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, KH₂PO₄ or NaH₂PO₄ 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, KH₂PO₄ or NaH₂PO₄ solutions were better than in H₂O. 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/dm³). EA of CPI in NaCl, KCl, NaH₂PO₄ or KH₂PO₄ 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 family 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 air-tight 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 50 minutes at room temperature. It was centrifuged at 3,500 rpm for 30 minutes. The resulting supernatant was adjusted to pH 4.5 with 1M HCl 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$^3$HCl or 1mol/dm$^3$NaOH. Each suspension was shaken (225rpm) on orbital shaker (Stewart Scientific 501) at room temperature for lhr and centrifuged at 3500rpm for 30min. Effect of KCl, NaCl, KH$_2$PO$_4$ and NaH$_2$PO$_4$ 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-
E mulsifying 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 20cm³ of supernatant and 20cm³ of vegetable oil with kitchen blender. After homogenization, triplicate 50µl aliquots of each emulsion were diluted with 10cm³ 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.

S t atistical 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.

R E S U LT S AND D I S C U SS I ON
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/ dm³ NaCl solutions; a two-fold increase in KCl at same concentration; KH₂PO₄ gave a two-fold increase in protein solubility at a concentration range of 0.6 — 1.0mol/ dm³; NaH₂PO₄ gave two-fold increase in the concentration range of 0.1 to 1.5mol/ dm³. All the salts considered (except 0.2 mol/ dm³NaCl) exhibited improved solubility compared to what was obtainable in salt free water. The ions in solution do neutralize the electric-
plasma protein concentrates (Arogundade \textit{et al.}, 2004; Arogundade \textit{et al.}, 2006; Oshodi and Ojokan, 1997). Electrical 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., 1.5NaCl and KH$_2$PO$_4$ may be responsible for the observed decrease in protein solubility (Yuan \textit{et al.}, 2002; Damodaran and Kinsella, 1982). The solubility trends in NaCl and KCl were similar to those of \textit{Colocynthis drurillus}, \textit{Vicia faba}, and bovine

![Graph](image-url)

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

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Protein solubility in most of the concentrations considered, was highest in NaCl, followed by KH$_2$PO$_4$ while KCl and NaH$_2$PO$_4$ ranked least in the concentration range of 0.1 — 0.4 and 0.6 — 1.5M, 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-

**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$_2$PO$_4$ and NaH$_2$PO$_4$, i.e., solubility in KH$_2$PO$_4$ was higher than that of NaH$_2$PO$_4$ (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$_2$PO$_4$ but NaCl gave a higher protein (p<0.05) solubility than NaH$_2$PO$_4$. Thus solubility in these solutions was neither a sole function of cations nor anions, but a combined effect of both anion and cation.

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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$_2$PO$_4$, in the range 0.2-0.6 mol/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$_2$PO$_4$). 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 breakdown was delayed until after 48 hours. KH$_2$PO$_4$ solution showed similar trend, except that the emulsion in 0.6mol/dm$^3$ KH$_2$PO$_4$ 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$_2$PO$_4$ 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$_2$PO$_4$
Figure 6: *Anacardium occidentale* protein isolate emulsion stability in NaH$_2$PO$_4$

Figure 7: *Anacardium occidentale* protein isolate emulsion stability in KCl
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, NaH₂PO₄ or KH₂PO₄ 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 NaH₂PO₄.

CONCLUSION
CPI solubility and emulsifying properties improved in chlorides and phosphates of Na and K. Addition of NaCl, KCl, KH₂PO₄ or NaH₂PO₄ 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 H₂O. These phosphates and chlorides concentrations in the range of 0.1 - 0.4mol/dm³, 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, 11 and 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.

REFERENCES


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