PRESERVATION OF FISH BY FREEZING AND GLAZING

I. BACTERIOLOGY OF FRESH, FROZEN AND GLAZED FISH

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Microbiological investigation of fresh and frozen fishes such as pomfret, surmai and mackerel was carried out under various conditions of preservation. Glazing, block-freezing and preservation in gunny bag were effected. Determination of bacterial load and isolation, identification and classification of the resistant bacteria were made. Spore-formers of *Subtilis mesentericus* group were found to be resistant to freezing as well as glazing by ascorbic acid, citric acid and sodium nitrite except a mixture of sodium chloride and glucose. Bacterial load was reduced to a good extent and maintained low till the end of frozen storage period.

INTRODUCTION

Hunter (1935) noted the route of bacterial attack in fish spoilage. Since then workers from different parts of the world have reported results as far as bacteriology of fresh and decomposing fish is concerned. However, information concerning effect of freezing, frozen storage and glazing of fish on bacteria associated with fish flesh is very much limited (Shewan, 1954). Hence the present piece of work was undertaken to study the effect of freezing i. e. quick-freezing, slow freezing, block freezing, glazing fish thereafter and frozen storage in different containers on bacterial load and bacterial types.

MATERIALS AND METHODS

White pomfret *(Stromateus cinereus)*, surmai *(Cybium commersonii)*, mackerel *(Rastrelliger kanagurta)* were selected for study. The white pomfrets and surmai were quick frozen and then glazed by spray with respective chemical substances while mackerel was slow-frozen along and by keeping water around the fish so that complete block was formed after freezing. Temperature of cold storage was - 15°C.

Pomfrets were divided in following six groups according to the conditions of preservation: 1. Control, 2. Ice-water glaze, 3. Preservation in gunny bag with

polythene lining, 4. Ascorbic acid glaze (0.1%), 5. Preservation in gunny bag having sodium chloride plus glucose glaze (0.5% each), 6. Sodium chloride plus glucose glaze (0.5% each) and maintained at -15°C.

Surmai were divided into following four groups according to the conditions of preservation: 1. Control, 2. Ascorbic acid glaze (0.1%), 3. Critic acid glaze (1.0%) and 4. Sodium nitrite glaze (1.0%).

PLATING

5 to 10g of fresh as well as preserved fish muscle according to the case was suspended in sterile 75% sea water (Zobell, 1946) in conical flasks. Later, the suspension was diluted in sterile test tubes containing 75% sea water and 1.0 ml therefrom was finally used for initial inoculation.

The plates were incubated at three different temperatures as follows: 1) 10° to 15°C, 2) Room temperature and 3) 37°C, till the bacterial growth was complete on Fischer's medium having the following composition: peptone 1.0%, fish extract 0.5%, ferric phosphate 0 01%, agar 1.5% and sea water 100 ml. Colonies were differentiated on the basis of their characteristics and later typical representative colonies were isolated on slants of the same medium.

IDENTIFICATION AND CLASSIFICATION OF CULTURES

The isolated cultures were identified and classified according to Bergy's Manual of Determinative Bacteriology. Biochemical reactions of the cultures were determined at the temperature of initial cultivation. Biochemical media prepared according to 'Handbook of Practical Bacteriology (Mackie and McCarteney, 1956) are given below:

1) Nutrient broth 2) Gelatin medium-for liquifaction, 3) Starch medium-hydrolysis, 4) Litmus milk-acidity, alkalinity, peptonization and coagulation, 5) Glucose-phosphate-peptone water-M. R. V. P. and Indol reactions, 6) Loffler's coagulated serum for liquifaction, 7) Citrate medium for citrate utilization, 8) Potato medium-chromogenesis, 9) Nitrate medium-reduction, 10) Hydrogen sulphide-medium production, 11) Ammonium phosphate medium-nitrogen requirement, and 12) Carbohydrate media such as mannose, xylose, lactose, sucrose, maltose, dextrose mannitol and arabinose.

In addition to the above mentioned biochemical reactions motility by hanging drop preparation, morphology by Gram's staining, Spore staining by Schaefer-Fulton Technique accompanied by physical measurements (Mackey and McCartney, 1956) were also studied.

RESULTS AND DISCUSSION

Since the fishes are affected worst by mesophilic micro-organisms only the counts of such organisms were taken into account. It has been stated by Shewan (1954) that freezing caused an initial drop of the order of 60% and 90% in the number of bacteria present provided the temperature of the storage was below the minimum for growth. As soon as freezing occurs the growth is arrested, probably due to the restricted moisture conditions. Normally growth of all description is suppressed below-10°C. Rate of freezing has no effect on the number of bacteria but repeated freezing and thawing is more lethal than single freezing and storage in the frozen state for the same time interval which is not possible on industrial scale.

In all cases of freezing i. e. quick, slow and block, it had marked effect on the bacteria associated with fish flesh. There was reduction by about 60% - 70% in the microbial flora in the case of white pomfret, 60% in mackerel and 70% in surmai.

The effect of frozen storage, glazing and block freezing can very well be made out from data given in Table I. In control group of each fish that was subjected to frozen storage it was observed that the number of colonies of bacteria went on decreasing till 2 to $2\frac{1}{2}$ months after which it remained more or less constant viz., 0.560 x 10^6 /g, in white pomfret, 0.552 x 10^6 /g in surmai and 0.815 x 10^6 /g in mackerel.

Total storage periods of white pomfret, surmai and mackerel were $4\frac{1}{2}$, 5 and 6 months respectively. In the case of white pomfret ice water glaze had reverse effect in that it added to the original bacterial load through the use of commercial ice, an observation consistant with the statement of Bedford, (1933). Preservation in gunny bag with polythene lining had little or no effect on the bacterial load. It was almost the same as the control group, i. e. 0.519 x 10^6 /g.

Mixture of sodium chloride and glucose was remarkably effective as a glazing material. Although its concentraation was 0.5% each no bacterium could survive. It may be due to the inhibitory action of sodium chloride in combination with the carbohydrate glucose, since both the substances have the property of disturbing osmotic pressure in the surrounding environment. This mixture was the most successful as direct glaze to the fish as well as to the gunny bag used as a container. No bacterial culture could be isolated from this group.

In the case of surmai, glazing solution of ascorbic acid (0.1%) had a similar effect as was noted in the case of white pomfret, restricting the bacterial population to 0 521 x 10^6 /g. Citric acid (1.0%) had a very good effect in that it reduced the bacterial load to a considerable extent reaching the final value of 0.515 x 10^6 /g. It may be due to its acidic characteristic which causes maintenance of low pH. Sodium nitrite reduced the bacterial population to 0.519 x 10^6 /g. Block freezing of mackerel in water decreased the growth to 0.800 x 10^6 /g.

BACTERIAL FLORA OF FRESH FISH:

Venkataraman and Sreenivasan (1955), while studying the qualitative flora of fresh shark and mackerel of West Coast of India reported presence of *Corynebacterium*, i. e., *C. helvolum, C. globiformie, C. simplex, Nocardia, Achromobacter, Flavobacter, Vibrio, Bacterium, Aerobacter, Micrococcus*, i. e., *M. lutea, M. flavus, M. citreus, M. aurantiacus, M. epidermidis, M. candidus*, and *Bacillus*, i. e., *B. subtilis, B. cereus var mycoides, B. circulans*, and *B. pumpitus*. Velankar (1956) reported the presence of *Bacillus* and *Micrococcus*, in pomfret and mackerel from Rameshwaram Road and Dhanushkodi.

In the present investigation the microbial flora of white pomfret comprised of the species in the genus *Bacillus., B. subtilis B. cereus, B. vulgatus:* in the *genus micrococcus; M. candidus, M. pikowskyi, M. luteus*; in the genus *Achrmobacter: A. marinoglutinosus A. halophilum*, and *Sarcina lutea*. Of the genera mentioned above *micrococcus* and *Sarcina* species were absent in white pomfret during frozen storage. However, in the case of ice water glazed pomfret, presence of *B. mycoides, B, megatherium B. mesentericus, B. adhaerens* and *B. simplex* was recorded.

Muscle of surmai revealed presence of genus *Bacillus* i. e, *B. subtilis, B. cereus*; *genus Achromobacter* i. e., *A. marinoglutinosus* when it was fresh. However, on frozen storage under different conditions of glazes, absence of genus *Archromobacter* was noted. Flesh of fresh mackerel revealed the presence of genus *Bacillus*,

TABLE I EFFECT OF FREEZING AND FROZEN STORAGE ON TOTAL PLATE COUNTS OF FISH

Fish	Group	Before freezing number x 106/g.	After freezing	After storage for months: Number in the Column X 10⁶/g.										
				1	1½	2	2½	3	3½	4	4½	5	5½	6
White pomfret	Fresh	1.665	0 666	–	–	–	–	–	–	–	–	–	–	–
	I	–	–	–	0.589	–	0.560	–	0.543	–	0.541	–	–	–
	II	–	–	–	0.729	–	0.685	–	0.680	–	0.673	–	–	–
	III	–	–	–	0.553	–	0.540	–	0.523	–	0.519	–	–	–
	IV	–	–	–	0.526	–	0.513	–	0.501	–	0.501	–	–	–
	V	–	–	–	0.000	–	0.000	–	0.000	–	0.000	–	–	–
	VI	–	–	–	0.000	–	0.000	–	0.000	–	0.000	–	–	–
Surmai	Fresh	2.225	0.667	–	–	–	–	–	–	–	–	–	–	–
	I	–	–	0.589	–	0.552	–	0.541	–	0.534	–	0.527	–	–
	II	–	–	0.554	–	0.540	–	0.538	–	0.527	–	0.521	–	–
	III	–	–	0.539	–	0.535	–	0.530	–	0.524	–	0.515	–	–
	IV	–	–	0.557	–	0.537	–	0.535	–	0.526	–	0.519	–	–
Mackerel	Fresh	2.115	0.846	–	–	–	–	–	–	–	–	–	–	–
	I	–	–	0.821	–	0.815	–	0.811	–	0.809	–	0.805	–	0.802
	II	–	–	0.819	–	0.814	–	0.810	–	0.808	–	0.803	–	0.800

White pomfret:

I = Control
II = Ice water glaze
III = Packed in gunny sac with polythene lining
IV = Ascorbic acid glaze
V = NaCl + Glucose glaze
VI = Nacl + Glucose gunny sac.

Surmai:

I = Control
II = Ascorbic acid glaze
III = Citric acid glaze
IV = Sodium nitrite

Mackerel:

I = Control
II = Block frozen

i. e, *B. subtilis, B. cereus*, genus *Achromobacter*, i. e, *A. marinoglutinosus* and genus *Micrococcus*, i. e, *M. candidus* and *M. luteus*. However, on frozen storage in control and block frozen mackerel absence of genera *Achromobacter* and *Micrococcus* was marked.

Of the bacterial genera mentioned, genus *Bacillus* could grow at all the three temperatures while genus *Achromobacter* could grow at room temperature and genus *Micrococcus* and *Sarcina* could grow at low temperature only.

It has been stated by Shewan *(loc. cit)* that when bacteria were frozen so me suffered immediate death, irrespective of the rate of freezing or its temperature. Some species are more cold sensitive than the others, e. g, *Ps. fluorescence* are more cold sensitive than the spore-formers of *subtilis mesentericus* group. These spore formers remain relatively unaffected. Freezing appears to have no effect on the characteristic cultural or otherwise of the marine types after thawing.

Genus *Bacillus* with its species and strains was found to be resistant to freezing and glazing solutions such as made of ascorbic acid, citric acid, and sodium nitrite in low concentrations except when made of a mixture of sodium chloride and glucose. This low concentration of mixture of sodium chloride and glucose, however, was found to be lethal to this genus of bacteria at that low temperature of -15°C as a direct glaze on the exposed surface of the fish and as a glaze applied to the container such as gunny bag.

Genus *Achromobacter* was found to resist freezing specially quickfreezing when associated with white pomfret but it was not evidenced in the case of quick frozen surmai and slow frozen and block frozen mackerel. It was recorded that *Achromobacter* was resistant to ascorbic acid when associated with white pomfret only as it was found to be suspectible to glazing solution used.

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