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CHANGES IN THE ELECTROPHORETIC PATTERNS OF WATER SOLUBLE PROTEINS OF FISH AND SHRIMP DURING STORAGE

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Changes that occur in the electrophoretic protein patterns of thirteen marine fish and two shrimp species during ice and frozen storage were studied using polyacrylamide gel electrophoresis. Difference in the electrophoretic mobility of proteins was attributed to different rates or mechanisms of protein denaturation. It may also be due to the addition and/or loss of small molecules. Merits of polyacrylamide gel electrophoresis technique as an index of spoilage during various stages of ice storage are discussed.

INTRODUCTION

Gel electrophoresis of water soluble proteins of fish muscle is now widely used for the identification of species[1–4]. It has been shown to be particularly sensitive method for differentiating species which are difficult to identify on morphological criteria alone.

The edibility of fish is determined mainly by their appearance, odour, texture and flavour. Although these attributes have proved useful in judging the quality of fish, they do not provide a sound basis for quality assessment these days for sound merchandizing, where increasingly large amounts of fish are being used for various processed products. It is therefore, imperative that processors have the means to make objective judgements of their raw material.

In their previous communication[5], the authors reported the practical application of polyacrylamide gel electrophoresis method for the identification of fish and shrimp in the absence of identifiable features. Electrophoretograms obtained were sufficiently reproducible to demonstrate the practical application of this method for distinguishing fillets of different fish varieties. It was of interest to evaluate this technique as an objective method for the determination of quality of fish and shrimp during storage.

The present studies have been undertaken to determine the changes in electrophoretograms of fish and shrimp after 1 week storage in ice (0°) and after 6 months storage at –15°C to –20°C. In addition attempts have also been made to evaluate the merits of this technique as an index of shrimp quality at various stages of spoilage.

MATERIALS AND METHODS

Harbour fresh edible fish and shrimp caught at the Karachi Makran coast were studied in the experiment. Ice Storage: Whole shrimp and fish after gutting and deheading were packed in polythene bags placed in crushed ice and stored in refrigerator maintained at 5 $\pm$ 1°. Melted ice was drained off every day and ice was continuously replenished. Samples were removed after one week storage for analysis. In another experiment shrimp stored in the similar way were removed after 1, 3, 5, 7, 9, 11, 13 and 15 day ice storage. Frozen Storage: Whole deheaded shrimp and gutted and deheaded fish were packed in polythene bags and frozen at –20° in a freezing cabinet. These were removed for analysis after six months.

Preparation of the Sample. The fish were skinned and filleted and masculature from both sides used for the study. For analytical purposes, fish and shrimp tissue was homogenized with distilled water. After centrifugation the supernatant was subjected to polyacrylamide gel electrophoresis[5].

RESULTS AND DISCUSSION

Electroporetic Patterns of One Week and Six Months Stored Samples of Different Species. At least three measurements were performed on different samples of fish of each variety. The reproducibility of the test was satisfactory. The electrophoretic patterns of fresh samples, ice stored samples and frozen samples are presented in Figs. 1, 2, and 3 respectively which are readily distinguishable. The position of band number in gel is ascending towards 'n' where 'o' is the origin as demonstrated thereon. In general, one week ice storage influenced the electrophoretic patterns more than six months frozen storage when compared with the fresh samples. For the purpose of discussion, changes in electrophoretic patems of individual samples are discussed separately.

1. Kergan *(Pelamys sp.):* Compared with the freshl

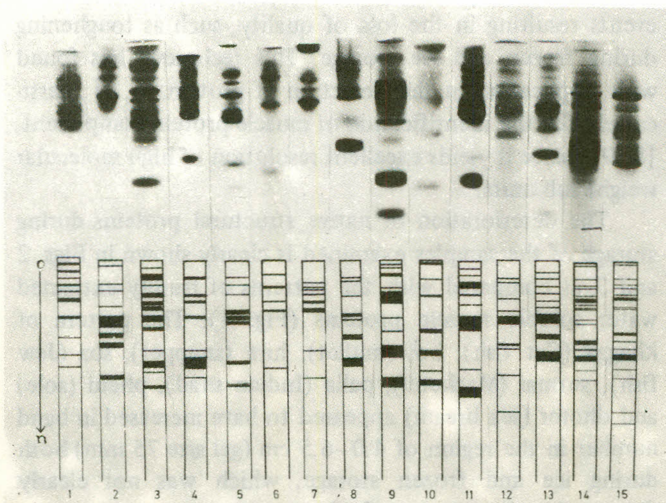

Fig. 1. Electrophoretograms of fresh muscle extracts of edible fish and shrimp found Karachi-Mekran coast.

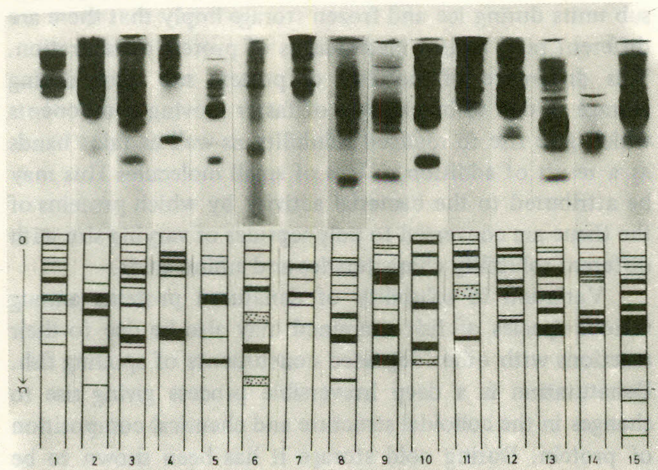

Fig. 2. Electrophoretograms of muscle extract of edible fish and shrimp found around Karachi-Mekran coast (Stored in ice for 1 week).

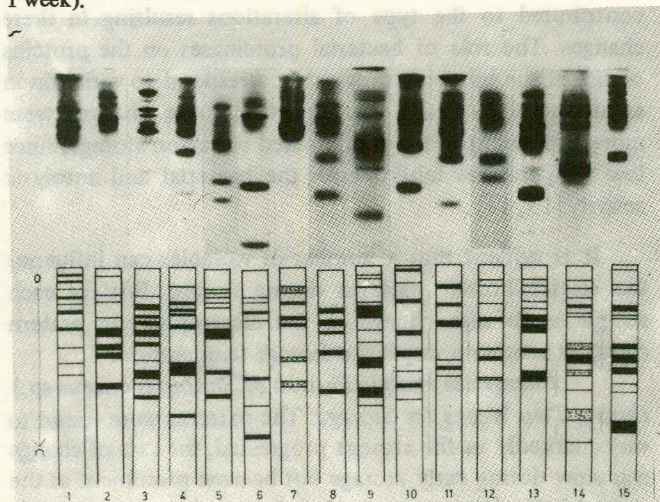

Fig. 3. Electrophoretograms of muscle extract of edible fish and shrimp found around Karachi-Mekran coast. (Stored at -20° for 6 months).

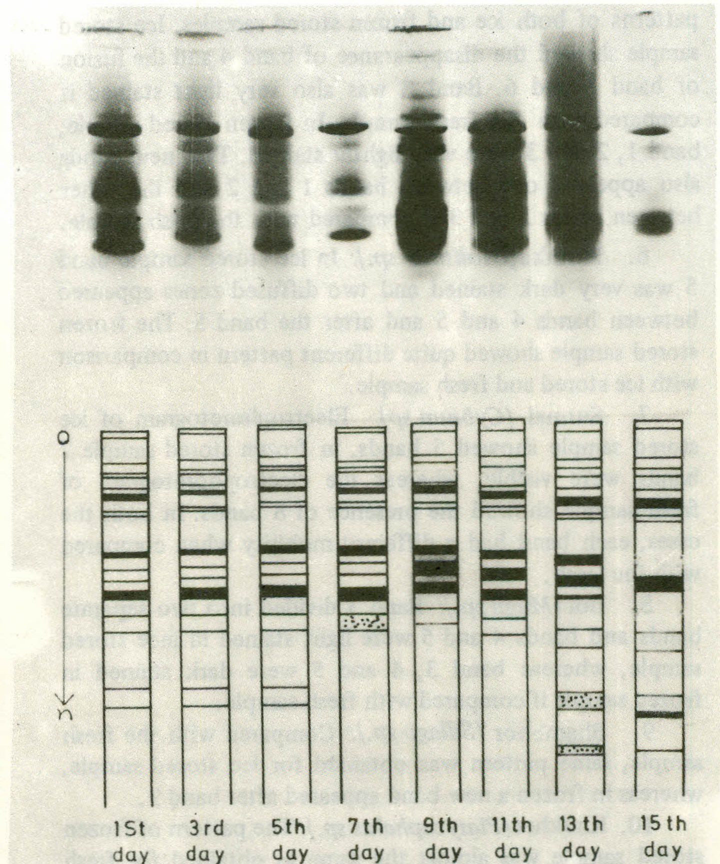

Fig. 4. Electrophoretograms of muscle extract of shrimp found around Karachi-Mekran coast. (During storage in ince) Jaira (Penaeus Sp.).

(Fig. 1) a remarkably similar pattern was obtained for the frozen stored sample (Fig. 3). In case of ice stored, bands 1 and 2 were light stained whereas band 7 was diffuse.

2. Sua *(Sciaena sp.)*: No difference was noticeable in the patterns of fresh and frozen samples. However, ice stored sample showed the disappearance of bands 1, 2 and 7 alongwith the appearance of a new band (after band no. 8 of fresh sample) if compared with the pattern of the fresh sample.

3. Dhoter *(Paristopoma sp.)*: Changes in electro–phoretic pattern were obtained both in ice and frozen stored samples if compared with fresh sample. Bands 2, 4 and 7 were very light stained in ice stored sample whereas bands 1 and 2 disappeared in frozen stored sample.

4. Khagga *(Arius sp.)*: Electrophoretograms of both the ice and frozen stored samples showed changes if com–pared with the fresh sample. Bands 1, 5 and 7 disappeared in ice stored sample, but a new band appeared between band 3 and 4 of fresh sample. In frozen stored sample band 1 was very light stained and two new bands appeared, one between band 3 and 4 and the other after band 8. Bands 5 and 7 completely disappeared.

5. Hira *(Lutijanus sp.)*: Changes were obvious in the

patterns of both ice and frozen stored samples. Ice stored sample showed the disappearance of band 4 and the fusion of band 5 and 6. Band 8 was also very light stained if compared with the fresh sample. In frozen stored sample, band 1, 2 and 3 were very lightly stained. Two new bands also appeared one between bands 1 and 2 and the other between bands 2 and 3 if compared with the fresh sample.

6. Mushka *(Otolithus sp.)*: In ice stored sample band 5 was very dark stained and two diffused zones appeared between bands 4 and 5 and after the band 5. The frozen stored sample showed quite different pattern in comparison with ice stored and fresh sample.

7. Surmai *(Cybium sp)*: Electrophoretogram of ice stored sample showed 5 bands, in frozen stored sample 7 bands were visible, whereas the electrophoretogram of fresh sample showed the presence of 8 bands. In both the cases, each band had a different mobility when compared with the fresh.

8. Boi *(Mugil sp.)*: Band 3 divided into two separate bands and bands 4 and 5 were light stained in ince stored sample, whereas band 3, 4 and 5 were dark stained in frozen sample if compared with fresh sample.

9. Bhambhor *(Sillago sp.)*: Compared with the fresh sample, same pattern was obtained for ice stored sample, whereas in frozen a new band appeared after band 9.

10. Khukher *(Platycephalus sp.)*: The pattern of frozen stored sample was almost the same as obtained for fresh sample except for the appearance of a minor band after band No. 7. In ice stored sample, band 2 was dark stained, band 6 disappeared and diffused zone appeared after band No. 7.

11. Sankh *(Muraena Sp.)*: In ice stored sample band 6 diffused and 8 disappeared. In frozen sample bands 1, 2, 4 and 5 disappeared and two minor bands appeared before and after band 8.

12. Palla *(Hilsa sp.)*: In frozen stored sample band 4 disappeared and two new bands appeared at band 8. In ice stored sample bands 1, 2 and 4 were dark stained and bands 8 and 9 disappeared.

13. Phani *(Synaptura sp.)*: The electrophoretic patterns of both ice and frozen stored samples were almost same as obtained for the fresh sample except some minor changes. In ice stored sample band 4 and 5 joined together whereas band 6 was very dark stained in frozen sample.

14. Karli *(Metapenaeus sp.)*: In frozen sample, bands 1, 3, 5, 9 and 11 disappeared, diffused zone appeared between bands 12 and 13 and band 15 and 16.

15. Jaira *(Penaeus sp.)*: Bands 4 and 5 disappeared and bands 11, 12, 15, and 16 were dark stained in the case of the frozen stored sample, whereas bands 7 and 8 were dark stained and band 10, 11, 12 joined togehter. A new dark band also appeared after band 8.

Polyacrylamide gel electrophoresis of different fish was carried out to gain some knowledge of the molecular events resulting in the loss of quality, such as toughening during frozen and ice storage. This technique has found wide application in the detection of proteins[6, 7], particularly for the identification of muscle protein component, [8, 9], since it yields excellent resolution of high molecular weight sub units.

The deterioration of native structural proteins during storage of the samples examined is clearly shown in Figs. 2 and 3 as compared with the patterns of freshly extracted water soluble muscle proteins (Fig. 1). The pattern of khagga (Cat fish), boi (mullet), hira (snapper), sua (Jew fish), surmai (Mackerel), palla (Indian shad), phani (sole) and dhoter (sea bream) appeared to have increased in band number in the region of 4.0–6.5 cm (gel size 75 mm) both during ice and frozen storage, which was not clearly discerned in the patterns of other species.

The variation in protein changes as indicated by the difference in the electrophoretic mobility of the protein sub units during ice and frozen storage imply that there are different rates and/or mechanisms of protein denaturation. This difference of mobility of protein sub units during storage is due to breakdown of faster moving components which give rise to reduced solubility as well as faint bands as a result of addition or loss of small molecules This may be attributed to the bacterial activity by which proteins of the tissue are converted to polypeptides of varying size with different solubility characterstics and amino acids.

Variation in solubility of denatured proteins among various species of fish examined may also be due to their reactions with other degraded constituents of spoiling fish. Denaturation is a deep irreversible process giving rise to changes in the colloidal structure and chemical composition of protein. During cold storage it has been shown to be dependent of the temperature and period of storage[10].

Since surface microorganisms were neither measured nor removed in this study before storage, they probably contributed to the type of alterations resulting in these changes. The role of bacterial proteinases on the proteins of fish tissue has been shown to correspond to variation in amino acids[11, 12]. As expected, these changes were more prominent in ice as compared to forzen storage, since low temperatures inhibit both the bacterial and autolytic activity[13, 14].

It is evident that a number of variables can influence the electrophoretic patterns during storage. But for each sample a definite change in the electrophoretic pattern could be seen with respect to storage temperature.

Changes in Protein Pattern of Shrimp (Penaeus sp.) *During Two Weeks Ice Storage.* The patterns were found to vary markedly as the storage progressed, the rate of change was slow during early storage but became prominent at the second half of the storage period. It was, therefore, possible to observe certain changes in the patterns as a function of storage time.

On electrophoresis water soluble muscle proteins of shrimp were resolved into 16 bands (Fig. 1). No change was noted on the 1st, 3rd and 5th day (Fig. 4). Significant changes were however, observed from the 7th day onwards. Diffused zones could be seen between bands 13 and 14. In addition, bands 13, 14, 15, and 16 disappeared. On the 9th day band 4 disappeared and bands 10 and 11 joined together to from a dark band. On the 11th day pattern was similar to the 9th day pattern except bands 10 and 11 were clearly separated. The 13th day pattern showed a decreasing concentration of band 12 and new band after band 3 along with two diffused zones after band 16. On 15th day band 9 and 12 disappeared and three new bands made appearance after band 16.

Because so many variables influence the electrophoretic pattern, it was not possible to use this method as a quality test. Each fish species examined gave a pattern characteristic of itself depending on the variables, the patterns changed with respect to storage time and temperature.

During storage of shrimp in ice for two weeks, changes do occur as a function of storage time. Such general changes in electrophoretic pattern may have some merits in the determination of quality of spoiling shrimp. It is, however, unreasonable to make judgement on the quality based on a single electrophoretic pattern influenced by so many variables. This technique alone cannot be recommended as a quality test for shrimp on the basis of these findings. Similar results have been reported by Moore *et. al.*] 15] for fish fillets using sepraphore III cellulose acetate strips and Gelman electrophoresis apparatus.

In summary, this study has clearly demonstrated that alterations in proteins with specific migratory rates are associated with so many complex changes occuring in shrimp and fish tissue during ice storage. Further studies in this area are required to show the merits of this technique as an index of freshness of ice stored shrimp and other fish species showing its relationship with sensory and biochemical changes.

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