

COMPARISON OF FUNCTIONAL PROPERTIES OF HEMOLYMPH PROTEIN FROM FRESHWATER CRAB, *BARYTELPHUSA CUNICULARIS* WITH CASEIN, EGG ALBUMIN AND BOVINE SERUM ALBUMIN

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ABSTRACT

The physico chemical and functional properties of hemolymph protein (HP) from freshwater crab, *Barytelphusa cunicularis* was compared with commercially available proteins viz. Casein, egg albumin and Bovine serum albumin (BSA) for possible use in food technology. The percentage solubility of proteins, observed were casein > HP > egg albumin > BSA at different pH. The emulsifying properties of casein, egg albumin, BSA and HP at pH 7 and 9, did not show any statistically significant difference. But, when compared with pH 7 and 9 between the groups, a statically significant difference was observed. The foam stability and capacity studies of protein were found in the order of casein > egg albumin = BSA > HP and casein < egg albumin < HP < BSA respectively. The foam stability and time showed a statistically significant relationship. The HP showed a less water binding capacity (WBC) and oil binding capacity (OBC) when compared to casein, BSA and egg albumin. Thus, the present study suggests that HP is a good candidate for use in food processing industries and this protein should not be simply ignored.

INTRODUCTION

Functional properties of proteins derived from plants, animals and microbial sources have been reviewed by various workers (Zayas 1997; Ha and Zamel 2003). These proteins are inexpensive and have good nutritional and functional properties. However, certain proteins, such as, plasma proteins from animal blood though have good have good emulsifying properties, but they are sensitive to heat, thus limiting their use in processed food (Tybor and et al., 1975; Saito and Taira 1987). Among the animal proteins, crustacean possesses a high nutritive values and their consumption has been encouraged worldwide. The hemolymph of freshwater crab contains various coagulative factors, which include respiratory protein, hemocyanin, proteins and enzymes. Among enzymes transglutaminase is picking up as a useful enzyme in food technology applications (Folk and Finlason 1977; O'sullivan, et al., 2002). Just as egg protein are extensively utilized as functional food products in food processing industries, because of the egg white's nutritional and wide range of functional properties. Similarly, crustacean constitute an important nutritional food and are mostly consumed by peoples around the world. However, attempt to analyze their functional properties so that they can be utilized in food processing was not carried out. Native proteins from single source may not have all desired characteristics; hence, modifications are normally achieved by enzymes for improving functional properties for food applications.

Many commercially available protein powders are widely used for their functional properties such as foaming capacity, emulsifying capacity, water and oil binding capacity and solubility. In an attempt to identify the potential areas for the utilization of hemolymph protein, this study was undertaken to compare the functional properties.

MATERIALS AND METHODS

Freshwater crab, *Barytelphusa cunicularis* were procured from local market and acclimated in the laboratory at 20°C and were fed *ad libitum* for two weeks prior to experiment. The hemolymph were drawn from the chelicerae by syringe and transferred to test tube. A 50 times diluted solution of the Hemolymph protein (HP) was used. The casein, egg albumin (Grade II), and Bovine Serum Albumin (BSA) for comparison was obtained from Loba Chemie, Mumbai.

Physico chemical property

Solubility and pH - A 5% protein HP, casein, egg albumin and BSA were prepared in distilled water and centrifuged at 12000xg for 10 minutes at 25°C. The supernatant was used for the analyses of solubility at different pH ranges viz. 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0 and 12.0. The percentage protein solubility at each pH was measured by Lowry's method (Lowry et al., 1951) at 660nm by using Bovine Serum Albumin as standard.

Functional properties

Emulsifying properties- The emulsion properties were measured by the method of Pearce and Kinsella (1978). Pure corn oil (2ml) and 0.1% protein solution were homogenized in a mechanical homogenizer for 1 minute. Aliquot of the emulsion (50ml) were pipetted out and diluted 100times in 0.1%SDS. Absorbance of these mixtures was measured at 500nm. The absorbance was measured immediately after emulsion formation (0 minute) was expressed as emulsifying activity of protein.

Foaming properties - Foaming capacities of proteins were determined by measuring the volume of foams immediately after introduction of air for 15 seconds in 5ml of 0.1%protein solution. Foaming stability was calculated by the method described by Kato et al., (1983).The foam stability was analyzed, at a time interval of 0,30,60,90,120,150and 180minutes

Water binding capacity (WBC) - An equivalent weight of 1g of protein of studies were hydrated to a paste like consistency with distilled de-ionized water and the WBC was determined according to the method of Beuchat (1977).

Oil bilding capacity (OBC) - An equivalent weight of 1g of protein of studies were hydrated to a paste like consistency with corn oil and the OBC was determined according to the method of Ahmedna et al., (1999).

Statistical analysis: All measurements were made in triplicates, and experiments were repeated once. All data were subjected to statistical analysis using univariate ANOVA on MS office.

RESULTS AND DISCUSSION

Solubility and pH: The solubility profile of HP, casein, egg albumin and BSA is shown in Fig.1. The HP and egg albumin were highly soluble at pH i.e pH 4 and 9 and least soluble at near pH 7. However, the percentage solubility of HP was higher than egg albumin and BSA. The ANOVA for the solubility of HP, casein, egg albumin and BSA to different ranges of pH and with individual pH were studied. The result showed a highly significant ($F(4,22) = 15.99; P < 0.05$) difference in solubility of proteins (HP, casein, BSA and egg albumin) at different ranges of pH, whereas, solubility of proteins (HP, casein, BSA and egg albumin) when studied separately with pH showed no significant difference ($F(5,21) = 0.421; P > 0.05$).

The potential use of proteins in food systems is determined by its ability to interact with other components and their physicochemical characteristics (Damodaran, 1990). Solubility is influenced by emulsification, gelation and foam formation (Kinsella 1976; Hettiarachchy et al., 1996).The reason for these difference in observed solubility may be associated with decrease in charge density, because of cross linking reactions or due to altered intra and inter molecular charge repulsions, hydrophobicity, pH and ionic strength. Similar, observations were reported by various workers (Cheftel et al., 1985; Kinsella and Whitehead 1989; Wagner and Anon 1990). A high solubility observed at non-iso electric pH (NIE pH), of HP and casein may be due to greater net charge then zero thus, favoring a high solubility in solvent. The protein solubility is

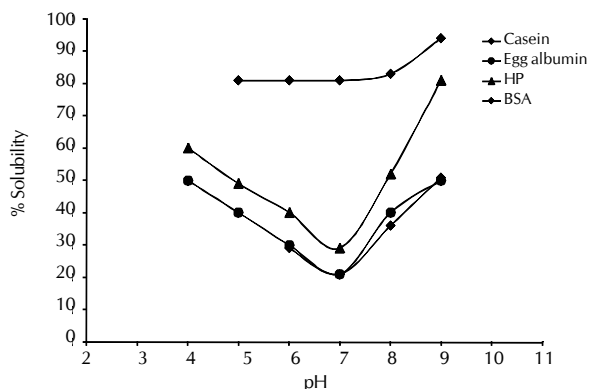


Figure 1: Solubility profile of HP, casein, Egg albumin and BSA.

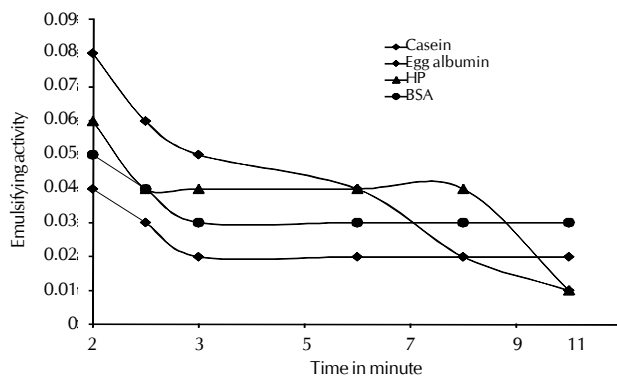


Figure 2: Emulsifying activity of HP, casein, egg albumin and BSA.

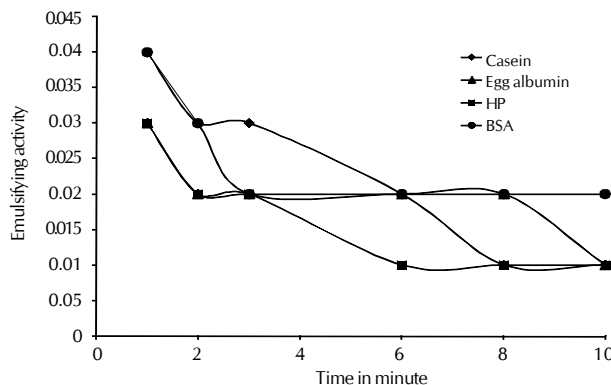


Figure 3: Emulsifying activity of HP, casein, egg albumin and BSA at pH 9

proportional to the square of the net charge on the protein (Tansford 1961), thus, the proteins are least soluble near their isoelectric point and the solubility increases as the pH is raised or lowered as the magnitude of the net charges increases. The BSA and egg albumin showed an almost same pattern of solubility in the present study.

Emulsifying properties - The emulsifying properties of egg albumin, HP, BSA and casein at pH 7 and 9 are shown in Fig. 2 and 3 respectively. The ANOVA studies of emulsifying activities of albumin, casein, BSA and HP at pH 7 and pH 9 showed no significant ($F(4,25) = 11.5; P > 0.05$) and ($F(4,25) = 11.54; P > 0.05$) difference between the proteins studied. When this observation was compared with pH 7 and 9 a significant ($F(4,30) = 4.194; P < 0.05$) difference between

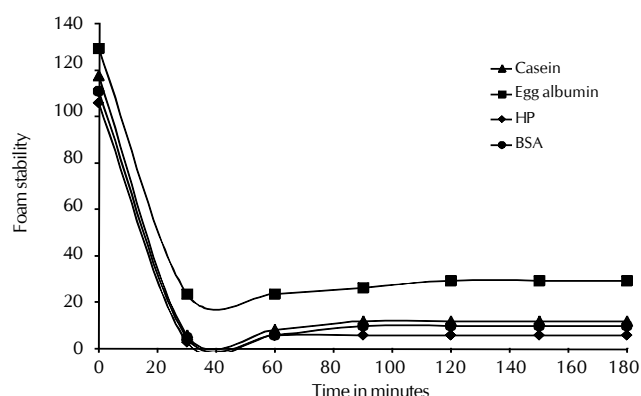


Figure 4: Foam stability of HP, casein, egg albumin and BSA at different time intervals.

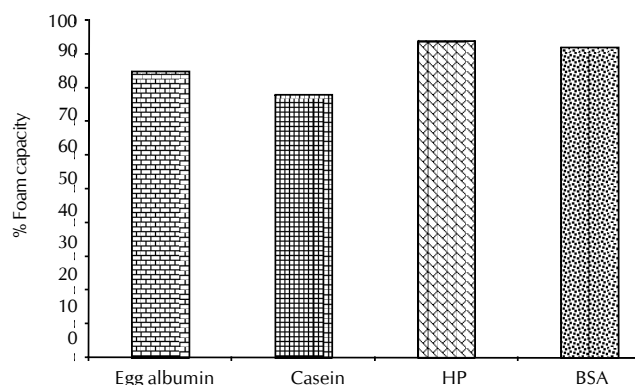


Figure 5: Foaming capacity of HP, casein, egg albumin and BSA.

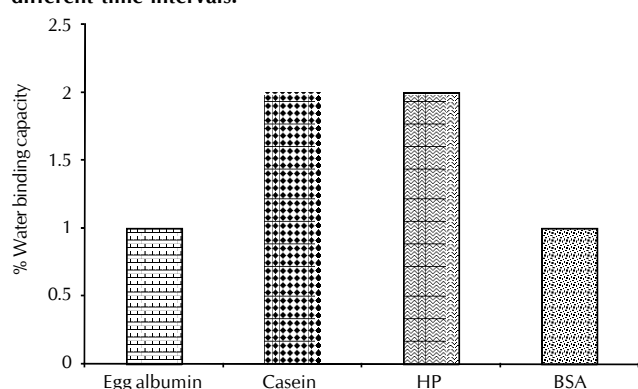


Figure 6: Water binding capacity of HP, casein, egg albumin and BSA.

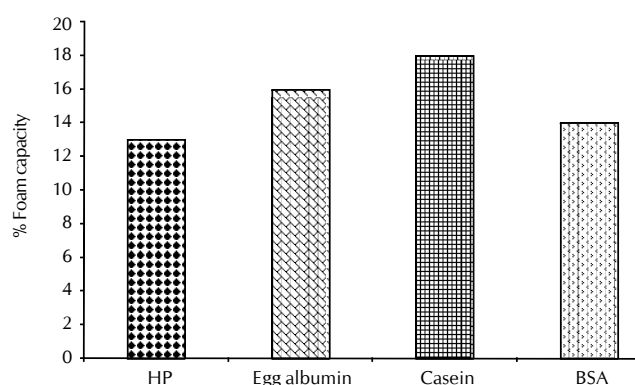


Figure 7: Oil binding capacity of HP, casein, egg albumin and BSA.

the groups was observed suggesting, that an interaction exist between treatments at different pH. When the individual protein emulsifying activity were compared with the pH 7 and 9; only, egg albumin and BSA did not show any significant ($F(2,20)=8.32;P<0.05$) difference. The casein and HP did not show any significant ($F(2,200)=6.40;P>0.05$; and ($F(2,20)=3.69;P>0.05$) difference with respective pH.

Thus, it was observed that the emulsifying profile for protein studied were different at pH 7 and 9 respectively. The differences observed may be attributed to an increase in pH towards alkalinity, which may be contributing charges to the protein molecules. Philip et al (1994) and Wong and Kitts (2003) had reported that the emulsifying property of a protein depends on various factors such as, amphipathic nature, solubility, pH, degree of denaturation, lipid to protein, emulsion viscosity and surface hydrophobicity This phenomenon may facilitate emulsification by promoting protein – to –fat interaction and thus, reduces protein-to-protein interactions through electrostatic repulsion. Similar, mechanism has been reported by various workers (Susheelamma and Rao 1974; Prinyawiwatkul et al., 1993).

Foam stability and capacity - The foaming stability and foam capacity are shown in figure 4 and 5. The foam stability and foam capacity was found in the order of casein >egg albumin = BSA >HP and Casein < egg albumin < BSA < HP respectively. The ANOVA for foam stability of albumin, casein and HP showed a statistically ($F(4,30)=2.88;P<0.05$) significant relation between time and foam stability among the proteins. However, when foam stability alone was studied

no statistically significant ($F(3,24)=0.0429;P>0.05$) difference was observed.

Kinsella and Whitehead (1989) reported that foaming stability is an important property of food systems. The foam stability for HP was low, this may be due to globular problem. The globular proteins generally have low free sulphhydryl group content, and stabilizes foam by unfolding the air / water interface due to hydrophobicity and serve as a physical barrier to bubble coalescence as reported by Trachoo and Mistry (1998). The difference in foaming capacity observed in casein, HP, BSA and egg albumin may be due to extensive uncoiling at the air water interface possessing relatively high surface hydrophobicity. Similar views were expressed by Kinsella (1979; and Zayas (1997).

Water binding capacity (WBC) - The WBC is shown in Fig.6. The WBC of proteins has an important role in physical, chemical and sensory attributes of foods. HP and BSA showed the least WBC among the proteins studied. No relationship between water binding capacity and solubility was established in the present study, this observation is consistent with the report of Kinsella(1981).However, an inverse relationship between WBC and solubility was confirmed by various workers (Wagner and Anon 1990;Vani and Zayas 1995; Wong and Kitts 2003).Ahmedna et al (1999) and Wong and Kitts (2003) reported that the greater WBC is due to partial denaturation, dissociation and unfolding of protein induced by heat treatment applied during preparation and drying. One of the explanation for this reasons may be the weak WBC of HP.

And this may be due to non-denaturation, non-dissociation and non-unfolding of the proteins contrary to the reports of Ahmedna *et al.*, (1999) and Wong and Kitts (2003).

Oil Binding Capacity (OBC) - The OBC of the proteins are shown in Fig.7. The oil binding capacity is the binding of the fat by non-polar amino acids present in the side chains of proteins (Susheelamma and Rao 1974). The affinity of protein to bind fat improves the texture and reduces yield losses in fabricated foods such as comminuted meat or bakery products.

According to Fig. 7 the OBC in descending capacity are casein > egg albumin > BSA > HP. The explanation for the difference in the OBC between the proteins may be due to lower initial fat content, then egg albumin and HP, which therefore, would result in an increase potential to bind more fat (Lin and Zayas 1987). Another possible reason for the weak OBC of HP may be due to non- denaturation and non unfolding of HP molecules with no hydrophobic regions available to interact with fat. The present study showed higher oil binding capacity in casein and a least capacity in HP. This observations corroborates the findings of Ahmedna *et al.*, (1999) and Wong and Kitts (2003).

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