

Full Length Research Paper

Chemical composition and functional properties of flour and protein isolate extracted from Bambara groundnut (*Vigna subterranean*)

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The objective of this research was to study the chemical composition of flour and protein isolate extracted from Bambara groundnuts. Protein, fat, ash, crude fiber and total carbohydrates were 17.70, 6.58, 4.22, 3.50 and 86.0% in flour and 85.97, 0.0, 3.37, 0.02 and 10.64% in protein isolate, respectively. Functional properties of Bambara groundnuts were also studied. Bambara groundnuts flour and protein isolate were high in water absorption, which recorded 281.35 and 221.83% dry sample, respectively. The highest foaming capacity was 210% at pH 9.0. The highest foaming stability was detected at pH 6.0 after 120 min. With protein isolate, foam stability (FS) was 40 min at different pH values (3.0 to 9.0). The highest emulsion stability (ES) was 70% after 48 h at pH 3.0 and 6.0. Data showed that gelation of Bambara flour and protein isolate were best at concentration of 20% (w/v), the gelation formation were 8 and 18%, respectively. The data revealed that bulking density (BD) in flour was higher than in protein isolate. Beside, protein solubility index (PSI) decreased as pH values increased until pH values 4.0 to 5.0 and then increased. Our results indicated that chemical and functional properties of Bambara groundnut may cause improvement in some food products when it is added to the foods.

Key words: Bambara groundnut, chemical properties, functional properties, flour, protein isolate.

INTRODUCTION

World demand for proteins is increasing and so more food protein is required from both conventional and new source of protein. Accepting that all proteins will have nutritional value, then in both cases successes in food industry requires that the protein have good functional properties to be acceptable as a food ingredient. Proteases are important in modifying food protein functional properties.

The Bambara groundnut (*Vigna subterranean*) is an indigenous African crop that has been cultivated for centuries from Senegal to Kenya and from the Sahara to South Africa and Madagascar. It originated in the Sahelian region of present day West Africa, which its name originating from the Bambara tribe who now live

mainly in Mali (Nwanna et al., 2005).

One of the main attributes of Bambara groundnuts is its tolerance of drought and poor soils and its ability to yield in conditions when Bambara groundnuts fails completely. It is also relatively pest and disease-resistant. For instance, it is tolerant to extreme weevil attack and allows the seed to be stored for long periods without loss (Collision et al., 2000).

Bambara groundnuts are the untraditional seeds; the new cultivated and promising crop is convenient to adapt in South Upper Egypt for increasing the local planted production and is currently being imported due to lack of legumes in particular in Egypt. They contain 7.3% moisture, 18 to 24% protein, 6.0 to 6.5% fat and 60 to 63% carbohydrates (Yusuf et al., 2008).

It is recognized that protein isolate offers immense possibility in the development of new class of formulated foods. The high concentration of protein with the

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advantage of colour, flavour and functional properties makes protein isolates an ideal raw material for use in beverages, infants and children milk food, textured protein products and certain types of specialty foods (Olaofe et al., 1998).

Seed proteins should also possess the essential requisite functional properties for successful utilization in various food products. These functional properties are intrinsic physico-chemical characteristics which affect the behaviour of properties in food systems during processing, manufacturing, storage and preparation. Critical functional properties necessary in protein ingredients include gelation which is an important function of proteins in food systems. The properties include emulsion capacity, activities and stability. It also includes foam capacity and stability. Other paramount functionalities are proteins solubility, water and fat absorption capacity organoleptic properties and bulk density (Aremu et al., 2007).

The objective of the present research was to study the chemical composition and functional properties of flour and protein isolate extracted from Bambara groundnuts (*V. subterranean*). This study could provide some basic information, which would help determine an application for Bambara groundnuts flour and protein isolate in food products.

MATERIALS AND METHODS

Materials

Plant samples

Bambara groundnuts (*V. subterranean*) were purchased from Nyala main market in Western Sudan and kept in a polyethylene bags on a dry place.

Chemicals

All the chemicals in this study (n-hexane, sodium hydroxide, hydrochloric acid and sodium chloride) were purchased from Merck (Darmstadt, Germany).

Methods

Methods preparation

Preparation of Bambara groundnuts flour: Bambara groundnuts seeds were cleaned manually to remove all foreign materials such as dust, dirt, small branches and immature seeds. The whole sample materials were blended to powder (0.60 mm) form with a high-speed blender (Braun KMM 30 mill), type 3045, CombiMax (Germany). The flour was defatted in Soxhlet apparatus, using n-hexane for 9 h at room temperature ($25 \pm 2^\circ\text{C}$). The defatted flour was air dried at room temperature ($25 \pm 2^\circ\text{C}$) and stored in an airtight polyethylene bag and kept in a refrigerator of about 4°C prior to analysis.

Preparation of Bambara groundnuts protein isolate: The protein isolate was extracted from flour according to the procedure of Nath

and Narasinga (1981). One hundred gram of flour was suspended in 1500 ml distilled water. The pH of the suspension was adjusted to 9.0 with 1 N NaOH. The materials were stirred for 20 min by using high-speed stirrer and then centrifuged at 1000 g for 20 min. The protein in the extract was precipitated at pH 4.0 using 1 N HCl. The precipitate washed once with distilled water and readjusted to pH 7.0 with 1 N NaOH and dried in an oven at 40°C .

Analytical methods

Proximate analysis (moisture, protein, fat, ash and crude fiber) was carried out on Bambara groundnuts flour and protein isolate, determined according to the methods described in AOAC (2000). Total carbohydrates were calculated by differences.

Functional properties of Bambara groundnuts flour and protein isolate

Water and oil absorption

Water and oil absorption capacities were determined according to the method of Adebawale et al. (2005). The results calculated as gram water or corn oil absorbed by 100 g dry sample.

Foaming properties

Foaming capacity (FC) and foam stability (FS) were determined according to the method of Makri et al. (2005). Foam capacity (FC) was measured in terms of volume increase on whipping expressed as percentage of original volume of the liquid. Foam stability (FS) was expressed as percentage of foam volume remaining, in relation to initial foam volume at room temperature ($25 \pm 2^\circ\text{C}$) after 5, 10, 20, 30, 40, 50, 60, 90 and 120 min.

$$\text{Foaming capacity (\%)} = \frac{\text{Vol. after homogenization} - \text{Vol. before homogenization}}{\text{Vol. before homogenization}} \times 100$$

$$\text{Foam stability (\%)} = \frac{\text{Foam volume after time (t)} \times 100}{\text{Initial foam volume}}$$

Emulsion properties

Oil emulsifying capacity (EC) was evaluated in 100 ml of 1% (w/v) aqueous dispersion of each sample at pH values of 3.0, 4.5, 6.0, 7.5 and 9.0 by titrating with corn oil to the break point of the emulsion (Naczki et al., 1985). Emulsifying capacity (EC) was expressed as ml oil emulsified by 1 g dry sample. The emulsion stability (ES) was recorded ($25 \pm 2^\circ\text{C}$) in term of the intervals of 15, 30, 50 min, 1, 2, 3, 24 and 48 h (Dipak and Kumar, 1986).

Gelation properties

Gelation properties of the samples were determined by employing the method of Adebawale et al. (2005). Samples suspensions of 2 to 20% (w/v) were prepared in 5 ml distilled water. The test tubes containing these suspensions were heated for 1 h in boiling water (100°C), followed by rapid cooling in ice. The test tubes were then cooled for 24 h at 4°C . The least gelation concentration (LGC) was taken as the concentration when the sample from inverted test tube did not fall down or slip.

Table 1. Chemical composition of flour and protein isolate extracted from Bambara groundnut.

Item	Chemical composition (% ± SD) of Bambara groundnut		LSD at 5%
	Bambara flour	Bambara protein isolate	
Protein	17.70 ^b ± 0.44	85.97 ^a ± 1.41	2.37
Fat	6.58	-	-
Ash	4.22 ^a ± 0.12	3.37 ^b ± 0.09	0.19
Crude fiber	3.50 ^a ± 0.15	0.02 ^b ± 0.00	0.24
Total carbohydrates*(%)	86.0 ^a ± 1.43	10.64 ^b ± 0.39	2.37

*Calculated by differences. All values are means of triplicate determinations ± standard deviation (SD). Means within rows different letters are significantly different ($P < 0.05$).

Bulking density

Bulking density was followed according to the method of Asoegwu et al. (2006). Samples were placed in a 25 ml graduated cylinder and packed by gently tapping the cylinder on the bench top 10 times from a height of 5 cm and the volume of the sample was recorded. The procedure was repeated two times for each sample and the bulk density was computed as g/ml of the sample.

$$\text{Bulking density} = \frac{\text{Weight of sample (g)}}{\text{Volume of sample (ml)}}$$

Protein solubility index (PSI)

Protein solubility index of Bambara groundnut flours and protein isolate were determined according to Clemente et al. (1998) in water or in 0.5 N NaCl at pH 7.0 and at ratio 1:2 (w/v). The pH was adjusted by 0.5 N HCl or 0.5 N NaOH. The suspension was shaken for one hour at room temperature and centrifuged at 6000 rpm for 15 min. Supernatant was analyzed for total nitrogen by microkjeldahl method (AOAC, 2000). Protein solubility is expressed as a percentage of the total protein content using factor of ($N \times 6.25$) in each sample.

Statistical analysis

The results were statistically analyzed by analysis of variance and means were compared using least significant difference (L.S.D.) at 0.05 levels according to the method described by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Chemical composition

The chemical composition of flour and protein isolate extracted from Bambara groundnut on dry weight basis are presented in Table 1. Data shows that the dry matter of Bambara groundnut flour and Bambara groundnut protein isolate were high in protein and carbohydrate contents. Regarding to the data, total carbohydrates was higher than protein in Bambara flour, in contrast with Bambara protein isolate, which protein content was higher than carbohydrates. These results confirmed by statistical analysis, for which highly significant differences ($p < 0.05$) were observed between the contents in the two

samples.

Although the fat content was 6.58% in Bambara flour, it was not detected in Bambara protein isolate. These results indicated that the fat component of the flours is largely removed during protein isolate preparation (Yusuf et al., 2008). Ash and crude fiber were also detected but at lower levels in both Bambara samples. However, a significant difference ($p < 0.05$) was observed between both Bambara samples, which ash and crude fiber contents were higher in flour than in protein isolate.

Similar results of Bambara flour were detected by Chikwendu (2007) and Yusuf et al. (2008) who showed that fat, protein, ash, crude fiber and total carbohydrates were 6.0 to 7.3, 17.40 to 22.36, 3.4 to 4.4, 3.30 to 6.10 and 53.1 to 70.52%, respectively.

Functional properties of Bambara groundnuts flour and protein isolate

Water and oil absorption

Water and oil absorption capacity of both flour and protein isolate were studied (Table 2). Data proved that both Bambara groundnuts flour and protein isolate were high in water absorption capacity (WAC) and lower in oil absorption capacity (OAC).

On the other hand, water and oil absorption capacity of Bambara groundnuts flour were significantly ($p < 0.05$) higher than the Bambara groundnuts protein isolate. The higher water absorption capacity of Bambara groundnuts flour may be due to the higher polar amino acid residues of proteins having an affinity for water molecules (Yusuf et al., 2008). The major chemical compositions that enhance the water absorption capacity of flours are proteins and carbohydrates, since these constituents contain hydrophilic parts, such as polar or charged side chains (Lawal and Adebawale, 2004).

The oil absorption of Bambara groundnuts flour was higher than those of proteins isolate. This suggested that Bambara flour may have more hydrophobic proteins flour; the more hydrophobic proteins demonstrate superior binding of lipids (Lawal and Adebawale, 2004). The major chemical component affecting oil absorption capacity is

Table 2. Water and oil absorption of flour and protein isolate extracted from Bambara groundnut (on dry weight basis).

Item	Bambara flour	Bambara protein isolate	LSD at 5%
	Dry sample (%)	Dry sample (%)	
Water absorption (%)	281.35 ^a ±2.27	221.83 ^b ±2.95	5.96
Oil absorption (%)	252.27 ^a ±4.49	102.29 ^b ±3.08	8.35

All values are means of triplicate determinations ± standard deviation (SD). Means within rows with different letters are significantly different ($P < 0.05$).

Table 3. Foam capacity (FC) of flour and protein isolate extracted from Bambara groundnut as affected by pH values

pH values	Volume increase (%) FC		LSD at 5%
	Bambara flour	Bambara protein isolate	
3.0	116 ^b ± 1.0	170 ^a ± 1.0	2.27
4.5	140 ^b ± 1.0	150 ^a ± 1.0	2.28
6.0	184 ^a ± 1.0	85 ^b ± 1.0	2.31
7.5	170 ^a ± 1.0	90 ^b ± 1.0	2.33
9.0	210 ^a ± 1.0	100 ^b ± 1.0	2.34

All values are means of triplicate determinations ± standard deviation (SD). Means within rows with different letters are significantly different ($P < 0.05$).

protein, which is composed of both hydrophilic and hydrophobic parts. Non-polar amino acid side chains can form hydrophobic interactions with hydrocarbon chains of lipid (Jitngarmkusol et al., 2008). These data showed highly significant differences ($p < 0.05$) between Bambara groundnuts flour and Bambara protein isolate of their water and oil absorption (%).

The values of Bambara groundnuts flour of water absorption are comparatively higher than the value of soybean flour (130%) and sunflower meal products reported by Padilla et al. (1996). African yam bean flour (118 to 179%) (Oshadi et al., 1997) and various lima bean flours (130 to 140%) (Adeyeye and Aye, 2005).

The higher water absorptivity reported in the present study suggested that Bambara groundnuts flour may be used in the formulation of some foods such as sausage, dough's, processed cheese, soups and baked products confirmed by Olaofe et al. (1998). Water retention capacity (WRC) is a critical function of protein in various food products like soups, dough and baked product (Adeyeye and Aye, 1998).

Oil absorption capacity is importance since oil acts as flavour retainer and increases the mouth feel of foods (Aremu et al., 2007). It has been reported that variations in the presence of non-polar side chains, which might bind the hydrocarbon side chains of oil among the flours, explain differences in the oil binding (Adebowale and Lowal, 2004).

However, the flours in the present study are potentially useful in structural interaction in food especially in flavour retention, improvement of palatability and extension of shelf life particularly in bakery or meat products where fat

absorption is desired (Aremu et al., 2007).

Foaming properties

Foam is a colloid of many gas bubbles trapped in a liquid or solid. Small air bubbles are surrounded by thin liquid films. Foam can be produced by whipping air into liquid as much and fast as possible (Sikorski, 2002). The reason why flours are capable of producing foams is that proteins in flours are surface active. Soluble proteins can reduce surface tension at the interface between air bubbles and surrounded liquid. Thus, the coalescence of the bubbles is obstructed. In addition, protein molecules can unfold and interact with one another to form multilayer protein film with an increased flexibility at the air liquid interface. As a result, it is more difficult for air bubbles to break, and the foams are more stabilized (Adebowale and Lawal, 2003).

Foaming capacity (FC) of flour and protein isolate extracted from Bambara groundnut as affected by pH values are presented in Table 3. Data showed that the maximum increase in foam volume (FC) of Bambara groundnut flour was 210% at pH 9.0. However, the lowest volume of the foam (116 %) was observed at pH 3.0. Results showed that FC increased as the pH values increased. While the maximum increase in foam volume (FC) of Bambara groundnut protein isolate (170%) was observed at pH 3.0. However, the lowest volume was recorded at pH 6.0 (8.5%). Data showed that FC of Bambara protein isolate decreased as pH values increased to pH 6.0 then increased again to pH 9.0.

Table 4. Foaming stability (FS) of flour and protein isolate extracted from Bambara groundnut as affected by pH values.

pH values	Foam volume (%) at room temperature (25 ± 2 °C) after different time (min.)										LSD at 5%	
	5	10	15	20	30	40	50	60	90	120		
3.0	a. 64 ^a ± 1.0	a. 59 ^b ± 2.0	a. 52 ^c ± 2.0	a. 40 ^d ± 1.0	a. 31 ^e ± 2.0	a. -	a. -	a. -	a. -	a. -	a. -	1.60
	b. 59 ^a ± 2.0	b. 56 ^b ± 2.0	b. 53 ^c ± 3.0	b. 53 ^c ± 3.0	b. 53 ^c ± 3.0	b. 53 ^c ± 3.0	b. -	b. -	b. -	b. -	b. -	1.52
4.5	a. 67 ^a ± 3.0	a. 40 ^b ± 3.0	a. 29 ^c ± 2.0	a. 21 ^d ± 2.0	a. 11 ^e ± 1.0	a. -	a. -	a. -	a. -	a. -	a. -	1.50
	b. 50 ^a ± 3.0	b. 27 ^b ± 2.0	b. 23 ^c ± 3.0	b. 23 ^c ± 3.0	b. 23 ^c ± 3.0	b. 23 ^c ± 3.0	b. -	b. -	b. -	b. -	b. -	1.32
6.0	a. 61 ^a ± 3.0	a. 60 ^b ± 2.0	a. 60 ^b ± 2.0	a. 43 ^c ± 1.0	a. 41 ^d ± 2.0	a. -	a. 35 ^e ± 2	a. 35 ^e ± 2.0	a. 30 ^e ± 2	a. 30 ^e ± 2	a. 30 ^e ± 2	1.32
	b. 59 ^a ± 3.0	b. 53 ^b ± 2.0	b. 47 ^c ± 2.0	b. 47 ^c ± 2.0	b. 47 ^c ± 2.0	b. 47 ^c ± 2.0	b. -	b. -	b. -	b. -	b. -	1.58
7.5	a. 41 ^a ± 3.0	a. 35 ^b ± 2.0	a. -	a. -	a. -	a. -	a. -	a. -	a. -	a. -	a. -	0.98
	b. 67 ^a ± 2.0	b. 67 ^a ± 2.0	b. 56 ^b ± 2.0	b. 56 ^b ± 2.0	b. 56 ^b ± 2.0	b. 56 ^b ± 2.0	b. -	b. -	b. -	b. -	b. -	1.60
9.0	a. 59	a. -	a. -	a. -	a. -	a. -	a. -	a. -	a. -	a. -	a. -	-
	b. 95 ^a ± 3.0	b. 80 ^b ± 2.0	b. 80 ^b ± 2.0	b. 80 ^b ± 2.0	b. 80 ^b ± 2.0	b. 80 ^b ± 2.0	b. -	b. -	b. -	b. -	b. -	1.81

All values are means of triplicate determinations ± standard deviation (SD). Means within rows with different letters are significantly different ($P < 0.05$). a. Bambara groundnut flour. b. Bambara groundnut protein isolate. (-): No foaming.

Statistical analysis proved that highly significant differences ($p < 0.05$) were recorded between FC in Bambara flour and Bambara protein isolate at different pH values 9.0 and 3.0, respectively.

The foaming stability (FS) of Bambara groundnut flour and Bambara protein isolate are presented in Table 4. Data shows that FS decreased gradually until 30 min in Bambara groundnut flour and 15 min in Bambara protein isolate and then stabilized until 40 min. The highest foam stability was recorded at pH 6.0 for 120 min in Bambara flour, which was about 50% from foam volume (%), was stabilized until the end of time (120 min). This could be due to the increase in protein solubility after removing lipids (Jitngarmkusol et al., 2008). Greater concentration of soluble proteins at aqueous phase could

enhance foam formation. On the other hand, the highest foam stability was observed at pH 9.0 for 40 min of Bambara protein isolate. After 40 min, about 84% of foam volume was resistant. Statistical analysis proved highly significant differences ($p < 0.05$) in Bambara flour as pH values (pH 6.0). However, no/or slightly differences observed with Bambara protein isolate at pH 9.0.

There was an inverse relationship between FC and FS. Flours with high foaming ability could form large air bubbles surrounded by thinner a less flexible protein film. This air bubbles might be easier to collapse and consequently lowered the foaming stability (Jitngarmkusol et al., 2008). These results suggest that the Bambara groundnut 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 similar to that reported by Lee et al. (1993).

Emulsion properties

The emulsification properties of protein-containing products like legume flours may result from both soluble and insoluble protein, as well as other components, such as polysaccharides. Protein can emulsify and stabilize the emulsion by decreasing surface tension of then oil droplet and of the oil droplet and providing electrostatic repulsion on the surface of the oil droplet (Sikorski, 2002), while some types of polysaccharides can help stabilize the emulsion by increasing the viscosity of the system (Dickinson, 1994).

Table 5. Emulsion capacity (EC) of flour and protein isolate extracted from Bambara groundnut as affected by pH values.

pH values	ml oil /g sample (EC)		LSD at 5%
	Bambara flour	Bambara protein isolate	
3.0	116 ^a ±1.0	111 ^b ±1.0	2.27
4.5	134 ^a ±1.0	120 ^b ±1.0	2.27
6.0	125 ^a ±2.0	89 ^b ±2.0	4.53
7.5	89 ^a ±3.0	76 ^b ±2.0	5.78
9.0	98 ^a ±3.0	71 ^b ±2.0	5.78

All values are means of triplicate determinations ± standard deviation (SD). Means within rows with different letters are significantly different (P < 0.05).

Table 6. Emulsion stability (ES) of flour and protein isolate extracted from Bambara groundnut as affected by pH values.

pH values	ES (%) aqueous phase separated after time								LSD at 5%
	15 min	30 min	50 min	1.0 h	2.0 h	3.0 h	24.0 h	48.0 h	
3.0	a. -	a. 39 ^g ± 0.01	a. 9 ^f ± 0.02	a. 18 ^e ± 0.3	a. 32 ^d ± 1.0	a. 60 ^c ± 3.0	a. 80 ^a ± 3.0	a. 70 ^b ± 2.0	1.30
	b. -	b. -	b. -	b. -	b. -	b. 30 ^c ± 2.0	b. 75 ^b ± 3.0	b. 80 ^a ± 3.0	1.22
4.5	a. 10 ^h ± 0.02	a. 15 ^g ± 0.03	a. 20 ^f ± 2.0	a. 28 ^e ± 3.0	a. 42 ^d ± 3.0	a. 46 ^c ± 2.0	a. 54 ^a ± 3.0	a. 50 ^b ± 3.0	1.27
	b. 5 ^h ± 0.3	b. 7 ^g ± 0.02	b. 17 ^f ± 2.0	b. 20 ^e ± 2.0	b. 35 ^d ± 2.0	b. 40 ^c ± 3.0	b. 48 ^b ± 3.0	b. 55 ^a ± 3.0	1.28
6.0	a. 5 ^h ± 0.01	a. 18 ^g ± 2.0	a. 36 ^f ± 3.0	a. 40 ^e ± 3.0	a. 60 ^d ± 2.0	a. 64 ^c ± 2.0	a. 80 ^a ± 3.0	a. 70 ^b ± 3.0	1.22
	b. -	b. 30 ^f ± 2.0	b. 75 ^e ± 2.0	b. 75 ^e ± 2.0	b. 80 ^d ± 3.0	b. 85 ^c ± 3.0	b. 90 ^b ± 3.0	b. 95 ^a ± 3.0	1.45
7.5	a. 20 ^h ± 0.03	a. 32 ^g ± 3.0	a. 40 ^f ± 3.0	a. 44 ^e ± 2.0	a. 56 ^d ± 3.0	a. 60 ^c ± 3.0	a. 69 ^a ± 3.0	a. 61 ^b ± 2.0	1.61
	b. 5 ^h ± 0.03	b. 13 ± 0.02	b. 17 ^f ± 2.0	b. 20 ^e ± 2.0	b. 25 ^d ± 2.0	b. 35 ^c ± 3.0	b. 50 ^b ± 3.0	b. 65 ^a ± 3.0	1.78
9.0	a. 2 ^h ± 0.01	a. 7 ^g ± 0.01	a. 12 ^f ± 0.03	a. 15 ^e ± 2.0	a. 32 ^d ± 3.0	a. 52 ^b ± 3.0	a. 60 ^a ± 3.0	a. 50 ^c ± 2.0	1.43
	b. -	b. 6 ^g ± 0.02	b. 15 ^f ± 1.0	b. 18 ^e ± 2.0	b. 25 ^d ± 2.0	b. 30 ^c ± 3.0	b. 55 ^b ± 3.0	b. 60 ^a ± 3.0	1.60

All values are means of triplicate determinations ± standard deviation (SD). Means within rows with different letters are significantly different (P < 0.05). a. Bambara groundnut flour. b. Bambara groundnut protein isolate (-): No emulsion stability.

Emulsion capacity (EC) of flour and protein isolate extracted from Bambara groundnut as affected by pH values are shown in Table 5. The results revealed that minimum emulsifying capacities of the Bambara groundnut flour were observed at pH 7.5 (89 ml oil/g sample) and markedly increased below and above pH 7.5 reaching their maximum at pH 4.5 (134 ml oil/g sample). The decrease in EC with time might be due to increased contact leading to coalescence reducing the stability (Aremu et al., 2008).

The emulsion capacity (EC) of Bambara groundnut protein isolate (Table 5) was more efficient in emulsifying the oil at pH 4.5 (120 ml oil/g sample). The minimum emulsifying capacities of Bambara protein isolate were detected at pH 9.0 (71 ml oil/g sample) and markedly increased above pH 9.0, reaching their maximum at pH 4.5 (120 ml oil/g sample), near the isoelectric point.

Statistical analysis proved that Bambara flour was highly significant differences (p < 0.05) than Bambara protein isolate as pH values affected. At the same time, EC in both Bambara (flour and protein isolate) was

affected significantly as the differences of pH values (pH 4.5).

Emulsion stability (ES) of flour and protein isolate extracted from Bambara groundnut as affected by pH values are shown in Table 6. The higher emulsion stability (ES), that is, the lower percentage of aqueous phase separated after 48 h of the flour was noticed at pH 4.5 being 50%. On the other side, the lower ES was found at pH 3.0 being 70%. These variations in the emulsifying properties of Bambara groundnut flour over the pH range of 3.0 to 9.0 were probably due to differences in their chemical composition.

These values are comparable to bennised 63% and pearl millet 89% reported by Oshadi et al. (1999). However, the values are much higher than soybean flour (18%) and wheat flour (11%) reported by Olaofe et al. (1998). The flours may be most useful as an additive for the stabilization of fat emulsion in the production of sausage, soup and cake (Aremu et al., 2008).

Regarding to Bambara protein isolate, the higher emulsion stability (ES) was noticed at pH 4.5 after 48 h

Table 7. Least gelation concentration (LGC) of flour and protein isolate extracted from Bambara groundnut.

Concentration (%)	Gelation formation (% \pm SD)		LSD at 5%
	Bambara flour	Bambara protein isolate	
2.0	20 ^b \pm 3.0	28 ^a \pm 3.0	1.21
4.0	18 ^b \pm 2.0	26 ^a \pm 3.0	1.19
6.0	17 ^b \pm 2.0	24 ^a \pm 3.0	1.17
8.0	15 ^b \pm 1.0	23 ^a \pm 3.0	1.13
10.0	14 ^b \pm 1.0	21 ^a \pm 3.0	1.11
12.0	12 ^b \pm 1.0	20 ^a \pm 2.0	1.12
14.0	11 ^b \pm 1.0	20 ^a \pm 2.0	1.09
16.0	10 ^b \pm 1.0	19 ^a \pm 2.0	1.08
18.0	9 ^b \pm 1.0	19 ^a \pm 2.0	1.05
20.0	8 ^b \pm 1.0	18 ^a \pm 2.0	1.05

being 55% (Table 6). On the other hand, the lower ES was found at pH 6.0 being 95%. Yusuf et al. (2008) reported that groundnut and soybean protein isolate were more efficient in emulsifying oil at pH 3.0 (100 ml oil/g sample) than at alkaline pH 8.0 (82 ml oil/g sample). The experimental conditions, such as equipment design, shape of the container, temperature, speed of blending, nature of blades in the blender, rate and mode of addition, pH, protein, solubility, concentration, presence of salt and water, would all individually contribute to the emulsifying capacities of protein (Jitngarmkusol et al., 2008).

The results obtained confirmed by statistical analysis showed highly significant differences between ES in Bambara flour and ES in Bambara protein isolate during different pH values (pH 4.5).

Gelation properties

The least gelation concentration (LGC) which is defined as the lowest protein concentration at which gel remained in the inverted tube was used as index of gelation capacity. The lower the LGC, the better the gelating ability of the protein ingredient (Akintayo et al., 1999).

Effects of Bambara flour concentration and protein isolate on gelation capacity are presented in Table 7. Generally increase in flour concentration and protein isolate from 2 to 20% w/v improved the gelation capacity of flours and protein isolate. Adebowale and Lawal (2004) have also reported improved on protein gel texture using carbohydrates. Improvement in gelation capacity following the increase in concentration of sample flours and protein isolate is because of decrease in thermodynamic affinity of proteins for the aqueous solution, which increased the interaction between proteins.

The results indicate that gelation of the Bambara groundnut flours and protein isolate were best at concentration 20% (w/v), which recorded 8 and 18%, respectively.

Variation in the values obtained might be linked to the relative ratio of different constituents- protein, carbohydrates and lipids as suggested by Aremu et al. (2007) that the interaction between such components may affect functional properties.

The ability of protein to form gels and provide a structure matrix for holding water, flavours sugars and food ingredients is useful in food applications and in new product development, thereby providing an added dimension to protein functionality (Oshadi et al., 1997). The low gelation concentration observed may be an asset in the use of these flours for the formation of curd or as an additive to other gel forming materials in food products (Aremu et al., 2007). Protein gelation is vital in the preparation and acceptability of many foods, including vegetables and other products (Lawal et al., 2007).

Bulking density

Bulking density of Bambara groundnut flour and protein isolate were studied (Table 8). The bulking density in Bambara groundnut flour is higher than the values of Bambara groundnut protein isolate. Statistical analysis proved that highly significant difference ($p < 0.05$) for both Bambara (flour and protein isolate).

These collected values under investigation are higher than the values reported for various samples of extrusion texturized soybean products with varied protein and soluble sugar contents (0.238 to 0.446 g/ml) (Aremu et al., 2007) and varied processed defatted fluted pumpkin seed flours (0.180 to 0.380 g/ml) (Fagbemi et al. 2006).

Protein solubility index (PSI)

The results for variation of protein solubility with pH values are presented in Figure 1. Generally, solubility decreased as the pH values increased until it reached minimum isoelectric point between pH 4.0 to 5.0.

Table 8. Bulk density (g/ml sample) of flour and protein isolate extracted from Bambara groundnut.

Sample	Bulk density (g/ml sample)
Bambara flour	0.621 ^a ± 0.02
Bambara protein isolate	0.556 ^b ± 0.01
LSD at 5%	0.04

All values are means of triplicate determinations ± standard deviation (SD). Means within column with different letters are significantly different ($P < 0.05$).

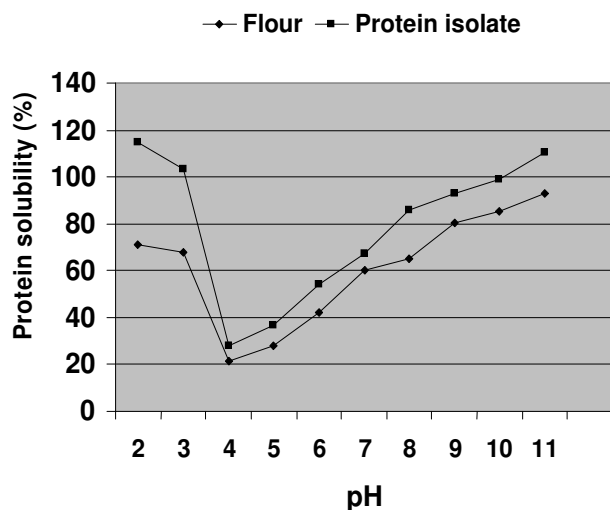


Figure 1. Effect of pH value on the protein solubility (%) of Bambara groundnut flour and protein isolate

Bambara groundnut flour had a minimum protein solubility of 21% around pH 4.0, increasing either side of this pH. About 71% of protein was soluble at pH 2.0 and at pH 11.0 was about 93% soluble. Beyond pH 11.0, no marked improvement in protein solubility was observed. The solubility behaviour was similar to that of soy bean flour and raw winged bean flour (Yusuf et al., 2008).

While Bambara groundnut protein isolate had a minimum protein solubility of 27.68% around pH 4.0, increasing with side of this pH. About 115% of protein was soluble at pH 2.0 and at pH 11.0, it was about 110% soluble. Similar observations have been presented earlier by Aremu et al. (2007). On the other hand, Fagbemi et al. (2006) reported that there is denaturation of pH which increases protein solubility. The solubility in both acid and alkali indicates that it may not be useful in formulating carbonated beverages (Olaofe et al., 1998).

Conclusion

It could be concluded that the chemical composition (high concentration of carbohydrates, protein and fat) beside the functional properties (water and oil absorption,

foaming, emulsion, gelation, bulking density and protein solubility index) of Bambara groundnut makes these seeds an ideal raw material for successful utilization in various food products and different beverages.

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