REVIEW OF LITERATURE
2. REVIEW OF LITERATURE

Fish is an essential food commodity consumed by human being throughout the world. Unlike temperate environment fish spoils quite rapidly in tropical conditions within a few hours of landing, if it is not properly preserved. Rapid handling result in a faster spoilage rate (Hobbs and Hodgkiss, 1982). The spoilage rate of fish may be reduced by good handling practices and better preservation methods from the very beginning (Sikorski and Sunpan, 1995).

Enzymes and bacteria do not cause any deteriorative changes in the living cell because of the natural defensive mechanism. In dead fish, enzymes released from lysosomes cause autolytic changes in the muscle. In addition bacteria also invade the muscle and proliferate there. Normally fish gut is rich in proteolytic enzymes. In dead fish it digests the gut and belly region making the fish very soft. Bacteria that are present on the surface, gills and gut of the fish invade other parts of the dead fish, decompose the tissue and bring about undesirable changes. Off odours and flavours, slime, gas production, discoloration and soft texture are the obvious signs of spoilage (Lakshmanan, 2000).
Main components involved in spoilage process are protein, lipids, carbohydrates, nucleotides and other non-protein nitrogen compounds. The rate of spoilage is temperature dependent. The effect of time / temperature storage conditions on product shelf life had been shown to be cumulative (Charm et al, 1972). Since freshwater fish are eaten immediately after catch, little information is available on extending its shelf life. Quality loss in fishery products may be attributed to three main causes viz. autolytic, bacterial and enzymatic actions (George and Gopakumar, 1998).

**Autolytic spoilage**

Autolysis means "self - digestion" (FAO, 1995). This type of spoilage is brought about by the enzymes contained within the fish itself at the time of death and is the first type of spoilage to take place. The enzymes concerned with this are of two types.

The first type of enzymes is that found in the living fish; it serves in a controlled manner to facilitate the digestion of food. Upon death, these enzymes act in an uncontrolled fashion and serve to bring about the breakdown of organs such as gut and surrounding tissues of fish that have been feeding heavily just prior to harvesting. As a result of this, along with the actual breakdown of organs is the production of discolouration and the breakdown of the surrounding flesh results “belly burst”.

The second type of enzymes is, those that are resident in the muscle tissue of fish. These enzymes have an effect on the flavour of the
fish by attacking the chemical compounds that bestow the sweet characteristic flavour of fresh fish and changing them first to more natural tasting compounds and eventually to the bitterness of spoiled fish. The flavour bearing compounds are produced by the nucleotide degradation in fish muscle, especially splitting of Adenosine triphosphate (ATP) by a series of dephosphorylation and deamination reactions.

\[
\text{Adenosine triphosphate} \rightarrow \text{Adenosine diphosphate} \rightarrow \text{Adenosine monophosphate} \rightarrow \text{Inosine monophosphate (IMP)} \rightarrow \text{Ammonia + Inosine} \rightarrow \text{Ribose} \rightarrow \text{Hypoxanthine.}
\]

At ambient temperature ATP breakdown occurs very rapidly and IMP accumulates in the fish tissue (Surette et al., 1988). In fresh fish, the level of IMP is very high and it imparts a desirable sweet, meaty and characteristic flavour to fish. As autolysis proceeds further, the level of IMP decreases and neutral tasting inosine or bitter tasting hypoxanthine accumulates in the tissue. As a result, fish becomes more insipid. Some of these compounds increase with time and have been used as indices of fish freshness.

In the dissolved gut components, bacteria proliferate and produce gases such as carbon-di-oxide and hydrogen. This gas production leads to belly burst after short storage period. Colour is an important factor in seafood quality and is an indicator of spoilage. Colour change in seafoods is caused by enzymic or non-enzymic action such as fat oxidation or by pigments (Lakshmanan, 2000).
Microbial spoilage

This is the most important type of spoilage in fish since it is the action of bacteria that creates the undesirable "sour" odours associated with spoiled fish. Bacteria present on the gills, in the surface slime, and in the intestines of live, healthy fish, but are precluded from invading the sterile flesh of the fish by the animal's normal defences.

Connell (1980) described the bacterial spoilage as follows: "The normal population or flora, on fish consists of several genera of bacteria. On death, the bacteria or the enzymes they secrete are free to diffuse into the flesh where they react with the complex mixture of natural substances present in the fish itself. The number of bacteria in the fish flesh grows initially slowly but then increasing rapidly. Their bacterial action results in a well-defined sequence of changes in odoriferous and flavorous compounds. At later stages of bacterial spoilage through the agency of secreted proteolytic enzymes attack the structural components like proteins resulting in a gradual softening of the flesh".

When the fish dies, the immune system collapses and bacteria are allowed to proliferate freely. During storage, they invade the flesh by moving between the muscle fibres. Murray and Shewan (1979) found that only limited number of bacteria invaded the flesh during iced storage. Microbial growth mainly takes place at the surface, spoilage is probably to a large extent as consequence of bacterial enzymes diffusing into the flesh and nutrients diffusing to the outside.
Closely related contemporaneous sequences of changes occur in the odour of the external surfaces and gills or organs. These odours are more intense than those in the flesh and can be used as excellent indices of degree of spoilage. In addition to changes in odour and flavour, the continued action of bacteria affects the appearance and physical properties of several components of the body. The slime on skin and gills, initially watery and clear, becomes cloudy, clotted and discoloured. The skin loses its bright iridescent appearance, bloom and smooth feel and becomes dull, bleached and rough to touch.

Oxidative spoilage

Enzymes reacting with the oily portions of fatty fish bring about oxidative spoilage. Oxygen reacting with the oily portions of fatty fish brings about this type of spoilage. The fatty fish are known to have high lipid content, and the action of oxygen reacting with the lipids results “rancidity”. As Hobbs (1982) notes: “rancidity is characterized in its early stages by a marked fishy odour and flavour followed by an unpleasant taint, described as like linseed oil or paint”. In addition, yellow to brownish discolouration known as “rusting” are evident in this type of spoilage. This discolouration “is believed to be due to the combination of some products of fat oxidation with substances in the flesh that contain nitrogen”. Lean fish have small amounts of fat and they do develop a cold storage odour and flavour due to the oxidation of fat that is present.
Factors affecting spoilage

Temperature

Temperature being a very important factor accelerating the process of spoilage, in a tropical country like India, the ambient temperatures are very conducive for causing quick spoilage in fish. The spoilage cannot be stopped completely; however, the best that can be done is to slow it down by means of some refrigeration technique. The most common method of prolonging shelf life and preservation of fresh meat is refrigeration, meaning storage at temperatures between -2°C and +5°C. Refrigeration can be mechanical (compressor and cooling pipes) or by adding ice to the medium (Connell, 1980).

Refrigeration storage not only impedes bacterial growth but also slows down the rate at which biochemical and physical changes take place in the muscle. With the increase in the use of refrigeration appliances for domestic purpose there is a need to provide information in detail about the storage life of different food products in refrigerators. The shelf life and safety of refrigerated fish and fish products are dictated by the presence of food spoilage and pathogenic microorganisms storage at around 5°C / 41°F for most foods is almost as good as storage at ice temperatures (Malhotra, 1965). Hess (1934) concluded that lowering of temperature becomes more and more effective in retarding bacterial decomposition.

The rate at which spoilage occurs varies with species of fish, sanitary conditions, methods of handling and storage. Increasing the
temperature from 0 to 5°C doubles the spoilage rate of many species of fish. Hence, chilling the fish immediately after catch, careful handling, gutting and maintaining good hygiene will retard the spoilage rate. The critical role of temperature control in atmospheric storage and keeping the temperature low affects both the microbiological and biochemical aspects of the changes in quality. Even if we can prevent microbial growth by various treatments other than temperature, the biochemical changes are most readily influenced by the choice of storage temperatures (Lakshmanan, 2000).

The microflora responsible for spoilage of fresh fish changes with storage temperature. At low temperature (0-5°C), *Shewanella putrefaciens*, *Photorbacterium phosphoreum*, *Aeromonas* sp. and *Pseudomonas* sp. cause spoilage. However, at high storage temperatures (15-30°C) different species of *Vibrionaceae*, *Enterobacteriaceae*, and gram-positive organisms are responsible for spoilage (Gram et al. 1987; 1990; Liston, 1992). Reasonable estimates of relative rate of spoilage are obtained for whole, packed and super chilled fresh fish products (Gibson and Ogden, 1987; Dalgaard and Huss, 1994). In spoilage of fish, it is very unlikely that all the bacteria are equally active (Adams et al. 1964). At ambient temperature, motile aeromonads are the specific spoilers of aerobically stored freshwater fish (Gorzyka and Pek poh Len, 1985; Gram et al. 1990). Psychrotrophic bacteria are the major groups of microorganisms responsible for spoilage of refrigerated seafood (Adams et al. 1964; Zhuang et al. 1996).
Durairaj and Krishnamurthi (1986) studied the quality changes in *Labeo rohita*, *Catla catla* and *Tilapia mossambica* during storage in ice and at ambient temperature (29-32°C). After ten hours at ambient temperature *Labeo rohita* and *Tilapia mossambica* were completely spoiled, while *Catla catla* became unacceptable within eleven hours. Bhattacharyya and Chaudhury (1990) reported the storage characteristics of *Clarius batrachus* at three different temperatures.

Bandyopadhyay et al. (1986) studied the ice-storage characteristics of *Catla catla* and *Labeofimbritus*. Based on their studies, both the fish could be stored in ice for eighteen days. Perigreen et al. (1979) worked out the technological aspects of production of fillets from different varieties of fish. It is generally agreed that spoilage of catfish fillets occur within five to eight days during refrigerated storage. (Kim et al. 1995; Przybylski et al. 1989 and Silva et al. 1993). Growth of undesirable spoilage bacteria in refrigerated fish causes deterioration of keeping quality and reduces consumer acceptance.

Marcel et al. (1996) investigated that dipping of the carp fillets in ice water did not have any influence on the microbiological quality of carp fillets. The packaging process (air, vacuum or modified atmosphere) had significant effects on the microbiology of the fillets. Good correlation was observed between sensory scores and physical/biochemical indices.

Gelman et al. (1986) conducted a study on the microbiological and biochemical changes in the dead fresh fish. They observed that the
organoleptic, microbiological, physical and chemical methods of evaluation were used to determine the shelf life and quality changes of carp (C. carpio) stored as eviscerated or whole at 0-2°C. Poulter and Nicolaides (1985 b) studied the quality changes in Bolivian freshwater fish species during storage in ice. Shelf life of carp (C. carpio) and trout (Salmo gairdneri) in crushed ice were twenty and fifteen to sixteen days respectively, based on the sensory evaluation regardless of whether they had been stored as whole or eviscerated.

**Chemical preservatives**

**Sodium chloride**

The use of common salt as a preservative is based on the fact that, at high concentrations, salt exerts a drying effect on both food and microorganisms. Some bacterial growth is inhibited by salt concentrations as low as 2%, whereas other salt-tolerant bacteria (Bacillus and Micrococi), yeasts and molds may be able to grow up to the saturation point. Halophilic organisms are involved in the spoilage of natural casings.

For effective preservation, the moisture content of the finished product should be 50-55 % and the salt content 9-11 %. Hallendoorn (1962) investigated the water retention of meat with polyphosphates and sodium chloride alone. Research also has indicated that sodium acetate and sodium citrate were effective in controlling microbial growth on fresh meat products in combination with ingredients such as glucose, potassium sorbate, phosphate, sodium chloride and acetic or lactic acids (Medonca et al. 1989a; Unda et al. 1990).
Several proteolytic enzymes have been discovered in the fish tissues. The cathepsins are acid proteases usually found packaged in lysozomes. In living tissue, lysozomal proteases are responsible for protein breakdown at sites of injury. Thus cathepsins are inactive in living tissue but become released into the cell juices upon physical abuse or upon freezing and thawing. The enzyme cathepsins play a major role in autolytic degradation of fish tissue, whose activity was inhibited strongly by the presence of salt. Virtually no activity remains after a 25-hour incubation in the presence of 5% sodium chloride. Cathepsins are believed to be responsible for major textural changes during the fermentation of salted preserved Japanese squid and Crucian carp (Makinodan et al. 1991, 1993). Chakrabarti (1993) studied that total volatile base nitrogen in unsalted dried fish was very high in comparison to salted dried fish indicating the arresting of the growth of putrefactive bacteria in the presence of common salt.

**Sodium acetate**

Sodium acetate is an approved U.S. Department of Agriculture (USDA) flavouring and pH control agent. It is a derivative of acetic acid (pka = 4.75) and its related salts are used as antimicrobial agent. Sodium acetate has also been an effective inhibitor of rope forming bacteria (*Bacillus subtilis*) in baked goods. Sodium acetate inhibits the growth of gram-negative bacteria (Kim and Hearsenger, 1994). Preservatives such as sodium acetate and potassium sorbate are found to extend the shelf life of refrigerated fish (Medonca et al. 1989).
Kim et al. (1995) reported that acetates are used for improving the microbiological quality of muscle foods and extending the shelf life. The aerobic plate counts of refrigerated catfish fillets were affected by increasing the levels of sodium acetate. The initial aerobic plate counts was significantly lowered by the use of 2% sodium acetate compared to control. Kim et al. (1995) also reported that sodium acetate either alone or combined with bifidobacteria extended the shelf life of refrigerated catfish fillets. Treated fillets odour and appearance resembled the fresh fillets for upto six days, while untreated fillets were unacceptable after three days. Kim et al. (1995b) recommended sodium acetate either alone or combined with monopotassium phosphate to extend the shelf life of refrigerated catfish fillets.

Zhuang et al. (1996) showed that treatment of catfish fillets with 2% sodium acetate significantly controlled the growth of psychrophilic bacteria. In shrimps treated with sodium acetate 2% w/v, (dip for 30 minutes) stored at 4°C, there was a decline in pH at the end of storage period. Kim and Hearsberger (1994) reported that the combination of sodium acetate and lactic culture could extend the storage life of refrigerated catfish fillets. The bacteriostatic effect of acetic acid increases at low pH values. Reasonably high concentrations must be employed in foods in order to preserve them effectively at room temperature. Shalini et al. (2000a) reported that the sodium acetate treated vacuum packed refrigerated Lethrinus lentjan fillets had a shelf life of two to three weeks whereas the untreated control fillets had around seven to eight days.
Potassium sorbate

Potassium sorbate is one of the by-products of sorbic acid. It is available as powder or in granules and highly soluble in water (58.2g / 100 ml). Microorganisms isolated from seafood exhibit varying degree of sensitivity to sorbate. Potassium sorbate is a Generally Recognised As Safe (GRAS) preservative widely used readily available, yet somehow expensive when compared to sodium acetate. Sorbic acid or potassium sorbate have a general antimicrobial activity against yeasts, molds and some highly aerobic bacteria. In general potassium sorbate inhibits highly the growth of catalase positive cocci than catalase negative organisms (Fey, 1980). The main advantage of potassium sorbate over sodium acetate is its greater solubility in water. At low concentrations, sodium acetate and potassium sorbate are non-toxic and metabolized by the human body in a similar manner to other fatty acids, via, beta-oxidation (Maclead and Onfrey, 1954). Currently the U.S. Department of Agriculture (USDA) does not permit preservatives in meats and poultry for human consumption, except a 2.5 % potassium sorbate to prevent surface mold in sausage.

Sorbates can be added to foods in a variety of ways including direct addition, dipping in a solution, spraying, dusting or use of sorbate-treated package wraps (Anon., 1977). Sorbates have been used for shelf life extension on fresh poultry meat and fresh fish (Sofos and Busta, 1983). Potassium sorbate at 0.1 % level also used for processing some of the food items (Fey, 1980). It was suggested that treatment would begin with the freshest fish possible after catch to maximize the antimicrobial effect. Robach (1979) used a 30 min. dip and recommended sorbate treatment of...
chicken to improve public health safety while extending shelf life. Greer (1982) indicated that dipping of fresh beef in a 10% potassium sorbate solution inhibited the growth of psychrophilic bacteria and extended the retail shelf life by two days.

Debevere and Voets (1972) reported that the addition of potassium sorbate inhibited the total volatile nitrogen and trimethylamine formation and decreased the number of spoilers in prepacked cod fillets. To prolong the keeping quality of prepacked cod fillets, an addition of 0.4% potassium sorbate is recommended to inhibit the trimethylamine formation. It can be assumed that the inhibition of trimethylamine oxide reducing bacteria increased the keeping quality of the fish to a large extent. This may be due to the inhibition of total number of aerobic bacteria, particularly of 'spoilers' which results complete inhibition of trimethylamine formation.

Sorbitol maintained good water retention properties. It may stabilize muscle proteins during frozen storage. Shanmugam et al. (2000) observed that the shelf life of fresh *Lethrinus lentjan* fillets was about seven to eight days in control samples and about two to three weeks in sodium acetate and potassium sorbate treated samples. They also concluded that *Bacillus* (72%) was the dominant initial microflora. Upon storage, *Coryneforms* formed the predominant species in all the samples.

Zamora and Zaritzky (1987) investigated the inhibitory activity of potassium sorbate on microorganisms growing on refrigerated fresh
beef. Sorbate increased the lag phase, decreased the growth rate of bacteria during the exponential phase and significantly lengthened the time for aerobic counts on vacuum packaged beef. 0.135 % potassium sorbate almost completely inhibited the spoilage of cod fillets for 6 days by slowing the growth of bacteria capable of producing trimethylamine. Medonca et al. (1989a) investigated that treatment of pork chops with 10% potassium sorbate solutions improved the pork colour.

Bremmer and Statham (1983) achieved suppression of spoilage and significant extension of shelf life by the addition of potassium sorbate to vacuum packed scallops. Sharp et al. (1986) studied the effect of potassium sorbate in extending the shelf life of modified atmospheric packed fresh lake white fish fillets. Doell (1962) reported that sorbate inhibited Salmonella typhimurium and Escherichia coli, the growth of Salmonella, Clostridium botulinum and Staphylococcus aureus in cooked, uncured sausage (Tompkins et al. 1974), Staphylococcus aureus in bacon (Pierson et al. 1979), Pseudomonas putrefaciens and Pseudomonas fluorescense in trypticase soy broth (Robach, 1979), Vibrio in crab meat and Salmonella, Staphylococcus aureus and Escherichia coli in poultry (Robach, 1980).

Packaging

Packaging is an art and a science of delivering a product to the consumer in an attractive manner with minimum cost and to protect the product from any physical, chemical or microbiological spoilage. Packaging has become an integral part of meat and meat products and forms a link between the meat products and the consumer (Dushyanthan
The material that is desirable for packaging should allow the passage of oxygen essential for the development of oxymyoglobin or blooming of the meat. Meat colour is the most important single prime factor in the appeal of packaging meat, which influences the consumer preference (Hessenbruch, 1986). Meekin et al. (1982) reported that the aerobically packed, refrigerated (4°C) sand flat head spoiled in eight to nine days and the shelf life of control air-pack was about seven days. Spoilage of vacuum-packed seafood is associated with anaerobic bacterial growth (Marcel et al. 1996). Tilapia fillets packaged under 100% air spoiled after nine days at 4°C (Reddy et al. 1994). These results are almost in agreement with earlier observations of Huss (1971).

**Sensory Characteristics**

Fish freshness is usually assessed by the general appearance, raw odour, colour of the gills, condition of eyes and firmness of the flesh. Appearance of the eyes of bony fish is a good guide to the degree of spoilage. The shiny and brilliant skin in fresh fish becomes dull, with lack of lustre and faded appearance as spoilage progresses. The eyes are bright with black, clear pupil and convex protruded cornea in newly caught fresh fish; the gills are bright blood red and have a fresh fishy odour. As the fish spoils, the colour of the gills changes to pale red to bleached white often covered with thick mucous. The firm, springy and elastic flesh of very fresh fish becomes soft when spoiled. Scoring is the most commonly used method for assessing the freshness of fish. The deterioration in fish quality is followed with the aid of a score sheet.
The strong off-odours associated with spoiled fishery products resulted when metabolites were released by bacterial action (Lannelongue et al. 1982). Reddy and Srikar, (1991) reported that the keeping quality of pink perch stored in ice was investigated by sensory, microbial and chemical analysis on the basis of organoleptic quality and it was found to have a shelf life of thirteen days in ice.

Ramachandran et al. (1990) reported that the results of the visual and olfactory assessment of *Hilsa toli* had a correlation between organoleptic changes and the microbiological quality. These results are almost in agreement with earlier observations of Huss (1971). Fey and Regenstein (1982) reported the freshness qualities of iced red hake and cod raw fish. Bremmer and Statham (1983) investigated the changes in odour of raw scallops, which were either air, vacuum packed or treated with potassium sorbate.

Kim and Hearsberger (1994) evaluated the changes in flavour, odour and appearance of refrigerated catfish fillets treated with the combination of food preservatives and/or lactic acid culture. Kim et al. (1995 a) evaluated the sensory characteristics of catfish fillets treated with sodium acetate and monopotassium phosphate and found that the fillets were sensorily accepted until twelve days. Reddy et al. (1994) reported that on sensory evaluation the fillets packaged in 100% air and they found that the fillets were spoiled after nine days. The shelf life of fillets in all atmospheres decreased with increase of storage temperature from 4°C to 16°C (Reddy et al.1997). Packaging techniques improved the microbial quality of iced and refrigerated channel catfish (Huang et al. 1994).
Organoleptically, the fish *Clarius batrachus* had shelf lives of six hours, eight and a half hours and fifteen days at 37°C, 22°C and 0°C respectively (Bhattacharyya and Chaudhury, 1990). Indian oil sardine stored in chilled seawater and kept in a chill room at 2 ± 1°C were in excellent condition for five days and acceptable condition upto eighth day (Shetty et al. 1992). Tilapia from freshwater showed an ice storage shelf life of thirteen days (Shenoy and James, 1972).

Durairaj and Krishnamurti, (1986) reported that the Indian freshwater fish such as rohu and mrigal were acceptable upto seven to eight days of storage in ice while tilapia was acceptable upto six days, whereas rohu, mrigal and tilapia when kept at ambient temperature (29-32°C) without ice spoiled within eleven hours. Kamasastri et al. (1986) investigated that the transported *Silver pomfret* fish stored in ice were in good condition only for two days. The fish samples showed definite spoilage after eight hours and were not in acceptable condition when stored at 28 ± 2°C.

**Chemical indicators of spoilage**

The evaluation of food quality depends upon the senses of smell, taste, sight and touch. These organoleptic subjective methods are qualitative and vary from person to person. The need for more adequate evaluation has focussed attention on chemical compounds (arising from the metabolism of the dominant spoilage organisms), which can be classified as ‘chemical indicators’ of food quality.
In the past, ammonia, nitrogen, reducing sugars such as dextrose, acidity of fat and bacteriological examination were used to detect spoilage (Fields et al. 1968). However, with the availability of newer precise measurement techniques, now-a-days several chemical indices like Total Volatile Base (TVB) (Cann, 1974); TVN total volatile nitrogen (Velankar and Govindan, 1959); Trimethylamine TMA (Cobb et al. 1973); indole, Hydrogen sulphide ($H_2S$), Peroxide value (PV), Free Fatty Acids (FFA), Alpha Amino nitrogen (AAN) (Cobb et al. 1977) and hypoxanthine (Flick and Lovell, 1972) were used successfully in practice for assessing the progressive spoilage.

The enzyme systems of microorganisms causing spoilage of protein include proteinase, peptidases, deaminases and decarboxylases. The aminoacids freed by the action of peptidases and the break down products of aminoacids resulting from deaminase or decarboxylase activity has been suggested as chemical indicators for the quality of protein rich foods. Thus during the process of putrefaction, various foul smelling compounds are produced. These arise as a result of bacterial action on aminoacids and include mercaptans, indole, hydrogen sulphide, ammonia, amines and organic acids (Fields et al. 1968).

The substrates for the production of volatiles are the carbohydrates (e.g. lactate and ribose), nucleotides (e.g. Inosine monophosphate and inosine) and other non-protein nitrogen molecules. Aminoacids are the important substrates for the formation of sulphides and ammonia. Microorganisms obtain far more energy from aerobic
oxidation than from an anaerobic fermentation. Trimethylamine is the reduced component, which is one of the dominant components of spoiling fish, has typical fishy odour. The level of TMA found in fresh fish rejected by sensory panels, varies between fish species, but is typically around 10-15 mg TMA-N/100g in aerobically stored fish (Dalgaard et al. 1993).

Many proteases like cathepsins have been isolated from fish muscle and the effects of proteolytic breakdown are often related to extensive softening of the tissue (FAO, 1995). The induction of bacterial spoilage in capelin by autolysis also resulted in the decarboxylation of aminoacids producing biogenic amines and lowered the nutritive value of the fish significantly (Aksnes and Brekken, 1988). Yamashita and Konogaya (1990) produced strong evidence implicating cathepsin-L rather than other cathepsins in the softening of salmon during spawning. Cathepsin-L had also been associated with the production of a jelly-like softening of flounder (Toyohara et al. 1993a) and the uncontrollable softening of pacific hake muscle, which has been parasitized by Myxosporidia. In addition to their detrimental effect on texture, catheptic enzymes induce invetional autolytic changes in fermented fish products.

A second group of intracellular proteases called calpains or calcium-activated factor (CAF) has recently been associated with fish muscle autolysis. It results in the tenderization process in several fish species including carp (Toyohara et al. 1985, and Wang et al. 1995). Relatively short shelf lives of chilled prawns due to softening of the tissue by the collagenase enzymes also cause gaping (Nip et al. 1985). The
reduction of Trimethylamine oxide (TMAO), an osmoregulatory compound in many marine teleost fish, is usually due to bacterial action, but in some species an enzyme trimethylamine oxidase is present in the muscle tissue which is able to break down TMAO into dimethylamine (DMA) and formaldehyde (FA).

\[(\text{CH}_3)_3\text{NO} \rightarrow (\text{CH}_3)_2\text{NH} + \text{HCHO}\]

The trimethylamine oxidase enzyme has been isolated from the lysosomal membrane in kidney tissue of hake muscle (Gill et al. 1992).

During prolonged storage of freshwater fish, catalase from erythrocytes enters the blood plasma and later diffuses into the tissues. This enzyme activity goes parallel with the cell destruction. The catalase activity of gills also runs parallel to the degree of spoilage (Tomiyama et al. 1950). This was established not only on seawater fish but also on freshwater fish, especially the common carp. In addition, there is a close relationship between the amount of volatile bases formed and catalase activity up to amount of 30 mg% volatile basic nitrogen in the flesh, the catalase activity remained almost constant.

**Volatile Bases**

Total volatile bases (TVB) is a relatively simple method and widely used to chemically assess freshness quality (colour / flavour) of seafood (Aitken, 1988). According to Venkataraman et al. (1967) a slight increase in the TVB-N and TMA-N values when the colour of the mucous was changed to red; the white mucous changed to red by one day storage.
of silver pomfret in ice. Durairaj and Krishnamurthy (1986) reported that the TVB-N value and total bacterial count at the time of spoilage of fish were closer to 30 mg% and 10⁷ cfu/g respectively. Spoilage indices like TVB-N and TMA-N contents were higher in controls than the irradiated fish like Silver pomfret and Bombay duck (Venkataraman et al. 1967) unwashed *S. leptolepis* surimi samples showed higher TVB-N levels compared to washed samples (Jantawat and Yamprayoon, 1990). During storage TVB-N levels increased significantly.

In cod and other gadoid fish, TMA constitutes most of the total volatile bases. However, in the spoiled fish where the TMAO supplies are depleted and TMA has reached its maximum level, TVB levels still rise due to the formation of ammonia and other volatile amines. A little ammonia is also formed in the first week of iced storage due to autolysis. In some fish that do not contain TMAO or where spoilage is due to a non-TMAO reducing flora, a slow rise in TVB is seen during storage, probably proteolysis commences resulting from the deamination of free amino acids (FAO, 1995).

Debevere and Voets (1972) reported that the addition of citrate buffer to prepacked cod fillets inhibited the TVB and TMA formation during storage period of six days at 0°C. They also studied the level of TVB-N formed in cod fillets treated with potassium sorbate and packed in film packs of different thickness. According to Velankar (1952), total volatile nitrogen gives a better index of fish spoilage than the TMA content. Lakshmanan *et al.* (1991) concluded that TVB-N values are high in samples
kept at elevated temperatures and an increase with storage period. Initial TVB-N value of the control sample was higher than that of the test sample of dried thelly prawn due to the preservative action of sodium chloride and citric acid thereby reducing the chances of spoilage in test samples during storage (Joseph et al. 1993).

Selvaraj et al. (1991) indicated that TVB-N increased gradually during frozen storage of ascorbic acid dip treated squid but the values are higher in control samples. Abraham et al. (1992) observed that though the total plate count of the fish mince was well within 7.0 log units upto seventh day, the sample exceeded the acceptable level of TVB-N (30mg/ 100g) on fifth day. The sharp increase in TMA-N and TVB-N values could be mainly due to the proteolytic action of bacteria in fish mince and 98% of the total population are of proteolytic in nature.

**Trimethylamine nitrogen (TMA-N)**

Trimethylamine is one of the dominant components of spoiling fish, has a typical fishy odour (Hebard et al. 1982). A level of 10-15mg/ 100g muscles is taken as the limit of acceptability in aerobically stored fish and 30mg/100g in packed cod (Dalgaard et al. 1993). Trimethylamine oxide is a natural component in muscle and many organs of marine fish, but is completely lacking or present in very small amounts in freshwater fish (Dyer, 1950). But according to Anderson and Fellers, (1952) and Hebard et al. (1982) this component found in all marine fish species in quantities from 1-5mg% of the muscle tissue but is virtually absent from fresh water fish and from terrestrial organisms. One exception was
recently found in a study of Nile perch and tilapia from Lake Victoria, where as much as 150-200mg TMAO / 100g of fresh fish was found (Gram et al. 1989).

Hebard et al. (1982) reported that the tilapia fillets packed in 100% air had low levels of TMA initially, and then accumulated rapidly, reaching high levels with that of spoilage during storage at 4°C. Low temperature storage also slows TMA production. Babbit (1986) reported that the TMA seems to be less in surimi compared to fish fillets since it is water-soluble. TMA formation increases considerably under the influence of packing.

Suseela et al. (1999) had also reported the usefulness of TMA determination in the muscle of Indian fish for detecting the onset of spoilage. Increase in TMA content was noticed during storage of fish by several workers like Lakshmanan et al. (1991); Reddy and Srikar, (1991); Shalini et al. (2000 b).

Both Jorgensen et al. (1988) and Dalgaard (1993) showed a linear correlation between the contents of TMA and hypoxanthine during iced storage of packed cod. Fieger and Friloux (1954) found that a significant increase in bacterial plate counts proceeded with increase in TMA values and aminonitrogen was negatively correlated with taste panel evaluation of flavour and quality. According to Banks et al. (1980) low levels of TVB-N in sodium acetate treated samples were a consequence of either a reduced bacterial population and /or a decreased capacity of bacteria for
oxidative deamination of non-protein nitrogen compounds or to reduce trimethylamine oxide to trimethylamine.

**pH: (Hydrogen ion concentration)**

The pH of flesh is of great importance to food technology. It is the most important factor governing the texture of the cooked flesh. Sengupta et al. (1972) reported that an increase in surface pH during storage indicates bacterial growth and spoilage of fish. Fresh tilapia fillets had a surface pH of 6.22 and an increase in pH during storage reported by Stammen et al. (1990).

The surface pH of fillets packaged in 100% air increased to >6.6 after nine days storage. The increased surface pH in spoiled fillets may be partly attributed to the production of alkaline compounds such as ammonia by spoilage bacteria (Stammen et al. 1990; Galli et al. 1993). Reddy et al. (1997) also reported higher surface pH on the day of spoilage for the 100% air packaged tilapia fillets stored at 4, 8 and 16°C.

Reddy et al. (1997) reported that the increase in pH of chicken patties during storage might be due to liberation of metabolites, resulting from bacterial activity. These results are in accordance with the findings of Thomson et al. (1983) in mechanically deboned pultry meat. Meekin (1982) reported the changes in pH values of untreated, citrate buffer and glucose treated vacuum packed sand flat head fillets. The pH of shrimp immediately after treatment with 2% sodium acetate was lower than the control. However differences were not significant.
Shalini et al. (2000a) reported that the initial pH of the refrigerated *Lethrinus lentjan* fillet was increased significantly on storage in control and sodium acetate treatment packs. During the end of storage period, there was a decline in pH of the treated samples. Zhuang et al. (1996) reported the similar results in shrimps treated with sodium acetate (2% w/v dip for 30 min.) and stored at 4°C. Bandyopadhyay et al. (1986) observed that the pH of *Catla catla* and *Labeo fimbriatus* increased significantly on ice storage. The pH of the flesh exerts a big influence on the strength of the tissue that holds the fillets together.

**Peroxide value (PV)**

Peroxide value test is the chemical measurement of oxidative rancidity, which occurs, especially in fatty fish. If the PV is above 10-20 mequ/g of fat then the fish will be in all probability smell and taste rancid. The widely used peroxide value usually correlates rather poorly to sensorial properties. As expected, the PV and FFA values increased during storage at 0°C. But the taste panel members could not detect rancid flavour and odour during storage of the fish. Bandyopadhyay et al. (1986) have made similar observations in the case of freshwater fish. In spite of quite high PV, the organoleptic evaluators did not report any rancidity possibly because of the nature of fat contents. Selvaraj et al. (1991) observed that the PV was found to increase gradually during frozen storage of ascorbic acid treated squid but the increase was less in treated samples than control. Bandyopadhyay et al. (1986) studied the ice storage characteristics of catla and labeo, PV increased from the initial values for both the fish.
Free Fatty Acids (FFA)

Oxidation and hydrolysis of lipids in fish during storage cause quality deterioration. Lipid hydrolysis results in the formation of free fatty acid (Huss, 1971). Shalini et al. (2000a) observed that the FFA value increased significantly with storage period and the increase in control was found to be more than that in sodium acetate treated samples. Husang et al. (1991) observed that vacuum packaging slowed down the lipid hydrolysis in farm raised catfish during storage in ice. However, concentration of FFA may not be considered as a reliable index of spoilage in vacuum packed refrigerated fish fillets, treated with sodium acetate.

During storage, a considerable amount of free fatty acids appears in herring stored at different temperatures. In lean fish like Atlantic cod, production of free fatty acids occurs even at low temperatures. FFA themselves may cause a 'soapy' off-flavour (FAO, 1995). Haung et al. (1994) reported that the free fatty acid content remained low in catfish fillets at the end of thirteen days storage at 4°C. Lassen et al. (1951) found a rise in free fatty acids in fish during spoilage from 1.1% after twenty four hours of storage to 2.5, 7.8, or 8.1% after one hundred and twenty hours of being kept at room temperature.

Shenoy and James (1972) analysed the tilapia muscle during ice storage; it showed a slow increase in FFA with increasing days of ice storage while PV also increased regularly. Joseph et al. (1993) reported that a gradual increase in free fatty acid contents was noticed both in control and test samples of ready to serve dried thelly prawn treated with
sodium chloride and citric acid during storage at ambient temperature. These results confirm the preservative action of sodium chloride and citric acid.

**Ammonia**

Ammonia is a metabolic waste product and toxic to the fish is eliminated through urinary excretion in freshwater fish. But in marine fish, as they cannot afford to lose sufficient water to eliminate all ammonia, the same is converted into urea and retained in the muscle and blood, which also helps in raising the osmotic pressure inside. Many nitrogen compounds are converted into off-smelling volatile bases by spoilage organisms and these compounds present in fish are good substrates. The free amino acid pool in the muscle of fish is readily utilised by typical spoilage organisms by the process of deamination results ammonia during decomposition of fresh fish.

Ammonia is the major component in the total volatile nitrogen fraction, which is often used as a quality indicator for fresh fish. Haaland and Njaa (1988) reported that the formation of trimethylamine is accompanied by the formation of ammonia during anaerobic storage of herring and mackerel. The very strong ammonia producers were found to be obligate anaerobes belonging to the family *Bacteroidaceae* genus *Fusobacterium* (Storroe *et al.* 1975,1977). According to Beatty and Collins (Debevere, 1970) deamination is the second step in the deterioration process of fish. This deamination of aminoacids is primarily caused by oxidative metabolism. The total volatile nitrogen (TVN) fraction in fish is
mainly composed of ammonia and primary, secondary and tertiary amines (Beatty, 1938).

**Alpha amino nitrogen (AAN)**

Changes in the dynamic balance between the production and breakdown of free aminoacids (FAA) by the associated muscle enzymes may lead to the FAA formation. According to Sakaguchi *et al.* (1984) low molecular weight peptides accumulate initially and were hydrolysed to free aminoacids by enzymes such as cathepsin A, B and C in the later period of ice storage. The probable reason for the constant alpha amino nitrogen content in the muscle of the test fish in spite of its continuous production may be due to its utilization or consumption by bacteria, as well as to leaching of water-soluble aminoacids along with the melted ice.

Joseph *et al.* (1977) reported that the alpha amino nitrogen content of squid became very low after two days storage. Organoleptic characteristics also showed marked changes after two days. The alpha amino nitrogen to be abundant in squid so long as the meat taste remained sweetish. Kamasastri *et al.* (1986) studied iced storage characteristics of pomfrets and found that there is a gradual increase in TVB-N, TMA-N, alpha amino nitrogen and bacterial count during storage both at room temperature and ice storage. Similar observations have been made by Bandyo padhyay *et al.* (1986) in *Catla catla* and *Labeo fimbriatus*.

AAN provide a source of nitrogen that can be readily assimilated by the microflora associated with fish spoilage. They contribute
substantially to the flavour of the fish also. Alpha-aminonitrogen content varied from 17 to 19-mg N/100g muscles in teleosts in fresh condition. Konosu and Watanabe (1976) reported a range of 50-75mg of free aminonitrogen per 100g found in cultured and wild red sea breams. Shellfish contain large amounts of extractives and free amino nitrogen, which may contribute to the sweet taste of the flesh.

**Changes in the proximate composition of fish during storage**

Information on the changes in the proximate composition of freshwater fish stored at ambient and reduced temperatures are very much limited. Occasionally, few workers have attempted to estimate one or two components.

**Moisture**

Moisture content in Indian fish generally vary between 70 and 80 percent, though occasionally figures as high as 90% are also encountered in fish like Bombay duck. In general, an adverse relationship is observed between the moisture and fat contents of fish.

Bandyopadhyay *et al.* (1986) observed that the moisture content increased from initial values for both catla and labeo during iced storage. The changes in the moisture content of the muscle of frozen sole fish during storage showed a decreasing trend initially with very little variations on prolonged storage (Anil Agarwal, 1984). The moisture content of common murrel increased during iced storage due to the uptake of water reported by Perigreen *et al.* (1987).
Williams et al. (1983) reported that the total moisture content of flounder, red snapper and ocean perch fillets remained virtually unchanged during cold storage at 2°C. Joseph et al. (1993) found that the initial moisture content of ready to serve fried Thelly prawns during storage at ambient temperature was slightly higher than the control, probably due to the higher equilibrium moisture content and lower rate of drying of the salted samples. A marginal increase in moisture content was noted during storage for both control and test samples. There is a direct relationship between the microbial counts and moisture content of the sample. Reddy and Srikar (1991) also observed that the proximate composition of the fish meat obtained from iced-storage whole fish showed significant fluctuations in moisture, total lipid and true protein levels. Increase in moisture could be because of the absorption of water by fish muscles.

**Microbiological aspects of fish spoilage**

Bacterial spoilage of fish was first recognised and reported by Anderson in 1907. Biological research on freshwater fish is scanty in contrast to the voluminous literature on marine fish. Some recent studies carried out on freshwater fish spoilage indicate that the general problem was not grossly different from that in marine fish (Durairaj and Krishnamurti, 1986; Mukundan et al. 1986). Handling of freshwater fish both in terms of keeping quality and of public health is important. During the past few years, attempts are being made in all the countries to increase the keeping quality of fish, both at room temperature as well as under refrigerated conditions. Several chemicals are also tried in fish preservation.
Icing has been practiced most widely for short time preservation of fresh fish. Khwaja (1966), Nair et al. (1971,1974), Gupta and Govindan (1975), Bhattacharyya et al. (1978), Nandeesha et al. (1984), Bandyopadhyay et al. (1986) and Durairaj and Krishnamurti (1986) had conducted studies on chilled freshwater fish using ice as preservative.

In addition to the microbes acquired from the environment, the gut microflora of the fish contributes greatly to its spoilage during transportation and storage periods. One of the probable reason remarked for higher gut microbial load could be due to the polluted water where the fish live (Lakshmanan et al. 1984). After death, invasion of flesh by bacteria from the surface and intestine could be the cause of bacterial spoilage of fish. Poor and unhygienic handling and transportation can greatly increase the microbial load. Among the spoilage organisms gram-negative bacterial types were found to be predominant. Very often Vibrio sp. being reported in freshly harvested fish as spoilage causing microbe (Vanderzant, 1970).

Apart from microbial etiology, fish spoilage is often initiated by autolysis, which pave way for the microbial entry. In most instances, the fish is refrigerated or chilled during transportation and storage. Hence, psychrophilic bacteria are the major groups of bacteria responsible for spoilage of fresh fish (Adams et al. 1964). The members of Vibrio sp. a common fish spoiler were reported to be an obligate psychrophile with optimum temperature between 0 to 5°C (Anand and Setty, 1977). Most of the biochemical indices used for the assessment of spoilage are the reflection of the extent of microbial spoilage.
Shetty et al. (1992) reported that the spoilage bacteria isolated from *Sardinella* stored in chilled sea water, irrespective of their primary isolation temperature ranging from $2 \pm 1^\circ$C to $28 \pm 2^\circ$C indicating their facultative psychrophilic nature. Different incubation temperatures had been recommended for the bacteriological examination of tropical fish (Surendran and Gopakumar, 1982; Devaraju and Setty, 1985). The lower counts at low temperature incubation are assumed to be due to the elimination of true mesophiles, which failed to grow at this temperature.

Several workers had studied the bacterial population in the gastrointestinal tracts of fish qualitatively and quantitatively. Sugita et al. (1983) observed that facultatively anaerobic bacteria, *Vibrio, Aeromonas* group and obligately anaerobic bacteria, *Bacteroids* type A and B were major components of the bacteria present in the gastrointestinal tract of cultured freshwater fish, tilapia, gold fish and carp. Sugita et al. (1983) also found that this bacterial flora was relatively stable in the fish cultured in freshwater. The catalase activity of the gills runs parallel to the degree of spoilage (Tomiyama et al. 1950). This was established not only on seawater but also on freshwater fish, especially the common carp. In addition, there is a close relationship between the amount of volatile bases formed and catalase activity. In the organoleptic tests, gills are the important indicators of spoilage. *Cyprinus carpio* var. *communis*, showed the presence of micrococci, gram-positive and gram-negative rods on the surface and intestine of the fish (Sen et al. 1977).

It is a common experience that the quality and storage life of many fish decreased if they have not been gutted. During feeding periods,
the fish contain many bacteria in the digestive system and strong digestive enzymes are produced. The latter will be able to cause a violent autolytic post mortem, which may give rise to strong off-flavour especially in the belly region or even cause belly-burst. This can be partially arrested by gutting. The volatile, foul-smelling compounds are mostly found in the gut and surrounding area whereas the amount of volatile acids and bases is relatively low in the fillet itself.

Native bacterial flora of fish

The flesh and body fluids of live healthy fish are generally free from bacteria (sterile) (Surendran, 2000). But even when the fish are alive, they harbour bacteria, mainly on three sites of their body—the slime on the skin surface, the gill tissue and the intestine. Bacteria, which are naturally present on fish, are called the native bacterial flora of fish. The population and nature of such flora depend on the waters from where the fish are caught, whether seawater, brackish water or freshwater. Generally, the bacterial populations on the skin surface are the least, while counts in the intestines are the highest and mediocre in gill tissue. Also, bacterial populations exhibit seasonal variations. During warmer months, the counts are higher than the colder seasons. Qualitatively, majority of the bacterial flora of marine fish are gram-negative, non-spore forming rods or cocci. Bacterial flora of fresh-water fish is predominantly gram-positive in nature. On the other hand the skin surface of the freshwater fish rohu and mrigal, the bacterial flora is mainly comprised of gram-negatives. The predominant bacterial species identified in gram-negative groups are *Pseudomonas, Acinetobacter, Coliforms* and gram-positives are *Micrococcus*
and *Bacillus*. The bacterial genera are quite limited for freshwater fish (Surendran, 2000).

Spoilage bacteria

The important class of spoilage microorganisms found in tropical species are *Pseudomonas*, *Flavobacteria*, *Acinetobacter*, *Aeromonas* and *Moraxella*. The spoilage bacteria are characterised by their ability to produce \( \text{H}_2\text{S} \), reduce TMAO to TMA and convert urea to ammonia. Many volatile sulphur compounds are also produced by *Pseudomonas*. A quantitative measurement of these compounds indicates the degree of spoilage. Fish flesh starts visibly to spoil when bacterial level rises above \( 10^7 \text{organisms/g} \). Some of the spoilage bacteria are proteolytic and undoubtedly contribute to the ammoniacal odour by producing ammonia from the protein breakdown. When the fish is left in ambient temperature, which is usually \( 28 \pm 2^\circ \text{C} \), tropical fish get spoiled within 6 to 12 hours depending on their size (Surendran, 2000).

Robach and Hickley (1978) suggested the use of sorbic acid to retard the growth rate of *Vibrio parahaemolyticus* in crabmeat and flounder homogenate. George and Gopakumar (1988) studied the spoilage changes in the muscle of crab stored at three different temperatures. They concluded that higher temperatures caused more rapid spoilage changes and faster growth of bacteria. Alur et al. (1971) isolated *Pseudomonas*, *Proteus*, *Aeromonas* and *Achromobacter* from spoiled freshwater fish *Labeo rohita*. Dalme et al. (1986) reported on the microbial quality of fish preparations in domestic trade. This studies carried out were mostly on
the incidence of specific pathogenic organisms such as Salmonella sp. and Staphylococcus sp.

Sanjeev et al. (1986), Lalitha and Iyer (1986) and Surendran and Gopakumar (1981b) reported that Pseudomonas, Vibrio, Moraxella, Acinetobacter, Flavobacterium, Achromobacter, Alcaligenes and Micrococcus were fish muscle spoilers at 28 ± 2°C. During fish spoilage, a considerable increase in the proteolytic bacterial population has been observed by Dyer et al. (1950), Lerke et al. (1967) and Listen, (1973). The rapid spoilage of fish at high ambient temperatures in the tropics is a well-known phenomenon and the fish spoils within a few hours.

Shewan (1971) stated that storing fish below -10°C could prevent bacterial spoilage. The discolouration of fish flesh due to spoilage ranges from yellow or greenish yellow caused by Pseudomonas fluorescens, yellow by Micrococi and others to red or pink by Sarcina, Micrococi or Bacillus or by molds or yeasts. Philip and Perumalsamy (1992) investigated the bacterial growth and protein degradation of selected strains of proteolytic bacteria in various fish flesh extracts.

According to Tarr (1954), the best single test for bacteriological quality would be the determination of total bacterial population. Farber (1965) related the spoilage of fish to the bacterial count and found a significant correlation. Newman et al. (1972) studied the physiological and biochemical properties of bacterial isolates of fish. The amylolytic bacterial occurrence was found to be high in mackerel and high incidence of
proteolytic bacterial population was observed in the alimentary canal of *Rastrelliger kanagurta* (Fatima et al. 1980).

Qualitatively, there is a selection of bacterial flora during iced storage of fish. Irrespective of the composition of the initial flora, the *Pseudomonas* / *Alteromonas* group emerge as the predominant group of bacteria at the time of spoilage. This is because most of the psychrotrophic bacteria capable of causing spoilage belong to these genera (Surendran, 2000).

Vishwanath and Lilabati (1995) and Lilabati and Vishwanath (1996) carried out some preliminary works on nutritional and microbiological quality of ice stored fish *Notopterus chitala*. According to them, highest count of total plate count of the iced fish *N. chitala* may be due to the growth of psychrotrophic bacteria. Ten genera of bacteria of *N. chitala* were psychrophiles. The dominant flora were *Pseudomonas* and *Moraxella*. *Pseudomonas* was an important freshwater fish spoilage bacteria. (Surendran and Gopakumar, 1981; Perigreen et al. 1987). Ali et al. (1992) also observed *Aeromonas* was another important spoilage microorganism.

Mahajan et al. (2000) noted that the total viable counts of cooked chicken meatballs during storage at 4 ± 2°C were influenced by season, treatments, packaging and storage period. The samples were considered spoiled, when the total viable counts exceeds log 7 cfu/g (Panda, 1971). Ayers et al. (1950), Essary et al. (1958) and Barnes and Thornley (1966) had already reported that when total viable counts on the meat tissue exceeded
log7 cfu/g off-flavour and slime developed, considering these criteria in view, it could be said that these products were well within the safety limits till nine days of storage ($4 \pm 2^\circ$C).

Dushyanthan et al. (2000) showed that the vacuum packaging showed a beneficial effect on chemical and microbial qualities of beef packed in different packaging materials and stored at refrigerated and frozen conditions. Steinhouser et al. (1988) observed lower number of spoilage bacteria on vacuum packaged beef.

Effect of delayed icing on the microbial quality and shelf life of *Hilsa tili* was studied by Ramachandran et al. (1990). They reported that the fish iced in rigor condition had a shelf life of eleven days and fish procured from the landing centre had a shelf life of only eight days. The results showed that the initial total bacterial count was low in all the samples and this may be due to very fresh nature of the samples. It was increased after storage, which coincided with the organoleptic unacceptability of the fish. Amu and Disney (1973), Poulter and Nicolaides (1985b) and Bandyopadhyay et al. (1986) also reported similar trend of low bacterial counts during the early stages of ice storage.

Lilabati *et al.* (1997) studied the biochemical and microbiological quality of smoked freshwater fish snakehead *Channa punctatus*. The bacterial flora of smoked fish were composed of *Bacillus*, *Staphylococcus*, *Micrococcus*, *Xanthomonas*, and *Aeromonas*. According to Graikoski (1973), the bacterial spoilage in smoked fish was mostly dominated with the nonspore forming rods. Moisture plays significant role in bacterial
spoilage and lowering of moisture retards the spoilage of fish (Stansby, 1963).

Lilabati and Viswanath (1999) studied the biochemical and microbiological quality of *Labeo gonius* stored in ice. They reported that the bacterial count was high in the gill of fish (log8.9 cfu/g) and lowest in the muscle tissue (log5.6 cfu/g). Nair et al. (1971) also made similar observation in *C. mirgula*. Russel and Fuller (1979) reported that the gill was an ideal site for microbial growth. Eight probable genera of bacteria were identified in iced *Labeo gonius*. Among those, six were psychrophiles. *Acinetobacter, Micrococcus* and *Pseudomonas* were found in higher percentage. Ali et al. (1992) had observed that *Aeromonas* and *Pseudomonas* were the dominant microorganisms associated with freshwater fish.

Hatha et al. (2000) studied the bacterial flora of the intestines of farm raised fish such as *Labeo rohita, Catla catla*, and *Ctenopharyngodon idella*. *Aeromonas* was the dominant genera in the intestine of all these fish. While *Corynebacterium* was not encountered in the intestine of *L. rohita* it was the second dominant genera in the intestines of *C. catla* and *C. idella*. *Moraxella* was present to the extent of about five percent in the intestine of all the three fish. Other genera present were *Micrococcus, Staphylococcus, Alcaligenes, Vibrio, Acinetobacter, Lactobacillus* and members of the family *Enterobacteriaceae*. *Aeromonas* and *Staphylococcus* were the first and second dominant genera in the intestine of *L. rohita*. It had been reported that the bacterial flora of the fish intestine was a reflection of ambient microflora and the presence of *Staphylococci* in farm raised fish was reported to be due to human activity around the culture pond. Total heterotrophic
bacterial population load in the intestine of C.i.della was one log higher
than THB population of the intestine of C. catla and L. rohita. THB
population of such magnitude were reported by Sugita et al. (1983) in the
gastrointestinal tract of fish collected from the Tama River, Tokyo.
However, the THB load was less than those reported in the intestines of
salmonids (Trust and Sparrow, 1974) as well as that of arctic charr in the
natural environment (Ringo and Strom, 1994). This may be due to the
controlled environmental conditions in the aquaculture farm as the fish
acquire their intestinal microflora from the ambient environment.

Till recently, it was believed that the larval gut was sterile until
first feeding (Muroga et al. 1987). However during the hatching process,
the water often contains heavy bacterial load (Hansen and Olafsen, 1992)
and during drinking, larvae ingest bacteria. The intestine of these fish
revealed nearly 70% of gram-negative bacterial types. Similar findings
were also noted among the various bacterial species occurred in other
fish such as chum salmon (Trust, 1975), salmonids (Yoshimizu et al. 1976)
and arctic charr (Ringo and Strom, 1994).

Bacterial isolates elaborate various enzymes. These enzymes
utilize various substrates like starch, gelatin and lipid. The beneficial effects
of enzymes released by microorganisms in the digestive process of
terrestrial animals were well documented (McBee, 1977). Some
investigators have also suggested that microorganisms exert a beneficial
effect on the digestive process of fish (Danulat and Kausch, 1984). Flora
of the digestive tract can act on lipids by way of contributing to triglyceride
breakdown. Lipolytic activity had been previously reported in bacterial
isolates from gastrointestinal tract of grass carp. Lipids such as lard, butter, olive oil and fatty acids stimulated bacterial lipase synthesis (Finnerty, 1989). Predominance of Aeromonas sp. was found in the intestines of freshwater fish Tilapia. Sugita et al. (1983) reported that Vibrio and Aeromonas were the dominant micro flora encountered in the intestine of freshwater fish such as carp, crucian carp, common minnow and brackish gobi. Their findings were well agreed with the observation of Hatha et al. (2000).

The production of hydrogen sulphide and other volatile sulphides is considered to be the characteristic of spoilage bacteria. Herbert et al. (1971), Herbert and Shewan (1976) had established the importance of bacteria in the production of volatile sulphides in fish. Several authors reported the dominance of Pseudomonas of ice stored mackeral and in spoiled fish (Surendran and Gopakumar, 1982) and in tropical white prawn Penaeus indicus at 4°C (Chandrasekaran et al. 1985). Miller et al. (1973a,b) reported that the microbial spoilage of fish muscle was due to the production of volatile nitrogen compounds, volatile acids, H₂S and mercaptans. They observed that during storage of air-packed fish TMAO-reducing Pseudomonas species and H₂S producing bacteria were favoured, which is in accordance with earlier investigations (Castell et al. 1949; Herbert et al. 1971 and Shewan 1977). Regardless of the packaging method, the growth of bacteria were accompanied qualitatively by an emergence of TMAO-reducing bacteria more or less endowed with H₂S-producing properties.