Effect of Blanching on Frozen Storage of Prawns (*Metapenaeus dobsoni*): Physicochemical and Functional Properties

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The effects of blanching on the physicochemical and functional properties of proteins from prawn (*Metapenaeus dobsoni*) during frozen storage have been investigated. The average increase in rate of drip loss was 0.72 ± 0.03% for every 30 days of storage at −18 °C up to a test period of 300 days. The gel filtration profile indicates complete dissociation of proteins as a result of blanching. The sedimentation velocity pattern of blanched sample showed a single dissociated peak with $s_{20,w}$ value of 2.5S. Polyacrylamide gel electrophoresis pattern showed fast-moving components in blanched sample. Fluorescence emission spectra showed a blue shift from 335 to 330 nm in blanched sample during frozen storage. The functional properties of proteins from blanched sample were altered as a result of the blanching process. Both water absorption and fat absorption values decreased as a result of blanching as compared to control values. Similarly, the foam capacity value, foam stability value, and emulsion properties of the blanched sample were considerably less in all of the blanched samples.

INTRODUCTION

The process of blanching is commonly practiced in the prawn-processing industry either in canning or in freezing operation. In many cases the reason for blanching on a commercial scale is to quickly enhance the quality and in some cases to generate the texture and color of prawns which are appealing. Further, it may also reduce surface microbial load and ultimately play a role in the keeping quality of prawns. However, there are certain disadvantages in the blanching process such as loss of flavor, solubility, and shape which would affect the end product. Recently increasing amounts of fish and fishery products are processed as ready-to-serve products which are precooked before freezing. Some of the undesirable changes of texture and flavor accompanying frozen storage could be overcome by sufficient prefreezing treatments (Sikorski et al., 1976). Changes in texture and juiciness of muscle foods as a result of blanching are as a result of denaturation (unfolding) and subsequent association of proteins. These emphasize the critical importance of these reactions to the chemistry of muscle foods (Foegeding, 1988). One of the many challenges in muscle protein chemistry is linking denaturation and association events with water holding and emulsification properties of fish/prawn meat products. Hence, understanding how protein structure determines denaturation and association–dissociation produces various changes in functional properties is of importance.

In the present investigation the effects of blanching of prawns and frozen storage on the physicochemical and functional properties their proteins are investigated. Also, the effects of different periods of blanching (95 °C for 30, 60, and 120 s) as well different lengths of frozen storage at −18 °C on functional properties have also been evaluated.

MATERIALS AND METHODS

Blanching. A large quantity of distilled water was heated to boiling (97 °C), and then prawns were dipped in the water such that the prawns reached a temperature of 95 °C. Three different blanching periods were chosen, *viz.* 30, 60, and 120 s. After the desired time of blanching was completed, prawns were taken out, air-cooled, and packed separately in polyethylene bags (100 g each) and processed for freezing as described earlier (Shamasunder and Prakash, 1994b). After thawing, representative samples were drawn from polyethylene bags for assessment of the different properties. About 15–20 prawns were drawn from different lots and then cut into small pieces. An aliquot of exact quantity of meat was drawn and then used for assessment of the various properties.

All other methodologies followed in both physicochemical and functional property evaluation are as described in the preceding papers (Shamasunder and Prakash, 1994a,c).

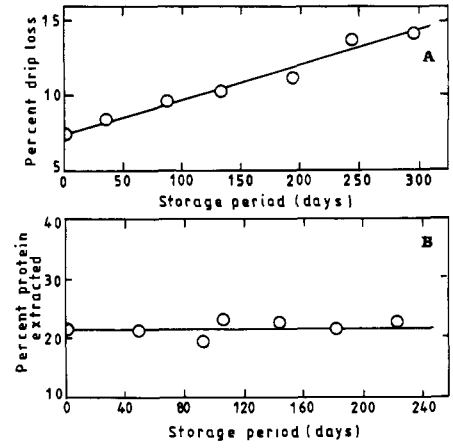

Figure 1. (A) Drip loss from blanched (95 °C for 120 s) prawns as a function of storage at −18 °C for different lengths of time. The drip loss is expressed as percentage based on frozen weight of prawns. (B) Effect of storage period on the protein extractability profile of blanched sample at −18 °C .

RESULTS AND DISCUSSION

The process of blanching on prawns has a significant effect on the drip loss with duration of storage at −18 °C (Figure 1A). The increase in rate of drip loss is 0.72 ± 0.03% for every month of storage at −18 °C, in comparison to unblanched sample which had a higher drip loss of 24% at the end of 300 days of storage at −18 °C (Figure 2A). In Figure 1B is shown the protein extractability at neutral pH as a function of storage period at −18 °C. There is a 4-fold decrease in solubility of protein as a result of

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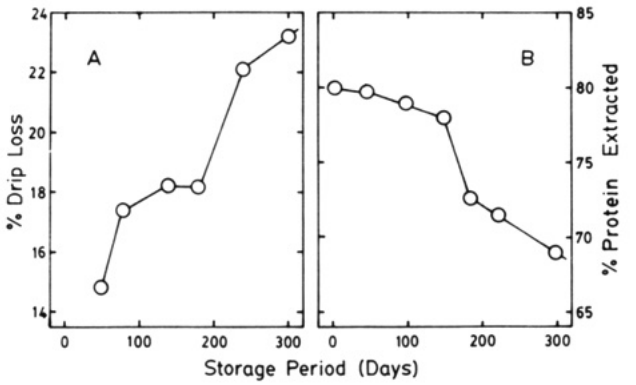

Figure 2. (A) Drip loss from unblanched prawns as a function of storage at −18 °C for different lengths of time. The drip loss is expressed as percentage based on frozen weight of prawns. (B) Effect of storage period on the nitrogen solubility profile of unblanched prawns at −18 °C.

blanching. Protein extractability in unblanched sample decreased to 65% after 300 days of storage (Figure 2B). This can be a result of heating and can arise from the process of aggregation as well as thermal unfolding of macromolecules resulting in decreased solubility. Such denaturation during the blanching process can change the physicochemical and functional properties of proteins from blanched prawn. To understand such a process, initially both ATPase and proteolytic activities in protein extract were determined. In the blanched sample neither ATPase nor proteolytic activity could be detected, whereas in unblanched sample the ATPase activity was 0.625 μg of phosphorus min^{-1} (mg of protein)$^{-1}$ and proteolytic activity was 0.70 μmol of tyrosine (mg of protein)$^{-1}$ h^{-1} (Shamasunder and Prakash, 1994a). The absence of enzyme activity in the blanched sample indicates thermal inactivation of these enzymes as a result of blanching.

The gel filtration profiles of proteins from blanched sample at different periods of storage at −18 °C are shown in Figure 3. The pattern is much different from that of unblanched sample in two ways. First, the percent fraction of high molecular weight component has decreased to a large extent, and second, there are no intermediate peaks in between the high molecular weight and low molecular weight proteins. However, due to the low solubility of proteins from blanched prawn, a large amount of protein could not be loaded onto the column due to limitations of loading volumes. The pattern throughout the storage period did not show any major changes except for the shift in the position of low molecular weight protein components.

Sedimentation velocity pattern of blanched sample up to 185 days of storage is given in Figure 4A. A single peak with $s_{20,w}$ value of 2.5S is seen for the blanched sample. This can also arise due to preferential solubilization of low molecular weight proteins only in the blanched sample, as compared to other fractions which might have undergone aggregation resulting in high molecular weight polymers which are less soluble. However, the 185-day-stored sample showed two minor peaks having sedimentation coefficients 6S and 8S, respectively (Figure 4A). The aggregation of proteins at low temperature may be responsible for these two peaks in the analytical ultracentrifuge. Such dissociation–reassociation induced by high temperature leading to denaturation process is reported for a number of proteins (Aoki and Sakurai, 1969; Oakes, 1976; Lakshmi et al., 1985).

The polyacrylamide gel electrophoresis (PAGE) pattern obtained for blanched samples stored for different periods

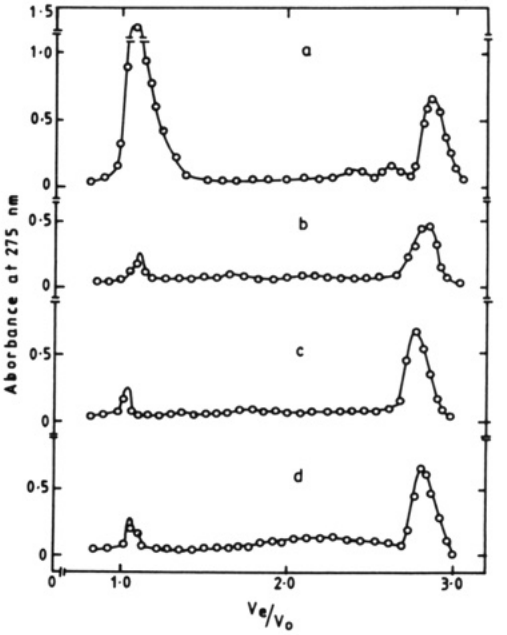

Figure 3. Effect of storage period of blanched prawn (95 °C for 120 s) frozen and stored at −18 °C on gel filtration profile in Sepharose 4B gel. The absorbance of each fraction was monitored at 275 nm. (a) Unblanched fresh [from Shamasunder and Prakash (1994a)]; (b) 50-day blanched prawn stored at −18 °C [after thawing, proteins were extracted in phosphate buffer (0.03 M, pH 7.8) containing 1 M NaCl]; (c) 148 days; (d) 290 days.

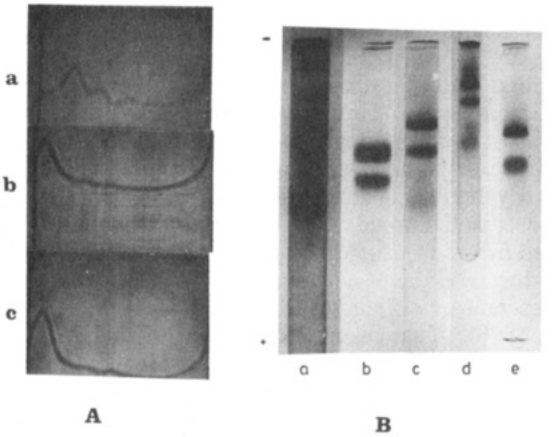

Figure 4. (A) Effect of storage period of blanched prawn (95 °C for 120 s) frozen and stored at −18 °C on the sedimentation velocity pattern of the total protein extract: (a) untreated sample; (b) 122-days stored; (c) 185-days stored. (B) Polyacrylamide gel electrophoresis pattern of total proteins from blanched prawn (95 °C for 120 s) frozen and stored at −18 °C for different periods of time up to 208 days: (a) untreated (fresh); (b) 55 days; (c) 120 days; (d) 164 days; (e) 208 days.

is given in Figure 4B. The pattern of blanched sample indicates more low molecular weight components. With progressive storage period, the generation of fast-moving components increases in concentration and the resolution of two major slow-moving bands is decreased, indicating possible aggregation of protein molecules. The dissociation–reassociation as a result of heating of both total proteins and multimeric homogeneous proteins from oil seeds are reported (Prakash and Narasinga Rao, 1986). Such a process can be accompanied by both aggregation and dissociation which will be in equilibrium as is shown for many proteins (Joly, 1965; Lakshmi et al., 1985).

The fluorescence emission spectra of blanched samples are shown in Figure 5. In blanched samples the emission spectra showed a blue shift of 5–8 nm up to 270 days of

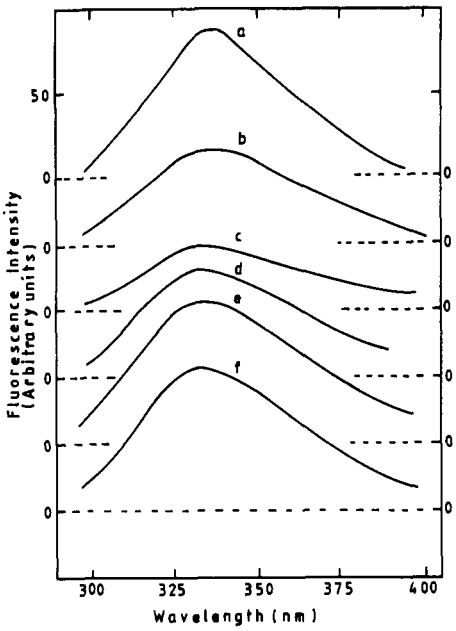

Figure 5. Effect of frozen storage of blanched (95 °C for 120 s) prawn on the fluorescence emission spectra in the range 300–400 nm of total proteins extracted. The proteins were extracted in phosphate buffer containing 1 M NaCl. (a) Unblanched fresh [from Shamasunder and Prakash (1993a)]; (b) 50 days of storage; (c) 103 days of storage; (d) 148 days of storage; (e) 200 days of storage; (f) 270 days of storage. To compare the fluorescence emission spectra (qualitatively), the ordinates are shifted such that there is no overlapping of individual spectra. The *Y*-axis is shown in arbitrary units.

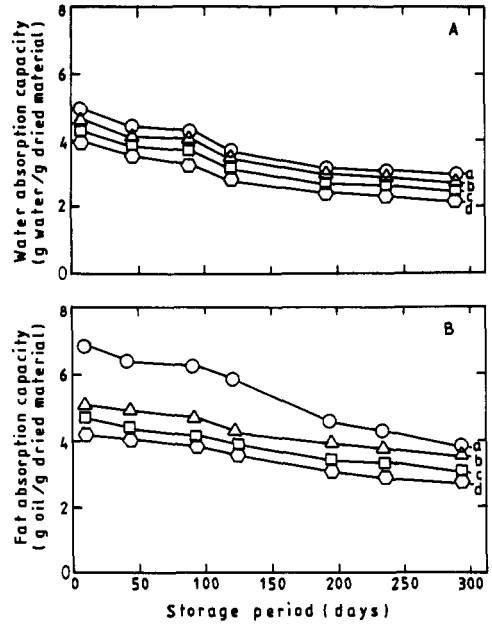

Figure 6. (A) Effect of storage period at −18 °C of prawn meat blanched for different periods on the water absorption capacity: (a) unblanched, frozen, and stored at −18 °C [from Shamasunder and Prakash (1994b)]; blanched at 95 °C for (b) 30, (c) 60, and (d) 120 s. The values are not significant ($P > 0.005$) between blanching treatments. (B) Fat absorption capacity of blanched prawns as a function of storage at −18 °C: (a) unblanched, frozen, and stored −18 °C [from Shamasunder and Prakash (1994b)]; blanched at 95 °C for (b) 30, (c) 60, and (d) 120 s. [The unblanched curve is shown for comparison taken from data from the preceding paper (Shamasunder and Prakash, 1994b).] The values are not significant ($P > 0.005$) between blanching treatments.

storage. The unblanched sample also shows a blue shift of 5 nm as a function of storage period (as reported in the preceding papers). Fluorescence emission spectra of blanched samples indicate that denatured protein has undergone association–dissociation phenomena, burying partly the exposed tryptophan groups. The fluorescence emission maximum of tryptophan or its ester is a sensitive parameter of solvent which surrounds tryptophanyl residue (Chabbret et al., 1991). Fish myosin, upon heating, undergoes initially a subtle change in conformation that promotes aggregation of hydrophobic residues (Wicker et al., 1989).

The viscosity of the extract from blanched sample does not show any significant change either in the value at zero protein concentration or of the slope of the line up to a concentration of 7 mg/mL. The value at zero protein concentration is reported to be 0.40 dL/g (Shamasunder and Prakash, 1994a). This gives an indication that the shape of dissociated and denatured protein does not alter during storage at −18 °C. The viscosity of the extract from unblanched sample at zero protein concentration is fairly constant (0.5 dL/g) during frozen storage (Shamasunder and Prakash, 1994b). However, the slopes of the lines are different with various storage periods, suggesting no significant alteration in the shape of protein during the process of storage.

Such changes in physicochemical properties may have a profound effect on surface active properties of proteins from prawn such as water absorption capacity (WAC), fat absorption capacity (FAC), foam capacity (FC), foam stability (FS), emulsification capacity (EC), and emulsion stability (ES).

The changes in WAC and FAC of blanched prawn during frozen storage are shown in Figure 6. One of the reasons for lower values of WAC and FAC in blanched sample as compared to unblanched sample is that the proteins from blanched sample are denatured and hence have lost the ability to hold water and fat. However, there are also reports that denaturation enhanced WAC in defatted sunflower meal (Huffmann et al., 1975; Tasneem and Prakash, 1994, unpublished results).

Foam capacity of the blanched sample was much lower than that of unblanched sample (Figure 7A). Prawns blanched for 30 and 60 s had higher foam capacity than prawns blanched for 120 s (Figure 7A). A stable foam results due to protein interactions resulting in a stable elastic membrane in solution (Kinsella, 1976). This possibly requires a three-dimensional network of proteins which is absent in the blanched sample as a result of heating. Hence, foaming properties of blanched sample are lesser as compared to those of unblanched sample. Foam stability was less in all blanched samples as compared to unblanched sample (Figure 7B). With increase in storage period up to 292 days there was no significant change in the foam stability values.

Emulsification capacity (EC) values of the blanched samples are shown in Figure 8A. The 120-s-blanched sample had higher EC value as compared to 30- and 60-s-blanched samples. Emulsion stability (ES) of the blanched samples was in the range 5–21 min (Figure 8 B). With increase in storage period at −18 °C the ES decreased in all samples, reaching a value of 4–6 min at 200 days of storage. The heat-treated proteins will have different ES values with a positive correlation between ES value and solubility of the protein (Voutsians et al., 1983).

The emulsifying ability of either protein or group of proteins is an intrinsic property and varies with the nature of the protein molecule and its surface properties. Being charged molecules, proteins lower the interfacial energy and retard droplet coalescence (Bull and Breese, 1976; MacRitchie, 1978). The surface properties of protein

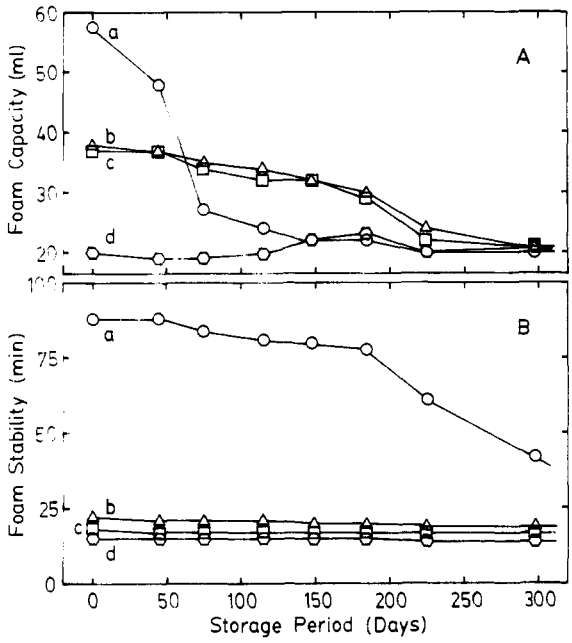

Figure 7. (A) Effect of storage period of prawns blanched for different periods on the foam capacity values: (a) unblanched, frozen, and stored at −18 °C [from Shamasunder and Prakash (1994b)]; blanched at 95 °C for (b) 30, (c) 60, and (d) 120 s. The values were significant ($P < 0.05$) between treatments. (B) Effect of storage period at −18 °C of prawns blanched for different periods on the foam stability: (a) unblanched, frozen, and stored at −18 °C [from Shamasunder and Prakash (1994b)]; blanched at 95 °C for (b) 30, (c) 60, and (d) 120 s. The values are not significant ($P > 0.05$) between treatments.

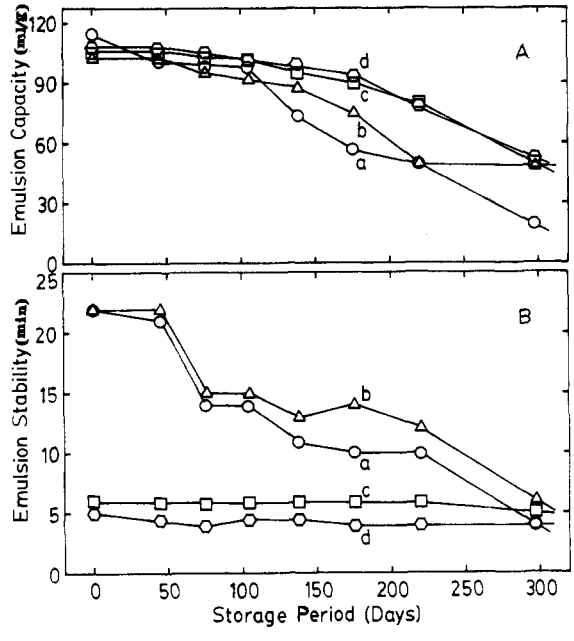

Figure 8. (A) Effect of storage period at −18 °C of the blanched sample on the emulsification capacity of total proteins extracted from prawns. The proteins were extracted in phosphate buffer (0.03 M, pH 7.8), and emulsification is expressed as milliliters of oil per gram of protein. (a) Unblanched, frozen, and stored at −18 °C [from Shamasunder and Prakash (1994b)]; blanched at 95 °C for (b) 30, (c) 60, and (d) 120 s. The values are not significant ($P > 0.05$) between treatments. (B) Emulsion stability of proteins extracted from blanched prawn as a function of storage period: (a) unblanched, frozen, and stored at −18 °C [from Shamasunder and Prakash (1994b)]; blanched at 95 °C for (b) 30, (c) 60, and (d) 120 s. The values are not significant ($P > 0.05$) between treatments.

molecules are sensitive to processing parameters such as blanching, which results in conformational changes. Such changes are known to alter emulsification properties (Graham and Phillips, 1975, 1977; Tarnberg, 1978). Although denaturation decreases EC, it need not be a general rule, since the process of denaturation many times results in increased emulsification properties as shown with other proteins (Smith et al., 1973; Wang and Kinsella, 1976; Aoki et al., 1980).

The results described in this paper indicate that alterations in the conformation of protein molecules as a result of blanching lead to changes in the functional properties of prawn proteins. An understanding of variation in physicochemical properties has a direct bearing on the prediction of functional properties and evaluation resulting from blanching. Also, blanching, even though it is a preprocessing step, alters the properties of the protein irreversibly, especially affecting the water and fat absorption capacities. These have a bearing on the end utilization of such pretreated prawns.

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