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Functional Properties of Hemolymph Protein from Freshwater Crab, *Barytelphusa cunicularis.*

Encily R. Martin

Department of Zoology, Dr. B.A.Marathwada University, Aurangabad 431004 (MS), India

Abstract: The hemolymph proteins (HP) of freshwater crab, *Barytelphusa cunicularis* were studied for their physico-chemical and functional properties. The HP showed a high percentage solubility at pH 4 and 9. The emulsifying properties of HP showed no significant difference at pH 7 and 9; however, when HP was compared with albumin and casein, only egg albumin showed a significant differences among groups, whereas, casein and HP did not show any significant difference. The foaming activity and stability of HP was less compared to casein and egg albumin. The HP showed a less water and oil binding capacity compared to egg albumin and casein.

Key words: Hemolymph protein · *Barytelphusa cunicularis* · Functional properties

INTRODUCTION

Interactions of physico-chemical characteristics with other components in foods determine the values of a protein in food systems. Each functional property of proteins requires different characteristics. Efficient formation of foams requires flexible molecules with few secondary and tertiary structures, whereas, intermolecular cohesiveness and elasticity are important to produce stable forms [1,2]. Native proteins from single source will not have all desired characteristics, hence, modification is necessary to attain appropriate balance. These modifications are normally achieved by enzymes for improving functional properties for food applications.

Functional properties of proteins derived from plants, animals and microbial sources have been reviewed by various workers [3,4].These proteins are inexpensive and have good nutritional and functional properties. The plasma proteins from animal blood have good emulsifying properties, but they are sensitive to heat, thus limiting their use in processed food [5,6]. Among the animal proteins, crustaceans possesss a high nutritive values and their consumption has been encouraged world wide. The hemolymph of freshwater crab contain various coagulative factors, which include respiratory protein, hemocyanin, proteins and enzymes. Among enzymes transglutaminase is picking up as an useful enzyme in food technology applications [7,8].

The present study was carried out to ascertain the physico-chemical and functional properties of hemolymph protein(HP) from freshwater crab, *Barytelphusa cunicularis.* These properties were compared with casein and egg albumin so that this protein can be used as potential food substitute in food industries.

MATERIALS AND METHODS

Materials: 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 chelecerae by syringe and transferred to test tube. A 50 times diluted solution of the HP was used in the studies. The pure casein and egg albumin (Grade II) for comparison was obtained from Loba Chemie.

Physico Chemical Property: Solubility and pH: A 5% protein solution of HP, casein and egg albumin were prepared in distilled water and these solution was centrifuged at 12000xg for 10 minutes at 25°C. The supernatant was used for the analyses of solubility at different pH ranges viz.4,5,6,7,8,9,10,11 and 12. The percentage protein solubility at each pH was measured according to Lowry *et al*. [9] at 660nm by using standard curve derived from bovine serum albumin.

Corresponding Author: Encily R .Martin,Department of Zoology, Dr.B.A.Marathwada University, Aurangabad 431004 (MS) India. E-mail: martin_encily@yahoo.com.

Functional Properties

Emulsifying Properties: The emulsion properties were measured by the method of Pearce and Kinsella [10]. Pure corn oil (2ml) and 0.1% protein solution were homogenized in a mechanical homogenizer for 1 minute. Aliquot of the emulsion (50µl) were pipetted out and diluted 100times in 0.1%SDS. Absorbance of these mixtures was measured at 500nm at t=0,1,3 and 3 hours. The absorbance measured immediately after emulsion formation (0 minute) was expressed as emulsifying activity of protein. The emulsion stability was estimated by measuring the half time of the turbidity measured immediately after emulsion formation.

Foaming Properties: Foaming capacities of proteins were determined by measuring the volume of foams immediately after introduction of air for 15 seconds into 5ml of 0.1%protein solution. Foam stability was calculated by the method described by Kato *et al.* [11]. The foam stability was analysed, at time intervals of 0,30,60,90,120,150,180 minutes.

Water Holding Capacity(WHC): An equivalent weight of 1g of protein of studies were hydrated to a paste like consistency with distilled deionised water and the WHC was determined according to the method of Beuchat [12].

Oil Binding 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*. [13].

Statistical Analyses: 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 hemolymph protein(HP), casein and egg albumin is shown in Fig. 1. The HP and egg albumin were soluble at wide range of pH i.e pH 4-9, however, the percentage solubility of egg albumin was less. HP showed a minimum solubility near pH 7. The ANOVA for the solubility of HP, casein and albumin to different ranges of pH and with individual pH were studied. The result showed a highly significant ($F(2,14)=15.99;P<0.05$ difference in solubility of protein at different ranges of pH, whereas, solubility of proteins when studied separately with pH showed no significant difference($F(5,11)=0.53;P>0.05$.

The potential use of proteins in food systems is determined by its ability to interact with other components and their physicochemical characteristics[2]. Solubility is influenced by emulsification, gelation and foam formation [14,15]. The plausible reason for the difference in solubility observed in the present study 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. A similar observations were reported by various workers[16-18]. 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 proportional to the square of the net charge on the protein[19],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.

Emulsifying Properties: The emulsifying properties of egg albumin, HP and casein at pH 7 and 9 are shown in Fig. 2 and 3, respectively. The ANOVA studies of emulsifying activities of albumin, casein and HP at pH 7 and pH 9 showed no significant ($F(2,15)=1.54;P>0.05$) and ($F(2,15)=1.5;P>0.05$) difference between the proteins studied. When this data were compared with pH 7 and 9 a significant ($F(5,50=3.194;P<0.05$) difference between the groups was observed suggesting 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 showed an significant ($F(1,10=6.23;P< 0.05$) difference. The casein and HP did not show any significant ($F(1,10=3.04;P>0.05$; and $F(1,10=2.59;P>0.05$) difference with respective pH.

In the present study, it was observed that the emulsifying profile for protein studies were different at pH 7 and 9, respectively. The differences observed might be attributed to an increase in pH towards alkalinity, which may be contributing charges to the protein molecules. Philip *et al*. [20] and Wong and Kitts [21] 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. A similar mechanism has been reported by various workers [22,23].

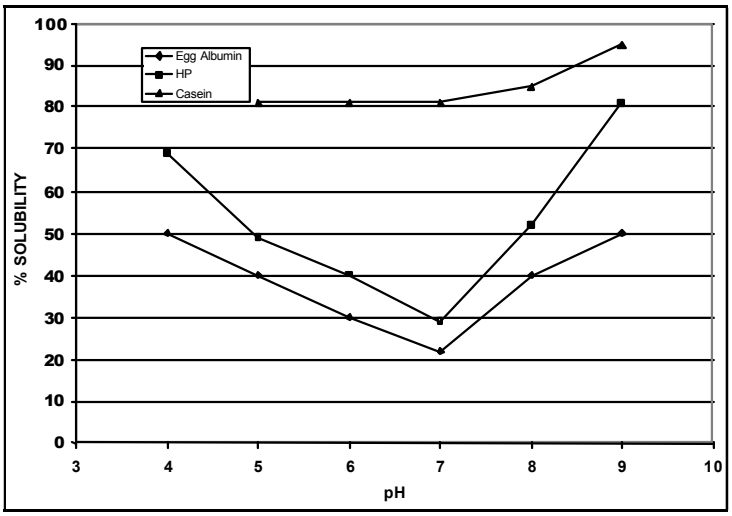

Fig. 1: Solubility profile of proteins viz. egg albumin , hemolymph protein casein at different pH

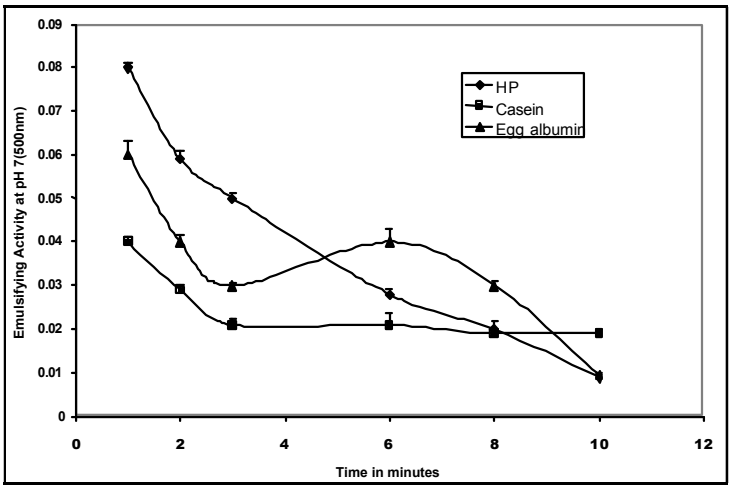

Fig. 2: Emulsifying activity of egg albumin, hemolymph protein and casein at pH 7

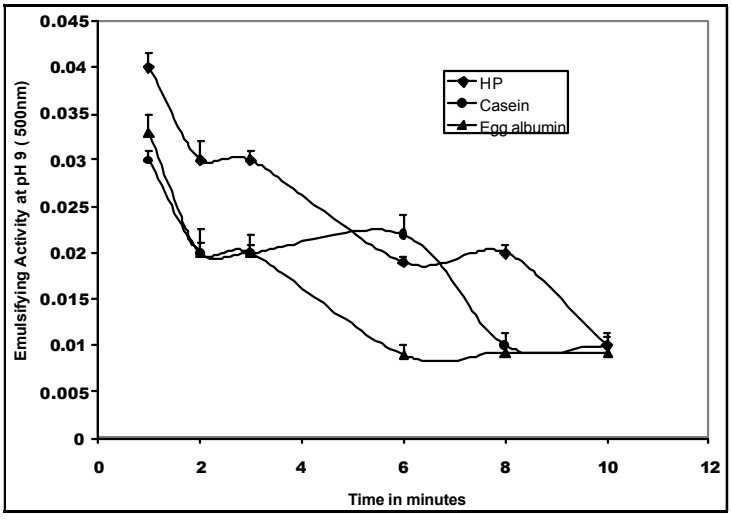

Fig. 3: Emulsifying activity of egg albumin, hemolymph protein and casein at pH 9

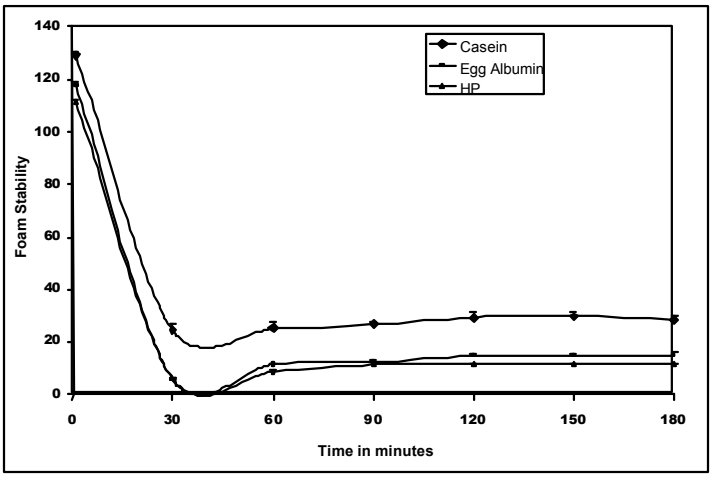

Fig. 4: Foam stability of different proteins, egg albumin, hemolymph protein and casein

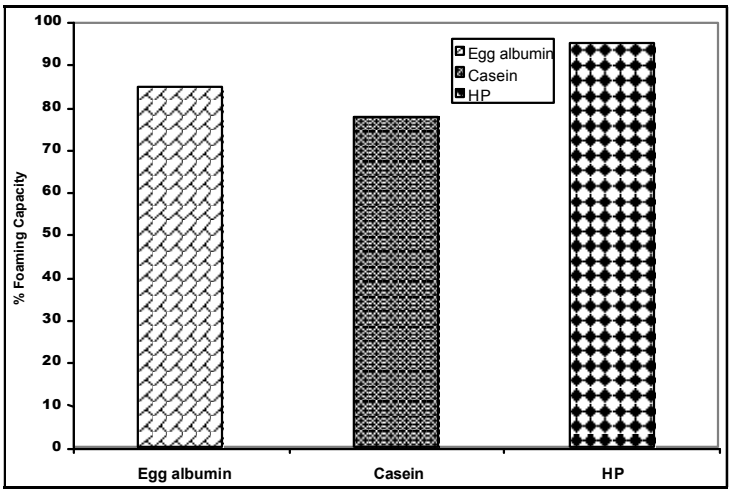

Fig. 5: Percentage of Foaming capacity of egg albumin, hemolymph protein and casein

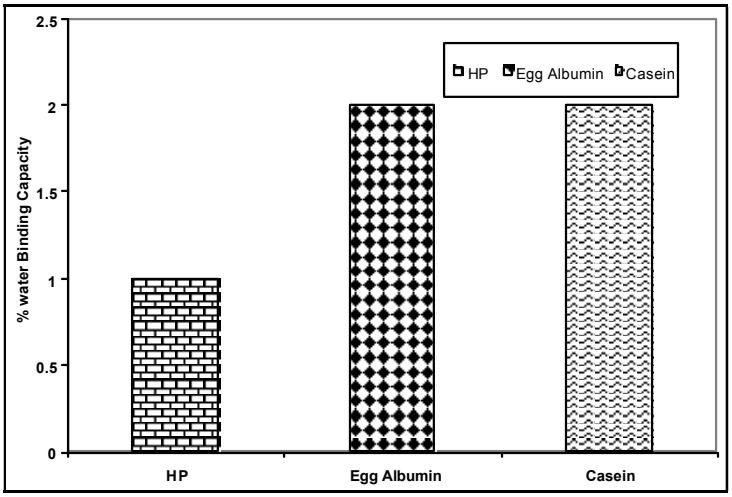

Fig. 6: Percentage of Water binding capacity of egg albumin, hemolymph protein and casein

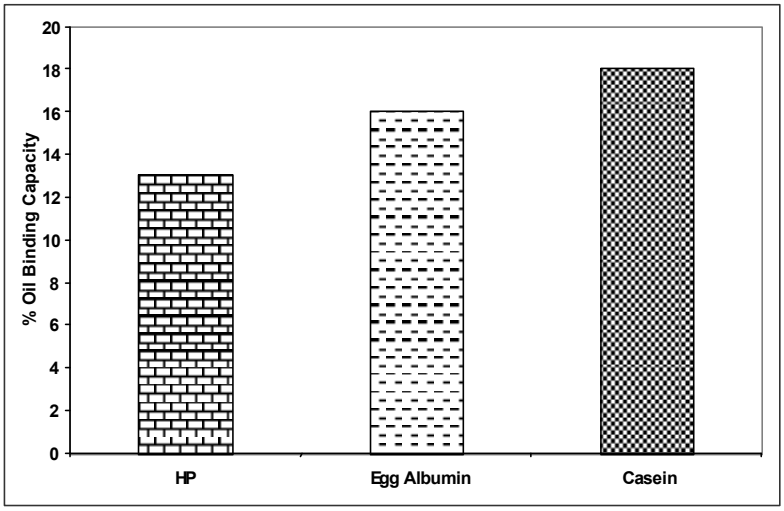

Fig. 7: Percentage of oil binding capacity of egg albumin, hemolymph protein and casein

Foam Stability and Capacity: The foaming stability and foam capacity are shown in Figures 4 and 5. The foam stability and foam capacity was found in the order of casein=egg albumin>HP and Casein>egg albumin>HP, respectively. The ANOVA for foam stability of albumin, casein and HP showed a statistically ($F(6,14)=4.10;P<0.05$ significant relation between time and foam stability among the proteins. However, when foam stability alone was studied no statistically significant ($F(2,18)=1.21;P>0.05$) difference was observed.

According to Kinsella and Whitehead [17] 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 sulphydryl 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, [24]. The difference in foaming capacity observed in casein, HP 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, [25] and Zayas [3].

Water and Oil Binding Capacity: The water and oil binding capacity is shown in Figs. 6 and 7. The water binding capacity plays an important role in physical, chemical and sensory attribution of foods. In the present study HP showed the least water binding capacity 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[1].However, an inverse relationship between WBC and solubility was confirmed by various workers [18, 21,26]. Ahmedna *et al.* [13] and Wong and Kitts [21] reported that the greater WBC is due to partial denaturation, dissociation and unfolding of protein induced by heat treatment applied during preparation and drying.

The oil binding capacity is due to the non polar amino acids present in the side chains of proteins [22]. This property improves the texture and reduces yield losses in fabricated foods such as comminuted meat or bakery products. 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.* [13] and Wong and Kitts [21].

In conclusion, the freshwater crab, *B.cunicularis* is the most widely consumed crab in these region. This crabs has a good organoleptic property. The functional studies suggest that this protein could have wide applicability in food technology because of their nutritional, rich minerals, immune stimulating properties. A detail study on the metal ions and globular protein accessibility and release mechanism will warrant a great potential in not only in food technology, but in health improvement programme also.

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