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**EFFECT OF RAW MATERIAL QUALITY  
ON THE SHELF LIFE  
OF FROZEN STORED FISH AND FISHERY PRODUCTS**

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CERTIFICATE

This is to certify that this thesis entitled "Effect of Raw Material Quality on the shelf life of Frozen Stored Fish and Fishery Products" embodies the result of original work conducted by Shri Jose Joseph under my supervision and guidance from 25-3-1985 to 12-7-1989. 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 Society. He has also passed the Ph.D. qualifying examination of the Cochin University of Science and Technology, Cochin-682022 held in July 1988.

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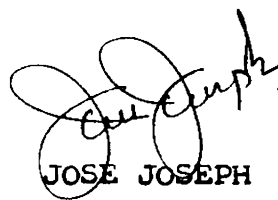
  
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DECLARATION

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

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## INTRODUCTION AND REVIEW OF LITERATURE

### 1.1 INTRODUCTION

Fish play a very important role in human nutrition. In developing countries fish provides the primary source of animal protein for over one billion people (FAO/World Bank/UNDP/CEC, 1989). Fishing is the main source of income for some 100 million people, about 80% of whom are in low income group.

The demand for fish has been constantly growing and is expected to be around 100 million tonnes by 2000 A.D. (FAO, 1981). The projected domestic demand by this time is between 12.5 and 20 million tonnes (Srivastava, 1985). The world fish catch rate, compared to 1960's and 1970's, has slowed down significantly inspite of increased fishing effort (FAO/World Bank/UNDP/CEC, 1989). Expanded research endeavours are badly needed in areas like improved management, avoidance of over fishing, market research for product development, post harvest losses and reduction of waste.

Freezing is considered to be one of the best method of preservation of fish. In this method the wholesomeness, nutritive value and sensory qualities are maintained to a

considerable length of time (Anon, 1972; Fennema, 1973). At the commercial frozen storage temperatures of  $-18^{\circ}\text{C}$  or below the bacterial growth is completely suppressed, but many chemical reactions are only retarded (Ronsivalli & Baker, 1981). These chemical changes cause insolubilization of proteins, oxidative deterioration, toughening, loss of juiciness and excessive thaw exudate (Fennema, 1973). Thus preservation by freezing and frozen storage is by no means perfect, although advantages outweigh disadvantages.

Frozen storage characteristics and shelflife vary considerably among species as well as within the species (Powrie, 1973; Fennema, 1973). This can be attributed to the variation in the composition of fish among various species. In certain species like sardines and mackerel, wide seasonal variation in chemical composition occur within the species. These variations affect the quality and shelflife. The nutritional level of water, spawning, method of catching, struggling etc. are found to have profound influence on the condition of the fresh fish.

Soon after death the deteriorative changes in fish start due to autolysis and bacterial growth. The rate of these changes depends mainly on temperature. The handling methods have great influence on bacterial contamination. Thus the type of handling, temperature control, period of chill storage, processing methods, type of freezing, condition of frozen



storage and period of storage affect the quality and shelflife of the fish.

In the present study extensive investigations were carried out on various factors affecting the quality of fish as well as their effect on the physical, chemical and sensory qualities of fish during frozen storage and the shelflife.

## 1.2 REVIEW OF LITERATURE

### 1.2.1 QUALITY

In fresh fish quality is almost synonymous with freshness (Wheaton & Lawson, 1985). Though everyone has a clear concept of the sense of fresh and spoiled fish it is difficult to give an exact expression of the grade of freshness (Jackson, 1971). In the fish trade there are many problems of decisive importance in determining the best method of utilization of fish. It is necessary to find out whether a certain quantity of fish is in a good and sound condition fit for human consumption, how good it is and how long will it keep. Until now, no methods available can provide an exact, reproduceable and quick answer to these problems (Connell, 1975; Damoglon, 1980). Such methods if available could be of tremendous importance to the fish trade and valuable tool in the work on quality improvement (Anderson, 1971).

By means of objective quality assessment methods the exact value of certain properties of the fish is measured (Connell, 1975). The results are independent of anything but the fish. Anyhow the quality of fish is directly connected with its organoleptic properties (Amerine, et al., 1965). In organoleptic quality assessment a judgement is made on the basis of experience of the appearance, odour, consistency and

flavour of fish taking into consideration all available information on the history of the fish, its catching, treatment, storage etc. (Connell, 1975; Anderson, 1965; Baines et al., 1965). Here the freshness is not measured but estimated. Much experience is required to estimate the freshness of fish and organoleptic testing is always defective like all subjective judgements. The sensibility of human senses is highly variable and to a high degree depends on the physiological well being and other unadjustable factors (Amerine et al., 1965; ASTM, 1968).

If two fishes are similar with regard to flavour, odour, texture and appearance they must be of the very same quality. If objective observations of the chemical and bacteriological properties are used to judge the quality, it is necessary to examine the connection between these observations, and simultaneous organoleptic observations (Baines, et al., 1965). This does not mean that objective methods are not of practical importance. If it is possible to agree upon the connection between certain objective methods and the organoleptic properties of the fish, these methods will furnish a means of discussing the freshness of fish along uniform lines. Several disagreements regarding experimental conclusions are due to different practices in quality assessment and also due to the difference in the raw material (Castell, 1971).

The production of frozen fish and fish products has been increasing steadily and considerably over the past several years (Bramsnaes, 1969). Along with commercial success of frozen seafood it has given rise to number of quality problems, mainly due to undesirable changes during storage and distribution of products. More knowledge about the changes is now available and frozen fish today is of much higher quality than it was a few years ago. The factors due to the quality of frozen seafood can be grouped under:

- 1) quality of the freshly caught fish.
- 2) quality changes during handling, chill storage, transportation and preparation prior to freezing.
- 3) packaging.
- 4) freezing rate and frozen storage conditions.

(Anon, 1972; Bramsnaes, 1969; Dyer & Dingle, 1961; Slavin, 1968)

The time for just noticeable difference to develop is usually referred to as high quality life of the product, while the practical storage life is the time to reach the acceptability limit.

#### 1.2.2 QUALITY OF FRESHLY CAUGHT FISH

There are many factors found to affect the condition or quality of fish just when it is taken out of water. They are:

- 1) feeding habits - the type of food and whether the fish is feeding or not feeding.

- 2) the geographic location of fishing grounds on which the fish are caught which includes a complex of environmental factors.
- 3) the sex and the changes in the habits and physiology of the fish associated with spawning.
- 4) specific difference in the chemistry of fish and
- 5) the method used for catching the fish and in particular, the degree to which the fish have been struggled immediately prior to death (Castel et al., 1959). Because of the above discribed factors the chemical, physical and morphological properties of the fish are found to show seasonal variation even among same species (Mathen et al., 1966; Venketaraman et al., 1968; Love, 1969; Solanki et al., 1976; Wheaton & Lawson, 1985). The variation in chemical properties among different species of fish are quite significant (Kuttiayappan et al., 1976; Devadasan et al., 1978; Wheaton & Lawson, 1985).

#### 1.2.2.1 Season

It is a well known fact that fatty species spoiled more rapidly than lean species. The fat content of many pelagic fishes vary considerably throughout the year. (Vasavan et al., 1960; Gopakumar, 1965; Mathen, 1968; Tashiro et al., 1981a; Venketaraman et al., 1968). Tashiro et al. (1981a) studied the variation of total lipids in horse mackerel and found that the lipid content was higher in spring and summer than in autumn

and winter due to increase in triglycerides. Gopakumar (1965) observed that in oil sardine the lipid content was high during November-December reaching more than 12% by weight of the muscle and the lipid was less than 2% in the month of May-June. He further observed that the changes in triglyceride content generally followed the pattern of total lipid content. So he concluded that the increase in lipid content was mainly contributed by the amount of triglycerides. Also the phospholipid content of the muscle did not change appreciably during different seasons. Vasavan et al. (1960) and Mathen et al. (1966) also reported the seasonal changes in lipid content of oil sardine. Venketaraman et al. (1968) observed that the lipid content of black pomfret changed with season. Tashiro et al. (1981b) found that in horse mackerel the compound lipids were constant throughout the year, but the main lipids, phosphatidyl choline and phosphatidyl ethanolamine, decreased in summer months. The meat lipids in horse mackerel were higher in saturated and monoenoic acids in summer and polyenoic fatty acids were less. Aguado (1979) observed that the neutral lipid content of trout was high in December and August and low in March, April and October.

Significant variations in the vitamin E content with season and species have been reported by several workers. Syvaaja et al. (1985) found that the vitamin E of all species

studied consisted of  $\alpha$ -tocopherol and the amount of this in muscle and roe were relatively high. They observed differences in tocopherol content between species. In high fat fish the tocopherol fat ratio was lower than in low fat fish. They noticed that fish caught in spring, the spawning season of most species had a higher tocopherol content (and a lower fat content) than those caught in autumn and the tocopherol and fat content in marine fish was higher than lake fish of the same species. Ackman (1967a) noticed variation in the stability of tocopherol depending on season during frozen storage. Tocopherols in sole caught in the period of June to early August were decomposed completely in four months under commercial conditions of frozen storage and the lipids were oxidised substantially, while the tocopherols and lipid in sole caught at other times were significantly more stable when stored under similar conditions. Tocopherols cannot be synthesized by the muscle tissue and are obtained through diets. It was found that the log of tocopherol content of tissue varied directly with the content of tocopherol in the diet (Griffitts, 1960; Rousseau et al., 1957).

Several studies on the effect of season on chemical composition showed changes in nutritive value, micronutrients and extractive nitrogen of fish flesh. Waters (1982) noticed maximum nutritive value and storage stability in spot caught between October and February. The extractive nitrogenous constituents in many fish vary with season. Watanabe et al.

(1985) found that extractive nitrogenous constituents of ascidian changed with season and observed a pronounced annual cycle with maximum in summer and/or autumn (414 mg) and minimum in winter (181 mg). Among the free amino acids of ascidian (Holocynthia roretzi), taurine was the most prominent and its content varied from collection to collection, being high in summer and autumn and low in winter and spring (Watanabe et al., 1983). The nucleotides content of ascidian also varied with season and Watanabe et al. (1985) found that the value in September (5.33  $\mu$  mol) was nearly four times high as that in January (1.33  $\mu$  mol). Trimethyl amine oxide content was higher in summer and autumn than in winter and spring. Afolabi & Oke (1981) reported that phenyl alanine and methionine were the limiting amino acids in January and April respectively caught blue whiting.

Love (1975a,b) reviewed the seasonal and environmental influences on the Atlantic cod flesh and observed that the sensory attributes like texture, flavour, odour, surface appearance and colour of the flesh varied according to season and the place where fish were caught. Several workers reported that season is one of the factor which affect the texture of fish meat with in a species (Sikorski et al., 1984; Dunajski, 1979; Howgate, 1977).

#### 1.2.2.2 Size

Size of the fish has some effect on the keeping quality



and it is a fact that large fish keep better than small fish (Connell, 1980). This can be attributed to the smaller surface area to volume ratio for larger fish so that in the same time period, less of the interior of larger fish is affected by spoilage microorganisms penetrated from the surface to the interior (Wheaton & Lawson, 1985). Another size effect is the flesh pH. Small fish of a given species tend to have a higher post-rigor pH than larger fish of the same species, thus giving rise to greater bacterial action (Connell, 1980).

#### 1.2.2.3 Food

The type of food that has been taken by fish is found to have significant effect on the flavour of its flesh. The influence of diet on flavour was demonstrated by Maligalig et al. (1973). They found that cat fish fed on a diet of turkey livers developed a liver like flavour within 19 days which became more pronounced as feeding continued and those fed on a cereal diet developed a cereal flavour in 33 days. The flavour described as "weedy" 'diesel' 'iodine' or 'sulphide' in flesh of certain fishes has been attributed to the presence of dimethyl sulphide (Connell, 1980). It generally occurs in the food of the fish. The planktonic bivalve mollusc known as petrophode is a food material for the fish. The petrophode contains dimethyl  $\beta$  propiothetin which is converted to dimethyl sulphide in fish. Ioda et al. (1985) isolated dimethyl sulphide and dimethyl  $\beta$  propiothetin from 20 species of

phaeophyta, 6 species of rhodophyta and 4 species of chlorophyta. Fresh water fish like tilapia and cat fish occasionally develop a muddy odour and flavour. Maligalig et al. (1973) noted that cat fish held in ponds developed a muddy taint associated with Anabaina ceratium and Rediastrum algae growing in the water. Lovell & Sackey (1973) reported that cat fish developed an "earthy-musty" taint within 2 days if held in tanks containing odour producing algae.

Ludovico-Pellago et al. (1984) noticed that overall degree of unsaturation of the fatty acid decreased as warm weather feeding occurred and less cold storage flavour developed. Artificial starvation of fish resulted in an increased proportion of polyunsaturated fatty acids in body lipids (Ramachandran Nai Gopakumar, 1981b). Takeuchi et al. (1987) studied the effect of starvation and consequent feeding in muscle components and fatty acid composition. They found that the muscle protein and visceral lipid decreased due to starvation and not so much muscle lipid during winter starvation. Following the periods of starvation, the adequate feeding leads to rapid, greater than normal growth of fish (Weatherly & Gill, 1981; Dobson & Holmes, 1984). Leo Margolis (1952) studied the effect of starving on the bacterial flora of the intestine of fish and found that in non-feeding fish bacteria is usually absent from the intestine.

#### 1.2.2.4 Fishing ground

Another factor which plays an indirect role on the quality of fishery product is the location of the fishing grounds (Jones, 1969). The flavour may vary from one ground to another and also in different seasons within the same species. This depends mainly on the nature of food available (Connell, 1980; Lovell & Sackey, 1973; Maligalig et al., 1973) and the physiological condition of the species. Winds, tides, water conditions and migratory patterns also have some influence on the condition and quality of fish before harvest. The time of year when spawning and subsequent poor condition develop in fish varies with the fishing grounds and may span a period of several months.

#### 1.2.2.5 Spawning

Sex plays a major role in quality soon after spawning. In certain species the females are of very poor quality soon after spawning while in some species such as salmon both sexes may be in poor condition after spawning (Wheaton & Lawson, 1985). During spawning the food reserves in the flesh are transferred for the development of gonads. Most fishes (except elasmobranches) do not feed during and for sometime after spawning and the flesh becomes depleted of fat, protein and carbohydrate. Once the fish resume feeding they normally recover their good condition. The quality changes brought by spawning are more pronounced in fatty fishes than lean fishes.

Connell (1980) found that the fat content in herring varied from less than 1% to over 25% between starvation period after spawning and resumption of feeding. During this change of the fat content the weight of the fish is maintained somewhat constant by a corresponding decrease in water content. Changes in the flesh composition as a result of spawning is noticed virtually in all species.

#### 1.2.2.6 Infection

Fish may be infected at times with parasites which may affect their quality. Most of these parasites and organisms fall in the category of protozoa, flatworms, round worms, bacteria and fungi (Wheaton & Lawson, 1985). Connell (1980) noticed that the protozoa infected Atlantic hake on storage in ice for few days appeared like toothpaste and the flesh was very soft. Generally flat worms appear in the viscera, but Connell (1980) found larval tapeworms in the flesh of halibut, weak fish and some white fish. Bacterial infections are wide spread and the lesions, nodules and festered areas found in commercially caught fish may be attributed to bacteria.

#### 1.2.2.7 Bacteria of Fresh fish

Fish are generally free from pathogenic bacteria except those fishes harvested from polluted waters. Spoilage of fish is caused by their naturally occurring microflora (Reay & Shewan, 1949; Liston, 1980). It is generally accepted that the flesh

of freshly caught healthy fish is sterile (Tarr, 1942; Shewan, 1962). The skin and gills may carry high loads of bacteria and the guts of fish which have been feeding. Marine fish have been found to have skin counts of  $10^3 - 10^7/g$ , gill counts of  $10^3 - 10^8/g$  and gut content counts of  $10^3 - 10^8/g$  (Shewan, 1962; 1977; Karthiayani & Iyer, 1967; 1971; Liston, 1980). Shewan (1962) found that bacterial loads on skin slime and gills changed with season and environment. The bacterial content of skate and sole (Liston, 1956), cod (Georgala, 1957) and sardine (Surendran & Gopakumar, 1982) showed seasonal changes while bacterial count of hake (Simmonds & Lamprecht, 1981) did not show any seasonal pattern of variation. Liston (1956) found markedly different bacterial load in different species of fish caught in the same place at the same time. Wood (1953) showed that the bacterial flora also changed with species of fish. Simmonds & Lamprecht (1985) remarked that where striking variations had been observed between different types of fish, they often reflected differences between broad classes such as flat fish versus round fish or cartilaginous fish as opposed to bony fish rather than narrow interspecies difference.

#### 1.2.2.8 Exertion

The rate and extent of glycolysis have profound effect on ultimate quality of fish. The rate of glycolysis varies with the species of fish and type of gear used. Struggling of fish during catching is sufficient to cause a partial depletion

of muscle energy reserves. Fraser et al. (1965) noted that cod from trap, boated and killed without apparent struggling had a high initial glycogen content (550 mg/100 g) while trawled offshore cod contained little or no glycogen, almost complete dephosphorylation and deamination of the nucleotide had occurred during the struggle involved in catching. Fishes that are very active in their normal habitat like tuna and mackerel may become excited and die in frenzied state in certain type of gears like seines (Dasow, 1976). He is of the opinion that certain type of gears eg. salmon gill nets may kill the fish after an exhausting struggle. Such activity before death results in rapid development of rigor mortis followed by earlier signs of deterioration during icing. The quality of fish caught by hook and line are better since they are brought to the boat swiftly and despatched quickly with a blow on the head. This type of clean kills are significant in extending freshness and quality. Tomlinson et al. (1965) found that the incidence of chalkiness encountered in fish taken by dragging was higher than that usually met within fish taken by longline. Tsuchimoto et al. (1985) studied the effect of catching under various operating conditions of trawlers in tropical water on the freshness of fishes. In general, the decomposition of glycogen and the accumulation of lactic acid are more rapid in fish subjected to struggle than instantly killed ones (Fraser et al., 1965; Dassow, 1976; Fukuda et al., 1979; Shimizu & Kaguri, 1986).

Botta et al. (1987a) found that caloric, moisture and protein constituents of Atlantic cod were significantly affected by the method of catching. Caloric and protein contents of cod caught by gill nets were significantly greater than those of cod caught by longline or trap. In contrast the moisture content of cod caught by longline or by trap was significantly greater than that of cod caught by gill net. This later phenomenon may be attributed to the hormone cortisol (17  $\alpha$ -hydroxy corticosteroid) which increases the water permeability of fish (Ranken & Bolis, 1984). Cortisol increases when fish struggle (Botta et al., 1987a). Gill net caught fish could therefore exhibit increased permeability which, because of the higher osmotic pressure of the surrounding sea water would result in a decreased moisture content of the muscle. Botta et al. (1987b) observed that method of catching significantly affected colour, discolouration/bruising, final overall grades and muscle pH. Love (1975 a,b) mentioned that difference in degree of struggling of fish caught by different methods probably produced difference in pH.

### 1.2.3 HANDLING AND CHILL STORAGE

#### 1.2.3.1 Post-mortem changes

Soon after death the blood circulation ceases and the cells are no longer supplied with oxygen. The tissue becomes depleted of vitamins and other important nutrients. Nerve and hormonal regulation of the metabolism also ceases. However,

the enzymes which normally carry out the various metabolic reactions are to a large extent, still active and undamaged. These enzymes cause the degradation of certain components in the muscle. The defence mechanisms against the invasion of microorganisms are unable to function in dead tissues. This results in the penetration and <sup>fr</sup> proliferation of bacteria into the dead fish.

During the early periods of post-mortem glycogen is converted to lactic acid through anaerobic glycolysis and phosphocreatin<sup>n</sup> is depleted. The adenosine triphosphate content decreases following depletion of phosphocreatin. When ATP content decreases to a critical level, chemical bonds are formed between actin and myosin. (Powrie, 1973). All these reactions are temperature dependant and can be slowed down by reducing the temperature. Species, seasons and biological variation affect the above factors.

#### 1.2.3.2 Spoilage factors

Many factors affect the spoilage of fish onboard. Castell et al. (1956) analysed various factors affecting the spoilage rate of fish in the trawlers at sea. They found that two conditions were the major causes of spoilage: treatment that resulted in very heavy initial contamination such as storing the fish directly against the slimy surface and treatments that resulted in a rise in temperature. When fish



are stored in contact with slime soaked wooden surface MacLean & Castell (1964) observed the development of bilgy odours due to the growth of an anaerobic bacteria in the slimy layer between the surface of the fish and wood.

Contamination occurs through different source, such as sea water, slime, faeces of fish, ice, landing centres and other incidental contamination. Some other factors which affect the spoilage rate are the care taken in handling, gutting and storing fish, atmospheric temperature and delay in chilling the fish. As season change some of these factors may lose or gain in importance. In general the spoilage of fish is a complex process and the pattern of spoilage is dependant on many factors (Stansby, 1958; 1963) already discussed.

#### 1.2.3.3 Bleeding

FAO (1977a) recommended the practices that should be followed in handling fresh fish intended for human consumption. Bleeding has been found to improve the colour and market acceptability (Connell, 1980). Wheaton & Lawson (1985) pointed out that the fish blood remain fluid for about 30 minutes after death at temperature just above freezing and tends to clot rapidly after this time or before at high temperatures.

#### 1.2.3.4 Gutting

Gutting is recommended for white fishes onboard and the purpose is to remove the guts which contain large number of

spoilage bacteria and enzymes (FAO, 1977a). Improper gutting is worse than no gutting. The product quality and shelflife is improved by bleeding and gutting (Anon, 1983; Scott et al., 1986; Vyncke, 1983; Ravesi et al., 1985; Castell & Greenough, 1956; Townley & Lauier, 1981).

#### 1.2.3.5 Slime

In live fish slime is immunologically active since it contains antibiotic substances and also represents a physical barrier against bacteria. But slime in the dead fish is an ideal growth medium for bacteria. Gillespie & Ostovar (1971) found that slime from freshwater fish was a good bacteriological medium and supported growth of  $10^9$  -  $10^{10}$  organism per gram. Growth of these bacteria on slime produces metabolic products with strong odours which penetrate into the muscle and make the flesh inedible even when the flesh contain few bacteria. Washing lowered the bacterial counts and obtained higher organoleptic ratings than unwashed samples (Gillespie & Ostovar, 1971).

#### 1.2.3.6 Chill storage of fish

Fish is preserved onboard mainly by stowing with ice. Methods of stowage vary but mainly consists of boxing, bulking and shelving (Anon, 1967; Eddie, 1980; FAO, 1977 a,b). These operations are possible when the catch is small but in trawlers the catch is large and hence the icing is difficult. Often

there is damage to the fish in the trawl due to crushing at the cod end and during discharge and handling on deck. Kordyl & Karnicki (1967) observed that deterioration of trawler catches were rapid and must be chilled immediately. In such cases, refrigerated sea water systems or chilled sea water systems are preferred (Merritt, 1974). This prevents further crushing and bruising.

Most of the spoilage reactions are temperature dependant. Autolysis, bacterial growth, other chemical reactions and the consequent decomposition of protein and fat increase progressively with rise in temperature. The effect of various chill storage temperatures on the degradative process and shelflife have been well documented (Dawood et al., 1986; Curran et al., 1980; Varma et al., 1983; de Book and Rommen, 1974; Londhal, 1981; Clucas, 1981; Ronsivalli & Baker, 1981; Lima dos Santos et al., 1981).

#### 1.2.3.7 Iced storage

The technical aspects of icing fish have been described by many workers (Ronsivalli & Baker, 1981; Lima dos Santos et al., 1981; Anon, 1967; Merritt, 1974). Small pieces of ice are more effective for cooling because of greater contact with fish and ice. Larger pieces of ice cause damage to the fish flesh by exerting point forces (Ronsivalli & Baker, 1981).

Biological factors and handling conditions affect the shelflife of iced fish. Wide variation in iced shelflife is

reported for the same fish from different locations. For cultured milk fish (Chanos chanos) an iced shelflife of 19 days (Gupta et al., 1980) and 14 days (Joseph et al., 1980) are reported. Yunizal & Arifuddin (1973) from Indonesia reported a shelflife of 11-16 days while Beza & Seson (1978) from Philipines found a shelflife of 21 days for milk fish. For tilapia (Tilapia mossambica) Varma et al. (1983) reported an iced shelflife of 11 days while for tilapia (Sarotherodon nilolica) Manthey & Karl (1984) found a shelflife of 21 days. For Indian mackerel (Rastrelliger kanagurta) the reported iced storage lives are 15 (Velenkar & Kamasastri, 1956), 6-8 (Perigreen et al., 1975), 21 (Banik et al., 1976), 7-8 (Hiremath et al., 1980), 8 (Kasemarn et al., 1967), 12 (Saisithi & Tipkong, 1978), 7 (Curran & Disney, 1979, Hussain, 1980) and 9 days (Rogers & Hoffman, 1980). In cod (Codrus moruha) iced storage lives varying from 11 to 15 days were reported (Cutting, 1953; Hansen, 1960; Shewan, 1962; Huss et al., 1974; Bramsnaes, 1965). Similar type of discrepancies in the iced shelflife of fresh water fishes are also reported. The fresh water fish marigal (Cirrhinus mrigala) had an iced shelflife of 36 days according to Nair et al. (1971) and 13-17 days by Bandhoyopathyay et al. (1985).

These discrepancies can be attributed to biological factors, handling methods and the techniques adopted for measuring spoilage. Despite these limitations the shelflife of many fishes

have been well established and several conclusions have been drawn (Lima dos Santos, 1981). In general flat shaped fish keeps longer than round shaped fish, red fleshed fish longer than high fat fish and teleost fish longer than elasmobranch fish (Bramsnaes, 1965). Also for round fish which is commercially the most important of the fish species the storage life on ice rarely extends beyond 15 days and is frequently less (Disney et al., 1974).

Fresh water fish are found to have a longer shelflife than marine fish. The shelflife of many fresh water fishes are more than 16 days (Nair & Dany, 1975; Bandhyopadhyaya et al., 1985; Pasteur & Herzberg, 1975; Disney et al., 1971; Joseph et al., 1988). These increased shelflife of fresh water fish can be attributed to the lower number of bacterial organisms in fresh water fish than in marine species (Shewan, 1977), differences in the microflora (Frazier, 1967; Kreuzer, 1954) and the absence of some components like trimethyl amine oxide (Dugal, 1967).

There are only limited studies on the effect of season on the ice storage life of fish. Lupen et al. (1980) found that hake had an iced storage life of 9-10 days during summer and in the remaining months the storage life under the same condition was upto 14-15 days. This was due to the difference in the biological condition of hake during and after spawning time, the shallow and temperate waters of the fishing grounds in summer and the heavy feeding after spawning. The studies of

Barassi et al. (1981) showed a great influence of biological factors on the keeping time in ice. The results suggested that in pre-spawning condition blue whiting could be stored upto 12 days while in post spawning condition the keeping time could not exceed 5 days. Mackerel also showed changes in iced storage characteristics and shelflife with season (Smith et al., 1980c). Love (1980) reviewed various biological factors affecting characteristics of fish.

#### 1.2.3.8 Chilled/Refrigerated Sea water

In chilled sea water (CSW) fish is surrounded by a mixture of ice and water while in refrigerated sea water systems (RSW) the heat from sea water is removed by mechanical refrigeration and the temperature can be reduced upto  $-1^{\circ}\text{C}$ . These systems are advantageous over using ice alone because cooling is fast, minimum damage to fish and maintenance of uniform temperature (Hulme & Baker, 1977; Ronsivalli & Baker, 1981). Baker & Hulme (1977) observed that in CSW many fishes may be scaled by agitation to which they are subjected by the rolling of the vessel. In many fishes such as herring, caplin, sardine etc. earlier development of discolouration and rancidity in CSW and RSW than iced fish were noticed (Roach & Tomlinson, 1969; Peter et al., 1965; Shaw & Botta, 1975; Perigreen et al., 1975). But fishes like perch, mackerel, seer, whiting, scad etc. were found to have better quality and shelflife in RSW/CSW (Licciardello et al., 1982; Perigreen et al., 1975; Smith et al., 1980 a,b,c).

#### 1.2.3.9 Vacuum Packaging

The merits and demerits of vacuum packaging have been thoroughly investigated (Johansen, 1965; Licciardello et al., 1967 a,b; Cann et al., 1965; Huss, 1972; Wilhelm, 1982). Vacuum storage is effective in reducing the growth of typical spoilage bacteria, but condition exist for the growth of Clostridium botulinum. Botulism can occur in products held at temperatures above 3°C. Vacuum packaging favours the growth of lactobacillai. Johansen (1965) found that the lactobacillai formed peroxides and acids which inhibited the growth of C. botulinum. Huss (1972) found that the shelflife of plaice can be extended by 6 days in vacuum storage at 0°C and Hansen (1964) noticed that herring and trout stored in ice became rancid in 6 days while under vacuum they did not become rancid during 20 days of storage

#### 1.2.3.10 Hypobaric and Hyperbaric storage

Hypobaric (low pressure system) and hyperbaric (high pressure system) storage are not generally used to fresh fish. The effect of hypobarric storage of Atlantic herring (Haard et al., 1979), cod, mackerel and herring (Varga et al., 1980) and shrimp and fresh water prawns (Mermilstein, 1979) were studied and reported. In all these studies the shelflife was found to be considerably extended. Charm et al., (1977) studied the hyperbaric storage of cod and reported that the shelflife was greatly extended.

### 1.2.3.11 Modified/Controlled Atmosphere storage

Modified Atmosphere Packaging - MAP - is a process by which the shelflife of a fresh product is increased significantly by enclosing it in an atmosphere which slows down the degradative process. The term "controlled atmosphere packaging" is also sometimes used. But this is an inaccurate description because the atmosphere inside any permeable package will change with time as gases diffuse into and out of the package at different rates as well as gases being absorbed and given off by the food in many instances (Wilhelm, 1982; Inns, 1987). Of the methods for modification of atmosphere,  $\text{CO}_2$  has been investigated most thoroughly (Killeffer, 1930; Coyne, 1933; Banks et al., 1980; Brown et al., 1980; Molin et al., 1983). Optimal results for prevention of spoilage bacteria were reported for  $\text{CO}_2$  concentration of 40-60% (Banks et al., 1980; Brown et al., 1980).  $\text{CO}_2$  stimulates the growth of gram negative bacteria such as *Lactobacillus* (Banks et al., 1980; Brown et al., 1980). Other gases such as nitrogen, ozone, ammonia etc. were also tried (Haraguchi et al., 1969; Subramanyan et al., 1965).

Extension of shelflife of chilled fish products by 4-10 days have been observed with good modified atmosphere package and temperature control (Brown et al., 1980; Banner, 1978; Bezanson, 1981; Banks et al., 1980). But highly pigmented fish in contact with  $\text{CO}_2$  may develop surface darkening probably due



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to oxidation of oxymyoglobin (Brown et al., 1980). Bezanson (1981) recommended that CO<sub>2</sub> packed aquatic products should be maintained below 1 to 2°C to get maximum benefit. The overwrap used should be impermeable to CO<sub>2</sub> and O<sub>2</sub> in order to maintain the desired atmospheric concentration in the package (Lindsay, 1981).

#### 1.2.4 BACTERIAL SPOILAGE OF CHILLED FISH

The spoilage of fish is caused mainly by bacterial activity. The level of proteolytic activity by muscle enzymes alone is relatively low (Liston, 1965). Proteolysis proceeds rapidly when caused by microbial growth. Shewan (1977) described a typical spoilage pattern for white fish during iced storage. For the first six days marked spoilage odours are virtually absent and the flesh is firm. This corresponds to the lag phase and the initial part of the logarithmic growth phase of bacteria. For the next four days the odour strengthens and becomes musty and the flesh becomes softer. This corresponds to the logarithmic phase. In the third stage the muscle becomes sour, sweet or bready and the flesh is definitely soft. At this stage the growth of microorganisms begins to slow down. In the fourth and final stage off odour producing substances like hydrogen sulphide and other sulphids are produced and the flesh is soft and slimy. This stage parallels the resting phase.

The rate of spoilage depends on the species of fish. During rigor mortis, the pH of many fish muscle drops to 6.2 - 6.4 which is possibly marginal for the growth of spoilage bacteria, while in flat fish the pH drops as low as 5.5 which is considered inhibitory (Simmonds & Lamprecht, 1985). Consequently flat fish have a longer shelflife than round fish. Shewan (1977) suggested that the slow spoilage in flat fish is due to the presence of large amounts of lysozyme in the surface slime of flat fish or that strains of bacteria causing spoilage are virtually absent from skin flora. The spoilage bacteria grows most rapidly in the gills and also grows in the surface slime, which is an ideal substrate for bacterial growth (Simmonds & Lamprecht, 1985). The organoleptic quality of fish flesh is not necessarily related to total bacterial population alone. The organoleptic quality is dependant upon the biochemical changes which are influenced by the strains of bacteria present and the type of fish. For example TMAO in the marine fish is reduced to TMA by some strains like Pseudomonas TMA has a strong offensive odour. Certain types of Pseudomonas produce sulphur compounds from sulphur containing amino acids (Shewan, 1977) Fruity odours arise from the degradation of amino acids glycine, serine and leucine to form esters of lower fatty acids (Simmonds & Lamprecht, 1985). Viscera is another important site of infection.

Spoilage at low temperature is caused by psychrophiles such as Pseudomonas and Achromobactor (Lerke et al., 1965). It is generally accepted that Pseudomonas dominates in cold water fishes as spoilage progresses (Shewan, 1977; Liston, 1980). In tropical fishes like sardine and mackerel, Surendran & Iyer (1976) and Surendran & Gopakumar (1981) found that though the number of Pseudomonas was low in the fresh fish it dominated after 21 days of iced storage.

#### 1.2.5 HISTAMINE IN FISH

Histamine is usually found in spoiled scombroid fish and other marine fish that have high levels of histidine in their muscle tissue (Arnold & Brown, 1978; Frank et al., 1981). The spoiled scombroid fish generally contain more than 100 mg histamine per 100 g muscle (Arnold & Brown, 1978). Many types of organisms belonging to proteus, enterobacter, vibrio, clostridium etc. contain histidine decarboxylase which convert histidine to histamine (Arnold & Brown, 1978; Yoshinaga & Frank, 1981). The effect of preprocess chilling on the production of histamine is studied by many workers (Putro & Saleh, 1985; Ames et al., 1987). They found that the histamine content of delayed iced fish was significantly higher than those immediately iced. The marine fish collected from local markets in India are found to contain the histamine well within the limits (Gopakumar et al., 1988; Vijayan et al., 1988).

### 1.2.6 PROCESSING OF FISH FOR FREEZING

Fish is usually packed as whole, gutted, gutted and headed, chunks, fillets and mince. Fish is converted into any of the above form depending on the convenience of handling, freezing, storage, transportation and the market demand. Many fishes find good market as fillets since it can be easily used for the formulation of many products.

#### 1.2.6.1 Fish fillets

Fillet is the strip of flesh cut parallel to the line of back bone. Fillet can be made as block fillet or single fillet. The details of filleting operations of different size and shape of fish are given by Roger et al. (1975) and Anon (1981). Zaitsev (1965) gave detailed account of various parameters involved in the production of chilled and frozen fish fillets. Zaitsev (1965) described the filleting methods, mechanisation of filleting, firming of fillets, packaging, chilling and freezing. The technological aspects of the production of fillet from different Indian fishes have been worked out by Perigreen et al. (1979). Curran et al. (1986 b) studied in detail about the effects of post-mortem state at the time of filleting and handling on the yield and quality of fillets.

#### 1.2.6.2 Minced fish

Minced fish is flesh separated in a comminuted form from the skin, bones, scales and fins of a wide range of fish species

It is a versatile but unstable commodity. It is mainly produced from underutilized species, fish by-catch and filleting waste. The flesh can be separated manually or mechanically. Grantham (1981) described the various type of mechanical methods of separation and their working principles. A belt and perforated drum system is used by Baader, Bibun, Prince etc. (Whitehorn, 1980). A screw feed and perforated cylinder system is used by Beehive (Taylor, 1972). Two concentric cylinders the inner perforated and rotating are used in Paoli (Miller, 1980).

Mincing is not a simple separation of flesh from bone and skin. The separation process fractionate the raw material into a range of distinct components which affect the texture, flavour and appearance of the mince. The bone fraction in the mince is found to be high in many cases. There are specifications for the limit of bone content (Krane, 1980) and many methods are suggested for its determination (Dawson et al., 1978; Patashnick, 1974; Bligh & Regier, 1976; Yamamoto & Wong, 1974).

A wide range of mince contaminants can damage texture stability. The enzymic breakdown product of TMAO, formaldehyde, causes degradation to texture by cross linking of proteins. Proteolysis of the minced fish causes considerable texture degradation. Even low levels of visceral contamination and catheptic enzymes cause extensive proteolysis of the mince

(Grantham, 1981; Cheng et al., 1979). High levels of polyunsaturated lipids dispersed in the mince predispose the products to problems of flavour and oxidative rancidity (Botta, 1974; Lee & Toledo, 1977; Tsukuda, 1978). The dispersion of the fat degrading enzymes, increased surface area and dispersion of fat degrading catalysts accelerate the oxidation of lipids. The haemoproteins from bone marrow, blood vessels and flesh catalyze the non-enzymic degradation (Lee & Toledo, 1977). Generally the mince has a darker colour than the raw material due to contamination by melanoid skin pigments, black belly membrane, blood and gut contents (King, 1973, 1977; Kiesvaara, 1979).

The operating conditions of the mechanical deboner has an important bearing on the quality of mince. A positive correlation is found between screen perforation size and bone content in the mince (Bligh & Reiger, 1976; Wong et al., 1978). An increasing pressure is found to reduce the water binding capacity (Belova, 1974; Webb et al., 1976) and damage protein functionality. Increasing pressure and shear are found to increase mince discolouration (FAO, 1977 b; Jauregin & Baker, 1980).

Many minces are subjected to a post-separation washing stage. The purpose is to remove inorganic salts, water soluble proteins, pigments, visceral contamination, bacteria and

decomposition products (Grantham, 1981). The fat content is found to reduce in many cases (Poulter & Disney, 1978), formaldehyde if present is completely eliminated and gel forming ability is improved. Washing improves the colour of the mince by removing the water soluble pigments, blood and visceral contamination but has little effect on muscle tissue that is inherently coloured (Grantham, 1981). Loss of substantial quantities of protein (Bligh & Regier, 1976) and soluble micro-nutrients such as vitamins, minerals and free fatty acids due to washing are reported (Sidewell, 1980).

#### 1.2.6.3 Chemical treatments

Many fish and fish products are treated with chemicals having <sup>a</sup> antioxidant, antimicrobial, cryoprotective or flavour enhancing activity to improve the retention of quality during frozen storage. Cryoprotectants such as sucrose (Chen & Sun 1984) glycerol (Love, 1962 c; Love & Elerian, 1965), polyphosphat (Mahon & Schneider, 1964; Tanikawa et al., 1963) and some amino acids (Peter & Srikar, 1981), antioxidants such as ascorbic acid, BHA, BHT, ethoxyquin, erythorbate, propyl gallate, sulphite and metabisulphate, thiodipropionic acid, tocopherol etc. (Lindsay, 1976; Bauerfeind & Pinkeri, 1970; Bilinski et al., 1979; Toyama & Shimazu, 1972), antimicrobial agents such as acetic acid and its salts, benzoic acid and its salts, propionic acid and its salts, sorbic acid and its salts and sulphur dioxide and

sulphites (Lindsay, 1976; Joslyn & Braverman, 1954) and flavour potentiators like disodium guanylate, disodium inosinate, disodium glutamate and sodium chloride (Lindsay, 1976) are used for fish and fish products.

#### 1.2.6.4 Antimicrobial agents

The use of antimicrobial agents depends on the nature of product, and type of preservation. For fresh fish Mitchell (1969) found that use of 0.1 to 1% by weight of ammonia preserved unevanescent anchovy in good condition for 2 weeks.

Subrahmanyam et al. (1965) dipped oil sardine in 1 N ammonia for 1 to 2 hours and kept in airtight container at a temperature of 30°C and found to have shelflife of 2 months. They found the treated fish were suitable for the preparation of fish flour. A chlorine dip is given to many fish and shell fish before freezing to reduce the bacterial load (Kosak & Toledo, 1981; Iyer et al., 1969). Many organic acids and their salts are used as antimicrobial agents.

#### 1.2.6.5 Antibiotics

Antibiotics had once importance in the preservation of fish particularly where it was incorporated into the ice used to chill the fish. oxytetracycline (OTC) and chlortetracycline (CTC) were found effective for reducing bacterial spoilage. They are both effective when dissolved in brine or in the ice used. The recommended concentrations incorporated into the ice



is 5 mg/L (Anon, 1968; Lueck, 1980). Anon (1968) found that whole fish remained fresh for 20 days under antibiotic ice while the control spoiled in 14 days. These antibiotics cannot be destroyed by the usual food processing techniques and this is considered as a disadvantage (Wheaton & Lawson, 1985).

#### 1.2.6.6 Antioxidants

Antioxidants are used to prevent oxidative rancidity in fish high in fats and may be applied as a dip or spray or as a glaze on frozen fish. They have little or no effect on preventing the growth of spoilage bacteria. When a single antioxidant is used it may not exceed 0.01% based on the fat content of the food and when more than one antioxidants are added the combined total may not exceed 0.02% of which no one antioxidant may exceed 0.01% (Dugan Jr., 1976). Antioxidants used in combination are sometimes more effective than an equal weight of single antioxidant. BHA and BHT, BHA and propyl gallate are synergistic but BHT and propyl gallate results in negative synergism (Dugan Jr., 1976). The use of antioxidants safely and effectively extends palatability, acceptability and nutrient value.

Certain acidic compounds such as citric acid, ascorbic acid are effective in sequestering metal prooxidant and provide increased keeping quality for seafoods (Anon, 1968).

#### 1.2.6.7 Chemical treatment and thaw drip

Many factors influence the amount of thaw drip. These may be inherent of the fish used, pre-freezing treatment, method of freezing and storage conditions (Dyer, 1969). Sodium chloride brine dips have been found to reduce thaw drip but is effective at higher concentrations (Holston & Pottinger, 1955). Various phosphates and polyphosphates have been effective in reducing thaw drip (Hallendoorn, 1962; Bendall, 1954; Ellis & Winchester, 1959; Dyer et al., 1964; Castell, 1966; Mathen, 1968, 1970; MacCallum et al., 1964). Sodium tripolyphosphate is the most commonly added phosphate in seafoods and it is often used along with sodium hexametaphosphate (Lindsay, 1976). The action may involve the influence of pH changes, ionic strength effects, and specific phosphate anion interaction with divalent cations and myofibrillar proteins (Hamm, 1971).

#### 1.2.6.8 Glazing and packaging of Fish and Fish products

Packaging has become an unavoidable necessity for frozen fish and fish products. Packaging serves many purposes. It protects the material from dehydration, avoids or reduces contact with air, prevents from external contamination and provides an aesthetic appearance. A good packaging material for frozen product should have high water vapour impermeability, high resistance to low temperatures, water repellent properties, satisfactory gas barrier, satisfactory aroma tightness, light

protection, good design for easy identification and consumer appeal (Almaker, 1965). In addition the packages must permit quick freezing and thawing.

Considerable extension of shelflife of cat fish fillets glazed and packed in polythene compared to unpacked fillets at  $-18^{\circ}\text{C}$  is reported (Perigreen & Joseph, 1980). Boyd et al. (1967) packed fish fillets in cartons with and without cellophane inner liner and found that loss in weight from frozen fillets packed with cellophane liners was approximately  $1/10^{\text{th}}$  amount of weight lost from frozen fillets without cellophane liners. Ahvenainen & Malkki (1985) found that packaging affected the quality of Baltic herring fillets under all storage conditions studied particularly under retailing conditions. Dehydration accelerates protein denaturation and oxidation of lipid and causes flavour deterioration. Dehydration causes freezer burn, an opaque dehydrated surface. This is due to the sublimation of ice on the surface region of muscle when the water vapour pressure of the ice is higher than the vapour pressure in the environmental air (Storey & Stainsby, 1970).

#### 1.2.6.8 Freezing rate

The freezing point of fish is about  $-1^{\circ}\text{C}$  and as the temperature is lowered below the freezing point, the ice content increases rapidly until a temperature around  $-5^{\circ}\text{C}$  is reached.

With further lowering of temperature the amount of water transformed to ice is relatively small (Dyer et al., 1957; Dyer, 1967). In haddock held at  $-1^{\circ}\text{C}$  about 10%, at  $-2^{\circ}\text{C}$  about 56%, and at  $-5^{\circ}\text{C}$  about 80% of the total water is frozen (Rudel, 1956).

The rate at which water is changed into ice is believed to have influence on the quality of frozen fish. This theory can be traced back to the work of Plank et al. (1916). But much experimental evidence available in this area suggest that apart from rarely used very slow freezing the rate of freezing has little influence on the quality of frozen fish (MacCallum et al., 1965; Nusbaum et al., 1983; Jul, 1984).

Freezing rate determines the location and size of ice crystals. Very quick freezing results in the formation of small ice crystals within the cell structures and comparatively little changes of the histological picture of the tissue (Plank et al., 1916; Bevilacqua & Zaritzky, 1980; Calvelo, 1981). At slow freezing rates, large ice crystals are formed partly inside the cell walls and seemingly causing rupture of these and partly between the cells (Plank et al., 1916; Bevilacqua & Zaritzky, 1980; Calvelo, 1981). In addition to the freezing rate the post-mortem conditions also influence the location of ice crystals. When Weld (1927) froze prerigor cod muscle intracellular crystals were formed, but when post-rigor muscle

was frozen at about the same rate extracellular crystals were evident. Love & Haraldson (1961) demonstrated that intracellular ice was formed in pre-rigor cod muscle regardless of the freezing rate.

MacCallum et al. (1965) studied the effect of fast freezing and slow freezing (0.7 and 2.6 h respectively through the temperature range of 32 to 5°F) on cod fillets and found that the quality of these fillets stored at -10°C were similar upto 22 weeks based on taste panel assessment. But Bilinski et al. (1977) found significant increase in crystal size in chum salmon frozen slowly (14.5 h through the temperature range of 0° to -5°C) compared with the quick frozen salmon (1 h). These effects were not suppressed by prolonged frozen storage. Sebranek (1982) quotes several cases where very rapid freezing of fish and shell fish leads to better texture. There are certain instances where very rapid freezing lead to quality deterioration For eg., when fish fillets are frozen in liquid nitrogen the surface layers are often frozen so rapidly that considerable internal strain develops, surface ruptures occur, and an inferior product is the result (Rasmussen & Olson, 1972).

One of the quality factors which show some relation to freezing rate is drip (Jul, 1984). In the normal range of freezing rates used commercially drip is practically independent of freezing rate (Jul, 1984). A detailed literature study of

freezing rate as related to drip and quality was given by Kondrup & Boldt (1960). Thomas & Mathen (1987) found no difference in thaw drip for block frozen prawns ~~freeze~~<sup>freeze</sup> in 1½ and 3 h respectively during 7 months storage at -18°C. Jul (1969) reviewed various experiments regarding the influence of freezing rate on drip and found that very differing results are obtained in different experiments.

Jul (1984) reported that no difference in taste was found between freezing rates of 0.15 cm/h and 5 cm/h for cod fishes and recommended a freezing rate of not less than 0.5 cm/h for fish. Love & Ironside (1958) reported that protein solubility values of cod muscle frozen at different rates were about the same when the muscle were thawed without storage.

#### 1.2.7 CHANGES DURING FROZEN STORAGE

Frozen fish and fishery products stored at subfreezing temperatures are not completely frozen and inert and they undergo slow deterioration during storage. The rate of deterioration during frozen storage depends on temperature and type of product and decreases as the temperature is lowered. However, different products stored at the same temperature can exhibit different rates of deterioration. Deterioration of frozen stored product can occur by physical or chemical means but not by the growth of microorganisms.

### 1.2.7.1 Physical changes

The major physical changes occur in frozen fishery products are freezer burn and recrystallisation (Fennema, 1973). Freezer burn occur on the surface of improperly packed fish products during storage. Freezer burn results in an opaque dehydrated surface. It is caused by the sublimation of ice on the surface of muscle when the water vapour pressure of ice is higher than the vapour pressure in the environmental air (Storey & Stainsby, 1970). Kreass (1961), Kreass & Weidemann (1969), Storey & Stainsby (1970) and Tehigeov & Vereshchagen (1970) found that the development of freezer burn on the muscle surface has been influenced by the difference in vapour pressure of ice and the partial pressure of water vapour, air velocity, storage temperature, post-mortem condition of muscle and rate of freezing. A histological study by Kreass & Weidemann (1967) revealed that the freezer burn region consisted of cavities which were previously occupied by ice crystals and which were enlarged by shrinkage of the fibers and bundles during dehydration.

Dehydration results in weight loss. It occurs during freezing and frozen storage. Kaminarskaya & Piskarev (1970) reported that when fish was frozen in air blast tunnels in trays with and without covers the weight losses were 0.5 and 1% respectively. In big fish like sturgeon freezing in tunnel causes weight loss of 1.3%. In frozen fish stored as blocks

in cartons the standard weight losses for 3 months are 0.32 and 0.26% in warm and cold seasons respectively. (Kaminarskaya & Piskarev, 1970). Glazing considerably retards the weight loss of fish because the evaporation of glaze takes place initially. When the initial glazing of fish is 3.5% about 30% of the glaze was lost in 5.5 months storage and complete glaze was evaporated after 8 months storage (Kaminarskaya & Piskarev, 1970). They also reported that during storage of frozen cod at  $-18^{\circ}\text{C}$ , a variation of  $\pm 2^{\circ}\text{C}$  resulted in defrosting-refreezing of 1.2% water, at  $-12^{\circ}\text{C}$  it will be 2.6% and at  $-25$  to  $-30^{\circ}\text{C}$  it will be only 0.15% and temperature variation of  $\pm 1^{\circ}\text{C}$  lead to changes in the amount of frozen out water by 2 times less. The amount of moisture loss by plate freezing, air blast freezing, fluidised bed freezing, liquid nitrogen and liquid freon freezing are 0.5, 3, 1, 1.4 and 0.1% respectively (Rasmussen, 1967; Brown, 1967; Lawler & Traubirman, 1969). In air blast freezing method the moisture loss may vary from 0.5 to 6% depending on the conditions. The moisture loss in liquid nitrogen freezing can be reduced to about 0.5% if nitrogen is used at a rate of 1.5 lb per pound of product (Astrom, 1971).

Ice crystals are unstable and they undergo metamorphic changes during frozen storage. The changes in the number, size, shape, orientation or perfection of crystals after completion of initial freezing are called recrystallisation (Burgers, 1963) Love (1962a) observed enlargement of ice crystals in cod exposed



to temperatures fluctuating between -14 and -7°C. Recrystallization occurs because the system has a tendency to attain a state of equilibrium wherein the free energy is minimized and chemical potential is equalized among all phases. The free energy is minimized when the crystal structure is perfect and its size is infinite. Fennema (1973) classified various types of recrystallisation as iso-mass, migratory, accretive, pressure - induced and irruptive. Fluctuating temperatures and associated vapour pressure gradients enhance recrystallisation.

#### 1.2.7.2 Chemical changes

The major chemical changes taking place in fish products during frozen storage are lipid oxidation and hydrolysis, protein denaturation, degradation of vitamin and THAO and enzymic discolouration. The chemical composition of fish muscle has great influence on the quality of frozen product. The amount and type of lipid govern the extent of rancidity during frozen storage. The thaw exudate is dependant on initial pH and concentration of inorganic ions. The toughness and juiciness of fish muscle is related to protein changes.

#### 1.2.7.3 Changes in lipids

The major chemical changes in frozen stored fish muscle lipids are autoxidation and hydrolysis. The development of rancidity is caused by the accumulation of carbonyl compounds

formed during autoxidation of muscle lipids (Ackman & Cormier, 1967; Awad et al., 1968; 1969; Lea, 1962). Enzymic hydrolysis of fish lipids liberates free fatty acids (Ackman & Cormier, 1967).

#### 1.2.7.4 Lipid oxidation

During frozen storage unsaturated fatty acids are oxidised in the presence of oxygen to hydroperoxides which decompose to aldehydes, ketones and acids, constituents responsible for rancid odour (Ackman & Cormier, 1967; Lea, 1962; Allen & Foegeding, 1981; Frankel, 1984). Both initiation and breakdown of oxidative degradation of lipids can be enzymatically or non-enzymatically mediated (Hsieh & Kinsella, 1986). Enzymes such as lipoxigenase (Josepson et al., 1984; German & Kinsella, 1985; German et al., 1985) peroxidase (Kanner & Kinsella, 1983) and microsomal enzymes from muscle tissues (McDonald et al., 1979; Rhee et al., 1984) can potentially initiate lipid peroxidation. The enzymes are found in different parts of fish. Hsieh et al. (1988) found the enzyme lipoxigenase at the gills and skin tissues. When fish is killed and tissue damaged certain enzymes such as lipoxigenase of fish gills and skin (German & Kinsella, 1985, 1986) peroxidase of fish blood (Kaner & Kinsella, 1983) and microsomal NADH peroxidase of fish muscle (Slabyj& Hultin, 1984) may become uncontrolled and initiate lipid peroxidation. These hydroperoxides are potential

precursors of many compounds such as hexanal, 4 heptanal and 2,4 heptadienal (Josephson et al., 1984).

Positive correlation between organoleptic rancidity and amounts of peroxides and malonaldehyde like compounds have been reported. Greig (1968) observed that the rancidity scores of a taste panel and peroxide and TBA values were highly correlated. Awad et al. (1969) found that as the level of organoleptic rancidity in white fish increased, the TBA value rose progressively. The rate and extent of autooxidation of lipids is dependent on the degree of fatty acid unsaturation, prefreezing holding period, the duration and temperature of frozen storage, oxygen content of the environment and the presence of pro and /or antioxidants (Kanner et al., 1988). The fatty acid composition of fish is mainly governed by the species and diet (Lovern, 1964; Ramachandran Nair & Gopakumar, 1981a).

Fish muscle in general contains high concentration of  $C_{16:1}$ ,  $C_{18:1}$ ,  $C_{20:5}$  and  $C_{22:6}$  fatty acids (Ackman, 1967b; Ueda, 1967; Lovern, 1964; Yamada & Hayashi, 1975). The proportion of these fatty acids varies from species to species. In Atlantic cod and pink salmon  $C_{20:5}$  and  $C_{22:6}$  fatty acids make up 32-34% of the total muscle lipid whereas lipids of Pacific halibut and herring contain 16-18% of these acids (Gruger et al., 1964). The rate of autoxidation of fatty acids increases exponentially with the number of methylene interrupted double bonds (Lundberg, 1962). The development of oxidative rancidity in fish muscle is

expected to occur at a fast rate. When unfrozen fish muscle is stored at 0°C fish lipids undergo autoxidation in a few days (Liston et al., 1961; Hansen, 1964).

The length of prefreezing storage influence the extent of rancidity development in frozen stored products (Hansen, 1964). But Connell & Howgate (1969) found that the development of off flavour in frozen haddock during storage at -14°C was not influenced by prefreezing storage in ice. The rate of autoxidation of fish lipids is directly related to the frozen storage temperature. Banks (1938) and Tarr (1947) reported that at -28°C the peroxide value increased very slowly with storage time in herring and salmon. According to Castell et al. (1966) the TBA Value of lipid in frozen cod stored at -23°C or -18°C did not change over a period of 270 days. Ke et al. (1977) found that the oxidation of skin lipids of mackerel was rapid at -15°C but was effectively inhibited by lowering the temperature to -40°C. Slavin (1968) compiled data on frozen pollock and haddock fillets and observed that rancidity is reduced and the shelflife was increased as the storage temperature was reduced to -29°C.

The peroxide value rose to a maximum and subsequently declined to a minimum during frozen storage of fish muscle (Awad et al., 1969; Dyer et al., 1950). Nair et al. (1979) observed that peroxide value reached maximum in skin and muscle lipids of sardine at -18°C after four weeks storage and then

decreased. The skin lipids showed a higher peroxide value than the muscle lipids. The decrease in PV is an indication that the peroxides are decomposed faster than they are being formed. But the TBA values in white fish muscle ( $-10^{\circ}\text{C}$ ), cod muscle ( $-12^{\circ}\text{C}$ ) and mackerel ( $-18^{\circ}\text{C}$ ) increase progressively with storage time (Awad et al., 1969; Castell et al., 1966; Pawar & Magar, 1966). But Nair et al. (1979) noticed that the TBA values in frozen stored ( $-18^{\circ}\text{C}$ ) sardine reached a maximum in 22 weeks in the skin and muscle lipids. Also they found that the development of TBA reacting substances is faster in the skin than the muscle.

Naturally occurring antioxidants like tocopherols in frozen muscle are effective to retard autoxidation of lipids (Ackman, 1967a; Sheltaway & Olley, 1966). Species and individual muscle differ in their ability to store dietary tocopherol. The dark muscle of cod contains about twice as much tocopherol on a lipid basis as white muscle (Ackman & Cormier, 1967). Tocopherole in sole caught in the period from June to early August were decomposed in four months frozen storage while tocopherols and lipids in sole caught in other times of the year were significantly more stable (Ackman, 1967a).

#### 1.2.7.5 Free fatty acids

The Free Fatty Acids (FFA) content of unfrozen muscle is dependent on factors like species of fish, type of muscle, post-mortem holding time etc. (Anderson & Ravesi, 1968; Lovern &

Olley, 1962). In iced storage studies of fish Lovern & Olley (1962) noticed that FFA content increased only slightly over the first 10 days and thereafter FFA accumulation increased sharply. Olley et al. (1962) found that FFA content in 11 species of marine fish stored unfrozen for a short period ranged from 0.6 to 5.9%. There is only few reports regarding the changes in FFA during freezing (Olley et al., 1962).

Literature on the FFA formation during frozen storage is plenty and many found that FFA accumulated during frozen storage of many fishes (Anderson & Ravesi, 1970b; Olley & Lovern, 1960; Awad et al., 1968, 1969; Dyer & Morton, 1956). The formation of FFA is directly related to frozen storage temperature (Olley & Lovern, 1960; Anderson & Ravesi, 1970b). But in some fish the FFA content does not change appreciably regardless of the frozen storage temperature. For example, Dyer et al. (1956) found that the FFA content of rose fish fillets did not change during 42 weeks of storage at either -12 or -23°C.

The formation of FFA in frozen stored muscle is caused mainly by enzymatic hydrolysis of phospholipids and partly by enzymatic hydrolysis of triglycerides. (Awad et al., 1968; 1969; Bilinski & Lau, 1969; Bligh, 1961; Nair et al., 1979). Olley et al. (1962) reported that phospholipids in muscles from cod, halibut and lemon sole were hydrolysed rapidly during the first 20 weeks of storage at -14°C. Bligh & Scott (1966) found a decrease in phospholipid content from 84 to 32% of the

total lipid after storing cod muscle at  $-12^{\circ}\text{C}$  for 12 months. A number of phospholipases have been detected in fish muscle (Bilinski & Jonas, 1966; Olley et al., 1962; Turkowski & Brockerhoff, 1965).

#### 1.2.7.6 Protein denaturation

Protein denaturation of frozen stored fish causes deterioration in quality as a result of textural changes such as the development of extra firmness, toughness, springiness, dryness, rubbery texture, lack of succulence, loss of water holding capacities or loss of juiciness (Shenouda, 1980). Freezing and frozen storage are believed to furnish favourable conditions for the irreversible denaturation of fish muscle proteins. Different methods are used to quantify the deteriorative changes in protein which involve the estimation of the extractability of fish proteins (total protein, protein groups or protein species), textural changes (thaw drip, water holding properties and objective texture measurements) properties of extracted proteins (viscosity, specific volume) enzyme activity and amount of low molecular weight degradation products.

Numerous studies show a direct relationship between decrease in protein extractability and increase in toughness in frozen fish muscle (Powrie, 1973). When compared to myofibrillar proteins, sarcoplasmic proteins are more stable

and their solubility remain unchanged except after a long storage time. In myofibrillar group myosin is more sensitive to denaturation and actin shows only a small change (Connell, 1960).

The rate and extent of decrease in protein extractability in frozen stored fish muscle is dependent on the species, prefreezing post-mortem state of the muscle, rate of freezing, storage temperature and time.

Changes in the protein extractability of various fishes showed that in lean fish such as lizard fish, cod, haddock and dog fish the protein extractability decreased fairly rapidly when compared to fatty fishes such as yellow tail, rose fish and sole (Dyer & Dingle, 1961; Simidu & Simidu, 1957). Because of the denaturation of myofibrillar proteins during frozen storage some fish cannot produce high quality frozen product (Tahata et al., 1975; Jiang, 1977; Nosaki et al., 1978; Chou & Chou, 1980). The protective effect of moderate levels of lipid is credited to the neutral lipid fraction such as triglycerides which diminish or counteract the detrimental effect of free fatty acids (Shenouda, 1980).

The protein extractability in frozen stored fish muscle is governed by the prefreezing state of the muscle and degree of muscle contraction during storage (Connell, 1968; Heen & Karshi, 1965; Love, 1966a). Love (1962b) found that protein in



pre-rigor cod muscle fillets was insolubilized at a slower rate than the protein in post-rigor muscle during frozen storage. Nikkila & Linko (1956) were unable to detect any changes in protein extractability for 35 days in Baltic herring at  $-20^{\circ}\text{C}$  but extensive insolubilization of protein occurred in pre-rigor muscle.

Cryogenic freezing of fish and shellfish does not cause a significant change in texture and protein extractability (Suyuki et al., 1964; Sebranek, 1982). Love & Ironside (1958) found that the protein solubility of cod muscle were about the same at different rates of freezing. Some studies showed that the rate of freezing has some influence on the protein extractability during storage (Love, 1958).

The rate of insolubilization of protein is dependent on storage temperature. Tomlinson & Geiger (1963) and Love & Elerian (1964) reported that maximum protein insolubilization occurred in the temperature range of  $-1$  to  $-5^{\circ}\text{C}$ . The protein stability is increased as the storage temperature is reduced (Love, 1966b). But Curran et al. (1980) did not find significant change in the percentage of protein soluble in 5% NaCl at  $-15$  and  $-30^{\circ}\text{C}$  for 20 months in the Bolivian fish, sabalo. Shaban et al. (1985) found that in Alaska Pollack surimi the protein denaturation gradually decreased as temperature is lowered and little or no denaturation occurred below  $-40^{\circ}\text{C}$ .

Many factors such as changes in moisture, lipids and activity of specific enzymes cause protein denaturation during frozen storage of fish (Shenouda, 1980; Sikoriski et al., 1976). The formation and accretion of ice crystals, dehydration of cells and increase in salt concentration in the tissue during freezing and frozen storage cause damage to the protein. Jarenback & Liljemark (1975) found a significant decrease between the contractile proteins after long frozen storage periods. The reduction in the distance between the filaments favours the formation of cross bridges between them and stiffens the fibers. Such aggregation denaturation is caused by the formation of hydrogen, hydrophobic and disulfide bonds (Jiang et al., 1988) Many hypothesis and postulations are currently proposed to explain the binding in aggregated proteins (Sikorski et al., 1976; Sikorski, 1978, 1980; Jiang et al., 1987). The removal of water from protein molecules through freezing result in the disruption of the natural net work of protein and leave the protein molecules unprotected and vulnerable. This causes hydrophobic-hydrophobic and hydrophilic-hydrophilic interreactions within the same protein molecule or between adjacent protein molecules inducing aggregation.

During freezing the concentration of solutes in the unfrozen water increases. At  $-20^{\circ}\text{C}$  more than 90% water is frozen leading to about tenfold increase in the concentration of soluble solutes (Shenouda, 1980). The increase in salt

concentration affect the secondary forces such as ionic, van der Waals, hydrogen and hydrophobic forces, which help to stabilize the tertiary and quaternary configuration of protein molecules. The net result will be a mixture of dissociational aggregational and conformational changes (Shenouda, 1980; Sikorski *et al.*, 1976). Myosin is a very sensitive myofibrillar protein at high ionic strengths (Godfrey & Harrington, 1970) and experiences a rapid reversible monomer-dimer equilibrium. Actin is relatively stable during frozen storage (Connell, 1960). But the presence of excess  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  cations changes the nature of actin and the molecules become more water repellant (Shenouda, 1980).

The action of fish lipids on protein depends on the state of the lipids. The presence of moderate levels of lipids has a protective effect on protein and presumed to diminish the detrimental effect of free fatty acids (Dyer, 1951; Shenouda, 1980). Glycerol has a cryoprotective effect on protein (Love & Elerian, 1965) and similar effect of lipids cannot be excluded. Lecithin is found to have a protective effect on actomyosin (Ikeda & Taguchi, 1967; Taguchi & Ikada, 1968). Studies of model system by Shenouda & Pigott (1974, 1975, 1976 & 1977) showed that lipid and protein extracted from the same fish when incubated together insoluble lipoprotein complexes were formed. This led to the hypothesis that lipid and protein from different localities in the cell form unconventional lipid-protein

complexes dissimilar to natural lipoprotein complexes.

Several studies found a correlation between decreased protein extractability and accumulation of FFA in frozen stored fish (Olley et al., 1962; Lovern & Olley, 1962; Shenouda, 1980). Lovern & Olley (1962) found maximum lipid hydrolysis at  $-4^{\circ}\text{C}$ . King et al. (1962) showed that polyunsaturated lipids insolubilized more fish myofibrillar proteins than did less unsaturated ones and shorter fatty acids were more powerful than high molecular weight FFA. The FFA protein interaction is considered to occur primarily through secondary forces - electrostatic, van der Waals, hydrogen and hydrophobic forces (Hanson & Olley, 1965; Anderson & Ravesi, 1970a; Sikorski et al., 1976).

The oxidised lipids interact with proteins causing undesirable changes in the nutritional and functional properties of protein. During frozen storage the product of lipid oxidation renders the fish tissue proteins into harder, more elastic insoluble complexes (Takama et al., 1972; Takama, 1974). The intermediate free radicals of lipid peroxidation may form protein free radical complexes which in turn could initiate various reactions with other proteins or lipids (Varma, 1967; Karel et al., 1975; Schaich & Karel, 1975). The stable oxidation products such as carbonyls react with side chain groups of protein (Varma, 1967). It is believed that the oxidised products of lipids attack specific susceptible functional groups of

proteins (Kuusi et al., 1975) and increase the hydrophobicity of protein making them less water soluble.

In certain marine fishes the TMAO present in their muscle are converted into formaldehyde and DMA by the enzyme TMAOase (Castell et al., 1971; Harada, 1975). Studies showed that the tissues containing formaldehyde became tougher and their ability to hold water increased but they lacked the juicy and moist mouth feel desired (Shenouda, 1980). The presence of formaldehyde caused a noticeable decrease in the extractability of proteins (Childs, 1973). Formaldehyde has the ability to bind covalently to various functional groups in the proteins. Walker (1964) identified various reaction sites in the protein molecule sensitive to formaldehyde attack. Ostyakova & Kosvina (1975) found that formaldehyde accelerated the hydrolytic decomposition of fish lipids.

Jiang et al. (1983a) reported the interaction of protein with amino acids. By studying the solubility, ATPase activity and electrophoretic evidence Jiang et al. (1983b) further suggested that the L forms of taurine, lysine and histidine accelerated actomyosin denaturation while glutamic acid, glycine and proline protected the proteins.

From the above review it can be seen that most of the studies on quality aspects and its impact on processing and frozen storage shelf-life have been carried out on fishes from

cold waters. Many information which are highly essential for proper handling, processing and preservation of the tropical fishes are lacking. This necessitates the need for detailed studies on the various aspects of chill and frozen storage of aquatic products. With this view, detailed investigations were carried out on the iced storage behaviour and shelflife of certain fishes from fresh and brackish water environments and molluscs. The effect of season and size of the fish on the iced shelflife were also conducted. Detailed studies were carried out to find out the impact of method of handling and duration of chill storage on the characteristics and shelflife of milk fish and squid. The effect of frozen storage temperature in retaining the characteristics, retarding rancidity and improving shelflife of mackerel were also investigated. The influence of antioxidants in reducing rancidity and improving shelflife of mackerel were studied. The effect of processing such as filleting, mincing, packaging, glazing etc. on the frozen storage characteristics was determined. The improvements in storage quality and functional properties of mince by washing as well as mixing with spices during frozen storage was investigated.

## 2. MATERIALS AND METHODS

### 2.1 MATERIALS

#### 2.1.1 FISH

The fishes used for the studies and <sup>their</sup> ~~its~~ scientific names are given below:

<u>Common Name</u>	<u>Scientific Name</u>
Catfish	<u>Trachysurus</u> spp.
Horse mackerel	<u>Megalaspsis cordyla</u>
Lizard fish	<u>Saurida tumbil</u>
Mackerel	<u>Rastrelliger kanagurta</u>
Milk fish	<u>Chanos chanos</u>
Mrigal	<u>Cirrhinus mrigala</u>
Rohu	<u>Labeo rohita</u>
Squid	<u>Loligo duvaucelli</u>
Threadfin bream	<u>Nemipterus japonicus</u>

Rohu and mrigal were collected from a fresh water pond ~~were~~ cultured fishes are grown and milk fish from CMFRI/State Fisheries pond in brackish water environment. These fishes were caught live and killed immediately by giving a blow to the head. A few samples were allowed to struggle. Squid was collected onboard the CIFT research vessel and was caught in experimental trawling. It was iced immediately after

catching. Mackerel was collected from country craft in rigor condition. Cat fish, horse mackerel, lizard fish and threadfin bream were collected from fisheries harbour Cochin soon after landing. All the samples were iced immediately. Fishes were sorted and only extremely fresh and uniform sized samples were used for experimental studies.

#### 2.1.2 CHEMICALS

Analytical grade reagents supplied by B.D.H. (India); E. Merck (Indi) and Sarabai M. Chemicals were used for experiments.

### 2.2 METHODS

#### 2.2.1 ICED STORAGE

All the fishes were iced immediately after capture or collection. Finely powdered block ice was used for the purpose. Only two layers of fish were packed in the box and each layer was iced in the bottom and above with sufficient quantity of powdered ice. Every day sufficient ice was added to supplement the loss due to melting. A part of the milk fish was bled immediately after capture and gutted and iced. Squid was iced as whole and as mantles.

#### 2.2.2 PROCESSING AND PACKING

Squid was divided into three batches and the following operations were carried out.



1. Dressed mantles were packed in  $\frac{1}{2}$  kg carton lined inside with 100 gauge polythene.
2. Dressed mantles were iced for one day and packed in  $\frac{1}{2}$  kg carton lined inside with 100 gauge polythene.
3. Iced whole squid after one day was dressed and the mantles packed in  $\frac{1}{2}$  kg carton with 100 gauge polythene lining.

Milk fish was frozen after gutting and washing properly as individual pieces. Each pieces were glazed in chilled water at 0 to 1°C after freezing and packed individually in polythene sheets of 100 gauge. Frozen samples were prepared after icing for 0, 4, 7, 10 and 14 days.

Whole mackerel after proper washing was packed in  $\frac{1}{2}$  kg cartons lined inside with 100 gauge polythene sheets and sufficient chilled water was added as glaze. Also mackerel was frozen individually, glazed in chilled water and packed in polythene sheets. A part of the mackerel was treated with 0.01% BHA solution for 15 minutes and packed as blocks of  $\frac{1}{2}$  kg in cartons lined with polythene sheets and glazed. The BHA was dissolved in minimum amount of oil and then dispersed in water.

Fillets were prepared by making a cut upto the back bone at the neck side and then cutting the flesh parallel to the back bone. The skin of the fillets were then removed using a

sharp knife. These were packed individually and as blocks with and without polythene lining in waxed cartons. One part of whole cat fish were frozen and stored at  $-20 \pm 1^\circ\text{C}$  for one month, thawed, filleted and refrozen.

Minces were prepared from cat fish, lizard fish, horse mackerel and threadfin bream. Mince from cat fish was prepared from the fillets using a meat mincer. The other fishes were headed, gutted and split opened and fed into meat <sup>bone</sup> ~~bone~~ seperater (Bader). These were frozen as blocks of  $\frac{1}{2}$  kg in polythene lined waxed cartons. Part of the mince from mackerel was mixed with 0.1% cloves powder and packed as above. One part of the minces from lizard fish, cat fish and threadfin bream were washed in chilled water (4 volumes), twice, filtered through a nylon mesh net and squeezed to a moisture level of 80-82%. These also were packed in  $\frac{1}{2}$  kg carton lined with polythene and frozen.

### 2.2.3 FREEZING AND COLD STORAGE

All the samples were frozen in a Jackston contact plate freezer at  $-40^\circ\text{C}$ . Approximately 2 hours were taken to freeze the sample. These frozen samples were stored at  $-20 \pm 1^\circ\text{C}$ . Part of the frozen mackerel blocks were stored at  $-20$  and  $-30^\circ\text{C}$  for frozen storage.

### 2.2.4 SAMPLING FOR ANALYSIS

In small fish about 10 fishes were taken and the complete

meat was separated and minced in a meat mincer. In fishes like rohu and mrigal half fillets from four fishes were taken, cut into small pieces and minced in a meat mincer. Sufficient amount of mince was prepared for all chemical analysis. All tests were carried in duplicate/triplicate and the average value was taken.

#### 2.2.5 CHEMICAL ANALYSIS

The samples were analysed for total nitrogen (TN), moisture, fat, ash, non-protein nitrogen (NPN),  $\alpha$ -amino nitrogen total volatile base nitrogen (TVBN), trimethylamine (TMAN), trimethylamine oxide (TMAO), peroxide value (PV), free fatty acids (FFA), thiobarbituric acid value (TBA), salt soluble nitrogen (SSN), sodium and potassium. Total nitrogen, moisture, fat, ash and non-protein nitrogen were determined by the method of AOAC (1984).  $\alpha$ -amino nitrogen was determined by the method of Pope and Stevens (1939). Total volatile base nitrogen and trimethylamine nitrogen were determined by the method of Conway (1947). Trimethylamine oxide was determined from the trichloroacetic acid extract after reducing it with Devardas alloy by the method of Dyer et al. (1952). The TMAN was determined before and after reduction by Conway (1947) method and the difference in the values gives the TMAO nitrogen.

The method of Lea (1952) was used for determining the peroxide value and FFA was determined by the method of AOCS

(1946). Thiobarbituric acid value was determined by the method of Tarladgis et al. (1960). Salt soluble nitrogen was determined by the method of Dyer et al. (1950).

To determine sodium and potassium the fish samples were ashed and they were dissolved in 1:1 HCl, filtered and filtrate was made upto 100 ml. The flame photometer was calibrated using 10 ppm sodium solution. The samples were diluted if necessary to bring the reading within the range of the standards. A few ml of the diluted sample was charged into the flame photometer and the reading was noted and from this the amount of Na was calculated.

A 5 ppm solution of potassium was used to calibrate for potassium in the flame photometer. A few ml of the appropriately diluted ash solution was charged into the photometer and from the reading the amount of potassium was calculated.

#### 2.2.6 PHYSICAL ANALYSIS

To determine pH about 10 gm of the muscle was blended with an equal quantity of water and the pH was determined using a pH meter.

To determine the weight loss during storage the weight of the sample was taken just before keeping it in the storage and the weight were taken at periodic intervals. The difference in weight was taken as the weight loss on storage.

Weight loss on thawing was determined by the following method. In unglazed samples the difference in weight before and after thawing was taken as the weight loss on thawing. In glazed samples the initial weight of the sample packed and the weight after thawing was used to determine the weight loss.

The weight loss in cooking was determined by taking the difference in weight before and after cooking for 10 minutes in boiling water. From the above value percentage of cook drip was calculated.

#### 2.2.7 SENSORY EVALUATION

A taste panel consisting of 12 members from the staff of CIFT was selected. The selection was made on the basis of their ability to discriminate the difference in various samples and also to repeat the judgements. 10 members were called for each experiment.

Samples for testing was prepared according to the size of the fish. Small fishes were dressed and cut into two pieces while big fishes were filleted and cut into pieces of suitable size. Precautions were taken to select materials so that it was representative of the product under study. These were then cooked in boiling 2% brine for 15 minutes and served hot (50 - 60°C) to the taste panel members to assess the appearance, texture and flavour and to assess the overall quality based on the above factors. They were asked to represent their liking on a hedonic scale consisting of nine points. Point 4 was

taken as the acceptability limit (Amerine et al., 1965).

The standard deviation was determined using the formulae.

$$S_D = \sqrt{\frac{N \sum X^2 - (\sum X)^2}{N(N-1)}} \quad (\text{ASTM, 1968})$$

N = number of panel members

X = is the score given by each members

The t-test was conducted to determine the significance of differences. The formulae is

$$t = \frac{\sum D}{\left[ \frac{N \sum D^2 - (\sum D)^2}{N-1} \right]^{1/2}}$$

where D = algebraic sum of the differences between the paired scores

D<sup>2</sup> = sum of the squares of the differences

N = number of pairs

By referring to the table values of t required for significance at various levels in ASTM (1968) the significance of difference is determined.

The raw samples were also analysed for its appearance, and odour of gill and muscle and texture of muscle. The taste panel members were asked to give the discription of the above properties.

#### 2.2.8 TOTAL BACTERIAL COUNT

Total plate count was determined as per IS:2237 (1971).

### 3. RESULTS

Table 3.1 gives the <sup>proximate</sup> ~~percentage~~ composition and the amount of sodium and potassium of the fishes used for the studies. It gives the upper and lower limits of these parameters obtained during different periods of the year. The composition of dark and white muscle of mackerel is presented in Table 3.2. The effect of the methods of initial handling on the pH of the muscle of rohu and mrigal are given in Table 3.3. The chemical composition of different portions of mrigal are presented in Table 3.4. Table 3.5 gives the extractive nitrogenous constituents in dark and white muscles of mackerel, horse mackerel and mrigal.

The changes in the chemical characteristics, sensory properties and total bacterial counts of rohu, mrigal, milk fish, mackerel and squid during iced storage were studied. Table 3.6 gives the changes in chemical composition and total bacterial count and Table 3.7 describes the sensory characteristics and presents the sensory score of mrigal during iced storage for 23 days. Table 3.8 gives the changes in chemical properties and sensory score of rohu collected during January and Table 3.9 presents the changes in the above properties of rohu of almost the same length and weight

collected during June. Table 3.10 gives the changes in total plate count and pH of rohu (June sample) during storage in ice. Table 3.11 gives the sensory characteristics of male and female rohu of average weight 560 g collected during September. The changes in chemical characteristics, bacterial count and sensory score of milk fish are given in Table 3.12. A comparison of the PV and sensory score of whole and bled and gutted milk fish in ice is presented in Table 3.13. Chemical changes during iced storage of squid is given in Table 3.14. Table 3.15 is a descriptive presentation of the sensory characteristics as well as sensory score during iced storage of squid. Tables 3.16 and 3.17 give the chemical and sensory changes respectively of mackerel during iced storage.

Fig. 3.1 gives the changes in salt extractable protein during storage in ice of squid, mackerel, milk fish, rohu and mrigal. Fig. 3.2 shows the sensory changes of the above fishes during iced storage.

Table 3.18 gives the changes in moisture, SSN and TMA of squid at  $-20\pm 1^{\circ}\text{C}$  storage. It also gives some of the sensory observations. Table 3.19 gives the changes in NPN, alpha amino nitrogen and TVN of squid subjected to 3 different conditions of initial handling during storage at  $-20\pm 1^{\circ}\text{C}$ . Fig. 3.4 shows the changes in salt extractable nitrogen of the three squid samples during frozen storage. Fig. 3.5 gives the changes in weight loss on thawing and cook drip loss of three samples of



squid. Fig. 3.6 shows the sensory score of three samples of squid and hence its shelflife. Fig. 3.3 gives a comparative study of the sensory score and shelflife of fresh squid, milk fish and mackerel.

Table 3.20 shows the chemical changes of milk fish at  $-20\pm 1^{\circ}\text{C}$ . The changes in NPN during frozen storage of milk fish iced for different period are presented in Table 3.21 while Table 3.22 gives the changes in PV. Fig. 3.7 shows the changes in soluble nitrogen of milk fish samples iced for different periods during frozen storage at  $-20^{\circ}\text{C}$ . Fig. 3.8 presents the sensory score of milk fish iced for different periods and frozen stored at  $-20\pm 1^{\circ}\text{C}$ .

Table 3.23 gives the chemical changes during frozen storage of mackerel, collected in June, during storage at  $-20\pm 1^{\circ}\text{C}$ . Table 3.24 shows the changes in PV and TBA of mackerel packed and stored as IQF and collected during June and December and having a fat content of 3.8 and 10.6% respectively during storage at  $-20\pm 1^{\circ}\text{C}$ . Fig. 3.9 presents the changes in sensory score and shelflife of mackerel collected in April, June and December. Table 3.25 shows the effect of type of packing viz. individually and blocks on PV, TBA and sensory score during frozen storage. The changes in SSN, PV, TBA and sensory score of mackerel stored at  $-20\pm 1^{\circ}\text{C}$  and  $-30\pm 1^{\circ}\text{C}$  are given in Table 3.26. Table 3.27 shows the effect of BHA treatment on PV, TBA and sensory score of mackerel at  $-20\pm 1^{\circ}\text{C}$ .

Table 3.28 gives the effect of filleting and freezing fresh cat fish and storing whole cat fish for one month, thawing, filleting and refreezing and then storing at  $-20\pm 1^{\circ}\text{C}$ . The weight loss during storage at  $-20\pm 1^{\circ}\text{C}$  of cat fish fillets in different styles of packing are given in Table 3.29. Table 3.30 shows the percentage yield of thawed cat fish fillets based on initial weight during storage at  $-20\pm 1^{\circ}\text{C}$  and Table 3.31 gives the changes in PV and FFA of the above samples. Fig. 3.10 gives the sensory score of different cat fish samples and hence the shelflife.

Fig. 3.11 gives the changes in the extractability of protein of fish mince from cat fish, threadfin bream and lizard fish at  $-20\pm 1^{\circ}\text{C}$  storage and Fig. 3.12 gives the changes in PV of the above samples and Fig. 3.13 gives the changes in sensory score of the above samples. Table 3.32 shows the effect of treatment with 0.1% cloves powder on PV, FFA and sensory score of minced horse mackerel during storage at  $-20\pm 1^{\circ}\text{C}$ . Table 3.33 gives the changes in certain chemical components of mince as a result of washing it. Fig. 3.14 shows the changes in the sensory score of the washed and unwashed minces during frozen storage at  $-20\pm 1^{\circ}\text{C}$ .

Table 3.1 Chemical composition of the fishes used for the studies

Common Name	No. of samples analysed	Moisture %	Protein %	Fat %	Ash %	Na mg/100g	K mg/100g
Cat fish	8	76.5-78.30	17.20-18.90	1.40-4.80	0.82-1.30	68- 93	209-368
Horse mackerel	6	74.2-77.90	18.60-21.40	1.60-6.20	0.91-1.50	80-162	150-270
Lizard fish	10	78.7-80.20	18.20-19.80	0.82-1.46	1.12-1.68	59- 84	266-313
Mackerel	10	66.3-75.60	18.80-20.70	3.20-11.8	1.16-1.92	126-178	236-371
Milkfish	4	71.4-74.60	19.86-21.18	1.80-4.60	1.27-1.53	160-180	280-336
Mrigal	4	76.8-79.10	20.60-22.50	2.30-5.70	1.30-1.90	63- 86	254-335
Rohu	4	75.4-77.00	21.20-23.17	0.50-1.40	1.00-2.10	68- 93	186-310
Squid	6	76.2-82.60	16.90-19.80	0.50-1.40	1.00-2.10	176	246
Threadfin bream	8	79.6-81.20	17.70-19.60	0.92-1.81	0.85-1.40	-	-

Table 3.2 Chemical characteristics of white and dark muscles of mackerel

Muscle	Moisture %	Protein %	Fat %	Ash %	NPN mg/ 100g	TMAO mg/100 g
White	76.9	18.9	3.2	1.13	481	120
Dark	74.2	17.8	8.6	1.21	245	168

Table 3.3 pH of rohu and mrigal 4 hours after catch

*meat*

	pH	
	Rohu	Mrigal
Instantly killed and iced	6.45	6.38
Strugglingly killed and iced	6.29	6.17

Table 3.4 Chemical composition of muscles in different parts of mrigal

Sample portion	Moisture %	Protein %	Lipid %	Ash %
Dorsal muscle	78.10 $\pm$ 1.00	22.17 $\pm$ 2.10	2.80 $\pm$ 0.90	1.7 $\pm$ 0.30
Ventral muscle	75.80 $\pm$ 1.40	22.24 $\pm$ 1.70	4.20 $\pm$ 0.70	1.6 $\pm$ 0.40
Caudal muscle	80.20 $\pm$ 0.90	21.43 $\pm$ 1.10	1.22 $\pm$ 0.50	1.4 $\pm$ 0.30
Dark muscle	74.10 $\pm$ 1.90	18.30 $\pm$ 1.30	6.82 $\pm$ 1.80	1.9 $\pm$ 0.60

Table 3.5 Extractive nitrogenous constituents in the dark and white muscles of certain fishes

Components	Mackerel		Horse mackerel		Mrigal	
	White	Dark	White	Dark	White	Dark
NPN mg%	481	345	459	323	390	279
TVBN mg%	15	11	14	9	18	13
TMA mg%	1	5	1	7	-	-
TMAO mg%	120	168	89	145	-	-

Table 3.6 Chemical changes and total bacterial count during iced storage of mrigal

Days	Moisture %	TN %	NPN mg %	SSN %	TVN mg %	camino nitrogen mg %	PV meq/kg fat	FFA % oleic acid	TPC/g
1	78.30	3.61	390	2.42	16.80	70	-	1.6	6.23x10 <sup>3</sup>
3	78.10	3.56	386	2.46	16.40	72	5.6	2.9	5.82x10 <sup>4</sup>
6	79.00	3.15	362	2.72	15.8	56	11.6	6.4	6.21x10 <sup>5</sup>
12	79.20	3.57	141	2.11	16.2	54	18.7	8.6	8.16x10 <sup>5</sup>
14	79.60	3.41	284	1.90	14.8	63	22.8	9.8	7.32x10 <sup>6</sup>
16	80.30	3.30	288	1.82	14.4	61	27.3	12.3	9.64x10 <sup>6</sup>
20	80.20	3.26	292	1.63	15.2	59	28.2	15.2	6.01x10 <sup>7</sup>
23	80.60	3.22	312	1.62	16.4	68	-	-	8.21x10 <sup>6</sup>

Table 3.7 Sensory characteristics and average score  
during iced storage of mrigal

Days		Observation	Score
1	Raw	Scales intact, bright surface, slightly brownish meat, gills red Rigor	8.5 $\pm$ 0.5
	Cooked	Sweet and muddy taste, firm muscle	
3	Raw	Rigor resolved, scales firm and intact, red gills	8.0 $\pm$ 0.6
	Cooked	Sweet and muddy taste, firm muscle	
6	Raw	Eyes slightly opaque, muscle soft, scales intact, gills slightly bleached, no off odour	7.3 $\pm$ 0.9
	Cooked	Muddy and slight sweet taste, muscle firm	
12	Raw	Sunken eyes, red colour around the pupil, gills slightly brownish, slight decayed weedy odour at the gills. Bone separated from the flesh at the belly portion. Yellowing in the flesh at the belly portion	5.0 $\pm$ 1.2
	Cooked	Bland taste, slightly pasty	
14	Raw	Gills dark, off odour at the gills, muscle soft, belly portion-yellowing, loosened muscle from bones	4.3 $\pm$ 0.6
	Cooked	Dull colour, slightly pasty, slight off taste	
16	Raw	Dark gills, loosened scales, off odour at the belly portion	3.8 $\pm$ 0.8
	Cooked	Pasty muscle, slight off taste	



Table 3.8 Chemical changes of rohu during iced storage (date of collection: 29-1-1986) Av. length 43 cm, weight 800 g

Days	Moisture %	TN %	NPN mg %	SSN %	α-amino nitrogen mg %	TVN mg %	PV m. eq/kg fat	FFA oleic acid %	Score
0	77.90	3.50	389	1.90	72	14.6	-	1.2	8.5±0.50
3	77.50	3.36	380	2.70	71	14.2	8.2	3.1	7.9±0.30
6	78.00	3.23	382	2.18	68	14.2	10.6	4.5	6.3±0.99
12	79.90	3.01	320	2.02	58	13.4	15.8	6.9	5.2±0.80
16	80.65	3.12	312	1.97	59	12.6	16.2	8.9	4.1±1.20
20	81.22	2.98	296	1.86	52	13.8	18.8	11.2	3.2±0.50

Ash 1.2% ; Fat 3.9%

Table 3.9 Iced storage characteristics of rohu collected in June 1986  
(average weight 810 g)

Days	TN %	WSN %	WSN % of TN	SSN %	NPN %	$\alpha$ -amino nitrogen mg %	TVN %	Moisture %	FFA oleic acid %	PV meq/kg fat	<i>Organoleptic</i> Score
0	3.69	0.87	23	1.68	0.35	66	11.2	76.45	0.8	-	8.5±0.4
1	3.52	0.68	25	1.76	0.41	67	11.6	77.39	1.3	-	8.5±0.3
5	3.63	0.87	24	2.55	0.39	67	11.8	78.6	4.2	6.1	7.6±0.6
7	3.60	0.86	24	2.65	0.26	62	9.8	78.4	5.2	7.2	6.5±1.1
11	3.50	0.92	26	2.40	0.36	67	9.2	78.2	6.6	11.3	5.2±0.8
13	3.70	0.94	25	2.25	0.37	60	8.6	79.2	8.2	14.3	4.8±1.2
15	3.64	0.77	21	1.96	0.31	61	10.2	89.3	9.7	17.9	4.5±1.1
18	3.56	0.79	22	1.81	0.32	67	9.8	79.0	11.2	19.6	3.5±0.8
20	3.42	0.88	23	1.64	0.34	83	9.4	79.1	12.1	21.3	

Fat 2.93; Ash 1.2

Table 3.10 Changes in pH and bacterial count during iced storage of rohu (June)

Days	pH	TPC g <sup>-1</sup>
1	6.30	9.35x10 <sup>3</sup>
5	6.39	9.38x10 <sup>4</sup>
7	6.42	1.80x10 <sup>5</sup>
11	6.57	3.36x10 <sup>5</sup>
13	6.59	6.98x10 <sup>6</sup>
18	6.69	7.50x10 <sup>7</sup>
20	6.74	6.09x10 <sup>7</sup>

Table 3.11 Sensory characteristics of male and female rohu in ice (Date of collection 4-9-1988)  
(Average weight 560 g)

Days	Male	Female
0	Bright red gills slimy surface, muddy flavour sweet taste  8.5 $\pm$ 0.3	Bright red gills, slightly muddy flavour, sweet  8.3 $\pm$ 0.6
4	Slight yellow discolouration at the belly, gills red, slightly sweet taste, muscle firm  7 $\pm$ 0.8	Slight yellow discolouration in the belly portion, slightly sweet and slightly soft  6.8 $\pm$ 0.6
8	Gills bleached, mucus, no off odour, opaque and sunken eyes, red colour around pupil. Yellow and black discoloura- tion at the belly portion. Ribs separated from muscle, guts started spoilng  5.5 $\pm$ 0.7	"           5.6 $\pm$ 0.9
13	Decay at the guts, muscle colour faded, bland taste  4.5 $\pm$ 0.6	Decayed belly muscle colour faded, bland taste  4.2 $\pm$ 0.9
16	Decayed weedy odour to the meat, belly completely spoiled, texture soft  3.9 $\pm$ 0.9	Belly completely spoiled, slight off taste to the muscle, slightly pasty  3.6 $\pm$ 0.4

Table 3.12 Changes during iced storage of milkfish

Days	Moisture %	TN %	NPN %	SSN % to TN	TVN mg %	$\alpha$ -amino nitrogen mg %	TPC g <sup>-1</sup>	Score	PV meq/ kg fat
1	71.69	3.22	0.560	67.57	7.0	69	$7.64 \times 10^2$	$8.0 \pm 0.4$	-
4	72.00	3.11	0.530	65.47	8.4	68	$1.13 \times 10^4$	$7.2 \pm 1.1$	3.5
7	72.45	3.18	0.504	64.62	11.2	65	$9.80 \times 10^4$	$6.3 \pm 0.7$	6.7
10	73.21	2.91	0.586	62.5	14.0	61	$2.50 \times 10^5$	$5.2 \pm 0.6$	12.14
14	73.60	3.12	0.568	60.7	18.2	76	$3.90 \times 10^5$	$4.1 \pm 1.1$	19.88
18	73.64	2.81	0.652	58.16	35.0	72	$4.2 \times 10^6$	-	22.21

Table 3.13 Changes in PV and sensory score of whole and bled and gutted milk fish

Days	Whole		Bled and gutted	
	PV meq/kg fat	Organoleptic Score	PV meq/kg fat	Organoleptic Score
1	-	8.0 $\pm$ 0.4	-	8.2 $\pm$ 0.3
4	5.5	7.2 $\pm$ 1.1	-	7.6 $\pm$ 0.4
7	6.7	6.3 $\pm$ 0.7	3.4	6.9 $\pm$ 0.7
10	12.14	5.2 $\pm$ 0.6	5.8	5.8 $\pm$ 0.9
14	17.88	4.1 $\pm$ 1.1	9.2	4.8 $\pm$ 0.5
18	22.12	3.3 $\pm$ 0.6	12.8	3.9 $\pm$ 0.7

Table 3.14 Chemical changes during iced storage of squid

Days	Moisture %	TN %	NPN mg %	SSN %	$\alpha$ -amino nitrogen mg %	TVN mg %
0	78.93	3.015	939.0	2.29	238	4.20
2	82.42	2.930	820.2	1.36	182	12.75
4	82.68	2.640	662.8	1.18	166	18.20
6	82.20	2.610	620.7	0.99	143	30.30

Table 3.15 Sensory changes during iced storage of squid

Days	Observations	Score
0	White muscle, firm, sweet taste	9.0
2	Mantle thickness reduced, sweetness considerably reduced, soft, pink discolouration on the mantles	6.2 $\pm$ 0.8
4	Intensity of pink discolouration on the mantles reduced, yellow discolouration on the belly portion. Decayed odour at the belly portion. Cooked meat: slight off taste at the belly portion. No sweet taste	4.3 $\pm$ 0.6
6	Intense decayed odour at the belly portion soft and thin mantles. Pronounced decayed taste at belly portion	3.0 $\pm$ 0.7



Table 3.16 Chemical changes during iced storage of mackerel

Days	Moisture %	SSN % to TN	NPN mg %	PV meq/ kg	TBA mg/kg meat	TVN mg %	TMA mg %
0	73.86	76.9	470	1.2	0.26	6.9	0.42
3	74.20	69.7	495	5.8	1.12	7.2	1.32
6	79.92	64.3	437	11.9	2.68	8.8	2.68
9	76.63	59.4	421	18.8	4.21	12.1	4.36
12	76.28	51.2	405	26.4	5.68	23.4	8.21

Table 3.17 Sensory changes during iced storage of mackerel

No. of days	Observations	Score
0	Eyes bright, red gills, firm flesh, bright skin, juicy and firm texture	8.3 $\pm$ 0.50
3	Eyes cloudy, gills slightly bleached, juiciness reduced muscle firm	6.9 $\pm$ 0.80
6	Sunken eyes, bleached gills, muscle slightly soft, scales loose, juiciness lost	5.2 $\pm$ 1.20
9	Sunken eyes, red colour around pupil, dark gill, off odour at the gills muscle soft, slightly rancid	3.7 $\pm$ 0.80
12	Highly rancid	-

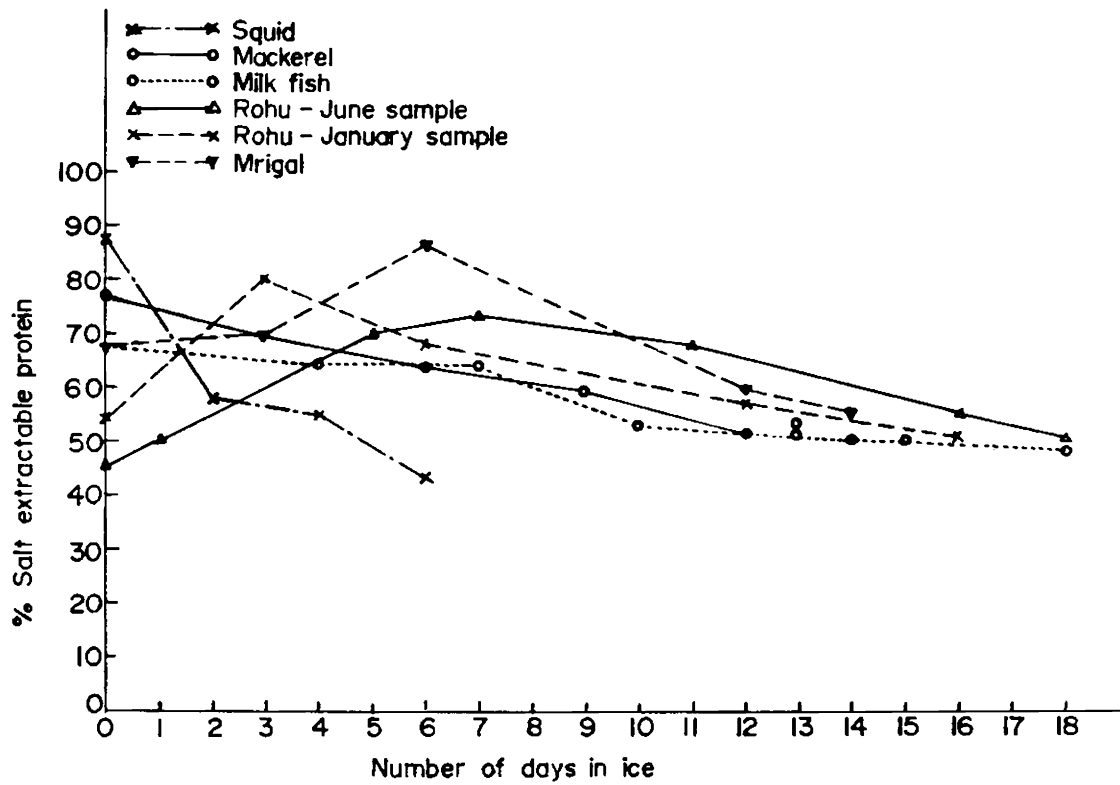


Fig.3-1 Changes in salt extractable protein during ice storage

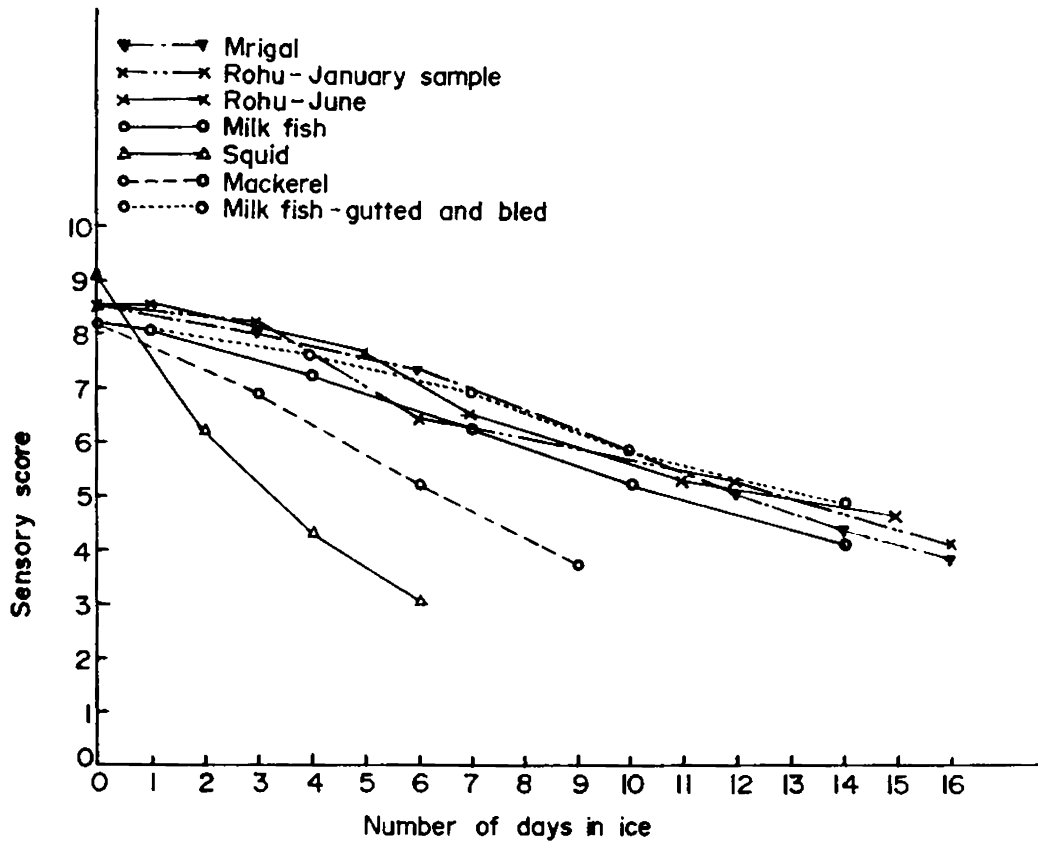


Fig.3:2 Average sensory score of different fishes during iced storage

Table 3.18 Changes in moisture, SSN and TMA of squid at -20±1°C (squid mantles frozen immediately after bringing to laboratory)

No. of weeks	Moisture %	SSN % to TN	TMA mg %	Sensory observation
0	78.93	85.19	-	white mantles, no desiccation
8	79.09	75.60	-	"
16	70.22	75.84	1.2	"
24	78.58	69.95	1.6	Very slight desiccation in few samples
32	78.45	67.39	1.6	Slight desiccation in few samples
40	77.96	64.45	2.0	"
48	77.12	61.29	2.8	Intensity of desiccation increased colour became dull excessive drip
56	76.23	58.48	3.2	"
62	75.50	55.26	2.4	"

Table 3.19 Changes in NPN,  $\alpha$ -amino nitrogen and TVN of squid during frozen storage  
( $-20 \pm 1^\circ\text{C}$ )

No. of weeks	NPN mg/100g			$\alpha$ -amino nitrogen mg/100g			TVBN mg/100g		
	I	II	III	I	II	III	I	II	III
0	730	568	720	236	92	141	5.6	4.0	9.6
8	911	492	680	221	90	112	6.2	4.4	10.2
16	750	412	589	194	76	97	7.2	5.6	12.6
24	723	352	552	190	75	88	8.6	6.4	12.8
32	698	351	528	184	66	83	10.2	7.2	14.2
40	712	328	486	176	62	80	11.8	7.6	13.6
48	672	318	442	168	61	76	12.4	8.4	15.2
56	536	306	434	146	58	65	13.8	10.2	16.4

I. Dressed and washed mantles frozen immediately.

II. Dressed mantles kept in ice for one day and frozen <sup>as dressed</sup> and frozen

III. Whole squid iced for one day and then dressed, and frozen

Table 3.20 Chemical changes during frozen storage of milk fish at -20±1°C

No. of weeks	Moisture %	SSN % of TN	NPN mg%	PV meq/kg fat	TBA mg/kg meat	TVBN mg/100g
0	71.69	67.59	561	-	-	7.0
2	71.30	64.27	512	6.82	-	11.2
10	71.23	63.39	542	11.18	1.06	11.9
20	70.92	61.40	488	12.12	1.11	12.6
30	70.69	59.34	428	20.28	1.46	13.3
40	70.61	57.40	433	24.34	1.77	14.7
50	70.23	55.32	418	21.26	2.34	16.8

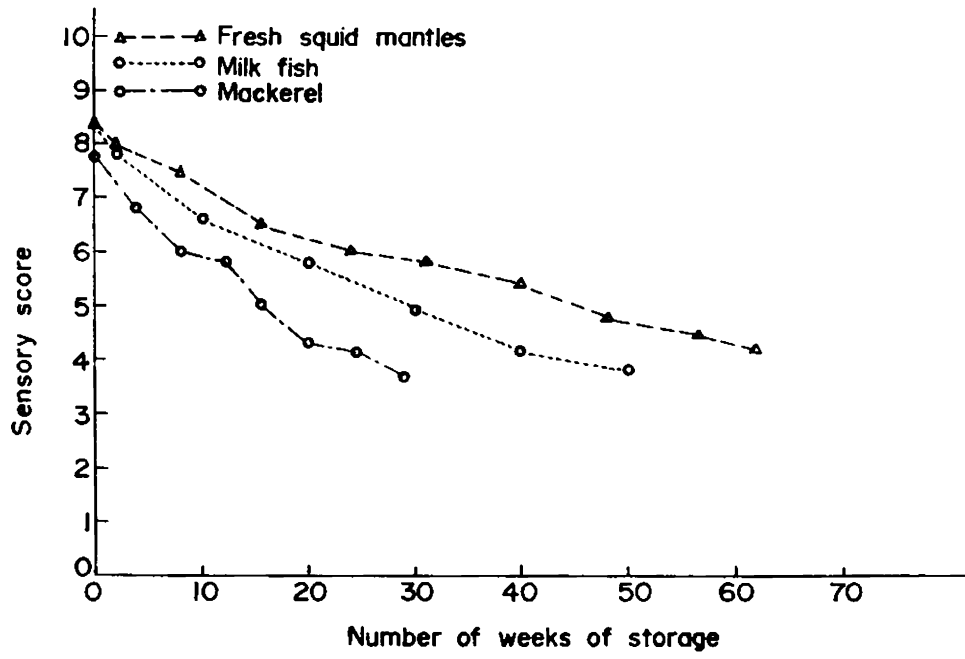


Fig.3:3 Average sensory score of frozen whole fish during storage at  $-20^{\circ}\text{C}$



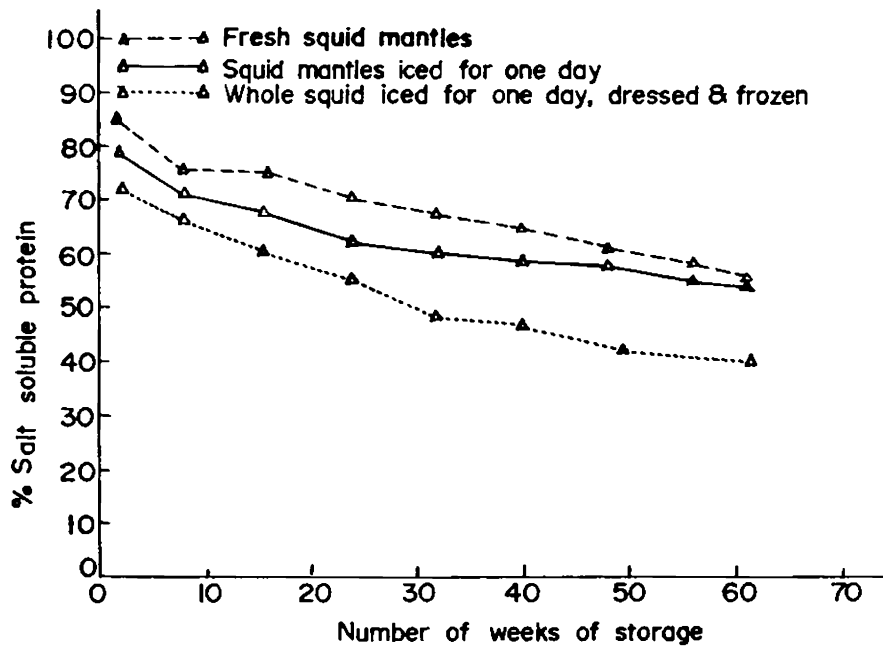


Fig.3-4 Changes in salt extractable nitrogen of squid samples at  $-20 \pm 1^\circ\text{C}$

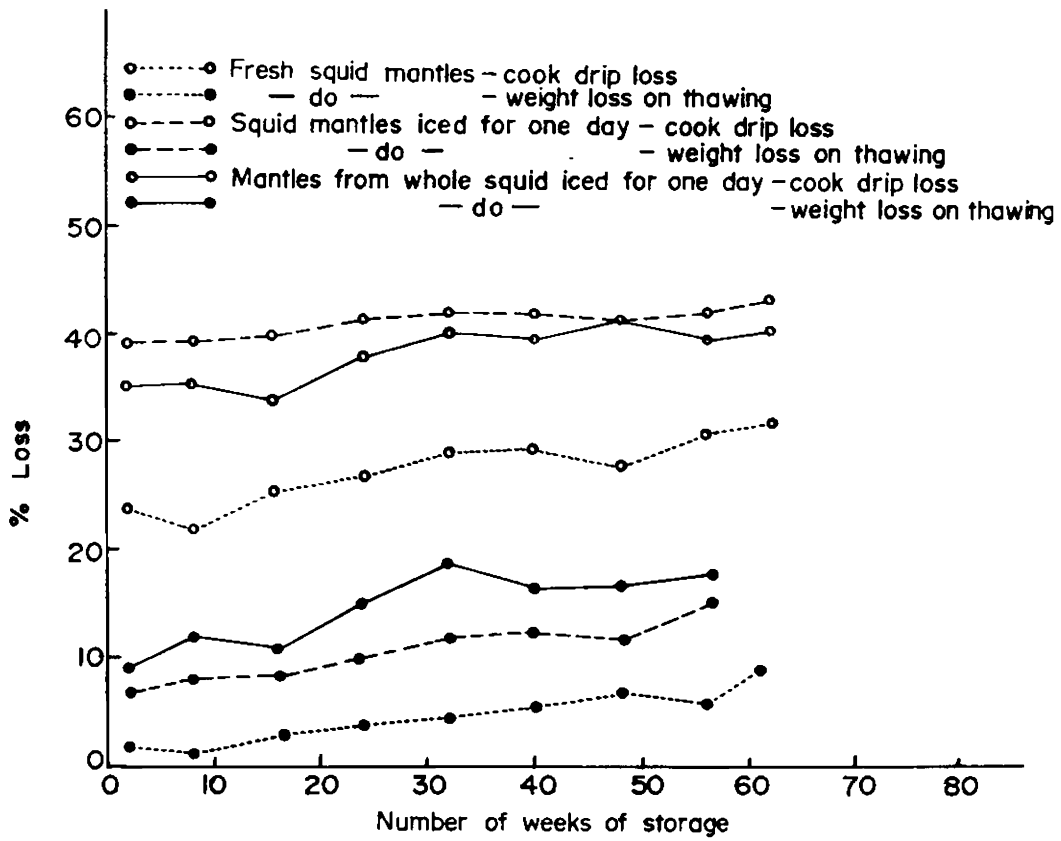


Fig.3-5 Changes in weight loss on thawing and cook drip loss of frozen squid

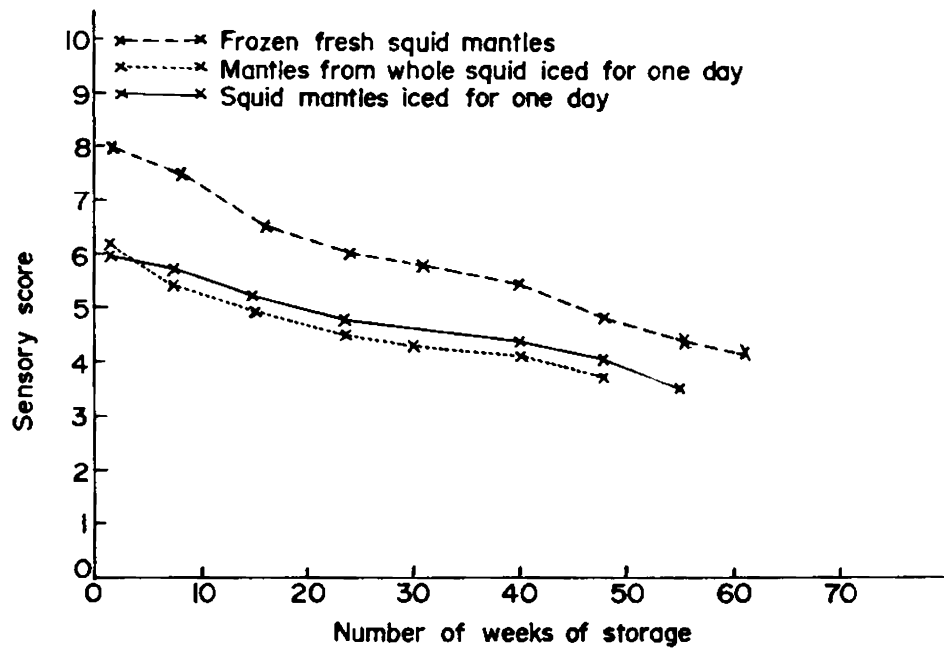


Fig.3-6 Sensory score of squid samples stored at  $-20 \pm 1^{\circ}\text{C}$

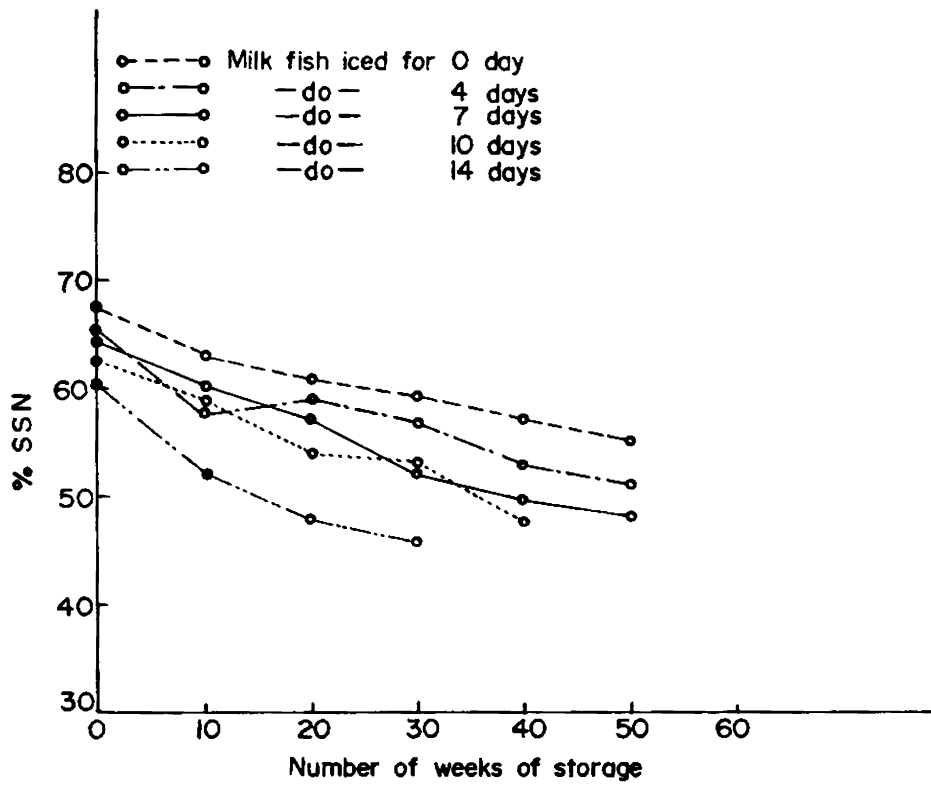


Fig.3-7 Percentage changes in soluble nitrogen of milk fish samples iced for different periods during storage at  $-20^{\circ}\text{C}$

Table 3.21 percentage changes in NPN during frozen storage of milk fish iced for different periods (mg %) stored at  $20 \pm 1^\circ\text{C}$

No. of weeks	No. of days in ice before freezing				
	0	4	7	10	14
0	560	530	504	536	568
2	574	504	501	475	448
10	616	510	476	458	420
20	532	490	420	402	393
30	476	448	364	346	378
40	490	420	336	304	350
50	392	364	322	-	-

Table 3.22 Changes in PV (m. eq/kg fat) of muscle during storage of milk fish iced for different periods, frozen and stored at 30 ± 1°C

No. of weeks	No. of days in the ice before freezing				
	0	4	7	10	14
0	-	5.50	6.70	12.14	17.88
2	4.82	8.86	12.24	16.80	26.38
10	11.18	10.78	28.29	26.15	24.42
20	12.12	16.52	26.15	21.15	18.39
30	20.18	22.61	23.82	22.11	16.21
40	24.34	21.12	21.69	18.26	-
50	21.20	20.64	19.20	-	-

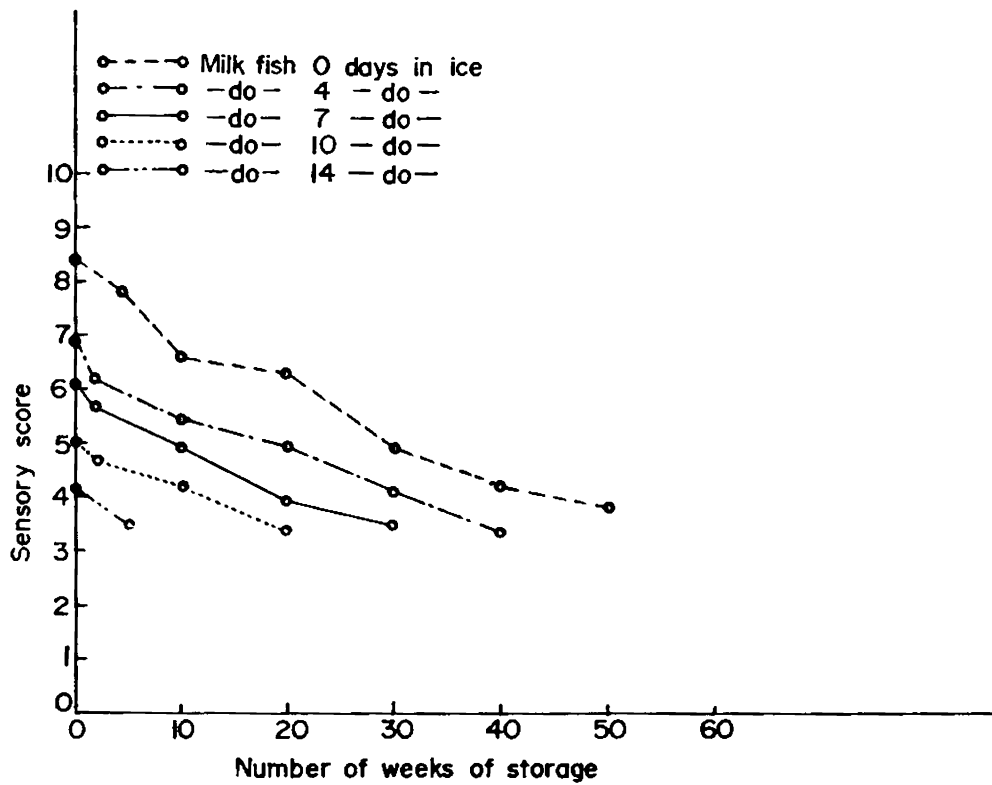


Fig.3-8 Sensory score of milk fish periodically frozen during iced storage and stored at  $-20 \pm 1^{\circ}\text{C}$

Table 3.23 Chemical changes during frozen storage of mackerel at -20±1°C (collected in June) and stored as IQF

No. of weeks	Moisture %	SSN % to TN	NPN mg %	TVBN mg %	TMA mg %	PV meq/kg	TBA mg/kg meat
0	73.86	73.12	476	7.00	0.42	1.6	0.39
4	73.47	65.47	490	9.80	1.40	6.3	1.10
8	73.32	61.39	470	11.20	1.12	13.6	1.79
12	72.82	56.72	448	12.60	0.70	16.2	2.34
16	72.43	54.12	437	14.70	0.98	21.2	3.51
20	71.12	51.30	423	14.30	0.98	26.9	4.68
24	70.26	48.21	406	15.12	1.40	34.3	7.02
28	69.92	47.32	392	15.40	1.12	43.8	8.58
32	69.15	47.03	364	16.94	1.70	36.6	10.92



Table 3.24 Changes in PV and TBA of mackerel (IQF) samples collected in June and December at -20±1°C (Lipid content: June sample 3.8%, December 10.6%)

No. of weeks	PV meq/kg fat		TBA (mg/kg meat)	
	June sample	December sample	June sample	December sample
0	1.6	2.8	0.39	0.31
8	13.6	9.4	1.79	1.56
16	21.2	16.9	3.51	2.42
24	34.3	26.7	7.02	5.46
32	36.6	32.9	10.92	8.58

Table 3.25 Changes in PV, TBA and sensory score of mackerel packed as block and individually and stored at -20±1°C (April samples)

No. of sample	PV meq/kg fat		TBA mg/kg meat		Score	
	IQF	BF	IQF	BF	IQF	BF
0	2.82	2.12	0.68	0.59	8.20±0.47	8.30±0.32
6	14.31	4.28	1.38	0.87	7.30±0.91	7.60±0.48
12	24.60	8.31	2.64	6.38	8.38±1.50	6.92±0.82
18	36.42	14.47	3.04	1.83	5.10±1.36	6.30±1.43
24	45.26	18.29	5.28	2.21	4.21±1.27	5.20±0.55
30	42.38	19.36	8.32	2.82	3.10±0.82	4.37±1.27

Table 3.26 Changes in SSN, PV, TBA and sensory score of mackerel packed individually and stored at  $-20\pm 1^{\circ}\text{C}$  (April)

No. of weeks	SSN % to TN		PV meq/kg fat		TBA mg/kg meat		Score	
	$-20^{\circ}\text{C}$	$-30^{\circ}\text{C}$	$-20^{\circ}\text{C}$	$-30^{\circ}\text{C}$	$-20^{\circ}\text{C}$	$-30^{\circ}\text{C}$	$-20^{\circ}\text{C}$	$-30^{\circ}\text{C}$
0	75.28	74.92	2.82	2.26	0.68	0.72	8.20 $\pm$ 0.47	8.0 $\pm$ 0.62
6	64.74	72.36	14.31	5.48	1.38	1.12	7.30 $\pm$ 0.92	7.62 $\pm$ 0.58
12	59.36	68.94	24.60	10.24	2.64	1.20	6.38 $\pm$ 1.51	7.38 $\pm$ 0.52
18	54.72	66.35	36.42	14.86	3.04	1.56	5.16 $\pm$ 1.36	7.04 $\pm$ 0.86
24	52.68	63.28	45.26	16.64	5.28	2.06	4.23 $\pm$ 1.27	6.51 $\pm$ 0.58
30	48.29	60.42	42.38	17.42	8.32	2.36	3.19 $\pm$ 0.82	6.20 $\pm$ 0.42

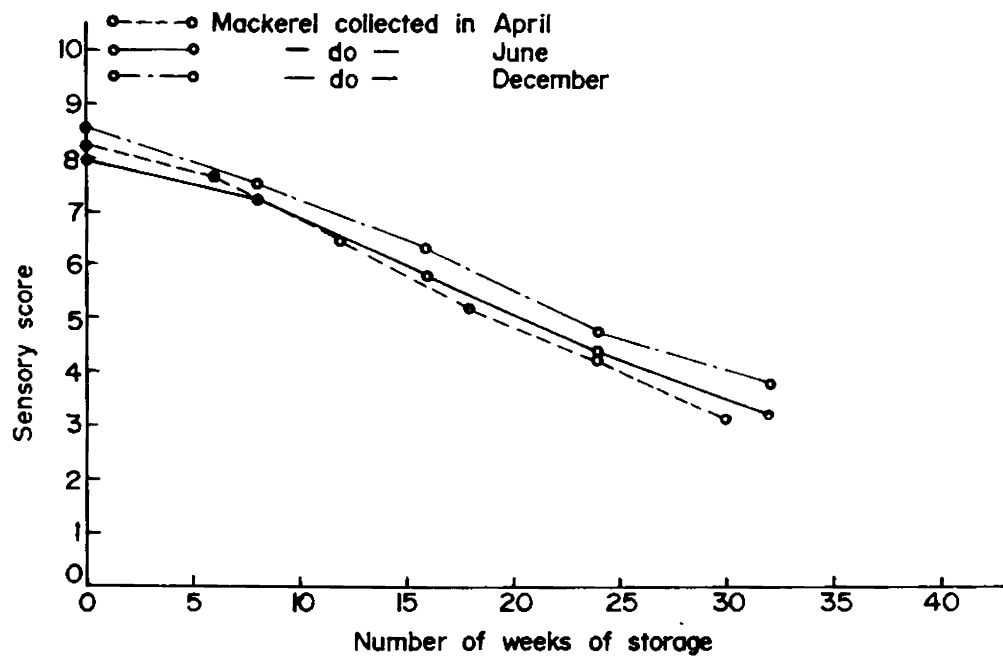


Fig.3-9 Changes in sensory score of mackerel collected during April, June and December

Table 3.27 Changes in PV and TBA of mackerel treated with BHA and untreated (Frozen as IQF and stored at  $-20\pm 1^{\circ}\text{C}$ )

No. of weeks	PV meq/kg		TBA mg/kg meat		Sensory score	
	BHA treated	Control	BHA treated	Control	BHA treated	Control
0	2.82	2.82	0.72	0.68	8.10 $\pm$ 0.38	8.20 $\pm$ 0.47
6	3.89	14.31	0.86	1.38	7.80 $\pm$ 0.87	7.30 $\pm$ 0.92
12	7.07	36.42	1.16	3.04	6.42 $\pm$ 0.86	5.16 $\pm$ 1.36
18	7.07	36.42	1.16	3.04	6.42 $\pm$ 0.86	5.10 $\pm$ 1.36
24	8.12	45.26	1.29	3.28	5.40 $\pm$ 0.92	4.20 $\pm$ 0.27
30	10.36	42.38	1.45	8.32	4.50 $\pm$ 1.36	3.19 $\pm$ 0.82

Control

Treated with BHA

Table 3.28 Changes in extractive nitrogen, PV and sensory score of fresh frozen fillets and refrozen fillets of cat fish

No. of weeks	Extractive nitrogen %		PV meq/kg		Score	
	Fresh	Refrozen *	Fresh	Refrozen *	Fresh	Refrozen *
4	68.2	66.4	4.18	3.12	7.0 $\pm$ 0.6	6.8 $\pm$ 1.2
8	62.3	58.7	6.23	9.32	6.5 $\pm$ 0.8	5.8 $\pm$ 0.7
16	55.7	54.3	9.32	14.86	5.6 $\pm$ 1.2	5.0 $\pm$ 1.4
24	51.9	51.6	12.48	16.34	4.8 $\pm$ 0.7	4.2 $\pm$ 1.4
32	49.6	46.4	14.32	16.82	4.1 $\pm$ 0.6	3.8 $\pm$ 0.6

\*stored for 4 weeks as whole and then filleted and refrozen

Table 3.29 <sup>Percentage</sup> Weight loss during storage at  $-20 \pm 1^\circ\text{C}$  of cat fish fillets in different style of packing

No. of weeks	Frozen fillets				
	1	2	3	4	5
5	0.68	0.33	0.25	Nil	Nil
10	2.75	0.38	1.05	Nil	Nil
16	6.14	0.48	1.37	Nil	0.32
22	-	0.59	1.62	0.25	0.44
28	-	0.68	1.86	0.50	0.64

1. Frozen fillets stored without any packing.
2. Wrapped in polythene sheets (200 gauge).
3. Packed in plain waxed carton.
4. Packed in waxed carton lined inside with polythene sheet.
5. Packed in waxed carton lined inside with polythene sheet and glazed.

Table 3.30 percentage yield of thawed cat fish fillets  
(based on initial weight)

No. of weeks	Yield				
	1	2	3	4	5
0	94.70	95.65	94.86	95.53	95.40
5	93.33	95.33	93.75	95.00	95.50
10	89.67	94.50	92.50	94.00	95.00
16	83.10	92.00	90.46	93.20	94.00
22	-	89.00	88.33	93.75	94.25
28	-	88.67	86.28	88.25	92.80

1, 2, 3, 4 & 5 are as in Table 3.29



Table 3.31 Changes in PV and FFA of cat fish fillets at -20±1°C in different type of packing

No. of weeks	PV (meq/kg)					FFA (as oleic acid %)				
	1	2	3	4	5	1	2	3	4	5
0	2.68	2.43	2.19	1.18	1.52	1.82	2.06	2.13	1.88	1.73
5	12.04	7.14	12.88	4.26	3.86	3.15	2.86	2.89	2.96	2.82
10	28.61	13.98	21.05	10.82	8.20	3.52	4.13	3.94	4.06	3.61
16	42.82	16.12	30.82	16.24	14.28	4.82	4.60	4.28	3.92	4.08
22	-	26.08	40.06	20.06	18.24	-	5.98	6.81	4.01	3.12
28	-	26.38	34.21	24.16	21.74	-	8.21	6.36	6.87	4.86

1, 2, 3, 4 & 5 as in Table 3.29

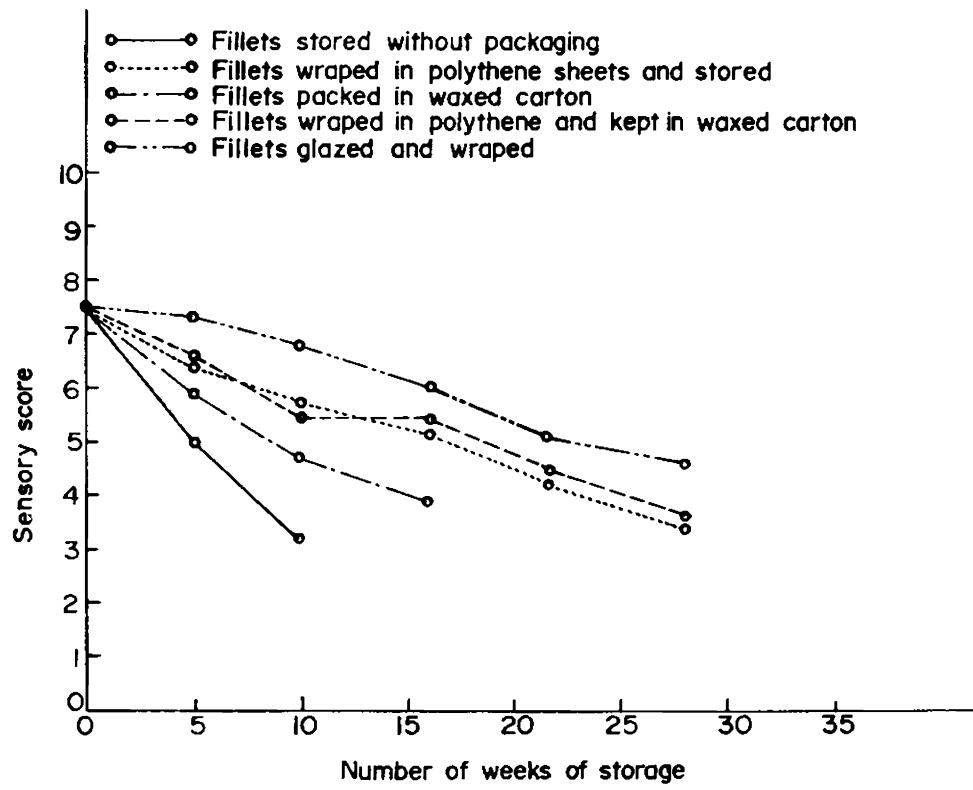


Fig.3-10 Sensory score of cat fish fillets

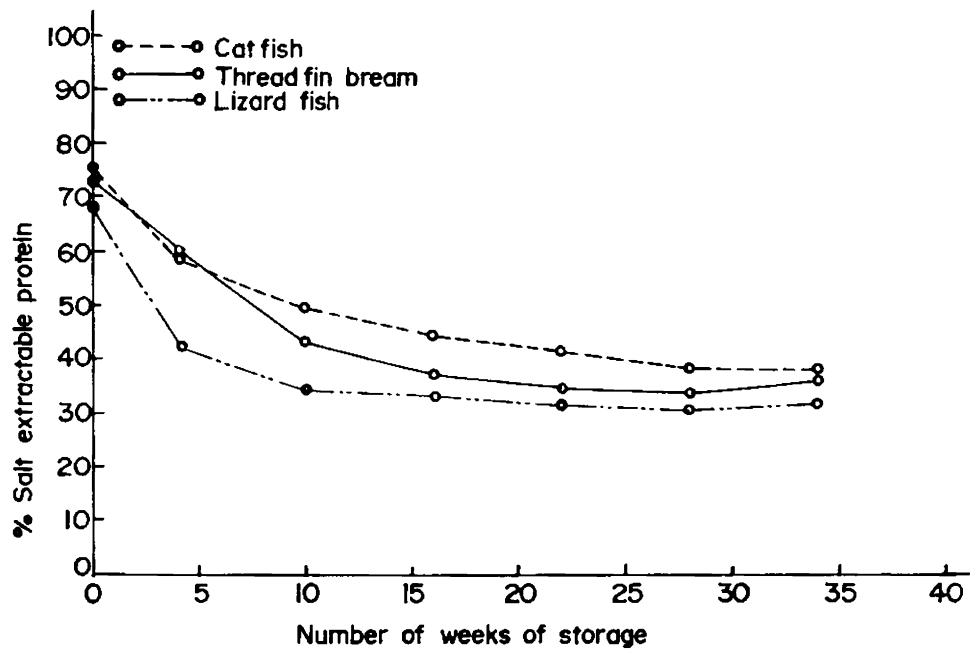


Fig.3-11 Changes in protein extractability of fish mince during frozen storage

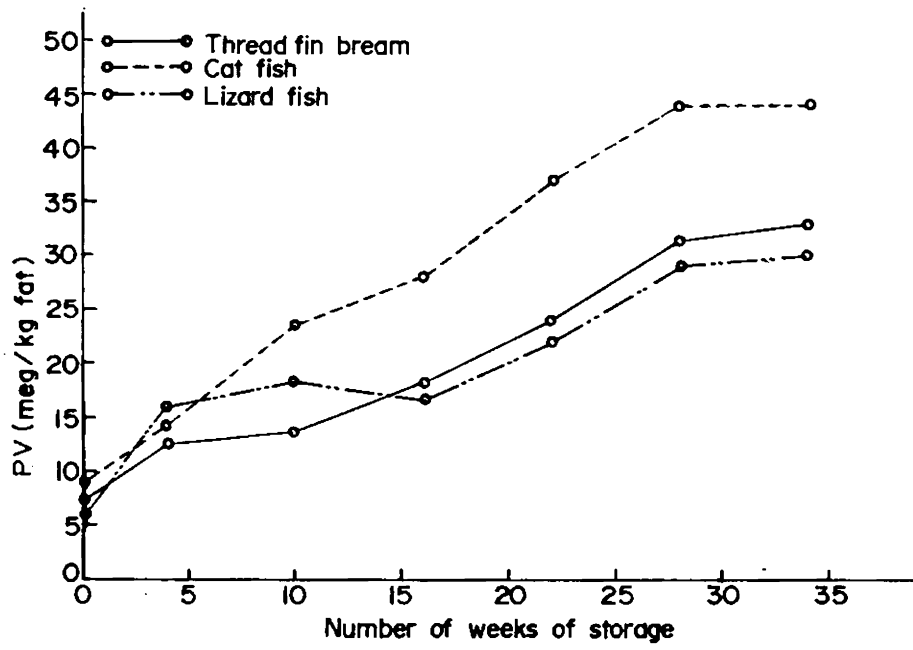


Fig.3.12 Changes in PV (mg/1000g) of fish mince *fat*

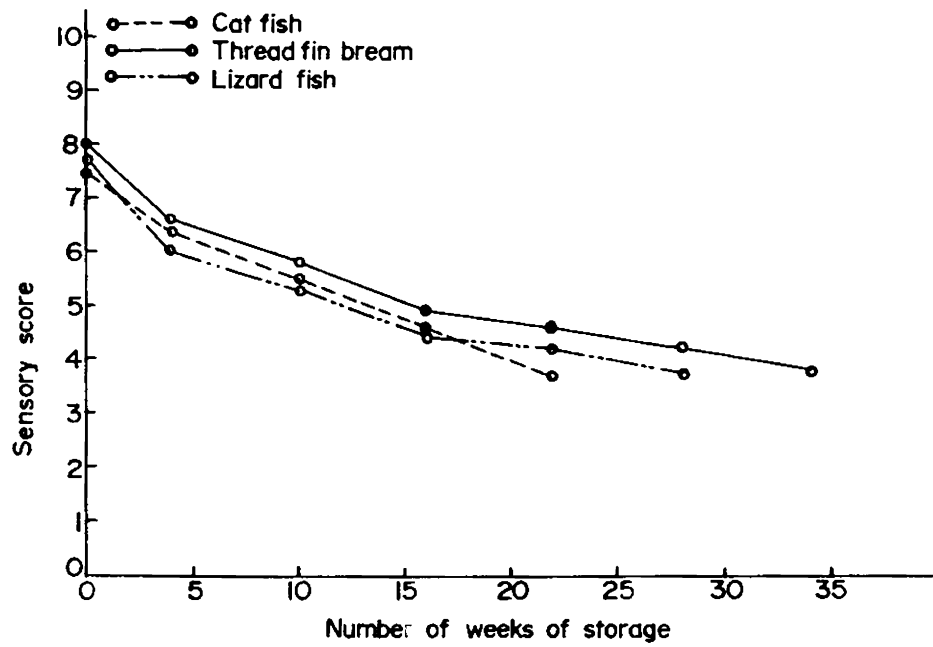


Fig.3.13 Changes in sensory score during frozen storage of fish mince at  $-20^{\circ}\text{C}$

Table 3.32 Changes in PV, FFA and sensory score of mince from horse mackerel treated with cloves powder and untreated at -20±1°C

No. of weeks	PV m.eq/kg fat		FFA (as oleic acid%)		Sensory score	
	C	T	C	T	C	T
0	12.8	8.9	1.02	0.430	7.2±0.90	7.5±0.6
3	22.7	11.2	2.92	0.554	5.5±1.20	7.0±0.8
21	47.3	13.8	3.20	0.480	4.1±0.65	6.4±0.5
35	58.2	27.9	2.80	0.670	3.3±0.92	4.8±1.2

C = Control

T = Mixed with clove powder

Table 3.33 Effect of washing the mince on certain chemical components

Fish	Moisture %		Fat %		NPN mg/100g		TVBN mg/100g	
	B.W	A.W	B.W	A.W	B.W	A.W	B.W	A.W
Cat fish	78.2	81.1	1.40	0.82	343	153	12.2	3.4
Threadfin bream	80.3	83.5	0.72	0.61	318	162	10.9	3.2
Lizard fish	79.5	82.5	1.18	0.86	325	146	8.6	2.8

B.W = before washing

A.W = after washing

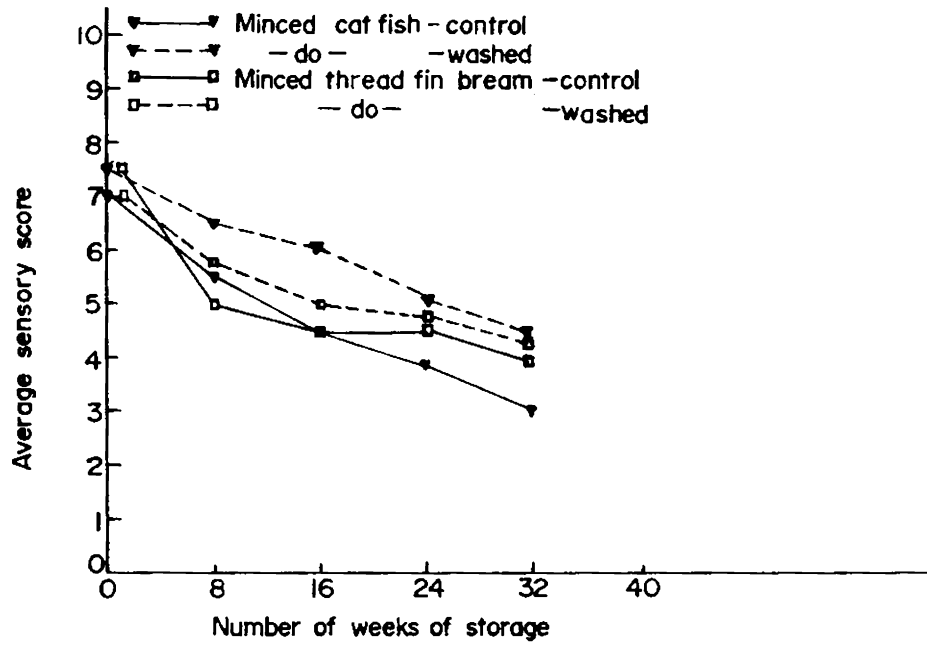


Fig.3-14 Average sensory score of washed and control mince during frozen storage



## 4. DISCUSSION

### 4.1 COMPOSITION

The variation in chemical composition was substantial in case of mackerel while other fishes used for the studies showed only marginal differences in composition (Table 3.1). Many reasons such as season, feeding, nutritional level of water, starvation, spawning etc. can be attributed for these variation. Usually lipid undergoes maximum changes with a concurrent change in moisture content while the protein content remains almost constant. Ravindranathan Nair et al. (1987) reported values of 4 and 11% for fat of mackerel collected during different periods. In certain other species more pronounced variations were reported; e.g. in sardine the fat content varies from 2 to 12% (Gopakumar, 1965) and in silver pomfret the variation is 5.5 to 8.3% (Solanki et al., 1976). The highest fat content and maximum variation was reported in lake trout (Cristivomer namaycush siscowet) and the fat in the fillet ranged from 21.3 to 67.2% (Thurston, 1962).

Spawning also affect the composition. Damberge (1964) observed that during the period of spawning in cod there was a decrease in fat (20%) and protein (5%) paralleled by an

increase of water solubles (+10%) and water (+5%). Idler et al. (1964) found that during prespawning period of scallop the total fat was low and this was attributed to the transfer of fat to the gonad tissue.

Another factor affecting composition is starvation. Satoh et al. (1984) reported that the amount of polar lipids were almost constant during starvation and the decrease of lipids was mainly due to decrease of triglycerides. He showed that the decrease in body weight was more severe in fish starved at 25°C than at 15°C. Takeuchi et al. (1987) observed a decrease of muscle protein and visceral lipids due to winter starvation and suggested that fish used mainly visceral lipids and muscle proteins as energy source and not so much muscle lipid during winter starvation.

The chemical and sensory properties are also influenced by the environment where the fish live. Fish reared in sewage water were tastier and had slightly higher fat and protein content than fish reared in fresh water (Giri et al., 1983). Differences were also noticed between cultured and wild fish. Lipid content was found higher in the cultured fish than in the wild fish (Ohshima et al., 1983 a,b; Satoh et al., 1986).

In all the fishes the amount of potassium was almost double or more compared to sodium. The amount of sodium was in the range of 59-180 mg/100 g fish meat while that of

potassium in the range of 150-371 mg/100 g meat. The ash content was found to be 0.82 to 2.1%.

The proximate composition of dark and white meat of fish muscle showed significant variation (Table 3.2). The dark muscle of mackerel had high lipid content (8.6%) with correspondingly lower moisture content and slightly low protein content. The white muscle contain 3.2% fat. The NPN content was higher in the white muscle. While TMAO was higher in the dark muscle (Tables 3.2 and 3.5). Considerably higher amount of lipid was noticed in the dark muscle than that in the white muscle of many fishes (Mannan et al., 1961; Obatake et al., 1985; Shindo et al., 1986). Many species of dark fleshed fish are known to have high levels of TMAO in the muscle (Takagi et al., 1967; Tokunaga, 1970; Murata et al., 1980).

Shindo et al. (1986) observed that the diameter of the dark muscles<sup>δ</sup> fibers was usually smaller than that of the white muscle and there were more connective tissues in the dark muscle than in the white muscle. They found that the carbohydrates were metabolised via lactic acid down to CO<sub>2</sub> in the dark muscles, while they were only glycolized to lactic acid in the white muscle. Dyer et al. (1966) found that the sequence of nucleotide changes occurred much earlier in the red muscle than in white muscle. Almost similar type of

observations were made by Obatake et al. (1988) and Obatake & Tometa (1988). Rehbein & Kundiger (1984) reported that sarcoplasmic proteins from dark and white muscle showed clear differences. The rigor contraction and isometric rigor tension were almost three times in dark muscle in comparison to white muscle of ling cod (Buttkus, 1963). Khan (1952) isolated a highly active enzyme capable of peroxidising non-conjugated unsaturated fatty acids from dark muscle. In general dark muscle is significantly more susceptible to deterioration in quality than white muscle.

The chemical composition of muscle in different parts of mrigal were analysed (Table 3.4). The moisture content was higher in caudal muscle than in other parts. The lipid was higher in the ventral muscle. The dark muscle contained high fat and lower moisture. Date & Yamamoto (1988) found that the chemical composition of yellow tail showed slight variations in chemical composition in different parts of muscle. Mannan et al. (1961) also noticed changes in composition of the various portions of edible flesh of Atlantic halibut. Damberg (1963) found that the head end of cod fillet was rich in protein, the middle section was rich in water solubles and the tale end contained more fat. The muscle from the tale section was found to become rancid faster than muscle from the head or central section of cod by MacLean & Castell (1964) and Castell & MacLean (1964).

Significant difference was found in the pH of strugglingly killed fish and instantly killed fish (Table 3.3). The lower value of pH in strugglingly killed fish in the initial stages of post-mortem can be attributed to the rapid glycolysis resulted in the production and accumulation of lactic acid. Fukuda et al. (1979) noted that the anerobic decomposition of glycogen was more rapid and more remarkable in strugglingly killed fish than in the instantly killed ones. The ultimate pH was found to be higher in fish caught by gill net compared to that by long line or handline (Botta et al., 1987b). Love (1975a) mentioned that difference in degree of struggling of fish caught by different methods probably produced differences in pH.

## 4.2 ICED STORAGE

### 4.2.1 CHANGES DURING ICED STORAGE

The chemical changes during iced storage (Tables 3.6, 3.8, 3.9, 3.12, 3.13 and 3.16) indicated some pattern. The moisture content was found to be almost constant for the first few days ( 3-4 days) and afterwards registered an increase. After about 14-16 days there were no increase in moisture content. The changes were 2-3% in rohu, mrigal, milk fish and mackerel. Squid showed a different pattern. In two days the moisture was increased by 4% which was higher than that for the other fishes. In the early stages of iced storage the skin of mackerel, milk fish, rohu and mrigal might be

impermeable to water penetration and this property was lost during the post-mortem changes and might have resulted in the absorption of water. The squid had a very thin skin and hence resulted in the absorption of water during the first day itself.

The total nitrogen content was found to reduce in all the fishes (Tables 3.5, 3.8, 3.9, 3.12, 3.14 and 3.16). The variation in TN in most fishes was somewhat significant and was found to be linked with the increase in moisture content. This might be attributed to the leaching of non protein nitrogenous compound originally present in the muscle as well as formed during autolysis and subsequent bacterial action with the concurrent absorption of moisture.

The non protein nitrogenous compounds were found to decrease during iced storage. The NPN compounds consists mainly of nucleotides and protein decomposition products. The nucleotides as well as amino acids contribute to the flavour of the fish (Hashimoto, 1965; Thomson et al., 1980). Though an increase was expected for NPN, decrease in the amount of NPN was noted during iced storage in melting ice. The decrease in NPN content and increase in moisture occurred almost simultaneously. This could be attributed to the loss due to leaching. The NPN contents in all the fish samples from fresh water were in the range 9 to 11% of total nitrogen

while for milk fish (brackish water) it was 17.4, mackerel (marine) 13.1 and squid 31.3%. The higher amounts of NPN in fish in the brackish and marine waters may be attributed to the osmotic regulation of fish with their environment. Differences in the amount of NPN in rohu collected in January and June also were noticed. The January sample contained 11.1% NPN while June sample had only 9.4%. Watanabe et al. (1983, 1985) noticed changes in extractive nitrogenous constituents and reported that they were maximum in summer and minimum in winter in the muscle of ascidian. The amount of NPN was found very high in molluscs. (Takagi et al., 1967; Konosu et al., 1958; Suyana & Kobayashi, 1980).

The  $\alpha$ -amino nitrogen content was in the range 69 to 76 mg/100 g of fish flesh in all the fishes studied except squid. Squid showed a very high value of 238 mg%. It was in the range of 16 to 19% of NPN in rohu, mrigal and milk fish but in squid it was 25.4% of NPN. High values of  $\alpha$ -amino nitrogen were reported in squid and cuttle fish (Konosu et al. 1958; Kreuzer, 1984; Suyama & Kobayashi, 1980). Among the fishes milk fish from brackish water environment showed a slightly less amount (16.01) while the fresh water fishes showed around 18% of NPN as  $\alpha$ -amino nitrogen. As in the case of NPN the  $\alpha$ -amino nitrogen also showed a decrease during iced storage. The decrease might be due to the leaching of  $\alpha$ -amino nitrogen alongwith other soluble components.

The pattern of changes in TVN content of different fish studied showed significant difference depending on the environment from where they were caught. The fresh water fishes in general were found to contain high amount of TVN (Tables 3.6, 3.8 and 3.9) and noticed no increase during storage and TVN content remained more or less constant. This indicated that only small amount of TVN was produced and was leached into the melting ice. In brackish water (milk fish) and marine species (mackerel and squid) the TVN content showed slight increase during early stages of iced storage and then showed a sudden increase (Tables 3.12, 3.14 and 3.16). The initial value of TVN content was low compared to fresh water species but showed a substantial increase during iced storage. This strongly pointed out clear difference in the microflora of fish in different environment (Frazier, 1967; Kreuzer, 1954). Another factor might be the lower number of bacterial organisms in fresh water fish than marine species (Shewan, 1977). pseudomonas which is the major cause for spoilage is found in significant number in marine fishes (Shewan, 1977; Liston, 1980; Surendran & Gopakumar, 1981).

No trimethyl amine was found in fresh water fishes during iced storage. The mackerel contained a very low level of TMA (Table 3.16) in the beginning of iced storage which increased gradually for few days and then showed a sudden increase.



The percentage of salt extractable protein were low in the pre-rigor muscles of rohu and mrigal (Tables 3.6, 3.8 & 3.9). It then increased and on the later stages of rigor or in the early post-rigor muscle reached a maximum and then decreased. The decrease in extractability was not very significant during iced storage. Mrigal had an extractability of 66% of protein after one day storage and reached the maximum value of 86% by 6 days and then registered a slow decrease (Table 3.6). Rohu (January sample) after few hours of iced storage had a value of 54% and reached maximum in 3 days (80%) and then decreased slowly (Table 3.8). Rohu collected in June had a value of 45%, 50%, 70%, 73% in 0, 1, 5 & 6 days of ice storage (Table 3.9). The nature of protein changes in rohu collected in January and June showed some variations. (Fig.3.2) Hatae et al. (1985) observed that the extractability of myofibrillar proteins were low in the beginning and reached a maximum value by two days and remained almost same in the remaining period in 5 species of fish at 4°C. Almost similar effects of rigor on the extractability of protein were noticed by Dyer (1951) in pollack, Nikkila & Linka (1954) in baltic herring and Perigreen et al. (1987) in common murrel.

The SSN values were 67% and 65% after 1 and 4 days of iced storage respectively for milk fish and then registered a very slow decrease (Table 3.12). Squid had a high extractability of 88% in the beginning of storage and reduced

to a value of 58% in two days storage. This could be attributed to the high solubility of proteins of squid in water (Migita & Matsumoto, 1954) and part of the soluble protein might have leached to the melt water. The enzymes in squid muscle were found to be very active (Stanely & Hultin, 1984) and this might have contributed to its denaturation during iced storage. Mackerel showed a slow decrease in protein solubility throughout storage (Table 3.16).

A comparison of the extractability of proteins of different fishes are shown in Fig. 3.2. The fresh water fishes rohu and mrigal registered a low value during pre-rigor and rigor period and then increased and afterwards decreased slowly. The milk fish did not show such a phenomenon. The sample were not analysed during the period between 1 and 4 days might be the reason for not observing it. The squid was typical in its solubility and the extractability decreased rapidly during iced storage. In other fishes the decrease in extractability was small compared with frozen stored fish muscle. Similar patterns were noticed in different fishes (Anderson & Ravesi, 1968; Hatae et al., 1985).

The changes in lipids were measured by PV, FFA and TBA. The peroxide values showed an increase during iced storage (Tables 3.6, 3.8, 3.9, 3.12, 3.13 and 3.16). No detectable amount of PV was noticed in rohu and mrigal in the initial

analysis (Tables 3.6, 3.8 and 3.9). In all the three fishes the PV showed a rapid increase upto 14-16 days and afterwards increased slowly. No distinguishable difference in PV values of rohu collected in January and June could be obtained. Almost a similar pattern was observed in milk fish (Table 3.12) The PV reached 19.88 meq/kg fat in 14 days. The PV in mackerel increased steadily and rapidly upto 12 days (26.4 mg/kg fat). By this time the material became rancid and hence rejected (Table 3.17).

Peroxides are primary products of lipid oxidation. Autoxidation can be initiated enzymatically or nonenzymatically (Hsieh & Kinsella, 1986). Enzymes such as lipooxygenase (Josephson et al., 1984; German & Kinsell, 1985; German et al., 1985), peroxidase (Kaner & Kinsella, 1983) and microsomal /<sup>pv</sup> enzymes (Mc Donald et al., 1979; Rhee et al., 1984) can potentially initiate lipid peroxidation. The peroxide may break down to secondary products such as aldehydes, ketones etc (Gray, 1978) or react with proteins (Gardner, 1979). Mackerel and mrigal showed slightly higher peroxide value during iced storage. These fishes contained higher content of fat and had dark meat. The dark muscle contains more organically bound iron than ordinary white muscle. These haematin compounds catalyze the oxidation of unsaturated compounds, predisposing the dark muscle to more rapid development of rancidity (Castell

MacLean, 1964). Even when the PV of mackerel was as low as 18.8 meq/kg fat, it showed organoleptic rancidity. Hence PV alone cannot be used as a measure of oxidised flavour. Melton (1983) reported that the relationship of PV to oxidised flavour in muscle food apparently varies with the type of meat. Pearson et al. (1977) reported that PV has not been used extensively in the study of oxidised flavour in muscle foods.

The free fatty acids showed a slow increase in the early stages of iced storage. One day iced mrigal contained 1.6% FFA which was increased to 15.2% in 20 days iced storage. The corresponding values for rohu (January sample) were 1.2 and 11.2% and for rohu (June samples) were 1.3 and 12.1%. No significant differences in PV between rohu collected in January and June were noticed. Olley et al. (1962) found that the FFA of many fishes ranged from 0.6 to 5.9. Lovern and Olley (1962) stored fresh fish in crushed ice and noted that FFA increased slightly over the first 10 days and thereafter FFA accumulation increased sharply. Addison et al. (1969) reported that FFA was formed mainly through hydrolysis of phospholipids and partly from hydrolysis of triglycerides in herring. Oshima et al. (1984) found that FFA levels in cod increased in step with the decrease of phosphatidyl choline and phosphatidyl ethanolamine which were decreased slowly during first 4 days of storage in ice then rapidly upto 20 days. In skipjack also the

phospholipids were found to decrease during iced storage with a concurrent increase in FFA (Oshima et al., 1983a).

The TBA values of mackerel showed a slow increase upto 6 days and afterwards a rapid increase (Table 3.16). By 12 days iced storage the value reached upto 5.68 mg/kg meat. The mackerel samples were rancid by 9 days iced storage (Table 3.17). Awad et al. (1969) indicated that as the TBA rose progressively, the organoleptic rancidity increased in fish. The TBA value is most widely used as a measure of the extent of oxidative deterioration of lipids (Gray, 1978; Rhee, 1978).

The changes in pH of rohu (June) during iced storage are given in Table 3.10. The pH value reached 6.3 by one day iced storage. By 5 days the value was 6.39 and then showed a steady increase and reached a value of 6.74 in 20 days. The decrease in pH is due to the post-mortem anaerobic glycolysis wherein glycogen is converted to lactic acid. The pH in fish muscle is found to drop between 5.5 to 6.6 in fish (Amlacher, 1961).

The total plate count in fresh mrigal, rohu and milk fish were low, i.e. in the order  $10^2 - 10^3$ /g (Table 3.6, 3.10 and 3.12). The average bacterial count of pond-reared fish at the time of harvest was found to be  $7.3 \times 10^2$ /g by Acuff et al. (1984). The values showed a slow increase and reached a value

of  $10^6$  in 14 days in mrigal and afterwards did not register any significant increase. In rohu the bacterial count was increasing upto 18 days; afterwards showed a slight decrease (Table 3.10). In the initial stages of iced storage of rohu it took 5 days to reach a count of  $10^4$  from  $10^3$ . In the milk fish the initial value was very low ( $7.64 \times 10^2$ /g) and by 18 days storage it reached only a value of  $4.2 \times 10^6$ /g. But by 14 days storage it was rejected by the taste panel. Barile et al. (1985) found a standard plate count of  $10^3$ /g when fish were rejected by a trained taste panel. The low count even at the time of rejection of the fresh and brackish water fishes showed that bacterial population might be predominantly mesophiles in these fishes.

The major organoleptic factors affecting the quality of ice stored fish are appearance, flavour and texture. In extremely fresh fish the surface appeared bright and shining and the scales were intact. The gills were found bright red for all fishes (Tables 3.7, 3.11, 3.17). In extremely fresh squid the skin chromatophores are found moving and the muscle was very white (Table 3.18). On 6th day of ice storage of mrigal the gills were slightly bleached, 12th day brownish and 14th day dark (Table 3.7). The development of off odour also was almost synonymous with the colour change of gills. The development of yellow colour at the belly

portion of mrigal were noticed by 12 days of storage. The bones were also found separated from the meat at the belly portion by 12th day. The texture was found soft to firm and tender in the early stages of storage. There was gradual loss of textural properties and became pasty by 16th day (Table 3.7). The appearance of eyes were also changing. It became sunken and developed red colour around the eyes by 12th day. The characteristics odour of the flesh was gradually lost on storage. It developed slight off taste by 16 days and it was prominent at the belly portion. The pattern of changes were almost similar in rohu (Table 3.11). It showed yellow discolouration at the belly portion on 4th day itself. The muscle of rohu was more firm than that of mrigal. The changes in sensory characteristics were more rapid in mackerel (Table 3.17). By 6th day the eyes were sunken, muscles soft, juiciness lost and the scales became loose. It developed rancidity by 9 days ice storage. Variations in texture among the four fishes were noticed. The textural changes during ice storage also varied considerably. Hatae et al. (1985) noticed differences in the rate of softening in the five species kept at 4°C.

The sensory characteristics in ice storage showed a different pattern for squid (Table 3.15). It had white muscle and the flesh was firm and chewy. During ice storage the mantle thickness was found to reduce, became flabby, soft and

the pink colour from the chromatophores spread on the mantle by 2 days ice storage. On 4th day the intensity of pink discolouration on the mantles were considerably reduced and yellow discolouration was spread on the belly portion. Decayed odour was developed at the belly portion of whole squid by 4 days storage and became intense by 6th day. The fresh muscle was sweet, and the sweetness was reduced considerably by 2 days storage and sweetness was completely lost by 4 days storage. The rapid spoilage at the belly portion of the squid may be due to greater amount of digestive enzymes found in the digestive tract (Morishita et al., 1974) Glycogen was found in small amounts in squid and hence the lactic acid production was low (Kreuzer, 1984). This account for the rapid onset of rigor mortis (Otwell, 1978) and an early spoilage could be expected.

Rohu and mrigal exhibited a peculiar phenomenon when they were iced immediately after capture. The rigor mortis sets in by 1½ - 2 h ice storage and the fishes were in full rigor in 4 hours while those fishes kept at ambient temperature (30°C) did not develop rigor by that time. The rigor started after 4 hours and about 30% fish were in almost full rigor while in others the rigor was only partial. Curran et al. (1980) noticed such a phenomenon, i.e. rapid development of rigor in the tropical fish, tilapa. They called it cold shock stiffening since the composition and



amount of nucleotides and glycogen break down products varied in normal rigor and cold shock stiffening. So it was concluded that cold shock stiffening and rigor mortis stiffening were different.

#### 4.2.2 SHELF LIFE

The shelf life of different fishes in ice could be obtained from Fig. 3.2. Among the fishes studied rohu had the highest shelf life in ice, about 17 days. Mrigal, milk fish, mackerel and squid had shelf lives of 15, 14, 8 and 4.5 d. respectively. The changes in the sensory scores of the fishes were almost identical. In the initial periods of ice storage they showed slow decrease in sensory score and afterwards the decrease was substantial compared to the initial changes. But squid showed a different picture. The sensory changes were rapid during the first two days. This could be attributed to the loss of characteristic sweet flavour as a result of the significant leaching. The leaching and the concurrent absorption of water caused an increase in water content and changes in texture. The enzymes present in gut (Kreuzer, 1984) and in the muscle (Kolotovkin et al., 1986) might have accelerated the spoilage.

Different shelf lives in ice had been reported for mrigal Nair et al. (1971) and Nair & Dani (1975) reported shelf lives of 36 and 27 days respectively. But Bandhoyopadhyay et al. (19

found a shelf of only 17 days for mrigal of size range 36-40 cm and 13 days for size range 30-33 cm. The vast difference in shelf life noticed for mrigal might be partly due to the difference in location and habitats and also due to the difference in the methods of icing as well as the method of assessing the shelf life. But this alone could not give a satisfactory explanation for the vast difference in shelf life. In the present study the shelf life was found to be only 15 days.

Gupta et al. (1980) reported a shelf life of 19 days for milk fish obtained from Andhra coast. The difference may be due to the changes in the location, bacterial flora of the waters and season. The assessing methods also caused variation in the shelf life. Yunizal & Arifudin (1973) reported a shelf life of 11-16 days for milk fish from Indonesia and Beza & Seson (1978) found a shelf life of 12 days for the brackish water milk fish.

A shelf life of 7-9 days were reported for Indian mackerel from India, Thailand, Brunei, Pakistan and Seychelles (Perigreen et al., 1975; Kasemsaran et al., 1967; Curran & Disney, 1979; Hussain, 1980; Rogers & Hoffman, 1980). However Velankar & Kamasastri (1956) reported a shelf life of 13 days and Banik et al. (1976) 21 days.

In squid also widely varying shelf life in ice have been reported. Botta et al. (1979) reported a shelf life of 8.5 days of squid, Illex illecebresus. Ampola (1980) found shelf lives of 10, 8 and 9 days respectively in three experiments in Loligo pealei. Ke et al. (1979a) iced squid in polythene bags and assessed the quality based on skin colour, texture condition of the mantle and flavour and odour. They found that squid could not be used for food for more than  $1\frac{1}{2}$  days. Ke et al. (1979b) considered that storing squid in direct contact with ice was not a proper means for handling squid since some disadvantages could arise such as skin discolouration changes in texture, flavour etc. In the present study the shelf life was found only 4.5 days. These variations in the shelf life may be due to the difference in catching, handling, icing and assessing methods. Trawling causes a high level of physical damage during the actual fishing operation and when the catch is handled on deck. Netted squid often exhibit ripped and torn mantle, poorer colour and a softer texture compared with jigged squid (Kreuzer, 1984). There is also an apparently increased susceptibility to bacterial action (Rathjen & Stanley, 1982). In the present study trawled squid was collected and iced directly in contact with ice. These might have caused low shelf life.

#### 4.2.3 SEX

The iced shelf life of male and female rohu showed only slight difference between them (Table 3.11). Statistical analysis of the sensory scores for t-by-difference for paired scores to determine the significance of difference gave a value of 2.52 which was not significant at 5% level. This indicated that there was statistically no difference in shelf life between the two samples of size 560 g collected in September. None of the samples was in the spawning condition. The results indicate that sex has no considerable effect during normal periods on shelf life. But after spawning shelf life in ice has been considerably reduced. Barasai et al (1981) found that in prespawning condition whole blue whiting could be stored upto 12 days while in postspawning condition the keeping time could not exceed 6 days. In certain species the females are of very poor quality soon after spawning while in some species such as salmon both sexes may be in poor condition after spawning (Wheaton & Lawson, 1985).

#### 4.2.4 BLEEDING AND GUTTING

The changes in PV and sensory score of whole and bled and gutted milk fish are shown in Table 3.13. The colour of the meat had been improved considerably in bled fish. Bleeding considerably reduced the blood stains in the flesh. Another important aspect was the reduction in the PV of the bled and

guttled fish. The development of peroxides was delayed and also reduced to half of that in whole fish during iced storage. This effect might be due to the removal of blood from the muscle which considerably reduced the amount of prooxidant iron from the meat. The sensory score also showed significant improvement. The t test gives a value of 5.59 which is highly significant (for 1% significance the value is 4.03). The increase in shelf life was due to the removal of blood from the muscle as well as the removal of gut which contains highly active proteolytic enzymes and large number of bacteria. Scott et al. (1986) found that there was reduced autolysis in the headed and gutted fish. In the present study the shelf life of milk fish in ice had been increased by 4 days by bleeding and gutting.

Many research workers have reported an extension in iced shelf life as a result of gutting fish such as gurnard (Vyneke, 1980), hake (Lupen et al., 1980), croaker and gray trout (Townley & Lauier, 1981), spiny dog fish (Bilinski et al. 1983), rainbow trout (Vyneke, 1983), spiny dog fish (Ravesi et al., 1985) and orange roughy (Scott et al., 1986). Bleeding and gutting have many advantages. Botta et al. (1982) reported that gutting reduced DMA production in round rose grenadier. Burt et al. (1974) demonstrated that delays in the gutting of Cape hake led to discolouration. Boyd & Wilson (1977

found that gutting reduced the rate of loss of texture quality in snapper caught during the period when the fish are actively feeding. Huss & Asenjo (1976) however, noted that while all fish they examined suffered some loss of quality if gutting were omitted, great differences were found between species. Some workers have reported that gutting did not significantly extend shelf life (Maia et al., 1981; Adebona, 1981; Avdalov & Repoll, 1981).

#### 4.2.5 SIZE

The size of the rohu collected in June and January had an average weight of 800-810 g while that collected in September had an average weight of 560 g. The shelf life of the former two samples were about 17 days while the smaller samples had a shelf life of around 14-15 days (Tables 3.8, 3.9, and 3.11). Since the fishes were collected from a fish culture pond and they were not under stress due to spawning or any other physical condition it could be considered that the decrease in shelf life was due to the size effect. According to Connell (1980) it is an established fact that large fish keep better than small fish. Bandhyopadhyaya et al. (1985) found significant lowering of shelf life of mrigal in ice as the size was decreased. One of the major cause of spoilage in fish is due to the action of bacteria which is penetrated into the muscle from the surface. Larger fish have a smaller surface

area to volume ratio so that in the same time period less of the interior of the larger fish is affected. During actively growing period the gut and muscle enzyme activity may be more in young ones which also contribute to the earlier spoilage.

#### 4.2.6 SEASON

Iced storage characteristics of rohu collected during January, June and September (Tables 3.8, 3.9 and 3.11 respectively) showed a shelf life of 17 days for rohu collected during January and June of weight 800-810 g while the September samples, had only 14-15 days (weight 560 g). This was mainly due to the size effect rather than seasonal effect. In fatty fishes changes in chemical composition and resultant changes in shelf life were noticed (Mathen et al., 1966). Connell (1980) reported that fish were in poor condition at the period after spawning. During this period the iced shelf life of fish was reduced considerably (Barassi et al., 1981).

### 4.3 FROZEN STORAGE OF FISH

#### 4.3.1 SHELF LIFE OF DIFFERENT SPECIES

During frozen storage at -20°C of mackerel, squid and milk fish moisture and protein showed changes in their contents as well as proteins and lipids showed changes in their composition. All the frozen stored samples showed a decrease in moisture content (Tables 3.18, 3.24 and 3.23).

The decrease in moisture content was maximum in mackerel among the three species studied. It showed an average decrease of 0.147% per week and the content was reduced by 4.71% in 32 weeks storage (Table 3.23). Squid showed an average rate of moisture decrease of 0.055% per week and in 62 weeks it was reduced by 3.43%. Upto 32 weeks there was practically no decrease in the moisture content in squid. After 32 weeks the decrease in moisture content was rapid and decreased by 2.95% in 30 weeks i.e. at a rate of 0.1% a week. Milk fish stored at  $-20^{\circ}\text{C}$  showed only a small decrease in moisture during 50 weeks storage. The decrease rate was 0.029% per week (Table 3.20). The effect of the difference in vapour pressure of the storage cabin and the product on moisture loss could be discarded since all the products were kept in the same storage cabin.

As in the case of moisture the SSN values also showed maximum decrease in mackerel (Table 3.23). The SSN decreased from an initial value of 73.12% to 47.03% in 32 weeks. The SSN values of mackerel decreased at a rapid rate, 1.37% per week for the first 12 weeks and afterwards showed a slow decrease (0.48% per week). The average rate of decrease was 0.82% per week. Squid showed a decrease of 30% in 62 weeks and decreased at an average rate of 0.48% per week. It did not show a definite pattern of decrease in extractability (Table 3.18). Milk fish showed the minimum changes in extractable protein and showed a decrease of only 12.27% in 50 weeks and the



average rate of decrease was 0.25% per week.

The lipid changes were studied in milk fish and mackerel. The average rate of formation of PV was more in mackerel, 1.09 m.eq/kg fat per week (Table 3.23) than in milk fish 0.43 m.eq/kg fat per week (Table 3.20). The net increase in PV was 21.24 m.eq/kg fat in 50 weeks in milk fish and 35 m.eq/kg fat in 32 weeks in mackerel. In milk fish an increase in PV was shown upto 40 weeks and afterwards decreased (Table 3.20) After 40 weeks more decomposition of peroxide took place than it was formed. The average rate of increase was 0.61 m.eq/kg fat upto 40 weeks and then decreased at a rate of 0.31 m.eq/kg. In mackerel the increase in peroxide formation was upto 28 weeks and increased at a rate of 1.51 m.eq/kg fat per week. After 28 weeks the decrease in PV was at a rate of 1.8 m.eq/kg fat per week. It showed a rapid decomposition or removal of peroxides than that took place in milk fish.

The rate of formation of peroxides in mackerel was  $2\frac{1}{2}$  times faster than that in milk fish. It can be attributed to the high amount of red meat in mackerel. The red meat in general contains more haematin compounds which are prooxidants (Hultin, 1976; Castell & MacLean, 1964). Also red meat contains high amount of lipid and the rate of lipid hydrolysis in fish was shown to be faster in dark muscle than in white muscle

(Olley et al., 1962). Though both fishes are individually packed and stored mackerel had more contact with air because of the smaller size than milk fish and hence more surface to volume ratio. Because of all these a faster rate of formation of peroxides in mackerel than in milk fish is expected and found true.

Very small amount of TBA reacting substances were formed in milk fish i.e. 2.34 mg/kg meat in 50 weeks (Table 3.20). The TBA value was found to increase with storage time. Mackerel also showed the same pattern but the rate of formation of TBA reacting substances were significantly higher in mackerel (0.33 mg/kg fat per week) than in milk fish (0.047 mg/kg fat per week). Since more amount of peroxides were formed in mackerel the decomposition product also might have shown a proportional increase.

From the above results it could be seen that various chemical changes are interconnected. Mackerel showed maximum decrease in water content and extractable protein. The decrease in extractable protein could be used as a measure of the extent of denaturation (Powrie, 1973). Hence mackerel showed maximum denaturation and resulted in a decrease in water holding capacity. Diminution in water holding capacity leads to excessive exudation of fluid (Powrie, 1973) and the moisture content also is reduced proportionally. King & Poulter (1985)

noticed a steady and rapid decrease in extractability of protein in mackerel at  $-14^{\circ}\text{C}$  storage. Jiang & Lee (1985) found that the muscle protein of frozen mackerel was the most unstable compared to amber fish and carp.

The results (Table 3.20) of extractability of protein studies showed that milk fish proteins were not very much susceptible to denaturation. In the present study the extractability reduced from 67.59 to 55.32% in 50 weeks. Jiang et al. (1988) observed a decrease in the salt soluble fraction of protein in milk fish from 73.3 to 58.9% in 10 weeks at  $-20^{\circ}\text{C}$ . They reported that aggregation denaturation in milk fish was mainly caused by the formation of hydrogen, hydrophobic and disulfide bonds during frozen storage.

The decrease in protein extractability was maximum in mackerel followed by squid and least in milk fish. Considerable variation in the salt extractability of proteins in different species was noticed by Olley et al. (1962). In some fishes quality frozen stored products cannot be produced because of the denaturation of proteins (Jiang, 1977; Nosaki et al., 1979; Kurokawa, 1979). Denaturation caused reduced moisture retention and increased muscle toughening and were associated with irreversible muscle contraction and matrix discontinuity due to ice crystal growth (Lee, 1982). Denaturation as measured

by decrease in salt extractable protein showed maximum in mackerel. This was closely related with the high rate of formation of peroxides (Table 3.23). Peroxides and their decomposition products are well known for causing changes in protein (Sikorski et al., 1976; Shenouda, 1980). Shenouda (1980) reported that oxidised lipids interact with proteins causing undesirable changes in the nutritional and functional properties of protein. Takama et al., (1972) and Takama (1974) observed that the products of lipid oxidation rendered the fish proteins into harder, more elastic and insoluble complex.

The nature of free amino acids present in the fish muscle also had effect on protein denaturation. Jiang (1985) and Jiang & Lee (1985) found that muscles containing high levels of free histidine and lysine had greater protein denaturation while those containing high levels of free glycine, alanine and proline had less protein denaturation. Jiang & Lee (1985) reported that the predominant amino acids in the muscle of frozen mackerel was histidine, lysine, alanine and taurine and also noticed that the muscle protein of frozen mackerel was very unstable. In squid muscle Endo et al. (1962) found a high amount of proline, glycine and alanine and a very low amount of histidine and lysine. So a low rate of protein denaturation is expected in the squid muscle. In the present study it has been found that the rate of protein denaturation in squid muscle as measured by changes in SSN values was about half as that in mackerel.

The NPN contents in the three species were found decreasing during frozen storage (Table 3.19, 3.20 and 3.23). The average rate of decrease per week was 3.5, 3.13 and 2.86 mg% respectively for mackerel, squid and milk fish. The decrease in NPN content was found to be proportional to the decrease in moisture content. Hence it could be assumed that the loss in NPN was due to the leaching of these into the thaw exudate and the more the thaw exudate the more would be the loss of NPN. The  $\alpha$ -amino nitrogen was also found decreasing during frozen storage. In squid the decrease was 70 mg% in 62 weeks (Table 3.19) and about 46% of the loss of NPN fraction was due to the loss of  $\alpha$ -amino nitrogen. The volatile base nitrogen content showed an increase in frozen storage. The increase was 8.2 mg% in 62 weeks in squid (Table 3.19), 9.8 mg% in 50 weeks in milk fish (Table 3.20) and 9.94 mg% in mackerel in 32 weeks (Table 3.23). This indicated that more TVBN was formed than it was leached into the solution. The TMA content in mackerel showed no noticeable change during frozen storage (Table 3.23).

A discription of the sensory changes in squid during frozen storage is given in Table 3.18. The white colour of the mantles was almost maintained upto 40 weeks, afterwards showed slight dull colour and the intensity was gradually increased on further storage. A few samples showed slight dessication by 24 weeks storage. By 48 weeks most of the

samples showed dessication and the amount of thaw drip was considerably increased by this time. The initial sweet taste of squid was gradually reduced on storage.

The shelf life of the three species could be obtained from Fig. 3.3. Squid had a shelf life of 62 weeks, milk fish 41 weeks and mackerel 25.5 weeks. All the samples showed a rapid change in the early stages of storage and afterwards showed a steady decrease. The rate of decrease in a 9 point hedonic scale was 0.11, 0.18 and 0.23 per week for squid, milk fish and mackerel respectively in the early stages and the corresponding rate of decrease afterwards was 0.05, 0.08 and 0.12. A lower value of shelf life was expected in mackerel from its chemical changes. The changes in protein and lipid was more than that in other two species. The major sensory changes in mackerel were toughing of muscle and development of off flavours. In milk fish no off flavour development was noticed but the gradual development of tough and fibrous texture limited its shelf life. Squid was found loosing its sweet taste on storage. Also it developed considerable dessication.

Shimizu & Kaguri (1986) reported that the shelf life of frozen fish was affected by the species of fish. They further observed that the shelf life of many fishes varied within the

species and this variation was due to the biological condition of fish such as season, age, death condition, freshness and fishing place.

#### 4.3.2. HANDLING ON FROZEN SHELF LIFE OF SQUID

The squid mantles were frozen after handling it in three different ways. The samples were frozen immediately after bringing it to the laboratory (I); after keeping the mantles in ice for one day (II) and keeping the whole squid in ice for one day (III). The changes in NPN,  $\alpha$  amino nitrogen and TVN are given in Table 3.19. NPN,  $\alpha$ -amino nitrogen and TVN decreased considerably in sample II compared to I. The flesh exposed to melting ice caused leaching of substantial quantities of these soluble substances. Between samples I and III, the amount of NPN was almost same. This was resulted by the substantial hydrolysis of proteins and nucleotides. The highly active gut enzymes (Gildberg, 1987; Kolotavkin et al., 1986; Sikroski & Kolodziejska, 1986) and the bacteria in the guts and those migrated to the squid muscle had accelerated the decomposition process. Also the skin prevented part of leaching of soluble components. Though NPN content did not reduce considerably between samples I and III substantial reduction in  $\alpha$ -amino nitrogen was noticed. This showed that  $\alpha$ -amino nitrogen was not produced in substantial quantities in muscle during one day iced storage of whole squid. But the volatile basic

nitrogen was increased considerably in sample III showing significant bacterial and enzymes action.

The changes in salt extractable nitrogen is shown in Fig. 3.4. Fresh squid showed high values of SSN (85% of TN) while sample II had a solubility of 79% and sample III, 72%. The decrease of SSN in sample II was due to the leaching of some of the soluble proteins in melting ice while in sample III enzyme action had caused consideration reduction of SSN. All the three samples showed a parallel decrease in SSN upto 25 weeks. After 25 weeks sample I showed a steady decrease but at a reduced rate, sample II did not show much reduction after 25 weeks showing a reduced enzyme action causing aggregation and cross linking of proteins. Part of the enzymes might have leached into the melting ice when the mantles were kept directly under ice. Sample III showed a steady decrease throughout storage.

Fig. 3.5 shows the changes in weight on thawing and also cook drip loss. The least thawing loss and cook drip loss were shown by sample I. The thawing loss exceeded 5% only after 35 weeks in Sample I. The samples II and III showed values of 7 and 9% weight loss during thawing in the initial periods of frozen storage itself. The high amount of weight loss in sample II could be attributed to the increased absorption of moisture during iced storage (Table 3.14) and partly due to the increased denaturation of protein (Fig. 3.4).



In sample III also increased water absorption during iced storage and denaturation during frozen storage would be the reason for such a high weight loss on thawing. Almost a similar pattern was shown in cook drip loss also. From these it is found that keeping of squid directly in contact with melting ice resulted in high absorption of moisture and loss of flavour producing components because of the peculiar nature of its flesh as well as protein and caused considerable changes in its chemical and sensory properties.

Fig. 3.6 showed that shelf life of three samples of squid. Sample I had a shelf life of 62 weeks, sample II had a shelf life of 48 weeks and sample III 43 weeks. This showed that sample II lost a shelf life of 14 weeks and III, 19 weeks as a result of iced storage. The shelf life of whole squid in ice was 4 days (Fig. 1 and Table 3.15) and so one day iced storage is expected to reduce the shelf life of whole squid by 25%. If the assumption is correct the expected shelf life during frozen storage of whole squid after one day icing is 46 weeks and by experiment it has been found to have a shelf life of 43 weeks. The slight variation may be due to the experimental error. Also when growth of microorganisms occur, the effect of time temperature treatment is unlikely to be additive (Jul, 1984). At ice storage temperature the growth of microorganisms occur and alter the additive effects. However, additivity of

effects, i.e. the defects of quality once produced in fish/fish products persist and are added to by all other defects that may be subsequently produced.

#### 4.3.3. ICED STORAGE AND CONSEQUENT FROZEN SHELF LIFE

Milk fish samples were frozen after keeping it in ice for different periods and the changes are given in Tables 3.21 and 3.22 and in Fig. 3.7. The NPN of all the samples showed a decrease during frozen storage. The rate of NPN decrease per week was increased as the storage period in ice increased. The rate of decrease for 0, 4, 7, 10 and 14 day iced samples were 1.75, 2.75, 4.2, 5.8 and 5.45 mg%/week respectively. This shows that the leaching of NPN in thaw drip increased in proportion to the period of storage in ice upto a certain period and afterwards remained almost constant. The thaw drip also was expected to show an increase.

The PV of all the samples showed an increase during frozen storage. All the samples showed a maximum PV of around 22 to 26 m.eq/kg fat. The time to attain the maximum value was somewhat proportionally reduced depending on the period of storage in ice. This indicated that the extent of oxidation remained almost same within a species of fish irrespective of icing periods. Depending on the duration in ice, the period to attain maximum PV is reduced.

The extractability of protein showed proportional decrease depending on the iced storage period (Fig. 3.7). The fresh milk fish showed remarkable stability of protein than the iced samples.

Fig. 3.8 showed the shelf life of milk fish during frozen storage after icing for different periods. Samples iced for 4 and 7 days showed a sudden decrease in sensory score in the early stages and afterwards showed a steady decrease. The shelf life for 0, 4, 7, 10 and 14 day iced samples were 42, 31, 22, 12 and 0 weeks respectively. The shelf life in ice was 14 days. Considering the additivity of defects the expected shelf life for 4, 7, 10 and 14 days iced samples are 30, 21, 12 and 0 weeks respectively and the corresponding experimental values were 31, 22, 12 and 0 weeks. The additivity effects revealed in squid samples were further confirmed in the storage studies of milk fish.

#### 4.3.4 SEASON

The shelf life of mackerel collected during April, June and December ~~were~~<sup>was</sup> determined (Fig. 3.9) and changes in some of the properties are given in Tables 3.23, 3.24 and 3.25. PV and TBA showed variations in the rate of changes in these samples. The average rate of increase of PV per week of April, June and December samples were 1.76, 1.14 and 0.92 respectively and in April sample PV reached the maximum in 24 weeks. Though the fat content was high in December samples it showed more stability.

against peroxidation than the other two samples. This might be attributed to the possible presence of high amount of tocopherols which might have accumulated from the food during the feeding period. The formation of TBA reacting substances was highest in June samples (0.33 mg/kg meat per week) than December and April samples (both showed a rate 0.26 mg/kg meat per week).

The shelf life of these samples could be determined from Fig. 3.9. The December sample showed a shelf life of 30 weeks while April and June samples showed shelf life of 25 and 26 weeks respectively. Such seasonal variation in shelf life has been noticed in many fishes. Waters (1982) found maximum nutritive value and storage stability in spot caught between October and February. Naturally occurring tocopherols in frozen muscle are effective antioxidants and the tocopherol content varies with season in most species (Syvaioja et al., 1985). Also the stability of tocopherols changes with season. Ackman (1967a) found that the tocopherols in sole caught in the period of June to early August decomposed completely in 4 months frozen storage while those collected at other times were significantly more stable. Thus a complex of many factors determine the shelf life and vary with season as well as species of fish.

#### 4.3.5 IQF VS BLOCK FROZEN FISH

Mackerel samples collected in April were packed and frozen

individually (IQF) and as blocks (BF), glazed and stored. The changes in fat and sensory properties of these two samples are given in Table 3.25. Highly significant variation in fat changes were shown between the samples. In IQF, PV showed an increase upto 24 weeks and then slowly decreased, while BF samples showed a slow increase in PV throughout the period of storage. The rate of increase of PV in IQF was 1.78 m.eq/kg fat/week while in BF it was only 0.57. Similar was the trend in TBA values too. In IQF samples the exposed surface area to air was significantly higher than that in BF samples. The loss of glaze in IQF would be more. Since in BF, the samples were embeded in ice there would not be much contact with air and significantly low PV and TBA was expected. The IQF sample were acceptable only upto 24 weeks while BF samples were in fair condition at 30 weeks storage. Statistical analysis for significance in shelf life showed a t value of 3.20 between the two samples which is significant at 5% level.

#### 4.3.6 STORAGE TEMPERATURE

The effect of storage temperature on SSN, PV and TBA and sensory properties are shown in Table 3.26. The samples were stored at -20 and -30°C. The SSN values showed an average rate of decrease of 0.9 and 0.48% per week <sup>respectively</sup>. This showed that a decrease of 10°C reduced the SSN changes to be almost half. The sensory studies also showed such a change. The samples

stored at  $-20^{\circ}\text{C}$  became tough, fibrous and rancid by 30 weeks storage, while the samples at  $-30^{\circ}\text{C}$  were juicy and not developed any rancid odour by that time. The PV and TBA values also confirmed this. The rate of increase in PV was 1.8 m.eq/kg fat per week at  $-20^{\circ}\text{C}$  while it was only 0.51 at  $-30^{\circ}\text{C}$  i.e. more than 3 fold decrease in peroxide formation by reducing the temperature by  $10^{\circ}\text{C}$  (from  $-20$  to  $-30^{\circ}\text{C}$ ). The TBA values were 0.25 and 0.05 mg/kg meat/week at  $-20$  and  $-30^{\circ}\text{C}$  respectively. Here the decrease was 1/5th. The sensory score also supported the above observations.  $-20^{\circ}\text{C}$  stored sample was in the limit of acceptability at 24 weeks storage, while  $-30^{\circ}\text{C}$  stored sample was in good condition even at 30 weeks storage.

Frozen storage temperature has profound influence on shelf life. Licciardello et al. (1982) found that red hake fillets had a shelf life of 150 weeks at  $-20^{\circ}\text{F}$ , 71 weeks at  $-5^{\circ}\text{F}$ . They observed that the deteriorative changes such as development of tough and fibrous texture proceeded at a faster rate at  $-5^{\circ}\text{F}$ . Lee (1982) reported that the freeze-thaw instability resulting from frozen storage at  $-20^{\circ}\text{C}$  was significantly improved by storing fish at  $-30^{\circ}\text{C}$ . Fukuda et al. (1982) found that the denaturation of myofibrillar protein was less at lower temperature regardless of the initial freezing temperature. But in the Bolivian fish sabalo, Curran et al. (1986a) could not find significant change at  $-15$  and  $-30^{\circ}\text{C}$

storage in pH or protein extractability. The storage life was in excess of 20 months at both  $-15^{\circ}\text{C}$  and  $-30^{\circ}\text{C}$ . Shaban et al. (1985) studied the extractability of protein of Alaska pollack and found that myofibrillar proteins were gradually denatured at  $-20^{\circ}\text{C}$  and denaturation proceeded much more slowly at  $-30^{\circ}\text{C}$  and no or little denaturation occurred below  $-40^{\circ}\text{C}$ . For Indian mackerel with an average fat content of 3.8% King & Poulter (1985) found a shelf life of about 16 months at  $-30^{\circ}\text{C}$  and 6 months at  $-15^{\circ}\text{C}$ . In general the temperatures of frozen storage influences the quality changes and shelf life in almost all fishes. By reducing the temperatures of the frozen storage by  $10^{\circ}\text{C}$  reduces considerably lipid peroxidation and related changes, improves protein stability and enhances the shelf life by more than double in mackerel.

#### 4.3.7 ANTIOXIDANT TREATMENT

The changes in PV, TBA and sensory score of mackerel treated with BHA and untreated are given in Table 3.27. Significant reductions in the rate of increase of PV and TBA were noticed between the samples. The rate of increase of PV per week of the treated and untreated samples were 0.28 and 1.8 respectively. The PV continued its slow increase even at 30 weeks storage in treated mackerel while it showed an increase upto 24 weeks and then afterwards decreased in the untreated samples. The TBA values also showed such significant difference between the treated and untreated samples. Both samples showed

an increase of TBA throughout storage. The treated samples were better organoleptically and was in fair condition even at 30 weeks storage while untreated sample was acceptable upto 24 weeks. But the reduction in temperature by 10°C was found to be more effective in maintaining the sensory properties than treatment with BHA (Tables 3.26 and 3.27).

Synthetic antioxidants such as BHA has been found effective to protect marine products from deterioration (Toyama & Shimizu, 1972). Many other antioxidants also have been successfully tried in fish. Sodium erythorbate (Bilinski et al., 1979; Iredale & York, 1977) tocopherol (Takama et al., 1978; Ackman & Cormier, 1967) etc. <sup>were</sup> ~~are~~ found to have very good antioxidant properties. In the present study BHA has been found to retard lipid ~~per~~oxidation and the formation of TBA reacting substances. The treatment improved the sensory qualities by retarding the development of rancid odour.

#### 4.3.8 REFREEZING

Table 3.28 shows the effect of filleting and freezing as well as freezing whole fish and thawing after a period of storage, filleting and refreezing. There was slight variation in the extractive nitrogen. The process of thawing, filleting and refreezing caused more denaturation of proteins but the change was not significant. The PV value also showed a slight



increase in the refrozen samples. The sensory scores showed variations. The refrozen samples were less acceptable and statistical analysis showed significant difference at 5% level.

In general refrozen materials have been found less acceptable than fresh frozen materials. Hiltz et al. (1977) found that refrozen silver hake processed as fillets underwent rapid deterioration during storage at  $-18^{\circ}\text{C}$  compared with once frozen control materials. Studies on cod refrozen as fillets have shown that the initial quality of the refrozen product is usually good, being undistinguishable from the once frozen materials (MacCallum et al., 1966; Peters et al., 1968), but on subsequent storage deterioration may be rapid compared with once frozen controls (Dyer et al., 1962). According to MacCallum et al. (1966) an acceptable and better twice frozen product was obtained by starting with material well handled and quickly chilled. Peters et al. (1968) found that freezing pre-rigor was preferable to freezing post-rigor for refreezing. Present study showed that freezing, thawing, filleting and refreezing of cat fish produced a product of inferior quality compared to freshly filleted and frozen cat fish.

#### 4.3.9 PACKAGING OF FILLETS

Effect of packaging on weight loss of cat fish fillets during storage at  $-20^{\circ}\text{C}$  is given Table 3.29. The frozen stored materials without any packaging showed a weight loss of 0.68%

in the first 5 weeks and in the next 5 weeks it was 2.07% and for the next 6 weeks it was 3.39%. This showed that the property of binding water molecules either by capillary forces or by proteins was decreased during storage and hence the weight loss increased as the period of storage increased. The removal of water vapour from the surface of fish accelerated the migration of water molecules from inside to the surface. Also the migration of water molecules disrupt the hydrogen bonding system and results in deconformation of the three dimensional structure. This induces interaction between the protein molecules and causes aggregation and denaturation.

Keeping the material without any polythene lining inside a carton was sufficient to reduce the weight loss considerably. But covering the material with a polythene sheet was sufficient to almost prevent weight loss. Keeping the wrapped material in a waxed carton practically stopped the weight loss for the first few weeks. Glazing and wrapping the material in a polythene sheet and keeping inside a carton also had the same effect. All the covered materials by a 200 gauge polythene sheets showed almost the same weight loss after 28 weeks storage.

The yield of the fillets after thawing is given in Table 3.30. About 5% weight loss was noticed by the process of freezing. The maximum loss of weight on thawing was noticed in unpacked fillets. This indicated that the water holding

capacity of the proteins were greatly affected by evaporation losses of moisture during storage. Keeping the materials in a waxed carton significantly reduced the quantity of weight loss on thawing. Further decrease was noticed by covering the material in a polythene sheet. Wrapping in a polythene sheet and keeping it in a carton did not make much difference from polythene wrapped material. But glazing the material had been found to reduce the weight loss on thawing considerably compare to samples 2 and 4.

Table 3.31 shows the changes in fat during frozen storage of fillets. The maximum changes in PV was noticed for sample I Sample 2 also showed significant increase in PV. The changes in PV in samples 2 and 4 were almost same during storage. Sample 5 showed significantly lower changes compared to all other samples. Glazing reduced the air contact considerably and hence a lower PV was obtained. FFA values did not show any significant pattern.

The shelf life of different samples are given in Fig. 3.1 Sample I showed a shelf life of only 6 weeks. By that time the product showed extensive dehydration and became rancid and the texture tough and fibrous. Just by keeping in a waxed carton the shelf life was improved and had a shelf life of 15 weeks. The edges of the fillet block showed dessication and considerable changes in PV because of the air contact. Samples 2 and 4 had

almost the same shelf life i.e. 24 and 26 weeks respectively. The reduction in the changes of lipid and also low evaporation losses might be the reason for it. Glazing further protected the material and the shelf life of the glazed material was 32 weeks. The experiments showed that wrapping the product in a suitable packaging material of low water vapour and air permeability significantly improved the quality and shelf life and this was further improved by glazing. Ahvenainen & Malkki (1985) found that packaging affected quality under all storage conditions studied.

#### 4.3.10 FROZEN STORAGE OF MINCED FISH

The percentage changes in SSN of minced fish from cat fish, threadfin bream and lizard fish are given in Fig. 3.11. Minces from all the three species showed a sudden decrease in SSN values and lizard fish showed the maximum decrease. After 5 weeks of storage the SSN showed only a slow decrease, the decrease being more in threadfin bream. But after 34 weeks storage both cat fish and threadfin bream showed almost the same values in SSN while that in lizard fish was the lowest. A corresponding change in the textural properties of mince from these fishes were also noticed. A rapid change in textural quality of minced lizard fish was observed. The texture became fibrous, tough and dry. The other two species were comparative better in textural properties. Certain species like lizard fish

can never produce high quality frozen stored minces. Shimizu & Fujita (1985) observed that the frozen storage tolerance of minced lizard fish was low. The gel forming ability of minced lizard fish was affected by the process of cooling and freezing (Kurokawa, 1982).

The changes in the PV of minces from cat fish, lizard fish and threadfin bream are given in Fig. 3.12. Maximum change in PV was noticed in cat fish while the other two did not show much variation. Though more peroxide was formed in cat fish a correspondingly higher decrease in SSN was not noticed. Also higher SSN decrease was found in minced lizard fish in which the peroxide formation was the lowest. Some other factors such as the decomposition products of TMAO, formaldehyde, might be playing a major role in denaturing proteins.

Fig. 3.13 gives the shelf life of mince from the three species. Threadfin bream had the highest shelf life of 29 weeks, while lizard fish had 24 weeks and cat fish 20 weeks shelf life. The limiting factor in minced cat fish was the development of off or rancid odour while in the other two mince it was the development of fibrous, tough and dry texture. The textural change was considerably greater in lizard fish than in threadfin bream. Intimate mixing of the black meat and white meat of cat fish might have accelerated the development of oxidative rancidity in cat fish. The rapid textural changes

in minces might have caused by the release and mixing of enzymes in various tissues as well as from the stomach.

The study indicated that various factors like the species, composition, variation within the species affected the frozen shelf life of mince. Even within lean species the quality changes were not uniform and changes in certain characteristics like protein extractability affected the frozen storage stability significantly. Fibrosity and granularity increased on frozen storage of all the samples but the rate and extent of these changes varied with species.

#### 4.3.11 TREATMENT OF MINCE WITH SPICES

The effect of mixing powdered cloves with mince from horse mackerel on the changes in PV and FFA during frozen storage is presented in Table 3.32. Substantial reduction in PV and FFA formation was found in treated samples compared to control. The rate of increase, in control and spiced mince of PV per week was 1.30 and 0.24 m.eq/kg fat and FFA 0.05 and 0.007% respectively. This effect was due to the antioxidants present in the cloves. Gallic acid and eugenol were identified as the major antioxidants in clove and the amounts of gallic acid and eugenol were 1.26 and 3.03 g respectively in 100 g of clove (Kramer, 1985). Many spices and vegetables extracts are reported to have antioxidant properties (Kihara & Inoue, 1962; Pratt & Watts, 1964; MacNeil et al., 1973; Saito et al.

1976; Lee et al., 1986): The sensory score also showed a corresponding increase in acceptability of spiced mince. The reasons for increased acceptability might be due to the lowering of fat oxidation and hence the rancid flavour and also by the imparting of a special acceptable flavour of the spices. Statistical analysis for t value showed significant difference at 5% level between the two samples in their shelf lives.

#### 4.3.12 WASHING FISH MINCE

Washing was found to <sup>improve</sup> increase colour, storage stability and jelling properties, but there was significant solid loss during washing. The solid loss on washing of cat fish, threadfin bream and lizard fish were 19, 22 and 23% based on the original weight of mince. Significant improvement in the colour of washed mince from cat fish was noticed. This was mainly due to the removal of blood, pigments and some colouring matter present in the mince. Mince from threadfin bream did not show any improvement in colour. If the muscle is inherently coloured washing would not have much effect to improve it. Okada (1964) found that washing removed colouring matter and unpleasant smell. Watanabe et al. (1983) found that the total amount of protein lost in the effluent was as high as 30-60%.

Table 3.33 gives the amount of moisture, fat, NPN and TVBN in the mince before and after washing. The moisture increase ranged from 2.9 to 3.2% and was found difficult to

reduce the moisture content further. Grantham (1981) reported an increase of about 3% moisture on washing and found it very difficult to reduce it further. The amount of fat lost was maximum in cat fish while threadfin bream showed only very little reduction in fat. Significant reduction was found in the NPN and TVBN content of all the three samples. Washing removed more than 50% of NPN and about 74% volatile bases.

Fig. 3.14 shows the average sensory score of washed and control samples of minces from cat fish and threadfin bream during frozen storage. Washing improved the shelf life of minced cat fish substantially. This may be due to the removal of colouring matter, blood, enzymes, fat and also the compound producing the characteristics cat fish odour. Shimizu and Fujita (1985) found that washing increased the storage tolerance of lizard fish and common mackerel mince, although the extent of increase was different with different species. Mince from cat fish improved the shelf life substantially while threadfin bream showed only marginal increase. Babbit (1986), Lanier (1986), Rasekh et al. (1980), Watanabe et al. (1982) etc. studied the effect of washing on various properties of mince and its effect on the keeping quality. All the above authors reported that the washed samples were superior in functionality and keeping quality than unwashed samples.



## 5. SUMMARY

Investigations were carried out on the following aspects.

1. Composition of the different fishes used for the studies.
2. Iced storage changes in rohu, mrigal, milk fish, mackerel and squid.
3. Effect of sex, season, size and bleeding and gutting on iced shelf life.
4. Frozen storage of milk fish, squid and mackerel.
5. Frozen storage life of ice stored squid.
6. Frozen storage life of ice stored milk fish.
7. Effect of season, type of packing, storage temperature and antioxidant treatment on the shelf life of mackerel.
8. Effect of packaging and refreezing on the shelf life of cat fish fillets.
9. Shelf life of frozen mince from cat fish, lizard fish and threadfin bream.
10. Effect of mixing with spices and washing on the shelf life of minced fish.

### 5.1 COMPOSITION OF FISH

Mackerel showed wide variation in the fat content

depending on season. Fat in mackerel varied from 3.2 to 11.8% on wet weight basis. The variation of fat in horse mackerel was 1.6 to 6.2% (wet weight basis). Mrigal, Milk fish and cat fish contained on an average 3% fat while rohu, threadfin bream and squid contained fat around one percent. The moisture content was found to decrease as the fat content increased. Rohu, mrigal and horse mackerel showed the highest protein content. The ash content in all the samples was around 1 to 2% of wet weight. All the samples showed a lower content of sodium than potassium. The highest sodium content was found in milk fish and squid while mackerel showed the highest potassium content.

The white and dark muscle of mackerel showed considerable difference in their chemical composition. The white muscle contained more moisture, protein and NPN while dark muscle contained more fat, ash and TMAO. Higher amount of NPN was found in the white muscle of horse mackerel and mrigal and the TVBN contents were also high in the white muscles of these fishes. TMAO and TMA were found high in the dark muscle. No TMAO was detected in fresh water fishes. Variations in the chemical composition of different parts of the fish were noticed. Lipid was found high in the ventral and dark muscle of mrigal while moisture was high in the caudal muscle. Protein was almost same in the muscle from three parts but

dark muscle contained low levels of protein.

Struggling after catch caused a sudden decrease in pH than instantly killed rohu and mrigal.

## 5.2 ICED STORAGE

During iced storage of rohu, mrigal, milk fish, mackerel and squid, all the samples showed an increase in water content. The increase in water content of squid was more than 4% and it increased to that level in two days. In all other fishes the water content did not increase for the first 3-4 days and afterwards it increased. The increase was only 2-3% from original moisture content. Corresponding to the increase in the moisture content the total protein content was found to decrease. The non protein nitrogen content also decreased during iced storage. The NPN content was highest in fresh squid and it contributed about 31% of the total nitrogen. The fresh water fish rohu and mrigal had an NPN content of 9 - 11% of total nitrogen while milk fish had 17.4% and mackerel 13%. The TVBN content in rohu and mrigal were comparatively high and remained more or less same during iced storage. Milk fish showed an increase in TVBN content during iced storage. The increase was low in early periods of storage and afterwards showed a sudden increase. An almost similar pattern was noticed in mackerel and squid. The decrease in salt soluble nitrogen was marginal in rohu, mrigal and milk fish, while

mackerel showed slightly more decrease. Squid showed a sudden decrease in SSN in 2 days iced storage and afterwards the decrease was marginal. The fresh water fishes rohu and mrigal registered a low value of SSN during pre-rigor and rigor period and then increased and in the end showed a decrease.

In all the fishes the PV showed an increase during iced storage. Fresh rohu and mrigal did not contain any detectable amount of peroxides. No distinguishable difference in PV of rohu collected in January and June could be obtained. Mackerel showed considerable increase in PV and the samples were found rancid by 9 days in ice. The free fatty acids showed only a slow increase in the beginning and afterwards increased somewhat rapidly. The TBA value of mackerel showed a very slow increase for the first 6 days and afterwards showed a sudden increase.

The pH of rohu slowly increased during iced storage and by 20 days the value reached from 6.3 to 6.74. The bacterial count was low in the beginning and reached a maximum of about  $10^6 - 10^7$  per gram muscle in 16 - 20 days storage.

The major factors affecting the organoleptic qualities of iced fishes were appearance, texture and flavour. All the fishes showed changes in the gut portion and the muscle separated from the bones in the belly portion. They developed yellow discolouration in the belly and intensity of

discolouration was varied among the fishes. The texture of all the samples became soft and pasty. Only mackerel showed development of rancidity. Rohu and mrigal became unacceptable due to the development of pasty texture and spoiled weedy like odour. Squid showed rapid decrease in the characteristic sweet taste and developed decayed odour at the belly portion in 4 - 6 days. The development of yellow discolouration also was found rapid in the belly portion. The mantle became thin and flabby. The iced shelf lives of rohu, mrigal, milk fish, squid and mackerel were 17, 15, 14, 4.5 and 8 days respectively.

### 5.3 SEX, SEASON, SIZE AND BLEEDING AND GUTTING ON ICE STORAGE LIFE

During normal periods the shelf life of male and female rohu showed only slight difference which was not significant statistically at 5% level.

In rohu of the same size collected from a fish pond during different periods did not show much difference in the shelf life. The fat changes in rohu were also not significant during various seasons. The small sized rohu (560 g) showed a shelf life of only 14 days while the big one (810 g) had a shelf life of 17 days.

Bleeding and gutting of milk fish was found to improve the shelf life significantly. Bleeding improved the colour of the fillets and the fillets were devoid of blood stains. Bleeding and

gutting reduced the rate of development of peroxides to almost half. The improvement in shelf life was highly significant. The shelf life in ice had been increased by 4 days by bleeding and gutting.

#### 5.4 FROZEN STORAGE OF MILK FISH, MACKEREL AND SQUID

The three fishes showed variation in the rate of decrease of protein extractability. Milk fish and squid protein underwent slow decrease in the extractability of protein while protein in mackerel showed rapid decrease in extractability. Water holding capacity of three fishes also reduced during storage as indicated by the decrease in moisture. The moisture content in squid did not show much decrease upto 32 weeks and afterwards decreased rapidly. Milk fish showed only very slow decrease while the moisture in mackerel decreased rapidly.

The rate of formation of peroxides was rapid in mackerel, about  $2\frac{1}{2}$  times faster than that in milk fish. Very small amount of TBA reacting substances were formed in milk fish while in mackerel significantly high amount of these substances were formed. Development of organoleptic rancidity in mackerel was also noticed.

Mackerel showed correlation between the decrease in protein extractability and formation of peroxides and its decomposition products. The limiting factor for shelf life in

mackerel was the textural deterioration and development of organoleptic rancidity. Milk fish muscle became tough and fibrous during prolonged frozen storage. In squid the characteristic sweet taste and white colour of the muscle was lost during storage. Mackerel, milk fish and squid had shelf life of 25, 41 and 62 weeks respectively.

#### 5.5 FROZEN STORAGE OF ICE STORED SQUID

Because of the rapid loss of flavour bearing components of squid mantles when held in contact with melting ice, it could not be kept for a longer time. The colour was not affected. In whole squid the mantles became slightly pink because of the leaching of colouring matter into the mantle. The gut contents showed considerable deterioration. During frozen storage all the three samples showed decrease in extractability. It was more in the samples stored at  $-20^{\circ}\text{C}$  after keeping it in whole condition in ice for one day.

The weight loss on thawing and cook drip loss showed considerably variations among the three sample. The fresh squid showed low values of weight loss on thawing and cook drip loss compared to the other two samples. The three samples showed shelf lives of 62, 48 and 43 weeks respectively. The additivity of defects was noticed. By one day iced storage  $\frac{1}{4}$ th of the shelf life was lost (iced shelf life of squid = 4.5

days) and hence expected a shelf life of 46 weeks and experimentally a shelf life of 43 weeks was found.

#### 5.8 FROZEN STORAGE OF ICE STORED MILK FISH

The amount of NPN in milk fish leached into the thaw exudate increased proportionally to the number of days in ice. The extractability of protein also showed a similar pattern. The fresh milk fish showed remarkable stability of protein than the iced samples during frozen storage. PV of all the samples showed an increase and reached a maximum value of 22 to 26 m.eq/kg fat. The period to attain the maximum value was proportionally reduced depending on the period of storage in ice. 0, 4 and 7 days iced samples showed a rapid decrease of sensory score in the early periods of frozen storage and afterwards showed a slow decrease. The frozen shelf lives of samples iced for 0, 4, 7, 10 and 14 days were 42, 30, 21, 12 and 0 and the frozen storage life calculated after reducing the loss in quality of milk fish kept in ice for periods of 4, 7, 10 and 14 days were 31, 22, 12 and 0 respectively. The experimental and calculated values are almost same which further confirms the additivity of effects i.e. the defects of quality persist and are added to by all other defects that may be subsequently produced.



### 5.7 EFFECT OF SEASON AND PROCESSING PARAMETERS ON SHELF LIFE DURING FROZEN STORAGE

The mackerel samples collected in April and June showed faster formation of peroxides than samples collected in December even though the fat was high in December samples. The formation of TBA reacting substances was highest in June. The shelf life of the April, June and December samples were 25, 26 and 30 weeks respectively.

Mackerel samples packed and frozen as blocks and individually showed wide variation in fat changes and shelf life. The rate of formation of peroxides in IQF sample was 3 times faster than that of block frozen mackerel. The shelf life at  $-20^{\circ}\text{C}$  for IQF samples was 25 weeks while BF samples were in fair condition at 30 weeks.

Treatment of mackerel with the antioxidant, BHA was found effective in reducing rancidity as measured by peroxide and TBA values and organoleptical scores. The untreated sample had only a shelf life of 25 weeks.

Lowering the storage temperature from  $-20^{\circ}\text{C}$  to  $-30^{\circ}\text{C}$  was found to be very effective for reducing lipid changes and maintaining organoleptic qualities. The rate of decrease in SSN at  $-30^{\circ}\text{C}$  was almost half as that at  $-20^{\circ}\text{C}$  while PV increase was only one third and TBA values one fifth. The samples stored at  $-20^{\circ}\text{C}$  became tough, fibrous and rancid by 30 weeks storage,

but the  $-30^{\circ}\text{C}$  stored samples were juicy and did not develop any rancidity. Reducing the storage temperature by  $10^{\circ}\text{C}$  was found more effective than treating with antioxidants in maintaining the original characteristics of the product.

#### 5.8 PACKAGING AND REFREEZING

Proper packaging showed significant improvement in quality and shelf life of frozen stored cat fish fillets. Unpacked product showed considerable weight loss, desiccation, discolouration and rancidity during frozen storage. It had a shelf life of only 6 weeks. Packaging the fillets prevented or retarded the above changes. The decrease in weight was only negligible and during prolonged frozen storage few spots of ice crystals were noticed. Glazing and packaging had further improved the quality. Retardation of peroxide formation was considerable. Packaged product had a shelf life 24 weeks while glazed and packaged product showed a shelf life of 32 weeks.

Thawing, filleting and refreezing of cat fish was found to affect the quality adversely compared to fillet from fresh cat fish and kept ~~streed~~ under similar conditions. The extractability of protein of refrozen product showed a decrease and the peroxides was increased in refrozen product.

#### 5.9 FROZEN STORAGE OF FISH MINCE

The frozen storage characteristics of mince from

threadfin bream, lizard fish and cat fish showed significant variations among each other. The minced threadfin bream yielded a frozen stored product reasonably stable during frozen storage and had a shelf life of 29 weeks at  $-20^{\circ}\text{C}$ . The limiting factor was the development of tough texture and loss of characteristic flavour. Minced cat fish developed rancidity by 20 weeks storage and the product became unacceptable. The minced lizard fish showed rapid changes in texture even in the early periods of storage and could not produce a frozen stored product with good gel properties. After a sudden change in texture, the product showed only slight decrease in characteristics and it had a shelf life of 24 weeks. Minces from these species showed significant variation in their characteristics during frozen storage.

#### 5.10 ADDITION OF SPICES AND WASHING THE MINCE

Mixing minced horse mackerel with 0.1% powdered cloves was found to reduce the formation of peroxides and organoleptic rancidity. The flavour of the product was improved considerably.

The solid loss on washing the minced cat fish, threadfin bream and lizard fish was 19, 22 and 23% of the original weight of the mince respectively. The colour of cat fish mince was improved while the other two did not show much change. There was 3 - 4% increase in moisture content. About 50% fat present

in cat fish was lost by washing. Other two samples also showed reduction in fat content. Non protein nitrogen content reduced significantly by washing. The gelling property showed improvement and shelf life increased. In general washing improved functionality and keeping quality of minces.

## CONCLUSION

The present studies have shown that the quality of fish is the most important parameter to determine the frozen storage life. The quality is found to vary with species, location, season, size, exertion, handling methods and processing technique. The studies gave an insight of the effect of these parameters into quality as well as iced and frozen shelf lives. This knowledge is of immense help for the fish processing industry since it provides the technologist a method to arrive at a conclusion of the possible frozen shelf lives from the type of raw material. If the percentage in loss of quality and the frozen shelf life of fresh fish is known, it is possible to estimate the approximate period to which the product can be preserved under commercial conditions.

Various processing operations effect the quality. Filleting the fish does not affect the quality significantly because the intact nature of the muscle is retained but the contact area of the muscle with air is significantly increased. But mincing completely disrupted the muscle structure and this results in extensive changes in chemical and sensory qualities and results in considerable reduction in shelf life. Yet it is possible to produce minced fish with a reasonable shelf life

and functionality from many species. These studies are quite significant by considering the fact that fillets and mince are fast becoming a base material for many culinary preparations. Further, preparation of mince is one of the best method of economically utilizing the trawl by-catches and other miscellaneous fish.

The present studies open a new field for detailed investigations with regard to the effect of biological<sup>e</sup> parameters and method of catching on the quality of fish. The indepth studies in these aspects may lead to draw general conclusions. This will help the fishing industry for suitable selection of location and period as well as catching methods so as to get the best quality fish.

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