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INFLUENCE OF pH, SOME CHLORIDE AND PHOSPHATES ON EMULSIFYING AND SOLUBILITY PROPERTIES OF CASHEW KERNEL *(Anacardium occidentale)* PROTEIN ISOLATE

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ABSTRACT

Effect of pH, NaCl, KCl, KH_2PO_4 or NaH_2PO_4 on solubility and emulsifying properties of cashew *(Anacardium occidentale L.)* protein isolate (CPI) were determined. The emulsifying properties of CPI were assessed turbidimetrically. Maximum soluble protein observed at pH 2 and pH 12 media were 62 and 95%, respectively. CPI solubility in NaCl, KCl, KH_2PO_4 or NaH_2PO_4 solutions were better than in H_2O. This solubility is salt concentration dependent. pH 5 gave a better emulsion activity {EA} than other pH values. Its emulsion appeared stable at pH 2, 11 or 12. CPI stabilized oil-in-water emulsion has longer shelf life at low chloride and phosphate concentrations (0.1 - 0.4mol/dm^3). EA of CPI in NaCl, KCl, NaH_2PO_4 or KH_2PO_4 showed positive correlation with solubility.

Keywords: Cashew kernel protein; phosphate; chloride; pH; solubility and emulsion properties.

INTRODUCTION

Oil - in - water emulsion can be stabilized in the food industries with protein as a surface active biopolymer. Proteins are adsorbed on to oil droplet surface during homogenization and form a membrane around the droplet thereby protecting them from coalescing (Kinsella and Whitehead, 1989; Morr and Ha, 1993; Dalgleish, 1996; Dickinson, 1997). The use of plant protein in food formulation in the food industries is gaining recognition as animal protein is in short supply. Pulses and oilseeds are protein -rich grains and their protein isolates may be exploited in the food industries e.g. soy bean protein isolate. Other oil seeds like Cashew nut which can serve the same purpose are underutilized in this respect.

Cashew nut *(Anacardium occidentale L.)* - a member of the *Anacardiaceae* is an energy – rich food with high fat (45-70%, w/w) and protein (20-25%, w/w). It enjoys worldwide acceptance and is valued for its sensory and nutritional attributes (Fetuga *et al*., 1974; Sathe, 1994). It is produced commercially in different countries in Asia, South America and Africa and has been rated as No.1 nut crop, since its production has surpassed that of almond by over 30,0000MT (FAO, 1998; Anonymous, 2007). Since protein is one of the major components of cashew nut kernel, it may be exploited as food functional ingredient.

The success of utilizing any seed protein as functional ingredients depends on their attributes and response to environmental factors or processing conditions like pH, ionic strength, (Myers, 1988; Nwanekezi et al., 1994; Aluko and Yada, 1995; Meng and Ma, 2002). Chloride salts and phosphates are acceptable food additives employed in meeting specific nutritional profile and/or to increase their shelf life (Fligner *et al.,* 1990 and 1991; Keowmaneechai and McClements, 2002; Rankumar *et al.,* 2000; Chang and Regenstein, 1997; Yapar *et al.,* 2005). Since amino acid constituents of protein which determine its functionality vary from one vegetable protein to the other. The effects of these substances on the functional performance of protein isolate depend on the protein under consideration (Kamat *et al.,* 1978; Mc Watters and Holmes, 1979; Shimizu *et al.,* 1985; Mitchell, 1989; Aluko and Yada 1995). This necessitated studies on individual vegetable protein functionality in the presence of different additives and pH.

Some studies on cashew kernel protein functional properties (*emulsifying, foaming, water and oil absorption properties*) had been reported (Arogba, 1999; Neto *et al.,* 2001), but the effect of phosphates employed in food industries has not been reported on cashew protein isolate emulsifying properties. This study determined the effect of phosphates and chlorides on cashew *(Anacardium occidentale* L.) protein isolate solubility, oil-in-water emulsion formation and stabilization.

MATERIALS AND METHODS

Sampling and Sample treatment

Cashew nut *(Anacardium occidentale)* seeds were obtained from Idere, Ogun State, Nigeria. The kernels were manually removed from the nut. The kernels were ground and extracted for 4 hours with *n*-hexane in a soxhlet apparatus. The hexane extracted meal residue was air-dried, re-ground to pass through 0.4mm sieve and stored in an airtight container as defatted cashew flour (DCF).

Preparation of Cashew Protein Isolate (CPI)

Cashew nut protein isolate was obtained by the method of Sanchez-Vioque *et al.* (1998) with modification. A suspension of DCF sample was made in distilled water at solid to solvent ratio 1:10. The mixture was stirred with magnetic stirrer for 10minutes and the pH adjusted to 8.0 with 1M NaOH and was further stirred for another 50minutes at room temperature. It was centrifuged at 3,500 rpm for 30 minutes. The resulting supernatant was adjusted to pH 4.5 with 1M HCI to precipitate the protein. The precipitate was separated by centrifugation, washed successively with distilled water at isoelectric point, ethanol and acetone. Each washing was followed by centrifugation and was dried at room temperature.

Protein Solubility

The solubility of the protein isolate (CPI) over a pH range of 2 to 12 was determined with 0.5% (w/v) dispersion in water. The dispersions were adjusted to desired pH with 1mol/dm^{3}HCI or 1mol/dm^{3}NaOH. Each suspension was shaken (225rpm) on orbital shaker (Stwart Scientific 501) at room temperature for lhr and centrifuged at 3500rpm for 30min. Effect of KCl, NaCl, KH_2PO_4 and NaH_2PO_4 in the concentration in a range of 0.1 - 15mol/dm^3 on the protein isolate was carried out as above. The soluble protein in the clear supernatant obtained in each case was determined spectrophotomet-

rically at 215 and 225nm (Aitken and Learmonth, 1996) in triplicates.

Emulsifying properties

The emulsifying activity (EA) was determined by the turbidimetrically (Pearce and Kinsella, 1978). An aqueous phase of 0.5% (w/v) CPI was prepared in the various media (water at pH range 2 - 12; chloride and phosphate solutions of 0 - 15M concentration). An oil - in - water emulsions were prepared by homogenizing 20cm^3 of supernatant and 20cm^3 of vegetable oil with kitchen blender. After homogenization, triplicate 50µl aliquots of each emulsion were diluted with 10cm^3 of 0.1 % sodium dodecyl sulfate (SDS) solution and the absorbance determined at 500nm on a Shimadzu UV-visible spectrophotometer (UV-1650PC) using the SDS solution in the reference cell. Plots of absorbance versus pH, chloride or phosphate concentrations were used in determining the emulsifying activity (Padilla *et al.,* 1996).

The emulsifying stabilities at 3, 18, 24 and 48hr were determined as described by Padilla *et al.* (1996). The emulsions were prepared in duplicate.

Statistical Analysis

One way analysis of variance (ANOVA) and Duncan's multiple range test of the data followed the procedure of Statistical Analysis System (SAS 1999). Least significant differences at $p<0.05$ were used for multiple mean comparison test. Protein solubility in the various media were statistically correlated $p<0.05$ with the emulsifying activity of the protein.

RESULTS AND DISCUSSION

Protein (CPI) Solubility

Solubility of CPI over a pH range of 2 – 12 are as shown in Figure 1. Minimum protein solubility of 3.7% was observed at pH 5. In the acidic end (pH 2), maximum protein solubility of 62% was observed; while in the alkaline maximum protein solubility of 95% was observed at pH 12. At pH 12, soluble protein was significantly ($p<0.05$) higher than all other pH ranges considered. Although, the minimum solubility was observed at pH 5, the solubility values in pH range of 4-9 were not significantly ($p<0.05$) different. This observation was in agreement with that of Sathe (1994), who reported pH 5 as the isoelectric point for defatted cashew flour and indicated that alkaline medium was more effective in solubilizing cashew protein compared to the acidic medium. Prakash and Narasinga (1986) reported similar observation for some oil seed protein. High CPI solubility in both alkaline and acidic pH is an important characteristic needed in food formulation and may serve as guide on the performance of the protein when applied to food systems.

Solubility of CPI in various salt media is as shown in Figure 2. Increase in solution concentration significantly increased the protein solubility ($p<0.05$) in all the solvents considered. The amount of soluble protein in the chloride and phosphate media is significantly ($p<0.05$) different from one another. A three -fold increase in protein solubility was observed in 1.0 and 1.5mol/dm^3 NaCl solutions; a two-fold increase in KCl at same concentration; KH_2PO_4 gave a two-fold increase in protein solubility at a concentration range of 0.6 — 1.0mol/dm^3; NaH_2PO_4 gave two-fold increase in the concentration range of 0.1 to 1 .5mol/dm^3. All the salts considered (except 0.2 mol/dm^{3}NaCl) exhibited improved solubility compared to what was obtainable in salt free water. The ions in solution do neutralize the electri-

cal charges on the protein by electrostatic attraction and their hydration sphere repulsion enhances protein solubility due to electrical double layer effect. While competition between proteins and the chloride/ phosphate for water at high concentration, i.e., 1.5NaC1 and KH_2PO_4 may be responsible for the observed decrease in protein solubility (Yuan *et al.,* 2002; Damodaran and Kinsella, 1982). The solubility trends in NaCl and KCl were similar to those of *Colocynthis citrullus, Vicia faba,* and bovine plasma protein concentrates (Arogundade *et al.,* 2004; Arogundade *et al.,* 2006; Oshodi and Ojokan, 1997).

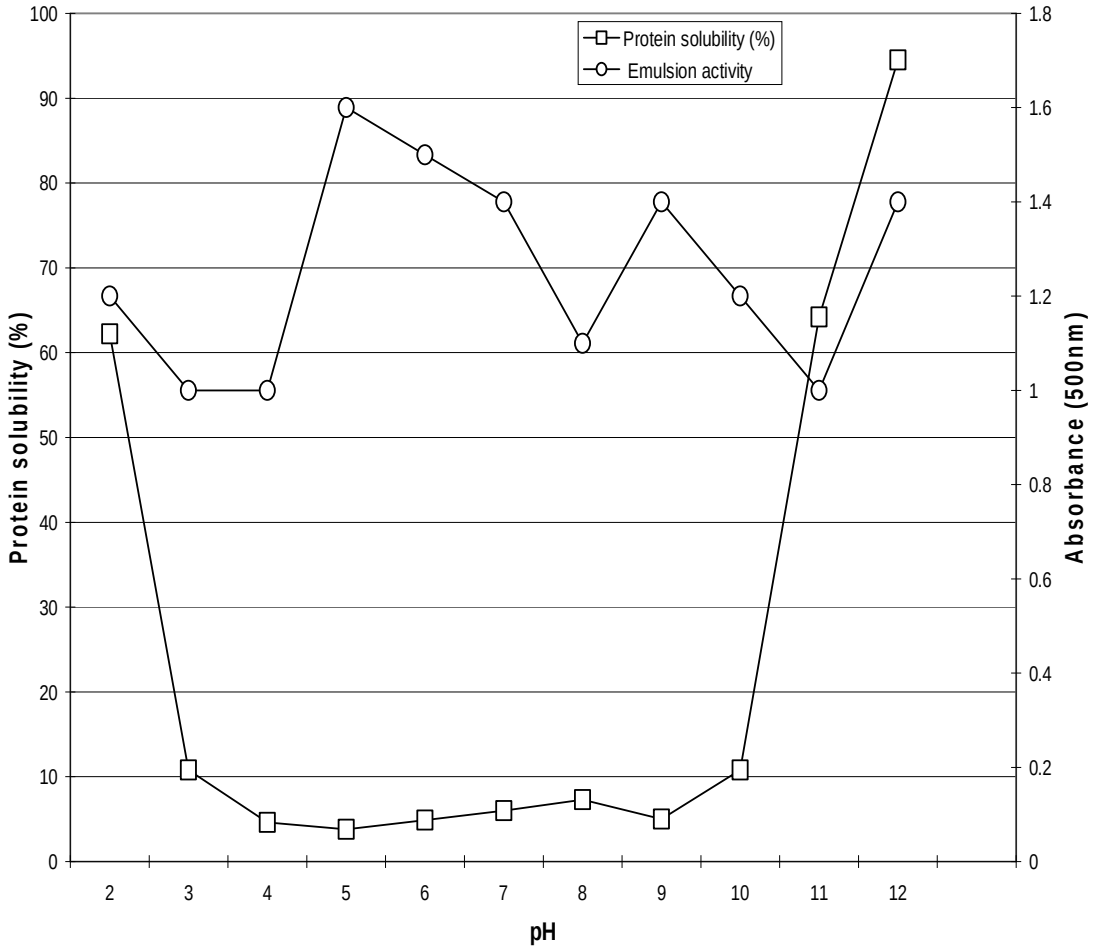

Figure 1:Cashew (*Anacardium occidentale*) protein isolate solubility and emulsion activity at varying pH

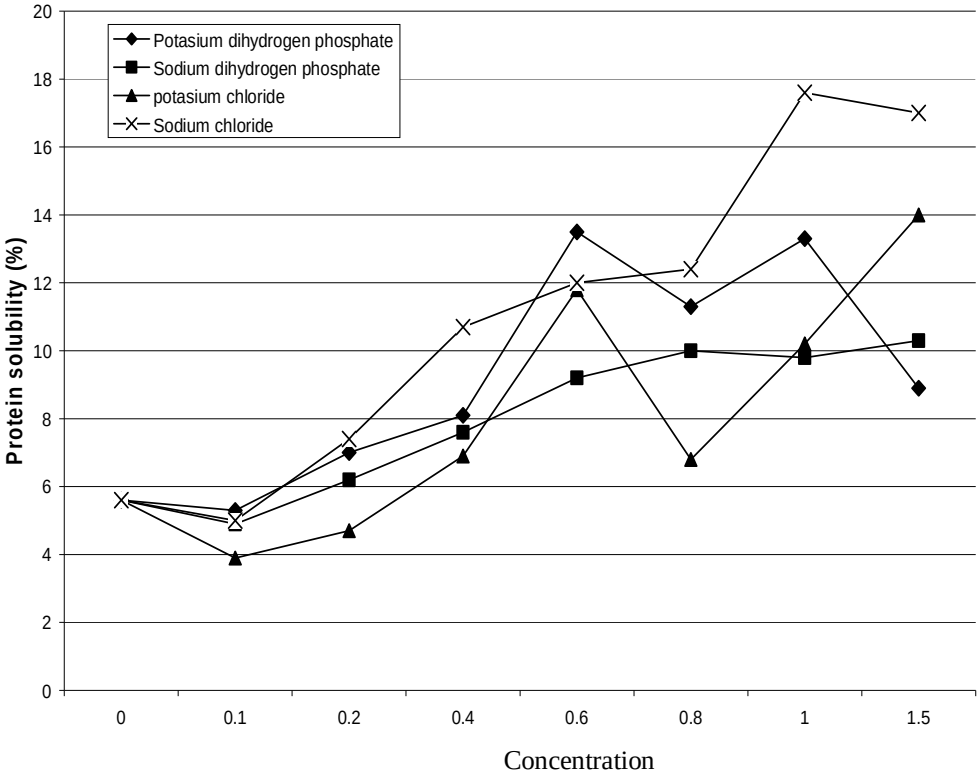

Figure 2: Cashew (*Anacardium occidentale*) protein isolate solubility in phosphate and chloride aqueous media

Solubility in NaCl was significantly ($p<0.05$) higher than in KCl in all concentrations considered but the reverse was the case in KH_2PO_4 and NaH_2PO_4, i.e., solubility in KH_2PO_4 was higher than that of NaH_2PO_4 (Figure 2). Thus cations may not be responsible for the solubility difference. On the other hand, the contribution of anions on protein solubility was significantly lower in KCl, than in KH_2PO_4 but NaCl gave a higher protein ($p<0.05$) solubility than NaH_2PO_4. Thus solubility in these solutions was neither a sole function of cations nor anions, but a combined effect of both anion and cation.

Protein solubility in most of the concentrations considered, was highest in NaCl, followed by KH_2PO_4 while KCl and NaH_2PO_4 ranked least in the concentration range of 0.1 — 0.4 and 0.6 — l.5M, respectively.

Emulsion Activity

The ability of cashew protein isolate to aid formation of oil-in-water emulsion was investigated by determining its emulsifying activity. The emulsifying activity (EA) profile against pH is shown in Figure 1. The emul-

sion activity in water was 0.3, increased 3 - 5 fold on adjusting the pH in the range of 2 - 12. The maximum EA occurred at pH 5.

The presence of chlorides and phosphates (Figure 3) significantly increased the EA. CPI emulsion property was best improved in NaH_2PO_4, in the range 0.2-0.6mol/dm^3 where greater than 6 fold increases in EA was observed compared to water. NaCl (0.8mol/dm^3) gave similar improvement in EA.

Yapar *et al*. (2005) also observed that phosphate addition to *Cyprinus carpio* L, significantly increased its emulsion capacity, Low concentration of NaCl (0.1-0.4 mol/dm^3) improved African locust bean protein EA, while greater than 0.4 mol/dm^3 decreased it (Lawal *et al*., 2005). The 0.3 EA value in water in the present study falls within the range (0.1- 10.9) reported for almond protein isolate, but lower than the values reported for *Caryodendran* and soybean flour (Sze-Tao and Sathe. 2000; Padilla *et al.,* 1996). Increased CPI solubility in the presence of chlorides and phosphates may be responsible for the improved EA compared to the observed in water (Li-chen and Nakai, 1984; Paterson *et al*., 1988; Yapar *et al*., 2005). The increase in EA at pH 5 may be due to the increased protein - protein interaction at isoelectric point which favours emulsion formation by increasing rheological properties of the interfacial protein films that encapsulate the oil droplets. Increased in rheological strength of the protein films could reduce mechanical deformation and desorption of the interfacial protein to give more emulsified droplet (Halling, 1981; Aluko and Yada, 1995).

Emulsion Stability

The emulsion stability (ES) trend with pH, chloride and phosphate media are shown in Figures 4 - 8. CPI emulsion stability (ES) at pH 3, 4, 6 - 10 were significantly ($p<0.05$) lower than the extreme pH values. Emulsion at high acidic and alkaline ends were stable, i.e. 96, 85 and 94% emulsion at pHs 2, 11 and 12 respectively remained after 24hrs. African locust beans albumin and globulin showed higher stability at extremes of the acidic and alkaline pH (Lawal *et al*., 2005). These stabilities at high alkaline and acidic pH may be caused by the level of solubilized protein which enhances cohesion between the oil and protein phases, thereby encouraging stability at the interfacial membrane through encapsulation. A balance of the attractive Vander Waals and repulsive electrostatic forces may add to the stability (Volkert and Klein, 1979). The observed emulsion stability of CPI in the presence of chloride and phosphates were concentration and time dependent. The ES decreased with time but the decrease was only significant ($p < 0.05$) at concentration equal to or greater than 1.0mol/dm^3 (except in KH_2PO_4). In NaCl (1.0 and 1.5mol/dm^3), the emulsion breakdown was observed after 18hr, while at concentration greater than 1.0mol/dm^3, the break down was delayed until after 48 hours. KH_2PO_4 solution showed similar trend, except that the emulsion in 0.6mol/dm^3 KH_2PO_4 broke down after 18hours. In NaCl, emulsion coalescence was delayed until after 48hours irrespective of the NaCl concentration used (0.1 - 1.5 mol/dm^3).

In all the media considered CPI emulsion stability were high (95-99%) at low concentration (0.1 - 0.4mol/dm^3) after 48hrs except in 0.4mol/dm^3 NaH_2PO_4 which dropped to 41 %. Yapar *et al.* (2005) observed that ≤0.5% phosphate concentration improved ES of *Cyprinus carpio*. Cowpea *(Vigna unguiculata)* globulin isolate showed increased ES

with increasing NaCl concentration up to 2.0M (Aluko and Yada, 1995). Improved ES in the presence of salts has been reported to be due to formation of charged layers around fat globules resulting in repulsion and / or formation of hydrated layer around the interfacial material, thereby lowering interfacial energy and retarding droplet coalescence (Kinsella *et al.*, 1985).

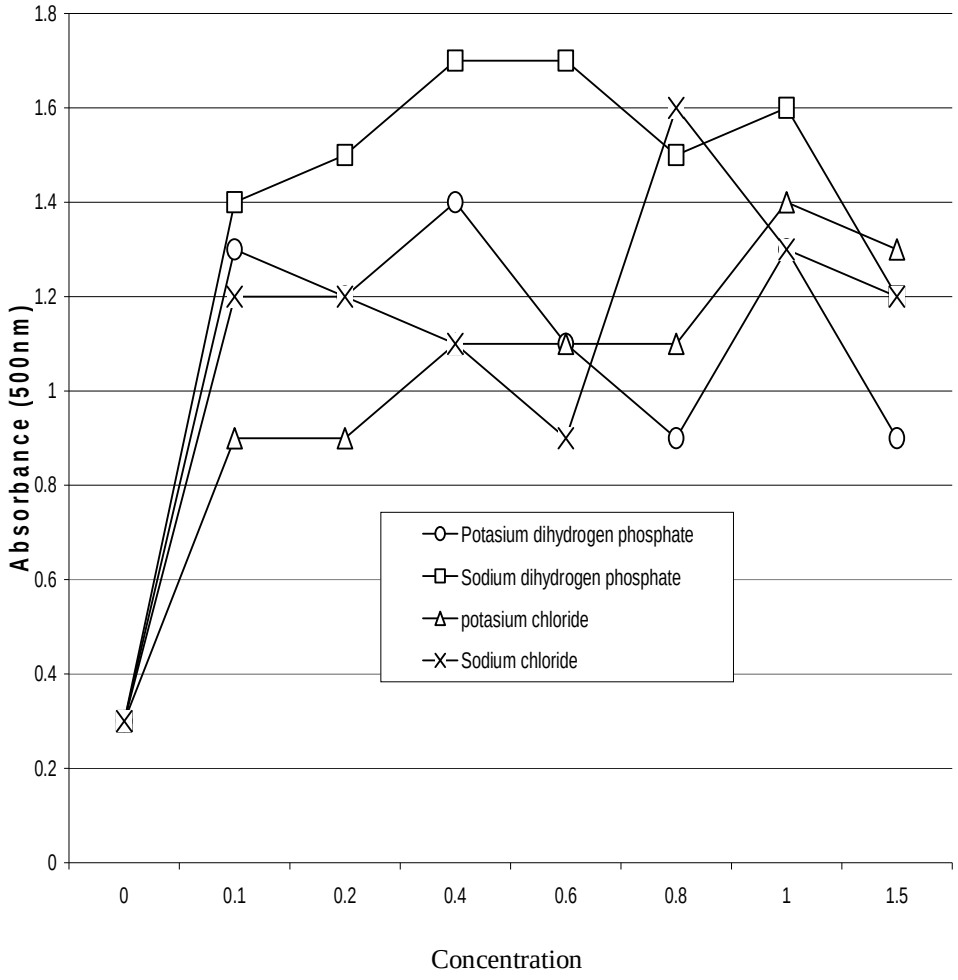

Figure 3: Emulsion activity of *Anacardium occidentale* in phosphate and chloride aqueous media

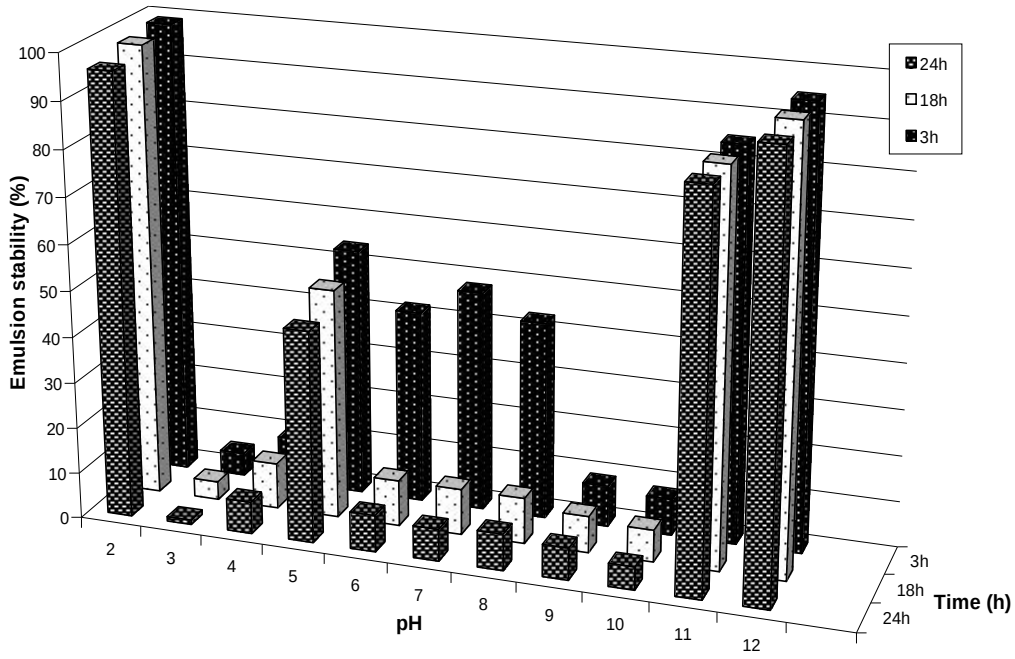

Figure 4: *Anacardium occidentale* protein isolate emulsion stability at different pH

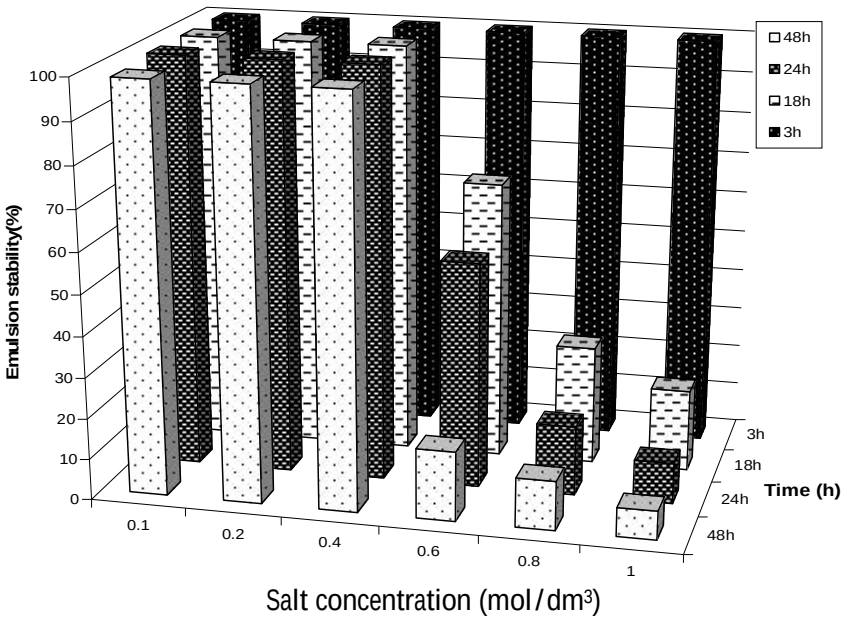

Figure 5: *Anacardium occidentale* protein isolate emulsion stability in KH_2PO_4

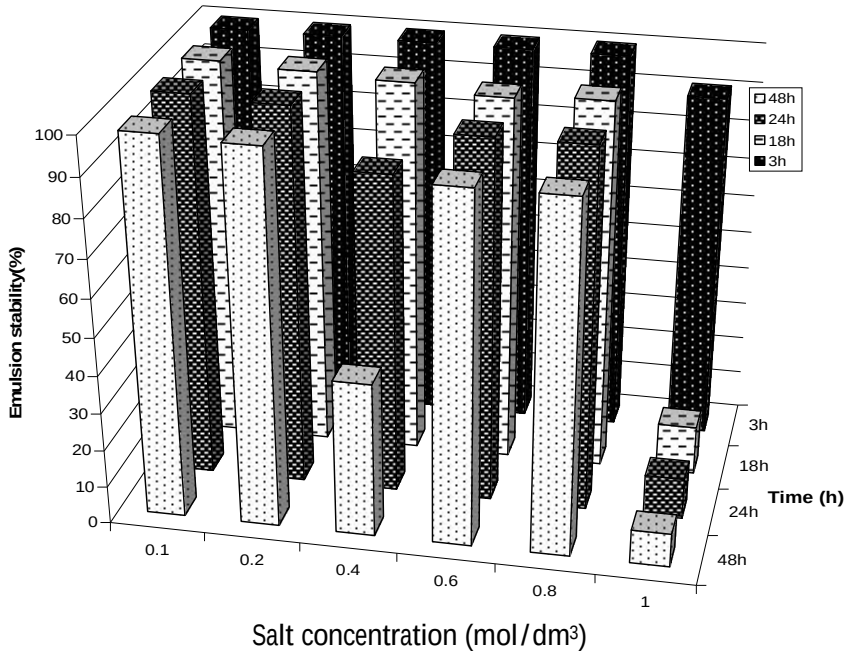

Figure 6: *Anacardium occidentale* protein isolate emulsion stability in NaH_2PO_4

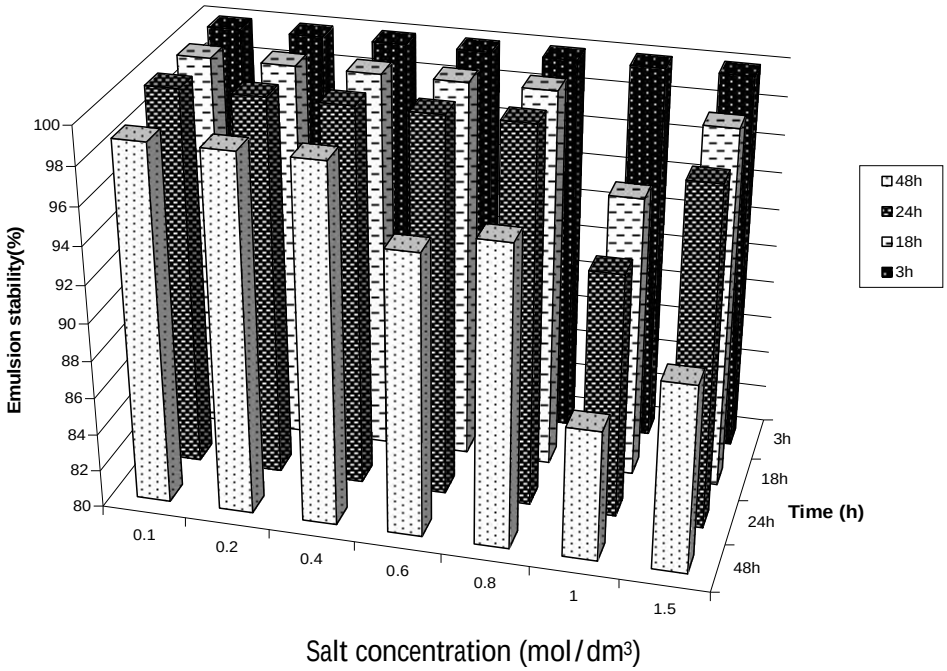

Figure 7: *Anacardium occidentale* protein isolate emulsion stability in KCl

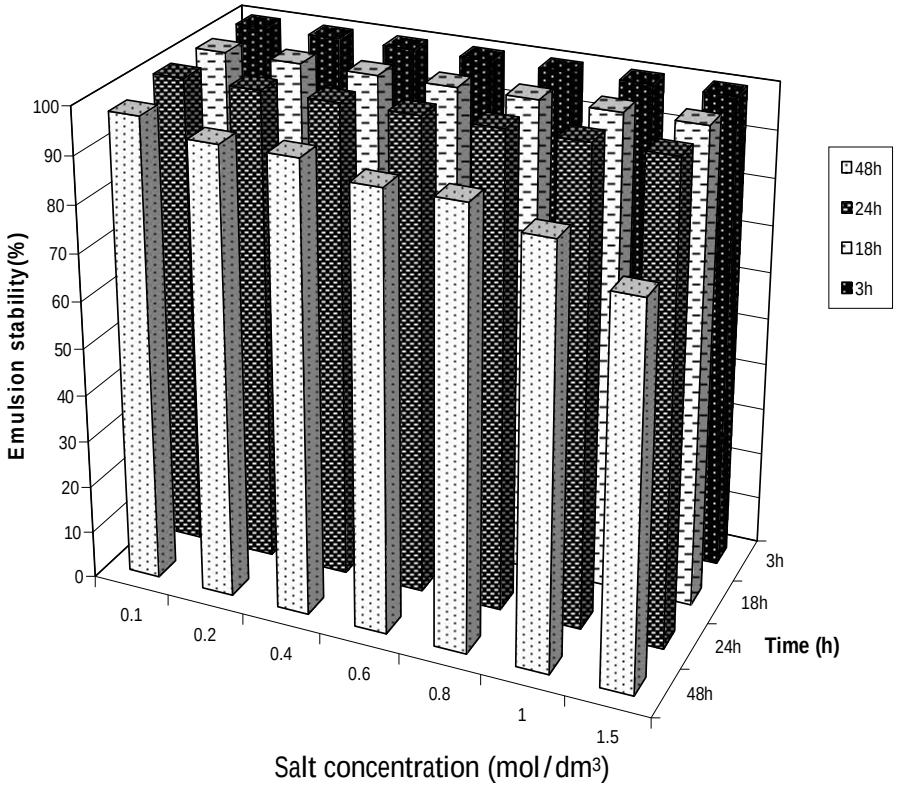

Figure 8: *Anacardium occidentale* protein isolate emulsion stability in NaCl

Interrelationship

As CPI solubility reaches the minimum at pH 5, the EA approaches its maximum (Figure 1). Jahaniaval *et al* (2000) reported that as the solubility of the heat-treated caseinate reached minimum at pH 3.4 - 4, EAI of soluble protein approached maximum. Almond *(Prunus dulcis L.)* protein isolate also showed a decrease in EAI with increasing protein concentration. Aluko and Yada (1995) reported that the emulsion activity of cowpea *(Vigna unguiculata)* globulin isolate in the pH range of 3 - 8 increased with increasing salt concentration did not correspond with increasing EA of the cowpea soluble protein.

Solubility of CPI in NaCl, NaH_2PO_4 or KH_2PO_4 showed positive correlation with EA, although not significant ($P<0.05$). But in KCl solution, a significant correlation ($r = 0.49$; $p = 0.015$) was observed between CPI solubility and EA.

The emulsion stability at 24hr showed a significant ($r = 0.85$; $p = 0.0001$) correlation with protein solubility at varying pH. A significant correlation was observed between CPI solubility and ES at 48hr in chloride and phosphate media except in NaH_2PO_4.

CONCLUSION

CPI solubility and emulsifying properties improved in chlorides and phosphates of Na and K. Addition of NaCl, KCl, KH_2PO_4 or NaH_2PO_4 to CPI gave at least a two-fold

improvement in its solubility and three fold increases in its emulsion activity compared to that observed in H_2O. These phosphates and chlorides concentrations in the range of 0.1 - 0.4mol/dm^3, stabilized CPI oil-in-water emulsion for a longer period. Thus for emulsified food products in which long shelf life is required and, thus, high emulsion stability, application of phosphates and chlorides at low concentration will be an advantage.

Protein has high solubility at pH 2, 11and 12, with minimum solubility at pH 5. Increase in pH did not affect the CPI emulsion activity, but its emulsion is significantly ($p<0.05$) stable at pH 2, 11 and 12 than at other pH values.

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