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IDENTIFICATION OF FISH AND SHRIMP BY POLYACRYLAMIDE GEL ELECTROPHORESIS

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Muscle extracts of fifteen fish and shell fish varieties belonging to different genera were examined by acrylamide gel electrophoresis in tris-glycine and tris-HCl buffers of pH 8.9 and 8.1 respectively. The electrophorogram of each fish examined had characteristic details making it possible to identify fish by means of an electrophoretic analysis of muscle extract made in prescribed conditions.

INTRODUCTION

Commercial species of fish can generally be identified by their appearance and morphological characters [1]. Specimen of different species that superficially look alike can normally be distinguished by closer examination, but sometimes result in diverse classification [2]. The most reliable identification of hybrids by virtue of characters intermediate to their supposed parents was first described by Regan [3]. The structure of pharyngeal teeth was considered as one of the most reliable characteristics, but Wheeler [1] pointed out that identification of certain hybrids by such characteristics is difficult. It has also been reported that most of the morphological characters with the exception of number of gill rakers are influenced by environmental factors [4]. Thus, there are certain difficulties in the identification of fish even when identifiable features are present. This difficulty is enhanced when fish have to be identified in the absence of characteristic features, i.e. when fish are filleted. The recent introduction of legislation in many countries, on the labelling of fish and fish products have emphasized the need for a reliable objective method of identification. At present Pakistani fish industry thrives only on the export of shrimp, great potential, however, exist for the development of other suitable fish products for export purposes.

It was, therefore, considered important to develop some method, which was sufficiently convenient for routine use, whereby individual species in mixed population in the fillet form could be identified. Method based on electrophoresis of water-soluble protein appeared likely to be suitable for the identification of fish in fillet form. Many workers have successfully used electrophoretic protein patterns for the identification of fish species [5–10].

In the present study we investigated the electrophoretic pattern of different varieties of edible fish and shrimp from Karachi–Mekran coastal water to characterize different protein components so as to find out the difference in their pattern to supplement quality characters for identifying varietal differences.

MATERIALS AND METHODS

Harbour fresh, edible fish (Table 1) and shrimp caught on the Karachi–Mekran coast were studied in the experiments. All fish used were of normal size and appearance. The fish were skinned and filleted and musculature 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.

Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis was carried out according to the technique described by Davis [11]. The apparatus used was that of Shandon, Gel (7.5%) was polymerized in tubes (5 × 55 mm), and the buffers for carrying out the electric conductivity being tris-glycine, tris-HCl pH 8.9 and 8.1 respectively. A constant current of 2mA (predetermined) per tube was applied. The current was allowed to pass until the tracking dye reached up to the bottom mark while the tubes were held at room temperature (20°).

Proteins were stained by immersing the gel in 0.25% Amido black dye in 7% acetic acid for 2 min and destained in 7% acetic acid with several washings.

RESULTS

Electrophorograms and diagramatic representation

Table 1. Systematic position of some examined marine fishes of Pakistan.
(Class: Teleostomi; Subclass: Actinopterygii)

Order	Suborder	Family	Genus	Common name	Local name	No. of bands
Cypinoformes	Siluroidei	Ariidae	*Arius* sp.	Cat fish	Khagga	7
Angulliformes	–	Anguillidae	*Muraena* sp.	Eel	Sankh	8
Mugiliformes	Mugiloidei	Mugilidae	*Mugil* sp.	Mullet	Boi	8
Pleuronectiformes	Pleuronectoidei	Bothidae	*Synaptura* sp.	Tongue sole	Phani	9
Perciformes	Percoidei	Sillaginidae	*Sillago* sp.	Lady fish	Bhambhor	9
”	”	Lutianidae	*Lutijanus* sp.	Snapper	Hira	8
”	”	”	*Paristopoma* sp.	Sea bream	Dhother	12
”	”	Sciaenidae	*Otolithus* sp.	Jew fish	Mushka	5
”	”	”	*Sciaena* sp.	Jew fish	Sua	8
”	Scombroidei	Scombridae	*Cybium* sp.	Mackerel	Surmai	8
”	Cottoidei	Platycephalidea	*Platycephalus* sp.	Flat head	Khukher	7
”	Scombridae	Cybidae	*Pelamys* sp.	Mackerel	Kergen	10
Clupeiformes	Clupeoidei	Clupeidae	*Hilsa* sp.	Indian Shad	Palla	9

Electrophoretograms of the muscle extracts of some edible fishes and shrimps found around Karachi-Mekran coast.

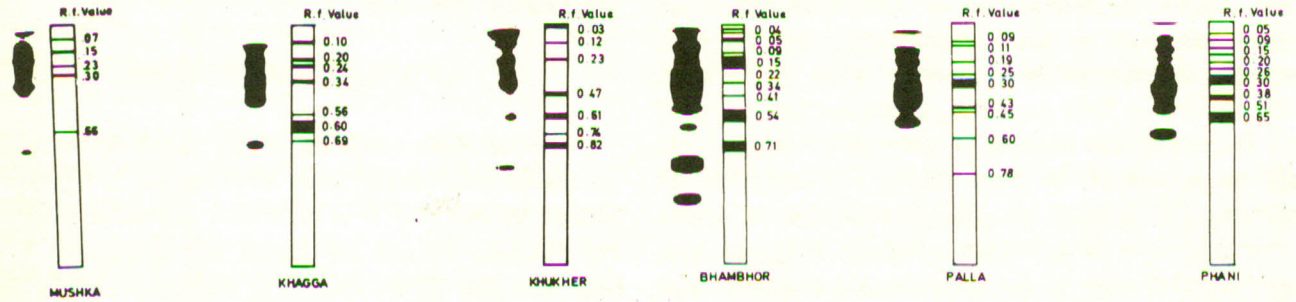
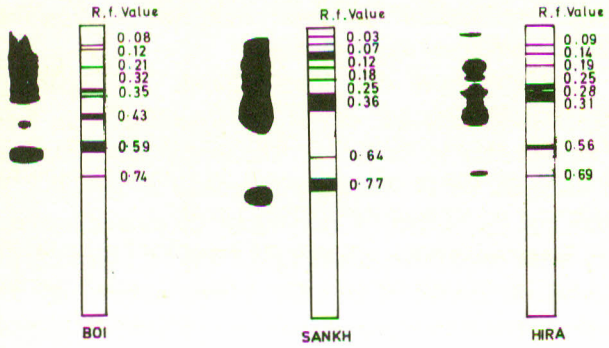


Fig. 1

Fig. 3



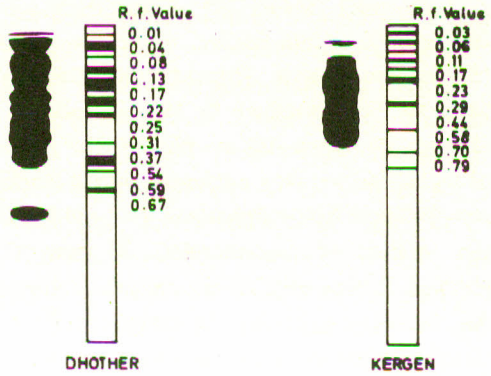


Fig. 4

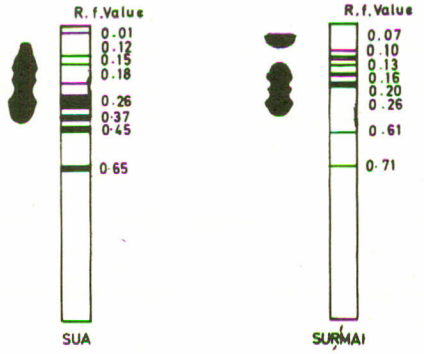


Fig. 2

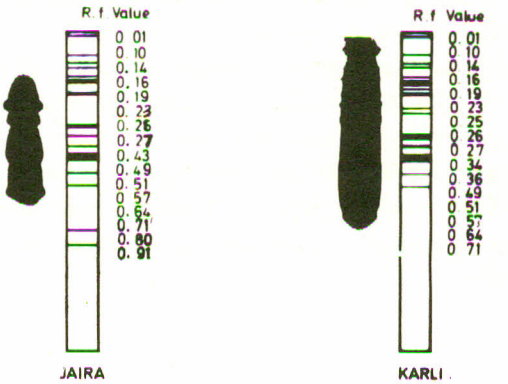


Fig. 5

showing R_f values for different fish examined under similar conditions are given in Figs. 1–5. The protein bands which were visually detected on gel have been numbered and R_f value recorded. Several measurements were performed on different samples from fish of each variety. The reproducibility of the test was satisfactory.

In general, the electrophorogram show that considerable difference exist between the electrophoretic pattern of the specimen examined.

Results obtained can be divided into 5 groups on the basis of number of bands. Electrophorogram are similar in each group in their main features but differ in detail characteristically from specimen to specimen.

Group I. In this group each fish's muscle proteins were separated into five to seven bands with different R_f values (Fig. 1). Three fishes namely Mushka (Jew fish), Khagga (Cat fish) and Khukher (Flat head) were placed in this group.

Group II. The muscle extract of Boi (Mullet), Sankh (Eel), Hira (Snapper), Sua (Jew fish) and Surmai (Mackerel) were resolved on eight bands with very different R_f values and each pattern was characteristic to its subject (Fig. 2).

On comparison the R_f 0.07 was found in Sankh (Eel) and Surmai (Mackerel), band of R_f 0.12 was found in Boi (Mullet), Sankh (Eel) and Sua (Jew fish). Similarly band with R_f 0.18 was found in Sankh (Eel), Sua (Jew fish). Band of R_f 0.25 was found in Sankh (Eel) and Hira (Snapper), the electrophoretic band of R_f 0.26 was found in Sua (Jew fish) and Surmai (Mackerel). Except these other R_f values were quite different from each other.

Group III. Bhambhor (Lady fish), Palla (Indian shad), Phani (Sole) were kept in this group because their proteins were resolved into 9 bands (Fig. 3).

A band of R_f 0.09 was found in all of them. A band of R_f 0.15 was found in Bhambhor and Phani whereas a band of R_f 0.30 was common in Palla and Phani.

Except these above R_f values other values of electrophoretic bands were quite different.

Group IV. In this group Dhother (Sea bream) muscle proteins were resolved into 12 bands and Kergen (Mackerel) muscle extract proteins were resolved into 10 components. On comparison there is no band of same R_f value.

Group V. Among the shell fish, muscle protein of shrimp locally known as Jaira (*Penaeus* sp.) and Karli (*Metapenaeus* sp.) gave very specific electrophoretic patterns. They were resolved into 16 bands (Fig. 5). The R_f 13 components were common in both the species, only three bands are different. There is another interesting point that the band with R_f 0.49 and 0.51 were dark-stained in Karli, but these bands were less intense in the Jaira, bands with R_f 0.43, 0.80, 0.91 were present in Jaira whereas R_f 0.25, 0.34, 0.36 bands were present only in Karli.

DISCUSSION

White muscle of the fish and shell fish revealed up to 16 bands on electrophoretic separation. The electrophoretic pattern obtained for each specimen show that each has a characteristic pattern distinguishable from the other in terms of relative mobility to be used as a means of identification. Factor such as diet, pollution, age and disease could have influenced the pattern, but these effects were not investigated in this study. The fish used were all from the same locality and were all handled in a similar way after capture and these factors could not have influenced the results. Some of the variation in electrophorograms could have been due to minor experimental differences.

Electrophorograms of muscle extracts of different fish examined showed consistent differences in detail between various groups. For instance, seven bands were revealed in two fish namely Cat fish (*Arius* sp.) and Flat head (*Platycephalus* sp.) but all the bands have a different R_f making the electrophorogram specific for each fish (Fig. 1). It may be concluded that there are no proteins which have similar molecular size and weight common among the two fishes. That is why these fishes were classified in separate order, class and genus. Other electrophorograms of different fishes also show great difference in the number of bands and their R_f (Figs. 2–5) making each electrophorogram specific to its own subject.

Apparently the smallest Mushka looks like Boi but they are classified into differnt order, suborder and genus (Table 1). Boi is a *Mugil* sp. and Mushka is one of *Otolithus* sp. The present study also confirmed their classification difference by their electrophoretic pattern.

Similarly Kergen (Mackerel) superficially looks like Surmai (Mackerel) they are also called by some common name but they have very different characteristics. It may be seen (Figs. 2, 4) that Kergen (Mackerel) muscle proteins were separated into 10 bands but Surmai (Mackerel) muscle proteins were resolved into 8 bands on polyacrylamide gel. Small Sua looks like Mushka and they also have the same common English name, i.e. Jew fish, but on electrophoresis their muscle proteins give characteristic pattern.

On the basis of electrophoretic pattern, Hean and O'Rourk [12] found marked differences between rudd and roach muscle proteins. Brassington and Ferguson [7] found that the roach, rudd bream and hybrids can be identified definitely on the basis of enzymes electrophoresis pattern. Similarly Metcalf *et al.* [6] identified many hybrid by their

distinctive electrophoretic pattern which differed from either of their parents.

Polyacrylamide gel electrophoresis has been widely used to examine genetic variation in fish and other animals [13–16].

Thuston [17] reported similar phenomenon with rainbow trout and concluded that the differences were due to a number of factors including hatchery, strain, sex, stress, gel pore size and scanning method.

From the results it is clear that electrophoretic pattern of fish white muscle extracts are species specific. The difference in pattern among fish specimen examined are sufficient to regard as specific separation compared with other taxonomical classifications.

This technique can be used to check the substitution of a cheaper fish in the form of fillets with an expensive one, thus establishing that any fish product offered for sale is correctly labelled and will also help a marine biologist in the identification of closely related fish species. Work on the electrophoretic separation of proteins of different species on fish and shell fish belonging to the same genus is in progress and will be the subject of another communication.

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