STUDIES ON THE MICROBIAL SPOILAGE OF *PENAEUS INDICUS*

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C E R T I F I C A T E

This is to certify that this thesis is an authentic record of the research carried out by Mr. M.Chandrasekaran, under our joint supervision and guidance in the School of Marine Sciences, University of Cochin, in partial fulfilment of the requirements of the Ph.D. Degree of the University of Cochin and no part thereof has been presented before for any other degree in any University.

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DECLARATION

I hereby declare that this Thesis entitled 'Studies on the Microbial spoilage of *Penaeus indicus*' has not previously formed the basis of the award of any degree, diploma or associateship in any University.

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CONTENTS

1. INTRODUCTION

1.1. PREFACE

Seafoods are not only nutritious and wholesome foods, but also an excellent source of high quality protein containing aminoacids in amounts and proportions required for human nutrition. The fats in fish are highly unsaturated as compared with those of pork, beef or mutton and they are good components of the prudent diet recommended by many nutritionists to reduce risks of circulatory diseases (Hegsted, 1978).

The rapid spoilage of fish at the high ambient temperatures in the tropics is a well known phenomenon. Fish spoil within a few hours unless some form of preservation is applied. Spoiled fish are not only a risk to human health, but also can endanger the health of the industry itself. It has been estimated that as much as 20% of the world catch of fish for human food is wasted because of spoilage and at today's prices this has serious economic implications as well as being an unexcusable loss of animal protein in a food-short world (Liston, 1980).

The most widely consumed shell fish within the group of crustacea, are shrimps, lobsters, crabs and cray fish. As a whole they contain far greater amounts of free

aminoacids than fish, which facilitates rapid bacterial growth resulting in quicker spoilage (Velankar and Govindan, 1957,'58; Ranke, 1959).

Prawn fishery is one of the major fisheries of India which is the largest supplier of shrimp in the world market. About 98% of our harvested seafood is exported or distributed as frozen product and in 1983-'84 the export value has risen to an all time high of 314.8 Rs crores (Table 1). For the production of high quality of frozen and canned prawns the raw material has to be absolutely in fresh condition. In order to assess the quality of the raw material suitable for the processing industry, a knowledge on the main biochemical and bacteriological changes, which occur in prawns from the time of catch from the sea till they are delivered to the factories, is necessary. Hence the study of prawn spoilage and bacteria involved in the process becomes very important.

Although considerable knowledge has been accumulated through several investigations on the biochemistry and processing technology of prawn, the role of native microorganisms on spoilage of prawns at ambient temperatures in unprocessed condition is not clearly understood. Therefore the present investigation has been

planned to study, in detail, the role of native bacteria during the process of spoilage of prawn, Penaeus indicus, stored as raw unprocessed prawns immediately after catch.

1.2. REVIEW OF LITERATURE

1.2.1. 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.

A chemical compound which indicates deterioration due to microorganism, may be defined as a metabolic by-product which is produced as a result of their growth on the food. The dominant spoilage flora is that group of microorganisms which persists and brings about deterioration in the quality of food under the usual handling and storage conditions. Since a specific spoilage situation may involve more than one organism, measurement of multiple compounds may be

superior to the use of a single metabolic by-product as an indicator of quality. Fields et al. (1968) suggested that some metabolic by-products of the dominant spoilage flora also might arise by autolysis, but the amount of the compound would be markedly lower than the levels associated with spoilage due to microorganisms.

In the past, ammonia, nitrogen, reducing substances such as dextrose, acidity of fat and bacteriological examination were used to detect spoilage (Fields et al. 1968). However with the availability of newer techniques, nowadays several indices like total volatile bases (TVB), total volatile nitrogen (TVN), ammonia (NH_3), total volatile reducing substances (TVR), trimethylamine (TMA), indole, hydrogen sulphide (H_2S), pH etc (Farber, 1965) are used in practice for detecting spoilage.

The enzyme systems of microorganisms causing spoilage of protein include proteinases, peptidases, deaminases and decarboxylases. The aminoacids freed by the action of peptidases and the breakdown products of aminoacids resulting from deaminase or decarboxylase activity have 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 mercaptons, indole, hydrogen sulphide, ammonia, amines and organic acids (Fields et al. 1968).

Various chemical indices such as TVB (Cann, 1974); TVN (Velankar and Govindan, 1959; Pillai et al. 1961; Vanderzant et al. 1973; Cobb et al. 1973,'76,'77); TMA (Velankar and Govindan, 1959; Pillai et al. 1961; Cobb et al. 1973); free amino acid nitrogen (AA-N) (Cobb et al. 1976,'77) and hypoxanthine (Flick and Lovell, 1972) were used successfully for assessing the progressive spoilage of shrimps.

1.2.1.1. Total volatile nitrogen (TVN)

TVN analysis in shrimp extracts is usually a measure of NH_3 and to a lesser extent volatile amines (Cobb et al. 1973). NH_3 is a metabolic by-product of several bacteria which hydrolyze protein. As described by Salle (1961), NH_3 is produced in conjunction with other products of enzyme action:

i) Production of a fatty acid and ammonia by deamination, decarboxylation and oxidation:

$$R\text{-}CHNH_2COOH + H_2O + 2\ O \rightarrow R\text{-}COOH + H_2O + CO_2 + NH_3$$

ii) Production of an alcohol and ammonia by deamination and decarboxylation

$$R - CHNH_2COOH + H_2O \rightarrow R + CH_2OH + CO_2 + NH_3$$

iii) Production of an acid and ammonia by reductive deamination

$$COOH\text{-}CHNH_2CH_2COOH \longrightarrow COOH\text{-}CH_2CH_2COOH + NH_3$$

Stansby and Lemon (1933) attributed the lower fish quality to the larger amounts of ammonia associated with decomposition by bacterial action. Crooks and Ritchie (1938) showed that the ammonia content increased with the length of storage of fillets of fish at 4-5°C. Ota and Nakamura (1952) established that the quantity of ammonia in raw and in pre-cooked meat of various fishes heated under pressure was proportional to the freshness of the raw meat. They inferred that the grade of freshness of the original raw meat could be estimated on the basis of the ammonia content.

Ammonia has been used successfully as an indicator of the quality of fresh and frozen crab meat (Burnett, 1965). He found that the NH_3 content increased uniformly and rapidly with the advancement of spoilage and it could be detected even before spoilage assessment by organoleptic procedure. Cobb et al. (1973) used NH_3 in addition to trimethylamine, dimethylamine and TVN for assessing quality of ice stored shrimp.

1.2.1.2. Trimethylamine (TMA)

Trimethylamine may be synthesized from creatine, betaine, choline, acetylcholine and trimethylamine oxide (TMAO).

TMA and other amines arise by the process of decarboxylation of aminoacids (Bramstedt, 1957). Much of the TMA apparently comes from TMAO. TMAO is a nitrogenous compound widely distributed in the marine environment and found in the tissues of marine fish and shellfish (Beatty, 1938; Yamada, 1967; Strøm and Larsen, 1979). TMAO is reduced to TMA by bacteria during spoilage (Watson, 1939; Tarr, 1954; Shewan, 1962) and the enzyme responsible is 'triamineoxidase' (Tarr, 1940). The enzyme from Vibrio parahaemolyticus (Unemoto et al. 1965) has been classified as NADH trimethylamine-N-Oxide oxidoreductase and the reaction may be represented by equation

$$NADH + H^+ + H_3C - \underset{\underset{CH_3}{|}}{\overset{\overset{CH_3}{|}}{N}} = O \rightarrow NAD^+ + H_3C - \underset{\underset{CH_3}{|}}{\overset{\overset{CH_3}{|}}{N}} + H_2O$$

TMAO TMA

It has earlier been suggested that TMA is a product of the early stages of spoilage (Collins, 1938; Hess, 1941) and that it may be lost indiscriminately during spoilage. The initial impetus to the use of trimethylamine as a measure of spoilage, was given by Beatty and Gibbons (1937) who also presented a simple Conway microdiffusion technique for its determination. Dyer (1943,'45) described a colorimetric procedure for the determination of TMA which was later

modified by Hashimoto and Akaichi (1957) and others Castell et al. 1971; Murray and Gibson, 1972). A conductance assay method for measuring the rate of TMAO reduction was described by Easter et al. (1982) and they stated that the method was advantageous over previous methods based on chemical estimation of TMA.

TMA was suggested as a chemical indicator of spoilage for shrimp by Campbell and Williams (1952). Fieger and Friloux (1954) found that significant increase in bacterial plate counts preceded with increases in TMA values and amino nitrogen was negatively correlated with taste panel evaluation of flavour and quality. Bailey et al. (1956) stated that in most cases TMA value of 1.5 mg/100 g shrimp tissue and a bacterial count of 10 x 10^6/g or higher, in headless-shelled shrimps indicated unacceptable products.

Velankar and Govindan (1959), have also reported the usefulness of TMA determination in prawns for detecting the onset of spoilage. Several workers like Velankar and Govindan (1959), Iyengar et al. (1960), Pillai et al. (1961), Velankar et al. (1961), Jacob et al. (1962), Lekshmy et al. (1962), Shaikhmahmud and Magar (1965), Kumta et al. (1970). Sajan George (1979) and Rajasekara Melanta (1980) in India and Bethea and Ambrose (1961,'62), Montgomery et al. (1970),

Cobb *et al.* (1973,'77), Flores *et al.* (1973) and Riaz and Qadri (1979) in other countries have noted increase in TMA content during storage of shrimps.

1.2.1.3. Hydrogen - ion concentration (pH)

Bailey *et al.* (1956) determined the relationships between glycogen, lactic acid or pH and the quality in Gulf shrimp. Kurtzman and Snyder (1960), Bethea and Ambrose (1962) conducted studies on raw headless brown prawn during ice storag and have shown that definite pH value could be assigned for different degrees of quality of prawn. Iyengar *et al.* (1960) observed that the pH of the muscle almost remained constant during the first 8 to 16 days (pH 7 to 7.2). Later during the next ten days, it registered an increase from pH 7.2 to 8 in ice stored *Penaeus indicus* and *Metapenaeus monoceros*. They have, further, suggested that a tentative pH value of 7.1 or less of the muscle of shrimp is indicative of prime quality, 7.2 to 7.6 indicate a presumptive spoilage stage and those greater than 7.6 indicates definite spoilage. Pillai *et al.* (1961) observed an increase of pH during spoilage of prawn at room temperature, normally exceeding 7.4 after 24 h of storage. Flick and Lovell (1972) observed a pH of 8.2 upto 10 days at $0^{o}C$ in penaeid prawns of Lousiana waters. Cobb *et al.* (1977) reported that there was no consistant pattern of pH change in the Gulf prawns

upto a period of 3 - 24 h at temperatures higher than ambient. Rajasekara Melanta (1980) observed a slow increase of pH in ice stored prawns treated with lactic acid bacterial culture.

1.2.2. CHANGES IN THE PROXIMATE COMPOSITION OF PRAWNS DURING SPOILAGE

Informations on the changes in the proximate composition of prawns stored at ambient temperatures and reduced temperatures are very much limited. Occasionally, few workers have attempted to estimate one or two components. However considerable literature on the frozen fish products are available (Huntsman, 1929; Dyer, 1950; Ironside and Love, 1958; Dingle and Hines, 1975).

Significant changes taking place in the prawn muscle during ice-storage were studied in detail by Govindan (1962,'69) and Lekshmy *et al*. (1962). They observed a rapid fall in total nitrogen, water soluble nitrogen and non-protein nitrogen during the first 8-10 days of storage. Nair *et al*. (1962) while studying the storage characteristics of prawns held in crushed ice and chilled seawater found that there was progressive loss in total solids, ash, sodium chloride, total nitrogen, non-protein nitrogen, alpha amino nitrogen and orthophosphate of meat during storage. Lekshmy *et al*. (1962) observed a gradual fall in water soluble nitrogen, non-protein

nitrogen and amino nitrogen in P. indicus during storage in ice. Pillai et al. (1961) also observed reduction in the values of non-protein and α-amino nitrogen contents with peeled and deveined samples stored in the chill room and after freezing. Pillai et al. (1965) suggested that the observed loss of characteristic flavour in the ice stored prawns is primarily due to the loss of soluble nitrogenous matter by the leaching action of ice. Devadasan and Nair (1970) have observed denaturation of major protein nitrogen fractions in Metapenaeus dobsoni and Penaeus indicus. Changes in levels of low molecular weight nitrogenous compounds in shrimp tails (Abdomen) during storage have been reported by several investigators (Campbell and Williams, 1952; Gagnon and Fellers, 1958; Woessner, 1961; Cobb and Vanderzant, 1971; Cobb et al. 1974,'76). Wilaichon et al. (1977) observed a decrease of non-protein nitrogen and protein nitrogen by an average of 50% and 25% respectively in ice stored shrimps. They also observed an increase of water content during ice storage. At room temperature (23-24°C) they further reported a decrease in protein content.

Reports on the changes in the lipid content of fishes during storage have been made by several workers

(Rajendranathan Nair, 1962; Bligh and Scott, 1966; Kinumakai et al. 1970; Takama et al. 1972; Viswanathan Nair et al. 1976,'78; Perigreen and Nair, 1974). However there is no report available for shrimps. It appears that no work has so far been done in India to record the changes in carbohydrate, ash and moisture content of unprocessed prawns stored at room temperature.

1.2.3. BACTERIOLOGICAL ASPECTS OF PRAWN SPOILAGE

Bacteriology of fresh and spoiling marine fish has been extensively reviewed by Griffithis (1937), Shewan (1949), Tarr (1954), Farber (1965), Shewan and Hobbs (1967). However, literature available on prawns are rather limited.

1.2.3.1. Natural flora of shrimps

Quantitative aspects:

Few reports are available on the bacterial content of prawn examined directly from the sea and most data presented are for freshly landed shrimps. Figures for total viable bacterial counts on temperate and cold water shrimps were between 10^3 - 10^7/g at an incubation temperature of 20°C while at 37°C a similar pattern but usually one order of magnitude lower was noticed (Cann, 1977).

The bacterial flora of tropical species of prawns differ from that of cold water species, it is largely mesophil:

due to an environmental temperature of 25^{o} - $30^{o}C$ (Cann, 1971; Newell, 1973). However initial total viable counts reported were 10^{3} - 10^{7}/g at an incubation temperature of 30 - $37^{o}C$. The bacterial population of freshly caught whole prawns from various regions of the world are presented in Table 2.

Qualitative aspects:

The major groups of bacteria associated with the prawns (whole) are Micrococcus, Corynebacterium, Moraxella, Acinetobacter (previously classified together as Achromobacter), Pseudomonas, to a lesser extent Flavobacterium/Cytophaga and Bacillus. The percentage composition varies widely. Williams et al. (1952) found that the main groups in Mexican Gulf shrimps were (as a whole) to be Achromobacter, Micrococcus, Pseudomonas and Bacillus. Harrison and Lee (1969) observed predominance of Acinetobacter - Moraxella in Pacific shrimp. Vanderzant et al. (1970) reported the dominance of coryneforms and species of Pseudomonas, Moraxella and Micrococcus in Mexican Gulf shrimps (whole) and Bacillus and Lactobacillus dominance in Pond reared shrimp. However Christopher et al. (1978) reported dominance of coryneform bacteria and Vibrio in the microflora of the Mexican Gulf shrimp. Sreenivasan (1959) indicated that prawns in India carried predominantly Micrococcus and Corynebacterium. Karthiayani and Iyer (1975) reported that the predominant

organisms found associated with marine animals (sardine, prawns, lobsters and seer fish) were the Gram-negative, asporagenous, rod-like organisms of the genera Pseudomonas, Achromobacter and Vibrio. Similar dominance of Gram-negative bacteria was also found in Penaeus indicus and P. monodon of Cochin backwater (I.C.A.R. Project Report No.4(11) 78-ASR-1 1983). Species of Vibrio was found to be dominant and other genera commonly present were Pseudomonas, Aeromonas, Acinetobacter, Alcaligenes, members of Enterobacteriaceae, Moraxella, Micrococcus, Bacillus and Corynebacterium. The freshly landed shrimps from North sea (Pandalus borealis, P. montagui and Crangon crangon) largely consisted of either Achromobacter spp. or the Gram-positive Micrococcus and Coryneform groups (Early, 1967). Gram-negative organisms of the Achromobacter and Pseudomonas groups were significantly present in the cold water types but were of lower incidence in the tropical prawns (Penaeus spp., Metapenaeus spp.) from Gulf of Thailand and Parapenaeopsis spp. from straits of Malacca (Cann, 1977).

1.2.3.2. Spoilage flora

Quantitative aspects

From the time of catching, bacteriological spoilage of shellfish is inevitable, unless they are either heat sterilized during processing or frozen stored below $-4^{o}C$.

The rate and type of spoilage will vary and they are mainly governed by factors such as time and temperature of storage. All the fish catches are either frozen or chilled to $0^{o}C$ immediately after catching. Because of the fact that ambient temperatures are higher, they are doubly susceptible to spoilage (firstly, by mesophilic bacteria until cooled from their initial temperature of 25 - $30^{o}C$ to below about $15^{o}C$; and secondly, by psychrophilic bacteria from 20^{o} to $0^{o}C$) unless the temperature is maintained at or near $0^{o}C$. However, if they are properly chilled, the shelf life could be considerably increased for tropical fish and shell fish when compared to cold water species (Shewan, 1977).

According to Tarr (1961), the best single test for bacteriological quality of fish would be the determination of total bacterial population and found a significant correlation. In a comprehensive review of the microbiology of shellfish by Fieger and Novak (1961), it is stated that shellfish spoil more readily than fish because the former contain more free amino acids. Spoilage starts immediately through marine bacteria on the surface of the shrimp as soon as they die. Bacterial counts vary with the place they are caught and with the amount of contamination from the bottom mud. While reviewing the work of several investigators, Farber (1965) related the spoilage of fish and shellfish to the total bacterial counts and found a significant

correlation. Shrimps on the market were graded as good, fair and poor quality on the basis of the total counts. Good shrimp contained 4.5 x 10^6 bacteria per g., fair 10.7 x 10^6/g and poor 19 x 10^6/g (Fields, 1979). Fieger and Novak (1961) stated that there was a definite correlation with taste panel score and log bacterial counts.

Fieger (1950) examined fresh and frozen prawns and considered spoilage of these products to be largely due to biochemical changes induced by the microbial population and to a lesser degree to enzymes and chemical compounds inherent in the prawns. Iyengar et al. (1960), Pillai et al. (1961), Velankar et al. (1961), Cann (1977) also reported an increase of spoilage in ice stored prawns with increase of bacterial population.

Onset of spoilage in frozen food was noted after bacterial colony counts, at 20°C, had exceeded 10^6/g to 10^7/g (Schmidt - Lorenz, 1982) when analysed after 3 and 7 to 12 weeks and 20 - 30 weeks periods of the samples stored at -2.5, -5 and -7.5°C respectively. With decreasing temperature lag phase increased and often a significant initial decrease in bacterial numbers was observed. When colony counts reached a level of 5 x 10^7 to 10^8/g, microbial spoilage became macroscopically visible. Similarly significant microbial spoilage of 30 different commercially

processed deep frozen foods stored at permanently controlled constant temperatures between 2.5 and -10±5°C was reported (Schmidt-Lorenz, 1963,'70, Schmidt-Lorenz and Gutschmidt, 1968,'69).

Studies on bacteria related to spoilage of prawns at room temperature (28±2°C) are rather scanty. Pillai et al. (1961) reported spoilage of prawns at room temperature and estimated the bacterial population in spoiled prawns. They reported a significant increase of bacterial population with increase of time from 10^4/g at 2 h to 10^5/g at 5 h in Metapenaeus monoceros, from 10^3/g at 0 h to 10^7/g at 24 h in Palaemon sp. and 10^6/g at 6 h to 10^8/g at 10 h in Metapenaeus dobsoni. Pillai et al. (1965) observed a similar trend of increase in bacterial counts along with increase of time recording a proportional increase from 2.08×10^4/g at 0 h to 4.75×10^6/g at 8 h at atmospheric temperatures. But reports on the nature of bacterial flora existing during spoilage of prawns at atmospheric or room temperatures (28±2°C) are not available.

However studies pertaining to prawns stored in chilled water, ice, refrigerated temperatures, freezing, frozen and canned ones are extensive (Green, 1949; Campbell and Williams, 1952; Fieger and Friloux, 1954; Fieger et al. 1956; Velankar and Govindan, 1959; Iyengar et al. 1960;

Govindan, 1962; Lekshmy et al. 1962; Carroll et al. 1968; Abdhurrahaman and Abdurrahim, 1973; Cann, 1974; Cobb et al. 1974; Koburger et al. 1975; Cobb et al. 1976; Riaz and Qadri, 1979; Surendran and Gopakumar, 1982).

Cann (1974) in his review on tropical shrimp indicated that penaeid shrimp from the Gulf of Thailand remained in acceptable condition for consumption even for 12-16 days in ice, whereas non tropical shrimp such as Pandalus sp. and Nephrops sp. were totally spoiled after 8-10 days under same conditions. The comparatively long shelf life of iced shrimps of tropical waters was also reported by Carroll et al. (1968), Clucas (1971) and Abdhurrahaman and Abdurrahim (1973). Prawns of India kept in ice had a shelf life of 16 days (Velankar and Govindan, 1959 and Iyengar et al. 1960). Total viable counts in shell fish after spoilage in ice are of in the order of 10^7 - 10^8/g at 20°C with the flora dominated by one or the other of the Gram-negative bacteria, Pseudomonas or Moraxella/Acinetobacter genera (Cann, 1977).

Delay in icing after catching can reduce the shelf life of fish and shellfish considerably. During their studies, Duggan and Strasburger (1946) allowed fresh shrimps to remain for 6 h at air temperatures before icing. They found that shrimps were spoiled after six days even if stored in ice as

indicated organoleptically. Fieger et al. (1958) observed that shrimp which remained at air temperatures for 2 h showed only a slight increase in count compared to freshly caught shrimp but after 6 and 11 days of ice storage the increase was two fold and three fold respectively. Counts of those held for 6 h at air temperature were twice that of fresh, but five fold after 6 days of storage in ice and seven fold after 11 days in ice.

Beheading of shrimp markedly reduced the initial count by about 75% (Fieger, 1950; Fieger et al. 1950; Fieger and Novak, 1961). Iced whole shrimp at the end of two days storage showed a seven fold increase in total bacterial population, while headless shrimp showed only a five fold increase. Washed, headless shrimp packed in alternative layers of ice showed steady increase in bacterial counts (Green, 1949a) Velankar et al. (1961) reported an increase in the bacterial count in whole and headless prawns immediately, whereas in peeled prawns a delay was noticed.

Storage of prawns (Penaeus spp.) without direct ice contact showed low bacterial counts, average being only 4.4×10^3/g after seven days storage (Fieger and Novak, 1961). However beheaded prawns wrapped in plastic bags and kept at

$0^{o}C$ showed considerable increase (10 to 10^{6}/g in 12 days) of total bacterial count (Shaikhmahmud and Magar, 1965).

Some bacteria are destroyed by freezing and during frozen storage, but the speed with which these bacteria are destroyed is controversial (Green, 1949b). Weiser and Osterund (1945) have found that there is an 'immediate' death caused by freezing and a 'storage' death which is a direct function of time and temperature. They have also indicated that death by freezing was marked, but did not vary with the intensity of freezing temperatures. When the temperature of incubation is lowered, there is initially an extension of lag phase growth. This is followed by a gradual elimination of various bacterial types as the temperature falls below their tolerance limit (Hess, 1934; Kiser, 1944; Ingraham, 1958). At about $5^{o}C$ mesophiles generally cease to grow and as the temperature is further lowered, various psychrophiles are also eliminated. Freezing causes a destruction of 60 to 90 percent bacterial population of pure cultures (Kiser and Beckwith, 1942; Pivnick, 1949). During storage a further fall in number occurs, exponentially for the initial period, then a more gradua decline. Exposure to low temperature causes an initial decrease in the number of living bacteria followed by a lag period before the development of survivors. The extent of the initial decrease and the lag depend on temperature (Stewart, 1934).

The initial decrease in bacterial counts due to freezing was observed by many investigators working on different fishes and shellfishes (Fieger, 1950; Pillai et al. 1961). Fieger and Dubois (1946) found that shrimp frozen immediately after catching maintained an excellent quality for 12 months in frozen storage. However shrimp frozen after 9-10 days in ice showed minor reduction in count through freezing and a gradual decline in the following 12 months (Green, 1949 a,b). Pillai et al. (1961) observed that frozen prawns did not undergo much changes during a frozen storage for a period of 13 weeks and the bacterial counts were 0.6×10^3 to 1.9×10^4 for fresh frozen and 3.2×10^5 for cooked frozen prawns. Pillai et al. (1965) also examined the microbiological quality of frozen prawn products by estimating the total counts and reported high bacterial count with the increase of spoilage.

Incorporating chemical preservatives and antibiotics in ice used for icing the fish or prawn has been extensively studied by many workers who observed significant reduction of total viable counts in the samples (Shaikhmahmud and Magar, 1965; Anand, 1976; Surendran and Gopakumar, 1981,'82).

Qualitative aspects

Vanderzant et al. (1970) stored fresh shrimp (Penaeus aztecus and Penaeus setiferus) at $1^{\circ}C$ for 7 days

and observed that although variations in microbial types were noted between samples, coryneforms and Pseudomonas, Moraxella and Micrococcus species predominated in fresh and stored shrimp. The microbial flora of fresh shrimp was usually dominated by coryneforms and that of stored shrimp by Pseudomonas species. Vanderzant et al. (1971) reported that when pond reared shrimp Penaeus aztecus stored at 3-5°C for 7 days, coryneform bacteria dominated the flora. Refrigerated storage caused increase in coryneform bacteria and Micrococcus and decrease in Vibrio, Flavobacterium, Moraxella and Bacillus species. Pseudomonas species were not significant in fresh or stored pond shrimp. While studying the bacteriological aspects of ice stored tropical shrimp Penaeus sp. of Gulf of Thailand, Mozambique and Malaysia and Metapenaeus sp. of Gulf of Thailand, Cann (1974) observed the predominance of Gram-positive bacteria. Over 50% of the flora comprised coryneforms and Micrococcus alongwith Cytophaga - Flavobacterium group, Achromobacter, Bacillus and Streptomyces. Members of the genus, Pseudomonas were not found. However after spoilage either Pseudomonas or Achromobacter species predominated. Cobb et al. (1976) reported that in ice stored Penaeus setiferus and P. aztecus, Vibrio, Pseudomonas and/or Moraxella - Acinetobacter species were initially dominant and after 12-15 days storage Vibrio sp. disappeared and Pseudomonas sp. predominated followed by

Moraxella - Acinetobacter sp. Christopher et al. (1978) observed some changes in the microbial flora of P. setiferus and P. vannamei when they were stored in ice for 8 days. Coryneform bacteria and Vibrio spp. together often constituted a major part of the microbial flora of fresh shrimp. However after storage, in both the samples, coryneform bacteria and Pseudomonas spp. predominated whereas Vibrio spp. were absent. In Indian prawn Metapenaeus dobsoni, Surendran and Gopakumar (1981,'82) stated that during ice storage, the microflora changed qualitatively in a significant pattern. They observed that the initial flora was constituted mainly by Pseudomonas, Moraxella, Acinetobacter, Vibrio, Flavobacter/Cytophaga and Micrococcus, and of these Moraxella and Vibrio formed major groups. However during storage with ordinary ice, Chlorotetracycline (CTC) - ice, and dip treatment in CTC and EDTA prior to ice storage, Vibrio decreased to 2% by 25th day of storage and Pseudomonas, Moraxella and Acinetobacter steadily increased till 25th day in ice. It is clear that members of Pseudomonas, Moraxella, Acinetobacter are found in prawns stored at lower temperatures.

1.2.4. HYDROLYTIC ENZYME PRODUCING BACTERIA

Physiological groups among bacteria have been studied by some workers in relation to regeneration of nutrients

in the marine environment (Sreenivasan, 1955; Seki, 1967; Hood and Meyers, 1973,'74; Sizemore et al. 1973). Few have studied physiological and biochemical properties of bacterial isolates of fish. (Colwell, 1962; Newman et al. 1972; Sera and Kimata, 1972) Mary (1977) studied physiological groups of gut microflora of fresh mullet (Liza dussumieri) and found the dominance of proteolytic(gelatinolytic) bacteria (45%) followed by chitinoclastic bacteria (44%). Starch and urea hydrolysers were found to be minimum (5.9 and 0.08% respectively Similar occurrence of high percentage of proteolytic (gelatinoly bacteria (91%) in the alimentary canal of Rastrelliger kanagurta was reported by Fatima et al. (1980). Amylolytic bacteria constituted higher percentage in mackerel (R. kanagurta) than mullet (Liza dussumieri). While working on the bacteriology of fresh fish and prawns in Cochin backwater (I.C.A.R. Project No.4(11) 78-ASR, 1983), it was found that proteolytic (gelatinolytic) bacteria were more in the gut of Mugil cephalus, Etroplus suratensis, Penaeus indicus and P. monodon. Besides, amylolytic bacteria were more in all the above fish and prawns when compared to earlier reports. Interestingly, in addition to ureolytic bacteria, chitinoclastic bacteria were very low in fish and prawns. But no such extensive report is available for spoilage flora of prawn occurring during storage period.

Among the numerous break down processes occurring in seafoods, proteolysis is one of the most prevalent.

Williams and his coworkers (1949,'52) indicated that 62% of the bacteria present in fresh shrimp were proteolytic and 78% of them came under the genera Achromobacter, Bacillus, Micrococcus and Pseudomonas. Same groups were observed by Magar and Shaikhamahmud (1956) in Bombay prawns, who also reported that proteolytic bacteria were responsible for spoilage of prawns.

Based on the comparative study of free amino acid pattern in sterile muscle and muscle inoculated with a pure culture of proteolytic Pseudomonad, Shewan and Jones (1957) have found that comparatively large amounts of lysine, valine and aspartic acid are produced and that alanine disappears as a result of bacterial action.

Liston (1973) based on his studies suggested that, proteolysis has importance in fish spoilage, primarily as a means of replenishing the amino acid content of the NPN (Non protein nitrogen) pool on which the spoilage bacteria are growing. He also observed from his studies that proteolytic organisms significantly increased in number during spoilage of English sole fillets stored at 0-2^{o}C.

Sajan George (1979) used total gelatinolytic bacterial counts for quality assessment of prawns during storage studies. Testing the bacterial isolates for gelatinase or caseinase enzym production is routinely done in taxonomic studies of bacteria.

However studies on proteolytic groups in storage conditions of prawn are very much limited. Surendran and Gopakumar (1981,'82) reported an increase of gelatin liquifiers in untreated and (CTC and EDTA) treated ice stored prawns (Metapenaeus dobsoni). They found an increase from 68% at O day to 90% at 25 day in untreated ice stored prawns. During initial storage, the percentage of gelatinolytic bacteria dropped to very low level (30%) and then increased to 90%. The CTC and EDTA treated samples also showed a reduction initially and later increased to 91% and 70% respectively.

Lipid hydrolysis by fish muscle lipases and bacterial lipases has been studied with regard to fish (Olley and Duncan, 1965; Hardy et al. 1979; Caselitz et al. 1980; Hanaoka and Toyomizu, 1981). But studies on the bacterial lipases and enumeration of lipolytic bacteria in spoilage of prawn are yet to be made. Similarly studies on other physiological groups and their hydrolytic activity have to be undertaken in a more systematic way.

1.2.5. SPOILAGE POTENTIAL OF BACTERIA

A series of biochemical reactions of food-borne microorganisms culminate in spoilage of flesh foods (Tomiyasu and Zenitani, 1957). The spoilage pattern is attributed to

predominant organisms and to the synergistic action between the weak and rapid spoilers under *in situ* conditions (Lerke *et al.* 1965). Thus, organisms were isolated from spoiling fish which could hydrolyze fat, reduce TMAO to TMA, decompose proteins like gelatin and casein (Castell and Mapplebeck, 1952) and those which could grow at low temperatures and exhibited proteolytic property (Shewan *et al.* 1960), produce off odours, volatile reducing substances (VRS), TMA, TVB (Lerke *et al.* 1965; Shaw and Shewan, 1968) and volatile sulphide compounds (Herbert *et al.* 1971) from flesh are considered to be potential spoilage bacteria.

The contribution by several types of bacteria to fish spoilage has been studied by inoculating sterile fish flesh and fish juice with a single pure culture (Castell and Greenough, 1957,'59; Castell *et al.* 1957,59; Lerke *et al.* 1965; Shaw and Shewan, 1968; Herbert *et al.* 1971; Cox and Lovell, 1973; Miller *et al.* 1973 a,b; Shewan, 1974; van Spreekens, 1977; Strm and Larsen, 1979; Surendran and Gopakumar, 1982). For this purpose fish muscle press juice or homogenate was sterilized by Seitz filtration (Lerke *et al.* 1963), ethylene dioxide (Shaw and Shewan, 1968), irradiation (Kazanas, 1968; van Spreekens, 1977) or autoclaving (Alur *et al.* 1971; Cox and Lovell, 1973; Strm and Larsen, 1979; Chandrasekaran *et al.* 1984 a) have been used as the test media.

It is evident from various studies that members of the genera Achromobacter and Pseudomonas groups predominate during advanced spoilage and indicate that these groups cause the spoilage. Many of the earlier workers have supported this view and also showed that a relatively small proportion of members within the groups consists of active spoilers (Shaw and Shewan, 1968; Herbert et al. 1971; Cox and Lovell, 1973; Liston, 1973; van Spreekens, 1977). Further, Lerke et al. (1965) noted that Aeromonas sp. and Vibrio sp. were found to be active spoilers at 5°C. Cox and Lovell (1973) classified the spoilers into 'rapid' and 'slow' spoilers. They observed that rapid spoilers belongs to Pseudomonas sp. with Achromobacter sp. a distant second. The genera containing no 'rapid spoilers' were Aerobacter, Bacillus, Flavobacterium, Lactobacillus, Micrococcus, Sarcina and Staphylococcus. The greatest number of slow spoilers belonged to the genus Pseudomonas with Achromobacter a close second. Other genera found to contain 'slow spoilers' were Alcaligenes, Flavobacterium, Aerobacter, Lactobacillus, Micrococcus and Staphylococcus. The genera containing no 'slow spoilers' were Bacillus, Proteus and Sarcina. van Spreekens (1978) observed Moraxella sp. Photobacterium sp. to be spoilers in addition to Pseudomonas and Alteromonas. Stromand Larsen (1979) reported Eneterobacter, Proteus and Aeromonas as spoilers. Surendran and Gopakumar (1982) while studying the spoilage potential

of selected bacterial cultures isolated from tropical fish and prawn found that most of the species of Pseudomonas, Vibrio, Moraxella, Acinetobacter, Flavobacterium/Cytophaga, Micrococcus, Archtrobacter and Alcaligenes were fish muscle spoilers at 28±2°C. At lower temperatures(-8±1°C and -1±1°C), only few Pseudomonas, Vibrio, Moraxella and Acinetobacter were found to be spoilers.

1.2.6. TRIMETHYLAMINE OXIDE (TMAO) REDUCING BACTERIA

Earlier investigators like Suwa (1909) and Poller and Linneweh (1926) found that TMAO in marine fish was reduced to the corresponding volatile amine during bacterial spoilage. TMAO reduction by bacterial strains have also been reported by Sasajima (1974), Sakaguchi and Kawai (1975).

Beatty (1938) indicated that atleast 94% of TMA in spoiling cod muscle came from TMAO and not from other possible precursors. Wood and Baird (1943) while studying the ability of bacteria to reduce TMAO to TMA devised a medium for easy assessment and observed that members of the family Enterobacteriaceae viz. Proteus, Escherichia, Aerobacter, and Salmonella were able to reduce TMAO to TMA. Further Kim and Chang (1974), Sakaguchi and Kawai (1976), Strom and Larsen (1979), Easter et al. (1982) also reported that members of Enterobacteriaceae where TMAO reducers. In addition, Vibrio parahaemolyticus (Unemoto et al. 1965; Easter et al. 1982), V. alginolyticus (Easter et al. 1982),

Pseudomonas (Laycock and Reiger, 1971; Liston, 1973; Lee et al. 1977), Alteromonas (Lee et al. 1977; Easter et al. 1982), Achromobacter (Liston, 1973), Aeromonas (Strom and Larsen, 1979) were reported to be TMAO reducers.

About 18% of the microflora initially present in fresh shrimp from Gulf of Mexico were constituted by TMAO reducing bacteria (Williams and his coworkers, 1949,'52a,'52b). According to Shewan (1961) several groups of marine bacteria can readily reduce TMAO to TMA. Laycock and Reiger (1971) reported that the percentage of TMA producing bacteria on haddock (Melanogrammus aeglejinus) fillets during refrigerated storage remained nearly constant upto spoilage stage and Pseudomonas putrefaciens was found to be the most numerous among the TMA producers. Sajasima (1974) also stated that there was no increase in bacterial cells nor any TMAO reducing activity at $-4^{o}C$ during a 25 day storage when initial cell counts were 10^{6}/ml, whereas reduction of TMAO has been observed when initial counts were 10^{8}/ml. However, Liston (1973) reported the increase in the percentage of TMAO reducers during ice storage of fish fillets and they were found to be Gram-negative bacteria.

Tarr (1938) reported an increase in TMA and viable bacterial population of lightly smoked sea fish fillets but

he could not find any constant relationship between the two values. He suggested that TMAO reduction was brought about by a specific dehydrogenase enzyme or enzymes which all bacteria may not possess. Further he concluded that TMA content alone may not prove to be a satisfactory criteria of the degree of bacterial contamination unless populations of TMA forming to non-TMA forming bacteria is fairly constant in all cases. Similarly Pivnick (1949) also recorded a greater percentage of TMAO reducing bacteria in cod frozen and stored at -12.3°C and 23.3°C.

In India, studies related to TMAO reducing bacteria are rather very much limited. Sajan George (1979) reported that with increasing bacterial counts the available oxygen level was depleted and hence the rate of TMA production increased. This rapid increase and decrease in TMA was found followed by increase and decrease of bacterial population suggesting a good correlation between TMA and bacterial population. Further he found out a significant correlation between TMAO reducing bacterial activity and TMA production. The data indicated that with the onset of incipient spoilage the rate of bacterial growth and the rate of accumulation of TMA increased significantly. Thus a change in the rate of bacterial growth or TMAO reduction may be a better choice of index of incipient spoilage rather than the qualitative level of these parameters.

1.3. RESEARCH APPROACH

Prawns caught by countryboats as well as smaller mechanised boats are generally landed in uniced condition. As the duration of the fishing trip is generally over 6 h, the prawns are known to undergo considerable changes in chemical and bacteriological composition. It is well known that prawns retain their prime quality only for about 4 h at atmospheric temperature (28°C) in India. Rapid deterioration sets in after 6 to 8 h (Pillai et al. 1965). According to the standards recommended by Indian Standards Institution, fresh fish are required to be graded according to size, properly iced and stored at temperature not exceeding 5°C (Moorjani, 1975). The International code of practice for fresh fish prepared by the Codex Alimentareous Commission (FAO/WHO/1977) recommends that fish should be chilled to the temperature of melting ice (0°C) as soon as possible after capture and should be maintained at this temperature until it reaches the consumer. The practice of icing, immediately after capture and throughout the distribution chain, retards spoilage and the shelf life of fresh fish can be extended quit considerably (Curran et al. 1980). However during transportat the temperature prevails around 5 to 6°C (Shewan, 1976). For frozen fish it has been recommended that the quick frozen product shall be uniformly glazed with water, packed in suitable containers and immediately transferred to cold

storage, the temperature of which shall not exceed $-18^{o}C$. In practice, the frozen and processed products are norma stored in the cold room, where the temperature is usually between -18 to $-20^{o}C$.

Literature reviewed in section 1.2 shows that practically all Indian works on the spoilage of prawn to date have been limited to biochemical and technological aspects. The bacteriological facet in raw and unprocessed prawns appears to be completely neglected. The aim of the present investigatic is to build up the knowledge on the role of commensal bacteria present on the prawns during storage at various temperatures. The aims of the present study are as follows:

(i) to evaluate the nature of spoilage of prawns during storage at three different temperatures ($28\pm2^{o}C$, $4^{o}C$ and $-18^{o}C$) by organoleptic assessment, accumulation of trimethylamine, ammonia content, changes in the flesh pH and total heterotrophic bacterial population at various time intervals.

(ii) to find out the changes in the proximate composition (protein, carbohydrate, lipid, ash and moisture) of the prawns during storage at various temperatures by estimating the contents at different time intervals along with spoilage assessment.

(iii) to study the occurrence and role of various bacterial genera which form the component of spoilage flora during storage.

(iv) to determine the distribution of various hydrolytic enzyme producing bacteria by evaluating their ability to produce enzymes such as caseinase, gelatinase, amylase, lipase and urease.

(v) to assess the spoilage potential of the bacteria by testing their ability to reduce trimethylamine oxide (TMAO) to trimethylamine (TMA) and to produce odour in flesh broth and halos in flesh agar media.

(vi) to study the growth kinetics of selected potential spoilers by growing them in different media and to assess the effect of sodium chloride concentrations, temperature and pH on their growth, survival and generation time.

(vii) to study the behaviour of selected strains in mixed cultures with varying populations and determine the recovery potential.

2. MATERIALS AND METHODS

2.1. AREA OF COLLECTION

The white prawns Penaeus indicus were collected from the Cochin backwater which forms a part of the Vembanad lake, a large backwater system situated along the southwest coast of India lying between the Lat. $9^{o}55'$ and 10^{o}N and Long. $76^{o}14'$ and $76^{o}20'$E and have an area of about 300 sq.kms (Fig.1). This area serves as a good nursery ground for a number of commercially important fish and prawns by virtue of its peculiar hydrographical features.

2.2. SAMPLE

Eventhough there are several species of prawns available in the study area only 'Penaeus indicus' H. Milne-Edwards, 1837; (K.H.Mohamed, CMFRI Bulletin 14, 1969) was selected for the present investigation considering its commercial importance and availability.

2.2.1. SYSTEMATIC POSITION

Phylum	:	Arthropoda
Class	:	Crustacea
Order	:	Decapoda
Suborder	:	Natantia
Family	:	Penaeidae
Genus	:	Penaeus
Species	:	indicus

2.2.2. THE DISTINGUISHING CHARACTERISTICS OF THE SPECIES

A close view of the specimen is shown in Fig.2 a. Body completely glaborus, rostrum slender, long with distinct double curve, 1.5 to 2 times in length of carapace in the juvenile stages, first five dorsal teeth close together, penultimate and distal teeth widely separated, position of latter variable. Rostrum becomes shorter with increasing size, equalling length of carapace in prawns of 80 mm, almost straight and with higher blade. Rostrum extending beyond tip of antenular scale in large prawns, blade high but not forming a triangular crest. Adrostral groove shallow, decreasing in depth backwards upto epigastric tooth. Eight to nine (some times seven) dorsal and four to five ventral teeth on rostrum. Carapace glaborus, thin, sulci and carina feebly defined. Gastro-orbital carina occupying the posterior 2/3 distance between hepatic spine and orbital angle. Orbitoantennal sulcus wide and ill-defined. Postantennular spine continued as an oblique ridge to the hepatic spine. Subhepatic ridge absent. Abdominal segments four to five keeled, keel on sixth segment ending acutely. Telson grooved, without lateral spines. Second and third joints of the first leg and second joint of the second leg provided with a spine.

General colour of live specimens is translucent whitish, with numerous small brownish, greyish or greenish

chromatophores scattered over the carapace and abdomen. The upper half of the rostrum, base of eyestalks, dorsal carina of the last three abdominal somites, telson and uropods are deeply pigmented with maroon and dull brown chromatophores. The antennae and the terminal portion of the exopods of the second and third maxillipeds are pinkish the tips of both uropods and the external margins of the outer pair are pinkish-red with similarly coloured setae. The antennular flagella are lemon yellow banded and dotted with maroon.

2.2.3. OCCURRENCE AND DISTRIBUTION

The occurrence of P. indicus has been reported from New South Wales: Off Broad water; Queensland: Gulf of Carpentaria; New Guinea: Port Moresby; North Borneo: Labnan, Sandakan; Indonesia: Palembang, East Kalimantan, Java; Phillippines: Manila Bay, St. Miquel Bay, Bulacan Province, Santa Cruz, Luzon; Singapore: Arnoy; Andamans; East Pakistan; Gulf of Aden; east coast of Africa and Madagascar. The species is fairly widely distributed in the Indo-Pacific, ranging from coasts of India and Ceylon to the west through Gulf of Aden to east coast of Africa and Madagascar, to the east to Andamans, Malaya, Singapore and Indonesia. As a commercial fishery the species exists in India, Ceylon, Malaya, Singapore, Mosambique and Madagascar, but it is reported as of scattered distribution

in Australia, New Guinea and Philippines. It is considered rare in waters of east and south east of Borneo. In India the species supports commercial fisheries in both the marine and estuarine environments on the east and west coasts.

In the backwaters of Kerala the species is fished almost throughout the year. However, marine fishery is largely seasonal and varies depending upon the region.

The estuarine backwater fishery for the juveniles of the species is carried out in every shallow waters not exceeding 10 m in depth. In the paddy fields where it is fished the depth of water is less than 1.5 metres. The commercial fishery for adults is generally carried out in the coastal waters upto a depth of 50 m along the Indian coast.

2.2.4. BIOLOGY

Eggs of *P. indicus* have been reported to occur in large numbers in subsurface plankton in marine environments. Larval forms in advanced stages are present in the inshore subsurface water particularly in the early hours of the morning. Postlarval stages are reported to occur in estuarine environments. Juvenile stages (30 to 120 mm total length) spend their life mostly in estuaries and backwaters.

On the south west coast of India,these juveniles support a good commercial fishery in the backwaters and paddy fields where they live till they attain lengths of 100 to 120 mm, after which they go back into the sea. The juveniles are bottom living and are obtained from the estuarine environment throughout the year. Sexually mature adults occur only in the sea. They are associated with shallow coastal regions and muddy sea bottom which are subjected to changes due to the physical conditions of the coast line and the nutrients obtained from the land and rivers. On the coasts of India the adults form part of the prawn fishery within 25 fathoms (45.8m) in the sea. Its occurrence in the sea is subjected to seasonal fluctuations.

2.2.5. FOOD AND FEEDING

These prawns feed on whatever suitable material they come across. Laboratory observations suggested that in nature the species is partly predatory in habits and chase smaller creatures of a size which can be seized between the appendages. Larger crustaceans, fish and others are attacked only in dead condition. They usually prefer small particles of food, which are grasped by the chelae of the pereopods and passed on to the mouth.

Food of young penaeids consist of organic detritus found in the mud, algal material and other extremely small

organisms contained in the mud. Food of vegetable matter includes diatoms like Coscinodiscus, Pleurosigma, Rhizosolenia, the planktonic alga Trichodesmium and cuttings of seaweeds. The crustaceans include copepods, ostracods, amphipods, tiny decapods and their larval stages.

2.2.6. GROWTH

The rate of growth is relatively high when they are in the estuaries and backwaters which act as a sort of nursery ground for the species. Vast majority of this species caught from the estuaries do not exceed 120 mm. Under the prevailing conditions in their brackish water habitat most of the prawns move out into the sea or are caught before they are about 100 mm in length although the adults may reach double that size. The size frequency distribution at 126-130 mm for males and 141-145 mm for females represent the first year class, those at 161-165 mm for males and 171-175 mm for females represent the second year class and those above 195 mm represent the third year class.

2.2.7. MIGRATION

The life cycle of the species is completed after passing through two distinct environments - the sea and the estuary. Larval development takes place in the sea, and

the migration into the estuaries, lakes and backwaters commences when they are in late mysis or early postlarval stages, before they are 10 mm long. This process of migration is continuous throughout the breeding period. The seaward migration begins after they attain 120 mm size. Further growth, attainment of sexual maturity and other life processes take place in the sea.

2.2.8. FISHING EQUIPMENT

In the backwaters the species is caught in large quantities in stake nets, cast nets, drag nets, dip nets and small scoop nets. Sluice nets are used in paddy fields. In the inshore marine fishery the principal types of gear employed in the capture of prawns are the boat seines and the shore seines. Along the Kerala coast and on the southern end of the west coast of India cast nets of various dimensions form an important gear for capture of prawns. From the deeper regions prawns are caught in trawls and stake nets only.

Small dug out canoes (4 to 6 m long) are the principal craft used in the backwater region. Larger dug-outs (6 to 10 metres long), canoes and catamarans are generally used in the inshore fishery in the west coast of India. On the east coast, plank built canoes and catamarans are in use. The shrimp trawls are operated from 7 to 11 metre pablo type

wooden hull boats powered by 10 to 30 hp diesel engines. A few larger steel built boats are also operating shrimp trawls.

2.2.9. COMMERCIAL SIGNIFICANCE

Penaeus indicus called locally as 'Naran chemmeen' and as 'White' in trade circles, being larger in size, like P. monodon and P. semisculcatus etc., enjoy an overriding commercial significance. By virtue of their high protein content they form a good source of animal protein and become one of the major commodity of export. They are known to be exported as frozen, canned and dried prawns. Of these, frozen prawns are reported to dominate the trade as it can be seen from the statistical figures reported by Marine Products Export Development Authority of India (1983) (Table 1) Both Japan and USA are reported to be the main markets for the frozen shrimps. The rest is shared by as many as 24 countries including France, West Germany, United Kingdom, Netherlands, Belgium, Australia etc.

According to Indian Standards Institution (1971) the frozen prawns shall be of the following types:

1. Whole	-	head and shell on
2. Headless	-	head removed, shell on
3. Fantail Round	-	head and shell removed except on the last segment and tail

4. Fantail Deveined - as in 3 but the dorsal tract removed
5. Fantail Butterfly - as in 4 in addition to splitting open
6. Peeled - head and shell removed completely
7. Peeled and Deveined - as in 6 in addition to removing the vein
8. Cooked and Peeled - peeled after cooking
9. Peeled, Deveined and Cooked - as in 7 but after cooking
10. Whole, Cooked - as in 1 but cooked
11. Peeled and Undeveined

However all these types fall into the following broad categories:

1. Whole
2. Headless
3. Peeled and Undeveined (PUD)
4. Peeled and Deveined(PD)

2.3. COLLECTION OF SAMPLES

Prawns were collected, using cast net, from Cochin backwater in the month of April 1981 (peak season of prawn fishery in this area). Both male and female

prawns were used for analyses. All prawns were in the same age group, size being 80-100 mm in length (total length). Prawns were kept alive in large plastic buckets containing the same water and transported to the laboratory.

2.4. TREATMENT OF PRAWN SAMPLES

On arrival in the laboratory the prawns were killed by shock treatment (dipping in ice cold water) and washed with sterile tap water to remove the adhering dirt. They were divided into four lots (Fig.2 b) as shown below.

i) Whole (W)
ii) Headless (H)
iii) Peeled and Undeveined (PUD)
iv) Peeled and Deveined (PD)

(ISI - 1971)

Proper aseptic conditions were strictly maintained and the processing was completed within 30 minutes.

2.5. FREQUENCY OF SAMPLING (Laboratory studies)

About 10-15 prawns, from each group stored at different temperatures (room temperature ($28\pm2^{o}C$), $4^{o}C$ and $-18^{o}C$) were used.

For various analyses samples were drawn at regular intervals as detailed below:

Sl. No.	Storage temperature		
	$28\pm2^{o}C$ (hours)	$4^{o}C$ (days)	$-18^{o}C$ (days)
1	0	0	0
2	4	2	4
3	8	5	12
4	12	10	21
5	24	15	34
6	-	30	60
7	-	-	100
8	-	-	210

The samples were analysed for spoilage, changes in the total heterotrophic bacterial counts (THB), chemical constituents and proximate composition. All the analyses were done in triplicate and also simultaneously. Temperature of $-18^{o}C$ was selected for storage studies since, ISI (1971) has recommended this temperature for cold storage.

2.6. ESTIMATION OF SPOILAGE

2.6.1. ORGANOLEPTIC ASSESSMENT

Organoleptic assessment or sensory evaluation of the prawns was performed by a panel of judges (5 persons), by

making observations on

i) general appearance
ii) slime formation on the body surface
iii) nature of eye
iv) appearance of gill
v) colour of gill
vi) presence of odour and
vii) texture

2.6.2. ENUMERATION OF TOTAL HETEROTROPHIC BACTERIAL POPULATION (THB)

Total heterotrophic bacterial population present in newly caught prawns and prawns stored at different temperatures were estimated following standard microbiological procedures as detailed below.

2.6.2.1. Preparation of samples (prawn):

The THB present on the shell surface, in gill, gut contents and in flesh were estimated.

2.6.2.1.1. Shell surface

From the 'whole' and 'headless' prawns the shell surface samples were prepared as follows. A portion of the carapace from all the specimens was removed and weighed

aseptically. This was transferred to a sterile suspension medium (50% seawater) which contained previously sterilised glass beads. The flask was shaken in a horizontal shaker (120 rpm/min.) for 10 minutes to achieve thorough mixing of the sample with the medium and to effect the release of bacterial cells adhering the samples into the medium. This sample was used for serial dilutions and plating.

2.6.2.1.2. Gill

Gill rackers from both sides of the whole prawns were aspectically removed, weighed and transferred to a presterilised tissue homogenizer. The homogenized sample was transferred to the conical flask containing sterile suspension medium. The flask was shaken in a shaker (120 rpm/min.) for 10 minutes. This sample was used for further serial dilutions and plating.

2.6.2.1.3. Alimentary canal (gut contents)

Alimentary canal was aspetically removed and homogenized in a presterilized tissue homogenizer. This was added to a conical flask containing sterile suspension medium. The flask was shaken thoroughly in a shaker (120 rpm/min.) for 10 minutes. This sample was used for serial dilutions and plating.

2.6.2.1.4. Flesh

The surface of the specimen was first sterilized with 90% alcohol for 5 minutes to avoid contamination of the surface area. A portion of the flesh from each specimen was aseptically removed. From this flesh pieces (10 g) were aseptically weighed, and homogenized with equal parts of sterilized suspension medium in a sterile tissue homogenizer. The content was then transferred to a sterile suspension medium in a conical flask. This sample was used for serial dilutions and plating.

2.6.2.2. Preparation of serial dilutions and plating procedures

Sterile suspension medium (100 ml and 9 ml) was prepared using 50% aged seawater as diluent, and sterilized in an autoclave at 15 lbs pressure for 15 minutes. Serial dilutions of the sample were made for inoculation into the media selected for the study.

ZoBell's 2216 e agar medium as suggested by Shewan (1980, personal communication) was used for the enumeration of total heterotrophic bacterial population in various samples.

Composition of ZoBell's 2216 e medium

Peptone	:	5.0 g
Yeast extract	:	2.5 g
Fe PO_4	:	0.1 g
Agar (BDH)	:	20.0 g
Aged seawater	:	750 ml
Distilled water (Copper free)	:	250 ml
pH	:	7.4 - 7.6

Conventional pourplate technique was employed. One ml aliquot of 10^{-4} to 10^{-7} dilutions was added into a sterile petridish (duplicate). About 15-20 ml of the molten medium ($\sim 40^{\circ}C$) was poured, mixed thoroughly by rotating the dishes clockwise and anticlockwise for 4-5 times and allowed to solidify. Plates were incubated at room temperatures ($28 \pm 2^{\circ}C$) for 7-15 days. The plates showing 30-300 colonies were selected for counting and referred as 'Total Heterotrophic Bacterial population' (THB). The population is expressed as number per gram wet weight of prawn after adding the number of colonies encountered in all the samples (shell+gill+ gut contents+flesh) and dividing by the total weight of the samples used.

2.6.3. ESTIMATION OF TRIMETHYLAMINE (TMA)

Trimethylamine content of the flesh was estimated following the modified Dyers Picrate method of Castell et al. 1974).

Flesh sample (1 g) was blended with 1 ml of 10% TCA (Trichloroacetic acid) for 45 seconds. The solution was allowed to stand at room temperature for 30 minutes with occasional shaking before filtering through Whatman filterpaper No.1 To 1 ml of filtered extract, 1 ml of 10% formalin was added, made upto 5 ml with distilled water in a screw capped tube and mixed well. To this 10 ml of toluene and 3 ml of 25% KOH were added. Then after shaking vigorously about 150 times the solution was allowed to settle until aqueous and toluene layers had separated (about 2 or 3 min.). From this 5 ml of toluene was transferred into a dry tube containing about 0.4 g of anhydrous sodium sulphate, stoppered and shaken gently. Dried toluene of 5 ml was transferred to another clear test tube and 5 ml of picric acid reagent (2 g of picric acid dissolved in 100 ml of moisture free toluene) was added and mixed well. The optical density was measured (within 10 min.) at 410 nm. Standard curve was prepared with analytical grade TMA (Sigma, U.S.A.).

2.6.4. ESTIMATION OF AMMONIA

Tissue homogenate was prepared by mixing flesh sample and distilled water in a tissue grinder in the proportion of 1:4 ($w/_v$) and made upto a known volume using distilled water. It was centrifuged at 12,000 g for 30 minute in a refrigerated centrifuge. From the supernatant 1 ml was

drawn and directly nesslerized (APHA, 1971) and optical density was measured at 410 nm. Standard curve was prepared with ammonium sulphate and calculations were made.

2.6.5. ESTIMATION OF pH

Tissue homogenate prepared as indicated under section 2.6.4. was used for estimation of pH in flesh. For all pH measurements a pH meter (ELICO model -L1-10) was used.

2.7. ESTIMATION OF PROXIMATE COMPOSITION

2.7.1. ESTIMATION OF PROTEIN

Protein was estimated following the modified Biuret method of Snow (1950).

Flesh (20 g) was blended with 100 ml of extractant solution containing 5% NaCl and 0.02 M $NaHCO_3$ with a final pH of 7 to 7.5 in a waring blender at $5^{o}C$ for about 3 to 5 minutes. The homogenate was centrifuged at 12,000 g for 30 minutes in a refrigerated centrifuge. The supernatant was used for protein estimation.

All the chemicals used in the present study were of analytical grade (BDH) unless and otherwise indicated. All optical density measurements were made in a Hitachi Model - 200 UV-Visible Spectrophotometer.

To 1 ml of the supernatant solution 5 ml of distilled water and 5 ml of Biuret reagent (NaOH, 180 g and $CuSO_4$, 2 g; dissolved separately in about 400 ml distilled water and the two solutions were mixed after NaOH had cooled and diluted to one litre) were added and mixed thoroughly. After development of violet colour (30 min.) the optical density was measured at 540 nm. Standard curve was prepared with Boving serum albumin (Sigma, U.S.A.) and calculations were made.

2.7.2. ESTIMATION OF CARBOHYDRATE

Carbohydrate content of prawns was estimated following Phenol-sulphuric acid method of Dubois et al. (1956).

Tissue homogenate was prepared as indicated under section 2.6.4. To 1 ml of the supernatant 1 ml of 5% phenol solution and 5 ml of conc. H_2SO_4 were added. The solution was mixed thoroughly with a glass rod and allowed to cool to room temperature (30 min.). The optical density of the solution was then measured at 490 nm. D-glucose was used as the standard and the unknown values were calculated.

2.7.3. ESTIMATION OF LIPID

Quantitative estimation of lipid was done according to the Gravimetric method of Folch et al. (1956).

Flesh (2 g) was ground in a tissue grinder with 5 ml of chloroform-methanol mixture (2:1 v/v) and was allowed to stand for 30 minutes at room temperature. The supernatant solution was transferred into a centrifuge tube. This process of homogenization and decanting the supernatant into the centrifuge tube was repeated for 3 times. Further, the tissue particles were removed by centrifugation: The supernatant was transferred to a weighing bottle of known weight and was dried in an oven maintained at 50°C. After complete drying the container with the fat was weighed, checked for constant weight and calculations were made.

2.7.4. ESTIMATION OF ASH

Ash content of the samples was estimated according to A.O.A.C. (1975). Known weight of flesh, previously dried at 100°C was treated with olive oil and heated slowly over flame to eliminate fat. The treated sample was heated to ash in a muffle furnace at 525°C until white ash was obtained. This ash was moistened with water, dried on a steambath and then on a hot plate. This was reached at 525°C to a constant weight.

2.7.5. ESTIMATION OF MOISTURE (IS: 1972)

Large pieces of flesh were cut into small sizes, mixed and ground into a fine paste using an electric grinder

to obtain a homogenous sample. The material was kept in an airtight container to prevent loss of moisture during subsequent handling and were used for analyses.

Five gram of the prepared sample was weighed and transferred to a petri-dish. The dish was kept in a hot air oven at 100±1°C for 6 h, cooled in a dessicator and weighed. This process was repeated until concordant values were obtained and calculations were made.

2.8. ISOLATION AND MAINTENANCE OF BACTERIAL CULTURES

Isolations were made at random from ZoBell's 2216 e agar medium plates containing countable number of colonies (30-300 colonies per plate). After recording the morphological characteristics of the colony, opacity and pigmentation, they were subcultured onto ZoBell's agar slants. The isolates were checked for their purity by repeated streaking on ZoBell's agar and were maintained on ZoBell's agar slants in the laboratory. One set of stock cultures were prepared in glass vials with rubber stopper, preserved under oil after growth, and stored at 20°C in a cold room.

2.9. IDENTIFICATION OF CULTURES

The isolated cultures were grouped into various genera based on their morphological and biochemical characters as suggested by, Shewan (1960), Cowan (1974) and Buchanan and Gibbons (1974).

2.10. PRODUCTION OF HYDROLYTIC ENZYMES BY BACTERIA

The bacterial cultures isolated from the fresh and spoiled prawns were tested for their ability to elaborate some of the hydrolytic enzymes such as gelatinase, caseinase, amylase, lipase and urease.

2.10.1. GELATINASE

Gelatinase producing capacity of various bacterial isolates was tested employing Frazier's gelation agar medium (modified) of Harrigan and McCance (1972).

Composition of the medium

Peptone	: 10 g
Meat extract	: 10 g
Gelatin	: 4 g
Sodium chloride	: 15 g
Agar	: 20 g
Tap water	: 1 l
pH	: 7.2

The prepared medium was autoclaved, poured into sterile petri-plates and allowed to solidify. Isolates were inoculated by surface streaking on the solidified agar medium and incubated at room temperature ($28\pm2^{o}C$) for 7 days. The plates were flooded with 8-10 ml of the test reagent

(mercuric chloride, 15 g; conc. HCl, 20 ml and distilled water, 100 ml). Gelatin hydrolysis was identified by clear halos around the colonies.

2.10.2. CASEINASE

Caseinase production by different cultures was detected by employing casein agar medium of Harrigan and McCance (1972).

Composition of the medium

Peptone	: 10 g
Meat extract	: 10 g
Casein (BDH)	: 30 g
Sodium chloride	: 15 g
Agar	: 20 g
Tap water	: 1 l
pH	: 7.2

The prepared medium was autoclaved, poured into sterile plates and allowed to solidify. Cultures were inoculated on these plates by surface streaking and incubated at room temperature ($28\pm2^{o}C$) for 7 days. Caseinase enzyme production was detected by the presence of clear zones around the colonies.

2.10.3. AMYLASE

Amylase production was tested on the agar medium

of Harrigan and McCance (1972) supplemented with starch as the substrate.

Composition of the medium

Peptone	: 10 g
Meat extract	: 10 g
Starch (soluble)	: 2 g
Sodium chloride	: 15 g
Agar	: 20 g
Tap water	: 1 l
pH	: 7.2

The prepared medium was autoclaved, poured into sterile petri-plates and allowed to solidify. The cultures were inoculated by surface streaking on the media and incubated at room temperature ($28\pm2^{o}C$) for 7 days. The production of amylase was tested by flooding the plates with Gram's iodine solution (Potassium iodide, 2 g; iodine, 1 g and distilled water, 300 ml). Unhydrolysed starch formed a blue colour with iodine. The amylolytic colonies developed clear zones around them.

2.10.4. LIPASE

Production of lipase was tested on Tween agar of Harrigan and McCance (1972).

Composition of the medium

Peptone	: 10 g
$CaCl_2$	: 100 mg
Tween-80 (Sorbitol monocleate)	: 10 ml
Sodium chloride	: 15 g
Agar	: 20 g
Tap water	: 1 l
pH	: 7.2

The prepared medium was autoclaved and poured into sterile plates. Isolates were streaked on this solidified media and the plates were incubated at room temperature ($28\pm2^{o}C$) for 7 days. Lipase production was detected by the appearance of opaque zones around the colonies. Appearance of a waxy material around the colonies was the indication of the liberation of insoluble oleic acid formed as a result of lipase action.

2.10.5. UREASE

Urease producing ability of the bacterial cultures was tested with Christensen's urea agar medium (1946).

Composition of the medium (Basal)

Peptone	: 1 g
KH_2PO_4	: 2 g
D-glucose	: 1 g
Sodium chloride	: 15 g
Phenol red (0.2% solution)	: 6 ml
Agar	: 20 g
Tap water	: 1 l
pH	: 6.8 - 7.0

The medium was autoclaved and cooled to 50°C. 20% urea solution, previously sterilized by filtration through a membrane filter (0.2μ) was then added to the basal medium to give a final concentration of 2% and poured into sterile plates. The plates were inoculated with bacterial cultures by streaking on the solidified agar medium and were incubated at room temperature ($28\pm2^{\circ}C$) for 7 days. Ureolytic activity was detected by the change in colour of the medium around the cultures from light yellow to pink.

2.11 ASSESSMENT OF SPOILAGE POTENTIAL OF BACTERIAL ISOLATES

Spoilage potential of bacterial isolates was tested by inoculating the culture into a sterile flesh broth and examining their ability to produce any off odours, or by forming clearing area around the colony in a flesh broth medium solidified with agar in plates.

2.11.1. STANDARDIZATION OF FLESH MEDIA

The method used in the present study was the modified procedure of Alur *et al.* (1971) and Strøm and Larsen (1979).

Fresh fish (*Etroplus suratensis*) and prawns *Penaeus indicus*, *P. monodon* and *Metapenaeus monoceros*) collected from Cochin backwater were washed with sterile tap water, eviscerated and the flesh was cut into small pieces. Homogenates were prepared separately for each species. 100 g of flesh was homogenized with equal volume of tapwater in a tissue homogenizer. The homogenate was made upto 1000 ml and was centrifuged at 12,000 g for 30 minutes at $0^{o}C$. The supernatant was divided into two portions. The first portion was boiled for 5 minutes and the coagulated protein was removed by centrifugation at 12,000 g for 30 minutes at $0^{o}C$ and the supernatant was used for the preparation of media. The second portion was used as such.

The prepared flesh broth was adjusted to pH 7 using 1N NaOH or 1N HCl and dispensed in 10 ml aliquots into test tubes. For solidified flesh media 1.5% agar (Difco) was added. The media were autoclaved at 15 lb pressure for 15 minutes.

Bacteria isolated from prawns stored at different temperatures during the spoilage studies were used for testing the efficiency of the prepared media. The pure strains were precultured in ZoBell's broth and from this, a loopful of 18-24 h old broth cultures was inoculated into flesh broth. In the solidified flesh agar media, the inoculum was planted at the centre of the prepared plate. The inoculated media were incubated at room temperature ($28\pm2^{o}C$) upto 48 h. Cultures showed rapid growth in both the flesh broth and flesh agar media within 18 h of incubation. They produced intense spoilage odour, fluorescent pigment and indole in flesh broth. In flesh agar media besides rapid growth of colony, clearing area around the colony was observed within 12-18 h of incubation (Fig.3). Clearing area around the colony was observed in the media prepared with and without coagulable protein using fish and prawn as source of flesh.

The biochemical analyses of protein and lipid of both the cleared and uncleared area in the plates showed reduction in the percentages of protein and lipid in the

cleared area (Table 3). From this test,it was inferred that media prepared using fish or prawn after removal of coagulable protein gave transparent solution. However both ways of preparation gave good results of growth. For spectrophotometric observations of growth, the transparent broth obtained after removal of coagulable protein was useful and hence in the determination of spoilage potential of bacteria and for growth studies flesh media was prepared after removing coagulable protein using prawn flesh.

2.11.2. PREPARATION OF FLESH MEDIA

A known quantity of grated prawn flesh (100 g) was homogenised with 100 ml of tapwater with 0.5% NaCl in a waring blender for 5 minutes at high speed. This solution was made upto 1000ml using distilled water. The homogenised solution was then centrifuged at 12,000 g at $0^{o}C$ for 30 minutes. The supernatant was boiled for 5 minutes. Once again the boiled supernatant was centrifuged at 12,000 g at $0^{o}C$ for 30 minutes to remove the coagulated protein. The supernatant was used as the medium.

pH of the flesh broth was adjusted to 7 and used directly as broth medium. For solidified medium 1.5% agar (Difco) was added. The media were autoclaved at 15 lb pressure for 15 minutes.

2.11.3. TESTING PROCEDURE

The autoclaved flesh broth (10 ml aliquots in test tubes) was inoculated with a loopful (10^4 cells/ml) of 12-24 h old broth culture and was incubated at room temperature ($28\pm2^{o}C$) for 24-72 h. Elaboration of spoilage odours was periodically checked at 24 h interval. Production of fluorescent pigment was clearly visible either in the presence or absence of ultraviolet radiation.

Growth was recorded by measuring the optical density at 600 nm. Indole production was tested, after recording spoilage odour, pigmentation and growth, in the same medium by the addition of Kovac's reagent (5 g of p-dimethyl amino-benzaldehyde dissolved in a mixture of 75 ml of amyl alcohol and 25 ml of concentrated H_2SO_4). Production of red colour was taken as positive.

On the surface of the flesh agar medium a loopful of 12-24 h old culture was streaked and incubated at room temperature ($28\pm2^{o}C$) for 24-72 h. Appearance of colony growth and characteristic clearing area around the colony formed were checked at intervals of 12 h and the results were recorded.

2.12. TRIMETHYLAMINE OXIDE (TMAO) REDUCTION TO TRIMETHYLAMINE (TMA) BY BACTERIA

Trimethylamine oxide reduction to Trimethylamine by bacteria was tested according to the methods of Wood and Baird (1943) and Laycock and Reiger (1971).

The composition of the medium is as follows.

Basal medium

Glucose	: 250 mg
Bacto peptone	: 500 mg
Sodium chloride	: 500 mg
Magnesium sulphate	: 100 mg
Dipotassium phosphate	: 100 mg
Water	: 100 ml
pH	: 7.2 (before autoclaving)

2.5 ml aliquots of the medium were dispensed in test tubes and autoclaved. To 5 ml of autoclaved basal medium, 0.15 ml of 5% filter sterilized TMAO solution was added so as to get a final concentration of 0.15%. TMA production was tested as follows.

To the test solution, 0.75 ml of 40% formaldehyde followed by 1.5 ml of saturated potassium carbonate were added. Immediately after the addition of the carbonate solution, the mouths of the tubes were covered with a petri dish lid containing a 9 cm diameter dried filter paper (Whatman No.1) which had been previously soaked in 0.05% bromothymol blue solution adjusted to pH 3 with 0.1 N sulphuric acid. The tubes were incubated in a water bath at 45°C.

Presence of TMA was detected by a change in the colour of the filter paper to blue (alkaline) at the mouth of the tube within 30 sec. to 2 min.

2.13. GROWTH AND PHYSIOLOGY OF SPOILAGE BACTERIA

2.13.1. SELECTION OF BACTERIAL ISOLATES

Eight isolates were selected based on their dominance showed during spoilage and certain characters like proteolysis, lipolysis, TMAO - TMA reduction, production of off odour from sterile flesh broth and formation of clear zones around the colony on flesh agar. The selected isolates belonged to three genera and were species of Pseudomonas (R8, L97 and F152), Vibrio (R42, L146 and F10) and Acinetobacter (L114 and F88). They were considered as potential flesh spoilers based on the above said characters.

2.13.2. PREPARATION OF INOCULUM

Inoculum was prepared by suspending 24 h old bacterial culture in sterile seawater and the optical density was adjusted to 0.2 at 600 nm. From this 0.5 ml was used as inoculum in the following experiments which were carried out in duplicates.

2.13.3. MEASUREMENT OF GROWTH

From the prepared inoculum 0.5 ml was transferred to culture medium in tubes and incubated at room temperature ($28\pm2^{o}C$) for 48 h. Turbidity resulted due to the growth of

the inoculated bacterial culture was measured at 600 nm. The growth was expressed as index of growth. Growth index is the average of turbidity in all the tubes.

2.13.4. ESTIMATION OF SURVIVING ORGANISMS

The effect of temperature, pH and NaCl concentrations on the survival of bacteria was determined as detailed below by subjecting the bacterial cultures to above said factors at various levels for different incubation period. Then the cultures were reinoculated into fresh peptone seawater medium and incubated at room temperature ($28\pm2^{o}C$) for 5 days. The growth of the survived organisms was determined as mentioned under section 13.3.

2.13.5. EFFECT OF TEMPERATURE ON GROWTH

The effect of temperature on the growth of the spoilage bacteria was assessed by growing them at different temperatures (-15, 5, 15, 30, 45, and $60^{o}C$). Growth was tested both in flesh broth and in ZoBell's broth. The prepared media were distributed in 5 ml aliquots into test tubes. After autoclaving they were inoculated with 0.5 ml of prepared inoculum, incubated and finally growth was measured (Sec.13.3).

2.13.6. EFFECT OF pH ON GROWTH

The effect of pH on the growth of spoilage bacteria was determined by growing them at various pH levels (2,4,6,8,10 and 12) in flesh broth and in ZoBell's broth separately adjusted to various pH with 1N NaOH or 1N HCl. 5 ml aliquots of the media were dispended into test tubes and autoclaved. The media were inoculated with 0.5 ml of prepared inoculum, incubated and growth was measured (Sec.13.3).

2.13.7. EFFECT OF SODIUM CHLORIDE CONCENTRATIONS ON GROWTH

Effect of sodium chloride concentrations on the growth of spoilage bacteria was determined both in flesh broth and in ZoBell's broth containing 0,1,3,6,10 and 15% NaCl. The media were dispensed in 5 ml aliquots into test tubes and autoclaved. They were inoculated, incubated and finally growth was measured (Sec.13.3).

2.13.8. EFFECT OF TEMPERATURE, pH AND SODIUM CHLORIDE CONCENTRATIONS AND THEIR INTERACTION ON GROWTH

Effect of temperature, pH and sodium chloride concentrations in various combinations on the growth of spoilage bacteria was determined in flesh broth and in ZoBell's broth. Media were prepared with different NaCl conc. (0,1,3,6,10 and 15%), dispensed in 5 ml aliquots in

tubes, and adjusted to various pH levels (2,4,6,8,10 and 12) using either 1N HCl or 1N NaOH. The media in tubes were autoclaved, inoculated with 0.5 ml of prepared inoculum and were incubated at different temperatures (-15,5,15,30,45 and 60°C) for 48 h and growth was measured at 600 nm.

2.13.9. EFFECT OF TEMPERATURE ON SURVIVAL OF SPOILAGE BACTERIA

The temperature tolerance of spoilage flora was determined by the following procedure.

The cultures used for this experiment were precultured in ZoBell's broth at room temperature (28±2°C). After 24 h of incubation the culture solution was distributed in 5 ml aliquots in different sterilized test tubes and were maintained at various temperatures as mentioned below.

Temperature (°C)	Incubation time (min.)	Sampling intervals (min.)
-15	180	30
5	180	30
45	90	15
60	60	10

At each sampling period, one loopful of culture broth from each tube was transferred into fresh ZoBell's broth tubes. They were incubated at room temperature (28±2°C) for a period of 5 days and finally growth was measured at 600 nm.

2.13.10. EFFECT OF pH ON SURVIVAL OF SPOILAGE BACTERIA

Effect of pH on survival of spoilage flora was tested as follows:

The cultures used for this test were precultured in ZoBell's broth at room temperature ($28\pm2^{o}C$). After 24 h of incubation, the culture solution was used as inoculum. Buffers of various pH levels (2.6,4.2,6.0,8.6,9.6 and 10.6) were prepared with citrate-phosphate and glycine-NaOH buffers. They were distributed into test tubes in 5 ml aliquots and were inoculated with 0.5 ml of the prepared inoculum. The tubes were incubated at room temperature for a period of 30 h and at intervals of 6 h one loopful of culture broth from each tube was removed and inoculated into fresh ZoBell's broth. After 5 days of incubation at room temperature the growth was measured at 600 nm.

2.13.11. EFFECT OF SODIUM CHLORIDE CONCENTRATION ON SURVIVAL OF SPOILAGE BACTERIA

Effect of sodium chloride concentration on survival on spoilage flora was tested by the following procedure. The cultures used for this test were precultured in ZoBell's broth at room temperature ($28\pm2^{o}C$) for 24 h and used as inoculum. Various concentrations of sodium chloride (0,1,3,6,10 and 15%) were prepared with double distilled

water and distributed in 5 ml aliquots into test tubes. These tubes were inoculated with 0.5 ml of prepared inoculum and incubated at room temperature ($28\pm2^{o}C$) for a period of 30 h. At intervals of 6 h one loopful of culture broth from each tube was removed and inoculated into fresh ZoBell's broth. After incubation at room temperature for 5 days, growth was measured at 600 nm.

2.13.12. GENERATION TIME OF SPOILAGE BACTERIA

The generation time of spoilage bacterium was estimated in flesh broth and in ZoBell's broth.

Media were prepared and distributed in 100 ml aliquots into 250 ml conical flask and were autoclaved. A known concentration of cells (10^{3}/ml) for each culture was inoculated into the prepared media and incubated at room temperature ($28\pm2^{o}C$) in a rotary shaker. At intervals of 15 minutes upto a period of 90 minutes and at intervals of 30 minutes upto a period of 6 h, aliquots were removed and the growth was measured. Generation time was determined from the exponential phase of the curve of a semilog arithmic plot.

2.14. STUDIES ON THE INTERACTIONS OF THE SELECTED BACTERIAL ISOLATES

2.14.1. SELECTION OF BACTERIAL ISOLATES

Of the eight cultures used in the growth kinetics study, five strains showing maximal growth and comparatively

higher participation in proteolysis, lipolysis, trimethylamine oxide reduction, production of off odours and clearing zones around the colonies in the flesh agar medium were selected. They include Vibrio spp. (1. R42; 2. L146), Pseudomonas spp. (3. R8; 4. L97) and Acinetobacter sp. (5. L114) and then subjected to interaction studies.

2.14.2. PREPARATION OF INOCULUM

Selected strains were precultured in ZoBell's 2216 e broth. The cell concentration of 24 h old broth culture was predetermined by plate counts and spectrophotometric measurement of turbidity. The culture was adjusted to the concentrations of 1 x 10^2, 1 x 10^4 and 1 x 10^8 cells per ml with sterile 1% saline and used as inoculum.

2.14.3. MEDIA USED

To simulate the natural environment when all the microbial population interact, flesh media in the form of broth (Sec.2.12.2) was used. The flesh broth was prepared in large quantities, autoclaved and stored in a refrigerator until used.

2.14.4. INOCULATION AND INCUBATION

Different combinations of the bacterial isolates with various concentrations of cells were worked out as below.

Batch	Culture combinations
I	*Vibrio* sp. R42+*Pseudomonas* sp. R8.
II	*Vibrio* sp. L146+*Pseudomonas* sp. L97.
III	*Vibrio* sp. L146+*Acinetobacter* sp. L114.
IV	*Pseudomonas* sp. L97+*Acinetobacter* sp. L114.
V	*Vibrio* sp. L146+*Pseudomonas* sp. L97+ *Acinetobacter* sp. L114

For each combination, 1 ml of inoculum of each culture at various concentrations (Sec.2.14.2) was added to 100 ml of the flesh broth. They were incubated at ($28\pm2^{o}C$) for a period of 48 h.

2.14.5. RECOVERY OF BACTERIA

After 24 h of incubation, the broth cultures were tested for the presence of inoculated populations. 1 ml of the flesh broth culture was serially diluted with sterile 1% saline and plated on ZoBell's 2216 e agar. They were incubated at $28\pm2^{o}C$ for 5 days. All colonies were picked from plates for each combination, checked for their identity and percentage recovery of each strain was calculated.

2.15. STATISTICAL ANALYSIS

2.15.1. VARIABLES

The variables used for the statistical analysis

include four different categories as follows.

1. Spoilage Indices

   a) THB b) TMA c) NH_3 d) pH

2. Proximate components

   a) Protein b) Carbohydrate c] Lipid

   d) Ash and e) Moisture

3. Spoilage flora

   a) Vibrio b) Pseudomonas c) Acinetobacter and d) Micrococcus

4. Hydrolytic enzyme producing bacteria

   a) Proteolytic b) Lipolytic c) Amylolytic and d) Ureolytic

2.15.2. CORRELATION COEFFICIENT ANALYSIS

To test the numerical differences of the above said variables noted, were significant or not, a Pearson correlation coefficient 'r' was calculated using the formula

$$\gamma = \frac{n\Sigma XY - (\Sigma X)(\Sigma Y)}{\sqrt{[n\Sigma X^2 - (\Sigma X)^2][n\Sigma Y^2 - (\Sigma Y)^2]}}$$

Calculations were done using the Micro-Computer BDP - 100 (ECIL of India) and the final outputs are presented in the form of tables.

3. RESULTS

3.1. STORAGE CHARACTERISTICS OF PRAWN

3.1.1. ORGANOLEPTIC ASSESSMENT

Organoleptic assessment is purely a subjective measurement of spoilage index. Although there exists many chemical estimations along with bacteriological analysis, organoleptic assessment is the prime measure of spoilage giving readily the state of spoilage. This assessment is made on the basis of general appearance of the sample, its colour, texture and nature of odour present.

Organoleptic assessment made on the fresh prawns revealed the presence of fresh appearance with characteristic prawn odour, slightly whitish in colour, eyes, gills and shell normal. But during spoilage, as a result of action of enzymes of both flesh and bacteria, the biochemical components of the prawn undergo deterioration which yield characteristic putrid spoilage odour.

Samples stored at room temperature:

During storage of the prawns at room temperature ($28\pm2^{o}C$) all the four types of samples viz. Whole, Headless, PUD and PD spoiled completely within 8 h of storage. As it can be seen from Table 4 spoilage started in the head and

rapidly spreaded to other regions in 'Whole' sample. In other types of samples the spoilage started directly in the flesh. During spoilage blackening or melanosis was observed first on the head and later in the flesh in 'Whole' samples. It was also observed in the flesh of 'Headless and Peeled' types. Further, rapid multiplication of bacteria resulted in the production of slime as thick yellow slimy layer over the body surface which marked progressive spoilage. Due to bacterial attack all the samples became red in colour. At the advanced stage of deterioration the shell became thinner and flesh turned into juice. Freshness of the samples did not remain after 4 h of storage at this temperature.

Samples stored at $4^{o}C$:

Organoleptic assessment made on the samples stored at $4^{o}C$ indicated that in unprocessed condition all the four types of prawns showed spoilage characters after 2 days of storage (Table 5). Later on the spoilage characters were prominent and progressed steadily. By the 5th day of storage they were found to be partly spoiled. However by 10-15 days time they got completely spoiled and became waste. Removal of head and shell to some extent, kept the shelf life of the samples longer. However the results suggest that they cannot be used for consumption after a period of 5 days when stored

at 4°C. Pattern of spoilage did not show any marked difference from that observed at room temperature.

Samples stored at -18°C:

Organoleptic assessment of the various samples stored at -18°C suggest that the pattern of spoilage observed is more or less similar to that of 4°C and 28±2°C (Table 6). But it was observed that the shelf life and quality of raw prawns remained fair until a period of a month. Later progressive spoilage set in and by 60 days they got partially spoiled. By 100 days of storage complete spoilage of the prawns was recorded with intense off odours. The results showed that the method of sample treatment for processing did not show any direct influence on the retardation of spoilage of prawn.

3.1.2. CHANGES IN THE TOTAL HETEROTROPHIC BACTERIAL POPULATION (THB) OF *PENAEUS INDICUS* DURING STORAGE AT VARIOUS TEMPERATURES

Whole:

It could be seen from the Fig.4 a.that THB of Whole samples increased significantly during storage at 28±2°C. Steady and gradual increase was observed till 8 h when a maximum was recorded (Log. 8.74). Later at 12 h a sudden decline was recorded (Log. 8.02). However, once again

an increase in THB was observed at 24 h. The final level of THB observed at 28±2°C was comparatively higher than that of 4°C and -18°C.

THB increased significantly during storage at 4°C. The increase was gradual till 5 days and then a rapid increase was noticed recording a very high population (Log. 9.16) on the 10th day. This was the maximum THB recorded for the sample during storage at various temperatures. However, on the 15th day, the population was found to be decreased (Log. 8.32). This final THB was comparatively lesser than that of 28±2°C and higher than that of -18°C.

Although there was a significant increase in the THB of the samples stored at -18°C, a regular pattern of increase was not observed. Increase and decrease in population were recorded till 60th day when the population reached a minimum (Log. 6.90). However, a sudden increase in THB was observed on the 210th day (Log. 8.19) recording the maximum level. Comparatively the THB was very low than that of 28±2°C and 4°C.

Headless:

During storage at 28±2°C, Headless samples showed a significant increase in THB (Fig. 4 b.) There was a gradual increase in THB till 4 h. After the sudden increase to a high level at 8 h, THB rapidly declined at 12 h. However

once again a rapid increase, to a maximum, was recorded at 24 h (Log. 8.85). Comparatively the THB observed at 28±2°C was lesser than that of 4°C and higher than that of -18°C.

During storage at 4°C, the THB increased to a highly significant level. An increasing trend was recorded till 10 days (Log. 9.07), while on 15th day a decline in the population was seen. However, again an increase was observed on the 30th day (Log. 9.25) which was the maximum THB level recorded during storage at various temperatures.

The samples stored at -18°C also exhibited a significant increase of THB. Till 21 days of storage the increase was gradual. However, it declined gradually to a very low level on the 60th day (Log. 6.94). But once again, an increase in THB was observed till 210 days, where a maximum THB was registered (Log. 7.87). Comparatively the THB level was very low than that of 4°C and 28±2°C.

Peeled and Undeveined (PUD):

From the Fig. 4 c it could be seen that during storage at 28±2°C the THB increased significantly. There was an increase of population at 4 h which decreased later at 8 h. However, a maximum population level was observed at 12 h (Log. 8.85). Again at 24 h, the THB level reduced significantly.

Comparatively this level of population was lower than that of 4°C and slightly higher than that of -18°C.

The samples stored at 4°C showed a gradual increase in THB initially and later a rapid increase to a maximum level (Log. 9.31) on the 10th day of storage. This population was at the highest level observed for the samples stored at various temperatures. However later, a gradual decrease was observed in THB, till 30 days (Log. 8.52). This level was comparatively higher than that of 28±2°C and -18°C.

A significant increase of THB was observed in the samples stored at -18°C. During the initial days of storage till 100 days, there was not any appreciable change in the THB level. Fluctuations in THB level was observed. However, after 60 days THB level increased steadily and a maximum THB (Log. 8.11) was observed on the 210th day. Comparatively the population level of THB observed at -18°C was lower than that of 4°C and 28±2°C.

Peeled and Deveined:

The data (Fig. 4 d) showed an initial increase of THB at 4 h, during storage at 28±2°C, and a subsequent decline at 8 h. However a gradual increase till 12 h and a sudden increase to a maximum (Log. 9.07) at 24 h, were also observed. Comparatively the THB level observed at 28±2°C was higher than that of 4°C and -18°C.

During storage at 4°C, the THB showed a significant increase in the population level. A sudden increase of THB to a maximum level on the 10th day (Log. 9.14) was recorded for the sample during storage at various temperatures. However unlike that of 28±2°C, there was a decline in the THB later on the 15th day. Once again there was an increase on the 30th day recording a very high THB level (Log. 9.01).

During storage at -18°C, except for two instances on the 21st day and 100th day, there was not appreciable change in THB level. The population in general was considerably low and the final population on the 210th day was very low (Log. 7.2).

3.1.3. CHANGES IN THE TRIMETHYLAMINE CONTENT OF VARIOUS PRAWN SAMPLES STORED AT DIFFERENT TEMPERATURES

There was no TMA present in the fresh flesh samples of all the four types of prawns. However, TMA increased steadily during storage at different temperatures in all the four types of samples.

Whole:

Results presented in the Fig. 5 a indicate that TMA increased significantly with the advancement of time during storage at 28±2°C, 4°C and -18°C. The final TMA level

was found to be high in samples stored at 28±2°C (20.45 µg/g) followed by that of -18°C (16.65 µg/g) and 4°C (12.59 µg/g).

When samples were stored at 28±2°C a rapid increase in TMA content was noticed till 4 h and its accumulation slowed down till 8 h (Fig. 5 a). Subsequently, there was a rapid increase almost twice that of 8 h (6.17 µg/g), at 12 h (15.60 µg/g) and by 24 h a maximum (20.45 µg/g) was recorded.

At 4°C, unlike that of 28±2°C, there was steady increase in TMA almost of linear fashion, till 10 days (11.76 µg/g) of storage. There was a slight increase on 15th day (12.59 µg/g). However, the final TMA level was comparatively very low than that of 28±2°C and -18°C.

Similar to 28±2°C, at -18°C after an initial lag, the increase of TMA was gradual and steady till 100 days and later the accumulation of TMA was slow. However additional storage till 210 days showed a significant increase. The final TMA level (16.65 µg/g) was comparatively higher than that of 4°C and lesser than that of 28±2°C.

Headless:

From the Fig. 5 b it could be seen that TMA content of the samples increased with advancement of time during

storage at different temperatures. Interestingly it was observed that the TMA level was almost same, with very little variation at 4° (16.91 µg/g) and at -18°C (16.66 µg/g).

During storage at 28±2°C, unlike that of 'Whole' samples, there was a steady increase in TMA level till 12 h (17.13 µg/g). Later the TMA increased slowly and reached a maximum of 19.06 µg/g at 24 h.

At 4°C, similar to 'Whole' samples, TMA content increased significantly showing a linear pattern of increase till 10 days (9.94 µg/g). However the later increase in TMA was only gradual and recorded a maximum of 16.91 µg/g on the 30th day.

TMA content increased slowly and steadily during storage at -18°C. The increase of TMA was gradual reaching a maximum of 16.66 µg/g on the 210th day which was lesser than that of 28±2°C and 4°C.

Peeled and Undeveined:

During storage at different temperatures TMA content was observed to increase rapidly with advancement of time. PUD samples recorded very high levels of TMA (Fig. 5 c) when compared with other samples at all storage temperatures.

TMA content was high (20.87 µg/g) at 28±2°C and 20.59 µg/g and 16.66 µg/g at 4 and -18°C respectively.

At 28±2°C an initial gradual increase at 4 h (2.06 µg/g) followed by a sudden increase in TMA, almost of seven times at 8 h (14.79 µg/g) was noticed. The rapid increase in TMA continued till 12 h (20.58 µg/g) and the concentration remained at the same level.

TMA content of the samples increased rapidly till 15 days of storage at 4°C (14.76 µg/g) and a further increase in TMA was recorded till 30 days (20.59 µg/g) of storage. The final TMA level was slightly lower than that of 28±2°C and higher than that of -18°C.

At -18°C, TMA content of the samples increased gradually till 21 days (2.79 µg/g). Later there was a rapid and steady increase of TMA till 100 days when it recorded almost four times of the TMA level of that observed at 21 days. This trend followed till 210 days and the TMA increased gradually and reached a maximum level of 16.66 µg/g.

Peeled and Deveined

TMA increased rapidly with the advancement of time in these samples during storage at different temperatures (Fig. 5 d). Comparatively there was a rapid and sudden increase of TMA in the early hours at 28±2°C besides

recording a maximum TMA (20.80 µg/g) at this temperature than that of other storage temperatures.

During storage at 28±2°C, the TMA recorded a sudden increase at 4 h (7.6 µg/g) and doubled at 8 h (16.38 µg/g). Later the rapid increase of TMA continued till 12 h (20.74 µg/g) and was constant till 24 h (20.80 µg/g). The final TMA level was comparatively higher than that of 4°C (19.89 µg/g) and -18°C (16.65 µg/g).

At 4°C, the TMA content in the samples increased significantly till 15 days of storage (16.42 µg/g). Subsequently the increase of TMA was gradual till 30 days where it recorded 19.89 µg/g.

Similar to other samples at -18°C, TMA content also showed gradual increase till 34 days (4.02 µg/g) and rapidly till 100 days (13.43 µg/g). Later the increase in TMA was gradual till 210 days (16.65 µg/g). The final value of TMA content was comparatively less than that of 28±2°C and 4°C.

The amount of TMA produced and the pattern of increase in Whole and Headless samples stored at various temperature was found to be apparently same. The samples PUD and PD also showed a similarity in the level and pattern of increase of TMA under stored conditions.

3.1.4. CHANGES IN THE AMMONIA CONTENT OF THE VARIOUS PRAWN SAMPLES STORED AT DIFFERENT TEMPERATURES

The changes in the ammonia content of various samples stored at different temperatures are given in Fig. 6. The data show that the samples on fresh condition was free of ammonia. However during storage at different temperatures, ammonia increased rapidly in all the four types of samples at all storage temperatures viz. $28\pm2^{o}C$, $4^{o}C$ and $-18^{o}C$. Very high level of ammonia was recorded in all the samples at $4^{o}C$ than at $28\pm2^{o}C$ and $-18^{o}C$.

Whole:

Results presented in Fig. 6 a indicate that ammonia content in the samples increased rapidly and significantly during storage at three temperatures.

During storage at $28\pm2^{o}C$, a rapid and sudden increase of ammonia was observed at 4 h (232.40 µg/g). Later, the increase of ammonia content was rather gradual and steady till 24 h (296.80 µg/g).

At $4^{o}C$, throughout the storage period, ammonia content increased rapidly and reached the maximum level (547.60 µg/g) on the 15th day.

Ammonia content of the samples stored at $-18^{o}C$ increased rapidly by 34 days (215.90 µg/g). Later there was apparently

no significant increase in ammonia till 100 days (223.50 µg/g). However subsequently a slight increase (249.10 µg/g) in ammonia was observed on 210th day.

Headless:

From the Fig. 6 b it could be seen that ammonia content in these samples increased rapidly with advancement of time during storage at different temperatures.

During storage at 28±2°C, ammonia content increased rapidly and suddenly by 4 h of storage (214.60 µg/g). However, the increase in ammonia at later period was only gradual till 24 h (287.60 µg/g).

At 4°C, there was a uniform, steady and rapid increase of ammonia till 15 days of storage (536.80 µg/g) recording almost a logrithmic pattern of increase along with increase of time. Later, there was only a slight increase till 30 days (576.80 µg/g) when compared with that of the earlier 15 days. The amount of ammonia measured was the maximum for the three temperatures.

At -18°C, there was a rapid increase of ammonia till 12 days (160.50 µg/g) of storage. Thereafter the increase in ammonia was gradual till 60 days (185.40 µg/g). However, an increase in the ammonia content reaching the maximum (235.90 µg/g) on 210 days of storage was recorded.

Peeled and Undeveined:

Ammonia content in these samples also increased considerably with advancement of time (Fig. 6 c) during storage at different temperatures.

As it was observed with other samples, an initial rapid increase of ammonia was recorded till 4 h (193.50 µg/g) at 28±2°C. Later till 12 h there was a steady increase of ammonia reaching 291.60 µg/g. Subsequently there was only an insignificant increase of ammonia till 24 h (298.40 µg/g).

At 4°C, similar to other samples, rapid and steady increase of ammonia was recorded till 15 days (557.30 µg/g) of storage. The increase of ammonia was in logrithmic proportion. However after 15 days apparently there was not much increase of ammonia (565.40 µg/g) till 30 days.

At -18°C, ammonia increased rapidly till 21 days (160.50 µg/g). Afterwards a slight increase of ammonia content (185.10 µg/g) on the 34th day and a marginal increase till 60 days (185.90 µg/g) was recorded. However later, a gradual increase of ammonia (280.30 µg/g) till 210 days was observed.

Peeled and Deveined:

Results presented in Fig. 6 d indicate that ammonia content in these samples increased significantly during storage at different temperatures.

During storage at 28±2°C ammonia level increased rapidly till 4 h (238.90 µg/g). Later, it increased gradually till 24 h (289.50 µg/g) of storage recording no fluctuation in the level of ammonia.

At 4°C, the increase of ammonia content was rapid and steady till 15 days (521.10 µg/g) of storage as it was observed with other samples and increased gradually till 30 days (571.90 µg/g) of storage.

At -18°C, the increase of ammonia was rapid upto 12 days (121.60 µg/g) and gradual until 34 days (159.20 µg/g). Later there was no significant increase of ammonia content till 60 days. Subsequently the ammonia level increased gradually and reached 271.60 µg/g by 210 days.

Comparatively, the ammonia level in the samples was almost double at 4°C than at 28±2°C and -18°C.

3.1.5. CHANGES IN THE pH OF FLESH OF VARIOUS PRAWN SAMPLES STORED AT DIFFERENT TEMPERATURES

The pH of the flesh was initially pH 6. During storage at various temperatures (28±2°C, 4°C and -18°C), pH of the flesh increased significantly in all the four types of prawns. The increase in pH was comparatively less at 28±2°C in all the samples and ranged between 7.3 - 7.55 at 24 h.

Whereas at 4°C and -18°C it was almost similar in level on the final day of storage (30 and 210 days respectively). The pH occurred in the range of pH 7.8 to 8.55 on the 30th day at 4°C and at pH 8.05 to 8.5 on the 210th day at -18°C.

Whole:

From the Fig. 7 a it could be seen that the increase in pH of flesh was rapid initially and gradual in the later periods of storage in all the three storage temperatures.

During storage at 28±2°C initial pH increased from pH 6 (0 h) to pH 6.8 at 4 h. Later, till 12 h there was only a slight increase in pH (pH 6.9). Thereafter the pH increased significantly recording a maximum of pH 7.55 at 24 h. This final pH was comparatively less than that of 4°C and -18°C.

At 4°C, the pH increased rapidly and suddenly from pH 6 to pH 7.55 on the 2nd day. Later the increase in pH was gradual and steady till 15 days and recorded a pH 8.25. This final pH was comparatively less than that of -18°C (pH 8.5). However the pH recorded in the intermediate period from 2nd day to 10th day was comparatively high than that observed for the samples at 28±2°C and at -18°C.

Similar to that of 4°C and 28±2°C, at -18°C the pH increased rapidly at the initial stages till 4 days (pH 7.05) and marginally till 12 days. Thereafter a gradual and steady increase reaching to the maximum of pH 8.5 by 210 days was observed.

Headless:

From the Fig. 7 b it could be inferred that pH of flesh of these samples increased rapidly during initial period of storage at all the three storage temperatures. Later a gradual increase in pH was observed till the final analysis. A maximum pH (pH 8.55) was observed at 4°C.

During storage at 28±2°C, pH increased rapidly from pH 6.0 (0 h) to pH 6.8 at 4 h and slightly at 8 h (pH 6.85). However, further increase in pH was gradual till 24 h and recorded a pH of 7.45.

At 4°C, the pH increased suddenly to 7.5 on the 2nd day and marginally on the 5th day (pH 7.55). A gradual raise of pH level was observed later till 30 days where a maximum of pH 8.55 was recorded. This final pH 8.55 was the maximum pH observed for the samples during storage at different temperatures.

At -18°C, the pH of flesh showed steady increase in a regular pattern from pH 6.0 (0 h) to pH 7.9 (34th day).

There was only marginal increase in pH till 100 days. However the pH increased gradually later till 210 days where it recorded a pH 8.2.

Peeled and Undeveined:

Results presented in Fig. 7 c indicate that pH increased significantly during storage at different temperatures. As seen in other samples, initial increase of pH was observed at all the three temperatures. Maximum pH was recorded at -18°C.

The samples stored at 28±2°C showed a similar trend of changes occurred in other samples. There was an initial rapid increase in pH from 6 to pH 6.8 at 4 h and a lag in the increase of pH at 8 h. Later the pH increased gradually till 24 h (pH 7.3). This final pH (7.3) was the lowest level of pH recorded during this storage study.

At 4°C, a rapid increase of pH was observed on the 2nd (pH 7.05) and 5th days(pH 7.5). Later, the increase of pH was gradual and steady till 30 days of storage (pH 8.2). This final pH was slightly lesser than that found at -18°C (pH 8.25).

The pH of the samples stored at -18°C increased initially from pH 6.0 (0 h) to pH 6.9 on the 4th day. After

4 days there was only a slight increase on the 12th day (pH 6.95) and there was no change till 21 days (pH 6.95). However, the pH increased rapidly and significantly at later stage till 210 days (pH 8.25) and recorded a maximum pH.

Peeled and Deveined:

From the Fig. 7 d it could be seen that pH of flesh increased rapidly and significantly during storage at different temperatures. Comparatively PD samples recorded low pH levels at all storage temperatures. The pH level was found high at -18°C when compared with that of 4°C and 28±2°C.

During storage at 28±2°C, an increase of pH as seen in other samples was observed. Also, similar to the samples stored at 4°C and -18°C, an initial rapid increase of pH at 4 h (pH 6.0) to pH 6.75) and a slight increase in pH at 8 h (pH 6.8) was noticed. Subsequently the pH increased rapidly once again at 12 h (pH 7.1) and then gradually till 24 h (pH 7.35).

At 4°C, pH increased initially to pH 7.05 from pH 6.0 (0 h) on the 2nd day and there was no significant increase on the 5th day (pH 7.1). Later the pH increased gradually till 15 days (pH 7.6) and very slowly till 30 days (pH 7.8) of storage.

The pH increased rapidly at -18°C. Although such increase was not in a regular pattern the final pH recorded

on the 210th day was the maximum pH (pH 8.05) for these samples under storage conditions.

3.2. PROXIMATE COMPOSITION

3.2.1. CHANGES IN THE PROTEIN CONTENT OF VARIOUS PRAWN SAMPLES STORED AT DIFFERENT TEMPERATURES

Protein content of fresh sample was 20.12%. During storage at different temperatures the protein content of all the samples decreased significantly. The protein content on the final analysis varied from 18.12% to 18.9% at $28\pm2^{o}C$, from 18.51% to 19.1% at $4^{o}C$ and from 18.81% to 19.1% at $-18^{o}C$. Comparatively higher level of reduction was recorded at $28\pm2^{o}C$.

Whole:

Protein content reduced significantly during storage at all temperatures (Fig. 8 a) and the reduction has taken place only during later period of storage.

At $28\pm2^{o}C$, there was no significant reduction in protein till 4 h (20.08%). However, rapid reduction at 12 h (18.88%) followed by a gradual reduction at 24 h (18.12%) was observed. The reduction in protein was high at $28\pm2^{o}C$ than at other temperatures studied for the 'Whole' sample.

At 4°C, a gradual reduction in protein till 10 days of storage (19.9%) and a high level of reduction in protein at a later stage (19.1%) was observed. The high protein content on the final day of analysis indicated a minimum level of reduction in protein at this temperature.

There was no reduction in protein content till 34th day of storage. However during later period of storage, protein reduced considerably and reached to a very low level (18.81%) when compared to other samples at this temperature.

Headless:

Protein content of headless samples decreased during storage at different temperatures (Fig. 8 b). High level of reduction in protein was observed at 4°C than at 28±2°C and -18°C.

During storage at 28±2°C, there was not considerable reduction in protein till 4 h (20.08%). Later the protein level reduced significantly to 18.69% at 24 h.

There was no significant reduction in protein till 5 days (20.06%) at 4°C. Later, a slight decrease (19.93%) on the 10th day and steep fall (18.56%) on the 30th day, in the protein level were recorded. Comparatively, at this temperature the protein content showed higher level of reduction than at 28±2°C and -18°C.

At -18°C, there was no change initially in protein level till 21 days. Subsequently it showed gradual reduction till 60 days (20.03%). Later there was a rapid and sudden decrease till 100 days (19.23%) of storage followed by a slight reduction on 210 days of storage (19.10%). At -18°C comparatively there was less reduction of protein in this sample.

Peeled and Undeveined:

Results indicate that (Fig. 8 c) protein content of PUD samples had undergone significant reduction during storage at different temperatures. Higher level of reduction in protein was observed at 4°C.

During storage at 28±2°C, there was not much reduction in protein till 4 h (20.09%). A rapid fall (19.45%) at 12 h and later a gradual decrease in protein reaching to 18.67% at 24 h was observed.

At 4°C, reduction in protein content was only marginal (20.03%) till 2 days, whereas, it was rapid till (19.26% 5 days). Subsequent storage for 30 days showed only a gradual reduction in protein (18.51%).

Similar to other samples, at -18°C, in PUD there was no change in the protein content till 34 days. Protein content

later came down to 18.87% on 210th day of storage. However, the reduction was not gradual during the period of storage.

Peeled and Deveined:

From the Fig. 8 d it could be seen that protein content of these samples got reduced significantly during storage at different temperatures. A higher level of reduction in protein was observed at 4°C.

During storage at 28±2°C, there was a slight reduction in protein content till 8 h (20.03%). However, a decrease in protein level to 18.9% was recorded at 24 h.

At 4°C, there was only a slight decrease in protein level initially (20.01% on the 5th day). Thereafter, a rapid reduction in protein was observed till 15 days (18.82%). However, the reduction in protein was less at later period (18.75%).

As seen in other samples,at -18°C, no initial change in the protein content oould be observed. The protein content reduced down to 18.97% on 210th days of storage. During storage period the reduction was either rapid or gradual.

Comparatively PD samples recorded less reduction in protein during storage at different temperatures than other samples.

3.2.2. CHANGES IN THE CARBOHYDRATE CONTENT OF VARIOUS PRAWN SAMPLES STORED AT DIFFERENT TEMPERATURES

The carbohydrate content of fresh sample was found to constitute 0.561%. However, during storage at different temperatures viz. 28±2°C, 4°C and -18°C, a significant reduction in carbohydrate content was recorded at all the three storage temperatures. Comparatively, the reduction in carbohydrate level was high at 4°C. The carbohydrate content of the samples varied from 0.341% to 0.390% at 28±2°C, from 0.290% to 0.377% at 4°C and from 0.466% to 0.505% at -18°C.

Whole:

Results indicate (Fig. 9 a) that carbohydrate content of these samples was considerably reduced during storage at 28±2°C, 4°C and -18°C. However the reduction was high at 28±2°C and at 4°C.

During storage at 28±2°C, there was no significant reduction in the carbohydrate content till 12 h (0.547%), whereas, after 12 h, there was a rapid reduction in carbohydrate level at 24 h (0.376%).

At 4°C, the carbohydrate content reduced gradually till 10 days (0.504%) and then suddenly to a level of 0.354% on the 15th day indicating a high reduction in carbohydrate content.

Initially there was only a slight reduction in carbohydrate level in the samples stored at -18°C till 12 days (0.555%). Later a gradual reduction in carbohydrate level was observed till 60 days (0.519%) of storage. Whereas after 60 days there was a rapid reduction till 100 days (0.472%) and then a low level of reduction till 210 days (0.466%). Comparatively a higher level of carbohydrate was observed on 210th day of analysis, when compared with that of other storage temperatures.

Headless:

Carbohydrate content reduced significantly in these samples during storage at different temperatures. Comparatively the reduction in carbohydrate was high at 4°C and at 28±2°C than at -18°C where there was only a slight reduction in carbohydrate level (Fig. 9 b).

During storage at 28±2°C, initially there was no significant reduction in carbohydrate till 4 h (0.557%). However, later there was a gradual reduction in carbohydrate till 24 h (0.359%).

At 4°C, there was no significant reduction in carbohydrate, initially, till 5 days (0.556%) of storage. Whereas, after 5 days reduction in carbohydrate was rapid till 10 days (0.416%) and gradual till 30 days (0.311%). The

The reduction in carbohydrate level was high at this temperature than that of other temperatures.

At -18°C, similar to that in other samples there was not much reduction in carbohydrate level till 4 days (0.559%). However there was gradual reduction in carbohydrate level from 4th day onwards till 210th day (0.505%). Comparatively the level of reduction was very low.

Peeled and Undeveined

As observed in other samples, PUD samples also showed a significant reduction in carbohydrate (Fig. 9 c) during storage at different temperatures. Comparatively the reduction in carbohydrate was high in these samples at 28±2°C than at 4°C and -18°C.

During storage at 28±2°C there was no significant reduction till 4 h (0.559%). However, later there was a rapid reduction in carbohydrate till 24 h (0.341%) recording a very low level of carbohydrate at 24 h.

At 4°C, there was not much reduction in carbohydrate content till 5 days (0.554%). Whereas, after 5 days there was a rapid reduction in carbohydrate level till 30 days (0.377%) recording significant reduction in carbohydrate during storage.

At -18°C, similar to that of other type of samples, there was only a slight reduction till 12 days (0.558%).

Later, there was a considerable reduction in carbohydrate till 210 days (0.501%).

Peeled and Deveined:

As it was observed in other types of samples, significant reduction in carbohydrate was observed in PD samples (Fig. 9 d) during storage at different temperatures. A high reduction in carbohydrate level was observed at $4^{o}C$ than at $28\pm2^{o}C$ and $-18^{o}C$.

During storage at $28\pm2^{o}C$, initially there was a slight reduction in carbohydrate level till 4 h (0.555%). Later, the carbohydrate content got reduced rapidly to 0.413% by 8 h. After 8 h the carbohydrate level got reduced slowly till 24 h (0.390%) and recorded significant reduction in carbohydrate content.

At $4^{o}C$, there was no significant reduction in carbohydrate till 5 days (0.559%). However, after 5 days reduction in carbohydrate level was rapid till 15 days (0.353%) and was gradual till 30 days (0.290%). A significant level of high reduction in carbohydrate was observed at this temperature. This amount of reduction in carbohydrate was not recorded in any other sample and at any other temperature during the storage study.

At -18°C, very much similar to that observed in other samples, initially there was not much reduction in carbohydrate and the carbohydrate level decreased slowly till 21 days (0.551%). Later there was a gradual reduction in carbohydrate level till 210 days (0.503%) of storage. However a significant reduction in carbohydrate level was recorded.

3.2.3. CHANGES IN THE LIPID CONTENT OF VARIOUS PRAWN SAMPLES STORED AT DIFFERENT TEMPERATURES

The fresh flesh contained 1.1% of lipid. During storage at 28±2°C, 4°C and -18°C, the lipid content reduced from initial level in all the four types of samples. In spoiled samples, the final lipid content of the samples varied from 0.75% to 0.95% at 28±2°C, from 0.75% to 0.85% at 4°C and from 0.65% to 0.85% at -18°C.

Whole:

Results presented in Fig. 10 a indicate that lipid content of 'Whole' samples got reduced during storage at different temperatures. Interestingly, it could be observed that the level of reduction in lipid at the three different temperature was same (0.85%).

During storage at 28±2°C, the reduction in lipid was high till 4 h (1%) and gradual till 8 h (0.95%). However, a rapid reduction was observed later till 12 h (0.85%).

Interestingly there was no further reduction in lipid after 12 h till 24 h (0.85%).

There was a rapid decrease in lipid content till 2 days of storage (1%) at 4°C. Later, the reduction in lipid was gradual till 10 days (0.95%) followed by a rapid reduction in lipid till 15 days (0.85%).

At -18°C, a rapid decrease in lipid level till 4 days (1.05%) could be noticed. Later till 34 days (0.95%) there was a steady decline in lipid level. However after 34 days the reduction in lipid level was low and gradual till 210 days (0.85%).

Headless:

From the Fig. 10 b,it could be seen that lipid content of these samples got reduced significantly during storage at different temperatures. The reduction in lipid at 28±2°C was comparatively less than that of 4°C and -18°C, where at both the temperatures similar level of reduction in lipid was observed.

During storage at 28±2°C, an uniform gradual reduction in lipid was observed till 12 h (0.95%). Further there was no reduction in lipid level.

At 4°C, similar to that of 28±2°C, a gradual and steady decline in lipid level was observed till 10 days (0.85%).

Later, the reduction in lipid level was observed to be slow and at the minimum till 30 days (0.75%). Comparatively a high reduction of lipid was observed at this temperature.

At -18°C, lipid content was found to be reduced rapidly till 34 days (0.88%), and gradually till 210 days (0.75%) of storage recording a low level of lipid.

Peeled and Undeveined:

Results presented in Fig. 10 c indicate that lipid content of these samples got considerably reduced during storage at different temperatures. In general a high level of reduction in lipid was observed at 28±2°C, 4°C and -18°C.

During storage at 28±2°C, a sudden and rapid reduction in lipid was noticed till 8 h (0.8%) of storage. Later, the reduction in lipid was gradual till 12 h (0.75%) and thereafter, there was no further reduction in lipid.

At 4°C, the level of reduction of lipid was gradual and reached to 0.8%. The value when compared to the values obtained for PUD in other temperatures was high.

At -18°C, initially there was also a rapid decline in lipid level till 21 days (0.92%). Later, the reduction in lipid was steady and gradual till 210 days (0.75%) of storage.

Peeled and Deveined

Lipid content of these samples was found to be reduced to the maximum level (Fig. 10 d) during storage at different temperatures. Comparatively a high reduction in lipid was observed at -18°C.

During storage at 28±2°C, the lipid content reduced steadily and significantly throughout the period of storage. However a higher level of lipid (0.78%) was observed finally at this temperature than that of 4°C and -18°C.

At 4°C, a decline in lipid level was observed till 2 days (1%) as it was in other samples. Later the reduction in lipid level was steady and gradual till 30 days of storage (0.75%).

At -18°C, the lipid level was found to be decreased steadily and rapidly throughout the period of storage without any marked fluctuation. A high reduction in lipid was observed on the 210th day (0.65%). The reduction was high indicating a higher reduction in lipid at -18°C than other temperatures.

3.2.4. CHANGES IN THE ASH CONTENT OF VARIOUS PRAWN SAMPLES STORED AT DIFFERENT TEMPERATURES

Ash content of the fresh samples recorded 1.3%. However during storage at different temperatures, the ash content of all the four type of samples showed significant reduction. Uniformly,

similar level of reduction in ash content was observed in all the types of samples and at all the three storage temperatures. The final ash content of the samples varied from 0.70% to 1.09% at 28±2°C, from 0.86% to 1.12% at 4°C and from 1% to 1.04% at -18°C. Comparatively a higher level of reduction in ash content was observed at 28±2°C.

Whole:

Results presented in Fig. 11 a.indicate that ash content significantly got reduced in 'Whole' type of samples during storage at 28±2°C, 4°C and -18°C. Comparatively the reduction was very high at 28±2°C.

During storage at 28±2°C, initially there was only a slight reduction in ash content till 4 h (1.19%). However, after 4 h the reduction in ash content was rapid till 24 h (0.7%). About 50% of ash content was reduced at 24 h recording a maximum reduction in ash content.

At 4°C, the reduction in ash content was gradual and significant throughout the period of storage till 15 days (1.03%).

At -18°C, very similar to that of 4°C, a reduction in ash content was observed to be low till 60 days (1.08%). The reduction in ash content was not so significant later, till 210 days (1.02%) when compared to that of the early 60 days of storage.

Headless:

From the Fig. 11 b,it could be seen that ash content got reduced during storage at different temperatures. Unlike in 'Whole' samples, a higher level of reduction in ash content was recorded at 4°C and the level of reduction was same at 28±2°C and -18°C.

During storage at 28±2°C, the ash content reduced gradually till 8 h (1.21%). Later the ash content reduced rapidly till 12 h (1.07%) and marginally till 24 h (1.02%).

At 4°C, a steady and significant reduction in ash content was observed till 10 days (1.10%). Later, the reduction in ash content was rapid till 15 days (0.9%). After 15 days, reduction in the ash content was only marginal (0.86%) till 30 days. However, a high level of reduction in ash content was observed at this temperature.

At -18°C, there was a gradual reduction in ash content initially till 34 days (1.1%). During the subsequent period the ash content got reduced slowly recording only meagre reduction in ash content in the later stages of storage till 210 days (1.02%).

Peeled and Undeveined

A significant reduction in ash content in these samples was observed during storage (Fig. 11 c) at all the three temperatures. However unlike that of

'Whole' and 'Headless' samples a high level of reduction in ash was recorded at -18°C.

During storage at 28±2°C, a significant reduction in ash content was observed till 4 h (1.2%). After 4 h a gradual reduction in ash content was observed till 24 h (1.09%).

At 4°C, a gradual and significant reduction in ash content was recorded till 15 days (1.1%). However later, ash content showed a minimum reduction in level till 30 days (1.02%).

Initially a rapid reduction in ash content was recorded till 12 days at -18°C (1.16%). However in the later period the reduction in ash content was gradual till 34 days (1.13%) and subsequently slow till 210 days (1%). However, a high level of reduction in ash content was recorded at this temperature.

Peeled and Deveined:

Ash content in PD samples showed significant reduction (Fig. 11 d) during storage at 28±2°C, 4°C and -18°C. However, the level of reduction was comparatively more at 28±2°C and -18°C than at 4°C where it was less.

During storage at 28±2°C, ash content reduced gradually till 8 h (1.2%) and rapidly till 12 h (1.1%). However again,

a gradual reduction in ash content was observed till 24 h (1.01%). Comparatively a high level of reduction in ash was observed at this temperature.

At 4°C, interestingly, the ash content showed an uniform and gradual reduction throughout the period of storage till 30 days (1.12%) of storage.

Initially, a gradual reduction in ash content of the samples stored at -18°C was observed till 34 days (1.12%). Later it was reduced to a minimum by 210 days (1.04%) recording a significant reduction during storage.

3.2.5. CHANGES IN THE MOISTURE CONTENT OF VARIOUS PRAWN SAMPLES STORED AT DIFFERENT TEMPERATURES

Moisture content was found to constitute 76.93% in the fresh samples. During storage at different temperatures, an increase in moisture content with advancement of time was observed in all the four types of samples. The increase in moisture content was almost similar in all the types of samples and at all the three storage temperatures. The final moisture content varied from 78.62% to 79.93% at 28±2°C, from 78.82% to 79.12% at 4°C and from 78.64% to 78.96% at -18°C.

Whole:

Results indicate (Fig. 12 a) that moisture content of these samples increased significantly with advancement

of time during storage at 28±2°C, 4°C and -18°C. Comparatively the increase in moisture content was high at 28±2°C than at 4°C and -18°C.

During storage at 28±2°C, initially there was a rapid increase in moisture content till 4 h (77.44%). Whereas after 4 h the increase in moisture content was only marginal till 8 h (77.76%). Subsequently there was a sudden increase in moisture content till 12 h (78.94%) which reached a maximum level (79.93%) by 24 h. For this sample, this was the maximum moisture level observed during storage at various temperatures.

At 4°C, the moisture content increased steadily and significantly till 5 days (77.54%). Later, increase in moisture content was marginal till 10 days (77.74%) and was rapid after 10 days till 15 days (78.93%) recording a significant increase in moisture level.

At -18°C, it was interesting to observe that moisture content increased regularly and gradually till 100 days (78.51%). However the increase in moisture content was insignificant after 100 days till 210 days (78.90%).

Headless:

In 'Headless' prawns,moisture content increased significantly during storage at different temperatures (Fig. 12 b). Comparatively the increase in moisture content was high at 4°C than at 28±2°C and at -18°C.

During storage at 28±2°C, moisture content raised markedly till 4 h (77.44%) and insignificantly till 8 h (77.50%). However, after 8 h, there was a rapid increase till 12 h (78.21%) followed by a gradual increase in moisture level till 24 h (78.62%).

At 4°C there was not significant increase in moisture as it was with other samples stored at this temperature during the initial period. After 2 days the moisture content increased significantly during storage and recorded a very high moisture level (78.82% on the 30th day).

When stored at -18°C, there was not significant increase in moisture content till 12 days (77.02%). Later, after recording a gradual increase till 34 days (77.32%), the moisture content showed a steady increase with advancement of time till 210 days (78.64%).

Peeled and Undeveined:

Moisture content in the PUD samples showed significant increase (Fig. 12 c) during storage at different temperatures. Comparatively a high level of moisture and an increase of moisture to the maximum was observed at 4°C than at 28±2°C and -18°C.

During storage at 28±2°C, significant increase in moisture till 4 h (77.14%) was not observed. Later moisture increased steeply till 12 h (78.2%) and gradually till 24 h

(78.97%) recording a significant level of moisture content.

At 4°C, interestingly, unlike 'Whole' and 'Headless' samples, the moisture content showed rapid increase till 10 days (78.43%) with a drop on 5th day. However later a gradual increase in moisture was observed till 30 days (79.12%) which was the maximum moisture level recorded for the sample.

The moisture content increased gradually till 34 days (77.27%) at -18°C and recorded significant increase in moisture in the subsequent period till 60 days (77.32%). Later, moisture content showed a steady and steep increase till 210 days (78.96%).

Peeled and Deveined:

Results indicate (Fig. 12 d) that moisture content of the samples increased significantly during storage at different temperatures. It was interesting to note that there was not much variation in the moisture level during the time of maximum spoilage of all the samples at all the three different temperatures. However the increase in moisture content was comparatively higher at 28±2°C and low at -18°C.

During storage at 28±2°C, there was not significant change in the moisture level till 4 h (77.10%). However after 4 h the moisture increased steadily and steeply till

12 h (78.63%) of storage and showed not much change in the moisture level later till 24 h (78.92%).

At 4°C, similar to that of PUD initially there was a rapid increase in moisture level till 2 days of storage (77.53%). Later only a gradual increase in moisture was observed till 10 days (77.73%). However after 10 days there was a sudden increase in moisture level till 15 days (78.62%) and later the increase in moisture was once again gradual till 30 days (78.90%).

There was not much appreciable change in moisture level till 21 days (77.08%) at -18°C. However after 21 days there was a steady increase in moisture till 34 days (77.19%) followed by a gradual slight increase till 60 days (77.32%) of storage. But interestingly the moisture level increased rapidly till 100 days (78.46%) and then gradually increased to 78.80% till 210 days.

3.3. GENERIC COMPOSITION OF TOTAL HETEROTROPHIC BACTERIA (THB) PRESENT DURING PRAWN SPOILAGE

3.3.1. Fresh prawns:

Bacterial flora of freshly caught prawns were identified to various genera and was found to show maximum generic diversity. About eight generic groups represented by species of *Vibrio*, *Pseudomonas*, *Acinetobacter*, *Moraxella*,

members of Enterobacteriaceae, *Micrococcus*, *Bacillus* and *Corynebacterium* were found. Of all the groups, species of *Vibrio* was found to be the dominant one (30.8%).followed by species of *Pseudomonas* (25.6%). All the other groups were represented by a minimum percentage of 8%. Gram-negative bacteria were more (77.2%) than Gram-positive bacteria.

3.3.2. Samples stored at 28±2°C:

During storage (24 h) at 28±2°C the diversity of bacterial genera was found to be reduced in prawns (Fig.13). However in both 'PUD' and 'PD' samples, reduction of generic diversity was higher than 'Whole' and 'Headless' samples. 'PD' samples showed maximum reduction in generic diversity at 4 h, 8 h and 12 h. 'PUD' samples showed maximum reduction in species diversity at 8 h and 12 h. 'Headless' showed maximum reduction in diversity at 12 h, when compared with other samples, 'Whole' samples showed maximum generic diversity at 4 h and 24 h with minimal reduction in the generic diversity observed for fresh prawns.

The data show that species of *Micrococcus*, *Bacillus* and *Corynebacterium* (Gram-positive bacteria) and *Moraxella Flavobacterium*-*Cytophaga* (Gram-negative bacteria) were eliminated during storage in all the four types of samples. Only *Vibrio* was found to be present in all the samples during the entire period of storage. Although *Pseudomonas*, members of Enterobacteriaceae and *Acinetobacter* were encountered

during storage, they occurred sporadically. While Acinetobacter could be recovered on few occasions in 'Whole and 'Headless' samples, Aeromonas was found to be present only on one occasion (24 h) in 'Whole' sample. Of all the groups, Vibrio formed the dominant group in all the types of samples.

Whole:

In the 'Whole' samples stored at 28±2°C as many as five genera of bacteria could be recorded. They were Vibrio, Aeromonas, Pseudomonas, Acinetobacter and members of Enterobacteriaceae. Vibrio was found to be the most dominant group throughout the storage period. However the maximum level (97.1%) was registered at 8h. Pseudomonas was found to be the next common group and it varied widely from 1.8% to 30.5%. Interestingly the level of Pseudomonas increased suddenly from 2.86% at 8 h to 27.7% at 12 h. The recovery of other genera such as members of Enterobacteriaceae, Aeromonas and Acinetobacter was erratic. They constituted the less dominant group. Gram-positive bacteria were not encountered throughout the storage period.

Headless:

Vibrio, Pseudomonas, Acinetobacter and members of Enterobacteriaceae were recorded in these samples during

storage. As in 'Whole' samples, Vibrio was the dominant group in these samples also during storage and was consistently recorded throughout the analyses. However the maximum level constituting as much as 100% was recorded at 12 h. Eventhough Pseudomonas formed the next dominant group (50%) at 4 h it was not recorded subsequently. Acinetobacter constituted very less population till 8 h and later they were not recorded. Members of Enterobacteriaceae were recorded at 8 h (25%) and at 24 h in minimal level (1.6%). Other groups were not recovered in these samples.

Peeled and Undeveined:

Vibrio, Pseudomonas and members of Enterobacteriaceae were present in these samples during storage at 28±2°C. Among them only Vibrio could be recorded throughout the storage period in maximal levels. Vibrio formed almost the entire flora throughout the storage period although members of Enterobacteriaceae and Pseudomonas were also present in less numbers only at 4 h and at 12 h respectively.

Peeled and Deveined:

Species of Vibrio and members of Enterobacteriaceae were recorded in these samples during storage at 28±2°C. As in other samples, Vibrio was found to be the dominant flora and was recovered constantly. Interestingly, Vibrio was found to be the only group forming the entire flora

during storage till 12 h. Although members of Enterobacteriaceae were also present at 24 h, they were in negligible percentage (4.6%).

3.3.3. Samples stored at 4°C:

The generic diversity observed in the initial flora was found to be considerably reduced during storage at 4°C (Fig.14). However the reduction was not so high as it was observed in 28±2°C. Among the samples, 'Whole' showed maximum generic diversity during the storage period and very high generic diversity was observed from 10th day onwards. Both in 'Headless' and 'PUD', generic diversity widely varied during storage. However a maximum generic diversity was observed in 'Headless' on the 5th and 30th day and very low diversity on the 10th day. In 'PUD' samples maximum generic diversity was recorded on the 2nd and 30th day and very low diversity on the 5th day. When compared with other samples, 'PD' samples showed very low species diversity. However, the maximum of generic diversity was observed on the 5th day in the 'PD' samples and very low diversity on the 15th day.

Almost all the genera found initially, when the samples were fresh, were found during storage, unlike that observed at 28±2°C. However, Alcaligenes which was not recorded in the initial flora was recorded in the 'Whole' and 'Headless' samples during storage. Further, members of

Enterobacteriaceae and Corynebacterium were not recorded in 'PD' samples during storage. Pseudomonas was consistently recorded throughout the storage period in all the samples as a dominant group constituting maximum population. All the other groups showed wide fluctuations in their occurrence.

Whole:

Eight genera could be recorded in these samples during storage at 4°C. They were Vibrio, Pseudomonas, Alcaligenes, Acinetobacter, members of Enterobacteriaceae, Micrococcus, Bacillus and Corynebacterium. Pseudomonas was found to be the dominant group throughout the storage period. However maximum level (68.4%) of this group was recorded on the 10th day. Acinetobacter and Micrococcus, which were also recorded throughout the storage period showed wide fluctuations. Acinetobacter was found to vary from 1.3% (10th day) to 26.9% (15th day). Micrococcus was found to vary from 1.8% (2nd day) to 32.1% (5th day). Interestingly their population increased on the 15th day (15.1%) after recording only 7.6%) on the 10th day. All the other genera were recorded only at very few occasions during storage. Vibrio was recorded at a later period on 10th day, when it formed the most common group (20.3%) next to Pseudomonas. They occurred in very low percentage on 30th day. Corynebacterium which was recorded till 10th day formed the next predominant group (17.9%) on the 2nd day of storage. Other genera such as

Alcaligenes, Bacillus and members of Enterobacteriaceae constituted the less dominant groups.

Headless:

As many as eight genera were recorded as in 'Whole' samples during the storage of these samples at 4°C. They were Vibrio, Pseudomonas, Alcaligenes, Acinetobacter, members of Enterobacteriaceae, Micrococcus, Bacillus and Corynebacterium. Among these, Pseudomonas was found to be the dominant group as in 'Whole' samples, throughout the period of storage. However maximum level (66.3%) was recorded on the 15th day while minimum level (35.9%) was recorded on the 10th day when it was observed to be the only highest population next to Vibrio. Unlike in 'Whole' sample, Vibrio formed the second highest population during later period of storage at 4°C. However their population varied from as low as 1.4% to as high as 62.5%. Although they were not recovered on the 5th day, they could record dominance on the 10th day (62.5%). Later, after recording a decrease on the 15th day (14%) they again increased to 36.5% on the 30th day. Acinetobacte was recovered on all occasions, except on the 10th day and it varied from 1.8% (5th day) to 18.6% (2nd day). Although they were not recorded on the 10th day, on subsequent days of storage their population slowly increased. All the other

groups were occasionally recorded during the storage period. Recovery of genera such as Alcaligenes, Bacillus, members of Enterobacteriaceae was erratic and they constituted the less dominant groups. Although Micrococcus and Corynebacterium were found to constitute the less dominant group, they increased to a maximum level on 5th day (33.9%) and 2nd day (22.9%) respectively.

Peeled and Undeveined:

Seven different genera could be recovered during storage at $4^{o}C$. They were Vibrio, Pseudomonas, Acinetobacter members of Enterobacteriaceae, Micrococcus, Bacillus and Corynebacterium. Among these, as in 'Whole' and 'Headless', Pseudomonas was found to be the dominant group throughout the storage period, except on the 15th day when it formed the second highest group next to Acinetobacter. However a maximum population of Pseudomonas (90%) was recorded on the 5th day. Although Pseudomonas was found to decrease on the 15th day it showed an increase (40%) on the 30th day. Next to Pseudomonas, Acinetobacter was recovered throughout the period of storage except on the 5th day. Their population varied from 32.2% on 15th day when they formed the dominant group, to 11% on the 30th day. All the other genera were recorded occasionally during storage constituting the less dominant group. However Corynebacterium which was recorded only on the

2nd day (20.6%) formed the second dominant group on that day. Vibrio, which was recorded only from 10th day onwards till 30th day, formed the second dominant group (38.6%) on the 10th day. Bacillus interestingly, formed the dominant group (40%) along with Pseudomonas on the 30th day although their occurrence on other days was erratic.

Peeled and Deveined:

Occurrence of all the genera recorded initially was found decreasing during storage of these samples at 4°C. Only five genera could be recorded during storage unlike that observed in the other types of samples. They were Vibrio, Pseudomonas, Acinetobacter, Micrococcus and Bacillus. Among these only Pseudomonas was recorded throughout the storage period and it formed the dominant group except on the 10th day when it formed the second dominant group. However maximum level of Pseudomonas population was recorded on the 15th day (96.2%), where, interestingly, they formed almost the entire flora. All the other genera such as Vibrio, Acinetobacter, Micrococcus and Bacillus constituted the less dominant group. However, Vibrio, which was recorded only on the 10th and 30th day formed the dominant group on the 10th day (55%) and the second dominant group on the 30th day (22.2%). Similarly Acinetobacter which was recorded only on the 2nd and 5th day formed the second dominant group on the 2nd day.

Micrococcus was also recorded only on the 2nd and 5th day and they formed the second largest group on the 5th day. The recovery of Bacillus was erratic.

3.3.4. Samples stored at -18°C

From the results it is clear that diversity observed in the initial flora was reduced during storage at -18°C (Fig.15). The reduction in diversity was similar to that observed at 4°C. Among the four types of samples, 'Whole' showed maximum diversity and 'PUD' and 'PD' showed low diversity. Very high generic diversity was observed only on the 4th day in all the samples. Later there was a reduction in the diversity throughout the storage period i.e. upto 210 days. Unlike other samples, 'PD' samples showed an increase in generic diversity on 210th day when compared to the low generic diversity observed on the 60th day.

All the genera, including Vibrio, Pseudomonas, Acinetobacter, Micrococcus and Bacillus were recorded in all the four types of samples. Interestingly, Staphylococcus which was not recorded in the initial flora was recorded in all the samples during storage. Alcaligenes which was also not found in the initial flora, was recorded during storage, in 'Whole' sample. Members of Enterobacteriaceae were not recorded in 'Headless' and 'PUD' samples during storage. Similarly Corynebacterium was also not recorded in 'PUD' samples. Moraxella which was recovered in the fresh samples

could not be reisolated in any of the samples during the storage period.

Of all these genera Pseudomonas was found to be the dominant one followed by Acinetobacter and Micrococcus in all the samples stored at -18°C.

Whole:

As many as nine genera could be recorded during storage. They included Vibrio, Pseudomonas, Alcaligenes, Acinetobacter, members of Enterobacteriaceae, Micrococcus, Staphylococcus, Bacillus and Corynebacterium. Among these, Pseudomonas, Acinetobacter, Micrococcus and Bacillus were consistently present. Of these four genera, Pseudomonas formed the dominant group and was found to vary from its maximum level of 54.2% on the 12th day to 3% on the 210th day. All the other three groups showed wide fluctuations during storage. Acinetobacter population varied from 35.5% (34th day) to 1.4% (60th day). However it formed the dominant group on the 21st day (33.2%). Here also the Acinetobacter group increased from its low level of 1.4% on the 60th day to 24.5% on the 100th day. However on 210th day their percentage occurrence dropped to 3.0. It can also be seen that this genus formed the second dominant group on the 34th day and 100th day. Micrococcus was found to vary from its maximum level of 59.1% on the 210th day, when it formed the dominant group, to 2.3% on the 4th day. Interestingly this

group increased on the 60th day of storage (34.3%) when compared to their low percentage occurrence (6.5%) on the 34th day. Vibrio which formed the dominant group in the initial flora was found to dominate on the 4th day also recording a maximum level of 33%. Also they formed the second dominant group on the 12th (22.2%) and 21st day (26.4%). Interestingly Vibrio which was not recorded on the 34th and 60th day of storage, reoccurred on the 100th day accounting for nearly 20% of the total population and subsequently disappeared. Although Bacillus could be recovered throughout the storage period, they formed only the less dominant group in most occasions. However this genera could form the second largest dominant group on the 4th (28.4%) and on the 210th day (25.8%) of storage. Occurrence of other genera such as Alcaligenes, Staphylococcus, Corynebacterium and members of Enterobacteriaceae was erratic and all these constituted the less dominant group during storage.

Headless:

As many as seven genera could be recorded in these samples during storage. They were Vibrio, Pseudomonas, Acinetobacter, Micrococcus, Staphylococcus, Bacillus and Corynebacterium. Pseudomonas formed the most dominant group. Alongwith Pseudomonas, Acinetobacter and Micrococcus were also recorded throughout the storage period as in 'Whole' samples.

All the three genera, showed wide fluctuations in their occurrence. Pseudomonas was found to vary from 13.7% on the 4th day to 79.3% on the 60th day. However very low population of this group was recorded on the 21st day and their population increased till 60th day and maintained almost at the same level till 100th day. However they could not be recovered on 210th day. Acinetobacter population was found to vary from 3.5% to 45.3%. In fact this group formed the second dominant group on many occasions during the storage period besides forming the dominant group on the 21st day (43.9%). However their maximum population level was recorded on the 210th day (45.3%). Micrococcus besides forming second dominant group on three occasions, was found to be the most dominant one on 210th day. Vibrio which formed the dominant group in the fresh samples, maintained their dominance only upto 4th day of storage. After 21 days, Vibrio were not found. Occurrence of other genera such as Staphylococcus, Bacillus and Corynebacterium was erratic and they formed the less dominant group.

Peeled and Undeveined:

It could be seen from the results only six genera could be recorded during storage. They were Vibrio, Pseudomonas, Acinetobacter, Micrococcus, Staphylococcus and Bacillus. Among these Acinetobacter formed the dominant group unlike that observed in 'Whole' and 'Headless' samples. The population

varied from 8.8% (100th day) to 95% (60th day). They formed the second dominant group on the 12th day (32.3%) and were not recovered from samples on the 210th day. Micrococcus which was recorded only on 12th, 21st, 100th and 210th day, was found to be the dominant group during storage on the 12th day (41.9%) and on the 210th day (88.9%). Also, they formed the second largest group on the 21st (27.8%) and 100th (14%) day. Eventhough Pseudomonas was recorded during storage, almost in all the occasions except on the 34th and 210th day, they formed the largest group, only next to Micrococcus, However they were found to be the dominant group on the 100th day (71.9%). Also they formed the second largest group on the 60th day (30.6%). Occurrence of other genera such as Vibrio, Bacillus and Staphylococcus was erratic and they constituted the less dominant group, except Bacillus which formed the second dominant group on the 4th day.

Peeled and Deveined:

As many as eight genera could be recovered from these samples during storage at -18°C. They were Vibrio, Pseudomonas, Acinetobacter, members of Enterobacteriaceae, Micrococcus, Staphylococcus, Bacillus and Corynebacterium. The data show that generic diversity was very low and interestingly no single group was found to be dominant throughout the period of storage. Instead various genera

showed dominance at different occasions. For example dominance was shown by Pseudomonas on the 12th (54.6%) and 100th day (56.3%), Micrococcus on the 60th (80%) and 210th (45.0%) day, Acinetobacter on the 21st day (74.1%), Vibrio on the 34th day (40%), Staphylococcus (30.8%) and Corynebacterium (30.8%) on the 4th day. Similarly no single genus could be assigned to second place in dominance throughout the period of study. Micrococcus was found to constitute about 80% of the flora occurred on 60th day. On 210th day Bacillus formed the dominant group. Members of Enterobacteriaceae were recovered only on the 100th day in low percentage (12.5%). However, of all the genera, frequency of occurrence made by Pseudomonas, and Acinetobacter was high when compared to other genera encountered in these samples during storage.

3.4. GROUPING OF HYDROLYTIC ENZYME PRODUCING BACTERIA (HEP)

The ability to produce various extracellular enzymes by bacteria isolated from stored prawns and assigning them into various physiological groups based on their hydrolytic properties i.e. caseinolytic, gelatinolytic, amylolytic, lipolytic and ureolytic has not been reported so far. In the present study such a grouping was performed with the isolates obtained from the prawns stored at 28 ± 2^{o}, $4^{o}C$ and $-18^{o}C$.

A total of 913 isolates were tested according to the methods described in section 2.10. 'Materials and Methods'. The general pool was constituted by 39 isolates from fresh prawns, 180 from samples stored at 28±2°C, 275 from samples stored at 4°C and 419 from samples stored at -18°C.

3.4.1. Fresh prawns:

The heterotrophic bacterial population present on the prawns, when they were fresh was dominated by gelatinolytic flora (61.67%) (Fig.16). Caseinolytic flora formed the second largest group (30%). lipolytic, amylolytic and ureolytic flora formed the less dominant group.

3.4.2. Samples stored at 28±2°C:

Majority of the heterotrophic bacteria isolated from the four types of samples stored at 28±2°C were proteolytic - both caseinolytic and gelatinolytic - (Fig.16). The results indicate that all the hydrolytic enzyme producing bacteria examined increased in their population from their initial level during storage. Among the hydrolytic flora examined, gelatinolytic bacteria formed the dominant group followed by caseinolytic bacteria in all the four types of samples. Other hydrolytic groups such as

amylolytic and lipolytic bacteria formed the less dominant flora in all the samples. In general ureolytic bacteria were the least encountered.

Whole:

From the results, it can be seen that all the bacteria examined for enzyme activities increased from their initial population level (Fig.16 a) and recorded a maximum at 8 h. Interestingly the percentage occurrence of hydrolytic bacteria were found to decrease at 12 h but subsequently increased at 24 h. Among the hydrolytic groups, proteolytic bacteria (both caseinolytic and gelatinolytic) were found abundant throughout the period of storage. Gelatinolytic bacteria were the most dominant group and their population was found to be uniformly higher throughout the period of storage. They increased from 66.67% at 0 h to 82.61% at 8 h and showed a decline in population level in subsequent period of storage. Caseinolytic bacteria constituted the second dominant group throughout the period of storage. They increased from 30% at 0 h to 73.33% at 24 h. In fact, unlike other groups, caseinolytic bacteria recorded their maximum population at 24 h, although a decrease in population was noticed at 12 h. The amylolytic group which was one among the less dominant groups showed an increase from 20.56% at 0 h to 56.62% at 8 h. However they decreased in their population in the later period of

storage. Lipolytic bacteria, formed another less dominant group during storage. However their occurrence was equal to gelatinase producers at 8 h (82.61%). They increased from 26.11% at 0 h to 82.61% at 8 h and later decreased suddenly at 12 h to 36.36%. Of all the hydrolytic flora examined,ureolytic flora accounted for the least dominan group during storage. But they showed an increase from 18.33% at 0 h to 39.13% at 8 h and a decrease at 12 h to a level of 22.73%.

Headless:

Proteolytic bacteria (caseinolytic and gelatinolytic) were found to be the abundant group throughout the period of storage. Like in the 'Whole' samples gelatinolytic bacteria were the most dominant group than other enzymatic groups. They increased from 61.67% at 0 h to 100% at 12 h. At 24 h a marginal reduction in their percentage occurrence was noticed. However fluctuations in the percentage occurrence during storage were also recorded. Caseinolytic bacteria showed a steap increase from 30% at 0 h to 87.5% at 4 h when they could reach their maximum population. Unlike other hydrolytic bacteria, they were found to decrease in population gradually during storage (Fig.16 b). Thus caseinolytic bacteria decreased from 87.5% at 4 h to 66.67% at 24 h. However they formed the second largest group during storage. Amylolytic bacteria showed wide fluctuations in the level of population during storage. Similar to caseinolytic

bacteria, they increased rapidly from 20.56% at 0 h to 75% at 4 h. But in the subsequent period of storage they decreased to a level of 16.67% at 24 h. In fact amylolytic bacteria formed the less dominant group during storage. Lipolytic bacteria exhibited a steady increase from 26.11% at 0 h to 75% at 24 h. There was not appreciable change within population during storage especially after 4 h. Further, lipolytic bacteria formed the dominant group next to caseinolytic bacteria during storage. As in 'Whole', in these samples too, ureolytic bacteria formed the least dominant group. However, an increase was observed from 18.33% at 0 h to 40% at 12 h. Later ureolytic bacteria decreased rapidly at 24 h to a level of 16.67% similar to amylolytic bacteria.

Peeled and Undeveined:

Results indicate that all the hydrolytic bacteria examined increased in their population during storage (Fig.16 c). However all the groups uniformly showed a decrease in their populations at 24 h. As in 'Whole' and 'Headless', proteolytic bacteria (both caseinolytic and gelatinolytic) were found to exhibit dominance over other hydrolytic groups. Gelatinolytic bacteria were found uniformly higher throughout the period of storage. They increased from 61.67% at 0 h to 100% at 12 h. and decreased to 69.23% at 24 h. Caseinolytic bacteria were the next predominant group during storage. They increased from 30% at 0 h to 75% at 8 h and remained same even at 12 h.

Subsequently they also decreased to 30.77% at 24 h. Amylolytic bacteria registered a sharp increase in their population from 20.56% at 0 h to 80% at 4 h. Interestingly this group decreased to 50% at 12 h of storage and finally were absent at 24 h. However they formed the dominant group at 4 h (80%). Lipolytic bacteria formed the less dominant group. They increased from 26.11% at 0 h to 62.5% at 8 h. Similar to caseinolytic bacteria this group also remained at the same level of population at 12 h (62.5%) and later marginally decreased to 53.85% at 24 h. At 24 h lipolytic bacteria formed the second major group. As observed in other samples, ureolytic bacteria formed the least dominant group here also. Unlike other hydrolytic groups, they were found to decrease at 4 h (10%) and later increased to 37.5% at 12 h. However once again it decreased to 23.08% at 24 h.

Peeled and Deveined:

As it was observed with other samples, the various hydrolytic bacteria increased in their occurrence during storage (Fig.16 d). Proteolytic bacteria (caseinolytic and gelatinolytic) were the dominant group throughout the period of storage. Gelatinolytic bacteria were found to show dominance over other hydrolytic groups throughout the period of storage. They increased from 61.67% at 0 h to 100% at 8 h and remained at the same level at 12 h. However their population decreased to 80% at 24 h. Caseinolytic

bacteria rapidly increased from 30% at 0 h to 75% at 4 h. Their population was found to be present at the same level till 12 h during storage and decreased to 60% at 24 h similar to gelatinolytic bacteria. Amylolytic bacteria also showed a similar pattern as that of caseinolytic bacteria. They increased from 20.56% at 0 h to 50% at 4 h and remained at the same level till 12 h. Later they decreased rapidly and reached 20% at 24 h. Lipolytic bacteria steadily increased from 26.11% to 75% at 12 h and later decreased to 40% at 24 h. However they formed the second most common group at 4 h and 12 h. Unlike in other samples ureolytic bacteria showed marked increase in their population from 18.33% at 0 h to 60% at 24 h. They could form the second largest group at 24 h. In general amylolytic, lipolytic and ureolytic bacteria formed the less dominant group of hydrolytic bacteria during storage of prawn.

3.4.3. Samples stored at 4°C:

From the results (Fig.17) it can be noticed that all types of hydrolytic bacteria increased in their population during storage from their initial levels. Among the different hydrolytic bacteria, gelatinolytic bacteria were found to be the abundant and showed dominance over other groups during storage. Lipolytic bacteria constituted the second predominant group during storage. Caseinolytic, amylolytic and ureolytic bacteria formed the less dominant groups.

Whole:

Results (Fig.17 a) show the increase of all types of hydrolytic flora during storage at 4°C. It was noticed that all the groups decreased on the 2nd day in all samples and later rapidly reached the maximum levels. Among the groups examined gelatinolytic bacteria were dominant on all occasions except the 10th day when lipolytic group was found to be dominant. The gelatinolytic population varied between 44.44% and 81.08%. They showed gradual decrease later. Caseinolytic bacteria was found to vary from 14.81% to 50%. Interestingly this group showed gradual increase in their population from 14.81% on the 2nd day to 50% on the 15th day. Lipolytic bacteria were the second dominant group next to gelatinolytic bacteria. They varied from 22.22% to 78.95%. This group showed a sudden increase in its population on the 5th day (70.27%) when compared to that on the 2nd day (22.22%). However after reaching a maximum population (78.95%) on the 10th day, the percentage of occurrence was found to decrease till 15th day (35.71%). Amylolytic bacteria formed the less dominant group and their population fluctuated from 14.81% to 45.95%. Ureolytic bacteria formed the least dominant group and varied from 5.26% to 25%. They attained the maximum level on 30th day of storage.

Headless:

All the hydrolytic flora recorded were found to be increased during storage in this sample (Fig.17 b). No single

group was found to be dominant over others throughout the period of storage as it was observed in 'Whole' samples. However gelatinolytic and lipolytic bacteria formed the dominant groups during storage. Interestingly both gelatinolytic and amylolytic bacteria were not recovered on the 30th day. Caseinolytic bacterial population was found to vary from 9.09% to 45.45% and they reached their maximum on the 15th day. After recording a low level of 13.64% on the 2nd day, they gradually increased to 45.45% on the 15th day and suddenly declined to 9.09% on the 30th day. The percentage occurrence of gelatinolytic bacteria was found to vary from 36.36 to 68.42. Although they formed a major group, after recording a maximum of 68.42% on the 5th day, they gradually decreased to 36.36% on 15th day and was completely absent on the 30th day. Amylolytic bacteria were found to vary from 18.18% to 45.45%. Very similar to gelatinolytic bacteria this group after recording a maximum percentage of population (45.45%) on the 2nd day, gradually decreased to 18.18% on the 15th day and was absent on the 30th day. Lipolytic bacteria which could form a dominant group was found to vary from 26.11% to 64.29%. They increased gradually till 10th day reaching a maximum level (64.29%) and later suddenly decreased reaching 36.36% on the 15th day. However an increase in percentage of population (54.56%) was recorded on the 30th day. Ureolytic

bacteria showed a uniform steady increase without any fluctuation during the storage period. They increased from 4.55% on the 2nd day to 72.72% on the 30th day which was the maximum percentage of population recorded in this sample.

Peeled and Undeveined:

Results indicate that all the hydrolytic groups of bacteria increased during storage (Fig. 17 c). Gelatinolytic bacteria constituted the dominant group followed by lipolytic bacteria. All the groups, except ureolytic bacteria, although increased in numbers during storage, were found to decrease on the 30th day. Wide fluctuations in their percentage of occurrence was observed. Caseinolytic bacteria was absent on the 2nd day. However they appeared on the 5th day and was recovered later. Their population was observed to vary from 16.67% to 60%.

A sudden decrease was registered on the 10th day (16.67%) when compared to their population (42.11%) on the 5th day. However they could form a dominant group on the 15th day (60%). Gelatinolytic bacteria showed variation from 36.36% to 78.95% and reached the maximum on 5th day. They suddenly declined on the 10th day (50%) and showed fluctuations in their percentage of occurrence later. Amylolytic bacteria showed a similar trend observed with gelatinolytic bacteria. They initially increased till 5 days

and recorded a maximum on the 5th day (52.63%). However, they suddenly decreased to a minimum (16.67%) on the 10th day. Although once again they increased on the 15th day (50.0%), again declined to 16.67% on the 30th day. Their population varied from 16.67% to 52.63%. Lipolytic bacteria showed an interesting observation. They increased rapidly to 100%.till 10th day and gradually declined to 41.67% on the 30th day. Ureolytic bacteria was found to vary from 18.18% to 33.33%. However they could not be recovered on the 10th day.

Peeled and Deveined:

Although all the hydrolytic groups showed increase during storage, they were not recorded at all occasions. No single group could be recorded as a dominant one throughout the period of storage (Fig.17 d). However gelatinolytic group showed dominance at many instances. Interestingly it was observed that on the 15th day except none other than ureolytic bacteria could be recovered. Similarly on the 10th day only gelatinolytic and lipolytic bacteria were recorded. Caseinolytic bacteria could be recorded initially till 5 days storage and later it reoccurred on the 30th day. Both caseinolytic and gelatinolytic bacteria varied from 9.09% to 100% during storage. Amylolytic bacteria could not be recovered after 5 days of storage. They registered an initial increase from 20.56% at 0 h to 36.36% on the 2nd day,

and made no further increase later. Similar to gelatinolytic bacteria, lipolytic bacteria varied from 9.09% to 100%. Ureolytic bacteria was not recorded on the 10th day. However they were recovered at later period and they varied from 9.09% to 100%.

3.4.4. Samples stored at -18°C:

Results presented in Fig.18 indicate that most of the bacteria examined for their hydrolytic enzymes production were proteolytic. In general gelatinolytic bacteria were the dominant hydrolytic flora in all the samples stored at this temperature. However the population of this group showed a decrease in 'Headless and 'PD' samples. Unlike in the samples stored at 4°C and 28±2°C, amylolytic bacteria formed the next dominant group in the samples at this temperature. Other groups such as lipolytic, caseinolytic and ureolytic formed the less dominant flora in the samples during storage.

Whole:

Results indicate (Fig.18 a) that all the hydrolytic bacteria increased during storage. Among them gelatinolytic bacteria were the dominant one in many occasions followed by amylolytic bacteria. All the groups showed wide fluctuations in their occurrence during storage. Caseinolytic bacteria could form the next predominant group to amylolytic bacteria. Their population varied from 10% to

70.83%. Although they were not recovered on the 12th day, later their increase in population was significant and gradual. However they decreased in numbers on the 210th day (64.29%) when compared to their maximum level of population (70.83%) on the 100th day. Gelatinolytic bacteria varied from 11.54% to 85.71%.and showed no significant increase till 60 days. Later they increased rapidly and registered a maximum (85.71%) on 210th day. Amylolytic bacteria varied from 20.56% to 50% and recorded a gradual increase throughout the period with a slight fall on 60th and 100th day. Lipolytic bacteria varied from 13.36% to 33.33%. They formed only a less dominant flora and showed wide fluctuations in the percentage occurrence. Ureolytic bacteria also varied from 13.79% to 57.14%. After showing fluctuations during the initial period of storage, they gradually increased later to the maximum on 210th day.

Headless:

As in other samples, all the hydrolytic flora except gelatinolytic, showed an increase during storage (Fig.18 b). Among the groups examined, gelatinolytic group was found to be the dominant group followed by amylolytic and caseinolytic as it was observed in 'Whole' samples. However wide fluctuations in the population were observed in all the flora. Caseinolytic bacteria could not be recovered on 21st day whereas they reappeared on 34th day. Their

population varied from 4.55% to 47.06%. Gelatinolytic bacteria did not show an increase, instead there was a slight decrease from their initial population. They were found to vary from 61.67% to 29.17%. Amylolytic bacteria varied from 11.76% to 57.14%. After recording a gradual increase from 22.73% on the 12th day to 42.11% on the 34th day, they showed a sudden decline on the 60th day (11.76%). However, steady increase in population was observed during the subsequent period of storage. Lipolytic bacteria formed the less dominant flora and their population varied between 8.33% and 31.58%. They were observed to decrease from 26.11% at 0 h to 13.36% on the 12th day and record a sudden increase on the 34th day (31.58%). However, later they declined gradually to 8.33% on the 100th day and was not recovered on the 210th day. Ureolytic bacteria varied from 9.09% to 31.58% and the maximum was recorded in 34th day.

Peeled and Undeveined:

Results indicate (Fig.18 c) that all the hydrolytic bacteria increased in their population during storage from the initial level. In general gelatinolytic bacteria were the dominant group followed by lipolytic bacteria. Caseinolytic bacteria which were not recorded on 12th and 21st days were recovered on the 34th day. They recorded a maximum percentage of 45.45% on 60th day. Later they gradually declined till 210th day (14.29%). Gelatinolytic bacteria varied from 15.38;

to 75%. A gradual decline in their initial population till 21 days and an increase till 100th day was observed. However on 210th day only a low level of their population (28.57%) was recorded. Amylolytic bacteria varied between 20.56% and 46.15%. They showed an increase in the percentage occurrence till 34 days (44.44%) and later, they decreased. Lipolytic bacteria were found to vary from 12.5% to 55.55%. Similar to amylolytic bacteria gradual increase till 34 days and gradual decrease till 100th day, in population was observed. They were not recorded on the 210th day. Ureolytic bacteria varied from 9.09% to 37.5%. The initial population after registering a reduced level (9.09%) on 12th day, increased gradually and reached the maximum (37.5%) on 100th day. However their population decreased on the 210th day (14.29%).

Peeled and Deveined:

All the hydrolytic bacteria showed more percentage of occurrence in few instances during storage as it was observed with 'Headless' samples (Fig.18 d). As in other samples, here too gelatinolytic bacteria exhibited dominance over other groups. Caseinolytic bacteria were in the range of 16.67% to 50%. After recording 16.67% of population on the 4th day, they were not recovered on the 12th and 21st days. Later they were recovered on the 34th day (25%) and they increased to their maximum (50%) on the 60th day. Subsequentl

their population decreased to 22.22% on the 100th day. In general, except on 60th day, the percent of caseinolytic bacteria was less than the initial level. Gelatinolytic bacteria varied from 12.5% to 61.67%. They recorded a rapid decrease on the 12th day and a sudden increase to 57.14% on the 21st day. Later their population was more or less constant till 210 day. Amylolytic bacteria were not recorded on the 12th day. Their population varied between 14.29% and 75%. Initially they increased on 34th day recording the maximum of 75%. However later, they decreased to the minimum of 14.29% on the 100th day and maintained the same level till 210 days. Lipolytic bacteria varied from 12.5% to 33.3%. After recording their maximal population of 33.33% on the 4th day, they suddenly declined to the minimum 12.5% on the 12th day. Then they showed an increase (28.57%) on the 21st day and once again decreased gradually to 14.29% on the 100th and 210th days. Ureolytic bacteria varied from 16.67% to 50%. Although they gradually increased to their maximum (50%) on 60th day later declined suddenly to 28.57% on 100th day and remained at the same level till 210 days. They were not recorded on 12th day as it was observed with caseinolytic and amylolytic .

3.5. DETERMINATION OF SPOILAGE POTENTIAL OF BACTERIA

3.5.1. Flesh spoiling bacteria:

Indirect methods of determining spoilage flora include estimation of proteolytic bacteria, lipolytic

bacteria, TMAO reducers, indole and NH_3 producers etc. But it has been suggested that a direct method of detecting spoilage flora by inoculating pure strains of bacteria in sterile muscle blocks or flesh juice and performing recovery of the inoculated bacteria, besides observing certain biochemical changes and odour production is more useful. In the present study the direct method of inoculating pure strains of bacteria into sterile prawn flesh broth and flesh agar media was performed to test the ability of them to spoil flesh.

The bacterial isolates obtained from the samples stored at various temperatures were selected randomly (219). The number of isolates selected for the study were as follows: 39 from $28\pm2^{o}C$, 96 from $4^{o}C$ and 84 from $-18^{o}C$. The isolates tested were species of Vibrio, Aeromonas, Pseudomonas, Alcaligenes, Acinetobacter, members of Enterobacteriaceae, Micrococcus, Bacillus and Corynebacterium. About 63% of the total isolates tested were potential spoilers (Table 7). Among the 138 positive strains Pseudomonas sp. were the dominant group (33.3%). Vibrio (24.7%)and Acinetobacter (21.0%) formed the next dominant groups in order. All the rest of the groups formed the lesser dominant ones. Gram-negative forms (65.4%) showed dominance over Gram-positive forms (52.5%).

Individually among Gram-negative forms 85% of Vibrio, 59.7% of Pseudomonas and 58% of Acinetobacter were potential spoilers. Other genera although showed significant percentages of spoilers were represented by less than 10 numbers. Among Gram-positive bacteria about 60% of Bacillus and Corynebacterium were potential spoilers whereas only 45% of Micrococcus showed spoilage activity when tested by this method.

Percentage composition and generic distribution of potential spoilers with respect to individual storage temperatures are presented in Fig.19. Higher percentage (68.8%) of potential spoilers were recovered in the samples stored at $4^{o}C$ when compared to that of $28\pm2^{o}C$ (64.1%) and $-18^{o}C$ (56%). In all the three temperatures the members of Vibrio, Pseudomonas, Acinetobacter and Enterobacteriaceae showing spoilage activity were recorded. Other groups showed sporadic occurrence.

Among the major groups tested, all the strains of Vibrio (100%) recovered from the samples stored at $-18^{o}C$ were potential spoilers when compared to that of $4^{o}C$ (89%) and $28\pm2^{o}C$ (72%). Spoilers among Pseudomonas sp. showed a decreasing trend along with reduction in the level of storage temperature ($28\pm2^{o}C$ to $-18^{o}C$). Maximum number of them (83.3%) were spoilers in the samples of $28\pm2^{o}C$ storage

and they could record 69.2% and 43.8% in the samples stored at 4°C and -18°C respectively. Whereas, Acinetobacter showed entirely a different trend when compared with Vibrio and Pseudomonas. The maximum percentage of spoilers (66.7%) in Acinetobacter sp. was present in the samples stored at 4°C and in moderate levels (65%) at -18°C and in very low level (22.2%) at 28±2°C. Similarly among members of Enterobacteriaceae, 60% potential spoilers were present in the samples of 4°C while 50% were present in the samples stored at 28±2°C and -18°C. Other generic groups did not represent spoilers in the samples stored at the three temperatures. Members of Micrococcus and Bacillus showing spoilage activity were recorded in the samples stored at 4°C and -18°C (60.5% and 33.3%; 54.1% and 62.5% respectively). While species of Alcaligenes with spoilage activity were present only in the samples stored at 4°C. Potential spoilage of Aeromonas sp. was recorded only in the samples stored at 28±2°C.

3.5.2. Trimethylamine oxide (TMAO) reducing bacteria:

Reduction of Trimethylamine oxide to Trimethylamine by bacteria was tested with 178 isolates obtained from samples stored at 28±2°C (26), 4°C (72) and -18°C (80). The isolates tested were species of Vibrio, Pseudomonas, Alcaligenes, Acinetobacter, members of Enterobacteriaceae, Micrococcus,

Bacillus and Corynebacterium. Results presented in the Table 8 indicate that 92.1% of the total isolates tested were capable of reducing TMAO to TMA confirming the presence of potential spoilers on the samples during storage. Interestingly, it was observed that all the isolates of Vibrio (100%) and Alcaligenes (100%), among the major groups of bacteria tested, were TMAO reducers. Among Pseudomonas sp. and Acinetobacter sp. about 89.2% and 92.1% respectively were TMAO reducers. TMAO reducers of the members of other genera varied between 83.3% and 100%.

Percentage composition and generic distribution of TMAO reducers with respect to individual storage temperatures are presented in Fig.20. Maximum percentage (96.2%) of TMAO reducing bacteria were present on the prawn samples stored at 28±2°C when compared with that of 4°C (88.9%) and -18°C (93.8%). Among various genera all the members of Vibrio, isolated from the samples stored at the three different storage temperatures, were TMAO reducers. Among Pseudomonas all strains isolated from the samples stored at 28±2°C were capable of reducing TMAO to TMA. When compared to that of -18°C (93.6%) and 4°C (83.3%). All the Acinetobacter strains isolated from the samples stored at 28±2°C were TMAO reducers, whereas, only 84.7% and 85.7% of them were represented on the samples stored at 4°C and -18°C. All the isolates of Enterobacteriaceae isolated from the samples stored at -18°C were TMAO reducers, whereas, only 75% of them isolated from

samples stored at 4°C were TMAO reducers. Among *Micrococcus* sp. the only isolate tested, recovered from 4°C storage, was TMAO reducer, whereas 90.9% of TMAO reducers were recorded for the samples stored at -18°C. On the other hand all the strains of *Bacillus* isolated from the samples stored at -18°C were TMAO reducers when compared to their number (83.3%) recorded for the samples stored at 4°C.

It is clear from the results, that all the isolates of *Pseudomonas*, *Vibrio* and *Acinetobacter* which were generally the dominant flora during storage, could have contributed to the spoilage of prawns at the three storage temperatures, by virtue of representing significant number of spoilers, although they varied in their number at different temperatures.

3.6. GROWTH AND PHYSIOLOGY OF SPOILAGE BACTERIA

3.6.1. Effect of temperature on the growth of spoilage bacteria:

The effect of temperature on the growth of spoilage bacteria was studied by growing them in flesh broth and Zobell's broth at different temperatures (-15, 5, 15, 30, 45 and 60°C) and their final biomass was estimated spectrophotometrically. Growth obtained is expressed in terms of growth index. Growth index is the percent of average turbidity of cultures grown for a specific period and at specific temperature. The results are presented in Table 9.

None of the isolates showed growth at higher temperature (60°C) in flesh broth and ZoBell's broth. Similarly all the isolates did not grow at -15°C in the flesh broth. However, three of them showed feeble growth (<3%) at this temperature in ZoBell's broth. Maximum growth was recorded at 30°C for all the cultures in ZoBell's broth. Whereas in flesh broth, 62.5% of the cultures recorded maximum growth at 30°C, 25% at 45°C and 12.5% at 15°C. All the isolates did not show similarity in growth, in both the media. However, optimum growth for all the cultures was found between 15° and 45°C in flesh broth as well as in ZoBell's broth.

All the isolates showed fair growth, in flesh broth when compared with ZoBell's broth at 5°C. Similar variation in the intensity of growth could be observed among cultures grown in a single medium and between the media. At 15°C, species of Pseudomonas (L97 and F152) and Vibrio (L146 and F10) showed fair growth in flesh broth than in ZoBell's broth. Whereas, species of Pseudomonas (R8), Vibrio (R42) and Acinetobacter (L114 and F88) recorded more growth in ZoBell's broth than in flesh broth at this temperature. At 30°C, comparatively, good growth for all the cultures was recorded in ZoBell's broth. The maximum growth was recorded, at this temperature, for 75% of the isolates and species of Pseudomonas R8 and L97 and Vibrio L146 recorded 67.35%,

94.54% and 89.89% of growth respectively. At 45°C species of Pseudomonas (R8,L97 and F152) showed fair growth in flesh broth than in ZoBell's broth. Whereas, all species of Vibrio and Acinetobacter showed fair growth in ZoBell's broth than in flesh broth.

Pseudomonas sp. (F152), Vibrio sp. (R42 and F10) and Acinetobacter sp. (L114 and F88) showed maximum growth (100%) at 30°C, in both the media. Only species of Acinetobacter (L114 and F88) showed maximum growth (100%) in flesh broth at 30°C. While strains of Pseudomonas sp. (F152) and Vibrio sp. (R42 and F10) preferred 30°C to grow maximum, in flesh broth, two strains of Pseudomonas (R8 and L97) and Vibrio (L146) showed preference for a lesser temperature (15°C) for maximum growth in flesh broth. Variation in growth pattern was observed within the same group of organisms in response to incubation temperatures.

3.6.2. Effect of pH on the growth of spoilage bacteria:

The effect of pH on the growth of spoilage bacteria was studied by growing them in flesh broth and in ZoBell's broth, adjusted to various levels of pH (pH 2 to 12). The results are expressed in terms of growth index and presented in Table 10.

All strains did not grow at extreme pH levels of pH 2 and pH 12 in both the media. Whereas feeble growth (<3%)

was noticed at pH 4. In general, optimum growth for all the isolates was observed between pH 6 and 10. About 50% of the isolates recorded maximum growth at pH 6, 37.5% at pH 8 and 12.5% at pH 10 in ZoBell's broth. Whereas in flesh broth, 25% showed maximum growth at pH 6, 37.5% at pH 8 and at pH 10.

None of the isolates, except Pseudomonas sp. R8 and Vibrio sp. R42, showed maximum growth at same pH level (pH 8 and 10) in both the media. At the same time, a variation in the growth pattern at other pH levels could be recorded. Pseudomonas sp. R8 showed higher percent of growth in ZoBell's broth at pH 6 and 8 than in flesh broth, whereas Vibrio sp. R42 showed fair growth in flesh broth than in ZoBell's broth. Pseudomonas sp. L97 and F152 showed maximum growth at pH 6 in flesh broth and at pH 8 in ZoBell's broth. Similarly, 100% growth was observed at pH 10 in flesh broth and at pH 6 in ZoBell's broth for species of Acinetobacter L114 and F88. Vibrio sp. L146 and F10 showed 100% growth at pH 8 in flesh broth and at pH 6 in ZoBell's broth. In general, Acinetobacter sp. showed maximum growth at pH 10 in flesh broth and at pH 6 in ZoBell's broth. Pseudomonas showed a preference to a wide range of pH (pH 6 to 10) to attain 100% growth in both the media, whereas Vibrio showed maximum growth at pH 8 in flesh broth and in ZoBell's broth.

Among strains of Pseudomonas sp., R8 showed maximum growth at pH 10 in both media, while, the maximum growth for L97 and F152 was observed at pH 6 in flesh broth and at pH 8 in ZoBell's broth. All the cultures of Vibrio recorded 100% growth at pH 8 in flesh broth, whereas, isolate R42 alone showed 100% growth at higher pH levels in ZoBell's broth and the other two isolates preferred pH 6 for their maximal growth. Both the isolates of Acinetobacter L114 and F88 showed a maximal growth at a higher pH (pH 10) in flesh broth, while in ZoBell's broth they preferred a lesser pH (pH 6) for their maximal growth. Variation in the pattern of growth of the isolates could be observed in the same medium and in various media.

3.6.3. Effect of sodium chloride concentrations on the growth of spoilage bacteria:

The effect of sodium chloride on the growth of spoilage bacteria was studied by growing them in flesh broth and in ZoBell's broth with various concentrations of NaCl (0 to 15%) and the results are expressed in terms of growth index and presented in Table 11.

The growth pattern of all the isolates was not similar in flesh as well as in ZoBell's broth. All the isolates did not grow at higher concentration (15%) of NaCl, added to flesh broth and ZoBell's broth. Optimum growth was recorded at 1 to 6% NaCl concentrations. However,

feeble to good growth was observed in both the media in the absence of NaCl also.

About 37.5% of isolates recorded 100% growth at 1 and 3% NaCl concentrations and 12.5% at 0 and 6% NaCl concentration in ZoBell's broth. Similarly 37.5% of the Isolates showed maximum growth at 6% NaCl, 25% at 1 and 3% NaCl and 12.5% at 0% NaCl. It was observed that the maximum growth for all the isolates was not accounted at a particular concentration of NaCl in both the media.

In general, all the cultures showed maximum growth at 1,3 and 6% NaCl concentrations, supplemented to flesh broth whereas such a similarity, in the requirement of NaCl concentration for maximum growth could not be observed in ZoBell's broth. The strains of Pseudomonas R8, Vibrio R42 and Acinetobacter F88 recorded maximum growth at 3,6 and 1% NaCl concentration both in flesh broth and ZoBell's broth. However isolates of Pseudomonas L97 and F152 and Vibrio sp. L146 and F10 showed maximum growth at 1 and 3% NaCl concentration in ZoBell's broth. Acinetobacter sp. L114 grew to the maximum in ZoBell's broth free of NaCl whereas in flesh broth they required 1% NaCl for maximum growth.

3.6.4. Effect of temperature on the survival of spoilage bacteria:

Temperature tolerance of spoilage flora was tested by exposing them to different temperatures (-15°, 5°, 45° and 60°C) for varying periods (<120 m for higher temperature, <240 m for lower temperature) and estimating the survival percentage. The results are presented in Table 12.

At higher temperatures, all the strains survived well at 45°C than at 60°C when exposed for a period of 120 minutes. At 45°C the survival percentage of various species tested varied from 28.2% to 70.2% at 120 m. Strains of Pseudomonas sp. recorded survival percentages in the range of 48% - 68.9% demonstrating their remarkable survival. Similarly Vibrio sp. also survived well (49.9% - 70.2%), whereas, Acinetobacter sp. showed lesser percentages of survival and ranged between 28.2% and 48.7%. When the temperature was increased to 60°C the survival percentages of all the species decreased with time. 60°C exposure for 120 m considerably affected the viability of the cells and the survival percentages were in the range of 0 - 4% Vibrio strains were highly sensitive than Pseudomonas to this temperature and one strain (Vibrio sp. F10) lost its viability after 45 m of exposure. Acinetobacter sp. was able to tolerate this temperature upto 90 m.

At reduced temperatures, it was observed that all the strains could survive at 5°C than at -15°C when exposed for a period of 240 m. The percentage of survival of bacteria was found to be decreased with increase in the time of exposure to 5°C and -15°C. About 9.3% to 66.6% of survival could be recorded when exposed to 5°C for 240 m. Except two strains (Pseudomonas sp. R8 and Vibrio sp. R42) which could record 50% survival, all the other strains could not survive well at 5°C for a prolonged exposure. Acinetobacter sp. (F88) could not survive after 120 m at this temperature. Exposure to -15°C considerably affected the longivity of the various species tested when exposed for 240 m. However, survival percentages ranging between 0% and 7.5% could be observed for the strains at this temperature. It was observed that Acinetobacter (F88) lost viability after 120 m and Pseudomonas (F52) after 180 m when exposed to -15°C.

3.6.5. Effect of pH on the survival of spoilage bacteria:

The effect of pH on the survival of spoilage bacteria was tested by incubating a known concentration of viable cells of the selected strains in buffers having different pH values (pH 2.6 to 10.6) and estimating the survival percentage at predetermined intervals upto 36 h. The results obtained are presented in Table 13. All the species were found to be more sensitive towards acidic (pH 4.2 and 2.6) and high

alkaline conditions (pH 10.6). All the isolates survived well at pH levels of 6 - 8.6. At pH 9.6, the survival percentages of all the species were comparatively less than at pH 8.6.

At pH 2.6 all the species could not survive after 18 h. It was observed that one Vibrio sp. L146 did not survive after 6 h of exposure to this hydrogen-ion concentration of 2.6. Similarly three other strains (Vibrio sp. F10, Acinetobacter sp. F88 and Pseudomonas sp. F152) were not found to survive after 12 h of exposure. Even at this short time the percentage of survival was very low. At pH 4.2, in general, viability of the cells of all the species was considerably affected after a period of 24 h exposure - and a gradual decrease could be seen from 6 h to 24 h of exposure. Among all the strains, Acinetobacter spp. L114 and F88 and Pseudomonas sp. F152 were highly sensitive and could not survive for 18 h of exposure to pH 4.2. At higher alkaline pH (pH 10.6), except Acinetobacter sp. F88 which lost its viability after 18 h of exposure, all the other strains could survive till 24 h of exposure.

At pH levels of 6,8.6 and 9.6 all the species survived well till 36 h of exposure and their survival percentages varied from 52.4% to 72.4% at pH 6, from 50.9% to 80.1% at pH 8.6 and from 25.5% to 48% at pH 9.6.

3.6.6. Effect of sodium chloride concentration on survival of spoilage bacteria:

Effect of sodium chloride, at various concentrations, on the survival rate of spoilage bacteria was tested by incubating a known quantity of actively growing cells with different concentrations of sodium chloride and estimating the survival percentage at predetermined intervals upto 36 h. The results are presented in Table 14.

In general concentrations of sodium chloride above 6% had considerably affected the survival of all the species tested. All the strains survived well at 0 to 6% NaCl concentrations. However, each species have responded differently at various concentrations of sodium chloride tested.

Among Pseudomonas sp., strain No.R8 showed maximum survival at 1% NaCl concentration. When compared with that observed for other NaCl concentration, strain L97 interestingly showed maximum survival percentage at 3% NaCl concentration and survived well at 0, 1 and 6% NaCl concentrations recording a tolerance towards a wide range of NaCl concentration, whereas, strain F152 showed reduced level of survival percentages at NaCl concentration 0 - 3%. However, it recorded maximum survival percentage at 0% NaCl concentration unlike the other two strains.

An interesting observation made with the *Vibrio* sp. was that all the strains of *Vibrio* sp. survived well at 0% and 1% NaCl concentration. Among the three isolates, strain R42 showed similar levels of survival percentage at 0% (70.8%) and at 1% (70.1%) NaCl concentration whereas strain L146 showed very high survival percentage at 1% (84.5%) NaCl concentration and strain F10 recorded maximum survival at 0% NaCl concentration. Comparatively at higher concentration of NaCl, survival of *Vibrio* sp. was observed to be considerably affected.

Among the *Acinetobacter* sp., strain L114 survived well at concentration of 0 - 6% NaCl whereas strain F88 could survive well at concentration of 0 - 1% NaCl. Strain L114 showed maximum survival percentage at 1% NaCl while F88 could record a maximum survival percent at 0% NaCl concentration. These two strains could not survive at higher NaCl concentrations.

3.6.7. Interaction of environmental factors on the growth of spoilage bacteria:

Bacterial cells of known number were grown at different temperatures (5°, 15° 30° and 45°C) with various concentrations of NaCl (0, 1, 3, 6, 10 and 15%) and various pH levels (pH 2, 4, 6, 8, 10 and 12) and the growth was measured after 48 h at 600 nm.

From the results it is clear that all the bacterial isolates could grow at pH 6 - 10 at all the temperatures and at moderate sodium chloride concentrations (1 - 10%) (Fig.21). However the maximum growth for all the isolates, except L146, was found to be at 30°C with a NaCl concentration of 1 and 3% and at pH 6 and 8. All the strains of Pseudomonas showed a requirement of pH 8 with 1% NaCl and and 30°C. Similarly, Acinetobacter showed a common requirement of 30°C and 1% NaCl, but varied in pH requirement (6 and 8). However, all Vibrio did not show any specific pattern of requirement except in pH, where they showed maximum growth at pH 6. The strains of Acinetobacter did show agreement in preference to concentration of NaCl and temperature, while they differed in pH requirement. All the strains showed no growth at all temperatures at lower and higher pH (pH 2, 4 and 12) and at 15% NaCl.

The influence of various environmental parameters on the growth pattern of Pseudomonas sp. R8 and Vibrio sp. R42 isolated from stored prawns at room temperature, is presented in Fig.21 a,b. These strains behaved differently. Pseudomonas sp. (R8) could grow at lower temperatures (5 and 15°C) at NaCl concentration of 1 - 6% and pH of 6 - 10 and at a higher temperature (45°C) with NaCl concentration between 1 and 10%. Whereas at 30°C it recorded growth in the

medium even without NaCl. The maximum growth at all the temperatures was observed at pH 8. However at 5° and 30°C the maximum growth was at 1% NaCl and at 15° and at 45°C it was at 6% NaCl. Increase of temperature showed influence on the tolerance to NaCl concentration (Fig.21 a).

Vibrio sp. (R42) showed entirely a different pattern (Fig.21 b). This strain showed growth at all temperatures between pH 6 - 10, but varied in NaCl requirement. It recorded growth at 0 - 10% NaCl concentrations at 5°, 15° and 30° while it could grow at 1 - 10% NaCl at 45°C. However an alkaline pH (pH 10) and higher temperature (45°C) affected the growth of Vibrio R42. The maximum growth was not recorded at a particular level of pH, NaCl and temperature. At 5°C, it was at pH 8 and 3% NaCl, at 15° and 30°C, it was at pH 6 and 3% NaCl concentration. Higher temperature (45°C) and pH 8 altered the requirement of NaCl concentration (6%) while growing to the maximum. Both the isolates did not show growth at lower pH 2 and 4 and higher alkaline pH 12 and also at 15% NaCl concentration, at all temperatures.

The isolates selected from samples stored at 4°C, when tested, showed a different pattern of relationship with environmental parameters. All the isolates Pseudomonas L97 (Fig.21 c), Acinetobacter L114 (Fig.21 d) and Vibrio L146 (Fig.21 e) could grow at all the temperatures tested, at a pH range of 6 - 10 and with NaCl concentration of 0 - 10%. They showed good growth in the medium without NaCl.

Vibrio sp. L146 showed maximum growth at 15°C with 3% NaCl and pH 6, while Pseudomonas sp. L97 and Acinetobacter sp. L114 recorded maximum growth at 30°C with 1% NaCl. But they differed in the requirement of pH, as the former preferred pH 8 and the latter, a pH 6. However, the percentage of growth varied with various combinations of NaCl, pH and temperature.

Vibrio sp. L146 showed a preference of <3% NaCl, pH 6 and lower temperatures (5° and 15°C) for maximal growth when compared with Pseudomonas sp. L97 which preferred lesser NaCl concentration (<3%) at lower temperatures (5° and 15°C) and moderately high NaCl concentration (6 - 10%) at 30° and 45°C and at alkaline pH levels. Such an influence was not noticed with Acinetobacter sp. L114. However, it showed preference towards pH 6, temperature <30°C and NaCl concentration of <3%.

At all the temperatures (<30°C) Vibrio L146 showed maximum growth at pH 6 with 3% NaCl whereas at 45°C, the pH requirement was increased to pH 8. The requirement of NaCl for maximum growth of Pseudomonas L97 differed in various temperatures. At 5° and 30°C, it preferred 1% NaCl concentration and pH of 8, whereas at 45°C, it was 6% NaCl with the same pH 8, while at 15°C, it was 3% NaCl with a pH 6. Similar to Vibrio (L146) and Pseudomonas (L97),

Acinetobacter (L114) did not show any regular pattern in preference to pH and NaCl at all temperature tested. At 5° and 30°C, it could grow to a maximum at 1% NaCl at a pH of 6. Whereas at 15°C, maximum growth was at 3% NaCl concentration and pH 8.

The growth pattern of the bacterial strains selected from the samples stored at -18°C, tested for the influence of temperature, pH and NaCl concentration on growth are presented in Fig.21 f-h. All the isolates showed growth at all the temperatures and at pH 6-10 and they varied in their NaCl requirement. Vibrio (F10) showed growth between 0-10% NaCl, whereas Acinetobacter (F88) and Pseudomonas (F153) showed growth at <3% NaCl at 30°, 15° and 5°C. At 45°C Acinetobacter (F88), 1-10% of NaCl and Pseudomonas (F153) preferred higher concentration of NaCl (6-10). It was observed that all the isolates showed maximum growth at 1% NaCl and at 30°C, while difference in pH requirement was noticed. Vibrio (F10) required pH 6, whereas Acinetobacter (F88) and Pseudomonas (F153) required pH 8 for maximal growth.

Optimum growth of Vibrio (F10) was influenced by all the parameters tested (Fig.21 f). At 5°, 15° and 30°C, maximum growth was at pH 6. But NaCl preference varied. Thus it required 6% NaCl at 5°C, 3% NaCl and 15°C and 1% NaCl at 30°C. Increase of temperature showed a requirement of reduced level

of NaCl concentration for this bacteria. However at 45°C, requirement of both pH and NaCl concentration was observed to be raised to pH 8 and NaCl 10% respectively.

Acinetobacter (F88) showed growth at 0-3% NaCl at 5°, 15°, 30°C and at 1-10% NaCl at 45°C (Fig.21 g). The maximum growth was at 5°C, at 1% NaCl with pH 6 and at 15° and 30°C, at 3% and 1% NaCl concentration respectively with a pH of 8. Interestingly, Acinetobacter (F88) showed maximum growth at 3% NaCl concentration with pH 10 which was not recorded with any other isolates tested. When tested at temperatures below 30°C, this isolate (F88) did not show growth at or above 6% NaCl, whereas at 45°C NaCl requirement was found to be increased to 10%. The impact of increased temperature on the requirement of NaCl was noticed for this bacteria (Acinetobacter F88).

Pseudomonas (F153) did not show growth at 0% NaCl at 5°, 15° and 45°C, while at 30°C it showed poor growth (8%) (Fig.21 h). At 5°C, the maximum growth was recorded at a pH of 6 and 1% NaCl. But at 15°C, pH 8 and 3% NaCl supported the maximum growth. Again at 30°C, the requirement of NaCl decreased to 1% while the preference of pH was same. For Pseudomonas (F153) at this temperature, optimal pH was between pH 6-8. At higher temperatures the preference of NaCl was found to be increased to 6%. This strain recorded

growth at 1-3% NaCl at 5°C, 1-10% NaCl at 15°C, 0-3% NaCl at 30°C and 6-10% NaCl at 45°C. The increase in temperature showed a definite influence on the NaCl concentration requirement.

3.6.8. Generation time of selected strains of spoilage bacteria:

The generation time of eight bacterial strains of Vibrio (R42, L146 and F10), Pseudomonas (R8, L97 and F152) and Acinetobacter (L114 and F88) was determined using ZoBell's broth and flesh broth.

Results presented in Table 15 suggest that the generation time of the strains was not similar in both the media. The generation time of Pseudomonas sp. R8 was short (33 min.) in the flesh broth whereas it was almost double in the ZoBell's broth (67 min.). However Vibrio sp. R42 recorded a reverse, showing 23 minutes in ZoBell's broth and 47.5 minutes in flesh broth. Similarly, Pseudomonas sp. L97 has taken 53 minutes for doubling the population in flesh broth and 30 minutes in ZoBell's broth. Acinetobacter sp. L114 recorded a very short generation time (30.7 min.) in flesh broth whereas in ZoBell's it was nearly three times that of flesh broth (90 min.). Vibrio sp. L146 showed more or less very similar time, 38.7 and 30 minutes in flesh broth as well as in ZoBell's broth respectively. Vibrio sp. F10 showed longer generation time (52 min.) in flesh broth and a

shorter generation time in ZoBell's broth (30 min.). Acinetobacter sp. F88 showed shorter generation time (31 min.) in flesh broth and a longer one in ZoBell's broth (60 min.). Pseudomonas sp. F153 also showed a shorter generation time in flesh broth (37 min.) and a longer one in ZoBell's broth (90 min.).

In general strains of Vibrio sp. showed a shorter generation time in ZoBell's broth than flesh broth. But strains of Acinetobacter sp. showed shorter generation time in flesh broth than in ZoBell's broth. Similarly strains of Pseudomonas sp., except one strain L97, showed shorter generation time in flesh broth than in ZoBell's broth.

3.7. INTERRELATIONSHIP AMONG VARIOUS BACTERIA (MIXED POPULATION) ON GROWTH

The relationship of growth between two bacterial genera (Vibrio Vs Pseudomonas; Vibrio Vs Acinetobacter; Pseudomonas Vs Acinetobacter) or more than two genera (Vibrio Vs Pseudomonas Vs Acinetobacter) was evaluated by inoculating a known number of bacteria in a liquid medium, allowing them to grow at a particular temperature ($28\pm2^{o}C$) for a definite period and recovering the cells by plating procedure. Two strains (Vibrio sp. R42 and Pseudomonas sp. R8) which were isolated from the samples stored at room temperature

were inoculated at various concentrations and the percentage recovery was made. In the second set of combinations, species of Vibrio (L146), Pseudomonas(L97) and Acinetobacter (L114) isolated from the samples stored at a lower temperature ($4^{o}C$) were selected and inoculated together at various concentrations, the percentage recovery was calculated and the results are presented in Tables 16-20. The data were analysed in various combinations and the observations were as below.

The data indicate that regardless of various concentrations of initial inoculum, Vibrio (R42) was able to supress the level of Pseudomonas (R8) population (Table 16), when they were mixed at various concentrations, inoculated into flesh broth and allowed them to grow.

Unlike isolates of Vibrio and Pseudomonas collected from the samples stored at $28\pm2^{o}C$, strains of Vibrio, Pseudomonas and Acinetobacter obtained from the samples stored at $4^{o}C$, when mixed in different proportions and combinations, showed interesting observations. It could be seen from the Table 17 that when Vibrio (L146) and Pseudomonas (L97) were mixed at various concentrations and inoculated into flesh broth, they were recovered at equal proportion. However, at equal concentration of their inoculum (10^{4} and 10^{8} cells/ml), Pseudomonas (L97) was found to show a slight dominance over

Vibrio (L146), whereas, at lower concentration of inoculum (10^2) the recovery was 50% each. This incidate that the size of the initial inoculum could have influenced the final population and hence none was found dominating to a maximum level.

Vibrio (L146) when mixed with Acinetobacter (L114), in equal concentration of initial inoculum the percentage recovery was in similar proportion (Table 18). Both the strains maintained their higher concentration with respect to the higher level of initial inoculum except at a concentration of 10^4 of Vibrio and 10^2 of Acinetobacter, where 35% and 65% of them respectively were recovered.

Pseudomonas (L97) and Acinetobacter (L114) strains showed a different pattern of growth when they were mixed at various concentrations. It could be seen from the Table 19 that Pseudomonas (L97) population was dominant over Acinetobacter (L114), when initial inoculum was in 1:1 ratio at concentration of 10^2 and 10^4 cells/ml, whereas, at a high concentration of 10^8 cells/ml recovery was 50% each. Interestingly, it was observed that Acinetobacter L114 could compete with Pseudomonas (L97) in maintaining an equal number of population in the flesh broth, when they were added in the ratio of 2:1 at a concentration of 10^4 and 10^8 cells/ml. At the same time, Pseudomonas (L97) when added at higher concentrations of 10^4 and 10^8 cells/ml with lower concentrations

of Acinetobacter (L114), 10^2 and 10^4 cells/ml respectively, Pseudomonas showed a clear dominance over Acinetobacter. However, when an initial inoculum concentration was reverse i.e. 10^8 cells/ml of Acinetobacter against 10^2 cells/ml of Pseudomonas, Acinetobacter showed dominance marginally over Pseudomonas.

From the Table 20 it could also be observed that when three species, viz. Vibrio (L146), Pseudomonas (L97) and Acinetobacter (L114) isolated from samples stored at lower temperature (4^oC), were added initially at equal concentrations of 10^2, 10^4 and 10^8 cells/ml each i.e. $10^2:10^2:10^2$, $10^4:10^4:10^4$ and $10^8:10^8:10^8$, the growth study demonstrated the dominance of Pseudomonas over others. At lower concentration of equal initial inoculum, the dominance of Pseudomonas (50%) followed by Acinetobacter (30%) and Vibrio (20%) was observed. Similarly at higher concentrations, a high percentage of Pseudomonas was recorded. At this concentrations Vibrio was recorded only in a minimal level (10%).

When the concentration of the inoculum of Vibrio was maintained low (10^2) and Pseudomonas and Acinetobacter were added at various concentrations (10^2,10^4 and 10^8), it was observed that even at a low concentration, Pseudomonas could show dominance. At the same time, the recovery of Acinetobacter was proportional to the size of initial inoculum

recording a percentage recovery, second to Pseudomonas and at one instance in the combination of 10^2:10^2:10^8 its recovery was 45% equalling Pseudomonas. Vibrio showed a low percentage of recovery. When the inoculum of Pseudomonas was maintained at 10^2 cells/ml and Vibrio and Acinetobacter were raised to various levels (10^2-10^8), the percentage recovery of Pseudomonas was high, except where the inoculum of Vibrio and Acinetobacter were 10^8 cells/ml. In the combination of 10^8:10^2:10^8, recovery of Pseudomonas was minimum (25%). Similarly when inoculum of Acinetobacter was at 10^2 and Vibrio and Pseudomonas were added at various proportions (10^2-10^8 cells/ml), the maximum recovery of Pseudomonas showing dominance followed by Acinetobacter was noticed, except at few instances were the inoculum of Vibrio was very high (10^8) and dominant second to Pseudomonas.

Further, the results on the combination of higher concentration (10^4) of Vibrio with various concentrations of Pseudomonas and Acinetobacter also indicated the dominance of Pseudomonas. The recovery of Acinetobacter was more, in general, than Vibrio, except in one instance when the inoculum size of the latter was higher. Similarly, while the inoculum of Pseudomonas was kept constant (10^4) and the concentration of the inoculum of Vibrio and Acinetobacter were altered, from 10^2-10^8, Pseudomonas was recovered at a higher proportion. Vibrio was recovered more in number than Acinetobacter when

the inoculum were mixed at $10^8:10^4:10^2$ range. In other combinations of higher concentration of Vibrio, the recovery was either same or less than Acinetobacter. While inoculum of Acinetobacter was maintained at 10^4 concentration and Pseudomonas and Vibrio were at various proportions, Pseudomonas was dominant. Whereas in the combinations where Pseudomonas was added in maximum level of concentration (10^8) the Vibrio was reduced to a very low (5%) level. Acinetobacter maintained second dominance in all combinations, except at $10^8:10^8:10^4$ of Vibrio, Pseudomonas and Acinetobacter respectively.

The concentration of the inoculum of Vibrio was raised to 10^8 and was added with various concentrations of Pseudomonas and Acinetobacter (10^2-10^8). In all the combinations, irrespective of the size of the initial inoculum, Pseudomonas was recovered in large number, except at the combination of $10^8:10^2:10^8$ where Acinetobacter was recovered at a maximum level (45%) followed by Vibrio (30%) and Pseudomonas (25%). When the initial inoculum of Pseudomon was maintained at 10^8 cells/ml and added with various concentrations of Vibrio and Acinetobacter, Pseudomonas showed dominance (65 to 75%). At some instances Vibrio was recovered in meagre numbers (0-5%). Acinetobacter maintained second dominance in all combinations, except at three instance where their initial inoculum concentration was low when compared to that of Pseudomonas and Vibrio.

When the concentration of the initial inoculum of Acinetobacter was made constant (10^8 cells/ml) and mixed with Vibrio and Pseudomonas at various concentrations, the dominance of Pseudomonas was noticed, except at one instance where the combination was 10^8:10^2:10^8 of Vibrio, Pseudomonas and Acinetobacter respectively. Eventhough, the inoculum of Acinetobacter was added at a higher (10^8) concentration, it could be recovered only at a population level second to Pseudomonas. The results indicate that Pseudomonas was dominant in all these instances except at one combination of 10^8:10^2:10^8 of Vibrio:Pseudomonas:Acinetobacter where its initial inoculum size was very low (10^2 cells/ml). Vibrio could not be recovered at one instance in these combinations when it was added at 10^2 cells/ml with 10^8 cells/ml of Pseudomonas and Acinetobacter.

Further, when the initial inoculum of two populations were maintained constant and one altered, Pseudomonas showed dominance and was followed by Acinetobacter and Vibrio. When Vibrio and Pseudomonas were maintained at same level (10^2:10^2 or 10^4:10^4) and Acinetobacter varying from 10^2 to 10^8, Pseudomonas showed dominance over the other two and Acinetobacter over Vibrio. Whereas, when the concentration of Vibrio and Pseudomonas were high (10^8:10^8) and the concentration of the inoculum of Acinetobacter was low (10^2 and 10^4) the percentage recovery of Acinetobacter was

less than 10%. Similarly, when the Pseudomonas and Acinetobacter inoculum were maintained constant (10^8:10^8) and Vibrio varied (10^2:10^4), Pseudomonas was dominant and the recovery of Vibrio was very low (0-5%).

When inoculum of Vibrio and Acinetobacter were maintained constant and Pseudomonas was altered, similar to all other combinations, Pseudomonas was found to show dominance over the other two at all combinations, except at one instance (10^8:10^2:10^8 of Vibrio:Pseudomonas:Acinetobacter) where it was recovered at a lesser number than Acinetobacter and Vibrio.

In general, the results obtained for the various combinations showed the dominance of Pseudomonas at all level irrespective of the initial inoculum concentration of these three isolates Vibrio, Pseudomonas and Acinetobacter isolated from samples stored at 4°C.

3.8. STATISTICAL ANALYSIS

The inter relationships between spoilage indices and THB are presented in Table 21. A highly significant positive correlation could be observed between spoilage indices themselves and with THB at all the three storage temperatures.

From the Table 22 it can be seen that significant negative correlation exist between spoilage indices (THB, TMA, NH_3 and pH) and proximate components (protein, carbohydrate, lipid and ash) excluding moisture which showed significant positive correlation.

Spoilage indices showed varied nature of correlation with the different major genera encountered during storage (Table 23). However, at 28±2°C, all the spoilage indices were positively correlated with Vibrio and negatively correlated with other genera (Pseudomonas, Acinetobacter and Micrococcus) at significant levels. At 4°C the correlation between spoilage indices and the major genera was not significant. Significant negative correlation between Vibrio and NH_3 and pH, and positive correlation between TMA and Micrococcus were observed at -18°C.

It can be seen from the Table 24 that all the spoilage indices recorded significant positive correlation with proteolytic, lipolytic and ureolytic groups at 28±2°C. Although, they showed positive correlation with the above said groups, the level of significance was not high at lower temperatures. Only TMA showed significant positive correlation with ureolytic at all the three temperatures.

Protein, carbohydrate, lipid and ash showed significant positive correlation with each other and all uniformly recorded

significant negative correlation with moisture at all the three temperatures (Table 25) except at one instance, at -18°C, where lipid recorded negative correlation with ash.

Nature of correlation between proximate components and spoilage flora was found to vary at the three temperatures tested (Table 26). At 28±2°C, all the proximate components, except moisture, showed significant correlation with Pseudomonas, Acinetobacter and Micrococcus and significant negative correlation with Vibrio. Whereas, moisture recorded positive correlation with Vibrio and negative correlation with others significantly. At 4°C no such significant relationship was observed. Interestingly lipid and ash showed significant positive correlation with Vibrio at -18°C.

From the Table 27 it can be seen that in general, a significant negative correlation existed between proximate components, excluding moisture, and ureolytic group at all temperatures. Moisture showed significant positive correlation with ureolytic at all the temperatures. Similar significant correlation was not observed for other hydrolytic groups.

Pseudomonas, Acinetobacter and Micrococcus had significant positive correlation with each other and all recorded a significant negative correlation with Vibrio at 28±2°C (Table 28). Interestingly no such significant relationship was observed at lower temperature.

Vibrio showed significant positive correlation with proteolytic, lipolytic and ureolytic bacteria at 28±2°C. Pseudomonas and Acinetobacter showed significant negative correlation with proteolytic, lipolytic and ureolytic bacteria at 28±2°C. At lower temperatures the relationship was not significant in most of the cases as is seen from the Table 29. However Acinetobacter maintained significant negative correlation with proteolytic group at lower temperatures.

Among the hydrolytic enzyme producing bacteria, proteolytic bacteria showed significant positive correlation with lipolytic and amylolytic group (Table 30.). Lipolytic bacteria maintained significant positive correlation with ureolytic at 28±2°C. No such relationship was observed at 4°C and -18°C in a significant level.

Table 1. Pattern of Marine Products Exports from India (1976-'77 to 1983-'84)

Items		1976-'77	1977-'78	1978-'79	1979-'80	1980-'81	1981-'82	1982-'83	1983-'84
Frozen shrimp	Q:	49375	50067	51162	51068	51358	52180	55002	54444
	V:	167.99	158.3	194.78	211.25	201.78	247.95	316.15	314.81
Frozen frog legs	Q:	3020	2899	4087	2926	3452	4065	1896	2428
	V:	7.17	6.67	9.92	6.34	8.41	11.2	4.72	6.68
Frozen Lobster tails	Q:	512	637	732	560	610	694	749	648
	V:	3.3	3.95	5.14	4.06	3.48	5.15	6.86	5.15
Frozen Cuttlefish and Fillets	Q:	752	977	1062	1551	1220	1819	2305	1526
	V:	1.26	1.42	1.91	4.29	1.94	4.12	6.27	3.38
Frozen squids	Q:	566	654	2755	2244	1705	1387	1222	2050
	V:	0.68	0.68	3.69	2.96	1.97	1.74	2.0	2.69
Fresh/Frozen fish	Q:	2753	3140	16757	22629	8769	6760	12847	22573
	V:	2.94	3.17	9.75	13.32	8.24	9.62	18.87	29.1
Canned shrimp	Q:	124	129	197	231	281	82	65	41
	V:	0.46	0.57	0.88	1.13	1.13	0.42	0.44	0.24
Dried fish	Q:	5372	4230	6909	3357	3887	1022	2597	6492
	V:	2.15	2.26	3.66	1.57	2.44	0.74	2.09	5.35
Dried shrimp	Q:	29	235	19	19	113	55	90	28
	V:	0.02	0.17	0.03	0.02	0.12	0.08	0.07	0.05
Sharkfins and Fish Maws	Q:	292	289	416	341	390	358	156	250
	V:	1.81	2.49	3.32	2.68	3.64	3.73	1.99	3.27
Misc. items	Q:	3955	2710	2798	1475	3806	1683	1246	2211
	V:	1.33	1.25	1.53	1.22	1.69	1.26	1.89	2.3
Total	Q:	66750	65967	86894	86401	75591	70105	78175	92691
	V:	189.12	180.95	234.62	248.82	234.84	286.0	361.36	373.02

Q: Quantity in tonnes V: Value in Rs Crores

Table 2. Bacterial population of freshly caught prawns (Whole) from various regions of the world

Species	Region	Place of collection	Incubation temperature	Bacterial population*	Reference
	Temporate waters				
Penaeus aztecus	Gulf of Mexico	Pond	28°C	10^4-10^7/g	Vanderzant et al. 1970
Penaeus setiferus	-do-	-do-	5°C	10^3-10^6/g	Vanderzant et al. 1970
Penaeus aztecus	-do-	-do-	28°C	10^4-10^6/g	Vanderzant et al. 1971
Penaeus aztecus Penaeus vannamei Penaeus setiferus Penaeus oxidentalis	-do-	-do-	28°C	10^4-10^6/g	Vanderzant et al. 1973
Penaeus aztecus Penaeus setiferus	-do-	Sea	25°C	10^4-10^6/g	Cobb et al. 1976
Penaeus setiferus	-do-	-do-	28°C	10^4-10^5/g	Cobb et al. 1977
Penaeus stylirostris Penaeus vannamei Penaeus setiferus	-do-	Pond	25°C	10^3-10^4/g	Christopher et al. 1978.

Cont.....

Table 2 Continued

Species	Region	Place of collection	Incubation temperature	Bacterial population*	Reference
	Tropical waters				
Palaemon sp.	India	Sea	28°C	10^4-10^7/g	Pillai et al. 1961
Metapenaeus dobsoni	-do-	-do-	28°C	10^5-10^6/g	Velankar et al. 1961
Penaeus indicus	-do-	-do-	28°C	10^4-10^5/g	Pillai et al. 1965
Penaeus indicus	-do-	Backwater	28°C	3.16 x 10^6/cm^2 (body surface) 102.2 x 10^6/g (gill) 164.23 x 10^6/g (Intestine)	I.C.A.R. Project Report, 1983
Penaeus monodon	-do-	-do-	28°C	6.5 x 10^6/cm^2 (body surface) 160 x 10^6/g (gill) 350.14 x 10^6/g (intestine)	I.C.A.R. Project Report, 1983

*Previous literature

Table 3. Protein and lipid content of halo and opaque area in the flesh agar medium tested (average of 10 samples)

Flesh agar media	Substrate	Content in the opaque zone (mg/g)	Content in the halo zone (mg/g)	Difference (mg/g)	Standard error	% of the content reduced
A. Medium prepared after boiling and removal of coagulated protein	Protein	15	13.5	1.5	±0.0071	10
	Lipid	4.7	1.9	2.7	±0.0003	58.8
B. Medium prepared directly	Protein	42.3	36.3	6.1	±0.0398	14.3
	Lipid	6.5	2.3	4.2	±0.0033	65.1

Table 4. Organoleptic assessment of Penaeus indicus stored at room temperature (28±2°C)

Storage period (hours)	Whole	Headless	Peeled and undeveined	Peeled and deveined
1	2	3	4	5
0	Prawns were fresh with characteristic odour of fresh prawns, eyes normal, gills normal with red colour, body firm, flesh firm and were consistant and white in colour.	Prawns were fresh with characteristic odour of fresh prawns, flesh firm and consistant and white in colour.	Prawns were fresh with characteristic odour of fresh prawns flesh firm and consistant and white in colour.	Prawns were fresh with characteristic odour and colour flesh firm and consistant.
4	Eyes were shrunken, rostrum and telson blackened, gills pale in colour, head red in colour, slight putrid odour, flesh slightly darkened and retained slightly finger impression.	Slight off odour, held finger impressions, slightly reddish in colour, lost freshness.	Slight loss of freshness in colour, no odour, slight loss of texture, held finger impressions.	Slight loss of freshness in colour, slight putrid odour, flesh held slightly finger impressions.
8	Eyes were shapeless, gills white juicy in nature, yellowish slime throughout the body head slightly blackened, intense putrid odour with ammoniacal smell, flesh paste like and was not firm to touch.	Intense putrid odour, yellow slime on the body surface, flesh lost firmness, became paste like, shell became red in colour.	Spoilage odour, flesh lost firmness became pasty and red in colour.	Loss of freshness, intense putrid odour, yellow slime over the reddened flesh which lost firmness and became pasty.

Table 4 continued

1	2	3	4	5
12	Eyes shapeless, gills white juicy, marked yellowish slime throughout the body surface, head area blackened completely, flesh juicy and slightly blackened, intense putrid odour, gut disfigured.	Intense putrid odour marked yellow slime formed on the body surface, shell intense red colour flesh was juicy.	Intense putrid odour yellow slime all over the body surface along with red colour of the body flesh juicy and lost firmness.	Intense putrid odour, thick yellow slime over the reddened flesh, flesh was juicy.
24	Eyes disfigured, gills white and juicy, marked yellowish slime throughout the body, head blackened completely, flesh juicy and slightly blackened, putrid odour was in high degree.	Intense putrid odour with smell of ammonia, thick yellow slime on the body surface, flesh juicy. Shell became red in colour.	Intense putrid odour, thick yellow slime all over the body surface, flesh turned completely juicy.	Intense putrid odour, thick yellow slime over the reddened flesh, flesh was completely juicy.

Table 5. Organoleptic assessment of *Penaeus indicus* stored at $4^{o}C$

Storage period (days)	Whole	Headless	Peeled and Undeveined	Peeled and Deveined
0	Fresh with characteristic colour and odour of fresh prawns, eyes normal, gills reddish fresh, body firm and was consistent.	Fresh with characteristic colour and odour of fresh prawns flesh firm and was consistent.	Fresh with characteristic colour and odour of fresh prawns, flesh firm and was consistent.	Fresh with characteristic colour and odour of fresh prawns, flesh firm and was consistent.
2	Slight loss of freshness in colour and fresh odour. Eyes normal, gills normal slightly red in colour, body firm and was consistent.	Fresh with characteristic colour and odour of fresh prawns, firm and was consistent.	Fresh with characteristic colour and odour of fresh prawns, flesh firm and was consistent.	Fresh with characteristic colour, odour and appearance of fresh prawns was consistent.
5	Eyes shrunken, head slightly blackened, gills pale in colour light yellowish slime all over the body surface, slight putrid odour, flesh slightly inconsistent and became darken in colour.	Slight loss of freshness in colour, light yellowish slime all over the body surface, flesh slightly inconsistent with loss of firmness. No putrid odour but contained slight ammoniacal odour.	Slight loss of freshness in colour and texture, no intense odours was observed.	Fresh, no odour, flesh firm and was consistent.

Cont.....

Table 5 continued

1	2	3	4	5
10	Eyes shrunken and disfigured, yellowish slime throughout the body, flesh not firm to touch and became pasty, gut disfigured, intense putrid odour was persistent.	Putrid odour, yellow slime all over the body surface, flesh lost firmness and paste like, shell became red in colour.	Spoilage off odours, surface became slightly blackened, firmness of flesh lost and became paste like.	Slight putrid odour, flesh not firm to touch and was slightly blackened.
15	Eyes shrunken and disfigured thick yellowish slime throughout the body, flesh inconsistent, pasty, body completely became black with intense unbearable putrid odour.	Intense putrid odour, thick yellowish slime on the surface, flesh lost firmness, juice like, shell became red in colour.	Intense putrid odour, surface became slightly blackened, flesh lost firmness and became juice like.	Intense putrid odour, flesh paste like and turned slightly black.
30	Became juice like in the form of a liquid, hence discarded.	Completely spoiled, intense unbearable putrid spoilage odour, flesh juice like, shell red in colour and became thin.	Intense unbearable putrid odour, flesh juice like, whole body became black in colour.	Intense putrid odour, flesh became juicy and completely blackened.

Table 6. Organoleptic assessment of *Penaeus indicus* stored at -18°C

Storage period (days)	Whole	Headless	Peeled and Undeveined	Peeled and Deveined
1	2	3	4	5
0	Fresh with characteristic colour, odour and appearance of fresh prawns, eyes normal gills normal with fresh colour flesh firm to touch and was consistent.	Fresh with characteristic colour, odour and the appearance was of fresh prawn.	Fresh with characteristic colour, odour and appearance was of fresh prawn.	Fresh with characteristic colour, odour and appearance was of fresh prawn.
4	-do-	-do-	-do-	-do-
12	-do-	-do-	-do-	-do-
21	-do-	-do-	-do-	-do-
34	Slight loss of freshness eyes shrunken, gill pale in colour, flesh paste like loss of freshness in colour, exhibited slight putrid odour.	-do-	-do-	-do-

Cont.....

Table 6 continued

1	2	3	4	5
60	Slight loss of freshness, eyes disfigured, gills pale in colour, flesh became inconsistent, pasty and slight putrid odour.	Slight loss of freshness in colour, odour and appearance, flesh became slightly inconsistent with loss of firmness to touch and very light putrid odour.	Slight loss of firmness in colour, flesh slightly reddish in colour with light putrid odour and showed loss of firmness.	Slight loss of firmness, flesh slightly reddish, very light putrid odour, firm to touch became inconsistent.
100	Freshness lost, eyes disfigured, gills and flesh juice like, blackened body surface and intense putrid odour.	Loss of freshness, became reddish in colour, intense putrid odour, flesh was paste like.	Loss of freshness in colour and appearance, intense putrid odour, flesh reddened and became juicy in nature.	Loss of freshness, flesh reddish in colour, intense putrid odour and turned pasty.
210	Eyes completely disfigured, gills and flesh juice like, body completely blackened, displayed intense putrid odour.	Body reddish in colour, intense putrid odour, flesh became juicy.	Intense putrid odour flesh reddish in colour and was juicy.	Intense putrid odour, flesh reddish in colour and became juice like.

Table 7. Generic distribution of potential spoilers isolated from spoiled prawns stored under various temperatures.

Genera	No. of isolates tested	No. of spoilers	Percentage of spoilers	Percentage of spoilers among the total positive strains
Pseudomonas	77	46	59.7	33.3
Acinetobacter	50	29	58.0	21.0
Vibrio	40	34	85.0	24.7
Micrococcus	20	9	45.0	6.5
Bacillus	15	9	60.0	6.5
Enterobacteriaceae	9	5	55.6	3.6
Corynebacterium	5	3	60.0	2.2
Alcaligenes	2	2	100.0	1.5
Aeromonas	1	1	100.0	0.7
Gram -ve	179	117	65.4	84.8
Gram +ve	40	21	52.5	15.2
Total	219	138	63.0	100.0

Table 8. Generic composition of TMAO reducing bacteria isolated from prawns stored at various temperatures.

Genera	Total No. of isolates	Total No. of TMAO reducers	Percentage of TMAO reducers
Pseudomonas	65	58	89.2
Acinetobacter	38	35	92.1
Vibrio	37	37	100.0
Bacillus	16	15	93.8
Micrococcus	13	11	84.6
Enterobacteriaceae	6	5	83.3
Corynebacterium	2	2	100.0
Alcaligenes	1	1	100.0
Gram -ve	147	136	92.5
Gram +ve			
Total	178	164	92.1

Table 9. Effect of temperature on the growth of spoilage flora grown in two different media

Sl. No.	Organism	Temperature (°C)											
		Flesh broth						ZoBell's broth					
		-15	5	15	30	45	60	-15	5	15	30	45	60
1	*Pseudomonas* R8	-	21.8	29.3	67.4	100.0	-	2.5	7.4	40.1	100.0	42.6	-
2	*Vibrio* R42	-	48.0	53.4	100.0	3.9	-	1.1	1.7	83.7	100.0	71.6	-
3	*Pseudomonas* L97	-	23.5	33.2	94.5	100.0	-	-	-	12.4	100.0	26.0	-
4	*Acinetobacter* F114	-	27.6	32.0	100.0	23.4	-	0.4	0.7	70.3	100.0	53.7	-
5	*Vibrio* L146	-	59.8	100.0	89.8	4.8	-	-	-	13.4	100.0	29.1	-
6	*Vibrio* F10	-	7.9	75.5	100.0	6.2	-	-	0.9	34.4	100.0	33.1	-
7	*Acinetobacter* F88	-	8.2	16.5	100.0	4.1	-	-	-	28.8	100.0	92.3	-
8	*Pseudomonas* F152	-	27.5	36.3	100.0	77.5	-	-	0.5	8.0	100.0	8.3	-

Growth expressed as percentage of growth index

Table 10. Effect of pH on the growth of spoilage flora grown in two different media

Sl. No.	Organism	Flesh broth						ZoBell's broth					
		2	4	6	8	10	12	2	4	6	8	10	12
1	*Pseudomonas* R8	-	-	28.7	53.0	100.0	-	-	1.6	66.7	83.3	100.0	-
2	*Vibrio* R42	-	-	81.8	100.0	13.6	-	-	1.4	49.6	100.0	20.0	-
3	*Pseudomonas* L97	-	3.2	100.0	98.1	49.1	-	-	2.7	72.2	100.0	45.8	-
4	*Acinetobacter* L114	-	-	50.6	62.0	100.0	-	-	0.4	100.0	82.1	40.9	-
5	*Vibrio* L146	-	-	42.4	100.0	51.2	-	-	0.2	100.0	30.6	15.1	-
6	*Vibrio* F10	-	-	73.7	100.0	80.3	-	-	0.6	100.0	96.9	72.5	-
7	*Acinetobacter* F88	-	3.2	32.3	96.8	100.0	-	-	-	100.0	94.9	44.4	-
8	*Pseudomonas* F152	-	-	100.0	94.7	80.3	-	-	0.7	60.9	100.0	81.2	-

Growth expressed as percentage of growth index

Table 11. Effect of sodium chloride concentrations on the growth of spoilage flora grown in two different media

Sl. No.	Organism	NaCl(%) Flesh broth						ZoBell's broth					
		0	1	3	6	10	15	0	1	3	6	10	15
1	*Pseudomonas* R8	53.7	85.3	100.0	91.2	36.0	-	35.9	49.6	100.0	42.7	15.4	-
2	*Vibrio* R42	10.1	82.2	81.1	100.0	19.5	-	45.8	45.6	46.1	100.0	30.6	-
3	*Pseudomonas* L97	49.6	28.7	100.0	41.1	20.9	-	48.4	100.0	43.4	41.8	8.2	-
4	*Acinetobacter* L114	8.7	100.0	38.4	19.6	14.7	-	100.0	83.7	44.9	75.5	9.3	-
5	*Vibrio* L146	16.5	81.7	74.2	100.0	1.1	-	53.9	75.0	100.0	34.6	5.8	-
6	*Vibrio* F10	26.9	89.3	89.3	100.0	8.6	-	34.1	80.3	100.0	63.7	-	-
7	*Acinetobacter* F88	6.8	100.0	6.9	5.7	4.6	-	49.8	100.0	88.2	9.2	-	-
8	*Pseudomonas* F152	100.0	81.4	97.1	94.3	65.7	-	74.2	100.0	76.3	65.0	44.3	-

Growth expressed as percentage of growth index

Table 12. Effect of temperature on the survival of spoilage bacteria.

Sl. No.	Organism	Temperature °C	Exposure time (minutes)							
			15	30	45	60	90	120	180	240
1	2	3	4	5	6	7	8	9	10	11
1	*Pseudomonas* R8	-15	ND	57.4	ND	48.8	38.2	18.2	10.1	5.2
		5	ND	100.0	ND	89.5	82.4	70.7	68.9	6.6
		45	98.2	92.2	85.3	77.6	68.4	66.4	ND	ND
		60	57.9	40.8	32.2	20.5	15.3	4.0	ND	ND
2	*Vibrio* R42	-15	ND	83.8	ND	55.6	46.5	35.8	20.2	3.2
		5	ND	100.0	ND	93.7	81.5	78.3	68.4	53.2
		45	92.6	91.3	87.1	85.3	76.2	70.2	ND	ND
		60	26.0	18.9	16.2	12.6	3.9	1.2	ND	ND
3	*Pseudomonas* L97	-15	ND	57.7	ND	45.6	30.2	22.4	15.8	3.2
		5	ND	100.0	ND	89.6	68.9	59.7	33.2	20.7
		45	97.7	91.9	82.8	75.7	71.1	68.9	ND	ND
		60	85.6	79.7	54.3	38.8	18.2	2.4	ND	ND
4	*Acinetobacter* L114	-15	ND	52.1	ND	45.8	32.9	28.4	14.6	7.46
		5	ND	61.4	ND	50.3	42.3	34.6	32.3	9.31
		45	100.0	92.5	90.2	88.8	56.8	48.7	ND	ND
		60	89.2	64.8	55.3	24.3	21.8	2.6	ND	ND

Cont.....

Table 12 Continued

1	2	3	4	5	6	7	8	9	10	1
5	*Vibrio* F146	-15	ND	88.8	ND	82.0	43.0	38.5	25.5	3.
		5	ND	92.9	ND	86.7	68.3	45.8	31.1	11.
		45	100.0	90.8	87.9	84.9	73.7	49.2	ND	ND
		60	10.6	10.2	8.4	3.9	2.6	0.2	ND	ND
6	*Vibrio* F10	-15	ND	88.5	ND	72.8	51.7	25.6	2.5	0.
		5	ND	92.9	ND	81.9	60.7	46.4	25.7	5.
		45	100.0	92.8	80.3	72.3	65.3	44.9	ND	ND
		60	5.5	0.6	0.0	0.0	0.0	0.0	ND	ND
7	*Acinetobacter* F88	-15	ND	53.6	ND	28.6	11.8	0.0	0.0	0.
		5	ND	77.4	ND	48.8	17.9	2.4	0.0	0.
		45	100.0	83.4	71.6	67.6	53.4	28.2	ND	ND
		60	64.2	59.3	47.1	35.7	11.8	0.0	ND	ND
8	*Pseudomonas* F152	-15	ND	85.0	ND	55.0	28.7	19.9	0.0	0.
		5	ND	95.9	ND	66.4	52.0	50.0	35.4	7.
		45	100.0	96.2	63.5	60.9	55.3	48.0	ND	ND
		60	82.4	62.5	51.3	18.9	2.9	0.6	ND	ND

Table 13. Effect of pH on the survival of spoilage bacteria

Sl. No.	Organism	Time (h)	pH					
			2.6	4.2	6.0	8.6	9.6	10.6
1	2	3	4	5	6	7	8	9
1	*Pseudomonas* R8	6	2.6	3.6	85.5	100.0	75.3	3.1
		12	1.7	2.1	77.4	93.2	72.9	1.7
		18	0.9	1.2	74.2	90.2	58.2	0.5
		24	0.0	0.6	70.4	88.5	51.4	0.3
		30	0.0	0.0	69.2	82.6	48.6	0.0
		36	0.0	0.0	68.8	80.1	48.0	0.0
2	*Vibrio* R42	6	8.2	14.8	100.0	79.5	67.9	3.6
		12	4.1	9.4	91.9	74.9	61.5	2.3
		18	0.6	1.7	85.6	73.8	60.5	0.6
		24	0.0	0.4	68.9	66.7	57.3	0.5
		30	0.0	0.0	65.2	55.4	41.2	0.0
		36	0.0	0.0	63.6	50.2	25.5	0.0
3	*Pseudomonas* L97	6	1.5	3.8	100.0	78.2	72.2	13.9
		12	0.5	2.5	83.2	74.2	58.4	4.0
		18	0.3	1.7	81.7	70.4	55.7	2.4
		24	0.0	0.1	77.2	59.9	51.5	0.6
		30	0.0	0.0	73.5	47.8	40.4	0.0
		36	0.0	0.0	72.4	41.4	38.6	0.0

Table 13 Continued 2

1	2	3	4	5	6	7	8	9
4	*Acinetobacter* L114	6	5.1	8.9	100.0	98.5	85.5	14.5
		12	2.2	2.5	88.8	82.7	84.6	5.8
		18	0.2	0.8	76.4	71.4	71.3	3.8
		24	0.0	0.0	73.8	68.5	66.4	0.9
		30	0.0	0.0	71.4	66.4	56.3	0.0
		36	0.0	0.0	68.3	59.2	24.5	0.0
5	*Vibrio* L146	6	14.9	22.5	94.8	100.0	68.2	45.3
		12	0.0	4.7	92.6	93.7	58.4	19.8
		18	0.0	0.6	84.6	89.5	41.6	5.6
		24	0.0	0.4	74.8	87.9	36.7	0.2
		30	0.0	0.0	64.3	74.4	36.2	0.0
		36	0.0	0.0	42.9	64.4	30.4	0.0
6	*Vibrio* F10	6	18.9	30.2	100.0	94.6	86.4	38.0
		12	14.6	23.9	99.8	87.4	83.1	20.5
		18	0.0	11.2	98.4	83.7	72.5	8.7
		24	0.0	0.8	90.3	78.9	68.3	0.4
		30	0.0	0.0	84.6	75.3	57.6	0.0
		36	0.0	0.0	76.7	68.9	44.6	0.0

Cont.....3

Table 13 Continued 3

1	2	3	4	5	6	7	8	9
		6	15.7	47.2	100.0	95.7	88.9	47.2
		12	9.6	19.1	96.8	85.3	83.4	8.9
7	*Acinetobacter* F88	18	0.0	7.6	95.8	81.3	80.6	0.2
		24	0.0	0.0	94.2	77.7	64.9	0.0
		30	0.0	0.0	64.9	61.9	49.5	0.0
		36	0.0	0.0	57.6	58.2	41.6	0.0
		6	13.3	23.9	91.4	100.0	95.2	17.1
		12	2.2	4.8	80.4	91.2	82.1	8.7
8	*Pseudomonas* F152	18	0.0	1.3	78.2	85.8	71.8	5.7
		24	0.0	0.0	67.6	82.4	65.7	0.2
		30	0.0	0.0	54.9	64.9	57.7	0.0
		36	0.0	0.0	52.4	50.9	27.2	0.0

Table 14. Effect of sodium chloride concentration on the survival of spoilage bacteria

Sl. No.	Organism	Time (h)	NaCl(%)					
			0	1	3	6	10	15
1	2	3	4	5	6	7	8	9
1	*Pseudomonas* R8	6	20.3	100.0	38.3	38.2	26.1	21.8
		12	17.9	85.6	35.4	32.6	17.1	5.2
		18	14.8	79.6	31.5	27.6	10.5	0.0
		24	7.2	69.7	28.2	21.4	4.7	0.0
		30	3.9	64.2	27.2	8.2	0.8	0.0
		36	3.1	58.2	26.1	4.8	0.0	0.0
2	*Vibrio* R42	6	100.0	99.6	85.7	83.6	81.8	78.9
		12	98.9	94.6	84.5	77.8	47.5	14.6
		18	90.4	87.5	80.9	70.8	17.2	0.0
		24	86.1	86.6	79.6	58.2	0.0	0.0
		30	77.8	75.8	71.7	12.4	0.0	0.0
		36	70.8	70.1	60.3	0.0	0.0	0.0
3	*Pseudomonas* L97	6	83.9	90.8	100.0	88.2	58.5	48.9
		12	75.4	88.2	94.2	84.3	41.5	11.5
		18	68.8	85.7	92.2	80.8	12.7	0.0
		24	61.6	75.4	88.6	70.3	8.4	0.0
		30	59.5	60.8	80.8	68.6	2.2	0.0
		36	51.4	55.6	78.6	58.6	0.0	0.0

Cont.....2

Table 14 Continued 2

1	2	3	4	5	6	7	8	9
4	*Acinetobacter* L114	6	61.8	100.0	92.4	66.2	56.2	34.7
		12	51.4	92.4	70.2	63.9	50.7	23.9
		18	43.2	70.2	66.1	59.3	39.6	0.0
		24	38.9	62.1	59.5	50.0	6.2	0.0
		30	30.6	50.0	38.9	47.9	0.0	0.0
		36	28.2	49.4	33.9	30.4	0.0	0.0
5	*Vibrio* L146	6	82.6	100.0	20.5	20.1	2.4	2.2
		12	78.7	98.6	19.4	18.6	1.2	1.1
		18	77.5	89.3	19.1	16.9	0.8	0.7
		24	76.5	87.2	17.0	13.6	0.0	0.0
		30	60.2	86.8	16.5	9.6	0.0	0.0
		36	58.9	84.5	13.8	1.9	0.0	0.0
6	*Vibrio* F10	6	100.0	74.8	69.6	54.1	48.9	43.9
		12	95.3	65.9	52.6	50.9	45.7	37.2
		18	76.0	52.6	44.8	18.1	31.1	0.0
		24	60.8	47.7	35.5	6.2	12.7	0.0
		30	54.4	44.4	25.3	5.4	0.0	0.0
		36	49.2	35.4	18.6	2.1	0.0	0.0

Cont....3

Table 14 Continued 3

1	2	3	4	5	6	7	8	9
7	*Acinetobacter* F88	6	78.2	100.0	19.1	9.6	9.6	9.6
		12	45.8	93.4	17.2	5.8	5.6	0.0
		18	40.0	87.6	16.1	0.0	0.0	0.0
		24	38.1	78.1	8.6	0.0	0.0	0.0
		30	37.2	71.4	2.0	0.0	0.0	0.0
		36	36.1	70.4	0.9	0.0	0.0	0.0
8	*Pseudomonas* F152	6	100.0	95.2	85.8	74.8	68.8	32.0
		12	97.2	75.8	72.3	67.8	55.0	25.1
		18	85.4	69.9	64.4	64.0	31.2	11.5
		24	66.0	67.9	63.2	51.4	2.0	0.0
		30	50.5	66.0	59.8	4.8	0.0	0.0
		36	48.5	41.8	35.6	3.1	0.0	0.0

Table 15. Generation time of the selected strains of spoilage flora at 28°C

Sl. No.	Organism	In flesh extract broth	In ZoBell's broth
1	*Pseudomonas* R8	33.0	67.0
2	*Vibrio* R42	47.5	23.0
3	*Pseudomonas* L97	53.0	30.0
4	*Acinetobacter* L114	30.7	90.0
5	*Vibrio* L146	38.7	30.0
6	*Vibrio* F10	52.0	30.0
7	*Acinetobacter* F88	37.0	90.0
8	*Pseudomonas* F153	37.0	90.0

Table 16. Percentage recovery of Vibrio R42 and Pseudomonas R8 when mixed in different proportions in the mixed population study.

Sl. No.	Organisms* R42	R8	Percentage recovery R42	R8
1	10^2	10^2	85	15
2	10^2	10^4	80	20
3	10^2	10^8	70	30
4	10^4	10^2	100	0
5	10^4	10^4	80	20
6	10^4	10^8	75	25
7	10^8	10^2	100	0
8	10^8	10^4	100	0
9	10^8	10^8	85	15

*Initial inoculum - cells/ml

Table 17. Percentage recovery of Vibrio L146 and Pseudomonas L97 when mixed in different proportions in the mixed population study.

Sl. No.	Organisms* L146	L97	Percentage recovery L146	L97
1	10^2	+ 10^2	50	50
2	10^2	+ 10^4	35	65
3	10^2	+ 10^8	0	100
4	10^4	+ 10^2	55	45
5	10^4	+ 10^4	45	55
6	10^4	+ 10^8	20	80
7	10^8	+ 10^2	60	40
8	10^8	+ 10^4	55	45
9	10^8	+ 10^8	40	60

*Initial inoculum - cells/ml

Table 18. Percentage recovery of *Vibrio* L146 and *Acinetobacter* L114 when mixed in different proportions in the mixed population study.

Sl. No.	Organisms* L146	L114	Percentage recovery L146	L114
1	10^2	+ 10^2	50	50
2	10^2	+ 10^4	30	70
3	10^2	+ 10^8	10	90
4	10^4	+ 10^2	35	65
5	10^4	+ 10^4	50	50
6	10^4	+ 10^8	25	75
7	10^8	+ 10^2	70	30
8	10^8	+ 10^4	60	40
9	10^8	+ 10^8	50	50

*Initial inoculum - cells/ml

Table 19. Percentage recovery of *Pseudomonas* L97 and *Acinetobacter* L114 when mixed in different proportions in the mixed population study.

Sl. No.	Organisms* L97	L114	Percentage recovery L97	L114
1	10^2	+ 10^2	60	40
2	10^2	+ 10^4	50	50
3	10^2	+ 10^8	45	55
4	10^4	+ 10^2	70	30
5	10^4	+ 10^4	65	35
6	10^4	+ 10^8	50	50
7	10^8	+ 10^2	100	0
8	10^8	+ 10^4	95	5
9	10^8	+ 10^8	50	50

*Initial inoculum - cells/ml

Table 20. Percentage recovery of *Vibrio* L146, *Pseudomonas* L97 and *Acinetobacter* L114 when mixed in different proportions in the mixed population study.

Sl. No.	Organisms* L146 + L97 + L114	Percentage recovery L146	L97	L114
1	2	3	4	5
1	$10^2 + 10^2 + 10^2$	20	50	30
2	$10^4 + 10^4 + 10^4$	15	65	20
3	$10^8 + 10^8 + 10^8$	10	65	25
4	$10^2 + 10^2 + 10^4$	10	50	40
5	$10^2 + 10^2 + 10^8$	10	45	45
6	$10^4 + 10^4 + 10^8$	10	55	35
7	$10^2 + 10^4 + 10^2$	20	60	20
8	$10^2 + 10^8 + 10^2$	10	70	20
9	$10^4 + 10^8 + 10^4$	5	70	25
10	$10^4 + 10^2 + 10^2$	20	60	20
11	$10^8 + 10^2 + 10^2$	35	40	25
12	$10^8 + 10^4 + 10^4$	25	50	25
13	$10^4 + 10^4 + 10^2$	15	65	20
14	$10^8 + 10^8 + 10^2$	25	70	5
15	$10^8 + 10^8 + 10^4$	20	70	10
16	$10^4 + 10^2 + 10^4$	25	40	35
17	$10^8 + 10^2 + 10^8$	30	25	45
18	$10^8 + 10^4 + 10^8$	15	45	40
19	$10^2 + 10^4 + 10^4$	15	60	25
20	$10^2 + 10^8 + 10^8$	0	65	35

Cont.....

Table 20 Continued

1	2	3	4	5
21	$10^4 + 10^8 + 10^8$	5	65	30
22	$10^2 + 10^4 + 10^8$	10	55	35
23	$10^2 + 10^8 + 10^4$	5	75	20
24	$10^4 + 10^2 + 10^8$	20	40	40
25	$10^8 + 10^2 + 10^4$	30	35	35
26	$10^8 + 10^4 + 10^2$	30	50	20
27	$10^4 + 10^8 + 10^2$	15	75	10

*Initial inoculum - cells/ml

Table 21. Pearson correlation coefficient of the various spoilage indices analysed at various temperatures

Storage temperature	Spoilage indices	TMA	NH_3	pH
	THB	+.7817*	+.9869*	+.9702*
$28\pm2^{o}C$	TMA		+.8019*	+.8718*
	NH_3			+.9484
	THB	+.9075*	+.8376*	+.8246*
$4^{o}C$	TMA		+.9679*	+.8565*
	NH_3			+.8250*
	THB	+.7514*	+.6482+	+.6367*
$-18^{o}C$	TMA		+.8428*	+.8703*
	NH_3			+.9777*

*Significant value at p 0.05

Table 22. Pearson correlation coefficient values of the various spoilage indices and proximate components analysed at various temperatures

Storage temperature	Spoilage indices	Proximate components				
		Protein	Carbohydrate	Lipid	Ash	Moistu
$28\pm2^{o}C$	THB	-.6706	-.7388	-.8809*	-.8441*	+.7633
	TMA	-.8941*	-.9257*	-.9761*	-.9874*	+.7573
	NH_3	-.6226	-.7016	-.8981*	-.8482*	+.9839
	pH	-.8151*	-.8454*	-.9227*	-9128*	+.8795
$4^{o}C$	THB	-.7751*	-.8073*	-.8693*	-.8760*	+.8359
	TMA	-.9624*	-.9647*	-.9817*	-.9859*	+.9796
	NH_3	-.9814*	-.9716*	-.9683*	-.9854*	+.9918
	pH	-.7682*	-.7409*	-.9234*	-.8957*	+.8701
$-18^{o}C$	THB	-.8332*	-.7132*	-.7068*	-.6351*	+.8045
	TMA	-.9505*	-.9618*	-.9410*	-.9143*	+.9897
	NH_3	-.6917*	-.8733*	-.9688*	-.9784*	+.8503
	pH	-.7191*	-.8744*	-.9640*	-.9679*	+.8594

*Significant value at p 0.05

Table 23. Pearson correlation coefficient values for the variou spoilage indices and spoilage flora analysed at varic temperatures

Storage temperature	Spoilage indices	Spoilage flora			
		Vibrio	Pseudomonas	Acinetobacter	Micrococ
$28\pm2^{o}C$	THB	+.9772*	-.8469*	-.9304*	-.9575
	TMA	+.8246*	-.7730*	-.8794*	-.6512
	NH_3	+.9823*	-.8177*	-.9448*	-.9733
	pH	+.9372*	-.8222*	-.9075*	-.8807
$4^{o}C$	THB	+.3164	+.2524	-.4728	-.3003
	TMA	+.0850	+.1777	-.2842	-.4201
	NH_3	+.0223	+.2335	-.1470	-.3993
	pH	+.2254	+.6204	-.0633	-.2206
$-18^{o}C$	THB	-.3910	-.1286	+.0340	+.4802
	TMA	-.6715*	+.0727	-.3091	+.7267
	NH_3	-.9029*	+.1950	+.1699	+.6045
	pH	-.8573*	+.1052	+.0826	+.6195

*Significant value at p 0.05

Table 24. Pearson correlation coefficient values for the spoilage indices and hydrolytic enzyme producing bacteria analysed at various temperature

Storage temperature	Spoilage indices	Hydrolytic enzyme producing bacteria			
		Proteolytic	Lipolytic	Amylolytic	Ureolytic
$28\pm2^{o}C$	THB	+.8417*	+.8774*	+.3603	+.7071
	TMA	+.5388	+.6301	-.0773	+.8790*
	NH_3	+.8977*	+.9107*	+.4405	+.7679*
	pH	+.7138	+.7595*	+.1596	+.7057
$4^{o}C$	THB	+.2418	+.8195*	-.4866	+.4689
	TMA	+.2284	+.6262	-.5861	+.7676*
	NH_3	+.2009	+.4346	-.4828	+.7773*
	pH	-.0261	+.5755	-.1824	+.5177
$-18^{o}C$	THB	+.1611	-.7324*	+.3981	+.3369
	TMA	+.5400	-.7749*	+.2620	+.7037*
	NH_3	+.0994	-.4747	+.5428	+.6638*
	pH	+.1965	-.5280	+.4870	+.6723*

*Significant value at p 0.05

Table 25. Pearson correlation coefficient values for the proximate components analysed at various temperatures

Storage temperature	Proximate components	Proximate components			
		Carbohydrate	Lipid	Ash	Moisture
$28\pm2^{o}C$	Protein	+.9779*	+.8415*	+.9034*	-.9584*
	Carbohydrate		+.9064*	+.9515*	-.9639*
	Lipid			+.9913*	-.9461*
	Ash				-.9770*
$4^{o}C$	Protein	+.9828*	+.9529*	+.9671*	-.9832*
	Carbohydrate		+.9335*	+.9499*	-.9650*
	Lipid			+.9959*	-.9904*
	Ash				-.9964*
$-18^{o}C$	Protein	+.8704*	+.8171*	+.7656*	-.9568*
	Carbohydrate		+.9639*	+.9428*	-.9642*
	Lipid			-.9938*	-.9430*
	Ash				-.9100*

*Significant value at p 0.05

Table 26. Pearson correlation coefficient values for the proximate components and spoilage flora analysed at various temperatures

Storage temperature	Proximate components	Spoilage flora			
		Vibrio	Pseudomonas	Acinetobacter	Micrococcus
28±2°C	Protein	-.6671	+.7278	+.7141	+.4493
	Carbohydrate	-.7673*	+.8465*	+.8223*	+.5290
	Lipid	-.9258*	+.8676*	+.9613*	+.7744*
	Ash	-.8848*	+.8596*	+.9286*	+.7049
	Moisture	+.7820*	-.7594*	-.8306*	-.5978
4°C	Protein	+.0409	-.1125	+.1632	+.4235
	Carbohydrate	-.1098	-.0229	+.1517	+.5599
	Lipid	+.0894	-.3382	+.1711	+.3539
	Ash	+.0545	-.3064	+.1940	+.3519
	Moisture	-.0816	+.2696	-.1403	-.3879
-18°C	Protein	+.4721	+.0357	+.3946	-.6876*
	Carbohydrate	+.6872*	-.1087	+.1537	-.6465*
	Lipid	+.8322*	-.1421	+.0135	-.6749*
	Ash	+.8754*	-.2092	+.0005	-.6600*
	Moisture	-.6649*	+.0183	-.2250	+.7392*

*Significant value at p 0.05

Table 27. Pearson correlation coefficient values for the proximate components and hydrolytic enzyme producing bacteria analysed at various temperatures

Storage temperature	Proximate components	Hydrolytic enzyme producing bacteria			
		Proteolytic	Lipolytic	Amylolytic	Ureolytic
$28\pm2^{o}C$	Protein	-.2268	-.3771	+.4249	-.6484
	Carbohydrate	-.3508	-.5287	+.3159	-.7599*
	Lipid	-.6828	-.7790*	-.0818	-.9133*
	Ash	-.5845	-.7012	+.0477	-.8840*
	Moisture	+.4338	+.5397	-.2077	+.7935*
$4^{o}C$	Protein	-.2526	-.4105	+.5682	-.8760*
	Carbohydrate	-.1623	-.4522	+.6693	-.8063*
	Lipid	-.1614	-.5542	+.4466	-.7588*
	Ash	-.2017	-.5353	+.4590	-.7677*
	Moisture	+.1826	+.4711	+.4779	+.8021*
$-18^{o}C$	Protein	-.5418	-.8723*	-.1554	-.5416
	Carbohydrate	-.5322	+.5923	-.4617	-.8475*
	Lipid	-.3275	+.5840	-.4859	-.7613*
	Ash	-.2925	+.5332	-.4698	-.7583*
	Moisture	+.4839	-.7640*	+.3386	+.6994*

*Significant value at p 0.05

Table 28. Pearson correlation coefficient values for the spoilage flora analysed at various temperatures

Storage temperature	Spoilage flora	Pseudomonas	Acinetobacter	Micrococc
28±2°C	Vibrio	-.9092*	-.9842*	-.9294*
	Pseudomonas		+.9354*	+.7118
	Acinetobacter			+.8583*
4°C	Vibrio	-.6271	-.5057	-.4069
	Pseudomonas		+.2162	+.3973
	Acinetobacter			-.3323
-18°C	Vibrio	-.3049	-.1585	-.6642*
	Pseudomonas		-.1665	-.2766
	Acinetobacter			-.2403

*Significant value at p 0.05

Table 29. Pearson correlation coefficient values for the spoilage flora and hydrolytic enzyme producing bacteria analysed at various temperatures

Storage temperature	Spoilage flora	Hydrolytic enzyme producing bacteria			
		Proteolytic	Lipolytic	Amylolytic	Ureolyt
$28\pm2^{o}C$	Vibrio	+.8621*	+.9343*	+.3589	+.8302
	Pseudomonas	-.6368	-.8344*	-.0758	-.8194
	Acinetobacter	-.8079*	-.9138*	-.2651	-.9100
	Micrococcus	-.9454*	-.9060*	-.6062	-.6382
$4^{o}C$	Vibrio	+.1026	+.4829	-.6184	-.2227
	Pseudomonas	-.1578	+.0657	+.6542	-.0720
	Acinetobacter	-.8281*	-.7196*	+.3046	-.2083
	Micrococcus	+.5366	-.1249	+.7555*	-.2185
$-18^{o}C$	Vibrio	+.0571	+.3848	-.3234	-.4954
	Pseudomonas	-.6913*	+.1061	-.1784	+.0392
	Acinetobacter	-.7359*	+.5274	+.7311*	-.0399
	Micrococcus	+.3210	-.7312*	+.0325	+.4209

*Significant value at $p\ 0.05$

Table 30. Pearson correlation coefficient values for the hydrolytic enzyme producing bacteria analysed at various temperatures

Storage temperature	Hydrolytic enzyme producing bacteria	Lipolytic	Amylolytic	Ureolytic
28±2°C	Proteolytic	+.9468*	+.7776*	+.6741
	Lipolytic		+.6026	+.7970*
	Amylolytic			+.1662
4°C	Proteolytic	+.3152	-.1037	+.4573
	Lipolytic		-.4831	+.2286
	Amylolytic			-.5218
-18°C	Proteolytic	-.3920	-.1281	+.6226
	Lipolytic		+.2321	-.1471
	Amylolytic			+.5980

*Significant value at p<0.05

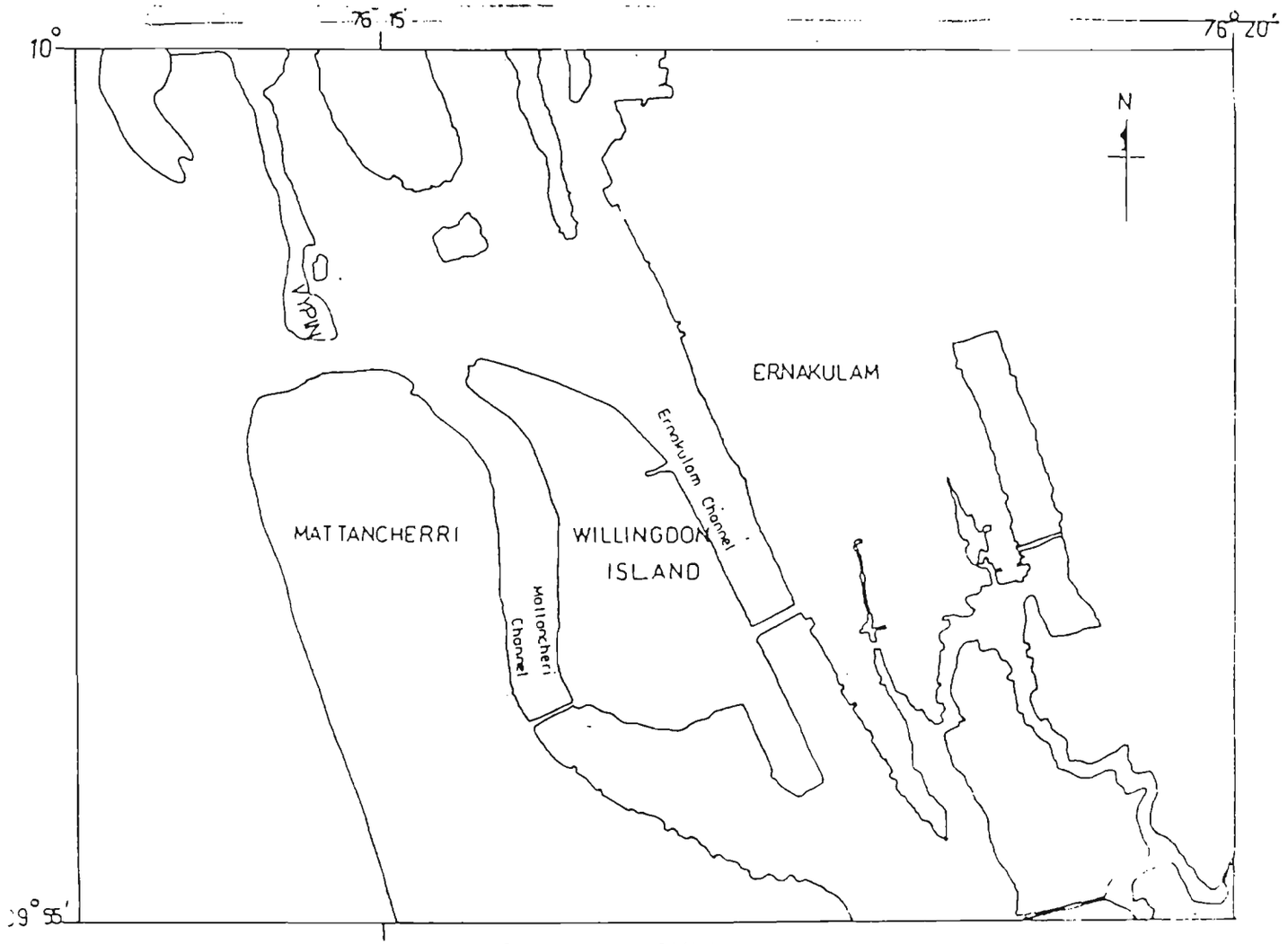


FIG.1. COCHIN BACKWATER

FIG. 2a. Penaeus indicus

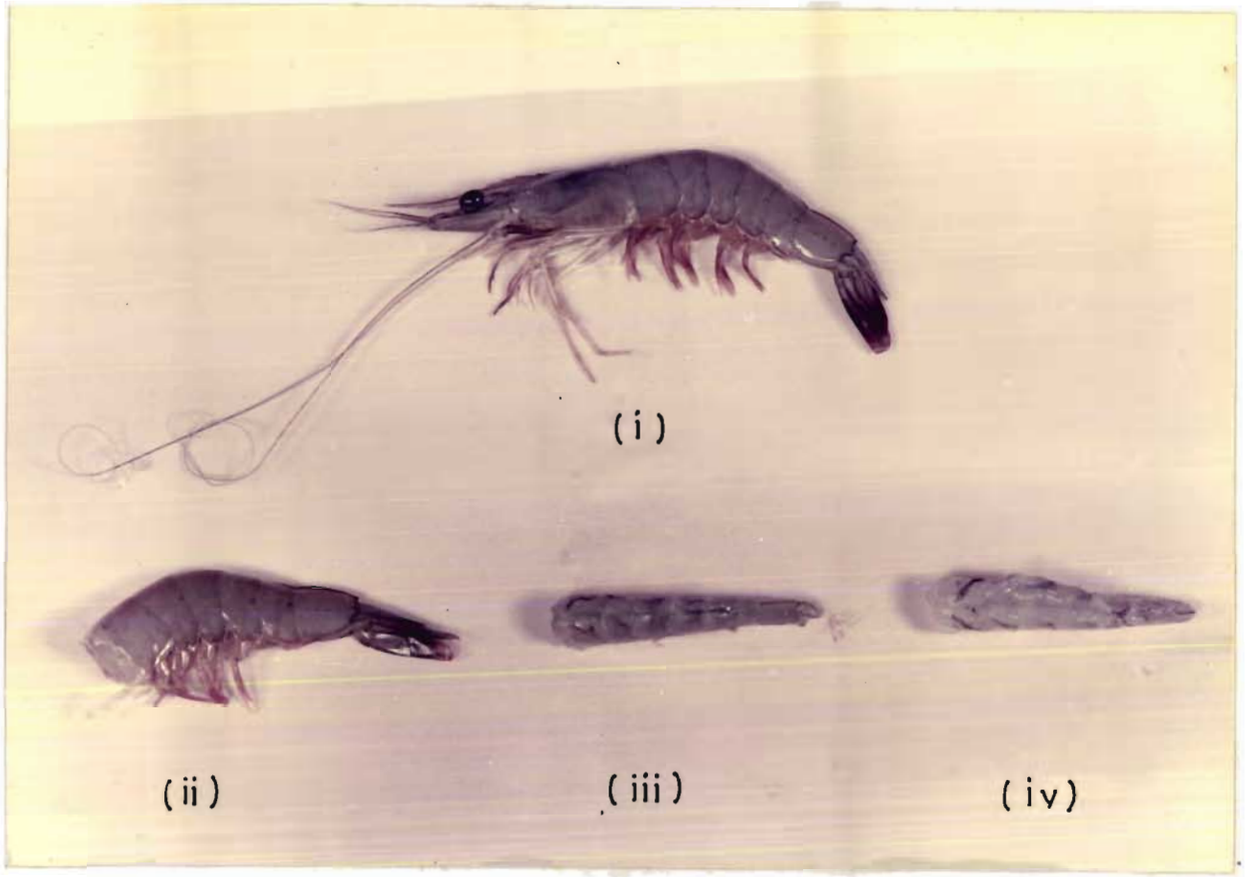


FIG. 2b. (i) Whole (ii) Headless (iii) PUD (iv) PD

Fig.3. Clearing zone produced by Pseudomonas sp. on flesh agar medium.

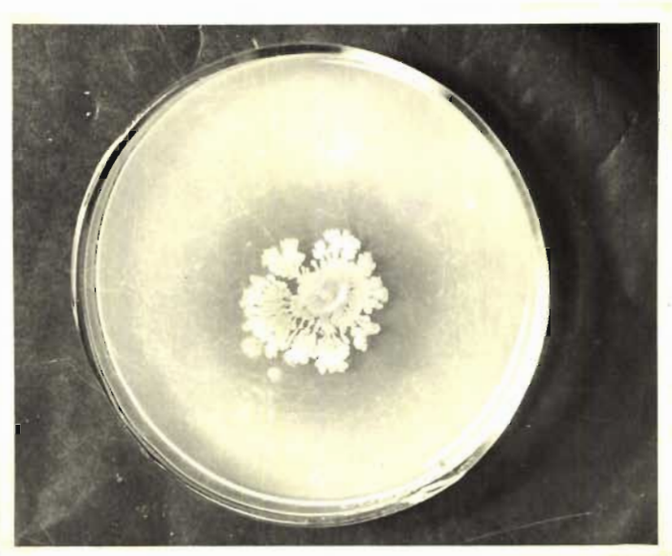


FIG. 3

Fig.4. Changes in the total heterotrophic bacterial population (THB) of the prawns during storage at different temperatures.

a) Whole

b) Headless

c) PUD

d) PD

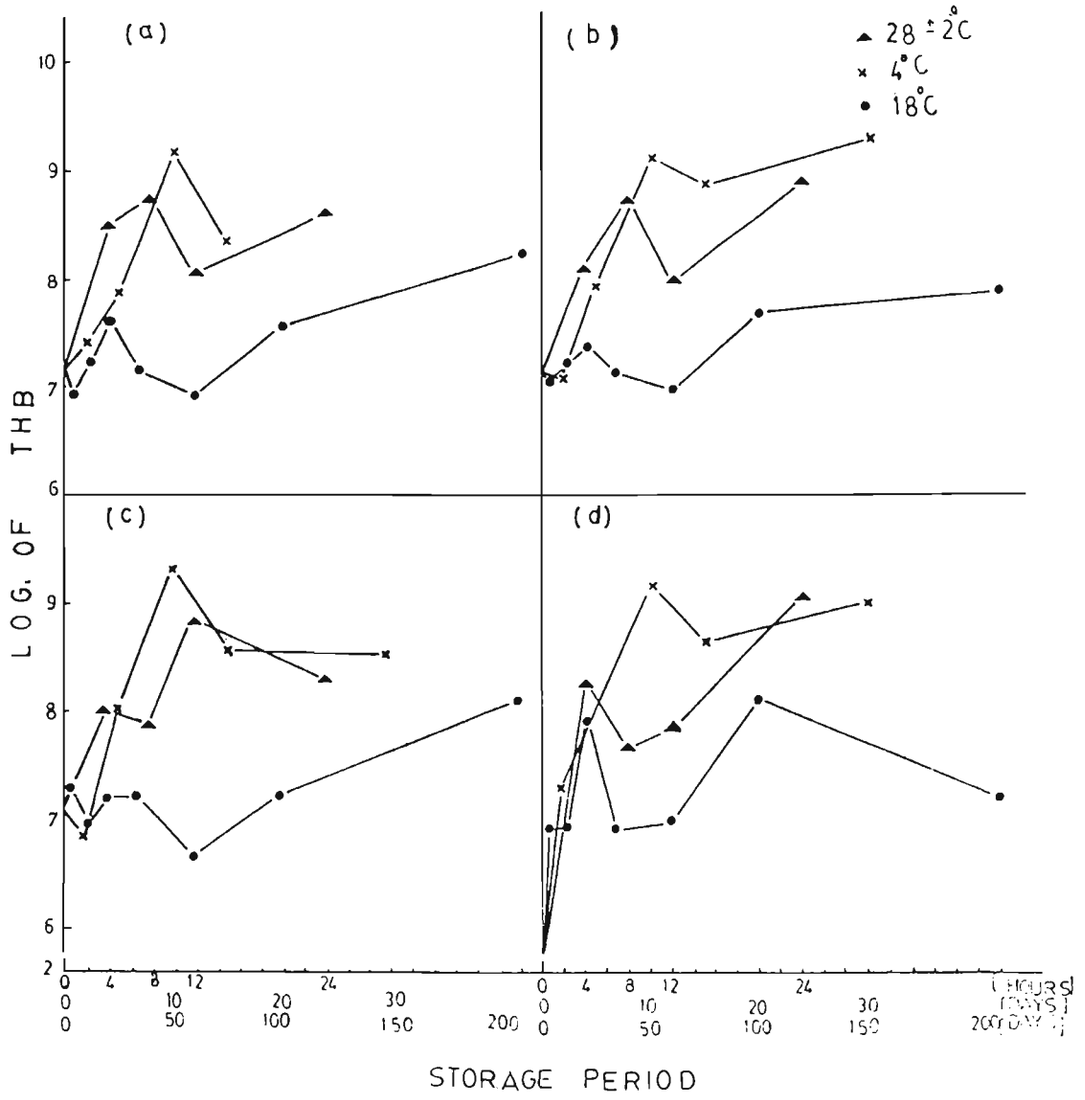

(a)
(b)
(c)
(d)
▲ 28 ± 2°C
× 4°C
● 18°C
LOG. OF THB
10
9
8
7
6
2
0 4 8 12 24
0 10 20 30
0 50 100 150 200
(HOURS)
(DAYS)
STORAGE PERIOD

FIG. 4

Fig.5. Changes in the Trimethylamine content of the prawns during storage at different temperatures.

a) Whole

b) Headless

c) PUD

d) PD

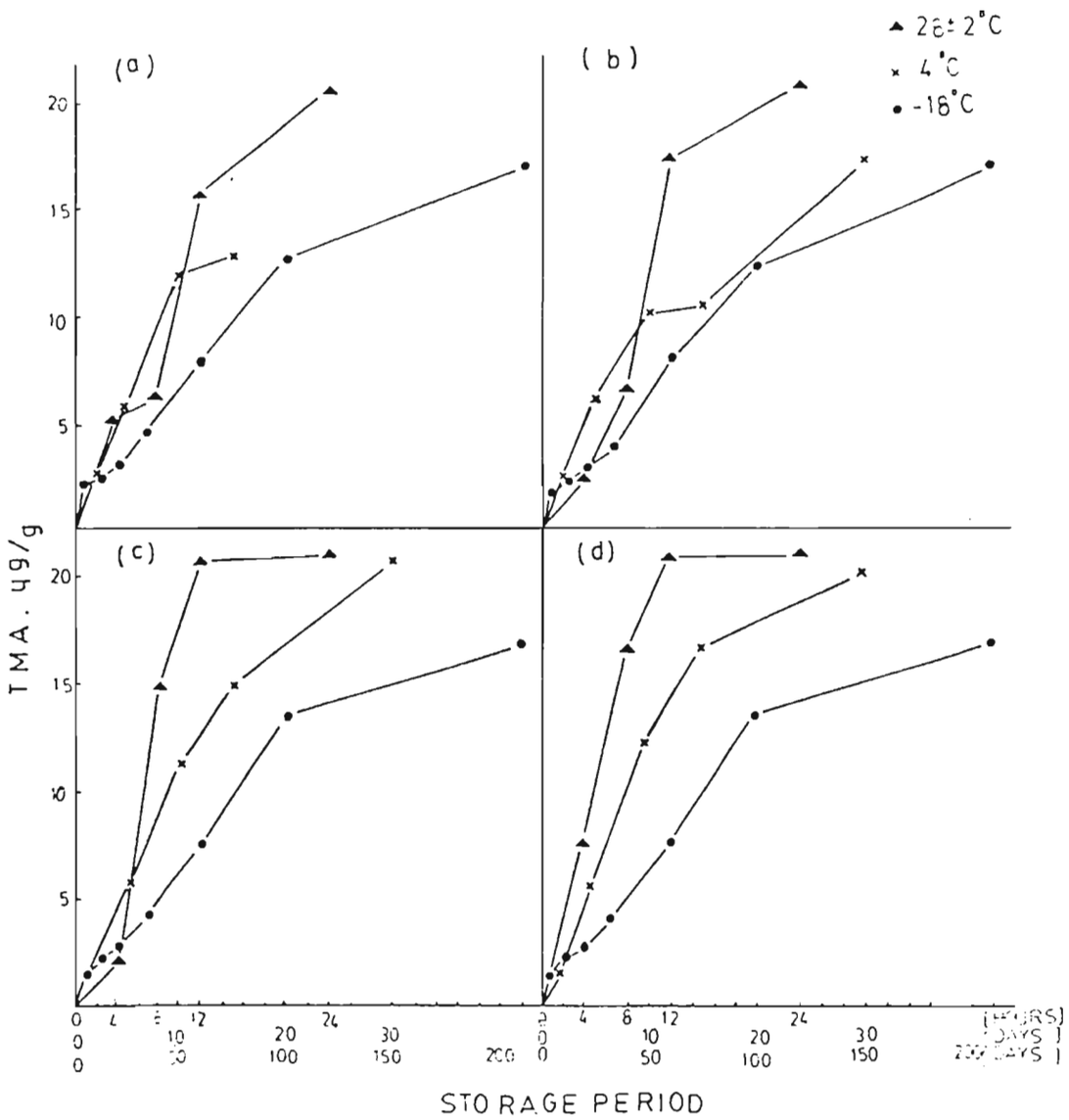


FIG. 5

Fig.6. Changes in the ammonia content of the prawns during storage at different temperatures.

a) Whole

b) Headless

c) PUD

d) PD

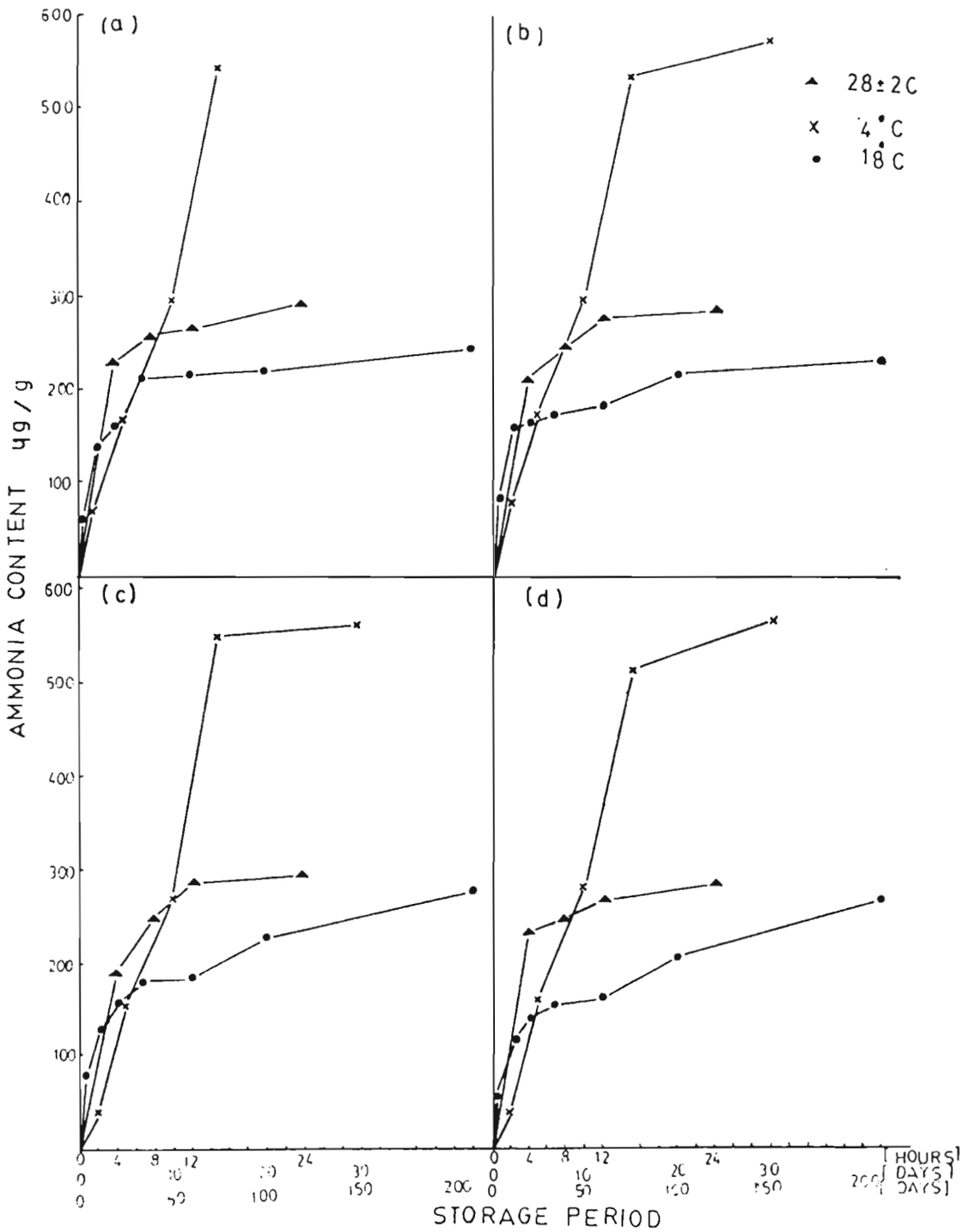

(a)
(b)
(c)
(d)
28±2C
4°C
18°C
AMMONIA CONTENT μg/g
600
500
400
300
200
100
0
4
8
12
24
30
10
20
50
100
150
200
[HOURS]
[DAYS]
STORAGE PERIOD

FIG. 6

Fig.7. Changes in the pH of the flesh of prawns during storage at different temperatures.

a) Whole

b) Headless

c) PUD

d) PD

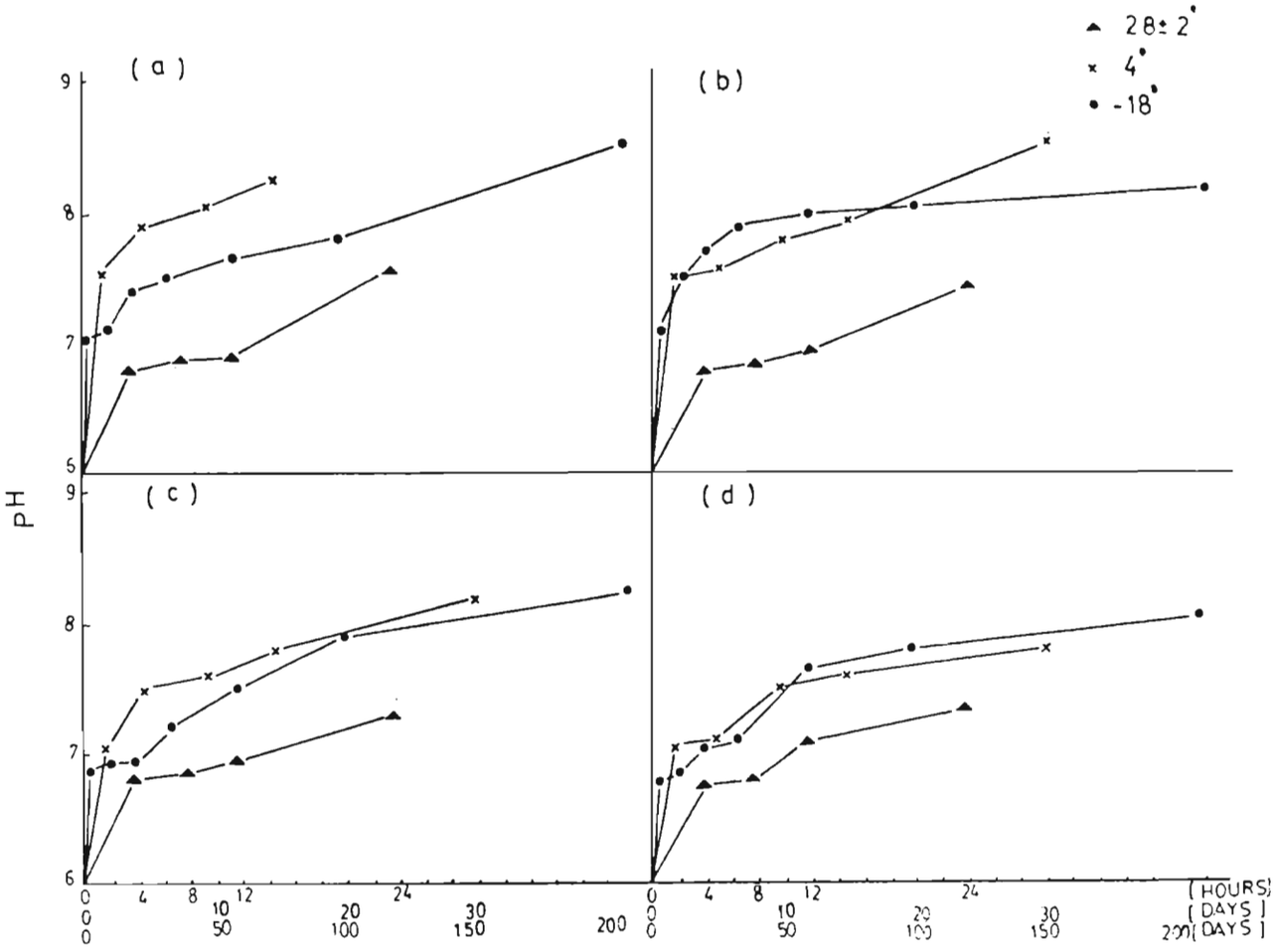

28± 2°
4°
-18°
(a)
(b)
(c)
(d)
pH
9
8
7
6
0 4 8 10 12 20 24 30
0 50 100 150 200
0 4 8 12 20 24 30
0 50 100 150 200
[HOURS]
[DAYS]
[DAYS]
STORAGE PERIOD

FIG. 7

Fig.8. Changes in the protein content of the prawns during storage at different temperatures.

a) Whole

b) Headless

c) PUD

d) PD

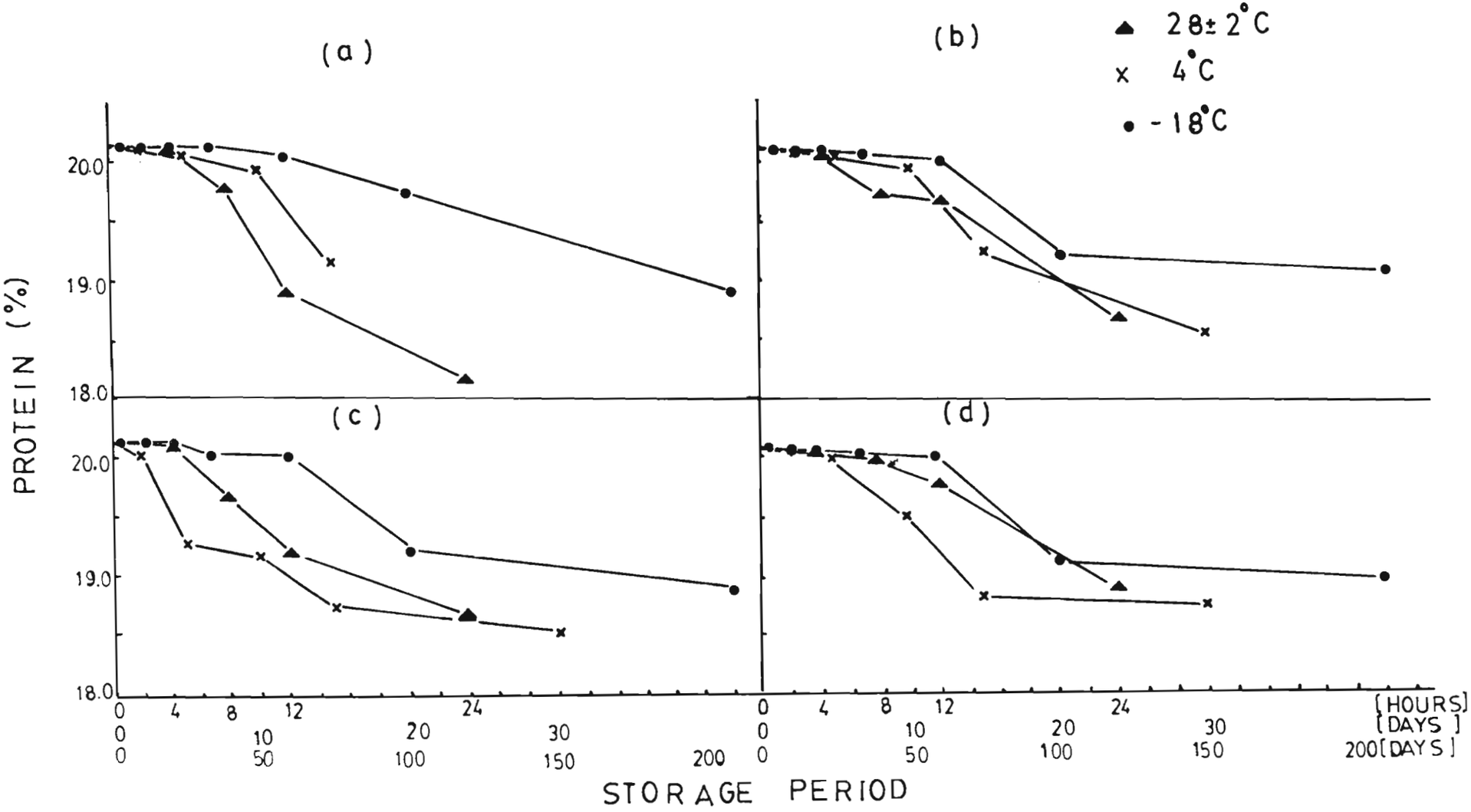

(a)
(b)
(c)
(d)
▲ 28± 2°C
× 4°C
● -18°C
PROTEIN (%)
20.0
19.0
18.0
0 4 8 12 24
0 10 20 30
0 50 100 150 200
[HOURS]
[DAYS]
200[DAYS]
STORAGE PERIOD

FIG. 8

Fig.9. Changes in the carbohydrate content of the prawns during storage at different temperatures.

a) Whole

b) Headless

c) PUD

d) PD

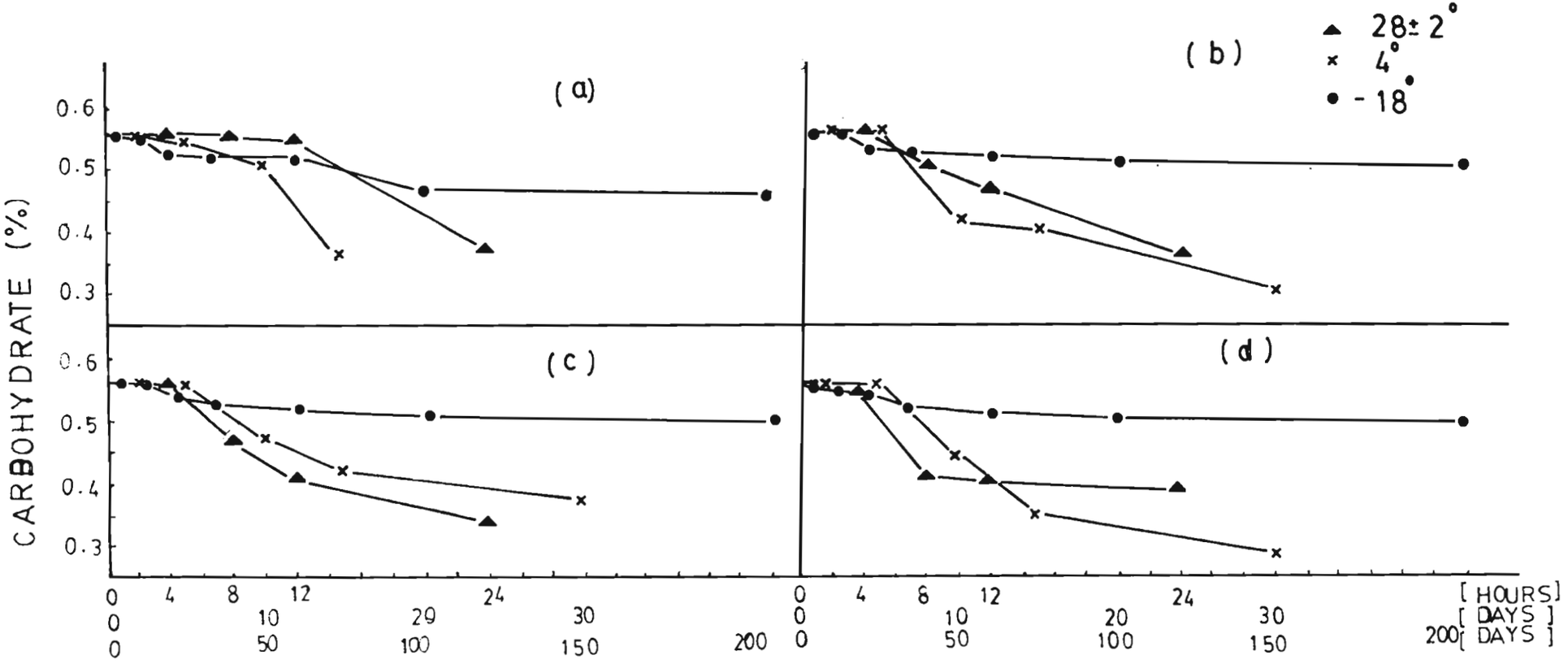

(a)
(b)
(c)
(d)
▲ 28± 2°
× 4°
● - 18°
CARBOHYDRATE (%)
0.6
0.5
0.4
0.3
0 4 8 12 24 30
0 10 20 30
0 50 100 150 200
0 4 6 12 24
0 10 20 30
0 50 100 150 200
[HOURS]
[DAYS]
[DAYS]
STORAGE PERIOD

FIG. 9

Fig.10. Changes in the lipid content of the prawns during storage at different temperatures.

a) Whole

b) Headless

c) PUD

d) PD

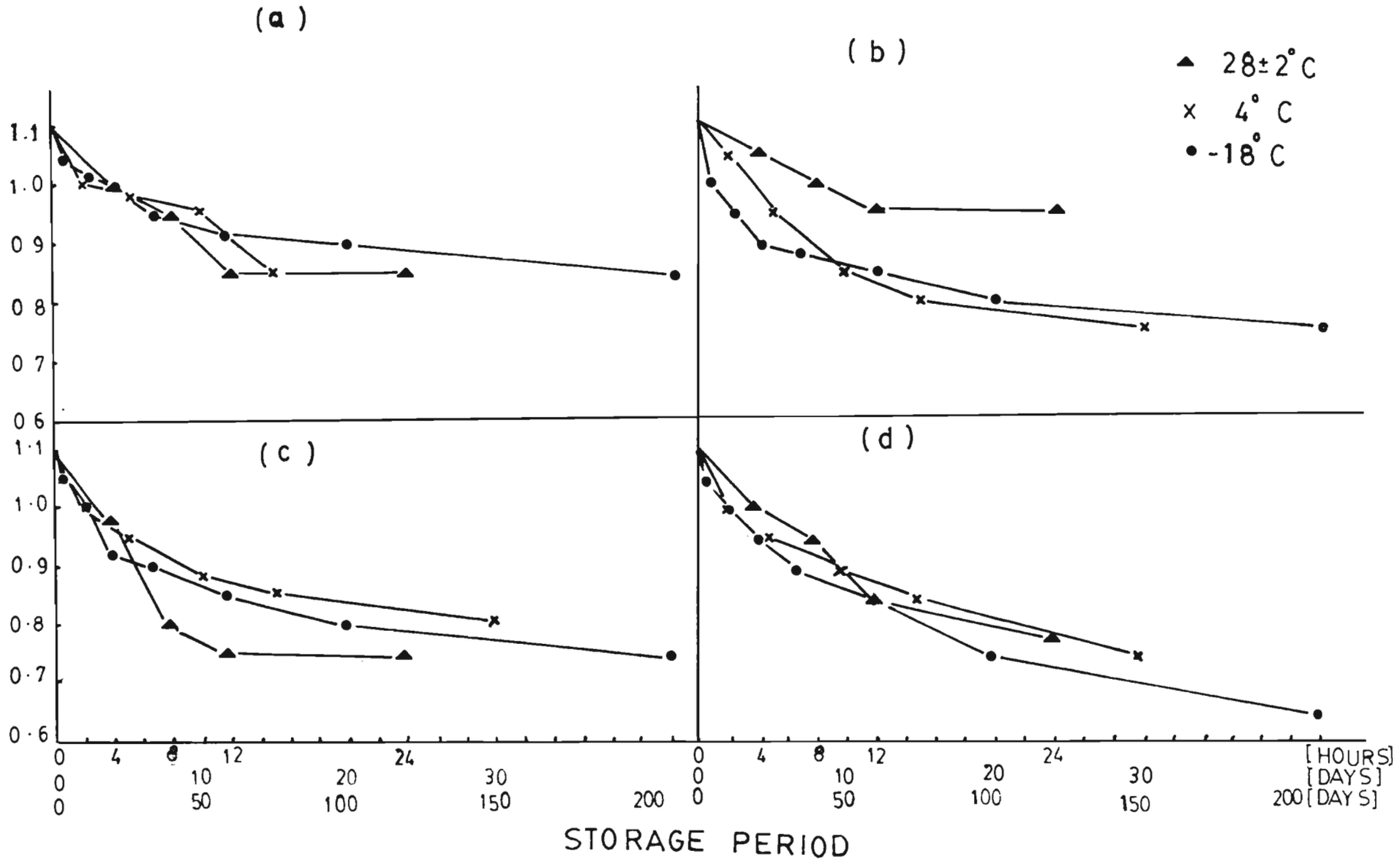

(a)
(b)
(c)
(d)
▲ 28±2°C
× 4° C
● -18° C
1.1
1.0
0.9
0.8
0.7
0.6
0 4 8 12 24
0 10 20 30
0 50 100 150 200
[HOURS]
[DAYS]
200 [DAYS]
STORAGE PERIOD

FIG. 10

Fig.11. Changes in the ash content of the prawns during storage at different temperatures

a) Whole

b) Headless

c) PUD

d) PD

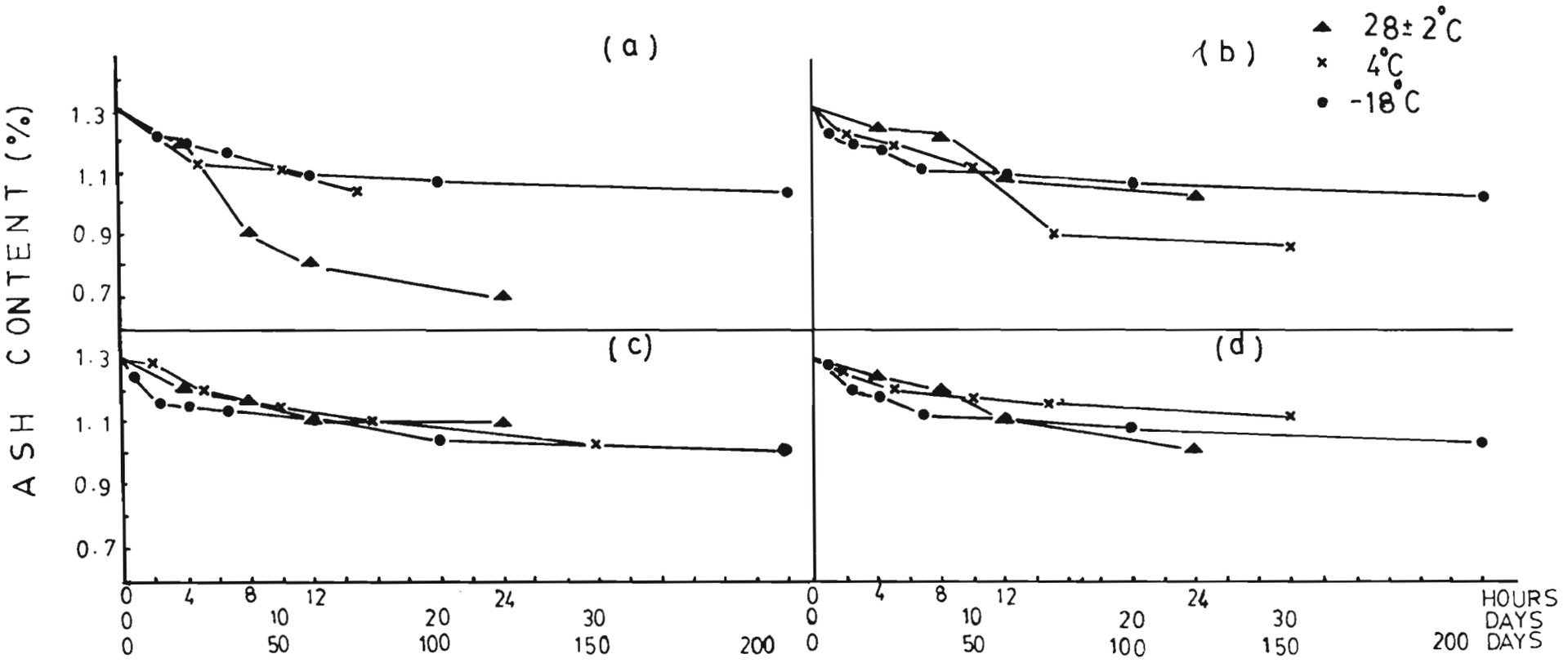

(a)
(b)
(c)
(d)
▲ 28± 2°C
× 4°C
● -18°C
ASH CONTENT (%)
1.3
1.1
0.9
0.7
0 4 8 12 24
0 10 20 30
0 50 100 150 200
HOURS
DAYS
200 DAYS
STORAGE PERIOD

FIG.11

Fig.12. Changes in the moisture content of the prawns during storage at different temperatures.

a) Whole

b) Headless

c) PUD

d) PD

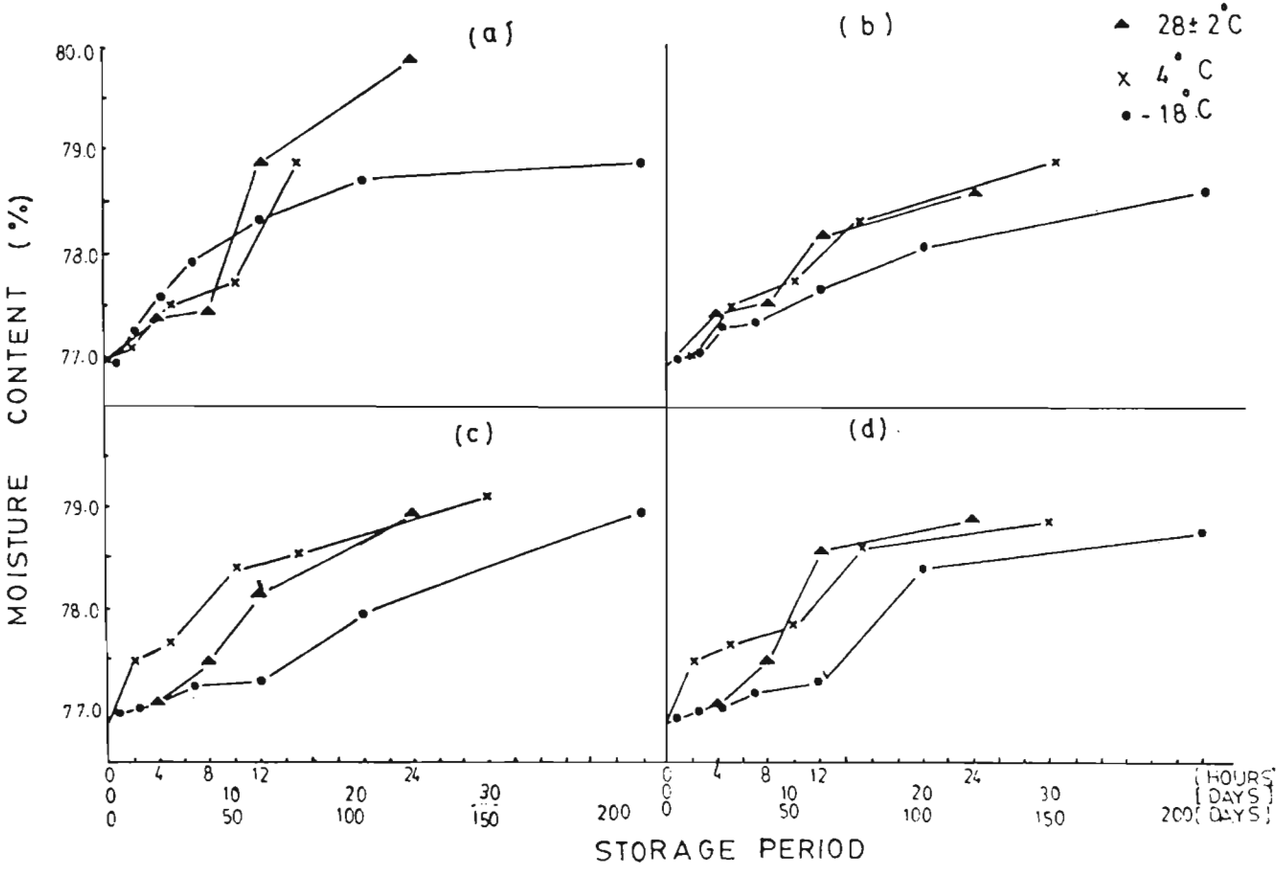
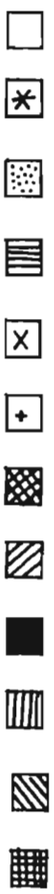

(a)
(b)
(c)
(d)
▲ 28± 2°C
x 4° C
• - 18° C
MOISTURE CONTENT (%)
80.0
79.0
78.0
77.0
0 4 8 12 24
0 10 20 30
0 50 100 150 200
(HOURS)
(DAYS)
STORAGE PERIOD

FIG. 12

Fig.13. Changes in the generic composition of the prawns during storage at 28±2°C



Vibrio

Aeromonas

Pseudomonas

Acinetobacter

Alcaligenes

Moraxella

Enterobacteriaceae

Micrococcus

Staphylococcus

Bacillus

Corynebacterium

Unidentified

FIG. 13

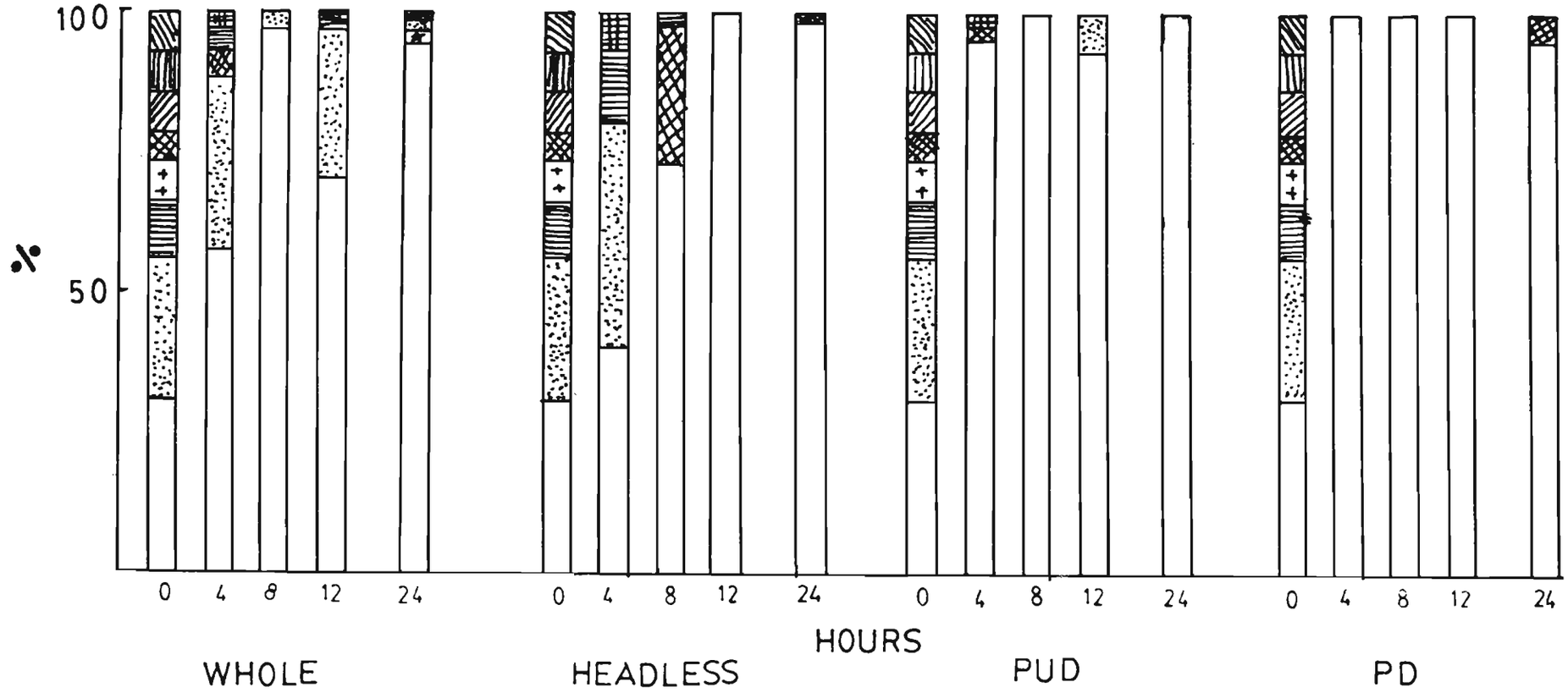


Fig.14. Changes in the generic composition of the prawns during storage at 4°C.

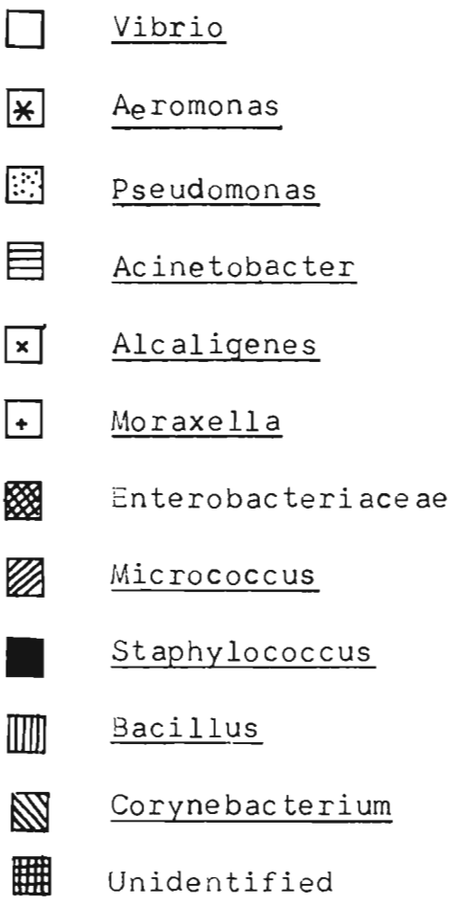


FIG. 14

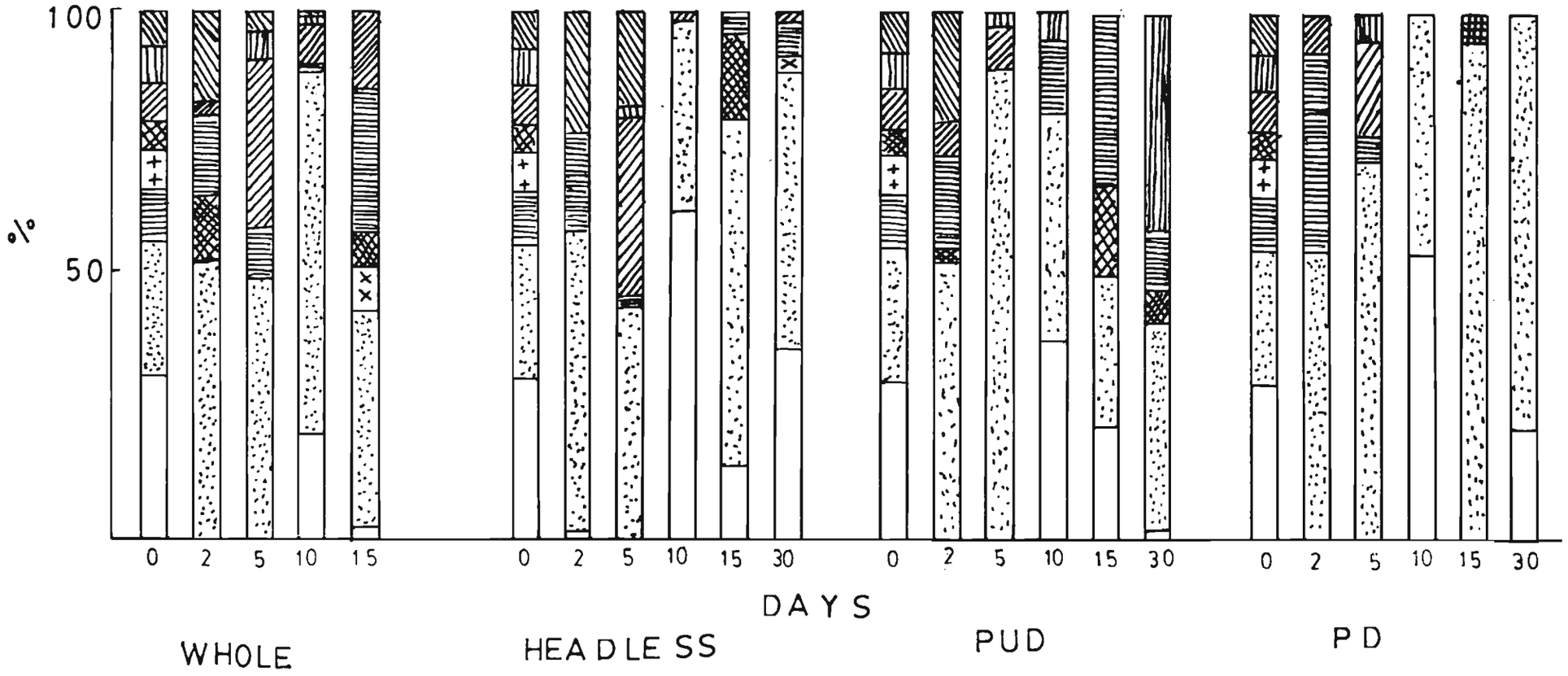

100
50
%
0 2 5 10 15
0 2 5 10 15 30
0 2 5 10 15 30
0 2 5 10 15 30
DAYS
WHOLE
HEADLESS
PUD
PD

Fig.15. Changes in the generic composition of the prawns during storage at -18°C.

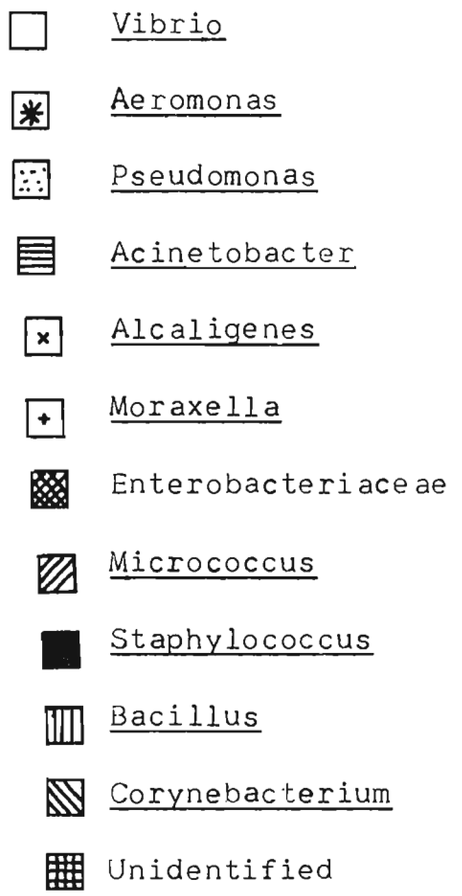


FIG. 15

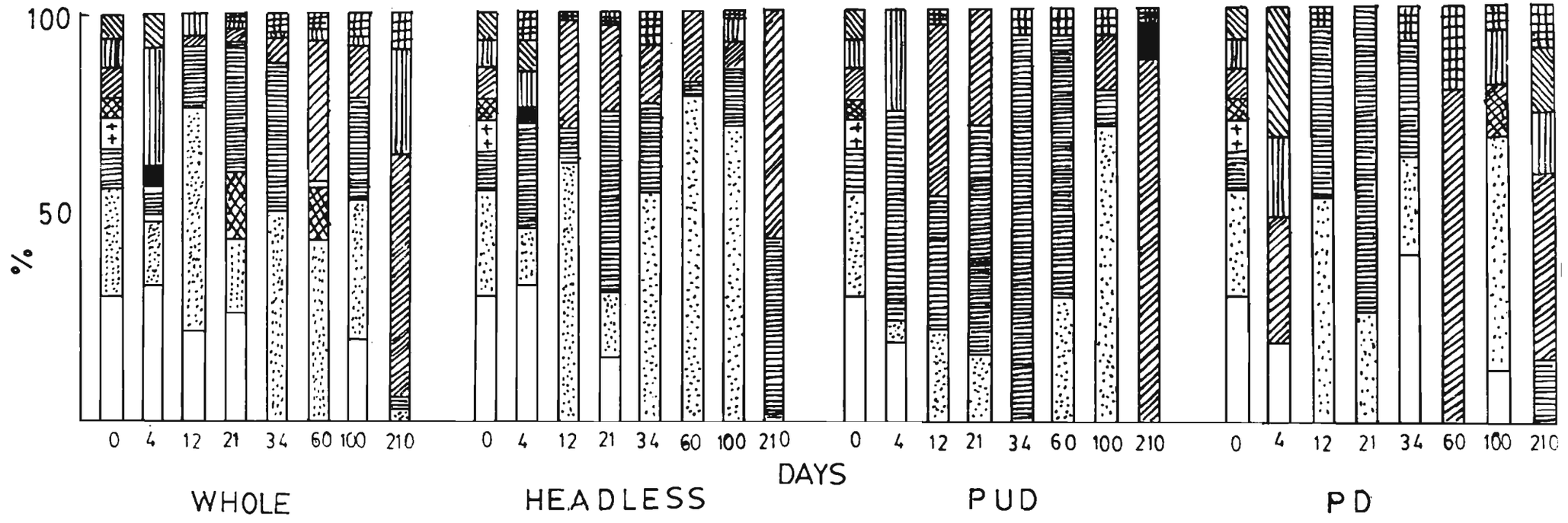

100
50
%
0 4 12 21 34 60 100 210
0 4 12 21 34 60 100 210
0 4 12 21 34 60 100 210
0 4 12 21 34 60 100 210
DAYS
WHOLE
HEADLESS
PUD
PD

Fig.16. Changes in the percentage composition of different hydrolytic enzyme producing bacteria in the prawns stored at $28 \pm 2^{o}C$.

C - Caseinolytic

G - Gelatinolytic

A - Amylolytic

L - Lipolytic

U - Ureolytic

FIG. 16

(a) WHOLE

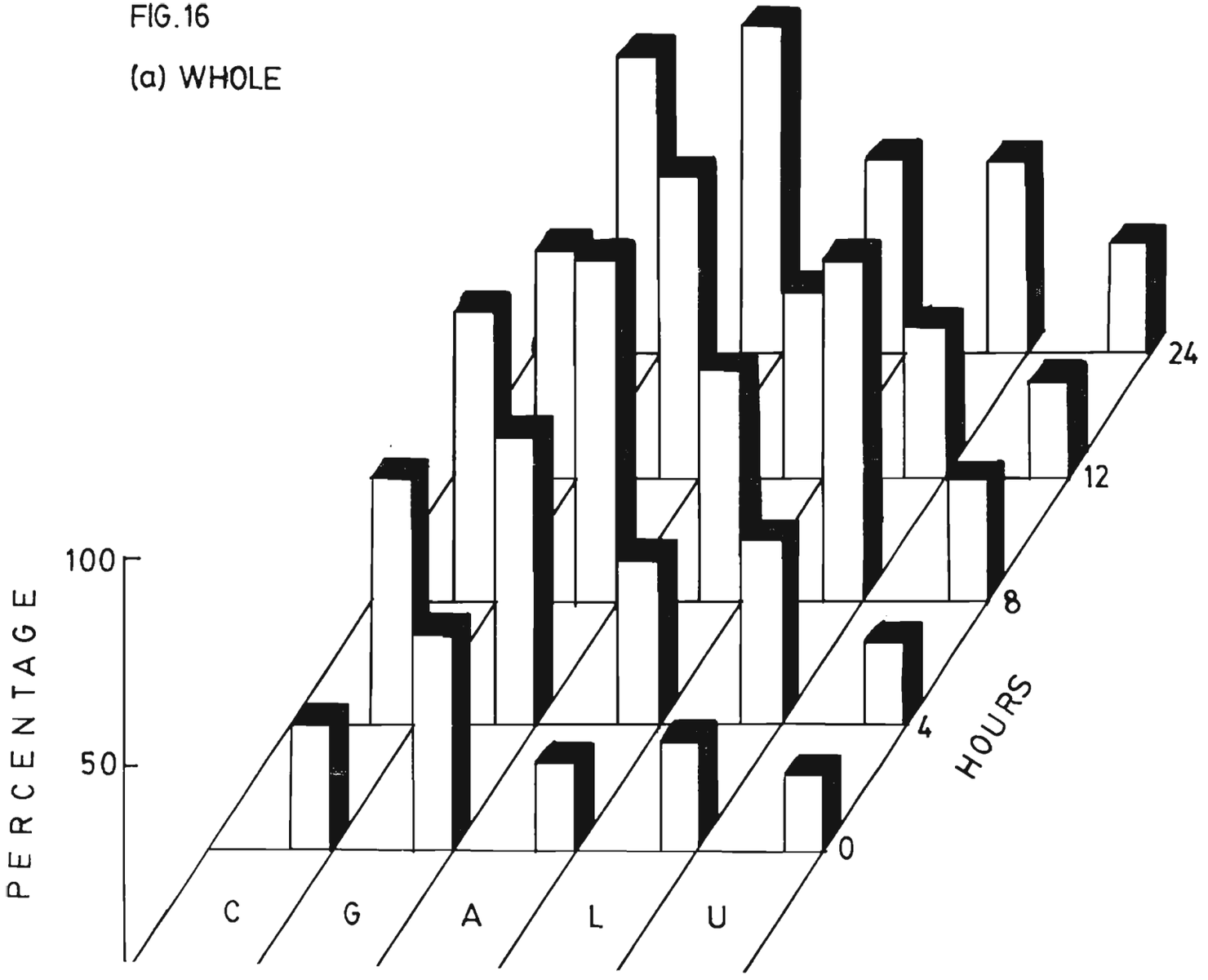


FIG.16

(b) HEADLESS

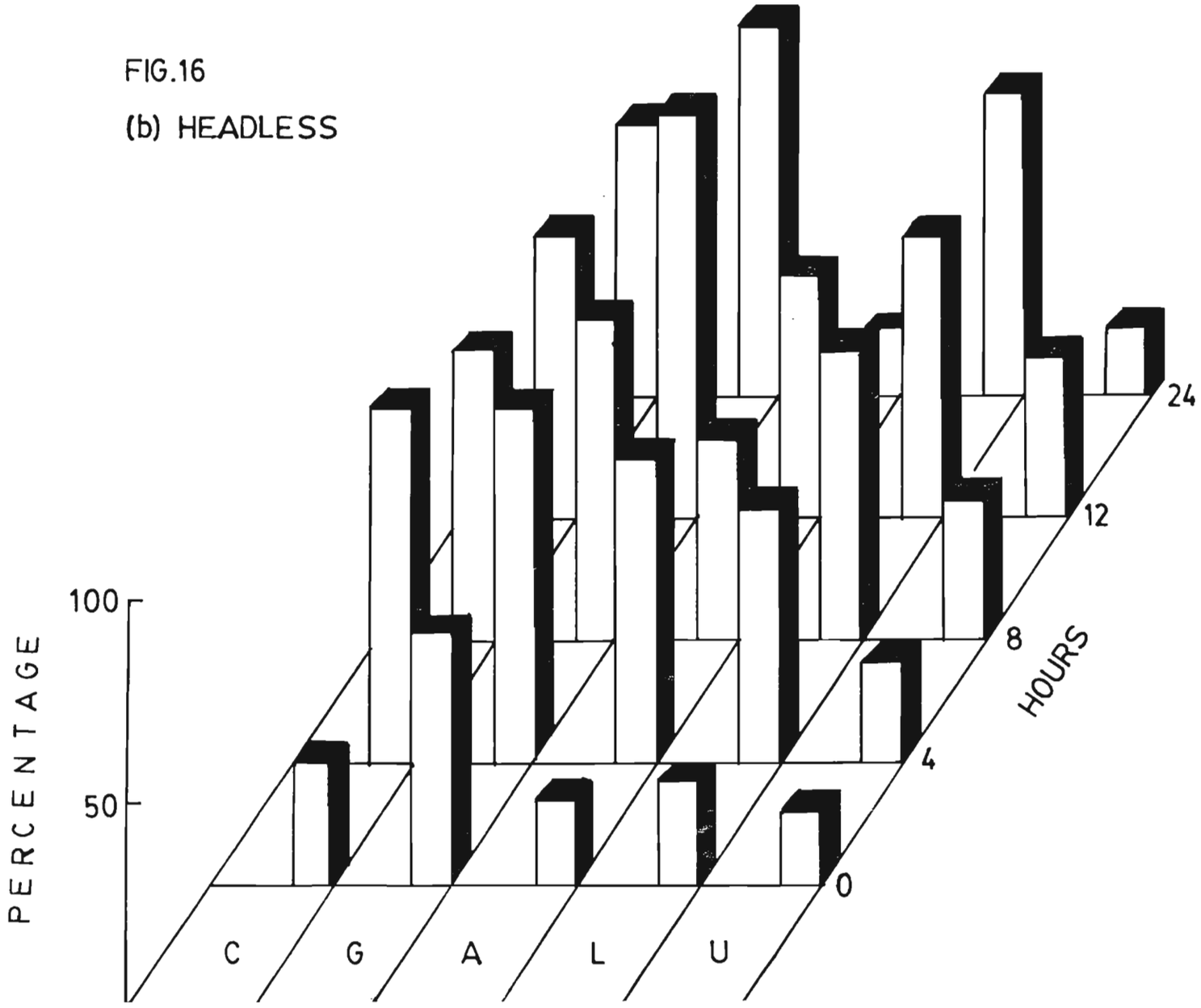


FIG. 16

(c) PUD

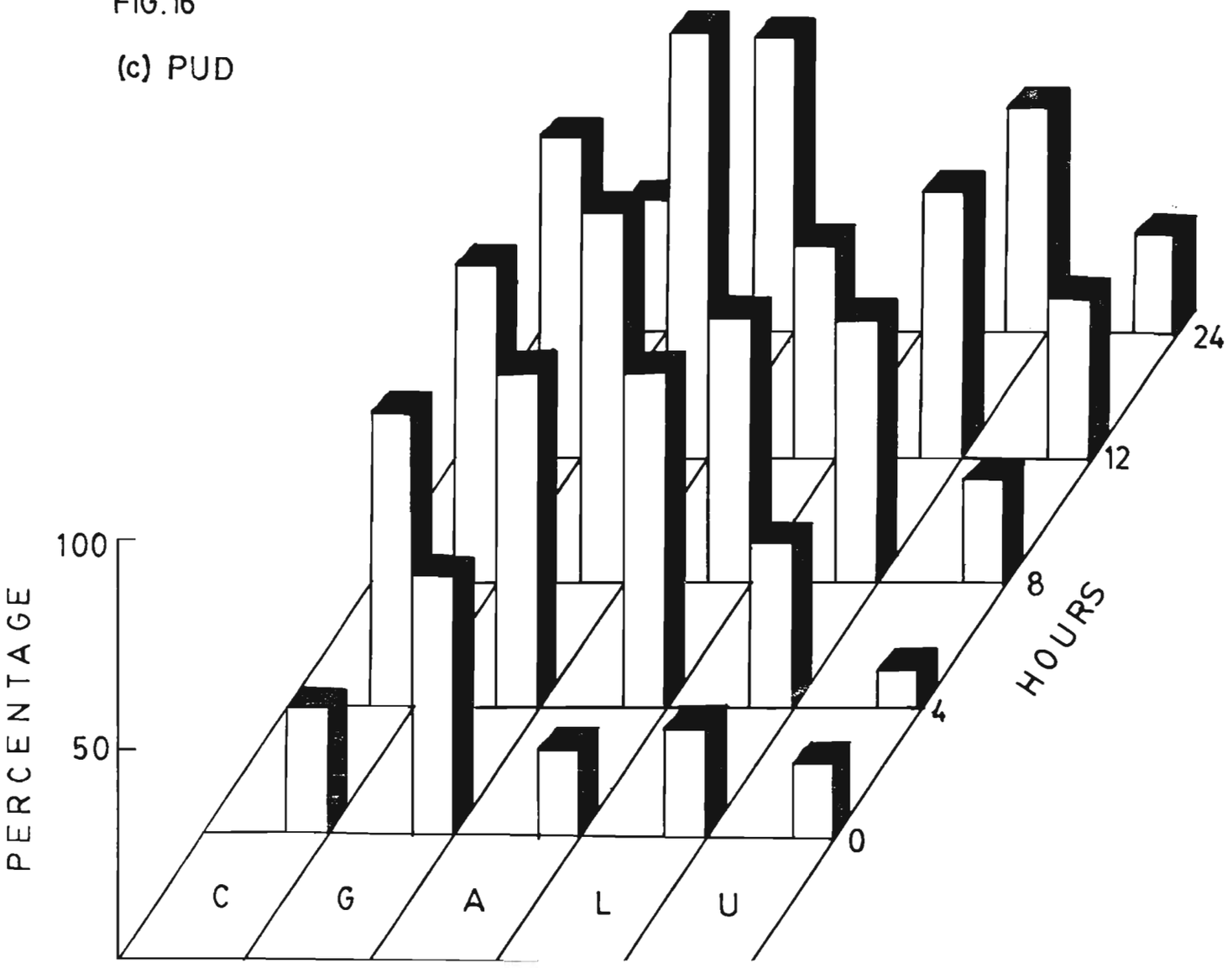


FIG. 16

(d) P D

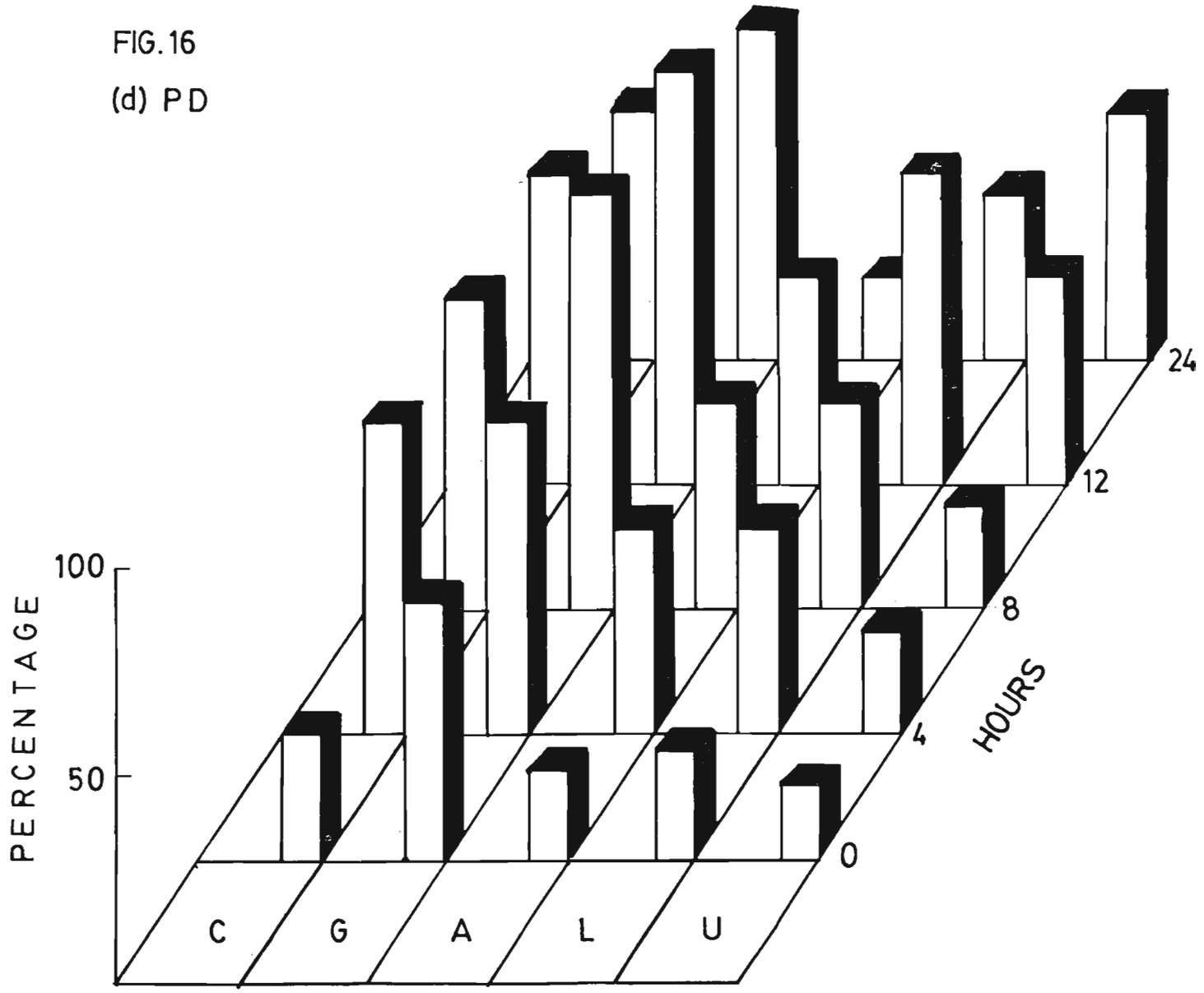


Fig.17. Changes in the percentage composition of different hydrolytic enzyme producing bacteria in the prawns stored at $4^{o}C$.

C - Caseinolytic

G - Gelatinolytic

A - Amylolytic

L - Lipolytic

U - Ureolytic

FIG. 17

(a) WHOLE

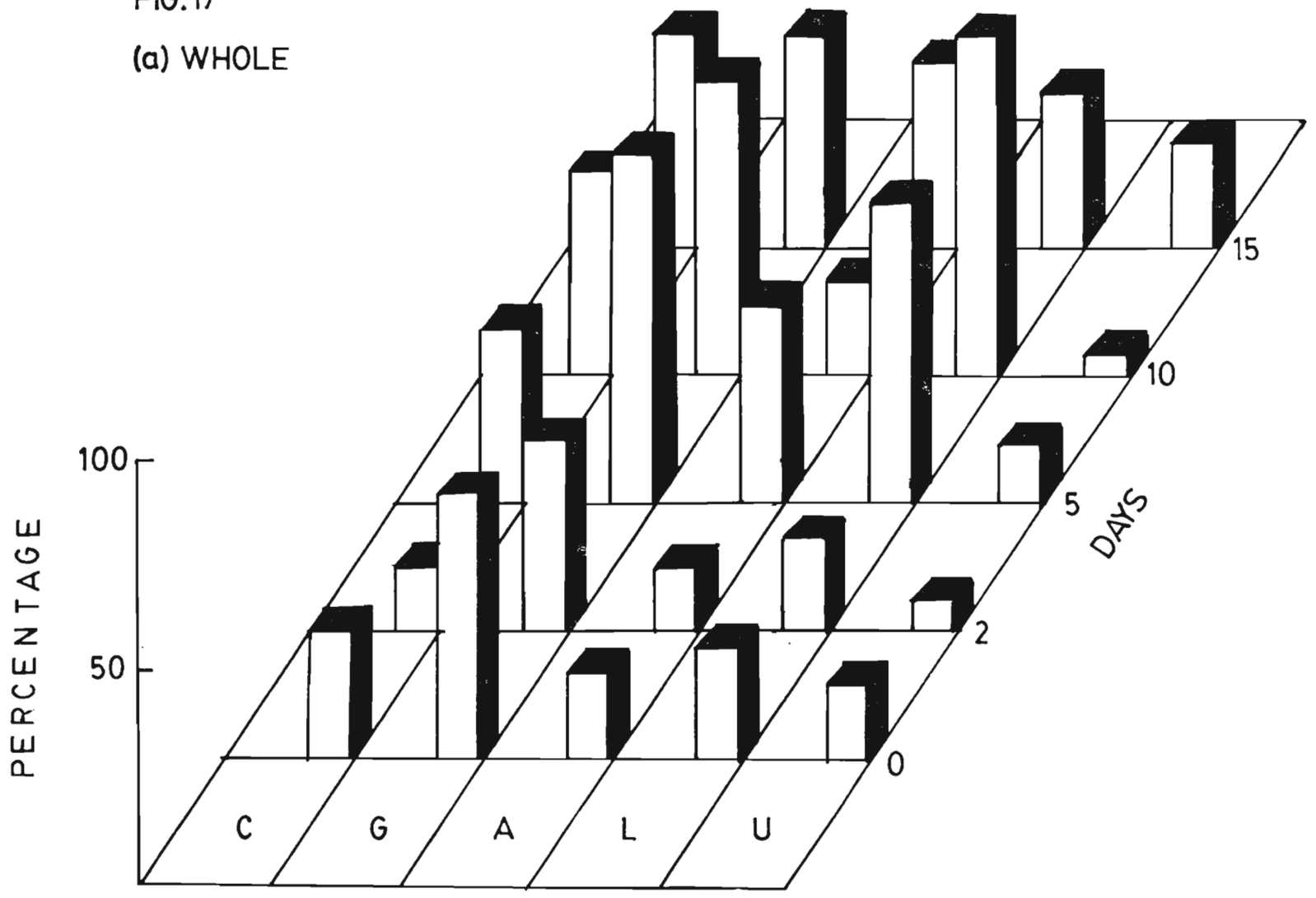


FIG. 17

(b) HEADLESS

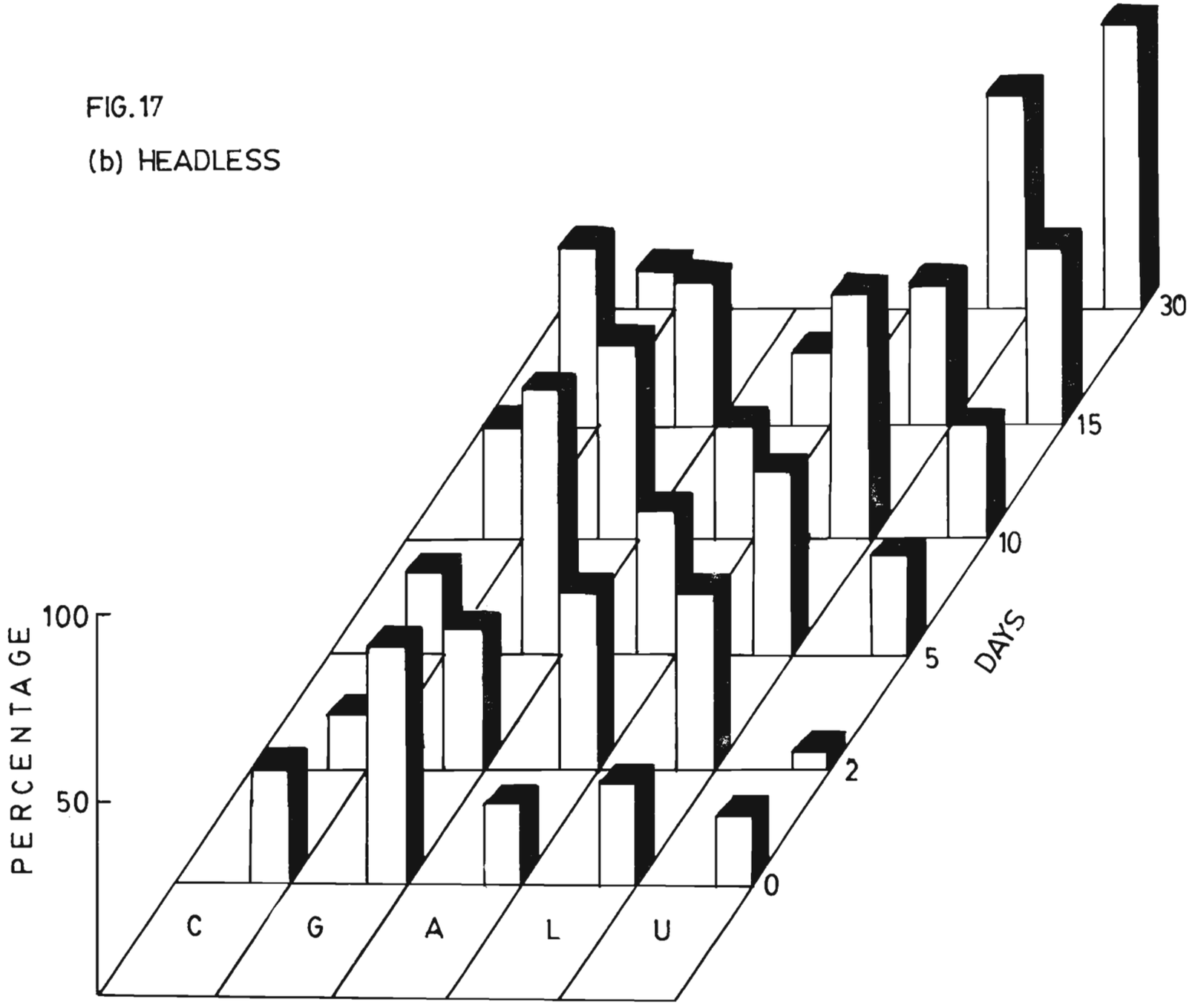


FIG. 17

(c) PUD

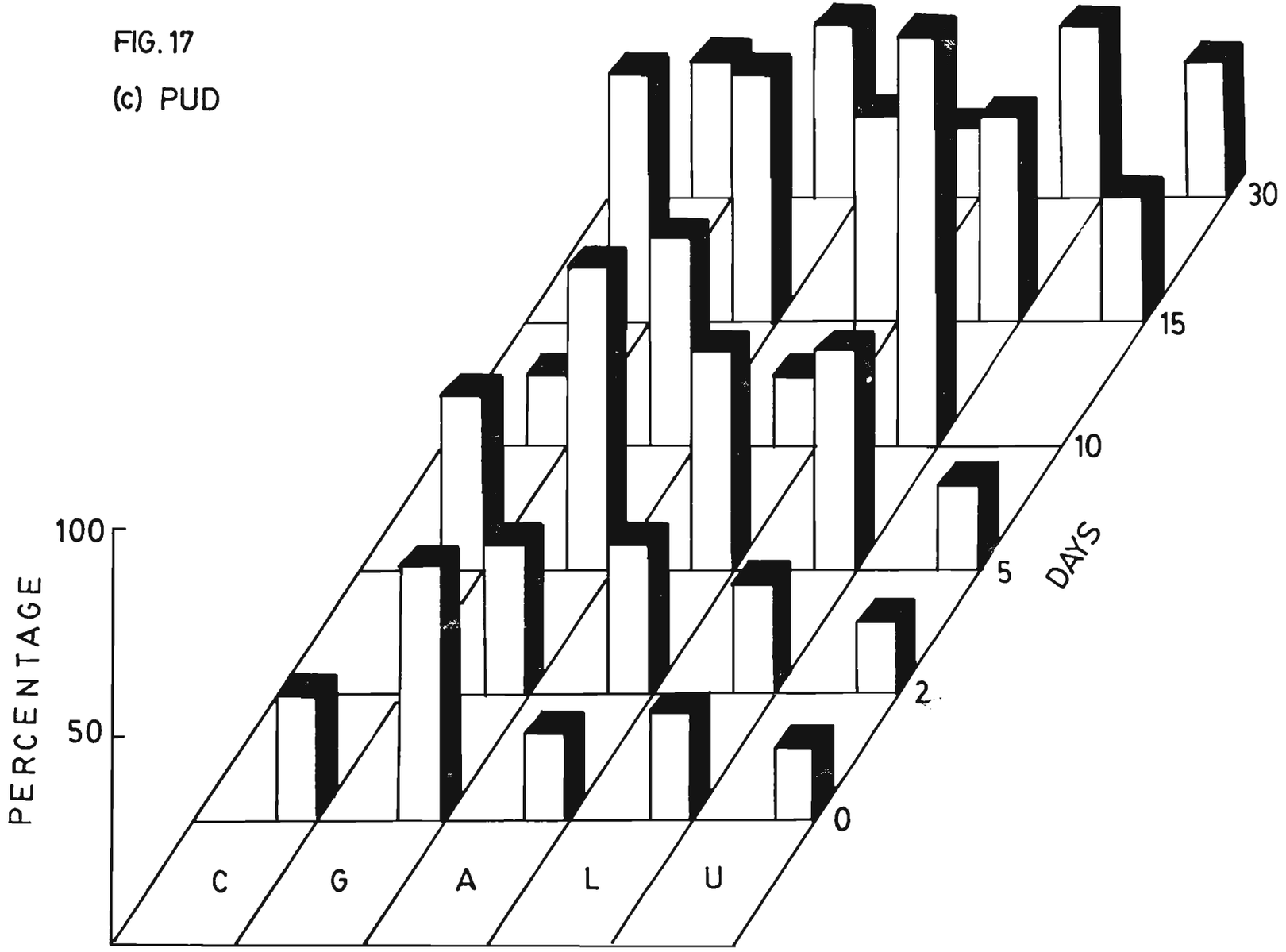


FIG.17

(d) P D

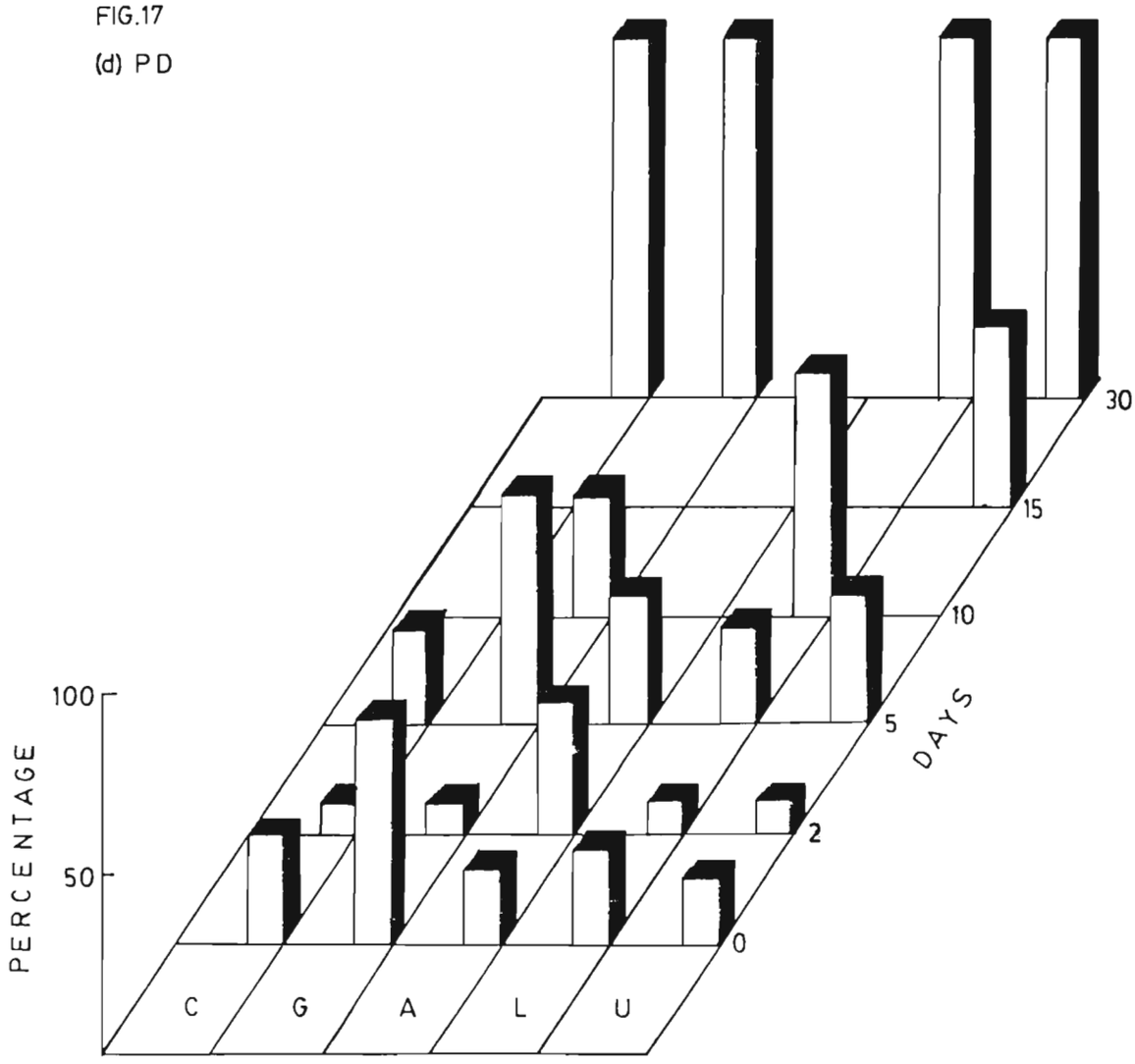


Fig.18. Changes in the percentage composition of different hydrolytic enzyme producing bacteria in the prawns stored at -18°C.

C - Caseinolytic

G - Gelatinolytic

A - Amylolytic

L - Lipolytic

U - Ureolytic

FIG.18

(a) WHOLE

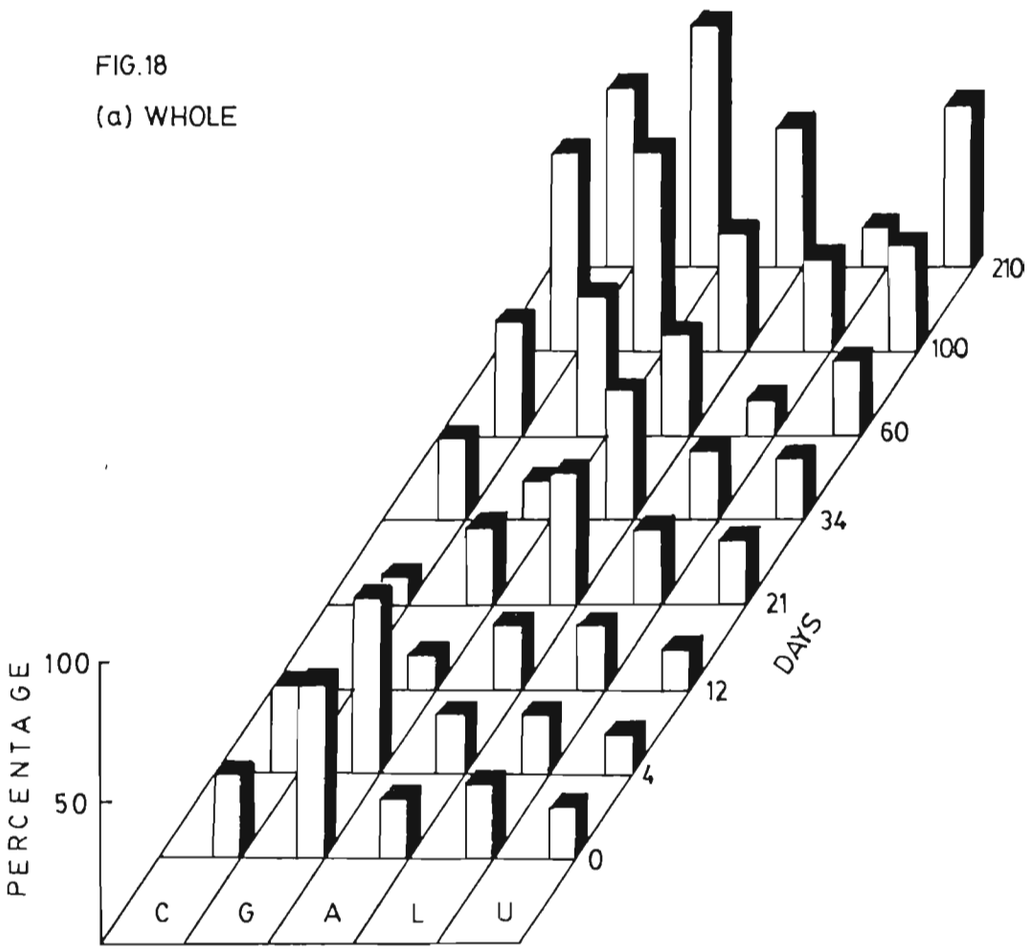


FIG.18

(b) HEADLESS

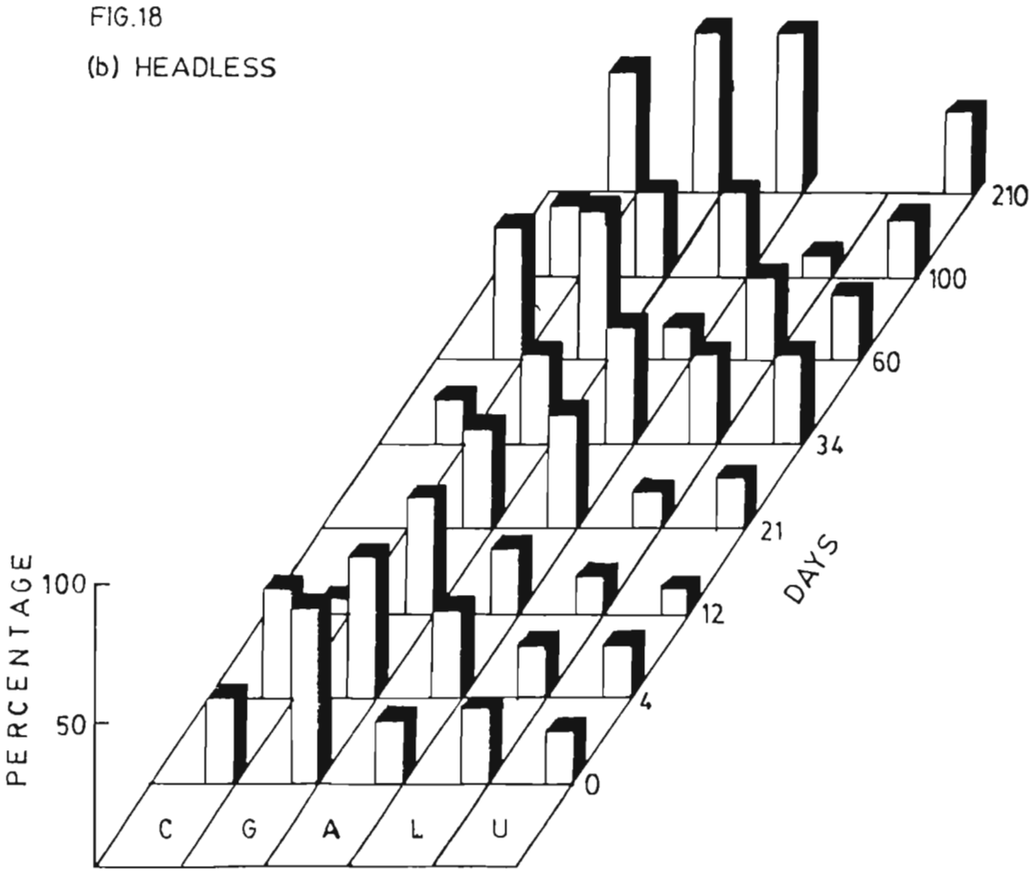


FIG.18
(c) PUD

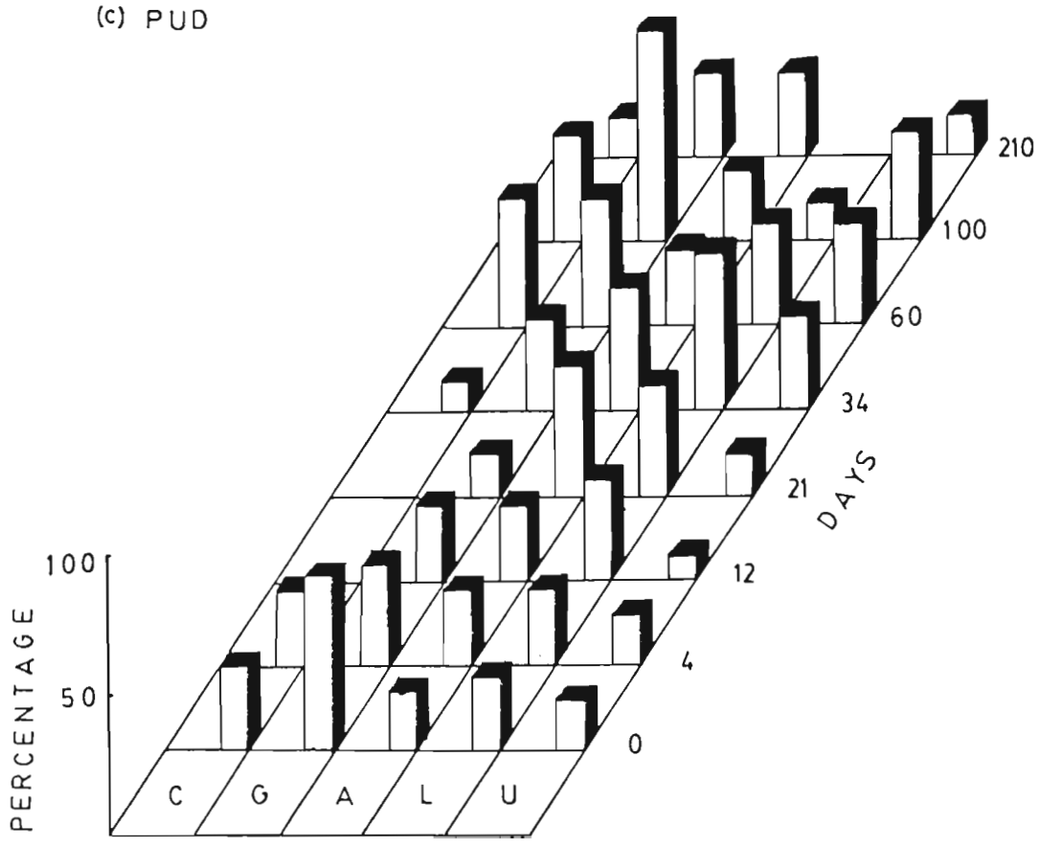


FIG.18

(d) PD

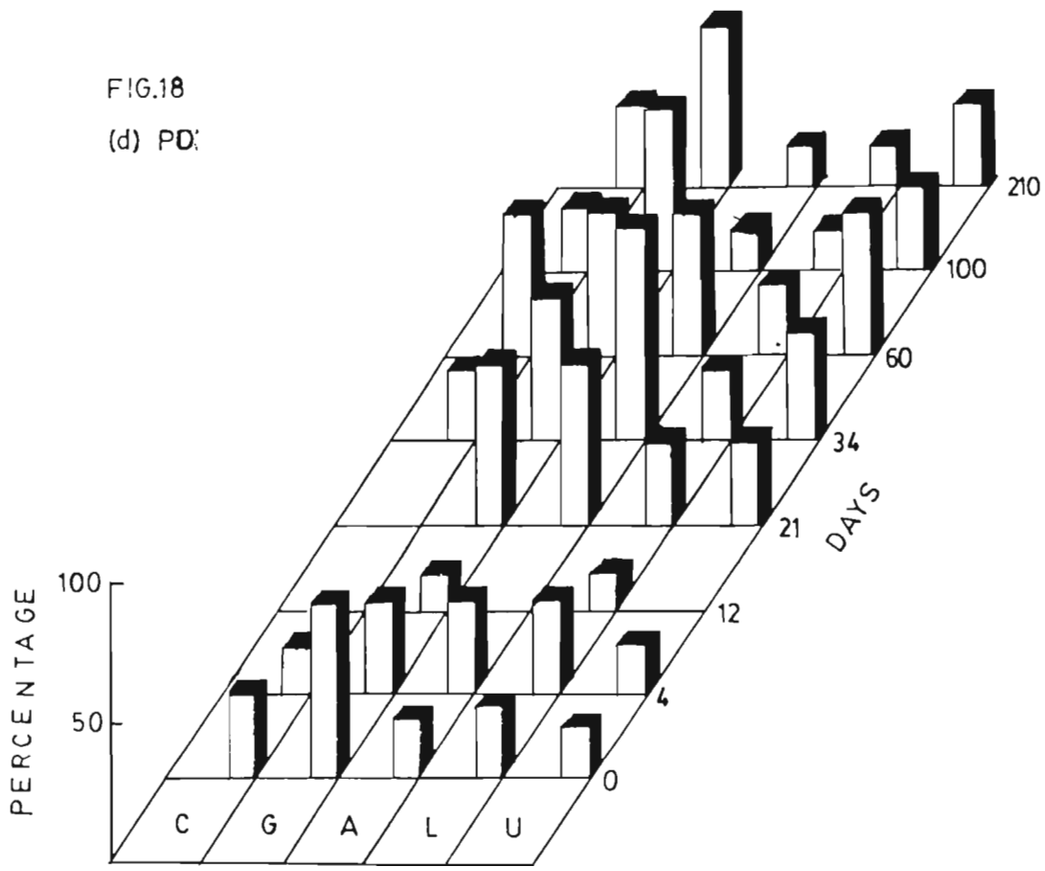


Fig.19. Percentage composition of potential flesh spoilers isolated from prawns stored at different temperatures.

Vi - *Vibrio*

Ae - *Aeromonas*

Ps - *Pseudomonas*

Al - *Alcaligenes*

Ac - *Acinetobacter*

E - Enterobacteriaceae

Mi - *Micrococcus*

Ba - *Bacillus*

Co - *Corynebacterium*

Fig.20. Percentage composition of Trimethylamineoxide reducing bacteria isolated from prawns stored at different temperatures.

Vi - *Vibrio*

Ps - *Pseudomonas*

Al - *Alcaligenes*

Ac - *Acinetobacter*

E - Enterobacteriaceae

Mi - *Micrococcus*

Ba - *Bacillus*

Co - *Corynebacterium*

FIG. 19

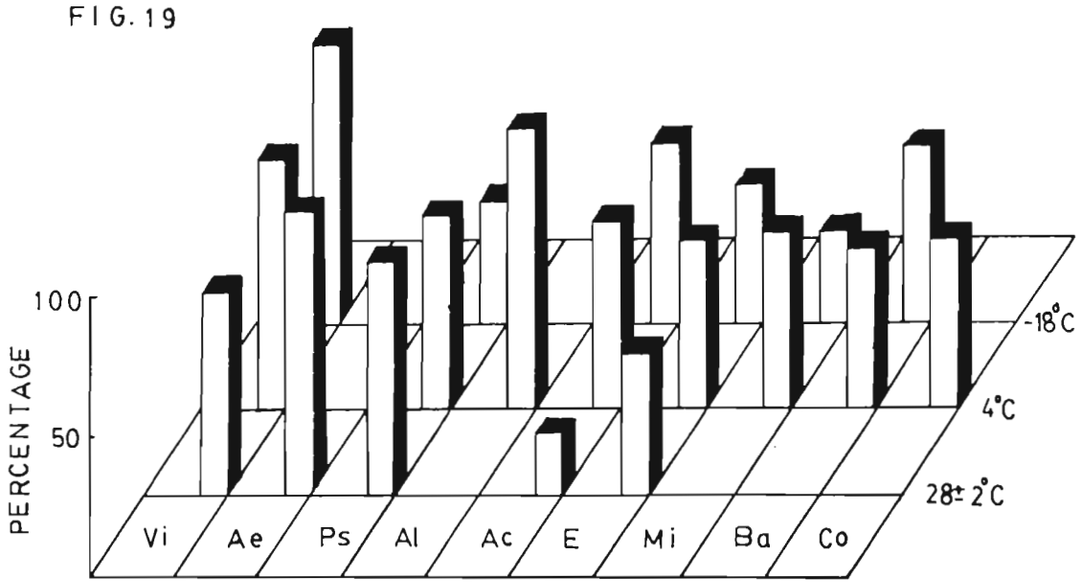

PERCENTAGE
100
50
Vi
Ae
Ps
Al
Ac
E
Mi
Ba
Co
-18°C
4°C
28± 2°C

FIG. 20

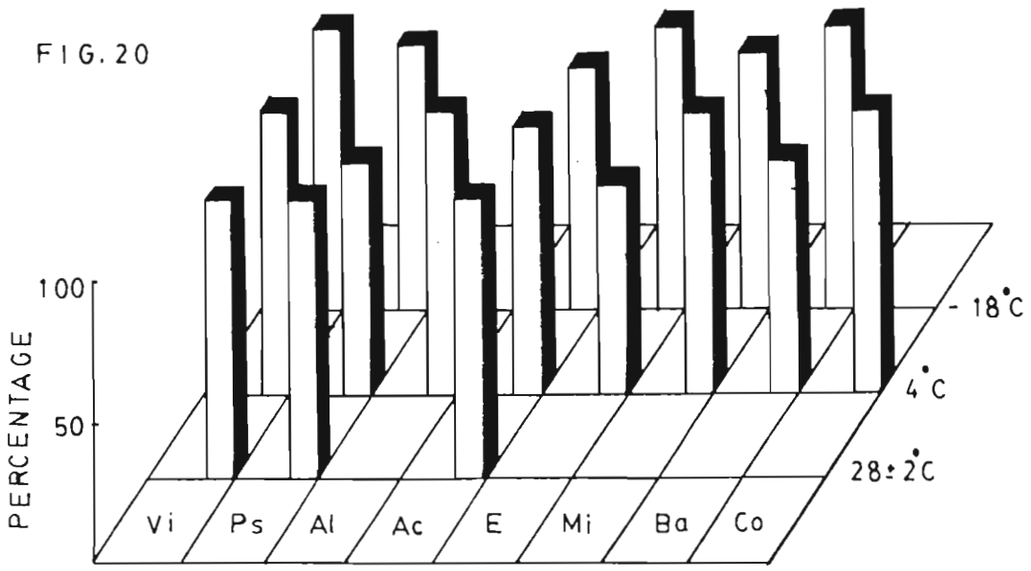

PERCENTAGE
100
50
Vi
Ps
Al
Ac
E
Mi
Ba
Co
- 18°C
4°C
28± 2°C

Fig.21. a - h. Combined effect of temperature, pH and Sodium chloride concentration on the growth of spoilage bacteria.

a) *Pseudomonas* R8

b) *Vibrio* R42

c) *Pseudomonas* L97

d) *Acinetobacter* L114

e) *Vibrio* L146

f) *Vibrio* F10

g) *Acinetobacter* F88

h) *Pseudomonas* F153

FIG. 21

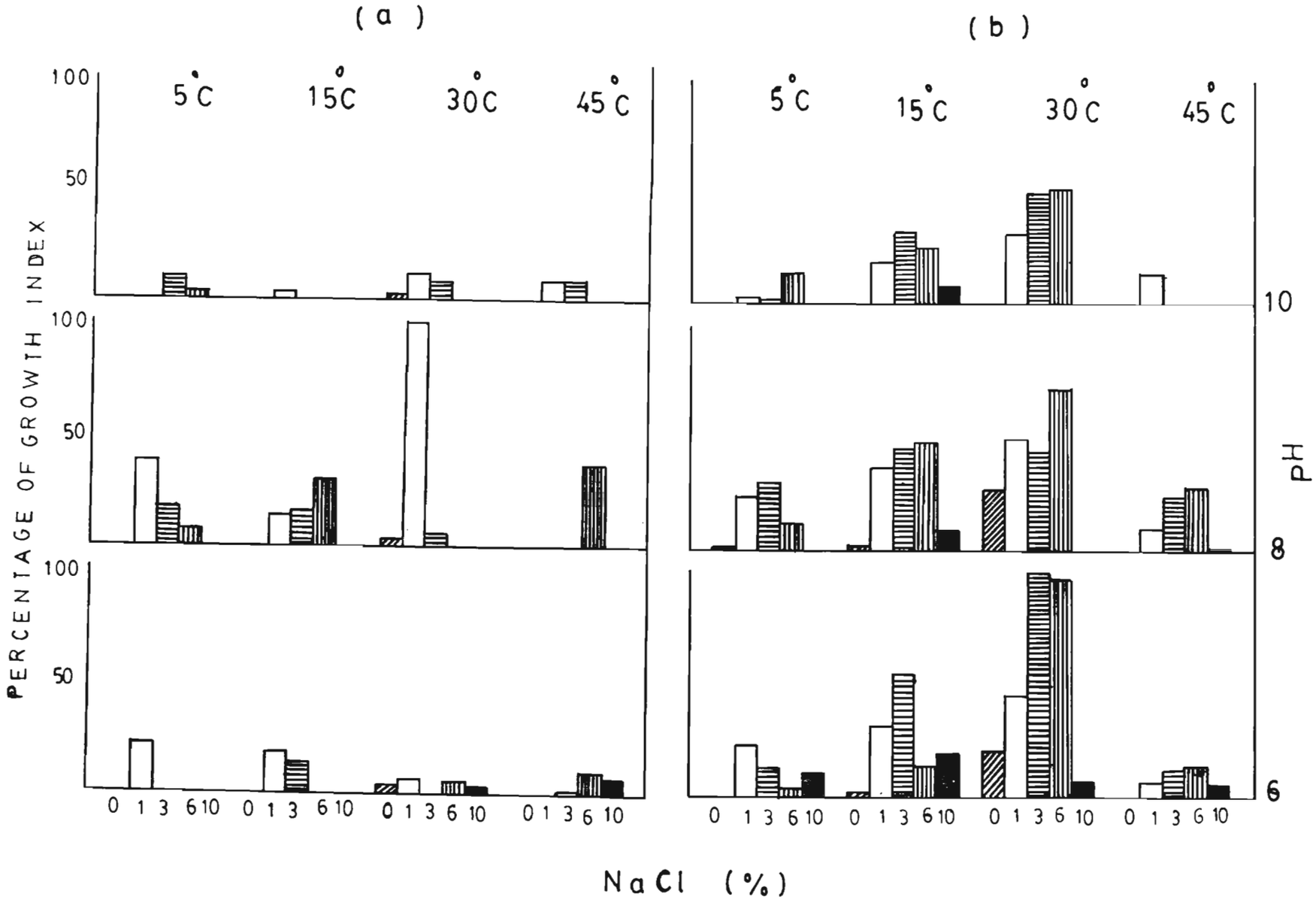

(a)
(b)
5°C
15°C
30°C
45°C
100
50
PERCENTAGE OF GROWTH INDEX
0 1 3 6 10
pH
10
8
6
NaCl (%)

FIG. 21

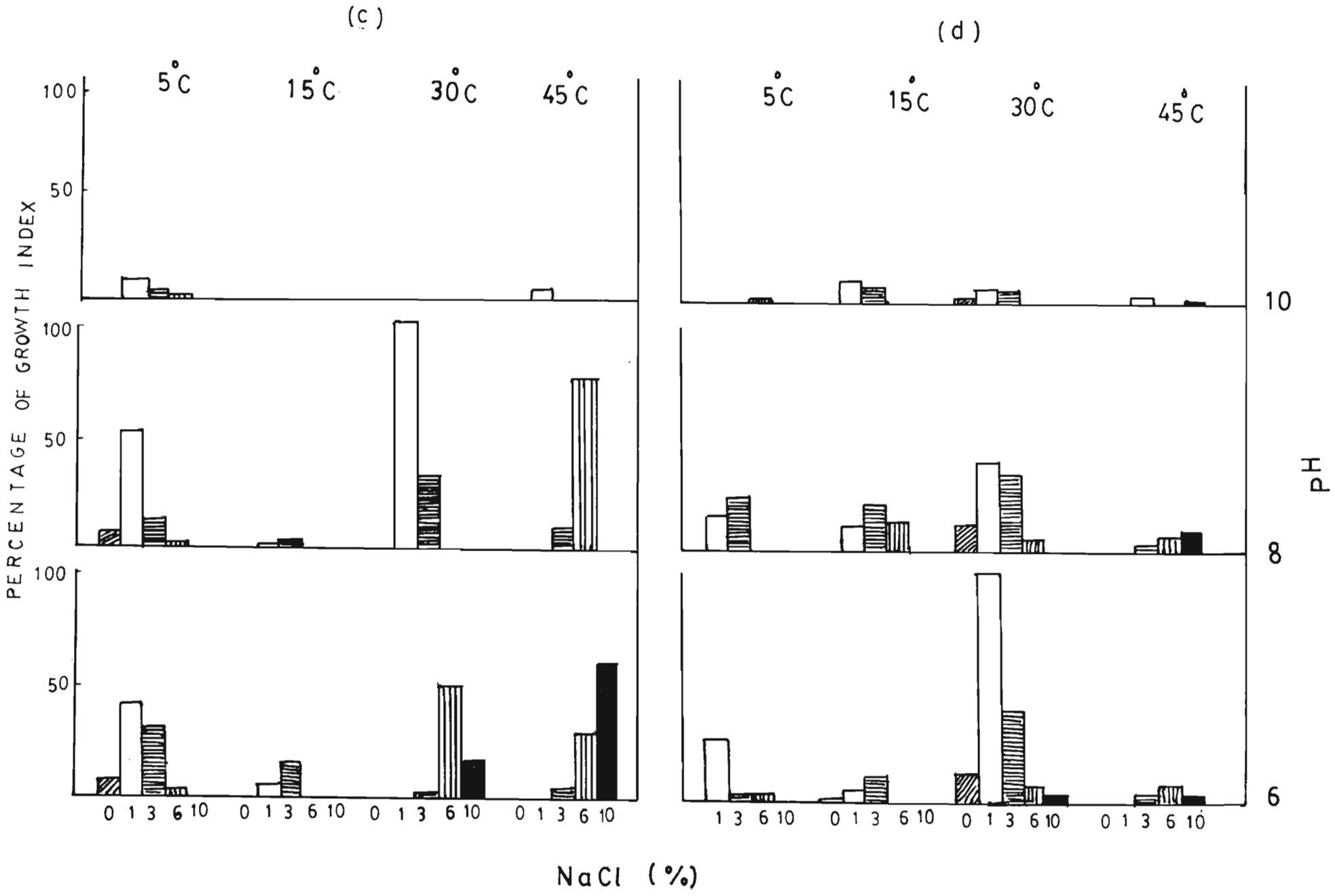

(c)
(d)
5°C
15°C
30°C
45°C
100
50
PERCENTAGE OF GROWTH INDEX
pH
10
8
6
0 1 3 6 10
NaCl (%)

FIG. 21

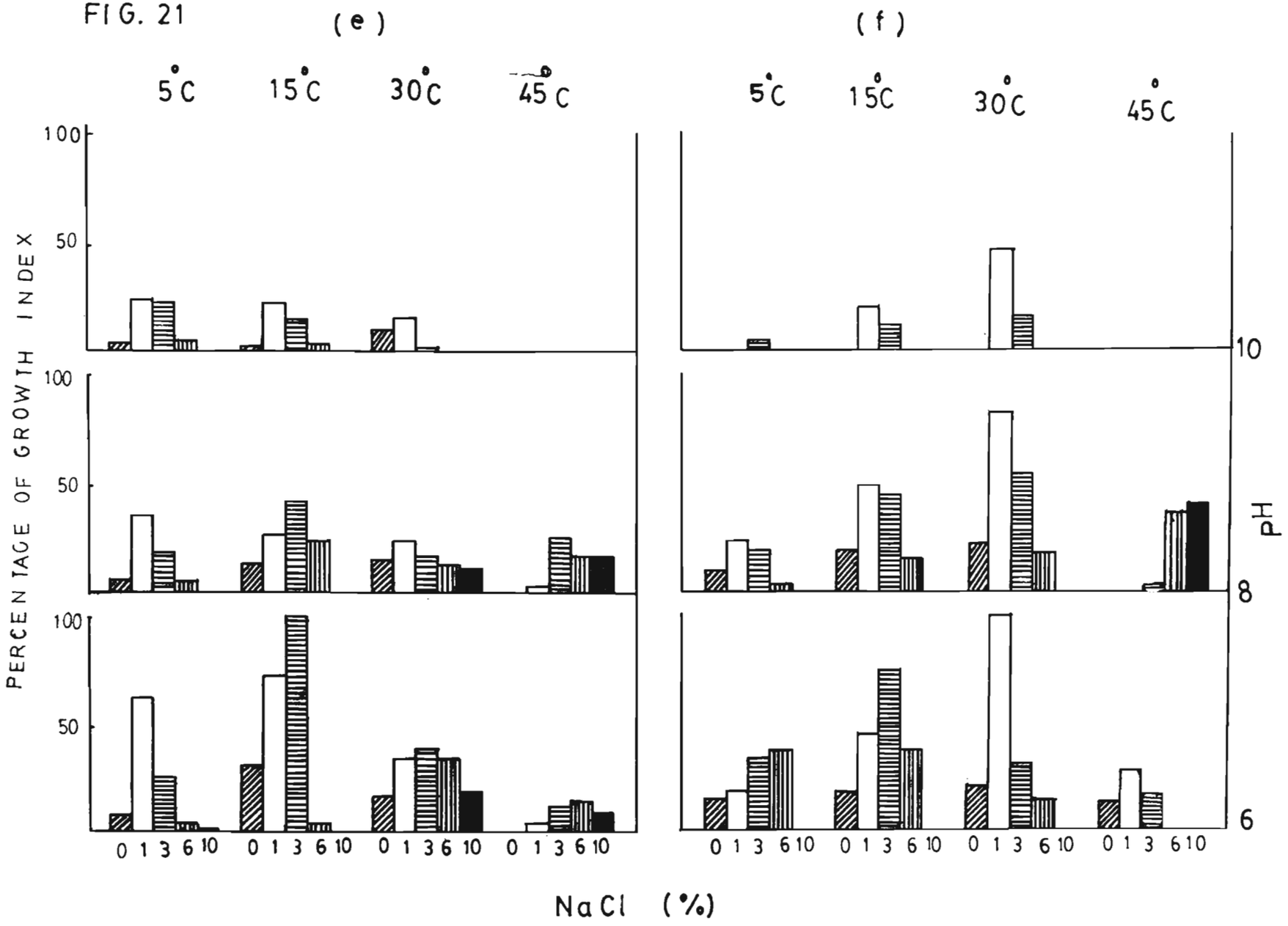
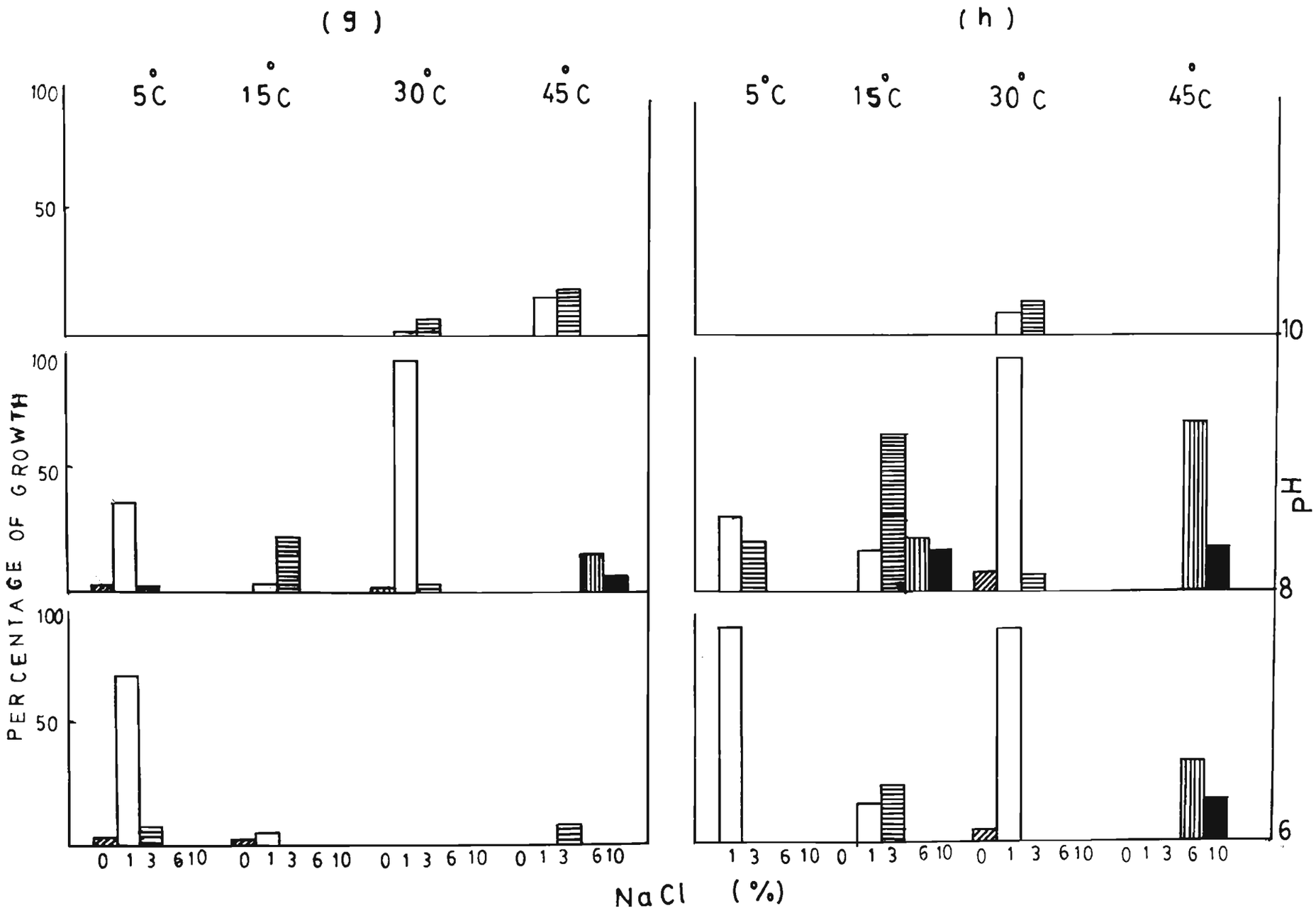

(e)
(f)
5°C
15°C
30°C
45°C
PERCENTAGE OF GROWTH INDEX
100
50
0 1 3 6 10
NaCl (%)
pH
10
8
6

FIG. 21


(g)
(h)
5°C
15°C
30°C
45°C
100
50
PERCENTAGE OF GROWTH
pH
10
8
6
0 1 3 6 10
NaCl (%)

4. DISCUSSION

4.1. STORAGE CHARACTERISTICS OF PRAWN

4.1.1. Organoleptic assessment:

Organoleptic assessment of the prawns stored at room temperature ($28\pm2^{o}C$) which is almost equal to atmospheric temperature of the fishing area, where the prawns are caught and stored immediately on the countryboats and nets, suggested that they spoil rapidly and get perished by the end of 8 h. All the samples 'Whole', 'Headless', 'PUD' and 'PD' showed similar changes organoleptically. The results show that freshness of prawn is not maintained after 4 h and spoilage sets in causing damage to the commodity. This suggests that rapid preservation should be exercised immediately after catch avoiding any delay in icing, so that the freshness can be extended considerably. This is in agreement with the earlier findings of Pillai *et al.* (1965) who reported that prawns *Penaeus indicus* retained their prime quality only for about 4 h at $28^{o}C$ (atmospheric temperature) and completely spoiled by 8 h.

Results also suggest that prawns stored at $4^{o}C$ in unprocessed form cannot keep their freshness beyond 2 days. Pattern of spoilage at $4^{o}C$ did not vary much from that of $28\pm2^{o}C$. Velankar *et al.* (1961) reported that whole prawns could be considered fresh only for a period of about 5 days after which spoilage sets in even at $0^{o}C$. Cann (1974) also

indicated that tropical shrimps start to lose their flavour as early as 2-4 days in ice-stored conditions. Vanderzant et al. (1973) also found that some deterioration in appearance had occurred in all species of Gulf shrimps except in Penaeus setiferus after 7 days of storage at refrigerated temperature.

Prawns stored at -18°C, the temperature at which usually the processed frozen prawns and fishes are stored in the cold storage, did not give an extended shelf life for more than a month. The results indicate that spoilage odours and characteristic appearance of red colour could be seen after a month and by 100 days they attained maximum deterioration.

Melanosis, a characteristic sign of progressive spoilage, was observed in prawns stored at three different temperatures. The colour varied from red to black and was rapid in sample 'Whole' at all the temperatures tested. Melanosis had set in at an earlier stage and became intense in samples stored at 28±2°C. The blackening brought down the quality of prawns from aesthetic view. The change of colour may be due to the active role of muscle enzymes which might have degraded the flesh. Sample 'Whole', which harboured high bacterial populations was found to be affected in a shorter period than other samples. This could be attributed

to the active participation of bacterial enzymes which might have contributed to melanosis. Similar observations of melanosis was reported in prolonged chilled or frozen stored raw prawns suggesting the active role of muscle enzymes in the process (Alford and Fieger, 1952; Bailey and Fieger, 1954 and Velankar, 1965). Incidence of melanosis was far less in the 'Headless' prawns when compared with 'Whole' prawns (Velankar et al. 1961). Cobb et al. (1977) reported that prawns held between 30 and 44°C for 3 h or less showed characteristic red and orange colours which are considered indicative of spoilage in raw white shrimp. Further they observed that melanosis occurred in only one lot of shrimp which was held for 6 h at 37 and 44°C.

4.1.2. Total Heterotrophic Bacterial Population:

The bacterial flora on fish is a function of the environment in which they are caught and subsequently stored (Disney, 1976). Also a general similarity is supposed to exist between the bacterial flora responsible for the spoilage of fish and that existing in its marine surrounding (ZoBell and Uphem, 1944). The prawns harboured 13.35 x 10^6/g of THB and the results indicate the influence of the environment on the distribution of bacteria associated with prawns. Also, the counts recorded were comparable to the total viable counts reported on shrimps by Venkataraman et al. (1953), Fieger et al. (1956), Pillai et al. (1961), Vanderzant et al. (1970,'71,'73) and Cobb et al. (1977). According to

Thatcher and Clark (1968), when fish harbours more than 10^6 microorganisms per gm or cm^2, it is considered to have a very short potential shelf life or may even to be at incipient spoilage. Results obtained from storage studies of the prawn at the three temperatures also suggest that the shorter shelf life observed might be due to the higher initial population. At 28 ± 2^oC, 'Whole' types showed maximum viable counts by 8 h suggesting definite spoilage which was supported by organoleptic assessment. 'Headless' types comparatively showed lesser counts than 'Whole', however the number of bacteria was significant. 'PUD' and 'PD' also recorded high counts showing rapid spoilage. The results indicate that the prawn was organoleptically spoiled, as determined by odour and other characters when a population of 10^8 - 10^9/g was reached.

Prawns stored at 4^oC also showed similar pattern of high THB during storage. Organoleptic and other chemical tests suggested that they were spoiled completely by 10th day itself. The total viable counts recorded in 'Whole', 'PUD' and 'PD' also agree with the observation made on organoleptic examination, by showing maximum viable counts on 10th day, 'Headless' types also showed similar result, however high counts were found only on the 30th day. The bacterial counts decreased during the initial days of storage in 'Headless' and 'PD'. The number of bacteria were found

to be higher than the counts reported by Velankar and Govindan (1959) who observed 10^6 bacteria/g after 7 days in Metapenaeus affinis and Penaeus indicus stored at 3°C. Vanderzant et al. (1973) and Cobb et al. (1976) have also observed only a very slight increase in the total viable counts, but in general they observed a decrease in total viable counts during the initial days of storage in ice. The higher viable counts and short storage life of raw prawns observed at 4° during the present study were definitely due to the higher initial counts of bacteria as Thatcher and Clark (1968) suggested. Also the decrease in bacterial population in the initial days of storage and an increase after 5 days was reported by Velankar et al. (1961). The differences in results reported earlier might be due to the low population observed in the fresh samples before subjecting them to storage, or plating method as suggested by Vanderzant et al. (1971).

Results obtained for the total viable counts of prawns stored at -18°C showed the influence of temperature on bacteria. A comparatively higher count during storage was noticed only in 'Whole' samples than other types. There was no change in population during the initial days. However, the population reached 10^9/g after 60 days of storage agreeing well with the organoleptic assessment and chemical scores indicating incipient spoilage of all the four varieties of prawn. Pillai et al. (1961) have recorded

counts less than 10^5/g during frozen storage upto a period of 13 weeks where the initial population did not exceed 10^4/g. Significant decrease in bacterial counts during freezing of various types of fishes and shell fishes have been reported by several workers (Kiser and Beckwith, 1942; Green, 1949 b; Shaikhmahmud and Magar, 1965; Bose, 1969; Radhakrishnan et al. 1973). However in the present investigation the TVC level did not show any specific trend but only fluctuated.

Varying results reported by many authors for iced, iced and frozen processed prawn and fishes are however, not observed in the present study, since the prawns were subjected to storage in raw unprocessed condition. The commensal flora with a high population, when subjected to sub ambient temperatures especially at -18°C showed an initial decrease. However, they were able to withstand the low temperature and multiplied during the later stages of storage, making the sample to deteriorate. The initial decrease might be due to an extension of lag phase growth and elimination of various groups as the temperature fell below the tolerance limit (Hess, 1934; Kiser, 1944; Ingraham, 1958). The population observed later might be caused by the survivors which could adapt to the new environment. As Kiser and Beckwith (1942) and Pivinik (1949) suggested, freezing causes a destruction of 60 to 90 percent bacterial population.

But freezing is usually done at -40°C, where most organisms die. But in the present study they were directly subjected to -18°C. Certain organisms which are psychrophilic and psychrotrophic might have withstood this temperature and survived. After passing through the initial lag phase, they could have proliferated and strenously multiplied utilizing the available substances as reported by Pablo et al. (1965). The initial counts reflected the survivors of reduced temperature, whereas, subsequent count reflected their growth pattern.

Higher bacterial counts associated with rapid spoilage of foods is a well known phenomenon. Spoilage is caused mainly as a result of bacterial activity over the flesh substrates in which most of the macromolecules are broken down into more readily usable metabolites. In such environments, normally one can expect a rapid increase in bacterial population. Such a pattern of spoilage is evident from the results obtained from the study, where incipient spoilage and loss of quality always accompanied increase of bacterial population in spite of the variation in storage temperature.

Incubation temperatures after plating are known to influence the total population enumerated. Nirmala Thampuran et al. (1981) have reported that spread plate technique yielded more counts than pour plate counts where most of the

psychrophiles may not develop. But they have suggested that a higher count was recorded when incubated at 30°C for fish and fishery products. However, in the present study, pour plate technique and incubation of inoculated plates at 28±2°C gave satisfactory results with higher viable counts. There was no chance of contamination of terestrial forms at any stage since aseptic condition was strictly enforced throughout the study to ensure the presence of native commensal flora and their role in spoilage. Beheading and peeling in fact, to some extent reduced the population level but did not extend the shelf life of the prawns at 28±2°C and 4°C. The present results support the findings of Koburger et al. (1974), who reported that bacterial counts on shrimp tails of Penaeus sp. stored with and without heads were essentially the same and a 15 member taste panel could not detect any significant variation in flavour, texture or overall acceptability between shrimp stored with or without heads throughout the 14 day storage period.

4.1.3. Chemical indicators:

4.1.3.1. Trimethylamine (TMA):

Trimethylamine is considered to be an useful index of quality assessment in shrimps and appears to be a good indicator of spoilage (Campbell and Williams, 1952; Velankar and Govindan, 1959; and Cobb et al. 1977). Bailey et al. (1956)

stated that in most cases a TMA value of 1.5 mg/100 g shrimp tissue on headless-shell on shrimps indicated an unacceptable product. In the present study similar or higher levels of TMA, in 'Whole' and 'Headless' at 12 h and in 'PUD' and 'PD' at 8 h, were observed when stored at room temperature suggesting their unacceptable levels. However, the level of TMA was found to be less than that reported by Pillai et al. (1965) who observed 2.33 mg/100 g at 8 h for P. indicus stored at 28±2°C. The maximum level of TMA recorded in this investigation was 2.1 mg/100 g at 24 h and did not show much variation between the four types of samples. High level of TMA indicated the complete spoilage of prawns which was in agreement with the organoleptic assessment.

At 4°C, the TMA levels showed an inconsistent pattern. TMA formation was rather slow and did not show any specific relation to organoleptic assessment. Also, the level of TMA was not significant enough to indicate the loss of quality. Similar observation on insignificant level of TMA in ice stored prawns was reported by Velankar and Govindan (1959). Velankar et al. (1961) reported that TMA content increased in both 'Whole' and 'Headless' prawns stored at 0°C and was detectable after 3 days, whereas, in peeled prawns it was detectable only after about 20 days. Sajan George (1979) observed very high TMA level (3.35 mg/100 g) in market fresh shrimps and low level (1.75 mg/100 g) on freshly caught

shrimps at the stage of incipient spoilage during ice storage. The low level of TMA obtained at 4°C may be due to the reduced activity of TMAO reducing bacteria at this low temperature which might have taken active role at room temperature in contributing to the spoilage.

All the four types of prawns stored at -18°C contained the same level of TMA concentration (1.666 mg/100 g) when stored for 210 days, suggesting complete spoilage (Bailey et al. 1956). However the increment in the TMA level was gradual. Organoleptic assessment suggested them to be spoiled after 60 days. But the TMA level around 60 days was only 0.7 mg/100 g. Pillai et al. (1961) observed the same level of 0.7 mg/100 g of TMA in headless fresh frozen samples during a storage period of 1 week. He reported a significant variation (0.7 to 2.8 mg/100 g) of TMA during prolonged storage and suggested that the fluctuation might have been associated with the level of bacterial population and initial substrate concentration. In the present study such a fluctuation in TMA level was not noticed, in spite of the variation in the bacterial population. Cobb et al. (1977) reported that TMN production appeared to be a good indicator of spoilage and high bacterial levels ($>10^6$/g). Fieger and Friloux (1954) also indicated that TMN levels in Gulf shrimp increased when bacterial levels were high.

4.1.3.2. Total volatile Nitrogen (Ammonia-NH_3):

Ammonia content of prawn tissue showed a steady increase during spoilage. The prawns (Whole, Headless, PUD and PD) stored at room temperature recorded an increase to a level of about 250 µg/g (25 mg%) by 8 h, when they were found to be spoiled organoleptically. The ammonia content was found to be the same in all the four samples at the time of complete spoilage. Cobb et al. (1973) while studying NH_3 formation in shrimp, in in situ conditions, found that spoiled samples recorded 13.2 mg% of ammonia and 1.5 mg% of TMA. The present study showed that the ammonia content in all the stored samples crossed that level at 4 h of storage, suggesting their progressive spoilage even before 4 h at 28±2°C. However, Pillai et al. (1961) reported that the change in TVN become significant only after 8 h of spoilage when prawns were stored at room temperature and the concentration of NH_3 was 31.57 mg% at 10 h in Metapenaeus dobsoni. Similar increase of TVN in shrimp was also recorded by Cobb et al. (1977). All shrimps with musty odour had TVN level ranging from 28 to 40 mg N/100 g and distinct putrid odours were not evident in shrimp with less than 40 mg N/100 g.However in P. indicus, stored at 28±2°C, strong putrid musty odour was recorded while NH_3 content was about 30 mg%.

At 4°C, NH_3 levels showed a steap rise during storage. It equalled the maximum amount of NH_3, recorded in the samples stored at other temperatures within 10 days suggesting the early onset of spoilage. The final value recorded was very high (547.6 µg/g to 576.8 µg/g). Similar high values of NH_3 was recorded by Cobb et al. (1973) on the 5th day of storage in all the samples suggesting the possible commencement of spoilage after 2 days itself, agreeing well with organoleptic and TMA assessment. In the present study, concentration of NH_3 was very low initially and from the 5th day onwards rapid increase was observed in all the samples. Whole prawns contained the least amount of NH_3. Velankar et al. (1961) reported that the TVN increased gradually in the first 3 days and rapidly after 10 days reaching a maximum of 48 mg N/100 g of prawn flesh on 24th day of storage at 0°C. He also recorded a significant rise of TVN occurring after two weeks of storage. Further he stated that TVN increased more rapidly in whole prawns than in the peeled prawns at 0°C. In the present investigation a slow increase, as indicated by Velankar et al. (1961) was found. However, the higher amount of ammonia content was recorded in other types, than in whole prawn.

Ammonia level in prawns stored at -18°C was found to be similar to that reported by Cobb et al. (1973)

who observed significant increase during storage, and also agreed well with the organoleptic and TMA evaluations. The increase of NH_3 content was minimum and the data suggest that the spoilage might have commenced from the 4th day. As in prawns stored at $28\pm2^{o}C$ the amount of NH_3 was less in Whole, but the maximum content of 280 µg/g was recorded in PUD.

Montgomery et al. (1970) indicated that TVN was an useful indicator of quality for Australian shrimp and the TVN content exceeding 30 mg N/100 g of the sample could be regarded as poor quality or spoiled. Further, spoilage studies on fin fish and shell fish (Crooks and Ritchie, 1938; Burnett, 1965) showed a rapid increase of NH_3 as seen in prawn P. indicus in the present study. Higher levels of NH_3 observed in prawns stored at $4^{o}C$ is quite intriguing. Yeh et al. (1978) reported that ammonia producing enzymes present in white shrimp tails (abdomen) may contribute NH_3 in spoilage of iced shrimp. However, Stansby and Lemon (1933) attributed the poor fish quality due to spoilage, for the larger amounts of ammonia associated with decomposition by bacterial action. Salle (1961) suggested that ammonia is a metabolic by-product of several bacteria which hydrolyze protein and urea. Urea produced by the action of arginase could serve as a source for ammonia production and bacterial breakdown of urea may be an important source of ammonia,

particularly during prolonged ice storage (Cobb et al. 1977).

In the present study the results suggest the possible association of different spoilage flora which might have taken active role along with muscle enzymes in the utilization of protein and non protein nitrogenous substances of prawn muscle, to the rapid accumulation of ammonia. Further higher amounts of ammonia produced might be due to bacterial action on the flesh substrates and urea as evidenced by the increase of proteolytic and ureolytic bacteria.

4.1.3.3. pH:

The initial pH of the flesh was found to be 6. During storage the pH increased to 7.55 at 24 h and there was no appreciable change in pH between the four type of samples stored at $28\pm2^{o}C$. However a gradual increase was seen in all the samples. Similar pH increase was also recorded in all the samples stored at $4^{o}C$ and $-18^{o}C$. At $4^{o}C$ the maximum pH was recorded (pH 8.55) at the time of complete spoilage and the variation between samples was about one magnitude. The pH of the sample stored at $-18^{o}C$ also showed a maximum pH of 8.5 and the variation between samples was found to be less than one magnitude. The prawns stored at higher temperature showed higher level of pH at 24 h indicating faster rate of spoilage at the higher temperature.

Iyengar et al. (1960) based on their studies have suggested a pH index for assessing spoilage. According to them a pH value of 7.1 or less indicates prime quality, 7.2 to 7.6 indicate a spoilage stage and greater than 7.6 indicate definite spoilage. As per this index, the values obtained for prawns stored at room temperature did not indicate complete spoilage but showed the onset of progressive spoilage. Since Iyengar et al. (1960) have formulated the index based on the studies made on ice stored shrimp, the values obtained may be suitable for reduced temperatures as it is observed at $4^{o}C$ and $-18^{o}C$ in the present study. But pH index of sample stored at room temperature gave a different picture and the pH values at the initial stages did not show any relation with spoilage of flesh. However Cobb et al. (1977) indicated that tissue pH was not a good indicator of the quality of shrimp stored for 3 - 24 h at high temperatures. Pillai et al. (1961) observed pH values prevailing around 6.7 - 6.9 at 5 h and 7.4 - 8.05 at the end of 24 h in some penaeid prawns stored at room temperature and suggested that pH can not be considered as an index of spoilage in prawn especially during the early stages of spoilage which supports the findings of the present study. However increase of pH during storage at lower temperature which is an indication of advancement of spoilage was found and the results are in agreement with

earlier reports (Bailey et al. 1956; Iyengar et al. 1960; Flick and Lovell, 1972 and Rajasekara Melanta, 1980). Further the present results also support the findings of Baron and Villanueva (1973) on the determination of freshness of Penaeus schmith and P. duorarum by estimating the pH of the exudate which when crossed pH >8 did not offer acceptable commercial condition on preservation by refrigeration.

pH assessment of the flesh in spoilage throws evidence for bacterial proliferation and active spoilage in the tissue, since reduced pH during riger mortis arrests bacterial activity and an elevated pH towards alkaline from neutral accelerates bacterial action. Hence the present study supports the basic principle that the moderate pH values greater than neutral pH observed, strongly indicate the influence of bacterial action on the rapid spoilage of prawns during storage since pH is an important factor influencing the growth of spoilage microorganisms.

4.2. PROXIMATE COMPOSITION

The proximate composition of fresh prawn Penaeus indicus recorded in the present study was almost similar to that reported earlier (Sriraman, 1978; Mukundan et al. 1981). During storage the proximate values

of this prawn showed varying results. As seen from the results, protein, lipid, carbohydrate and ash contents showed significant reduction with increase of time, at all the three storage temperatures in all the samples, whereas moisture content showed significant increase during storage in all the samples.

Decrease in protein and non protein fractions of prawn muscle stored in ice was reported earlier (Govindan, 1962, '69 and Lekshmy et al. 1962). Nair et al. (1962) indicated progressive loss in total nitrogen, non protein nitrogen, alpha amino nitrogen, ash and total solids in prawns held in crushed ice and chilled seawater. Devadasan and Nair (1970) observed denaturation of major protein fractions in Metapenaeus dobsoni and Penaeus indicus in ice storage. They observed that denatured protein increased along with a decrease in protein fraction confirming denaturation of protein during storage. Wilaichon et al. (1977) also observed decrease of NPN and protein nitrogen by an average of 50% and 25% respectively in ice stored shrimps by the time spoilage had occurred and decrease in protein content at 23-24°C. Significant increase in water content ($p<0.01$), in shrimps, was also observed by them during ice storage. Similar increase in moisture and decrease in protein content was observed in spotted seer (Scomberomorous guttatus)

during ice storage by Shenoy and James (1974). Devadasan and Nair (1977) also reported decrease in protein fraction in fish muscle during ice storage. Rajendranathan Nair (1962) observed lipid break down in oil sardine during storage at refrigerated temperatures. Viswanathan Nair *et al.* (1976,'78) showed such lipid hydrolysis during frozen storage of mackerel (*Rastrelliger kanagurta*).

Changes in protein during storage at 28±2°C, 4°C and -18°C observed in the present study suggest that, prawn, which contain high protein, lose its nutritive value due to spoilage considerably, despite the influence of storage temperatures. Lipid which was reported to decrease in fishes was found to show similar degradation in prawn also. Proteolytic and lipolytic muscle enzymes might have played an active role in the reduction of the contents in the initial stages. However, the results show that a major reducti was seen only in the later stages of spoilage, where the bacterial enzymes would have played an active role in the deterioration. Reduction in carbohydrate and ash levels at incipient spoilage stages also suggested that they were attacke by certain group of bacteria which prefer them as substrates fo their growth. Increase in the moisture content during spoilage indicated a direct evidence for microbial action over the flesh substrates as water molecules were formed in many of the

metabolic reactions. Also increase of pH favoured the activity of the organisms which might have enhanced the amount of accumulation of water molecules.

A number of factors could affect the proximate composition of freshly killed shrimp, such as size and salinity of the water from which the prawns were caught (Lockwood, 1968; Wilaichon et al. 1977). The type and level of microorganisms on the shrimp and endogenous enzymes or the enzymatic contamination during the processing of the shrimp might have influenced post mortem biochemical changes as suggested by Cobb and Vanderzant (1971). Bauer and Eitenmiller (1974) reported intracellular catabolism of proteins by enzymes (arylamidase) occurred in white shrimp. The loss of non-protein and protein nitrogen during the ice storage suggested that these components were probably contained in interstitial fluid and/or that there was considerable cellular breakdown during the storage period.

4.3. GENERIC COMPOSITION OF SPOILAGE FLORA

The available literature show that the microbial flora associated with higher organisms (quantitatively and qualitatively) is a function of the environment in which they are caught (Shewan, 1977). This is agreeable in Cochin backwater also, where species of Gram-negative bacteria were found to be dominating in freshly caught prawns. Species of

Vibrio, *Pseudomonas*, *Acinetobacter*, *Bacillus* and *Micrococcus* were found in order of predominance (Chandrasekaran *et al.* 1984 b). Occurrence of *Vibrio* sp. as predominant group in fresh pond shrimp has been documented by Vanderzant *et al.* (1971) and Christopher *et al.* (1978). Cobb *et al.* (1976) reported predominance of *Vibrio* along with *Pseudomonas* and *Moraxella*, *Acinetobacter* species in fresh Gulf shrimps.

Spoilage at room temperature ($28\pm2^{o}C$) in all the four types was mainly accompanied by rapid increase of *Vibrio* sp. which was found to be dominant during storage. The commonly considered spoilers like *Pseudomonas* and *Acinetobacter* were, however, found to be less dominant. The unusual observation made on the occurrence of *Vibrio* sp. and spoilage activity at $28\pm2^{o}C$ particularly on this prawn and generally on any fish or prawn is not reported so far. It could be suggested that the initial bacterial flora of fish or prawn, which is a function of the environment, may have a definite influence on the existence of spoilage bacteria and the pattern of spoilage at this temperature. *Vibrio* sp. which was found dominant initially in prawn was found to play a major role in the spoilage of prawn at room temperature. The temperature, alkaline pH and availability of suitable flesh substrates released during spoilage would have definitely contributed to the higher percentage incidence of *Vibrios* and their activity.

Bacterial flora of prawns stored at 4°C did not show much variation from other reports made especially on iced or chilled and stored fish (Vanderzant et al. 1970; Cobb et al. 1976). In general Pseudomonas sp. was found to dominate in all the types of prawns during storage, followed by Acinetobacter, Vibrio, Coryneforms and Micrococcus, Vibrio sp. showed dominance on few occasions. They could not be recovered at many instances but could be recorded at a later stage. Although Pseudomonas and Acinetobacter were known to attain dominance and predominance during refrigerated storage in fish and prawn (Shewan et al. 1960; Cann et al. 1971 Cann, 1977; De Leon Fajardo and Marth, 1979; Surendran and Gopakumar, 1981,'82), the occurrence of Vibrio sp. in the later stages of spoilage which could be considered as late spoilers were reported by Kartar Singh (1978) and Chandrasekaran et al. (1984 b). However it was reported that Vibrio sp. decreased during iced or refrigerated storage (Surendran and Gopakumar, 1981,'82). Recovery of Vibrio sp. during the later stages of spoilage might be due to its ability to adapt to the reduced temperatures. They may be psychrotrophic in nature to withstand the reduced temperatures. While temperatures of 3 to 13°C have been shown to support growth of Vibrio parahaemolyticus, the lowest growth temperature in laboratory media was 5°C (Beuchat, 1973). However the survival of V. parahaemolyticus for 8 days when inoculated to whole, peeled and deveined shrimp and stored at 3,7,10 and -18°C was reported by Vanderzant and Nickelson (1972).

Hence the presence of Vibrio sp. at lower temperatures is possible as reported here.

Species of Vibrio, Pseudomonas, Alcaligenes, Acinetobacter, Enterobacteriaceae, Micrococcus, Staphylococcus, Bacillus and Coryneforms were all encountered during storage at -18°C in the prawn samples. The percentage composition varied considerably during storage. Although all the genera failed to survive throughout the storage period, they could be recovered at few instances. The dominance of Gram-negative bacteria in the earlier period of storage (< 100 days) and of Gram-positive forms in the later period (210 days) was observed in these stored prawns (-18°C). Adaptation to an extended period of storage at -18°C by Gram-positive forms than Gram-negative bacteria might be the cause of this observation. In general Pseudomonas, Acinetobacter and Micrococcus were found to be dominant flora, one dominating the others, at different occasions in the four types of samples stored. Dominance of a single species in all the samples throughout the entire period of storage was not observed at this temperatures as it could be seen at 4°C and 28±2°C.

In the present study, storage at three temperatures, 28±2°C, 4°C and -18°C showed characteristic observations with regard to generic composition of the heterotrophic bacterial flora. Vibrio sp. were the dominant bacteria in the freshly caught prawns and at room temperature storage and

formed almost the entire flora during the course of spoilage. There was no difference in the flora with respect to different types of prawns stored and also no succession of flora could be observed. At 4°C also Pseudomonas sp. was dominant which is reported often as the dominant and principal spoilage agent at reduced temperatures (Liston and Matches, 1976). Succession of flora was observed only in the case of predominant groups such as Acinetobacter, Vibrio and Micrococcus which followed Pseudomonas. Whereas, at -18°C a definite trend of succession of flora at different timings was observed. Pseudomonas, Acinetobacter, Micrococcus, Bacillus and Vibrio showed dominance and predominance at varying times marking the floral succession during spoilage. Recovery of Vibrio parahaemolyticus in frozen seafoods has been reported by Temmyo (1966), Liston et al. (1971), Johnson et al. (1971). The species of Vibrio may be able to tolerate the low temperature. Since identification was not carried out upto species level, species diversity could not be discussed. Liston (1973) suggested that psychrotrophic strain belonging to Pseudomonas and possibly Achromobacter organisms outgrow other bacterial types in the spoilage microflora by virtue of their efficient utilization of non protein nitrogenous compounds in fish flesh. Increase in bacterial load, decrease in major proximate components, protein and fat, with increasing ammonia and TMA level observed during the present study supports

Liston's (1973) hypotheses, that bacteria have direct relationship with spoilage. All the groups observed at the time of spoilage might possess the ability to utilize the non protein nitrogen constituents for their survival and propagation.

4.4. HYDROLYTIC ENZYME PRODUCING BACTERIA

Spoilage of fish and prawn is presumed to occur due to activity of enzymes present in the muscle during autolysis and by microbial enzymes after rigor mortis. However, bacterial enzymes contribute much to the degradation and decomposition of the major components of flesh during incipient and complete spoilage. These microbial enzymes elaborated by heterotrophic bacteria present on the sample during spoilage are hydrolytic in nature. Enzymes such as proteases, lipases, decarboxylases, deaminase etc. released by bacteria attack specific substances available and degrade them. The complex molecules are broken down to simpler substances and later they are utilized by the bacteria for the biosynthesis of cellular material.

Physiological grouping of bacteria (proteolytic-gelatinolytic and caseinolytic, lipolytic and amyolytic) in relation to marine environments have been reported by few workers (Sreenivasan, 1955; Sizemore, 1973) and to freshly caught fishes and prawns (Williams et al. 1949,'52;

Colwell, 1962; Sera and Kimata, 1972; Mary, 1977; Natarajan et al. 1982 and ICAR report 1983) and proteolytic and gelatinolytic bacteria in relation to storage of fish (Shewan and Jones,1957; Shewan, 1961 and Liston, 1973) and in prawn (Sajan George, 1979) have been reported. However informations on various hydrolytic enzyme producing bacteria occurring during storage of prawn is not available.

In the present investigation an attempt was made to find out the various hydrolytic enzyme producing bacteria, by screening the isolates obtained during storage of prawns at three different temperatures. The results suggest a relationship which could exist between the hydrolytic enzyme producing bacteria present during spoilage and degradation and subsequent reduction in the major proximate components like protein and lipid. Further, an increase and higher percentage of gelatinolytic and lipolytic groups observed in all the samples during storage suggest the possibility of involvement of hydrolytic enzyme producing bacteria in the degradation of protein and lipid in these samples. Similarly carbohydrate has also been degraded by saccharolytic bacteria which were recorded in higher level. Ureolytic bacteria although present in less numbers at many instances, showed a gradual increase on few occasions.

Results observed in the case of gelatinolytic and caseinolytic groups agree well with reports of Liston (1973) for fish and Sajan George (1979) for prawn, who observed similar increase in proteolytic bacterial counts during storage.

Participation of bacterial enzymes in degradation of major flesh components and subsequent reduction in proximate composition which leads to loss in nutritive value of the product is an universally accepted phenomenon. The present study strongly supports this phenomenon.

4.5. SPOILAGE POTENTIAL OF HETEROTROPHIC BACTERIA

4.5.1. Action of bacteria on flesh:

The results obtained from the spoilage potential test conducted in flesh medium showed that 64.1%, 68.8% and 56% of the isolates from prawns stored at $28\pm2^{o}C$, $4^{o}C$ and $-18^{o}C$ respectively were active spoilers. They produced all the characteristic spoilage odour and halo zone in flesh agar medium. Of the different bacteria tested, species of *Vibrio*, *Pseudomonas*, *Acinetobacter*, *Bacillus* and *Corynebacterium* were confirmed as active spoilers. *Vibrio* sp. generally known as pathogenic and food poisoning organisms, are also found to be flesh spoilers. Since identification of the isolates upto species level could not be performed, the exact

species of Vibrio involved in spoilage could not be ascertained. Lerke et al. (1965) have reported Vibrio sp. and Aeromonas sp. besides species of Pseudomonas and Achromobacter (presently grouped as Moraxella-Acinetobacter) to be fish flesh spoilers. Bacillus and Micrococcus were also reported to be spoilers (Cox and Lovell, 1973).

Lerke et al. (1963) suggested that sterile muscle press juice as a suitable medium for differentiating spoiler and non-spoiler bacteria based on their activity in the medium. Adams et al. (1964) concluded after testing the pure cultures in sterile muscle press juice, that only a small proportion of the bacterial population were spoilers and there was no selection for spoilers as spoilage progresses. Their results suggest that the bacterial flora of spoiling fish consists of wide variety of organisms, of which, only a small percentage cause spoilage. The rest of the groups probably exist as free riders or perhaps are involved in some synergism with weak spoilers. Lerke et al. (1965) while characterizing the spoilers with the newly formulated medium reported that the importance of weak or inapparent spoilers in fish spoilage should not be arbitrarily discounted. Spoilage under natural conditions may be quite different, involving interaction of numerous factors steming from concurrent or successive activities of various microorganisms. According to them one should not say an

organism isolated from spoiled fish, as a non spoiler just because in pure culture it is unable to spoil fish. That same organism may, in mixed culture, play an important role in fish spoilage. Hence, in the present study, although, nearly 60% of the isolates tested showed them to be active spoilers, the remainder also might play an unknown role in the spoilage which needs further investigation.

4.5.2. Trimethylamine oxide reduction to Trimethylamine (TMAO-TMA):

Trimethylamine oxide reduction and trimethylamine production by bacteria is one among the reliable tests for detecting fish spoilage bacteria. In the present study, of the 178 isolates tested 93.1% were TMA producers. From the results it is also evident that species of Vibrio, Pseudomonas, Alcaligenes, Acinetobacter, Enterobacteriaceae, Micrococcus, Bacillus and Corynebacterium were capable of reducing Trimethylamine oxide. These TMAO reducers cause the increase in TMA content during spoilage as observed by several workers like Laycock and Reiger (1971), Liston (1973) and Sajan George (1979). Sasajima (1973) also suggested that a limited number of viable cells (7.5 to 8.0) in log count were required to produce 16.2 µg of TMA, an arbitrary amount of TMA-N/ml of cultured medium containing 0.05% TMAO. The results obtained in the present study for TMAO reducers suggest the participatio of these forms in the increase of TMA content of the prawns during storage at various temperatures.

4.6. GROWTH AND PHYSIOLOGY OF SPOILAGE BACTERIA

4.6.1. Effect of environmental factors on the growth and survival of spoilage bacteria:

In the present investigation three strains of the genus Pseudomonas (R8,L97 and F152), three strains of the genus Vibrio (R42,L146 and F10) and two strains of the genus Acinetobacter (114 and F88) were tested for growth and survival to the most common environmental factors such as temperature, pH and sodium chloride concentration. These three genera were selected since they were the dominant group during spoilage and these strains exhibited higher activity of spoilage potential, proteolytic, lipolytic and trimethylamine oxide reducing properties. From the results it is clear that extreme temperatures such as -15 and 60°C arrested growth of these bacteria. They were able to grow at temperatures between 5 and 45°C. However maximum growth was observed at 30°C. The percentage of growth varied in the broths (flesh broth and ZoBell's broth) at different temperatures. Maximum growth was observed at 30°C in ZoBell's broth for all the isolates, whereas, in flesh broth, it was recorded at various temperatures. All the isolates grew well in flesh broth at 30°C except two Pseudomonas sp. which showed preference to 45°C. The percentage growth of majority of the isolates were drastically retarded at the other levels of higher or lower temperatures from optimum and growth trend

was not gradual. Alur et al. (1971) reported that Gram-negative bacteria, Pseudomonas sp., Proteus vulgaris and Aeromonas hydrophila grew rapidly at 0 and 10°C in fish homogenate while the Achromobacter grew well at 10°C but poorly at 0°C. They showed maximum growth at 30°C except Pseudomonas sp. whose growth was considerably retarded.

The isolates were able to tolerate higher temperatures (45 and 60°C). Only Vibrio sp. (F10) was not able to tolerate 60°C. Majority of them showed different percentage of survival at various temperatures when exposed for various length of time. Isolates of Vibrio sp. F10, Acinetobacter F88 and Pseudomonas sp. F152 were sensitive to freezing temperatures (-18°C). However all the isolates showed survival at refrigerated temperature (4°C) even for 240 minutes. It has been reported that (Iemmyo, 1966) there was no survivors of V. parahaemolyticus in peptone water or fish extract after 5 days at 4, -2 and -18°C. Vanderzant and Nickelson (1972) also indicated that V. parahaemolyticus was sensitive to refrigeration and freezing. Iemmyo (1966) reported that V. parahaemolyticus was destroyed after 5 minutes at 60°C in peptone water. In the present study, two of the three Vibrio sp. could tolerate 60°C for about 120 minutes. However percentage survival was less than 2% at 120 minutes. It is possible that prawn flesh homogenate might have altered their sensitiveness to higher temperatures since nutrients influence growth behaviour of bacteria.

All the bacterial isolates did not grow at pH 2, 4 and 12, demonstrating the undesired effect of extreme acid and alkaline pH on growth. pH is a powerful parameter regulating microbial growth and its direct effect is seen on these bacteria. Optimum growth was found at pH 6 - 10 in both the media and maximal growth for each strain varied with pH irrespective of the media. However, 4 of the isolates recorded maximum growth at pH 6 in ZoBell's broth and they showed maximum growth at different pH in flesh broth. Except the two strains of Pseudomonas (L97 and F152), all the other strains showed maximal growth at an alkaline pH in flesh broth than in ZoBell's broth. This confirms earlier findings (Vanderzant and Nickelson, 1972) that bacteria prefer alkaline pH for their growth in flesh. Also better growth in flesh broth suggests that the available nutrients in prawn flesh may favour proliferation and propagation of these isolates at alkaline pH. This could be helpful in active participation of these bacteria in spoilage of prawn during storage.

The tested isolates survived well at pH 6 to 8.6 and were highly sensitive either to highly acidic (pH 2.6 and 4.2) or highly alkaline condition (pH 10.6). However they could survive for few hours (12 to 18 h) initially at extreme pH conditions. Vanderzant and Nickelson (1972) reported that V. parahaemolyticus in shrimp homogenates was very sensitive to pH values below pH 6 and no survivors could be detected in

homogenates adjusted to pH 1 to 4. Little change in viable population occurred when stored for 2 h at pH values ranging from pH 6 - 10. Generally the pH of the spoiled flesh exceeds pH 7 and may be favourable for these bacteria to record maximum population and degrade flesh.

The optimal requirement of sodium chloride varied from one strain to another. Two strains of Pseudomonas (R8 and L97) showed preference of 3% NaCl concentration for maximal growth in both the media whereas Pseudomonas sp. (F152) preferred 1% NaCl concentration for maximum growth. All the strains of Vibrio sp. (R42, L146 and F10) showed maximal growth at 6% NaCl concentration and recorded more than 74% of growth at 1 - 6% NaCl concentration in both the media, suggesting their euryhaline nature. However Acinetobacter behaved differently in these media. They recorded maximum growth at 1% NaCl in flesh broth whereas Acinetobacter sp. L114 showed maximum growth in ZoBell's broth without NaCl and Acinetobacter sp. F88 at 1% NaCl concentration. As suggested earlier prawn flesh which generally contain rich nutrients might have influenced the growth of these bacteria.

All the isolates were capable of growing in the medium without sodium chloride and could survive also. However concentrations above 6% of NaCl drastically affected the growth of all bacteria except Pseudomonas L97 and

Acinetobacter L114 by 18 to 24 h. This study confirms that spoilage flora are sensitive to high concentration of sodium chloride.

4.6.2. Influence of Interaction of environmental factors on the growth of spoilage bacteria:

Effects environmental factors like temperature, pH and sodium chloride concentration on the growth of spoilage bacteria were studied separately in flesh and ZoBell's broth. Influence of more than one of these factors on the growth of spoilage bacteria was also studied to find out the level of combinations of factors which support maximum growth or suppress growth. By maintaining one of the factors as a constant, the other two factors were altered and different combinations were obtained. Flesh media was adjusted to such combinations and bacteria were inoculated in known concentrations and incubated for specific period. Growth obtained is expressed in terms of growth index. Results obtained from this study indicate that at extreme conditions each individual factor has an independent influence on the growth of the microorganisms.

Incubation at temperatures of -15 and 60°C, pH 2, 4 and 12, and 10% and 15% sodium chloride concentrations were found to arrest the growth of bacteria although when two factors were at optimum and one factor was at extreme. Growth

was observed at temperatures of 5, 15, 30 and 45°C, at pH 6, 8 and 10 and at 0 - 6% sodium chloride concentrations. Maximum and minimum growths occurred at different combinations and certain combinations did not favour the growth of these spoilage bacteria.

The bacterial isolates showed growth in pH 6 - 10 at different temperatures and sodium chloride concentrations. Strains of Pseudomonas and Acinetobacter recorded feeble growth at pH 10. It is well known that alkaline pH above 9 may not be conducive for these bacteria to grow normally. It could be seen that pH has less influence over temperature and NaCl concentration towards enhancing the growth of the bacteria. Also 30°C temperature was found to be favourable for all the isolates at a NaCl concentration of 3% and pH 6 and 8. However Vibrio sp. R42 recorded better growth at 30°C at all pH 6 - 10 with 6% NaCl concentration. Requirement of sodium chloride is increased when temperature is elevated above room temperature. At the same time, at reduced temperature such as 5°C, very low NaCl concentration i.e. 1% is enough, as in 30°C, for enhancing the maximal growth. Sodium chloride concentration and temperature are observed to have some relationship in enhancing maximal growth of the bacteria in spite of pH change within the range pH 6 - 10. Goatcher and Westhoff (1975) reported that salt content and pH of oysters would appear to be important factors which

influences growth. Sensitivity of Vibrio parahaemolyticus may be increased under adverse conditions, such as low salt content and low temperatures. Survival of V. parahaemolyticus in fish homogenates has been shown to be affected by salt content (Covert and Woodburn, 1972).

This study indicates that the physiological properties of strains vary. Their interaction with multiple environment factors differ from one another and they behave independently.

4.6.3. Generation time of spoilage bacteria:

It is observed that the nature of nutrients available in the medium influences to a greater extent the multiplication of bacteria since none showed same generation time in both the media (flesh broth and ZoBell's broth) under same temperature. Two strains of Pseudomonas sp. (R8 and F152) and strains of Acinetobacter sp. (L114 and F88) and one strain of Vibrio sp. (L146) showed shorter generation time in flesh broth (33, 37, 30.66, 31 and 38.66 min. respectively) and longer generation time in ZoBell's broth, except Vibrio sp. L146 which showed shorter generation. Other strains exhibited shorter generation times in ZoBell's and longer generation times in flesh broth. In general, most of them showed shorter generation time and could multiply at faster rate and the chemical composition of the medium certainly influences the growth and multiplication of these bacteria.

4.7. MIXED POPULATION STUDIES

Bacteria existing as a commensal and spoilage flora on fish or prawn is usually comprised of various generic groups. Preference to specific substrates and to environmental conditions is generally noticed among bacteria in all habits. In normal conditions, organisms those are capable of utilizing the available substrates efficiently at specified environmental conditions dominate over the rest of the genera. During spoilage, bacteria which could utilize the available flesh components grow to the maximum in spite of its initial population level before storage. Only few bacteria like Pseudomonas spp. and Acinetobacter spp. are often encountered as spoilage flora during spoilage, and record high population levels. The rest of the groups may play a synergistic role at that time of spoilage (Lerke et al. 1965).

It is found that, the level of initial population encountered during storage study also had some influence in deciding over dominance and predominance of the genera. Vibrio sp. which was dominant in the fresh prawn maintained its level during spoilage at 28±2°C. Pseudomonas and Acinetobacter showed predominance. However these genera showed dominance and predominance in samples stored at

different temperatures for longer duration. This suggested the need to find the behaviour of these dominant bacterial cultures in flesh broth added with different proportions of inoculum.

In spite of varying population levels of inoculum of Vibrio (R42) and Pseudomonas (R8) which were isolated from spoiled prawns stored at 28±2°C, Vibrio sp. usually usually held down the number of Pseudomonas sp. This supports the results obtained in the storage studies of prawn at room temperature (28±2°C) that species of Vibrio dominated during storage at higher temperature. Also the readily available nutrients in the flesh broth and the favourable temperature (28±2°C) might have supported them to attain maximum number. Further Pseudomonas may be less active and less repressive at this temperature. Seminiano and Frazier (1967) reported that when 19 Pseudomonas cultures were tested with Staphylococcus with initial inoculum of 1:1, only one strain (which resembled P. convexa), markedly repressed the growth of S. aureus at 30°C. However at lower temperatures (25 and 18°C) all were found to be inhibitory. Hence, it could be possible that the temperature may not be favourable to Pseudomonas tested in this investigation while it favoured Vibrio. This might be the reason for high number of Vibrio recovered from prawn sample stored at 28±2°C.

When Vibrio sp. (L146) and Pseudomonas sp. (L97) were mixed, the influence of the level of the inoculum in determining the dominance was seen. At very low concentration of equal proportion (10^2) they maintained equal ratio on recoveries whereas, at higher concentrations of equal size of inoculum, Pseudomonas sp. dominated Vibrio sp. Similarly, the number of cells of inoculum decided the dominance of Vibrio sp. (L146) and Acinetobacter sp. (L114) and at equal concentration, no suppression of either bacterium was noticed.

Pseudomonas sp. (L97) showed dominance over Acinetobacter sp. (L114) at all combinations except when they were added in 10^2+10^8 respectively. This suggest that the Pseudomonas isolated from sample stored at lower temperature (4^oC) proved to be dominant and suppress the growth of other genera in pure culture study also. This underscores the behaviour of Pseudomonas at lower temperatures. Seminiano and Frazier (1967) tested eight cultures, representing Pseudomonas, Alcaligenes, Achromobacter and Flavobacterium for their effect on growth of S. aureus by means of detailed growth curves at 10, 15 and 22^oC and with ratios of effectors to staphylococci in inocula of 1:100, 1:1 and 100:1. In general inhibition increased with increased proportions of effector bacteria in the inocula and with decreasing temperature, but inhibition was not as great as with the other

bacteria studied. Psychrophiles and particularly low temperature tolerant Pseudomonas are believed to play an important role in the repression of S. aureus at temperatures below those of the room temperature and to cause changes in foods that make them unfit to eat before the number of staphylococci become significant (Seminiano and Frazier, 1967).

Vibrio sp. (L146), Pseudomonas sp. (L97) and Acinetobacter sp. (L114) isolated from spoiled prawns stored at 4°C, when grown together at various initial concentrations, it was observed that Pseudomonas sp. which originally showed dominance during storage, maintained the same order indicating its active participation and utilization of flesh substrates during spoilage at refrigerated temperatures. When all the above three cultures were mixed together at equal concentrations, Pseudomonas sp. dominated followed by Acinetobacter sp. suggesting the active participation of Pseudomonas sp. and Acinetobacter sp. Pseudomonas and Acinetobacter were reported to be dominant spoilage bacteria at lower temperatures (Cobb et al. 1976). The present study supports the earlier findings. The results for various inocula level however suggest that when a particular bacteria like Pseudomonas sp. is very active and efficient in utilizing the available substrates it can outgrow in spite of varying concentration of inoculum or the other

bacteria present at the same time in varying population level on the animal.

4.8. STATISTICAL ANALYSIS

It is well known that the concentration of spoilage indices increase during storage and are known to be influenced by the biochemical constituents of the flesh and the type of bacterial population. In this investigation to understand those influences, a number of factors were monitored. The correlation between the spoilage indices, proximate composition and bacteria were drawn by a series of correlation coefficient matrices (Pearson's Correlation). The data showed that there existed a significant correlation between these variables.

Proximate components excluding moisture, got reduced during storage at all the three temperatures, indicating quality loss and reduction in nutritive value. When spoilage indices and proximate components were related, proximate components i.e. protein carbohydrate, lipid and ash showed reduction in contents along with the increase in the level of spoilage indices. A significant negative correlation between spoilage indices and the proximate components and a significant positive correlation of spoilage indices and moisture in all the temperature indicated the dependent relationship of proximate composition and the spoilage indices.

The degradation of major components of flesh, after rigormortis, may be either due to autolysis, or bacterial action or both together (Shewan 1974). This results in the reduction of proximate composition and the increase of spoilage indices. It has long been established that the rapid spoilage of shellfish and fish even at chill temperatures is due to chiefly the action of bacteria (Pillai et al. 1961; Shaw and Shewan, 1968; Cobb et al. 1977) and the major chemical changes resulting from microbial spoilage are the production of TMA, NH_3 and volatile fatty acids (Liston 1973). Cobb et al. (1977) suggested that TMN and TVN levels appeared to be good indicators along with high (10^6/g) bacterial levels. Fieger and Friloux (1954) also indicated that TMN levels in Gulf shrimp increased when bacterial levels were high. In the present study, such increase was recorded during storage. Also THB was observed to record significant positive correlation with the TMA and NH_3. It is imperative that the bacteria take active part in the break down and subsequent reduction of proximate components resulting in an increase in the concentration of spoilage indices.

It is a known fact that bacteria reduce the proximate level in the flesh by elaborating hydrolytic enzymes, such as proteolytic, lipolytic, amylolytic and

ureolytic. The most important enzymes which are directly involved in the degradation are proteolytic, ureolytic and at a lesser extent lipolytic. Increases in the percentage of hydrolytic enzyme producing bacteria and spoilage indices were observed during storage at all the three temperatures. However significant correlation was not noticed at all temperatures. There existed significant positive correlation between TMA and ureolytic bacteria at $28\pm2^{o}C$ and $4^{o}C$, NH_3 and ureolytic bacteria at $28\pm2^{o}C$ and $4^{o}C$ and NH_3 and proteolytic and lipolytic at $28\pm2^{o}C$.

Urea produced by the action of arginase could serve as a source for ammonia production. Bacterial break down of urea may be an important source of ammonia, particularly during prolonged storage. Also the production of TVB (NH_3) by proteolytic bacteria may be due to the replinishment of the aminoacid substrate pool by proteolysis. The increase of ammonia during prolonged storage of all samples at the three temperatures suggests the possible contribution of proteolytic and ureolytic bacteria in the accumulation of ammonia. The positive significant correlation made by NH_3 with ureolytic and proteolytic bacteria at $28\pm2^{o}C$ indicated the significance of such hydrolytic enzyme producers in spoilage. However such significant relationship was not seen at $-18^{o}C$. At $4^{o}C$ proteolytic bacteria did not show significant

relationship with ammonia. This suggests that, the enzyme activity of proteolytic bacteria might be affected by a lower temperature. Cobb et al. (1977) also reported the bacterial breakdown of urea and suggested that it may be an important source for accumulation of ammonia. Shewan (1977) stated that some of the spoilage bacteria were proteolytic and undoubtedly contributed to the ammoniacal odours by producing ammonia, although proteolysis was not a prerequisite of spoilage.

The pH of the flesh of freshly killed shellfish and fish is usually close to 7 falling within a few hours of death, when rigormortis has set in, to between 6.1 and 6.9 (Cuttings, 1953). An increase in pH during storage was recorded in the present study. The degradation of flesh might have resulted in the accumulation of amines and ammonia causing an increase in the pH level (Cuttings, 1953). The increase in pH was high at $28\pm2^{o}C$ when compared to $4^{o}C$ and $-18^{o}C$. pH showed significant positive correlation with THB and the hydrolytic bacteria like proteolytic, ureolytic and lipolytic at room temperature. This suggests that at higher storage temperature various bacterial groups readily attack the flesh, resulting in more accumulation of amines and ammonia.

However Cobb et al. (1977) stated that tissue pH was not a good indicator of quality of shrimp stored for 3 - 24 h. at higher temperature, When prawns were stored in ice after

capture, there was usually little or no further change in pH (after full riger) for about 10 days. By this time the prawns were fairly stale. After this, the production of ammonia and amines caused the pH to rise from 6 to 8.55. Cuttings (1953) reported that the production of ammonia and amines caused the pH to rise between 7.5 and 8 after 20 or 25 days when the fish were quite putrid and inedible. In Dog fish, breakdown of urea, released additional ammonia (Shewan, 1938) and raised the pH to 8.5. These increases could be completely accounted for on the basis of the buffering capacity and the quantities of the base produced. Similarly Flick and Lovell (1972) found an increase of pH from 7.4 to 8.2 in 10 days of storage in shellfish and suggested that advanced bacterial spoilage increased the level of pH and they became unacceptable in the pH range 7.5 to 8, by more frequently when the pH was near 8.

Vibrio sp. was the dominated bacteria at $28\pm2^{o}C$ and was recorded at a moderate to low percentage at $4^{o}C$ and at a very low level at $-18^{o}C$. The number of Pseudomonas was least at $28\pm2^{o}C$ and dominant at $4^{o}C$ and $-18^{o}C$. Acinetobacter and Micrococcus recorded higher percentage at $-18^{o}C$. The tropical prawns harbour mainly of mesophilic bacteria and they may be sensitive to lower temperatures.

The high number of Vibrio at 28±2°C indicated the dominance of the mesophilic bacteria throughout the period of storage and their active participation in spoilage. Pseudomonas and Acinetobacter are generally considered to be main active agents of ice stored prawns and fish. In the present investigation they were found to be dominant at lower temperature. Significant positive correlation between spoilage indices and Vibrio and negative correlation between spoilage indices and Pseudomonas, Acinetobacter and Micrococcus at 28±2°C indicated that Vibrio generally of mesophilic nature took an active role in spoilage at ambient temperatures. At -18°C, Vibrio showed significant negative correlation with NH_3 and pH showing that they are highly sensitive to lower storage temperatures. Cobb et al. (1977) also found that Vibrios were highly sensitive to lower temperatures. However Pseudomonas and Acinetobacter which are considered to be the spoilers at lower temperature did not show any relationship with any of the spoilage indices. Cox and Lovell (1973), Cann (1974) and Disney (1976) also reported similar findings, that the mesophiles which were generally found in freshly caught tropical fish would not be active at lower temperature. Hence a reduced spoilage could take place until a psychrophilic flora develops. The type and levels of

microorganisms on the shrimp and the endogenous enzymes or enzymatic contamination during the beheading process of the shrimp influences the post mortem biochemical changes (Cobb and Vanderzant, 1971). The positive significant correlation of Vibrio with moisture indicated that the increase of moisture may be due to the biochemical changes initiated by Vibrio. However such relationship was not recorded at 4 and -18°C.

Vibrio sp. showed a tendency to either dominate or occur at very low number or absent. Sometimes they were suppressed by other groups. A negative correlation of Vibrio with other genera at -18°C suggested that suppression of one bacteria by other exists during spoilage. However these bacteria did not show any significant relationship at lower temperature.

Correlation between spoilage bacteria and hydrolytic enzyme producing bacteria suggest that Vibrio which were in higher percentage at 28±2°C could have formed the major hydrolyting enzyme producing group. Although Pseudomonas and Acinetobacter were dominant at 4 and -18°C, they did not show any significant relationship with proteolytic group. However it should not be overlooked that Pseudomonas are efficient in utilizing non protein nitrogen components such as free aminoacids (Liston, 1973). Prawn which contains more aminoacids (Arul James, 1969) naturally forms a good source

of non protein nitrogen too, supporting the dominance of Pseudomonas. Since analysis of non protein nitrogen was not performed, no conclusion could be drawn presently.

4.9. CONCLUDING REMARKS

In the present investigation it was found that prawns, immediately after catch undergo rapid spoilage at a faster rate. At ambient temperatures the spoilage was rapid. When stored at reduced temperatures ($<5^{o}C$) the raw unprocessed prawns showed an extended shelflife for 10 days at $4^{o}C$ and 60 days at $-18^{o}C$. Styling of prawns into Whole, Headless, Peeled and Undeveined and Peeled and Deveined forms did not give significantly a satisfactory extended period of shelf life at room temperatures. Of course, at lower temperatures, beheading, peeling of the shell and deveining extended the storage life.

Dishey (1976) while reviewing the reports on the spoilage of tropical fish indicates that tropical fishes get spoiled rapidly at ambient temperatures by 11-24 h, recording a bacterial level of 10^{3}-10^{6} per g of tissue. According to him, it is reasonably assumed that the deterioration is due to rapid bacterial growth but the relationship between bacterial enzymes and autolytic enzymes is largely unknown. The present study clearly indicates the participation of bacterial enzymes in the rapid deterioration of prawns, since THB showed

significant positive correlation with the spoilage indices. Besides proteolytic, lipolytic and amylolytic bacteria too recorded significant positive correlation with spoilage indices. Further, significant reduction in the proximate components such as protein, carbohydrate, lipid and ash and increase in moisture was observed. All these give ample support for the role of bacterial enzymes than autolytic enzymes.

5. SUMMARY

1. *Penaeus indicus* collected from Cochin backwater (size 80-100 mm in total length) were stored as Whole, Headless, Peeled and Undeveined (PUD) and Peeled and Deveined (PD) styles in fresh unprocessed condition at $28\pm2^{o}C$, $4^{o}C$ and $-18^{o}C$ for varying periods.

2. Samples were randomly drawn for each type and analysed. About 10-15 prawns, for each type, were analysed at each sampling point. Analysis was performed upto 24 h, 30 days and 210 days for the samples stored at $28\pm2^{o}C$, $4^{o}C$ and $-18^{o}C$ respectively.

3. The aspects dealt with are (i) evaluation of nature of spoilage by organoleptic assessment, estimation of total heterotrophic bacterial population (THB), Trimethylamine (TMA), total volatile nitrogen in the form of ammonia (NH_3) and flesh pH at various time intervals; (ii) evaluation of changes in the proximate composition by estimating protein, carbohydrate, lipid, ash and moisture contents of flesh at various intervals along with spoilage assessment (iii) distribution and percentage occurrence of heterotrophic bacterial flora which formed the components of spoilage flora during storage (iv) determination and distribution of various hydrolytic bacteria such as caseinolytic,

gelatinolytic, amylolytic, lipolytic and ureolytic forms present during storage (v) assessment of spoilage potential of the bacteria by testing their ability to produce off odours and halo zones in flesh broth and flesh agar, trimethylamine oxide (TMAO) reduction to trimethylamine (TMA), (vi) growth and physiology of selected strains of potential spoilers: effect of temperature, pH and sodium chloride on growth and survival (vii) combined effect of temperature, pH and sodium chloride on the growth of spoilage bacteria in flesh broth (viii) generation time of spoilage bacteria and (ix) behaviour of mixed cultures in flesh broth.

4. Organoleptic assessment suggested that all the four types of prawns stored were spoiled completely by 8 h at $28\pm2^{o}C$, by 10 days at $4^{o}C$ and by 100 days at $-18^{o}C$.

5. Total heterotrophic bacterial population varied for each type of sample and at different temperatures during storage. Although there was a significant increase in THB of the samples stored at $28\pm2^{o}C$, $4^{o}C$ and $-18^{o}C$, a regular pattern of increase was not observed. THB of $-18^{o}C$ was comparatively very low than that of $28\pm2^{o}C$ and $4^{o}C$.

6. Trimethylamine was found to increase significantly during storage in all the four types of samples stored. In general TMA was found to be high at $28\pm2^{o}C$ and all the four types recorded almost same level of TMA at $-18^{o}C$.

7. Ammonia content of the flesh recorded significant increase during storage in all the four samples. Ammonia was found to be high in prawns stored at 4°C than at 28±2°C and at -18°C.

8. pH of the flesh showed significant increase (pH 8.55) from the initial level (pH 6) in all the four types of samples during storage. However higher alkaline pH was recorded for the samples stored at 4°C and -18°C.

9. Protein content of all the samples showed significant reduction in their level from their initial content during storage at the three different temperatures. Reduction in protein was more at 28±2°C than at 4°C and -18°C.

10. Carbohydrate content of the flesh of all the four types of prawns showed significant reduction from their initial level during storage. The reduction in carbohydrate level at -18°C was very less when compared to 28±2°C and 4°C.

11. Lipid content showed significant decrease from their initial level in all the four samples during storage. The level of reduction in lipid was almost similar in all the samples at all the three temperatures.

12. Ash content was found to record significant reduction from their initial level in all the four samples during storage. Also, the reduction in ash content showed

a similar magnitude in all the samples and at all the three temperatures.

13. Moisture content showed a significant increase during storage in all the four types of samples. Increase in moisture content was comparatively high at 28±2°C than at 4°C and -18°C.

14. Bacterial flora of freshly caught prawns were represented by species of Vibrio, Pseudomonas, Acinetobacter, Moraxella, members of Enterobacteriaceae, Micrococcus, Bacillus and Corynebacterium. Vibrio (30.8%) was found to be the dominant genus and followed by Pseudomonas (25.6%) in the fresh flora.

15. During storage at 28±2°C, Vibrio sp. outnumbered all the other species and showed dominance in all the four types of prawns. They were found to vary from 94.7% to 100% in the samples eliminating most of the other groups. Among the four types of prawns stored PD recorded only Vibrio sp. as the spoilage flora and PUD, Headless and Whole recorded higher percentages of Vibrio sp. along with other groups in lesser percentage.

16. At 4°C, Pseudomonas sp. showed dominance over Acinetobacter sp., Vibrio sp., and Micrococcus sp. and

others in all the four types of samples. Only on few occasions Vibrio sp. showed dominance over others.

17. During storage at -18°C, Pseudomonas sp., Acinetobacter sp. and Micrococcus sp. showed dominance over one another and others at different occasions. Vibrio sp. and Bacillus sp. were found to be dominant on few occasions. However, in all the samples moderate number of Pseudomonas sp. were recorded when compared with other species.

18. A total of 913 isolates obtained from the prawns stored at the three temperatures were tested for various hydrolytic enzyme production. The fresh prawns were found to harbour maximum number of gelatinase (61.67%) and caseinase (30%) producers. During storage at 28±2°C, the proteolytic bacteria (both gelatinolytic and caseinolytic) were found to dominate others in all the four types of samples. They were followed by lipolytic, amylolytic and ureolytic bacteria. At 4°C, comparatively the percentage occurrence of the various hydrolytic enzyme producers were less. However gelatinolytic and lipolytic bacteria were found to dominate followed by caseinolytic, amylolytic and ureolytic bacteria in all the four types of samples. At -18°C, moderate level of hydrolytic enzyme producers were recorded. Gelatinolytic and amylolytic bacteria showed dominance over lipolytic, caseinolytic and ureolytic bacteria

19. About 219 isolates, randomly selected, were tested for their activity in flesh broth and flesh agar. They were species of Vibrio, Aeromonas, Pseudomonas, Alcaligenes, Acinetobacter, Micrococcus, Bacillus, Coryhebacterium and members of Enterobacteriaceae. About 63% of the total isolates tested were potential spoilers.

20. About 178 isolates, randomly selected, were tested for their ability to reduce trimethylamine oxide (TMAO) to trimethylamine (TMA). They were species of Vibrio, Pseudomonas, Alcaligenes, Acinetobacter, Micrococcus, Bacillus, Corynebacterium and members of Enterobacteriaceae. Among them 92.1% were TMAO reducers.

21. Eight isolates were selected and their growth and physiology was studied. The isolates were species of Pseudomonas (R8, L97 and F153), Vibrio (R42, L146 and F10) and Acinetobacter (L114 and F88). Effect of different temperatures (-15, 5, 15, 30, 45 and 60°C), pH (2, 4, 6, 8, 10 and 12) and sodium chloride concentration (0, 1, 3, 6, 10 and 15%) on the growth and survival of bacteria was tested in flesh broth and ZoBell's broth. All the strains did not grow at -15° and 60°C in both the media. Optimum growth for all the isolates was found to vary from 15° to 45°C in both the media. Variation in growth was observed for the same group of organisms. All the isolates were sensitive to

acidic and alkaline pH in both the media. In general optimum growth for all the isolates was observed at pH levels from pH 6-10. The growth pattern of all the isolates in response to various sodium chloride concentrations was not similar in flesh as well as in ZoBell's broth. All the isolates did not grow at 15% NaCl concentrations in both the media. Optimum NaCl was found to vary from 1 to 60% concentration. However, feeble to good growth was observed in the absence of NaCl in both the media.

22. At higher temperatures all the strains survived well at 45°C than at 60°C when exposed for a period of 120 minutes. At reduced temperature all the strains could survive at 5°C than at -15°C when exposed for 240 minutes. The percentage of survival of bacteria was found to decrease with increase of time. All the species were sensitive towards acidic (pH 4.2 and 2.6) and high alkaline (pH 10.6) conditions. They survived well at pH levels 6 to 8.6. Concentrations of sodium chloride above 6% affected the survival of all the species tested. All the strains survived well at 0-6% NaCl concentrations. However each species have responded differently at various concentrations of sodium chloride.

23. Influence of more than one environmental factor such as temperature, pH and sodium chloride concentration on the growth of spoilage bacteria was tested by combining these factors at various levels. Thus by maintaining one

of the factors as a constant and altering the other two, different combinations were obtained. The study was performed in flesh media. Results suggested that at extreme conditions each individual factor has an independent influence on the growth of bacteria.

24. Generation time of all these eight strains was estimated in flesh broth and in ZoBell's broth at room temperature. It was found that the generation time of the strains were not similar in both the media for most of the strains tested. Strains of Vibrio sp. showed a shorter generation time in ZoBell's broth than in flesh broth. Whereas, Acinetobacter sp. showed shorter generation time in both the media. Of the three strains of Pseudomonas sp. two showed shorter generation time in flesh broth than in ZoBell's broth whereas the third one recorded vice versa.

25. In interactions between two or three genera were studied by inoculating a known number of cells of each species in flesh broth and by recovering the cells by plating for a definite period at $28\pm2^{\circ}C$. When Vibrio R42 was mixed with Pseudomonas R8, Vibrio sp. outnumbered Pseudomonas sp. When Vibrio sp. L146 and Pseudomonas sp. L97 were mixed at various combinations, they were recovered at equal proportions. At equal concentration of their initial inocula size (10^4 and 10^8 cells/ml) Pseudomonas dominated.

The inoculum size decided the dominance of Vibrio sp. L146 and Acinetobacter sp. L114 and at equal size of inocula, none outnumbered the other. Pseudomonas so. L97 showed dominance over Acinetobacter sp. L114 at all combinations except when they were added at 10^2 and 10^8 cells/m respectively. When Vibrio sp. L146, Pseudomonas sp. L97 and Acinetobacter sp. L114 were grown together, Pseudomonas sp. L97 dominated others on most occasions.

26. Pearson correlation coefficient 'r' was calculated for all the variables analysed in the present study. The 'r' was calculated with BDP-100 (ECIL of India) microcomputer. The variables include spoilage indices THB, TMA, NH_3-N and pH; proximate components - protein, carbohydrate, lipid, ash and moisture; spoilage flora - Vibrio sp., Pseudomonas sp. Acinetobacter sp. and Micrococcus sp. and hydrolytic enzymes producing bacteria - Proteolytic, lipolytic, amylolytic and ureolytic.

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