solid from adhering to the

bottom of the flask. At the end of 30 minutes, the mixture was allowed to stand for one minute, and filtered immediately through the Buchner funnel lined with a muslin cloth. The insoluble matter was washed into the flask for alkali digestion using 0.3M sodium hydroxide. The digest was boiled for 30 minutes and was allowed to cool for one minute and then filtered using a muslin cloth as before. The residue was then washed successively with 0.1MHCl and finally with boiling water until it was free of acid. It was then washed twice with alcohol and thrice with ether. The residue or insoluble matter was then transferred into a crucible and dried at 105^0C in an oven to a constant weight, cooled and weighed. It was then ashed at 550^0C, cooled and weighed. The difference in weight after ashing was then calculated as the fibre content of the sample and was expressed as a percentage of the original weight. The percentage crude fiber content was thus calculated:

% crude fibre = $\frac{(w2-w3)}{W1}$x 100

Where W1 = Weight of sample
W2 = Weight of sample and crucible after drying at 105^0C
W3 = Weight of sample (as ash) and crucible after ashing

2.3.5 Crude protein content

The dried ground sample (1g) was weighed into an already dried kjeldahl flask. A few drops of water was added to the sample to moisten it, using a burette, 3ml of conc. H_2SO_4 acid was added into the flask followed by the addition of 0.5g of $CuSO_4$. The content of the flask was then digested in a fume cupboard with occasional stirring until a clear solution was obtained. The flask was allowed to cool and a small quantity of distilled H_2O added. The digest was then transferred into 100ml volumetric flask and the initial volume recorded. The mixture was shaken thoroughly to obtain a homogenous solution.

The mixture was now ready for distillation. The distillation apparatus was steamed for 30 minutes as to get rid of traces of alkali left in the flask. With the aid of a pipette, 10ml of the digest was added to the micro distillation apparatus using a funnel. 10ml of 50% NaOH solution was put in the funnel with measuring cylinder, with stopper glass rod in place. A water condenser set was connected with a 100ml conical flask used as a receiver which contained 10ml of 4% boric acid and two (2) drops of mixed indicator (bromocressol green/methyl red). The drop end of the condenser was immersed well into the boric acid. The stopper glass rod was gradually removed to allow the NaOH solution to thoroughly mix with the sample digest solution. The funnel was filled with distilled H_2O and the steam generator was closed at the top and steam passed into the distillation set. NH_3 was liberated and was distilled into 10ml 4% boric acid for 15 minutes. 50ml of the distillate of blue/green colour was collected and the drip end of the condenser was washed with distilled water into the 100ml conical flask containing the distillate. The distillate was then titrated against 0.1N hydrochloric acid till it changed to pink colour.

A reagent blank was run as a control and the protein content was then calculated by multiplying Nitrogen obtained with the factor of 6.25, expressed on dry basis. The experiment was carried out in triplicates. The formula for % crude protein is given below:

% Protein = % Nitrogen x 6.25

% N = $\left(\frac{100}{W}\right)$ x $\left(\frac{N \text{ x } 14}{1000}\right)$ x $\left(\frac{V}{Va}\right)$ T − B

Where W= weight of sample
N= Normality of titrant
Vt= volume of digest volume
Va= volume of digest analyzed
B= Blank
T= sample titre value

2.3.6 Carbohydrate content

Carbohydrate content was determined by the difference method. This was done by summing up the (% moisture, % protein, % fat, and % ash and % crude fibre) contents and then subtracting their sum from 100. It was also expressed in percentage (%).

2.4 DETERMINATION OF FUNCTIONAL PROPERTIES

The functional properties of Asparagus bean flour samples were determined using the method specified by [8] and [9].

2.4.1 Bulk Density

The method of [8] was used. Two gram of flour sample was measured into a calibrated measuring cylinder. The bottom of the cylinder was tapped repeatedly on a pad placed on a laboratory bench. Tapping was done until there was no further reduction in the volume occupied by the sample. The bulk density was determined as the ratio of the weight of the sample to its volume calculated as shown below;
Bulk density = w/v where
w = weight of sample in gram
v = volume of sample in ml

2.4.2 Water Absorption Capacity

This is determined as the weight of water absorbed and held by one gram of sample [8]. One gram of the sample was weighed and put into a test tube. 10mls of distilled water was added into the sample and mixed. The mixture was allowed to stand for 30minutes at room temperature. The mixture was centrifuge at 3500rpm for 30mins. The supernatant was decanted and measured.
Therefore WAC = v1- v2
V1= initial volume of distilled water
V2= final volume of the distilled water

2.4.3 Oil Absorption Capacity

This was determined in the same way as water absorption capacity. However, a refined vegetable oil was used in place of water and the time allowed for absorption was longer (1hour at room temperature as against 30 minutes for water). The oil absorption capacity was determined by difference, as the volume of oil absorbed and holds as 1gram of the sample shown below;
Oil absorption capacity = (initial volume of oil) – (final volume of the oil).

2.4.4 Gelation Capacity

5grams of sample was weighed into a beaker with 20mls of water and heated until gelling point. The temperature at which it gels was measured using thermometer.

2.4.5 Emulsion Capacity

The method used was described by [9]. One gram of sample was mixed with 10mls of distilled water in a test tube and shake for 30 seconds. 10mls of refined oil was also added and shake continuously until properly mixed. The test was left to stand for 30 minutes. The height of oil separated from the sample was measured. The emulsion capacity was expressed as the amount of oil emulsified and held per gram of the sample. It is shown below;
Emulsion Capacity = Emulsion height / water height x 100

2.4.6 Swelling Index

Swelling index was calculated using the method of [10]. One gram of the processed sample was weighed and dispersed into a test tube, leveled and the height noted. Distilled water (10mls) was added / stirred and allowed to stand for 1 hour. The height was then recorded and the swelling index calculated as the ratio of the final height and the initial height.
Swelling index = H2/H1 where
H2 = final height
H1 = initial height

2.4.7 Wettability

This was determined as the time in seconds taken by a unit weight (1g) of the flour sample to get completely wet on the sample of water under labouratory conditions. The method used was described by [8]. About 500mls of water was measured into a clean glass beaker (600mls capacity). With the aid of retort stand, it was arranged such that a clean test tube was clamped in an inverted position over the

water in the beaker. The clamped position was adjusted such that the distance from the mouth of the test tube to the surface of the water in the beaker was exactly 10cm. both the water in the beaker and the clamped position were marked with masking tape. Subsequently, 1 gram of the sample was weighed into the marked test tube and its mouth covered with a thumb. It was carefully inverted over the water and clamped with the retort stand at the marked spot without removing the thumb. With the stop watch set to read, the thumb was removed and the sample allowed to fall into the water surface as the stop watch was put to stop simultaneously. The flour samples were observed and the stop watch stopped as the last few samples got wet. The experiment was repeated three times for each sample and the mean values taken.

2.5 Statistical Analysis

Experimental data were analyzed using analysis of variance (Anova) and the fisher' least significant difference (LSD) was used to determine significant difference among the means at 0.05 level of confidence.

3. RESULTS

Table 1: Proximate composition of full-fat, defatted flour, protein concentrate and protein isolate

Samples	Moisture	Protein	Fibre	Ash	Fat	Carbohydrate
Full fat flour	7.83^{a} ±0.42	21.83^{b}±0.16	2.41^{a}±0.66	2.18^{b}±0.24	6.32^{a}±0.03	59.44^{a}±0.12
Defatted flour	7.12^{c}±0.01	23.10^{b}±0.15	2.69^{a}±0.08	2.46^{c}±0.08	0.61^{b}±0.64	64.03^{a}±0.15
Protein concentrate	6.98^{b}±0.07	61.78^{a}±0.07	0.00^{c}	2.82^{a}±0.13	0.00^{c}	28.43^{b}±0.06
Protein isolate	6.47^{b}±0.36	89.18^{a}±0.23	0.00^{c}	2.19^{a}±0.18	0.00^{c}	1.46^{b}±0.21
LSD	0.561	0.326	1.477	0.337	3.072	0.292

All values are expressed as mean ± SD of their evaluation. Mean values within column with Superscripts are significantly different at ≤ 0.05

Table 2: Functional properties of full- fat flour, defatted flour, protein concentrate and protein isolate

Samples	BD	SW1	WAC	OAC	GT	EC	FC	W
Full fatted flour	0.35c± 0.10	1.13b± 0.42	1.34c± 0.11	1.51b±0 .70	16.00a± 0.02	90.20a± 0.16	7.27a±0 .63	0.50b±0 .74
Defatted flour	0.50c± 0.01	1.73c±0 .07	1.83a± 0.16	2.57b±0 .09	13.00a± 0.29	96.10a± 0.19	9.73a±0 .21	0.44b±0 .78
Protein concentrate	0.58a± 0.03	2.12a± 0.16	1.30b± 0.14	3.21a±0 .28	11.00b± 0.87	30.71b± 0.19	3.42b±0 .16	2.53a±0 .43
Protein isolate	0.67a± 0.08	2.43b± 0.33	1.71±0. 10	3.62a±0 .51	10.00±0 .01	35.32b± 0.16	4.28b±0 .11	3.67a±0 .11

LSD	0.136	0.560	0.258	0.920	2.646	0.349	2.888	1.588

Where: BD= Bulk density
SW1=Swelling Index (ml/ml)
WAC= Water Absorption Capacity (ml/g)
OAC= Oil Absorption capacity (ml/g)
GC = Gelling capacity (^{0}C)
EC= Emulsion capacity (%)
FC= Fomaing capacity (%)
W= Wettability (sec)

4. DISCUSSION

4.1 Proximate Composition of the flour samples

Analysis was carried out on the proximate composition of the flour samples as soon as they were ready, in order to prevent loss of value due to deterioration. The proximate compositions of the test are shown in table 1. The result revealed a high protein content of 21.83±0.16% of the full fat flour. [11] reported a protein content of 21.0- 29.0% for AYB full fat flour, although lower than most major legumes like soy bean (38- 44%), and African locust bean (23-27%).The result was found to fall within the range of other legumes including groundnut (21- 26%), pigeon pea (24.46± 0.31%) as reported by ([12]; [13]). On the other hand, the African yam bean protein concentrate and isolate had an average protein content of 61.78% and 89.18% respectively.

This also compared favourably with (81- 91%) obtained for winged bean and 96.5% for the soy bean as reported by [9]. The ash contents of the protein concentrate (2.82±0.13%) and isolate (2.91±0.18) are significantly different at $p<0.05$. however, that of protein isolate is within the range by the report given by [9} for winged bean (3.4%) and lower for soy bean (5.5- 7.5%).

The full fat flour and the defatted flour contain fibre of (2.41±0.66) and (2.69±0.82%) respectively which slows down the release of glucose into the blood stream, hence high legume diet is recommended for diabetic patients. There was little or no traces of fibre and fat found in the protein concentrate and isolate of African yam beans. African yam bean concentrate an isolate was found to have values which were significantly different at ($p<0.05$) from those of full fat and defatted flour.

4.2 Functional properties of the flour sample

The functional properties of the full fat flour and the defatted flour samples are shown in Table 2. The result revealed high bulk density of (0.50± 0.01) for the defatted flour.The higher bulk density of the defatted more than the full fat flour (0.35± 0.10) can be attributed to increase in density during processing bringing about significant differences of $p>0.05$ between the two samples. The foam capacity of the protein concentrate flour and protein isolate flour (4.28%) is significantly lower than that of the full fat flour (7.27%) and the defatted flour (9.73%). This means that the protein concentrate and protein isolate flour does not have the ability to retain stable foam when whipped and may not be useful as an aerating or foaming agent in some food formulations like ice cream. The water absorption capacity of the protein concentrate and flour and protein isolate flour were significantly lower than that of the full fat flour and the defatted flour but the oil absorption capacity is higher. These results show that the capacity of food protein depends upon intrinsic factor like amino acid composition, protein conformation and surface polarity or hydrophobicity [14]. The emulsion capacity of the protein concentrate and protein isolate flour was found to be (30.7±0.19%) and (35.32± 0.16%) respectively and they are comparatively lower than the full fat flour and defatted flour samples. The relatively low emulsion capacity of the protein concentrate and protein isolate flours could be due to the nature and type of protein materials and its constituents [15]. [16] reported that emulsion capacity and stability is higher in protein with globular nature and also the concentrate and isolate flour was found with a wettability of (2.53s) and (3.67s) per gram respectively. The full fat flour had a lower swelling capacity when compared with the protein concentrate and protein islolate flours which could be attributed to the extent of starch damage due to thermal and mechanical processes. According [17] the extent of swelling in the presence of water depends on the temperature,

availability of water, starch species, extent of starch damage and other carbohydrates and protein. Also the swelling capacity of flours depends on size of particles, types of varieties and types of processing methods or unit operations. The gelling capacity of the full fat, defatted flour, protein concentrates and protein isolate samples were found to have no significant difference ($p > 0.05$). The full fat, defatted flour, protein concentrate and protein isolatefull fat and defatted flours were found to gel at temperature 95^0C and 92^0C, Nil and Nil respectively [16] associated the variation in gelling properties to different constitutes – proteins, lipids, and carbohydrate that make up the legume. Protein was attributed to globulin fraction and gelling point is indeed an aggregation of denatured molecules. This property suggests that the full fat and defatted flours samples will be suitable in food systems such as pudding, sauces and moin-moin which require thickening and gelling properties and also important in the baking of bread and other flour products where it contributes to the desired bread crumb texture and structure of the product [18]. The affinity of the flour samples for water also showed the water capacity of the four flour samples while the low values of the water absorption capacity of the different flour samples suggest that African yam bean flour is less hydrophobic than other legume flours. Therefore, African yam bean flour have more useful functional ingredient in viscous foods like baked products, gravies, soup to increase viscosity. The oil absorption capacities of 1.51g/ml and2.57g/ml for the full fat and defatted flour samples respectively are far lower when compared to other legumes. This result shows that African yam bean have lower flavor retention than other legumes of higher oil absorption capacity such as soy bean flour. This may be due to low hydrophobic protein in the African yam bean flour. Consequently, the low oil absorption capacity shows that it decreases the mouth feel when used food preparations such as meat analogues [14].

5. CONCLUSION

The result obtained from this study revealed great potential of African yam bean. The protein isolate was found to be very high in protein content, thus making it a potential source of quality protein comparable to those of legumes such as soy bean for the possibility of replacing animal protein with its protein. The proximate and functional obtained indicate that the starch will have useful technological properties for many applications both in food processing and non-food applications such as in paper and textile industries. It can also be said that African yam bean represents a source of alternative protein supplement and its protein isolates possess characteristics which show that it could find its uses in different products as protein enrichment or texturizer .With the potential contribution of African yam bean to nutrition, it is therefore recommended that cultivation and utilization of this bean be encouraged while maximizing its processing. African yam bean should be incorporated into flour samples to form composite flours other foods, processed into flours as a complement to cereal flour while further work should be done on the shelf stability of the its flour and the suitability of the flour used in baking products like bread and biscuits.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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