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FULL PAPER

Effects of lyotropic series salts on the functional properties of bambara groundnut (*Voandzeia subterranean*) protein isolate

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Abstract

Lyotropic series salts have profound effects on the stability and the biochemical equilibrium of proteins colloids. The influence of kosmotropic and chaotropic salts on some functional properties of a protein isolate prepared from *Voandzeia subterranean* was investigated. Experimental results showed that the foaming, emulsifying and gelation properties were better in dispersions prepared with chaotropic salts (NaI, $NaClO_4$ and NaSCN) than those prepared with kosmotropic salts (Na_2SO_4, NaCl and NaBr). However, kosmotropic salts improved water holding capacity more than the chaotropic salts. Increase in the water holding capacity followed the lyotropic or Hofmeister trend: NaSCN<$NaClO_4$<NaI,<NaBr<NaCl<Na_2SO_4 while the reverse was observed in foaming, emulsifying and gelation properties. This study provides relevant information in understanding the bio-physical behaviour of the seed protein colloids in chaotropic or kosmotropic solutions.

1. Introduction

Studies on the hydrodynamics of protein molecules in a three-component system like protein-water-salt have gained a lot of attention in recent years. Salt solutions have remarkable effects on the structure and functional properties of hydrocolloids (Yin *et al*., 2008). These effects are sensitive to the nature of the salt which may be chaotropic or kosmotropic. Kosmotropic salts stabilize the structure of proteins while chaotropic salts destabilize protein structures. These terms were later extended to the apparently correlating property of increasing or decreasing, respectively, the structuring of water. Chaotropes interrupt the hydrogen-bonded network of water, allowing macro-molecules 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 and Uedaira, 2001).

The stabilizing or destabilizing effects of salts on proteins generally follows the lyotropic or Hofmeister series which is a qualitative ordering of ions based originally on their propensity to salt out proteins from aqueous solutions (Kunz *et al*., 2004). Generally, the ranking of the ions in descending order of effectiveness in driving processes such as folding, subunit assembly, crystallization and precipitation of protein is as follows; anions: SO_4^{2-}> $H_2PO_4^-$> CH_3COO^-> Cl^-> Br^-> I^-> ClO_4^-> SCN^- and cations: NH_4^+ > Cs^+ > K^+ > Na^+ > Li^+ > Mg^{2+} > Ca^{2+} > Ba^{2+}

The Hofmeister phenomenon is more pronounced for anions than cations. In homogeneous protein systems, the predominant attractive forces linking protein molecules are short-range hydrogen bonding, hydrophobic, electrostatic and van der Waals interactions (Halasz and Lasztity, 2017). Salts such as Na_2SO_4, NaCl, NaBr, NaI, $NaClO_4$ and NaSCN affect the physicochemical properties and interactions between proteins by their concentration and the ionic strength effects they have on charged groups of the protein molecules. Previous studies have shown that the two effects of salts make major contributions to the structure-

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stabilizing properties of the proteins, and these are their effects on solvent (water) structure and electrostatic interactions with the charged groups of the protein (Sarabia *et al*., 2000; Li *et al*., 2013). Ion effect on protein stability is assumed to be employed through two operative forces: (i) surface tension and (ii) preferential hydration. The net free energy of stabilization, $\delta(\Delta G^o)_{stability}$ (Equation (1)) depends on the change in cavity formation, $\delta(\Delta G^o)_{cavitation}$ (surface tension effect) and the free energy contribution from protein-solvent interaction, ($\delta(\Delta G^o)_{salvation}$ (hydration).

$$\delta(\Delta G^o)_{stability} = \delta(\Delta G^o)_{cavitation} + \delta(\Delta G^o)_{solvation} \quad (1)$$

The measure of the efficiency of different ions in affecting proteins in solutions depends on a combination of free energy of cavitation, which is positive (favourable) for a solvent with increased surface tension and free energy of solvation which depends upon the nature of the protein-solvent interaction (Breslow and Guo, 1990). Hofmeister salt effects on proteins were attributed to the favourability of cavity formation for a cosolute ion in the protein solvation shell. It was demonstrated that the surface tension increment σ of salt can give a quantitative amount of the measure of the salting-in and salting-out effects of electrolytes on proteins. The surface tension increment σ is defined from Equation (2):

$$\gamma = \gamma_o + \sigma m \quad (2)$$

where γ is the solution surface tension and m is the salt concentration.

In addition, a previous study posited that the surface charge density of ions correlates well with the stability of nucleic acid triplexes and it was suggested that the same correlation would apply to protein stability. The position of ions in the Hofmeister ranking correlates with the Jones-Dole equation (Equation 3), which establishes a relationship between these salt-solvent effects and the viscosity of the salt solution (η) with salt concentration c, relative to the viscosity of water (η_o):

$$\frac{\eta}{\eta_o} = 1 + A\sqrt{c} + Bc + Dc^2 \quad (3)$$

The coefficient A is an indication of the strength of electrostatic interaction of the salt in solution while the B -viscosity coefficient indicates ion – solvent interactions. The term D is only needed at very high salt concentrations. $A\sqrt{c}$ represents the electrostatic Debye – Hückel effects. Chaotropic ions have negative B-viscosity coefficients which make them deactivate proteins, while kosmotropic ions have positive B-viscosity coefficients and are expected to be stabilizers. In addition, preferential interactions of cosolutes with the protein surface have been suggested for the kosmotropic and chaotropic effects (Lin and Timasheff, 1996).

In previous work, the effects of anions on thermally induced whey protein isolate were reported and findings revealed that monovalent salts have a universal effect on the fracture properties of thermally induced whey protein isolate gels regardless of anion valence or Hofmeister effects (Bowland and Foegeding, 1995). The effect of the Hofmeister series on gluten aggregation and strength have been reported and the findings have shown that aggregation time followed the order of the Hofmeister series, with minor effects at salt concentration <0.3 M and increasing differences at higher salt concentrations (Melnyk *et al*., 2011). The effects of Hofmeister series salts on emulsions, foams and gels of jack bean protein has been reported and findings have revealed that functional properties of the protein isolate were significantly affected by the Hofmeister salts (Lawal, 2005). The functional properties of native and chemically modified *Voandzeia subterranean* concentrates have been reported and studies showed that functional properties such as foaming, emulsifying and gelation properties were dependent on the temperature, pH and ionic strength of the solutions (Lawal *et al*., 2007).

The applications of seed proteins in food systems or any other relevant application depend extensively on their functional properties which are controlled by the forces linking protein molecules. These forces influence functional properties such as solubility, foaming, emulsifying, and gelation capacity. Seed proteins are very important for diverse applications and the understanding of the forces in an aqueous solution that controls their physico-chemical processes is crucial in fields such as food processing, process design in the chemical industry, drug design in the pharmaceutical industry and the modelling of biological processes. Plant protein isolates have vital applications particularly as additives in food systems (Bainy *et al*., 2010). Isolation and characterization of some seed proteins from lesser-known sources have been reported in the literature. They include mucuna (Adebowale and Lawal, 2003; Lawal and Adebowale, 2004), African locust bean (Lawal, 2004; Lawal *et al*., 2005), Lablab bean (Lawal, 2005), broad bean (Arogundade *et al*., 2006), Jack bean (Lawal and Adebowale, 2006), bayberry (Cheng *et al*., 2009), kidney bean (Yin *et al*., 2008), and field pea (Adebiyi and Aluko, 2011). In the present investigation, seed protein was isolated from *Voandzeia subterranean* also known as bambara groundnut. *Voandzeia subterranean is* an underutilized legume (Adebowale *et al*., 2002), it is widely cultivated throughout tropical Africa, India, Sri Lanka, Indonesia and Malaysia (Hillocks *et al*., 2011). Being underutilized, the functional properties of *Voandzeia subterranean* seed proteins are not well known. This study would provide an understanding of

the functional properties of the *Voandzeia subterranean* seed protein under the influence of kosmotropic and chaotropic anions. This is the first report on the effect of cosolutes or additives in the aqueous colloidal system of *Voandzeia subterranean* seed protein and the findings are important for its applications.

2. Materials and methods

2.1 Materials and reagents

Voandzeia subterranean seeds were obtained from Bodija Market, Ibadan, Nigeria. Na_2SO_4, NaCl, NaBr, NaI, $NaClO_4$, NaSCN and NaOH, all analytical grades were purchased from Sigma-Aldrich (St. Louis, MO). The average yield of protein isolate from the flour was 28.4%, whereas, the percentage protein content of the protein isolate was 92.2%. These were determined using the Kjeldahl method. Crude nitrogen of the samples was determined in triplicate by the standard micro-Kjeldahl method then converted to protein content using a 6.25 conversion factor (AOAC, 1985).

2.2 Preparation of protein isolate

Protein isolates were prepared using the method previously described by Lawal *et al*. (2007). Alkaline solubilisation was used because the process facilitates higher yield based on preliminary studies. *Voandzeia subterranean* seeds were dehulled manually after soaking for 3 hrs. It was air-dried for 48 hrs at room temperature (30±2°C), and then dry-milled to fine powders before sieving using 75 µm sieve. Flour (1 kg) was dispersed in distilled water (10 L) and the pH was adjusted to 8.0 with 1 mol/L NaOH, to facilitate protein solubilisation. It was stirred at a stirring speed of 250 rpm for 4 hrs at 30±2°C. The pH of the supernatant obtained after centrifuging at 4000×*g* (Type GLC-1 Ivan Sorvall Inc., USA) for 30 mins was adjusted from 8.0 to 4.0 with 0.5 mol/L HCl to precipitate the protein isolate, which was recovered by centrifugation at 5000 *g* for 30 mins. The protein isolates in cake form were dried with a freeze dryer.

2.3 Foaming capacity and foam stability

Foaming capacity and foam stability were studied using the method of Akintayo *et al*. (1999). The protein isolate (2 g) was dispersed in 100 mL of 0.1, 1.0 and 2.0 mol/L solutions of Na_2SO_4, NaCl, NaBr, NaI, $NaClO_4$ and NaSCN. The solutions were whipped vigorously for 2 min using Phillips kitchen blender. The volumes of the solutions were recorded before and after whipping. The percentage volume increase, which serves as the index of foam capacity, was calculated using Equation 4.

$$Volume\ change\ (\%) = \frac{V_2 - V_1}{V_1} \times 100 \quad (4)$$

Where V_1 is the volume of solution before whipping; V_2 is the volume of protein solution after whipping. The volume of foam that remained after 8 hrs standing (30±2°C) was expressed as the percentage of the initial volume for the foam stability.

2.4 Emulsifying properties

Emulsifying properties were determined using the method of Neto *et al.* (2001). Protein solution (5 mL, 10% w/v) prepared in 0.1, 1.0 and 2.0 mol/L solutions of Na_2SO_4, NaCl, NaBr, NaI, $NaClO_4$ and NaSCN were homogenized with 5 mL of corn oil (Executive Chef Unilever, Lagos, Nigeria) for 1 min using Vari-Whirl mixer set at speed 2 (A901, Salver Chem, Chicago, IL, USA). Thereafter, the emulsions were centrifuged at 1100×*g* for 5 mins. The height of the emulsified layer and that of the total contents in the tube was measured. The emulsifying activity (EA) was calculated using Equation 5.

$$EA(\%) = \frac{Height\ of\ emulsified\ layer\ in\ the\ tube}{Height\ of\ the\ total\ content\ in\ the\ tube} \times 100 \quad (5)$$

The emulsion stability (ES) was determined by heating the emulsion at 80°C for 30 mins before centrifuging at 1100×*g* for 5 mins and was calculated using Equation 6.

$$ES(\%) = \frac{Height\ of\ emulsified\ layer\ in\ the\ tube}{Height\ of\ emulsified\ layer\ before\ heating} \times 100 \quad (6)$$

2.5 Gelation properties

Gelation properties were studied using the method of Akintayo *et al.* (1999). Protein sample suspensions of 2 – 20% w/v were prepared in 0.1, 1.0 and 2.0 mol/L solutions of Na_2SO_4, NaCl, NaBr, NaI, $NaClO_4$ and NaSCN. A 10 mL volume of each of the prepared dispersion was transferred into a test tube, which was heated in a boiling water – bath for 1 hr, followed by rapid cooling in a bath of cold water. The test tubes were cooled further at 4°C for 2 hrs. The least gelation concentration was taken as the concentration when the sample from the inverted tube did not fall or slip.

2.6 Water holding capacity

The water holding capacity was determined using the method of Lawal (2004). Protein concentrate sample (1 g) was mixed with 10 mL of 0.1, 1.0 and 2.0 mol/L solutions of Na_2SO_4, NaCl, NaBr, NaI, $NaClO_4$ and NaSCN for 30 s. The samples were allowed to stand at room temperature (30±2°C) for 30 mins before centrifuging at 5000×*g* for 30 mins. The volume of the supernatant obtained after centrifugation was determined in a 10 mL graduated cylinder. Water holding capacity was calculated per gram of total protein.

2.7 Statistical analysis

Measurements were carried out in triplicates and the

mean values with their standard deviations are reported. Analysis of variance (ANOVA) was used 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 Foaming properties

Foaming capacities and foam stabilities of *Voandzeia subterranean* protein isolate studied in the presence of Hofmeister ions and at different concentrations are presented in Figures 1 and 2, respectively. The results indicated that both foaming capacity and foam stability reduced as the ionic strength of the solution increased and the reductions in the values of foaming capacity and foam stability were significant at all ionic strengths ($p<0.05$). The highest foaming capacity (86.08%) and foam stability (78.1%) were recorded in protein solutions prepared with NaSCN (0.1 mol/L). The chaotropic salts (NaI, $NaClO_4$ and NaSCN) significantly ($p<0.05$) increased the foaming capacity and foam stability of the protein isolates than the kosmotropic salts (Na_2SO_4, NaCl and NaBr). The results also show that the increase in foaming capacity and foam stability followed the ranking in the Hofmeister series in the order: Na_2SO_4<NaCl<NaBr<NaI<$NaClO_4$<NaSCN. According to the Jones–Dole equation (Equation 3), salts with chaotropic properties have negative B-viscosity coefficients which make them deactivate proteins. It is reasonable that kosmotropes, with positive B-viscosity tend to improve the inter- and intramolecular structure-making interactions in aqueous solutions of protein while chaotropes weaken such interactions. In this sense, weakening the binding forces among the protein molecules could lead to increased flexibility among the macromolecules, thereby facilitating various changes in their kinetic behaviour as well. This enhanced flexibility accounts for the better foamability observed in protein solutions prepared with chaotropic salts. It has been reported that chaotropic salts may accelerate reactions of proteins by loosening their structure (Der and Ramsden, 1998; Zhao, 2016).

The instability of foams in colloids interfaces may be due to either foam coalescence or disproportionation. Coalescence involves the irreversible combination of two or more foam bubbles when the interfacial films drain and the bubbles are eventually ruptured to form a bigger bubble. Foam disproportionation is the process that involves the diffusion of air molecules through the disperse phase between bubbles. As a result of the increased Laplace pressure of smaller bubbles, the diffusion flux generally leads to the shrinking of smaller bubbles and the growth of larger gas bubbles, thereby leading to the instability of foams (Dickinson, 1992; Zhu *et al*., 2018). Generally, the denaturant action of Hofmeister ions among the chaotropes is due to the salting–in of the peptide groups and the improved interactions with the unfolded form of the protein (Kang *et al*., 2020). This process facilitates protein solubility and increases the concentration of the protein at the air-water interface which improves foam stability. The results also indicate that both foaming capacity and stability reduced as the ionic strength of the solution increased which is consistent with a report on the foaming properties of broad bean protein isolate (Arogundade *et al*., 2006).

3.2 Emulsifying properties

Both EA (Figure 3) and ES (Figure 4) showed ion specificity and were better in protein solutions in chaotropic environments than in kosmotropic environments. Accordingly, both the EA and ES reduced significantly ($p<0.05$) with an increase in salt concentration. Similarly, the increase in EA and ES followed the Hofmeister trend: Na_2SO_4<NaCl<NaBr<NaI<$NaClO_4$<NaSCN although the results obtained in Na_2SO_4 and NaCl solutions were not significantly different at all ionic strengths for EA and ES ($p<0.05$). An emulsion is a heterogeneous system of at least two immiscible liquid phases, one of which is dispersed in the other, in the form of droplets (Sadoc and Rivier, 2013; Tadros, 2016). Proteins act as a surfactant in many food emulsions diffusing to and being adsorbed at the oil-water interface. The migration of the protein

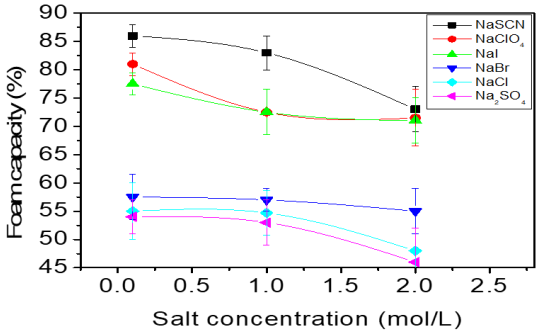

Figure 1. Foaming capacities of *Voandzeia subterranean* protein isolates

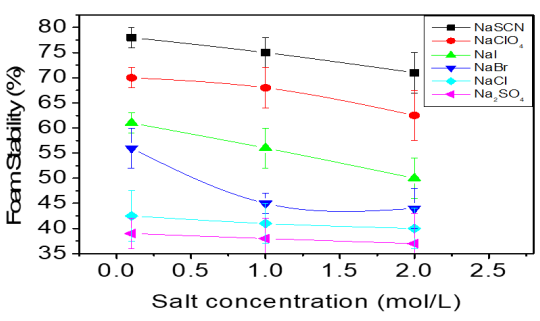

Figure 2. Foaming stabilities of *Voandzeia subterranean* protein isolates

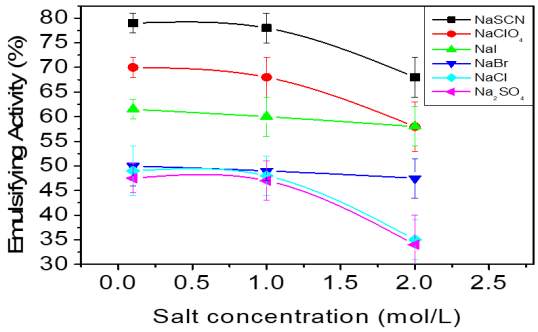

Figure 3. Emulsifying activities of *Voandzeia subterranean* protein isolates

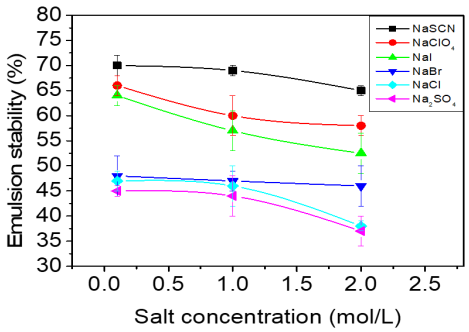

Figure 4. Emulsifying stability of *Voandzeia subterranean* protein isolates

molecules from solution to the interface is thermodynamically favourable because the conformational and hydrational energy of the protein decreases at the interface (Kulmyrzaev and Schubert, 2004). Chaotropic anions improved emulsifying activities because, in water, they are usually associated with large positive entropies (Damodaran, 1990; Hu *et al.*, 2014), leading to the de-structuring of water molecules. Consequently, water gets more disordered and lipophilic in the presence of these anions, thus improving water-oil emulsions. In addition, the processes of the protein unfolding caused by protein denaturation facilitated the hydrophobic hydration which enhanced emulsifying activity. Emulsion systems are usually stabilized through physical entrapment of fat globules within the protein matrices largely via protein-protein interactions followed by the formation of an interfacial protein film that surrounds and stabilizes fat globules (Barbut, 1995; Su *et al*., 2000). The processes which cause emulsion instability are creaming, flocculation, coalescence and oiling off (Benhura and Chidewe, 2004). Low protein concentration often causes limited protein availability at the oil-water interface thereby causing flocculation and instability of emulsions. The extent of flocculation is dependent on the structure of the adsorbed layer and the thermodynamic quality of the intervening solvent. If the stabilizing film at the oil-water interface ruptures, this leads to coalescence, making the oil droplets merge into larger spherical globules. In creaming, oil rises in an oil-in-water emulsion (O/W) and this occurs when the density of oil droplets is less than that of the continuous phase. In this sense, improved ES in chaotropic protein solutions is a result of increased protein solubility which led to a higher concentration of protein in the oil-water interface.

3.3 Gelation properties

The gelation properties of *Voandzeia subterranean* protein isolates under various lyotropic salt concentrations are presented in Figure 5. Using the least gelation concentration (LGC) as the index of gelation. At various salt concentrations, the lowest LGCs were observed in protein solutions prepared in chaotropic salts. Progressive and significant ($p<0.05$) increase in LGC were observed as the ionic strength of the solution increased, following the Hofmeister trend: Na_2SO_4 > NaCl > NaBr > NaI > $NaClO_4$ > NaSCN. A gel is a continuous network of macroscopic dimensions immersed in a liquid medium exhibiting no steady-state flow. In gelation, a three-dimensional network is developed by progressive aggregation of macromolecules or particles through chemical bonds or physical interactions under suitable conditions. The following processes occur in the heat-induced gelation process: Protein unfolding, water binding, protein-protein interactions and water immobilization. Low salt concentration (0.1 mol/L) enhanced gelation better than higher salt concentrations (1.0 and 2.0 mol/L). At low salt concentrations, charge screening of protein molecules occurs as a result of the presence of counter ions thereby leading to decreasing electrostatic free energy of the protein. These processes may increase the activity of the solvent and protein solubility. However, when the salt concentration is high, the abundance of the salt ions reduces the solvating power of the solvent and this may reduce the protein solubility. In this sense, the reduction in protein solubility at higher salt concentration is as a result of the neutralization of charges on the protein molecules by the ions, which lead to weaker gel formation. Reduction in gel firmness of whey protein when the NaCl content of the mixture was increased has been reported (Otte *et al*., 1999). Similarly, an increase in the least gelation concentration has been reported for pigeon pea after the ionic strength of the medium was increased from 0.5 mol/L to 1.0 mol/L (Akintayo *et al*., 1999). These findings are also consistent with previous reports on soy protein (Li *et al*., 2007; Li *et al*., 2009). The denaturant action of chaotropic anions is due to the fact that they salt-in the peptide group and consequently they interact much more strongly with the unfolded form of a protein than with its native form, thereby pulling the protein unfolding reaction. It is reasonable that the enhanced gelation in protein solutions prepared with chaotropic salts could be attributed to an increase in the protein unfolding which allowed more effective networking among the protein

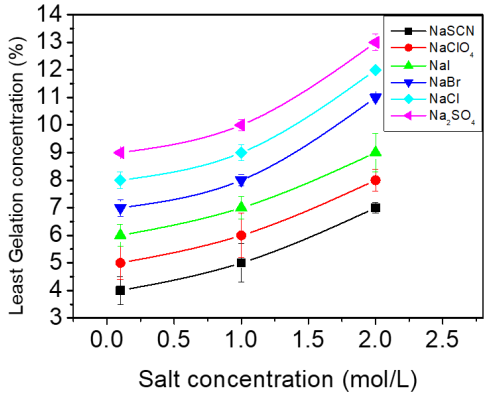

Figure 5. Gelation properties of *Voandzeia subterranean* protein isolates

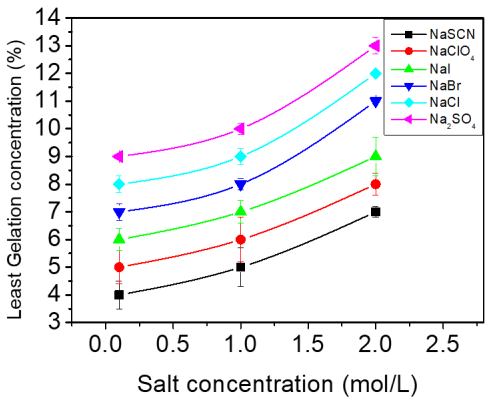

Figure 6. Water holding capacity of *Voandzeia subterranean* protein isolates

molecules for gel development.

3.4 Water holding capacity

The water holding capacities of *Voandzeia subterranean* protein isolates obtained at various ionic strengths of Hofmeister salts are presented in Figure 6. In the dispersions prepared in 0.1 mol/L salt solutions, the highest water holding capacity (9.7 g/g) was observed in the solution of Na_2SO_4 while the least was recorded in NaSCN solution. Generally, water holding capacity reduced progressively and significantly ($p<0.05$) as the concentration of the ions increased. This observation was consistent for both the chaotropic and the kosmotropic salts.

The results also indicate that kosmotropic salts facilitated improved water holding capacities over the chaotropic salts and generally, the reduction in the water holding capacity followed the Hofmeister trend: Na_2SO_4 > NaCl > NaBr > NaI > $NaClO_4$ > NaSCN. Kosmotropic salts facilitate water structure stabilization while the reverse is observed with chaotropic salts (Uedaira and Uedaira, 2001; Bye *et al.*, 2014). It has also been reported that direct ion binding to the peptide backbone by kosmotropic ions may facilitate structural stabilization while the chaotropic ions cause destabilization (Sarabia *et al.*, 2000). As indicated in Equation 2, an increase in surface tension (γ) is enhanced by an increase in salt concentration (m). It is reasonable that the reductions in water holding capacity could be due to an increase in surface tension as the salt concentration increased. This is consistent with a previous report on the increase in surface tension with salt concentration (Persson *et al.*, 2003; Jawerth *et al.*, 2018). In addition, as water is more structured in the presence of kosmotropes, this may act as a driving force for its absorption by the protein molecules. Chaotropes may be considered as "sticky" ions with weakly structured water surrounding them, driven to non-polar surface and this explains the weaker water holding observed with the chaotropes.

4. Conclusion

Studies on the effect of various lyotropic salts and their concentrations on the functional properties of protein isolates of an underutilized but rich protein resource of *Voandzeia subterranean* is presented. The studies provide information about the influences of the various kosmotropic and chaotropic anions on the protein colloids which may be relevant in diverse applications such as food and pharmaceutical industries. Protein colloids prepared in chaotropic salts solutions (NaI, $NaClO_4$ and NaSCN) exhibited better foaming, emulsifying and gelling properties than in kosmotropic salts solutions (Na_2SO_4, NaCl, NaBr). However, water holding capacities were higher in protein dispersions in kosmotropic salts solutions than in chaotropic salts solutions. Findings reveal that *Voandzeia subterranean* protein isolates may be formulated for specific end – uses by adjusting its co-additives. Being an underutilized seed protein resource, that is readily available in developing countries, it is expected that the results presented here would facilitate its applications in several industries where protein colloids are relevant. The salts used in this study have been used to understand the chemistry of *Voandzeia subterranean* protein isolates in food systems. Therefore, it is appropriate that food safety and regulatory standards should be consulted for the use of chemicals in food systems.

Conflict of interest

The authors declare no conflict of interest.

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References

Adebiyi, P.A. and Aluko, R.E. (2011). Functional properties of protein fractions obtained from commercial yellow field pea (*Pisum sativum* L.) seed protein isolate. *Food Chemistry*, 128(4), 902-908. https://doi.org/10.1016/j.foodchem.2011.03.116

Adebowale, K.O., Afolabi, T.A. and Lawal, O.S. (2002). Isolation, chemical modification and physicochemical characterisation of bambara groundnut (V*oandzeia subterranean*) starch and flour. *Food Chemistry*, 78(3), 305-311. https://doi.org/10.1016/S0308-8146(02)00100-0

Adebowale, K.O. and Lawal, O.S. (2003). Foaming, gelation and electrophoretic characteristics of mucuna bean (*Mucuna pruriens*) protein concentrates. *Food Chemistry*, 83(2), 237-246. https://doi.org/10.1016/S0308-8146(03)00086-4

Akintayo, E.T., Oshodi, A.A. and Esuoso, K.O. (1999). Protein isolates from Chick pea (*Cicer arietinum* L). Chemical composition, functional properties and characterization. *Food Chemistry*, 66(2), 51-56. https://doi.org/10.1016/S0308-8146(98)00155-1

AOAC (1985). Official methods of analysis. 14th ed. Washington DC, USA: Association of Official Analytical Chemists.

Arogundade, L.A., Tshay, M., Shumey, D. and Manazie, S. (2006). Effect of ionic strength and/or pH on Extractability and physico-functional characterization of broad bean (*Vicia faba* L.) protein concentrate. *Food Hydrocolloids*, 20(8), 1124-1134. https://doi.org/10.1016/j.foodhyd.2005.12.010

Bainy, E.M., Corredig, M., Poysa, V., Woodrow, L. and Tosh, S. (2010). Assessment of the effects of soy protein isolates with different compositions on gluten thermosetting gelation. *Food Research International*, 43(6), 1684-1691. https://doi.org/10.1016/j.foodres.2010.05.010

Barbut, S. (1995). Importance of fat emulsifications and protein matrix characteristics in meat batter stability. *Journal of Muscle Foods*, 6(2), 161-177. https://doi.org/10.1111/j.1745-4573.1995.tb00564.x

Benhura, M.A.N. and Chidewe, C.K. (2004). The emulsifying properties of a polysaccharide isolated from the fruit of *Cordia abyssinica*. *International Journal of Food Science and Technology*, 39(5), 579-583. https://doi.org/10.1111/j.1365-2621.2004.00809.x

Bowland, E.L. and Foegeding E.A. (1995). Effects of anions on thermally induced whey protein isolate gels. *Food Hydrocolloids*, 9(1), 47-56. https://doi.org/10.1016/S0268-005X(09)80193-8

Breslow, R. and Guo, T. (1990). Surface tension measurements show that chaotropic salting-in denaturants are not just water-structure breakers. *Proceedings of the National Academy of Sciences of the United States of America*, 87(1), 167-169. https://doi.org/10.1073/pnas.87.1.167

Bye, J.W., Platts, L. and Falconer, R.J. (2014). Biopharmaceutical liquids formulation: a review of the science of protein stability and solubility in aqueous environments. *Biotechnology Letters*, 36(5), 869-875. https://doi.org/10.1007/s10529-013-1445-6

Cheng, J., Zhou, S., Wu, D., Chen, J., Liu, D. and Ye, X. (2009). Bayberry (*Myrica rubra* kernel: A new protein source. *Food Chemistry*, 112(2), 469-473. https://doi.org/10.1016/j.foodchem.2008.05.106

Damodaran, S. (1990). Interfaces, protein films and foams. *Advances in Food Nutrition Research,* 34, 1-79. https://doi.org/10.1016/S1043-4526(08)60006-6

Der, A. and Ramsden, J.J. (1998). Evidence for loosening of a protein mechanism. *Naturwisenschaften*, 85, 353-355. https://doi.org/10.1007/s001140050515

Dickinson, E. (1992). An introduction to food colloids. Oxford, United Kingdom: Oxford University Press.

Halasz, A. and Lasztity, R. (2017). Use of biomass in food production. Boston, USA: CRC press. https://doi.org/10.1201/9780203734551

Hillocks, R.J., Bennett, C. and Mponda, O.M. (2011) Bambara nut: A review of utilisation, market potential and crop improvement. *African Crop Science Journal*, 20(1), 1-16.

Hu, Y., Yang, J., Jia, R., Ding, Y., Li, S. and Huang, H. (2014). Chemical modification with functionalized ionic liquids: a novel method to improve the enzymatic properties of Candida rugosa lipase. *Bioprocess and Biosystems Engineering*, 37(8), 1617-1626. https://doi.org/10.1007/s00449-014-1134-4

Jawerth, L.M., Ijavi, M., Ruer, M., Saha, S., Jahnel, M., Hyman, A.A., Julicher, F. and Fischer-Friedrich, E. (2018). Salt-dependent rheology and surface tension of protein condensates using optical traps. *Physical Review Letters*, 121(25), 258101. https://doi.org/10.1103/PhysRevLett.121.258101

Kang, B., Tang, H., Zhao, Z. and Song, S. (2020). Hofmeister series: Insights of ion specificity from amphiphilic assembly and interface property. *ACS Omega*, 5(12), 6229-6239. https://doi.org/10.1021/acsomega.0c00237

Kulmyrzaev, A.A. and Schubert, H. (2004). Influence of KCl on the physicochemical properties of whey protein stabilized emulsions. *Food Hydrocolloids*, 18(1), 13-19. https://doi.org/10.1016/S0268-005X(03)

00037-7

Kunz, W., Nostro, P.L. and Ninham, B.W. (2004). The present state of affairs with Hofmeister effects. *Current Opinion in Colloid and Interface Science*, 9 (1-2), 1-18. https://doi.org/10.1016/j.cocis.2004.05.004

Lawal, O.S. (2004). Functionality of African locust bean (*Parkia biglobosa*) protein isolate: Effect of pH, ionic strength and various protein concentrations. *Food Chemistry*, 86(3), 345-355. https://doi.org/10.1016/j.foodchem.2003.09.036

Lawal, O.S. (2005). Functionality of native and succinylated Lablab bean (*Lablab purpureus*) protein concentrate. *Food Hydrocolloids*, 19(1), 63-72. https://doi.org/10.1016/j.foodhyd.2004.04.015

Lawal, O.S., Adebowale, K.O. and Adebowale, Y.A. (2007). Functional properties of native and chemically modified protein concentrates from bambara groundnut. *Food Research International*, 40(8), 1003-1011. https://doi.org/10.1016/j.foodres.2007.05.011

Lawal, O.S., Adebowale, K.O., Ogunsanwo, B.M., Sosanwo, O.A. and Bankole, S.A. (2005). Functional properties of Globulin and Albumin protein fractions and flours of African locust bean *Parkia biglobosa*. *Food Chemistry*, 92(4), 681-691. https://doi.org/10.1016/j.foodchem.2004.08.043

Lawal, O.S. and Adebowale, K.O. (2004). Effect of Acetylation and succinylation on solubility profile, water absorption capacity, oil absorption capacity and emulsifying properties of Mucuna bean (*Mucuna pruriens*) protein concentrate. *Nahrung/Food,* 48(2), 129-136. https://doi.org/10.1002/food.200300384

Lawal, O.S. and Adebowale, K.O. (2006). The acylated protein derivatives of *Canavalia ensiformis* (Jack bean). A study of functional characteristics at various protein concentrations, ionic strength and pH of solution. *LWT-Food Technology*, 39(8), 918-929. https://doi.org/10.1016/j.lwt.2005.06.016

Li, X., Cheng, Y., Yi, C., Hua, Y., Yang, C. and Cui, S. (2009). Effect of ionic strength on the heat-induced soy protein aggregation and the phase separation of soy protein aggregate/dextran mixtures. *Food Hydrocolloids*, 23(3), 1015-1023. https://doi.org/10.1016/j.foodhyd.2008.07.024

Li, X., Li, Y., Hua, Y., Qiu, A., Yang, C. and Cui S. (2007) Effect of concentration, ionic strength and freeze-drying on the heat-induced aggregation of soy proteins. *Food Chemistry*, 104(4), 1410-1417. https://doi.org/10.1016/j.foodchem.2007.02.003

Li, Z.R., Wang, B., Chi, C.F., Zhang, Q.H., Gong, Y.D. and Tang, J.J. (2013). Isolation and characterisation of acid soluble collagens and pepsin soluble collagens from the skin and bone of Spanish mackerel (*Scomberomorous* and *Niphonius*). *Food Hydrocolloids*, 31(1), 103-113. https://doi.org/10.1016/j.foodhyd.2012.10.001

Lin, T.Y. and Timasheff, S.N. (1996). On the role of surface tension in the stabilization of globular proteins. *Protein Science*, 5(2), 372-381. https://doi.org/10.1002/pro.5560050222

Melnyk, J.P., Dreisoerner, J., Bonomi, F., Marcone, M.F. and Koushik (2011). Effect of the Hofmeister series on gluten aggregation measured using a high shear based technique. *Food Research International*, 44 (4), 893-896. https://doi.org/10.1016/j.foodres.2011.01.053

Neto, V.Q., Narain, N., Silva, J.B. and Bora, P.S. (2001). Functional properties of raw and heat processed cashew nut (*Anarcardium occidentale*, L.) kernel protein isolate. *Nahrung/Food*, 45(4), 258-262. https://doi.org/10.1002/1521-3803(20010801)45:4<258::AID-FOOD258>3.0.CO;2-3

Otte, J., Schumacher, E., Ipsen, R., Ju, Z.Y. and Qvist, K.B. (1999). Protease induced gelation of unheated and heated whey proteins: effects of pH, temperature and concentration of protein, enzyme and salts. *International Dairy Journal*, 9(11), 801-812. https://doi.org/10.1016/S0958-6946(99)00151-X

Persson, C.M., Jonsson, A.P., Bergstrom, M. and Eriksson, J.S. (2003). Testing the Gouy-Chapman theory by means of surface tension measurements for SDS-NaCl-H_2O mixture. *Journal of Colloid and Interface Science*, 267(1), 151-154. https://doi.org/10.1016/S0021-9797(03)00761-6

Sadoc, J.F. and Rivier, N. (2013). Foams and emulsions, Vol. 354. United Kingdom: Springer Science and Business Media.

Sarabia, A.I., Gomez-Guillen, M.C. and Montero, P. (2000). The effects of added salts on the viscoelastic properties of fish skin gelatin. *Food Chemistry*, 70 (1), 71-76. https://doi.org/10.1016/S0308-8146(00)00073-X

Su, Y.K., Bowers, J.A. and Zayas, J.F. (2000). Physical characteristics and microstructure of reduced-fat frankfurters as affected by salt and emulsified fats stabilized with nonmeat proteins. *Journal of Food Science*, 65(1), 123-128. https://doi.org/10.1111/j.1365-2621.2000.tb15966.x

Tadros, T.F. (2016). Emulsion: Formation, stability, industrial applications. Boston, USA: Walter de Gruyter GmbH and Co KG. https://doi.org/10.1515/9783110452242

Uedaira, H. and Uedaira H. (2001). Role of hydration of

polyhydroxy compounds in biological systems. *Cell Molecular Biology*, 47(5), 823-829.

Yin, W., Zhang, H., Huang, L. and Nishinari K. (2008). Effects of the lyotropic series of salt on the gelation of konjac glucomannan in aqueous solutions. *Carbohydrate Polymers*, 74(1), 68-78. https://doi.org/10.1016/j.carbpol.2008.01.016

Zhao, H. (2016). Protein stabilization and enzyme activation in ionic liquids: specific ion effects. *Journal of Chemical Technology and Biotechnology*, 91(1), 25-50. https://doi.org/10.1002/jctb.4837

Zhu, X., Verzicco, R., Zhang, X. and Lohse, D. (2018). Diffusion interaction of multiple surface nanobubbles: shrinkage, growth, and coarsening. *Soft Matter*, 14(11), 2006-2014. https://doi.org/10.1039/C7SM02523H

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