Effect of Sodium Acetate on Physicochemical Properties of Proteins from Frozen Prawns (*Metapenaeus dobsoni*)

B. A. Shamasunder and V. Prakash*

Department of Protein Technology, Central Food Technological Research Institute, Mysore 570 013, India

The effect of prefreezing dip treatment with 10% sodium acetate on the physicochemical properties of frozen prawn was investigated. The drip loss was higher in treated sample as compared to untreated sample during frozen storage. Sodium acetate favored initial aggregation and later dissociation of the prawn proteins. The dissociated molecules had a sedimentation coefficient of 6S as estimated from sedimentation velocity experiments. The gel filtration and polyacrylamide gel electrophoresis experiment suggests dissociation of high molecular weight to low molecular weight fractions as a result of sodium acetate treatment. The fluorescence emission maxima showed a blue shift from 335 to 330 nm with the untreated sample and from 335 to 328 nm with the treated sample during frozen storage. No significant change in intrinsic viscosity of salt-soluble protein extracted from both treated and untreated prawns was observed during frozen storage.

INTRODUCTION

The perishable nature of seafoods is a focal point of many investigations (Shenouda, 1980; Matsumoto, 1980). Prawn meat, which is considered to be a delicacy among aquatic foods, is susceptible for alterations in texture and organoleptic qualities during frozen storage. The changes in biochemical properties, subunit association of myofibrillar and sarcoplasmic proteins of shrimp muscle as a result of either partial freezing or storage in ice, have been studied (Fatima et al., 1988). During ice chilling the endomysium and perimysium intact structure can be degraded gradually (Nip and Moy, 1988). The changes in prawn proteins during frozen storage as measured by different physicochemical and biophysical techniques mainly reflect such alterations that occur in myosin–actomyosin equilibrium (Godavaribai et al., 1987).

The effects of certain salts from the Hofmeister series on proteins are known to be protective agents for denaturing proteins (Jencks, 1969). The nature of salt has an overriding effect on the concentration of salt itself (Jencks, 1969). These salts can be described as those that contain sulfate, phosphate, acetate, or fluoride, which tend to precipitate proteins and protect them against denaturation and dissociation, whereas salts such as thiocyanate, iodide, perchlorate, lithium, calcium, or barium tend to dissolve, denature, and dissociate proteins (von Hippel and Wong, 1964; Robinson and Jencks, 1965). In the present investigation the effect of the sodium salt of acetate is investigated in detail as a prefreezing dip treatment of prawn. The physicochemical properties of total proteins during frozen storage at −18 °C after treatment with sodium acetate are reported.

MATERIALS AND METHODS

Fresh prawns (*Metapenaeus dobsoni*) were used for the study and were caught fresh off the Mangalore Coast of western India.

Acrylamide, bis(acrylamide) [*N,N'*-methylenebis(acrylamide)], *N,N,N',N'*-tetramethylethylenediamine (TEMED), sodium dodecyl sulfate, 2-mercaptoethanol, amido black, adenosine triphosphate disodium salt, and Sepharose 4B gel were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Hemoglobin powder was obtained from Worthington Biochemicals (Freehold, NJ). Dialysis tubings with molecular weight cutoff of 3000 were obtained from Thomas Scientific Co. (Philadelphia, PA).

Sodium Acetate Treatment. To determine the optimum concentration of sodium acetate for prefreezing dip treatment of prawns, four different concentrations of sodium acetate, *viz.* 5%, 10%, 15%, and 20% (w/v) solutions, were used. The prawns were dipped in sodium acetate solutions of pH 8.3 at 10 °C for 15 min. The ratio of dip solution to prawn was 2:1. The excess solution was drained off by gentle pressure on the prawn. Sodium acetate was estimated in meat as described later in this section. The optimum dip time (Figure 1B) was arrived at after the sodium acetate content of the meat dipped in various concentrations of the salt was estimated and the drip loss was monitored for various lengths of time of storage using a pilot study.

Estimation of Acetate in Prawns. The acetate content in the prawn meat was estimated by conductivity method using a Consort Model K-220 conductivity meter calibrated with sodium acetate salt. Prawn meat (3.0 g) was macerated well in a pestle and mortar with 30 mL of distilled water. The slurry was centrifuged in a Sorvall RC-5B refrigerated centrifuge at 3500*g*. The conductivity of the clear solution was measured. A standard graph was obtained using sodium acetate at different concentrations. Sodium acetate in prawn meat was calculated from the standard graph.

Freezing and Storage of Prawns. The sodium acetate dipped prawns (treated) along with untreated prawns were packed in small trays (60-mm height) and frozen in a contact plate freezer (Toyo make) with a plate temperature of −30 °C. The freezing time was 4 h. After freezing, the samples were stored at −18 °C and removed periodically for measurement of the physicochemical properties of proteins. The frozen blocks after being taken out from the freezer were weighed immediately. Thawing was performed at 7 ± 2 °C for 6 h. After thawing, the temperature of prawns was 8 °C. The samples were weighed again, and the difference in weight was expressed as percentage of drip. The drip was also collected for analysis of protein, analytical ultracentrifugation, PAGE, and amino acid composition.

Extraction of proteins from prawn, determination of protein concentration, fluorescence spectra, polyacrylamide gel electrophoresis, gel filtration, sedimentation velocity experiments, viscosity, and enzyme activity measurements (both adenosine triphosphatase and proteolytic activity) are as described in the preceding manuscript (Shamasunder and Prakash, 1994).

Statistical Analysis. The data on the drip loss were subjected to the Wilcoxon signed ranks test (Fowlie, 1969). ANOVA was applied to test the significance of treatment on ATPase and proteolytic enzymes.

* Author to whom correspondence should be addressed (contact telephone 91-821-20131; contact telex 846-241-FTRI-IN; contact fax 91-821-27697).

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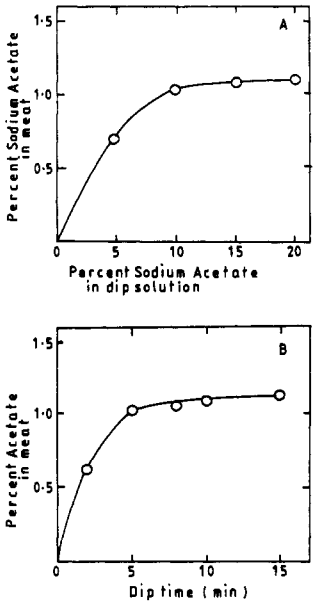

Figure 1. (A) Effect of increasing concentration of sodium acetate in dip solution for 15 min on the acetate uptake in meat at 27 °C with a prawn to dip solution ratio of 1:2. (B) Effect of dip time in sodium acetate solution on the percent uptake in meat at 27 °C. The concentration of sodium acetate in dip solution was 10%.

RESULTS AND DISCUSSION

The effect of different concentrations of sodium acetate present in dip solution as a function of sodium acetate uptake in meat is shown in Figure 1A. During a 15-min dipping, the uptake was linear up to 10% sodium acetate (Figure 1A). When the prawn was dipped in 10% sodium acetate, the uptake was almost kept at a constant level after 5 min of dipping (Figure 1B). Accordingly, the concentration of sodium acetate and dipping time were fixed at 10% and 5 min, respectively, in the following experiments.

The drip loss as a function of storage period for untreated and treated prawns is given in Table 1. The results show that increase in drip loss was nearly the same both in the untreated and in the treated samples up to 240 days of storage. At the end of 300 days of storage, a drip loss of 27.8 ± 1.9% was recorded for treated sample, whereas in untreated prawn it was 23.2 ± 1.6%. Hence, the drip loss is significantly different as analyzed by the Wilcoxon signed rank test (Fowlie, 1969). These results suggest that acetate dip treatment prior to storage at −18 °C increases the drip loss.

The calcium-activated ATPase activities of protein extract of both untreated and treated prawn are given in Table 2. In both samples the loss in ATPase activity was about 80% at 0-day value with a storage period of 40 days. The proteolytic activity of prawn muscle extract during frozen storage decreased in both samples, but the activity of the enzyme could still be detected even at the end of the 300 days of storage. Fish muscles are known to contain several lysozymal enzymes including proteinases, and the progressive decrease in proteolytic activity of mackeral, shark, and pompfret meat during frozen storage has been reported (Warrier et al., 1988). In the present study the ATPase and proteolytic activities are not affected by the addition of sodium acetate ions.

Such changes in drip loss and enzymatic activity can also be accompanied by changes in physicochemical properties of proteins.

The gel filtration profile of proteins extracted from untreated prawn over a period of 220 days is shown in Figure 2. As a function of storage at −18 °C, there is no major change in gel filtration profile except for a decrease in concentration of peak eluting at V_e/V_0 of 2.8 (Figure 2Ac). Possibly this is the fraction that is leached out with the drip solution. At 80 days of storage the pattern is different, with an additional shoulder resolving at V_e/V_0 of 1.30. Above this period of storage, such as at 174 and 220 days, the trailing edge of a peak eluting at V_e/V_0 of 1.0 is observed. These results indicate that during the first 80 days of storage there is a depletion of low molecular weight proteins after which the dissociation of high molecular weight proteins occurs.

The effect of sodium acetate dip treatment on the gel filtration profiles of frozen stored prawn sample is shown in Figure 2B. Unlike in the untreated sample, a shoulder exists at V_e/V_0 of 1.30 (Figure 2Bb). With further increases in storage periods to 98 and 146 days, the gel filtration pattern shows a decrease in the area under the major component eluting at V_e/V_0 of 1.2 with the trailing edge present. The pattern of samples stored for 183 and 223 days shows an increase in low molecular weight components eluting at V_e/V_0 of 2.85 with a concomitant decrease in high molecular weight proteins. These results suggest that sodium acetate brings about dissociation of the protein as compared to untreated sample. In the present study the proteins are separated into two major components which can be attributed to the actomyosin complex (eluting just after the void volume) and the another component which is a low molecular weight component eluting near the bed volume of the column. With the increase in storage time the dissociation of actomyosin complex is rapid up to 146

Table 1. Effect of Storage Period of Untreated and Treated Prawns on Drip Loss

	% drip loss[a]					
treatment	50 days	80 days	140 days	180 days	240 days	300 days
untreated	14.8 ± 1.5	17.4 ± 1.8	18.2 ± 1.1	18.2 ± 1.4	22.1 ± 1.8	23.2 ± 1.6
10% sodium acetate treated	18.1 ± 0.85	20.2 ± 1.7	21.3 ± 1.1	23.0 ± 1.4	23.0 ± 1.7	27.8 ± 1.9

[a] Significant ($P < 0.05$) between treatments; ± standard deviation, $n = 3$.

Table 2. ATPase and Proteolytic Activity of Sodium Acetate Treated and Untreated Prawn Muscle Extract during Frozen Storage

	ATPase activity[a] after frozen storage of							proteolytic activity[b] after frozen storage period of						
sample	0 days	40 days	80 days	120 days	180 days	240 days	300 days	0 days	50 days	100 days	150 days	200 days	250 days	300 days
untreated prawn	100[c]	28[c]	25[c]	24[c]	26[c]	23[c]	22[c]	0.70[c] ± 0.08	0.72[c] ± 0.10	0.60[c] ± 0.08	0.60[c] ± 0.14	0.58[c] ± 0.13	0.4[c] ± 0.12	0.45[c] ± 0.10
10% sodium acetate treated prawn	100[c]	27[c]	24[c]	23[c]	23[c]	22[c]	22[c]	0.70[c] ± 0.08	0.75[c] ± 0.18	0.75[c] ± 0.14	0.70[c] ± 0.19	0.6[c] ± 0.20	0.6[c] ± 0.15	0.6[c] ± 0.15

[a] ATPase activity expressed as μg of phosphorus per mg of protein per min. The initial reading is taken as 100% activity. [b] Proteolytic activity expressed as μmol of tyrosine per mg of protein per h. [c] Values are not significantly ($P > 0.05$) different.

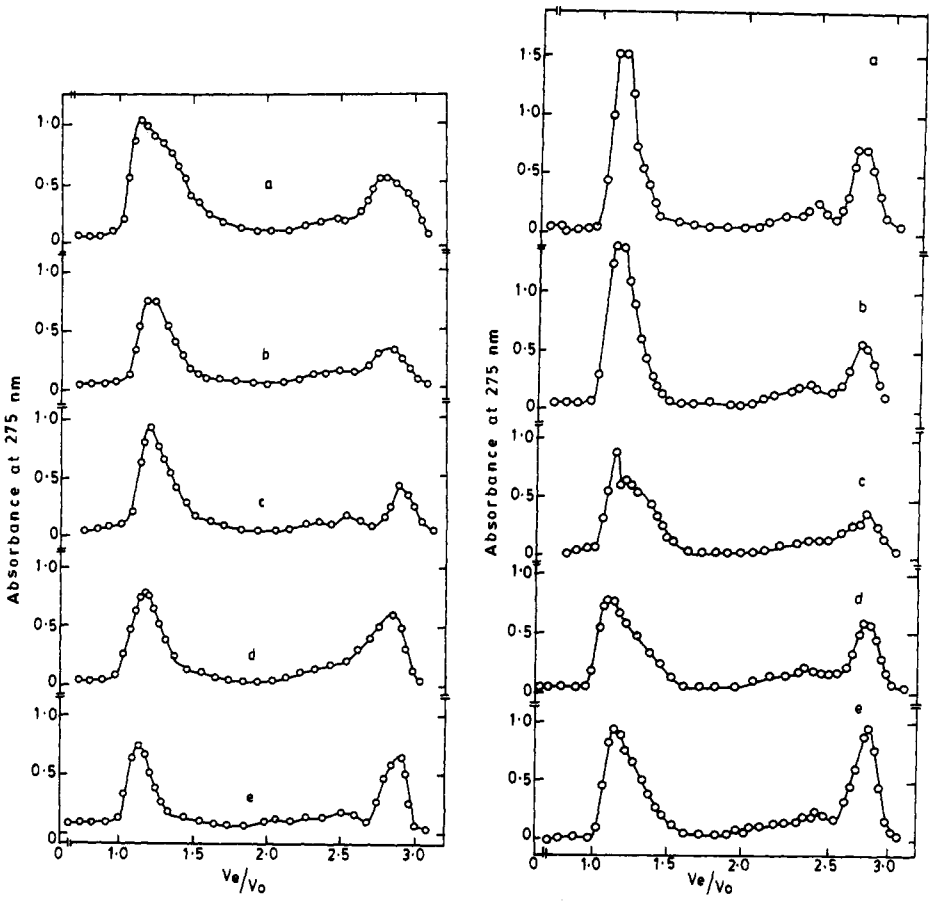

Figure 2. Effect of storage period at −18 °C on the gel filtration profile in Sepharose 4B gel of protein extracted from untreated and 10% sodium acetate treated prawns. X-axis represents the ratio of elution volume (V_e) of each fraction to that of void volume (V_0) of the column. The absorbance of the fractions was monitored at 275 nm. (A, left) Untreated prawns stored for (a) 38, (b) 80, (c) 128, (d) 174, and (e) 220 days. (B, right) 10% acetate treated prawns stored for (a) 48, (b) 98, (c) 146, (d) 183, and (e) 223 days.

days of storage in treated sample, and thereafter the dissociation rate was the same in both untreated and treated samples. The pattern of gel filtration profile of other fishes such as cod also suggests a dissociation process of high molecular weight components during frozen storage (Ohnishi and Rodgar, 1980).

The effect of storage period at −18 °C on the sedimentation velocity pattern of untreated and treated prawns is shown in Figure 3A. The sedimentation velocity pattern of proteins extracted from fresh untreated prawn shows four components with $s_{20,w}$ values of 6S, 11S, 14S, and 20S consisting of 60%, 25%, 10%, and 5%, respectively (Figure 3Aa). With progressive storage period, the concentration of fast-moving components, especially 14S and 20S, decreases with a concomitant increase in the 6S component (Figure 3Ab–d). By 185 days of storage the entire sedimentation velocity pattern is represented by the 6S component, indicating progressive dissociation of high molecular weight components during storage at −18 °C. The effect of sodium acetate on the sedimentation velocity pattern as a function of storage period is given Figure 3Ae–g. The results indicate that even at 112 days of storage the pattern is completely represented by the 6S component. The pattern is much different from that of untreated samples. The fast-moving component was observed in treated sample after 195 days of storage. The results from sedimentation velocity experiments suggest that acetate alters the association–dissociation phenomenon of total proteins from prawn as a function of storage at −18 °C. The extent of the effect depends on the length of storage period at −18 °C.

The native PAGE pattern of proteins extracted from untreated prawns stored for different periods of time at −18 °C is shown in Figure 3B. The pattern is dominated by fast-moving components, and two bands representing high molecular weight proteins represent myosin. With progressive storage period the concentration of fast-moving component increases with a concomitant decrease of slow-moving component. By 164 days of storage three faint bands are seen (Figure 3Bd). The concentration of these bands increases, as can be seen in the pattern of 208 days of storage. The PAGE pattern of total protein of 10% sodium acetate treated prawns at different periods of storage at −18 °C is also shown in Figure 3C. The pattern of 120 and 208 days has more fast-moving components in it, very similar to that of the protein extract from untreated prawn. However, one should be cautious in interpreting the PAGE pattern of proteins treated with salt, since salt can bind to protein and alter the overall charge along with the association–dissociation phenomenon (Cann, 1970) even though experiments are performed by dialyzing out the excess salt at low ionic strength. Such results are reported in the literature for many protein systems (Catsimpoolas et al., 1970). This aggregation process might have arisen from a dissociated protein molecule as evidenced from analytical ultracentrifuge and gel filtration experiments. It is also possible that part of the tryptophanyl residues which are exposed to solvent during the process of dissociation might be exposed to higher dielectric constant generated by the microenvironment of charged groups. This is monitored by fluorescence spectroscopy.

In Figure 4 is shown the fluorescence emission spectra of proteins extracted from untreated and treated prawns

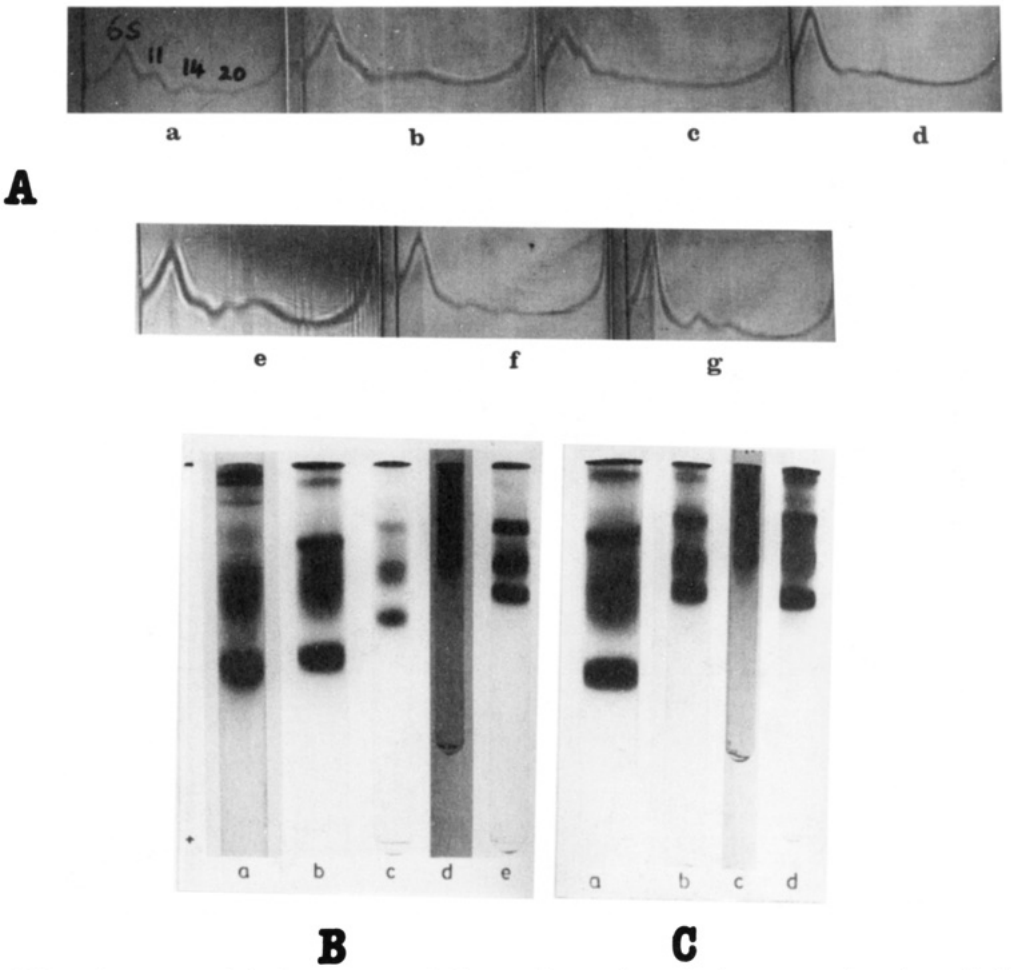

Figure 3. (A) Effect of storage period of prawns at −18 °C on sedimentation velocity pattern of total protein from prawns. The sedimentation proceeds from left to right. The photographs were taken 60 min after two-thirds maximum speed was reached at a bar angle of 55°. The prawn samples were (a) untreated fresh; untreated prawns stored at −18 °C for (b) 43, (c) 110, and (d) 185 days; 10% sodium acetate treated for 5 min before freezing and storage at −18 °C for (e) 50, (f) 112, and (g) 195 days. (B) Polyacrylamide gel electrophoresis pattern of total proteins extracted from untreated prawn stored at −18 °C for different periods of time up to 260 days of storage. The prawn samples were untreated sample stored for (a) 0, (b) 55, (c) 120, (d) 164, and (e) 208 days and (C) 10% sodium acetate treated sample for 5 min before freezing and stored at −18 °C for (a) 55, (b) 120, (c) 164, and (d) 208 days.

stored at −18 °C for different lengths of time. The fluorescence emission spectrum of fresh untreated proteins from prawn has an emission maxima of 335 nm and arises principally from tryptophanyl residues. With progressive increase in storage period up to 280 days, the emission maximum shifts to a lower wavelength of emission (blue shift). The shift of 5 nm in untreated sample during storage at −18 °C indicates that tryptophan residues are being embedded inside the protein molecule during storage due to either aggregation or temperature-induced association phenomena. By 42 days of storage, the spectrum showed a blue shift to 332 nm and remained constant up to 220 days of storage, after which time the emission maxima further shifted to 328 nm. This sharp change in emission maximum might have arisen due to rapid association–dissociation of protein by acetate ions resulting in embedding of tryptophanyl residues as shown by gel filtration and analytical ultracentrifuge experiments. The present study demonstrates that in sodium acetate treated sample the process of initial aggregation is very minimal and the process of dissociation is predominant during extended periods of storage as judged by gel filtration profile, sedimentation velocity pattern, and PAGE pattern. These results of fluorescence suggest that the microenvironment around tryptophanyl residues is altered as a result of sodium acetate treatment and storage at −18 °C.

The viscosity values of total proteins from untreated prawns as a function of storage period are shown in Figure 5. The viscosity at zero protein concentration of fresh untreated protein from prawn is 0.4 dL/g and does not vary during frozen storage up to 298 days (Figure 5). However, the slope of the line appears to be different with storage period, suggesting a change in shape and size of the protein molecule during the process of storage. The result does not exclude the constancy of shape of protein during storage since preferential hydration phenomenon can counteract and still one can obtain the same viscosity value (Jencks, 1969). The effect of sodium acetate ions on the viscosity of total proteins from prawn stored at different periods of time is shown in Figure 6. The extrapolated value at zero protein concentration is 0.5 dL/g. The viscosity results are also analyzed by plot of apparent viscosity as a function of storage period from the data at 1% protein concentration (Figure 7). In the untreated sample a large difference from 0 to 50 days of storage was observed (Figure 7a). In the case of sodium acetate treated sample the increase in reduced viscosity was maximum at 220 days of storage (Figure 7b). This derivative graph clearly indicates that there are minimal changes in reduced viscosity of 1% protein solution treated with sodium acetate as compared to untreated sample.

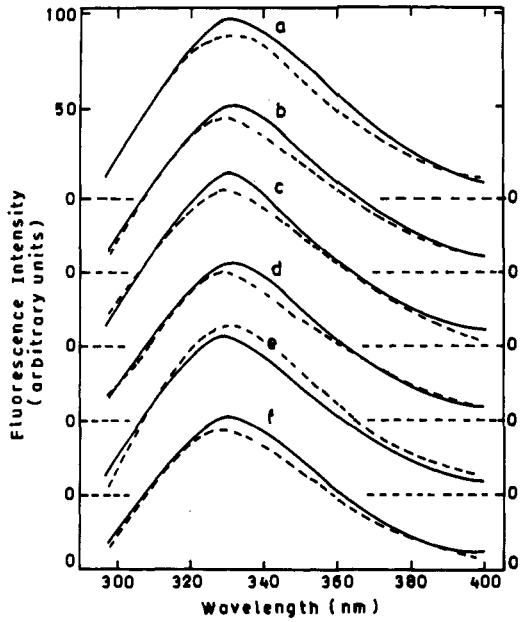

Figure 4. Effect of storage period at −18 °C on the fluorescence emission spectra in the range 300–400 nm of total protein from untreated and acetate treated prawn. (—) Untreated prawns stored at −18 °C for (a) 38, (b) 80, (c) 128, (d) 174, (e) 210, and (f) 280 days. (- - -) 10% sodium acetate treated sample for 5 min before freezing and storage at −18 °C for (a) 48, (b) 102, (c) 140, (d) 180, (e) 220, and (f) 302 days. To compare the fluorescence emission spectra (qualitatively), the ordinates are shifted such that there is no overlapping of individual spectrum. The *Y*-axis hence is shown in arbitrary units.

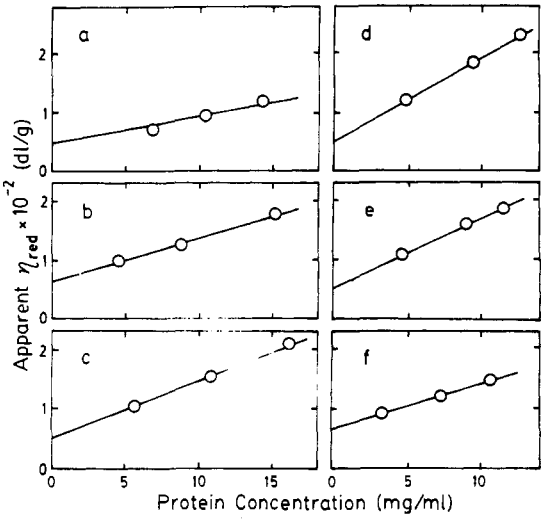

Figure 5. Effect of storage period of prawn frozen and stored at −18 °C on the apparent reduced viscosity at 25 ± 0.5 °C of the total protein from untreated and sodium acetate treated prawn: untreated prawn stored for (a) 38, (b) 80, (c) 120, (d) 174, (e) 220, and (f) 298 days.

It is well-known that proteins in solution undergo physicochemical changes as a result of addition of solutes. A change in protein structure is favored in the presence of different salts through salt–peptide interaction. These effects are opposed by the salting out effect of amino acid side chains, especially nonpolar groups (Nandi and Robinson, 1972). One approach is to extract total proteins from the system to which is added the desired concentration of salt and to monitor the effects using various physicochemical techniques with proper controls. Such experiments would generate data under controlled conditions and will help in understanding the effects of salts

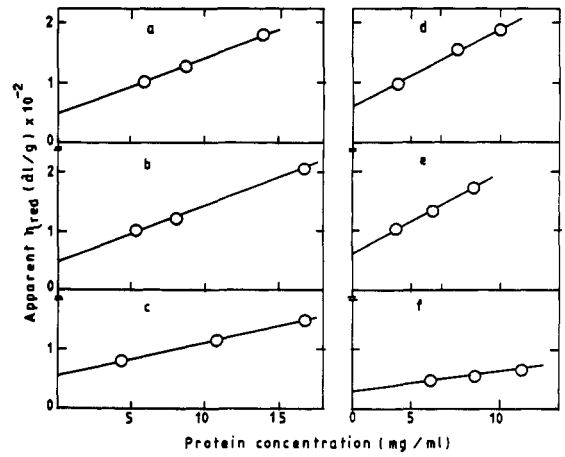

Figure 6. Effect of storage of prawn dipped in 10% sodium acetate for 5 min and stored at −18 °C as the apparent reduced viscosity at 25 ± 0.5 °C of the total protein: (a) 48, (b) 98, (c) 146, (d) 183, (e) 220, and (f) 302 days.

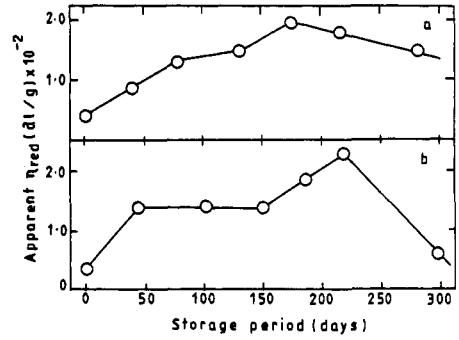

Figure 7. Effect of storage period of frozen prawns at −18 °C on the apparent reduced viscosity 1% at 25 ± 0.5 °C protein solution: (a) untreated prawns frozen and stored at −18 °C; (b) 10% acetate solution treated for 5 min before freezing.

in vivo from both mechanistic and energetics points of view.

The sedimentation velocity profiles of untreated dialyzed sample and sample equilibrated with different concentrations of sodium acetate are shown in Figure 8. The untreated protein in the presence of 1 M NaCl sediments into four components with $s_{20,w}$ values of 6S (60%), 11S (25%), 14S (10%), and 20S (5%). Upon dialysis to remove NaCl, the pattern is predominantly that of the 6S component (Figure 8Aa). This indicates that salt removal has resulted in either poor solubilization of fast-moving components, that is 10S, 14S, and 20S, or the above fractions in low ionic strength might have dissociated to the 6S component. In the presence of different concentrations, of acetate the sedimentation velocity pattern does not show any appreciable change (Figure 8Ab,c) as compared to control.

The electrophoretic mobility of the control (protein solution dialyzed vs phosphate buffer) and treated (protein solution dialyzed vs different concentrations of acetate) samples is given in Figure 8B. With an increase in the concentration of acetate there is a decrease in mobility (Figure 8C). It is also possible that the binding of acetate ions to the protein itself would alter the electrophoretic mobility. The representative gel filtration patterns at 2% sodium acetate concentration and the control are given in Figure 9. The control pattern is represented by low molecular weight proteins eluting near the bed volume of the column. In the presence of 2% sodium acetate, the pattern is almost similar to that of the control except for a small shoulder seen at V_e/V_0 of 1.2. These results are in good agreement with sedimentation velocity and PAGE

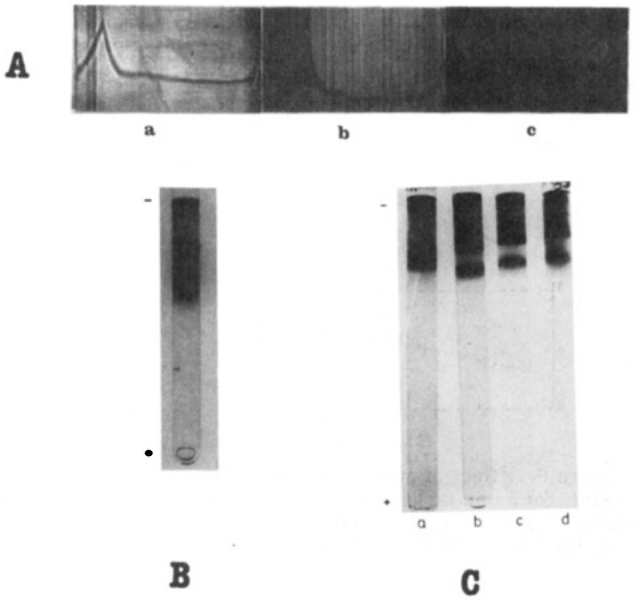

Figure 8. (A) Effect of sodium acetate at different concentration on sedimentation velocity profile of protein extracted from prawns (*in vitro* studies). The clear supernatant of the dialyzed protein solution was taken for the experiment. The sedimentation proceeds from left to right. The photographs were taken 60 min after two-thirds maximum speed was reached at a bar angle of 55°: (a) untreated (dialyzed vs phosphate buffer); (b) 1% acetate treated; (c) 3% acetate treated. (B) PAGE pattern of proteins extracted from prawn in phosphate buffer (0.03 M, pH 7.8) and dialyzed vs the same buffer. (C) Effect of sodium acetate dip at different concentration on the PAGE pattern of proteins extracted from prawn in phosphate buffer (0.03 M, pH 7.8) and dialyzed vs phosphate buffer (0.03, pH 7.8) containing desired concentration of acetate for 20 h. The clear supernatant of the dialyzed protein solution was taken for the experiment; 100 μg of protein was loaded to the gel for electrophoresis. The acetate concentrations used were (a) 0.5%, (b) 1%, (c) 2%, and (d) 3%.

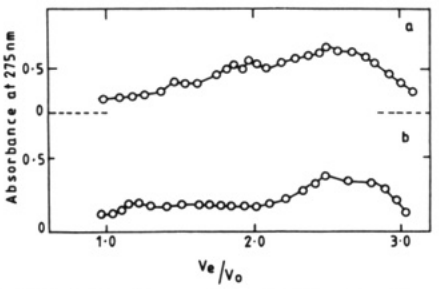

Figure 9. Effect of sodium acetate at 2% level on the gel filtration profile in Sepharose 4B gel of total proteins extracted from prawn studies. The proteins were extracted in phosphate buffer (0.03 M, pH 7.8) containing 1 M NaCl and dialyzed vs the same buffer containing 2% concentration of acetate: (a) untreated; (b) 2% sodium acetate treated protein extract.

pattern, suggesting there is no significant change in protein pattern as compared to control.

These data show that acetate ions favor dissociation of the prawn proteins initially and the dissociated proteins reaggregate. This is also supported by *in vitro* studies. However, unless information on the extent of conformational changes of individual specific proteins, the effect of proteolytic enzymes, and the effect of individual Hofmeister series salts on water structure is generated, a total interpretation of the observed results in terms of the effect of acetate ions would be only an initial attempt to understand the system. Explaining the detailed mechanism of the behavior of various ions as reflected by Hofmeister series salts at present is difficult if not impossible. According to von Hippel and Hamabata (1973), such information would be valid when information regarding the equilibrium structure of water around various groups of macromolecules becomes available.

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