STUDIES ON MINCED FISH TECHNOLOGY

THESIS
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by

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INTEGRATED FISHERIES PROJECT

(Government of India)

Cochin - 682 016

DECLARATION

I hereby declare that this thesis is a record of bonafide research carried out by me under the supervision of Dr. P.K. Surendran, my supervising teacher and it has not previously formed the basis for award of any degree, diploma, associateship, fellowship or other similar title or recognition to me, from this or any other University or Society.

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This is to certify that this thesis entitled **"Studies on Minced Fish Technology"** embodied the results of the original research work done by Mrs. S. Girija under my supervision and guidance from 1-11-1984 to 30-11-1992. I further certify that no part of this thesis has previously been formed the basis of the award of any degree, diploma, Associateship, Fellowship or other similar titles of this or any other University or Societies. She has passed the Ph.D qualifying examination of the Cochin University of Science & Technology in August, 1985.

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dedicated to my

mother

ABBREVIATIONS USED IN THE THESIS

g	gram
hr.	hour
I.U	International unit
kg.	kilogram
l.	litre
mg	milligrams
ml	millilitres
no.	number
rpm	revolutions per minute
µg	micrograms
n.saline	normal saline
ppm	parts per million
sq.cm.	square centimetre

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INTRODUCTION

C H A P T E R - 1

1.1. INTRODUCTION

While increasing fish production is very important to the economy, equally important is the maximum utiilization and economical distribution of the entire catch. Since each species of fish caught differs considerably in appearance, taste and hence consumer acceptance, fishes with less consumer appeal have to be converted into different products and distributed to consumers in stable acceptable forms at prices which they can afford. It is in this context that fish mince assumes importance as a product of great potential.

Low consumer appeal and acceptance of whole fishes could be due to a variety of reasons. Unfamiliarity, grotesque shapes and forms, unattractive colours, presence of too much of bones and scales, inconvenient sizes, poor taste, etc., are some of them. To make these varieties acceptable to the consumers, they have to be converted into suitable, stable products with good appearance and made available at reasonable prices. Grotesque

shapes have to be changed or masked, bones and scales removed, size made suitable, tastes improved using additives etc., in order to make them appealing to the consumers. Fish mincing is a very effective method to achieve the above goals. It has the further advantages of effecting better utilization of catch and by catch, maximum extraction of flesh from bones with the least loss of edible parts and utility in the production of numerous products of very good consumer demand. The maximum utilisation of catches result in better returns to the producers and affordable prices to consumers. The fish mince can easily be stored, and since the storage is after the elimination of scales, gills, bones, fins, guts and skin, cost of storing unnecessary bulk can be saved.

Fish mince is the flesh, in a comminuted form, separated from unwanted inedible parts. It can form an intermediary in the preparation of a variety of end-products.

1.1.1 MINCED FISH IN THE WORLD MARKET

(a) From commercial species

In the case of commercial species, fish mincing is mainly employed world over for two reasons: (1) to obtain higher yield from headed and gutted whole fish and (2) to extract more flesh from fillet wastes. Block frozen fish mince forms a major commodity in the world market. In the developed countries cods, hakes,

haddocks pollacks and croakers are the main species among commercial fish used for fish mince.

(b) From underutilized species

FAO estimates that the World consumption of fish could be more than doubled if the presently underutilized or unused resources were brought to the human food. There is a major incentive to apply mince technology to many of these species, when the intractable problems of their processing and marketing by other means are considered (FAO Fisheries Technical Paper No.216, 'Minced Fish Technology - A Review').

Even in the developed world, many varieties remain under-exploited and unexploited due to reasons like unfamiliarity, processing difficulties, bad connotations to the names of some fishes, grotesque appearance of the whole fish etc. To overcome these difficulties and to utilize these fishes, mincing is the only option. This also allows the correction of intrinsic quality shortfalls in some varieties, like high moisture contents, soft texture etc. Acceptable mince products have thus been developed from smoothhead, rabbitfish, grenadier etc. Fish mince production again, allows the use of many additives to overcome some quality problems, for example the use of antioxidants to reduce oxidation of fat in fatty fishes.

(c) From by-catch

Millions of tons of fish which are caught and discarded into

the sea have been wasted every year. This unwanted by-catch has been wasted over years due to many reasons, like extremely adverse price differences with the main catch, bulk species and size mixing, low yield of product, adverse consumer preferences and also to save fish hold space for the more sought-after species etc. Attempts are being successfully made in the developed nations to utilise these fish through various methods, of which fish mince preparation is the most important.

1.1.2 RELEVANCE OF MINCED FISH IN THE INDIAN CONTEXT

In the context of the protein deficient Indian diet, the importance of maximum utilization of the fish catch cannot be overemphasised. It is a sad paradox that the underdeveloped tropical and sub-tropical countries with huge fish catch from their waters, where the need for protein food is the greatest, do not have the technology or capital to maximise catch and its utilisation, and that, the least attention has been given to have concentrated effort in achieving this goal.

In India, where consumption of processed fish has not yet become popular, the major bulk of fish consumed is in the 'fresh form'- the word 'fresh' only indicates that they are not in any processed form. The processing industry mainly caters to the export markets and that too only with highly priced fishes, crustaceans etc.

In the fishing effort for popular and highly priced fish, there will always be by-catches and incidental catches of less preferred fishes which are totally discarded and wasted. The inefficient and insufficient infrastructure available in the country for preserving, storing and transporting causes wastage of thousands of tons of even well accepted types of fishes. Development of fish mince technology for various types of fish wasted or under-utilized can lead to a better and efficient utilization of fish catches.

1.1.3 THE PRESENT STUDY

The quality of minced fish, as mentioned earlier depends largely on the type and quality of the raw material used, as well as on the processing methods employed. Moreover, fish mincing involves cutting up of tissues thereby increasing surface area to a great extent and releasing of enzymes and nutrients from the tissues. Due to these factors fish mince is relatively more prone to chemical, autolytic and microbial spoilage. Hence study of minced fish with these factors in focus is very important. Equally important is the availability, price and preference of the raw material vis-a-vis the end products and the storage period it passes through.

In the present study, changes in the bacterial flora, both quantitative and qualitative of the dressed fish, viz. Nemipterus japonicus,

and mince from the same fish during freezing and frozen storage have been investigated in detail. The effect of a preservative, viz., EDTA on the bacteriological and shelf life characteristics of the minced fish has also been investigated.

Attempts have also been made to develop various types of products from mince and to study their storage life.

1.1.4 ABOUT THIS THESIS

In this thesis, the study has been divided into three parts:-
(i) the bacteriological and organoleptic studies of minced meat from Nemipterus japonicus during freezing and frozen storage (ii) changes in protein extractability and texture of minced meat from Nemipterus japonicus during freezing and frozen storage. and (iii) Development of consumer products from minced meat from Priacanthus species.

In the first part (Chapter-II), bacteriological investigation on the minced fish, effect of freezing and frozen storage on the bacterial flora and the organoleptic changes during mincing, freezing of the minced fish and its frozen storage have been presented.

In the second part (Chapter-III) studies on protein extractability of the minced fish and changes in the textural qualities of the mince have been reported.

In the third part (Chapter-IV), investigation on the development of various consumer products from minced fish are presented.

CHAPTER - 2

C H A P T E R -2

BACTERIOLOGICAL AND ORGANOLEPTIC STUDIES OF MINCED MEAT FROM NEMIPTERUS JAPONICUS DURING FREEZING AND FROZEN STORAGE

2.1 REVIEW OF LITERATURE

2.1.1 BACTERIOLOGY OF FROZEN FISH AND SHELL FISH

The major factors that should be considered in the production of good quality frozen fishery products are quality of the raw material, handling and processing conditions and post-process contamination.

2.1.1.1 **Quality of raw material**

The quality of the raw material, to a great extent, determines the quality of the final products (Cann, 1977). Most of the freshly landed fish and prawn have their bacterial load within the limits of 10^3/g to 10^6/g (Andrews et al 1977; Zuberi & Quadri 1980; Abeta, 1983; Lakshmanan et al 1984). But the bacterial count increases considerably during handling after catch (Shewan, 1961 Lee and Pfeifer, 1977; Gillespie & Macrae, 1975). The method of catching (Watanabe, 1964; Novak, 1973) and the seasonal variations in bacterial and biochemical compositions of fish (Raj & Liston, 1963; Iyer et al 1970) affect the final quality.

2.1.1.2 **Effect of pre-process handling on bacterial quality**

Apart from the bacteria natrually associated with the fish and shrimp considerable, number of bacteria get access to fish flesh as contaminants during various stages of processing. Some of them are of public health significance (Iyer et al, 1966). While primary contamination may occur upto the time of catching, secondary contamination begins from catching till the fish is finally processed.

Secondary contamination begins on the fishing vessel. Bacteria present on the deck, utensils, fishholds, ice and fish boxes get entry into fish flesh and multiply there on (Watanabe, 1964; Castell 1973).

Washing, if carefully done can reduce the surface load of bacteria by 80-90% and with very careful hand-washing, upto 99% reduction is possible (Spencer 1956; Shaik Mahzud and Magar, 1956; Sreenivasan, 1959; Georgala, 1957 a, b; Cann, 1977, Lee and Pfeifer, 1977; Ray et al (1976). But washing with polluted inshore water may raise bacterial load on fish (Watanabe, 1964). Also, washing generally did not have much effect on generic distribution of Microorganisms s (Shewan 1971, a).

The use of chilled water has been advocated in washing process. Between chilled sea water at 0°C and tap water at 25°C, an

increase in bacterial count was noted when washing was done with tap water. Chilled water arrested bacterial multiplication, (Pillai & Lakshmy, 1961). Other workers have found that chilled water used for washing retarded bacterial growth, but the low temperature apparently favoured the selection of psychrotrophic spoilage bacteria (Shewan, 1971 a, Lee & Pfeifer, 1977).

Ice is applied to reduce the temperature thereby delaying the spoilage of fish. But it can act as a dangerous vehicle in allowing microbial contamination. This possibility was studied by Georgala (1957 a,b) and Iyer & Choudhuri (1966). Contact with ice directly from factory or unused ice from the fish hold does considerably alter the flora. In one of the experiments (Georgala, 1957 a) the uniced fish contained about 6% Coryneformes and 6% Flavobacteria at 20°C and only a few (Less than 1%) of either of these groups at 0°C. After icing with trawler's ice, the Coryneformes formed 27% of the flora at 20°C and the Flavobacteria 26% at 0°C.

Iyer & Choudhari (1966) have studied the influence of ice on the bacteriological quality of the procured fishery products. In almost all the cases ice had been traced to be a major source of contamination depending on the nature of water used for preparing it. The bacterial count of the material during transportation with ice increased from 3.5×10^5/g to 7.6×10^6/g, *E. coli* from nil to 310/g and faecal streptococci from

12 to 310g in 5 hours.

Compared to fish, more information is available on the handling of cursteceans. The beneficial effect of heading the Shrimp on microbial quality has been reported widely. Green (1949) Williams et al (1952), Novak (1973) and Cann (1974) observed that for shrimp that was landed alive heading immediately after catch was essential to prevent autolytic changes. Contrary results had also been reported (Koburger et al, 1973; Alvarez and Koburger 1978).

The processing practices influenced the microbial load as well as the generic composition of the flora. (Harrison & Lee, 1969, Cann et al 1971; Lee & Pfeifer, 1977; Zuberi et al (1983), Philip & Peeler 1972, Lee & Pfeifer, 1975; Ray et al 1976). These studies indicated that microbial load increased after washing, brining or cooking. The Gram positives were recovered with increasing frequency after each step (Harrison & Lee, 1969).

Farber & Lerke (1961) noted a positive correlation between diversity of the microbial flora in sea food and its freshness. This observed diversity indicated the lack of opportunity for a specific micro organism to dominate.

2.1.1.3 Bacteriology of freezing of fish

2.1.1.3.1 Quantitative aspects

Shewan (1954) summarized the results of freezing as follows: "Freezing causes an initial drop in the numbers of bacteria present, of the order of 60-90% and provided the temperature of storage is below the minimum for growth, there is an exponential drop followed by a more gradual decline during storage. The heavier the initial load, the greater the number of survivors."

Microorganisms such as the Gram positive types, can survive freezing while others cannot, and freezing does not seem to change the properties of microorganisms. Vegetative cells are more resistant to freezing than spores. It appears that the process of freezing and thawing is more lethal than storage in the frozen state (Jorgenstein, 1962).

There is more microbial killing between -1°C and -10°C than at -20°C or lower. At -195°C there appears to be no freezing death at all.

The general conclusion is, therefore, that the freezing process destroys microorganisms to a certain extent, depending chiefly on the temperature, which in turn determines the freezing rate. Freezing storage further destroys bacteria due to denaturation of protein and is more lethal at the temperature interval between 0°C and -10°C than at lower temperature. Some Micro organisms may therefore survive long storage at low temperatures.

The salient features of the microbiology of freezing of fish were discussed by Tressler & Evers (1957). Freezing does not sterilize the fish; neverthless there is considerable reduction in bacterial population due to freezing.

Kiser & Beckwith (1942), studying the effect of fast freezing and storage of mackerel at -28°C noticed that the total bacterial count of muscle exhibited a decrease of 43.3%, while the bacterial count of intestines showed a more striking reduction (97.9%). A reduction of 40-60% in bacterial population was noted by Pivnick (1949). Similar values were reported for tropical fishes also. (Sreenivasan, 1959, Bose, 1969, Jadhav & Magar, 1970, Cann 1974; Zuberi et al, 1983)

While rate of freezing had no effect on bacterial death repeated freezing and thawing was found to be more lethal to bacteria (Pivnick, 1949, Shewan, 1961).

The temperature of freezing had been the subject of some studies. But most of the workers used pure cultures of bacteria. It was found that the lower the temperature of freezing, the greater the destruction (Huss, 1934; Kiser 1944, Wieser and Osterud, 1945). During frozen storage also, the residual bacterial population experienced reduction. During storage a further fall in number occurs, exponentially for the first period, followed by a gradual decline. The heavier the initial load, the greater the number of survivors.

Kiser & Beckwith (1942) noticed that marked reduction in bacterial number occurred only after 48 hours during frozen storage (Pivnick, 1949) reported a reduction of 40-60% in bacterial counts in 24 hours of frozen storage at -20°C.

The method of packing, freezing and storage of headless shrimp was investigated by Fieger et al (1956). The study indicated freezing caused a greater reduction in bacteria in peeled shrimp than in whole shrimp.

Effect of different glazes and packaging methods on the bacterial quality of the fishes from Bombay coast was studied by Jadhav and Magar (1970). They found that both packaging methods and glazes exerted some effect on bacterial counts of the frozen fish. While ice water glaze increased the bacterial numbers, others like citric acid, ascorbic acid and sodium chloride - glucose mixture tended to reduce the number of bacteria. Mathen et al (1970) could not find any significant difference in bacterial count between glazed and unglazed frozen shrimp. They suggested that for short term preservation (2 months) by freezing, glazing was not necessary.

The difference between laboratory scale and commercial scale processed frozen prawn, with regard to the bacterial population

was studied by Novak (1973). The percentage reduction of bacterial counts in laboratory frozen samples were fairly constant (89-99%) whereas in commercially processed samples, larger variations were noted. The author attributed this to storage conditions and delay in freezing. Also for the factory frozen samples wide variations were noted among factories for similar products (Cann, 1977; Zuberi et al (1983).

2.1.1.3.2 Qualitative changes in bacterial flora on freezing of fish

Earlier studies indicated that freezing imparted selective action on the microbial flora of fish. Various species of bacteria were affected at different levels.

The Gram positive bacteria were found to be more resistant to freezing and frozen storage. Kiser & Beckwith (1942) observed that Micrococcus and Moraxella - Acinetobacter group were frequently encountered in frozen mackerel. Pure culture studies showed that freezing and storage at -20°C for 20 days, resulted in almost 100% reduction in Achromobacter spp; while Micrococcus spp. withstood the temperature much better.

During one month's storage of Ocean perch at -15°C, Lee et al (1967) noted the loss of all Gram negatives. The Gram positive species showed different sensitivity to freeze damage. Among Gram positives, Bacillus, Lactobacillus and Micrococcus species were more susceptible and Coryneforms the least affected.

Jadhav & Magar (1970) studying the bacterial flora of tropical fishes from Bombay coast found that spore-formers like Bacillus mesentericus were very resistant to freezing as well as glazing by solutions of ascorbic acid, citric acid and sodium nitrite.

Microbiological characteristics of the frozen prawn imported from tropical countries were investigated by Kawabata et al (1975). The study revealed that for shrimp almost 70% of flora was constituted by Gram positives belonging to the genera Micrococcus, Streptococcus, Staphylococcus, Microbacterium and Corneybacterium. The Gram negatives were few in number and belonged to the genera Flavobacterium, Cytophaga, Pseudomonas, Moraxella and Acenetobacter. However, Zuberi et al (1983) noted a predominance of Pseudomonas spp. in frozen shrimp followed by Micrococcus spp.

2.1.1.4 **Effect of handling and processing on microorganisms of public health significance.**

Effect of various steps in handling and processing of sea foods with regard to bacteria of public health significance have been studied by many scientists. Raj and Liston (1963) and Raj (1970) showed that the raw material entering the processing plant carried comparatively lower level of bacteria of public health significance and each step of handling and processing introduced

significant number of coliforms, enterococci and cogulase positive staphycococci, The effect of processing, distribution and storage on the Vibrio parahaemolyticus count in oysters was studied by Thompson et al (1976). Karunasagar et al (1984) studied the shrimps undergoing processing for export for the presence of Vibrio parahaemolyticus. They noted that 54 out of 56 raw, 42 out of 50 processed and 54 out of 57 frozen samples contained Vibrio parahaemolyticus in quantities of 10/g. None of the sample contained Vibrio parahaemolyticus greater than 10^2/g.

2.1.2 BACTERIOLOGY OF MINCED FISH

The process of mechanical deboning of fish causes considerable maceration of the tissue. Microbial contamination may get easily blended throughout the tissue. Also, rise in temperature during the deboning process may enhance bacterial growth. Several studies have been conducted on bacterial proliferation in mechanically deboned fish. Liston (1980) has opioned that the extreme susceptibility of this product to bacterial contamination should be clearly recognised.

2.1.2.1 **Bacterial quality of minced fish**

Cann & Taylor (1976) have found out that the major determinant of the microbiological quality of the minced fish is the raw material itself. Declerik (1979), Giletz et al (1977 b) and

Nickelson et al (1980) have found that the holding period of filleting wastes before mincing or poor storage of whole fish raw materials caused increase in total counts in the minced products and increased the risk of spoilage.

It was reported that bacteriologically, the minced fish produced in North America was very poor in quality (Table 2.1) and that this was found to be due to unhygienic practices such as accumulating fillet wastes over a day's run before deboning it.

TABLE - 2.1.

Bacterial quality of raw commercial frozen minced fish

(Figures as % of the total samples taken)

	SPC/g at 25°C (Standard plate count)			Coagulase ±ve Staphylococci/g.			Faecal coliform/100g.		
Frozen minced fish	$<10^5$	10^5-10^6	$>10^7$	$<10^2$	10^2-10^3	$>10^3$	<100	100-300	>300
	31%	28%	41%	96%	4%	0%	67%	7%	26%

Later, Licciardello & Hill (1978) found that there was considerable improvement in bacterial quality of these products.

TABLE - 2.2

Microbial quality of minced fish blocks in U.S. Markets

(1975/1976)

Mince from	SPC/g	Coagulase±ve Staphylococci/g.	Coliforms/g.
Alaska Pollack	$10\text{-}10^{5}$	0-16	0-3
Cod frames	$10^{4} - 10^{7}$	0-16	0-15
Cod	$10^{3.5} - 10^{6}$	0-24	0-43
Other North Atlantic fish	$10^{3}\text{-}10^{6}$	0-24	0-43

Blackwood (1973) found that 40% of the minced fish samples he examined had a bacterial count of more than 10^{6}/g. Voluntary bacteriological limits for minced fish in some countries set a maximum SPC level of 10^{5}/g (Bond, 1975).

Nickelson et al (1980) had stated that preprocess treatment of the material by scaling, heading and evisceration had very little effect on the final bacterial quality of the mince. It was noted that the presence of even gut in material lead only to a small increase in the bacterial counts and no pathogenic bacterial species were found in the viscera of most of the raw fish (Young, 1978).

The microbial flora and the spoilage problem of minced fish are reported to be similar to those of intact fillets. Still there are certain peculiarities noted in minced fish. Nickelson et al (1980) had found that mincing led to substantial increase in microbial flora by dispersion of surface contamination through out the muscle tissue. Mince of high levels of initial contamination had an extreme risk of spoilage on handling, storage and further processing.

Raccah & Baker (1978) noted that the mechanical deboning of several traditional and under-utilized fish species increased the microbial count tenfold. The shelf life of mince was 5 days at 2°C and 3 days at 12°C. Total bacterial and coliform counts showed little change during frozen storage. They also studied some microbiological aspects of minced fish from underutilized species and indicated that the challenge of microbial growth and spoilage was similar to that of traditional fish products with no apparent unique microbial problems. The study stated that during the frozen storage of the fresh water white sucker mince, a gradual decrease of the mesophilic population was observed while the psychrotrophic population remained in a steady state. No change in the coliform population were observed during the period of the frozen storage.

The growth of both the psychrotrophic and mesophilic population of minced white sucker was faster at 12°C than at 2°C. The study revealed that the dominant flora at the onset of spoilage was merely psychotrophic. The shelf life of white sucker mince decreased from 7 days at 2°C to less than 3 days at 12°C.

In the Japanese industry where minced fish produced under closely controlled high sanitary conditions was used in the manufacture of surimi and fish sausage, the bacterial quality was reportedly quite good. In a study of minced fish microbial quality Licciardello & Hill (1978) compared minced, washed Alaska pollack (Surimi) produced in Japan with minced fish from North Atlantic. They found that Surimi produced in Japan contained lower number of aerobic heterotrophs, coliforms, faecal streptococci and coagulase positive staphylococci than found in blocks of minced pollack, cod and other fish produced in Canada, Greenland and Europe. The total plate counts at 21°C for surimi ranged from 10 to 100,000/g while the coliforms (MPN) per gram was less than 3. Ninety seven percent of the surimi blocks contained fewer than 25 faecal streptococci and fewer than 3 coagulase positive staphylococci per gram. One hypothesis given to explain the difference in bacterial counts was that washing steps of Japanese surimi production decreased the microbial load while minced fish samples from North Atlantic were not washed and thus contained more bacteria.

Another explanation, also offerred by Licciardello & Hill (1978) was that coliforms present in fresh surimi died off during frozen storage or were injured and not detectable by using selective enrichment media.

Elliot (1986) has studied the microbial profiles of Alaska pollack surimi. In his study an on-line survey of the processing revealed that the microbial load of the surimi increased during processing from less than 3 x 10^3 c f u/g in skinned fillets to 8.9 x 10^5 c f u/g in the finished product. The coliform number increased from 0.6 MPN per gram in fillets to more than 100 MPN/g in refined mince and surimi.

TABLE - 2.3

@ Changes in Aerobic plate counts and total coliforms during various stages of surimi preparation from Alaska pollack

Sample at different stages	Aerobic plate count c f u/g	Total Coliforms MPN/g
Raw Alaska pollack	3.0 x10^3	0.6
Minced meat	2.6 x 10^3	0.6
Washed mince	2.8 x 10^4	10
Refined mince	8.5 x 10^4	54
Dehydrated refined mince	6.6 x 10^5	250
Surimi § Cryoprotectant	7.2 x 10^5	610
Surimi block	8.9 x 10^5	320

@ Elliot (1986)

Bacterial profile of fresh and spoiled fish mince from Johnius dussumieri at refrigerated storage had been studied by Abraham et al (1992). The percentage composition of bacterial flora was found to vary in fresh fish, fish mince and spoiled fish mince. Acinetobacter and Aeromonas which were dominant in fresh fish decreased drastically upon mincing, washing and storage. In fresh mince 71.0% of the bacterial population comprised of Gram positive groups, of which Micrococcus was the dominant group. Flora of the spoiled mince was dominated by Gram negative groups (80.9%) comprising mainly of Vibrio, followed by Pseudomonas spp.

2.2.2.2 Bacteriology of fish mince based products

Delvalle (1975) has evaluated the salted and dried fish cake for proximate composition, protein quality and bacterial counts. The salted cakes were freshly made and stored for 18 months at ambient tropical temperature. Total plate counts and halophilic counts were found to decrease to almost zero after 18 months.

Studies on the frozen storage characteristics of frozen fish fingers has been made by Reddy et al (1992). They found that there was decrease in bacterial counts in the fish fingers through out the frozen storage period. Food poisoning organisms were not found in any of the samples.

Microbiology of Surimi based products had been studied by Matches et al (1986). This study revealed that during storage of Surimi based products both quantitative and qualitative changes took place in the micro flora. At low temperature, the population changed to Gram negative rods. At higher temperature Bacillus species predominated.

2.3 MATERIALS AND METHODS

The study was designed to measure the qualitative and quantitative changes of bacterial flora during the frozen storage of minced fish in comparison with treated minced and dressed fish.

2.3.1 MEDIA AND SOLUTION

The following media were used in this study

1. Sea water agar (SWA)

Bacteriological Peptone	10 g
Ferric phosphate	50 mg
Agar powder	15 g
Sea water	1 litre
pH	7.1 ± 0.1

Sterilized at 121°C for 15 minutes.

2. Seawater peptone (SWP)

Bacteriological peptone	10 g
Potassium nitrate	2 g
Sea water	1 litre
pH	7.1 ± 0.1

Sterilized at 121°C for 15 minutes.

3. Tryptone Glucose Agar (TGA)

Tryptone	5 g
Beef extract	3 g
Sodium chloride	5 g
Distilled water	1 litre
pH	7.1 ± 0.1

Sterilized at 121°C for 15 minutes

4. Skim Milk Agar (SMA)

A sterile solution of skim milk powder in distilled water (Sterilized at 0.07 kg/Cm^2 for 20 minutes) was added aseptically to nutrient agar, melted and cooled to 50°C. The final concentration of skim milk powder in nutrient agar was adjusted to 10%.

5. Iron Agar (IA)

Beef extract	3 g
Yeast extract	3 g
Peptone	20 g
Sodium chloride	5 g
Lactose	10 g
Glucose	1 g
$Fe\ So_4\ 7H_2O$	0.2 g
$Na_2\ S_2\ O_3$	0.3 g
Phenol red	12 ml of 0.2% solution
Agar	15 g
Distilled water	1000 ml
pH	7.4 ± 0.1

Sterilized at 121°C for 15 minutes.

6. Na2 EDTA Solution

Ethylene Diamine Tetra Acetic Acid, disodium salt, Na_2 EDTA (BDH-INDIA) dissolved in distilled water (1% W/V).

7. Stock Buffer Solution

Potassium dihydrogen orthophosphate (KH_2 PO_4)	35 g
Distilled water	1000 ml

Dissolved the KH_2 PO_4 in 1000 ml. water. Adjusted the pH to 7.2. Stored in a well-stoppered bottle in refrigerator.

8. Working buffer

12 ml. of the stock buffer solution was diluted to 1 litre. Adjusted the pH to 7.2. Distributed in 9 ml. quantities in 18 x 150 mm. chemically cleaned test-tubes and 90 ml. quantities in 150 ml conical flasks.

Steriilized at 120°C for 20 minutes.

9. Dehydrated media

The following dehydrated media from Oxoid (England) and Difco (USA) were used. They were prepared as directed by the manufacturers.

Baird Parker Agar (BP) - (OXOID)

Disoxy cholate lactose Agar (DLA) - (DIFCO)

Thiosulphate Citrate Bile salt - Sucrose Agar (TCBS) - (OXOID)

Tergitol - 7 Agar (T_7) - (DIFCO)

Chemicals used were BDH (England), SISCO (India), E. Merck (Germany) brands and were of analytical reagent (AR) grade.

2.3.2 RAW MATERIAL

The raw material used was the fish belonging to the species Nemipterus japonicus. Fish selected for the study was taken from the catches of the vessels belonging to the Integrated Fisheries Project (IFP), Kochi-16. The catches were from the fishing grounds off the South West Coast of India.

2.3.2.1 **Sample preparation**

Fresh fish meant for the study were washed thoroughly in potable water, iced using crushed ice in 1:1 ratio and kept in chilled room at +8°C till the time it was taken for dressing/mincing.

2.3.2.2 **Dressed Fish**

The fish were then beheaded, eviscerated and washed. This fish was packed in 500 g. lots as dressed fish samples in polythene bags, frozen at -40°C in a contact plate freezer and stored at -20°C in cold storage of IFP.

2.3.2.3 **Minced Fish**

For preparing mince, the remaining dressed fish were split open from the ventral side to expose maximum surface area.

The fish were thoroughly washed again. The washed fillets were minced in a Badder 694 deboning machine. Mince was divided into two parts. One part was apportioned into 500 g lots, packed in polythene bags and frozen at -40°C in a plate freezer and stored at -20°C.

2.3.2.4 **Treated Minced Fish**

The remaining half of the above was mixed with sufficient volume of 1% Na_2 EDTA solution (W/V) to give resultant concentration of 0.01% of NA_2 EDTA in the mince. The treated mince was also packed in 500 g. lots in polythene bags and frozen at -40°C and stored at -20°C.

2.3.2.5 **Storage**

The frozen samples were stored at -20°C at the Integrated Fisheries Project's Cold Storage for a period of six months. The samples were subjected to various quality tests at different intervals during the storage period. The schedule of such sampling and tests was:

1. before freezing 2. after freezing 3. after 10 days frozen storage 4. after 2 month's frozen storage 5.after 4 month's frozen storage and 6. after 6 month's frozen storage.

2.3.3 METHODS

The quantitative and qualitative changes in the bacterial flora during the frozen storage of the minced fish in comparison

with treated mince and dressed fish were assessed.

Chemical and organoleptic changes were also monitored simultaneously.

2.3.3.1 **Enumeration of total bacteria (TPC)**

Muscle homogenate of the raw material and frozen samples were prepared by grinding about 10 g. of the sample with 100 ml. of working buffer solution. Subsequent dilutions were prepared in the same diluent by mixing 1 ml. of the sample dilution with 9 ml. of diluent in a vortex mixer (Remi India), and so on for further dilutions. One ml. of each dilution was pipetted into petridishes in triplicate. Tryptone Glucose Agar (TGA) was used as plating media. TGA media previously molten and maintained in molten state by keeping in a thermostatically controlled water bath at 45°C was used for plating.

One set of plates was kept at 29 ± 2°C (Room Temperature, RT) for total plate count at RT and another set of plates at 37°C in the incubator for mesophilic count. The third set of plates were kept at 8°C for psychrophilic count.

The plates were incubated at RT for 48 hrs., 37°C for 48 hrs. and 8°C for 10 days.

The total aerobic bacterial count/gram was calculated by the relation:

$$\text{Total bacterial count/g} = \frac{\text{Average count x dilution factor}}{\text{Weight of the sample}}$$

Counts falling between 30 and 300 and agreeing by 10% between duplicates at the particular temperature of incubation were used for calculation of total plate count (TPC).

2.3.3.2 **Enumeration of pathogenic/indicator bacteria**

Total Coliform Bacteria and Escherichia coli.

The appropriate dilutions from the sampling for TPC were pour-plated with Desoxycholate Lactose Agar (DLA). Plates were incubated at 36 ± 1°C for 24 hours., and pink colonies were counted as total coliforms. Plating was also done with Tergitol - 7 Agar (T-7 Agar) by spread plate method, and incubated at 36 ± 1°C for 24 hours. Yellow, circular, mucoid colonies were counted. This gave the E. coli count. For confirmation, typical colonies from the Tergitol - 7 Agar were inoculated into the E.C. medium, incubated at 45°C and examined for production of acid and gas at the end of 24 hours. Positive cultures were further confirmed by studying their Indole, Methyl Red, Voges - Prauskar and Citrate utilization reactions (IMVIC Tests).

2.3.3.3 **Total Faecal Streptococci**

Appropriate dilutions of the samples as mentioned in the case of coliforms, were plated with KF Agar. Plates were incubated at 36 ± 1°C for 48 hours and dark pink colonies counted as faecal streptococci.

2.3.3.4 **Staphylococci count**

Sampling as above was done using Baird - Parker Agar (Spread

plate method) incubated at 36 ± 1°C for 36-48 hours. Black colonies with narrow white margins and zones of clearance around were counted as staphylococci colonies.

2.3.3.5 **Salmonella**

25 g. of the sample was used for detection of salmonella by the AOAC (1970) methods.

2.3.3.6 **Isolation of the Bacterial cultures**

About 30-50 colonies were picked from the TPC plates taking care to include colonies of differing morphology in the ratio of their relative distribution in the plate. Colonies were transferred to tryptone glucose broth (TGB). They were incubated at the corresponding incubation temperature used for initial isolation ie. for e.g. the colonies isolated from the plates at RT were incubated at RT and those from 37° at 37°C. The period of incubation was 2 days for cultures kept at RT and 37°C and 7 days for those kept at 8°C. These cultures showing visible growth after the completion of incubation period, were taken onto Nutrient Agar (NA) slants.

The cultures were purified by repeated streaking on the nutrient agar plates. Finally the pure cultures were maintained on NA slants at 5°C for further studies.

2.3.3.7 **Morphological studies**

For Gram staining, smears were made from 18 to 20 hour

old cultures, grown on NA slants at RT (29 ± 2°C) and 8°C. Gram Stain as modified by Hucker was used for staining (Salle, 1954).

The dimension of the cells were measured on Gram stained preparation using calibrated ocular micrometer. Presence of spore was detected usually by staining 48-72 hours old cultures and examining microscopically. Mobility was observed by hanging drop method (Anon 1957) using phase contrast microscope (Carl Zeiss Jena, Germany).

2.3.3.8 **Biochemical characteristics of the cultures**

The ability of the culture, to reduce nitrate, to produce indole from tryptone, to liquify gelatin to ferment surcose, maltose, manitol, arabinose, raffinose and rhamnose and to produce H_2S was studied by the standard methods (Salle 1954).

The mode of attack of glucose by cultures was determined by using Hugh and Leifson's oxidative and fermentative test medium (Hugh & Leifson 1953). The presence of cytochrome oxidase in the cultures was detected by the modified Kovac's test (1956), catalase by observing gas when a drop of 10% H_2O_2(V/V) was mixed with a speck of young culture on a clean slide, pigmentation by observation on skimmed milk peptone agar (Surendran 1980) after a week's incubation at 28 2°C, luminiscence by examining the cultures in a dark room daily for four days after incubation and sensitivity to penicillin and 0/129 compound, by the pad plate

method. Filter paper of 5 mm dia, impregnated with the test compound at the appropriate levels, were placed on agar plates already seeded with the test culture and then incubated at 28 ± 2°C for 18 to 24 hours and zone of clearance around the filter paper pads noted. In the case of penicellin each disk contained 2.5 I.U. penicillin and for 0/129 compound, 10 µg.,

The differentiation of the bacterial cultures upto the generic level was done by the scheme (Surendran 1980) outlined in Fig. I. .

2.3.3.9 **Chemical indices**

pH was measured in a slurry of 10 g. of the material in 100 ml. distilled water, using a combined electrode pH meter.

Degradative changes in fat was assessed by peroxide value (P.V). P.V. was determined by the AOAC method (1970).

2.3.3.10 **Organoleptic evaluation**

For organoleptic evaluation 3 to 5 packet of each of the test samples were used at a time. The mince fish portion (100g) were sealed in a polythene bag and kept dipped in boiling water for 15 mts., by which time they were cooked. They were served to six panelists immediately on opening. Flavour, odour, texture and taste were recorded using scalar system

of scoring. Representative score sheet is given in Table 2.4.

Acceptance level	Score
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Table 2.4. Representative score sheet used for assessing organoleptic qualities of the test sample.

2.3.4 RESULTS AND DISCUSSIONS

2.3.4.1 **Bacteriological changes in minced fish - Quantitative aspects**

2.3.4.1.1 Effect of pre-process handling on total bacterial counts

Quantitative changes in bacterial count of (A) dressed fish (B) Minced fish are given in Table 2.3.4.1 for 3 sets of experiments.

The bacterial count (TPC) at room temperature of dressed fish in Expt.I was 2.2×10^5 /g., before freezing. In the

case of minced fish the corresponding TPC was one log. higher ie., 1.5 x 10^5/g. Similar increases had been obtained in the other two experiments also. This increase in TPC was on expected lines because during the process of mechanical mincing of the dressed fish using Baader 694 deboner, there was ample chance of contamination from handling as well as from the machine. There was total mixing up of bacteria, which in the case of the whole fish would be mainly concentrated on surfaces, in comminuted meat. Such mixing could provide a highly nutritious environment to the bacteria which in turn would have multiplied by atleast one generation, before the product was processed. So actually mincing had helped in enhancing the total bacterial count of fish muscle, a change which was not desirable from the bacteriological stand point.

Nickelson et al (1980) had made similar observations in the case of croaker and tilapia mince. Also Liston (1980) found that fish minces prepared even under the most controlled conditions were extremely susceptible to such contaminations. Raccah and Baker (1978) in their studies using several traditional and under utilized fish species noted that mechanical deboning of various species resulted in a ten fold increase in the microbial load. In India the observations of Abraham et al (1992) on the mincing of several tropical fishes also

were in conformity with the results obtained in the present study.

Elliot (1986) in his study on an in-line survey of the mincing of Alaska pollak revealed that the microbial load increased during processing from less than 3 x 10^3 cfu/g. in skinned fillets to 8.5 x 10^4/g. in refined mince.

Use of chemical preservatives to control the multiplication of bacteria in the mince had some effects. In this study, the treating the minced fish with EDTA had the effect of controlling the increase in TPC before freezing.

Table 2.3.1 shows that while the total plate count of the minced fish was 1.5 x 10^6/g, the corresponding count of the EDTA treated fish mince was only 40% of the TPC of the minced fish. This indicated that even though the TPC of the treated mince was 2.5 times higher than the dressed fish it was far less than the TPC of the minced fish and hence EDTA treatment has definitely controlled bacterial multiplication in the minced fish before freezing.

Table 2.3.2 gives data on the TPC of dressed fish, fish mince and treated minced fish at 37°C. While there is not much difference in the TPC of dressed fish incubated at 37°C, from that at RT, there was about one log cycle difference in the TPC of the minced fish at RT and at 37°C.

Quantitative changes of bacterial count in

A. Raw dressed fish
B. Minced fish
C. Treated minced fish

TABLE - 2.3.1

EFFECT OF PRE-PROCESS HANDLING OF FISH MUSCLE ON BACTERIAL POPULATION AT RT

		Total plate counts/g at RT		
		A	B	C
Experiment	I	2.2×10^5	1.5×10^6	6.1×10^5
	II	4.3×10^3	7.6×10^4	7.4×10^3
	III	3.3×10^4	2.2×10^5	6.6×10^4

TABLE - 2.3.2

EFFECT OF PRE-PROCESS HANDLING OF FISH MUSCLE ON BACTERIAL POPULATION AT 37°C

		T P C at 37°C		
		A	B	C
Experiment	I	1.3×10^5	2.3×10^5	2.4×10^5
	II	2.4×10^3	1.3×10^4	1.34×10^3
	III	1.6×10^3	3.8×10^4	1.6×10^4

Quantitative changes of bacterial count in

A. Raw dressed fish
B. Minced fish
C. Treated minced fish

TABLE - 2.3.3

EFFECT OF PRE-PROCESS HANDLING OF FISH MUSCLE ON BACTERIAL POPULATION AT 8°C

		T P C at 8°C		
		A	B	C
Experiment	I	7.4×10^4	4.6×10^5	3.8×10^5
	II	2.3×10^3	7.4×10^3	1.2×10^2
	III	1.4×10^4	2.3×10^4	3.2×10^3

Table 2.3.3 shows the total plate count of the three samples A,B,C at 8°C. The counts were in general lower in all the three types of samples compared with those at RT. In the case of minced fish there was one log cycle reduction in recovery.

The most reasonable explanation could be that the flora recovered at the ambient temperature of 29 ± 2°C included both mesophilic and psychrotrophic bacteria. At 37°C the psychrotrophic are not recovered and hence a reduction to that extent was apparent in TPC at 37°C. Similarly mesophilic bacteria cannot grow at 8°C and hence the TPC at 8°C would be lower to that extent.

Lee and Pfeifer (1974) has found that psychrotrophic bacteria associated with fish often grow poorly or not at all at 35°C. Decrease in aerobic plate counts of minced fish caused by freezing were more extensive, in counts at 25°C than at 35°C. This is probably so because injury or death of psychrotrophic bacteria would not have been as apparent at 35°C as at 25°C.

Nickelson et al (1980) has shown that aerobic plate count (APC) of freshly minced flesh at 35°C was markedly lower than those determined at 25°C. The counts were always 1.6 - 2.6 logs lower at 35°C than those obtained at 25°C. APC of Sheepshead was 3.0×10^4/g. at 35°C while it was only

1.4 x 10^5/g. at 25°C. In mullet it was 7.0 x 10^4/g. at 35°C and 3.7 x 10^6/g. at 25°C. In black drum it was 1.3 x 10^6/g. at 35°C and 1.9 x 10^8/g. at 25°C.

2.3.4.1.2 Effect of freezing on the total bacterial counts.

Table 2.3.4). shows the effect of freezing and frozen storage of dressed fish, minced fish and treated minced fish on the TPCs at RT.

Freezing has brought about a drastic reduction in the TPCs of all the three samples. In the case of dressed fish the reduction was to the tune of 91.5% compared with the TPC before freezing.

The bacterial count of minced fish suffered a reduction of 91.3% which is almost the same as the dressed fish, but the reduction brought about in treated minced fish was quite high ie. 97%.

Freezing always brings about considerable death to the bacteria and this has been very much pronounced in the case of bacterial population of the aquatic organisms, particularly fish.

This has generally been explained to be due to the predominant Gram negative nature of their flora (Shewan 1961).

In the present study an interesting observation was that in spite of the fact that the untreated minced fish had initial bacterial count which was almost about 10 times higher than that of dressed fish, the rate of reduction brought about by

TABLE - 2.3.4

Changes in total bacterial counts at RT of

A. Raw dressed fish

B. Minced fish

C. Treated minced fish

DURING FREEZING AT -40°C AND FROZEN STORAGE AT -20°C

Storage period	T P C at RT		
	A	B	C
Initial (Before freezing)	2.1×10^5	1.5×10^6	6.0×10^5
After freezing at -40°C	1.7×10^4	1.3×10^5	1.8×10^4
After storage at -20°C			
For 10 days	1.9×10^4	2.7×10^5	1.31×10^4
two months	1.0×10^4	1.9×10^4	1.29×10^4
four months	1.1×10^4	1.0×10^4	1.301×10^4
six months	3.3×10^3	1.5×10^4	8.1×10^3

freezing was almost similar (91%). This would naturally indicate that they had similarly susceptible bacterial population. In the case of EDTA treated minced fish the freezing had almost reduced the bacterial population to 3% of the initial count. This drastic reduction can be explained due to only to the combined effect of freezing and EDTA treatment. Effect of EDTA could be that it had rendered the bacterial cells more susceptible to freezing thereby causing higher reduction in TPC.

Table 2.3.5 gives the effect of freezing on the total bacterial counts at 37°C of dressed fish, minced fish and treated minced fish. In the case of dressed fish and minced fish comparable reduction of 71.2% and 70.48% respectively were observed, from the corresponding TPCs at 37°C. But in the case of EDTA treated mince the reduction was still higher at 95.35%. The explanations relevant in the case of the observations on the effect of freezing on the TPCs at RT are relevant here also.

Table 2.3.6 shows the effect of freezing on the total bacterial count at 8°C of dressed fish, minced fish and treated minced fish. In this case, a drastic reduction in the bacterial count are seen for dressed fish and untreated mince, the reduction being 99% and 97% respectively. However, for EDTA treated mince the reduction was only 73% from the initial TPC at 8°C.

TABLE - 2.3.5

Changes in total bacterial count at 37°C of

A. Raw dressed fish
B. Minced fish
C. Treated minced fish

DURING FREEZING AT -40°C AND FROZEN STORAGE AT -20°C

Storage period	T P C at 37°C		
	A	B	C
Initial (Before freezing)	3.2×10^5	3.32×10^5	2.8×10^5
After freezing at -40°C	9.2×10^4	9.8×10^4	1.3×10^4
After storage at -20°C			
For 10 days	9.0×10^3	9.4×10^4	1.24×10^4
two months	4.2×10^3	6.7×10^4	1.5×10^4
four months	3.40×10^3	5.3×10^4	1.3×10^4
Six months	2.46×10^3	1.2×10^4	8.7×10^3

TABLE - 2.3.6

Changes in total bacterial count at 8°C of

A. Raw dressed fish

B. Minced fish

C. Treated minced fish

Storage period	T P C at 8°C		
	A	B	C
Initial (Before freezing)	4.4×10^5	5.96×10^5	3.8×10^5
After freezing at -40°C	2.1×10^3	1.76×10^4	1.1×10^5
After storage at -20°C			
For ten days	2.8×10^3	1.3×10^4	1.2×10^5
two months	3.2×10^3	1.28×10^4	9.4×10^4
four months	2.8×10^3	1.32×10^4	8.1×10^4
Six months	2.1×10^3	6.8×10^3	9.6×10^4

Effect of freezing and frozen storage on the bacterial population associated with raw as well as minced fish from the temperate waters had been the subject of a lot of detailed investigations. Pivnick (1949), Tressler and Evers (1957), Kiser and Beckwith (1942) and Borgstrom (1955) had studied the effect of freezing and frozen storage on the bacterial counts of whole fishes.

Pivnick (1949) found that freezing and frozen storage of the North Sea Fish, ocean perch, stored at (-) 15°C resulted in 17% reduction of the bacterial count after one month. Kiser and Beckwith (1942) observed a 43.3% reduction in the bacterial count of mackerel after 10 days of frozen storage, (-) 28°C. Tressler and Evers (1957) had also reported a considerable reduction in bacterial population of oil sardine during freezing and frozen storage at (-) 23°C.

Jadhav and Magar (1970) found that freezing at (-)40°C and subsequent storage at (-) 15°C for two and half months brought about 60% reduction in the bacterial population of mackerel and 60-70% reduction in the bacterial count of White Pomfret.

In this study, the total bacterial population of dressed fish had suffered a reduction of more than 90% from the initial count at RT. Compared with the reduction in TPC reported for temperate water fishes, this value appears to be quite on the higher side. Also the findings of Jadhav and Magar in the case of fishes

from the Bombay coast are still nearer to our reported observations. The maximum reduction in bacterial count was observed in the early period of frozen storage after which the reduction was more or less gradual. This trend was noticed by Jadhav and Magar (1970).

Death of bacteria during freezing and frozen storage happens in two stages viz. (i) the inactivation occuring during the actual process of freezing and (ii) the inactivation during the process of frozen storage. Freezing commonly begins at (-) 1°C to (-)3°C depending on the substrate in which the bacteria is frozen. At a temperature immediately below 0°C the substrate may contain enough water for the maintenance of bacteria. However, as the process of freezing continues, more and more water is removed from the substrate as ice crystals, so that the substrate gets dehydrated and the water activity goes down, below the critical level for bacterial viability. The conventional theory regarding the destruction of bacteria due to freezing was that the death of bacteria at freezing resulted mainly through the action of extracellular ice crystals (Borgstrom 1955). But now, bacterial death due to freezing is reported to be due to damage caused to semipermeable properties of cytoplasmic membrane. Above (-)10°C freezing occurred only externally and a bacterial cell which is capable of making osmotic adjustment would escape death. Below this temperature, cell membrane failed to act as a barrier to the proliferation of the ice crystals already formed outside

the cell, resulting in intracellular ice formation. This would result in solute concentration. Thus the death or injury to the bacterial cell was due to the ice formation and consequent solute concentration which resulted in the damage to the semipermeable properties of cell membrane (Ingram & Mackey, 1976).

According to Tanaka & Yoh (1980) damage to the DNA was also involved in the death of bacteria due to freezing.

In the present study the freezing was done at (-)40°C and frozen storage at (-)20°C both temperatures being far below the critical temperature of (-)10°C upto which the bacterial cell wall would have insulated the bacteria from injury and death. This explains the significantly high reduction in bacterial population during freezing. As indicated earlier in Table 1.3.4. after the initial drastic reduction in the TPC at RT, of the minced fish and treated mince there was not much decrease in the bacterial count during storage at (-)20°C for a period of six months.

Raccah and Baker (1978) and Elliot (1986) had also found that there was little decrease in the bacterial numbers in minced marine fish during storage at (-)20°C.

In the course of his investigation on freezing and frozen storage of minced fish, Nickelsen II (1980) had observed that even though there was some decrease in the aerobic plate count at 35°C, the reduction during storage was not very extensive.

2.3.4.1.3 Effect of frozen storage on the total bacterial count

Table 2.3.4 presents the data on the changes in bacterial count at RT of dressed fish, minced fish and treated minced fish during frozen storage at -20°C. In the case of dressed fish by the 10th day of frozen storage there was not much change in the surviving bacterial counts. During subsequent storage upto 4 months also the TPC remained more or less same at 10^4/g. indicating that the flora which resulted after freezing at -40°C were capable of survival during frozen storage at -20°C to 4 months. However, after six months of frozen storage the viable counts were only 1/3 of the flora that remained after four months.

In the case of minced fish the bacterial count after 10 days of frozen storage at -20°C was more or less the same as the residual flora after freezing at -40°C. However, during further storage the flora was reduced by about 90% in two months time. Subsequent frozen storage upto six months did not appear to bring about a drastic reduction in the TPC. This observation in the case of minced fish is at variance from the effect of frozen storage of the dressed fish in which case, storage at -20°C beyond 10 days did not drastically reduce the bacterial count upto 4 months.

The bacterial count of the treated minced fish during frozen storage at -20°C did not show any significant reduction upto the entire period of storage upto six months. This indicated

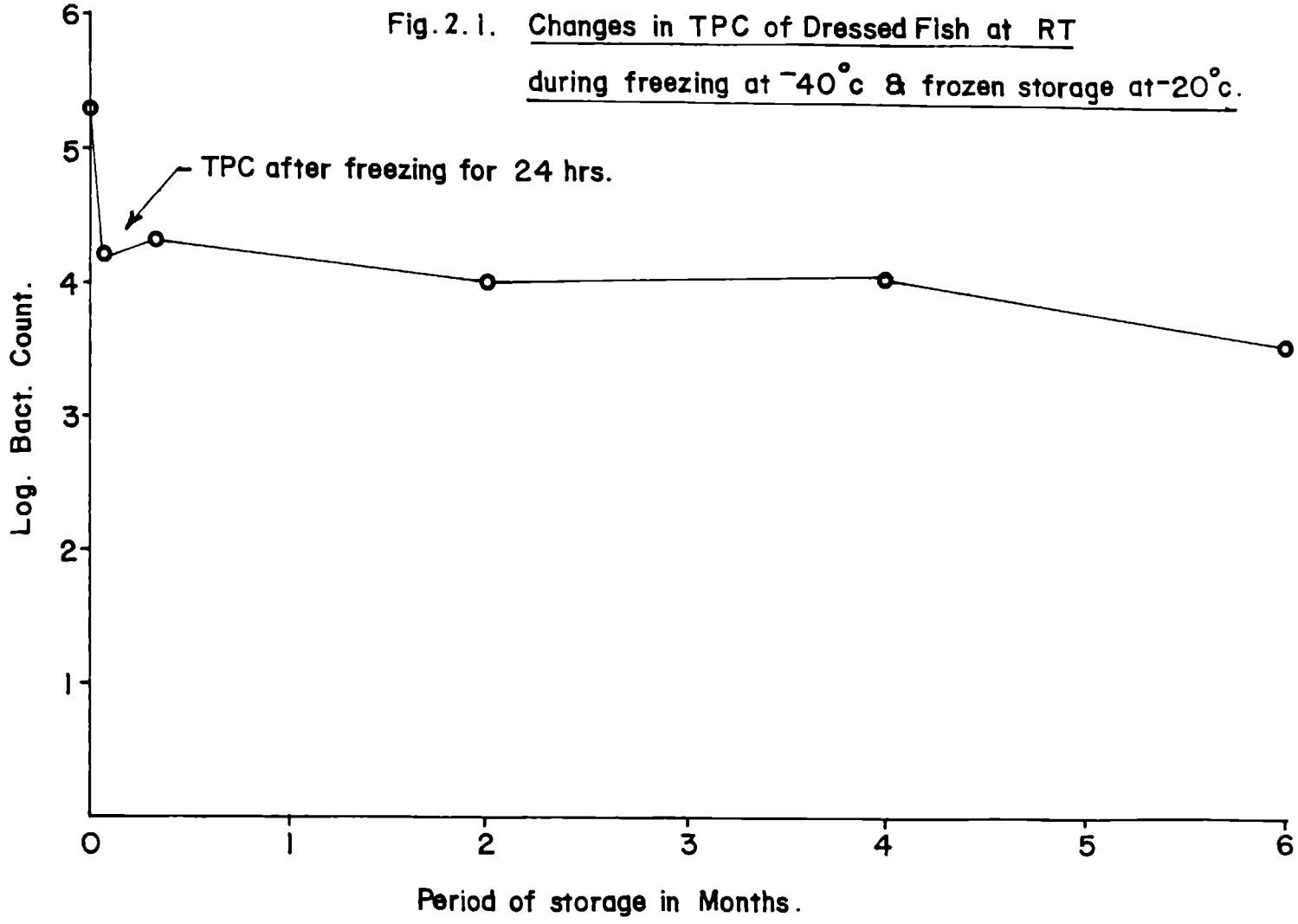
that almost the entire bacterial cells which survived freezing at -40°C were capable of tolerating frozen storage at -20°C.

In Table 2.3.5 the changes in the bacterial population recovered at 37°C of frozen dressed fish, minced fish and EDTA treated minced fish are presented.

In the case of dressed fish by the 10th day of frozen storage at -20°C, the TPC has decreased from 9.2×10^4/g. to 9×10^3/g. ie. one log cycle reduction. However, during subsequent storage upto six months the TPC remained more or less constant at that level indicating that the residual bacterial flora are not further affected by continued frozen storage at -20°C. However, the picture is different in the case of minced fish and EDTA treated minced fish. After the initial reduction due to freezing at -40°C there was not much perceptible decrease in the total bacterial count during the entire storage period of six months at -20°C.

Table 2.3.6. gives the changes in the bacterial population at 8°C of dressed fish, minced fish and treated minced fish during frozen storage at -20°C for a period of six months. As evident from the Table, in all the three cases, the bacterial count did not show any significant reduction during the entire period of frozen storage indicating, that the residual flora which were recovered at 8°C were more or less stable during the frozen storage.

Fig. 2. 1. Changes in TPC of Dressed Fish at RT during freezing at $-40^{\circ}c$ & frozen storage at $-20^{\circ}c$.



Flg 2.2. Changes in TPC of Minced Fish at RT during freezing at $-40^{\circ}c$ & frozen storage at $-20^{\circ}c$.

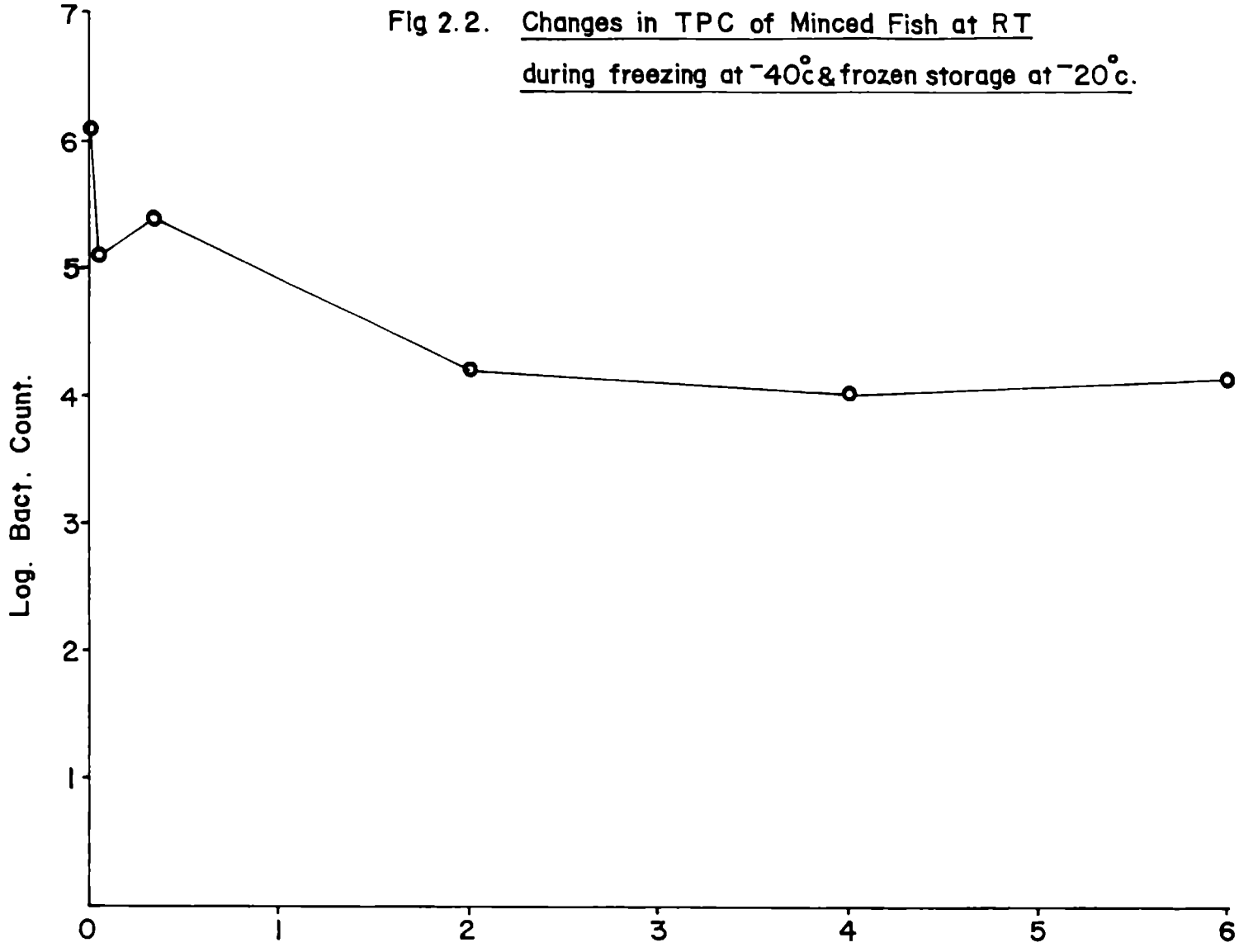


Fig. 2. 3. Changes in TPC of Treated Mince Fish at RT during freezing at $-40^{\circ}c$ & frozen storage at $20^{\circ}c$.

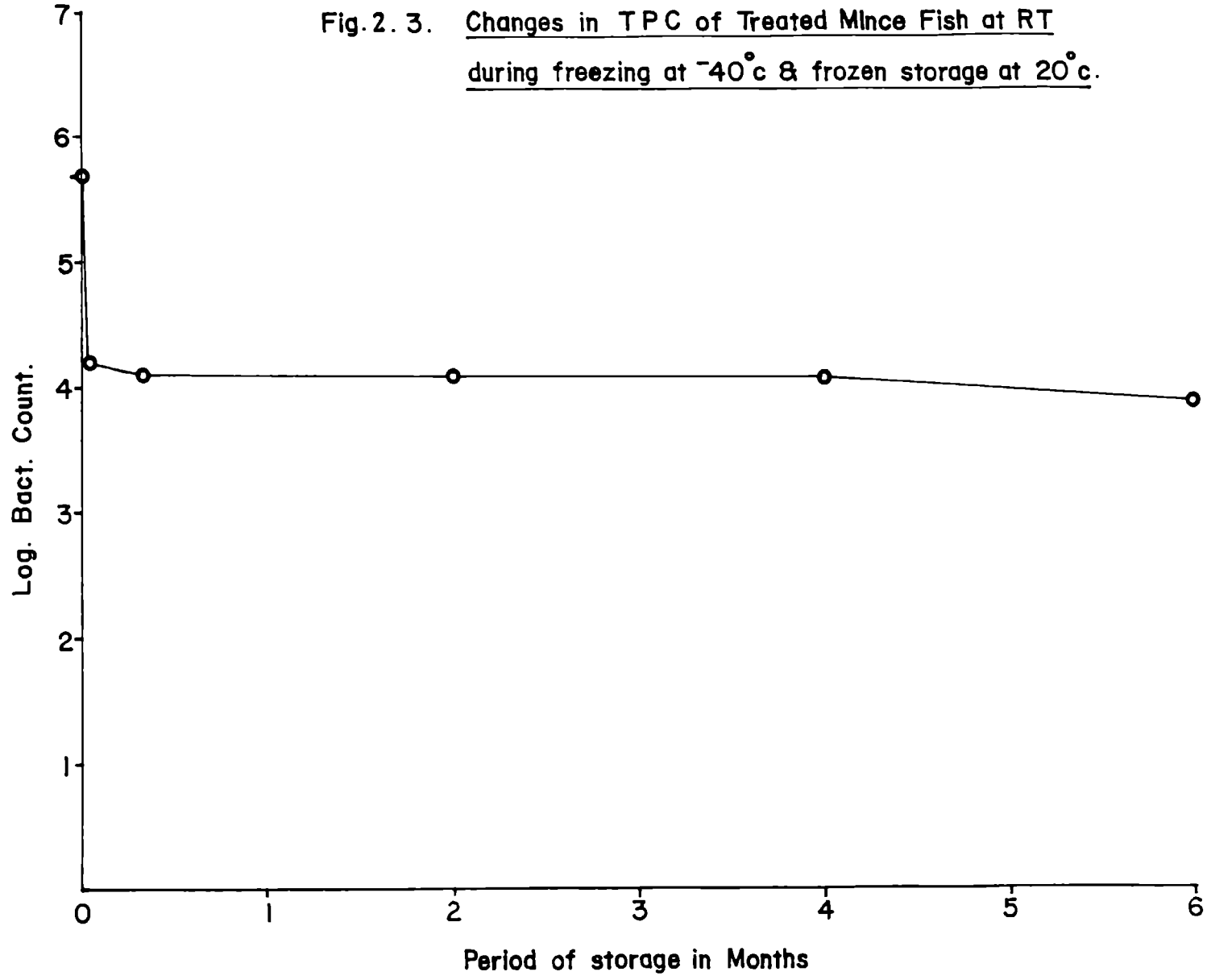


Fig. 2. 4. Changes in TPC of Dressed Fish at 8°c during freezing at -40°c and frozen storage at -20°c

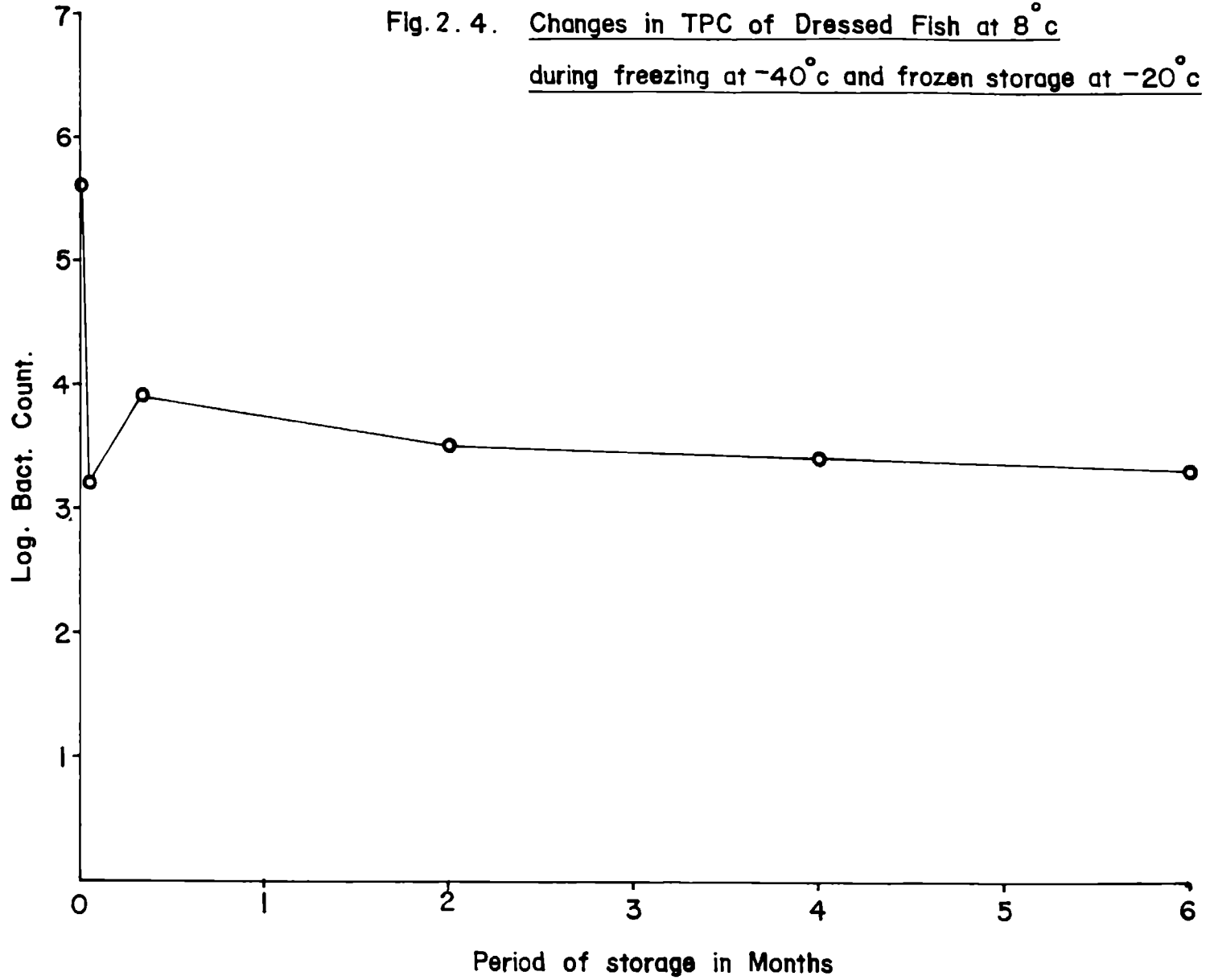


Fig. 2. 5. Changes in TPC of Minced Fish at 8°c.
during freezing at −40°c and frozen storage at −20°c

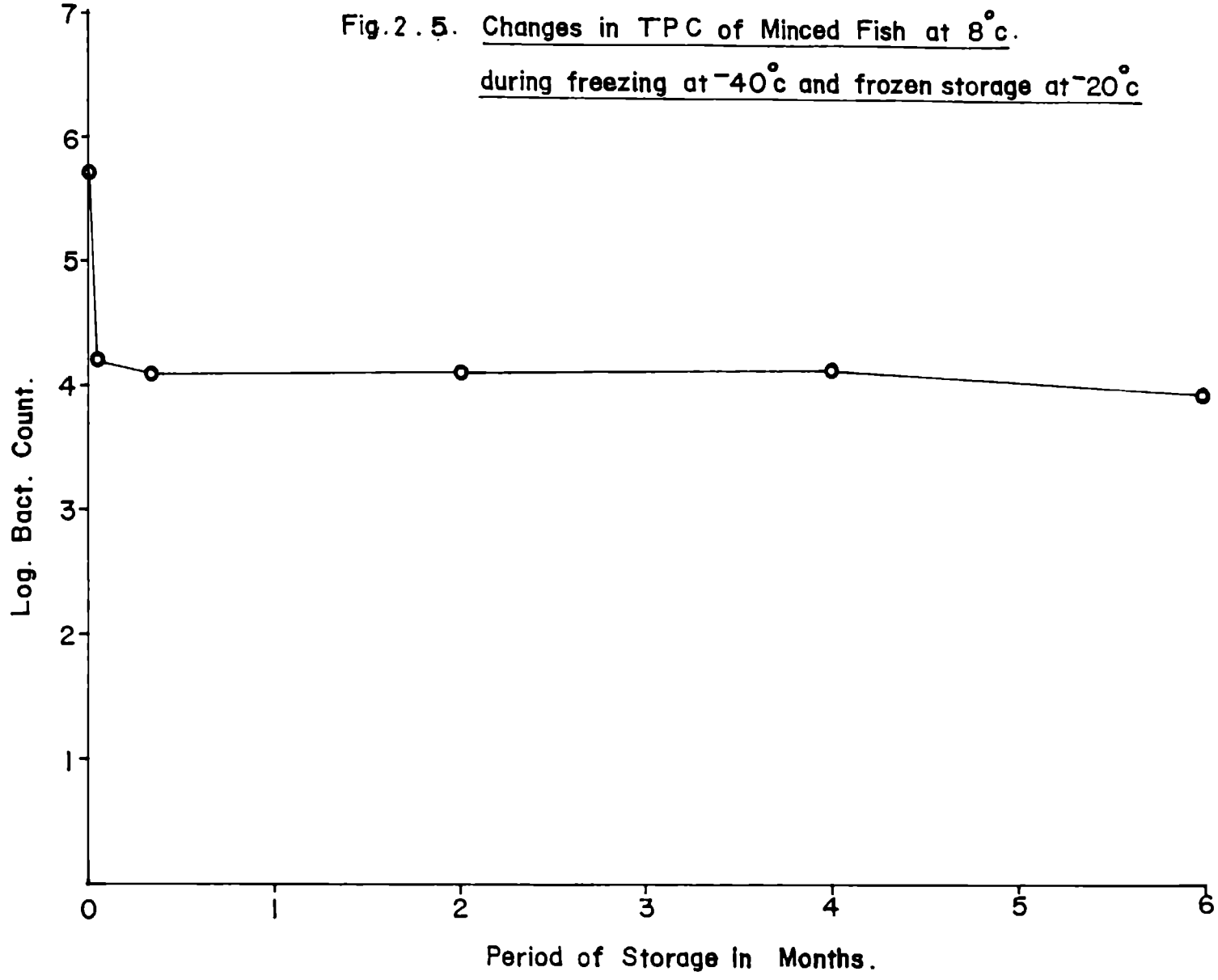


Fig.2.6. Changes in TPC of Treated Minced Fish at 8°c during freezing at -40°c and frozen Storage at -20°c

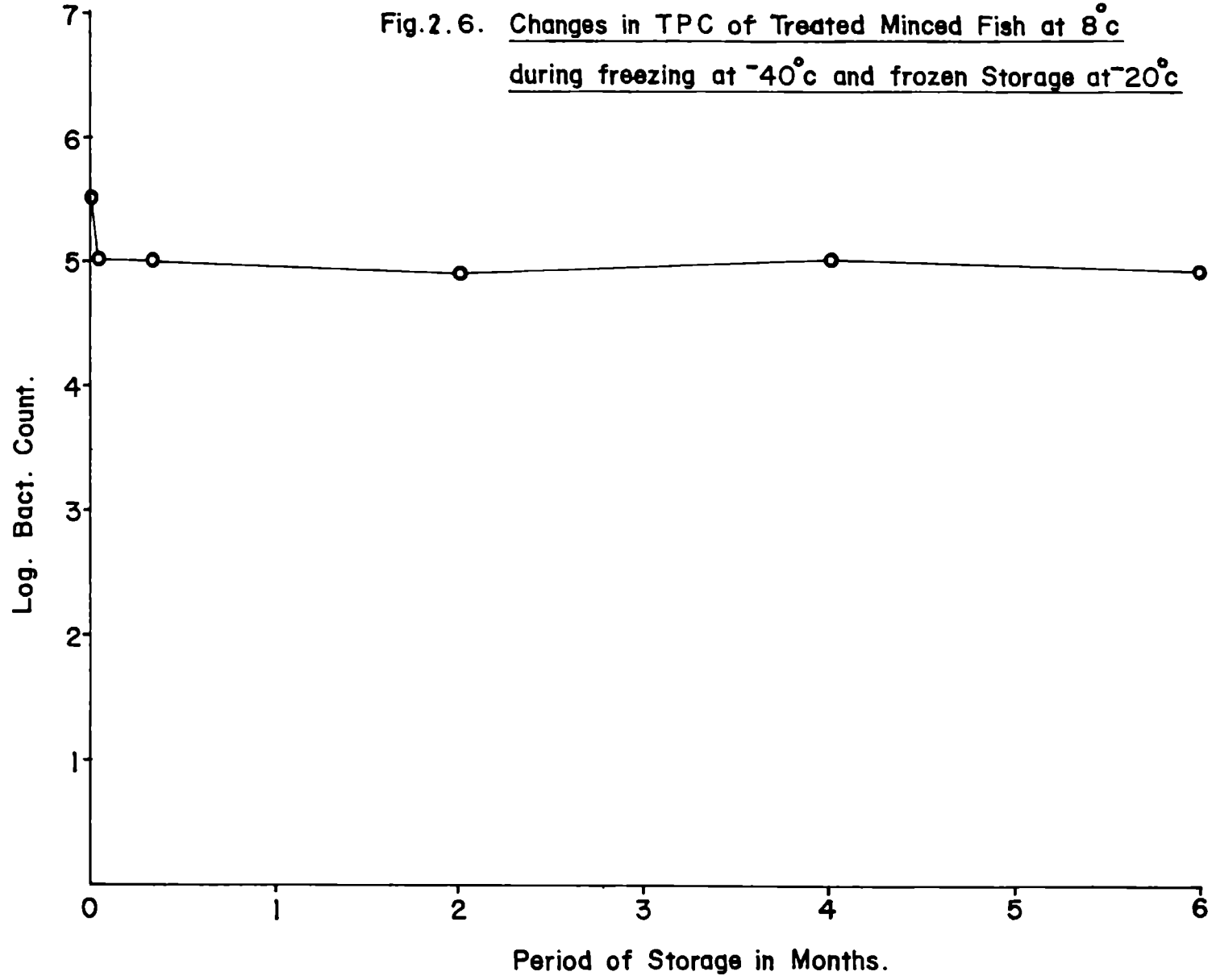


Fig. 2. 7. Changes in TPC of at 37°c of Dressed Fish during freezing at -40°c and frozen Storage at -20°c

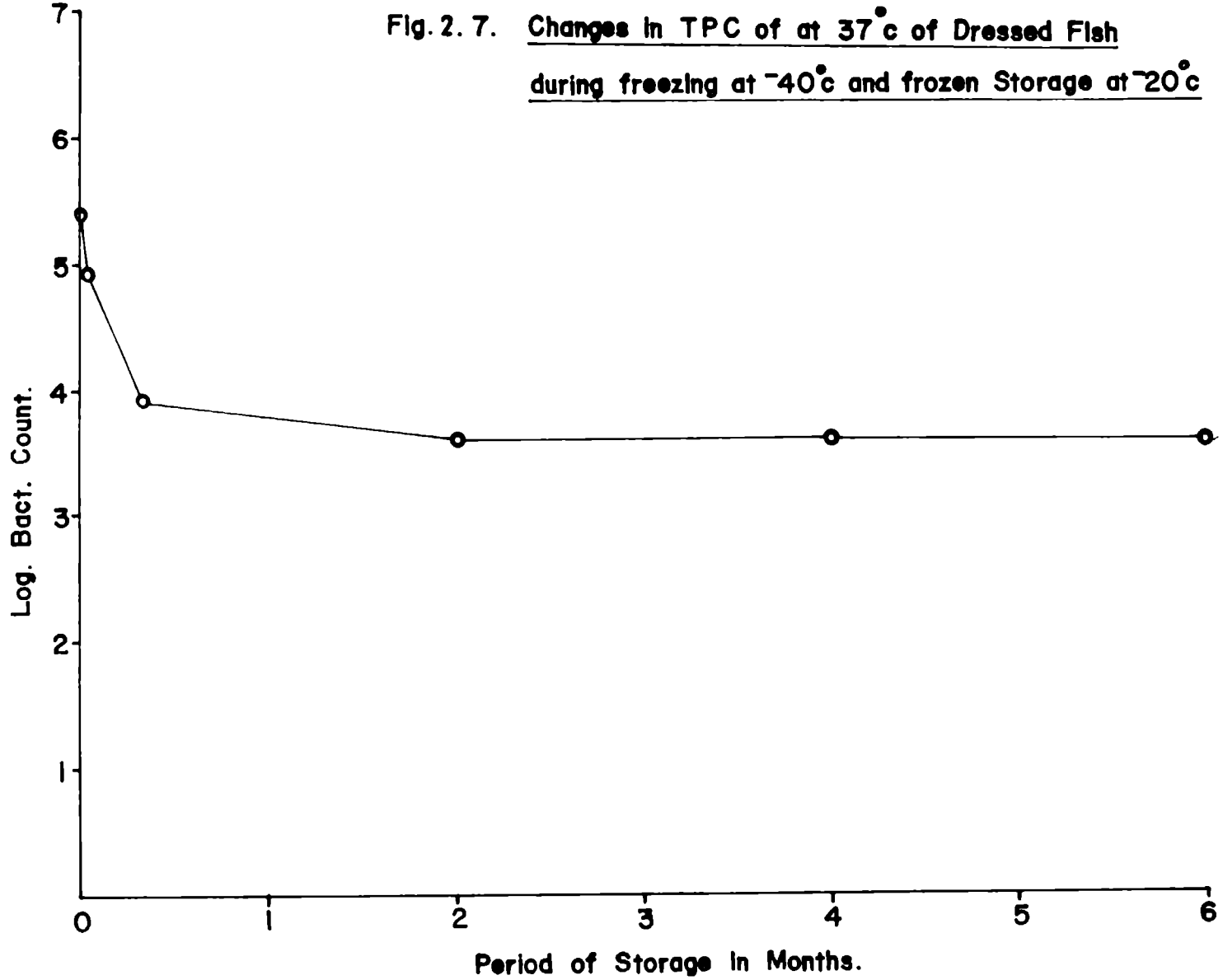


Fig. 2.8. Changes in TPC of Minced Fish at 37°c during freezing at -40°c and frozen storage -20°c

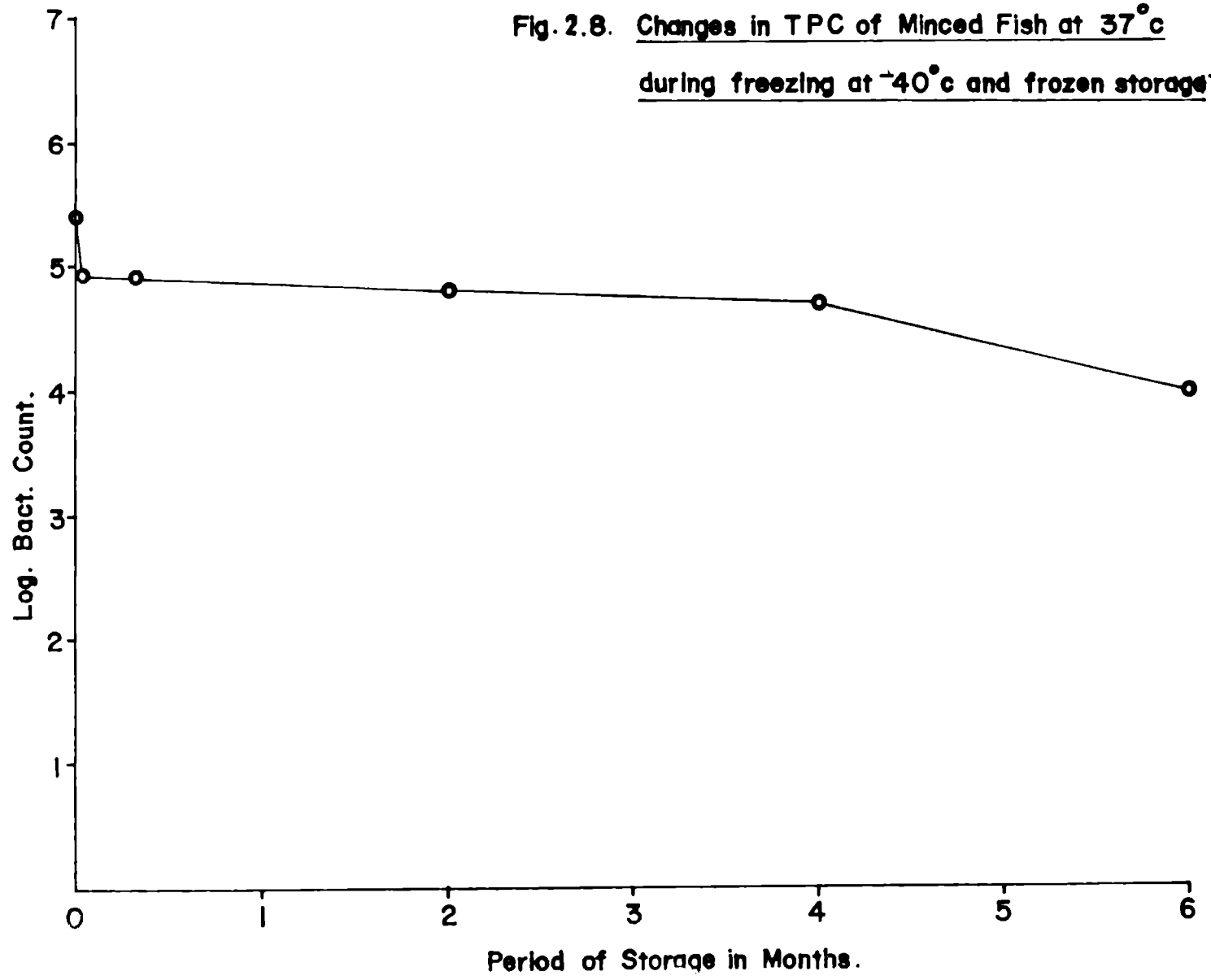
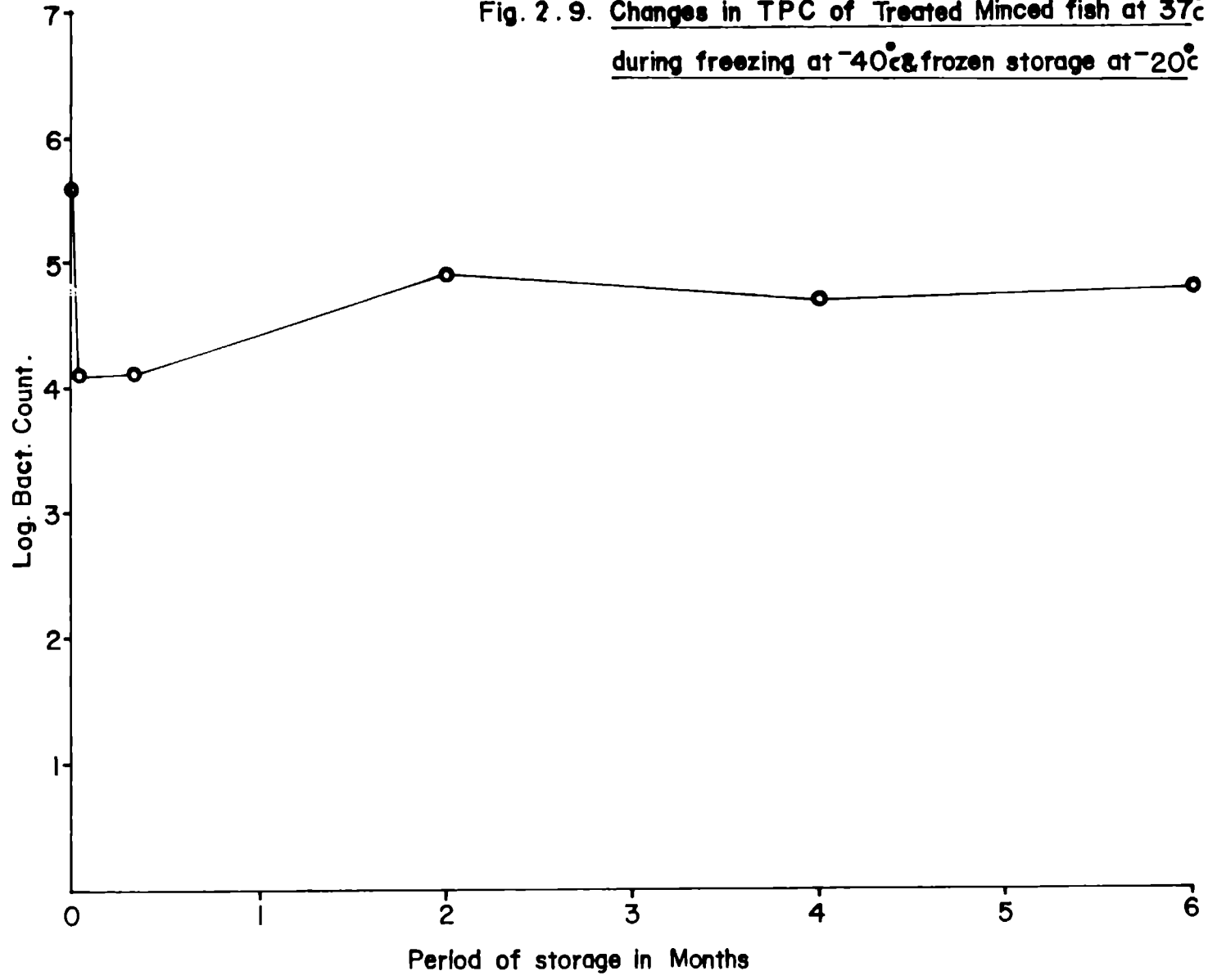


Fig. 2.9. Changes in TPC of Treated Minced fish at 37°c during freezing at -40°c & frozen storage at -20°c



The observation made in our study that after the initial drastic reduction in the count there was no perceptible decrease during frozen storage is in full agreement with the observation made by Raccah & Baker (1978), Elliot (1986) and Nikelson.II (1980).

A recent study by Sorinmade et al (1985) from Nigeria had indicated that contrary to the usual expectation of a declining bacterial population during freezing and frozen storage, there was a more or less steady increase in the total viable count when minced fish was frozen and stored at (-) 17°C. This observation has been attributed to disintegration of clumped bacterial cells due to freezing. Also such phenomena had been reported for pure culture by Postgate and Hunter (1961). However in none of the three series of experiments which was conducted as part of this study, no such increase in the bacterial population of frozen mince during frozen storage at (-) 20°C was observed.

2.3.4.1.4 Effect of freezing and frozen storage on the H_2S producing bacteria.

Table 2.3.7 and 2.3.8 show the effect of freezing and frozen storage on H_2S producing bacteria of dressed fish, minced fish and treated mince at RT and 8°C respectively.

Before freezing the dressed fish harboured 1.1×10^4/g of H_2S producing bacteria which were detected as black colonies on Iron Agar.

TABLE - 2.3.7

Effect of freezing at -40°C and frozen storage at -20°C on the H_2S producing bacteria at RT in

A. Raw dressed fish
B. Minced fish
C. Treated minced fish

Storage period	H_2S producing bacterial count at RT		
	A	B	C
Initial (Before freezing)	1.1×10^4	6.1×10^5	1.2×10^5
After freezing	6.2×10^3	4.1×10^4	6.06×10^3
After ten days	2.2×10^2	1.01×10^2	95
After two months	Nil	Nil	Nil
After four months	Nil	Nil	Nil
After six months	Nil	Nil	Nil

TABLE - 2.3.8

Effect of freezing at -40°C and frozen storage at -20°C on the H_2S bacteria at 8°C in

A. Raw dressed fish
B. Minced fish
C. Treated minced fish

Storage period	H_2S producing bacterial count at 8°C		
	A	B	C
Initial (Before freezing)	8.1×10^3	7.2×10^3	7.4×10^4
After freezing	1.5×10^3	1.2×10^3	1.4×10^3
After ten days	1.8×10^2	Nil	Nil
After two months	Nil	Nil	Nil
After four months	Nil	Nil	Nil
After six months	Nil	Nil	Nil

In the minced as well as EDTA treated minced fish there is a ten fold increase in H_2S bacteria. Immediately after freezing at (-) 40°C there was a 45% reduction in the H_2S producing count in the case of dressed fish, 98% in the case of minced fish, and 95% in the case of treated mince.

On storage at (-) 20°C the counts have drastically falled further by the first 10 days and this drastic reduction had been very significant in the case of EDTA treated fish, the reduction being almost 98.5% of the residual count after freezing.

After two months of frozen storage the H_2S producing bacteria had been completely absent indicating their susceptibility to frozen storage. The trend in the H_2S producing bacterial counts and changes during freezing and frozen storage were almost the same at 8°C also, except that within the first ten days of storage, the H_2S producing bacteria had died out in the case of minced fish and EDTA treated minced fish.

A comparison of the H_2S producing bacterial count and the total plate count at RT in the case of dressed fish, minced fish and EDTA treated mince are presented in Tables 2.3.9, 2.3.10 and 2.3.11 respectively.

A similar comparison at 8°C are presented in Table 2.3.12, 2.3.13 & 2.3.14 respectively.

Before freezing the H_2S producing bacterial count of the raw

dressed fish was only 5.5% of the TPC at RT. But immediately after freezing its ratio increased to 36.9%. This indicated that the death of H_2S producing bacteria due to freezing of the dressed fish at (-) 40°C was comparatively less in relation to the non H_2S producing bacterial component of the TPC. However, within 10 days of storage at (-) 20°C the H_2S producers had drastically died out and by two months time at (-)20°C there was none left. This was indicative of the fact that eventhough H_2S producers could better survive quick freezing at (-) 40°C, they became eventually susceptible to cold storage at (-) 20°C.

H_2S producing bacteria formed 40% of the TPC at RT in the case of minced fish before freezing. The higher incidence of the H_2S producers in minced fish could have been due to the contamination during the mincing process. Due to freezing at (-) 40°C the ratio had come down to 30% of the TPC. Within ten days of frozen storage at (-) 20°C the percentage of H_2S producers in the TPC at RT had been reduced to a very insignificant level 0.03%. This also completely vanished by the 2nd month of frozen storage.

In the case of EDTA treated mince also more or less similar trends as the minced fish could be seen in the ratio of H_2S producing bacteria to the TPCs at RT. Eventhough a slight increase has been noticed in the ratio of H_2S producing

TABLE - 2.3.9

Comparison of total bacterial count and H_2S producing bacterial Count at RT for dressed fish

	T P C at RT (a)	H_2S producing bacterial count at RT (b)	Ratio of $\frac{b}{a}$ %
Initial (Before freezing)	2.1×10^5	1.1×10^4	5.3
After freezing	1.7×10^4	6.2×10^3	36.9
After ten days	1.9×10^4	2.2×10^3	11.5
After two months	1.0×10^4	Nil	Nil
After four months	1.1×10^4	Nil	Nil
After six months	3.3×10^3	Nil	Nil

TABLE - 2.3.10

Comparison of total bacterial count and H_2S producing bacterial count at RT for minced fish

Storage period	T P C at RT (a)	H_2S producing bacterial count at RT (b)	Ratio of $\frac{b}{a}$ in %
Initial (Before freezing)	1.5×10^6	6.1×10^5	40
After freezing	1.3×10^5	4.1×10^4	30
After ten days	2.7×10^5	1.01×10^2	0.03
After two months	1.9×10^4	Nil	0
After four months	1.0×10^7	Nil	0
After six months	1.5×10^4	Nil	0

TABLE - 2.3.11

Comparison of total bacterial count and H_2S producing bacterial count at RT for treated minced fish

Storage period	T P C at RT (a)	H_2S producing bacterial count at RT (b)	Ratio of $\frac{b}{a}$ in %
Initital (Before freezing)	6.3×10^5	1.2×10^5	19
After freezing	1.8×10^4	6.06×10^3	30
After ten days	1.31×10^4	95	0.75
Afteer two months	1.29×10^4	--	--
After four months	1.301×10^4	--	--
After six months	8.1×10^3	--	--

TABLE - 2.3.12

Comparison of total bacterial count and H_2S producing count at 8°C for dressed fish

Storage period	T P C at 8°C (a)	H_2S producing bacterial count at 8°C (b)	Ratio of $\frac{b}{a}$ in %
Initial (Before freezing)	4.4×10^5	8.1×10^3	1.8
After freezing	2.1×10^3	1.5×10^3	74
After ten days	2.8×10^3	1.8×10^2	6.4
After two months	3.2×10^3	Nil	0
After four months	2.8×10^3	Nil	0
After six months	2.1×10^3	Nil	0

TABLE - 2.3.13

Comparison of total bacterial count and H_2S producing bacterial count at 8°C for minced fish

Storage period	T P C at 8°C (a)	H_2S producing bacterial count at 8°C (b)	Ratio of $\frac{b}{a}$ in %
Initial (Before freezing)	5.96×10^5	7.2×10^3	1.2
After freezing	1.76×10^4	1.2×10^3	6.
After ten days	1.30×10^4	Nil	0
After two months	1.28×10^4	Nil	0
After four months	1.32×10^4	Nil	0
After six months	6.8×10^3	Nil	0

TABLE - 2.3.14

Comparison of total bacterial count and H_2S producing bacterial count at 8°C for treated minced fish

Storage period	T P C at 8°C (a)	H_2S producing bacterial count at 8°C (b)	Ratio of $\frac{b}{a}$ in %
Initial (Before freezing)	3.8×10^5	7.4×10^4	19
After freezing	1.1×10^5	1.4×10^3	1.2
After ten days	1.2×10^5	Nil	0
After two months	9.4×10	Nil	0
After four months	8.1×10^4	Nil	0
After six months	9.6×10^4	Nil	0

bacteria after freezing they had died out rapidly during frozen storage.

The H_2S producers in dressed fish in the case of TPC plates incubated at 8°C were only 1.8% of the total plate count. Their percentage increased to 74% of the total after freezing at (-)40°C which further reduced to 6.4% on frozen storage at (-) 20°C for ten days and completely disappeared by two months of frozen storage.

In the case of minced fish the H_2S producers recovered at 8°C was only 1.2% of the total plate count at 8°C. Their percentage increased to 6% on freezing at (-)40°C. However, within ten days of frozen storage at (-) 20°C H_2S producers were completely destroyed.

In the case of EDTA treated minced fish, the percentage of H_2S producers formed 19% of the initial count which was drastically reduced to 1.2% by freezing at (-) 40°C and by the end of 2 months storage H_2S producers were totally absent.

Bacteria capable of production of H_2S are considered to be capable of spoilage of fish muscle particularly at low temperatures. They are detected by plating the sample using Iron Agar medium and characteristic H_2S producers form black colonies on the medium. Most of those bacteria producing

black colonies on Iron Agar have been identified as Alteromonas putrefaciens which are also psychrophilic in nature (Thampuran and Iyer 1990, Gram et al 1986). The incidence of H_2S producers in fish used for the study indicated that they were mostly present as part of the native flora itself. In the case of dressed fish the percentage of H_2S producers were as low as 5.5% only. However, after mincing the percentage of H_2S producers had increased to 40% of the flora, which was an indication that most of the contaminants from the handlers and the mincing machine were H_2S producers. In the minced fish treated with EDTA the number of H_2S producers had come down to 19% of the flora. This reduction can only be due to the effect of the EDTA treatment.

EDTA was found to selectively eliminate spoilage flora like the Psuedomonas groups in the case of oil sardine, Indian mackeral and prawns (Surendran 1980). He had found that in the EDTA treated fish TMAO reducers were selectively eliminated by EDTA treatment. He had also reported that 75% of Psuedomonas strains, 20% of Moraxella strains and 12% of Vibrio strains isolated from fish and prawn were susceptible to 0.1% EDTA. Pelroy and Seman (1969) have found that in EDTA treated fish fillets stored at 0.5°C, EDTA had preferentially eliminated the Psuedomonas group. Such preferential elimination of spoilage group which were shown to

be producers of H_2S had been reported to be the major preservative effect of EDTA treatment. In this study also the significant reduction of H_2S producers by EDTA treatment had been noticed. Further the drastic death of H_2S producers in EDTA treated fish mince as a result of frozen storage could be due to the fact that EDTA treatment might have rendered the bacterial cell more susceptible to cold injury leading to death.

2.3.4.2 **Bacteriological changes in Minced fish - Qualitative aspects**

Table 2.3.15 presents the initial bacterial flora of the dressed fish, minced fish and treated minced fish at RT. The initial flora of the dressed fish consisted of only Gram negative asporogenous rods mainly constituted by Psuedomonas and Acinetobacter species. Out of these 65 colonies examined from a typical experiment, 69% was Psuedomonas and the rest Acinetobacter.

Usually the bacterial flora of ocean fresh tropical fishes are mainly composed of Gram negative non sporeforming rods or cocci. They are constituted by the major genera like Psuedomonas, Vibrio, Acinetobacter, Moraxella and Flavobacterium. Also some Gram positive groups like Micrococcus and Arthrobacter have also been reported. (Surendran and Iyer 1976, Karthiayani & Iyer 1967). In this studies even-

though the initial flora of the dressed fish was composed entirely of Gram negative Asporogenous rods, they comprised only two major genera viz. Psuedomonas and Acinetobacter. However, the fact that the fresh fish was dressed and washed for this experiments had to be taken into account because this operation invariably would have caused major alterations in the initial native flora of the fish. The relatively small groups had hence been not apparent in the number of colonies picked from the TPC plate for qualitative studies. Hence the presence of only Psuedomonas and Acinetobacter was well accounted for as the major flora associated with the dressed fish before freezing.

The Table 2.3.15 also gives the initial flora of the minced fish, which consisted mainly of Psuedomonas (71%), Acinetobacter (11%), Vibrio (9%) and Micrococcus (9%). In this case also the dominant bacterial group was the Gram negative asporogenous rods or cocci. The percentage of Psuedomonas was almost the same as those in the dressed fish before mincing. However, the Acinetobacter group had come down to 11%. Vibrio (9%) and Micrococcus (9%) had been detected as two additional genera in the minced fish. The presence of the Gram positive cocci viz. Micrococcus might have invariably been a contamination from mincing process. Also

TABLE - 2.3.15

Initial bacterial flora of dressed, minced and treated minced fish at RT (In percentage)

Bacterial group	dressed fish	Minced fish	treated minced fish
Psuedomonas	69	71	50
Moraxella	0	0	0
Acinetobacter	30%	11	29
Vibrio	0	9	0
Arthrobacter	0	0	0
Flavobacterium	0	0	0
Staphylococus	0	0	0
Micrococcus	0	9	9
Enterobacteriaceae	0	0	9
No. of cultures identified	65	62	54

Vibrio could be a contamination during the processing or could have been present in the native flora itself but failed detection during examination of the flora of the dressed fish.

Nickelson et al (1980) had studied the bacterial profile of minced fish. He found that, in general, the bacteria flora of the dressed fish and freshly minced fish were very similar. He obtained Moraxella- Acinetobacter group, Pseudomonas, Flavobacterium, Lactobacillus, Aeromonas and Micrococcus as the major genera, common in both cases.

Abraham et al (1992) had made a detailed study of the bacterial profile of fresh fish, minced fish and spoiled mince. He observed a multitude of both Gram negative and Gram positive bacteria in the fresh fish mince. They comprised mainly of Pseudomonas, Acinetobacter, Aeromonas, Vibrio, Micrococcus, Staphylococcus and Bacillus. In his findings, 73% of the bacterial flora of fresh minced fish was composed of Gram positives, and Pseudomonas and Acinetobacter together constituted only 20% and Vibrio 3%. However, in the present study 91% of the flora were found to be Gram negative asporogenous rods and only 9% was constituted by the Gram positive cocci, Micrococcus. Hence these findings on the bacterial flora of fresh minced fish before freezing were totally at variance from the observations of Abraham et al (1992) but

the findings were in total corraboration with the findings of Nickelson et al (1980).

In Table 2.3.15 is also presented the initial bacterial flora of the treated minced fish. The flora consisted of 50% Psuedomonas 29% Acinetobacter, 9% Enterobacteriaceae and 9% Micrococcus. In comparison with both dressed and minced fish the percentage of Psuedomonas had come down quite drastically, while Acinetobacter remained more or less at the same proportion. The presence of Enterobacteriaceae could only be explained to have come from the manual process of mixing the minced fish with the EDTA solution.

Comparatively lower percentage of Pseudomonas in the treated mince before freezing definitely indicated the effect of EDTA on Pseudomonas. The action of EDTA treatment appeared to be distinctly selective towards Pseudomonas and this has been amply confirmed by the fact that not only the percentage of the Acinetobacter had not diminished, but has increased to 29% from the 11% level in the minced fish. The Micrococcus did not appear to be affected by EDTA treatment because it maintained the same level of 9% as was in the minced fish.

Surendran (1980), Surendran & Gopakumar (1982) investigated the effect of EDTA treatment on the bacterial flora of fish

and prawns during iced storage. They found that the Pseudomonas content of both oil sardine, Indian mackerel and prawn (Metapenaeus dobsonii) underwent significant reduction from the very beginning itself due to EDTA treatment.

Pelroy and Seman (1969) have made similar observations in the case of EDTA treated petrale sole and ocean perch fillet.

Work on the effect of EDTA treatment on the bacterial flora of fishes are rather scanty. The present observations on the effect of EDTA treatment on the microflora of minced fish appear to be pioneering one. However, the effect of EDTA on Pseudomonas was more or less the same, in fresh fish as well as minced fish.

Table 2.3.16 presents data on the initial microflora of raw dressed, minced and EDTA treated fish mince at 8°C. The general trend on the incidence of major microbial groups in all the three types of samples was the same. In dressed fish only Pseudomonas and Acinetobacter were detected, Pseudomonas being 80%. In the minced fish, the percentage of Pseudomonas was only 70% and that of Acinetobacter 9%. Arthrobacter and Micrococcus were the two genera, newly added to the flora. Both these groups are Gram positive and could have come only as a result of the mincing process by way of contamination. In the case of EDTA treated mince the percentage of Pseudomonas

TABLE - 2.3.16

Initial bacterial flora of raw dressed, minced and treated minced fish at 8°C (in %)

Bacterial group	Dressed fish	Minced fish	Treated minced fish
Psuedomonas	80	70	44
Moraxella	0	-	-
Acinetobacter	20	9	19
Vibrio	0	-	8
Arthrobacter	0	9	-
Flavobacterium	0	0	0
Staphylococus	0	0	0
Micrococcus	0	9	27
Enterobacteriaceae	0	-	-
No. of cultures identified	45	53	52

has drastically come down to 44% and Micrococcus has increased to 27%. Acinetobacter accounted for 19% of the flora and Vibrio to the tune of 8% have also been obtained. The drastic reduction in the percentage of Pseudomonas by EDTA treatment has already been explained in connection with the discussion on the initial flora at RT (vide infra).

2.3.4.3 Changes in the Bacterial flora at RT during Freezing and Frozen storage

2.3.4.3.1 Dressed fish

The changes in the bacterial flora at RT of dressed fish during freezing and frozen storage is presented in Table 2.3.17

As pointed out in the table, the initial flora before freezing consisted only of Pseudomonas (69%) and Acinetobacter (30%). After freezing at -40°C percentage of Pseudomonas came down to 50% and that of Acinetobacter 11%. Also Micrococcus (39%) was found as part of the flora after freezing. As is well known (Thampuran 1987), Kiser and Backwith (1942), Jadhav & Magar (1970) Gram negative bacterial groups were more susceptible to freezing, while Gram positives survived. The sudden decrease of Pseudomonas and Acinetobacter during freezing has brought down the total bacterial population by 91%) (Table-1.3.4). Consequently the Gram positives which were relatively in small percentage in the initial flora, happened to be detected during the sampling of frozen fish and that is how 39%

TABLE - 2.3.17

Pattern of changes of bacterial flora in dressed fish during a storage period of 6 months - Plates at RT

Bacterial group	Before freezing	After freezing - 40°C	On frozen storage at -20°C			
			10 days	2 months	4 months	Six months
Pseudomonas	69%	50%	45%	35%	15%	9%
Moraxella	0	0	0	0	0	0
Acinetobacter	30%	11%	15%	10%	10%	8%
Vibrio	0	0	0	0	0	0
Arthrobacter	0	0	0	0	0	0
Flavobacterium	0	0	8%	20%	20%	32%
Staphylococus	0	0	0		0	0
Micrococcus	0	39%	31%	30%	45%	50%
Enterobacteriaceae	0	0	0	0	0	0
No of cultures identified	86	72	96	108	112	106

of the bacterial flora of the dressed fish after freezing happened to be Micrococcus.

During frozen storage for a period of six months at (-)20°C the qualitative composition of flora showed further changes. By the 10th day of frozen storage, Pseudomonas still came down to 45% and Micrococcus remained at 31% of the flora. The percentage of Acinetobacter was slightly higher at 15% 8% of the flora was constituted by Flavobacterium. By two months of frozen storage, even though there was some relative changes in the percentages of these groups of bacteria, the qualitative composition remained more or less same. While Pseudomonas and Acinetobacter, showed some decrease, there was a relative improvement in the percentage of Flavobacterium. Micrococcus remained steady at 30%. On future storage upto four months, drastic reduction in the percentage of Pseudomonas became apparent. Consequently Micrococcus rose to 45%. Acinetobacter and Flavobacterium remained at the same percentage of 10% and 20% respectively. By six months of storage Micrococcus became the most dominant flora at 50% level. Flavobacterium constituted 32% of the flora. The percentage of Pseudomonas fell down to 9%. But Acinetobacter was more or less steady at 8%.

Freezing had been found to impart a selective action on the

bacterial flora of fish. Various bacterial species had been affected at different levels. Generally Gram positive bacteria were found to be more resistant to freezing and frozen storage.

Kiser & Beckwith (1942) found that Micrococcus and Achromobacter (the present Acinetobacter - Moraxella group) were frequently encountered in frozen mackerel. Lee et al (1967) noted that during one month's storage of ocean perch at 15°C all the Gram negatives were destroyed.

Jadhav & Magar (1970), studying the bacterial flora of tropical fishes from the Bombay coast found that Gram positives like Bacillus were very resistant to freezing.

In the present study, freezing had substantially reduced the population of Gram negative flora (mainly Pseudomonas and Acinetobacter) of the dressed fish. The major surviving group after freezing was Micrococcus. During frozen storage also, the death of the residual Gram negatives continued. By the sixth month the percentage of Pseudomonas had come down to mere 9% from the initial level of 69% and that of Acinetobacter to 8% from the initial 30%. Thampuran (1987) had made similar observations in the case of Mackerel, frozen at -40°C and further stored at -20°C. She found that the Gram negatives which constituted 59% of the initial flora of the Indian mackerel before freezing, decreased to 47% after six months of storage

at -20°C. And the Gram positives which was 34% of the initial flora increased to 49% after six months of frozen storage. In the case of Pseudomonas, freezing and frozen storage had brought about similarly considerable changes. In the present study also, the major effect of freezing and frozen storage was felt in the Pseudomonas group. The Micrococcus which was below the detectable level in the initial flora had reached the level of 39% of the flora immediately after freezing. The percentage more or less remained steady during the first two months of storage at -20°C and further increased to 50% of the total flora by sixth month of storage. An interesting observation was the behaviour of Flavobacterium which was below detectable level in the dressed fish before freezing. By 10 days of the frozen storage at (-) 20°C, the Flavobacterium had been 8% of the flora which appeared to have resisted destruction during further frozen storage so that by the end of six months, the proportion of Flavobacterium was quite high at 32%. The observation of Thampuram (1987) in the case of Indian mackerel frozen stored at (-) 20°C is at variance from the present finding, in the case of survival of Flavobacterium. She found that eventhough the Flavobacterium group remained viable upto two months of frozen storage at (-)20°C, they finally vanished from the flora.

2.3.4.3.2 Minced Fish

The changes in the bacterial flora of minced fish during freezing and frozen storage are presented in Table 2.3.18. The bacterial genera of the minced fish before freezing composed of Pseudomonas (70%), Acinetobacter (9%), Vibrio (9%) and Micrococcus (10%).

After freezing at (-) 40°C, the level of Pseudomonas came down to 49%, while Acinetobacter and Micrococcus increased to 20% of the flora and Vibrio remained at 10% itself. On frozen storage at (-) 20°C for 10 days, there was significant changes in the relative composition of these bacterial groups. More of Pseudomonas died out as has been the case with Vibrio. Their proportion came down to 41% and 6% respectively. Micrococcus increased to 32% of the flora.

On consequent storage the percentage of Pseudomonas consistently reduced, until it reached 6% of the total flora by the sixth month. In the case of Vibrio within two months it was completely destroyed. Micrococcus on the other hand progressively increased in proportion and attained the level of 83% of the flora by six months time.

Acinetobacter appeared to be remaining almost at the same level of 10% during the same period of frozen storage.

TABLE - 2.3.18

Pattern of changes of Bacterial flora in Minced fish during a storage period of 6 months - Plates at RT

Bacterial group	Before freezing	After freezing -40°C	On frozen storage at -20°C			
			10 days	2 months	4 months	Six month
Pseudomonas	70%	49%	41%	32%	20%	6%
Moraxella	0	0	0	0	0	0
Acinetobacter	9%	20%	19%	10%	8%	10%
Vibrio	9%	9%	6%	0	0	0
Arthrobacter	0	0	0	0	0	0
Flavobacterium	0	0	0	0	0	0
Staphylococcus	0	0	0	0	0	0
Micrococcus	10%	20%	32%	56%	72%	83%
Enterobacteriaceae	0	0	0	0	0	0
No. of cultures identified	80	102	84	106	120	90

The changes in the bacterial flora of minced fish were more or less similar to the changes in the case of dressed fish. The Pseudomonas group were the most susceptible and this group steadily got destroyed during the course of frozen storage. Micrococcus appears to be resistant to destruction during freezing and frozen storage. Its percentage progressively increased until it attained the position of the most dominant bacterial group by the end of the frozen storage period of six months.

Nickelson et al (1980) investigated the bacterial flora of minced fish flesh from nontraditional Gulf of Mexico fin fish species like sheepshead, mullet, croakers, sandtrout and tilapia. They found that, the microbial flora of the whole fish before processing, were comprised of both Gram negatives and Gram positives, viz. Flavobacterium, Moraxella, Acinetobacter, Lactobacillus, Micrococcus and Microbacterium species and Staphylococcus and cornyeformes. During the process of mincing some of the original species were replaced by some other species like Bacillus and the percentage of Micrococcus increased considerably. After freezing Flavobacterium, Moraxella, Acinetobacter, Lactobacillus and Microbacterium disappeared and the residual flora was mainly a mixture of Pseudomonas and Corynebacterium in most cases, eventhough in some of the fishes Moraxella Acinetobacter group and Flavobacterium had also survived to some extent. These authors have not reported

the changes in the flora during frozen storage of these minces.

Much work does not appear to have been done to determine the qualitative changes in the bacterial groups of minced fish during freezing and frozen storage. However, the present study indicated that the behaviour of the bacterial genera in the minced fish during freezing and frozen storage was more or less the same as those in the case of fresh fish under frozen storage.

2.3.4.3.3 Treated Mince

Pattern of changes of bacterial flora of EDTA treated minced fish during freezing and frozen storage is given in Table 2.3.19.

Before freezing the flora consisted mainly of Pseudomonas (49%), Acinetobacter (29%), Micrococcus (11%), and Enterobacteriaceae (11%). After freezing, the flora presented a very interesting picture. The decline in the Pseudomonas group was very drastic and it formed only 20% of the flora after freezing.

Acinetobacter increased to 39% and Micrococcus 31%. The Enterobacteriaceae were completely destroyed during freezing. On frozen storage at - 20°C, Pseudomonas experienced further destruction and was reduced to 9% of the flora, by the 10th day of frozen storage. By two months frozen storage, Pseudomonas came down to 8%. By the 4th month, Pseudomonas completely

TABLE - 2.3.19

Pattern of changes of Bacterial flora in treated minced fish during a storage period of 6 months - Plates at RT

Bacterial group	Before freezing	After freezing -40°C	On frozen storage at -20°C			
			10 days	2 months	4 months	Six month
Pseudomonas	49%	20%	9%	8%	0	0
Moraxella	0	0	0	0	0	0
Acinetobacter	29%	39%	50%	10%	0	0
Vibrio	0	0	0	0	0	0
Arthrobacter	0	0	0	0	0	0
Flavobacterium	0	0	0	0	0	0
Staphylococcus	0	0	10%	10%	0	0
Micrococcus	11%	31%	29%	79%	100%	100%
Enterobacteriaceae	11%	0	0	0	0	0
No. of cultures identified	70	98	108	126	78	76

vanished from the flora. In the case of Acinetobacter there was an improvement in the relative percentage after 10 days of frozen storage. It reached the level of 50% of the flora but by two months it was reduced to 10%. By the 4th month of frozen storage Acinetobacter disappeared just like the Pseudomonas.

Micrococcus on the other hand steadily increased in percentage and by the 2nd month of frozen storage it attained the level of 79% of the flora. After four months of storage the entire bacteria that had survived were constituted exclusively by Micrococcus.

The behaviour of the bacterial flora of the treated mince during freezing and frozen storage were due to the combined effect of freezing, frozen storage and the inhibitory effect of EDTA. EDTA selectively inhibited the survival and growth of Pseudomonas. The studies on EDTA treated fish and prawn in iced storage have amply confirmed the selective inhibitory effect on Pseudomonas (Surendran 1980), Surendran and Gopakumar (1982), Pelroy and Seman (1969).

The Micrococcus has already been found to survive freezing and frozen storage (Thampuran, 1987). Further, the EDTA has little bacteriostatic action on Micrococcus, even at 2% level (Surendran 1980). Hence in the treated mince the survival of Micrococcus was accounted for. The susceptibility of the

Gram negative bacteria to freezing and frozen storage and the selective destruction of Pseudomonas by EDTA had ultimately resulted in a bacterial flora, exclusively comprised of Micrococcus, during prolonged frozen storage of the EDTA treated mince.

2.3.4.4 **Changes in the Bacterial flora at 8°C during Freezing and Frozen storage.**

In Tables 2.3.20, 2.3.21 & 2.3.22, the changes in the bacterial flora, recovered at 8°C, of the dressed, minced and treated minced fish during freezing and frozen storage are given.

2.3.4.4.1 Dressed fish

In the case of dressed raw fish, the initial bacterial flora before freezing recovered at 8°C were mainly composed of Pseudomonas (80%) and the rest Vibrio. This flora, comprised of only Pseudomonas and Vibrio might be due to the fact that the dressed fish was washed and kept at chilled temperature before the mincing process. This washing and chilling operations had resulted in the elimination of relatively less incident bacterial groups leaving behind the only two major groups viz. Psedomonas and Vibrio.

Immediately after freezing, the percentage of Pseudomonas

TABLE - 2.3.20

Pattern of changes in Bacterial flora recovered at 8°C in Dressed raw fish during freezing and frozen storage

Bacterial group	Before freezing	After freezing at -40°C	On frozen storage at -20°C			
			10 days	2 months	4 months	6 months
Psuedomonas	80%	50%	21%	9%	15%	15%
Moraxella	0	0	0	0	0	0
Acinetobacter	0	0	0	0	0	0
Vibrio	20%	30%	31%	9%	10%	10%
Arthrobacter	0	0	0	0	0	0
Flavobacterium	0	0	21%	21%	35%	40%
Staphylococcus	0	0	0	0	0	0
Micrococcus	0	20%	31%	30%	40%	40%
Enterobacteriaceae	0	0	0	0	0	0
No. of cultures identified	90	120	78	86	118	124

had substantially come down to 50%. While Vibrio had increased to 30% and there was the recovery of a new genus - Micrococcus (20%). Generally Gram negatives are very susceptible. This had resulted in a reduction of about 99% in the TPC at 8°C of the frozen dressed fish due to freezing. Consequently less incident groups like Micrococcus had been detected in abundance, in this case 20% of the total flora. During frozen storage at -20°C, the Pseudomonas had been in a declining trend. By the 10th day of storage at -20°C Pseudomonas was only 21%, while Vibrio and Micrococcus had increased to 31% each. Also there was the recovery of Flavobacterium upto 21% by this time. The trend of decline of Pseudomonas had been reversed after about 4 months of frozen storage. Pseudomonas had attained the level of 15% of the flora and remained at that level upto sixth month of frozen storage. Flavobacterium and Micrococcus had steadily increased in their percentage and reached 40% each at the end of six months of frozen storage. Vibrio gradually declined to 10% at the end of that period.

2.3.4.4.2 Minced Fish

The pattern of changes in the initial flora of minced fish recovered at 8°C during freezing and frozen storage is presented in Table 2.3.21.

The bacterial flora before freezing consisted of Pseudomonas

TABLE - 2.3.21

Pattern of changes of Bacterial flora recovered at 8°C in Minced fish during freezing and frozen storage

	Before freezing	After freezing at -40°C	On frozen storage at -20°C			
			10 days	2 months	4 months	6 months
Psuedomonas	69%	51%	36%	20%	0	0
Moraxella	0	0	0	0	0	0
Acinetobacter	10%	9%	6%	9%	11%	10%
Vibrio	0	0	0	0	0	0
Arthrobacter	10%	0	0	0	0	0
Flavobacterium	0	0	0	0	0	0
Micrococcus	10%	40%	58%	68%	89%	90%
Enterobacteriaceae	0	0	0	0	0	0
No. of cultures identified	36	43	45	54	56	62

(69%), Acinetobacter (10%), Arthrobacter (10%) and Micrococcus (10%). The incidence of Micrococcus and Arthrobacter in the minced fish could be a contamination in the mincing process. Immediately after freezing the Pseudomonas had come down to 51% and Acinetobacter to 9%. The Micrococcus had increased to 40%. As already pointed out in the previous discussion, Pseudomonas group were susceptible to freezing while Micrococcus was very resistant to freezing. Acinetobacter remained more or less at the same level during freezing. During subsequent storage at -20°C there was a further decline in the percentage of Pseudomonas to 36% and a consequent increase in the percentage of Micrococcus to 58%. On further storage Pseudomonas decreased to 20% by the 2nd month of frozen storage and later completely disappeared by the fourth month of frozen storage. Micrococcus steadily increased in proportion. By the end of six months of frozen storage 90% of the residual flora recovered at 8°C was constituted by Micrococcus alone.

2.3.4.4.3 Treated mince

Initial flora of the treated minced fish as presented in Table 2.3.22 was more or less similar to those of the untreated mince. Majority of the bacterial genera before freezing were Gram negative, comprising of Pseudomonas (45%), Acinetobacter (21%) and Vibrio (9%). Micrococcus (Gram positive) constituted 27% of the flora.

TABLE - 2.3.22

Pattern of changes of Bacterial flora at 8°C in treated minced fish during Freezing and Frozen storage

Bacterial groups	Before feez-ing	After freez-ing at -40°C	On frozen storage at -20°C			
			10 days	2 months	4 months	6 months
Psuedomonas	45%	24%	11%	0	0	0
Moraxella	0	0	0	0	0	0
Acinetobacter	21%	11%	8%	12%	6%	0
Vibrio	9%	20%	20%	18%	12%	0
Arthrobacter	0	0	0	0	0	0
Flavobacterium	0	0	0	0	0	0
Staphylococcus	0	0	0	0	0	0
Micrococcus	27%	42%	58%	69%	79%	100%
Enterobacteriaceae	0	0	0	0	0	0
No. of cultures identified	66	65	59	56	43	55

Immediately after freezing at -40°C Pseudomonas decreased to 24%, while Vibrio increased to 20% and Micrococcus 42%. The percentage of Acinetobactercame down to 11%, on frozen storage at -20°C, by the 10th day, the proportion of Pseudomonas had drastically come down to only 11% while Acinetobacter and Vibrio more or less remained at the same level. The drastic reduction of Pseudomonas should have been due to the combined effect of EDTA treatment and the frozen storage. EDTA has a very selective destructive effect on Pseudomonas (Pelroy & Seman 1969), Surendran (1980). On further storage at -20°C, by two months period, Pseudomonas had completely vanished. Micrococcus attained 69% and the rest of the flora was composed of Vibrio (18%) and Acinetobacter (12%). By 4th month, Micrococcus attained 79% of the flora with a proportionate decrease in the percentage of Vibrio and Acinetobacter. At the end of six months storage at -20°C the entire residual flora of the EDTA treated mince was constituted by Micrococcus alone.

2.3.4.5 **Effect of Freezing and Frozen storage on pathogenic/indicator Bacteria.**

Table 2.3.23 gives the changes in pathogenic and indicator bacteria in the dressed fish during freezing and frozen storage. Only coliforms and E. coli were the two groups of pathogenic/indicator bacteria present in the dressed fish. Initial count

TABLE - 2.3.23

Quantitative changes in the Pathogenic/Indicator bacteria in the dressed fish during freezing and frozen storage

Period of storage	Coliforms @	Faecal Strepto cocci @	Staphylococci @	E. Coli @	Salmonella @
Initial	446	ND	ND	13	ND
After freezing at -40°C	ND			ND	
Frozen storage at -20°C					
After ten days					
After two months					
After four months					
After six months					

@ Count/gram

ND - Not detected

of coliforms before freezing was 446/g. Streptococci, Staphylococci and Salmonella were absent. The count of E. coli was only 13/g. Immediately on freezing both coliforms and E. coli were completely killed and on no occasion during further storage upto six months, any of the pathogenic/indicator bacteria were detected.

Table 2.3.24 shows the changes in pathogenic/indicator bacteria in minced fish during freezing and frozen storage. Initially the coliform count/g. was 4111 and Streptococci count was 5672/g. The coliform counts in the mince was almost 10 times those obtained in raw dressed fish. This increase in coliforms could be due only to the contamination during the mincing process. Elliot (1986) had also made similar observation. He found that the coliform number increased from 0.6 MPN/g. of fillet to more than 100 MPN/g in refined mince.

During the freezing process coliforms were completely destroyed. Nickelson (1980) had observed that due to freezing and frozen storage the coliforms count registered substantial decreases.

In the case of faecal streptococci, the initial count of 5672/g was reduced to 888/g. due to freezing, which on frozen storage for 10 days were completely destroyed.

Table 2.3.25 shows the changes in pathogenic/indicator bacteria

TABLE - 2.3.24

Quantitative changes in the Pathogenic/Indicator Bacteria in the minced fish during Freezzing and Frozen storage

Period of storage	$ Coliforms	$ Faecal Streptococci	$ Staphylococci	$ E. Coli	$ Salmonella
Initial (Before freezing)	4411	5672	ND	ND	ND
After freezing at -40°C	ND	888			
Frozen storage at -20°C					
After ten days		ND			
After two months					
After four months					
After six months					

$ Count/Gram

ND - Not Detected

TABLE - 2.3.25

Quantitative changes in the Pathogenic/Indicator Bacteria in the treated minced fish during Freezing and Frozen storage

Period of storage	$ Coliforms	$ Faecal streptococci	$ Staphylococci	$ E. coli	$ Salmonella
Initial (Before freezing)	3769	1682	ND	ND	ND
After freezing at -40°C	446	669			
Frozen storage at -20°C					
After ten days	ND	ND			
After two months					
After four months					
After six months					

$ Count/gram

ND - Not Detected

in treated minced fish during freezing and frozen storage. The initial count of coliforms were 3769/g and that of faecal streptococci 1682/g. Staphylococci, E. coli and Salmonella were absent. During freezing the coliforms were substantially reduced to 446/g. which on further frozen storage for 10 days were completely killed. Similarly in the case of faecal streptococci, freezing brought about considerable reduction in count from 1682/g to 669/g. On frozen storage for 10 days the faecal streptococci were completely killed as observed in the case of untreated minced fish.

2.3.4.6 **Changes in Chemical indices and organoleptic assessment**

Changes in the peroxide value and free fatty acid during freezing and frozen storage of minced fish

In Tables 2.3.26 & 2.3.27 are presented the changes in peroxide value (P V) and free fatty acid (FFA) value during freezing and frozen storage of dressed fish, minced fish and EDTA treated minced fish.

The PV of the dressed fish, fish mince and treated mince were 7.15, 7.16 and 7.5 milli equivalent/kg respectively in the samples before freezing. Immediately after freezing the values showed a slight decrease in the case of dressed fish and treated mince while the PV of minced fish increased to 8.58 milli equivalent/kg. However, during storage the PV

registered almost steady decrease in all the three cases. This decrease in PV during frozen storage would indicate the progressive decomposition of the peroxides to degradation products like aldehydes aand ketones.

Tasukuda (1978) noticed an initial increase followed by a decrease in the PV of sardine minced meat frozen stored at -20°C. Joseph and Perigreen (1983) on the other hand obtained a steady increase in the PV in the case of threadfin bream mince frozen stored at -23°C. But in the present study, on the frozen storage of minces from the same species of fish, only a steady decrease in the PV was observed.

Table 2.3.27 gives the changes in free fatty acid (FFA) values during frozen storage of dressed fish, minced and EDTA treated minced fish.

The initial values of FFA in the dressed fish and minced fish were 0.88 mg/100 g. each and for EDTA treated mince, 1.03mg/100g. The initial value of FFA itself in the samples was far too low, indicating very low level of lypolytic enzyme activity in the raw material itself. Immediately after freezing the FFA values in the raw fish as well as the treated mince increased slightly to 1.11 and 1.17 mg/100 g respectively. But the value obtained for untreated mince was at 0.82 mg/100g. indicating

TABLE 2.3.26

Storage period	PV milli equivalent/kg.		
	A	B	C
Initial (Before freezing)	7.15	7.16	7.5
After freezing	6.8	8.58	6.7
After storage for			
two months	4.0	3.76	6.33
four months	4.56	4.9	4.32
Six months	3.5	4.8	3.32

A Dressed fish

B Minced fish

C Treated minced fish

TABLE - 2.3.27

Storage period	F F A mg/100g		
	A	B	C
Initial (Before freezing)	0.88	0.88	1.03
After freezing	1.11	0.82	.07
After storage for two months	1.41	1.59	1.76
four months	1.46	1.49	2.7
Six months	1.52	1.80	2.7

A Dressed fish

B Minced fish

C Treated minced fish

that there was no change in the FFA content. On further storage at -20°C, the FFA values showed gradual increase. The increase in the case of dressed fish as well as the untreated mince was not very significant.

After six months of frozen storage at -20°C the FFA value in the case of dressed fish was only 1.52 mg/100g. while that of untreated mince in the 1.80 mg/100g. However, the changes in the FFA values of the EDTA treated mince were more pronounced. After six months of frozen storage the EDTA treated mince was 2.7 mg/100g. about 50% higher than the FFA value of the corresponding untreated mince.

Joseph and Perigreen (1983) has reported slow but steady increase in the FFA values of minced threadfin bream during frozen storage at -23°C. In their studies, they obtained an initial FFA value of 2 mg/100g. which on frozen storage reached 12 mg/100g. in 9 months.

Deng et al 1977) also observed similar changes in the FFA values of whole mullet. In the present study, FFA values were not showing changes, indicative of higher lipase activity at -20°C.

2.3.4.7 **Changes in organoleptic scores during Freezing and Frozen storage of Dressed, Minced and treated minced fish.**

Changes in organoleptic scores are presented in Table 2.3.28.

TABLE - 2.3.28

CHANGES IN THE ORGANOLEPTIC CHARACTERISTICS OF RAW DRESSED FISH, MINCED FISH & TREATED MINCE AFTER FREEZING AT -40°C AND FROZEN STORAGE AT -20 ± 2°C

Period of storage	Organoleptic score		
	Dressed raw fish	Minced fish	Treated mince
Initial	7.0	7.5	7.0
After freezing at -40°C	6.5	7.0	6.5
Frozen storage at -20°C			
After 2 week	6.5	7.0	6.5
After 8 weeks	6.5	6.5	6.5
After 16 weeks	5.0	4.5	5.0
After 24 weeks	4.5	4.0	3.5

The organoleptic score in respect of dressed raw fish was initially seven showing the prime quality of the raw material. After freezing at -40°C the score showed an initial drop to 6.5. On subsequent storage at -20°C, upto eight weeks, the organoleptic score remained the same for the raw fish, followed by a decrease to 5 after sixteen weeks. By the end of six months frozen storage the score was 4.5 indicating that the fish was still organoleptically acceptable.

In the case of minced fish the initial score was 7.5 which on freezing at -40°C decreased to 7 units for one week's frozen storage at -20°C the quality remained steady. After eight weeks of storage the organoleptic score came down to 6.5 which further decreased to 4.5 after sixteen weeks of frozen storage. By the end of 24 weeks the score was only 4 which is the level of rejection.

The changes in the treated mince was similar to those of dressed fish. The initial organoleptic score was seven, which on freezing came down to 6.5 and remained at that level upto 8 weeks frozen storage. At the end of 16 weeks, the score lowered to 5. However, by the end of 24 weeks the organoleptic score of the treated mince was only 3.5 which was below the threshold of rejection.

The mincing process always interferred with the compact protein

structure of the fish muscle. One has to expect a much faster decrease in the organoleptic qualities of the minced fish. In this study also the decrease in the organoleptic scores of the minced fish was always at a rapid pace, in comparison with the dressed fish. However, upto six months of frozen storage, the minced fish was organoleptically acceptable. But in the case of EDTA treated mince, on organoleptic scores the level of rejection had been reached much before 24 weeks of frozen storage. Eventhough EDTA treatment had substantial effect on the residual bacterial flora of the fish mince, it indeed did not prevent the decrease in organoleptic qualities. This could be explained to be due to the presence of added chemicals in the EDTA treated mince.

Joseph and Perigreen (1983) have also reported similar decrease in the sensory scores of minced fish from threadfin bream during frozen storage.

Devadasan and Venkatraman (1978) studying the storage life of minced fish have found that at (-)20°C storage life of minced fish is only 12 weeks based on organoleptic scores.

However, in this study, level of rejection based on sensory scores was at the 24th week, during frozen storage at -20°C.

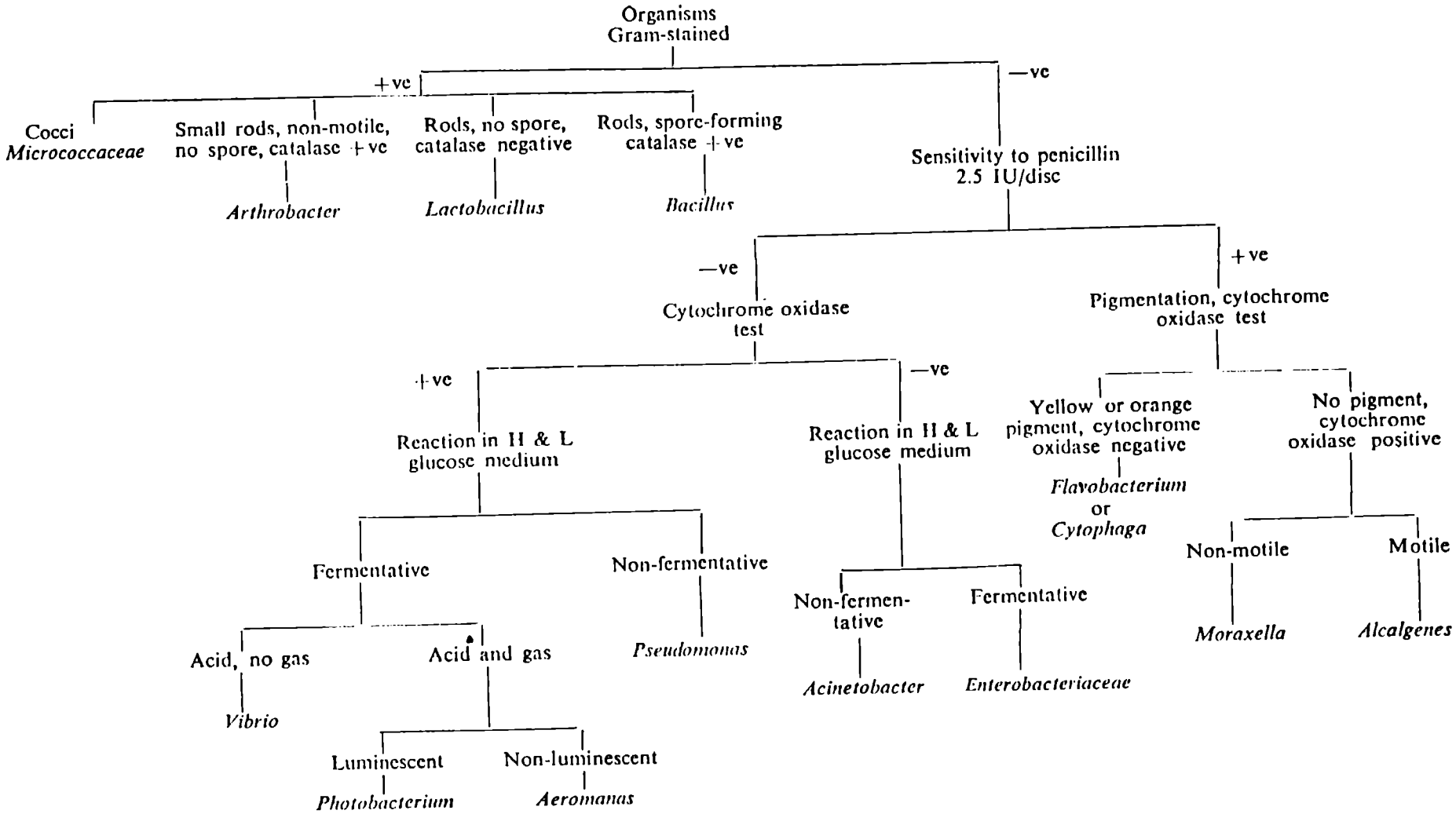


Fig. 1. Scheme used for classifying the cultures

Note:- (1) Gram negative, sensitive to 2.5 I. U. penicillin, no pigment, but cytochrome oxidase negative are *Acinetobacter*-like.
(2) Penicillin sensitive, Gram negative cytochrome oxidase positive, pigmented yellow or orange, are also grouped as *Cytophaga/Flavobacterium*.

CHAPTER - 3

C H A P T E R - 3

CHANGES IN PROTEIN EXTRACTABILITY AND TEXTURE OF MINCED MEAT FROM NEMIPTERUS JAPONICUS DURING FREEZING AND FROZEN STORAGE

3.1 INTRODUCTION

Freezing is the best method for achieving long term preservation of fish. However, after prolonged holding of fish and fishery products at frozen storage temperatures of at -20°C or above, significant undesirable sensory changes take place. In lean fish species, quality loss often involves alteration in protein structure accompanied by loss of water holding capacity and toughening of the muscle (Sikorski et al 1976).

3.2 CHANGES IN FISH PROTEINS DURING FREEZING AND FROZEN STORAGE

The occurrence of deterioration in frozen fish products had been known for many years (Huntsman 1929). Frozen fish frequently was dry and tough and comparatively flavour less. Such changes are generally referred to be due to denaturation of protein in fish muscle.

Protein denaturation is a change in the protein, such that it is no longer soluble in or extractable by salt solutions under conditions in which the native proteins are soluble or extractable (Borgstrom, 1960). In frozen fish, the decrease

in protein extractability was noted as early as 1935 by Reay, Bate & Smith (1934). They found that the decrease in extractability of protein by salt solution was greater at high storage temperature than at low temperature and that it increased with storage time. The major decrease was found in the globulin fraction of the protein.

When fish muscle is cooled below 0°C at about 0.8°C to -5°C water freezes out as crystals of pure ice to the maximum. This leads to concentration of salt in the liquid phase of fish muscle. The removal of water from the fish muscle as ice crystals during freezing leads to considerable dehydration of the fish protein. Earlier it was thought that the ice crystals formed during freezing of fish muscle ruptured the cell wall allowing the fluid to escape on thawing ie, the damage due to freezing was mechanical (Dyer & Dingle, 1961).

However, it was later realised that the mechanical damage alone was not responsible for freezing damage of the fish muscle. When there was removal of the water from the muscle as ice crystals the increased salt concentration could denature the fish protein. Consequently there was rapid release of the large part of moisture which was

previously held by the swelled protein at slightly lower salt concentration.

When the protein is denatured its water holding capacity is very much decreased (Dyer 1951). Other factors that are involved in protein damage during freezing is pH. This would render proteins to be hydrophobic.

Water extractable protein, which are mainly albumins in nature are the least affected by frozen storage (Dyer 1953).

Myofibrillar proteins which are extracted with solutions of moderate ionic strength are the most affected during frozen storage (Dyer & Dingle 1961

On a practical level, it has been found that cryoprotectants reduce the rate of deteriorative changes in fish and fishery products during frozen storage. Fish fillets are commonly dipped in sodium tripolyphosphate (STP) and sodium hexameta phosphate to reduce drip and increase tenderness (Ellinger, 1972, Mahon et al 1971). The addition of sucrose, sorbitol polyphosphate and/or sodium glutamate to surimi has been shown to maintain protein functionability during long periods of frozen storage (Suzuki 1981).

Krivchenia and Fennema (1988) have studied the effect of cryoprotectants on frozen white fish fillets. White fish fillets

were treated with sodium tripolyphosphate (STP) monosodium glutamate, MSG or a high pH buffer by high pressure injection, then frozen and stored at -20°C. Control samples included untreated fillets stored at -12°C and -60°C. It was noted by them that protein extractability decreased significantly during the early stages of frozen storages, exhibiting values of about 300-400 mg. protein/0.1 ml. at 10 week and the remaining relatively constant thereafter. The -60°C control sample exhibited significantly greater protein extractability values than those of other samples at each time period though it did not remain constant throughout the storage period, but rather followed the same pattern exhibited by other samples.

Samson et al (1985) also observed similar changes in minced cod during storage for 35 days at -40°C.

Awad et al (1969) measured the protein extractability of white fish and observed a steady decrease to 43% from 93% of total fish protein during 16 weeks of storage at -10°C. He also found a linear relationship between protein extractability and the sensory texture of baked white fish with the fish becoming tougher as PE decreased.

However, Krivchenia found a week negative corelation between protein extractability and texture.

Protein extractability often but not always co-relates well with textural changes in frozen fish (Connel & Shewan, 1980).

Noguchi & Matsumoto (1975) have studied the preventive effect of some aminoacids, peptides, acetyl amino acids and sulfur compounds on the freezing denaturation of carp actomyosin at -30°C for 4 to 8 weeks using in vitro models.

Additives like proline, cystine and glutamyl cysteinyl glycine showed marked effect on controlling the solubility loss of protein.

O Guniet al (1975) states that the solubility and intrinsic viscosity of actomyosin decreased with the length of frozen storage and the ultra centrifugal pattern showed aggregation proceeding during frozen storage.

Noguchi and Matsumoto (1975) have studied the preventive effect of carboxylic acids on freezing denaturation was followed by estimating solubility, viscosity, ATP ase activity as well as the degree of super precipitation. The overall results can be summarised as follows:

Additives like malonic, lactic, malic, tartaric, gluconic, glycolic, methyl malonic acids gave marked preventive effect on denaturation.

Moderate effect was displayed by formic, acetic and propionic acids

Their results suggested that the preventive effect of an additive was influenced by the nature position, number and configuration of the functional group of the compounds.

Proteins of picked fish mince have great inherent functional properties which contribute to the texture and flavour and binding properties of the mince. But, they are particularly susceptible to degrdation. To prevent the decrease in protein stability in fish mince, degradative changes have to be minimised. The changes occurring during frozen storage of fish mince have been extensively reviewed by Arai (1975) and Sikorsky et al (1976). Laird et al (1980), Motohiro and Numakura (1974) have found that mincing accelerated the deconformation, aggregation and cross linking of the myofibrillar protein. Bremner (1977 a and 1977 b) and Dingle (1978) have studied the loss of extractability and contractability of actomyosin. Grabowska and Sikorski (1973) have said that loss of extractability of actomyosin resulted in the increase in objective toughness, granularity and drip loss and a decrease in water binding capacity and rheological properties. Nowlan & Dyer (1974) have found that frozen denaturation is also accelerated by the rapid pH drop resulting from the accelerated glycolysis on mincing. Calvo & Bordarias (1979) have reported that temperature cycling during frozen storage had accelerated frozen denaturation.

Sensory effects of frozen degradation had been reported by Sorensen (1976) and Weddle (1980).

Much work has been carried out for stabilizing the frozen mince. Most extensive work had taken place on the use of polyphosphate

In presence of salt, phosphates stabilise the muscle against actomyosin aggregation (Borderias et el, 1978). They also enhanced the water binding capacity of the muscle and increased the rate and extent of salt solubilization of the proteins (Bennet, 1976, Ghadi & Lewis, 1977)

Standards for the phosphates for use in mince blocks have been reviewed by Antonacopoulos (1980), several sugars synergistically enhance the effect of phosphate, particularly under allkaline conditions. (Ajinomoto 1965 Miyauchi 1977, Nikken 1980, Sorensen 1976 and 1980) Sugars seem more effective on pre or post-mince than on mince on rigor (Sorensen, 1976 & 1980).

Sugar alcohols such as sorbitol were also effective cryoprotectants (Ueno Seivaku 1979) and also higher polysaccharides such as starches (Grabowska et al, (1975) (Moral et al, 1978) cellulose glycolate (Nikken, 1980) and amylopectins (Nishida,1979).

Gabrowska et al (1975), Kibun (1978-a, 1978-b & 1978-C)

have reported that the incorporation of protein fat emulsions into mince and the emulsification of mince with fats would minimise frozen degradation. Clycerides with a high mono-ester content such as glycerine monostearate are also reported to be effective (Shimizer et al, 1973).

Rodger et al (1979) reported that texture changes could result from the changes in the protein of mince and the major part of solubility loss occurred during the first week of storage and the subsequent solubility loss was very low.

Borderias et al (1980) attributed the rapid loss of protein solubility due, perhaps, to the fact that large amount of intracellular liquid with strong enzyme activity were released during the extrusion process which induced protein denaturation.

Powrie (1973) pointed out the dependence of juiciness with water holding capacity and protein denaturation.

Prabhu et al (1988) have studied suitability of lesser sardine meat for preparation of fish sausages. A significant reduction in the salt soluble nitrogen content of the minced meat after second month of storage was noticed (71.33% to 62.09% at the end of second month) thereafter it was steady.

Suzuki (1981) had reported that the protein in minced fish

meat, stored at -20°C without additives showed a marked denaturation and the meat was spongy in texture.

Agarwal et al (1986) have studied the frozen storage characteristics of Composite Fish Mince from Dhoma (Sciaenid sp.) Lactarius (Lactarius lactarus). He found that initial decreases in salt soluble nitrogen (SSN) were fairly rapid upto 16 weeks of storage followed by a gradual decrease in the subsequent period upto 8 weeks. After this period the solubility losses were very low and reached almost to a constant level.

Similar trends were observed in frozen storage of thread fin bream mince (Joseph & Perigreen, 1983).

Tableros et al (1981) have studied the behaviour of mechanically separated minces of five common fish species of the Mexico during storage at -20°C. The changes in the extractable protein nitrogen was investigated during 10 months of storage at -20°C. There was a gradual reduction in extractable protein nitrogen for all species although this was more marked in the samples of flat fishes and washed mince showed minimal changes in EPN through out the storage period. The range of EPN values encountered and the extent of the reduction were found to vary between samples from different species. Such changes are particularly marked in frozen minced fish due to the surface area available for reaction. A variety

of possible mechanism existed causing protein aggregations in fish muscle, when stored at low temperature. These phenomenon might in turn alter the texture of fish minces leading to low acceptability.

Park et al (1987) had studied freeze-induced protein denaturation of pollack surimi, as affected by the addition of sugar and/or polyol; including a starch hydrolysate product, and/or phosphates during 8 months frozen storage. Polydextrose appears to substitute for the sucrose/sorbitol now used in surimi manufacture without changes in cryoprotective effect. The maltodextrin adversely affect gel forming properties, although it maintained, the salt soluble protein extractability nearly as well as done by sucrose/sorbitol or polydextrose. The cryoprotective effects of phosphate addition seemed to depend upon the pH and/or specific phosphate ion used.

He found that phosphate addition, alone or in combination with sugar/polyols, appeared to have little if any, protective effect on protein extractability of the salt soluble nitrogen during frozen storage. Addition of STP alone seemed to be slightly protective, while NPP addition was detrimental to maintenance of protein extractability whether alone in combination with sugar/polyol. The addition of sugar/polyol and orthophosphate had no effect on salt soluble protein extractability of surimi stored for 8 months.

Joseph and Perigreen (1986) have studied the effect of washing minced cat fish in water, sodium chloride solution (1%) and ascorbic acid solution (0.1%) in improving the quality and frozen shelf life. Washing improved the colour and reduced the non-protein nitrogen contents and extractable nitrogen. Denaturation was more in samples washed in salt and ascorbic acid solution.

The difference in extractability among the samples was significant. The water washed samples showed 26% loss in solubility compared with control. This was attributed to the loss of water soluble proteins and non-proteins. The values of protein extractability in samples washed with Nacl solution and ascorbic acid solution might be due to the denaturation of proteins as a result of washing in salt solution and ascorbic acid solutions.

Holmquest et al (1984) had studied the properties of Kamaboko made from red hake fillets, mince or surimi. Salt soluble nitrogen reduced from 90mg/100 mg of the sample to 10 mg/100 mg in the hake mince stored at -5°C.

Shama Sunder et al (1988) had studied the effect of washing on nutritional and gel characteristic of three marine fish flesh viz. pink perch, croakers and ribbon fish. Salt soluble nitrogen reduced from 2.19 to 1.80 in pink perch and from 1.81 to 1.00 in croaker.

Devadasan & Venkatraman (1978) had studied the frozen storage characteristics of ribbon fish mince. The study showed that in 12 weeks storage the SSN% came down from 58.50 to 33.00% of TN and organoleptic rejection came on the 12th week.

Dingle & Hines (1975) studied the protein instability in minced flesh from fillets and frames of several commercial Atlantic fishes during storage at -5°C. Minced flesh of Atlantic cod and pollock, recovered by means of meat-separation machines from frames left after filleting operations, suffered a rapid loss of protein solubility during storage at -5°C. This was due to the presence of kidney tissue which caused the formation of dimethylamine (DMA) and formaldehyde (FA) from the trimethylamine oxide of the muscle. The minced flesh from flounder, American plaice and Atlantic mackeral were relatively stable when mixed with homogenates of their own kideny tissue, but cod kidney caused the same changes in gray sole as it did in minced cod flesh. The exclusion of gadoid kidney and blood from minced fish preparations was recommended.

There was evidence that DMA and FA are formed by an enzymic degradation of the TMA normally found in most marine fish muscle. The enzymatic activity occurred in several visceral organs and also in the dark muscle of Alaska pollack and red hake (Amano & Yamada, 1964).

Castel (1971) and Castel et al (1973) showed that the loss

of extractability could be related to the amount of FA formed in storage. Dyer (1973) had postulated that a reaction between FA and protein was the major mechanism of protein aggregation on gadoid species, particularly in Alaska pollock and red hake, where its effect far surpasses that of other proposed mechanisms such as salting out or reaction with fatty acids.

The decrease in EPN in fish muscle was related to increased toughness when cooked and a consequent decline in acceptability by taste panels. Dyer (1951), Tokunaga (1964) and Babbit et al (1972) have reported that the formation of DMA and FA occurred more rapidly in flesh than in intact fillets.

Noguchi et al (1975) had studied the technological application of cryoprotective substances on the frozen minced fish. The effect of 6 amino acids, 7 carboxylic acids/sorbitol and glucose on the salt added and salt free frozen horse mackerel meat mince was examined. The samples with the above additives produced good quality. Kamboko gelly after frozen storage for six week at -20°C. The amino acids and carboxylic acids tested showed a synergistic effect with sorbitol and glucose on the extractability of protein.

The study reported in this chapter aims at assessing some of the degradative changes occurring during frozen storage of minced fish and the efficacy of some of the means available to minimise the degradative changes specifically the possibility of using washing and non-protein additives to prevent loss of quality.

3.3. MATERIALS AND METHODS

3.3.1 RAW MATERIAL

The raw material for this study was collected from the landings of Integrated Fisheries Project, owned vessels operating along the south west coast. These vessels were daily going vessels. The main species employed for the study was Nemipterus japonicus or pink perch as it is commonly called.

The freshly landed fish was gutted, packed in crushed ice and kept in the chill room (+5°C) until used for mincing Usually, the mincing was done before 2 to 3 days in ice.

Salt solubility

Salt solubility of the protein was determined by extraction of salt soluble proteins from fish muscle by the method of Dyer (1950). Buffer containing Na Cl containing 0.02 m sodium bicarbonate (pH 7.6) was used for extraction.

The minced meat was blended with buffer cooled to 0°C in 1:10 (v/v) proportion for 2 minutes in a warring blender. Blending was done in a chill room at +5°C. The homogenate was transferred to centrifuge tubes (100 ml) and centrifuged at 5000 rpm for 30 mts at 0°C using a refrigerated centrifuge.

The supernatant was separated by decanting. The dissolved nitrogen was determined by the biurette method (Work & Work, 1969).

Each sample was given a code for avoiding confusion and they are outlined below:

1st symbol	W or U	If mince was washed or not. U - Unwashed W - washed
2nd symbol	X,L,M or S	Type of additive X - no additive L - Lactose M - Monosodium glutamate S - Sodium citrate
3rd symbol	-5, -18, -12°C	Temperature of storage
4th symbol	0,4,8,12,16,20	Time in weeks of storage

3.3.2 METHODS

Samples of the stored fish minces were withdrawn periodically and analysed for protein extractibility pH and texture. Periodicity of examination was (1) before freezing (2) One week (3) 4 weeks (4) 12 weeks (5) 16 weeks and (6) 20 weeks after frozen storage at -20°C.

The fish were headed, split open, cleaned, washed and passed through a Baader 694 deboner with a drum pore size

of 5 mm. The mince so obtained was treated as described below:

The mince was divided into two parts. One part was subjected to washing by mixing well with 4 to 5 parts of chilled water and stirred for 10 mts. with hands. Meat was allowed to settle and supernatant was decanted off. The process was repeated 2 to 3 times. The meat was strained in a net and pressed using a muslin cloth. This was the washed mince. The other was used unwashed. Additives were added in both washed and unwashed mince. The additives used were lactose, monsodium glutamate (MSG) and sodium citrate. Both the minces were divided into 4 different lots. Each additive was added in the dry state to separate lot of the minces, to give a final concentration of 3% (w/w). Mixing was done thoroughly to spread the additives homogenously. One lot from each lot of mince to which additives were not added was the control.

FLOW DIAGRAM FOR THE PREPARATION OF THE SAMPLE

Ice stored fish

Headed, split open, cleaned washed

BAADER 694

(drum pore 5mm. dia)

MINCE

WASHED MINCE	UNWASHED MINCE
(1) Control (No additives)	(1) Control (No additives)
(2) Mince ± lactose (3%)	(2) Mince ± lactore (3%)
(3) Mince ± MSG (3%)	(3) Mince ± MSG (3%)
(4) Mince ± Sodium citrate(3%)	(4) Mince±Sodium Citrate 3%)

Packing: The mince were packed in polythine bags each bag containing 500 gms. mince and was frozen at -40°C.

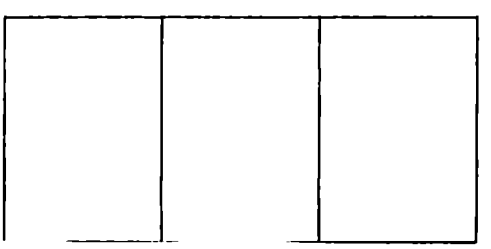
Storage: Storage was done at three different temperatures viz; (-) 25°C, (-) 18°C, (-) 5°C.

REPRESENTATIVE SCORE SHEET FOR

TEXTURE EVALUATION

9	8	7	6	5	4	3	2	1
Like extremely			On the fence					Dislike extremely

SAMPLE CODE



Baker, R.C., 1977 Creamy fish bites. New York Sea Grant Institute, USA, December 1977 (NTIS PB 277094/9SL).

3.4 RESULT & DISCUSSION

3.4.1 EFFECT OF WASHING AND FROZEN STORAGE TEMPERATURE ON PROTEIN DENATURATION OF THE FISH MINCE

The fish mince from Nemipterus japonicus was from at -40°C as blocks, both without washing and after washing with potable water and subsequently frozen stored at 3 different temperatures, viz., -25°C, -18°C and -5°C

3.4.1.1 Effect of Washing on salt solubility of proteins

Table 3.3.1 and 3.3.2 show the effect of washing on the salt solubility of proteins from fish mince during frozen storage.

Immediately after freezing of the unwashed mince, 90% of the protein fraction of the mince was salt extractable (Table-3.3.1) But the salt extractable fraction of the protein had significantly come down to 70% as a consequence of washing in potable water (Table 3.3.2).

The fact that 90% of the proteins from the fish mince were extractable, in the case of unwashed fish mince immediately after freezing, indicated that the raw material used for the study was in quite prime condition and the proteins were

TABLE - 3.3.1

SALT SOLUBILITY OF UNWASHED MINCE WITH NO ADDITIVE (CONTROL UX) FROZEN STORED AT 3 DIFFERENT TEMPERATURES

Storage period in weeks	-25°C	-18°C	-5°C
	Salt Solubility, as protein in mg/100 mg		
0 (immediately after freezing)	90	90	90
1	66	48	36
4	52	38	18
8	54	30	20
12	50	32	22
16	51	30	19
20	49	31	22

TABLE - 3.3.2

SALT SOLUBILITY WASHED MINCE WITH NO ADDITIVE (CONTROL, WX) FROZEN STORED AT 3 DIFFERENT TEMPERATURES

Storage period in weeks	-25°C	-18 °C	-5 °C
	Salt solubility of protein in mg/100 mg		
0 (Immediately after freezing)	70	70	70
1	48	33	23
4	43	34	20
8	40	30	19
12	32	26	18
16	33	20	18
20	33	19	18

not at all denatured. Still the value of 90% would appear to be quite on the higher side since such high level of salt extractability is seldom obtained. But in the case of very low fat fishes with only white meat in the body such extractability is not an isolated observation. Awad et al (1969) found that 93% of the proteins were extractable from white fish. Krivchenia & Fennema (1988) also obtained a very high protein extractability for fresh white fish Coregonus cuplaformis. Rodger et al (1980), obtained about 80% protein extractability for 5 days ice-stored, unwashed fish mince from cod (Gaddus morhua).

The fish species Nemipterus japonicus used for the present study has a very low fat content (0.72%) and the fish flesh is completely white. Hence the observed value of 90% protein extractability was quite natural.

The 20% decrease in the salt solubility of protein in the case of washed fish mince indicated that 20% of the protein fractions of the muscle of Nemipterus japonicus was water soluble. Usually the water soluble fraction of the muscle protein are mainly albumins and to a small extent low molecular weight globulins. Rodger et al (1979) has reported that washing the mince reduced 15 to 20% of the salt soluble protein of freshly caught inshore cods. Adu et al (1983)

while studying the nutritional and quality characteristics of dried minced rock fish flesh had observed that most of the sarcoplasmic proteins were lost during washing but 77% of the total protein (N x 6.25) was recovered in the washed fraction.

3.4.1.2 **Effect of washing and storage temperature on salt solubility of protein**

Table - 3.3.1 (Fig. 3.1) and Table - 3.3.2 (Fig. 3.2) give the changes in salt solubility of proteins of unwashed and washed fish mince, frozen stored at three different temperatures for a period of 20 weeks. In the case of unwashed mince, the initial value for salt solubility immediately after freezing was 90 mg/100 mg (90 mg%).

The salt solubilities after one week of frozen storage at three different temperatures viz. -25°C, -18°C and -5°C were found to differ greatly being 66%, 48% and 36% respectively.

This meant that the denaturation of proteins of the fish mince stored at -25°C was the least, while it was maximum in the case of fish mince stored at -5°C, indicating that the storage temperature had a significant effect on the denaturation of proteins of the frozen fish mince. During storage for a period of 20 weeks, salt solubility of protein decreased from 66%

after one week to 49% after 20 weeks of storage at -20°C. The corresponding values in the case of unwashed frozen mince stored at -18°C were 48% and 31% and for mince stored at -5°C, 36% and 22% respectively. The effect of temperature of storage on salt solubility was that the solubility was the least affected at storage temperature of -25°C and the most affected at the storage temperature of -5°C.

Table - 3.3.2 and Fig. 2.2 give the changes in the salt soluble protein content of washed fish mince frozen stored at the three different temperatures viz., -25°C, -18°C and -5°C. Immediately after freezing the salt soluble protein content was only 70%. On storage at -25°C for a period of one week this came down to 48%. The corresponding value of salt soluble proteins of -18°C was 33% and at -5°C 23%.

These values indicated that the protein denaturation was the least when the fish mince was stored at -25°C and the greatest at -5°C. During further storage for a period of 20 weeks the protein solubility in salt solution has gradually decreased. At -25°C the decrease was quite gradual, the salt solubility after 20 weeks being 33%. The corresponding value for mince stored at -18°C was 19% and at -5°C, 18%.

During the entire period of frozen storage of the washed

mince the effect of frozen storage temperature was almost the same as observed after one week of frozen storage.

The effect of washing of the fish mince on the salt solubility of protein had already been discussed earlier. Other than the initial drop of about 20% in the salt soluble protein due to washing, there was no other apparent difference in the salt solubility of both unwashed and washed mince during the entire period of frozen storage in three different temperatures. The effect of storage temperature on protein denaturation observed in the case of washed fish mince was the same as that of the unwashed fish mince. This indicated that other than the initial removal of the water soluble fraction of the protein from the fish mince, washing of the mince did not bring about any material change in the solubility properties of the fish proteins held in frozen storage.

Krivechenia & Fennema (1988) found that the temperature of frozen storage had a significant effect on the protein extractability of white fish fillet. In their study involving storage of frozen fillets of white fish at -60°C and -12°C, they found that for fillets stored at -60°C, the protein extractability was the most during storage upto a period of 28 weeks, while

for samples stored at -12°C, there was a considerable reduction in protein extractability. Awad et al (1969) observed that the protein extractability of white fish frozen stored at --10°C for a period of 16 weeks decreased from 93% to 43%.

Rodger et al (1980) had made extensive studies on the effect of temperature and time of storage of quality of fish mince from inshore cod. They found that the major part of the solubility loss of protein during frozen storage occurred during the first week and the subsequent loss of solubility was very slow.

Agarwal et al (1986) studying the frozen storage characteristics of composite fish mince from dhoma and lactarius had noted that salt soluble nitrogen reduced from 68% to 37% within 44 weeks of storage. During the initial 16 weeks of loss of solubility was rather rapid.

Tableros & Young (1980) studying the behaviour of the mechanically separated flesh of three common fish species of the Mexican shrimp catch during storage at -20°C for a period of 10 months found that there was a gradual reduction in extractable protein fraction This was more marked in the samples of flat fishes (Bothidae). However, washed mince showed minimal change in extractable protein nitrogen throughout the storage period.

Tejada & Borderias (1988) had found significant changes in protein solubility of frozen hake during frozen storage at -18°C. The solubility decreased from about 85% to about 39%. Devadasan & Nair (1970 & 1977) had observed that in the case of tropical fishes and prawn, the muscle contained about 30% sarcoplasmic proteins which were mainly the water soluble components of the fish protein. In the present study on Nemipterus japonicus, the water soluble protein content which was almost equal to the reduction in salt soluble protein during washing was also about 22% which is in agreement with the findings of Rodger et al (1980) and Devadasan & Nair (1970 & 1977).

Holmquest (1984) while studying the properties of Kambaboko made from red hake fillets, mince or surimi had observed that a -5°C salt extractable proteins in minced fish steadily from 90% to 12% during a storage period of 10 weeks.

The results of the present study, presented in Table 3.3.1 & 3.3.2 had amply confirmed this observation. The loss of solubility in the first week of frozen storage was more than 20% in both unwashed and washed mince in all the three storage temperatures, while subsequent decreases in protein solubility over a period of 19 weeks of storage were more or less steady

and slow.

Rodger et al (1980) had also observed that there was no linear relationship between the rate of salt solubility of protein and the storage temperature. But in the present study, the decrease in salt solubility of proteins in minces stored at -25°C have been found to be less compared with those stored at -18°C and at -5°C, in both washed and unwashed minces. This behaviour was on expected lines, since storage at sub zero temperatures nearer to zero causes a greater denaturation of the protein than storage at still lower temperature. This observation has been supported by the studies of Awad et al (1969) and Krevechenia and Fennema (1988).

3.4.1.3 **Effect of washing and storage temperatures on the textural changes of fish mince.**

Table 3.3.3, 3.3.4 and Fig. 3.3 and 3.4 show the textural changes of unwashed and washed fish mince, frozen stored at -25°C -18°C and -5°C respectively. Immediately after freezing the textural scores of both unwashed and washed fish mince were the same. In spite of the fact that washing has reduced the salt solubility of protein in the mince by about 20-30% the texture of both washed and unwashed fish mince scores were comparable.

TABLE - 3.3.3

TEXTURAL CHANGES OF UNWASHED FISH MINCE WITH NO ADDITIVE (CONTROL, UX) FROZEN STORED AT 3 DIFFERENT TEMPERATURES

Storage period in weeks	-25°C	-18°C	-5°C
	Textural scores		
0 (Immediately after freezing)	8	8	8
1	7.5	7	6.7
4	7	6	5
8	7	6.5	4
12	7	6	3
16	6	6.5	2
20	5	4	2

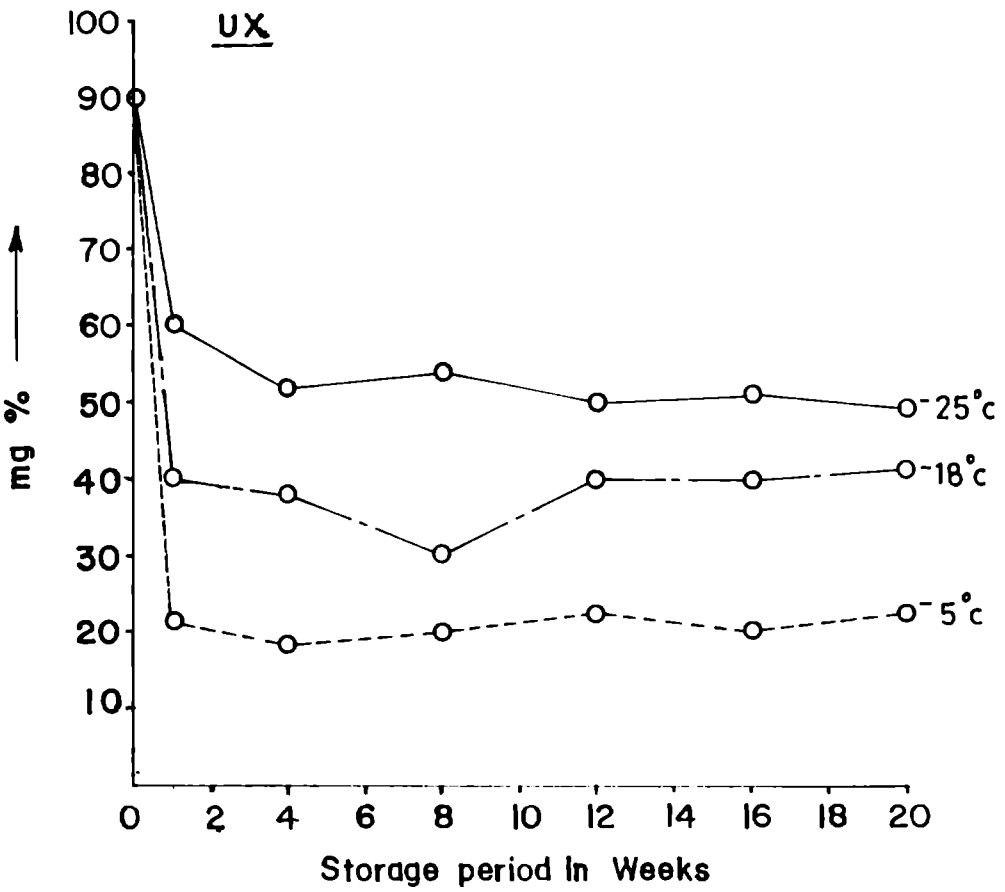


Fig. 3.1 Salt solubility of unwashed mince with no additive frozen stored at 3 different temperatures

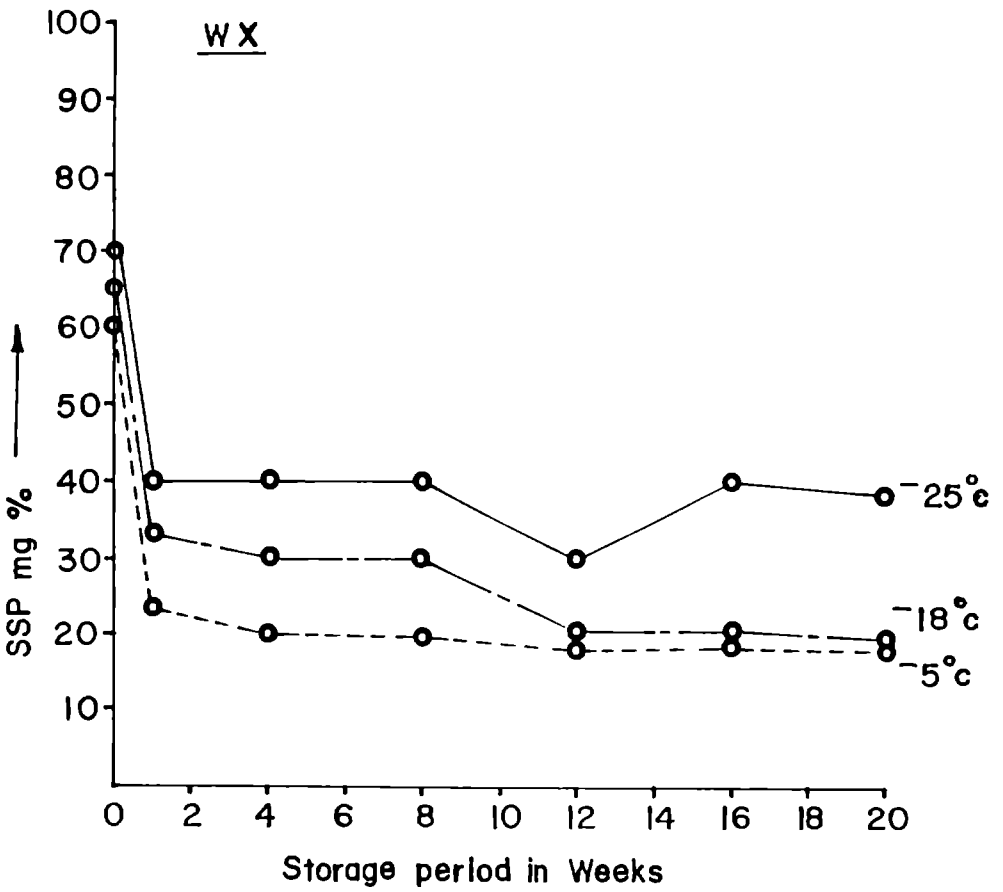


Fig. 3.2. Salt solubility of washed mince with no additive (control, WX) frozen stored at 3 different temperatures.

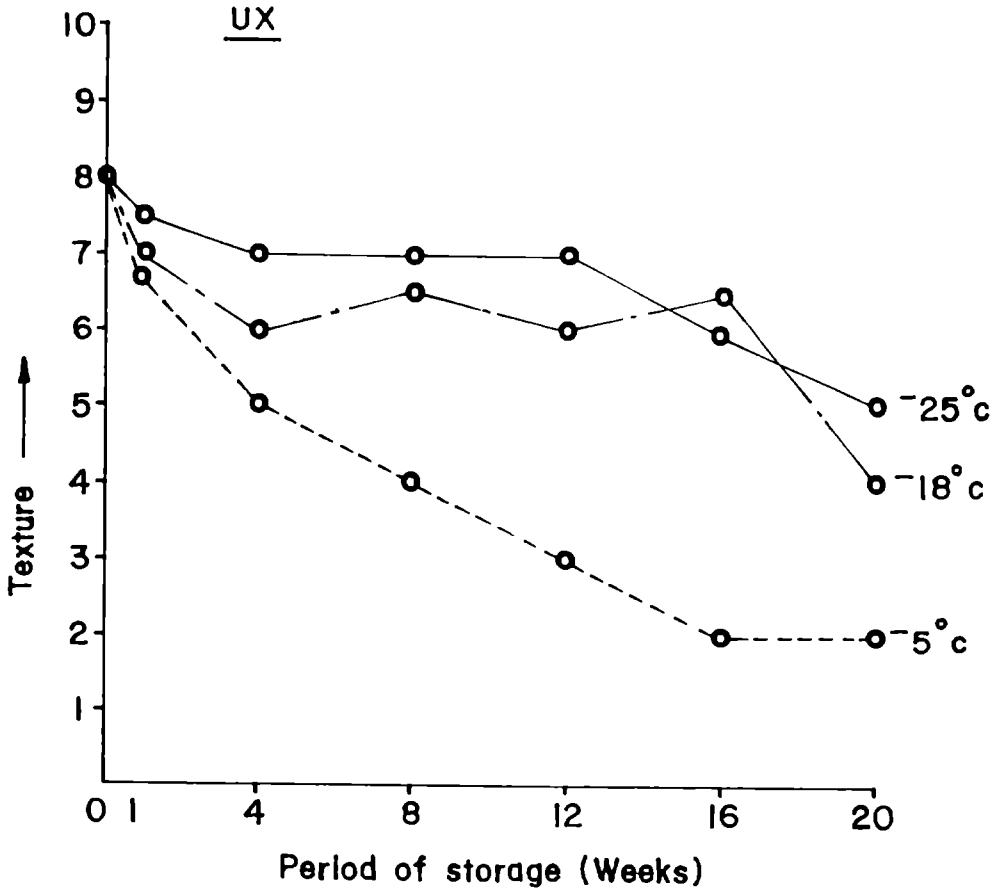


Fig. 3.3. Textural changes of unwashed fish mince with no additive (control UX), frozen stored at 3 different temperatures

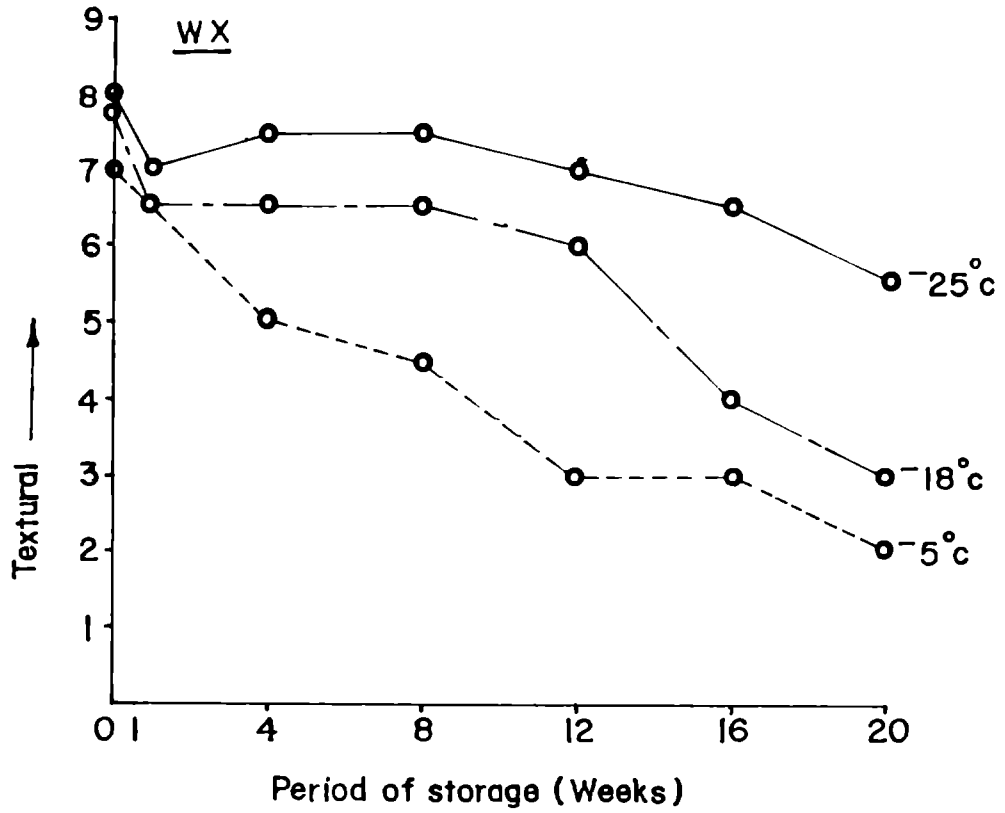


Fig. 3.4. Textural changes of washed fish mince with no additive (control, WX) frozen stored at 3 diffent temperatures

TABLE - 3.3.4

TEXTURAL CHANGES OF WASHED FISH MINCE WITH NO ADDITIVE (CONTROL, WX) FROZEN STORED AT 3 DIFFERENT TEMPERATURES

Storage period in weeks	-25°C	-18°C	-5°C
	Textural scores		
0 (immediately after freezing)	8	8	8
1	7	6.5	6.5
4	7.5	6.5	5
8	7.5	6.5	4.5
12	7	6	3
16	6.5	4	3
20	5.5	3	2

Frozen storage at -25°C has vastly protected the fish muscle from textural deterioration. But such protection was not uniformly seen in minces stored at a higher temperature viz. -5°C. Between -25°C and -18°C, such difference was not apparent in the texural scores. Probably both the temperatures were well below the critical frozen storage temperature above which denaturation of fish protein could be faster.

Nakayama and Yamamoto (1977) while studying the physical, chemical and sensory evaluation of frozen stored deboned fish flesh had found that in rock fish species the texture of the minced flesh on frozen storage increased in firmness slightly after a few months of frozen storage.

Patashnik et al (1970) have reported that during frozen storage the texture of the flesh of black rock fish (Sebastes spp.) increased in firmness.

Tableros & Young (1980) had observed that changes in solubility of proteins of fish flesh from several species of fish during frozen storage at -20°C was seen to influence the textural properties to a minimal extent.

Rodger et al (1979) had followed the textural changes of fish mince from inshore cod using a texturometer during frozen storage at different temperatures for various length of time. He had found that scrcoplasmic protein mostly water soluble, had little to contribute towards the textural properties of

cod muscle. Hence when they were removed by washing, no perceptible change could be expected to take place in the texture of the residual fish mince. This indicated that the texture of the fish muscle was not influenced by the presence of water soluble proteins in the flesh. During frozen storage for one week the textural score slightly decreased by about 1 to 1.5 unit in both unwashed and washed fish mince. The decrease in textural scores was least apparent in minces stored at -25°C and such decrease was mostly felt in fish mince stored at -5°C. During subsequent storages the textural scores diminished very gradually in the case of minces stored at -25°C and -18°C and comparatively at faster rate in minces at -5°C. Based on textural scores the fish minces, both unwashed and washed were in acceptable state even after 20 weeks of storage at -25°C. In the case of samples stored at -18°C, the unwashed minces were acceptable upto 20 weeks and washed mince upto 16 weeks. However, based on the decrease in texture scores, the minces stored at -5°C became unacceptable by the 8th week itself. The temperature of storage appears to have greatly influenced the deterioration in the muscle texture of both unwashed and washed fish minces, when we take into account the changes in textural scores of fish minces stored at -25°C and -5°C. The findings obtained in this study that the textural scores were not affected by washing

of the fish mince is therefore in full agreement with the observation of Rodger et al (1980).

The texture of the frozen stored fish muscle hardens because of the reaction between fish protein and the formaldehyde formed in the fish mince during frozen storage by the enzymatice reaction. TMAO - Trimethyl amine oxide; DMA - Dimethyl amine + Formaldehyde (FA). Hardening of the texture of the fish muscle decreases textural scores, when evaluated by sensory scoring system. The progressive deteriorative changes in the muscle texture in fish minces on frozen storage could be due to the combination of fish protein with formaldehyde (Rodger et al, 1980).

Dingle & Hines (1975), Tokunaga (1964) and Babbit et al (1972) have reported that the formation of DMA and formaldehyde occured more rapidly in minced flesh than in in-tact fillet of Alaska pollack and Pacific hake. Hence one should expect that the minced fish under frozen storage would rather rapidly undergo textural deterioration than intact fillet.

3.4.1.4 Effect of additives on the salt solubility proteins of fish mince held in frozen storage.

3.4.1.4.1 Effect of lactose

Tables 3.3.5 and 3.3.6 give the details on the salt extractability of proteins of unwashed and washed minced fish, treated with lactose and frozen stored at three temperatures, viz. -25°C, -18°C and -5°C.

The salt solubility of fish proteins decreased from the initial 90% to 78%, 60%, and 31% at -25°C, -18°C and -5°C respectively by one weeks' storage. During further frozen storage upto 20th week, there was a gradual decrease in solubility from 90% to to 60% in samples stored at -25°C 90% to 43%, at -18°C and from 90% to 20%, at -5°C. When compared with the unwashed mince with no additive (Table 3.3.1), the reduction in solubility is less at lower temperatures namely -25°C and -18°C. However, at -5°C it was more or less the same.

Table 3.3.6 gives the salt solubility of proteins in washed mince treated with lactose. The same patterns of changes could be observed as in unwashed mince treated with lactose. The difference in the initial values of protein extractability had been the effect of only the washing process and consequent removal of water soluble protein. In this case also the effect of treatment with

TABLE - 3.3.5

SALT SOLUBILITY OF UNWASHED MINCE MIXED WITH LACTOSE (UL), FROZEN STORED AT 3 DIFFERENT TEMPERATURES

Storage period in weeks	-25°C	-18°C	-5°C
	Salt solubility of protein mg/100 mg		
0 (immediately after freezing)	90	90	90
1	78	60	31
4	70	58	29
8	70	52	28
12	63	50	30
16	63	46	23
20	60	43	20

TABLE - 3.3.6

SALT SOLUBILITY OF WASHED MINCE MIXED WITH LACTOSE (CONTROL, WL) FROZEN STORED AT 3 DIFFERENT TEMPERATURES

Storage period in weeks	-25°C	-18°C	-5°C
	Salt solubility as protein mg/100 mg.		
0 (immediately after freezing)	75	60	65
1	45	40	38
4	50	20	18
8	60	23	18
12	50	23	20
16	50	25	19
20	52	20	19

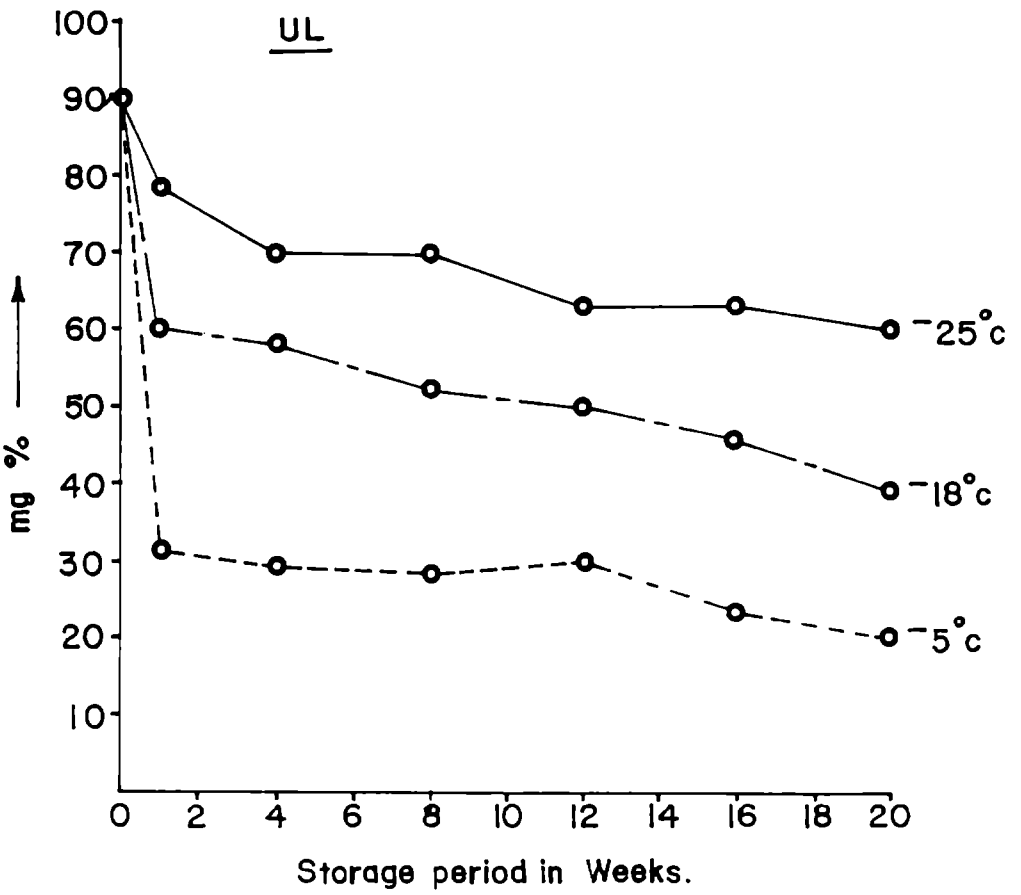


Fig. 3.5. Salt solubility of unwashed mince mixed with lactose (UL) frozen stored at 3 different temperatures.

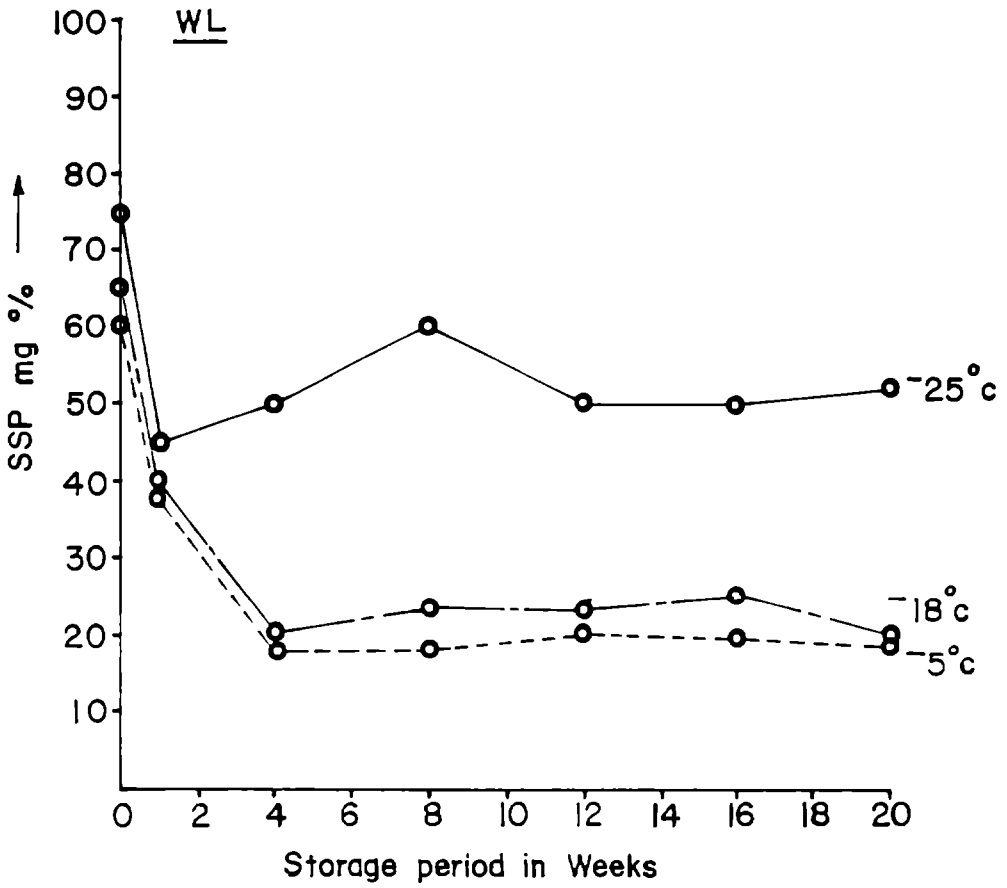


Fig. 3.6. Salt solubility of washed mince mixed with lactose (WL) frozen stored at three different temperatures.

lactose was more pronounced at lower temperatures.

Table 3.3.7 gives the effect of lactose treatment on unwashed mince on its textural properties. When compared with unwashed mince with no additive (Table 3.3.3) no perceptible difference is seen in the textural changes in both case. It can be inferred from the above that lactose played no role in influencing the textural changes of fish mince frozen stored at the three temperatures. In washed miince with lactose, a similar score reduction due to textural change can be observed (Table.3.3.8). The effect of lactose on textural changes in minced fish had been the same as in the case of unwashed mince.

3.4.1.4.2 Effect of monosodium glutamate (MSG)

In Table 3.3.9 and 3.3.10 are presented effect of treatment of unwashed and washed mince with MSG on the salt extractability of proteins. Initial salt solubility value of the unwashed mince mixed with MSG was 90%. It was reduced to 49%, 30% and 27% respectively at -25°C, -18°C and -5°C during a frozen storage period of 20 weeks. On comparison with unwashed mince with no additives (Table 3.3.1) it can be seen that the changes in protein extractibilties were more or less the same. It shows that MSG was not effective in controlling the loss in extractability of protein in unwashed mince.

TABLE - 3.3.7

TETURAL CHANGES OF UNWASHED FISH MINCE WITH LQCTOSE (UL) FROZEN STORED AT 3 DIFFERENT TEMPERATURES

Storage period in weeks	-25°C	-18°C	-5°C
	Textural scores		
0 (immediately after freezing)	8	8	8
1	7	7.5	6
4	8.5	6.5	4
8	7.5	6	3.5
12	7.5	6.5	3
16	6.5	5	3
20	5.5	5	3

TABLE - 3.3.8

TEXTURAL CHANGES OF WASHED FISH MINCE LACTOSE (WL) AT 3 DIFFERENT TEMPERATURES

Storage period in weeks	-25°C	-18°C	-5°C
	Textural scores		
0 (immediately after freezing)	8.8	8.8	8.8
1	7	6.5	6
4	6.8	7	5
8	6.75	6.5	4
12	7	6.5	3
16	6.5	4	3
20	6	4	2

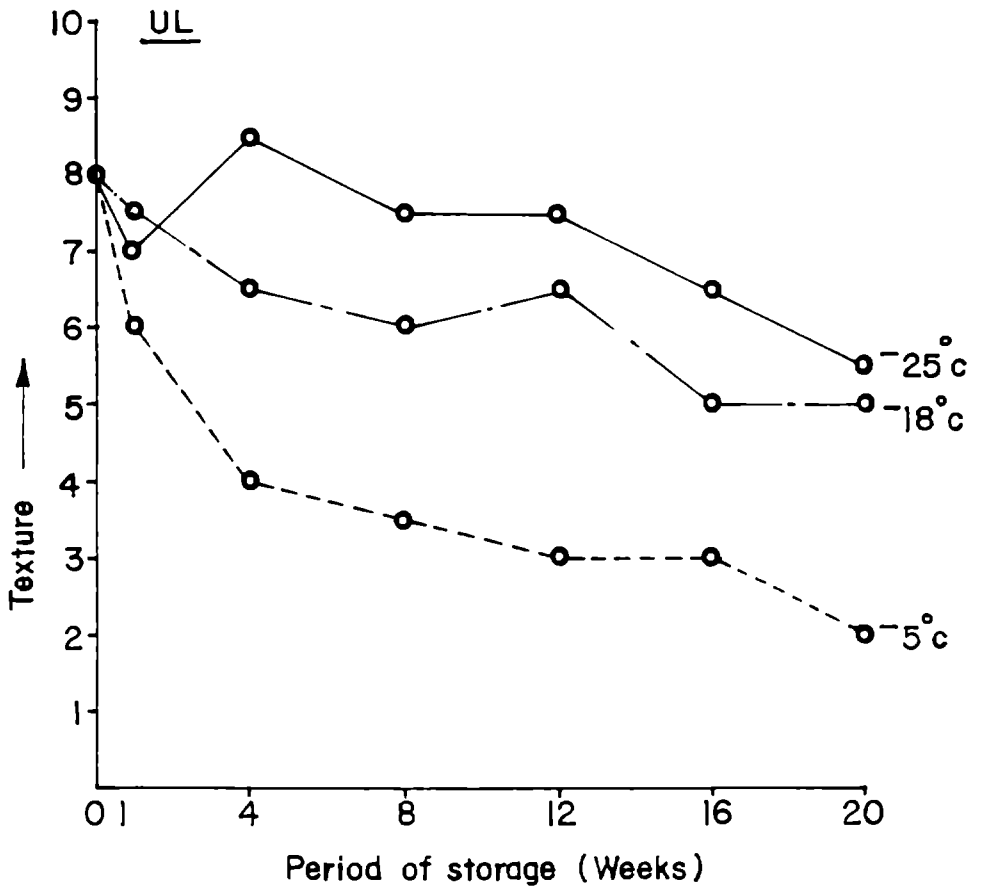


Fig. 3.7 Textural changes of unwashed mince with lactose as additive frozen stored at 3 different temperatures.

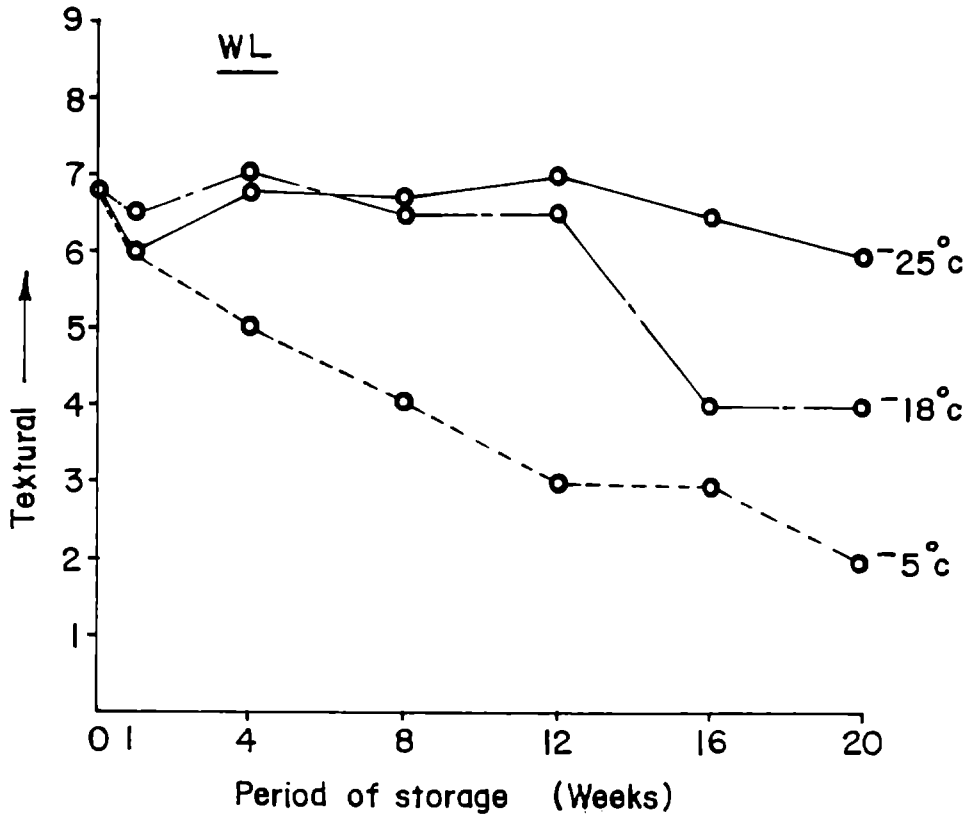


Fig. 3.8 Textural changes of unwashed fish mince with lactose (WL) frozen stored at 3 different temperatures.

TABLE - 3.3.9

SALT SOLUBILITY OF UNWASHED MINCE WITH MONOSODIUM GLUTAMATE (UM) FROZEN STORED AT 3 DIFFERENT TEMPERATURES

Storage period in weeks	-25°C	-18°C	-5°C
	Salt solubility as protein in mg/100 mg		
0 (immediately after freezing)	90	90	90
1	70	50	30
4	62	31	30
8	70	32	30
12	60	34	32
16	50	31	29
20	49	30	27

TABLE - 3.3.10

SALT SOLUBILITY OF WASHED MINCE WITH MONOSODIUM GLUTAMATE (WM) FROZEN STORED AT 3 DIFFERENT TEMPERATURES

Storage period in weeks	-25°C	-18°C	-5°C
	Salt solubility as protein in mg/100 mg		
0 (immediately after freezing)	70	70	70
1	50	31	25
4	58	37	19
8	59	30	18
12	58	30	20
16	49	28	18
20	38	21	18

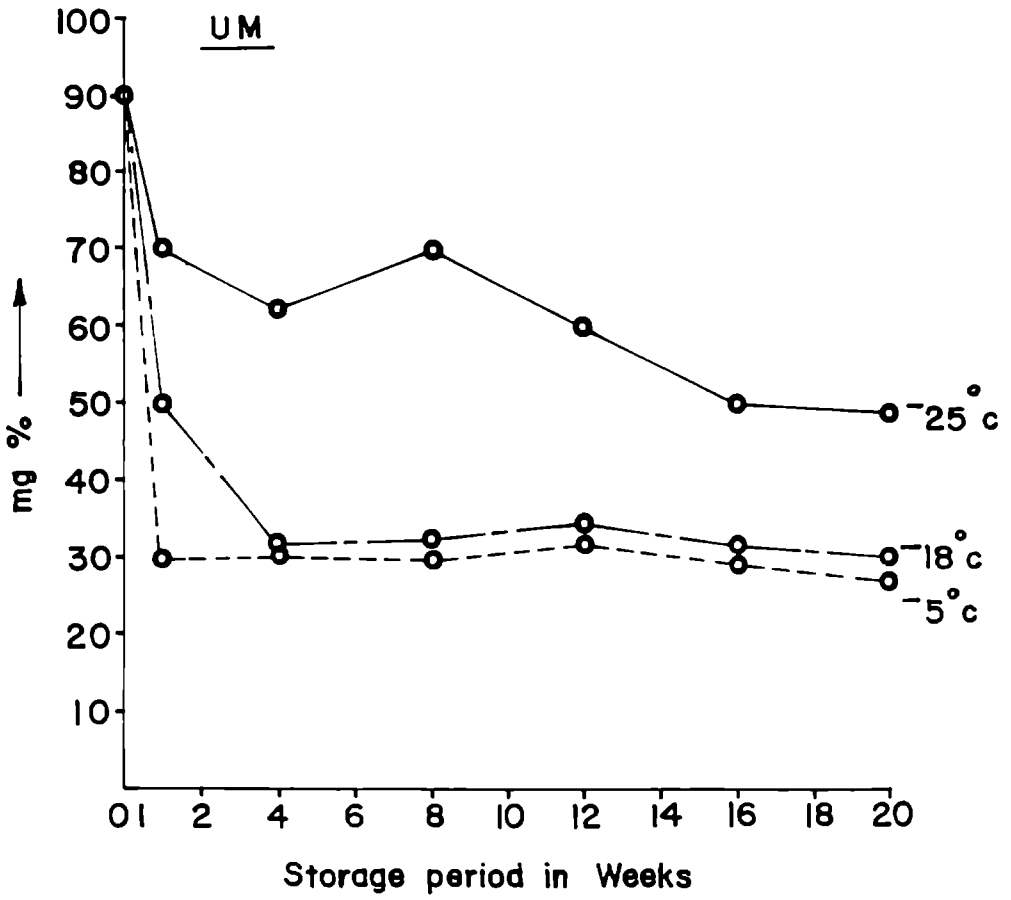


Fig. 3.9. Salt solubility of unwashed mince with monosodium glutamate frozen stored at 3 different temperatures

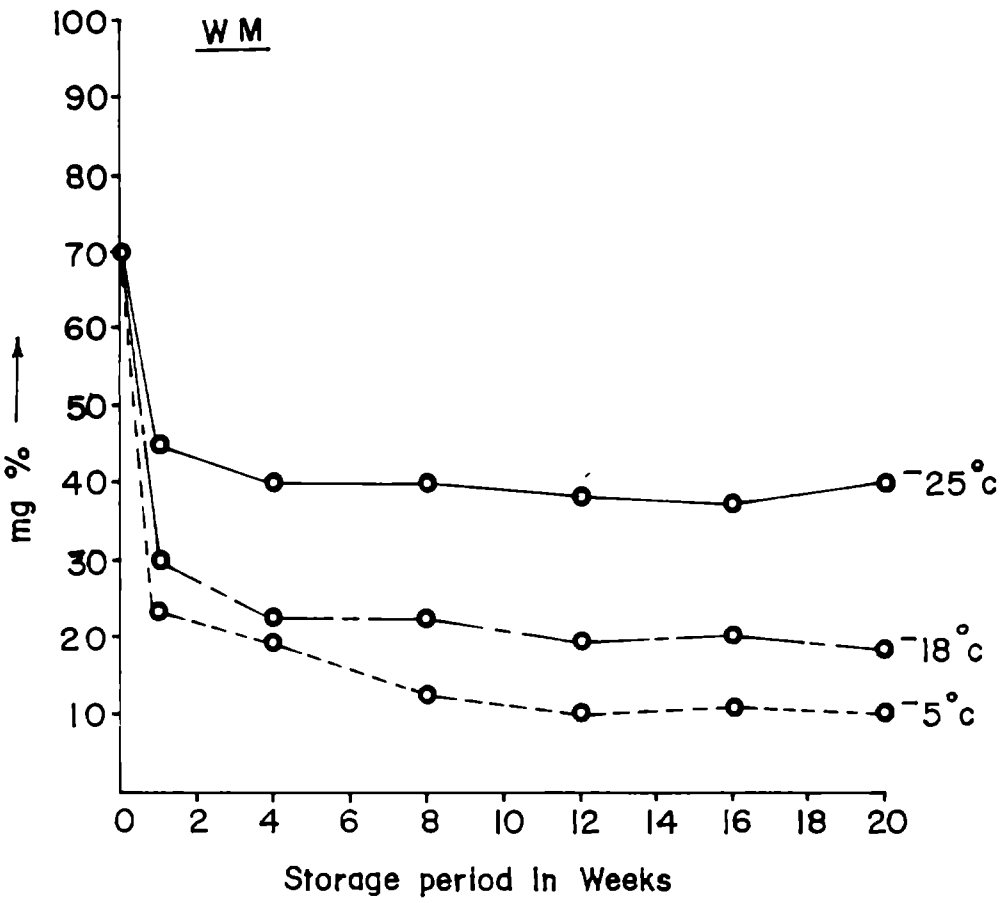


Fig. 3.10. Salt solubility of washed mince with monosodium glutamate frozen stored at three different temperatures.

Similar results were obtained for MSG treated washed mince (Table 3.3.10) also. Other than the initial loss of water soluble nitrogen caused by washing, no effect of MSG could be seen on the salt extractability of protein on washed mince.

Effect of MSG on textural properties of unwashed and washed mince can be seen from Tables 3.3.11 & 3.3.12. Initial textural score of washed mince was 8 and the scores after 20 weeks of frozen storage were 4.3 and 4.2 at -25°C, -18°C and -5°C respectively. These values indicated that MSG did not have any effect on the textural changes of fish mince during frozen storage. The reduction in textural scores were entirely related to the frozen storage temperatures.

In the case of washed mince (Table 3.3.12), it can be seen that though initially the textural score was only 7.5 the rate of reduction in scores were more or less the same as the frozen stored unwashed mince (Table. 3.3.3).

3.4.1.4.3 Effect of sodium citrate.

Tables 3.3.13 & 3.3.14 give the salt solubilities of proteins of washed and unwashed mince treated with sodium citrate and frozen stored at three different temperatures namely -25°C, --18°C and -5°C. Tables 3.3.15 and 3.3.16 give the corresponding changes. The decrease in salt solubility of proteins were more pronounced in the case of minces stored at -5°C and the less

TABLE - 3.3.11

TEXTURAL CHANGES OF UNWASHED FISH MINCE WITH MSG(UM), FROZEN STORED AT 3 DIFFERENT TEMPERATURES

Storage period in weeks	-25°C	-18°C	-5°C
	Textural scores		
0 (immediately after freezing)	8	8	8
1	7.5	7	6.4
4	6	6	4
8	6	6	3
12	5.5	6	2
16	4	4	2
20	4.3	4.2	1

TABLE - 3.3.12

TEXTURAL CHANGES OF WASHED FISH MINCE WITH MSG (WM), FROZEN STORED AT 3 DIFFERENT TEMPERATURES

Storage period in weeks	-25°C	-18°C	-5°C
	Textural scores		
0 (Immediately after freezing)	7.5	7.5	7.5
1	6.5	7	6
4	6	6	4.5
8	6	5	4
12	6,5	4	4
16	5.5	4	3
20	5.5	4.3	2

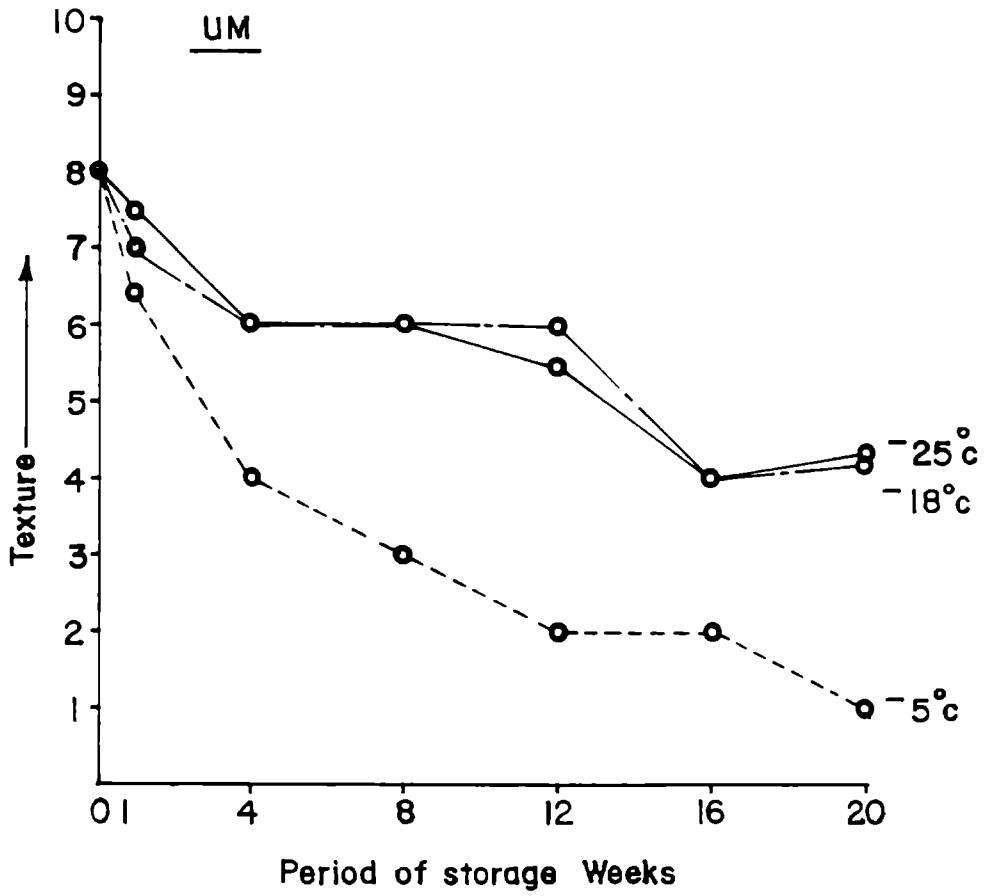


Fig. 3.11 Textural changes of unwashed fish mince with MSW (Wm) frozen stored at three different temperatures.

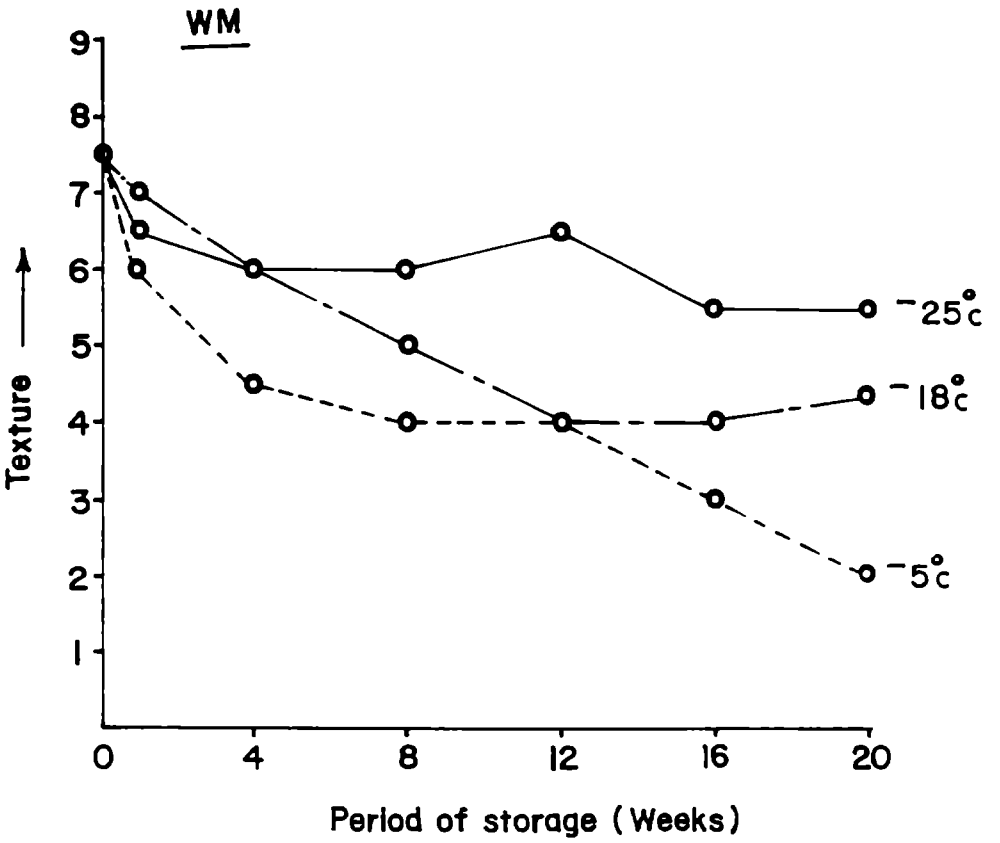


Fig. 3.12. Textural changes of washed mince with MSG(wm), frozen stored at 3 different temperatures.

TABLE - 3.3.13

SALT SOLUBILITY OF UNWASHED MINCE MIXED WITH SODIUM CITRATE (US) STORED AT 3 DIFFERENT TEMPERATURES

Storage period in weeks	-25°C	-18°C	-5°C
	Salt solubility as protein in mg/100 mg		
0 (Immediately after freezing)	90	90	90
1	50	40	20
4	48	30	20
8	47	30	22
12	50	40	22
16	50	30	18
20	45	27	18

TABLE - 3.3.14

SALT SOLUBILITY OF WASHED MINCE WITH SODIUM CITRATE (US) FROZEN STORED AT 3 DIFFERENT TEMPERATURES

Storage period in weeks	-25°C	-18°C	-5°C
	Salt solubility as protein in mg/100 mg		
0 (Immediately after freezing)	70	70	70
1	50	31	25
4	58	37	19
8	59	30	18
12	58	30	20
16	59	28	18
20	62	21	18

TABLE 3.3.15

TEXTURAL CHANGES OF UNWASHED FISH MINCE WITH SODIUM CITRATE (US) FROZEN STORED AT 3 DIFFERENT TEMPERATURES

Storage in weeks	-25°C	-18°C	-5°C
	Textural scores		
0 (Immediately after freezing)	8	8	8
1	7	7	7.5
4	6.5	7	6
8	6.5	7.5	6
12	6.5	7	4
16	6	6	4
20	5	5	3

TABLE - 3.3.16

TEXTURAL CHANGES OF WASHED FISH MINCE MIXED WITH SODIUM CITRATE (US) STORED AT 3 DIFFERENT TEMPERATURES

Storage period in weeks	-25°C	-18°C	-5°C
	Textural measurement		
0 (Immediately after freezing)	8	8	8
1	7.9	7.6	6
4	8	7	5
8	7	6.5	4
12	6.5	5	4
16	6	5	3
20	6	4	2

at -25°C. The trends were mostly the same as those observed in corresponding control samples (Tables 3.3.1, 3.3.2, 3.3.3 and 3.3.4). The results indicated that the treatment with sodium citrate had no effect on either protein extractability changes or textural changes of minced fish during frozen storage.

Lot of studies had been carried out on the use of non-protein additives to help preserve the required functional properties of the fish mince. On a practical level, it had been found that cryoprotectants reduce the rate of deteriorative changes in fish and fishery products during frozen storage. The addition of sucrose, sorbitol, polyphosphate and/or sodium glutanate to surimi had been shown to maintain protein functiionality during long periods of frozen storage (Suzuki, 1981).

Tecry and Miyauchi (1972) developed a modified minced fish block. Sodium choride and sodium tripolyphosphate were utilized to partially solubilize the muscle protein in order to bind the particles of coarse - minced muscle from black rock fish and to remove water extractable consistutents and added a binder mixture to improve the textural attributes of comminuted fish muscle. It was shown that in the absence of salt, textural strength was extremely weak with respect of shear and compression strength. Low compression strength was due to lack of cohesion between particles. When 0.5% polyphosphates were added it showed significant increase in textural strength. These workers found that

polyphosphates increased the solubilization of muscle protein and improved the water binding characteristics of comminuted fish muscle.

Cobb and Yoh (1974) discussed the effect of several additives on the texture of minced Atlantic croakers. Sodium chloride increased the firmness of the croaker patties, with 2% salt content being preferred for both taste and texture. The addition CaH PO_4 to croacker patties (mince based product) had a positive effect on firmness but was less effective than sodium chloride. A bland taste was observed if CaHPO4 exceeded 3% in the patties. Sugar improved the texture slightly. Egg albumin at the 2% level was the most effective additive in improving the texture of croacker patties. The addition of starch was shown to be beneficial in water-washed croaker, redeeming the problem of rubbery texture.

However, the three additives used in the present study viz., lactose, MSG and sodium citrate were not showing any remarkable effect on salt solubility of protein or on textural properties. Lactose alone showed a small effect in controlling salt solubility of proteins of fish mince frozen stored at -25°C.

However, Rodger et al (1980) observed, in his studies using the same additives ie., lactose, MSG and sodium citrate that all additives used had some effect in preserving the solubility of the fish mince proteins. Lactose performed best, but for all the additives used, the effect was most pronounced at the storage temperature -29°C. The effect at -17°C, and -14°C was not very much apparent in relation to the control sample without additives.

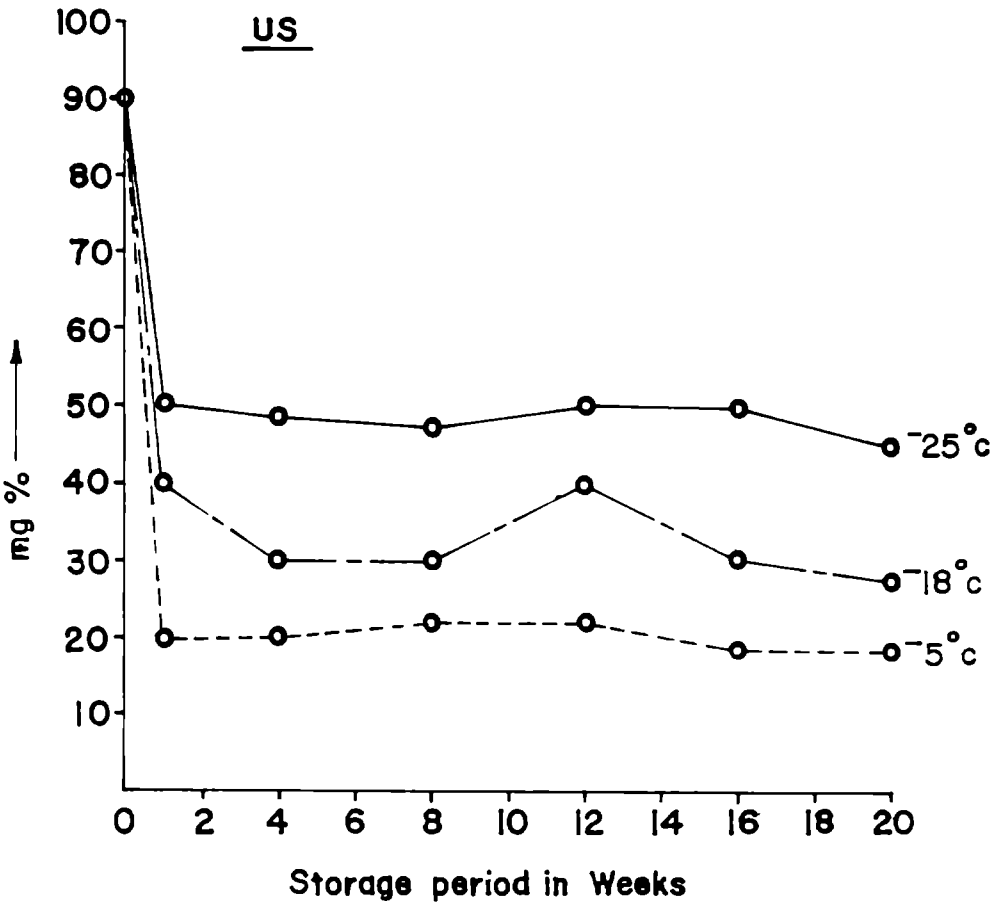


Fig. 3.13. Salt solubility of unwashed mince mixed with sodium citrate stored at 3 different temperatures.

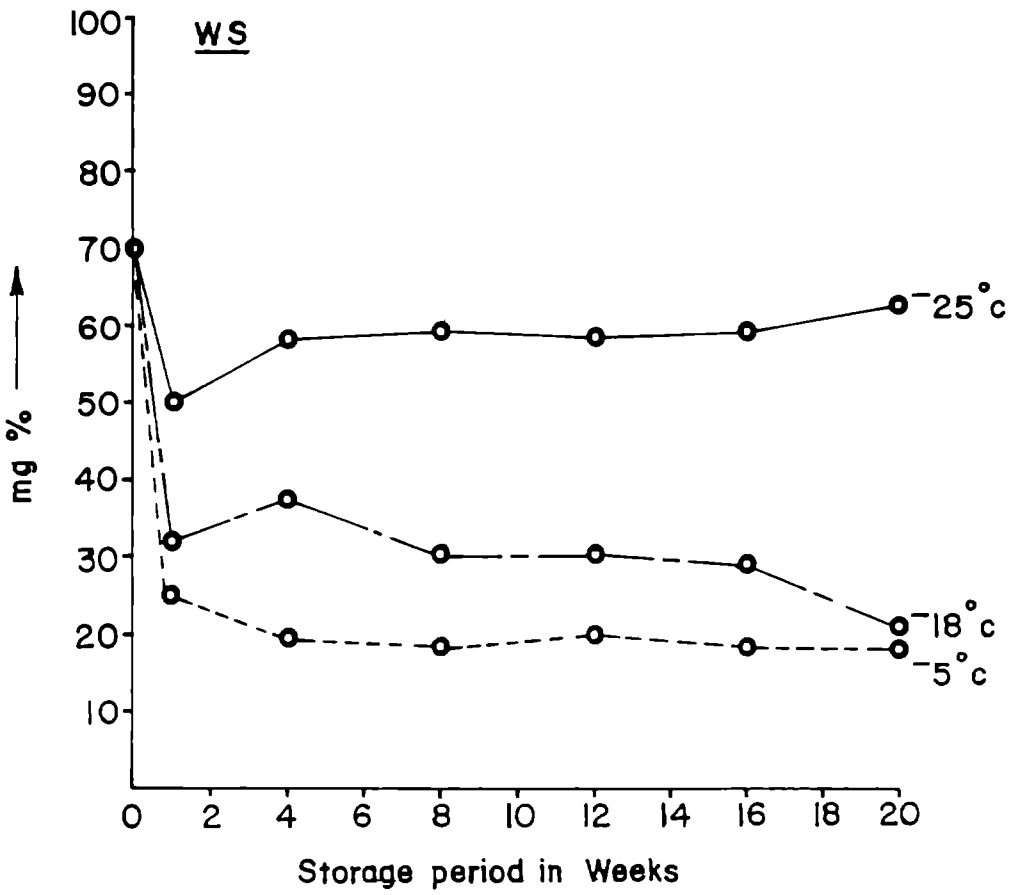


Fig.3.14. Salt solubility of washed mince with sodium citrate (us) frozen stored at 3 different temperatures.

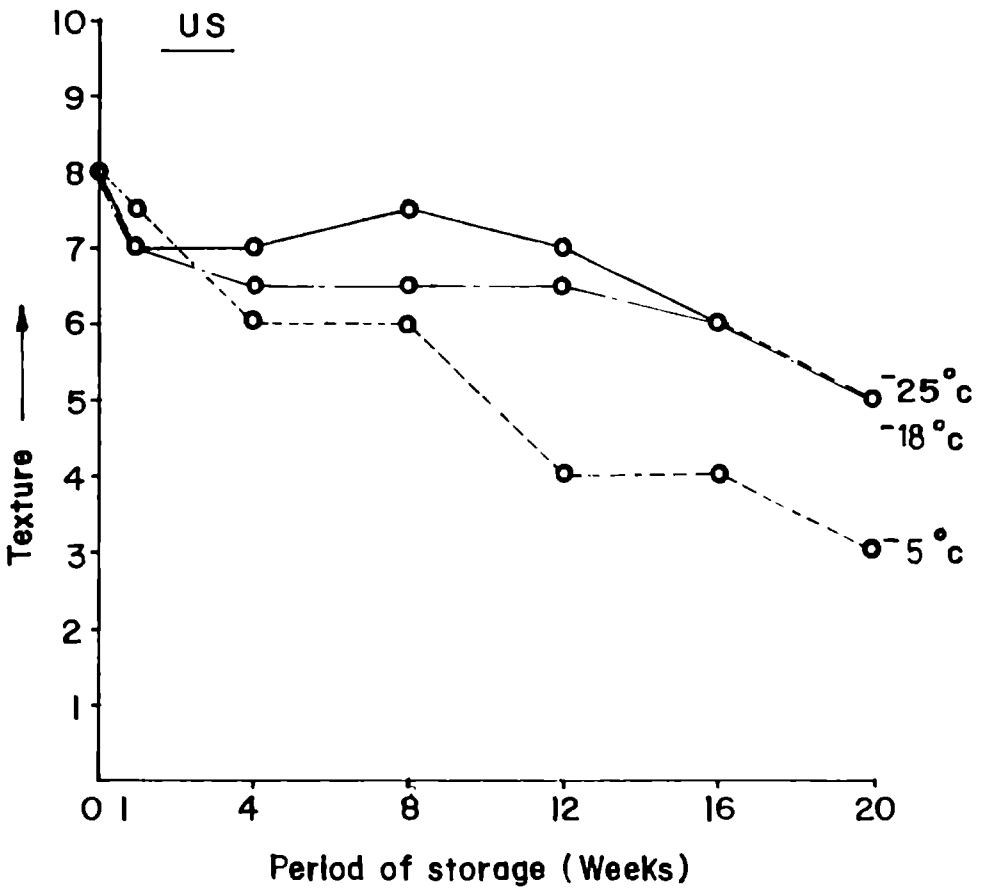


Fig. 3.15. Textural changes of unwashed fish mince with Sodium citrate (US) frozen stored at 3 different temperatures.

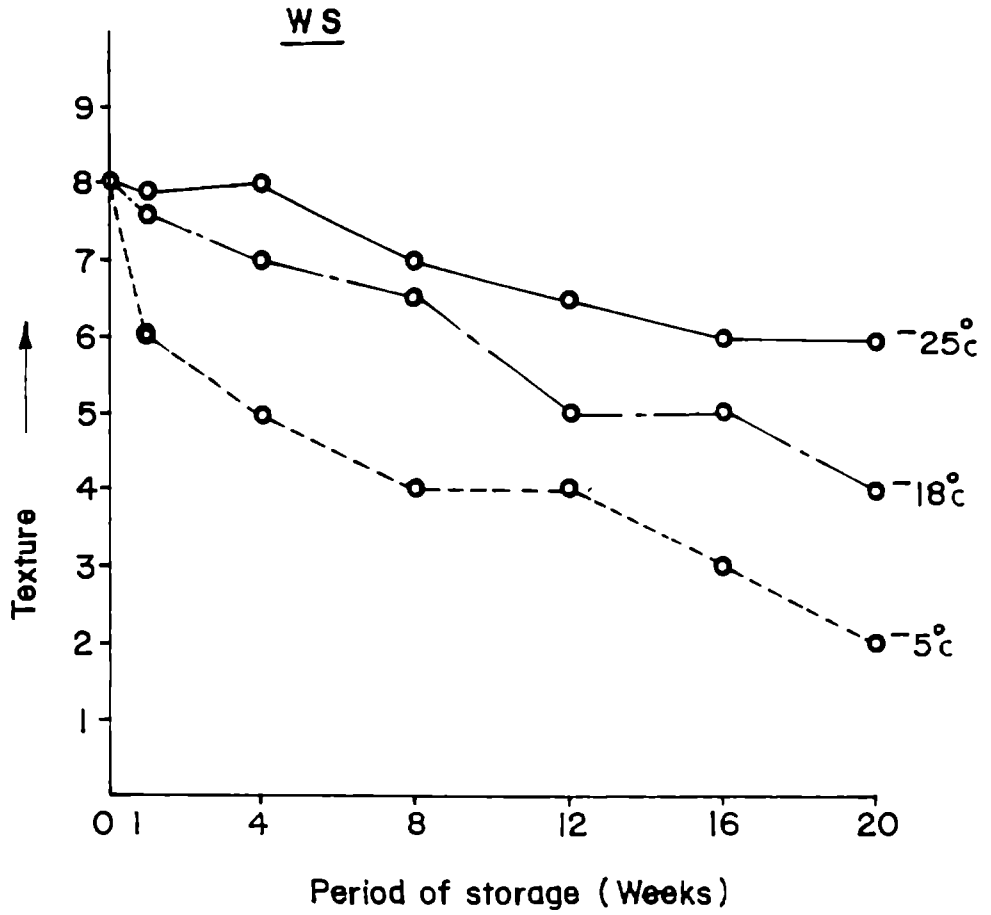


Fig. 3.16. Textural changes of washed fish mince mixed with sodium citrate stored at three different temperatures.

C H A P T E R - 4

C H A P T E R - 4

DEVELOPMENT OF CONSUMER PRODUCTS FROM MINCED MEAT FROM PRIACANTHUS SPECIES

4.1 INTRODUCTION

Minced fish being an intermediary product and since it is not very popular in India except the market reaches of the Integrated Fisheries Project whose product range has fish mince as one among the scores of different fish and fish products, its utility in the production of different consumer products, their acceptance, and nutritive value needs be studied. Minced fish is a mass of fish flesh in a comminuted form and devoid of bones, scales, guts, gills etc., and usually presented in the form of frozen blocks. The potential of minced fish in the production of various highly acceptable end products have to be demonstrated for the consumers, to fully establish a long chain of end products, and wide and ramified market. Only establishment of such an offtake of minced fish can ensure maximum utilization of catch and bycatch. The bland, formless fish flesh must be given suitable additives and flavours and made into food stuffs of good acceptance.

In the present study a few products had been developed from the minced meat of Priyacanthus species, the quality of these products, the organoleptic scores, and nutritive value through promixate composition have been analysed and reported to examine them for acceptability as consumer products.

4.2 REVIEW OF LITERATURE

There are a limited range of mince products established in the world market. In the West, they are dominated by frozen mince while in Japan, by 'Surimi' 'Surimi' is a historical fish paste product of Japan which has high preservation qualities and is a highly refined fish meat protein. It is a preliminary product to be advantageously used for diverse fish meat fabrication processes. The most important functional property of fish meat as the material of 'Surimi' is its gel forming ability because textural qualities are always essential for 'Surimi' based products.

A number of products are being developed in the developed and developing countries from minced fish.

3.2.1 FROZEN PRODUCTS

Eventhough adequate statistics are not available, the annual production of frozen mince blocks amounts to well over 500,000 tonnes (Steinberg 1980). Blocks are produced either exclusively from

minces or as mixtures of mince and fillets generally in moulds in plate freezers, generally incorporating additives like salt, sugars, phosphates etc. The commercial methods of such productions had been reviewed by Bond (1975) and Lanier & Thomas (1978).

In the international trade, mince blocks are a major commodity but they are only intermediaries in the manufacture of retail products. Some of the retail products produced from frozen mince are battered fingers, sticks, steaks and cakes (Licciardello et al 1980).

Freezing also can be used in the production of freeze-textured products (Stanley & Deman 1980, Takamura 1979) and pellet frozen minces (Loodahl & Astroe 1979).

Use of alginate gels for texturing frozen mince into sheets followed by layering to simulate myotome flackes had been studied by Keay & Hardy (1978), Babbit et al (1974), Law (1976) and Ruello (1978) and reported on the improvement of acceptability and oxidative stability of mince by incorporation of shrimp into mince portions. Functional additives like salt, phosphate, soyprotein and gums and their use in obtaining optional rheological-characteristics and texturisation have been reported by Brotsky (1980), Clark (1980) and Decker et al (1980). Use of colours, flavours and seasonings were studied by Pannel (1976).

4.2.2 CANNED PRODUCTS

Among all preserved minced products, canned mince products are the most stable. In spite of the vast potential of mince in a range of products, only a few applications were reported.

Canning was the main preservation method for Scandinavian mince balls. Mullet mince balls along with some canned chowders had been tested by Backer (1978 & 1980), Baker & Darfler (1980) and Regenstein (1980). Young (1979) reported on the canned smoked mince chunks and mince and vegetables from Mexican by-catch. Studies on many consumer accepted composite canned products had been done by many workers, viz., loaf products (Herborg 1976) Indian speciality products (Kuriyan 1977) pastes and spreads (Young.R. 1980). Poulter & Trevino (1978) have developed a canned paste product using deboned minces from five fish species commonly found in shrimp by-catch from the Gulf of California.

Canning of unprocessed fish mince had also been reported. Mince from channel catfish (Botta, 1974) carp (Lovell & McCoy (1979) hake (Mendelsohn, 1977) are some of such products with good acceptability.

4.2.3 SURIMI AND KAMABOKO

More than one million tonnes of kneaded mince products are

produced in Japan annually. Kamaboko, Chikuwa, and Satsumagae and their intermediate surimi are unique to Japan.

Preparation of surimi from Alaska pollack and use of additives have been studied by Miyauchi et al (1973) and Steinberg (1980). Its quality aspects including whiteness, rheological properties and gel forming abilities had been studied by Kaloh et al (1979). Mijake (1973), Umemoto & Muraki (1969) had reported on the use of sugars and sugar alcohols for gel forming and protection against frozen denaturation.

Kamaboko, a fine textured elastic fish sausage prepared from surimi by grinding, blending, shaping and heat sterilization had been studied by Yamamoto & Miyake, (1980). Surimi is being increasingly used as a raw material for mince products elsewhere in the world, as reported by Chambers (1980) and Thrash (1980).

4.2.4 DRIED AND INTERMEDIATE MOISTURE PRODUCTS

Many dried and cured products are possible from minced fish. The reduced water activity below 0.90 inhibits most bacteria, below 0.87 most of the yeasts and below 0.65 most moulds (Leistner & Rodel, 1975, Mossel, 1975). Use of different methods of drying and the retension of many qualities of minced fish have been reported by many workers. Freeze-drying (Calve and Borderias, 1979, Jensen, 1979) warm-air fluidised drying (Lagunov & Krivchenia, 1973) mild drying with protective sugars (Niki et al, 1978), Noguchi 1980, Snowbrand 1980) are some of them.

Suzuki (1981) had studied the production of marine beef from minced fish. Many traditional formed product like sausages, cakes, patties, balls, loaves and burgers are yielded by minced fish. Webb (1975) had studied the development of sea food patties utilizing mehcanically separated fish flesh tissue. On the basis of these experiments it was concluded that mechanically separated fish muscle tissue could effectively be used to prepare cat fish patties and sea food patties provided that supplemented ingredients were used in formulating the products. He had found that the use of steamed and flaked fish tissue was effective in texture ratings in seafood patties made with relatively high amounts of minced fish tissue. Laslet & Bremner (1979) had evaluated acceptability of fish fingers from sensory variables.

Gopakumar (1987) has given a review of mince based products. Zain (1979) has developed spice minced fish from Tilapia and studied its acceptability.

Joseph et al (1984) have prepared and stored cutlets from low priced and observed is quality character.

Basu (1984) has demonstrated the preparation of fish cubes from cutla and studied its shelf life.

Raj (1987) has carried out heat penetration study on fish sausage.

Raj & Chandrashekhar et al (1987) have made observation on some of the storage characteristics of high temperature processing of fish sausages.

Sudhakara (1979) briefs on the product developed at Mangalore Fisheries College, specifically dehydrated products, fried and ready to eat products, sweet products and canned products.

Angel et al (1979) has employed minced, whole fish fillets, remains after filleting and connective tissue of silver carps in various combinations to produce an emulsion based products. It was observed that a fairly firm textured emulsion products could be obtained with 30% minced whole silver carp and 70% minced or ground fillet.

Poulter (1983) had studied the acceptability of canned paste product based on some Gulf of California shrimp by-catch.

Victor Raj (1986) has developed an improved technique for the preparation of fish sausage over the conventional method.

Vasanth Shenoy (1988) has made a comparative study of the suitability of five species for the production of textured meat. Study has indicated that all three species are good source for the purpose.

Venugopalan (1967) has prepared fish flakes from trash fish mince.

Patel (1972) has studied the effect of certain preservatives on the shelf life of fish sausages.

Herborg (1977) developed noval fish products from the fishes of the Carribean area. He studied the development of marinated

products and cooked/minced products from various fish species. He has also examined the possibility of producing sausage from the meat of Whiting, Croaker and Shark.

Herbort et al (1974) described of the attempts to make fish cheese.

Rudrashetty (1974) had introduced a new method for incorporation of fish meat into domestic products.

Rudrashetty (1975) had shown a modified method for the preparation of canned fish paste from trash fish.

Muddanna et al (1975) have developed fish jam, chakkuli, sevu and new fish concentrates from mince fish.

Seno (1978) had studied the processing and utilisation of mackeral comminuted meat for the production of fish paste and different products to the frying taste.

Durairaj et al (1985) have studied the production of quick salted Lethrinus (Sea bream) fish cakes. The products were good from organoleptic and bacteriological point of view and after desalting can be used for preparations in the usual style.

Sankar et al (1993) had studied the effect of washing the mince from Indian oil sardine with chilled water on the quality of sausages prepared from it.

Sankar et al (1993) had studied the effect of sodium bicarbonate treatment on the minced meat characteristics of Indian Oil sardine and the quality of sausages prepared from it.

Reddy (1990) has used an extrusion technique to make fish fingers. The recipe has been standardised through taste panel studies.

Basen (1990) has studied the use of tripoly phosphate in the development of products from minced fish meat. It lowers the water activity of the product and has got bacteriocidal properties. Reddy et al (1992) have studied the storage behaviour of frozen fish fingers from croaker and perches.
Nair (1982) has observed the biochemical changes of fish fingers held at frozen storage.

Studies on quality of minced products.

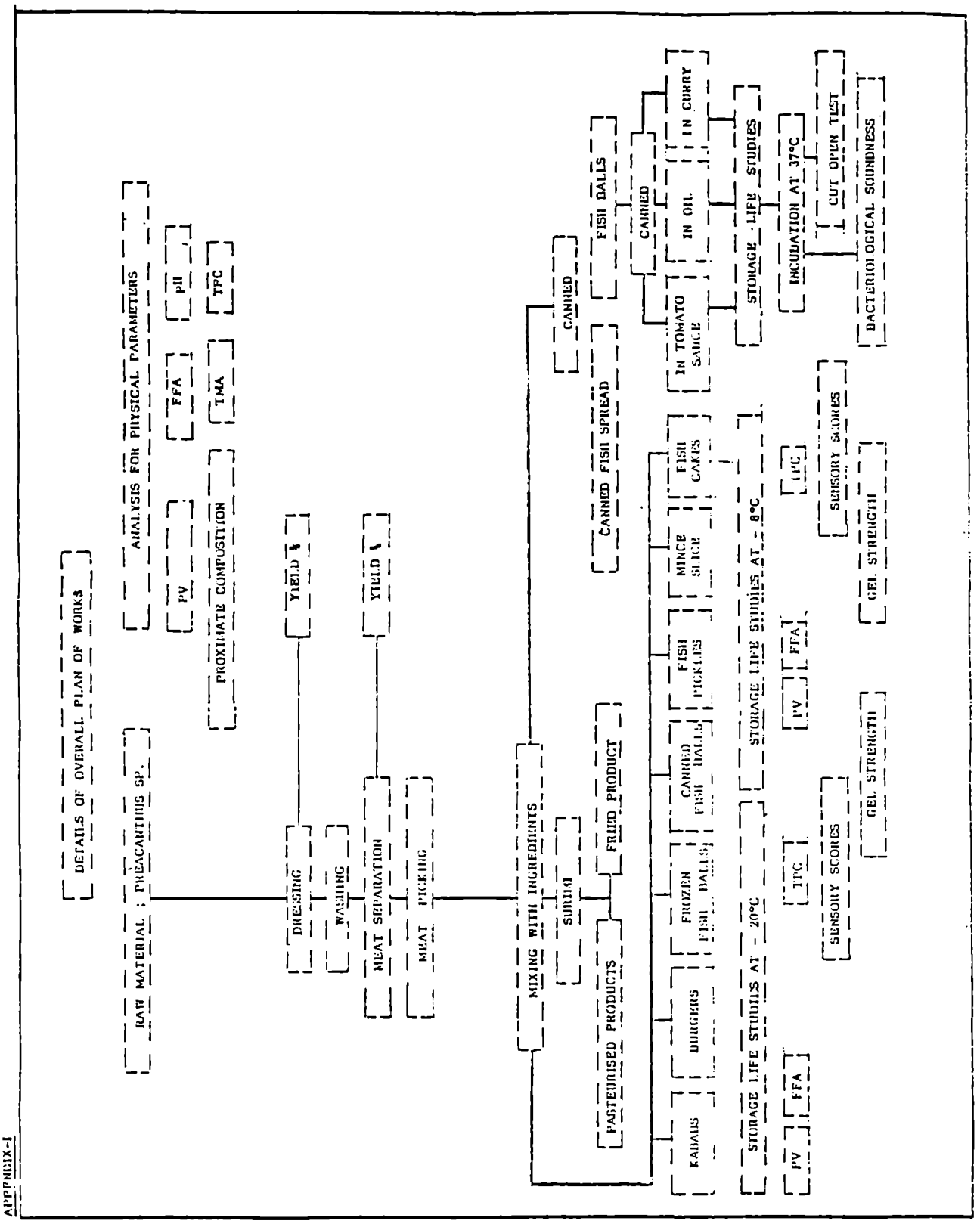
Deng (1979) has tried surface methodology to determine the effects of salt, tripolyphosphate, and sodium alginate on the quality of fish patties from minced fish croaker.

Gopakumar (1979) has described preparation of fish soup powder and fish flakes and fish hydrolysate. A study on the preparation and preservation of fish ball has been made by Chandrasekhar 1977

Steinborg (1976) had studied the fish mince as partial replacement for lean beef in commercially procured meat products such as frank furters, meat loaves and pork sausages held in frozen storages.

In the present study by employing mince from the low valued priyacanthus species, value added products have been formulated, quality evaluated and for a selected few products storage characteristics also has been evaluated. All these products were test marketed from the retail out lets of Integrated Fisheries Project, Cochin. Based on the marketing experience also it could be confidently stated that the products were nutritionally good and had very good consumer acceptance.

APPENDIX-I


DETAILS OF OVERALL PLAN OF WORKS
RAW MATERIAL : PRECANNING SP.
ANALYSIS FOR PHYSICAL PARAMETERS
PV
FFA
pH
PROXIMATE COMPOSITION
TMA
TPC
DRESSING
YIELD %
WASHING
MEAT SEPARATION
YIELD %
MEAT PICKING
MIXING WITH INGREDIENTS
SPICES
PASTEURISED PRODUCTS
FRIED PRODUCT
CANNED
CANNED FISH SPREAD
FISH BALLS
CANNED
IN TOMATO SAUCE
IN OIL
IN CURRY
MINCE SLICE
FISH CAKES
FISH PICKLES
FROZEN FISH BALLS
CANNED FISH BALLS
KABABS
BURGERS
STORAGE LIFE STUDIES AT - 20°C
STORAGE LIFE STUDIES AT - 8°C
STORAGE LIFE STUDIES
INCUBATION AT 37°C
CUT OPEN TEST
BACTERIOLOGICAL SOUNDNESS
PV
FFA
TPC
PV
FFA
TPC
SENSORY SCORES
SENSORY SCORES
GEL STRENGTH
GEL STRENGTH

4.3 MATERIAL AND METHODS

4.3.1 RAW MATERIAL

One of the varieties of fish located recently in considerable quantities by the survey vessels from the Indian waters is the deep sea fish Priacanthus species. They are represented by the four different species viz., P. hamur, P. tayemus, P. cruenteus, P. arentus.

Fisheries survey undertaken by the Government of India vessels in recent years has located this new variety. Philip et al (1984) stated that there was no demand for these varieties in the local market. Priacanthus species are deep sea water fish, available in considerable quantities in the Indian waters (Joseph, 1984) is popularly known as "bullseye" or "big eye".

The 'bullseye' is found on the south west coast from Goa to Mandapam and from Point-Calimore to Vishakapatanam on the east coast at 50-400 m. depth with peak concentration at 100 - 200 m.

4.3.1.1 Quality of fish used

Length and weight of fishes used for the study were recorded. Moisture, total protein, crude fat and crude ash contents of the samples were determined by the AOAC methods (AOAC.1978).

For peroxide value (PV) iodimetric method of estimation was followed (AOAC 1970).

Free fatty acid value (FFA)

The method described by Olley and Lovern (1960) was adopted. The values were expressed as percentage of oleic acid in total lipids.

Trimethyl amine and total volatile nitrogen were estimated by the Conway's micro diffusion method (Beatty & Gibbons 1937). The values were expressed as mg.N/100 g. meat.

Physical parameters

The yield percentage of dressed fish and picked meat were recorded. Percentage weight of the fish to ingredients is also recorded in the data sheet. pH was measured in a slurry of 10 gm. of the products in 100 ml. distilled water.

4.3.2 PRODUCTS DEVELOPED

The products developed were kababs, fish burger, fish balls, canned fish balls, two products from surimi, fish cakes, fish fingers and canned fish pastes.

4.3.2.1 **Recipe ingredients**

Recipe ingredients used in the preparation of products were of food grade quality. Ingredients included salt (Nacl), Sugar

(Sucrose), soda crackers, milk powders, Mono Sodium Glutamate, white of egg, fats, oil, butter, garlic, chemical seasonings, pepper, bread powder, spices etc. The composition of ingredients was standardised by trial and error method by judging the appearance, texture and taste of the final product. The composition of the recipe for each product is given under the corresponding product head.

4.3.2.2 **Equipments**

Mincing machines (Baader 674)

Silent cutter for grinding and mixing (Kramer and Grebe)

pH metre Agronic - 511

Moisture balance Egatube

Rheometer

Thermometer

Weighing balance

Knives

Frying pans

Moulds etc.

4.3.3 METHODS

Fishes were washed thoroughly, dressed, washed and fed through the meat bone separator (Baader 695). The picked meat was used for further preparation. The detailed plan of work is

shown in Appendix-I. pH was measured using a pH meter (Agronic - 511) Moisture was analysed using a moisture balance (Egatube) by heating the material at 70°C for 30 minutes. The gel strength was measured by rheometer

Microbiological quality

Total plate count (TPC) Total plate counts were estimated according to the method given in APHA (1976). The values were expressed as numbers/g of meat.

The samples, after freezing were analysed for E.coli, total coliforms, faecal streptococci and coagulase positive staphylococci. Total coliforms were estimated using desoxy cholate agar, E.coli using T_7 agar, faecal streptococci using KF media, and coagulase positive Staphylococci using Baird Parker agar.

Organoleptic evaluation

Organoleptic evaluation of all products was done by a group of 10 panellists who were instructed to evaluate the product by giving scores for different attribute like appearance, colour, taste, texture, odour and overall acceptability on the basis of 5 point scale. The scores for each attribute were pooled and average scores were presented. The five points stood for the following grades:

5	- Very Good	2	- Not good
4	- Fairly good	1	- Bad
3	- Good		

Cut open tests were conducted for the canned product. Cans were analysed after storage at ambient temperature for 2 weeks, three months, six months and nine months. Those cans examined for thermophiles were incubated at 55°C and mesophiles at 37°C for 48 hours. For detecting aerobic and anaerobic bacteria they were inoculated into thioglycollate medium and proceeded as per standard methods (AOAC. 1978)

Two products viz., fish cakes and fish burgers were subjected to frozen storage life studies at -20°C and at -18°C. Both the products were subjected to organoleptic assessment as well as biochemical tests. Bacteriological analysis was also done at regular intervals.

4.3.3.1 **Preparation of products from fish mince**

4.3.3.1.1 Fish Burger

The fish was washed, gutted and split open. Belly membrane and kidney tissues were removed. The material was passed through a meat bone separator; Baadar 694. Mince so obtained was washed once, lightly and then mixed with the ingredients in a silent cutter for 5 mts. (Details in P.D.1). The material was shaped into burgers of weight 84 gm, 2 inch dia in wooden moulds and were grilled at 90°C for 15-20 mts., and steamed for 4 mts. to make cooking complete. The product was cooled to room temperature in about 3 hrs. using fans and subsequently

frozen at -40°C. It was packed and kept in frozen stores at -20°C. During the entire procedure except while cooking the temperature was never allowed to raise above 10°C. Precooling the silent cutter and adding of ice instead of water while grinding ensured this temperature even during grinding (Product detail-1 - Flow chart-1)

4.3.3.1.2 Frozen Fish Balls

The mince meat is ground using a wet grinder with salt and milk for two minutes. Afterwards it is mixed well with starch, spices, ice water, vegetable fat and more milk was added (Details in P.D.2). The entire process was completed within 6 mts. The temperature of ground material was not allowed to rise above 10°C.

The materials were made into balls of size 2-3 cms diameter by hand and cooked in 1.5% brine at 90°C. The cooked balls would float on the surface of brine which can be separated using a perforated ladle and then cooled and packed in polythene bags and frozen at -40°C and stored at -20°C.

4.3.3.1.3 Canned Fish Balls

Minced meat was prepared by passing the dressed fish through a meat bone separator, Baadar 694. This minced meat was directly mixed with sub materials like lightly fried onion, raw egg,

bread, salt, MSG, dried spices like pepper powder, chilly powder, coriander powder and green chillies (Details in P.D.3). This was ground in a silent cutter for 5 minutes. The fish balls were formed by hand (1 cm^3 balls). Balls were fried in oil at 150-180°C or alternatively steamed at 100°C for 5 minutes. This product was canned.

For canning it was packed in 200 gms. lots in ½ Hansa aluminium cans with brine or tomato sauce oil as media, sealed, sterilized at 115°C for 50 minutes. Cans were cooled, wiped and stored at ambient temperature.

Cut open test was conducted for all the three varieties of cans.

Composition of the tomato sauce medium was as follows:

Tomato puree	5.95 kg
Water	10 litres
Ground nut oil	2.5 kg
Salt	350 gms.
Vinegar	600 ml.
Green chilly	1 kg.
Pepper powder	50 gms.
Sugar	100 gms.

All the above ingredients were mixed in a silent cutter for 5 minutes. Care was taken to avoid frothing.

4.3.3.1.4 Preparation of products from Surimi

The fish was scaled, headed, gutted and split open, belly flap was cut off, washed well and passed through a meat bone separator. The mince so obtained was washed with double the quantity of chilled water and constantly stirred for 30 minutes. The meat was allowed to settle and the supernatant decanted off. The meat was then taken on to a nylon webbing and pressed to remove water. The moisture content level was made 60%. It was blended in a silent cutter without allowing the temperature to rise above 10°C. After the initial grinding sugar was added and ground for 1 minute.(Details in P.D.4). A mixture of sodium tripolyphosphate and sodium pyrophosphate (1:1 w/w) was ground with this for 4 minutes. The surimi so obtained was further processed to develop the following products.

(i) Fried product

To the surimi obtained, the following materials were added in the proper proportion - Carrot, onion, egg, cooked potato, sugar, starch and spices like green chillies, pepper powder, ginger, garlic and also ice (Details in P.D. 5). The most used ingredients were added first and so on and ground for 2 mts.

It was shaped in the form of cutlets and flash fried in ground nut oil at 150°C. The product was stored at -8°C in a refrigerator.

(ii) Pasteurised product

To the 'surimi' obtained, 3% salt was added, and ground. To this ground material, cut vegetables (Details in P.D. 5) like carrot, onion, cooked potato, green chillies, ginger, garlic, pepper powder, egg etc., was added and ground again for 2 mts. It was filled in cylindrical polybags and tied at both ends. It was dipped in water at 90°C for 40 mts. This was kept in chilled store at 8°C.

4.3.3.1.5 Fish Cake

Minced meat was ground in a silent cutter with salt, starch powder and spices for 8 to 10 mts.(Details in P.D 6). It was moulded in metallic or wooden moulds of different shapes. The thickness was not allowed to exceed 1 cm. The moulded fish cakes were cooled in a freezer till it becomes semifrozen.

The Batter was made by mixing wheat powder or starch with chilled water in the ratio 1:1. Breading was made by powdering dried bread. First the semifrozen fish cake was dipped in the butter and bread powder was uniformly spread over it. Then the battered and breaded fish cakes were quick frozen in a plate freezer at -40°C and stored at -20°C.

The frozen fish cakes without thawing was fried in hot oil till golden yellow colour was attained before use.

4.3.3.1.6 Battered and breaded mince slices

The frozen mince blocks were sliced into thin pieces of size 6x5x1 cm each weighing 60-70 gms. These were spread over trays and dipped in batter made from wheat powder and chilled water (1:1 w/w) and bread powder was spread over uniformly on all sides.(Details in P.D. 7). Afterwards it was spread on the trays and frozen in an IQF machine. The frozen battered and breaded mince slices were individually packed in polythene bags and stored at -20°C. The frozen product was fried in hot oil (220°C) for 45-50 seconds till golden colour was attained and served hot to the paneliests for organoleptic assessment.

4.3.3.1.7 Fish Kababs

Minced meat was mixed with all the green and dry spices and bengal gram (C hanna dal) (Details in P.D. 9). The mixture so formed was cooked with water till such time the meat and dal were cooked. The cooked mixture was then ground in a silent cutter for 30 seconds. Eggs were added to the paste and mixed well. The ground material was flattened into kababs and filled with finely chopped onions and green chillies. Kababs were individually frozen at -40°C and was packed in poly bags and stored at -20°C.

4.3.3.1.8 Canned Fish spread

Mince meat was boiled with fat and water till the meat got cooked. Proportion between fish mince, fat and water was 2:1:2.1. It was ground well in a silent cutter adding the ingredients one by one (Details in P.D. 8). The most used ingredients was added first. It was thoroughly ground to make a semifluid paste. This was filtered through a micro pulveriser. Minute bone fragments were sieved in this operation. This was filled in cans. Cans were seamed and sterilized at 115°C for 45 mts. On cooling of the cans the fluid sets to a butter like consistancy. Cans were stored at ambient temperature.

4.4 RESULTS AND DISCUSSION

4.4.1 RAW MATERIAL CHARACTERISTICS

Priacanthus species was employed in the experiments. The average length of the fish employed was 10-19 cms. and weight of 15-200 g.

4.4.1.1 Proximate composition

The proximate composition of the fish before and after mincing is given in Table 4.4.1

The moisture content of the Priacanthus species was 76.87% which on mincing increased to 81.33%. This increase is expected because while determining the moisture content of the whole fish, the weight of bones, scales and skins are also taken into account while the moisture content of the mince is exclusively that of the muscle.

The protein content of the raw fish was 17.43% which for the mince was 17.93%. The fat content was 0.78% and 0.83% respectively for the whole fish and the mince. The ash content in the whole fish was 1.62% compared with 0.60% for the mince.

The proximate composition of Priacanthus species were comparable with similar marine species. Perigreen and Joseph (1983) have

reported a proximate composition 80.24% for the moisture, 18.13% for the protein, 0.72% for the fat and 0.55% for the ash content of mince from Nemipterus japonicus. The jew fish (Sciaenia claucus) a comparable proximate composition has been reported (Gopakumar, 1993). The higher ash content for the fish is because of the fact that bones have also been accounted for while in mince only the mineral content of the muscle is taken care of. Knzynowek et al (1984) obtained the following ranges of values for proximate composition expressed as percent of wet weight for three Gadoids protein 14-17%, fat 0.4-2%; moisture 81-84%; ash 0.5-1.9%. These values are comparable with that of the present study.

The values of the chemical indices like TMA., TVBN, PV and FFA for both whole fish and mince are indicative of the freshness of the material used for the study. The pH values of 6.8 for the whole fish and 6.5 for the minced fish indicated that the muscle had not undergone any proteolytic/deaminative changes by the associated bacteria. Also the total bacterial count in the range of 10^3/g for both raw fish and the mince showed that the bacterial population was very minimum.

4.4.1.2 Meat yield at different stages of processing

During the processing of fish into mince, yield at different stages were determined Table 4.4.2 gives the percentage recovery

TABLE - 4.4.1

PROXIMATE COMPOSITION OF THE RAW MATERIAL

PRIACANTHUS SPECIES

	Raw fish	Mince
Moisture %	76.87	81.33
Protein %	17.43	17.93
Fat %	0.78	0.83
Ash %	1.62	0.66
TMA	0.45	0.42
PV	6.3	5.03
FFA	0.73	0.78
pH	6.8	6.5
TPC/g	3×10^3	5×10^3

TABLE - 4.4.2

MEAT YIELD FROM PRIACANTHUS SPP. AT DIFFERENT STAGES OF PROCESSING

Yield after Dressing	50%
Yield after Mincing	40%
Meat recovered from frames	3%

of the meat at different stages.

The yield of meat from Priacanthus species after dressing was 50% which on mincing reduced to 40% ie. the yield of mince was slightly more than 1/3rd of the whole fish. Menon (1975) have studied the yield of picked meat for 11 different species of fish and they reported values ranging 29% to 60%.

Samuel et al (1986) obtained almost similar values for the dressed and the minced meat yield of Priacanthus species. Perigreen and Joseph (1983) had obtained 44-46% minced meat yield for Nemipterus japonicus.

The fish being full of bones, comparatively bigger head portion, thick and leathery skin does not have much consumer acceptance in whole form (Philip et al 1984). Hence eventhough the minced meat yield of this species was only 40% this is the best way of having economic utilisation.

4.4.1.3 **The quality of the water used**

The chilled water used for the study was of potable quality, pH and total plate count/ml., of water were found to be 6.5 and 1.7 x 10^2 respectively. Coliformes were not detected in the water.

4.4.1.4 **Fish Burger**

The product detail (P.D.1) gives the processing detail

and the recipe for the fish burger. The given recipe is for 140 kg. of the finished product for which 225 kg. of raw fish is needed to begin with, which yields 100 kg. of minced fish. Weight of ingredients to fish meat was 28% approximately.

Out of the 40 kg. of ingredients added at various stages of grinding, starch, sugar and white of egg and fat are meant for obtaining the desisred binding and consistency for the fish burger.

Sodium polyphosphate is a seasoning agent added which would sustain the water holding capacity of the fish muscle. The spices and salt added are meant for providing the desired taste and flavour to the finished product. The quality required were arrived at by trial and error method.

The characteristics of fish burger are presented in Table 4.4.3 both before and after freezing. The moisture content of the fish burger was 70% before freezing and 68.01% after freezing. Gel strength was 300 gm/Cm^2 and the pH 7. The bacterial count before freezing was 3 x 10^3/g, which was completely destroyed during the freezing process.

The values for the organoleptic characteristics like form and taste, colour and hue, aroma and texture on 5 point scale were on the very good side which would indicate that the

fried fish burger was organoleptically very much acceptable.

From the nutrition stand point the fried product had a carbohydrate level of 13.9% protein, 11.43% fat, 5.63% and 1.03% ash. Calorific value of the fried fish burger worked out to 151 K -calories which shows that the product is nutritionally excellent.

4.4.1.5 **Frozen fish balls - (Cooked in brine)**

The product detail (P.D.2) gives the processing details and the recipe for fish balls. The given recipe is for 18.4 kg. of finished product for which around 32 kg. of raw material was used which yielded 10 kg. of minced meat. In the finished product 54.5% formed fish and 45.5% was the ingredients.

Out of the 8.4 kg. of ingredients added salt, milk and vegetable were meant for ensuring the desired binding and texture for the fish balls. Salt and pepper powder are to impart the desired taste and flavour. The quantity of ingredients to be added was arrived at by conducting several trials, taking into account the opinion of the taste panel.

The characteristics of fish balls cooked in brine are given in Table 4.4.4 both before and after cooking.

The moisture content before cooking was 80% and after cooking 66.26%. Gel strength was 230 g/Cm^2 before cooking and after

TABLE - 4.4.3

CHARACTERISTICS OF FISH BURGER

Before freeing		After frying the product	
p H	7	i) Organoleptic quality	
Moisture %	70%	Form & taste	5
		Colour and hue	5
		Aroma	4
		Texture	4
		ii) Proximate composition	
		Moisture	68.01%
		Ash	1.03%
		Fat	5.63%
		Protein	11.43%
		Carbo hydrate	13.90%
T P C	$3x10^3$/g	iii) T P C	Nil
Gel strength	300g/CM^2		450g/CM^2

TABLE - 4.4.4

CHARACTERISTICS OF FROZEN FISH BALLS COOKED IN BRINE

Before cooking		After cooking
Moisture	80%	66.26%
T P C	3.9×10^4	3×10^2
Gel strength	230g/CM²	310g/CM²
pH	6.5	--
Organoleptic qualities		Calorific value 161 Kcalories
Flavour and taste		4
Colour and hue		5
Aroma		4
Texture		5
Proximate composition		
Carbohydrate		15.35%
Moisture		66.26%
Ash		1.01%
Fat		5.95%
Protein		11.43%

cooking it increased to 310 g/Cm². The product had a pH of 6.5.

The values for organoleptic characteristics were 4 for flavour and taste, 5 for colour and hue, 4 for aroma and 5 for texture, which indicated that panelists rated the quality attributes as good or very good.

From the nutritional point of view qualities were as follows: The product had a carbohydrate level of 15.35%; fat content of 5.59% and protein 11.43%. The calorific value per 100 gm of the cooked fish ball worked out to be 161 K. calories which meant that the product is fairly nourishing.

4.4.1.6 **Fish balls - Steamed**

The product detail (P.D.3) gives the processing details and the recipe for the product. The given recipe is for 130.5kg of finished product for which 100 kg. of minced fish is required. 325 kg. of raw fish yielded 100 kg. of minced fish meat. 30.5 kg. of ingredients were added Raw egg and bread powder are for effective binding and spices, M.S.G and onion are for taste.

The characteristic of the steamed fish balls before and after steaming are given in Table 4.4.5.

The product had a moisture content of 78.5% before steaming and it reduced to 68.58% on steaming. TPC before steaming

TABLE - 4.4.5

CHARACTERISTICS OF FISH BALLS TYPE II PREPARATION STEAMED

	Before steaming	After steaming
Moisture	78.5%	68.58%
T P C	4 x 10^3/g	3 x 10^2/g
Gel strength	250g/CM²	320g/CM²
pH	6.5	
Organoleptic qualities		
Flavour and taste		5
Colour and hue		4
Aroma		5
Texture		4
Proximate composition		
Moisture		68.58%
Carbohydrate		8.60%
Ash		2.04%
Fat		6.28%
Protein		14.50%
Calorific value		149 K calories

was 4 x 10^3/g which on steaming was reduced to 3 x 10^2/g. Gel strength initially was 250 g/CM^2 which on steaming increased to 320g/CM^2. pH of the product was 6.5.

Sensory scores were either very good or good for flavour, colour, aroma and texture.

Proximate composition analysis showed that the product had a carbohydrate content of 8.6%; fat content of 6.28% and protein content of 14.50%. The calorific value of the product worked out to be 149 K. calories. The product was comparatively of less energy value compared with the brine cooked fish balls which had a calorific value of 161 K. calories.

4.4.1.7 **Fish balls - Fried**

This preparation was used for canning. However, it was also amenable for freezing.

The processing details and ingredients employed are the same as in (P.D.4). The only difference was that this product was fried instead of steaming.

The characteristics of this product is presented in Table 4.4.6. The water content was 77% which on freezing was reduced to 60.87% TPC which was 4 x 10^3/g, reduced to NIL on freezing. Gel strength increased from 320 g/Cm^2 to 450 g/Cm^2 on freezing. pH was 6.9.

TABLE - 4.4.6

CHARACTERISTICS OF FISH BALLS (PREPARATION.II) FRIED

	Before frying	After frying
Moisture	77%	60.87%
T P C	4 x 10^3/g	Nil
Gel strength	320g/CM^2	450g/CM^2
pH	6.9	
Organoleptic qualities		
Flavour and taste		5
Colour and hue		5
Aroma		5
Texture		4
Proximate composition		
Moisture		60.87%
Carbohydrate		9.17%
Ash		2.54%
Fat		11.08%
Protein		16.34%
Calorific value		202 K calories

Organoleptically this product was more preferred by the panelists than the blanched or cooked ones. Nutritionally also this product had 202 K. calories.

4.4.1.8 **Fried product from Surimi**

The processing details and proportion of ingredients are given in (P.D.5). To obtain 150 kg. of finished product approximately 100 kg. of fish mince and 50 kg. of ingredients were employed. Out of the 50 kg. of ingredients, egg and cooked potato were used for ensuring proper binding. Chemical seasonings like sodium tripolyphosphate and sodium pyrophosphate were for sustaining the gel forming property of the product.

Vegetables and spices were added for obtaining the desired taste and flavour. Ingredient proportion and choice of ingredients were arrived at by conducting several formulation trials.

The characteristics of this fried product from 'Surimi' is given in Table 4.4.7. Moisture content, gel strength, TPC and pH were 77.88%, 390g//Cm^2, 4.3 x 10^4/g and 6.9 respectively. On freezing the values changed for moisture content to 65.33%. TPC after freezing was NIL, and gel strength increased to 433.25 g/Cm^2

Organoleptically the product was rated good. Nutritionally

TABLE - 4.4.7

CHARACTERISTICS OF THE FRIED PRODUCT DEVELOPED FROM SURIMI

	Before frying	After frying
Moisture	77.8%	65.33%
T P C	4.3×10^4/g	Nil
Gel strength	390g/CM²	433.25g/CM²
pH	6.9	
Organoleptic qualities		
Flavour and taste		4
Colour and hue		5
Aroma		5
Texture		5
Proximate composition		
Moisture		65.33%
Carbohydrate		11.43%
Ash		1.00%
Fat		10.08%
Protein		10.98%
Calorific value		177 K calories

also the product was maintaining good standards, its calorific value being 177 K. calories per 100 gm. Proximate composition of the product was 11.43% carbohydrate moisture content of 65.33%; fat 10.08%, protein 10.98%.

4.4.1.9 **Pasteurized product from 'Surimi'**

The processing details and proportion of ingredients used is given in (P.D.6) To obtain approximately 140 kg of finished product 100 kg fish mince and 40 kg of ingredients were used.

Out of the ingredients, starch, egg and cooked potato were used for obtaining the desired texture. Vegetables and green and dry spices were added for getting the desired taste, colour and aroma. Use of chemical seasonings like pyrophosphate and sodium tripolyphosphate were employed for sustaining the gel strength of the product.

The product characteristics are presented in Table 4.4.8 It gives the values before and after pasteurization. Before pasteurization moisture content was 74.76% and it remained the same even after pasteurization. TPC decreased from the count of 3.3×10^4/g before pasteurization to 1.2×10^2/g after pasteurization. pH was 6.88.

Sensory score was 4 (good) for flavour and taste. Only for its colour it scored 5.

Proximate composition of the product was analysed carbohydrate content, fat content and proteiin content were 11.39%;

TABLE - 4.4.8

A PASTEURIZED **PRODUCT FROM SURIMI**

	Before pasteurization	After pasteurization
Moisture	74.76%	74.76%
T P C	3.3×10^4/g	1.2×10^2/g
Gel strength	230 g/cm²	320 g/cm²
pH	6.88	
Organoleptic qualities		
Flavour and taste		4
Colour and hue		5
Aroma		4
Texture		4
Proximate composition		
Moisture		74.76%
Carbohydrate		11.39%
Ash		2.03%
Fat		1.01%
Protein		10.81%
Calorific value		97.89 K calories

1.01% and 10.81% respectively. Calorific value of the product was only 98 K calories, falling in the low calories foods category.

4.4.1.10 **Battered and breaded fish mince slices**

Processing details and composition of ingredients are given in (P.D.7). A total quantity of 56 kg. of finished product was obtained from 30 kg. of minced fish. The weight of ingredients was 26 kg.

The product characteristics before frying and after frying are given in Table 4.4.9. The moisture content was 80% in the raw product which was reduced to 65.33% on frying. TPC was 3 x 10^4/g. This was NIL after frying Gel strength increased from 358.6 g/Cm² to 459 g/Cm² on frying.

Organoleptic scores were comparatively less since there was lack of spices and hence the taste was bland.
Proximate composition of the product was carbohydrate 11.01%; fat 16.08% and protein of 16.09%. Calorific value of the product was 253 K calories.

4.4.1.11 **Fish Cakes**

Processing details and composition of ingredients of fish cakes are given in (P.D. 6). For 10 kg. mince, 4.5 kgs. ingredients were used. Starch and vegetable oil were the binding agents.

TABLE - 4.4.9

CHARACTERISTICS OF BATTERED AND BREADED FISH MINCE SLICES

	Before frying	After frying
Moisture	80%	65.33%
T P C	3×10^4/g	Nil
Gel strength	358.6g/cm²	459g/cm²
pH	6.9	
Organoleptic qualities		
Flavour and taste		4
Colour and hue		4
Aroma		4
Texture		4
Proximate composition		
Moisture		65.33%
Ash		1.08%
Fat		16.08%
Protein		16.09%
Calorific value		119.08K calories

The proportion of ingredients and the proper batter uptake was standardised through trials.

Characteristics of the Projects are given in Table 4.4.10.

Before freezing the product had a moisture content of 79% which was reduced to 69.14% on frying. Gel strength increased from 243 g/cm² to 320 g/cm² on frying TPC was 3 x 10^4/g which on frying reduced to NIL.

Sensory scores rating was good. Proximate composition analysis showed a fat content of 8.65%, protein content of 14.55% and carbohydrate content of 4.12% for the product. The calorific value was 152 K.calories/100 kg.

4.4.1.12 **Fish Kababs**

(P.D 9) gives the details of the recipe composition and processing.

Other than fish, bengal gram was the main ingredient of this product. Besides egg, bengal gram also helps in effecting the binding. All other ingredients are for taste improvement.

Chracteristics of this product is given in Table 4.4.11. Moisture content reduced from 54.54% to 44.63% on frying Similarly TPC was NIL in fried samples. Gel strength increased from 235 g/Cm² to 319.33g/Cm² on frying.

TABLE - 4.4.10

CHARACTERISTICS OF FISH CAKE

	Before frying	After frying
Moisture	79%	69.14%
T P C	3×10^4/g	Nil
Gel strength	243g/cm²	320g/cm²
pH	6	
Organoleptic qualities		
Flavour and taste		4
Colour and hue		5
Aroma		4
Texture		4
Proximate composition		
Moisture		69.14%
Ash		2.12%
Fat		8.65%
Protein		14.55%
Carbohydrate		4.12%
Calorific value		132.93K calories

TABLE - 4.4.11

CHARACTERISTICS OF FISH KABAB

	Before frying	After frying
Moisture	54.54%	44.63%
T P C	3.9×10^5/g	Nil
Gel strength	235g/cm²	319.33g/cm²
pH	6.5	
Organoleptic qualities		
Flavour and taste		5
Colour and hue		5
Aroma		4
Texture		4
Proximate composition		
Moisture		44.63%
Ash		3.15%
Fat		19.50%
Protein		16.35%
Carbohydrate		7.76%
Calorific value		272.94K calories

Organoleptically this was rated very good by the panelists. The proximate composition of the fish kabab showed carbohydrate, protein and fat contents as 7.76%, 16.35% and 19.50% respectively. The fried product had a calorific value of 272K. calories.

4.4.2 STORAGE STUDIES OF PRODUCTS

4.4.2.1 **Frozen storage studies**

4.4.2.1.1 Fish Burgers

Fish burgers prepared as per recipe P.D. 1 were frozen at -40¶C in a plate freezer and subsequently subjected to frozen storage studies by keeping them at -20¶C and -8¶C. Changes in chemical, bacteriological and organoleptic parameters were followed at intervals 2,6,10,14,18 and 22 weeks.

Table 4.4.12 gives the changes in TVBN, PV, FFA, organoleptic scores, TPC and gel strength of fish burger stored at -20°C.

The TVBN value was 6 mg/100 gm immediately after freezing which on frozen storage at -20°C gradually rose to 21 mg/100gm in 22 weeks.

Peroxide value which was only 2.5 milli equivalents initially rose to 10 milli equivalents in six weeks. However, on further storage the peroxide values rapidly declined which on 22nd

TABLE - 4.4.12

CHANGES IN CHEMICAL, BACTERIOLOGICAL AND ORGANOLEPTIC PARAMETERS IN FISH BURGERS DURING FREEZING AT -40°C AND STORAGE AT -20°C

Storage period in weeks	TVBN mg/100g	PV milli equivalent/kg.	FFA as % oleic acid	Organoleptic scores	TPC/g	Gel strength g/cm²
0	6	2.5	2.4	9	$5x10^4$/g	400
2	6.5	8.5	5.5	8	$5x10^3$g	348
6	12	10	7	6	$3x10^3$/g	239.33
10	14	3.5	5	5.5	$3.5x10^3$/g	200.12
14	14.5	3	4	5	$3.5x10^2$/g	182.44
18	19.5	1.5	4.5	5.5	$3x10^2$/g	161.31
22	21	1	3	4	$3x10^2$/g	153.33

week was only 1 milli equivalent per Kilogram.

The decline of the PV values could only be due to the decomposition of the lipid peroxide to component aldehydes or ketones. The free fatty acid content which was 2.4 in the beginning though showed sliight increase initially later started to show poorer values. However, this fluctuations in the values of FFA appeared to be not very significant.

Organoleptic evaluation of the fish burger during the frozen storage studies were made by a panel of six persons on a 9 point hedonic scale as detailed in Chapter-I.

In this scale the fish burger immediately after freezing was rated as having a score of 9 indicating 'like extremely' On frozen storage the sensory score declined progressively. After two week the score was only 8. After six week 6, and by 14 weeks the score reached the point of 5. However, the score reached only 4 at the point of rejection at the end of 22 weeks of frozen storage. The total bacterial count of the fish burger after freezing at -40°C was 5×10^4 /g which on frozen storage at -20°C showed gradual decrease. Until at the end of 22 weeks frozen storage the TPC was only 3×10^2/gm. E. coli, faecal streptococci and coagulase positive staphylococci were absent.

The gel strength of the fish burger was initially 300 gm/Cm². On frozen storage at -20°C after about 2 weeks storage considerable decrease in the gel strength was noted. After six weeks it sscame down to 239 g/Cm² which by the end of 22 weeks of storage was only 153 g/cm². Gel strength is an indication of the binding properties of the product.

Nair et al (1982) prepared fish fingers from threadfin bream and mackerel. The protein content of their products had a higher value of above 30%. They had used monosodium glutamate and studied the storage characteristics of six months at frozen storage. They had not incorporated any spices in the product.

No study has been reported on the preparation of fish burgers from India so far. From Canada, Blackwood (1974) had reported the preparation of fish burger in which he recommended that at least 60% of the weight of the fish burger ought to be fish muscle.

On the fish burger reported in this study 72% of the product was fish meat itself.

Herborg (1974) had reported the preparation of fish burgers from cat fish. He suggested that red meat of fish was not suitable for fish burgers. But in the present study the lean meat from priyacanthus had been found to give a very palatable product when fat was incorporated as an ingredient.

Baker (1977) had suggested that soy protein fibre added to fish balls would provide a fibrous texture and bite to the finished product, bread crumbs, would improve the absorptive qualities and tendering effect.

In the present study bread crumbs and raw eggs had been incorporated in the recipe for fish balls for necessary textural

improvements.

Table 4.4.13 gives the changes in TVBN, PV, FFA, Organoleptic scores, TPC and gel strength of the fish burger frozen at --40°C and stored at -8°C.

The TVBN value was 6 mg/100 mg immediately after freezing, which on frozen storage at -8°C gradually rose to 2.5 mg/100g in 22 weeks.

Peroxide value which was only 2.5 milli equivalent/kg initially rose to 10.1 milli equivalent/kg in six weeks. However, on further storage the peroxide value rapidly decreased which on 22nd week was only 2 milli equivalent per Kilogram.

Free fatty acid value which was 2.4 immediately after freezing showed slight increase initially and then decreased on further storage. The changes in FFA values were very insignificant.

Organoleptic scores however, showed drastic changes. The level of rejection was reached on the 6th week. The scores reduced from 9 to 3 during this period indicating that the product became very rapidly unacceptable during frozen storage at -8°C.

Though other quality indices remained within limits, the organoleptic score showed that the samples stored at -8°C had only less than half the shelf life when compared to the samples

TABLE - 4.4.13

CHANGES IN CHEMICAL, BACTERIOLOGICAL AND ORGANOLEPTIC PARAMETERS IN FISH BURGERS FROZEN AT -40°C AND STORED AT -8°C

Storage period in weeks	TVBN mg/100g	PV milli equivalent/kg.	FFA as % oleic acid	Organoleptic scores	TPC/g	Gel strength g/CM²
0	6	2.5	2.4	9	$5x10^7$/g	400
2	12.5	10.11	2.7	5	$4x10^3$/g	284.34
6	16	3.5	1.75	3	$4x10^2$/g	159.34
10	19	3.5	1.92	3	$3x10^2$/g	142.39
14	20.5	2.5	1.9	2	$3x10^2$/g	130.33
18	24.5	2	1.0	2	$2.3x10^2$/g	129.38
22	25	2	1.5	2	$2.3x10^2$/g	129.00

stored at -20°C where the rejection level was reached only on the 22nd week.

Gel strength drastically decreased initially upto six weeks to 159.34/g Cm^2 from 300 g/Cm^2. Further reduction was gradual. But by the sixth week itself the product started disintegrating on freezing.

4.4.2.1.2 Fish Cakes

Table 4.4.14 gives the changes in PV, FFA, Organoleptic scores, TPC and gel strength of the fish cakes stored at -20°C.

Peroxide value which was 8.7 milli equivalents/kg initially decreased to 7 milli equivalents/kg. by the sixth week and then reduced gradually to 4 milli equivalent/Kg on 22nd week.

Free fatty acids (FFA) progressively decreased from 3.03 to 1.50 during 22 weeks frozen storage.

TPC was initially 5×10^4/g, which gradually declined to 3×10^2/g frozen storage for 22 weeks at -22°C.

Sensory scores showed gradual reduction from 9.5 to 4 during the storage period. The level of rejection was reached on the 22nd week.

Gel strength decreased from an initial value of 303 g/Cm^2 to 153.33 g/Cm^2 in 22 weeks of storage.

On frozen storage at -20°C after about 2 weeks storage considerable decrease in the gel strength was noted. After six weeks it came down to 239 g/Cm² which by the end of 22 weeks of storage was only 153 g/cm². Gel strength is an indication of the binding properties of the product. A decrease in gel strength indicated the increasing chances of the product getting disintegrated during cooking or frying. In the case of fish burger also even though organoleptically and chemically the product was good after 10 weeks of storage, the product developed cracking on frying after 8-10 weeks of storage. Much work has not been reported on this development of value added products from fish mince.

Reddy et al (1990) had reported the details of new recipes for the preparation of fish fingers from the fish mince from croakers. The product was reported to have an oriental taste, the spices having been incorporated in both fish and the batter. Usually the batter alone had the spicy taste while the inner fish mince part of the fish finger usually was bland in taste. But in the preparation reported by Reddy et al had the advantage of both the fish mince as well on the batter having the spicy taste.

The protein content of their preparation was higher at 22.4% while the battered and breaded mince slice reported in this study had only a lower protein content namely 16%.

TABLE - 4.4.14

CHANGES IN CHEMICAL, BACTERIOLOGICAL AND ORGANOLEPTIC PARAMETERS OF FISH CAKES DURING FREEZING AT -40°C AND STORAGE AT -20°C

Storage periods in weeks	PV milli equiva-lent	FFA % oleic acid	Organoleptic scores	TPC	Gel strength g/cm²
0	8.7	3.03	9	$5x10^4$/g	303.00
2	6.84	2.03	9	$5x10^3$/g	298.00
6	7.0	2.76	8.5	$3x10^3$/g	239.33
10	4.56	2.82	4.5	$2.5x10^3$/g	200.12
14	4.50	1.72	6	$3.5x10^2$/g	182.44
18	4.50	1.60	5	$3.5x10^2$/g	161.31
22	4.00	1.50	4	$3x10^2$/g	153.33

Table 4.4.19 shows the changes in PV, FFA, Gel strength, TPC and organoleptic scores of fish cakes frozen at -40¶C and stored at -8°C.

The PV immediately after freezing was 8.9 milli equivalent/Kg. It gradually decreased on frozen at -8°C to 1.59 milli equivalent Kg. in 22 weeks.

FFA gradually decreased from an initial value of 2.08 to 1.23 at the end of 22 weeks. TPC decreased from 6 x 10^4/gm to 2 x 10^4/gm during the same period of storage.

Reduction in gel strength was drastic. The value reduced from 303 g/Cm² to 180 g/Cm² in 10 weeks. The same value was reached in fish cakes stored at -20°C only by the 14th week.

Oranoleptically the level of rejection was reached on the 10th week of storage at -8°C. Hence it can be seen that the fish cakes stored at -8°C had only half the shelf life when compared to those stored at -20°C.

Storage studies of Canned Fish Balls

Fried fish balls prepared as per (methodology No. 4.3.3.1.3) were canned in three different media namely in brine, vegetable oil and tomato sauce.

TABLE - 4.4.15

CHANGES IN CHEMICAL, BACTERIOLOGICAL AND ORGANOLEPTIC PARAMETERS OF FISH CAKES DURING FREEZING AT -40°C AND STORAGE AT -8°C

Storage periods in weeks	PV milli equivalent/ Kg.	FFA % Olcic Acid	Organole- ptic scores	TPC	Gel strength
0	8.9	2.08	7.5	6×10^4/g	303 g/Cm²
2	8.00	1.23	7.5	5×10^3/g	208
6	4.54	1.08	6	3×10^3/g	182
10	2.59	1.92	4	2.5×10^3/g	180
14	2.00	1.00	3	2.5×10^3/g	161
18	1.59	1.11	3	2×10^4/g	149
22	1.59	1.23	2	2×10^4/g	121

PV Millimoles oxygen/100 extracted oil

FFA as % Oleic Acid

Organoleptic Scores on 9 point hedoine scale

TPC Total bacterial count/g

Gel strength g/Cm²

TABLE - 4.4.16

CHANGES IN PHYSICAL AND ORGANOLEPTIC CHARACTERISTICS OF CANNED FISH BALLS IN BRINE DURING STORAGE AT RT (28± 4°C)

Can used: ½ Hansa Aluminium can (SR. lacquered)

Details	Period of observation			
	After incubation for 15 days	After 3 months	After 6 months	After one year
1. Can condition	Normal	Normal	Normal	Normal
2. Std. Net Wt.	200 gm	200 gm	200 gm	200 gm
3. Std. Solid Wt.	145 gm	145 gm	145 gm	145 gm
4. Gross Wt.	228 "	227	228	227
5. Empty Can Wt.	27	27	27	27
6. Solid + can Wt.	172	170	174	171
7. Water/Liquid(Ml)	56 ml.	57 ml.	54 ml.	56 ml.
8. Solid wt.	145 gm.	143 gm.	147 gm.	144 gm.
9. +Solid wt.	+0 "	-2	+2 "	-1
10. Appearance	A+	A	A	A
11. Colour	B+	B+	B	C+
12. Flavour	B+	B	B	C+
13. Texture	A+	A+	A	A
14. No.of pieces	16	17	15	15
15. pH	6.2	6.1	6.1	6.1
16. Sulphide blackening	--	--	--	--
17. Saltiness	Normal	Normal	Normal	Normal
18. Colour of the brine	White	White	White	White
19. Turbidity	Turbid	Turbid	Turbid	Turbid
20. Brine strength	2.10	2.00	2.00	1.98
Overall score	B+	B+	B	B

A+ Excellent A Very Good B+ Good

B Fair C+ Average

Mesophilic and thermophilic aerobes and anaerobes were absent.

The canned fried fish balls were stored at the ambient temperature (RT), 28 ± 4°C) for a period of one year. The cans were examined after 15 days, 3 months, six months and one year for changes in physical and organoleptic characteristics. The results are summarised in Tables 4.4.16, 4.4.17 and 4.4.18 respectively for fried fish balls in brine, oil and tomato sauce.

The fish balls canned in all the three ways remained in acceptable condition for one year based on sensory qualities. In the case of fish balls canned in brine turbidity was observed even after the 15 days of storage. But it did not affect the sensory qualities of the canned fish ball. There was no disintegration.

The pH of contents were consistently on the acidic side in all the cases. In the case of brine the pH was always above (6.1.-6.2). In the cans packed in oil pH value varied from 5.9 to 6 and those packed in tomato sauce 4.8 - 4.9.

The appearance, colour, flavour and texture in all the three cases were above the acceptable levels. Also the conditions inside the can in all the three types were normal.

On microbiological examination of the contents mesophilic and thermophilic aerobes and anaerobes were always absent.

TABLE - 4.4.17

CHANGES IN PHYSICAL AND ORGANOLEPTIC CHARACTERISTICS OF CANNED FISH BALLS IN OIL DURING STORAGE AT RT (28±4°C)

Can used : ½ Hansa Aluminium can (SR. lacquered)

Details	Period of observation			
	After incubation for 15 days	After 3 months	After 6 months	After one year
1. Can condition	Normal	Normal	Normal	Normal
2. Std. Net. Wt.	200 gm	200 gm	200 gm	200 gm
3. Std. Solid Wt.	140 gm	140 gm	140 gm	140 gm
4. Gross Wt.	220	218	221	223 "
5. Empty Can Wt.	27	27	27	27
6. Solid can wt.	168	165	167	170
7. Water/Liquid	0.2/61 ml	0.3/62 ml	0.1/61 ml	0.3/60 ml
8. Solid Wt.	141 gm.	138 gm	140 gm	143 gm.
9. Solid wt.	1 "	-2	0 "	3
10. Appearance	A+	A+	A+	A+
11. Colour	A	A	A	A
12. Flavour	A	A	A	B+
13. Texture	B+	B+	B+	B+
14. No.of pieces	5	4	4	4
15. pH	6.0	5.9	5.9	5.9
16. Suphide blackening	--	--	--	--
17. Saltiness	Normal	Normal	Normal	Normal
18. Colour of Oil	Yellow	Deep yellow	Deep yellow	Deep yellow
19. Turbidity	Transparent	Transparent	Transparent	Transparent
20. Brine strength	-	--	--	--
Overall score	A	A	A	A

A+ Excellent; A Very Good B+ Good

Mesophilic and thermophilic aerobes and anaerobes were absent

TABLE 4.4.18

CHANGES IN PHYSICAL AND ORGANOLEPTIC CHARACTERISTICS OF CANNED FISH BALLS IN TOMATO SAUCE DURING STORAGE AT RT (28±4°C)

Can used : ½ Hansa Aluminium can (SR. lacquered)

Details	Period of observation After incubation for 15 days	After 3 months	After 6 months	After one year
1. Can condition	Normal	Normal	Normal	Normal
2. Std. Net.Wt.	200 gm	200 gm	200 gm	200 gm
3. Std. Solid Wt.	145 gm	145 gm	145 gm	145 gm
4. Gross Wt.	230	228	225	226
5. Empty Can Wt.	27	27	27	27
6. Solid + Can Wt.	180	180	175	177
7. Water/Liquid	50 ml	51 ml	50 ml	49 ml
8. Net Wt.	153 gm	150 gm.	148 gm.	150 gm.
9. Solid Wt.	153 "	150	148	150 "
10. +Net Wt.	+8	+5	+3	+5
11. + Solid wt.	+8	+5	+3	+5
12. Appearance	A+	A+	A+	A+
13. Colour	A	A	A	B+
14. Flavour	A	A	A	A
15. Texture	A+	A+	A	A
16. No.of pieces	15	18	16	15
17. pH	4.8	4.8	4.9	4.9
18. Sulphide blackening	--	--	--	--
19. Saltiness	Normal	Normal	Normal	Normal
20. Colour of Tomato sauce	Reddish	Reddish	Reddish	Reddish
21. Turbidity	Normal	Normal	Normal	Normal
22. Brine strength	--	--	--	--
Overall score	A	A	A	A

A+ Excellent A Very Good; B+ Good

Mosophilic and thermophilic aerobes and anaerobes were absent

Based on organoleptic characteristics the fried fish balls packed in tomato sauce had a better score compared with the other two.

The fried fish balls packed in vegetable oil stood next in sensory qualities and those packed in brine had the lowest organoleptic rating. However, all the three types of products were organoleptically acceptable throughout the one year storage period at ambient temperature.

In all the frozen products E. coli, faecal streptococci and coagulase positive staphylococci were absent.

Canned fish paste

Processing details and ingredients composition are given in (P.D. 8).

Fish spread made from priacanthus had a light brown colour, good odour and taste. This compares with the products made from white bait. Consistency of the product was also excellent without any traces of smaller particles of scales and bones.

The canned spread were subjected to shelf life analysis for a period of one year. Cans were subjected to cut open tests after 15 days, after 3 months, after 6 months and after one year.

Table 4.4.10 shows the observations over a period of one

TABLE - 4.4.19

CHANGES IN THE PHYSICAL AND ORGANOLEPTIC CHARACTERISTICS OF THE CANNED FISH SPREAD DURING STORAGE AT RT

PRODUCT: CANNED FISH SPREAD

Details	Period of observation			
	After 15 days	After 3 months	After 6 months	After one year
1. Can condition	Normal	Normal	Normal	Normal
2. Std. Net Wt.	200 gm	200 gm	200 gm	200 gm
3. Std. Solid wt.	145 gm	145 gm	145 gm	145 gm
4. Gross wt.	230 gm	228 gm	225 gm	226 gm
5. Empty can wt.	27	27	27	27
6. Solid + Can wt.	230 gm	228 gm	228 gm	227 gm
7. Net wt.	200 gm	200 gm	200 gm	200 gm
8. Appearance	A+	A+	B+	B
9. Colour	A	A	A	A
10. Flavour	A	A	B	B
11. Texture	A+	A+	B+	B
12. pH	6	6	6.2	6.1
13. Sulphide blackening	Nil	Nil	Nil	Nil
14. Saltiness	Normal	Normal	Normal	Normal
15. Consistency	Butter like	Butter like	Separation of V. oil. Hence dilute consistency.	

Bacteriological examination results

Mesophilic and thermophilic- aerobes and anaerobes were absent

year. Upto till the sixth month the organoleptic qualities were rated excellent or very good, after which it was only good or fair (B or B+). At the end of six month original butter like consistency was lost. There was separation of vegetable oil and hence dilute consistency was observed. No sulphide blackening was observed in any of the cans. pH was also consistent.

The canned paste were anlysed for mesophilic and thermophilic aerobes and anaerobes and nil results were obtained.

On the whole the product was found good and were produced on a semi commercial basis at Integrated Fisheries Project and sold through its marketing outlets and the product mustered appreciable clienteele.

Begueras (1985) had prepared fish balls from unwashed and washed mince from sprats and fish balls prepared from the same and its sensory and storage characteristics in relation to washing had been studied. Washing showed marked effect in extending the shelf life. But unwashed samples showed better sensory properties.

Bremner (1974) had reported a recipe for fish cakes which consisted of fish meat, quinna (locally grown seed in Peru) Pork fat, salt and water. A totally different formulation was used by the present author for preparation of fish cake from

priyacanthus in which spices, salt, vegetable oil were added and mixed with the fish mince to prepare the fish cakes.

Del valle (1968) prepared salted and pressed fish cake from fish mince and studied their proximate composition, protein quality and microbial count during storage.

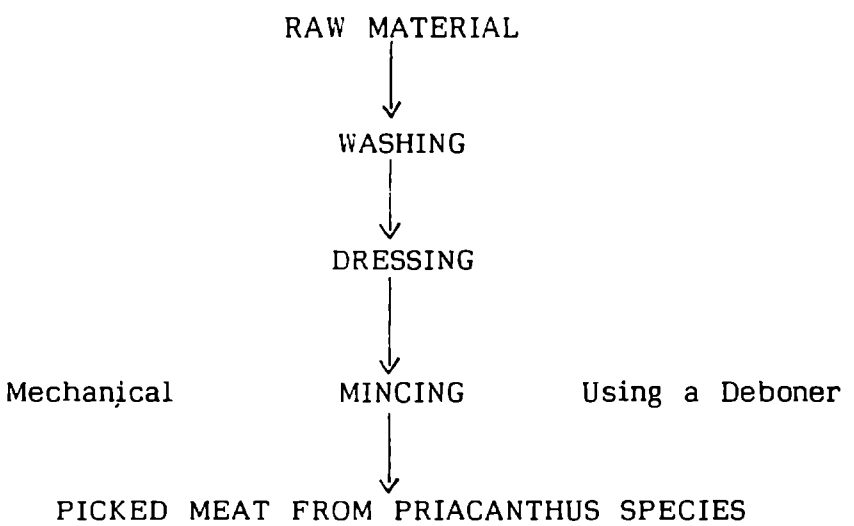
Moreland in (1974) studied breaded fish cakes prepared from fish mince. He has recommended that the fish tissue should be mixed with other texture improving ingredients to obtain a good quality fish cake.

Wood et al (1985) had reported the development of fish cake from mackerel, silver belly and sardine. The taste panel evaluation revealed the suitability of mackerel for producing good quality fish cakes. Fish cakes of silver belly and sardine were not acceptable by the panelists. But the fish cake made in the present study using mince from priyacanthus species had been judged as very good by taste panelists.

Poulter & Trevino (1983) have studied about canned fish paste from five different species of fish commonly found in shrimp by catch from the Gulf of California. They observed that major differences in quality existed particularly with respect to colour for those pastes prepared from certain fish species. However, the product formulation that is mentioned in this study yielded a product of very good sensory properties.

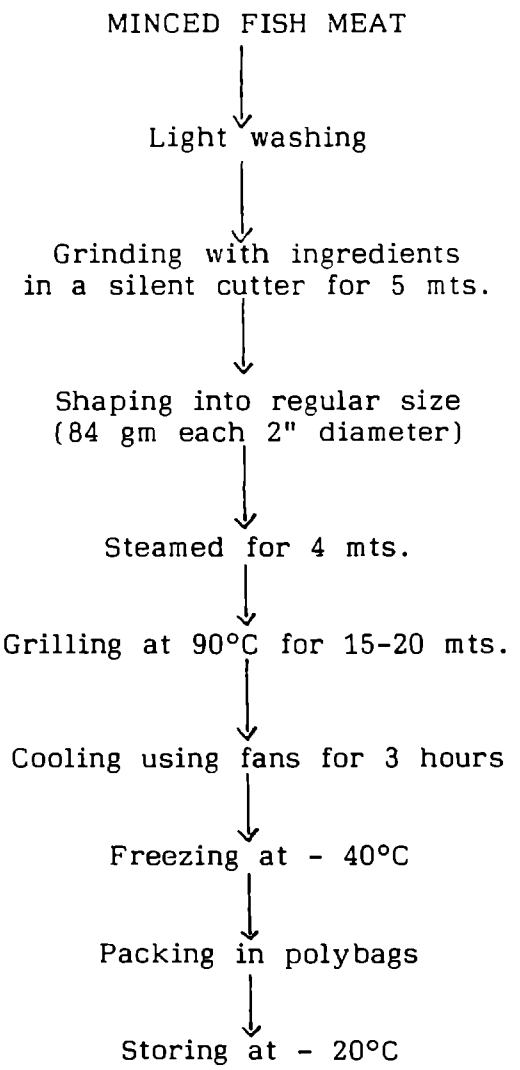
PROCESS FLOW CHART - I

FLOW SHEET FOR MINCED MEAT FROM PRIACANTHUS SPECIES



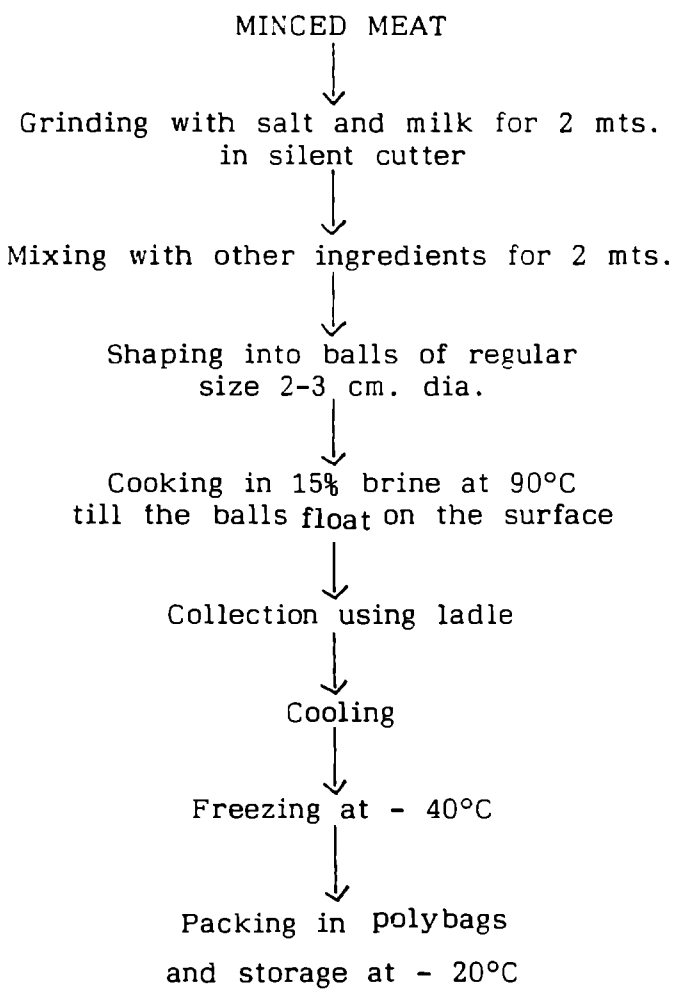
PROCESS FLOW CHART-II

F I S H B U R G E R



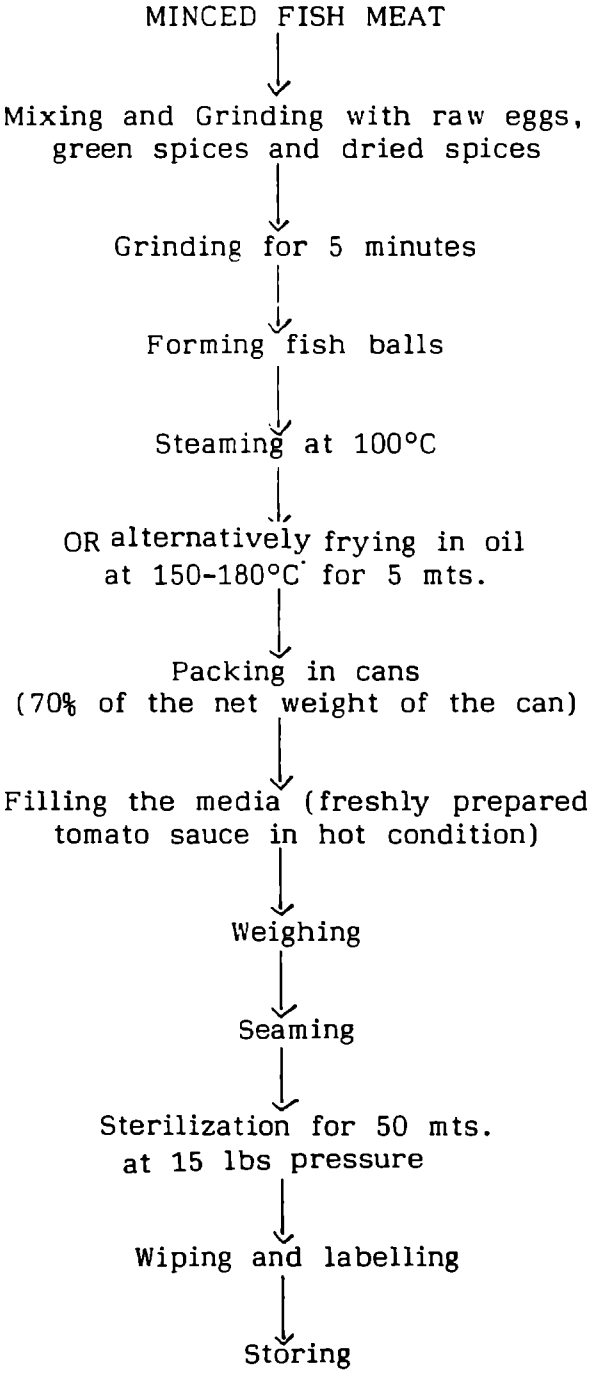
PROCESS FLOW CHART-III

FROZEN FISH BALL



PROCESS FLOW CHART-IV

CANNED FISH BALLS



PROCESS FLOW CHART-V

PREPARATION OF SURIMI & PRODUCTS

RAW MATERIAL (FISH)
↓
Washing
↓
Dressing (Butterfly style)
↓
Meat picking
↓
Washing in chilled water
↓
Mixing with additives
↓
(Polyphophate (0.15-0.3%) Sugar(5-6%)
↓
Mixing in silent cutter for 4-5 Mts.
↓
Surimi packed in trays
↓
Freezing at -40°C
↓
Stored - 20°C

Fried products from Surimi

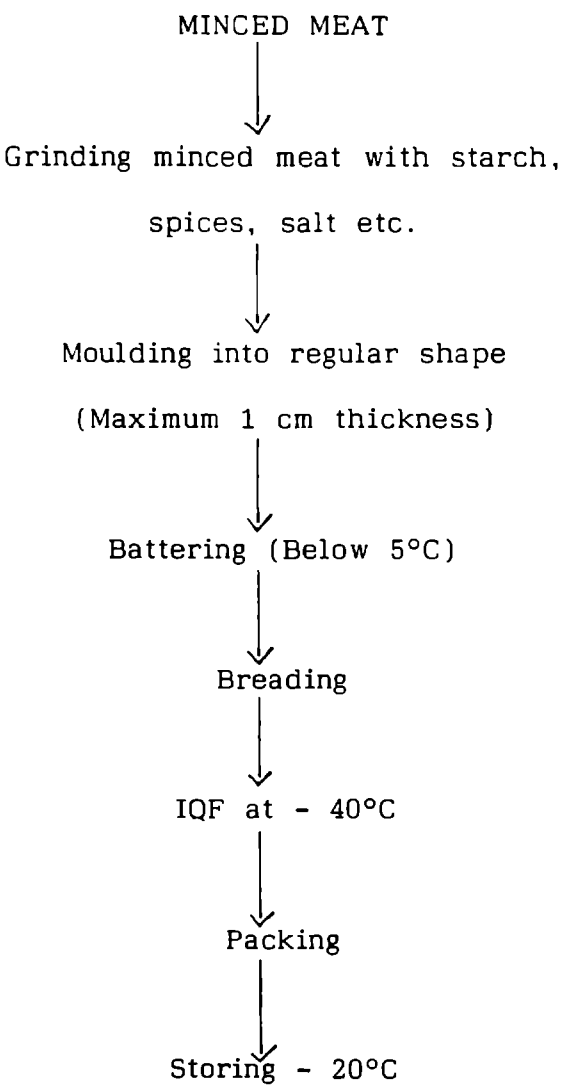
Adding cut vegetables, dried spices, chopped green vegetables
↓
Grinding for 3 Mts.
↓
Shaping
↓
Frying in oil at 150°C

Pasteurized products

Thawing Surimi
↓
Adding 3% Salt
↓
Grinding with cut vegetables
↓
Filling in cylindrical polybags and tying at both ends
↓
Dipping in water at 90°C for 40 mts.
↓
Chilled storage

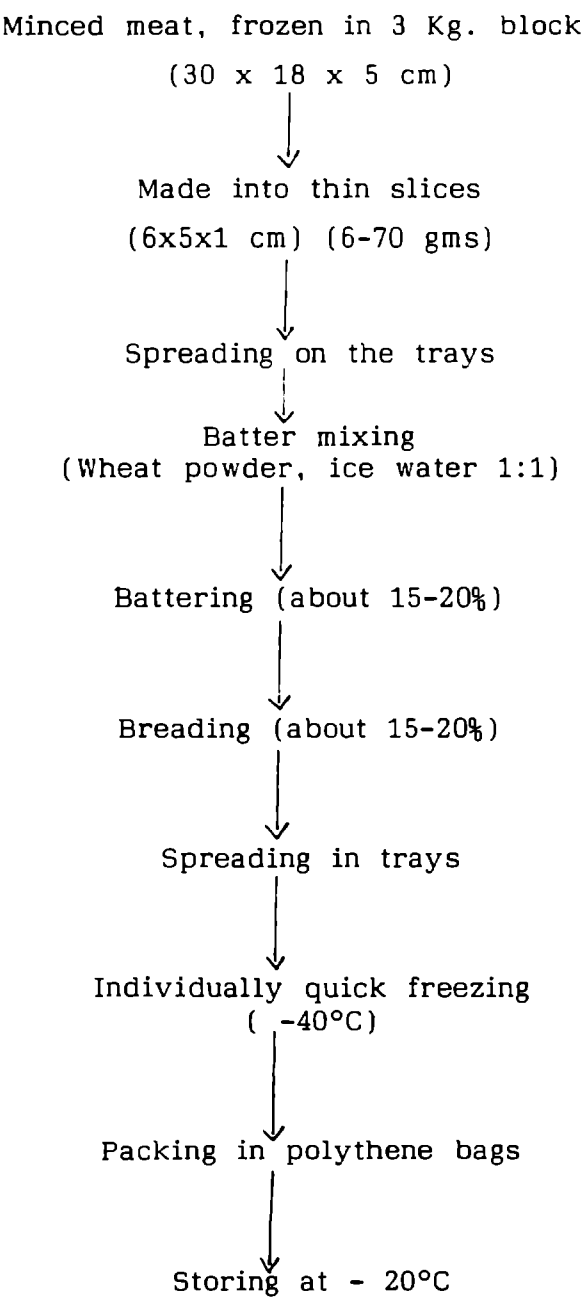
PROCESS FLOW CHART-VI

FISH CAKE



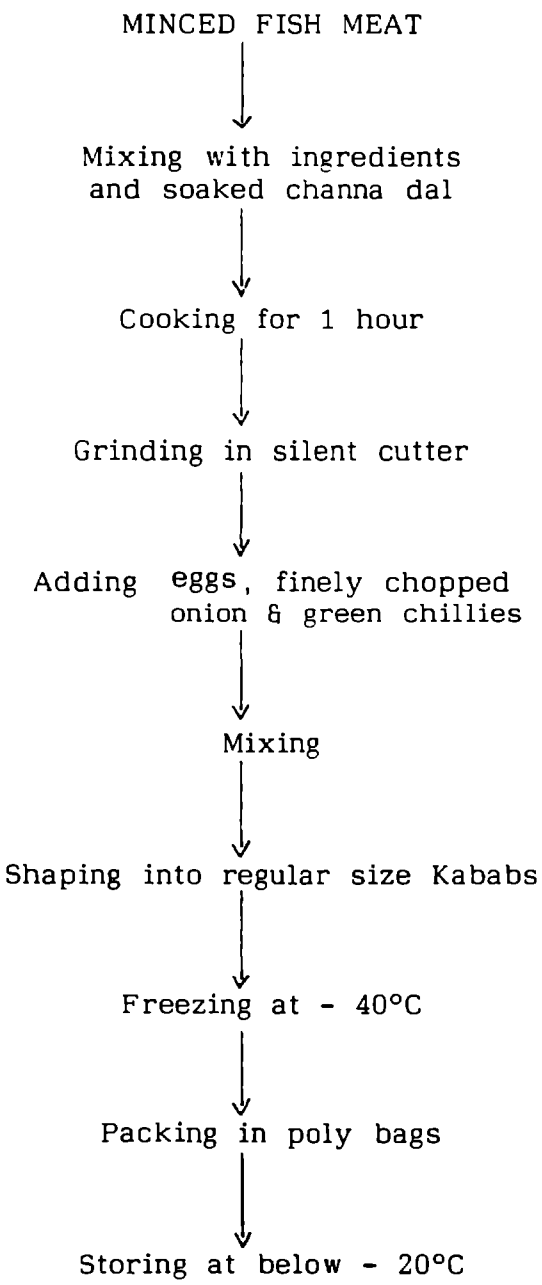
PROCESS FLOW CHART-VII

BATTERED AND BREADED MINCE SLICES



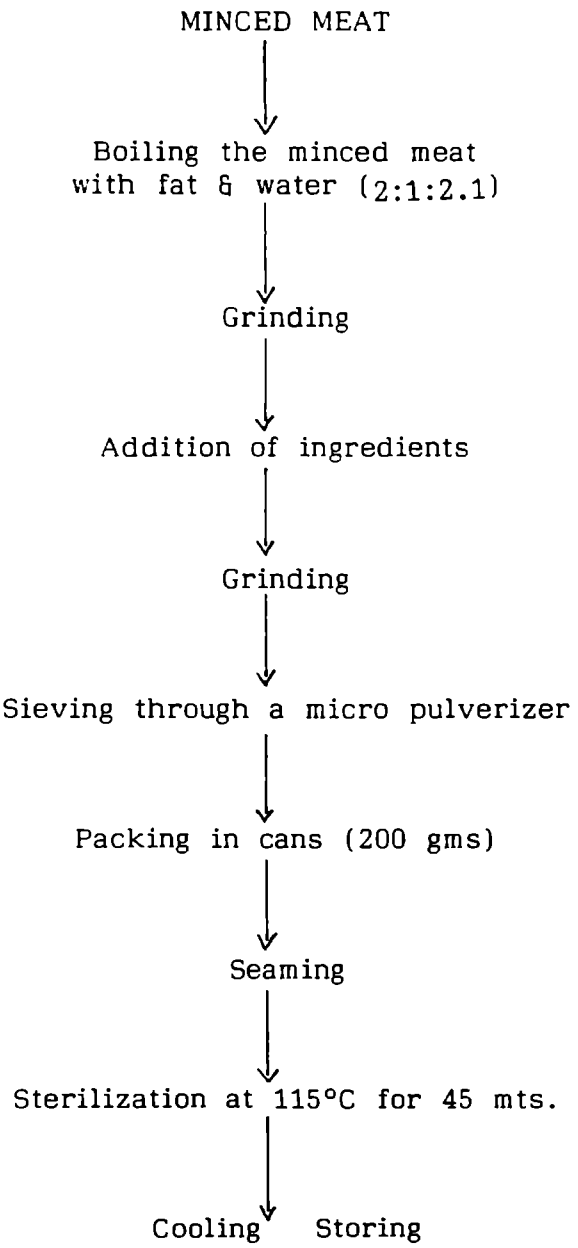
PROCESS FLOW CHART-VIII

FISH KABAB



PROCESS FLOW CHART-IX

CANNED FISH SPREAD



PRODUCT DETAIL - 1

FISH BURGER

1. Raw fish weight	225 kg.
2. Fish minced weight	100 kg.
3. 1st grinding time	5 Mts.
4. 2nd grinding time	Nil
5. 3rd grinding time	Nil
6. Temperature at final stage	10°C

Weight of ingredients to fish meat	40.200 kg. (28.17%)
Refined salt	1.800 kg.
Starch	1.000
Sugar	1.000
Chemical seasonings:	
Na Polyphosphate	0.200 kg.
White of egg	5.000
Pepper	0.100
Bread crumbs	6.000
Milk powder	5.000
Mono sodium Glutamate	0.900
Onion	10.800
Fat	2.000
Butter	2.000
Garlic	0.400
Spices	2.000 "
Oil	2.000

PRODUCT DETAIL - 2

FISH BALL - TYPE -1

1. Raw fish weight	22 kg.
2. Fish minced weight	10
3. 1st grinding time	10 Mts.
4. 2nd grinding time	Nil
5. 3rd grinding time	Nil
6. Temperature at Final stage	10°C

Weight of ingredients to fish meat	8.400 kg.(45.6%)
Refined salt	0.300 kg.
Starch (Wheat)	1.000
Water (ice)	0.500 "
Milk	6.00 litre
Vegetable fat	0.400 kg.
Pepper powder	0.200

PRODUCT DETAIL - 3

FISH BALL - TYPE-II

1. Raw fish weight	225 kg.
2. Fish minced weight	100
3. 1st grinding time	5 Mts.
4. 2nd grinding time	Nil
5. 3rd grinding time	Nil
6. Temperature at Final stage	10°C

Weight of ingredient to fish meat	30.500 kg.
Refined salt	0.500 kg.
Onion (slightly fried)	20.000
Raw egg	2.500
Bread powder	3.000
M.S.G	0.500
Spices	4.000

PRODUCT DETAIL - 4

FRIED PRODUCT FROM SURIMI

1. Raw fish weight	225 kg.
2. Fish minced weight	100
3. 1st grinding time	30 Mts.
4. 2nd grinding time	1 Mt.
5. 3rd grinding time	2 mts.
6. Temperature at final stage	10°C

Weight of ingredient to fish meat

Starch	2.400 kg
Sugar	5.200
Sodium tripolyphosphate } 1 1 Sodium Pyro Phosphate }	0.03
Water	12.00Kg(Ice)
Carrot	5.000 "
Onion	5.000
Cooked potato	5.000 "
Green chillies	1.200
Pepper powder	0.200
Green ginger	1.200
Garlic	0.600

PRODUCT DETAILS - 5

A PASTEURISED PRODUCT FROM SURIMI

1. Raw fish weight	225 kg.
2. Fish minced weight	100
3. 1st grinding time	25 Mts.
4. 2nd grinding time	nil
5. 3rd grinding time	nil
6. Temperature at final stage	10°C

Weight of ingredients to fish meat

1. Chemical seasoning	0.030 kg.
2. Water	12 kg.(Ice)
3. Carrot	5
4. Onion	5
5. Egg	10 Nos.
6. Cooked Potato	5 Kg.
7. Green chillies	1.2 "
8. Pepper powder	0.600 "
9. Green ginger	1.200
10. Garlic	0.600 "

PRODUCT DETAILS - 6

MINCED FISH CAKE

1. Raw fish weight	32 Kg.
2. Fish minced weight	10 kg.
3. 1st grinding time	8-10 Mts.
4. 2nd grinding time	Nil
5. 3rd grinding time	Nil
6. Temperature at final stage	10°C

Weight of ingredients to fish meat — 4.550 kg. (31.07%)

1. Refined salt	0.150 kg.
2. Starch	1.250
3. Vegetable oil	0.700
4. Pepper powder	0.050
5. Garlic	0.050
6. Mint leaves	0.050
7. Green chillies	0.100
8. Batter	1.000
9. Bread	1.200

PRODUCT DETAILS - 7

BATTERED AND BREADED MINCED FISH SLICE

1. Raw fish weight	86 kg.
2. Fish minced weight	30
3. 1st grinding time	nil
4. 2nd grinding time	
5. 3rd grinding time	
6. Temperature at final stage	10°C

Weight of ingredients to fish meat	26.000 kg. (46.66%)
1. Refined salt	1.500 kg.
2. Water	8.500 kg.
3. Spices	0.500
4. Wheat powder	8.000
5. Bread powder	8.000

PRODUCT DETAILS - 8

CANNED MINCED FISH SPREAD

1. Raw fish weight	65 kg.
2. Fish minced weight	25 kg.
3. 1st grinding time	7 Mts.
4. 2nd grinding time	Nil
5. 3rd grinding time	Nil
6. Temperature at at final stage	10°C

Weight of ingredients to fish meat — 23.840 kg.(48.7%)

1. Refined salt	0.750 kg.
2. Water	10.500
3. Fat	12.000
4. Garlic	0.360
5. White pepper powder	0. 180
6. Mint leaves	0.050 "

PRODUCT DETAIL - 9

FISH KABABS

1.	Raw fish weight	3.5 kg.
2.	Fish minced weight	1.0 kg.
3.	1st grinding time	30 seconds
4.	2nd grinding time	1 Minute
5.	3rd grinding time	1 Minute
6.	Temperature at final stage	10°C

Weight of ingredients to Fish Weight	: 400 gm(28.5%)
Channa Dal	250 gms
Garlic	20 pearls
Cardamon	2 Nos.
One inch piece cinnamon	10 Nos.
Eggs	2 Nos.
Jeera	1 Table spoon
Ginger	2 inch piece
Onion (big)	2 Nos.
Pepper Corns	8 Nos.
Red chillies	10 Nos.
Gloves	8 Nos.
Salt	To taste
	400 gms)

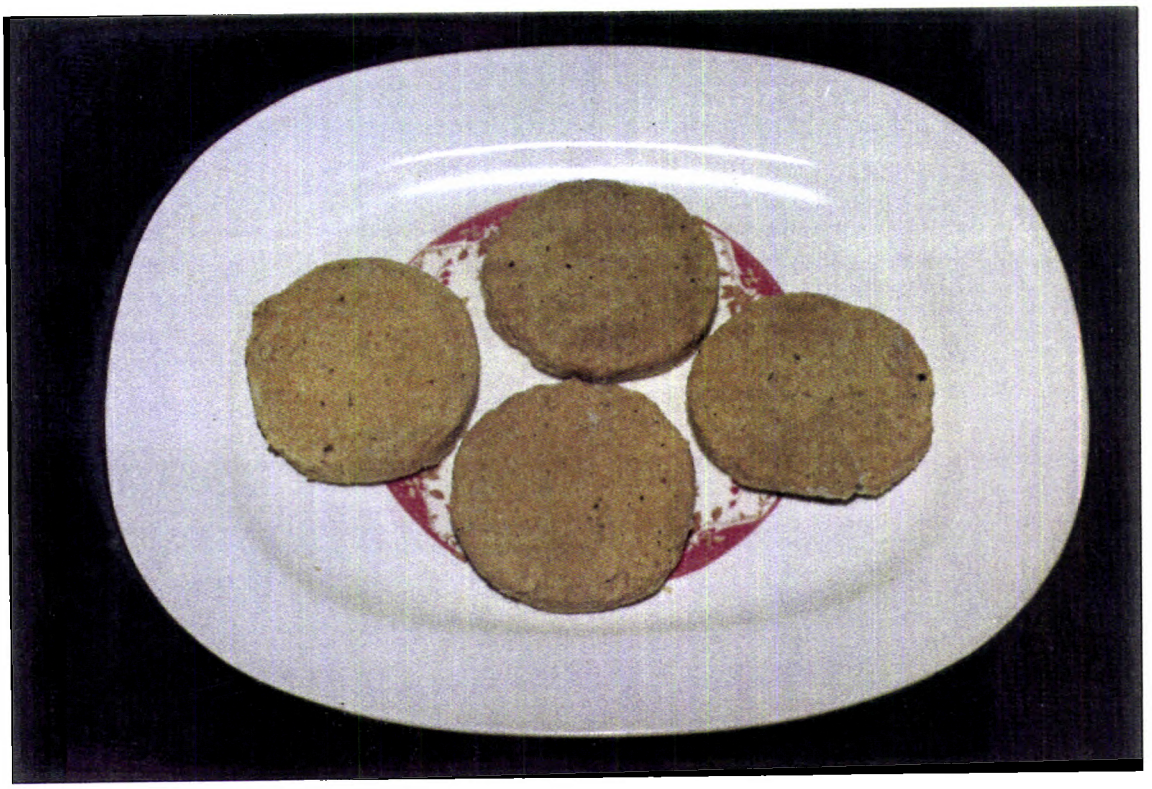


PLATE - A FISH BURGER (BEFORE FRYING)

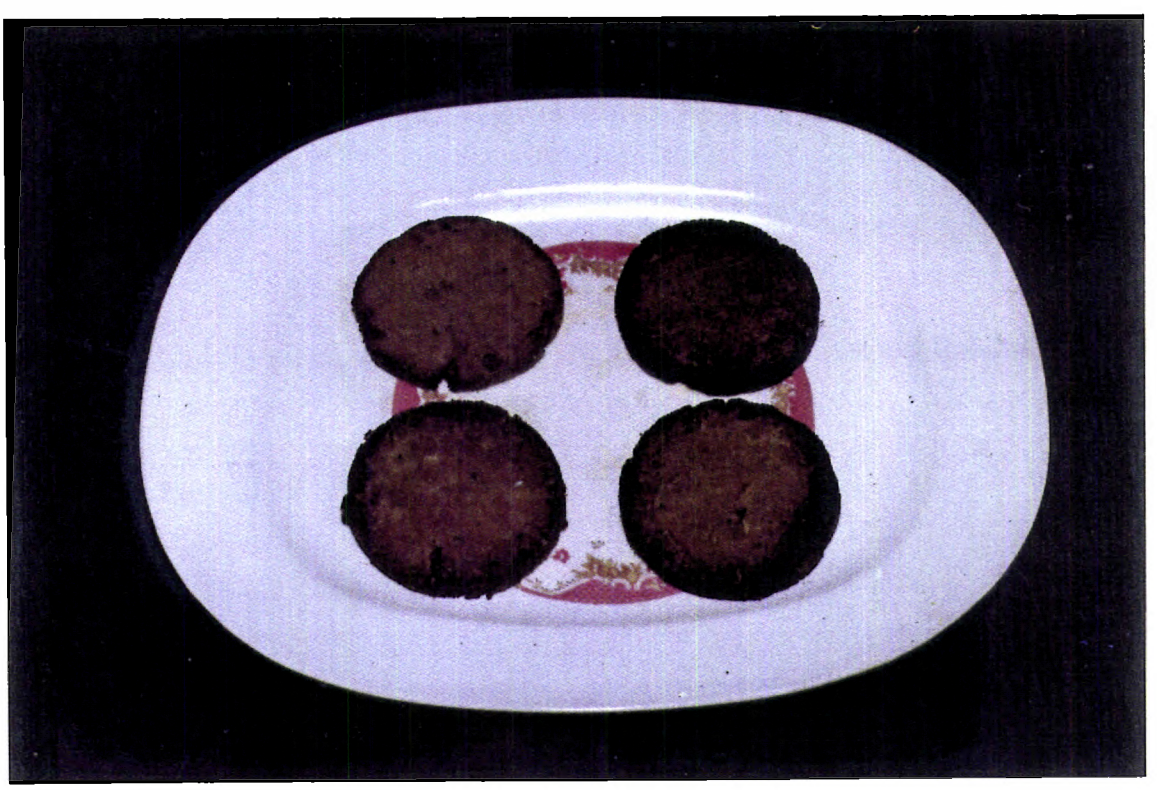


PLATE - B FISH BURGER (AFTER FRYING)

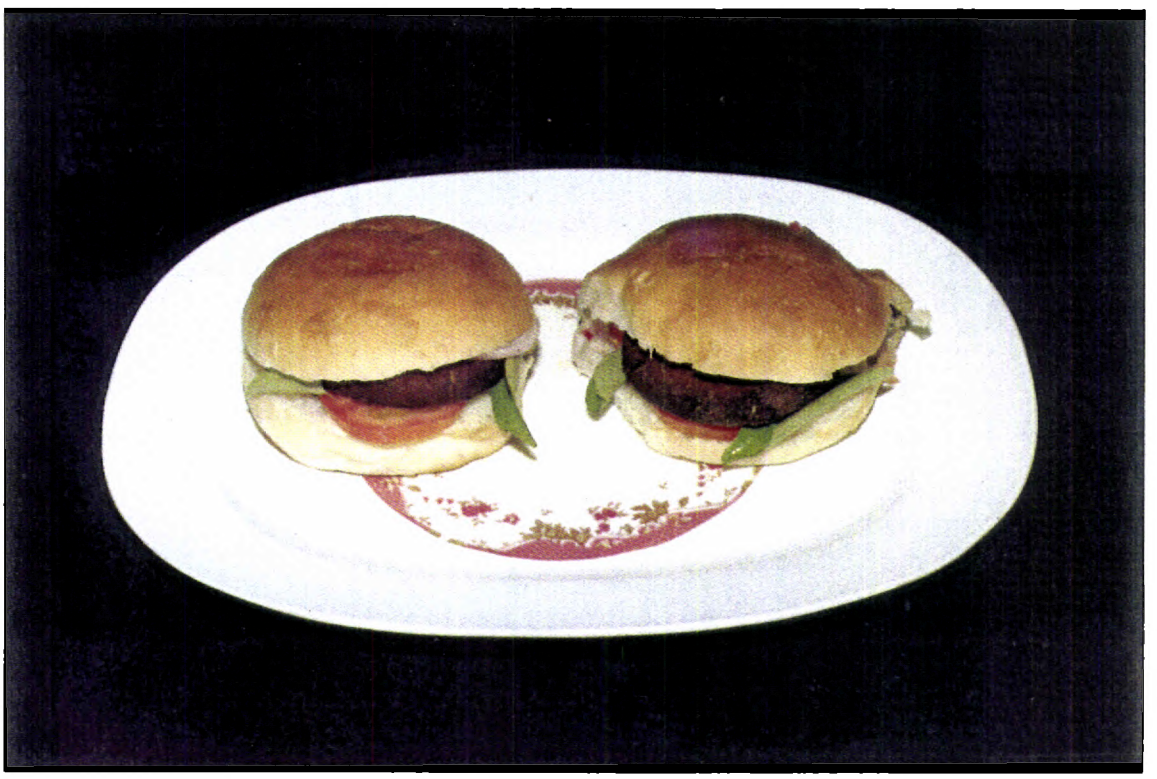


PLATE - C DRESSED FISH BURGER

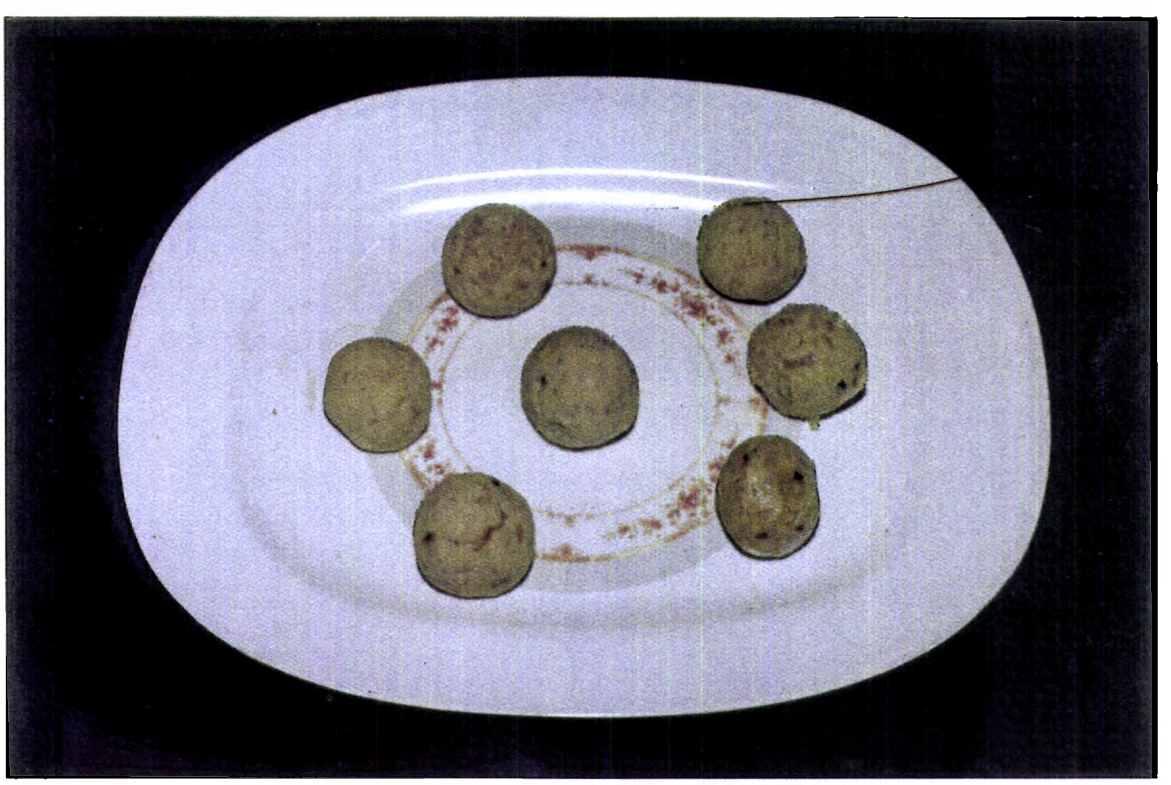


PLATE - D FISH BALLS (BEFORE FRYING)

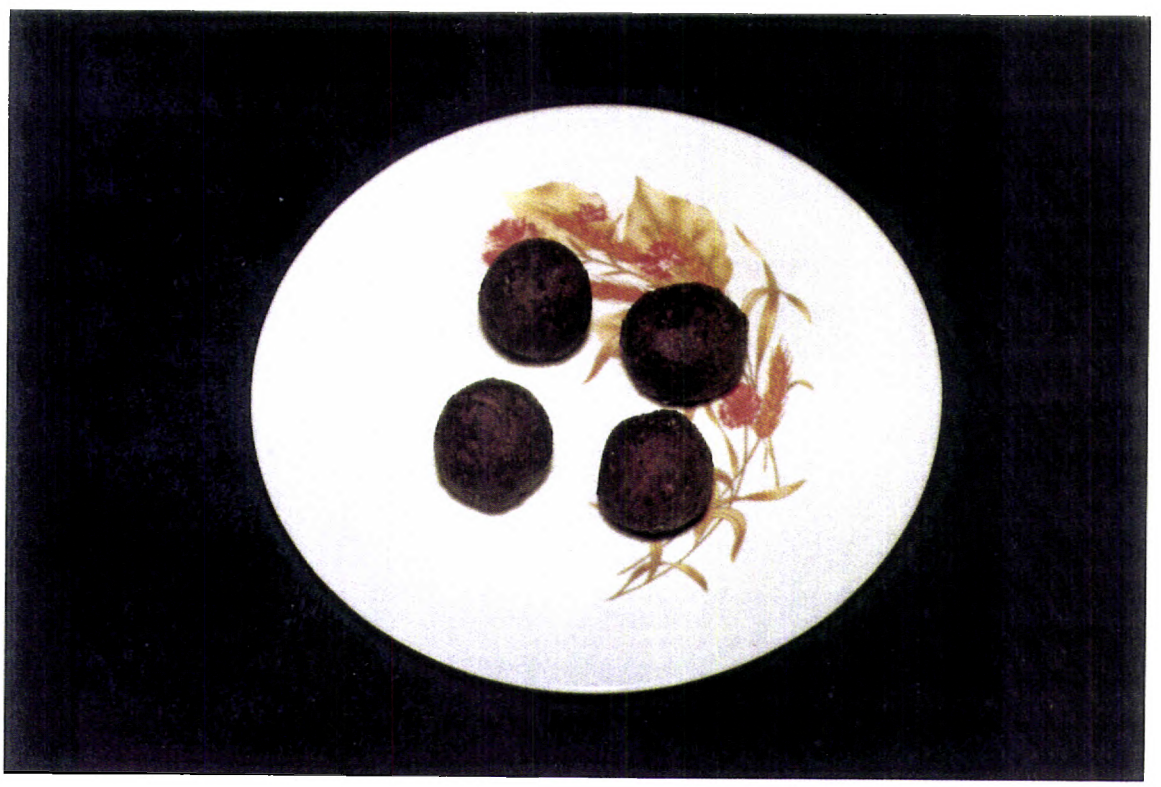


PLATE - E FISH BALLS (AFTER FRYING)

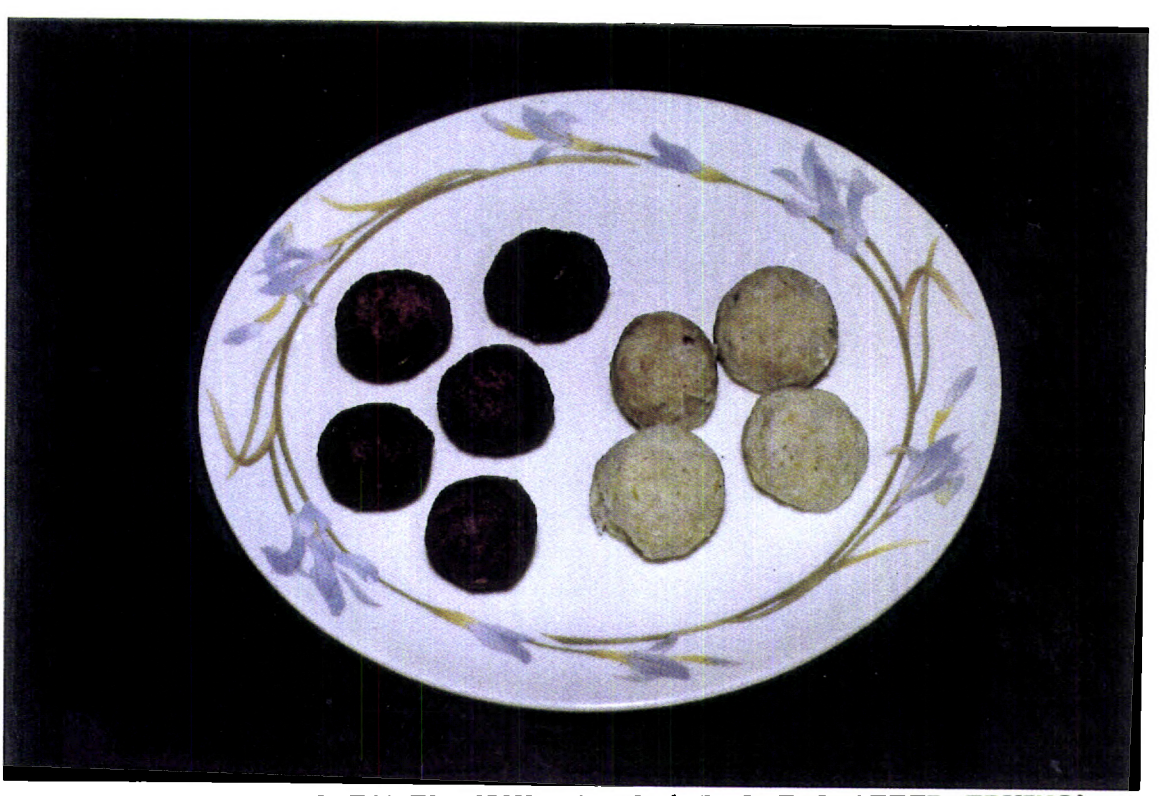


PLATE - F STEAMED FISH BALLS (BEFORE & AFTER FRYING)

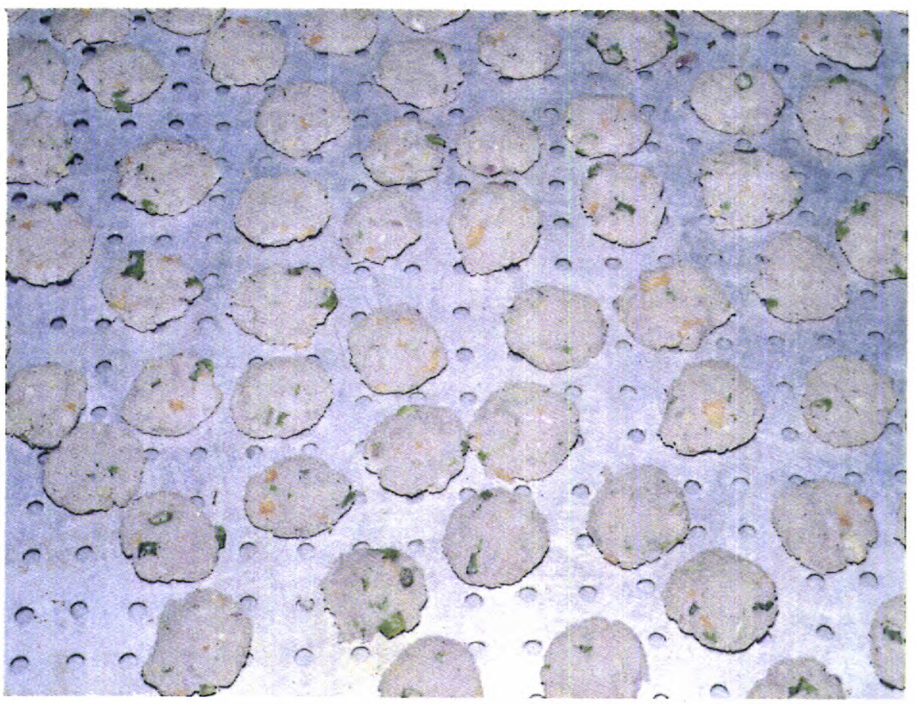


PLATE - G : A PRODUCT FROM SURIMI
(BEFORE FRYING)

PLATE - H AFTER FRYING

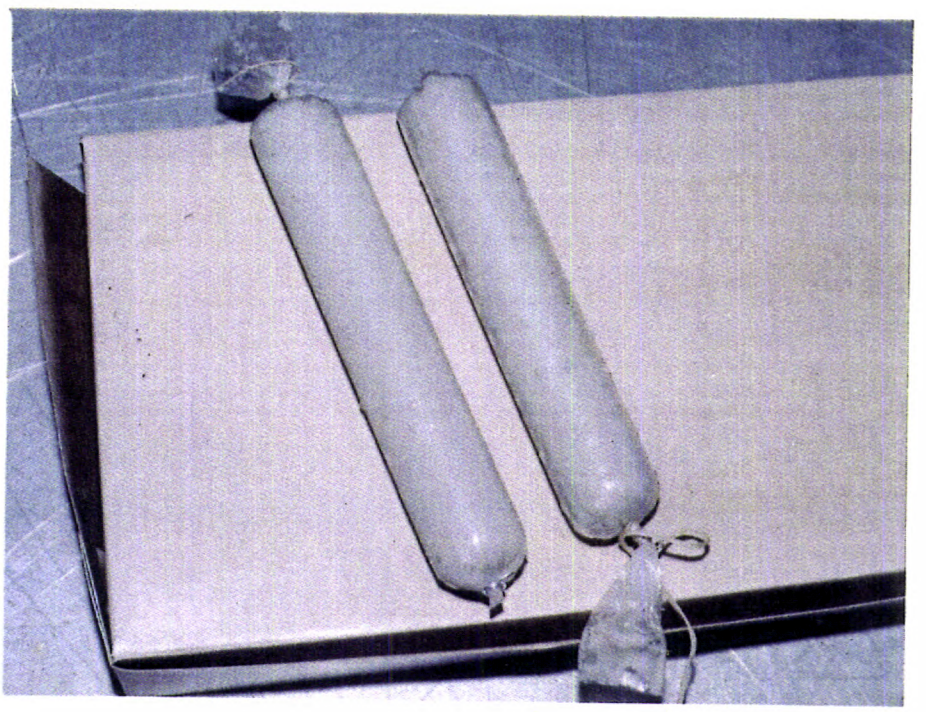


PLATE - I A PASTEURIZED PRODUCT FROM SURIMI

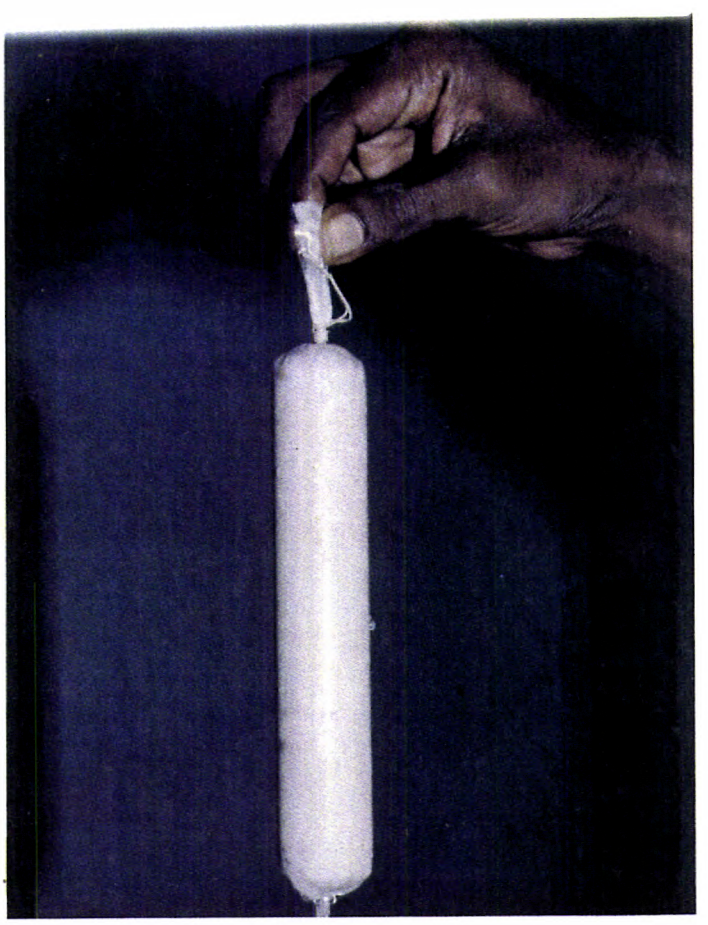


PLATE - J FISH CAKES (BEFORE FRYING)

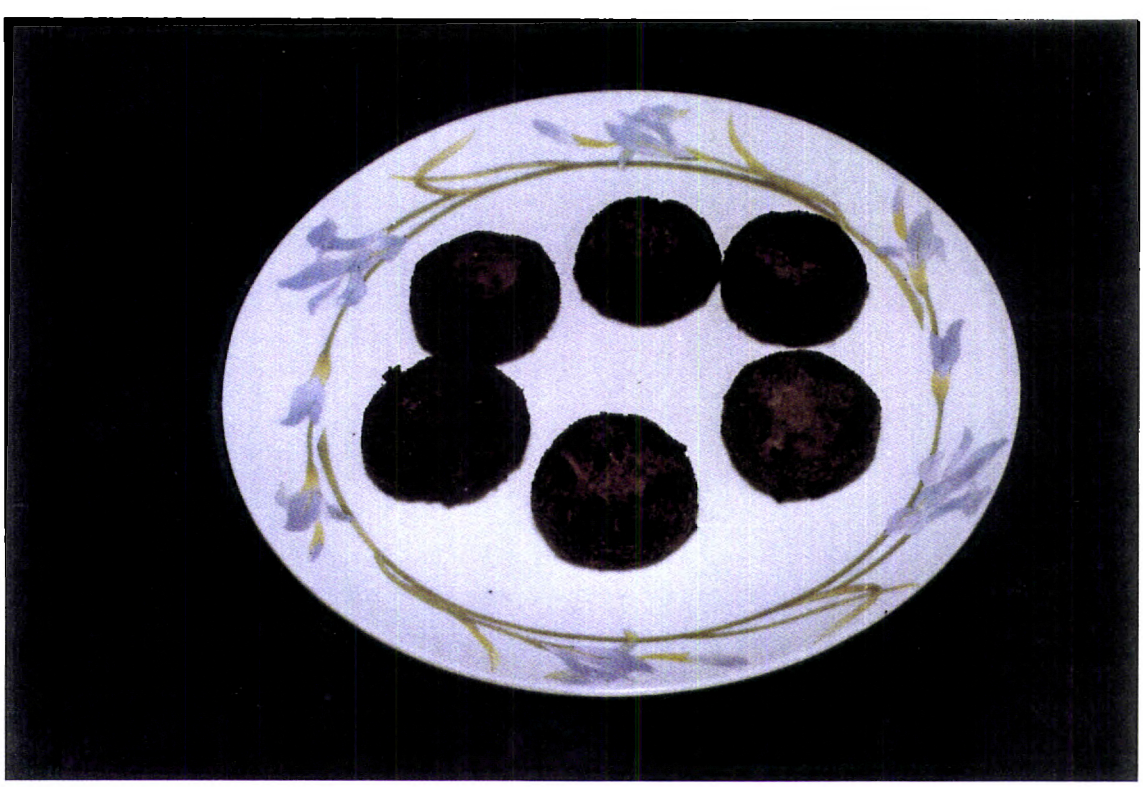


PLATE - K FISH CAKES (AFTER FRYING)

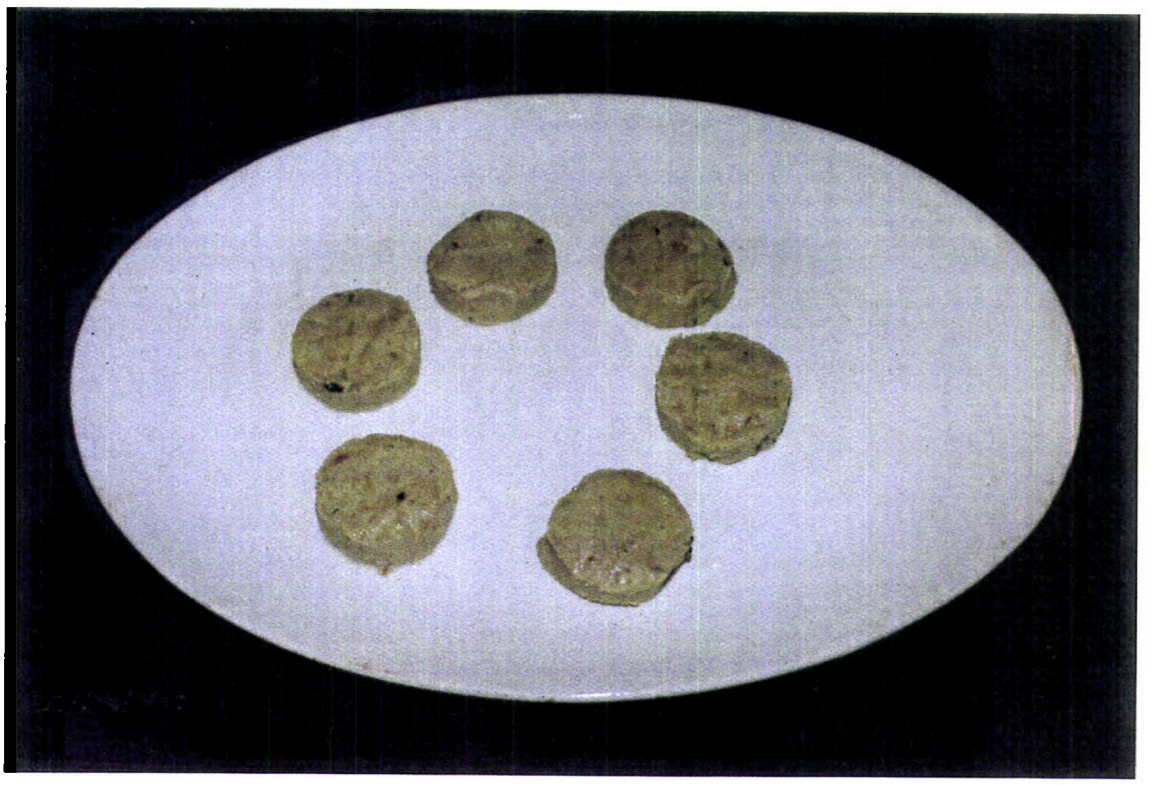


PLATE - L FISH KABABS

PLATE - M CANNED FISH BALLS IN TOMATO SAUCE

PLATE - N: CANNED FISH BALLS IN BRINE

PLATE - O CANNED FISH BALLS IN OIL

PLATE - P FROZEN MINCE BLOCK

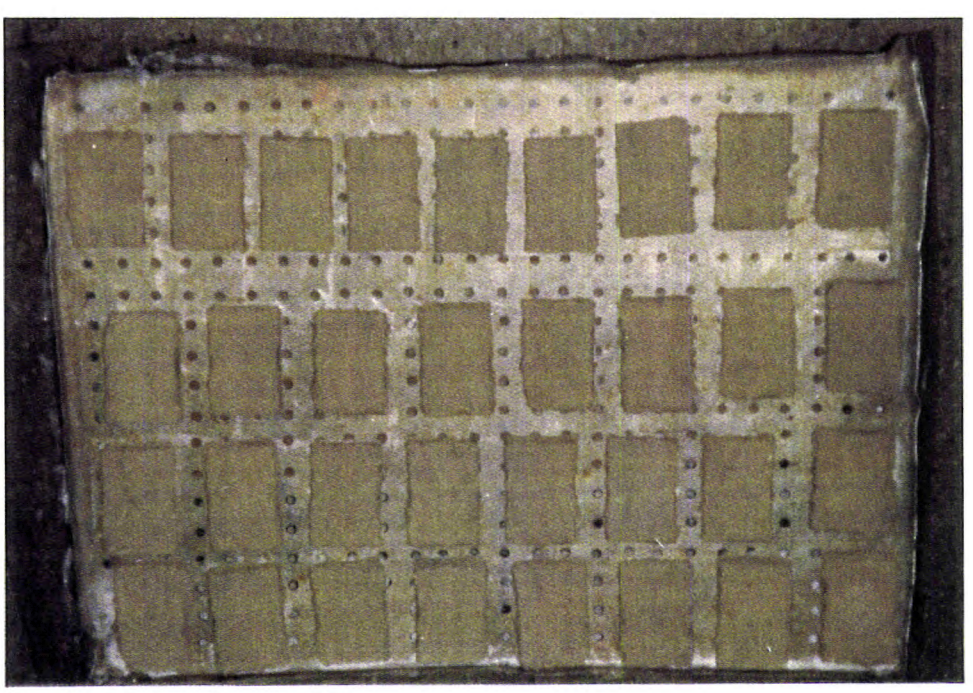


PLATE - Q BATTERED & BREADED FISH MINCE SLICES (BEFORE FRYING)

PLATE-6 BATTERED & BREADED MINCE SLICES
(AFTER FRYING)

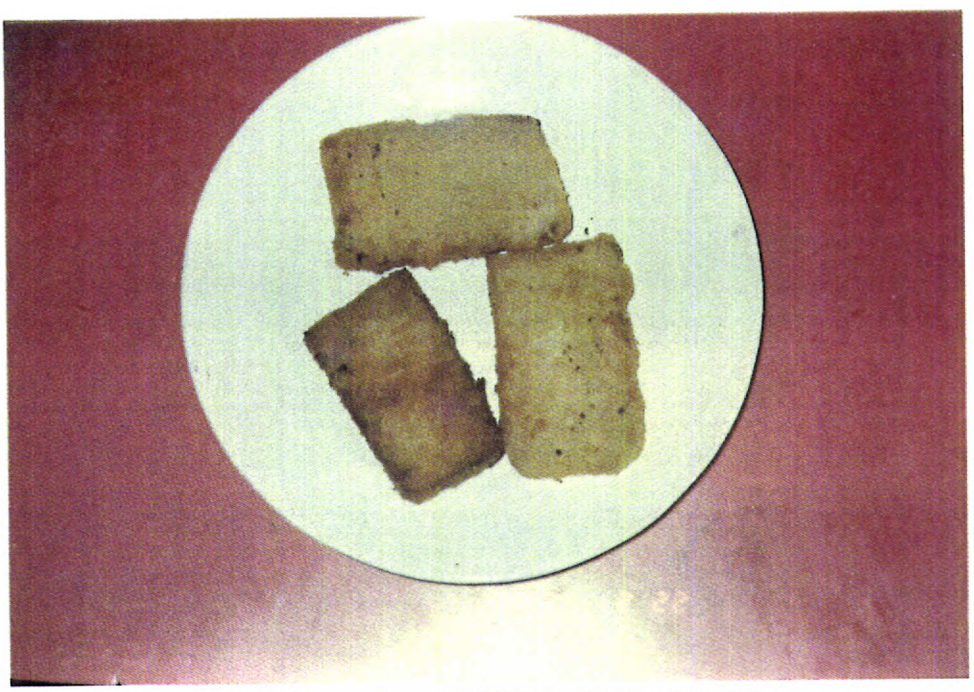


PLATE - 5 . CANNED FISH SPREAD

APPENDIX-2

PROJECT FOR DEVELOPMENT OF VALUE ADDED MINCE BASED PRODUCTS FROM LOW VALUE FISH LIKE PRIACANTHUS & NEMIPTERUS JAPONICUS

CAPACITIES MAXIMUM 5000 kgs. of Raw material per day of one eight hour shift.

Assumed practical capacity 4000 kgs.

PROCESS:

Stage 1 Development of minced fish meat.

Stage 2 Development of consumer products like frozen fish kheema blocks, Burger, Balls, Cakes, Kababs, Fingers, , Pasteurised and frie products.Canned fish balls and fish paste.

Facilities, machineries & equipments essentially required.

1) Plant and building
2) Cold storage of 50 T capacity
3) Chill room of 20 T capacity
4) I.Q.F. of 300 kg/hour capacity.
5) Dressing tables, knives, handling boxes and other equipments.
6) Meat bone seperator of capacity 500 kg./hour.
7) Seamer
8) Autoclave
9) Silent cutter
10) Equipments for a small quality control laboratory.

Products developed

1) Frozen Fish kheema 500 gm. pkts.
2) Frozen Fish Burger 100 gm. Pkt.
3) Frozen Fish Balls 100 gm. Pkts.
4) Fish Cakes 100 gm. Pkts.
5) Frozen Mince slice 100 gm. Pkt.
6) Fried product from surimi 100 gm. Pkt.
7) Frozem pasteurised products 100 gm. pkt.
8) Frozen fish Kabab 100 gm. pk t.
9) Canned fish Ball in Tomato sauce 200 gm can.
10) Canned fish paste 200 gm. can.

DAILY PRODUCTION PATTERN - PROCESS

RAW FISH - 4000 Kgs.

3000 Kgs. DRESSED FISH

1600 Kgs. MINCED FISH MEAT

FREEZING		CANNING
Frozen Products R.M. 1500 Kg. Mince + 500 kgs. other ingredients	50 kg Minced meat	50 kg.Minced meat
	Can Fish Ball (200 gm) 500 Nos.	Can Fish Paste (200 gm) 550 Nos.
FISH KHEEMA 500 g. pkts. R.M. 800 kg. - Product : 1600 Pkt.	Battered & breaded fish mince slices- 100 gm. Pkt. R.M Mince 100 kg. Others 115 Kg. Product 2000 pkt.	
FISH BURGER 100 gm. pkt. R.M. Mince 100 kg. Others 50 kg. Product: 1400 pkt.	FRIED PRODUCT : 100 gm pket. R.M. Mince 100 kg. Others 50 kg. Product 1500 pkt.	
FISH BALL 100 gm.pkt. R.M Mince 100 gk. Others 90 kg. Product: 1800 pkt.	PASTEURISED PRODUCT 100gm.Pkt R.M Mince 100 kg. Others 50 kg. Product 1500 pkt.	
FISH CAKE 100 gm. Pkt. R.M. Mince 100 kg. Others 85 kg. Product 1800 pkt.	FISH KABAB 100 gm.pkt. R.M. Mince 100 kg. Others 60 kg. Product 1500 pkt.	

COSTS & REVENUE STATEMENT

(FOR ONE YEAR)

Assumptions:	Daily production	Raw material 4000 kg.
	Working hours	8 hour shift per day
	No. of days/year	250
	Raw material fish	Priacanthus & Nemipterus japonicus
		Price Rs. 5/- per Kilogram
		Price/Kg. of other ingredients
		Rs. 20/-
	Packing material	Best appealing packets.

Price assumptions:

A. Frozen Kheema	500 gm. Pkets.	Rs. 15/-
B. Fish Burger	100 gm. Pkts.	Rs. 6/-
C. Fish Ball		Rs. 5/-
D. Fish Cake	"	Rs. 5/-
E. Fish Finger		Rs. 6/-
F. Fried product		Rs. 5/-
G. Pasteurised		Rs. 5/-
H. Fish Kabab		Rs. 5/-
I. Fish Ball/Tomato	Can 200 gm.	Rs. 15/-
J Fish Paste		Rs. 12/-

Market

Kheema has an established and definite market among all target groups. Other items will definitely find target with restaurants, hotels, fast food counters and other caterers and also with daily home based consumers.

Distribution will be effected by engaging insulated/refrigerated vehicles.

Price assumed are very reasonable as revealed by test marketing.

Working Capital One month's complete variable expenditure.

Plant installation, commissioning and production:

Plant will be commissioned within the 1st half year and production will start at beginning of 2nd half.

A. CAPITAL COST

1) Land 800 m^2 @ Rs. 1250/m^2	Rs. 10,00,000
2) Plant & buildings 450m^2 @ Rs. 2000/m^2	9,00,000
3) Cold storage 50 T with machinery and installation	15,00,000
4) Chillroom 20 T	6,00,000
5) IQF Unit 250 kg/hr.	15,00,000
6) Seaming machine (small unit) Semi auto	1,00,000
7) Autoclave	1,00,000
8) Silent Cutter	2,00,000
9) Meat bone seperator 500kg/hr.	4,00,000
10) Dressing tables - 10 Nos.	50,000
11) Fish handling boxes - 200 Nos.	40,000
12) Vehicles - 2	16,00,000
13) Other equipments for quality control etc.	2,00,000
Sub total	81,90,000

Depreciation on 2 to 13 above assuming 15 year life.		4,79,500

B. VARIABLE COSTS

1) Cost of fish 1000 tons	50,00,000	
2) Cost of other ingredients	31,00,000	
3) Packing materials, can etc.	61,60,000	
4) Cleaning materials	4,000	
5) Plant uniforms etc.	20,000	
6) Liquids & gases	75,000	
7) Quality control	10,000	
8) Electricity and water	5,50,000	
9) Labour 80 workers/shift per day x Rs. 40/- x 250	8,00,000	
10) Salaries to supervisory & Management staff	2,88,000	
11) Added benefits to employees	2,72,000	
12) Reapirs & maintenance 3.5%	2,53,000	
13) Waste removal & pollution control	1,00,000	
14) Marketing expenses & vehicle operation	7,00,000	
15) Other unforeseen and sundry expenses	1,00,000	
Total	174,32,000	
Grand total including depreciation		179,11,500
Net revenue 99,000/day x 250		225,00,000
Net gain		Rs. 45,88,500/-

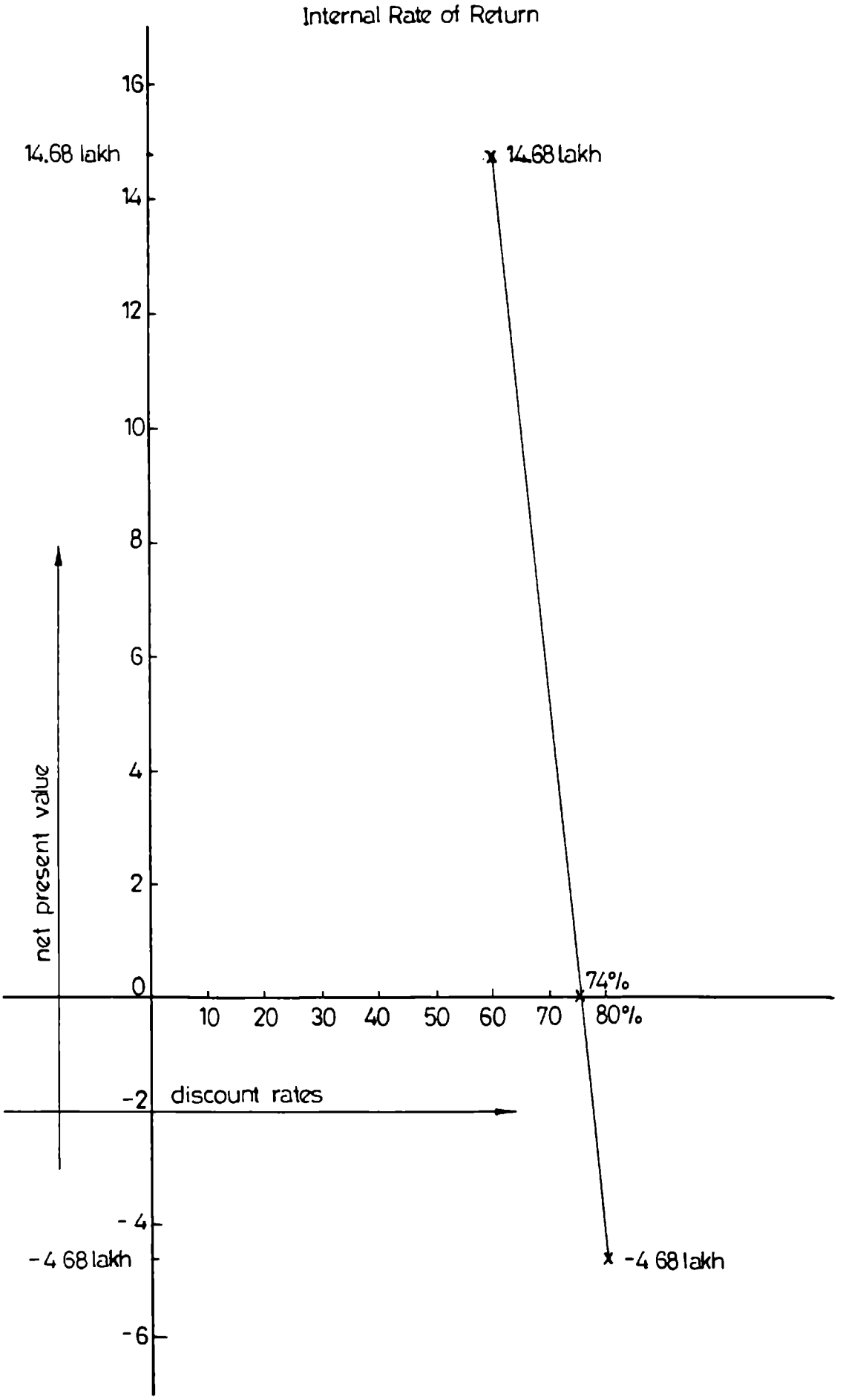
FINANCIAL STATEMENT OF THE OPERATION OF THE PROJECT

IN D.C.F. CHART

Discount rate 13%

(Rs.in lakhs)

Cost particulars	Year 0	1	2	3	4
A. CAPITAL COSTS					
i) Land	10.00				-10.00
ii) Plant, building machineries & equipments	71.90				-57.52
iii) Working capital	15.00		-15.00		
Total Capital costs	96.90		-15.00		-67.52
B. VARIAIBLE COSTS	88.66	174.32	174.32	174.32	174.32
Total costs	185.56	174.32	174.32	174.32	106.80
C. REVENUE	112.50	225.00	225.00	225.00	225.00
D. NET PROFIT	-73.06	50.68	65.68	50.68	118.20
E. PRESENT VALUE	-73.06	44.85	51.44	35.12	72.49
NET PRESENT VALUE	130.84				
	=========				
Internal Rate of Return	74%				
Net Present Value on contingency when variable costs increases by 5%					
NPV	100.20				


Internal Rate of Return
16
14.68 lakh
14
12
10
8
6
4
2
0
-2
- 4
-4 68 lakh
-6
net present value
14.68 lakh
74%
10
20
30
40
50
60
70
80%
discount rates
-4 68 lakh

SUMMARY

5. S U M M A R Y

This thesis is mainly concerned with the studies on freezing and frozen storage of minced fish flesh from Nemipterus japonicus, its degradation and some of the means available for control of such degradation. Also the amenability of minces from Priacanthus spp. for product formulation has been studied. In 2 chapters the thesis describes the salient features of the bacteriology of freezing and frozen storage of minced fish flesh from Nemipterus japonicus, the changes in protein extractability of minced fish during freezing and frozen storage and in one chapter, utility of minced flesh from Priacanthus spp. for product formulations.

5.1 BACTERIOLOGICAL AND ORGANOLEPTIC STUDIES OF MINCED MEAT FROM NEMIPTERUS JAPONICUS DURING FREEZING AND FROZEN STORAGE

Bacteriology of dressed fish, minced fish and minced fish treated with EDTA during pre-process handling, freezing and frozen storage is detailed in Chapter-2.

Fresh fish meant for the study was washed thoroughly. One lot was dressed and one lot was minced. The minced flesh was divided into two. One half was treated with 1% Na_2 EDTA solution to

give a resultant concentration of 0.01% of Na_2 EDTA in the mince. All the samples were packed in 500 gm. lots, packed in polythene bags, frozen at -40°C and stored at -20°C.

Effect of preprocess handling on total bacterial count was as follows:

Average bacterial count at room temperature (RT) for dressed fish, minced fish and treated minced fish were 2.2×10^5/g, 1.5×10^6/g and 6.1×10^5/g respectively. Mincing has actually enhanced the total bacterial counts of fish muscle by about one log cycle. EDTA treatment was found to have definite control on the bacterial multiplication in the minced fish before freezing. The TPC of the treated mince was only 40% of the TPC of the untreated mince.

Fresh dressed fish, minced and treated mince showed significant difference in bacterial count between incubation temperatures; RT (29 ± 2°C) giving a higher bacterial count than 37° or 8°C. While there was not much difference in the TPC of dressed fish incubated at 37°C from that at RT there was about one log cycle difference in the TPC of the minced fish at RT and at 37°C.

Freezing has brought about a drastic reduction in the TPCs of all the three samples. In the case of dressed fish the reduction

was to the tune of 91.5% compared with the TPC before freezing.

The bacterial count of minced fish suffered a reduction of 91.3% which is almost the same as the dressed fish, but the reduction brought about in treated minced fish was quite high ie. 97%. In the case of EDTA treated minced fish the freezing has almost reduced the bacterial population to 3% of the initial count.

The effect of freezing on the total bacterial counts at 37°C of dressed fish, minced fish and treated minced fish was also studied. In the case of dressed fish and treated minced fish comparable reduction of 71.2% and 70.48% respectively were observed, from the corresponding TPCs at 37°C. But in the case of EDTA treated mince the reduction was still higher at 95.35%.

The effect of freezing on the total bacterial count of dressed fish, minced fish and treated minced fish at 8°C was also observed. In this case, a drastic reduction in the bacterial count were seen for dressed fish and untreated mince, the reduction being 99% and 97% respectively. However, for EDTA treated mince the reduction was only 73% from the initial TPC at 8°C.

The effect of frozen storage was not very extensive during storage though freezing caused drastic reduction. In all the three cases after the initial reduction due to freezing at -40°C, there was

not much perceptible decrease in the total bacterial count during the entire storage period of six months at -20°C.

In dressed fish by the 10th day of frozen storage there was not much change in the bacterial counts. During subsequent storage upto 4 months also the TPC remained more or less same at 10^5/g. indicating that the flora which resulted after freezing at -40°C were capable of survival during frozen storage at -20°C upto 4 months. However, after six months of frozen storage the viable counts were only 1/3 of the flora that remained after four months.

In the case of minced fish the bacterial count after 10 days of frozen storage at -20°C was more or less the same as the residual flora after freezing at -40°C. However, during further storage the flora was reduced by about 90% in two months time. Subsequent frozen storage upto six months did not appear to bring about a drastic reduction in the TPC.

The bacterial count of the treated minced fish during frozen storage at -20°C did not show any significant reduction upto six months.

The changes in the bacterial population recovered at 37°C, of frozen dressed fish, minced fish and EDTA treated minced fish were also studied. In the case of dressed fish by the 10th day

of frozen storage at -20°C, the TPC recovered at 37°C had decreased from 9.2 x 10^4/g to 9 x 10^3/g. However, during subsequent storage upto six months the TPC remained more or less constant. In the case of minced fish and EDTA treated mince after the initial reduction due to freezing at -40°C there was not much decrease in the total bacterial count during the six months of storage at -20°C.

In all the three cases, the bacterial count at 8°C did not show any significant reduction during the entire period of frozen storage. The maximum reduction in bacterial count was observed in the early period of frozen storage after which the reduction was more or less gradual.

Before freezing the dressed fish harboured 1.1 x 10^6/g of H_2S producing bacteria. In the minced as well as EDTA treated mince, there was a ten fold increase in H_2S producing bacteria. Immediately after freezing at -40°C there was 45% reduction in the H_2S producing bacterial count in the case of dressed fish, 98% in the case of minced fish and 95% in the case of treated mince.

On storage at -20°C the counts have drastically fallen further by the first 10 days and this drastic reduction had been very significant in the case of treated mince, the reduction being 98.5% of the residual count after freezing.

After two months of frozen storage the H_2S producing bacteria had been completely destroyed, indicating their susceptibility to frozen storage. The trend in the H_2S producing bacterial counts and changes during freezing and frozen storage were almost the same at 8°C also except that within the first ten days of storage the H_2S producing bacteria had died out in the case of minced fish and EDTA treated minced fish.

Before freezing the H_2S producing bacterial count of the dressed fish was only 5.5% of the TPC at RT. But immediately after freezing its ratio increased to 36.9%. However, within 10 days of storage at -20°C the H_2S producers had completely died out and by two months time at -20°C there was none left.

H_2S producing bacteria formed 40% of the TPC at RT in the case of minced fish before freezing. Due to freezing at -40°C the ratio came down to 30% of the TPC and within 10 days of frozen storage at -20°C the percentage of H_2S producers in the TPC at RT had been reduced to a very insignificant level of 0.03%. This also completely vanished by the 2nd month of frozen storage.

In the case of EDTA treated mince also more or less similar trends were observed.

The H_2S producers at 8°C in dressed fish, minced fish and treated minced fish were 1.8%, 1.2% and 19% of the total plate count

respectively. After two months of storage at -20°C the H_2S producers were not recovered at all at -8°C.

Qualitative aspects of bacteriological changes in dressed fish, minced fish and treated minced fish during freezing and frozen storage were studied in detail. The initial flora of the dressed fish consisted only Gram negative asporogenous rods mainly constituted by Psuedomonas and Acinetobacter species. 69% of the total colonies examined were Psuedomonas and the rest Acinetobacter. Initial flora of minced fish consisted mainly of Pseudomonas (71%), Acinetobacter (11%), Vibrio (9%) and Micrococcus (9%). The flora of the treated mince was 50% Psuedomonas, 29% Acinetobacter, 9% Enterobacteriaceae and 9% Micrococcus.

Comparatively lower percentage of Psuedomonas in the treated mince before freezing definitely indicated the effect of EDTA on Psuedomonas. The action of EDTA treatment appeared to be distinctly selective towards Psuedomonas and this has been amply confirmed by the fact that not only the percentage of Acine tobacter has not reduced, but has increased from 11% to 29%.

The general trend on the incidence of major microbial groups in all the three types of samples was the same at -8°C. In dressed fish only Psuedomonas and Acinetobacter were detected, Psuedomonas being 80% In the minced fish, the percentage of Pseuedomonas was 70% and that of Acinetobacter 9%. In the case

of EDTA treated mince the percentage of Psuedomonas has drastically come down to 44% and Micrococcus had increased to 27%. Acinetobacter accounted for 19% of the flora and Vibrio to the tune of 8% have also been obtained.

After freezing at -40°C percentage of Psuedomonas came down to 50% and that of Acinetobacter 11%. in dressed fish incubated at RT. By six months storage Micrococcus became the most dominant flora at 50% level. Flavobacterium constitued 32% and the percentage of Psuedomonas fell down to 9%.

The bacterial genera of the minced fish before freezing composed of Psuedomonas (70%), Acinetobacter (9%), Vibrio (9%) and Micrococcus (10%). After freezing at -40°C, the level of Psuedomonas came down to 49%, while Acinetobacter and Micrococcus increased to 20% of the flora and Vibrio remained at 10% itself. On subsequent storage, at the end of six months Psuedomonas reduced to 6%. Micrococcus on the other hand progressively increased in proportion and reached the level of 83% of the flora by six months' of storage.

In treated mince before freezing the flora consisted mainly of Psuedomonas (49%), Acinetobacter (29%), Micrococcus (11%) and Enterobacteriaceae (11%). The decline in the Psuedomonas group was very drastic and it formed only 20% of the flora after freezing.

By the 4th month Psuedomonas completely vanished from the flora. Micrococcus on the other hand steadily increased and at the end of six monthsthe entire bacteria that had survived were constituted exclusively by Micrococcus.

The changes in the bacterial flora, recovered at 8°C, of the dressed, minced and treated minced fish during freezing and frozen storages were also studied.

In the case of dressed fish, the initial bacterial flora before freezing were mainly composed of Psuedomonas (80%) and the rest Vibrio. Immediately after freezing, the percentage of Pseudomonas had substantially come down to 50% and the Vibrio had increased to 30%. Micrococcus (20%) was also recovered. Flavobacterium and Micrococcus had steadily increased in their percentage and reached 40% each at the end of six months frozen storage.

The bacterial flora of the minced fish at RT before freezing consisted of Psuedomonas (69%), Acinetobacter (10%), Arthrobacter (10%) and Micrococcus (10%). By the end of six months of frozen storage 90% of the residual flora recovered at 8°C was constituted by Micrococcus alone.

In treated mince majority of the bacterial flora before freezing were Gram negative, comprising of Pseudomonas (45%), Acinetobacter (21%) and Vibrio (9%). Micrococcus (Gram positive) constituted

27% of the flora. At the end of six months storage at -20°C, the entire residual flora of the EDTA treated mince was constituted by Micrococcus alone.

During freezing there was drastic reduction of pathogenic and indicator bacteria in dressed, minced and treated minced fish. The incidence of pathogenic/indicator bacteria was very low in dressed, minced and treated mince. Complete destruction of these bacteria was observed in all the three samples during freezing and frozen storage.

Organoleptically the dressed fish was acceptable even at the end of six months of frozen storage while the minced fish reached the level of rejection at the end of 24 weeks. The treated mince was below the threshold of rejection at the 6th month of storage.

5.2 CHANGES IN PROTEIN EXTRACTABILITY AND TEXTURE OF MINCED MEAT FROM NEMIPTERUS JAPONICUS DURING FREEZING AND FROZEN STORAGE

The fish mince from Nemipterus japonicus was frozen at -40°C as blocks after the following treatments (a) without washing (b) after washing in potable water (c) without washing and with additives (d) with washing with additives.

The above samples was stored at three different temperatures

namely -25°C, -18°C and -5°C. The additives employed were lactose Mono Sodium Glutamate (MSG) and sodium citrate.

The loss in solubility of protein of the minces and their textural changes were followed in order to estimate the effect of time, temperature, additives and the processing variables like washing on salt extractability of protein and textural qualities of the minced fish.

Immediately after freezing of the unwashed mince with no additives, 90% of the protein fraction of the mince was salt extractable. But the salt extractable fraction of the protein have significantly come down to 70% as a consequence of washing in potable water. The 20% decrease on the salt solubility of protein in the case of washed mince indicated that 20% of the protein fraction of the muscle of Nemipterus japonicus was water soluble.

The initial solubility of 90% in the case of unwashed mince, frozen stored at three different storage temperatures was reduced to 66%, 48% and 36% at -25°C, -18°C and -8°C respectively on one week of storage.

The effect of temperature of storage on salt solubility is that it is affected the least at storage temperature of -25°C and the most affected at the storage temperature of -5°C.

The salt solubility loss of protein in washed mince stored at the three different storage was also assessed. Immediately after freezing the extractability was only 70% at all the three temperatures of storage. At the final observation it was 38%, 19% and 18% at -25°C, -18°C and -5°C respectively. Other than the initial drop of about 20% in the salt soluble protein due to washing there was no other apparent difference in the salt solubility of both unwashed and washed mince without additives during the entire period of frozen storage in three different temperatures. This indicated that other than the initial removal of water soluble fraction of the protein from the mince, washing of the mince did not bring about natural change in the solubility properties of the fish protein during frozen storage.

Textural score immediately after freezing was the same for both washed and unwashed fish mince. The score was 8 for all the three samples. At the final observation it was 5, 4 and 2 at -25°C, -18°C and -5°C respectively. In spite of the fact that washing had reduced salt solubility of protein in the mince by about 20-30%, the texture of both washed and unwashed fish mince was comparable.

Frozen storages at -25°C had vastly protected the fish muscle from textural deterioration due to storage. But such protection

was not seen in minces stored at a higher temperature viz. -5°C. Between -25°C and -18°C, much difference was not apparent in the textural scores.

The effect of lactose on the salt extractability of protein and textural properties of unwashed and washed mince stored at three different temperatures was studied. Reduction in saltsolubility of protein of the unwashed mince with lactose addition, when compared with the control (unwashed mince with no additive) was less at lower temperature namely -25°C and -18°C. However, at -5°C it was more or less the same as the control. The reduction was to the extent of 30% at -25°C whereas the reduction in untreated sample at -25°C was 40%.

As far as textural scores were concerned, no perceptible effect was caused by lactose irrespective of the storage temperature. In washed mince also no protective effect was exhibited by lactose on salt extractability and textural score at all the three different temperatures.

Monosodium glutamate (MSG) was used as an additive in washed and unwashed mince stored at three different temperatures. The MSG had no on effect of salt solubility of protein and textural properties of the minced fish irrespective of the temperature of storage.

Sodium citrate was the third additive employed. The result regarding the effect of the additive on salt solubility of protein and textural properties was that it has no effect on salt extractability of protein as well as textural changes in minces.

Hence among the three additives used only lactose showed some effect at -25°C in controlling the salt extractability of protein.

5.3. DEVELOPMENT OF CONSUMER PRODUCTS FROM MINCED MEAT FROM PRIACANTHUS SPECIES

This chapter encompasses studies on the formulations of a few products from the minced meat of Priacanthus species and studies on the physical, chemical, bacteriological and nutritional characteristics of the products so developed. Additives and flavours were suitably incorporated for the production of various consumer products from the bland, formless fish flesh. The products developed were fish burgers, fish balls, two products from surimi, fish cakes, battered and breaded fish mince slices, fish kababs and canned fish paste.

Two frozen products namely fish burgers and fish cakes were subjected to frozen storage life studies at -20°C for a period of six months. Two canned products namely canned fish balls (canned in three different media) and canned fish paste were

also subjected to storage life study for a period of one year, at ambient temperature.

All the products were organoleptically sound as per the taste panel scores. Proximate composition analysis showed that the products have excellent nutritional properties. The Calorific value ranged from 68 to 271 k. calories. All the products were test marketed through the marketing outlets of Integrated Fisheries Project and met with appreciable consumer acceptance.

Fish burgers were frozen stored at -20°C as well as -8°C. Organoleptically the threshold of rejection came on the 22nd week of storage for the samples stored at -20°C. Chemically the samples were sound at the 22nd week. However, the samples had lost their binding properties and developed cracking on frying after 8-10 weeks of storage. Storage at -8°C lead to a lesser shelf life. Organoleptically the samples were rejected at the 3rd month. Physical and chemical changes were rather drastic when compared to the samples stored at -20°C.

Fish cakes were also subjected to shelf life studies. Storage was done at -20°C as well as -8°C. For the samples stored at -20°C the values for chemical changes remained within limits during the entire period of storage and it reached the threshold

of organoleptic rejection at the end of 22nd week. However, the cakes stored at -8°C had a lesser shelf life. By 14th week, organoleptically it was rejected.

Two canned products namely fish balls and fish paste were studied for their storage characteristics for a period of one year. The fish balls canned in all the three different media namely brine, oil and tomato sauce remained in acceptable condition for one year based on sensory qualities. In the case of fish balls canned in brine though turbidity was observed, it did not affect the sensory qualities. Bacteriologically the product was safe during the entire period of storage. The appearance colour, flavour and texture in all the three cases were well above the acceptable levels.

Canned fish paste was subjected to storage studies for a period one year. Until the sixth month the organoleptic qualities were rated excellent or very good. Bacteriologically the product was safe during the entire period of storage. However, at the end of the sixth month, the original consistency of the product was lost, since vegetable oil separated.

The present study is an important investigation in the field of minced fish technology from tropical fish. In addition to obtaining

some fundamental data on the bacteriology and protein stability of minced fish, this study will be useful for the prospective entrepreneurs of commercial sector for implementing quality control monitoring as well as development of value added products from rather cheaper variety of fish.

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