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Functionality of African locust bean (*Parkia biglobossa*) protein isolate: effects of pH, ionic strength and various protein concentrations

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Abstract

A protein isolate was prepared from African locust bean (ALBPI) and the functional properties were investigated under the influence of pH, ionic strength and varying protein concentration. Protein solubility diminished as the pH increased from 2 to 4.5, after which further increase in pH increased it progressively. The oil absorption capacity of ALBPI was 1.97 ml/g protein. Water absorption capacity increased when ionic strength of the medium increased to 0.2 M but diminished with further increase in ionic strength. Gelation properties improved at pH 4. The results indicate improvement in gelation capacity as the ionic strength of protein solution increased from 0.1 to 0.2 M while further increase in ionic strength caused the gelation tendency to decline. Increase in concentration favoured emulsifying activity (EA) and stability (ES) up to 4% (w/v) concentration, while further increase from 6% to 10% (w/v) diminished emulsifying properties. Also, initial increase in ionic strength, up to 0.2 M, improved both emulsifying activity and stability while further increase in ionic strength progressively reduced emulsifying properties. Maximum emulsifying activity and stability were at pH 10 while minimum values were obtained at pH 4. Foam capacity and stability increased as the protein concentration increased. Increase in ionic strength from 0 to 0.2 M increased the foam capacity (FC) and stability (FS) but foam capacity was reduced as the ionic strength increased further. Foam capacity and stability were pH-dependent. A minimum value of 32.7% was recorded at pH 4 while further increase in pH increased the foam capacity progressively until it reached a maximum (86.8%) at pH 10. Contrarily, maximum foam stability was recorded at pH 4 while foam stability diminished as the pH of the protein solution increased.

1. Introduction

In recent years, there has been a concerted effort toward harnessing the potential of lesser-known legumes to minimize problems of protein malnutrition in Africa (Adebowale & Lawal, 2003). This is because animal protein is beyond the reach of a large percentage of the people in developing countries. This quest for alternative sources of protein has prompted research on proteins from various legumes, such as beach pea (Chavan, Mc Kenzie, & Shahidi, 2001), cowpea (Aluko & Yada, 1995), *Cajanus cajan* (Akintayo, Oshodi, & Esuoso, 1999; Mwasaru, Muhammad, Bakar, & Cheman, 2000), rapeseed (Dua, Mahajan, & Mahajan, 1996; Gruener & Ismond, 1997), chickpea (Liu & Hung, 1998), lupin seed (Pozami, Doxastakis, & Kiosseoglou, 2002) and faba bean (Krause, Mothes, & Schwenke, 1996).

The seeds of African locust bean (*Parkia biglobossa*) offer an unexploited source of protein. It is a legume that grows naturally in the forests of Africa and produces abundant seeds. In Nigeria, particularly in the southwest, the seeds are processed into a local condiment to improve the tastes of various local dishes.

Flavour and functional properties of food systems often get impaired when legumes are used in large quantities as food supplements (Rooney, Gustafson, Clark, & Cater, 1972). In view of this development, preparation of protein isolate from these legumes appears to be a panacea. The technology involved in preparation of protein isolates, which includes the

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solubilisation of protein in alkaline media (pH 8–12) and subsequent preparation at the isoelectric point (IEP), purifies it from non-protein substances, such as sugars, fibre, lipids and other non-desirable components (Lqari, Vioque, Pedroche, & Millan, 2002). This gives protein isolates superior functional properties (Fernandez-Quintela, Larralde, Macarulla, Marcos, & Martinez, 1993; Kolar, Richert, Decker, Steike, & Vander Zanden, 1985). The use of plant protein isolates in foods as functional ingredients to improve the nutritional quality of the products, or for economic reasons, is increasing (Sanchez-Vioque, Clement, Vioque, Bautista, & Millan, 1999). Despite this, the ultimate success of utilizing any seed protein as a food ingredient depends largely on its functional properties (Chavan et al., 2001; Aluko & Yada, 1995). Functional properties include emulsification, foam formation, viscosity, gelation, and water and oil absorption capacity. A very important consideration, in the study of protein isolate functionalities, is their responses to the operating environment; these include the ionic strength and pH of the medium (Myers, 1988). Molecular forces operating within the matrices of protein molecules, such as hydrophobic and electrostatic interactions, can be manipulated by pH and ionic strength to produce proteins with varying structural conformations and functional properties (Aluko & Yada, 1995). Also, hydrodynamics of protein molecules in food systems are greatly influenced by prevalent pH, ionic strength and concentration.

The author is not aware of any previous work on functional properties of protein isolate from African locust bean; therefore, the present work was undertaken to investigate the functional properties of a protein isolate from African locust bean under the influence of varying sample concentration, pH and ionic strength.

2. Materials and methods

2.1. Materials

African locust bean seeds were obtained from Bodija market, Ibadan, Nigeria. All chemicals used were of analytical grade.

2.2. Preparation of African locust bean flour

The seeds were screened to eliminate stones and other foreign materials, following which they were ground, to pass through a BS-60 mesh screen, using a flourmill (Braun multimix Deluxe, Germany). The flour was then defatted with hexane for 16 h under constant shaking with four changes of hexane. The hexane was decanted and the flour was air-dried and used for protein isolate preparation.

2.3. Preparation of protein isolate

The defatted flour was dispersed in distilled water at a flour to water ratio of 1:5 (w/v); the pH was increased to 9.5 with 1 M NaOH and stirred for 3 h at 30 ± 2 °C. Extract was separated by centrifugation at 4000*g* for 30 min. The residues were re-extracted twice more, under the same conditions. The extracts were then combined and protein was precipitated by adjusting the pH to 4.5 with 1 M HCl before centrifugation at 4000*g* for 20 min. The protein isolate (pH 4.5) was washed twice with distilled water. It was then resuspended in distilled water and the pH was adjusted to 7.0 with 1 M NaOH prior to freeze-drying. For subsequent analyses, dry protein isolate was stored in airtight glass containers at 30 ± 2 °C. The protein content of dry ALBPI was 90.2% ($N = 6.25$), as determined by the Kjeldahl method. The schematic diagram is outlined in Fig. 1.

2.4. Protein solubility profile

The method of Were, Hettiarachchy, and Kalapathy (1997) was employed for the determination of pH-dependent solubility profile of ALBPI. One hundred and twenty five milligram of the sample was dispersed in 25 ml 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:

$$\text{Solubility}(\%) = \frac{\text{Amount of nitrogen in the supernatant}}{\text{Amount of nitrogen in the sample}} \times 100.$$

2.5. Water and oil absorption capacity

Water and oil absorption capacities were determined by using the method of Beuchat (1977).

One gram 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 5000*g* 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

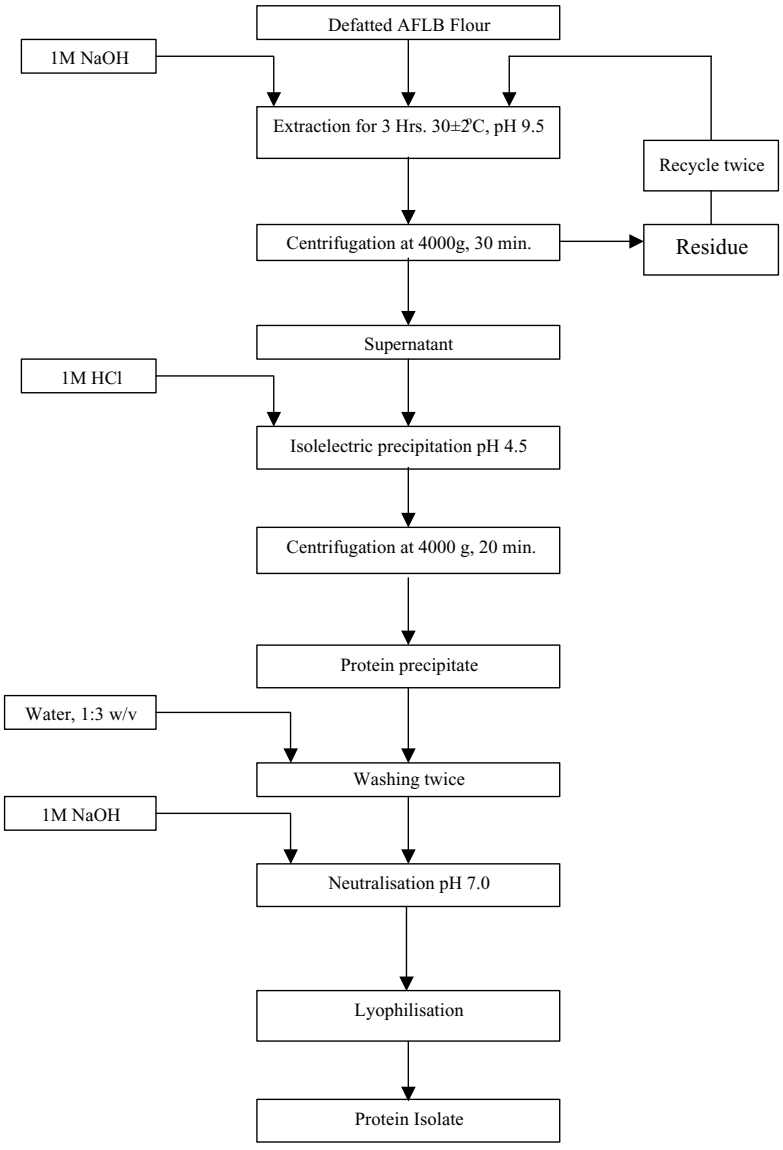

Fig. 1. Schematic diagram for preparation of African locust bean protein isolate.

solutions. Studies were conducted as described above and each experiment was conducted in triplicate.

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. Ten millilitre of each of the prepared dispersions was transferred into a test tube. Each 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 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). Five millilitre portions of protein solution (10 mg/ml) was homogenized with 5 ml oil (Executive Chef Unilever Plc) for 1 min. The emulsions were centrifuged at 1100*g* for 5 min. The height of emulsified layer and that of the total contents in the tube were measured.

The emulsifying activity was calculated as

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

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

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

Effect of concentration on emulsifying activity and stability of protein isolate was 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 pH 2–10. Effect of ionic strength was studied by preparing protein solutions (10 mg/ml) at ionic strengths of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 M NaCl solutions.

2.8. Foaming capacity and stability

The method of Coffman and Garcia (1977) was employed for foam capacity studies. A protein solution containing a weighed amount of protein isolate dispersed in 100 ml distilled water was prepared. The 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, which serves as the index of foam capacity, was calculated as follows:

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

where V_2 is the volume of protein solution after whipping, V_1 is the volume of solution before whipping.

Studies were conducted to investigate the effect of concentration on the foaming properties by whipping 2–10% (w/v) of the protein solution as described above. Influence of pH on foaming properties was found by adjusting 2% (w/v) dispersions to the desired pH range, from 2.0 to 10.0, using either 1 M HCl or 1 M NaOH, followed by whipping vigorously as described above.

Influence of ionic strength was evaluated by dispersing 2 g of protein concentrate in 100 ml NaCl solution of known ionic strength. Studies were conducted in solutions of ionic strength of 0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 M, following which they were whipped vigorously.

Foam stability was determined as the volume of foam that remained after 8 h (30 ± 2 °C), expressed as a percentage of the initial foam volume.

2.9. Statistical analysis

Statistical analysis of the results was done with statistics for windows 5.0 (1995) and the *t*-test was used to determine significance of differences between means. Trends were considered significant when means of compared sets differed at $P < 0.05$.

3. Results and discussion

3.1. Protein solubility profile

pH-dependent solubility profile is presented in Fig. 2. The results indicate that protein solubility diminished as the pH increased from 2 to 4.5, which is the isoelectric point, after which subsequent increase in pH increased protein solubility progressively. The highest protein solubility of 96.7% was observed at pH 10. This result is similar to the observation on solubility profile of chick pea (Sanchez-Vioque et al., 1999), soy protein (Achouri, Zhang, & Shying, 1998), cowpea (Prinyawiwatkul, Beuchat, Mc Watters, & Phillips, 1997), peanut (Beuchat, 1977) and lentil (Bora, 2002); pH-dependent protein solubility characteristics could be explained by the prevalent charge on the constituent amino acids of proteins at various pH values

$$\underset{\text{II}}{\mathrm{H_2NCHRCOO^-}} \underset{\mathrm{OH^-}}{\overset{\mathrm{H^+}}{\rightleftarrows}} \underset{\text{I}}{\mathrm{{}^+H_3NCHRCOO^-}} \underset{\mathrm{OH^-}}{\overset{\mathrm{H^+}}{\rightleftarrows}} \underset{\text{III}}{\mathrm{{}^+H_3NCHRCOOH}}$$

I is a zwitterion or dipolar ion which predominates at the region of isoelectric point in protein. At this pH, minimum solubility occurs because of minimum repulsion among the constituent amino acids. The balance in positive and negative charges reduces the electrostatic repulsion, and this limited solubility of ALBPI at pH 4.5. As pH of the solution diminished further, cation III predominates while, in alkaline medium, anion II as-

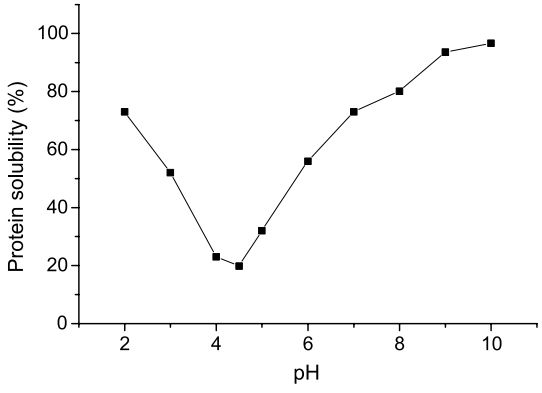

Fig. 2. Protein solubility profile of African locust bean protein isolate.

sumes preponderance. In either of the two cases, electrostatic repulsion occurs and this facilitates enhanced solubility as is observed at pH 2 and 10.

3.2. Water and oil absorption capacity

The oil absorption capacity of ALBPI was 1.97 ml/g of protein. The value observed here is lower than that of commercial soy isolate (3.29 ml oil/g), as reported by Mwasaru et al. (1999). However, it is comparable to 1.7 ml/g protein reported for chickpea (Paredes-Lopez, Ordorica-Falomir, & Olivares-Vasquez, 1991), 1.59–2.58 ml/g for adzuki bean isolates (Tjahjadi, Lin, & Breene, 1988) and 2.00–2.22 ml/g for cowpea IP isolates (Sefa-Dedeh & Yiadom-Farkye, 1988). Oil absorption has been attributed to physical entrapment of oil within the protein isolates (Kinsella, 1976). It is also due to the presence of non-covalent bonds, such as hydrophobic, electrostatic and hydrogen bonding forces, that are involved in lipid–protein interactions.

Water absorption capacity (WAC) as a function of ionic strength is presented in Fig. 3. Increase in ionic strength of the medium from 0.0 in control to 0.2 M increased the WAC of ALBPI by 51.6%, while further increase in ionic strength of the medium reduced the WAC progressively. A minimum value of 1.8 ml/g of protein was observed when ionic strength of the medium reached 0.8 and 1.0 M, which correspond to 42% reduction in WAC when compared with the control. At low salt concentrations ($\leqslant 0.2$ M), hydrated salt ions were weakly bound to charged groups on proteins. In this case, binding of ions to proteins did not affect the hydration shell of the charged groups on the proteins; consequently, increase in water binding was from water associated with the bound ions. When salt concentration increased further, much of the existing water was bound to salt ions and this caused dehydration of protein and reduction in water-binding capacity.

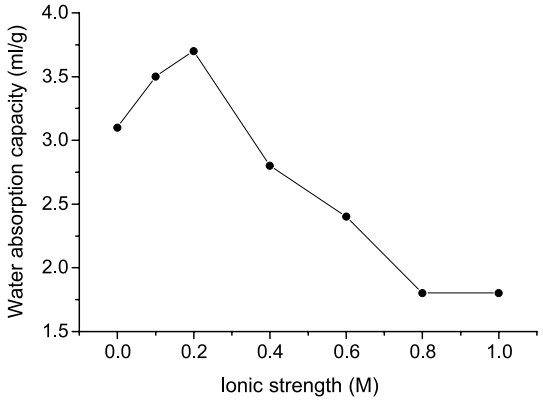

Fig. 3. Effect of ionic strength on water absorption capacity of African locust bean protein isolate.

3.3. Gelation properties

Effects of concentration and pH on gelation capacity are presented in Table 1. Taking the least gelation concentration (LGC) as the index of gelation capacity, lower LGC means better gelation capacity. Gelation properties were pH-dependent. Concentration [14% (w/v)] was required to form a gel when protein solution was prepared at pH 2. Marked reduction in LGC was observed at pH 4 as the value reduced to 6% (w/v). Progressive increase in LGC was observed as the pH of the protein solution increased further from 4.0 to 10.0. In all, the highest LGC (16%) was observed at pH 10. Previously, LGC values of 12% and 14% (w/v) had been reported for mucuna protein (Adebowale & Lawal, 2003) and lupin seed protein (Sathe, Desphande, & Salunkhe, 1982), respectively. In the present study, the LGC of ALBPI at pH 7, which indicates the control, is lower than values reported for mucuna protein and lupin seed proteins. The result suggests that ALBPI will be a better gelating food hydrocolloid than mucuna or lupin proteins. Gel formation of protein is the result of a two-step process involving the partial denaturation of individual proteins to allow more access to reactive side groups within the protein molecules, and the aggregation of these proteins by means of reactive side groups into a three-dimensional network structure capable of retaining significant amounts of water. Differences in gelation capacity at various pHs are due to the prevalent surface charge of the proteins. At pH close to the isoelectric point, the net surface charge is close to zero, which significantly reduces the repulsive interactions between the protein molecules (Elofsson, Dejmek, Paulson, & Burling, 1997). In this sense, gelation is enhanced at this pH, due to greater interaction of protein molecules. On the other hand, at pH far removed from the isoelectric point, the surface charge is large and significant repulsive forces prevent aggregation of protein molecules and formation of an ordered network structure.

Effects of concentration and ionic strength on the gelation of ALBPI are presented in Table 2.

The result indicates improvement in gelation capacity as the ionic strength of the protein solution increases from 0.1 to 0.2 M, where the minimum LGC of 6% (w/v) was observed.

Increasing the ionic strength of the medium to 0.4 M increased the LGC to 10% (w/v), which is the LGC obtained in the control protein solution. However, a difference was observed in the physical appearance of the gel at 14% (w/v). Further increase in ionic strength of the medium increased the LGC progressively until the highest value of 16% (w/v) was observed at 1.0 M protein solution. This result lends credence to our observation on increase in LGC of mucuna protein concentrate with increase of ionic strength (Adebowale &

Table 1
Effects of concentration and pH on the gelation capacity of African locust bean protein isolate

Sample concentration (%)	pH 2	pH 4	pH 7	pH 8	pH 10
2	−liquid	−liquid	−liquid	−liquid	−liquid
4	−liquid	−liquid	−liquid	−liquid	−liquid
6	−liquid	+ gel	−viscous	−liquid	−liquid
8	−viscous	+ gel	−viscous	−liquid	−liquid
10	−viscous	+ gel	+ gel	−viscous	−viscous
12	−viscous	+ firm gel	+ gel	−viscous	−viscous
14	+ gel	+ firm gel	+ firm gel	+ gel	−viscous
16	+ gel	+ v. firm gel[b]	+ firm gel	+ gel	+ gel
18	+ firm gel	+ v. firm gel	+ v. firm gel	+ firm gel	+firm gel
20	+ firm gel	+ v. firm gel	+ v. firm gel	+ firm gel	+ firm gel
LGC[a]	14	6	10	14	16

Determinations carried out in triplicate. (−) No gelation; (+) gelation.
[a] Least gelation concentration.
[b] Very firm gel.

Table 2
Effects of concentration and ionic strength on the gelation capacity of African locust bean protein isolate

Sample concentration (%)	Control	Ionic strength (M)					
		0.1	0.2	0.4	0.6	0.8	1.0
2	−liquid	−liquid	−liquid	−liquid	−liquid	−liquid	−liquid
4	−liquid	−liquid	−liquid	−liquid	−liquid	−liquid	−liquid
6	−viscous	−viscous	+ gel	−liquid	−liquid	−liquid	−liquid
8	−viscous	+ gel	+ gel	−viscous	−liquid	−liquid	−liquid
10	+ gel	+ gel	+ firm gel	+ gel	−viscous	−viscous	−liquid
12	+ gel	+ firm gel	+ firm gel	+ gel	+ gel	−viscous	−viscous
14	+ firm gel	+ firm gel	+ firm gel	+ gel	+ gel	+ gel	−viscous
16	+ firm gel	+ firm gel	+ v. firm gel	+ firm gel	+ firm gel	+ gel	+ gel
18	+ v. firm gel[b]	+ v. firm gel	+ v. firm gel	+ v. firm gel	+ firm gel	+ gel	+ gel
20	+ v. firm gel	+ v. firm gel	+ v. firm gel	+ v. firm gel	+ v. firm gel	+ firm gel	+ firm gel
LGC[a]	10	8	6	10	12	14	16

Determinations carried out in triplicate. (−) No gelation; (+) gelation.
[a] Least gelation concentration.
[b] Very firm gel.

Lawal, 2003). Also, Otte, Schumacher, Ipsen, Ju, and Qvist (1999) reported a reduction of gel firmness of whey protein, when the NaCl content of the mixture was increased. Castimpoolas and Meyer (1970) had earlier given an account of reduction in gelation properties of soybean globulin in solutions of high ionic strength. Initial increase in ionic strength, up to 0.2 M, facilitated a shielding effect on the surface charges; this development reduced the repulsive forces acting among the protein molecules, creating an identical situation to the isoelectric region, whereas further increase in ionic strength influenced the gel forming process negatively by decreasing the protein unfolding. A similar observation has been reported for whey protein (Boye, Alli, Ismail, Gibbs, & Konishi, 1995; Van Camp, Messens, Clement, & Huyghebaert, 1997).

3.4. Emulsifying properties

Both emulsifying activity (Fig. 4) and emulsion stability (Fig. 5) were concentration-dependent. Increase in concentration favoured emulsifying activity up to 4% (w/v) concentration, while further increase in concentration, from 6% to 10% (w/v), reduced emulsifying activity. Similarly, initial increase in protein concentration favoured increased emulsion stability up to 4% (w/v), after which a decline was observed with further increase in concentration. A similar observation on emulsifying properties has been earlier reported (Phillips, 1981). Initial increase in protein concentration facilitated enhanced interaction between the oil phase and the aqueous phase. However, as the concentration increased, a point was reached where further increase in protein concentration led to accumulation of proteins in the aqueous phase, this development resulted in decrease of emulsifying activity. Emulsion stability diminished after 4% (w/v), presumably because of increase in protein–protein interaction at the expense of protein–oil interaction.

Effects of ionic strength on emulsifying activity and stability are presented in Figs. 6 and 7, respectively. Initial increase in ionic strength, up to 0.2 M, improved

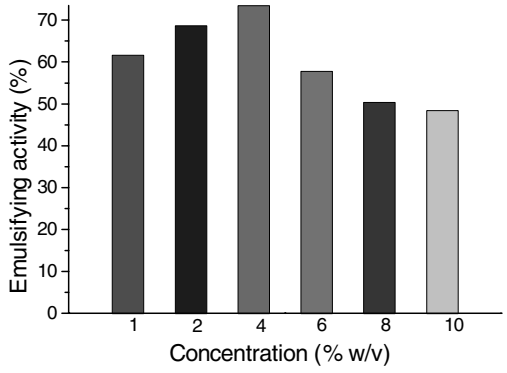

Fig. 4. Effect of concentration on emulsifying activity of African locust bean protein isolate.

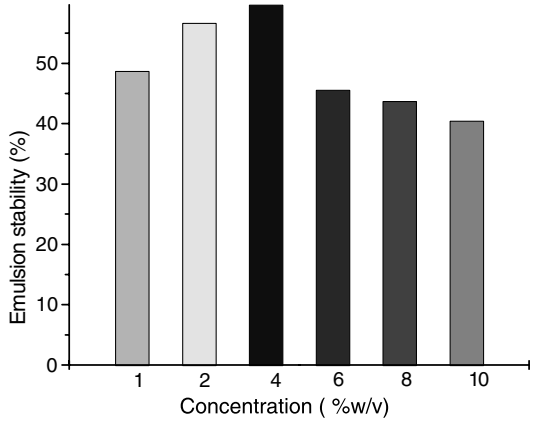

Fig. 5. Effect of concentration on emulsion stability of African locust bean protein isolate.

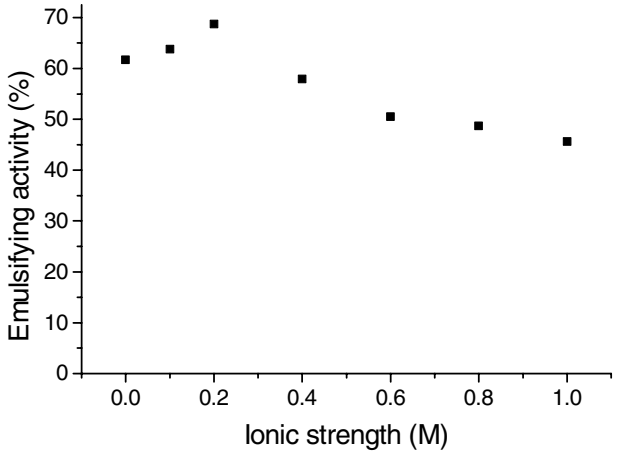

Fig. 6. Effect of ionic strength on emulsifying activity of African locust bean protein isolate.

both emulsifying activity and stability. However, further increase in ionic strength progressively reduced both emulsifying activity and emulsion stability. Increase in ionic strength, up to 0.2 M, encouraged unfolding of protein molecules and subsequent increase in protein

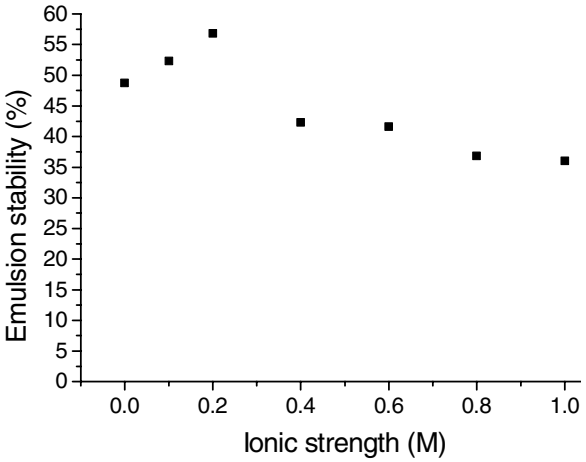

Fig. 7. Effect of ionic strength on emulsion stability of African locust bean protein isolate.

solubility (Aluko & Yada, 1995). This increase in protein solubility enhanced a rapid migration to the oil–water interface and improved emulsifying activity of the protein. With further increase in ionic strength (>0.2 M), screening of the surface charges increased and this encouraged protein–protein interaction but reduced protein–oil interaction.

Emulsifying activity (EA) was 76.3% at pH 2 (Fig. 8) and this was followed by marked reduction at pH 4, where minimum EA was recorded. Subsequent to that, emulsifying activity increased as pH increased up to pH 10, where a maximum value of 78.4% was observed.

Following a similar trend, minimum emulsion stability was observed at the isoelectric region (pH 4). Emulsion stability improved at extremes of the acidic range (pH 2) and alkaline range (pH 10) (Fig. 9). Similarly, with EA, maximum ES was observed at pH 10. Going by the pattern of these results, it is reasonable that protein solubility should influence emulsifying properties.

Observations on the direct relationship between protein solubility and emulsifying properties support

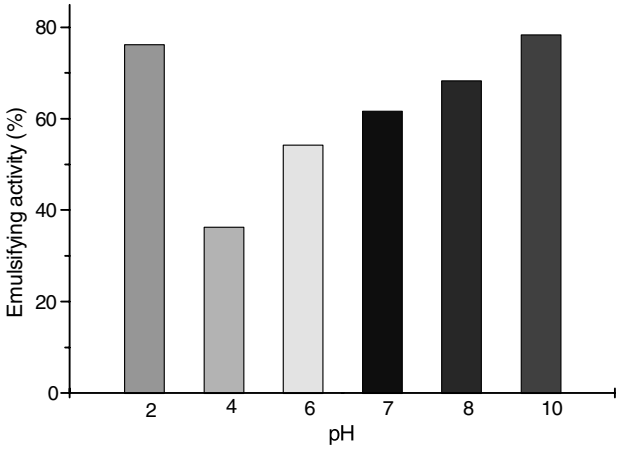

Fig. 8. Effect of pH on emulsifying activity of African locust bean protein isolate.

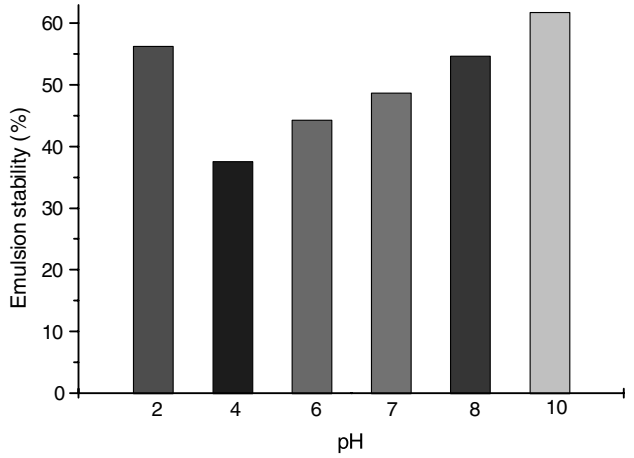

Fig. 9. Effect of pH on emulsion stability of African locust bean protein isolate.

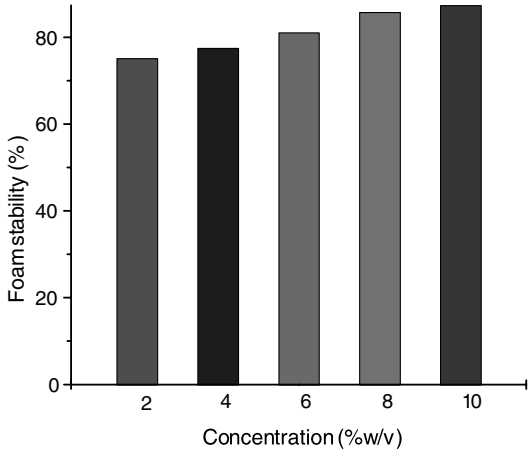

Fig. 10. Effect of concentration on foam stability of African locust bean protein isolate.

similar previous results (Kinsella, 1979; Narayana & Narasinga Rao, 1984). Chavan et al. (2001) also postulated that increase in EA with increase in pH suggests that droplet size decreased as the pH increases beyond the isoelectric point. At extremes of alkalinity and acidity, protein molecules unfolding exposed most of the buried lipophilic functional groups on the protein molecules and limited interfacial energy between oil phase and aqueous phase, which resulted in improved EA. Low ES at the isoelectric pH may be attributed to lack of electrostatic repulsive interactions among particles, which promoted flocculation and coalescence.

3.5. Foaming properties

Foam capacity (FC) increased as the concentration of protein increased in the solution (Table 3). Also, foam stability (FS) increased as the level of protein concentration increased (Fig. 10). As standing time progressed, foam volume decreased. A similar trend has been reported for Great Northern bean proteins (Sathe & Salunkhe, 1981), mucuna bean protein concentrate (Adebowale & Lawal, 2003) and glandless cottonseed flour (Cherry and McWatters, 1981). Increase in foam stability with increase in concentration is a result of formation of stiffer foams. Foam stiffness develops from small bubble size and high viscosity. Foam stability improved by greater protein concentration because this increases viscosity and facilitates formation of a multiplayer cohesive protein film at the interface.

Effect of ionic strength on foam capacity of AFLBPI is presented in Table 4. From the results, increase in ionic strength, from 0.0 to 0.2 M, increased the foam capacity from 57.8% to 66.4%, while further increase in ionic strength, from 0.4 to 1.0 M, reduced the foam capacity until a minimum value of 42.2% was reached at 1.0 M solution. Similarly, foam stability increased from 75.2% in control protein solution to 77.7% in 0.2 M NaCl protein solution but, afterwards, FS diminished as the ionic strength of the solution increased (Fig. 11). In a previous study, Akintayo et al. (1999) reported initial increase in foam capacity and stability of pigeon pea protein concentrate up to 0.5 M NaCl protein solution, while further increase in ionic strength markedly reduced foam capacity and stability. Similarly, in our previous study (Adebowale & Lawal, 2003) on mucuna bean protein concentrate, improvement in foam capacity and stability in solutions of ionic strength 0.1–0.4 M

Table 3
Effect of concentration on foam capacity of African locust bean protein isolate*

Sample conc. (% w/v)	Vol. after whipping (ml)	% increase in vol.	Volume (ml) at room temperature (30 ± 2 °C) at time intervals (h)							
			0.5	1.0	1.5	2.0	2.5	3.0	6.0	8.0
2	158 ± 2.7^{a}	57.8	143 ± 1.6^{a}	137 ± 1.1^{a}	128 ± 3.1^{a}	128 ± 4.7^{a}	127 ± 2.3^{a}	126 ± 2.1^{a}	121 ± 1.8^{a}	119 ± 3.2^{a}
4	169 ± 4.1^{b}	69.4	160 ± 1.8^{b}	143 ± 1.0^{a}	141 ± 2.0^{b}	138 ± 1.2^{b}	136 ± 2.5^{a}	134 ± 5.4^{a}	133 ± 1.3^{b}	131 ± 2.4^{b}
6	174 ± 2.1^{bc}	73.6	169 ± 1.0^{b}	164 ± 2.1^{b}	157 ± 2.4^{b}	148 ± 2.1^{c}	146 ± 2.2^{b}	143 ± 2.4^{b}	143 ± 1.9^{c}	141 ± 4.4^{b}
8	175 ± 2.4^{bc}	74.8	172 ± 2.7^{bc}	170 ± 2.4^{bc}	169 ± 3.3^{c}	158 ± 2.2^{d}	157 ± 3.3^{c}	154 ± 3.4^{c}	150 ± 1.4^{cd}	150 ± 3.7^{c}
10	181 ± 1.8^{c}	80.7	180 ± 3.2^{c}	177 ± 4.0^{c}	171 ± 4.0^{c}	169 ± 2.4^{e}	168 ± 2.5^{d}	164 ± 1.3^{d}	159 ± 2.4^{d}	159 ± 3.8^{c}

* Results reported as means ± SD of triplicate determinations. Means within columns with different letters are significantly different ($P \leqslant 0.05$). Values were obtained at pH 7.

Table 4
Effect of ionic strength on foam capacity of African locust bean protein isolate*

Ionic strength	Vol. after whipping (ml)	% Increase in volume	Volume (ml) at room temperature (30 ± 2 °C) at time intervals (h)							
			0.5	1.0	1.5	2.0	2.5	3.0	6.0	8.0
0.0	158 ± 3.0^{a}	57.8	143 ± 3.0^{a}	137 ± 2.1^{a}	128 ± 4.0^{a}	128 ± 2.9^{a}	127 ± 6.1^{a}	126 ± 1.0^{a}	121 ± 3.1^{a}	119 ± 2.5^{a}
0.1	160 ± 1.5^{ab}	60.7	157 ± 1.0^{b}	148 ± 3.7^{b}	140 ± 3.6^{b}	138 ± 4.9^{b}	132 ± 5.5^{a}	128 ± 3.0^{a}	125 ± 6.0^{a}	124 ± 5.5^{ab}
0.2	168 ± 5.0^{b}	66.4	169 ± 1.9^{c}	167 ± 4.8^{c}	160 ± 3.5^{c}	153 ± 3.4^{c}	149 ± 1.6^{b}	138 ± 3.6^{b}	136 ± 4.0^{b}	129 ± 2.0^{b}
0.4	154 ± 3.9^{a}	54.4	141 ± 6.1^{a}	135 ± 2.8^{a}	127 ± 2.0^{a}	124 ± 2.9^{a}	123 ± 4.7^{ac}	123 ± 1.0^{a}	120 ± 3.5^{a}	115 ± 4.0^{a}
0.6	149 ± 6.0^{ac}	49.1	139 ± 3.5^{ad}	133 ± 4.5^{a}	120 ± 4.8^{a}	120 ± 2.1^{a}	119 ± 3.6^{c}	116 ± 3.7^{b}	114 ± 6.3^{ac}	110 ± 4.0^{ac}
0.8	145 ± 3.0^{c}	45.2	131 ± 2.0^{d}	130 ± 2.6^{a}	128 ± 6.0^{a}	127 ± 3.1^{a}	116 ± 2.0^{c}	115 ± 3.5^{b}	111 ± 2.8^{c}	106 ± 5.0^{c}
1.0	142 ± 5.6^{c}	42.2	130 ± 2.7^{d}	129 ± 2.7^{a}	127 ± 2.5^{a}	120 ± 2.5^{a}	115 ± 3.6^{c}	109 ± 4.0^{b}	108 ± 3.9^{c}	103 ± 3.2^{c}

* Results reported as means ± SD of triplicate determinations. Means within columns with different letters are significantly different ($P \leqslant 0.05$).

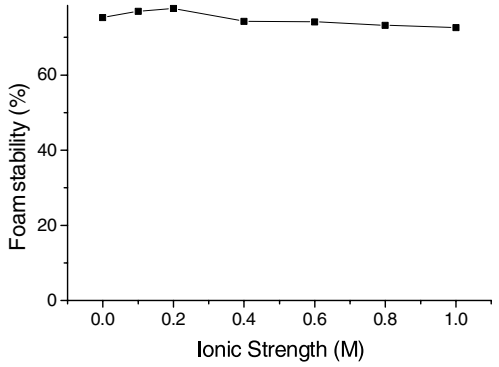

Fig. 11. Effect of ionic strength on foam stability of African locust bean protein isolate.

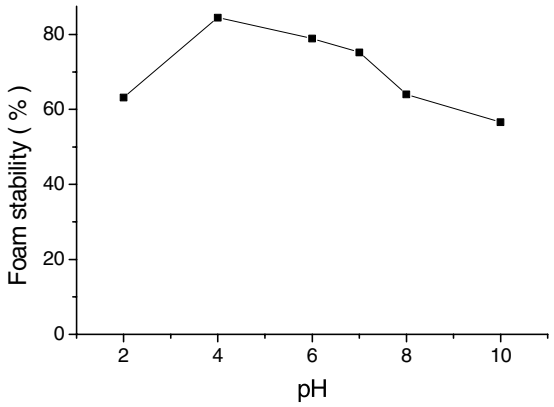

Fig. 12. Effect of pH on foam stability of African locust bean protein isolate.

was reported, while further increase in ionic strength reduced foaming properties. Increase in ionic strength of the protein isolate to 0.2 M improved solubility and protein dispersion, thus leading to enhanced whippabilty and formation of stable cohesive films around the air vacuoles. As the ionic strength increased further, charge-screening occurred and thus hydrophobic interaction and intermolecular cohesion between protein molecules improved and these led to reduction in flexibility of the protein surfactant molecules.

Foam capacity (Table 5) and stability (Fig. 12) were also pH-dependent. The volume (72.3%) increase was observed at pH 2, followed by a marked reduction at pH 4, where a minimum value was obtained. Subsequently, a progressive increase in foam capacity was observed as the pH increased progressively until a maximum value of 86.8% volume increase was observed when pH reached 10. Contrarily, maximum foam stability was recorded at pH 4, while foam stability decreased as the pH of the protein solution increased further. Lin, Humbert, and Sosulski (1974) have reported pH-dependent foaming properties for sunflower meal products. In addition, pH-dependent foaming properties have also been reported for *Phaseolus lunatus* and *Canavalia ensiformis* (Chel-Guerrero, Perez-Flores, Bentacur-Ancona, & Davila-Ortiz, 2002). The high foaming capacity at pH 2 and 10 may be due to an increase in the net charge of the protein

Table 5
Effect of pH on foam capacity of African locust bean protein isolate*

pH	Vol. after whipping (ml)	% increase in volume	Volume (ml) at room temperature (30 ± 2 °C) at time intervals (h)							
			0.5	1.0	1.5	2.0	2.5	3.0	6.0	8.0
2	172 ± 2.8^{a}	72.3	169 ± 4.0^{a}	160 ± 2.3^{a}	156 ± 3.4^{a}	138 ± 2.8^{a}	125 ± 1.0^{a}	120 ± 2.5^{ab}	117 ± 3.9^{a}	109 ± 4.2^{a}
4	143 ± 3.9^{b}	42.7	137 ± 2.4^{b}	131 ± 4.4^{b}	129 ± 5.1^{b}	128 ± 3.6^{b}	126 ± 3.0^{a}	125 ± 2.0^{a}	123 ± 4.5^{a}	121 ± 3.0^{b}
6	151 ± 3.0^{b}	50.7	144 ± 3.8^{b}	141 ± 2.7^{b}	139 ± 2.0^{c}	137 ± 2.3^{a}	129 ± 2.6^{a}	121 ± 3.8^{a}	120 ± 3.0^{a}	119 ± 2.1^{b}
7	158 ± 2.6^{b}	57.8	143 ± 2.0^{b}	137 ± 3.6^{b}	128 ± 2.9^{b}	128 ± 5.1^{b}	127 ± 2.7^{a}	126 ± 2.5^{a}	121 ± 4.0^{a}	119 ± 3.0^{b}
8	178 ± 2.3^{c}	77.8	176 ± 3.2^{c}	157 ± 3.0^{a}	141 ± 1.0^{c}	132 ± 2.8^{ab}	124 ± 3.3^{a}	120 ± 3.2^{ab}	116 ± 3.8^{a}	114 ± 2.3^{ab}
10	187 ± 4.5^{c}	86.8	180 ± 4.0^{c}	175 ± 3.8^{c}	152 ± 3.0^{a}	134 ± 4.0^{ab}	120 ± 4.0^{a}	115 ± 2.4^{b}	110 ± 4.0^{a}	106 ± 4.0^{a}

* Results reported as means ± SD of triplicate determinations. Means within columns with different letters are significantly different ($P \leqslant 0.05$).

molecules, which weakens hydrophobic interactions and increases protein flexibility. This allows them to spread to the air water interface more quickly, thus encapsulating air particles, and increasing foam formation.

Protein stability is higher in the neighbourhood of the isoelectric pH than at any other pH. This observation lends credence to similar results that have been reported earlier (Buckingham, 1970). Repulsive interaction at the isoelectric region was minimized and this favoured protein–protein interactions and formation of a viscous film at the interface. This development led to the formation of stable molecular layers at the air–water interface, which imparted stability to the foam.

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