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Influence of altered solvent environment on the functionality of pigeonpea (*Cajanus cajan*) and cowpea (*Vigna unguiculata*) protein isolates

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Abstract

Functional properties of pigeonpea and cowpea isolates were determined as a function of pH and NaCl concentrations. At low pH, nitrogen solubility decreased with increasing NaCl concentration whereas, at high pH, it increased. Addition of NaCl to the solvent medium resulted in a marginal improvement and a significant improvement, in the emulsifying activity and emulsion stability of pigeonpea isolate, respectively. The above treatment decreased these properties for the cowpea isolate. Varying both the pH and NaCl concentrations resulted in significant improvements in the emulsifying properties of the isolates relative to the control treatment. NaCl concentrations higher than 0.1 M significantly ($P < 0.05$) increased the expansion of pigeon and cowpea protein–stabilized foams but reduced foam stability, and adjusting pH from 2 to 8 improved foam expansion but decreased foam stability. Varying both the pH and salt concentration had similar effects on the foaming properties of the isolates. The least gelation concentration (LGC) of the isolates decreased with increasing salt concentration. Adjusting the pH to values away from the apparent isoelectric point, elicited similar responses in LGC. In 0.5 M NaCl solution, adjusting the pH to 2, 6 and 8 resulted in significant reductions in LGC relative to the distilled water protein suspensions.

1. Introduction

The effective use of soy proteins in engineered foods is dependent on the tailoring of one or two functional properties to meet the complex needs of the product to be manufactured (Arce, Pilosof & Bartholomai, 1991). The functional properties of proteins can be manipulated by chemical or enzymatic modification but, for household and retail applications, it may be more cost effective to modify the functionality by altering the solvent environment in terms of pH, ionic species and strength. Salts affect physicochemical properties and interactions between proteins and at higher concentration, may alter water structure with subsequent changes in hydrogen and electrostatic bonding and hydrophobic interactions (Phillips, Yang & Kinsella, 1991). Sodium chloride concentration has been shown to affect oilseed protein properties related to functionality in emulsions and foams (Cherry, McWatters & Beuchat, 1979; McWatters & Holmes, 1979), lupin and winged bean proteins (Sathe, Deshpande & Salunkhe, 1982a, 1982b), cowpea flour (Giami, 1993; Okaka & Potter, 1979), and cowpea globulin isolate (Aluko & Yada, 1995). Perusal of literature reveals that, although the effects of altered solvent environment on functionality have been studied extensively for soybean proteins, little work has been done on other legume protein isolates. The present study was designed to investigate the influence of altered solvent environment, in terms of pH and sodium chloride concentration, on the solubility and properties related to functionality in emulsions, foams and gels of pigeonpea and cowpea protein isolates with a view to assessing the functional performance of these isolates in model food systems.

2. Materials and methods

Isoelectrically precipitated protein isolates extracted at pH 8.5 from pigeonpea and cowpea seeds were used.

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The crude protein contents (AACC, 1983 method 46-12) were 83.4 and 92.9% for pigeonpea and cowpea protein isolates, respectively.

For all the functional tests, the control treatment refers to the suspension in distilled water adjusted to pH 7.

2.1. Effect of sodium chloride concentration on the pH-solubility

The pH-solubility indices of the protein isolates were determined in solutions containing 0.1, 0.2, 0.3, 0.4, and 0.5 M NaCl adjusted to pH values between 2 and 12 using 0.1 M HCl or 0.1 M NaOH according to AACC (1983) method 46-23.

2.2. Effects of pH and sodium chloride concentration on the emulsifying and whipping properties

Emulsifying and whipping properties of the isolates were determined under pH conditions of 2, 4, 6, and 8 and salt concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 M, individually and in combination using procedures described by Yasumatsu, Sawada, Moritaka, Toda and Ishii (1972) and Kabirullah and Willis (1982), respectively.

2.3. Effects of pH and sodium chloride concentration on the gelation properties

The least gelation concentrations of the protein isolates were determined under pH conditions of 2, 4, 6, and 8 and salt concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 M, individually using procedures described by Coffmann and Garcia (1977). The effects of pH and 0.5 M salt concentration on protein gelation were also studied.

2.4. Statistical analysis

Analysis of variance and Duncans multiple range test were done according to the Statistical Analysis System package (SAS, 1987).

3. Results and discussion

3.1. Effects of NaCl concentration on protein pH-solubility

Sodium chloride concentration had significant effects on the pH-solubility profiles of both pigeonpea and cowpea protein isolates as shown in Figs. 1 and 2, respectively. For both isolates, the presence of NaCl significantly ($P < 0.05$) decreased the solubility of the proteins in the acidic pH range (2–4). In the alkaline pH extremes, the solubility of the isolates increased relative to the control. Increase in the molality of NaCl caused the solubility profiles to change. The solubility curves became sharper with a narrower range of low solubility.

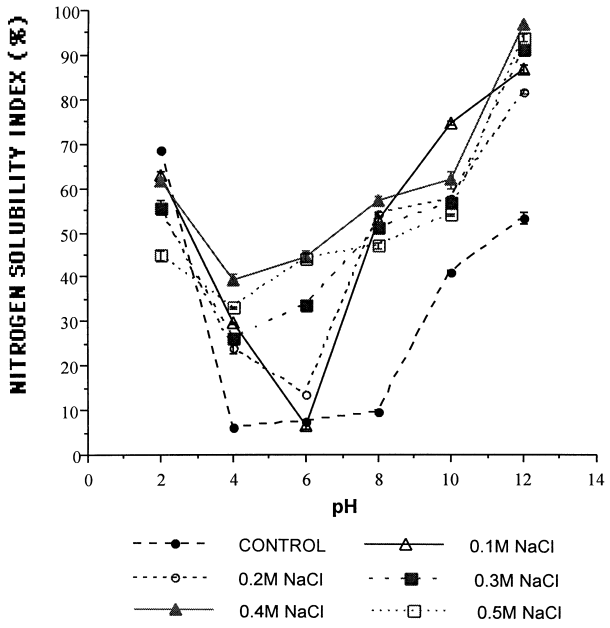

Fig. 1. Effects of sodium chloride concentration on the pH-solubility of pigeonpea protein isolate. Means of duplicate determinations.

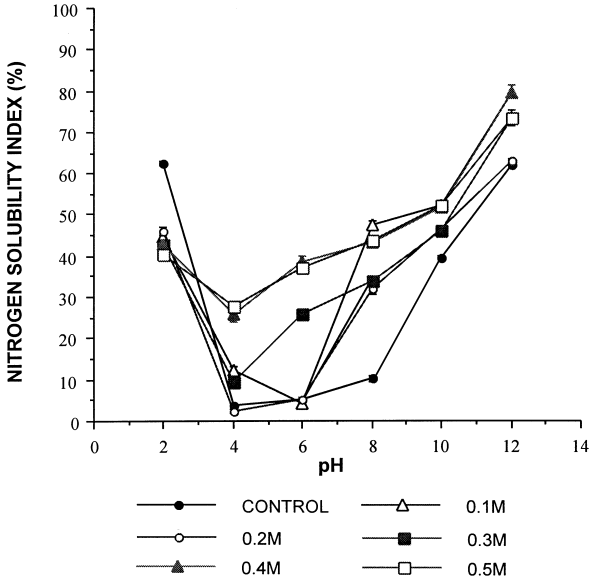

Fig. 2. Effects of sodium chloride concentration on the pH-solubility of cowpea protein isolate. Means of duplicate determinations.

This observation was indicative of the enhanced sensitivity of the solubility of the isolates to changes in pH in the presence of salt. For both isolates, the solubility minima in 0.1 and 0.2 M NaCl shifted from pH 4 to 6. At NaCl concentrations above 0.2 M, the solubility curves adopted a flatter profile with solubility minima $> 30\%$. The protein suspensions in 0.5 and 0.2 M NaCl exhibited the lowest solubility in the acidic and alkaline pH extremes, respectively. The highest solubility was observed in 0.4 M NaCl at pH 12 for pigeonpea (96.9%) and for cowpea (79.5%) isolates, compared to

53.4% and 61.8%, respectively for the controls. It was also evident that NaCl concentration affected the pH-solubility characteristics of pigeonpea isolate more than those of cowpea isolate. Aluko and Yada (1995) had shown that at low pH (3–4) the solubility of cowpea globulin isolate decreased with increasing ionic strength but, as pH was increased, the effect of NaCl diminished; the solubilities increased with pH with each salt concentration. The findings in this study are in contrast to the above study probably due to differences in isolate composition and ionic strengths used. The findings in our study are, however, similar to the findings of previous investigations on the effect of NaCl concentration on the pH-solubility of soy isolate (Hermansson, 1979; Kinsella, 1979). The effects of ionic strength on protein solubility presumably involve electrostatic, solvation, and salting-in and salting-out phenomena (Kinsella, 1979). The structure–function relationship approach of Aluko and Yada explained that, at low pH, all carboxyl groups are protonated and the protein acquires a net positive charge, resulting in decreased repulsion of the Cl^- ions and enhanced hydrophobic interactions leading to the formation of insoluble aggregates. At high pH values the increased negative charge on the proteins, combined with the salting-in effect of NaCl, serve to dissociate the protein aggregates and thus increase solubility. The results obtained in the present study demonstrate that the deleterious effects of denaturation of the isoelectric precipitation extraction process on protein solubility (Mwasaru, 1996) may be ameliorated by dispersion in an appropriate pH and ionic strength solvent environment.

3.2. Effects of NaCl concentration and pH on emulsifying properties

As shown in Table 1, a significant ($P < 0.05$) reduction in the emulsifying activity of pigeonpea isolate was evident in 0.3 M NaCl in comparison with other salt concentration studied. The improvement in this property in the presence of NaCl was, however, marginal relative to the control. Increasing NaCl concentration generally resulted in significant increases in the stability of pigeonpea protein–stabilized emulsion but no consistent trends in the response were apparent. The highest emulsion stability was recorded in 0.3 M NaCl (57.36%) compared to 44.89% for the control. Addition of NaCl decreased the emulsifying activity of cowpea isolate, particularly at 0.3–0.5 M levels. Scant information was available in literature on the effects of NaCl concentration on the properties of protein–stabilized emulsions at neutral pH. Aluko and Yada (1995) reported that the emulsifying activity of cowpea globulin isolate was higher at low NaCl concentration at various pH conditions. Kinsella (1979) reported that the emulsifying activity of soy protein was better in 0.05 than in 0.03 ionic strength solvent environment. Halling (1981) reported significant correlations between protein solubility and emulsion stability. The effects of NaCl on the emulsifying activity of pigeonpea isolate paralleled those on solubility but no clear-cut relationships were evident for cowpea isolate. This finding suggests that NaCl probably affected the emulsifying activity of the isolates by other mechanisms, in addition to its effects on protein solubility. The observed differences in the response of emulsifying properties to changes in salt concentration between pigeonpea and cowpea isolates may be due to differences in their subunit molecular weight distribution and amino acid composition.

The effects of pH on the emulsifying properties of pigeonpea and cowpea protein isolates are presented in Figs. 3 and 4, respectively. For pigeonpea isolate, adjusting the pH to 2, 4 and 8 resulted in significant ($P < 0.05$) increases in the emulsifying activity. The highest emulsifying activity of 48.05% was recorded at pH 2 compared to 39.50% for the control. The stability of pigeonpea protein–stabilized emulsions was also increased at these pH values but was significantly lowered at pH 6. Protein suspensions at pH 4 exhibited the highest emulsion stability (58.57%) compared to 44.89% for the control. For the cowpea isolate, adjusting the pH resulted in a reduction in the emulsifying activity and emulsion stability relative to the control.

Table 1
Effect of sodium chloride concentration on the emulsifying properties of pigeonpea and cowpea protein isolates[a]

NaCl concentration (M)	Emulsifying property (%)			
	Pigeonpea isolate		Cowpea isolate	
	Activity	Stability	Activity	Stability
Control	39.50 ± 0.38b	44.98 ± 1.56c	48.16 ± 0.05ab	54.90 ± 1.54b
0.1	43.09 ± 0.33a	47.86 ± 1.12bc	49.92 ± 2.02a	50.68 ± 0.95c
0.2	42.26 ± 0.23a	50.67 ± 0.95b	48.83 ± 0.17bc	55.72 ± 0.23b
0.3	39.34 ± 2.60b	57.36 ± 0.31a	43.05 ± 0.27c	58.90 ± 0.80a
0.4	43.75 ± ± 0.00a	51.47 ± 2.08b	38.57 ± 2.02d	58.43 ± 0.76a
0.5	42.42 ± ± 0.00a	56.35 ± 1.12a	44.29 ± 0.21c	54.42 ± 0.18b

[a] Means in a column followed by the same letter are not significantly different ($P < 0.05$). Values given are means of duplicate determinations.

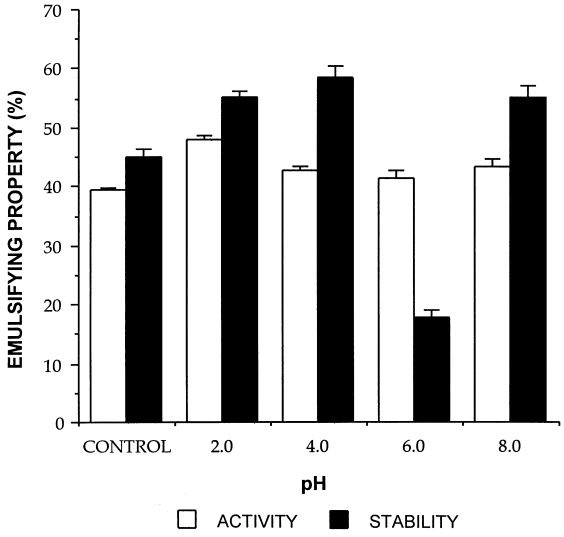

Fig. 3. Effect of pH on the emulsifying properties of pigeonpea protein isolate. Means of duplicate determinations.

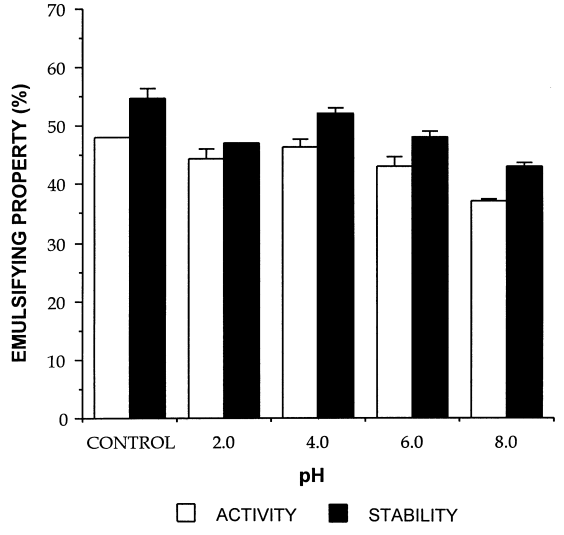

Fig. 4. Effect of pH on the emulsifying properties of cowpea protein isolate. Means of duplicate determinations.

The results of this study indicated that the emulsifying properties of pigeonpea isolate were more responsive to changes in the solvent pH than those of cowpea isolate. Kamat, Graham and Davis (1978) reported that proteins near the isoelectric point should perform well in terms of emulsifying properties because protein adsorption and viscoelasticity at the oil–water interface are maximum and repulsive forces between proteins are minimum. The results obtained in this study were consistent with the above observations in terms of the stabilities of pigeonpea and cowpea protein–stabilized emulsions. Studies on the emulsion capacity of lupin protein concentrate showed that it was pH-dependent and that this property was improved more at acidic than at alkaline pH ranges (Sathe *et al.*, 1982a). The observation is consistent with the results obtained in this study. However, the reverse effect has been reported for winged bean protein isolate (Sathe *et al.*, 1982b). The emulsion capacity of safflower protein isolates exhibited slight variations in response to solvent pH changes except at pH 4 and 6, where significant reductions were noted (Paredes-Lopez & Ordorica-Falomir, 1986). Halling (1981) has suggested that pH exerts its effects on emulsification properties primarily by altering the charge on protein molecules.

The combined effects of varying both the NaCl concentration and pH on the emulsifying activity of pigeonpea and cowpea isolates are presented in Table 2. For pigeonpea isolate, increasing the pH from 2 to 8 at all salt concentrations (0.1–0.5 M) generally resulted in significant ($P < 0.05$) increases in the emulsifying activity, reaching a maximum at pH 6. The highest emulsifying activity of 47.30% was noted at pH 6 in 0.2 M NaCl compared to 39.50% for the control. For the cowpea isolate, increasing the pH at lower salt concentrations (0.1–0.2 M) resulted in significant increases in the emulsifying activity with maxima at pH 4. At higher salt concentrations (0.3–0.5 M), increasing pH also resulted in significant increases in emulsifying activity but the maxima shifted to pH 6. The maximum emulsifying activity of 49.38% was noted at pH 6 in 0.3 M salt compared to 48.16% for the control, a negligible improvement. As shown in Fig. 5, the pigeonpea protein–stabilized emulsions were most stable at pH 4 and least stable at pH 8 at low salt concentrations (0.1–0.2 M). The sensitivity of emulsion stability to increasing NaCl concentration was highest at pH 2. The highest emulsion stability of 63.20% was observed at pH 4 in 0.3 M NaCl compared with 44.89% for the control. Fig. 6 shows that the stability of cowpea protein-stabilized emulsions responds to changes in pH and NaCl concentrations similarly to those observed for the pigeonpea isolate. However, the maxima were centered in the 4–6 pH range at low salt concentrations and at higher salt concentrations (0.3–0.5 M), the maximum stability was noted at pH 2. The highest emulsion stability of 69.28% was noted at pH 2 in 0.5 M NaCl compared to 54.90% for the control. The emulsifying activity of cowpea globulin isolate in the presence of NaCl was reported to be higher at low pH, and to decrease progressively with increasing pH (Aluko & Yada, 1995). This observation is at variance with the results obtained in this study, probably due to differences in the composition of the proteins involved. The effects of pH on the emulsifying properties of soy flour in 1.0 M NaCl (McWatters & Holmes, 1979) were, however, similar to the results obtained in this study. It has been postulated (Aluko & Yada) that, at low pH, proteins, carry a net positive charge. Addition of NaCl, therefore, causes the oppositely charged Cl^- to interact with the proteins, thereby decreasing the repulsion and enhancing hydrophobic interactions; the emulsifying activity of the

Table 2
Effects of sodium chloride concentration and pH on the emulsifying activities of pigeonpea and cowpea protein isolates[a]

NaCl concentration (M)	Emulsifying activity (%) at pH							
	Pigeonpea				Cowpea			
	2.0	4.0	6.0	8.0	2.0	4.0	6.0	8.0
0.1	40.25 ± 1.10b	43.99 ± 1.06a	43.42 ± 0.25a	42.14 ± 1.56ab	38.12 ± 1.08c	48.75 ± 1.77a	45.95 ± 0.00a	41.76 ± 0.14c
0.2	41.57 ± 0.76b	39.18 ± 0.42c	47.30 ± 0.11a	38.66 ± 1.15c	38.88 ± 0.00b	46.59 ± 0.90a	45.83 ± 0.17a	45.95 ± 0.00a
0.3	36.11 ± 0.00c	41.33 ± 1.11b	46.56 ± 1.15a	38.04 ± 0.28c	39.16 ± 2.86c	46.11 ± 1.57ab	49.38 ± 0.88a	41.79 ± 0.46bc
0.4	40.54 ± 0.00c	42.68 ± 0.80b	44.64 ± 0.14a	38.34 ± 0.71d	42.83 ± 0.58a	43.59 ± 3.01a	45.83 ± 0.17a	45.95 ± 0.00a
0.5	39.31 ± 0.61b	40.27 ± 0.38b	45.77 ± 0.08a	37.67 ± 0.24c	45.71 ± 0.00ab	43.20 ± 2.71bc	47.82 ± 1.07a	41.11 ± 0.11c

[a] Means in a row for each protein isolate followed by the same letter are not significantly different ($P < 0.05$). Values given are means of duplicate determinations.

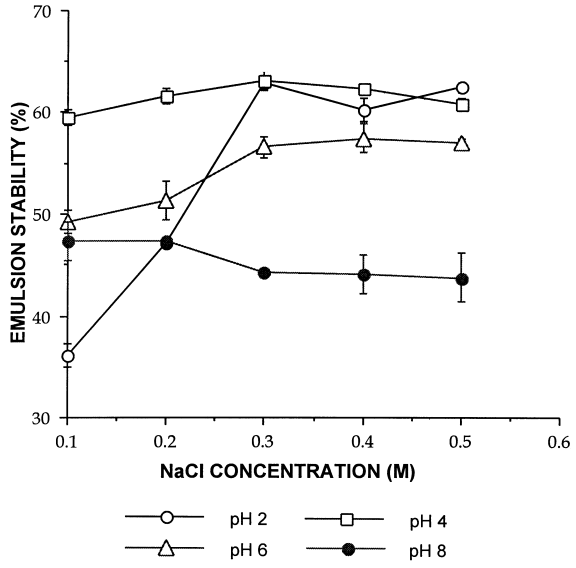

Fig. 5. Effects of sodium chloride concentration and pH on the stability of pigeonpea protein-stabilized emulsion. Means of duplicate determinations.

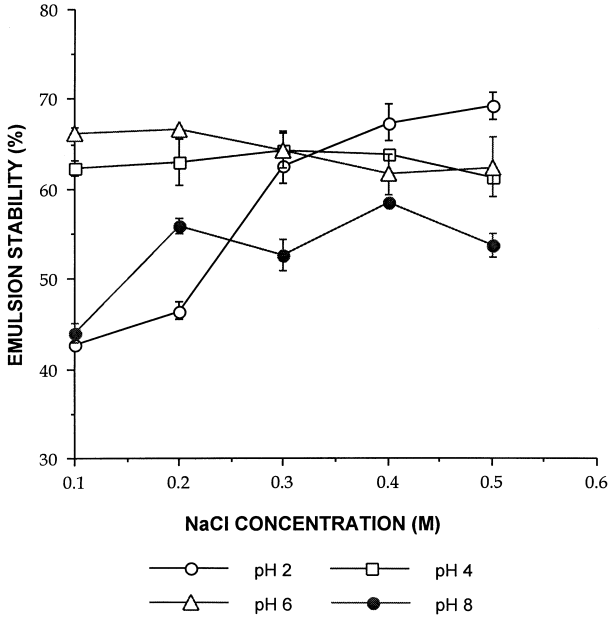

Fig. 6. Effects of sodium chloride concentration and pH on the stability of cowpea protein-stabilized emulsion. Means of duplicate determination.

proteins is thus increased with increasing salt concentration. The observed stability of the legume protein–stabilized emulsions, as a function of salt concentration, at pH values below 8 in this study, may be due to the formation of charged layers around the oil globule resulting in mutual repulsion as previously reported (Aluko and Yada; Chung & Ferrier, 1992; McWatters & Holmes, 1979). The inferior emulsion stability at pH 8 was presumably due to the fact that, at this pH, the protein molecules acquire a net negative charge and interaction with Na^+ destabilized the interfacial films through reduction of electrostatic repulsive forces.

3.3. Effects of NaCl concentration and pH on whipping properties

The volume expansion of pigeonpea and cowpea protein-stabilized foams significantly ($P < 0.05$) increased with increasing NaCl concentration as shown in Table 3. For both isolates, significant improvement in foam expansion relative to the controls was observed at all salt concentrations, except at 0.1 M. The maximum expansion was observed in 0.5 M NaCl, with values that were almost twice the values noted for the controls. However, the presence of NaCl resulted in significant reductions in the stability of pigeonpea and cowpea protein–stabilized foams. No consistent trends were apparent in the response of foam stability to increasing salt concentrations but stability was lowest in 0.2 and 0.3 M NaCl for pigeonpea and cowpea isolates, respectively. Aluko and Yada (1995) observed no significant differences between NaCl levels and their effect on the foam stability of cowpea globulin isolate, which is similar to the results obtained for cowpea isolate in this study. Addition of NaCl was reported to improve the foaming properties of lupin and winged bean proteins (Sathe *et al.*, 1982a, 1982b) with maximum improvement occurring at 0.6

Table 3
Effect of sodium chloride concentration on the whipping properties of pigeonpea and cowpea protein isolates[a]

NaCl concentration (M)	Foam property			
	Pigeonpea isolate		Cowpea isolate	
	Expansion	Stability	Expansion	Stability
Control	34.00 ± 0.85e	77.80 ± 0.00a	35.30 ± 0.99d	79.40 ± 1.56a
0.1	21.00 ± 0.95f	29.29 ± 1.01c	24.00 ± 2.83e	37.98 ± 0.68b
0.2	40.24 ± 1.75d	21.67 ± 2.35d	42.00 ± 2.83c	34.00 ± 0.68c
0.3	54.33 ± 3.30c	24.70 ± 1.26d	68.00 ± 2.83b	33.79 ± 0.64c
0.4	59.00 ± 1.41b	32.05 ± 1.81bc	79.00 ± 1.41a	36.00 ± 1.41bc
0.5	64.17 ± 1.18a	35.56 ± 0.00b	83.00 ± 1.41a	36.50 ± 2.12bc

[a] Means in a column followed by the same letter are not significantly different ($P < 0.05$). Values given are means of duplicate determinations.

and 0.8% levels, respectively. The foaming properties of cowpea flour were improved in 0.2 M salt (Giami, 1993). Kinsella (1976) reported the formation of high capacity-low stability foams when NaCl was added to soy isolates, an observation consistent with the results obtained in this study. The improvement in the expansion of protein–stabilized foams on addition of NaCl has been attributed to increased solubility of proteins (Halling, 1981; Kinsella, 1979; Sathe *et al.*, 1982b) which, inter alia, increases the adsorption of protein films at the interface, resulting in improvement in foaming capacity, but causes a decrease in the rheological properties of the films resulting in lower foam stability. NaCl reduces the surface denaturation of protein, necessary to impart the required rheological properties for foam stability (Kinsella, 1981).

The effects of varying pH on the whipping properties of pigeonpea and cowpea protein isolates are presented in Figs. 7 and 8, respectively. For both isolates, the expansion of the protein–stabilized foams increased significantly ($P < 0.05$) with changes in pH (2–8), compared to the control. For pigeonpea isolate, the highest expansion (77.6%) occurred at pH 2, followed by a drop at pH 4 (55.0%) and a recovery at pH 6 (63.0%) and 8 (65.0%). The stability of the foams was, however, significantly reduced as the pH was adjusted from 2 to 8 compared to the control. The response of foam stability was relatively indifferent to changes in pH. The cowpea isolate exhibited similar trends to those of the pigeonpea isolate, but maximum foam expansion occurred at pH 2 and 6, and foam stability was more responsive to changes in pH, particularly between pH 6 and 8. Studies by Sathe *et al.* (1982a) had shown that the foaming capacities of lupin and winged protein were highest at pH 2 and decreased progressively as the pH was increased, which agrees with the results in this study. Kinsella (1979, 1981) reported maximum expansion and stability of soy protein–stabilized foams at pH 2 and 9, with minima occurring between pH 4 and 6, the point of minimum solubility. Maximum stability and minimum

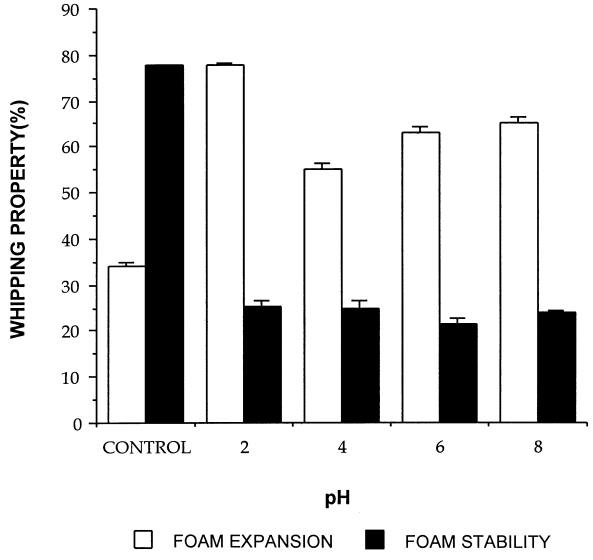

Fig. 7. Effect of pH on the whipping properties of pigeonpea protein isolate. Means of duplicate determinations.

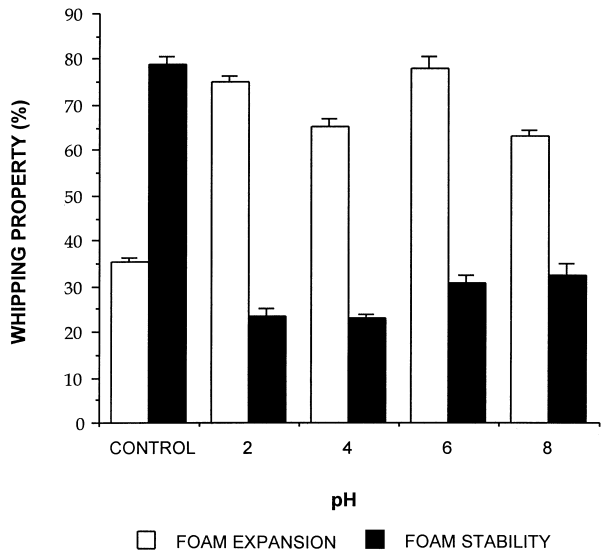

Fig. 8. Effect of pH on the whipping properties of cowpea protein isolate. Means of duplicate determinations.

solubility occur in the isoelectric pH range of the protein. For foam formation, reasonable solubility is desirable to enable surface adsorption to occur. Many proteins easily coagulate in the isoelectric region, resulting in reduced film stability. Although film strength is maximum in this pH range, concurrent coagulation may reduce foam stability and this may account for the observed optimal foam expansion of the isolates at pH values away from the apparent isoelectric point (pH 4) in the current study. Proteins of different origins are known to vary immensely in their foaming properties, reflecting differences in their composition, conformation, structure, and interactions with other compounds and their immediate environment. The effects of varying pH (2–8) and NaCl concentration (0.1–0.5 M) on the expansion of pigeonpea and cowpea protein–stabilized foams are presented in Fig. 9 and 10, respectively. Foam expansion was higher in the acidic than the alkaline pH ranges for the pigeonpea isolate but the reverse was true for the cowpea isolate. Increasing NaCl concentration had significant ($P < 0.05$) but varying effects on the whipping properties depending on the type of protein and pH. All combinations of pH and NaCl concentration significantly improved the expansion of pigeonpea protein-stabilized foams relative to the control (34.0%) and the highest value was observed at pH 2 in 0.2 M NaCl (91.0%). For cowpea isolate, significant increases in foam expansion were observed as the pH was increased from 6 to 8 at all salt concentrations. The highest foam expansion was observed at pH 8 in 0.5 M NaCl (106.0%) compared to 35.30% for the control.

As shown in Figs. 11 and 12, the respective responses of the stability of pigeonpea and cowpea protein–stabilized foams tended to resemble their pH-solubility in the presence of salt (Figs. 1 and 2). For both isolates, increasing the pH resulted in significant ($P < 0.05$) reductions in foam stability, at all NaCl concentrations. Minimum values were noted at pH 4 followed by increases at higher pH except at pH 6 in 0.4 and 0.5 M

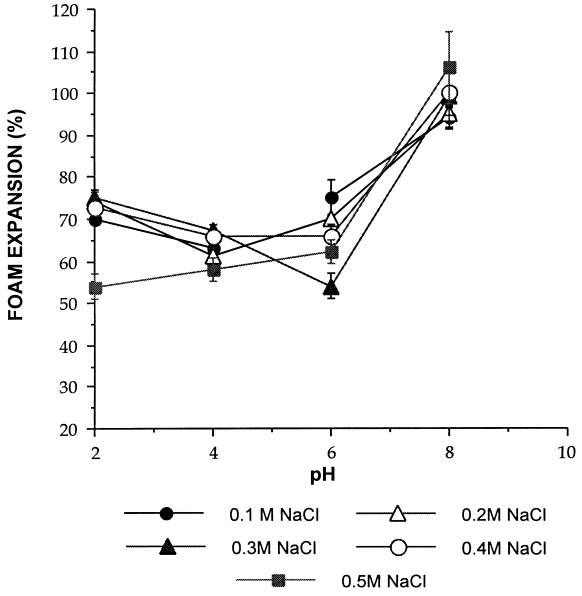

Fig. 10. Effects of sodium chloride concentration and pH on the expansion of cowpea protein-stabilized foam. Means of duplicate determinations.

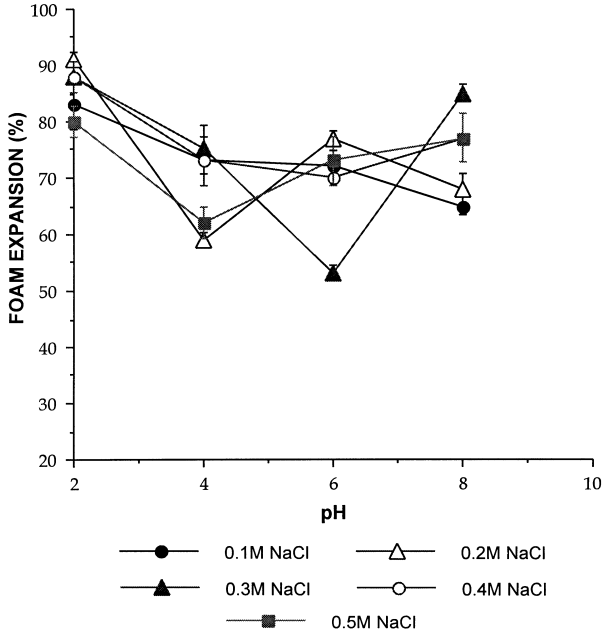

Fig. 9. Effects of sodium chloride concentration and pH on the expansion of pigeonpea protein-stabilized foam. Means of duplicate determinations.

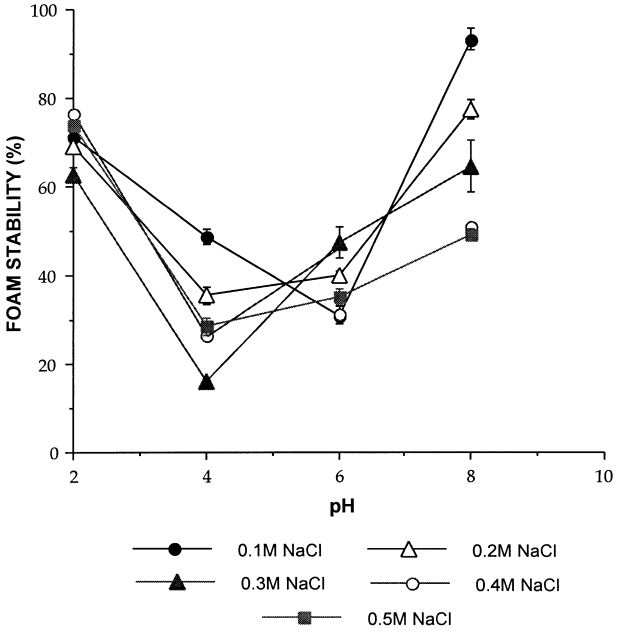

Fig. 11. Effects of pH and sodium chloride concentration on the stability of pigeonpea protein-stabilized foam. Means of duplicate determinations.

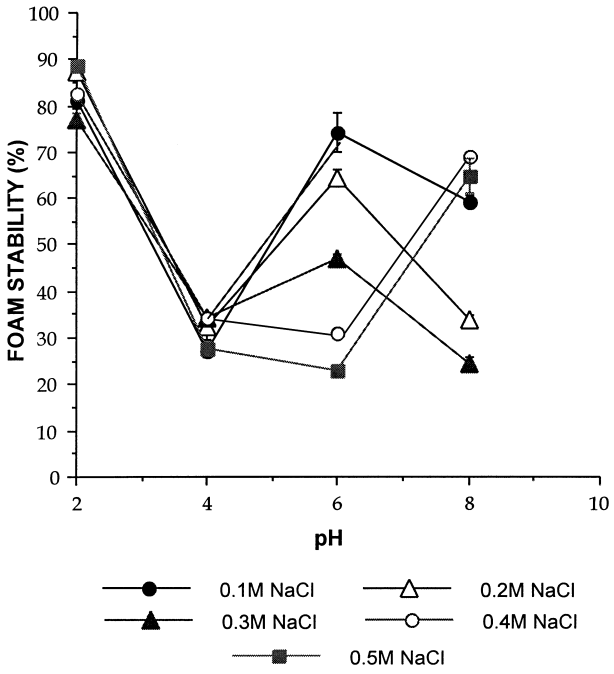

Fig. 12. Effects of pH and sodium chloride concentration on the stability of cowpea protein-stabilized foam. Means of duplicate determinations.

and at pH 8 and 0.5 M concentrations for cowpea isolate, and at pH 6 in 0.1 M NaCl for pigeonpea isolate in which significant reductions occurred. All combinations of pH and salt concentration resulted in significant reductions or no improvement in emulsion stability except for pigeonpea isolate at pH 8 in 0.1 M, and at pH 2 in 0.2 and 0.5 M NaCl for cowpea isolate, in which the values were higher than the corresponding controls. Foam stability tended to be higher in the alkaline and acidic pH range for the pigeonpea isolate and cowpea isolates, respectively. Aluko and Yada (1995) observed a general increase in the foaming capacity of cowpea globulin isolate as the pH and NaCl concentration were increased, and better foam stability at higher pH regardless of ionic strength. Addition of salt increased foaming away from the isoelectric point of the protein (Halling, 1981) which is consistent with the results obtained in this study. The mechanism of the combined effects of NaCl concentration and pH on the whipping properties of protein isolates has been elucidated on the basis of their effects on the electrostatic repulsion between protein molecules (Aluko & Yada), the effects of salt on protein aggregation (Kinsella, 1981) and the shielding effects of Na^+ and Cl^- ions on protein–protein association (Poole, West & Walters, 1984).

3.4. Effects of NaCl concentration and pH on gelation properties

Increasing pH and NaCl concentration individually had varying effects on the least gelation concentration (LGC) of pigeonpea and cowpea protein isolates as shown in Table 4. Adjusting the pH to 4 significantly ($P < 0.05$) decreased the LGC relative to the other pH values which were similar, whereas increasing NaCl from 0.1 to 0.5 M resulted in significant reductions in the LGC values. The cowpea isolate generally exhibited higher LGC than the pigeonpea isolates at low (0.1–0.2 M) salt concentrations. Aluko and Yada (1995) reported that cowpea globulin isolate was extensively coagulated at pH 3 and that coagulation decreased gradually as the pH was increased. An inverse relationship was also observed between LGC and solubility. It had been shown earlier in this study (Figs. 1 and 2) that pigeonpea and cowpea isolates exhibited minimum solubility at pH 4 in distilled water and this coincided with the point of lowest LGC. It has been suggested (Poole *et al.*, 1984) that pH exerts its effects on protein gelation via its influence on charge and electrostatic balance within and between protein molecules. Electrostatic bonds may have a great effect in gel formation as the pH of the protein is moved away from the isoelectric point (Schnepf, 1992): at pH lower than the pI, there may be too many positively charged groups and above the pI too many negatively charged groups to allow gel formation. Thus, the LGC of the isolates is expected to increase as the pH is moved away from the pI, as was the case in the present study. Arntfield, Murray and Ismond (1990) reported that optimum network characteristics were obtained with 300 mM NaCl for ovalbumin and 200 mM for faba bean vicilin; at higher salt concentration the storage moduli and tan decreased indicating disruption of the network due to the masking of protein charges by the salt. As shown in Table 5, when the gelation test was performed in 0.5 M NaCl, adjusting the pH to 2 δ resulted in significant reductions in LGC relative to the distilled water control (Table 4) for pigeonpea isolate. The LGC values for the cowpea isolate exhibited significant reductions at pH 2, 6, and 8 h. The biggest reduction occurred at pH values away from the apparent isoelectric point (pH 4), as expected; neutralization of the repulsive forces by the Na and Cl ions enhanced protein–protein interactions resulting in gelation occurring at lower concentrations of protein.

In summary, the results obtained in this study indicated that the poor functionality of oven-dried protein isolates could be improved by altering the solvent environment in terms of pH and NaCl concentration. The solubility of pigeonpea and cowpea protein isolates was significantly improved by the presence of salt. This in turn resulted in the improvement of their emulsifying, foaming and gelation functionalities. Further improvements in the functionality of the protein isolates were achieved by altering both pH and NaCl concentration. Most food preparations involve solvent environments that contain salt and pH in the range 4 to 8. The present study is relevant to these situations and in further applications, especially in the domain of product devel-

Table 4
Effects of pH and sodium chloride concentration on the least gelation concentration of pigeonpea and cowpea protein isolates[a]

	Least gelation concentration (%)	
	Pigeonpea isolate	Cowpea isolate
pH		
2.0	14.0b	14.0b
4.0	8.0d	10.0c
6.0	14.0b	14.0b
8.0	14.0b	14.0b
NaCl concentration (M)		
0.1	14.0b	16.0a
0.2	14.0b	16.0a
0.3	10.0c	10.0c
0.4	6.0e	6.0e
0.5	6.0e	6.0e

[a] Means followed by the same letter are not significantly different ($P < 0.05$). Values given are means of triplicate determinations.

Table 5
Effects of pH and 0.5 M NaCl on the least gelation concentration of pigeonpea and cowpea protein isolates[a]

pH	Least gelation concentration (%)	
	Pigeonpea isolate	Cowpea isolate
2.0	4.0c	4.0c
4.0	8.0a	8.0a
6.0	6.0b	4.0c
8.0	8.0a	4.0c

[a] Means followed by the same letter are not significantly different ($P < 0.05$). Values given are means of triplicate determinations.

opment. Some potential applications include the protein fortification of fruit juices, in which the natural acidic pH will enhance protein solubility, fermented milk products, and fermented vegetables.

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