M.S.94. NIRMALA THAMPURAN – Quantitative and Qualitative Studies on the Bacteriology of frozen fishes and prawns–1988–Dr. K. Gopakumar.

The thesis is concerned with a detailed study of the bacteriology of freezing of mackerel (*Rastrelliger kanagurta*) and prawn (*Metapenaeus dobsoni*).

True assessment of the bacterial load and accurate interpretation of the results depend on the methodology employed for the recovery of bacteria. Hence, factors affecting the recovery of bacteria from fresh/frozen fish and prawn were standardized for maximum recovery. The effect of plating methods, effect of temperature of incubation and period were studied in detail. The effect of diluent and the time for holding the homogenate in these diluents were also investigated. Fishes like oil sardine (*Sardinella longiceps*), mackerel (*Rastrelliger kanagurta*), mullet (*Mugil cephalus*), jew fish (*Johnius dissumeri*), prawn (*Metapenaeus dobsoni*) etc. were used for this part of the study.

The main purpose of the investigation was to understand the gross bacteriological changes occuring during freezing of fish and prawn. This necessitates a thorough knowledge of the background microbial flora of the fresh fish and prawn. Hence, a detailed study of the bacteriology of the newly caught fish and prawn was made. This revealed that the tropical fish and prawn harboured higher numbers of *Vibrio* spp. than the cold water fishes. The total bacterial population was also comparatively high.

The survival pattern at freezing temperatures $-39 + 2°C$ and $-20 + 2°C$ of major species of bacteria isolated from fish and prawn was investigated. Also the influence of interrelated factors such as pH, salt concentration, cell density and cold shock which govern survival at low temperature were studied. The study showed that maximum death occurred in the freezing phase. Death of lesser proportion occurred in the next ten days during frozen storage. Thereafter death was very gradual and almost linear curves resulted. Even after storage for up to one year at $-39 + 2°$ C or $-20 + 20°C$ sufficient numbers of bacteria persisted in many instances. The behaviour at these temperatures of pathogens such as *Salmonella anatum* was very much comparable to that of marine isolates. The Gram-negatives in general and *Vibrio* species in particular showed high sensitivity to low temperature. The Gram-negatives suffured death during freezing and subsequent storage period. In Gram-positive types death was more evident

in the storage period.

Selected bacteria in mixed populations were frozen and stored at −39 + 2°C, and their survival was compared to that of individual cultures. *Pseudomonas* and *Micrococcus* exhibited similar pattern of survival individually and in mixtures. *Moraxella* showed a difference in survival pattern.

The freezing menstrum exerted a major influence on survival at −39 ± 2°C. It was found that fish muscle medium was very protective. The effect of initial cell number of bacteria on the survival at low temperature was evident only at high initial cell densities of the order $10^7$to 10^9. The pH of the medium and age of cells also affected the survival. The minimum concentration of sucrose and glycerol which protected the bacterial cells varied with species. The metabolic injury occuring to selected bacterial isolates were determined at −39 ± 2°C and −20 ± 2°C. The growth rate during thawing of frozen cultures was also investigated.

Changes occurring both quantitatively and qualitatively in the native flora of mackerai and prawn during freezing and subsequent frozen storage were investigated.

Washing the raw material caused 52-98% reduction in the total bacterial count, while icing for short period caused no significant change in the bacterial flora of both mackerel and prawn. Removal of head of prawns decreased the bacterial count considerably. Icing resulted in a slight increase in the *Pseudomonas* species. Peeling and deveining resulted in increase in the number of Gram-positive cocci, especially *Micrococcus* species.

Freezing of the fish and prawn at −40°C caused 70-80% reduction in the total bacterial count of the muscle. The intestine also registered ccorresponding decrease. Storage of fish and prawn at −20 ± 2°C for period up to one year caused a steady decline in the bacterial count. But the decrease was less (about 30%) and the values varied considerably. Among the various genera encountered in fish and prawn, the *Vibrio* species faced maximum destruction. Even though this group surpassed all other types in the native flora of mackerel, within one month they were completely eliminated from the system. The Gram-positive *Micrococcus* species showed significant resistance to death during freezing. The *Moraxella* species remained stationery, while *Pseudomonas* showed decrease in their relative proportion.

The changes in the various physiological groups of bacteria during freezing and frozen storage of makerel and prawn were also followed.

Thawing studies of frozen fish and prawn at temperature +4°C, +15°C and room temperature (29 ± 2°C) indicated a delay on the onset of growth and multiplication of bacteria in thawed samples. Quantitatively as well as qualitatively, the bacterial profile of the thaw drip was a reflection of the bacterial flora of the thawed muscle. *Pseudomonas* species were the terminal flora in mackerel while in prawn *Moraxella* was the main group at the time of spoilage of thawed fish.

The biochemical and spoilage characteristics of the isolated cultures from frozen and thawed mackerel and prawn showed *Pseudomonas* to be the most active. No relationship was observed between spoilage potential and biochemical activity. Influence of temperature on the biochemical activity was also studied.