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Characterization of Proteins in Defatted Flour and Protein Isolate of Baobab (*Adansonia Digitatal*) Seeds

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Abstract: This study sought to analyze the proteins of baobab seeds present in the defatted flour and protein isolate in terms of their functional properties, the profile of their fractions, and the in vitro digestibility and electrophoretic pattern. Baobab seeds were milled into flours and sieved, defatted with hexane and extracted at the pH of higher protein solubility to obtain the protein isolate. The defatted flour was high in protein (37.79 ± 1.17), protein concentrate and isolate were 52.58 ± 2.46, 88.14 ± 079 respectively but moderate in available carbohydrates. The mineral (ash) decreased insignificantly; fiber and fat contents decreased significantly. Globulin ($56.11 \pm 0.95\%$) followed by the albumins ($28.12 \pm 0.24\%$), are the major fractions of the flour and protein isolate respectively. In vitro protein digestibility was greater for the protein isolate (88.91 ± 0.20) than for the defatted flour (78.9 ± 0.43).The electrophoretic profile of the protein fractions in full fat, defatted and isolate was evaluated in SDS-PAGE with major bands corresponding to molecular weights in the range of 42 – 34, 42 -14, 26 - 14, 34 - 42 kDa respectively. The functional properties of the proteins indicate the possibility of their use in various foods providing water absorption capacity of 2.86 ± 0.06gg-1, oil absorption capacity of 1.97 ± 0.05gg-1, bulk density 0.43 ± 0.01(g/ml), protein solubility of isolate is 97.34%, minimum foaming capacity of 55.00% and emulsion activity of 20% at iso-electric point. The results, suggest that baobab seeds protein has potential for use in food applications due to nutritional and good technologically functional properties.

Keywords: Baobab Seeds, Protein Isolate Functional Properties, Electrophoretic Pattern, Protein Fractionation and In Vitro Protein Digestibility.

1. Introduction

Africa has abundant native plant species and although little explored, some fruits have been proved to be excellent sources of nutrients required by the body for growth and prevention of diseases. Baobab (*Adansonia digitata L.*), member of the family Malvaceae is one of the most intriguing trees growing on the African continent. Every part of the baobab tree is reported to be useful [1]. The fruit consists of large seeds embedded in a sour acidic pulp and shell. The consumption of baobab seeds in different forms has therefore been going on for quite a long time in different regions of Africa.   Fermented seeds are used as flavouring for soup, and the roasted seeds are used as a side dish, substituting peanut [1-3]. The seeds are also pressed for oil but the by-product, baobab seed cake is typically underutilized [4]. Baobab seed nutrient composition has been investigated by several investigators in different geographical locations because plants nutrient and mineral contents [4, 5]. The results all indicated that it could serve as an alternative source of human food and could find immediate utility in mixed animal feed. The employment of protein fraction as a food ingredient can improve the value attributed to this fruit and diversify its applications in binary and tertiary food preparations.

The applications of defatted flours as functional ingredients in food systems play an important role due to their lower production cost compare to that of concentrate and isolate [6]. The use of these flours depends on their performance as functional ingredients and their behavior in particular food system. In order to contribute to a better use of baobab seeds to produce value-added products, the objective of the study were to evaluate the functional properties, the profile of protein fractions and in-vitro digestibility of the defatted flour, protein concentrate and isolate.

2. Materials and Methods

2.1. Material
The fruits were collected directly from baobab trees in three different areas in Plateau state, North Central, Nigeria. The pulp were separated manually from the seeds by the use of mortar and pestle and and the flour was sifted. The pulp was removed manually by soaking the baobab seeds for 12 h in distilled water (1:10w/v).

2.2. Flours Preparation
Seeds of baobab were pulverized in a mill and were sieved with a 200 mesh. The flours obtained were defatted with n-hexane, following a small-scale hexane extraction method described by Tzeng, et al. [7]. The oil-free flours was desolventized and stored in desiccator at room temperature for subsequent uses.

2.3. Production of Baobab Seed Protein Concentrates and Isolate
The proteins from defatted flour of baobab seeds were isolated by alkaline extraction and isoelectric precipitation method described by Kaur and Singh [8]. The lyophilization of the resulted protein concentrate was performed with Alpha 1- 4 LD Plus freezer at 1 mbar and - 20ºC followed by a final drying for 20 min. The Protein Isolates were kept in air tight polythene bags.

The protein concentrate determination was done following the method described by [9]. Ten grams of the defatted sample was mixed with 100ml of NaCl solution (0.15m) and stirred at 35^0C for 120 minutes. The pH was adjusted to 9 with 1N NaOH, and was further stirred for 30 minutes. The slurry was centrifuged at 2000 rpm for 30mins using a centrifuge. The supernatants was treated with 95% (v/v) ethanol and the pH was adjusted to 4.5 using 1N HCl under stirring and the precipitated proteins recovered by filtration. The protein concentrate was dried at 50^0C for 48hours in an air convection oven.

2.4. Chemical Proximal Analysis
The protein content of the whole flour, defatted flour, protein isolate was determined by the micro-Kjeldhal method with a conversion factor of 6.25 [10]. Ash content was determined using a muffle furnace at 550 °C to a fixed mass, and the moisture content of the defatted flour was determined by drying in an oven at 105 °C [10].

2.5. Extraction of Principal Proteins Fractionations
The extraction and fractionation of the baobab seeds proteins were carried out according to the method described by Morales, et al. [11]. A sequential extraction was performed obtaining soluble proteins in water, salt, alcohol and alkaline as globulin, albumin, prolamin, glutelin, and an insoluble residue fraction. The protein content in each protein fraction was determined using the micro- Kjeldahl method with conversion factor of 6.25 [12].

2.6. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
The electrophoretic profile of the protein fractions in the defatted flour and the protein isolate was obtained in polyacrylamide gel using a discontinuous system [13]. The gel was run at constant voltage of 160 V for approximately one hour or until the dye reached the bottom of the gel. The gels were dyed by immersion into a solution of 0.025% Coomassie Blue. A standard protein molecular weight marker (Fermentas Life Sciences Spectra[TM] Multicolor Broad Range Protein Ladder MW 10 – 260 kDa) was run concurrently with the sample and used to estimate apparent molecular weight of the different bands detected.

2.7. In-vitro Protein Digestibility (IVPD)
The *in-vitro* protein digestibility for both full fat, defatted flour and isolate was determined in triplicate following the multi-enzyme technique of [14] with some modifications using porcine pancreatic trypsin , bovine pancreatic chymotrypsin and porcine intestinal peptidases. Each sample was dissolved in 50ml distilled water each (6.25mg protein/ml) was used to determine the activity of the enzymes in digesting casein. The pH of the resulting solution was adjusted to pH 8.0 and incubated in water bath at 37^0C with constant stirring. A five millimeter (5ml) of fresh multi-enzyme solution prepared to contained 1.6mg trypsin, 3.1mg chymotrypsin and 1.4mg peptidase dissolved in 1ml distilled water was added to

each sample suspension with constant stirring at 37^0C. The pH drop of the samples from pH 8.0 was recorded after 20 min of incubation. The IVPD was calculated according to the regression equation Y= 234.84 – 22.56 X, where Y is the % digestibility and X the pH drop.

2.8. Functional Properties
2.8.1. Protein Solubility
Protein solubility of sample flours, concentrate and protein isolate were determined according to the method described by [15] with modifications. 200mg of sample were dispersed in 20ml of de-ionized water and pH of the mixture was adjusted to 2, 3 ,4, 5 ,6, 7 ,8, 9, 10, 11 by 0.5 N HCl or 0.5 N NaOH. The mixture was centrifuged at 6000 rpm for 15 min. Supernatant was analyzed for total nitrogen by micro-kjeldahl method as described above. Protein solubility expressed as a percentage of the total protein content using factor of (N $\times$ 6.25) in each sample.

2.8.2. Water and Oil Absorption
Water (WAC) and oil (OAC) absorption capacities were determined according to the method of Okaka and Potter [16]. Density of distilled water assumed to be 1 g mL^{-1} and that of oil (Grand pure soya cooking oil, Nigeria) was found to be 0.89 g mL^{-1}. Results were expressed on a dry weight basis.

2.8.3. Foaming Capacity
Foaming capacity (FC) and foam stability (FS) were determined according to the method of Makri, et al. [17]. Foam capacity (FC) was measured in terms of volume increase on whipping expressed as percentage of original volume of the liquid.
Foaming capacity (%) = Vol. after homogenization - Vol. before homogenization x 100/ Vol. before homogenization.

2.8.4. Emulsion Capacity
Emulsifying activity was determined by the method of Okezie and Bello [18]. A suspension was prepared with protein samples and soybean oil followed by mechanical emulsification. Emulsifying activity was obtained as the ratio between the emulsified layer and the total volume expressed as percentage. The emulsion was centrifuged, and the emulsion stability was considered as the volume of emulsified layer remaining in relation to the volume of initial layer.

$$\text{Emulsion capacity} = \frac{Emulsion\ height}{Water\ height} \times 100$$

The emulsion stability (ES) was recorded (30 $\pm$ 2°C) in term of the intervals of 15, 30, 60, 90 minutes.

2.8.5. Bulk Density
Bulk density was determined after measuring the weight and volume of a sample of powder according to the method of Ocloo, et al. [19]. A calibrated plastic micro centrifuge tube was weighed (W1) and filled to 2ml with powder. Constant tapping was used to pack the powder into the tube until there was no change in volume. The tube was weighted again (W2).the bulk density is calculated from the difference in weights and expressed as grams per milliliter (g/ml) as follows:

$$\text{Bulk density (g/ml)} = \frac{W2 - W1}{ml}$$

2.9. Statistical Analysis
All experimental analyses in this study were carried out in triplicates. The data were analyzed using SPSS version 17.00 .The mean and errors were reported as standard deviation from the mean. The analysis of variance (ANOVA) was performed to determine significant differences between the means (p $\leq$ 0.05). Means were separated using Duncan multiple range test and significance was acceptable at the 0.05 level of probability.

3. Results and discussion
3.1 Characteristics of the Flours, Protein Concentrate and Protein Isolate

The proximate composition of full fat, defatted, protein concentrate and isolate are depicted in Table 1. The protein contents on wet basis of whole baobab seeds flour, defatted flour, protein concentrate and isolate were $31.29 \pm 0.21\%$, 37.79 ± 1.17, $52.58 \pm 2.46\%$ and $88.14 \pm 0.79\%$ respectively which differ significantly ($p < 0.05$). The difference in protein content for full fat, defatted, protein concentrate and isolate may be attributed to the extraction method used. Production of protein isolate significantly reduced the carbohydrate, fat and crude fibre contents to 0.00% and increases the composition of protein in the finished product. Present results regarding the protein content corroborated the findings of Guimarães, et al. [20] who reported protein content of $28.45 \pm 0.43\%$, $57.65 \pm 4.92\%$ for flour and protein concentrate of baru nut. It was suggested that baobab seed protein concentrate and protein isolate could be considered as an additional source of plant protein in food products. The moisture and ash content varied between 6.37% to 4.94% and 7.35% to 6.05% respectively, these varied significantly ($p < 0.05$) among the four samples. The full fat sample still had higher ash content than protein concentrate and isolate. The ash content of the defatted flour was close to that reported by Okafor, et al. [21] in defatted black bean at 4.98%, and Moses, et al. [22] in the defatted flour of the lima bean (*P. lunatus*) at 5.53%, but it was higher than that found by Shoshima, et al. [23] in cowpeas (*Vigna unguiculata* L. Walp) with a content of 3.59%. Higher moisture contents were found in defatted flours of other species, such as pigeon pea (*Cajanus caja* (L) Millsp.) with 12.15% Mizubuti, et al. [24].

3.2. Fractioning of the Principal Baobab Seed Proteins

Fractionation of proteins is often employed to quantify protein types within food materials. The results of the extracted protein fractions from defatted baobab seed kernel flour are summarized in Table 2. Globulin ($53.50 \pm 1.11\%$) were the most dominant protein fraction recorded, followed by albumin ($30.05 \pm 0.41\%$), Prolamine (9.66 ± 1.07) and glutelin ($3.87 \pm 0.38\%$) in protein isolate.

The globulin and albumin yield in this work were higher than those of prolamin and glutelin for both samples. The present results are in agreement with those of Tounkara, et al. [25] who reported significant difference ($P > 0.05$) in albumin (91.50%) and globulin (93.77%) fraction of roselle seed protein isolate respectively. Baobab protein isolate presents a protein profile similar to that of the majority of the legumes, such as baru nut defatted 52.88 % globulin, 11.66% albumin Guimarães, et al. [20], cowpea (*Vigna unguiculata* L.) which contains 41.99% globulin, 10.11% albumin, and 7.81% glutelin Shoshima, et al. [23]. Chan and Phillips [26] reported that the relative proportion of each protein fraction in the seed strongly affects the nutritional and functional quality of the total seed protein. Therefore, baobab seed protein having globulin and albumin as a dominant protein fraction is indicative of good quality protein and could be used in food formulations due to its essential amino acid.

3.3. SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) Pattern of Baobab Seed Proteins

The electrophoretic profile of protein of boiled baobab seeds (BBS), raw baobab seeds flour (RBS), defatted baobab seeds flour (DBS) and baobab seed kernel protein isolate (BPI) is shown in Figure (1). There was a noticeable difference on SDS-PAGE profiles among BBS, RBS, DBS and BPI in the range of 42 – 34, 42 -14, 26 - 14, 34 - 42 kDa respectively. The molecular weight of the proteins in this study varies from 14.00 kDa to 42.00 kDa which is similar to the proteins of various plant species. Molecular weights of many other legumes and nuts have been reported such as cashew nut protein, 24.83 and 31.99 kDa Sathe [27], black mucuna pruriens proteins,21.0 and 30.0 kDa Machuka [28]; groundnut proteins, 12.0,13.2, 18.6, 33.1, and 39.8 kDa [29]. The 34 – 72 kDa bands were lightly visible in protein isolate probably due to the presence of disulphide linked to protein aggregates that are cleaved to smaller bands on reduction with mercaptolethanol. This may further suggest that the proteins were probably lost during the preparation of the protein isolates as reported by Byaruhanga, et al. [30]. Belitz, et al. [31] reported that the molecular weight of different proteins influences their suitability for use in processes like protein texturization in the range of 10 to 50 kDa. They further maintained that the proteins less than 10 kDa are considered weak fiber builders while those with molecular weight higher than 50 kDa are disadvantageous due to their high viscosity and the tendency to gel in alkaline pH range. Nehete, et al. [32] maintained that proteins' molecular weight variation depends on the number of amino acid residues. A substantial number of protein bands were within the range 14-34 kDa (Figure 1) suggesting that baobab seed protein is potentially good for texturization.

3.4. In-Vitro Multi-Enzyme Protein Digestibility (IVPD)

The *in-vitro* protein digestibility of full fat and defatted flours were compared with protein isolate extracted from baobab seeds kernel as presented in Table 7. The values ranged from 87.10± 0.41, 89.95± 0.43, 95.8 ±0.20 for full fat flour, defatted flour and protein isolate respectively were significantly different ($P < 0.05$). The protein isolates showed highest digestibility compare with the full fat and defatted flours. It has been reported that the presence of trypsin and chymotrypsin inhibitors and the unfolding of the native protein structure during the cause of hydrolysis is yet another factor that likely facilitates digestibility. In addition, seed proteins are denatured during isolation, rendering the protein isolates more accessible to digestive enzymes and improve the hydrolysis [33]. The *in-vitro* protein digestibility of *baobab* protein isolates (95.81%) corroborated with the range of values (96.70 ± 0.30) for white melon [34] and (95.6- 96.1%) for chickpea [35], but higher than the range of 86.3- 93.9% for lupin protein isolates [36]. Baobab seed protein isolates have potential to be used in food system.

3.5. Functional Properties
3.5.1. Water and Oil Absorption Capacity

The water absorption capacity (WAC) and oil absorption capacity (OAC) of baobab seeds flour and its isolate are reported in Table 2. These capacities represent the amount of water and oil, respectively, that can be bound per unit weight of the protein material and constitutes useful indices of the ability of the protein to prevent fluid leakage from a product during food storage or processing Kiosseoglou and Paraskevopoulou [37]. The WAC results recorded in this study for full fat flour (2.13±0.02%g/g) was lower compared to defatted (2.90%g/g) and isolate (2.86%g/g) samples. Thus, higher than those reported by Butt and Batool [38] as 0.97, 1.38, 1.63, and 1.52 g g^{-1} for pigeon pea, cow pea, mung bean, and cow pea protein isolates respectively. The OAC of the isolate (1.97± 0.05) in this study was higher than values reported for the flours samples (1.30±0.01 1.88±0.05) suggesting that baobab seeds protein isolate would absorb more oil in a frying process. Khalid, et al. [39] reported OAC value of 1.90 g g^{-1} for cow pea while Butt and Batool [38] 1.68, 1.45, 1.13 and 1.40 g g^{-1} for pigeon pea, cow pea, mung bean, and pea protein isolates, respectively. The differences between the WAC and OAC of protein isolate from baobab seeds and other legumes can be attributed to both species and variety, and the technique employed for protein recovery which may also influence the absorption capacity values. The influence of interactions of water with proteins on the food product texture and succulence are important in food systems Amadou, et al. [40]. The high WAC and moderate OAC values of the baobab seeds protein isolate would suggests improved product texture succulence potential in meats, sausage, bread and cakes due to moisture and oil retention.

3.5.2. Bulk Density

The bulk density of the isolate (0.43±0.01 gml^{-1}) in this study was lower compare to values reported for the full fat and defatted flour (0.76 and 0.74 gml^{-1}) respectively. Butt and Batool [38] reported bulk densities of 0.71 and 0.68 g cm^{-3} for proteins isolates of cowpea and pea, respectively. Bulk density is known to affect the packaging requirements of the product after processing.

3.5.4. Protein Solubility

The protein pH-solubility profiles of full fat, defatted and protein isolate is shown in Figure 2. The region of least solubility occurs at pH 4.0 with 17.15%, 18.60% and 27.34% for full fat, defatted flour and protein isolate respectively. The maximum protein solubility at pH 11.0 with values of 95.71%, 97.34% and 126.23% for full fat, defatted flour and protein isolate respectively. The protein solubility of full fat, defatted and protein isolate followed a definite trend of decreased as pH increased until it reaches the isoelectric point and then increases which showed U-shaped curves, similar to the protein solubility profiles reported for walnut [41] and cashew nuts Ogunwolu, et al. [42]. Protein solubility is one of the most important functional properties of a protein due to its effect on all other functional properties of the protein [43]. At the isoelectric point the protein-protein interactions increase because the charge on the protein is zero. This means that electrostatic repulsive forces are lowest and the attractive van der Waals forces will predominate [44]. In contrast the highest solubility for all three protein samples occurred at high pH. The increase in solubility either side of the isoelectric point can be explained by the protein having negative or positive net charge under these conditions and therefore a net repulsive electrostatic interaction between the proteins under these conditions. Similar results were also observed in walnut protein as reported by Sze-Tao and Sathe [45]. The protein solubility of protein isolate was significantly

higher ($p < 0.05$) than that of defatted and full fat samples at all pH; these results were similar to the behavior observed in the protein solubility of defatted cashew nut powder, cashew nut protein concentrate and isolate reported by Ogunwolu, et al. [42]. The protein solubility of baobab seed protein was higher than that of peanut protein isolate (60.5%), soy protein isolate (71.7%) as reported by Yu, et al. [46], and Molina [47], respectively. The protein solubility in both acid and alkali is influenced by amino acid composition and sequence which may indicates poor performance in formulating carbonated beverages [48]. This may be enhanced by increasing the net.

3.5.5. Emulsifying Properties

Emulsion activity (EA) reflects the ability of a protein to aid the formation of an emulsion. EA results of all the samples are presented in Figure 3a. The protein isolates exhibited relatively lower EA compared to other samples at pH 4.5 (isoelectric point) were 31.26%, 35.50 % and 19.56% for full fat, defatted and protein isolate respectively. The slightly higher values of EA for crude sample than for the purified samples at all pH values may be attributed to the presence of non-protein components. The EA values of the samples increase with increasing pH values for full fat, defatted and protein isolate respectively. Similar trends was reported by Byaruhanga, et al. [30] for yam bean seed protein isolate. Butt and Batool [38] reported a higher emulsion activity values of 49.5, 47.5, 41.1 and 45.5% for pigeon, cowpea, mung bean, and pea protein isolates, respectively.

Emulsion stability (ES) reflects the ability of the protein to impart strength to emulsion for resistance to stress [30]. The effects of pH on emulsion stability (ES) of full fat, defatted and protein isolate are shown in Figure 3b. The ES of all samples was low at the pH 4.5, 19.86%, 23.53%, 36.63%, respectively and increase with increasing pH values for full fat, defatted and protein isolate respectively. Butt and Batool [38] reported emulsion stability of 83.3%, 52.2%, 21.0% and 43.2% for pigeon, cowpea, mung bean, and pea protein isolates, respectively. Nassar [49] posits that proteins with high emulsifying capacity are good for salad dressing, sausages, bologna, soups, confectionery, frozen dessert and cakes. However, the low EA and moderate ES values at isoelectrict point of the baobab seeds protein isolate would suggests its used as an emulsifier possibly with modification of its properties.

3.5.6. Foaming Properties

Foaming properties are used as indices of whipping characteristics of protein isolates [38]. Foaming capacity (FC) of flours and protein isolate extracted from baobab seed kernel as affected by pH values are presented in Figure (4). The maximum increase in foam volume (FC) of the samples were 152.67%, 131.00% and 116.33% for protein isolate, defatted and full fat samples respectively at pH 2.0. However, the lowest volume of the foam 70.00%, 74.00% and 85.67% were observed at pH 11.0. The result showed a concurrent decrease in foam capacity as the pH increased until it reaches the minimum at isoelectric point pH 4.50 and then increase again. These values are higher than the FC values reported for other leguminous species such as *Sesamum indicum* (82%) and *Vigna subterranean* (70%) [50]. However, the lowest volume of the foam capacity recorded in this study were higher than the FC of peanuts (10-13%) [8], peanut protein concentrate (20- 40%) [51].The low foam ability exhibited by the full fat and defatted flour samples respectively shows that fat and protein affects foaming capacity. Acidic pH also favour the foaming capacity of the extracted protein isolate more probably due to the increase in protein solubility after removing lipids. The increased net charge on the protein molecules at the acidic and alkaline regions weakens the hydrophobic interactions, resulting in better structural flexibility which aids in their diffusion to the air-water interface.

Flours with high foaming ability could form large air bubbles surrounded by thick and more flexible protein film. This air bubbles might be more stable and consequently increased the foaming stability. These results suggest that the defatted baobab seed flour may be useful in food system to improve textural and leavening characteristics such as ice-cream, cakes or topping and confectionery products where foaming properties is important. Foaming properties of the protein isolate can be advantageously utilized in whipped toppings, angel cakes and confectionary.

4. Conclusions

Results from this study indicate that the chemical composition of baobab defatted seeds flour were different when compared with protein concentrate and isolate. The profile of protein fractions was predominantly globulin followed by the fractions of albumin both in defatted flour and protein isolate . Also, protein isolate attain better digestibility compared to defatted flour and whole flour. Furthermore, the functional properties of protein products were improved when baobab protein isolate were produced

from defatted flour by isolation. Baobab protein isolate exhibit good technologically functional ingredient properties in food systems such as required in cake, meat and ice cream product processing. The production of baobab protein isolate and concentrate from defatted baobab seeds flour could also add-value to the low value by-product of baobab oil production.

Table 1. Chemical composition of full fat flour, defatted flour, protein concentrate and isolate extracted from baobab seed kernels

Composition %	Full fat Flour	Defatted Flour	Protein Concentrate	Protein Isolate
Crude Protein	31.29^d ±0.21	37.79^c ±1.17	52.58^b ±2.46	88.14^a ±0.79
Moisture	6.37^a ±0.01	5.67^a ±0.13	4.94^b ±0.07	5.81^a ±0.21
Fat	29.37^a ±5.80	3.18^b ±0.01	2.45^b ±0.04	0.00^c±0.00
Ash	7.65^a ± 0.04	6.74^b ±0.24	6.44^b ±0.09	6.05^c ±0.99
Crude Fibre	7.12^b ±0.02	7.30^a ±0.04	4.28^c ±0.26	0.00^d±0.00
NFE	18.38^c ±0.31	39.32^a±1.25	28.36^b ±0.47	0.00^d±0.00

Source: Field work 2017

Each value in the table represents an average of three repetition, $\pm SD$, a, b, c, d, Treatment means with different superscripts are significantly (p<0.05) different from each other, according to Duncan's Multiple Range Test (DMRT). NFE: Nitrogen free extract.

Table 2. Protein fractions of the defatted flour and protein isolate of baobab seeds

Protein fraction	Defatted flour	Protein isolate
Albumin	30.05^a ±0 .41 (+30.65)	28.12^b ± 0.24 (+22.26)
Globulin	53.50^b ±1.11 (+17.27)	56.11^a ±0.95 (+22.99)
Prolamine	9.66^a ±1.07 (+64.00)	10.48^a±0.19 (+77.92)
Glutenin	3.87^b ±0.38 (0.00)	5.09^a ±0.72 (+31.52)

Source: Field work 2017

Each value in the table represents an average (±SD) of three repetitions. Values with the same superscript letters in the same row not significantly different at p < 0.05

Table 3. Water absorption capacity, oil absorption capacity, bulk density, swelling index and *In vitro* digestibility

| Properties	Differently	Processed	Flour
	Full Fat Flour	Defatted Flour	Protein Isolate
WAC(%g/g)	2.13 ± 0.02	2.90 ± 0.05	2.86 ± 0.06
OAC(%g/g)	1.30 ± 0.01	1.88 ± 0.01	1.97 ± 0.05
BD(g/ml)	0.76 ± 0.01	0.74 ± 0.01	0.43 ± 0.01
IVPD	67.10 ± 0.41	78.95 ± 0.43	88.91 ± 0.20

Source: Field work 2017

Key: WAC-Water Absorption Capacity, OAC - Oil Absorption Capacity, BD – Bulk Density, IVPD – In vitro Protein Digestibility.

Figure 1. Electrophoresis profile of the proteins of P: reference pattern for molecular weights, 1: boiled baobab seeds flour; 2: raw baobab seeds flour; 3: defatted baobab seeds flour; 4: baobab seeds protein isolate kDa with accumulation of protein bands.
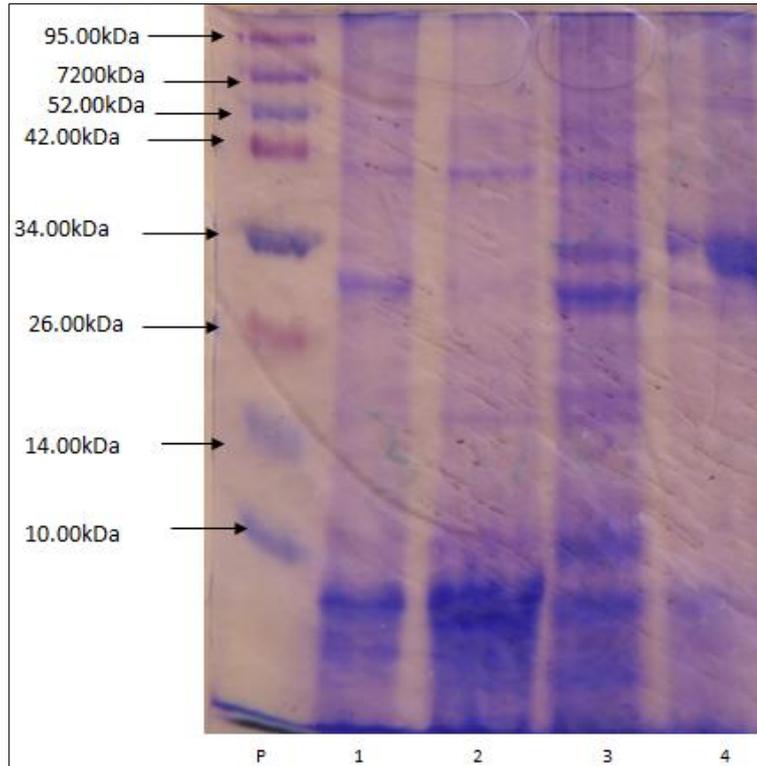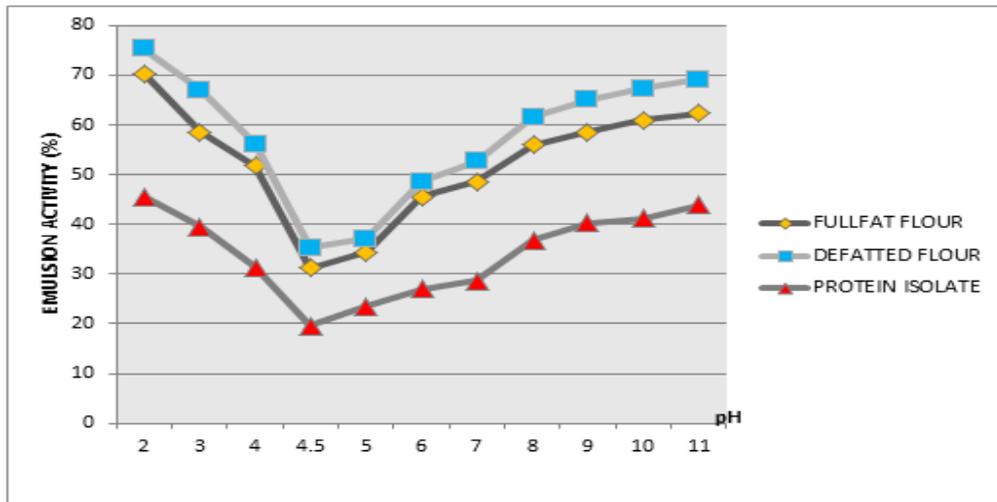

Source: Field work 2017

Figure 2. Effect of pH value on the protein solubility (%) of baobab seed kernel full fat flour, defatted flour and protein isolate
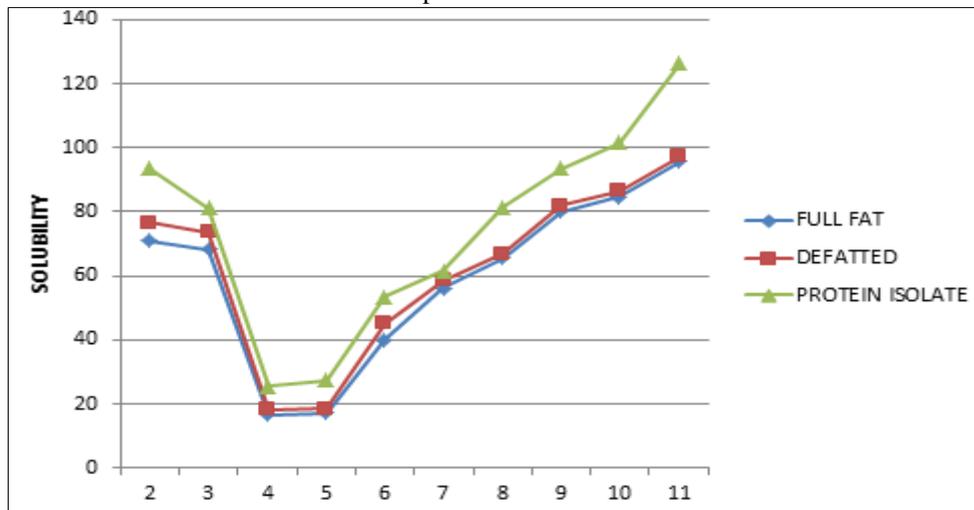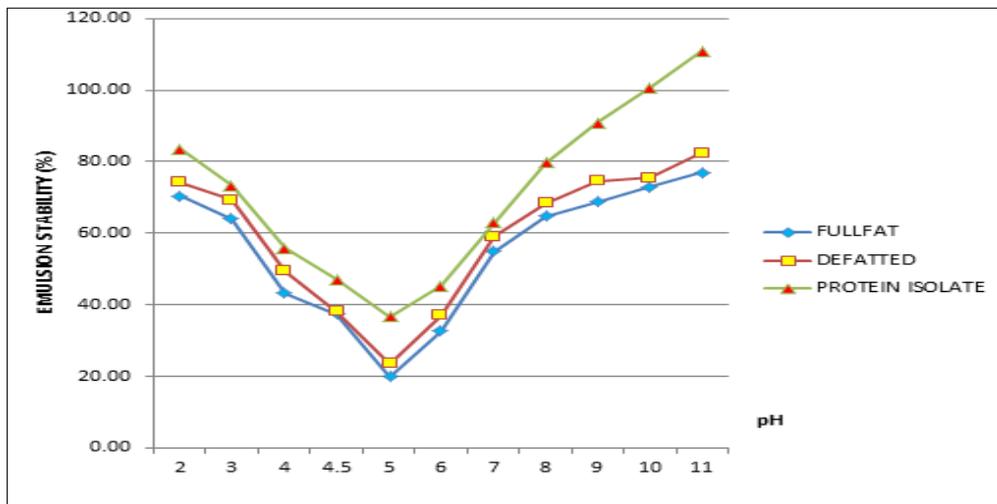

Source: Field work 2017

Figure 3a. Effects of pH on Emulsion Activity (EA) of Fulfill Flour, Defatted Flour and Protein Isolate Extracted from Baobab Seed
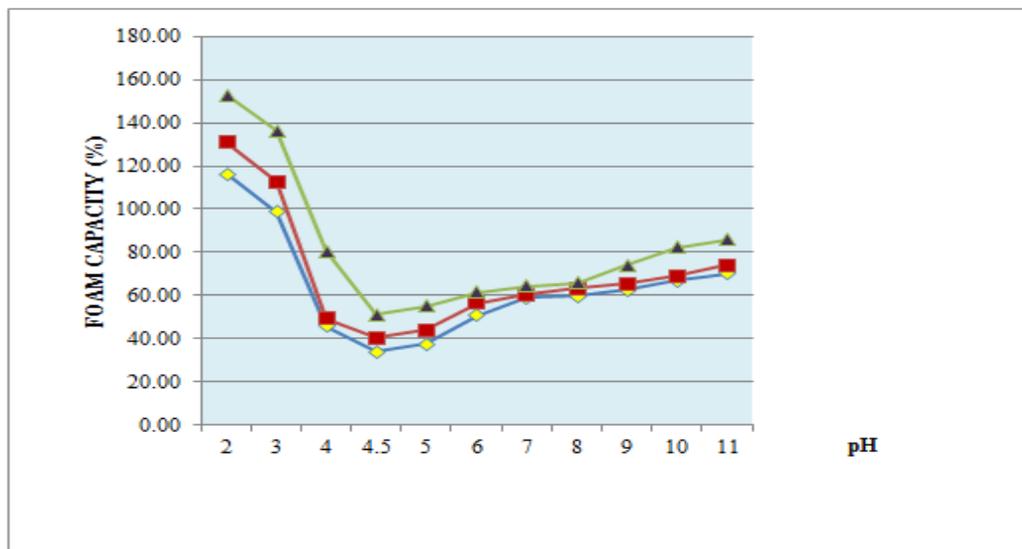

Source: Field work 2017

Figure 3b. Effects of pH on Emulsion Stability (ES) of Fullfat, Defatted and Protein Isolate Extracted from Baobab Seed

Source: Field work 2017

Figure 4. Effects of pH on Foam Capacity (FC) of Full fat, Defatted and Protein Isolate Extracted from Baobab Seed Kernel

Source: Field work 2017

References

[1]   M. Sidibé and J. T. Williams, "Baobab Adansonia digitata. International Centre for Under-utilised Crops, UK," 2002.

[2]   E. O. Addy and E. Eleshola, "Nutritive value of a mixture of tigernut tubers and baobab seeds," *J Science Food Agric,* vol. 35, pp. 437- 440, 1984.

[3]   N. M. Nnam and P. N. Obiakor, "Effect of fermentation on the nutrient and antinutrient composition of baobab (Adansonia digitata) seeds and rice (Oryza sativa) grains," *Ecology of Food and Nutrition,* vol. 42, pp. 265-277, 2003.

[4]   M. A. Osman, "Chemical and nutrient analysis of baobab (Adansonia digitata) fruit and seed protein solubility," *Plant Food Human Nutrition,* vol. 59, pp. 29-33, 2004.

[5]   I. I. Nkafamiya, S. A. Osemeahon, D. Dahiru, and H. A. Umaru, ": Studies on the chemical composition and physicochemical properties of the seeds of baobab (Adansonia digitata)," *African Journal of Biotechnology,* vol. 6, pp. 756-759, 2007.

[6]   S. H. Wang, S. H. Fernandes, and L. C. Cabral, "Solubilidade de nitrogenio, dispersibilidade de protein e propriedades emulsificantes dos extratos hidrossoluveis desidratetados de arroz e soja," *Cienciae Technologia de Alimentos,* vol. 20, pp. 12-17, 2000.

[7]   Y. M. Tzeng, L. L. Diosady, and L. J. Rubin, "Production of canola protein materials by alkaline extraction, precipitation and membrane processing," *Journal of Food Science,* vol. 55, pp. 1147-1156, 1990.

[8]   M. Kaur and N. Singh, "Characterization of protein isolates from different Indian chickpea (Cicer arietinum) cultivars," *Food Chem,* vol. 102, pp. 366 -374, 2007.

[9]   M. M. A. Mune, S. R. Menka, and I. L. Mbome, "Chemical composition and nutritional evaluation of cowpea protein concentrate," *Global Advance Research Journal of Food Science and Technology,* vol. 2, pp. 35-43, 2013.

[10]   AOAC, "Official Methods of Analysis (18th edn.). Association of Official Analytical Chemists. Washington. DC.," 2006.

[11]   A. G. Y. Morales, L. A. Chagolla, L. O. Y. Paredes, and P. Barba de la Rosa, "Characterization of yam bean (Pachyrhizus erosus) proteins," *J. Agric. Food Chem,* vol. 49, pp. 1512-1516, 2001.

[12]   AACC, "(1983). Approved Methods of the AACC. Method 10–91, approved April 1968, revised October, 1982. St. Paul, MN: The Association," 2000.

[13]   U. K. Laemmli, "Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature; 227," pp. 680-685, 1970.

[14]   H. W. Hsu, D. L. Vavak, L. D. Satterlee, and G. A. Miller, "A multi-enzyme technique for estimating protein digestibility," *J. Food Sci,* vol. 42, pp. 1269-1273, 1977.

[15]   A. R. S. Clement, J. Vioque, T. A. J. Bautis , and F. Millan, "Effect of cooking on protein quality of chickpea (Cicer arietinum L.) seeds," *Food Chem,* vol. 62, pp. 1-6, 1998.

[16]   J. C. Okaka and N. N. Potter, "Functional properties of cowpea-wheat flour blend in bread making," *Food Sci,* vol. 42, pp. 828-833, 1977.

[17]   E. Makri, E. Papalamprou, and G. Doxastakis, "Study of functional properties of seed storage proteins from indigenous European legume crops (lupin, peas, broad bean) in admixture with polysaccharides," *Food Hydrocolloids,* vol. 19, pp. 583-594, 2005.

[18]   B. O. Okezie and A. B. Bello, "Physicochemical and functional properties of winged bean flour and isolate compared with soy isolate," *J. Food Sci,* vol. 53, pp. 450-454, 1988.

[19]   F. C. K. Ocloo, D. Bansa, R. Boatin, T. Adom, and W. S. Agbemavor, "Physico-chemical, functional and pasting characteristics of flour produced from Jackfruits (Artocarpus heterophyllus) seeds," *Agriculture and Biology Journal of North America,* vol. 1, pp. 903-908, 2010.

[20]   R. d. C. A. Guimarães, S. P. Favaro, A. C. A. Viana, B. N. J. A., V. A. Neves, and M. R. Honer, "Study of the proteins in the defatted flour and protein concentrate of baru nuts (Dipteryx alata Vog). Ciênc. Tecnol. Aliment., Campinas," vol. 32, pp. 464 – 470, 2012.

[21]   D. C. Okafor, R. O. Enwereuzoh, J. C. Ibeabuchi, A. E. Uzoukwu, S. O. Alagbaoso, and C. Udenkwo, "Production of flour types from black bean (PhaseolusVulgaris) and effect of pH and temperature on functional physico-chemical properties of the flours," *European Journal of Food Science and Technology,* vol. 3, pp. 64-84, 2015.

[22]   O. Moses, I. Olawuni, and J. O. Iwouno, "The Proximate Composition and Functional Properties of Full-Fat Flour, and Protein Isolate of Lima Bean (Phaseolus Lunatus)," *Open Access Scientific Reports,* vol. 1, pp. 1-349, 2012.

[23]   A. H. R. Shoshima, O. I. Tavano, and V. A. Never, "Digestibilidade in vitro das proteínas do caupi (Vigna unguiculata L. Walp) "Var. Br-14 Mulato:" Efeito dos fatores antinutricionais," *Brazilian Journal of Food Technology,* vol. 4, pp. 299-304, 2005.

[24]   I. Y. Mizubuti, O. B. Júnior, L. W. De-Oliveira-Souza, R. S. Dos, S. F. Da –Silva, and I. Elza – Iouko, "Functional properties of pigeon pea (Cajanus cajan (L.) Millsp) flour and protein concentrate. Archivos," *Latinoamericanos Nutricion,* vol. 50, pp. 274-280, 2000.

[25]   F. Tounkara, T. Amza, C. Lagnika, L. Guo-Wei, and S. Yong-Hui, "Extraction, characterization, nutritional and functional properties of roselle (Hibiscus sabdariffa Linn) seed proteins," *Songklanakarin J. Sci. Technol,* vol. 35, pp. 159-166, 2013.

[26]   C. Chan and D. R. Phillips, "Amino acid composition and subunit constitution of protein fractions from cowpea (Vigna unguiculata L. Walp) seeds," *Journal of Agricultural Food Chemistry,* vol. 42, pp. 1857-1860, 1994.

[27]   S. K. Sathe, "Solubilization and electrophoretic characterization of cashew nut (Anacardium occidentale) proteins," *Food Chemistry,* vol. 51, pp. 319-324, 1994.

[28]   J. Machuka, "Characterization of the seed proteins of velvet bean (Mucuna pruriens) from Nigeria," *Food Chemistry,* vol. 68, pp. 421–427, 2000.

[29]   B. V. Mnembuka and B. O. Eggum, "The nutritive value of some selected Tanzanian plant food sources," *Plant Foods for Human Nutrition,* vol. 44, pp. 1-10, 1993.

[30]   Y. B. Byaruhanga, A. Kisambira, J. H. Muyonga, P. Tukamuhabwa, S. Tumwegamire, and W. Gruenberg, "Physicochemical Characteristics of Yam Bean (Pachyrhizus erosus) Seed Proteins," *Journal of Food Research,* vol. 3, pp. 736-746, 2014.

[31]   D. H. Belitz, W. Grosch, and P. Schieberle, "Food Chemistry (4th ed.). Berlin: Springer-Verlag," 2009.

[32]   J. Y. Nehete, R. S. Rhambar, M. R. Narkhede, and S. R. Gawali, "Natural proteins. Sources, isolation, characterization and applications," *Pharmacogn Rev,* vol. 7, pp. 107-116, 2013.

[33]   C. J. Lynch, C. K. Rha, and N. Catsimpoolas, "Tryptic hydrolysis of glycinin and its subunits," *J. Sc. Food and Agric,* vol. 28, pp. 971-979, 1977.

[34]   M. E. Ogunbusola, N. T. Fagbemi, and F. O. Osundahunsi, "In-vitro protein digestibility, amino acid profile, functional properties and utilization of white melon (Cucumeropsis mannii) protein isolates," *African Journal of food science and technology,* vol. 4, pp. 153-159, 2013.

[35]   R. Sanchez-Vioque, A. Clement, J. Vioque, J. Bautista, and F. Millan, "Protein Isolates from chickpea (Cicer arietinum L). Chemical composition, functional properties and protein characterization," *Food Chem,* vol. 64, pp. 237-243, 1999.

[36]   H. Lqari, J. Vioque, J. Pedroche, and F. Millan, "Lupinus angustifolius protein isolates: chemical composition, functional properties and protein characterization," *Food Chem,* vol. 76, pp. 349-356, 2002.

[37]   V. Kiosseoglou and A. Paraskevopoulou, *Functional and physicochemical properties of pulse proteins in K. B. Tiwari, A. Gowen and B. McKenna (Eds.). Pulse Foods: Processing, Quality and Nutraceutical Applications. London: Elsevier Inc*, 2011.

[38]   S. M. Butt and R. Batool, "Nutritional and functional properties of some promising legumes protein isolates," *Pakistan Journal of Nutrition,* vol. 9, pp. 373-379, 2010.

[39]   I. I. Khalid, S. B. Elhardallou, and A. E. Elkhalifa, "Composition and functional properties of cowpea (Vigna unguiculata L. Walp) flour and protein isolates," *American Journal of Food Technology,* vol. 7, pp. 113-122, 2012.

[40]   I. Amadou, A. Tidjani, M. B. K. Foh, M. T. Kamara, and L. Guo-Wei, "Influence of Lactobacillus plantarum Lp6 fermentation on the functional properties of soybean protein meal," *Emirates Journal of Food and Agriculture,* vol. 22, pp. 456-465, 2010.

[41]   Y. Hua and X. Mao, "Composition, structure and functional properties of protein concentrates and isolates produced from walnut (Juglans regia L.)," *Int. J. Mol. Sci,* vol. 13, pp. 1561-1581, 2012.

[42]   S. O. Ogunwolu, F. O. Henshaw, H. P. Mock, A. Santros, and S. O. Awonorin, "Functional properties of protein concentrates and isolates produced from cashew (Anacardium occidentale L.) nut," *Food Chem,* vol. 115, pp. 852-858, 2009.

[43]   J. E. Kinsella, "Functional properties of food proteins: a review," *Critical Reviews in Food Science and Nutrition,* vol. 7, pp. 219-280, 1979.

[44]   D. H. G. Pelegrine and C. A. Gasparetto, "Whey proteins solubility as function of temperature and pH. Lebensm.-Wiss. U.-Technol," vol. 38, pp. 77-80, 2005.

[45]   K. W. C. Sze-Tao and S. K. Sathe, "Functional properties and in-vitro digestibility of almond (Prunus dulcis L.) protein isolate," *Food Chemistry,* vol. 69, pp. 153-160, 2000.

[46]   J. Yu, M. Ahmedna, and I. Goktepe, "Peanut protein concentrate: Production and functional properties as affected by processing," *Food Chem,* vol. 103, pp. 121-129, 2007.

[47]   E. Molina, "Papadapoulou, A and Ledward, D. A : Emulsifying properties of high pressure treated soy protein isolate and 7S and 11S globulins.," *Food Hydrocolloids,* vol. 15, pp. 263-269, 2001.

[48]   O. Olaofe, L. A. Arogundad, E. I. Adeyey, and O. M. Falusi, "Composition and food of the variegated grasshopper," *Trop. Sc,* vol. 38, pp. 233-237, 1998.

[49]   G. A. Nassar, "Chemical composition and functional properties of prickly pear (Opuntia ficus indica) seeds flour and protein concentrate," *World Journal Dairy and Food Science,* vol. 3, pp. 11-16, 2008.

[50]   Y. A. A., H. Ayedun, and L. O. Sanni, "Chemical composition and functional properties of raw and roasted Nigerian benniseed (Sesamum indicum) and bambara groundnut (Vigna subterranean)," *Food Chem,* vol. 111, pp. 277-282, 2008.

[51]   H. Wu, Q. Wang, T. Ma, and J. Ren, "Comparative studies on the functional properties of various protein concentrate preparations of peanut protein," *Food Research International,* vol. 42, pp. 343-348, 2009.