CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Post mortem degradation of fish muscle proceeds through rigor-mortis, resolution of rigor-mortis, autolysis and various other chemical/microbial changes. Most of the past studies on freshness of fish were based on the view that freshness of fish is mainly decreased by bacterial action. Shigeo Ehira (1976) experimentally elucidated the differences between bacterial freshness and freshness lowering due to bio-chemical changes. He has shown by experiments carried out on plaice and skipjack on ice storage that even while bacterial count, the amounts of total volatile nitrogen, trimethyl amine nitrogen and pH did not register any marked change, adenosine triphosphate and its degradation products exhibited drastic changes before putrefaction of fish. Similar experiments were performed on aseptic fish muscle which was iced and its freshness lowering examined as compared to non aseptic sample. Changes in the biochemical indices of freshness of fish were almost same for aseptic and nonaseptic samples. The author has confirmed that freshness lowering of fish was caused by rather auto-degradation of its tissues than bacterial action. Freshness of fish therefore must be considered to be closely related with biochemical changes in fish before putrefaction.

Sikorski (1980) has described the main processes occurring in the proteins of fish after catching under the influence of endogenous and bacterial enzymes as well as changes in the characteristics of fish. These changes are given schematically below. It is shown that as a result of the hydrolytic reactions catalysed by enzymes from tissues themselves, the penetration of micro-organisms is facilitated and nutrients are formed which would promote bacterial growth.
MUSCLE OF FRESHLY CAUGHT FISH

Relaxed extensible sarcomeres, actin and myosin uncoupled, pH about 7

\[ \text{ATP} \rightarrow \text{ADP} \rightarrow \text{release of Ca}^{2+} \rightarrow \text{from sarcoplasmic reticulum} \]

FISH MEAT IN RIGOR MORTIS

Partly contracted sarcomeres, actomyosin, pH about 6

\[ \text{Cathepsins, Ca}^{2+} \text{ activated glucuronidase and other lysosomal proteinase, collagenase} \rightarrow \text{enzymes} \]

TENDER FISH MEAT

Partly hydrolysed sarcoplasmic proteins, slightly disintegrated sarcomeres, disrupted collagen structure, pH about 7

\[ \text{endogenous enzymes} \rightarrow \text{bacterial enzymes} \]

AUTOLYSED FISH MEAT

Partly hydrolysed proteins, non-protein nitrogenous compounds, pH about 7

Many enzymes of carbohydrate metabolism have been employed in the past for investigations related to the post mortem deterioration of fish quality. Much work has been carried out in the past employing succinic dehydrogenase assay in testing the freshness of fish. Other enzymes of carbohydrate metabolism such as malic dehydrogenase, \( \rightarrow \) glycerophosphate dehydrogenase, glutamate oxaloacetate transaminase, etc. were also employed in the past as indices of freshness of fish. Activity of proteolytic enzymes such as cathepsin D, alkaline protease etc. was determined in fish in order to assess freshness during storage.

1.1 ICE STORAGE STUDIES

Less work has been reported on employing enzyme activity as a test for measuring freshness of ice-stored fish. Some work were done in the past employing enzymes associated with the break down of nucleotides. Post mortem biochemi-
cal changes of nucleotides are important autolytic reactions taking place in fish. In fish muscle adenine nucleotides (5-8 μmole/g) occupy more than 90% of total nucleotides (Ikeda, 1980).

In fish muscle rigor-mortis is induced with a decrease in the ATP level of muscle (Partman 1965). The intensity of rigor-mortis is dependent upon the amounts of ATP decomposed per unit time (Yamanaka et al, 1978). Heber et al (1973) reported that the cause for the rapid disappearance of ATP in the fish muscle stored below freezing point might be the activation of adenosine triphosphatase by Ca\(^{2+}\) released as a result of cellular destruction caused by ice crystal formation.

Actin is a water soluble protein. One molecule of ATP is bound to it. Myosin exhibits ATPase activity. The ATPase activity of both actin and actomyosin is stimulated by Ca\(^{2+}\). Mg\(^{2+}\) inhibits myosin-ATPase activity but stimulates actomyosin activity. ATPase (EC. 3.6.1.8, ATP pyrophosphohydrolase) catalyses the following reaction,

\[
\text{ATP} + \text{H}_2\text{O} \rightleftharpoons \text{AMP} + \text{Pyrophosphate}
\]

Ca\(^{2+}\) ATPase activity of myofibrillar proteins of the two species of fish such as carp and sardine gradually decreased during ice storage (Seki & Narita, 1980). Seki et al (1980) observed that the myofibrillar EDTA-ATPase of fish decreases by 70% after one day storage in ice. Ca\(^{2+}\) ATPase activity decreased to 50% of their initial level after 6 days storage in ice. However, in the case of stoneflounder and plaice, total actomyosin Ca\(^{2+}\) ATPase activity changed little during 14 days ice storage (Ehira & Uchiyama, 1979). Seki et al (1979) reported that Ca\(^{2+}\) ATPase activity of carp myofibril stored in an alkaline medium increased during storage.
The effect of storage of carp post mortem muscle at room temperature (25°C) for 4 days on myofibrillar ATPase activity was determined by Seki & Watanabe (1982). It was found that Mg$^{2+}$ ATPase activity first increased rapidly, then decreased. Little change was observed in Ca$^{2+}$ ATPase activity.

Lowering of freshness and thermal stability of actomyosin ATPase activity in the dorsal muscle of various fish from Ryuku fishing ground at different temperatures were studied by Nishimoto & Miki (1979). Freshness was measured by estimating K values of the samples at 0°C, 10°C, 20°C & 30°C whereas thermostabilities of actomyosin was measured at 25°C, 30°C and 35°C by way of liberated inorganic phosphate. Freshness estimation index, K value was calculated from the following formula:

$$K (\%) = \frac{HxR + Hx}{ATP + ADP + AMP + IMP + HxR + Hx} \times 100$$

where HxR represents inosine and Hx stands for hypoxanthine. Values in μmoles/g will be used for this calculation. It is not necessary to determine separately ATP degraded compounds for the purpose of calculating K value, but determining the total amounts of ATP related compounds and that of inosine and hypoxanthine is sufficient for this purpose.

ATP degraded compounds are formed in fish muscle by the degradation of ATP through the auto catabolic path way,

$$ATP \rightarrow ADP \rightarrow AMP \rightarrow IMP \rightarrow HxR \rightarrow Hx$$

Rate constants of freshness lowering and inactivation of actomyosin-Ca$^{2+}$ ATPase activities were evaluated in the above study. Regardless of the species the freshness lowering rate increased with temperature. The thermostabilities of actomyosin-Ca$^{2+}$ ATPase activities of the samples were found
to vary with species.

Lipoamide reductase (EC 1.6.4.3 NADH : lipoamide oxido reductase) is a flavoprotein component of the multi enzyme pyruvate dehydrogenase complex and 2-oxo glutarate dehydrogenase complex. The mammalian enzymes are of mitochondrial origin. This enzyme catalyses the following reaction

$$\text{NADH} + \text{lipoamide} \rightarrow \text{NAD}^+ + \text{dihydrolipoamide}$$

Lipoamide oxidoreductase functions physiologically in the re-oxidation of dihydrolipoic acid, bound in amide linkage to the amino group of a lysine residue in the transacetylase or trans succinylase, the electron acceptor is NAD$^+$. The lipoic acid is reduced as a consequence of the thiamine pyrophosphate dependent oxidative decarboxylation of $\alpha$-keto acids, pyruvate and $\alpha$-keto glutarate yielding acetyl-CoA and succinyl CoA respectively.

Substrate specificity of lipoamide reductase is very broad. A large number of catalytic reactions are possible between pairs of hydrogen donors and acceptors. Suitable hydrogen donors are dihydrolipoyl derivative, NADH and oxidised pyridine nucleotide analogs and suitable acceptors are oxidised lipoyl derivative, NAD and oxidised pyridine nucleotide analogs, $K_3 \text{Fe (CN)}_6$, 2,6, dichlorophenol indophenol and to an extent $O_2$. NADH - 2,6 dichlorophenol indophenol reductase activity is known as diaphorase activity and this reaction was employed throughout the present study. Little work is found reported in literature employing lipoamide reductase activity as an index of spoilage of fish.

Although many workers have reported that some of the dehydrogenases of carbohydrate metabolism could be employed for investigations related to the
post-mortem deterioration of fish quality, little has been reported on lactate dehydrogenase. Of the many enzymes studied much work has been reported on succinic dehydrogenase. A gradual disappearance of succinic dehydrogenase activity was observed during ice storage of fish. Fukuda (1957) has proposed the assay of succinic dehydrogenase for determining freshness of fish. Mochinga (1969) has correlated the degree of freshness of shucked oysters with the gradual disappearance of succinic dehydrogenase activity from the gill tissue. Frigerio et al (1980) have found that chilling of fish reduces this enzyme activity.

In a study of iced haddock, the reactivity of glycerophosphate dehydrogenase to differing concentrations of magnesium ions added to the assay mixture was used as a measure of actual time elapsed from death and was interpreted as an indicator of leaching of the flesh (Gould, 1969). Reportedly a very sensitive gauge of ice storage age, it was accurate only to the first week of ice storage at 4°C. Because of the cellular disruption that occurs in frozen and thawed fish tissue, glycerophosphate dehydrogenase test as an index of leaching was not considered suitable for use with frozen stored fish.

Mitochondrial malate and glutamate dehydrogenase activities of muscle were found to increase during ice storage. Vana et al (1981) reported that in beef Psoas major muscle stored at 0 - 4°C mitochondrial malate dehydrogenase and glutamate dehydrogenase activity increased with storage period.

LDH activities of different species of fish and shellfish have been reported. Lactate dehydrogenase (EC 1.1.1.27 L - lactate: NAD oxido reductase) catalyses the final step in muscle glycolysis, i.e. the reduction of pyruvate to lactate with the oxidation of NADH to NAD⁺.

\[ \text{L - Lactate} + \text{NAD}^+ \rightleftharpoons \text{Pyruvate} + \text{NADH} \]
In post mortem fish muscle in the absence of citric acid cycle which is the aerobic pathway of glycogen degradation, pyruvate is alternately reduced to lactic acid anaerobically. In the LDH molecule there are two different poly peptide chains, in five different permutations. The distribution of these two poly peptide chains was dependent whether the extract originated in aerobic tissue such as heart (where the $H_4$ isozyme predominates) or in anaerobic tissue as in skeletal muscle (where the $M_4$ isozyme predominates).

In tuna and flounder skeletal muscle lactate dehydrogenases are quite similar. However, the heart enzymes are quite different in the two species. Cahn et al (1962) obtained data indicating that the heart and muscle LDH of sole and halibut are identical. They reported that flat fish are the only group of vertebrates for which identical types of LDH were found in heart and muscle in the adult stage.

Effect of storage at accelerated conditions on lobster muscle LDH has been reported earlier. Kaloustian et al (1969) has observed that when the crude extract of lobster muscle in Tris-Cl (0.01 M) EDTA (0.001 M, PH 7.6) were incubated at 37°C, LDH activity rapidly diminished as a function of time.

The role of cathepsin D which is a major lysosomal acid proteinase involved in the intracellular protein degradation in altering the post-mortem textural attributes resulting in the accentuated formation of free amino acids, peptides and non protein nitrogen during storage has been recognised in recent years (Parrish et al, 1969., Reddi et al, 1972., Caldwell, 1970). Several attempts have been made to purify cathepsin from the skeletal muscle of fish such as tilapia (Doke et al, 1980); Salmon (Ting et al, '1968) and albacore (Grominger, 1964). Makinodan et al (1982) purified cathepsin D from carp muscle. Cathepsin D is physio-
logically considered to take part in intracellular digestion of protein. Proteolytic activity in mackerel stored in ice has been correlated to the rapid metabolic rate of the fish muscle (Brackar 1956). However, the relation between fish muscle cathepsin D and proteolysis after the death of fish is uncertain. Its participation in autolysis is doubtful.

Fish muscle proteinases maximally active at acid and alkaline pH values have been often described. It is of considerable interest to note that a proteinase active at neutral pH is also present (Makinodan et al, 1979). It is yet to understand whether this neutral proteinase has any observable activity under commercial conditions of storage. Proteases and peptidases have been shown to be active at 5°C and 37°C in sterile muscle (Makinodan et al, 1980). Further more using radio labelled substrate it has been suggested that both cathepsins and alkaline proteases could contribute to proteolysis under normal post mortem pH (Lin et al, 1980). The loss of elasticity in kamabako has been attributed to the activity during processing of an alkaline protease in the muscle (Iwata et al, 1979). Makinodan et al (1980) suggested the existence of endo and exo peptidases in carp muscle during autolysis.

It was shown that storage at elevated temperatures enhanced the post-mortem ageing effect of muscle connective tissue and resulted in an increased release or depolymerisation of collagen components from collagen fibres. Lysosomal glycosidases may have an important function in collagenolysis by degrading the associated proteoglycan components of the tissue matrix and ground substance and improving the susceptibility of collagens to collagen degrading enzymes. On a study on the influence of post mortem time and incubation temperature on the release of lysosomal enzyme, it was found that high temperature storage (37°C) caused greater release of lysosomal enzymes (Wu et al, 1981).
Davey & Gilbert (1976) studied the effect of temperature on muscle tenderisation and have observed that the maximum rate of tenderisation take place at 60°C. They postulated that the proteolytic enzyme cathepsin-C may be responsible for the greater tenderisation effect at 60°C. However, at 60°C fish muscle paste showed lower gelstrength. This was caused by muscle alkaline protease activity. Alkaline protease has optimum activity at pH 8.0 & 65°C (Makinodan & Ikeda, 1969) which differs from the properties of cathepsins.

Some work has been reported on studies conducted on enzymes involved in the breakdown of ATP. Fish muscle ATP is decomposed principally in the following course (Tarr, 1966, Eskin et al, 1971).

\[
\text{ATP} \rightarrow \text{ADP} \rightarrow \text{AMP} \rightarrow \text{IMP} \rightarrow \text{Inosine} \rightarrow \text{Hypoxanthine} \rightarrow \text{Hypoxanthine} + \text{Ribose} + \text{Ribose} + \text{IP} \rightarrow \text{Hypoxanthine} + \text{Ribose} + \text{Ribose} + \text{IP} + \text{PO}_4
\]

Accumulation of IMP was observed in lobster (Dingle et al, 1968) shrimp (Suryanarayana et al, 1969) and crab (Sasano & Hirata, 1973). Unlike in fishes, AMP was found to accumulate in squid before conversion to IMP. (Langille & Gill, 1984). In the early stages after death the reaction IMP→Inosine→Hypoxanthine occurs more slowly than those in the sequence ATP→AMP→IMP. This leads to the accumulation of IMP in fish meat in the early stage after death.

AMP deaminase activity has been reported in fish muscle. The precise function of AMP deaminase (EC.3.5.4.6 AMP amino hydrolase) is not yet well known (Raffin, 1984). It catalyses the reaction,

\[
\text{AMP} + \text{H}_2\text{O} \rightarrow \text{IMP} + \text{NH}_3
\]
Among the many physiological roles proposed to this enzyme, the assumption that AMP deaminase may stabilise the adenylate energy change seems to be well established (Chapman & Atkinson, 1973). Pathways for the degradation of adenine nucleotides in the invertebrates have not been clearly established. Arai (1966) found no IMP in certain invertebrates and demonstrated that in many species AMP is dephosphorylated to adenosine which is then deaminated to inosine.

AMP deaminase is regarded as an allosteric enzyme activated by ATP and monovalent cations and some cases inhibited by GTP. Besides its function in the balance of the level of adenine nucleotide, which may itself be involved in control of glycolysis, the production of ammonia may enhance the activity of phospho fructo kinase both by direct activation and by a pH effect on inhibition.

Adenylic acid has been found in several animal tissues such as heart muscle, liver and kidney but the highest activities are often found in white skeletal muscle (Conway and Cooke, 1939). High enzyme activities are also measured in fish gill (Raffin & Leray, 1980).

Activity determination of adenosine 5 monophosphate aminohydrolase was carried out in cod muscle (Dingle and Hines, 1967). It was found that activity of the enzyme in the extract decreased on post rigor. In the transition to post rigor the enzyme appeared to become associated with the myosin fraction.

The nucleotide degradation pathway in shrimp and stability of the enzymes catalysing the reactions involved have not been clearly established. Arai
(1966) observed two pathways for the breakdown of adenine nucleotides in Japanese prawn (Pandalus hypsinotus). One involves the direct deamination of adenosine monophosphate to inosine monophosphate while the second involves dephosphorylation of adenosine monophosphate to adenosine which is followed by deamination to inosine. Both these pathways will subsequently lead to hypoxanthine. Cheuk et al (1979) investigated the stability of AMP deaminase and adenosine deaminase during post mortem ice storage of pink (Penaeus deorarum) and brown shrimp (Penaeus cuztecus) caught in the Gulf of Mexico. AMP deaminase activity was lost rapidly during the early stages of ice storage and no activity could be detected after ten days for pink and sixteen days for brown shrimp. Even though there was a gradual loss in activity adenosine deaminase could be detected in both species through the entire storage period of 21 days. The authors suggested that the activity of adenosine deaminase & AMP deaminase could potentially be used as quality indices for fresh shrimp held on ice.

1.2 FROZEN STORAGE STUDIES

Many enzyme systems potentially useful as a probe to quality control are present in fish muscle or in the tissue fluid obtained by pressing or centrifuging the flesh. Freezing and thawing the flesh solubilises even more enzyme activity, previously latent (Gould and Peters, 1971). The properties of certain enzymes in the sarcoplasm have been reported to change measurably under conditions of frozen storage.

Nucleotides undergo a predictably rapid and considerable change after death. Their pattern of breakdown has been studied in fish muscle undergoing frozen storage (Jones & Murray, 1961., Jones, 1965., Tomlinson & Geiger, 1963). Rates and patterns of nucleotide degradation in frozen fish differ widely from those
in iced fish, as do the specific activities of the enzymes involved (Jones & Murray, 1961., Fraser et al, 1961). Only traces of labile phosphates were found in fish muscle after frozen storage at different temperatures (Heen, 1953) and the breakdown of nucleotides seemed to be an objective index for only the first five to six days of storage, depending on the storage temperature. There is also a difference among species, the degradation in carp and trout flesh frozen in liquid air and stored at -8°C was complete in 10 days (trout) to four weeks (carp) (Partman, 1961).

ATPase was one of the first objects of concentrated enzymic investigation, because of its intimate association with the contractile elements and functions of muscle. Connell (1960) reported that although no change in ATPase activity could be detected in fish stored at -7°C, a definite drop occurred at -14°C and even at -22°C. The decrease in activity, which was slight, bore no apparent relation either to the rate of loss of solubility or to the development of toughness.

Partmann (1954) has shown that the activity of ATPase, though apparently accelerated below 0°C was erratic and did not parallel freezing denaturation changes and was therefore not a suitable index for decomposition of fish either during iced or frozen storage. Saito and Hidaka (1955) and Sawant & Magor (1961) confirmed Partmann's findings.

The ATPase activity in the muscular extracts of fish is greater than in homogenates. On storage of extracts, homogenates and also fish of different species, Kangur (1977) observed that the ATPase activity decreases differently in the different species. However, no significant changes in ATPase activity could be established season wise or species wise. Fukuda et al (1981) studied the dena-
\textsuperscript{13} Denaturation of muscle during freezing and subsequent frozen storage of deep sea fish muscle in terms of myofibrillar—ATPase activity and solubility of muscular proteins. ATPase activity was found high and the extent of denaturation was most effectively reduced by storage at \(-40^\circ\text{C}\) as compared to storage at other temperatures such as \(-20^\circ\text{C}\) or \(-30^\circ\text{C}\).

Frozen storage studies were carried out on eviscerated and gilled fresh mullet (\textit{Mugil cephalus}) at \(-20^\circ\text{C}\) for 1 year (Jiang, 1977). It was found that the solubility and \(\text{Ca}^{2+}\) ATPase activity of salt soluble proteins during frozen storage at \(-20^\circ\text{C}\) decreased markedly.

Thermal stability of fish myofibril was influenced by the environmental temperature at which the species live. Arrhenius plots for ATPase activity in the presence of \(\text{Ca}^{2+}\) was non linear whereas in the absence of \(\text{Ca}^{2+}\) the plot was linear over the same temperature range (Seki et al, 1979). The parameters such as enthalpy of activation, the entropy of activation and the free energy of activation increased from cold water to warm water habitats. A positive correlation existed between the above parameters and the environmental temperature.

Enzymes of carbohydrate metabolism were often employed as analytical tool in assessing quality of fish. Studies carried out on trout gave positive correlation of succinic dehydrogenase activity with decrease in freshness (Frittoli & Ruggeri, 1968). This enzyme undergoes a reduction in activity when subjected to freezing and hence qualitative and quantitative succinic dehydrogenase methods are useful when performed simultaneously on a fresh sample of the same product to recognise fresh sea foods from frozen ones (Parisi \textit{et al}, 1978). Cooling and freezing reduce but not completely destroy this enzyme.
activity (Frigerio et al, 1980). Frittoli and Ruggeri (1968) reported that succinic dehydrogenase could be used to distinguish between fresh and frozen fish. Similarly freezing and thawing of rat mitochondria resulted in decrease in succinic hydrogenase activity (Ivanov, 1979).

Freezing and thawing of fish flesh has been reported to solubilise a labile, latent form of malate dehydrogenase causing an increase in specific activity (Gould, 1968). During a five month period of frozen storage, cod, pollock and dab showed a progressive decline in malate dehydrogenase activity when stored at - 7°C but no loss at all when stored at - 29°C (Gould, 1964). It was suggested that the relative lability of latent malate dehydrogenase might be turned to advantage in determining loss of freshness in frozen stored fish. On 3 months frozen storage of bovine muscle at - 20°C, more or less sharp reduction in total extractable activity of glutamate dehydrogenase was observed. Total activity of succinate dehydrogenase did not alter very much. Frozen storage caused no significant increase in the activity of glutamate dehydrogenase and succinate dehydrogenase in the muscle press juice (Hamm & El-Badawi, 1984). Ciani & Salerni (1965) have suggested that the dehydrogenase activity of the muscle tissue estimated using triphenyl tetrazolium chloride can be used as a method in distinguishing fresh fish from thawed frozen fish.

Aldolase which catalyses the break down of hexose phosphate into two triose phosphates is found in most fishes (Burt & Jones, 1961). In a study of the enzyme in cod & haddock, Connell (1966) found that some activity was lost during freezing and thawing and that the remaining activity gradually diminished during a 60 week period of storage at - 14°C. For all practical pur-
poses, the decline in aldolase activity appeared to be regular enough to serve as a possible objective test of frozen storage deterioration. There are two possible causes for decline in aldolase activity during storage at -14°C, denaturation of the enzyme and progressive lack of availability of the enzyme in the homogenates. The first of these causes is the most obvious for it has been widely invoked to explain the behaviour of other proteins during the frozen storage of fish.

Lipase release from lysosomes of rainbow trout (Salmo gairdnerii) muscle subjected to low temperatures was studied by Geromel and Montgomery (1980). Storage on ice and fast and intermediate rates of freezing did not cause the release of acid lipase from the lysosomes. However, slow freezing and fluctuation of temperatures (-12 to -35°C) of the frozen fillet resulted in appreciable release of acid lipase from lysosomes. During frozen storage most of the release of acid lipase from the lysosomal fraction occurred within the first month of storage. Olley et al. (1962) studied lipolytic activity during cold storage at 7°C and 14°C in gadoid and related species, flat fishes and elasmobranchs. Phospholipase activity found to be negligible in the three elasmobranchs studied, but in all the other species phospholipase was at least as important as lipase in producing FFA. Lipase activity has been reported to bear no relationship to the fat content of fish.

In order to develop an analytical method for differentiating fresh fish fillets from frozen and thawed ones, Hamm & Masic (1971) measured the release, on freezing and thawing of mitochondrial form of glutamate oxaloacetate transaminase in carp fillets. This attempt was unsuccessful because the mitochondria were already destroyed to a high degree with concomitant
release of GOT iso enzyme during normal storage of carp fillets in ice. Based on studies conducted on haddock and cod, Gould (1971) reported that it is possible to determine whether or not the flesh of certain food fish has been frozen and thawed by measuring changes in the properties of soluble malate dehydrogenase activity.

The process of freezing and thawing animal tissues causes a remarkable release of cytochrome oxidase from mitochondria. The activity of cytochrome oxidase in extract of tissues after freezing and thawing increases by fifteen times in chicken liver, 2.5 times in trout and four times in beef muscle as compared to extracts of unfrozen sample. (Barbagli & Crescenzi, 1981). These results suggest that the determination of the release of cytochrome oxidase can be used as a method to distinguish fresh animal tissues from those which have been frozen and thawed.

The process of freezing animal tissues leads to the disruption of cellular organelles like mitochondria, lysosomes etc. releasing into the cell sap the enzymes bound to these structures. Hamm & Kormandy (1969) studying on bovine and porcine muscle found that freezing and thawing causes a remarkable increase in glutamic oxalacetic transaminase (localised in mitochondria) activity in the muscle press juice. Chhatbar and Velankar (1977) observed that freezing and thawing of four species of tropical fish lead to an increase in the total activity of aspartate amino transferase in tissue fluid due to the release of bound form of mitochondrial enzyme.

Barbagli and Crescenzi (1981) reported that the process of freezing and thawing of animal tissues causes a remarkable release of cytochrome oxidase
from mitochondria. The activity of cytochrome C oxidase in extracts of tissues after freezing increases by 2.5 times in trout as compared to extracts in frozen sample. The authors suggested that the release of cytochrome C oxidase can be used as a method to distinguish fresh animal tissues from those which have been frozen and thawed.

Cattaneo et al. (1982) reported that the specific activity of the lysosomal enzyme ß-glucosidase in press juice from trouts increased significantly on freezing and thawing while no difference was noticed in the activity of ß-N-acetyl glucosaminidase. A similar method to distinguish between fresh and thawed pork was reported (Demmer & Werkmeister 1985). Fresh and thawed frozen meat is identified from the ratio of ß-hydroxy acyl CoA dehydrogenase activity of a partially disintegrated sample to the total extracted enzyme activity. However Hamm & Badawi (1984) observed that frozen storage of bovine muscle at -20°C caused no significant increase in the activity of mitochondrial aconitase, fumarase, glutamate dehydrogenase in the muscle press juice.

Literature survey has shown that many enzyme systems potentially useful to quality control are present in the tissue of fishes. Iced and frozen storage of fish and shellfish reported to cause substantial changes in the activity of many enzymes. However, little change or even increase., was observed in some enzymes. The present study was undertaken to screen some of the fish muscle enzymes for their potentiality as indices of freshness of tropical fish. Activity of the enzymes in press juice was also determined simultaneously in different species of fish/shellfish stored in ice and in frozen condition.

Four enzymes were chosen for the present study. They were Ca\(^2+\) ATPase and AMP deaminase, enzymes associated with the breakdown of ATP, lactate de-
hydrogenase, an enzyme of carbohydrate metabolism and lipoamide reductase. Ca$^{2+}$ ATPase is an enzyme associated with the rapid disappearance of ATP in fish muscle on post mortem storage. Stability of this enzyme has been studied in different species of tropical fishes during ice and frozen storage. AMP deaminase is an enzyme which catalyses the deamination of adenosine monophosphate to inosine monophosphate.

In post-mortem fish muscle in the absence of citric acid cycle which is the aerobic path way of glycogen degradation, pyruvate is reduced to lactic acid anaerobically. Lactate dehydrogenase catalyses this final step in muscle glycolysis. Although many workers reported that some of the dehydrogenases of carbohydrate metabolism could be employed for investigations related to the post-mortem deterioration of fish quality, little has been reported on lactate dehydrogenase. Hence, this enzyme was chosen for the present study.

Little work has been carried out in the past employing lipoamide reductase as an index of spoilage of fish. The simple and rapid method of assay of this enzyme is a second reason for choosing it for the study reported in this thesis.

Several objective chemical tests were carried out simultaneously, along with enzyme assays to determine the degree of freshness of fish. Such tests carried out on fish muscle in the present study were, total bacterial count, pH, $\infty$amino nitrogen, total volatile nitrogen, trimethyl amine nitrogen, free fatty acid, peroxide value and thiobarbituric acid number and the subjective method of sensory evaluation.

Six species of tropical fish/shell fish were employed in the present study. Mainly fresh water and brackish water species of fish which are economically important
were chosen. The different species chosen are mrigal, mullet, pearlspot, milk fish, tilapia and _P. indicus._

Mrigal (*Cirrhinus mrigala (Ham.)*) is one of the Indian major carps. A natural inhabitant of the Gangetic river system, mrigal contributes to a major share of the fisheries of this river system and the connected impoundments. A bottom feeding omnivore, mrigal subsists mainly on decomposing plants and animal matter, algae, detritus etc. In natural waters it is reported to grow to a length of about a meter attaining a weight of 8-10 kg. Because of the ease in producing the seed in controlled conditions and the good growth attained in culture ponds, it is probably the most widely cultivated fish species in India. Normal growth rate in culture ponds is one Kg/year.

Mullet (*Liza parsia (Ham. Buch)*)) is one of the most desirable groups of fishes from the consumer point of view. They are hardy, low in food chains being herbivores and highly tolerant to salinity fluctuations. In culture ponds they accept supplementary feeds.

Pearlspot (*Etroplus suratensis (Bl.)*) is another popular table fish, particularly in Kerala. It is one of the best suited fish for brackish-water culture and can be acclimatised to fresh water. This is the only cultivated brackish water fish that breeds in confinement. In nature, it is reported to grow up to 30 cm reaching a wt of 1 1/2 kg. Although the growth rate in culture ponds is much less as compared to that in nature, because of the high consumer preference, it occupies an important place in brackish water culture.

The milk fish (*Chanos chanos (FORSKAL)*)) is cultivated in brackish waters throughout the world particularly in South East Asia. It is cultured on a large
scale in Indonesia, Philippines and Taiwan. It is reported to reach a maximum size of 150 cm and 20 Kg in nature. In culture ponds usually a weight of 500-1000 g is attained in a year in normal stocking densities. In India it commands a place next only to the mullets. It is found to grow faster in culture ponds either in monoculture or in polyculture.

Tilapia (Oreochromis mossambicus (Peters)) a natural inhabitant of the rivers on the east-coast of Africa, is now well established in different parts of the world and is probably the most widely cultivated fish the world over. It is cultivated in various environments like fresh and brackish water ponds, paddy fields, sewage-fed ponds and reservoirs. It is an omnivore, very hardy and tolerant to a wide range of salinity. It breeds throughout the year. It is reported to attain a weight of 850 g under favourable conditions at the end of the first year. Production rate of 4980 Kg/ha/year has been reported from fresh water ponds, while 7000 kg/ha/year is reported from the culture in sewage water ponds. Tilapia has low commercial value as a fresh fish and is considered as a poor man's food. Several high protein foods such as salted dried tilapia, tilapia canned in tomato sauce, curry sauce or chilli sauce can be successfully prepared from tilapia and are well accepted.

About 62 species of prawns and shrimps of the family penaeidae are present in the Indian waters. Of these Penaeus indicus Milne Edw. is an important species which is commercially exploited. West-coast of India accounts for more than 85% of the total marine prawn landing. Frozen prawn is exported to countries such as Japan, USA, France and the quantity exported during 1986 was 89000 tonnes valuing Rs.370 crores. Because of its tremendous significance in the sea food export trade of this country, Penaeus indicus
was chosen as a representative of the marine species, for the study reported here.