SUMMARY AND CONCLUSION

This thesis reports the findings of a study on the potentiality of certain endogenous enzyme systems as tools of quality control of fish and shellfish. Activity determinations of the four enzymes viz., Ca$^{2+}$ ATPase, lipoamide reductase, lactate dehydrogenase and 5'AMP deaminase were carried out in the species mrigal, mullet, pearlspot, milkfish, tilapia and P. indicus. Stability of the enzymes were studied in fish and shellfish during the period of iced and frozen storage. In addition to this, the effect of freezing and thawing on the activity of the enzymes in press juice of muscles were determined. Results of a study on cold shock reactions in tropical fishes are also given in this thesis.

Ice storage of fish and shellfish caused considerable and significant loss in the activities of the cytoplasmic enzymes lactate dehydrogenase and 5'AMP deaminase in the muscles of fish and shellfish. Critical values of the activities of LDH and 5'AMP deaminase in the muscle extract of ice stored fish and shellfish were computed based on the result obtained for sensory evaluation and other tests evaluating freshness of fish. Fishes having enzyme activity values below the critical values indicated here are considered unacceptable.

In the species such as mrigal, mullet, pearlspot and tilapia stored in ice, critical values of LDH specific activities (expressed as NADH $\mu$ mole/min/mg protein) ranged from 312.1 to 545.0. Limiting values of 5'AMP deaminase
specific activity (expressed as units/mg protein) ranged from 1.38 - 2.92. High limiting value of LDH specific activity was observed in the species milkfish during ice storage. 5'AMP deaminase activity in the species milkfish decreased to 0.03 at the end of 6 days storage in ice and at the end of 9 days storage total loss in enzyme activity occurred. Limiting values of specific activities of the enzymes LDH and 5'AMP deaminase in the species _P. indicus_ were 21.7 NADH\textsubscript{U} moles/min/mg protein and 0.07 units/mg protein respectively.

Although loss in activities of the enzymes Ca\textsuperscript{2+} ATPase and lipoamide reductase were observed in a few species subjected to storage in ice, this trend was not consistent and significant in the remaining species. Highly significant correlations were observed between activities of the enzymes LDH and 5'AMP deaminase in ice stored fish and shellfish and freshness indices such as total volatile nitrogen, \textalpha-amino-nitrogen, free fatty acid and overall acceptability score. The phenomenon of leaching out of the water soluble nitrogen and \textalpha-amino nitrogen from the muscle into the ice melt water has been reported. Highly significant positive correlations were observed between fall in activities of the enzymes LDH and 5'AMP deaminase and freshness test values such as \textalpha-amino nitrogen and total volatile nitrogen.

Effect of freezing and thawing on enzyme activity in press juice of muscles were determined. This study shows that the substantial increase in the activity of the enzyme lipoamide reductase in the press juice of muscles on freezing and thawing can be employed in distinguishing fresh
fish from that which has been frozen and thawed. The increase in activity is caused by releasing of the mitochondrial enzyme on freezing and thawing. Slight increase in the activities of the enzymes LDH and 5'AMP deaminase in press juice of muscles were also observed on freezing and thawing. However, this was not so significant as in the above case.

Prolonged cold storage of fish and shellfish resulted in a steady decrease in the activities of the enzymes Ca\(^{2+}\) ATPase and lactate dehydrogenase. Although decrease in 5'AMP deaminase activity was observed in some of the species, this trend was not significant in the remaining samples.

In the frozen stored fishes critical values of enzyme activities are determined, below which the samples will be considered unacceptable. These values were computed based on the corresponding sensory score and results of freshness tests. In the case of fish muscle Ca\(^{2+}\) ATPase, limiting values in the range of 0.011 - 0.088\(\mu\)mole Pi/min/mg protein has been observed for all the five species of fishes studied. In most species of fishes studied, limiting values of LDH activity ranged from 174.7-204.3 NADH \(\mu\)mole/min/mg protein. In the species, _P. indicus_, disappearance of LDH activity could be taken as an early sign of loss in freshness.

This study has shown highly significant negative correlation between free fatty acid content in frozen stored fish muscle and activities of the enzymes Ca\(^{2+}\) ATPase, and to some extend LDH. The presence of FFA is reported to cause aggregation of myofibrillar proteins and can be one of the causes of fall in enzyme activity. Another cause for fall in
enzyme activity can be denaturation due to increase in solute concentration caused by freezing which in turn will lead to changes in ionic strength and pH.

A study was conducted on cold shock reactions in several species of fresh water, brackish water and marine species of fish. When compared to storage at room temperature, chilling resulted in rapid onset of stiffening in the various species studied. A comparative study on the biochemical characteristics of cold shock reactions at 0°C and rigor mortis at room temperature was carried out to find out the subtle differences between the two phenomena. A definite lowering in muscle pH was found associated with stiffening at 0°C. Muscle glycogen content has also undergone similar pattern of change. Muscle ATPase activities was determined to find out the extent of denaturation of myofibrillar proteins. Although ATPase activity at the time of onset of stiffening at 0°C was higher than activity at the time of death, it was found lower than in fish muscle undergoing rigor at room temperature. Studies on the effect of exposure to different temperatures on the onset of stiffening in tropical fishes have shown an intense thermal shock in fishes exposed to 37°C.

Cold shortening was found to more closely resemble thaw rigor than normal post rigor changes. This phenomenon is explained by the influence of lowering of temperature on the membrane system of the sarcoplasmic reticulum triggering a series of changes which ultimately initiate the onset of stiffening.
Enzymes such as Ca$^{2+}$ ATPase, LDH and AMP deaminase have shown potentialities in the determination of freshness of fish and shellfish. Many of the chemical tests for freshness are based on the quantitative determination of one or a group of substance formed as a result of post mortem spoilage reactions. One of the problems associated with these tests is that by the time a detectable concentration of these substances are formed the product might have undergone considerable loss in freshness. This problem can be overcome by employing enzyme assay.

One of the limitations of employing enzyme assays in testing the freshness of fish and shellfish is the variation in the initial level of enzyme activity in different species. The critical values of enzyme activity depend on the initial levels of activity. Determinations of variations in enzyme activity level in the same species during different seasons are prerequisites in developing any enzyme assay as a freshness test. However, the above work was beyond the scope of this thesis and hence not attempted.

Interesting results were obtained in the study conducted on cold shock reactions in various species of tropical fishes. Chilling resulted in a rapid onset of stiffening in the various species studied. This contradicts earlier observation that icing delays the onset of rigor mortis. It will be of interest to the fish processor to know the effect of cold shock on keeping qualities of fish. With this in mind an investigation was taken up to study the biochemical characteristics of cold shortening. It was observed that cold shortening resembles more closely to thaw rigor than normal post rigor changes.
Commercial application of enzyme assay as a freshness test depends on its simplicity in adoption for routine work. Assay procedures of most of the enzymes studied in this work are complex requiring sophisticated equipments. Further work may be taken up for developing simple assay techniques for these enzymes studied such as LDH, Ca$^{2+}$ ATPase, and 5'AMP deaminase which have been found useful in testing the freshness of fish.

Another area requiring future investigation is the phenomenon of thermal shock observed in many species of fishes exposed to 30°C. It will be interesting for the fisheries scientist to know the bio-chemical differences between thermal shock and cold shock reactions and also the effect of these phenomena on post mortem deteriorative changes in fish. Hence, further research on this topic is recommended.

It may be concluded that in this study major stress was given for screening some of the muscle enzymes for their application in testing the freshness of fish and shellfish. This is an area promising immense potentialities for future investigations. The problem of variation in enzyme activity level in each species can be overcome by recommending critical values of enzyme specific activity for various species. With rapid progress in analytical techniques, it is not unreasonable to expect the development of simple enzyme assay techniques for routine application. It is believed that this study will stimulate research in this direction and new techniques and new insight will lead to greater achievements in this area.
REFERENCES


