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Kosmotropes and chaotropes as they affect functionality of a protein isolate

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Abstract

Influences of kosmotropic and chaotropic anions on functional properties of a protein isolate prepared from African locust bean were investigated. The pH-dependent protein solubility profile showed that solubility was minimal at the isoelectric point of the protein isolate (pH 4.5). At all pHs, protein solutions prepared in chaotropic anions (SCN^-, ClO_4^- and I^-) had better solubilities than those prepared in kosmotropic anions (SO_4^{2-}, Cl^-, I^-). Increase in protein solubility followed the Hofmeister series: Na_2SO_4, <NaCl, <NaBr, NaI,<$NaClO_4$,<NaSCN. Water absorption capacity was maximal in solutions prepared at 0.1 M concentrations and it was reduced when the concentration increased further. Generally, the result indicates that kosmotropic salts had improved water absorption capacity over the chaotropic salts. Both foaming capacity and stability were better in chaotropic salts than in kosmotropic salts. Emulsifying activity and stability were reduced as the concentration of salts in each protein solution increased. Among the various protein solutions, maximal emulsifying activity and stability were observed in protein solutions prepared in NaSCN and protein solution prepared in $NaSO_4$ had the lowest emulsifying properties. Chaotropic salts had better emulsifying properties than had kosmotropic salts and the emulsifying potentials increased according to the Hofmeister series. The result indicates that least gelation concentration increased as the concentration of salts increased and LGC values were lower in chaotropic anions than in kosmotropic anions.

Keywords: Chaotropes; Kosmotropes; Hofmeister series; Protein isolate

1. Introduction

Over a century ago, ions were arranged into a series based on their ability to salt out proteins (Hofmeister, 1888) and since then this approach has been extended to characterise the ability of particular ions to nature or denature biological material (Baldwin, 1996; Collins & Washabaugh, 1985; Eagland, 1975; Lawal & Adebowale, 2004; Von Hippel & Schleich, 1969). The term 'kosmotrope' refers to salts that stabilize protein structures while 'chaotropes' are salts that destabilize protein structures (Wiggins, 2001). The terms were later extended to the apparently correlating property of increasing, or decreasing, respectively, the structuring of water (De Xammar Oro, 2001). Chaotropes break down the hydrogen-bonded network of water, so allowing macromolecules more structural freedom and encouraging protein extension and denaturation. Kosmotropes are stabilizing solutes, which increase the order of water, whereas chaotropes create weaker hydrogen bonding, decreasing the order of water, increasing its surface tension and destabilizing macromolecular structures (Uedaira & Uedaira, 2001).

It has been reported that the effect of salt concentration on protein stability is very ion-specific, with stabilizing or destabilizing effects typically following the Hofmeister series (Von Hippel & Wong, 1962):

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$SO_4^{2-} > H_2PO_4^- > CH_3COO^- > Cl^- > Br^- > I^- > ClO_4^- > SCN^-$. Salts such as Na_2SO_4, NaCl, NaBr, NaI, $NaClO_4$, NaSCN influence the physicochemical properties and interactions between proteins by ionic strength effects, binding to charged groups. Also, at high concentrations, they may alter water structure with subsequent changes in hydrophobic and electrostatic interactions (Damodaran & Kinsella, 1982). Studies on the effects of salts on the structural properties of proteins have revealed that at least two effects of salts make major contributions to the structure-stabilizing properties of the proteins, which are their effects on solvent (water) structure and electrostatic interaction with the charged groups of the protein (Muller, 1988). This reasoning has also been expounded by certain workers, who believe in the possibility of direct ion binding to the peptide backbone of proteins while others believe that ions affect protein folding, indirectly, by interacting with structurally-bound water molecules (Asghar & Henrickson, 1982; Sarabia, Gomez-Guillen, & Montero, 2000).

The extent of such changes depends on the nature and concentration of the solute. In the Hofmeister series, I^-, ClO_4^-, and SCN^- are categorised as chaotropic while SO_4^{2-}, Cl^-, Br^- are kosmotropic.

Effective utilization of a seed protein in food systems depends extensively on its functional properties (Chavan, Mc Kenzie, & Shahidi, 2001). These include emulsification, foam formation, viscosity, gelation, and water and oil absorption capacity. It is also vital to study functional properties of protein isolates in response to the operating environment; these include the ionic strength and pH of the medium (Myers, 1988). Binding forces within the matrices of protein molecules, such as hydrophobic and electrostatic interactions, can be influenced by ionic strength to produce proteins with varying structural conformations and functional properties (Aluko & Yada, 1995). In addition, hydrodynamics of protein molecules in food systems are subject to prevalent ionic strength and concentration. In a three-component system, such as protein–water–salt, knowledge of the preferential interactions of the proteins with the solvent components can give an insight into the manner by which additives affect the solubility and stability of proteins. Effect of salts, particularly NaCl, have been studied on the functional properties of some protein concentrates such as oil seed (Mcwatters & Holmes, 1979; Cherry & Mc Watters, 1989) lupin and Great Northern bean proteins (Sathe & Salunkhe, 1981; Sathe, Desphande, & Salunkhe, 1982), pigeon pea and cowpea protein isolates (Akintayo, Oshodi, & Esuoso, 1999; Mwasaru, Muhammad, Bakar, & Cheman, 2000) and mucuna protein concentrate (Lawal & Adebowale, 2004).

The present study was targeted at investigating the influence of various concentrations of Na_2SO_4, NaCl, NaBr (kosmotropic salts) and NaI, $NaClO_4$, and NaSCN (chaotropic salts) on functional properties of a protein isolate prepared from African locust bean (*Parkia biglobossa*).

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. The seeds were ground to pass through a BS 60-mesh screen, using a household flourmill (Braun Multimix Deluxe, Germany). The flour was kept in a refrigerator at about 4 °C until used.

2.2. Preparation of protein isolate

Two kilogramme of African locust bean flour were dispersed in 10 l of distilled water. The pH was adjusted to 8.0 with 0.5 M NaOH to enhance protein solubilization and the dispersion was stirred for 5 h at 30 ± 2 °C. The pH of the supernatant obtained after centrifuging at 4000*g* for 30 min was adjusted to 4.5 with 1.0 M HCl. The protein concentrate was recovered by centrifugation at 5000*g* for 30 min. It was dispersed in distilled water at pH 7, following which the pH was readjusted to 4.5 and protein concentrate recovered by centrifuging at 5000*g*. The average yield of protein concentrate from African locust bean flour was 30.2% while the percentage protein content of the concentrate was 80.4% using the Kjeldahl method (AOAC, 1985).

2.3. Protein solubility profile

The method of Were, Hettiarachchy, and Kalapathy (1997) was employed for the determination of pH-dependent solubility profile of protein isolate. One hundred and twenty-five milligrammes of the sample were dispersed in 25 ml of 0.1 M separate solutions of Na_2SO_4, NaCl, NaBr, NaI, $NaClO_4$, NaSCN and the solutions pHs were adjusted from 2 to 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 (AOAC, 1985). 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:

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

2.4. Water absorption capacity

Water absorption capacity studies were conducted using the method of Beuchat (1977).

One gramme of sample was mixed with 10 ml of 0.1 M, 1.0 M and 2.0 M solutions of Na_2SO_4, NaCl, NaBr, NaI, $NaClO_4$, and NaSCN 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.

2.5. Foaming capacity and stability

The method of Coffman and Garcia (1977) was employed for foam capacity studies. Two gramme of protein isolate were dispersed in 100 ml of 0.1 M, 1.0 M and 2.0 M solutions of Na_2SO_4, NaCl, NaBr, NaI, $NaClO_4$, and NaSCN. The solutions were 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 and V_1 is the volume of solution before whipping.

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.

2.6. Emulsifying properties

Emulsifying activity and stability were determined using the method of (Neto, Narain, Silva, & Bora, 2001). Five millilitre portions of protein solution (10 mg/ml), prepared in 0.1, 1.0 and 2.0 M solutions of Na_2SO_4, NaCl, NaBr, NaI, $NaClO_4$, and NaSCN, were homogenized with 5 ml oil (Executive Chef Unilever Plc, Nigeria) for 1 min. The emulsions were centrifuged at 1100*g* 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 1100*g* for 5 min

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

2.7. 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 0.1, 1.0 and 2.0 M solutions of Na_2SO_4, NaCl, NaBr, NaI, $NaClO_4$ and NaSCN. Ten milli litre of each of the prepared dispersions were transferred into test tubes. There were 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.

2.8. Statistical analysis

All determinations were replicated three times and mean values and standard deviations reported. Analyses of variance (ANOVA) were performed to calculate significant differences in treatment means, and the mean separations were performed 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 profile

The pH-dependent protein solubility profile of African locust bean in various Hofmeister salts is presented in Table 1. Minimum protein solubility, in all salt solutions, was observed at pH 4.5, which corresponds to the isoelectric pH of the protein isolate. Maximum protein solubility was observed at pH 10 in all salt solutions. Chaotropic salts, within the pH range studied, had better protein solubilities than had kosmotropic salts. It is also noteworthy that, at all pH values studied, increase in protein solubility followed the Hofmeister series accordingly: Na_2SO_4, <NaCl, <NaBr, NaI, <$NaClO_4$, <NaSCN. This result suggests that Hofmeister salts retain their kosmotropic or chaotropic properties regardless of the pH of the solution. At the extreme of acidic or alkaline range, electrostatic charge repulsion facilitated improved solubility and this accounts for increases in solubility as observed at pH 2 and 10 in all salt solutions. At the acidic end, constituent amino acids assume predominant positive charges due to NH_3^+, while COO^- takes preponderance at the alkaline end of the pH. Electrostatic repulsion under these conditions improved protein dispersability and solubility of the protein isolate. Similar observations have been reported in previous publications on soy protein (Achouri, Zhang, & Shying, 1998) chick pea (Achouri, Zhang, & Shying, 1999) peanut (Neto et al., 2001) and cowpea (Prinyawiwatkul, Beuchat, Mc Watters, & Phillips, 1997).

Table 1
Protein solubility profile of African locust bean protein isolate in various salts[a]

pH	Control	Na_2SO_4	NaCl	NaBr	NaI	$NaClO_4$	NaSCN
2	72 ± 1.9	60 ± 3.2	60 ± 4.4	54 ± 6.1	75 ± 2.5	77 ± 3.8	79 ± 2.8
3	55 ± 4.3	50 ± 2.2	50 ± 4.1	48 ± 0.4	58 ± 4.1	59 ± 2.7	62 ± 3.7
4	24 ± 3.3	21 ± 3.9	19 ± 4.2	18 ± 0.6	28 ± 2.9	31 ± 2.6	33 ± 3.0
4.5	20 ± 1.1	18 ± 3.9	17 ± 1.9	16 ± 1.6	23 ± 3.7	27 ± 2.7	29 ± 3.8
5	32 ± 1.2	30 ± 2.2	28 ± 1.6	24 ± 1.7	35 ± 1.6	38 ± 2.8	41 ± 3.0
6	52 ± 1.5	49 ± 1.9	45 ± 2.6	42 ± 4.1	56 ± 3.2	58 ± 3.2	59 ± 1.2
7	78 ± 2.2	70 ± 2.2	67 ± 0.9	62 ± 2.6	82 ± 3.3	84 ± 3.2	85 ± 2.7
8	80 ± 2.7	75 ± 2.7	70 ± 3.2	67 ± 3.1	85 ± 3.1	87 ± 3.3	88 ± 2.6
9	90 ± 3.9	80 ± 3.1	80 ± 2.3	76 ± 3.2	92 ± 2.9	93 ± 1.8	95 ± 3.1
10	92 ± 3.4	85 ± 3.2	83 ± 2.2	80 ± 1.5	94 ± 2.7	95 ± 1.9	96 ± 3.4

Results are means ± SD.
[a] Studies were conducted at 0.1 M solutions of each salt.

3.2. Water absorption capacity

Water absorption capacity of African locust bean protein isolate, studied at various concentrations in different salts, is presented in Fig. 1. In all the salts, water absorption capacity was maximal in solutions prepared at 0.1 M concentrations and it was reduced when the concentration increased to 1.0 M. Further reductions in water absorption capacity were also observed when the concentrations increased to 2.0 M. Water absorption capacity was maximal in Na_2SO_4 and least tendency for water absorption was observed in NaSCN solutions. Generally, the result indicates that kosmotropic salts (Na_2SO_4, NaCl, NaBr) had improved water absorption capacity over the chaotropic salts (NaI, $NaClO_4$, NaSCN).

Ions may be classified as "water structure formers" or water structure breakers on the basis of their ability to alter the net structure of water by its polarizing power, which results in modification of water viscosity (Fennema, 1977). In previous works, neutron diffraction studies on two important chaotropes (guanidinium and thiocyanate ions) show very poor hydration, supporting the suggestion that they preferentially interact with protein rather than with water (Mason, Neilson, Dempsey, Barnes, & Cruickshank, 2003). The observed preferential hydration is the consequence, primarily, of the perturbation of the surface free energy change at the protein–solvent interface, induced by the addition of the salts. Reductions in water absorption concentration with increase in salt concentration could be explained by the effect of salts on charged groups on protein molecules. At low salt concentrations, 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.

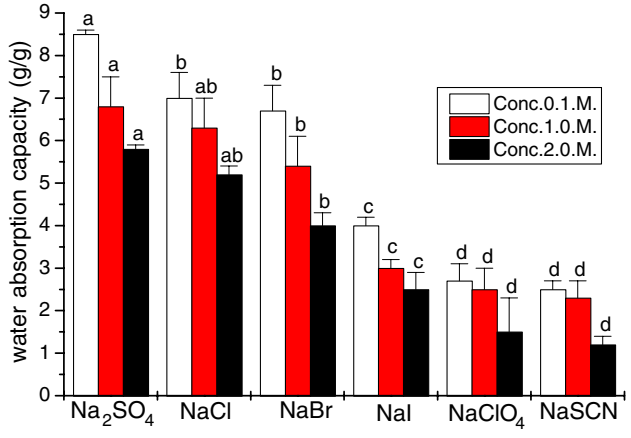

Fig. 1. Water absorption capacities of African locust bean protein isolate prepared in salts of different concentrations. Error bars: standard deviations. Results are means of triplicate determinations. Values followed by the same letter at the same concentration are not significantly different ($P < 0.05$). Values are presented in gg^{-1}.

3.3. Foaming properties

Foam capacities and stabilities of African locust bean protein isolate, prepared in salts of different concentrations, are presented in Figs. 2 and 3, respectively. Both foaming capacity and stability were better in chaotropic salts than in kosmotropic salts. Except in protein solutions prepared in 0.1 and 1.0 M NaSCN and $NaCO_4$ where equal values of foam capacities were observed, maximal foam capacities in all other salts were observed in protein solutions prepared at 0.1 M concentration and these decreased progressively as the concentration increased. Chaotropic salts facilitate protein denaturation by altering the hydrophobic interactions, which stabilise protein structures. This process minimises the hydrophobic effect, which ordinarily prevents interaction of non-polar moieties in protein structure with aqueous solutions. In the presence of chaotropic anions, non-polar moieties are exposed and protein solubility improves. Increase in protein solubility facilitated

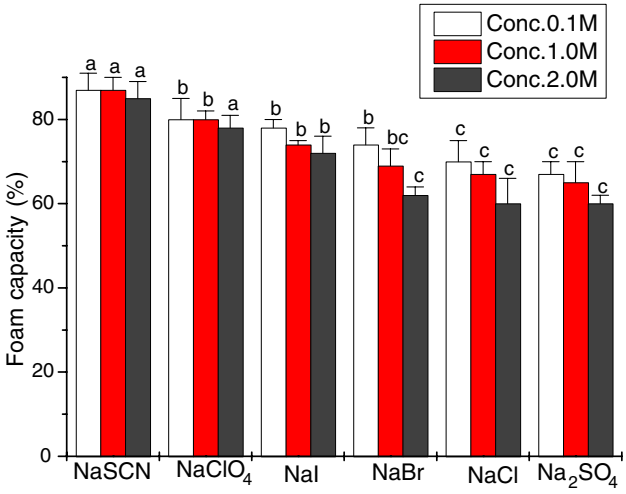

Fig. 2. Foam capacities of African locust bean protein isolate prepared in salts of different concentrations. Error bars: standard deviations. Values followed by the same letter at the same concentration are not significantly different ($P < 0.05$). Results are means of triplicate determinations.

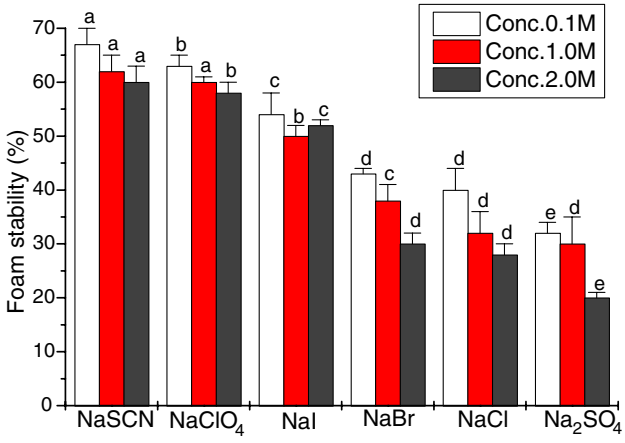

Fig. 3. Foam stabilities of African locust bean protein isolate prepared in salts of different concentrations. Error bars: standard deviations. Values followed by the same letter at the same concentration are not significantly different ($P < 0.05$). Results are means of triplicate determinations.

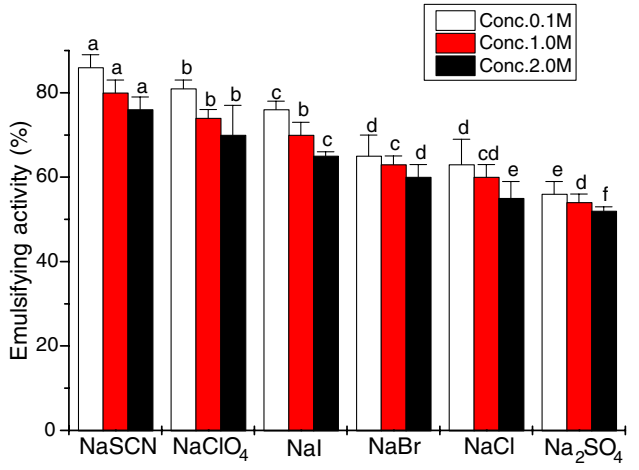

Fig. 4. Emulsifying activities of African locust bean protein isolate prepared in salts of different concentrations. Error bars: standard deviations. Values followed by the same letter at the same concentration are not significantly different ($P < 0.05$). Results are means of triplicate determinations.

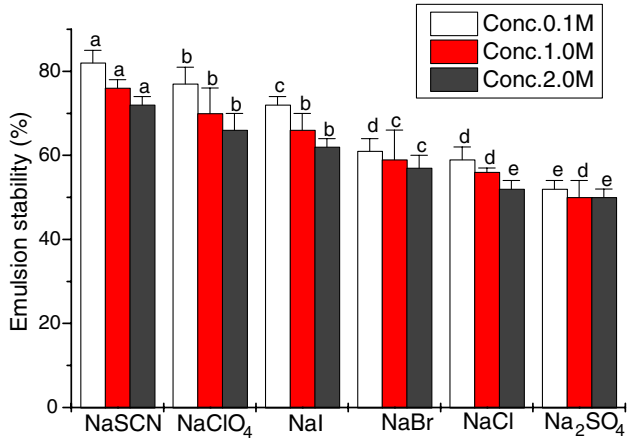

Fig. 5. Emulsion stabilities of African locust bean protein isolate prepared in salts of different concentrations. Error bars: standard deviations. Values followed by the same letter at the same concentration are not significantly different ($P < 0.05$). Results are means of triplicate determinations.

improved foam capacity in chaotropic salts compared with the kosmotropic salts. Increased protein solubility also enhanced better foam stability as a result of increase in protein concentration at the air water interface, which is a vital tool in limiting protein foam coalescence. Similar reasoning has been advanced on the influence of altered solvent environment on the foaming properties of pigeon pea (Mwasaru et al., 2000).

3.4. Emulsifying properties

Emulsifying activities and emulsion stabilities are presented in Figs. 4 and 5, respectively. The results indicate that emulsifying activity and stability were reduced as the concentration of salts in each protein solution increased. It was also observed that, among the various protein solutions, maximal emulsion activity and stability were observed in protein solutions prepared in NaSCN and protein solution prepared in $NaSO_4$ had the lowest emulsifying properties. In addition, generally, chaotropic salts had better emulsifying properties than did kosmotropic salts and the emulsifying potentials increased according to the Hofmeister series. Dissolution of chaotropic anions (SCN^-, ClO_4^-, I^-) in water is usually associated with large positive entropies (Hatefi & Hanstein, 1969). In this process, water structure is broken down, or melted, or depolymerized as compared to ordinary water. Consequently, water gets more

disordered and lipophilic in the presence of these anions, thus improving water–oil emulsions. It is also reasonable that enhanced protein solubility in chaotropic salts solutions, as stated earlier, facilitated improved emulsifying properties, as increase in protein concentrations enhanced surfactant properties and reduced oil–water interfacial tensions.

3.5. Gelation properties

Gelation properties of the protein isolate are presented in Fig. 6. Using least gelation concentration (LGC) as the index of gelation, the result indicates that least gelation concentration increased as the concentration of salts increased and LGC values were lower in chaotropic anions than in kosmotropic anions. The sequence of increase in LGC at various salt concentrations, followed the Hofmeister series.

Protein gels are formed by intermolecular interactions, which produce a continuous, three-dimensional network exhibiting structural rigidity. Gelation of a protein is directly dependent on its solubility. At low concentrations of salt, solubilities of proteins usually increase slightly (salting in). But at high concentrations of salt, the solubility of the proteins drop sharply (salting out). Initial salting at low concentrations is explained by the Debye–Huckel theory. At low salt concentrations, proteins are surrounded by the salt counter ions (ions of opposite net charge) and this screening results in decreasing electrostatic free energy of the protein and increasing activity of the solvent which, in turn, leads to increasing solubility. But, at high concentrations, the abundance of the salt ions decreases the solvating power of the solvent and the solubilities of proteins decrease. This is as a result of neutralization of charges on the protein molecules by the salt ions and subsequent reduction in electrostatic repulsion. In previous works, Akintayo et al. (1999), reported increase in least gelation concentration for pigeon pea, when the ionic strength of the medium was increased from 0.5 to 1.0. Otte, Schumacher, Ipsen, Ju, and Qvist (1999) reported reduction of gel firmness of whey proteins when the NaCl content of the mixture was increased. In a similar development, Castimpoolas and Meyer (1970) also reported a reduction in gelation properties of soybean globulins in solutions of high ionic strength.

When chaotropic salts cause protein denaturation, the resultant effect is the exposure of the buried functional groups which facilitate its native conformation. However, upon exposure, these functional groups are used in building the intermolecular three-dimensional network necessary for protein gel formation. A reversal of the process occurs in the presence of kosmotropic salts, and this explains increases observed in LGC of protein solutions prepared in kosmotropic salts.

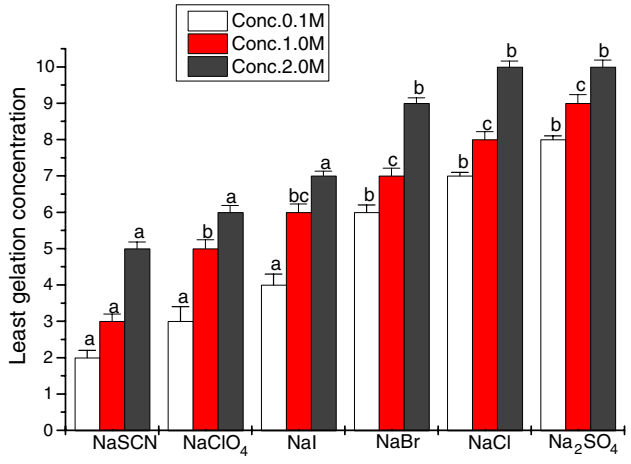

Fig. 6. Least gelation concentration of African locust bean protein isolate prepared in salts of different concentrations. Error bars: standard deviations. Values followed by the same letter at the same concentration are not significantly different ($P < 0.05$). Results are means of triplicate determinations.

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