A STUDY OF THE EFFECT OF PRESERVATION METHODS ON DEGRADATION OF MUSCLE PROTEINS OF FISH, *CYPRINUS CARPIO*.

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Abstract

In the present investigation, the effect of preservation methods / media on degradation of fish proteins has been tested by taking *Cyprinus carpio* as a model. The average relative concentration of polypeptides obtained after densitometric scanning showed that storage in salt-mixed ice is the best preservation method for fish proteins. The addition of salt to ice depresses the freezing point of water and the preserved fish gets cooled to a lower temperature. Enzymatic activity as a cause of spoilage is restricted during ice preservation methods. Salt preserved fishes reported loss of activity of several proteins and decline in intensity of existing bands. This preliminary test can be very useful in determining the comparative value of different methods employed for fish preservation.

Key words: Relative mobility, preservation, polypeptides and densitometry.

Introduction

Fish is a highly perishable food commodity. Harvested fish lose quality because of bacterial or enzymatic activity or both. Reduction of storage temperature retards these degradative activities significantly, thus delaying spoilage and autolytic deterioration. Proper use of preservation methods and adherence to good sanitary practices ensure maintenance of fish freshness during unloading from the vessel at the shore plant, during processing and transport throughout the distribution chain. Fish landed in good quality will spoil rapidly if these practices are not carried out. The difference in composition of many species of fish and in marketing requirements necessitate considerations of the specific product with regard to quality maintenance and methods of preservation. In the present investigation, the effect of preservation methods / media on degradation of fish proteins has been studied in *C. carpio*. The average relative concentration of polypeptides obtained after densitometric scanning showed that storage in salt-mixed ice is the best preservation method for fish proteins. The present analysis is a pioneering attempt to study the comparative degradation of proteins of fish preserved by different methods.

Materials and methods

C. carpio, belonging to family Cyprinidae and order Cypriniformes, has been analyzed for general proteins (total muscle proteins) in fresh as well as preserved tissue (preserved in ice, salt and ice-salt mixture at one hour and two hour duration). The specimens of these fishes were collected from ponds and ditches near Kurukshetra University, Kurukshetra. The fishes were brought alive to the laboratory and acclimatized in well-aerated aquaria. The extract of the muscle tissue of each fish (fresh as well as preserved one) was prepared in 0.125 M Tris-HCl buffer (pH 6.8) containing 2% sodium dodecyl sulphate (SDS) and stored in eppendorf tubes at –10°C.

14% SDS-PAGE (sodium dodecylsulphate-polyacrylamide gel electropho- resis), using discontinuous system of Davies (1964) and Ornstein (1964) was used for the analysis of tissue extracts. Electrophoresis was carried out under non-dissociating conditions using formulations of Laemmlie (1970).

The gels were stained in Commassie Brilliant Blue (0.05 %), dissolved in a mixture of solvents containing methanol, acetic acid and distilled water in the ratio of 50: 7: 48 (v/v). The gels were destained (in case of over staining) in the above solvents lacking in Commassie Brilliant Blue. Molecular weights of polypeptides separated on SDS gels were determined following the method given by Laemmlie (1970). Polypeptides constituting various proteins and separated as bands by SDS-PAGE were scanned at 580nm using Pharmacia-1 KB ultra scan spectrophotometer.

Results

The sodium-dodecyl sulphate acrylamide electrophoretic profile of general proteins of *C. carpio* showed a large number of bands with very high density. Relative mobilities of all the bands have been calculated and molecular weights have been estimated from the calibration curve (a curve obtained by plotting relative mobilities of different standard proteins of known molecular weights against the log of their respective molecular weights). The densitometric measurement showing relative concentration of polypeptides of *C. carpio* have also been analyzed.

The polypeptide patterns of protein fraction of fresh muscle tissue of *C. carpio* on SDS-PAGE can be seen in Fig.1 (lane 1) and the protein patterns of the fish preserved in plain ice for one hour and two hour, have been presented in Fig.1 (lane 2 & 3). The protein patterns of the fish preserved in salt-mixed ice for one hour and two hour, have been presented in Fig.1 (lane 4& 5) and the protein patterns of the fish preserved in salt for durations of one hour and two hour, have been presented in Fig.1 (lane 6 & 7). The staining intensity of all the bands is very high. The approximate molecular weights and gel densitomeric measurements showing relative concentration of polypeptides have been presented in Table 1. Gel densitometric scanning profiles of lane 2 to lane 6 have been presented in Fig.2 to Fig.7 respectively.

In case of fish, preserved in plain ice, the bands corresponding to molecular weights 53,000, 36,500 and 27,000 daltons have been found to be absent or very light in intensity as compared to the profiles obtained in the case of freshly killed fish. In salt-mixed ice preserved fish, all the bands similar to the fresh sample were present but the bands were somewhat diffused in contrast to the sharp bands observed in the fresh sample. The total number of peaks observed was same for fresh as well as preserved samples. The staining intensity of the bands was very poor in salt preserved fish as compared to staining intensity observed in other bands from extracts, which were preserved in other media. Relative concentration of various protein polypeptides is very less as is evident from the table.

Discussion

Fishery products undergo undesirable changes in flavour, odour, appearance and texture during frozen storage and salt drying. These changes are attributable to dehydration of the fish, oxidation of the oil or pigments, and enzymatic activity in the fish. The rate at which these changes occur depends on the

a) fish species composition.
b) level of constancy of temperature and humidity.
c) packaging materials

The composition of a particular species of fish affects its storage life considerably. Fish having high oil content, such as some species of salmon, tuna, mackerel and herring, have a comparatively short storage life because of the development of rancidity as a result of the oxidation of the oils and pigments in the flesh. The development of rancidity is less pronounced in the fish with low oil content. Therefore, lean fish such as haddock, cod and carps, if handled properly, can be kept in storage in frozen state for many months without serious loss of quality (Holston & Pottinger, 1954).

The present analysis is a pioneering attempt to study the comparative degradation of proteins of fish preserved by three methods. The present results indicate that the loss of quality of frozen fish due to storage depends primarily on storage temperature. Temperature above –20^{o}C even for a short period, resulted in rapid loss of quality. The protein bands were intense but somewhat diffused when the fish was preserved at temperatures less than –10^{o}C. According to Peters (1964), continuous storage at temperatures lower than –20^{o}C reduces oxidation, dehydration and enzymatic changes, resulting longer product shelf life. A high relative humidity in the storage room tends to reduce the evaporation of moisture from the product.

The proteins deteriorated rapidly when the fishes were preserved only in salt. The bands were observed less in number and concentration of polypeptides was very low. This rapid deterioration of proteins during salt preservation could be due to enzymatic degradation of proteins as the enzymes are active during this storage. During cold storage, temperature is the most important factor enhancing the storage life of frozen fish. At a temperature below freezing, bacterial activity as a cause of spoilage is limited. However, fish proteins are permanently altered during freezing and cold storage. This denaturation occurs quickly at temperature not far below freezing and even at –18^{o}C, fish deteriorates rapidly. Badly stored fish are easily recognized; the thawed product is opaque, white and dull and juice can easily be squeezed from it. The properly stored product is firm and elastic.

The present results indicate that the fishery products preserved in salt-mixed ice have maximum storage life. The bands on the gels were found to be clear and intense. The relative concentration of polypeptides was also very high. Test conducted on the storage of haddock in salt water ice and the present results indicate that fish iced with salt-mixed ice cooled faster and to a lower temperature than fish iced in plain ice (Nelson, 1963). The addition of salt to ice/water depressed the freezing point and this resulted in lower ice temperatures. An adequate amount of ice made from a 3% solution of sodium chloride is enough to cool the fish faster (Pesters et al., 1963).

To be effective, plain ice or salt-mixed ice must be clean when used aboard the vessel or in cold storage. Bacteriological tests on ice, taken from various fish storage sites, showed high bacterial counts. These studies indicate that chlorinated water should be used in making the ice at the ice plant. The present results point to the superiority of fish preserved in salt-mixed ice to the preserved in only ice or only salt.

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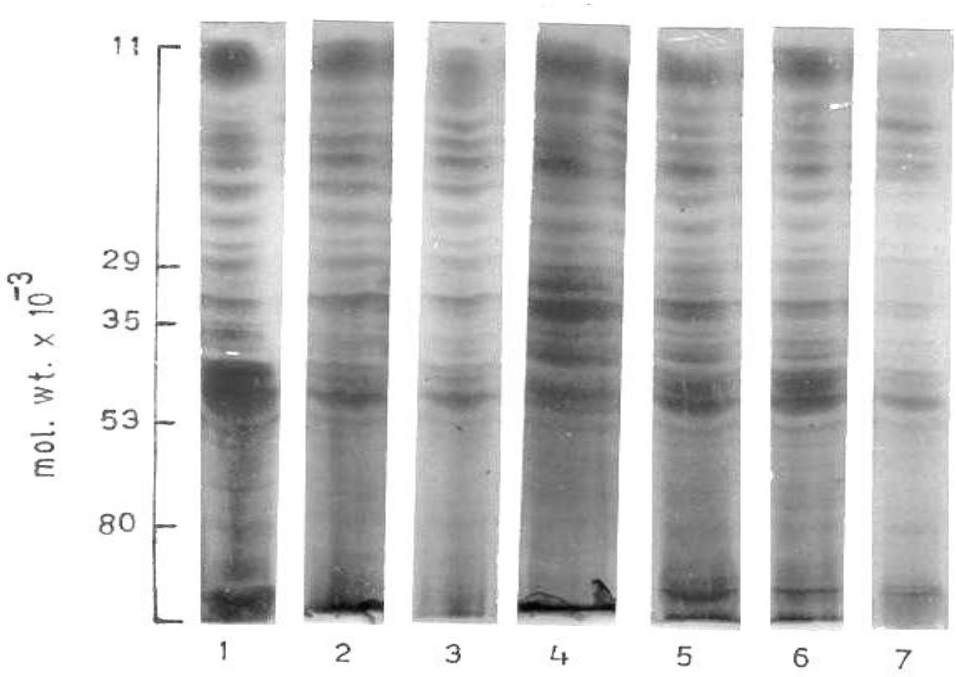
LEGENDS

Fig. 1 General proteins of *C. carpio* (lane 1), the poplypeptide patterns obtained after one hour and two hour interval from the fish preserved in plain ice (lane 2 & 3), salt-mixed ice (Lane 4 & 5) and salt (lane 6 & 7).

Fig. 2-7 Gel densitometric profiles of lanes 2-7 from fig. 1 respectively.

Table 1 showing relative mobilities, relative concentrations and approximate molecular weights of various polypeptides from muscle extracts of *C. carpio*.



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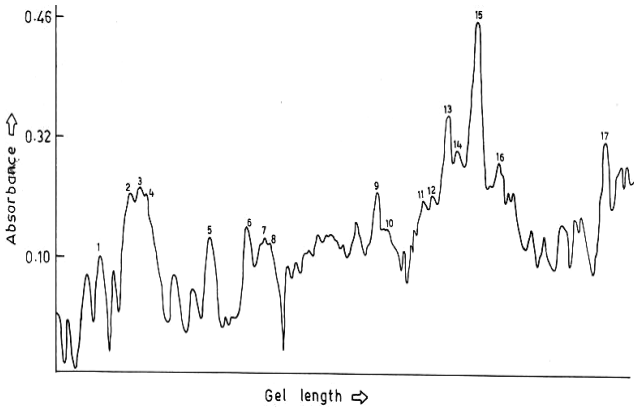

Fig. 2

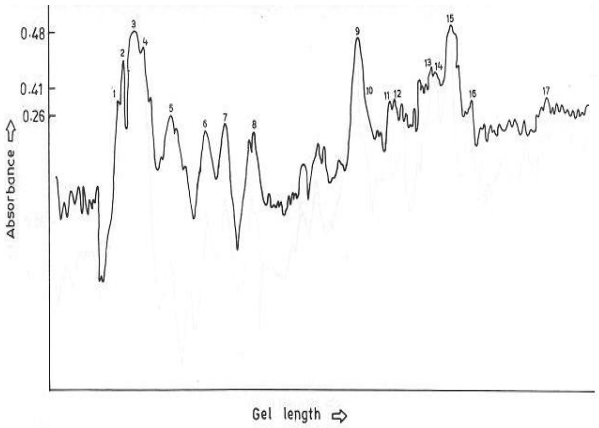

Fig. 3

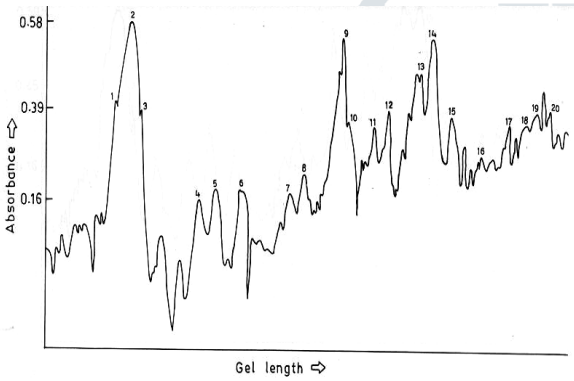

Fig. 4

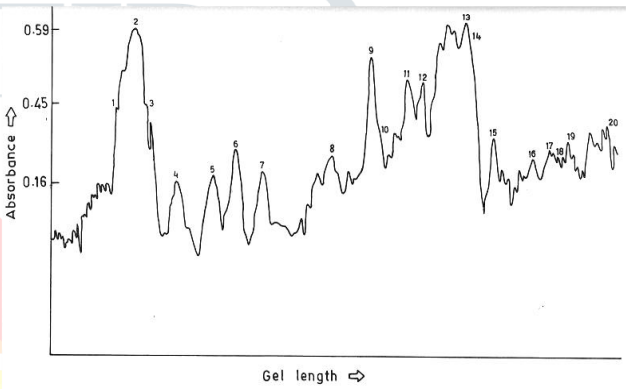

Fig. 5

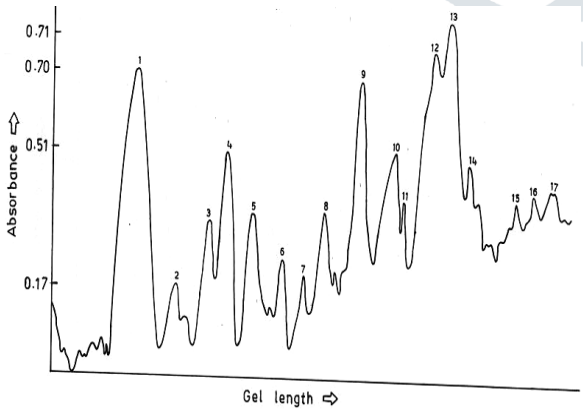

Fig. 6

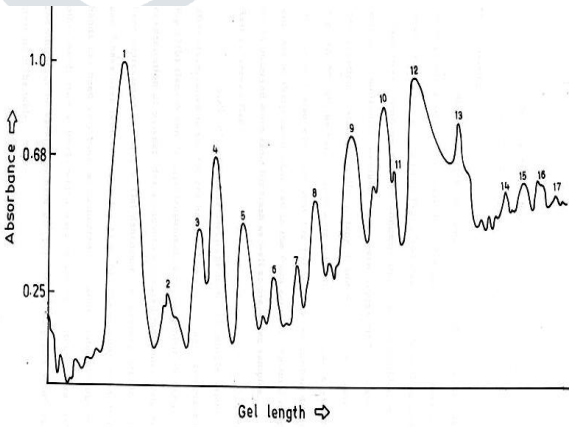

Fig. 7

Table No. 1

Relative mobilities (R. m.), relative concentrations (R. c.) and approximate molecular weights (M. w.) of various polypeptides from muscle extracts of *Cyprinus carpio*.

R. m.	R. c. (Lanes)							M. w.
	1	2	3	4	5	6	7	
.97	5.6	3.5	3.0	2.9	2.9	3.6	3.5	11,500
.93	5.7	4.4	2.8	3.4	1.6	5.6	5.2	12,000
.92	3.7	3.2	3.2	2.8	1.5	3.3	2.2	12,500
.88	4.5	3.5	2.6	3.3	2.9	3.0	4.3	13,500
.81	13.7	13.5	11.6	1.9	3.1	5.9	5.9	14,500
.76	8.7	8.6	8.5	5.9	2.5	2.2	2.1	18,500
.70	8.0	2.1	2.0	7.6	7.0	2.5	2.4	21,500
.66	8.2	7.2	5.7	6.9	5.8	3.6	3.0	24,000
.64	9.2	8.1	8.0	6.5	6.0	7.7	2.9	25,000
.60	4.9	-	-	4.8	4.8	-	-	27,000
.58	3.8	3.7	3.5	2.2	1.6	2.0	1.8	29,000
.54	10.1	1.5	1.4	9.9	9.9	3.7	2.7	31,000
.53	3.2	1.7	1.4	2.9	2.0	4.3	2.4	32,000
.49	4.6	3.9	2.9	2.7	2.3	4.2	2.3	35,000
.47	4.5	-	-	3.5	3.3	2.3	-	36,500
.44	6.0	5.0	4.0	2.6	2.1	-	3.0	40,000
.42	2.5	2.8	2.4	2.4	2.2	5.6	2.3	42,000
.39	3.9	1.3	1.2	3.2	1.9	2.8	1.3	45,500
.36	14.9	14.0	13.0	14.7	12.3	1.8	1.4	49,000
.32	2.8	-	-	2.6	2.3	-	-	53,000
.28	2.2	x	x	x	x	x	x	59,000
.21	2.0	x	x	x	x	x	x	69,000
.14	1.8	x	x	x	x	x	x	80,000

x- densitometric scanning attempted from mol. wts. 11,500 to 53,000.