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On the functional properties of globulin and albumin protein fractions and flours of African locust bean (*Parkia biglobossa*)

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Abstract

Albumin (ALBa) and globulin (ALBg) fractions of African locust bean were isolated and the functional properties were compared with its defatted (ALBdf) and undefatted flours (ALBf). Albumin had minimum % solubility (56.7%) at pH5, while minimum solubility was observed at pH4 for globulin and the flours. In all the samples studied, maximum solubility was observed at pH 10. A pH-dependent gelation study revealed that all of the samples had the highest least gelation concentration at pH10 apart from ALBf which had 16% w/v LGC at pH 2. Initial increase in ionic strength of the medium, to 0.4 and 0.6 M, enhanced the gelation capacity of protein fractions and flours, respectively, while further increase in ionic strength reduced it. Oil absorption capacity was maximal in ALBa while ALBf had the least value of 1.05 ml/g. Initial increase in ionic strength, up to 0.4 M, increased the water absorption capacity (WAC) of albumin fractions while WACs of the globulin fraction and flours were reduced when the ionic strength of the media reached 0.4 M. Foam capacity increased as the concentration of protein solution increased but was reduced by 6% w/v in ALBf. Initial increase in ionic strength enhanced both foam capacity and stability. Maximum EA was observed at pH 10 in all samples apart from ALBf, which reached a peak EA value at pH 2. ES (emulsion stability) was maximal at pH 10 for ALBa and ALBg while the same values were observed for ALBdf and ALBf at pH 2 and 10. Increasing the ionic strength, to 0.4 M, enhanced the EA and ES of ALBa while further increase in ionic strength, to 0.7 M, improved EA of ALBf but reduced the ES. Both EA and ES of ALBf reached peak values in 0.2 M solutions but no fixed pattern was observed in the response of ALBdf to various ionic strengths of the solutions.

Keywords: Functional properties; Albumin; Globulin; African Locust bean

1. Introduction

In Africa, where the per capita consumption of essential diet any components is generally very low, legumes represent the major source of proteins (Araujo et al., 2002). Although the production of grain legumes is relatively small when compared to cereals, they make a greater relative contribution to human nutrition (FAO, 1980). However, previous studies have revealed that some legumes are still underutilized (Adebowale & Lawal, 2003, 2004; Lawal & Adebowale, 2004). African locust bean, a leguminous tree plant that grows naturally in the tropical forest and savannah belt of Nigeria, is known to produce abundant seeds that are only utilized as food condiment (Lawal, 2004).

In legume seeds, globulins (7S vicilins and 11S legumins) account for 30–80% of the total seed protein, with albumin constituting the remainder (Derbyshire, Wright, & Boulter, 1976; Mackuka, 2000; Peterson,

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1987; Borroto & Dure, 1987; Gorinstein, Zemser, Fiedman, Vasco-Mendez, & Paredes-Lopez, 1996).

A typical seed storage protein occurs as oligomers composed of two or more subunits that are in turn made up of a number of polypeptide chains. In the report of Bewley and Black (1994), 7S vicilins and 11S legumins in legumes were found to consist largely of multisubunit complexes with molecular masses of 145–190 and 320–400 kDa, respectively. Also, the legumins consist of six non-identical subunits (52–65 kDa), with each subunit containing an acidic (33–42 kDa) and a basic (19–23 kDa) polypeptide. Proteins within the albumin class are more diverse, both structurally and functionally (Ward, Uknes, & Ryals, 1994).

The use of plant proteins as food hydrocolloids is based on their functional properties, such as emulsification, solubility, foaming properties, water and oil absorption capacities and gelling properties (Kinsella & Phillips, 1989). Also, it is expedient to study these functional properties in relation to the prevalent environment of the protein, and pH and ionic strength are paramount (Myers, 1988; Aluko & Yada, 1995). Extensive literature has been published on protein functionality in relation to pH, ionic strength, varying protein concentration, presence of various salts and presence of other food hydrocolloids (Schwenke, 2001; Prakash, 1986; Mitchell, 1986; Shimizu, Saito, & Yamauchi, 1985; McWatters & Holmes, 1979). These efforts were aimed at effective utilization of inexpensive proteins for nutritional and functional purposes.

In previous studies, functional properties of plant protein fractions (globulin and albumin) have been reported. These include: pea (Koyoro & Powers, 1987), cowpea (Aluko and Yada, 1985), lentil (Bora, 2002), rapeseed (Gueguen, Bollecker, Schwenke, & Raab, 1990), canola seed (Gruener & Ismond, 1997) and great northern bean (Sathe & Salunkhe, 1981a; Sath & Salunkhe, 1981b).

The purpose of this study was to determine the functional properties of globulin and albumin fractions of African locust bean protein and its flours. Specifically, protein solubility, gelation, water and oil absorption capacity, foaming and emulsifying properties were studied in relation to the effects of pH, ionic strength, and various protein concentrations.

2. Materials and methods

2.1. Materials

African locust beans were bought from Bodija market in Ibadan, Nigeria. The seeds were identified at the International Institute of Tropical Agriculture Ibadan. All other chemicals used were of analytical grade.

2.2. Preparation of flours

Foreign objects in African locust bean were removed by picking, following which they were ground to pass through a BS-60 mesh screen. A flour mill (Braun multimix Deluxe Germany) was used for the milling; 0.5 kg of the flour was defatted with hexane for 16 h under constant shaking. The hexane was changed four times to enhance removal of fat, following which the solvent was decanted and the flour air-dried. Another 0.2 kg flour was left undefatted for comparative experiments.

2.3. Fractionation of albumin- and globulin-enriched fractions

Globulin and albumin were prepared using the method described by Rudger (1993), as modified by Mackuka (2000). Defatted African locust bean flour was extracted with 0.05 M tris-HCl buffer (pH 8.0) containing 0.1 M NaCl, 1 mM $CaCl_2$ and 1 mM $MgCl_2$ in a ratio of 10 ml buffer/g of flour for 3 h at 4 °C. The slurry was centrifuged at 20,000*g* for 30 min at 4 °C and the supernatant filtered through Whatman number 1 paper. The pH of the filtrate was adjusted to 4.5 by slowly adding 1 M acetic acid and the proteins were recovered by centrifugation at 19,000*g* for 20 min at 4 °C. The precipitate obtained was resuspended in distilled water and lyophilised as the globulin fraction. The albumin-enriched supernatant solution was adjusted to pH 8.0 with 1 M NaOH, dialysed against distilled water for 48 h, and lyophilised.

2.4. Protein solubility profile

The method of Were, Hettiarachchy, and Kalapathy (1997) was employed for the determination of pH-dependent solubility, profile. One hundred and twenty five milligrammes (125 mg) of the sample were dispersed in 25 ml of distilled water and the solution pH was adjusted to 2–10, using 0.5 M NaOH or 0.5 M HCl. The slurries were mixed for 1 h at 24 °C, using a magnetic stirrer before centrifuging at 12,000*g* for 20 min at 4 °C. The supernatant was filtered through glass wool to obtain a clear solution. Protein content in the supernatant was determined by the Kjeldahl method. Triplicate determinations were carried out and solubility profile was obtained by plotting averages of protein solubility (%) against pH. The percentage soluble protein was calculated as follows:

$$\text{Solubility (\%)} = \frac{\text{Amount of nitrogen in the supernatant}}{\text{Amount of nitrogen in the sample}}.$$

2.5. Foaming capacity and stability

The foaming capacity and stability were studied according to the method of Coffman and Garcia (1977). A weighed amount of protein concentrate was dispersed in 100 ml of distilled water. The resulting solution was whipped vigorously for 2 min in a Phillips Kitchen blender set at speed 2. Volumes were recorded before and after whipping. The percentage volume increase was calculated according to the following equation:

$$\text{Volume } (\%) = \frac{(V_2 - V_1)}{(V_1)} \times 100,$$

where V_2 is the Volume of protein solution after whipping and V_1 is the Volume of solution before whipping.

Effects of concentration on the foaming properties were investigated by whipping 2%, 4%, 6%, 8% and 10% w/v of the dispersions as described above. The effect of pH on foaming properties was carried out by adjusting a 2% w/v dispersion to the desired pH range from 2.0 to 10.0, using either 1 M HCl or 1 M NaOH, followed by vigorous whipping as described above.

Influence of ionic strength was evaluated by dispersing 2 g of protein concentrate in 100 ml KCl solution of known ionic strength. Studies were conducted in solutions with ionic strength (μ) of 0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0, after which they were whipped vigorously.

2.6. Gelation properties

Gelation properties were investigated, using the method described by Coffman and Garcia (1977). Sample suspensions of 2–20% w/v were prepared in distilled water. 10 ml of each of the prepared dispersions were transferred into a test tube. This was heated in a boiling water bath for 1 h, followed by rapid cooling in a bath of cold water. The test tubes were cooled further at 4 °C for 2 h. The least gelation concentration was taken as the concentration when the sample from the inverted tube did not fall or slip.

Studies on the effect of pH were conducted on the sample at various concentrations by adjusting the pH to the desired value from 2.0 to 10.0, prior to heating, using either 0.5 M HCl or 0.5 M NaOH. Least gelation concentration was determined as described above.

Effect of ionic strength was investigated by preparing sample suspensions (2–20% w/v) in NaCl solution of known ionic strengths of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 M.

2.7. Emulsifying properties

Emulsifying activity and stability were determined using the method of Neto, Narain, Silva, and Bora (2001); 5 ml portions of protein solutions and flour dispersions (10 mg/ml) were homogenized with 5 ml oil (Executive Chef Unilever Plc) for 1 min. The emulsions were centrifuged at 1100g for 5 min. The heights of emulsified layer and that of the total contents in the tube were measured.

The emulsifying activity was calculated as

$$\text{EA } (\%) = \frac{\text{Height of emulsified layer in the tube}}{\text{Height of the total content in the tube}} \times 100.$$

Emulsion stability was determined by heating the emulsion at 80 °C for 30 min before centrifuging at 1100g for 5 min

$$\text{ES } (\%) = \frac{\text{Height of emulsified layer after heating}}{\text{Height of emulsified layer before heating}} \times 100.$$

Effects of concentration on emulsifying activity and stability of protein isolate were studied by preparing 1–10% w/v solutions before conducting experiments as described above.

Influence of pH was studied by preparing protein solutions (10 mg/ml) at various pHs (2–10). Effect of ionic strength was studied by preparing protein solutions (10 mg/ml) at various ionic strengths of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 NaCl solutions.

2.8. Water and oil absorption capacities

Water and oil absorption capacities were measured by the method of Beuchat (1977). 1 g of sample was mixed with 10 ml distilled water or oil (Executive Chef Unilever Plc) for 30 s. The samples were then allowed to stand at room temperature (30 ± 2 °C) for 30 min before centrifuging at 5000g for 30 min. The volume of supernatant was noted in a 10 ml-graduated cylinder.

Effect of ionic strength on water absorption capacity was investigated by varying the ionic strength of the medium, using 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 M NaCl solutions. Studies were conducted as described above and each experiment was conducted in triplicate.

2.9. Statistical analyses

All determinations triplicated and mean values and standard deviations reported. Analyses of variance (ANOVA) were achieved to calculate significant differences in treatment means, and the mean separations were achieved by Tukey's HSD test ($p < 0.05$) using Sigmatstat® Version 2.0 (Jandel Scientific/SPSS Science, Chicago, IL, USA).

3. Results and discussion

3.1. Protein solubility

Protein solubility profile of the samples is presented on Fig. 1. The protein solubility profiles indicate higher

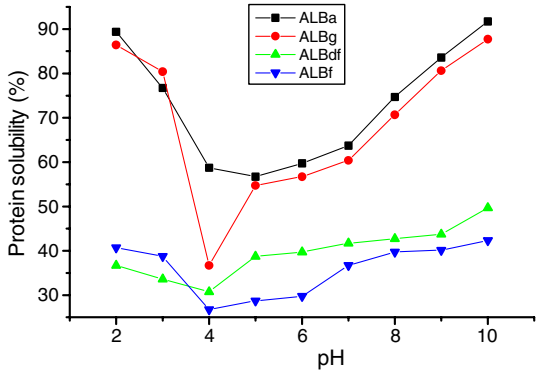

Fig. 1. Protein solubility profile of albumin (ALBa), globulin (ALBg), defatted (ALBdf) and undefatted flours (ALBf) of African locust bean.

solubilisation at pH 10 than at other pHs. Albumin had minimum solubility at (56.7%) at pH 5, while minimum solubility was observed at pH 4 for globulin and the flours. For all the samples, % solubility increased as the pH increased at pH values alkaline to the isoelectric pH. In all the samples studied, maximum solubility was observed at pH 10. A similar observation on solubility of proteins of great northern bean has previously been reported (Sathe & Salunkhe, 1981a, 1981b). In a similar way, Gruener and Ismond (1997) have also reported a pH-dependent solubilisation of canola 12 s globulin; high solubility was reported for the proteins at the pH extremes 2 and 9. Also, Bora (2002) reported high solubility for native and succinylated lentil globulins.

Electrostatic interactions which involve ionisation of the interior non-polar groups by alkali and acid lead these groups to disrupt the native structure of the proteins, thus shifting the equilibrium towards the unfolded form and subsequently to expose the buried functional groups in the protein molecules. This development causes intermolecular repulsion and enhances protein solubilisation.

3.2. Gelation

pH-dependent gelation is presented in Table 1. Apart from ALBf, all other samples have the highest least gelation concentration at pH 10. The lowest LGCs, for all of the samples, were observed at pH 4. At pH 7, which serves as the control, the minimum LGC (8) was observed in protein solution prepared with defatted African locust bean flour, while the globulin did not form gel until it reached 14% w/v. Observations presented in this work agree with the report of Sathe and Salunkhe (1981a, 1981b) on gelation properties of great northern bean flour and protein fractions, in which case 10%, 18% and 20% w/v were recovered for flour, albumin and globulin, respectively. However, using the LGC as the index of gelation, then, the African locust bean flour and its protein fractions will be better gelating food components than great northern bean flours and its protein fractions. In the present study, among the samples investigated, the results suggest that defatted ALB flour will be a better gelating food component than other samples. The presence of carbohydrates of higher quantity in the defatted flour probably facilitated its better gelating property. Earlier, we have observed improvement in the gelating property of Mucuna bean protein concentrate in the presence of other carbohydrates, such as sucrose, maltose, lactose and starch (Adebowale & Lawal, 2003). Fat probably interfered in the building of the molecular network needed for gel formation and this explains why ALBdf has a lower LGC than ALBf. Electrostatic repulsion was minimal at pH 4 and this probably enhanced formation of the necessary intermolecular forces among the protein molecules which led to better gelation in this region while, at pH 2 and 10, the lowering of gel strength could be a result of increased electrostatic repulsion among the protein molecules (over the electrostatic attraction forces that form part of the bonding forces in the molecular network). This observation lends credence to similar reports soybean globulins (Castimpoolas & Meyer, 1970).

Table 2 presents the effect of ionic strength on gelation properties. Increasing the ionic strength to 0.4 and 0.6 M reduced the least gelation concentration of protein fractions and their flours, respectively. However, further increase in ionic strength increased the least gelation concentration compared with the control protein solution. The minimum LGC was showed in ALBdf while ALBg showed the highest LGC at all ionic

Table 1
Effect of pH on least gelation concentrations of albumin (ALBa), globulin (ALBg), defatted (ALBdf) and undefatted flours (ALBf) of African locust bean[a]

Sample	PH					
	2	4	6	7	8	10
ALBa	16	10	12	12	16	18
ALBg	16	12	14	14	18	18
ALBdf	14	4	6	8	10	16
ALBf	16	6	8	10	12	14

[a] Values are least gelation concentration expressed as % w/v.

Table 2
Effect of ionic strength on least gelation concentrations of albumin (ALBa), globulin (ALBg), defatted (ALBdf) and undefatted flours (ALBf) of African locust bean[a]

Sample	Ionic strength (M)						
	0.0	0.1	0.2	0.4	0.6	0.8	1.0
ALBa	12	10	10	8	14	14	16
ALBg	14	14	12	10	16	18	18
ALBdf	8	6	6	4	4	10	12
ALBf	10	10	8	8	6	12	14

[a] Values are least gelation concentration expressed as % w/v.

strengths. Protein gels are formed by intermolecular interactions, which produce a continuous three-dimensional network exhibiting structural rigidity. The mechanism of cross-linking involves formation of multiple hydrogen bonds (Eldrigde & Ferry, 1954), sulphuryl–disulphide bonds (Higgins, Tapley, & Jensen, 1951) and interchange and formation of peptide groups (Bello, 1965). At low ionic strength, unfolding of buried functional groups within protein matrices was enhanced and this facilitated improved interaction among the protein molecules. This development accounts for better gelation in the 0.2 M solution compared with the control. However, further increase in ionic strength caused a shielding effect on the protein molecules and this phenomenon caused salting-out and subsequently, gelation was impaired compared with the control solution.

3.3. Water and oil absorption capacities

The oil absorption capacity is presented in Fig. 2. The albumin protein fraction, which has the highest oil absorption capacity, absorbed 3.4 ml/g of protein while minimum OAC was observed in ALBf. In a recent study, Lqari, Vioque, Pedroche, and Millan (2002) reported a higher oil absorption capacity for proteins of Lupinus angustifolius than for the flour. Similarly higher oil absorption capacities of proteins of *Phaseolus lunatus*, *Canavalia ensiformis* and soybean than flours have been reported (Chel-Guerrero, Perez-Flores, Bentacur-Ancona, & Davila-Ortiz, 2002). Probably, the albumin and globulin fractions, following the process of isolation, have better surface area and hydrophobicity, which improves the oil absorption capacity. The difference between oil absorption capacities of albumin and globulin is possibly due to conformational characteristics of these proteins. This development influences the capacity of the protein to entrap oil (Kinsella, 1976).

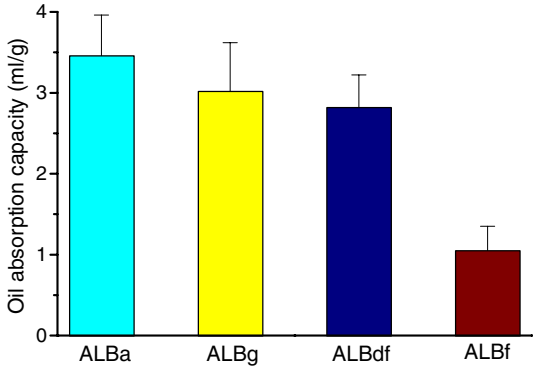

Fig. 2. Oil absorption capacity of albumin (ALBa), globulin (ALBg), defatted (ALBdf) and undefatted flours (ALBf) of African locust bean. Error bars: Standard deviations results are means of triplicate determinations.

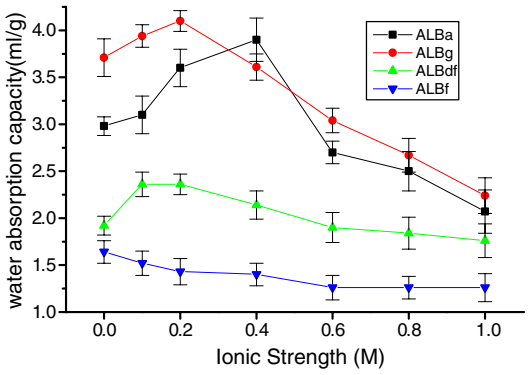

Fig. 3. Effect of ionic strength on water absorption capacity of albumin (ALBa), globulin (ALBg), defatted (ALBdf) and undefatted flours (ALBf) of African locust bean. Error bars: Standard deviations results are means of triplicate determinations.

Effect of ionic strength on water absorption capacity is presented in Fig. 3. For the albumin protein fraction, increase in ionic strength up to 0.4 M improved the water absorption capacity compared with the control, while further increase in water ionic strength reduced the water absorption capacity progressively. For the globulin protein fraction and the flours, increase in water absorption capacity was recorded up to 0.2 M following which a decline in value of WAC occurred. It is noteworthy that, from 0.6 M solution to 1.0 M, increase in ionic strength did not affect the WAC of ALBf, as the value of 1.26 ml/g remained constant. Better response of protein fractions to water absorption compared with the flours may reasonably be attributed to some external factors during the process of isolation. These may include pH and stirring velocity, which may affect the nature of the water-binding sites in the protein molecules. Initial increase in ionic strength facilitated improved water absorption capacity. At low salt concentration, hydrated salt ions bind weakly to charged groups on proteins and binding of ions to proteins did not affect the hydration shell of the charged groups on the protein. Therefore, increase in water binding essentially came from water associated with the ions. However, as the salt concentration increased, much of the existing water was bound to salt ions and this led to dehydration of the proteins and subsequent reduction in water-binding capacity.

3.4. Foam properties

Effect of concentration on foam capacity is presented on Table 3. For all the samples studied, foam capacity increased as the concentration of protein solution increased, except that a decline was observed in FC of ALBf above 6% w/v. However, it is noteworthy that, even at these concentrations (8% and 10% w/v), FC was still higher than the value obtained at 20% and

Table 3
Effects of concentration on foam capacity of albumin (ALBa), globulin (ALBg), defatted (ALBdf) and undefatted flours (ALBf) of African locust bean[A,B]

Sample	Concentration (% w/v)				
	2	4	6	8	10
ALBa	$76.8^{a} \pm 0.9$	$81.7^{a} \pm 1.7$	$94.17^{a} \pm .7$	$97.8^{a} \pm 1.9$	$109.1^{a} \pm 2.0$
ALBg	$70.3^{a} \pm 1.5$	$80.2^{a} \pm 0.9$	$81.7^{b} \pm 0.8$	$81.9^{b} \pm 0.9$	$107.4^{b} \pm 2.3$
ALBdf	$66.4^{b} \pm 0.8$	$74.3^{b} \pm 0.9$	$79.6^{b} \pm 1.2$	$81.0^{b} \pm 1.6$	$93.6^{b} \pm 2.1$
ALBf	$47.8^{c} \pm 0.9$	$56.8^{c} \pm 0.7$	$79.1^{b} \pm 1.9$	$69.6^{c} \pm 1.0$	$59.4^{c} \pm 2.8$

[A] Each value represents the mean of three determinations ± standard deviation.
[B] Samples followed by the same letter are not significantly different ($p < 0.05$).

4% w/v. Probably, the high lipid content of ALBf compared with other samples is responsible for the decline in foam capacity above 6% w/v. Lipids, when present at concentrations greater than 0.5%, markedly impair the foaming properties of proteins because lipids are more surface active than are proteins; they readily adsorb at the air–water interface and inhibit adsorption of proteins during foam formation.

Foam stability was concentration-dependent (Table 4). Foam stability increased as the concentration increased in ALBa, ALBg, and ALBdf. However, foam stability decreased with increase of concentration of ALBf. Previously, Akintayo, Oshodi, and Esuoso (1999) reported increase in foam stability as the concentration of pigeon pea protein increased. Also, in previous work, Sathe and Salunkhe (1981a, 1981b) had reported increase in foam stability as the concentration of globulin, albumin and flours of great northern bean increased. Protein–protein interaction was improved by increase in protein concentration. This improves viscosity and facilitates formation of multilayer cohesive protein film at the interface. Coalescence of bubbles is minimized by formation of cohesive multilayer films. Also, increase in concentration probably led to formation of thicker films, which limit the effect of drainage of proteins from films. Because lipid films lack the cohesive and viscoelastic properties of the foam bubbles, the rapidly expand and then collapse; this explains why foam stability decreases with increase in concentration of the undefatted flour.

Foam capacity was pH-dependent (Fig. 4). All samples investigated recorded minimum foam capacity when pH reached 4 apart from ALBa, which had its minimum foam capacity at pH 5. Maximum foam capacity, in all cases, was recorded at pH 10. The pattern of foam capacity increase follows the trend of pH-dependent solubility of the sample. In this sense, solubility plays a significant role in the foaming properties. This observation lends credence to the report of Hermannson (1973), in which protein solubility was found to make an important contribution to foaming behaviour. At pH 7, which serves at the control, maximum foam capacity was noticed in ALBa, followed by ALBg. In a previous report, Grahams and Phillips (1976) attributed food foamability to flexible protein molecules that can reduce surface tension, while highly ordered globular proteins, which are relatively difficult to surface – denature give low foamability. High flexibility of albumin might have been responsible for better foamability compared with the globulin fraction. The high foaming capacity at alkaline pHs may be due to an increase in the net charge of the protein which weakens hydrophobic interactions and increases protein flexibility, allowing protein to spread to the air–water interface more quickly, encapsulating air particles and thus increasing foam formation (Chau, Cheung, & Wang, 1997).

Maximum foam stability for ALBa was observed at pH 5 while ALBg and the flours recorded maximum foam stability at pH 4 (Fig. 5). In all samples, foam stability reached a minimum when pH reached 10. It is

Table 4
Effects of concentration on foam stabilities of albumin (ALBa), globulin (ALBg), defatted (ALBdf) and undefatted flours (ALBf) of African locust bean[A,B]

Sample	Concentration (% w/v)				
	2	4	6	8	10
ALBa	$76.4^{a} \pm 1.7$	$77.8^{a} \pm 2.1$	$81.7^{a} \pm 1.6$	$82.3^{a} \pm 1.0$	$84.7^{a} \pm 1.7$
ALBg	$57.4^{b} \pm 1.4$	$61.8^{a} \pm 2.4$	$62.3^{a} \pm 1.9$	$64.7^{b} \pm 1.5$	$69.7^{b} \pm 1.0$
ALBdf	$41.6^{b} \pm 1.9$	$43.7^{b} \pm 1.5$	$44.5^{b} \pm 1.4$	$46.7^{b} \pm 1.4$	$47.4^{c} \pm 1.2$
ALBf	$36.8^{c} \pm 1.4$	$34.7^{c} \pm 1.0$	$30.9^{b} \pm 1.9$	$30.1^{d} \pm 1.2$	$29.2^{d} \pm 1.8$

[A] Each value represents the mean of three determinations ± standard deviation.
[B] Samples followed by the same letter are not significantly different ($p < 0.05$).

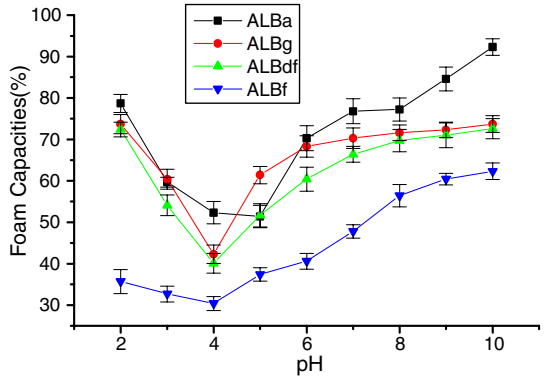

Fig. 4. Effect of pH on foam capacities of albumin (ALBa), globulin (ALBg), defatted (ALBdf) and undefatted flours (ALBf) of African locust bean. Error bars: Standard deviations results are means of triplicate determinations.

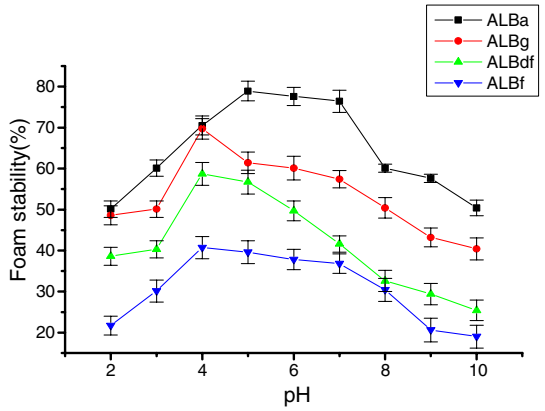

Fig. 5. Effect of pH on foam stability of albumin (ALBa), globulin (ALBg), defatted (ALBdf) and undefatted flours (ALBf) of African locust bean. Error bars: Standard deviations results are means of triplicate determinations.

noticeable from this study that a minimum foam stability, at all pHs, was recorded in ALBf; this suggests interference of lipids in foaming properties at all pHs. Previous investigations have revealed that protein-stabilized foams are more stable in the neighbourhood of the isoelectric point of the protein than at any other pH (Buckingham, 1970; Aluko & Yada, 1995). At the isoelectric point, formation of a viscous film at the interface is facilitated by lack of repulsive interactions; this may have contributed to the formation of a stable molecular layer in the air–water interface, which imparted stability to the foam.

Ionic strength has a pronounced effect on the pattern of foam capacity of the samples, as presented on Table 5. Increase in ionic strength, to 0.4 M, improved the FC of both ALBa and ALBg, significantly, after which further increase in ionic strength reduced the FC of the samples progressively until minimum values of 74.7% and 60.4% were reached at 1.0 M for ALBa and ALBg, respectively. ALBdf and ALBf followed the same pattern but maximum FC was observed at 0.2 M after which FC also declined progressively. Initial increase in foam capacity may be attributed to increase in protein solubility at these salt concentrations. Narayana and Narasinga Rao (1982) reported a similar observation in winged bean flour. Recently, Akintayo et al. (1999) also reported that low concentration of salt enhanced solubility of pigeon pea protein, which decreased at high concentration.

In a similar way, ionic strength affected the foam stability of the samples under investigation (Table 6). FS of ALBa increased as the ionic strength increased from 0.1 to 0.4 M, following which it decreased progressively. Also, the initial increase in ionic strength enhanced foam capacity of ALBg, ALBdf and ALBf, up to 0.2 M, after which further increase in ionic strength reduced FS progressively. Initial increase in ionic strength, which enhanced foam stability facilitated unfolding and rearranging at the interface, this process enabling the proteins to form a viscous cohesive film through intermolecular interactions and thus improve foam stability.

3.5. Emulsifying properties

Effects of concentration on emulsifying activity and stability are presented on Tables 7 and 8, respectively. Emulsifying activity of ALBa increased as the concentration of protein increased until it reached 8% w/v, when the EA was reduced compared with 1% w/v.

Table 5
Effects of ionic strength on foam capacities of albumin (ALBa), globulin (ALBg), defatted (ALBdf) and undefatted flours (ALBf) of African locust bean[A,B]

Sample	Ionic strength (M)						
	0.0	0.1	0.2	0.4	0.6	0.8	1.0
ALBa	$76.8^{a} \pm 0.8$	$81.4^{a} \pm 0.7$	$87.6^{a} \pm 1.4$	$89.4^{a} \pm 0.8$	$75.3^{a} \pm 1.2$	$75.1^{a} \pm 1.5$	$74.7^{a} \pm 1.0$
ALBg	$70.3^{a} \pm 0.5$	$71.6^{a,b} \pm 1.6$	$72.3^{b} \pm 1.0$	$74.6^{b} \pm 1.0$	$68.7^{b,c} \pm 1.0$	$65.1^{b} \pm 1.2$	$60.4^{b} \pm 1.8$
ALBdf	$66.4^{b} \pm 1.0$	$68.4^{b} \pm 1.2$	$69.7^{b} \pm 1.2$	$60.4^{c} \pm 1.6$	$56.7^{c} \pm 1.8$	$55.7^{c} \pm 1.3$	$52.7^{c} \pm 1.3$
ALBf	$47.8^{c} \pm 1.9$	$48.1^{c} \pm 1.3$	$43.1^{c} \pm 1.2$	$40.6^{d} \pm 1.0$	$40.1^{d} \pm 1.2$	$40.0^{d} \pm 1.7$	$38.2^{d} \pm 1.1$

[A] Each value represents the mean of three determinations ± standard deviation.
[B] Samples followed by the same letter are not significantly different ($p < 0.05$).

Table 6
Effects of ionic strength on foam stabilities of albumin (ALBa), globulin (ALBg), defatted (ALBdf) and undefatted flours (ALBf) of African locust bean[A,B]

Sample	Ionic strength (M)						
	0.0	0.1	0.2	0.4	0.6	0.8	1.0
ALBa	76.4^{a} ± 1.2	77.6^{a} ± 1.9	79.4^{a} ± 1.0	81.6^{d} ± 1.4	70.5^{a} ± 1.4	68.2^{a} ± 1.2	66.7^{a} ± 1.6
ALBg	57.4^{b} ± 1.6	58.9^{b} ± 2.1	62.3^{b} ± 1.9	50.4^{c} ± 1.0	47.3^{b} ± 1.5	46.1^{b} ± 1.3	40.5^{b} ± 1.3
ALBdf	41.6^{b} ± 1.1	43.7^{c} ± 1.1	45.8^{c} ± 1.0	40.5^{d} ± 2.9	39.6^{b,c} ± 1.9	30.4^{c} ± 1.6	30.0^{c} ± 1.5
ALBf	37.7^{c} ± 1.9	39.1^{d} ± 1.4	43.7^{c} ± 1.7	40.1^{d} ± 1.6	36.7^{c} ± 1.0	35.2^{c} ± 1.5	30.5^{b} ± 2.3

[A] Each value represents the mean of three determinations ± standard deviation.
[B] Samples followed by the same letter are not significantly different ($p < 0.05$).

Table 7
Effects of concentration on emulsifying activities of albumin (ALBa), globulin (ALBg), defatted (ALBdf) and undefatted flours (ALBf) of African locust bean[A,B]

Sample	Concentration (% w/v)					
	1	2	4	6	8	10
ALBa	57.8^{a} ± 0.4	62.7^{a} ± 1.7	69.7^{a} ± 0.7	75.6^{a} ± 1.6	51.4^{a} ± 1.5	50.6^{a} ± 0.7
ALBg	55.3^{a} ± 1.8	60.5^{a} ± 0.4	61.4^{b} ± 1.5	61.5^{a} ± 0.6	61.5^{b} ± 1.0	61.5^{b} ± 1.9
ALBdf	35.4^{b} ± 1.0	30.7^{b} ± 2.0	29.7^{c} ± 1.0	29.0^{b} ± 0.9	28.0^{c} ± 1.2	26.0^{c} ± 1.1
ALBf	30.3^{b} ± 1.2	29.7^{b} ± 1.3	28.1^{c} ± 1.5	28.0^{b} ± 1.3	24.0^{c} ± 0.7	20.0^{d} ± 1.7

[A] Each value represents the mean of three determinations ± standard deviation.
[B] Samples followed by the same letter are not significantly different ($p < 0.05$).

Table 8
Effects of concentration on emulsion stabilities of albumin (ALBa), globulin (ALBg), defatted (ALBdf) and undefatted flours (ALBf) of African locust bean[A,B]

Sample	Concentration (% w/v)					
	1	2	4	6	8	10
ALBa	58.4^{a} ± 0.4	59.4^{a} ± 1.2	62.4^{a} ± 1.8	64.3^{a} ± 1.9	50.1^{a} ± 1.9	47.2^{a} ± 1.0
ALBg	63.6^{a} ± 0.9	61.4^{a} ± 0.2	62.3^{a} ± 1.4	74.5^{b} ± 1.8	60.4^{b} ± 1.9	56.7^{b} ± 1.2
ALBdf	52.3^{a,b} ± 1.8	50.4^{b} ± 1.8	48.7^{b} ± 1.9	47.3^{c} ± 1.3	47.3^{a,c} ± 1.0	46.5^{a} ± 1.0
ALBf	39.6^{b} ± 1.2	38.5^{c} ± 1.0	38.5^{c} ± 1.0	38.4^{d} ± 1.0	38.3^{c} ± 1.9	38.2^{c} ± 1.6

[A] Each value represents the mean of three determinations ± standard deviation.
[B] Samples followed by the same letter are not significantly different ($p < 0.05$).

Increase in protein concentration enhanced EA activity of ALBg as it increased from 55.3% in 1% w/v solution to 61.5% in 6% solution, after which further increase in concentration did not improve or reduce the EA. For ALBdf and ALBf, increase in concentration reduced the EA progressively, from 1% solution to 10% w/v. Following a similar pattern with EA, emulsion stability of ALBa increased with increase in protein concentration until it reached its peak at 6% w/v, after which further increase in protein concentration reduced the ES compared with the control. The improvement in emulsion stability as a result of the increase in protein concentration was explained by an increase in rigidity of interfacial lamella (Wagner & Guenguen, 1999). No fixed pattern was noticed in the ES of ALBg while ES was reduced with concentration increase in ALBdf and ALBf. However, it is noteworthy that reduction in ES, with increase in concentration of ALBf, was insignificant. Variation in EA and ES might be due to differences in conformation of the constituent proteins.

Emulsifying activity and stability were pH-dependent, as depicted in Figs. 6 and 7, respectively. ALBa showed minimum EA at pH 5 while ALBg and the flours showed minimum EA at pH 4. EA increased as the pH decreased at pHs acidic to the isoelectric point while it increased as pH increased at pH values alkaline to the isoelectric region. Generally, ALBa had better EA than ALBg, while ALBdf had better EA than ALBf at all pHs studied. Emulsion stability followed a pattern similar to the EA. Also, minimum ES for ALBa was recorded at pH5 while other samples had minimum ES at pH4. In a previous work, Aluko and Yada (1995) have also reported pH-dependent emulsifying properties for cowpea globulin isolate. Earlier, Koyoro and Powers (1987) had reported pH-dependent emulsifying properties for pea globulin fractions. Although McWatters

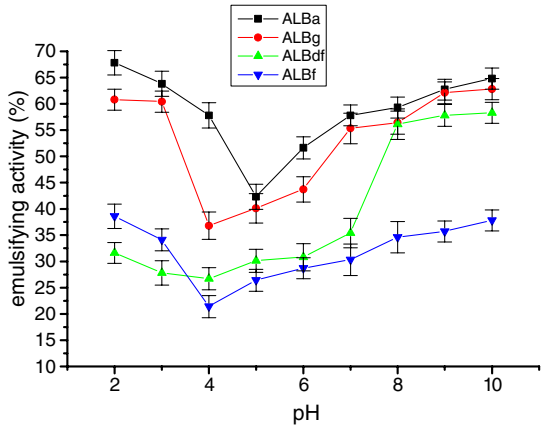

Fig. 6. Effect of pH on emulsifying activity of albumin (ALBa), globulin (ALBg), defatted (ALBdf) and undefatted flours (ALBf) of African locust bean. Error bars: Standard deviations results are means of triplicate determinations.

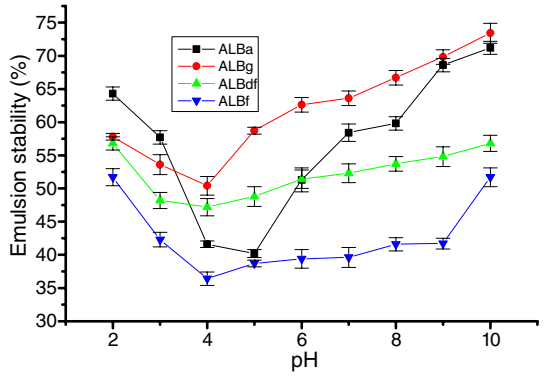

Fig. 7. Effect of pH on emulsion stability of albumin (ALBa), globulin (ALBg), defatted (ALBdf) and undefatted flours (ALBf) of African locust bean. Error bars: Standard deviations results are means of triplicate determinations.

and Holmes (1979) showed that large concentrations of soluble nitrogen were not necessarily related to maximum emulsifying capacities, Nakai (1983), reported that solubility, surface hydrophobicity and molecular flexibility influence the emulsification behaviour of globular proteins, such as pea proteins that have extensive quaternary structures. The high emulsion stability at extremes of the acidic range and alkaline range may be caused by protein denaturation that enhances mutual cohesion between oil phase and protein, which encourages stability at the interfacial membrane.

Effects of ionic strength on emulsifying activity and stability (Tables 9 and 10) were investigated. Under the conditions studied (0.1–1.0 M NaCl), the results indicate that emulsifying activity of ALBa increased as the ionic strength increased until it reached 0.4 M, followed by subsequent reduction of EA with further increase in ionic strength. EA of ALBg progressively increased as ionic strength increased and it reached a maximum of 0.6 M. However, no fixed pattern was observed in EA of ALBdf. The results obtained for EA of ALBf at various ionic strengths indicate that initial increase in ionic strength, up to 0.2 M, favoured EA while further increase reduced the emulsifying activity, although no difference was observed between the EA at 0.4 and 0.6 M. Similarly, emulsion stability was ionic strength-dependent and initial increase in ionic strength, up to 0.4 M, enhanced increases in EA of ALBa, ALBg and ALBdf, reaching maximum values of 67.7%, 66.8% and 56.8%, respectively, while ALBf reached a peak value of 48.9% in 0.2 M NaCl solution. Among the samples, at all ionic strengths, ALBg had the best ES, followed by ALBa. Fat content in ALBf appeared to have impaired its ES as it had the lowest ES at all levels of ionic strength. Differences in ES and EA between ALBa and ALBg are due to different conformations of the constituent proteins at various ionic strengths.

Earlier, it had been observed that the ionic strength of the medium, by inducing association–dissociation of the protein, influenced the adsorption kinetics at the air–water interface (Wagner & Gueguen, 1995). It was also observed that the dissociation of the oligomeric structure of 11S-glycilin at low ionic strength and consequently the improvement of surface behaviour, led to higher emulsion stability for glycilin. In their own account, Aluko and Yada (1995) attributed the increase in ES of cowpea globulin isolate at low ionic strength to formation of charged layers around the fat globules, which resulted in mutual repulsion and forming a hydrated layer around the interfacial materials, which

Table 9
Effects of ionic strength on emulsifying activities of albumin (ALBa), globulin (ALBg), defatted (ALBdf) and undefatted flours (ALBf) of African locust bean[A,B]

Sample	Ionic strength (M)						
	0.0	0.1	0.2	0.4	0.6	0.8	1.0
ALBa	$57.8^{a} \pm 0.6$	$58.3^{a} \pm 0.7$	$59.6^{a} \pm 0.8$	$64.2^{a} \pm 0.9$	$56.1^{a} \pm 1.9$	$53.2^{a} \pm 2.0$	$50.4^{a} \pm 2.5$
ALBg	$55.3^{a} \pm 0.7$	$59.7^{a} \pm 1.3$	$63.8^{b} \pm 1.8$	$69.7^{a} \pm 1.2$	$71.8^{b} \pm 1.0$	$54.3^{a} \pm 1.8$	$53.2^{b} \pm 1.8$
ALBdf	$35.4^{b} \pm 0.6$	$39.7^{b} \pm 1.0$	$41.2^{a} \pm 1.0$	$30.4^{b} \pm 1.0$	$30.5^{c} \pm 1.7$	$30.5^{b} \pm 1.9$	$31.2^{c} \pm 1.8$
ALBf	$30.3^{b} \pm 1.5$	$33.6^{a} \pm 1.3$	$33.7^{c} \pm 1.8$	$30.1^{c} \pm 1.8$	$30.1^{c} \pm 1.9$	$29.7^{a} \pm 1.0$	$20.4^{d} \pm 0.6$

[A] Each value represents the mean of three determinations ± standard deviation.
[B] Samples followed by the same letter are not significantly different ($p < 0.05$).

Table 10
Effects of ionic strength on emulsion stabilities of albumin (ALBa), globulin (ALBg), defatted (ALBdf) and undefatted flours (ALBf) of African locust bean[A,B]

Sample	Ionic strength (M)						
	0.0	0.1	0.2	0.4	0.6	0.8	1.0
ALBa	$58.4^{a} \pm 0.3$	$62.8^{a} \pm 1.2$	$63.4^{a} \pm 1.8$	$64.7^{a} \pm 1.6$	$50.6^{a} \pm 1.9$	$48.7^{a} \pm 0.4$	$49.7^{a} \pm 0.6$
ALBg	$63.6^{b} \pm 1.4$	$64.7^{a} \pm 1.0$	$65.7^{a} \pm 0.8$	$66.8^{b} \pm 1.9$	$61.2^{b} \pm 1.5$	$58.3^{b} \pm 1.4$	$50.8^{a} \pm 0.9$
ALBdf	$52.3^{a} \pm 2.1$	$52.9^{b} \pm 1.4$	$54.7^{a} \pm 1.6$	$56.8^{b} \pm 2.1$	$50.4^{a} \pm 1.0$	$48.6^{a} \pm 1.6$	$47.2^{a} \pm 1.5$
ALBf	$39.6^{c} \pm 2.1$	$48.4^{b} \pm 1.6$	$48.9^{b} \pm 1.0$	$46.4^{c} \pm 2.0$	$40.6^{a} \pm 1.2$	$35.7^{c} \pm 1.5$	$31.6^{b} \pm 1.9$

[A] Each value represents the mean of three determinations ± standard deviation.
[B] Samples followed by the same letter are not significantly different ($p < 0.05$).

lowered the interfacial energy and retarded droplet coalescence.

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