BIO-CHEMICAL CHANGES ASSOCIATED WITH PROCESSING OF SHELL FISHES AND FLAVOUR CONSTITUENTS OF BODY MEAT AND CLAW MEAT OF CRAB

THESIS SUBMITTED TO
THE UNIVERSITY OF COCHIN
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FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

By
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Dedicated

to

my Parents

DECLARATION

I hereby declare that this thesis entitled "Bio-chemical changes associated with processing of shellfishes and flavour constituents in body meat and claw meat of crab" is a record of bonafide research carried out by me under the supervision of Dr. K.Gopakumar M.Sc., Ph.D., A.R.S., Scientist S3, and that it has not formed the basis for award of any degree, diploma, associateship, fellowship or other similar titles from this or any other University or Society.

Cochin-29
13th September, 1984

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This is to certify that this thesis entitled "Bio-chemical changes associated with processing of shellfishes and flavour constituents in body meat and claw meat of crab" embodies the results of original work conducted by Mrs. Chinnamma George under my supervision and guidance from 24-10-1978 to 20-7-1984. I further certify that no part of this thesis has previously been formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles of this or any other University or Society. She has also passed the Ph.D. qualifying examination of the University of Cochin held in December 1981.

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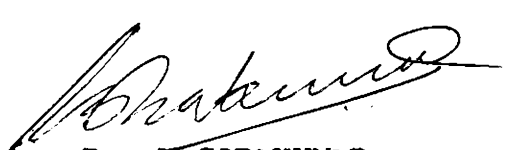

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ABBREVIATIONS USED IN THE THESIS

ATP	..	Adenosine triphosphate
ATP-ase	..	Adenosine triphosphatase
cm	..	centimetre
EDTA	..	Ethylene diamine tetra acetic acid
E. coli	..	Escherichia coli
µ gm	..	micro gram
µ g/pi	..	micro gram inorganic phosphorus
lbs	..	pounds
α-NH_2-N	..	alpha amino nitrogen
M	..	Molar
mg	..	milligram
ml	..	millilitre
nm	..	nanometre
NPN	..	non-protein nitrogen
Phos (in)	..	Phosphorus (inorganic)
PN	..	Protein nitrogen
PSI	..	Pound per square inch
rpm	..	revolutions per minute
TCA	..	Trichler acetic acid
TN	..	Total nitrogen
WEN	..	water extractable nitrogen
WSN	..	water soluble nitrogen

INTRODUCTION

1. INTRODUCTION

Acute shortage of protein foods and rapidly growing population particularly in the developing countries in Asia, Africa and Latin America have pointed out the need for a rapid development and exploitation of the food resources from the sea and other water areas which cover over 70% of the earth surface contributing to less than 1% food supply. According to the data of the United Nations Organisations, about 2000 million of the 3,700 million people on earth fail to get the minimum requirement of 30 grams of protein per day (Anon, 1971), and that nearly three million people die every year due to protein malnutrition (Menon, 1967). Thus the World's challenging task today is to find out ways and means to irradicate hunger and malnutrition and to provide enough protein rich food to man.

India with a coast line of nearly 5000 Km and shelf-area of more than 2,50,000 square kilometers into which numerous large and perennial rivers discharge their silt-laden waters and with a number of small gulfs and bays all along the coasts offers almost an unlimited scope for the development of fisheries (Prasad & Thampi, 1971). The scientific exploitation of the fishery resources is not only important for meeting the acute shortage of protein foods in India but also necessary for raising the socio-economic status of the fishermen who constitute one of the poorest and most backward communities.

Growth and development of the fishery and fish processing industry in India during the post independent era, have been remarkable. This resulted in a rapid and steady growth of the fishery export trade of the nation. The striking feature of India's fishery export industry is that it is basically shrimp oriented with more than 85% of earnings from shrimp exports when this item constitutes only 10-12% of the total marine fish landings in the country (Anon, 1979).

Shell fishes comprising of crustaceans and molluscs do not come under the general classification of fish. Only a few species amongst them were regarded as food, but nowadays their popularity is increasing due to their

high delicacy as well as their high food value.

Next in importance to fish and prawn is the crab, mussel and clam which have attracted the attention of man probably because of their sedentary habits and easy accessibility. No separate statistics is available regarding the landings of these varieties and they are grouped among crustaceans other than prawn. Even in India there is a steady increase in the utilization of crustaceans other than prawn. Of crustaceans, crab plays a prominent role.

1.1 The crab fishery resources of India

According to Vedavyasa Rao et al. (1973), 640 species of crabs occur in Indian waters of which only eight species are at present considered to be commercially exploitable. Such crabs, as listed by him, include Scylla serrata (Forskal), Portunus pelagicus (Linnaeus), Portunus sanguinolentus (Herbst), Matuta lunaris (Forskal) (Calappidae), Charybdis natator (Herbst), Charybdis annulata (Fabricius), Charybdis crusciata (Herbst) and Varuna literata (Fabricius) (Grapsidue).

Crabs are exploited in appreciable quantities along the coasts of Gujarat, Maharashtra, Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, Pondicherry and Goa and small quantities from Orissa and West Bengal. Crabs are also available from the Gangetic delta, Chilka lake and Ennur

backwaters. The peak fishing season in Gujarat is from July-September, Maharashtra from August-October, Karnataka from December to January, Kerala from July-September, Tamil Nadu from March-June and October to December and in Andhra Pradesh from April-September.

1.1.1 Scylla serrata

Among the edible crabs occurring in the coastal waters, estuaries and backwaters of India, Scylla serrata, popularly called the 'Green crab', is the most valued species because of its large size (150-200 mm carapace width) and high quality meat. In general, the productive backwaters of Cochin and Vembanad backwaters of south-west coast and the Vellar estuary and killai backwater of Tamil Nadu are well known areas of this crab fishery.

Scylla serrata enjoys a wide distribution all over the Indo-pacific region from east coast of Africa through Red sea, coasts of India and Pakistan to Japan, Australia, Tahiti and New Zealand. According to Vedavyasa Rao et al. (1973), the estimated potential of crab resources of the inshore waters of the entire coast of India is 25,347 tonnes. Their seasonal variability mainly appears to be an inhibiting factor for organised fishery in India.

1.1.2 Gear

A variety of fishing implements such as stake net,

CRAB (SCYLLA SERRATA)

cast net, gill net, drag net, siene net, hoop net, hooked iron or steel rod, line with baits, shore seine, boat seine and crab trap are used.

1.2 Mussel Fishery Resources of India

According to Jones and Alagaraswami (1973), the world production of sea mussels stood at 2,82,900 metric tonnes in 1966. The major mussel producing countries are Holland, Spain, France, Denmark and Germany. Two species of sea mussels occur in Indian waters, the green mussel (Perna viridis) and the brown mussel (Perna indica). The brown mussel which is considered as one of the greatest delicacies by the coastal people has a characteristic distribution being confined to south of Quilon in Kerala upto Cape Comerin along the west coast and from there upto Tinnevely district on the east coast of India. The green mussel has a much wider distribution all along both the coasts. But it is found in abundance off Cochin, Malabar and north of Kerala. The green mussel occurs not only in the coastal waters, but also in the backwaters and bays as in some parts of orissa, Madras and Kerala. At Fort Cochin in Kerala thick growth of green mussel is seen in the backwaters. A remarkable feature observed in the recent years is the thick carpet like spattering of mussels on the rocks laid along the shore for protection against

MUSSELS (PERNA VIRIDIS)

erosion. Apart from the coastal rocks, submarine rocks at a distance of about 300 m from the shore and at depth upto 15 m bear dense growth of mussels. Appreciable quantities of mussels are exploited from Ratnagiri, Malwan, Karwar, South Canara, Calicut, Canannore, Cochin, Vizhinjam, Kolachal, Muttam, Cape Comerin, Madras and Kakinada.

1.2.1 Fishing on the west coast is usually by handpicking at low tide, but larger specimens from deeper water are taken by divers. The fishing implements are an iron chisel or a wooden wedge sharpened at both ends for detaching the mussels from the substrata, and a coir bag to receive the catch.

1.3 Clam Fishery Resources

The species that contribute to clam fisheries of commercial importance in India are Meretrix meretrix (Linnaeus), Meretrix casta (Chemnitz), Kateleysia opima (Gmelin), Katelysia marmorata, Paphia laterisulea black clam - Villorita cyprinoids (Gray) and Gafrorium tumidum (Roding) (Alagaraswamy & Narasimham, 1973).

The rivers of Goa have abundant clam resources particularly Meretrix meretrix and Villorita cyprinoids. The clam season is throughout the year excepting the

CLAMS (VILLORITA CYPRINOIDS)

monsoon months (June-September). The annual production of clams from Kalinadi river alone would be around 1000 metric tons. On both sides of the estuary at Honavar and Kasserkod clams are collected regularly by about 100 persons. The important markets for clams are Bombay, Ratnagiri, Malpe, Mangelore and Mulki.

Along Kerala and Madras coasts Meretrix casta is one of the important clams occurring in almost all the estuaries and backwaters.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Crab

2.1.1 Biological aspects, catching and handling

The seasonal variability mainly appears to be an inhibiting factor for organised fishery in India, Reports by Rai (1933), Hora (1935), Chopra (1936, 1939), Anon (1951), Prasad & Thampi (1951, 1953), Jones & Sugasingani (1953), Menon (1952), Wealth of Indian Raw materials, C.S.I.R. (1950), Radhakrishnan (1979, 1980), Mohanty (1973) and Vedavyasa Rao et al (1973) provide valuable information on the nature and prospects of crab fishery in India including the bionomics, species composition, seasonal variations, methods of fishing and marketing. According to Rao et al. (1973), 640 species of crab occur in Indian waters of which only 8 species are at present considered to be commercially exploitable. They stated that crabs are cheap food consumed mostly by the coastal inhabitants and do not fetch high

prices as other edible crustaceans and fishes do. This obviously is a reason for the scattered and unorganised nature of the fishery. This condition has totally changed during the last decade.

Shelton Duane, D. (1965) recorded spectacular growth rate in the king crab fishery industry in the United States with the overall catch for 1963 was 244 million pounds. The fishing season in India and methods implemented for their exploitation are given by Vedavyasa Rao *et al* (1973) and Radhakrishnan (1980) and in U.K. by Edwards & Earley (1967). Radhakrishnan (1980) gave a descriptive account of crab landings in 10 states of India for the years 1973-1978.

Crab Landings in metric tons*

Year	1958	1960	1965	1970	1975	1980
Quantity M. tons	1,508	2,571	2,391	10,662	19,893	25,496

The commercial production of blue crabs increased from about 7.5 million pounds in 1880 to almost 155 million pounds in 1960 in New Jersey (David H.B. Ulmer, 1964). The information given by Edwards & Earley (1967),

*Source: Reports of the Central Marine Fisheries Research Institute

Marlin E.Tagatz (1965), George H.Rees (1963) and Robert Young (1957) about moult cycle and relative growth rate in crabs, its fishery, fishing methods and gear used in U.K., Florida etc are worth mentioning. According to George Rees (1963) the number of times that a crab moults during its life time and the length of time between moults varies among species and is affected by such factors as temperature and amount of food available.

2.1.2 Nutritive value of crab meat

The work of Heath (1970) on the biochemical composition of crab - Carcinus maenas during moult cycle, Badawi (1971) on the chemical composition of Portunus pelagicus, and of Addison et al. (1972) on the lipid content of the queen crab - Chinocetes opilio are worth mentioning.

Robert N.Farragert & Mary H.Thompson (1966) observed variations in body constituents according to season in body meat and claw meat. They reported that the oil and moisture changed inversly in the body meat but not in claw meat.

Nelson Richard & Claude E.Thursten (1965) observed high protein and low oil contents and higher quantities of sodium and potassium in Dungeness crab (Cancer magister).

Velankar & Mahadeva Iyer (1961) studied the amino acid pattern of crab - Neptunus pelagicus and Chatbar &

Velankar (1979) studied the vitamin B_{12} content in crab Scylla serrata.

Allen (1971) gave an account of the amino acid and fatty acid composition of the tissues of Dungeness crab.

The studies of George (1968) on the effect of salinity changes on the weight and respiratory rate on Portunus sanguinolentus and Venkatachari & Vasantha (1973) on the variationsin the protein content in different tissues of freshwater crab as a function of salinity adaptation are worth mentioning.

De Koning (1970) studied the phospholipids extracted from marine and freshwater crabs, Porter (1968) studied the acid soluble nucleotides in King crab muscle, Kanupandi & Paul Pandian (1975) studied the electrophoretic pattern of blood and muscle proteins of the crab Scylla serrata. The work of Radhakrishnan & Natarajan (1979) on the nutritive value of crab Podophthalmus vigil reveal that in young ones more protein and less fat and carbohydrate and vice versa in older ones. Mukundan et al. (1981) studied the nutrient content and calorific value of crab - Scylla serrata in comparison with fish and prawn.

2.1.3 Biochemical changes during preservation and processing

When shellfish is decomposed ammonia is formed which

is used as an index of spoilage in crab meat (Burnett James, 1965).

2.1.4 Canning is an important method of food preservation throughout the world. It requires thorough knowledge of its technological aspects. Four factors are to be controlled to get a canned product (Dungeness crab) with the taste, colour and texture of freshly cooked crab meat was given by Farber (1953) and Tanikawa et al. (1953) made an attempt to can crab meat -(Erimacrus isenbeckii) from different parts of the body separately and compared its quality. He concluded that in the processing of canned crab meat it is better to use sea water or freshwater with an addition of salt than freshwater alone.

Previous works on handling and processing methods employed in U.S.A. (Empey, 1954) and in British Columbia (Dewberry, 1959) clearly indicated that the success of operation depends upon using live healthy and vigorous crabs with prompt butchering and processing in a hygienic condition. All surfaces and utensils that come in contact with the meat should be made of Stainless steel, Aluminium or Monelmetal to prevent contamination (Herman S.Groninger & John A.Dassow (1964), Melvin E. Waters (1970), Edwards & Early (1967). The brine floatation method or U.V. light was employed to remove or identify shell

particles in the picked crab meat (Empey, 1954), Dewberry, B. (1959), Dewberry, E.B. (1970).

The optimum precook time was worked out in order to get maximum yield for canning by Blackwood et al. (1969), Dewberry, B. (1959) and Dewberry, E.B. (1970).

The work of Gangal & Magar (1967) on the effect of canning and storage on the loss of water soluble constituents and flavour bearing compounds and prevention of deleterious effects by the incorporation of antioxidants and of Varga et al. (1970) on the effect of post-mortem spoilage on the quality of heat processed crab meat are worthy to mention.

Several workers observed blueing, blackening and browning in canned crab meat and studied the possible causes responsible to such phenomenon and tried preventive methods (Herman S.Groninger & John A.Dassow, 1964), Keiichi Tanikawa (1971) and Inoue & Motohiro (1970a,b,c,d,e 1971, 1971a). The cause and mechanism of blue discolouration in canned king crab meat was studied in detail by Inoue & Motohiro (1970a,b,c,d,e, 1971a) and established a relationship between the copper content in the meat and incidence of the blue discolouration.

The effects of citric acid for the prevention of blueing in heat processed crab meat was established

(Varga et al. 1969) Edwards & Early 1967, Eiichi Tanikawa (1971) and Dewberry, E.B. (1970).

Some workers recommended fractional cooking as a means to prevent formation of blue discolouration (Dewberry, E.B. (1970), Blackwood et al. (1969).

The preprocess age of the raw material is one important factor affecting the shelf-life of the canned product. This aspect was studied in detail by Varga et al. (1969; 1970). Eiichi Tanikawa (1971) had given an account of the formation of abnormal odour, flat sour and swelling during storage of canned crab meat and also remedial steps to avoid such quality defects.

2.1.5 Studies on freezing of crab meat

With reference to freezing preservation of crab meat earlier works provide much information. The studies of Dassow (1950) on freezing and canning of king crabs, Gangal and Magar (1963) on freezing of crab meat, Collins and Brown (1965) on the effects of freezing of the meat of P. camtschatica and the deteriorative changes taking place in crab meat during long periods of frozen storage resulting in loss of characteristic flavour, appearance of stringy texture, yellowing of the bright red pigment and drip loss (Anon, 1966) are worth mentioning.

Blackwood et al. (1969) used live crabs for processing and worked out the advantages compared to dead crabs and studied the quality loss and defects observed in ice stored and frozen stored crab meat. Varga et al. (1970) studied the quality loss in frozen and heat processed meat of crab (Geryon quinquedens).

Strasser & King (1971) reported the effect of heat processing on crab meat. Paparella et al. (1971) studied the keeping qualities of blue crab claws. The method of extracting crab meat using centrifugal force by Lockerby (1971) provide valuable and useful information in this field.

2.1.6 Microbial changes during crab meat processing

It is a general practice in food microbiology to test for certain human pathogens before the material is certified as fit for human consumption. Most of these pathogens are present either as carriers or as normal flora of certain parts of the body of man or animals. Berry (1942) studied the growth of E. coli in crab meat and found that this organism grew well at 25°C and that although the numbers decreased at 5°C, viable organisms were present at 5°C even after 15 days.

E. coli has been isolated from the hands of workers handling crabs (Tobin & Mc Cleskey, 1941).

Presence of faecal streptococci on the utensils and on the palms of workers in a crab meat processing plant has been reported by Ostrolenk et al. (1947).

In a study conducted by Olson & Shelton (1973), in 46 crab meat processing factories, the log average MPN coagulase positive Staphylococci per gm of the 'lump' and special crab meat was 38 and 29 respectively in the processing factories having 'good' hygienic status.

Occurrence of Salmonella in the incoming picked crab meat has been reported by Andersen et al. (1971).

In a study comprising 140 crabs caught in England (off the Devon & Cornish coasts) Cann (1977) found V. parahaemolyticus to be present only in one of the samples, although it was present in 10 out of the 47 samples of sand and seawater examined from the same area. Isolation of this organism (V.P.H.) from frozen crab meat stored at -15°C and -30°C after 30 days and 60 days respectively has been reported by Johnson & Liston (1973).

2.2 Mussels

2.2.1 Biological aspects and fishery

Because of the sedantary habits and easy accessibility, the attention has been diverted to mussels, which is

next in importance to fish and prawn. Information regarding the resources and fishing methods are givey by Hornell (1917), Rao, K.V. (1958), Jones, 1950, 1968a,b) and Jones & Alagaraswamy (1973). Holland & Spain the two leading countries in mussel production yield about 80% of the total world catch. But in India that much importance is not given. Two species of mussels are available in India, the green mussel and brown mussel of the family Perna. The former is widely distributed on the east as well as the west coast, but the latter has a restricted distribution in the Kanyakumari-Tinnevely coast of the Madras State and South Kerala coast. Almost all the rockey stretches including backwaters and piles laid by man along the coast from the shoreline to a depth of 6-8 m harbour mussels and the west coast contains more mussel beds than the east coast because of the existence of vast areas of creeks, muddy bays, rocky inshore regions estuaries and backwaters suitable for them to thrive well.

Mussels attain the commercial size of 75 mm in an years time under culture conditions (Andreu, 1968).

2.2.2 The fishing methods are comparatively simple and can be grouped under three categories.

1. Collecting mussels from the rocks on the shore.
2. Swimming to reach the rocks and

3. By going in boats or canoes and diving.

The fishing implements are an iron chisel or a wooden wedge.

2.2.3 Nutritive value and seasonal variation

Very little information is available on the nutritional quality of mussel meat. According to Waterman (1969) the quality of the meat varies during the year, They are at their best in the late autmn and winter months but become poor during and after spawning in March or April. The work of Waterman (1969) on proximate composition, Suryanarayan & Alexander (1972) on chemical composition and calorific value, Gopakumar & Nair (1972) on the fatty acid composition of mussel lipid, Joyner & Spinelli (1969) on mussel meat as potential source of high quality protein for FPC preparation and isolation of peptidases from Mytilus edulis by Berg (1954) are worthy to mention.

2.2.4 Preservation and processing of mussel meat

The studies on changes due to freezing and cold-storage of cooked frozen mussel by Waterman (1969) and Banks & House (1958), effect of processing on nutritive value of mussels by Korobkina et al. (1970) are worth mentioning.

The details of the process employed for the

utilization of mussel meat as canned, smoke cured and pickled products are given by Balachandran & Unnikrishnan Nair (1975) and Muraleedharan et al. (1979 & 1982).

2.2.5 Bacteriological aspects

Food poisoning appears to be the major microbiological problem associated with mussels (Ernest A. Fieger & Arthur F.Novak, 1961). A single instance of mussel pollution near Calicut was reported (Venkataraman & Sreenivasan, 1955), in that case faecal pollution has been observed during southwest monsoon period. Quantitative estimation of the bacterial load of the brown mussel (Perna indica) cultured at Vizhinjam has been shown as 10^6. The occurrence of Coliforms, Escherichia coli, Faecal streptococci and coagulase positive staphylococci are reported. Pseudomonas, Vibrio and Micrococcus are seen as normal flora of mussel and seawater (Thankappan Pillai, 1980).

Enteropathogenic E. coli has been isolated from freshwater mussels by Stephen et al. (1975). Buttiaux (1962) stated that edible oysters, mussels and shellfishes were very often infected with salmonellae because the most essential sanitary precautions were neglected in the culture areas. Gevaudan & Gay (1958) came to the conclusion that the concentration of salmonella in mussels varied with the number of salmonella present in the surrounding water.

A simplified system of mussel purification was outlined by Reynolds (1956), Dewberry, E.B. (1953), Waterman (1969) and Connell (1980). The method is based on the natural action of the bivalves cleaning their alimentary canals in clean chlorinated seawater during a period of 48 hours. The mussels rid themselves of all harmful organisms thus making them safe for human consumption by this process of depuration.

2.3 Clams

Clams are one of the most popular and important shellfish in the world. They are produced abundantly in Canada, United States, Argentina, Chile, China, Japan and Korea.

2.3.1 Biological aspects and fishery

A survey of the literature on the Indian mulluscan resources, shows that Mr. James Hornell has contributed much to our knowledge. His works (1917, 1922 & 1949) on the Indian molluscs remain todate the most authentic report on many aspects of Indian shell fisheries. Rai (1932) made a survey of the coast of the then Bombay Presidency and made available valuable data on the oyster and clam industry of that area. There are several other publications that give some information on the abundance and the economic importance of the clams. Some of which are Rao, H.S.

(1941), Rao, K.V. (1952, 1958 & 1963), Abraham (1953) Nayar (1955), Joshi (1963), Ranade (1964), Alagaraswamy (1966) and Jones (1968).

Among clams those belonging to the family Veneredae are by far the most important in the Indian waters, nine species contribute to the fisheries of commercial importance. There is no system at present of collecting data for the molluscs, as for the fishes and crustaceans.

Along the Kerala coast, Meretrix casta is one of the important clams occurring in almost all the estuaries and backwaters. Villorita cyprinoids is available in the Cochin area and at several other places to its south. According to Sebastian (1970) nearly 2,400 metric tons of clam meat is obtained annually from the Vembanad lake alone.

2.3.2 The clams are exploited in Kerala for their shell. It is extensively used in cement industries. The clam meat is infact a byproduct of this industry. Clams are usually hand picked in shallow waters at low tides. Very rarely any mechanical devices are employed for them except small bagnets or dredges operated from canoes (Alagarawamy & Narasimham, 1973).

Molluscan shells for lime are gathered from the estuaries and backwaters in considerable quantities. During

monsoon when large quantities of dead shells are drifted ashore by the currents, they are collected and made use of for the purpose (Rao, K.V., 1958). In Kerala its importance emerges from the occurrence as extensive subfossil deposits in the Vembanad lake, which are used as raw material in the cement industry at Kottayam (Algaraswamy & Narasimham, 1973).

2.3.3 Nutritive value of clam meat

India faced with the problem of food shortage sometime back, being systematically investigating the resources around her to solve the problem. Clams are a good source of protein, fat, glycogen and minerals, with all the essential amino acids are easily digestible. Only very limited information is available on nutritive value of clam meat and the seasonal influence on chemical composition. The chemical composition of clam meat by Venkataraman & Chari (1951) clearly indicates variations in moisture, protein, fat and minerals. Viswanathan Nair et al. (1976) studied the lipid and fatty acid composition of clams - Villorita cyprinoids. 50-71% of the total lipid content was phospholipids and about 6% C_{17} saturated fatty acids. Of the phospholipids, phosphatidyl choline (40.7%) and phosphatidyl ethanolamine (28.4%) are found to be the major constituents.

2.3.4 The studies of Baruch Rosen (1966) on soft clams frozen in plug-top metal cans, then cold stored revealed an increase in lactic, acetic, propionic and pyruvic acids and bacterial count increased at an exponential rate.

Studies on causes of can swelling and blackening in canned baby clams by Tanikawa et al. (1966 & 1969) and Hardy (1953) about colour change observed in canned razor clams and precautions to be taken for obtaining good quality product and Motohiro (1974) on the utilization of shellfish in Japan such as boiled, seasoned, smoked and canned clams are worth mentioning.

The processing of clams in the United States as canned minced clams, clam nectar, clam chowder, clam extract, in Japan as canned whole hen clams and in Canada about the process employed for preparing good quality canned clam meat was described by Tanikawa & Doha (1965).

2.3.5 Microbiology of clam meat processing

Bacteriological study of the coliform group in clams stored under normal conditions was made by Sandholzer & Arcisz (1946). Rice (1929) isolated 38 species of organisms from clams; the Bacillus and Pseudomonas groups were the most common bacteria present. In an investigation of several Japanese baby clam canneries Amano (1948) found

heat-tolerant microorganisms of the genera Bacillus and Clostridium in the soils, rubbish, factory wastes, sand and sea water surrounding the processing plants. No Clostridium botulinum or any related toxins were present. Apparently anaerobic bacteria are the principal viable organisms in the shells of the living baby clams. A heat resistant anaerobe related to Clostridium bifermentans was isolated from fresh clam meat of the species Venerupis Philippinarum.

The Occurrence of paralytic shell fish poisoning on the United States west coast was summarised by Pivnick (1951).

The polluted shellfish can be made safe for human consumption by the process of purification has been adequately demonstrated by Fabre-Domergue (1916), Wells (1916), Dodgson (1928) and Connell (1980).

2.3.6 The effect of radiation pasteurization on shelflife and changes in free amino acid contents and organoleptic qualities of soft shell clam meats was illustrated by Brooke & Steinberg (1964), Connors & Steinberg (1964) and radiation processing and storage effects on head gas components in clam meats was studied by J.M.Mendelsohn & Brooke (1968). According to them the diemthyl sulphide was found to be the most dominant head gas component and to be the source of the typical clam odour.

2.4. Proximate chemical composition of fish

Water, protein and fat are the major constituents of fish with non-protein nitrogenous substances, carbohydrates and salts occurring in small amounts. Since muscle proteins are the main solids of fish, it is a protein food stuff, particularly if it is lean.

Moisture, protein, fat and calorific value of 44 species of fish and shell fish in the British coasts was given by Reay et al. (1943). A detailed account of chemical composition and changes due to season in different varieties of fish and shell fish muscle was given by Raymond Jacquot (1961). Chemical constituents such as high protein and low fat, moderate quantities of calcium, phosphorus, iron and B group vitamins were analysed in Sepia orientalis and Loligo vulgaris by Pandit & Magar (1972). The variations in composition of Atlantic halibut, mackerel, tuna and sword fish during spawning migration was observed by Mannan et al. (1961); while low oil and sodium contents and high protein content in halibut meat was noted by Anon (1959). The proximate chemical composition and seasonal and local variations in chemical composition, the effect of salinity of water on chemical composition in oyster meat was given by Paul S.Galtsoff (1964).

2.4.1 Proteins

Proteins are perhaps the most important constituent of fish muscle, constituting more than 80% of the dry weight.

Based on the differences in their physico-chemical properties, the proteins are broadly categorised as sarcoplasmic and myofibrillar proteins, stroma and denatured fractions (Warrier et al. (1975); Paul et al. (1966); Baliga et al. (1962 & 1969); Sayre (1968); Carpenter & Saffle (1965); Connell (1962); Sayre & Briskey (1963) and Yuji Maruyama & Taneko Suzuki (1968).

The sarcoplasmic proteins forming approximately 15-20% of the total proteins depending on the fish species, are generally soluble in water or buffers of low ionic strength. To this class of proteins belong enzymes of the glycolytic pathway (Tarr, 1966), Nagayama (1967), Gould (1965) Martin & Tarr (1961) and autolytic reactions (Siebert, 1958); Siebert & Bottke (1963); Bird et al. (1969) Warrier et al. (1972a,b). Most of these are low molecular weight proteins.

The myofibrillar proteins consisting 60-80% of the total proteins are soluble only in salt solutions of high ionic strength and have molecular weight in the range of $4x10^5$ to $6x10^5$. The remaining portion about 3-10% is

insoluble even in dilute solutions of hydrochloric acid or sodium hydroxide and has been called stroma. It is derived from connective tissue. Fibrillar proteins play an important role in contributing textural quality of the flesh.

The textural qualities associated with muscle such as fibrousness, water holding capacity, plasticity and gell forming ability are controlled by the myofibrillar proteins

All the major myofibrillar proteins isolated from meat namely, actin, myosin and tropomyosin have been found in fish also. Preparation of fish myosins in pure state is very difficult since actin gets extracted easily with myosin (Connell, 1962; Mackie & Connell, 1964). The contamination with actomyosin can be minimised by using acidic extraction media (Hamoir, 1955) or extractants containing Adenosine triphosphate (Hamoir et al. 1960) or pyrophosphate (Connell, 1954, 1960, 1962). The most important biochemical characteristics of myosin is its enzymatic activity with respect to hydrolysis of adenosine triphosphate (ATP) and is related to the ATP-ase activity of reconstituted actomyosin (Barany, 1967) and to the interaction between actomyosin and ATP (that is molecular contraction (Mommaerts, 1950, 1966; Davies, 1963). At higher temperatures there will be an appreciable decrease in activity (Connell, 1960; Sawant & Magar, 1961).

Interaction of actin with myosin is the main reaction involved in muscle contraction. The relaxing protein (tropomyosin-troponin complex) also plays a significant role in this by regulating the Ca^{+2} and Mg^{+2} concentration. Globular (g) actin to Fibrous (F) actin transformation has been extensively investigated by different workers (Mommaerts, 1951; Laki et al. 1951; Strohman (1959). The interaction of actin and myosin forming actomyosin and the dissociation of actomyosin in presence of ATP was soon recognised as the fundamental reaction involved in muscle contraction. Fish actomyosin have been prepared from different species of fish by different workers and its properties studied (Shizunori Ikeda & Takeshi Taguchi, 1968; Horie et al. 1975; Murozuka et al. 1976; Dingle & Hines (1960).

The third group of proteins in fish muscle is the connective tissue which is insoluble in 0.1 N sodium hydroxide or hydrochloric acid, which is constituted mainly by collagens which are rich in hydroxy proline (Sayre, 1968; Paul et al. 1966). Fish muscle contains very little stroma or connective tissue compared to meat; and are noted for their heavy gelatinization. The low content of stroma in fish muscle and its easy gelatinization are important properties which confer the characteristic texture to fish muscle.

2.4.2 Non-protein nitrogenous constituents

This fraction is said to account for 10-20% of the total nitrogen content in the fish. The compounds occuring in this fraction have been grouped as follows:-

a) Volatile bases (ammonia, mono-di and trimethyl amine).
b) Trimethylammonium bases (trimethyl amine oxide, betaines).
c) Guanidine derivatives (creatine and arginine).
d) Imidazole or glyoxaline derivatives (histidine, carnosine and anserine).
e) Miscellaneous (urea, amino acids and purine derivatives) (Shewan, J.M. (1951) and Raymond Jacquot (1961).

The non-protein nitrogen in fish muscle was measured after trichloracetic acid (TCA) precipitation (Sayre & Briskey (1963) and Wood (1958) studied the non-protein nitrogenous constituents of the muscle of sockeye salmon during spawning migration.

2.4.3 Fat or Lipids

The chief storage form of available energy in the animal cell is the lipid molecule. When the calorie intake exceeds utilization excess food is invariably stored as fat.

Jafri (1973) made an attempt to describe the variation in total fat and water contents of the flesh of a popular cat fish and Gopakumar & Nair (1966, 1967 and 1972) studied the fatty acid composition of the lipids extracted from oil sardines, mackerel, pomfret, kilimeen, jew fish and eight other species of Indian marine fish. The concentration of fat is subjected to seasonal variations. Sen & Gracy Mathew (1973-74) reviewed the work on fish lipids, fatty acid composition and phospholipids of fishes and shell fishes of Indian waters.

2.4.4 Sugar and sugar phosphates

The storage of polysaccharide of animal tissues is glycogen. The occurrence of glycogen in fish muscles was investigated first by Dill (1921) and subsequently in more detail by MacLeod & Simpson (1927). Tomlinson & Geiger (1962) had pointed out that many species of fish have a muscle glycogen content which compares favourably with that of warm-blooded mammals.

Tarr & Leroux (1962) studied the free sugar contents in fish skeletal muscle and the possible mechanism for their formation. The studies on seasonal variations in glycogen contents in oyster muscle by Paul S.Galtsoff (1964), the concentration of ribose, glucose, ribose-1-phosphate, glucose-1-phosphate, glucose-6-phosphate,

fructose monophosphate and fructose 1,6-diphosphate in muscle extracts of aquarium kept cod by Burt (1961); liver glycogen levels of salmon during spawning migration by Violet M. Chang & Idler (1960); the free sugar contents in fish skeletal muscle and the possible mechanism for their formation by Tarr & Leroux (1962) are worth mentioning.

2.4.5 Phosphorus

Our knowledge on phosphorus compounds in fish muscle is comparatively recent and their study was initiated by Tarr (1950a) who determined these compounds in the skeletal muscle of starry flounder, lingcod, tomcod, whiting and blue perch.

Phosphorus is essential to cell metabolsim and has got more functions than any other single mineral. Most of the phosphorus is concentrated to the nucleus. It combines to form phosphoproteins which initiate muscle metabolism and phospholipids are essential in lipid metabolism. The blood of fish is rich in organic acids of soluble phosphorus compounds, but flows as inorganic phosphoric acid in the blood stream.

There are three closely related phospholipids, these being esters of phosphatidic acids and nitrogen containing alcohols (Choline, ethanolamine and serine). The one derived from choline is the well known lecithin isolated

from a great number of fishes. Glycerophosphatides have been identified in several fishes and are characterized by the presence of appreciable and frequently high proportions of C_{20} and C_{22} highly unsaturated acids chiefly arachidonic and clupanodonic (Lovern & Olley, 1953a,b).

2.4.6 Minerals

Conor Reilly (1977) had given an account of the role of minerals in muscle metabolism. Calcium and magnesium which are the principal metals in bone and sodium and potassium which are concentrated in blood and other body fluids are included among the major elements.

Paul S.Galtsoff (1964), based on his studies on American oysters, reported that many bivalves have the ability to accumulate various heavy metals such as zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), lead (Pb) and arsenic (As). The problem is of importance because in polluted coastal waters shell fish may store substances that may be dangerous to human health.

Connell (1975) stated that mercury (Hg), cadmiun (Cd), lead (Pb), selenium (Se) and arsenic (As) are cumulative poisons, repeated ingestion of small amounts cause injury to health.

2.5 Fish flavours

Flavour is a complex concept involving primarily aroma and taste but also appearance, behaviour on manipulation, feel in the mouth and even the sounds emitted in chewing (Nursten, 1975). The sense of taste is relatively simple, there being four basic qualities, bitter salt, sour and sweet.

While much information has accumulated concering the chemistry of fish much of it has not been correlated directly to flavour (Jones, 1961). Much of the sweetness of fresh fish results from the initial concentrations of glucose. The loss of sweetness and meatiness from very fresh fish correlates well in some species to the disappearance of free glucose, the hexose phosphates and inosine-5-monophosphate which possess those flavour characteristics from the muscle. The progressive development of fishiness, pungency, sourness, bitterness etc. can be accounted for by the presence of well characterised compounds in the fish. The importance of inosinic acid in enhancing the flavours of flesh foods is well known principally as a result of Japanese investigations (Kuninaka et al. 1964; Wagner et al. (1962).

The loss of flavour commonly associated with the short-term chill storage of fish derives partly from leaching losses into ice melt water, and partly due to a great

extent from the actions of autolytic systems which cleave flavourous compounds present in the muscles (Jones, 1962). Inosine-5'-monophosphate, a major flavourous component is cleaved rapidly with formation of Inosine. This compound is hydrolysed or phosphorylated in cod muscle to form hypoxanthine which contribute much of the bitterness characteristic of staling fish; Glycolysis in chill stored muscle produces changes in the concentrations of hexose phosphates which are also important to flavour. Sugar-amino reactions occurring in processed fish muscle produce, meaty and bitter flavours (Jones, 1962). Dimethyl sulphide is an important odour constituent in edible shell fish and it is derived from dimethyl-β-propiothetin, a compound found in certain of the algae ingested by filter-feeders (Anon, 1967, 1968).

2.6 Causes of deterioration

When the fish dies, the balance between the process of body maintenance is upset. The enzymes instead of acting on the food normally taken in, continue actively to digest any of the particular type of materials such as fats, carbohydrates or proteins - thus a reversal of normal process of digestion and assimilation is occurred. Enzymes are secreted by the bacteria which act in the same general manner and their attack is facilitated by the fish enzyme actions.

A second cause for deterioration in quality of fish results from oxidation and rancidity. The oxidation and rancidity of fats can be caused by the simple or combined action of tissue enzymes, bacterial enzymes and exposure to air. Oxidation, besides causing rancidity can cause other changes in fish, the fading of pigments, and the development of off colour and browning.

Bacteria are usually the most important causes for deterioration in fish as in other protein foods. They are present in air, water and soil in innumerable forms, shapes and species each with a characteristic method of attacking, which although we do not see with the naked eye can be noted by the odours, flavours or colours imparted to the material on which they are acting.

2.6.1 Thus a proper evaluation of the factors involved in spoilage is essential to the proper handling, storage, transportation and proper processing of food products (H.O. Triebold & L.W. Aurand).

2.6.2 One of the main factors determining the onset of spoilage in freshly caught fish is rigor mortis, a stiffening of the body which develops some 1-7 hours after death. (Ludorff, 1957). Rigor mortis passes quickly in very active fish and slowly in inert fish. Rigor mortis passes

quickly in fishes which resisted the catch than in fishes caught without struggle (Ludorff, 1957).

When an animal is slaughtered, Mg-ATP (mangnesium-adenosine triphosphate) which is present in the muscle fibres is decomposed by an enzyme present in the sarcoplasm. There is a large release of energy which is used up in causing the actin filaments in the myofibrils to slide in between the myosin filaments. As this interdigitation takes place the actin filaments became rigidly attached to the myosin filaments causing a large decrease of extensibility and giving rise to the well known phenomena of rigor mortis (Ferguss Hill, 1967).

2.6.3 As fish spoils, an easily recognisable spoilage pattern can be noticed according to the development of a regular succession of different odours. Four stages can be recognised in the spoilage of fish.

1) the muscle has a characteristic fresh fish or sea fish odour.
2) the muscle loses some of the fresh fish odour but has no spoilage odours.
3) development of the first spoilage odours, which vary according to the season of the year.
4) fish is rotten or putrid according to the development of spoilage compounds such as hydrogen sulphide (H_2S), indole, ammonia etc (Castell, 1957).

The loss of sweetness or meatiness from very fresh fish correlates well in some species to the disappearance of free glucose, hexose phosphates and inosine-5' monophosphate which possess those flavour characteristics from the muscle (Jones, 1960).

Fish muscle contains very active cathepsins capable of splitting protein and also very rich in peptidases. Proteolytic enzymes in fish muscle are abundant enough to suggest that they play an important role specially in the early stages of spoilage by degrading fish muscle proteins and by furnishing amino acids and peptides for the growth of microorganisms (Siebert, 1961).

Fraser et al. (1961) showed that struggling reduced the muscle glycogen with accumulation of lactic acid and concluded that the time of onset, the degree and duration of rigor mortis were dependent upon a number of factors, the most important being the method of catching (MacLeod & Simpson, 1927; Sharp, 1934; Black et al. (1961). An enzymic breakdown of adenosine-triphosphate (ATP) by actomyosin ATP-ase or apyrase occurs in fish muscle (Partman, 1954).

After death lactic acid is produced by anaerobic glycolysis and creatine phosphate (Cr-p) concentration decrease (Buttkus & Tomlinson, 1966). Jones (1959)

estimated about 0.67 mg% of pyruvic acid in the muscle of freshly killed trawled codling . It is the penultimate stage of glycolysis in muscle, a key compound yielding energy through the tricarboxylic acid cycle and an intermediate in the biosynthesis of alanine.

The post-mortem degradation of glycogen undoubtedly contribute to both the flavour and texture of fish and the free ribose in fish muscles arises largely from the post-mortem degradation of ATP (Tarr, 1966).

The freshness of fish depends principally on its temperature and the time that has elapsed since death. The higher the temperature the faster the bacteria living in the fish multiply (Anon, 1960).

The muscle protein solubility was grossly altered by the conditions of both temperature and pH which existed at the onset of rigor mortis or during the first few hours after death (Sayre & Briskey, 1963).

2.6.4. Though sensory methods are likely to remain the most versatile and sensitive way of measuring freshness, chemical tests have a role also (Anon, 1977).

A continuing problem in fisheries research is the lack of an objective test for the freshness of fish (Edith Gould, 1969).

A satisfactory test for the detection of spoilage in fish must meet certain definite requirements. It should provide an accurate measure of the degree of spoilage and should be based on the most characteristic change occurring in the product as spoilage progresses. Finally the test should be rapid and simple to perform (Dyer et al. 1944).

2.5.4.1 Hypoxanthine concentration rises as fish stales, so the increase in hypoxanthine concentration in muscle during storage has been suggested as an objective measure of quality (Jones, 1962, 1964; Edith Gould, 1959; Anon, 1977; Fraser et al. 1968).

2.6.4.2 Histamine developed 40-50 hours after death, and this is used as a quality index by Hughes (1959).

2.6.4.3 The increase in tyrosine value was taken as a measure of spoilage in fish meat by Ota & Ajiska (1953) and Dyer et al. (1944).

2.6.4.4 The pH of fish muscle has been proposed as an index of spoilage by Van Deurs & Hoff-Jorgensen (1936), Shaikmahamud & Magar (1965) and Nazir & Magar (1963).

2.6.4.5 The amount of trimethylamine (TMA) which is the reduction product of the oxide TMAO is widely adopted as an index of spoilage of fish (Velankar et al. 1961);

Venkataraman & Chari, 1953; Wierzchowski et al. 1953); Shaikhmahamad & Magar, 1965; Nazir & Magar, 1963); Bose, A.N., 1954; Dyer et al. 1944.

2.6.4.6. A combination of trimethylamine, volatile acid number and bacterial count indicates the potential keeping quality more accurately than visual examination of fish (Velankar et al. 1961).

2.6.4.7 Among physical tests, refraction of the eye fluids and redox potentials were useful tests (Joseph Denfel, 1963).

2.6.4.8 Nazir & Magar (1963) followed pH, glycogen, lactic acid, inorganic phosphorus, creatine phosphorus adenosine triphosphate, trimethylamine and barium acetate non-precipitable ribose, in order to study the biochemical changes in fish muscle during rigor mortis.

2.6.4.9 F. Shaikhmahamud & Magar (1965) found that the suitable tests for freshness of fish were determinations of pH, total bacterial count, trimethylamine, glycogen lactic acid and vitamin B contents.

2.6.4.10 Muscle protein solubility appeared to be one of the factors affecting the juice retaining properties of the muscle (Sayre & Briskey, 1963).

2.6.4.11 Geetha Ramanathan & Moorjani (1973-74) estimated peroxide value, carboxyl value, dicarbonyl compounds and malonaldehyde in order to study the oxidative action in fish products and found that malonaldehyde is an index of rancidity.

2.7 Studies on spoilage pattern of fish and shellfish during different storage conditions.

2.7.1 Room temperature storage

Velankar et al. (1961) studied the spoilage pattern of prawns at ambient temperature by following chemical, bacteriological and organoleptic changes. They found that a combination of trimethylamine, volatile acid number, bacterial count and organoleptic conditions indicate the potential keeping quality more accurately than the visual examination.

2.7.2 Refrigerated storage at 0°C

No significant changes occurred in the phosphorus content of the phospholipid, ribo nucleic acid or deoxyribo nucleic acid fractions of sterile lingcod muscle stored at 0°C, but in the acid soluble fraction, the portion of total phosphorus accounted for by inorganic phosphorus increased to 96% from 75% (Neil Tomlinson et al. (1960).

Jones & Murray (1962) observed during the course of their studies on degradation of adenine and hypoxanthine nucelotides in the muscle of chill stored trawled codling that the adenosine 5' triphosphate remaining in the muscle at the time of death was rapidly converted to inosine -5' monophosphate. This is dephosphorylated to inosine which is itself cleaved to hypoxanthine and either ribose or ribose 1' -phosphate.

Burt (1961) studied free sugars and sugar phosphates in muscle of chill stored aquarium cod. He observed that rested cod muscle contains more free sugars and sugar phosphates than trawled cod muscle.

The studies on free sugars in chill stored codling by Jones (1958) revealed that glucose is the only free sugar present in fresh codling muscle, and ribose appears during chill storage.

2.7.3 Ice storage

Icing is the most common method of preservation of fishery products. For transportation of fresh fish over long distances to the interior parts of the country icing is preferred.

Very little information is available on the changes in protein fractions of fish muscle during ice storage.

In India Moorjani et al. (1962) and Baliga et al. (1962, 1969) have attempted to follow the changes in muscle proteins of freshwater fishes during ice storage.

Moorjani et al. (1962) followed the changes in protein fractions such as fibrillar, non-protein nitrogen and stroma in freshwater fish during storage in crushed ice.

Baliga et al. (1962) followed the changes in soluble protein nitrogen in Ophicephalus sp. stored in crushed ice.

Baliga et al. (1969) fractionated the muscle proteins of freshwater fish stored in ice. They observed that the amount of actin that was not reconvertible to 'F' actin increased during storage of the fish. Also viscosity of the buffer extracts increased during the period of development of rigor and decreased on further storage.

Devadas & Nair (1970, 1971) followed changes in the major protein nitrogen fractions such as sarcoplasmic, myofibrillar and stroma of prawns, sardines, mackerel and lactarius during ice storage. Myofibrillar proteins were found to get denatured at a rapid rate than sarcoplasmic protein fraction; and the presence of free fatty acids in the muscle which can inhibit the extraction of muscle proteins.

Sakaguchi et al. (1982) observed little change in

the levels of most free amino acids in white muscle of yellow tail during ice storage for over 40 days, but in the dark muscle the levels of almost all free amino acids except taurine increased significantly over a period of 33 days.

Liston et al. (1961) followed organoleptic and chemical tests to study the spoilage pattern of Pacific coast rock fish stored in ice. Odour, flavour, rancidity, total volatile acid and total volatile base agreed with spoilage and a sharp cut off point was apparent between the organoleptically edible and inedible fish.

Shewan & Jones (1956) had given an account of the chemical changes occurring during spoilage of chilled fish and their relation to freshness tests. Changes in some of the constituents for example, anserine, some amino acids, nucleotides and sugars are due to autolysis, while changes in volatile bases, other amino acids and trimethyl amine oxide are the results of bacterial action, and considerable leaching losses occurred during ice storage.

Lahiry et al. (1963) studied the factors influencing the keeping quality of freshwater fish in ice.

2.7.4 Changes during freezing and storage

2.7.4.1 The most noticeable change in frozen stored fish

is the development of a tough texture. This is attributed to protein denaturation, that leads to loss in water holding capacity (WHC). The term WHC is used to express the ability of meat or fish to hold water during the application of force like pressing, centrifugation etc (Warrier et al. 1975).

Hamm (1960) has suggested that only 4-5% of the total water of muscle is tightly bound to the muscle proteins and is not influenced by changes in the structure and charges of proteins. Most of the remaining water is termed as free water and is retained within the protein structure.

According to Love (1962) when an animal is frozen, the constituent water usually separates out as pure ice. At first, before much denaturation has taken place, such separated water is reabsorbed by the protein gel. When the tissue is thawed, the ability to reabsorb water diminishes during the course of denaturation.

Awad et al. (1969) observed a decrease in water holding capacity of freshwater white fish muscle during frozen storage at -10°C.

2.7.4.2 The changes due to freezing and storage make the fish less palatable, and vary according to species, freshness, treatment prior to freezing, freezing method and the storage conditions (Nikkila & Linko, 1954). The changes

caused by a denaturation of proteins results in a drier and coarser muscle texture than fish muscle. The denatured proteins lose their ability to swell, retain muscle juice and return to their original condition after defrosting. He observed that the muscle frozen in rigor mortis and subjected to cold storage is more likely to become denatured during defrosting than that muscle frozen after the resolution of rigor mortis. Freezing causes a change in the condition of the native proteins and make them more liable to denaturation. If the storage time and defrosting temperature were increased, the myosin became increasingly less soluble in salt solution.

Experimentally the denaturation of proteins was studied best by changes in the solubility characteristics of proteins (Dyer et al. 1950). Anderson et al. (1963) have cast serious doubts on accepting solubility as a criterion of protein denaturation. The best approach so far to the problem of determination of protein denaturation in frozen fish has been to study the loss of solubility in a neutral salt solution of high ionic strength. Bate Smith (1934, 1937) and Reay (1933, 1934, 1935) observed that during frozen storage of fish, there is a progressive loss in solubility of its muscle proteins, especially the globulins. Dyer et al. (1950) improved the extraction procedure by the introduction of a blending technique, which

has subsequently become a standard method for determining protein solubility in frozen fish.

Variations were considerably diminished in the percentage of total protein soluble in 5% sodium chloride solution when certain myotomes freed from myocommata were assayed rather than whole minced fillets (Ironside & Love, 1958).

Love & Ironside (1958) followed changes in the percentage of soluble proteins during frozen storage of fish and found that during 20 weeks at -14°C the value declined steadily from 85% to 28%, after reaching this stage no further decrease was observed.

Love (1958) measured the proportion of soluble protein in cod muscle which had been frozen at various speeds and storage for different times at different temperatures.

Love (1962) followed opacity measurements to study the denaturation of proteins, but the seasonal variation in fat content interferes in the opacity measurements.

Love & Mackay (1962) followed the development of cell fragility method during cold storage. The cells of fish muscle during cold storage gradually develop an increasing resistance to destruction by a mild homogeniser. The homogenate contains a greater or lesser number of

intact cells according to the extent of cold storage denaturation and the proportion of these is assessed by measuring the optical density of the homogenate in a colorimeter. An increase in the number of intact cells results in a decrease in optical density. Here also fat affects the opacity of the homogenate by forming an emulsion with the formaldehyde.

Love (1962) studied the effect of onset and resolution of rigor mortis on protein denaturation.

Love & Elerian (1962) studied the temperature of maximum denaturation in cod and found that the rate of denaturation was maximal at a temperature close to -15°C. According to Love et al. (1965) the changes in extractability are the consequence of a binding together of the structural protein molecules and perhaps myofilaments, while a binding together of myofibrils is the agent causing changes in cell fragility readings.

Love (1970) stated that the properties of the myofibrillar proteins gradually change during freezing and frozen storage of fish, that is an uncoiling of the molecular helix leading to cross linking between the adjacent parallel molecules; the actomyosin complex becomes steadily less soluble in 5% sodium chloride solution after increasing time of storage.

Love & Maslemuddin (1972) studied the protein denaturation by measuring the cell fragility based on pH effects.

Connel (1960 & 1962) studied the changes in the actin and myosin of cod flesh during storage at -14°C. 70-80% of the myosin became non-extractable at a rate similar to that at which the total myofibrillar protein of the flesh became non-extractable. The remainder became non-extractable at a very much slower rate. Upto 52 weeks pure myosin was extractable from cod muscle stored at -14°C, and actin had been prepared for upto 127 weeks. The amount of sarcoplasmic proteins remained virtually unchanged during prolonged storage.

According to Connell (1962) the principal mechanism involved in the development of toughness, rubberry texture and loss of water holding capacity during storage of frozen cod is the formation of increased numbers or increased strength of bonds between the constitutent myofibrillar proteins.

The actomyosin insolubilisation in fish held in frozen storage is due to free fatty acid accumulation in the muscle as a result of lipid hydrolysis (June Olley & Duncan, 1965; Dyer & Fraser, 1959; Devadasan & Nair, 1971; Seagram, 1958); but according to Raymond Jacquot (1961)

denaturation was usually more rapid in the non-fatty species than in the fattier species. Elerian (1965) followed the changes in the refractive index of muscle juice or whole muscle tissue of cod to measure the deterioration. According to King (1966) myosin was more sensitive to freezing induced denaturation than actin; Awad et al. (1969) followed the solubility criterion of actomyosin to study the frozen storage deterioration in freshwater white fish muscle and Moritoshi Oguni et al. (1975) studied the physicochemical characteristics of freeze-denatured carp actomyosin. Banks et al. (1977) suggested that the protein damage may be due to a number of different interrelated physical and chemical changes, that vary with species and methods of processing storage and handling.

2.7.4.3 Adenosine triphosphatase activity (ATP-ase) of Myosin and Actomyosin

The most important biochemical characteristics of myosin is its enzymatic activity with respect to the hydrolysis of ATP (Engelhart et al. 1939). The activity of purified myosin is related to the ATP-ase activity of reconstituted actomyosin (Barany, 1967) and to the interaction between actomyosin and ATP in vivo (that is muscular contraction) (Mommaerts, 1950; 1966 and Davies, 1963). By using the loss of enzyme activity as a measure of denaturation trout myosin was about 23 times more

stable than cod myosin, but lost its activity about 25 times more rapidly than rabbit myosin (Buttkus, 1966). Substantial differences in the ATP-ase activity of myosins were observed from the skeletal muscle of various species (Bailey, 1942; Perry, 1960; Quass & Briskey, 1968; Morey et al. 1968).

Connell (1962) suggested that cod flesh stored at -14°C was becoming denatured that is undergoing a confi-gurational change in structure that leads to loss of enzy-mic (ATP-ase) activity. Adenosine triphosphatase exerts its effect on the muscle energy mechanism by cleavage of the terminal phosphate from its substrate adenosine tri-phosphate. The liberated chemical energy is then utilised in other organic reactions, transformed into mechanical energy, or it can be dissipated as heat (Tonzetich, 1954). According to Buttkus (1966) denaturation measured by the loss of myosin ATP-ase activity proceeds with positive entropy changes and can be looked upon as an opening or unfolding of the secondary and tertiary structure of the myosin molecule. Takashi Taguchi & Shizunori Ikeda (1968) studied the effect of lecithin on ATP-ase activity of actomyosin of pre and post rigor cod muscle.

Akihiko Hashimoto & Ken-ichi Arai (1978) found that the rate of inactivation of ca-ATP-ase of sardine myofi-brils at pH 5.8 and 5°C was found to be comparable with that of ca-ATP-ase at pH 7.6 and 26°C.

Buttkus (1966) found out that myosin had two enzymic activities, an adenosine triphosphatase and an acetyl choline esterase, the rate of inactivation of acetyl choline esterase activity at 45°C was approximately equal to the rate of inactivation of ATP-ase activity at 25°C.

2.7.4 Freezing as a method of food preservation comes closer to preserving the food in the natural state than other methods of preservation. Of the various factors that influence the course of changes in the quality of fish muscle during frozen storage, the condition of the fish at the time of freezing appears to be the most important (Love, 1962a,b); and the temperature of storage is the most important single factor affecting the storage life of frozen fish (Anon, 1965; Dyer et al. 1968); and the temperature and humidity of the cold storage room have long been known to be important factors in determining the storage life of frozen foods (John A. Peters, 1970).

The first change noticed during cold storage of fish is surface drying, second closely related change is the denaturation of labile soluble fish proteins; this results in a toughening of muscle texture and an increase in drip loss during thawing, a third change is the appearance of off flavours and odours resulting from lipid spoilage (Beaumariage et al. 1969).

The amount of drip formed on thawing of frozen fish varies with a large number of factors involving both freezing and storage conditions and pre-freezing condition of the fish (Dyer et al. 1968; Empey & Howard, 1954). Dyer & Fraser (1961) suggested that when pH or acidity of the muscle was changed by lactic acid formation, the moisture binding capacity of the protein was reduced and drip was formed.

Glycolysis proceeds in fish muscle at subzero temperature (Tomlinson et al. 1963 and Burt, 1971).

It has been stated by Shewan (1954) that bacteria when frozen some suffered immediate death, irrespective of the rate of freezing or its temperature. Radhakrishnan et al. (1973) observed a gradual decrease of total and pathogenic bacterial counts in Bombay duck as the frozen storage period increased.

2.7.5 Use of glazes, preservatives and packagings. Ice glaze is inexpensive, can be easily applied and adopted in a production line and provides a satisfactory covering for a variety of fishery products, inspite of its susceptibility to cracking, its brittleness and high vapour pressure. According to the literature, ice by itself does not prolong the shelflife but chemical and antibiotic ices appear to appreciably extend the storage life of prawns (Tarr et al.

1950; Fieger et al. 1956). The effectiveness of various water soluble antioxidants for retarding the development of rancidity in frozen lake herring products was studied by Greig et al. (1967) who observed that ascorbic acid was found to be more effective than propylgallate, monosodium glutamate or sodium tripolyphosphate. The effectiveness of ascorbic acid or ascorbic citric acid mixture for controlling yellow discolouration in frozen pomfrets, balck spots in shrimps, inhibition of growth of the natural mixture of flora at temperatures between -18 and 28°C, for protecting the meat colour and for preventing discolouration in tuna meat and for improving the general quality of frozen stored pomfrets, surmai and mackerel were reported (Jadav & Magar, 1970; Bailey & Fieger, 1954; Shaikhmahamud & Magar, 1965; Fieger et al. (1956); Tressler (1957); Tanikawa, 1971 and Sawant & Magar, 1961).

Several varieties of fishes and shellfishes had better keeping qualities when coated with sodium alginate (Pillai, 1964). Glazing with a salt sugar (1%) solution was found to be superior to ordinary water glaze for frozen prawns (Mathen et al. 1970) and protective coatings such as agar-agar and dipping in hydroqunone solutions enhance the storage life of frozen oil sardines (Mathen et al. 1966). Shaikhmahamud & Magar (1965) tried boric acid, dipotassium hydrogen phosphate, sodium bisulphite, ascorbic acid,

citric-ascorbic acids, acronised, ferrimycin and penicillin for room temperature preservation of prawns. Tanikawa (1971) suggested the use of sodium nitrite, citric acid, ascorbic acid, butylated hydroxy anisole or butylated hydroxy toluene with ascorbic acid for preventing discolouration in frozen tuna meat and black spots in prawns. Sawant & Magar (1961) attempted to study the effect of chemical glazes such as sodium chloride, citric acid and sodium nitrite in frozen pomfrets, surmai and mackerel and found that the deleterious changes during frozen storage were slowed by these glazes. A dip in butylated hydroxy anisole (0.005%) for 15 minutes and subsequent storage in polythene lined gunny bag at -15 to -18°C was recommended to enhance the keeping quality and to prevent dehydration and discolouration in frozen pomfrets (Kamasastri et al. 1967). The polyphosphate and sodium citrate treatments reduced thaw drip and oxidative rancidity in frozen fish (Anon, 1963-64).

2.7.6 Changes during canning

Canning is that method of preservation of food where spoilage is averted by killing the microorganisms present by application of heat. Therefore canning can be called the process of heat sterilization of foods in hermetically sealed containers. Since sterilization implies complete destruction of all living organisms and since this condition

may not be attained in some processed foods, the term 'commercial sterilization' has been introduced in the canning industry. Commercially sterile cans may be defined as cans which have been so processed that the food under ordinary storage conditions, will neither spoil nor endanger the health of the consumer. This definition requires that the temperature applied should have been adequate for the destruction of clostridium botulinum spores (Hersom & Hulland, 1969). Exclusion of air after sterilization prevents any further contamination by organisms.

The important factors controlling the drained weight of canned prawn are concentration of brine used for blanching and blanching time. Other factors such as acidity of brine used for filling the can, volume of brine used, time of sterilization and time of cooling the blanched meat are also to some extent found responsible for fluctuations in drained weight (Varma et al. 1969).

Probable sources of contamination of raw blanched and processed meat at various stages of handling of prawns and methods for their rectification have been described by Choudhury et al. (1970) and Choudhury & Bose (1971) gave an account of the bacteriological spoilage of canned prawn and its methods of prevention. The prime factor of spoilage was post process contamination which accounted for 92.3%

of the defective cans, while under processing accounted for only 7.7% of the cans. Importance of bacteriological quality of water, ice and other materials with which prawn comes in direct or indirect contact has been emphasized (Choudhury & Bose, 1971).

According to Tarr & Bisset (1954) the brown discolouration which frequently occurs during the canning of certain white fleshed fish is due largely to maillard (Sugar-protein) types of reaction. The browning reaction is liable to occur whenever foods containing proteins or amino acids and reducing sugars are heated and are stored for long periods without refrigeration. The production of stale and otherwise unpleasant tastes, the varying degrees of brown discolouration, the loss of protein solubility leading to a deterioration in texture and the failure of foods to reconstitute properly are deleterious. The nutritional value of proteins has been seriously impaired by reaction with carbohydrates. Evidence is presented by Tarr (1954), which indicates that only the free and not the combined ribose in fish muscle takes part in maillard reations. About 5 times as much glucose as ribose is required to cause the same degree of browning in fish flesh.

Fresh silver and black pomfrets and hilsa were canned at fresh and iced conditions and the qualities of the canned products were studied by Venkataraman et al. (1970).

Under identical conditions a maximum quantity of cook drip and nitrogen contents were found to be lost in black pomfrets and minimum in hilsa.

The effect of canning and storage in the presence and absence of antioxidants such as nordihydro guairetic acid and ascorbic acid on the nutrients in black and white pomfrets and prawns was studied by Sawant & Magar (1961). Canning denatures the proteins; the amino acids contents are not adversely affected. On the other hand they observed significant loss in vitamins. On storage there is marked loss of nutrients and the extent of losses increases with rise in storage temperature. The antioxidants prevent discolouration of the canned product.

2.7.7 Changes in dried products

Sun drying of fish has been a traditional process of fish preservation in most of the countries of the world. The process, however, is inordinarly slow and gives a product lower in nutritional value and high percentage of moisture which decreases its storage life. Dehydrated foods are subjected to deteriorative reactions during storage resulting in discolouration, off flavours and changes in texture. It is generally agreed that these reactions are kept to a minimum if residual moisture is low and storage is in an inert atmosphere, preferably at a low temperature.

Nazir & Magar (1965) observed that dried Bombay ducks stored in tin containers kept better and longer than those stored in polythene bags. Hunt & Matheson (1958) studied the effect of dehydration on actomyosin in cod muscle. Actomyosin became insoluble in salt solution, the muscle fibres may or may not lose their power to contract, although about half the adenosine triphosphatase activity was not destroyed. Tarr & Gadd (1965) observed discolouration in freeze dried lingcod and sole muscle and stated that it was due to Maillard-sugar amino reactions. Toyomizu et al. (1963) suggested that oxidation of the oil was largely responsible for the brown discolouration of freeze dried dark muscle of horse mackerel.

2.8 Aim and scope of the present work

The above literature survey clearly indicated that the literature with regard to the processing parameters and consequent loss in quality of shellfishes like crab, mussel and clam are scanty and inadequate for application to processing establishments.

In this thesis all these aspects are taken into consideration. Extensive studies were conducted on all aspects of processing of crabs, mussels and clams. The species taken for studies are commercially used ones namely, Scylla serrata, Perna viridis and Villorita cyprinoids.

In Chapter 4.1 with regard to crab, the following aspects on their handling and processing are reported:- seasonal variation in chemical constituents, changes taking place during ice storage, freezing, canning etc.

In Chapter 4.2 with regard to mussel, the relation between age (size) and chemical constituents, changes taking place during ice storage, freezing, canning etc. are reported and in Chapter 4.3 the changes taking place in clam muscle during icing and freezing are reported and the amenability of ice stored clams for canning purpose is reported.

The interference of high concentration of glycogen in mussel and clam muscles during the colour development of ribose (Mejbaum's method) is observed and remedial steps are taken to minimise the interference.

Industrial processing of crabs, mussels and clams although taken widely in India, has not shown rapid strides as expected in terms of quantity inspite of heavy demands from overseas markets.

The results of the investigation proves that a number of parameters have to be considered and evaluated both at harvesting and subsequent post harvesting and processing of these valuable food commodity if it is to be an economically viable proposition industrially.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Crab

3.1.1.1 Sampling

Live crabs caught from the fishing grounds of Cochin were brought to the laboratory. They were washed thoroughly in chlorinated water to be free of adhering slime and dirt. Size of the samples varied from 12±1 cm length. For the determination of proximate composition and its changes in seasonal variation fully grown female samples were taken. For the studies on seasonal variations in proximate composition, samples were collected during full-moon and newmoon days of every month.

3.1.1.2 Extraction of meat

Crabs were deshelled, cleaned properly by removing

the gills and intestines. Muscle flakes were removed by cutting open the intermediate thin shells. Meat was also taken from the claws and legs by cutting open the thick shell and kept separately. The liquid content obtained from the claw (claw juice) was collected and analysed. The muscle was cooled to 0°C and minced well at that temperature. This minced muscle was taken for the different analysis.

3.1.1.3 Different samples of muscle (separated as above) was kept stored at 37°C, 25-28°C, 6.5-7.5°C and 0°C (out of contact with ice) and subjected to detailed analysis.

3.1.1.4 Ice storage studies

Live crabs were brought to the laboratory and washed well. They were kept in crushed ice (1:1). They were removed at different intervals of time and weight loss was determined. The meat was separated as described earlier and subjected to detailed analysis after mincing at 0°C. Fresh ice was added to the ice boxes as and when needed to maintain the ratio 1:1.

3.1.1.5 Freezing of crab meat

The crab meat separated as described earlier was packed in aluminium trays, covered with polythene paper

and the meat quick frozen in plate freezer at -40°C for 2½ hours with required quantity of water as glaze. The frozen blocks were taken out packed in thick paper cartons and kept stored at -23°C. At definite intervals of time samples were taken out from the frozen storage and subjected to detailed evaluation of quality by biochemical and organoleptic methods.

3.1.1.6 Addition of different chemicals on glaze water and its effect on quality

Different chemicals were added to water glazes to study its effect on quality of frozen crab meat. 1% solution of the following chemicals were used.

(a) ascorbic acid

(b) citric acid

(c) ascorbic acid, citric acid mixture (1:4)

(d) sodium bisulphite

(e) glycine

(f) disodium salt of ethylene diamine tetra acetic acid (EDTA)

Crab meat was frozen at -40°C with the glaze water containing above chemicals and storage studies conducted by keeping samples at -23°C as described above.

3.1.1.7 Effect of cooking of crab meat on quality

Crabs collected as above was cooked in steam at

atmospheric pressure (760 mm of Hg) at 100°C at different intervals of time to determine the optimum time required for cooking. This was evaluated by noting colour changes during cooking and organoleptic evaluation of the cooked crab meat by an expert team of taste panel members of the Institute

Cooked and raw crabs were stored in insulated ice boxes with ice (1:1) and at intervals equal quantities were removed from both series, the raw crabs were then cooked and immediately cooled. The meat was picked from both sets, quick frozen at -40°C with water as glaze and stored at -23°C. The frozen stored samples were drawn at intervals, thawed and analysed for physical, organoleptic and chemical characteristics.

3.1.1.8 A comparative study was also conducted between the cooked and raw crab meat packed in the following way.

a) with shellon

b) meat alone

c) claw with shellon

3.1.1.9 Canning

Blanching and sterilization conditions were carefully worked out and optimum requirements were standardised. The crab meat was packed in cans (301x206) and heat pro-

cessed at 15 pounds pressure for 30 minutes. The cans cooled immediately with potable water and subjected to detailed testing.

3.1.2 Mussel

3.1.2.1 Green mussels (Perna viridis) collected from the landing places of Calicut and from Central Marine Fisheries Mussel culture centre at Calicut were transported to laboratory in refrigerated Van or in iced condition. The material was cleaned properly free of mud and byssus thread.

3.1.2.2 Their size-weight measurements were taken, the shell was opened with a scalpel and meat separated and its yield was calculated on the basis of whole weight. The intestinal part was cut off and the muscle was cooled to 0°C and minced well. This minced muscle was used for the different analysis.

3.1.2.3 Nine size grades of mussels collected from mussel culture centre were used for the studies on relation between age (size) and chemical composition. Muscle was separated as described earlier.

3.1.2.4 Ice storage studies of Mussels

Live mussels collected from the landing centres of

Calicut were brought to laboratory in a refrigerated Van. It was kept in insulated ice box with crushed ice (1:1) after proper cleaning. They were removed at different intervals of time and weight loss was determined. The muscle was picked up as described earlier, intestines cut off and minced. This minced muscle was taken for different analysis.

3.1.2.5 Freezing of mussel meat

The mussel meat separated as described earlier was packed in Aluminium trays covered with polyethylene paper and quick frozen in a plate freezer at -40°C for 2½ hours with the required quantity of water as glaze. Frozen blocks were taken out, packed in thick cartons and kept stored at -23°C. At definite intervals of time samples were drawn from the frozen storage thawed at 0-4°C and subjected to detailed evaluation of quality by microbiological, biochemical and organoleptic methods.

3.1.2.6 Effect of cooking on quality of frozen mussel meat

Mussels collected as above was cooked in steam at atmospheric pressure at 100°C for different intervals of time to determine the optimum time required for cooking as judged by appearance and flavour.

Then half of the lot was cooked for 15 minutes and allowed to cool. This and the raw mussels were stored in insulated ice boxes with crushed ice (1:1). At definite intervals equal quantities were removed from both sets. The raw mussels were then cooked and immediately cooled. The meat was scooped out from both, intestines cut off, packed in aluminium trays covered with polyethylene paper, quick frozen at -40°C with water as glaze and stored at -23°C.

The frozen stored samples were drawn and analysed as above.

3.1.2.7 Canning of mussel meat

Canning procedure was standardised in order to get good quality canned mussel with minimum weight loss. The brine was usually cloudy due to higher concentrations of glycogen and colloidal proteins.

3.1.3 Clams

3.1.3.1 The most important variety of clams (Villorita cyprinoids) collected from Cochin backwaters was brought to laboratory in live condition. After thorough washing the physical measurements were taken and the shell was opened with a stainless steel knife and meat was scooped out and its yield calculated.

3.1.3.2 The meat scooped out from clams collected and handled as above was strained in a stainless steel strainer kept at 0°C for a definite interval of time collecting the free liquid. The intestinal part was cut off and minced properly. This minced meat and the clam liquor was subjected for detailed analysis.

3.1.3.3 The cleaned material was stored in insulated ice boxes with intermittant layers of ice (1:1). The samples were drawn at intervals, muscle was scooped out as described earlier and analysed. The experiment was continued till the material reached border line quality.

3.1.3.4 The muscle separated from the iced material was quick' frozen in a plate freezer at -40°C with sufficient water to form a thick glaze over the frozen block. The blocks were wrapped in polyethylene film and stored at -23°C. At intervals the blocks were drawn, samples were taken for bacteriological analysis, then thawed at 4°C in a cold room, meat and drip separated and their biochemical and organoleptic analysis were carried out.

3.1.3.5 Canning of clam meat

Canning procedure was standardised for preparing good quality canned clams. The brine was usually cloudy due to higher concentrations of glycogen and colloidal proteins.

3.2 Experiments

3.2.1 Size-weight measurements

3.2.1.1 In the case of crab the carapace length and breadth was measured using a scale. In mussels and clams height is the distance between the umbo and the ventral valve margin, length is the maximum distance between the anterior and posterior margin measured parallel with the hinge axis and width is the greatest distance between the outsides of the closed valves measured at right angles to the place of shell commissure.

Whole weight is the total weight of the animal. After removing the carapace in crabs, the orange red coloured eggs in the intestinal cavity was picked up and its weight determined. Crab body was cleaned free of gills, intestines and by cutting off the intermittant shells the meat was picked up and weighed. The claw shell was cut open with scissors, the liquor retained inside the cavity was collected, its volume and weights determined and the weight of the claw meat was determined.

3.2.1.2 In the case of mussels and clams, with the help of a stainless steel scalpel, the shells were detached and the muscle was picked up, intestinal portion was removed and the material was strained in a strainer for two minutes, then the meat weight and the volume of drained liquor

were
~~was~~ taken. An aliquot of the shell liquor was used for chemical analysis.

3.2.2 Moisture, protein, ash and lipids were estimated according to the procedure of the Association of Official analytical Chemists (1975).

3.2.3 Fractionation of protein

Protein fractions from the shellfishes were accomplished by the preferential solubility technique of Frederick J.King (1966) and Paul (1966). Potassium phosphate (KH_2PO_4) - sodium phosphate (Na_2HPO_4) buffer of μ -0.05 and pH 7.45 was used for extraction of sarcoplasmic proteins in all cases. All operations were done in a cold room maintained at 0-3°C. The myofibrillar proteins were extracted using KCl-NaH_2PO_4-Na_2HPO_4 buffer (μ -0.6, pH 7.5) at 0-3°C. For extraction of nucleoproteins 80% ethylalcohol was used and for extraction of inextractable denatured protein 0.1 N sodium hydroxide at room temperature (25-27°C) was used. Residue after these extractions was directly digested to estimate the stroma or connective tissue.

Minced muscle sample (about 10 gm) was taken in a conical flask, 100 ml phosphate buffer (μ -0.05) was added and shaken in an electric shaker for 15 minutes at

1-4°C. It was transferred to centrifuge tubes and centrifuged in a refrigerated centrifuge at 0°C at 5000 r.p.m. for 30 minutes. The supernatant was transferred to another conical flask, and the residue was taken in the original conical flask with another 100 ml phosphate buffer. It was shaken and centrifuged as before and the supernatant was transferred to the first extract. The residue was transferred to the original conical flask. It was extracted with 2x100 ml portions of Kcl-phosphate buffer (μ-0.6 pH 7.5) as before. The supernatant (myofibrillar fraction) after centrifuging was combined. The residue was shaken with 100 ml 80% ethylalcochol to extract the nucleo protein. The residue after centrifuging was treated with 200 ml 0.1 N sodium hydroxide to dissolve the denatured proteins in the original conical flask and kept at room temperature for one day. The solution was then centrifuged at room temperature at 8000 r.p.m. to effect a clear settling of the connective tissue. The solution was decanted and the residue was directly transferred to digestion flask. It was digested and its nitrogen content determined.

An aliquot sample from each extract (1st Sarcoplasmic protein, 2nd myofibrillar protein, 3rd nucelo-protein and 4th denatured protein) was digested and its nitrogen content determined.

For comparing this successive extraction technique with direct extraction 10 gm portions of the muscle from each shell fish sample was homogenised directly with 200 ml Kcl-phosphate buffer (μ-0.6, pH 7.5) in a waring blender - centrifuged and the protein nitrogen content was determined in the supernatant.

3.2.3.1 All samples were similarly extracted with Dyer's (1950) buffer (5% sodium chloride and 0.02 M sodium bicarbonate μ-0.874, pH 7.2), then precipitated the actomyosin fraction by dilution of the centrifugate 10 times with ice cold distilled water. The precipitate was dissolved in cold potassium chloride solution and its enzyme activity (adenosine triphosphatase) was determined as per Perry's (1952) method.

The assay of myosin adenosine triphosphatase (ATPase) is most simply carried out by estimating colorimetrically the inorganic phosphate liberated by the enzyme from ATP in the presence of Ca^{++} under specified conditions.

The incubation medium containing glycine buffer pH 9.1, 0.1 M $Cacl_2$, 0.05 M ATP and actomyosin in Kcl. The reaction was stopped by the addition of trichloracetic acid and the phosphorus liberated by myosin from ATP was determined by the method of Fiske Subbarow (1925).

Unit of activity was calculated based on the quantity of phosphorous liberated.

Total nitrogen in the muscle was determined on each sample by digesting one gram portion of the minced muscle with sulphuric acid and determining the nitrogen content. Total non-protein nitrogen was determined by precipitating all protein from an aqueous extract of the muscle (10 grams) by blending with 20 ml 20% TCA and filtering off the precipitate. The filtrate was made up to 250 ml and its nitrogen content was determined to calculate the total non-protein nitrogen in the muscle. Non-protein nitrogen of the sarcoplasmic protein extract was also separately determined each time in a similar way by precipitating the protein with TCA. Content of the different protein nitrogen fractions were then calculated and expressed as percentage of total protein nitrogen;and non-protein nitrogen fraction as percentage of total nitrogen.

3.2.4 Water extractable nitrogen (WEN)

10 grams of the minced muscle was blended with 200 ml distilled water in a waring blender for one minute, filtered, an aliquot of the filtrate was digested and its nitrogen content was determined.

3.2.5 Free alpha amino nitrogen was estimated by the

method of Pope & Stevens (1939) and the extraction of free amino acids has been made according to Jones (1959) and the determination of amino acids by the microbiological assay methods of Schockman (1963).

3.2.6 Trimethylamine (TMA) and total volatile nitrogen (TVN) were determined by microdiffusion method of Conway & Byren (1932). Glycogen was estimated by the method of Van de Kleiy (1951). Lactic acid was determined according to the procedure of Barker & Summerson (1941) and Ribose by the method of Mijbaum (1939). Phosphorus (total and inorganic) was estimated by the procedure of Fiske & Subbarow (1925).

3.2.7 The pigment colour of claw meat was not evident in the fresh condition, but when subjected to heat a characteristic pink colour was developed (characteristic colour of crustacean shellfishes). This pigment is soluble in chloroform or acetone. The extraction of pigments in body meat and claw meat of crab was effected using acetone and measuring the optical density at 470 mμ. (Roussean, Jr. 1960; Lusk et al. 1964; Stewart & James, 1970).

3.2.8 Sodium, potassium and calcium were estimated by flame photometry (Vogel, A.I, 1961).

3.2.9 Thawed yield and weight loss

The flesh of the thawed fish will consist of two phases:- the solid phase plus a fluid known as drip which is not reabsorbed by the flesh. The frozen block was kept in a strainer over a steel tray at 0-4°C, the drip separated on thawing was simultaneously collected in the tray. The weight of the thawed material and the volume and weight of the drip collected was determined and from the packed weight the percentage loss of weight of the frozen thawed material can be calculated. The drip was analysed for chemical constituents.

3.2.10 Organoleptic scoring

Organoleptic scoring was made on the fresh or thawed material after cooking in 2% sodium chloride solution for 10 minutes and the quality was judged by the panel members of the Institute who are regularly determining the fishery products for organoleptical scoring. Testing was done according to the official methods of the ASTM*.

1. Characteristic colour, smell, texture and flavour .. 10 marks or Good
2. Negligible loss of characteristic qualities .. 7-9 or Good - Fair
3. Appreciable loss of characteristic qualities but still in an acceptable condition .. 5-7 or Fair

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*Manual on Sensory testing Methods, ASTM E-18, of the American Society for testing Materials, STP 434, 1968.

4. Change of colour, hardening
of texture and loss of flavour .. 3-5 or F-P

5. Off colour and stale flavour .. 3-0 or poor

Samples scoring below 4 marks were regarded as unacceptable.

3.2.11 Sodium chloride in brine was estimated by official methods of AOAC (1975).

A known excess of standard silver nitrate was added to a measured volume of brine to precipitate all the chlorides as silver chloride and the excess silver nitrate was determined by titration against standard ammonium thiocynate solution using ferric alum as indicator.

3.2.12 Bacteriological examination of samples

About 10 gms of the sample was taken in a sterile petri dish by means of a sterile scissors. It was then put into a sterile mortar, sterile sand was added, thoroughly ground, added 100 ml of sterile sea water gradually and stirred the solution to get an uniformly concentrated sample. One ml of the supernatant solution was pipetted out into two sterile petri dishes and another one ml solution was added to nine ml sterile sea water which gave 1/1000 dilution (sterile pipettes were used). The samples were mixed properly by rotating the tubes between the palms of the hands. Using a fresh pipette transfer one ml of the 1/1000 solution to two sterile petri dishes

and to another 9 ml sea water tube. Likewise made dilution up to 1:1 million. Pipetted out one ml each from 1:10,000 dilution onwards to sterile petri dishes for total plate count. For coliforms and streptococci 1:100 and 1:1000 dilutions were used.

3.2.12.1 Total or standard plate count

Tryptone Glucose Extract Agar (Difco code No.B2) was used for the determination of total plate count. The petri dishes were incubated at 37°C for 48 hours. All the colonies were counted and calculated.

3.2.12.2 *E. coli*

Desoxycholate - Lactose Agar (Difco code No.B420) was used for enumerating coliforms and *E. coli*. Red colonies were counted as coliforms. Ten colonies from each of the selected plates were streaked into Eosine Methylyene Blue Agar (Difco Code No.B76) and the inoculated plates were incubated at 37°C for 24 hours. The organisms giving metallic sheen in each of the streaks were separately inoculated into Escherichia coli medium (Difco code No. B314) and the inoculated tubes were held in a water bath maintained at 44±0.5°C for 48 hours. Cultures producing gas in the medium were regarded as *E. coli* of faecal origin.

3.2.12.3 Faecal streptococci

KF agar of Kenner et al. (1961) was used for the enumeration of faecal streptococci. All the pink colonies were counted as faecal streptococci and calculated as the number of colonies per gm muscle.

3.2.13 Canning of crab meat (Scylla serrata)

Live crabs were used for the canning purpose. Washed the crabs properly free of mud. Put each one in boiling water for one minute in order to facilitate deshelling, then cooled. The claws and other legs cut off, the outer carapace removed and the body was cleaned free of gills and intestines.

The whole lot was blanched in 7% brine containing 0.1% citric acid for 7 minutes and cooled. The body meat and claw and leg meats were picked. The cans were packed with claw meat at the bottom and top and body meat in the middle portion (25:75%). The cans were filled with 2% brine containing 0.1% citric acid and exhausted for 2 minutes, seamed and sterilized at 10 lbs pressure for 45 minutes. Cooled, dried the outside and kept at room temperature.

3.2.13.1 Canning of mussel meat (Perna viridis)

The mussels were allowed to starve in clean water

for a day (for depuration). Then the water was chlorinated at 5 p.p.m. level and allowed to stand for another 2 hours. Boiled in open vat and shucked out the meat. The meat was blanched in 5% brine for 5 minutes, drained and cooled. The meat was packed in cans and filled with hot brine (containing 2% salt plus 0.1% citric acid). (Oil or tomato sauce can be substituted as filling medium). The cans were exhausted for 10 minutes in steam and seamed immediately and heat processed under 10 p.s.i. for 30 minutes. Cooled, washed and wiped the surface dry. Stored at room temperature.

3.2.13.2 Canning of clam meat (Villorita sp.)

The clams were allowed to starve in clean water for a day, then allowed to stand in chlorinated water (5 ppm) for another 2 hours.

The material was steamed with shell on for one minute, the meat was shucked out. Blanched the meat in 7% brine for 3 minutes, drained and cooled. The material was packed in cans and filled with hot brine (containing 2% salt plus 0.1% citric acid) (oil or tomato sauce can be substituted as filling medium). The cans were exhausted for 10 minutes in steam and seamed immediately. The cans were heat processed under 10 p.s.i. for 20 minutes, cooled, washed and wiped the surface dry. Stored at room temperature.

3.2.14 Estimation of ribose by Mijbaum's method

Heat treatment of the trichloroacetic acid (TCA) extract of clam and mussel meats with 1% orcinolin concentrated hydrochloric acid containing 0.1% ferric chloride, black particles are often formed. These particles are insoluble in organic solvent or hydrochloric acid, but soluble in concentrated nitric acid and sulphuric acid (charring occurs). The solution gives negative reaction for metals. It is found that orcinol, ferric chloride and hydrochloric acid are essential ingredients for the characteristic colour development of ribose. The precipitate was tested for inorganic salts or organic compounds (aliphatic or aromatic) and found out to be aliphatic aldehyde. The formation of the precipitate was suspected to be due to the presence of higher concentration of glycogen or colloidal protein in TCA extract of mussel and clam meats. A clear TCA extract was never obtained in the case of mussel or clam meats. In standard ribose solutions small quantities of albumin and glycogen were incorporated and it was heated with orcinol reagent. In both cases precipitate was obtained while heating, but in the glycogen incorporated sample the particle size was more or less same of that obtained in TCA extract of clam or mussel meat. The solubility characteristics of the precipitate was similar to that obtained in glycogen incorporated ribose solution.

According to Baruch Rosen (1966) basic zinc is a better protein precipitant in preference to the common acidic protein precipitants such as perchloric and trichloroacetic acid, because these latter reagents extract much glycogen from the tissue. So attempts were made to compare both methods for protein removal and found that a clear extract was obtained in the case of basic zinc, hence the problem of precipitation ~~was~~ did not occur~~red~~, but the extractability of ribose was below 50% of that with TCA.

Remedial steps were worked out to prevent the formation of black particles during colour development (heating) by:

(1) initial hydrolysis of glycogen in TCA extract using 2 N hydrochloric acid for 5 minutes then proceeding as per capital method.

(2) the colour development at 90°C (instead of boiling water) without hydrolysis. The standard also should be heat treated at 90°C.

In both cases the precipitation was prevented with 95±5% accuracy.

LIST OF MANUFACTURERS WHOSE CHEMICALS WERE USED

All reagents and chemicals used were of highest purity and purchased from the following companies.

1. B.D.H. (Glaxo laboratories, India, U.K.)
2. E. Merck, India
3. Sarabhai M. Chemicals, Baroda, India
4. E. Merck A.G., W. Germany
5. Sigma, Saint Louis, MISSOURI, U.S.A.

The grades used were Analar or Guaranteed quality (99.9% and above purity).

List of equipments

1. Spectronic 20 Spectrophotometer Colorimeter, Bosh and Lomb, U.S.A.
2. Evelyn photoelectric colorimeter, minneapolis - Honeywell Reg. Co., Rubicon Instruments, Philadelphia, U.S.A.
3. International High speed Refrigerated centrifuge (International Equipment Co., Needham, H.T.S. Mass, U.S.A.)
4. Ultracentrifuge (60,000 r.p.m.) Janetzki, Vac 601, East Germany.
5. Atomic absorption Spectrophotometer model - Varian, U.S.A.
6. Flame Photometer (Systronics, India).

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

4.1 CRAB (*Scylla serrata*)

4.1.1 Morphometric and weight measurements

Tables 1a, b, & C give the size weight measurements, the ratio of breadth and length and whole weight and meat weight of 61 crabs collected from Cochin backwaters. It is known that the animal grows by successive moults and time lag between moulting is less in the earlier stages of growth. The ratio of length Vs breadth is between 1.27 and 1.45 and meat weight Vs whole weight is between 0.104 and 0.314. The data was subjected to statistical analysis. According to Marlin E.Tagatz (1965) the length-width ratio decreased as the animals grew. George H.Rees (1963) reported that the number of times that a crab moults during its life time, and the length of time between moults, varies among species and is affected by such factors as temperature and amount of food available.

Table 1a Size weight ratio of crab (Scylla serrata) (1978)

No.	Month	Sex	Length cm	Breadth cm	Ratio	Whole wt. g	Meat wt. g	Ratio
1	June	M	16.5	11.6	1.41	484	155	0.3143
2	Dec.	F	10.5	7.2	1.45	147	30	0.2040
		F	10.3	7.4	1.39	140	23	0.1640
		F	11.2	8.1	1.38	189	38	0.2010
3	Feb.	F	19.0	14.0	1.35	479.5	115	0.2400
4	Mar.	F	11.4	8.7	1.31	212.0	38	0.1790
		M	10.6	7.7	1.37	151	33	0.2100
5	Apr.	F	10.2	7.5	1.36	159	26.8	0.1688
		F	10.5	8.5	1.23	179	28.5	0.1592
6	May	M	11.7	8.7	1.34	215	47.5	0.2210
		M	11.9	8.7	1.36	220	54	0.2455
7	June	F	12.0	8.3	1.44	219	59	0.2695
		F	10.1	7.8	1.29	147	28	0.1905
8	July	F	12.0	9.0	1.33	243	64	0.2634
		M	11.7	8.5	1.37	212	60.5	0.2854
9	Aug.	F	12.5	8.7	1.43	215	55	0.2550
		F	13.2	9.4	1.40	253	54	0.2130
10	Aug.	F	11.5	8.6	1.33	202	43	0.2140
		F	11.6	8.5	1.36	207	59	0.2850

Number of samples analysed 61.

Table 1b Size weight ratio of crab (Scylla serrata) (1978)

No.	Month	Sex	Length cm	Breadth cm	Ratio	Whole wt. g	Meat wt. g	Ratio
11	Sept.	F	13.5	10.0	1.35	355	66	0.1860
		F	14.0	10.5	1.33	396	72	0.1810
12	Sept.	F	12.0	8.4	1.42	218	61	0.2790
		F	11.2	8.1	1.38	201	41	0.2030
13	Oct.	F	11.5	8.3	1.38	182	39.5	0.2170
		F	11.5	8.4	1.37	184.5	38.5	0.2080
14	Oct.	F	10.3	7.6	1.35	146	27	0.1840
		F	10.1	7.8	1.29	143	21	0.1460
15	Nov.	F	8.8	6.5	1.35	96.5	21	0.2176
		F	10.5	7.6	1.38	139	27	0.1943
		F	9.5	6.9	1.37	101	22	0.2178
16	Dec.	F	10.7	7.8	1.37	169	34	0.2012
		F	10.6	8.2	1.29	174	34	0.1954
		F	10.0	7.7	1.30	134	24	0.1791
17	May	F	11.5	8.3	1.38	186	36	0.1936
		F	12.1	9.0	1.34	233.5	51	0.2184
18	June	F	11.55	8.50	1.35	198	50	0.2525
		F	10.54	7.74	1.36	154	37	0.2403
19	July	F	10.9	8.3	1.31	189	37	0.1957
		F	10.1	7.6	1.33	142	42	0.2957

No. of samples analysed 61.

Table 1e Size weight ratio of crab (Scylla serrata) (1978)

No.	Month	Sex	Length cm	Breadth cm	Ratio	Whole wt. g	Meat wt. g	Ratio
20	Dec.	F	13.0	9.4	1.383	273	84	0.3077
		F	10.3	7.7	1.338	152	36	0.2369
		F	9.7	7.3	1.329	120	28	0.2333
21	Dec.	F	12.5	9.1	1.374	253	63	0.2490
		F	11.9	8.3	1.433	192	41	0.2135
		F	10.7	8.4	1.274	171	30	0.1754
22	Jan.	F	10.2	7.9	1.291	156	27	0.1731
		F	10.8	8.1	1.333	186	34	0.1828
		F	9.8	7.3	1.343	137	24	0.1752
23	Jan.	F	10.9	7.7	1.416	171.5	34.5	0.2012
		F	10.7	8.0	1.328	173	30.5	0.1783
		F	10.9	8.1	1.346	183.5	34	0.1852
24	Feb.	F	11.9	8.1	1.469	295	42	0.1423
		F	11.1	8.0	1.383	252	39	0.1547
		F	10.9	7.5	1.453	259	36	0.1390
25	Mar.	F	9.5	7.0	1.357	166	24	0.1445
		F	10.4	7.2	1.444	206	27	0.1311
		F	9.7	7.5	1.293	135	22	0.1629
		F	8.9	6.6	1.349	93	19.5	0.2090
26	Apr.	F	10.3	7.7	1.338	250.5	26	0.1040
		F	10.8	8.0	1.350	165.5	36	0.2175
		F	10.2	7.6	1.342	143.5	27	0.1882

Number of samples analysed 61.

The study of length-weight relationship is an important tool in fishery biology and according to Le Creen (1951) it is pursued with two objectives, namely, (1) to establish a mathematical relationship between two variables namely, to the length and the weight,. so that if one is known the other could be computed and (2) to know whether variations from the expected weight for the known length groups are indication of fatness, general "well being", gonad development and suitability of environment. Dawan et al. (1976) studied the length weight relationship in Portunus pelagious (crab) from Zuary estuary and obtained exponent values above 3 for both the sexes. In the study of Varikul et al. (1972) on Scylla serrata (crab) from Thailand, the exponent values were 2.1377 for males and 1.6619 for females. Hamai (1934) found that the growth of Meretrix meretrix at different localities and also under different substrata is influenced by temperature, salinity and other parameters of the environment.

Statistical analysis on crab experiments

Sixty one observations each on length, breadth, whole weight, meat weight and yield of crabs were subjected to statistical analysis. The crabs selected for the study ranged between 8.8 and 16.5 cm in length. The length measurements were found to have +ve and highly significant

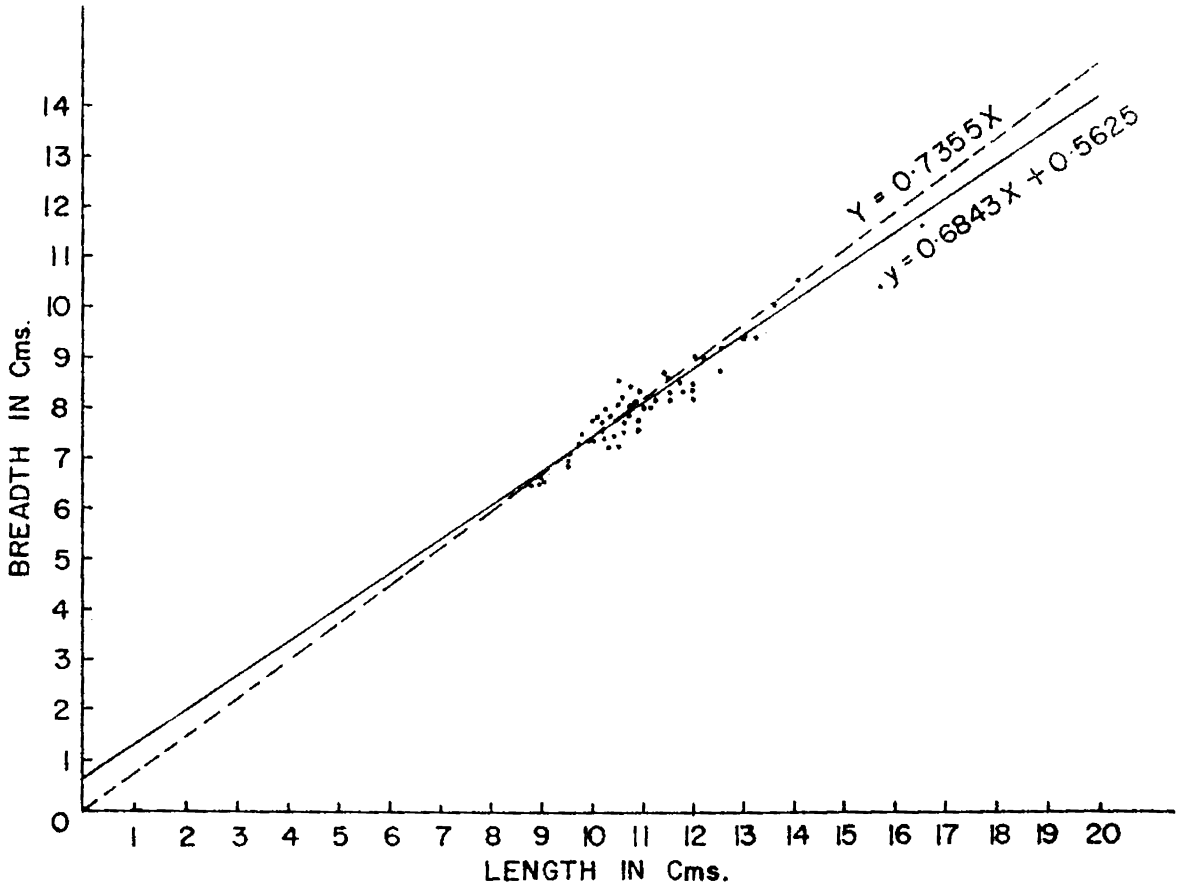


Fig. 1. REGRESSION OF BREADTH OF CRAB ON LENGTH

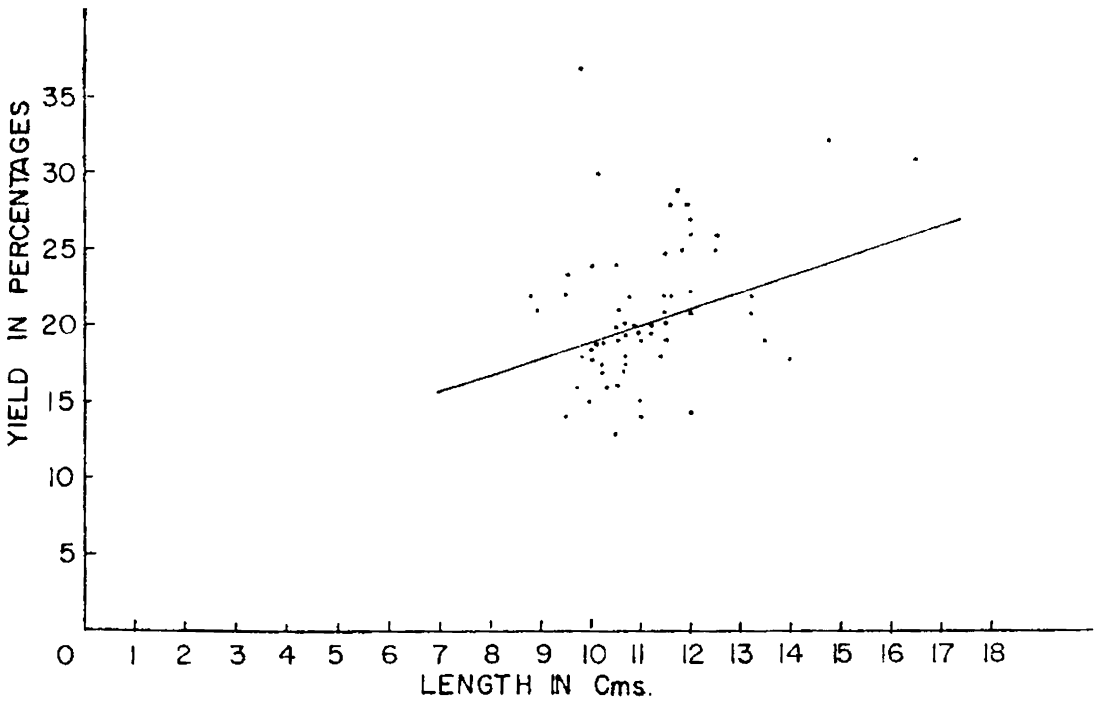


Fig. 2. REGRESSION OF YIELD OF CRABS ON LENGTH

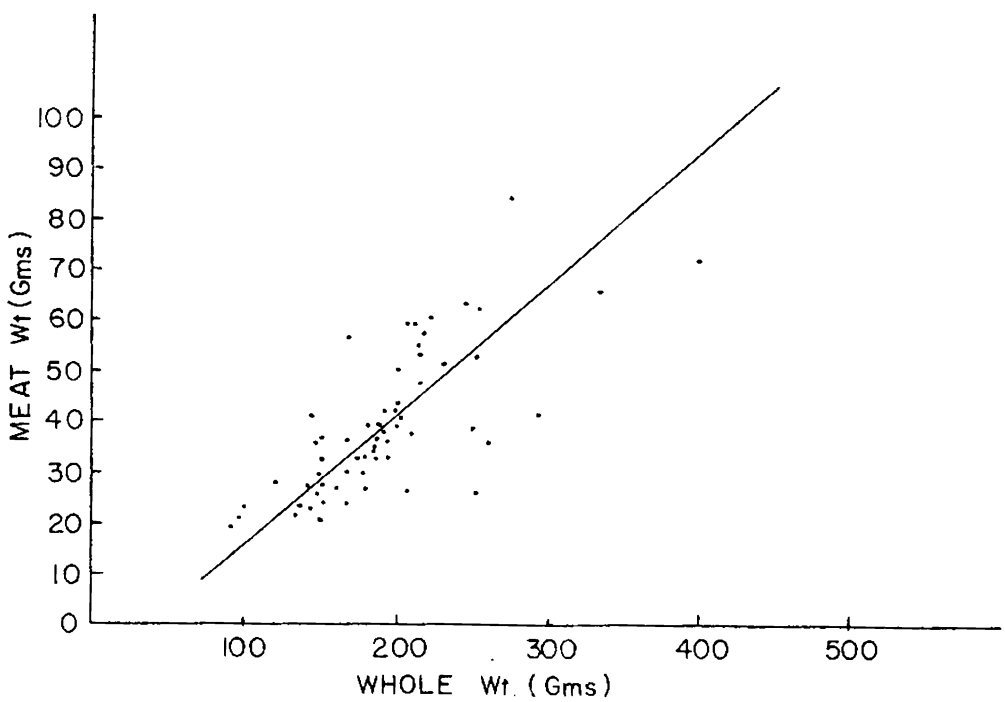


Fig. 3 REGRESSION OF MEAT WEIGHT OF CRABS ON WHOLE WEIGHT.

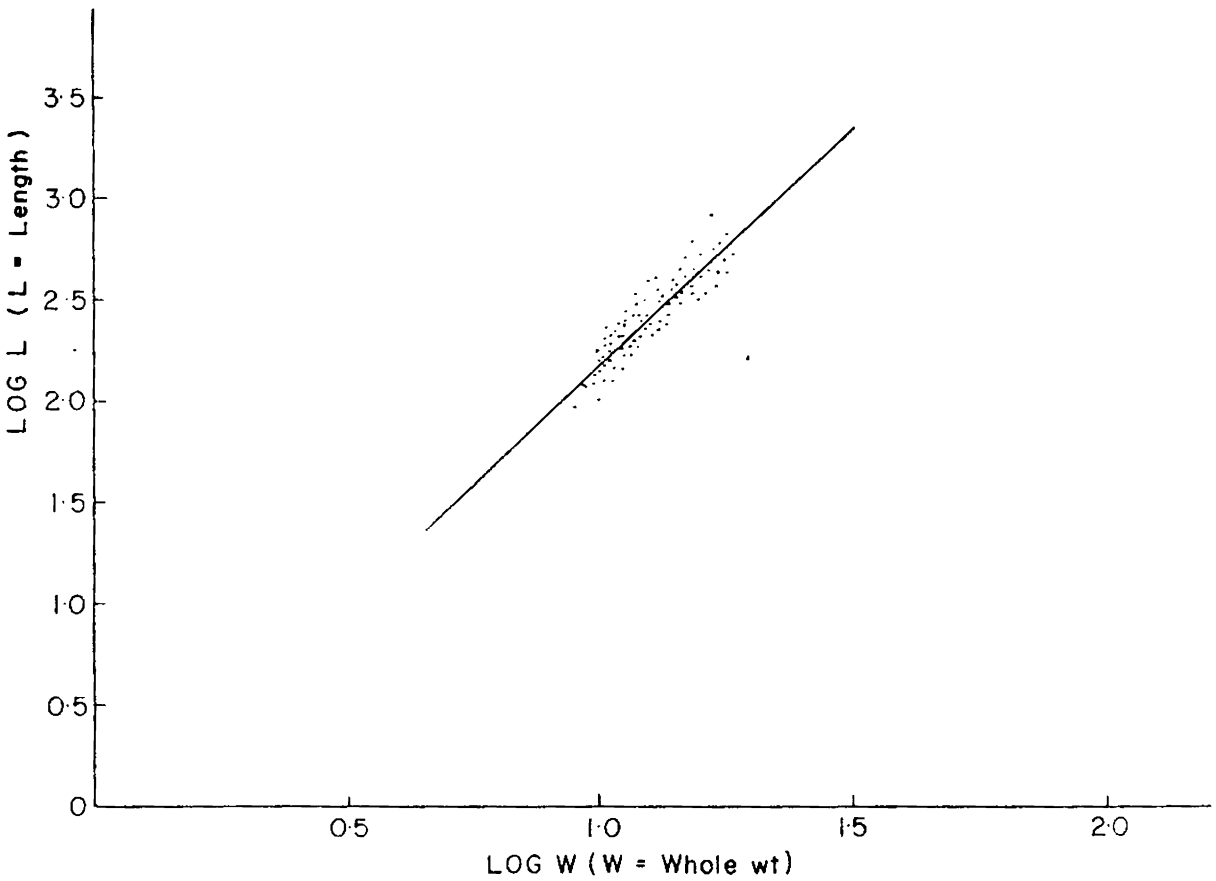


Fig. 4. REGRESSION OF WHOLE WT ON LENGTH (CRAB)

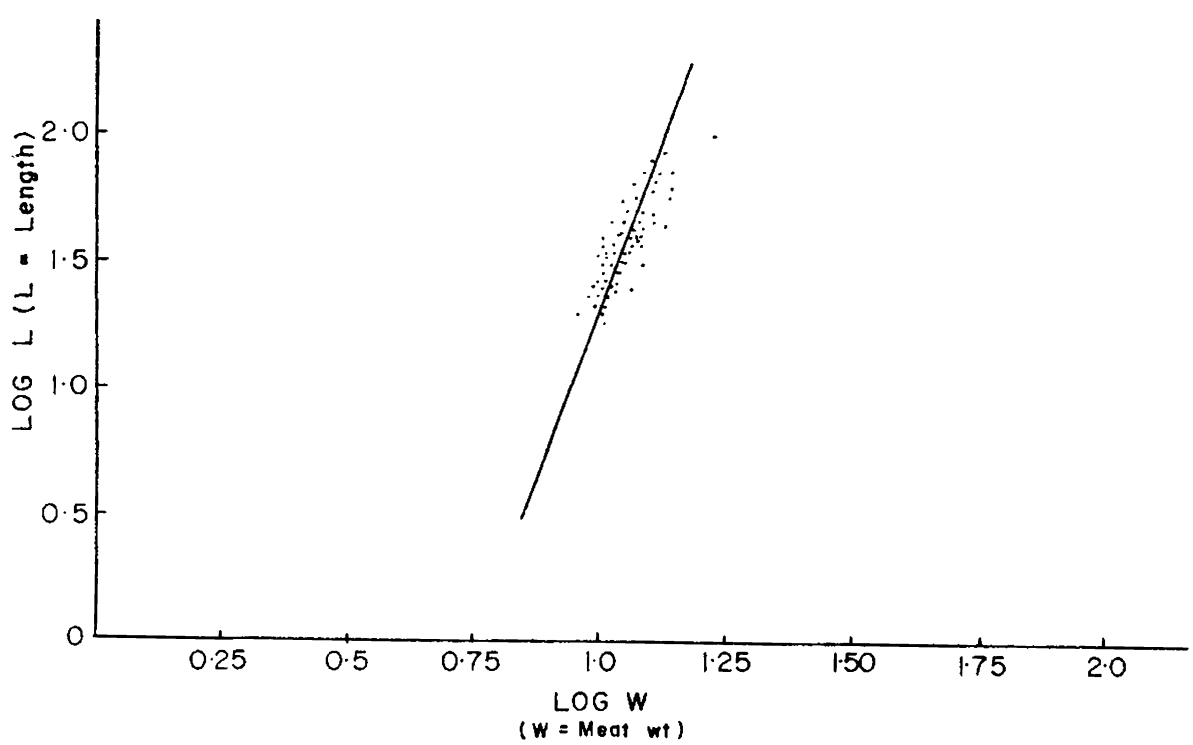


Fig. 5. REGRESSION OF MEAT WT. OF CRAB ON LENGTH

($P \leq 0.001$) correlation with other measured variables. The values of r, the correlation coefficient between pair of variables are given below:

	r
Length and breadth	0.9704**
Length and whole weight	0.9112**
Length and meat weight	0.9200**
Length and yield	0.4125**
Whole weight and meat weight	0.8740**

** highly significant, $P \leq 0.001$

Treating the length of crab as independent variable (x), linear regression equations for dependent variable (y) were formed.

These equations are

$$y = 0.6843X + 0.5625, \text{ y is the breadth cm} \quad (1)$$

$$y = 0.1134X + 0.0792, \text{ y is the yield ratio} \quad (2)$$

For whole weight x (in gms) we have

$$y = 0.2599X - 9.8338, \text{ y is the meat weight (gms)} \quad (3)$$

These regression lines are presented in Figs. 1,2 & 3 respectively. From the figures it is clear that the regression line fit very well with the plotted points in respect of length, breadth and whole weight-meat weight relations.

As regards to the length - yield relation, the correlation is 0.4125 and is highly significant. But the regression line (Fig.2) does not appear to have much linearity since the plotted points are scattered. Moreover, only about 17% of the total variation is explained by the regression, though the correlation is highly significant. Similarly, regarding the length - breadth relationship (Fig.1) suggests that they are directly proportional. Allowing some sampling fluctuations, we can safely modify the regression equation as

$$Y = BX; \text{ when } B = \frac{Y}{X}$$

Thus the regression equation becomes

$Y = 0.7355X$ which means that the breadth is about 73.6% of that of the length of the crab. From the above fitted regression equations, for any given value of X, the corresponding Y value can be obtained.

As regards to the length-weight relationship, they are connected by the hypothetical law

$$W = al^n \text{ or}$$

$\log W = \log a + n \log L$; where W is the weight and L is the length.

This is a linear relationship of the form

$$Y = a + bx$$

If the length (cm) is known, the whole weight (gm) can be computed by the equation,

$$\log W = -0.1586 + 2.3300 \text{ Log } L \quad (4)$$

Similarly for any given length (cm), the meat weight (gm) can be found by the relation,

$$\log W = -1.5745 + 3.0148 \log L \quad (5)$$

The goodness of fit of the regression are shown in figures 4 & 5 respectively.

4.1.2 Studies on biochemical aspects of body meat and claw meat of crab and claw liquor

Table 2 gives an account of the proximate composition of the body meat of crab picked from female crabs of same size group with one having egg (1) and other without egg (2). Meat content in sample one is only 21.9% while in the other 26.4% yield was obtained. This difference in yield was due to the presence of egg in the first sample. Protein content is on the higher side in crabs (19.16 - 20.92%) and high percentages of free alpha amino nitrogen and phosphorus were observed and glycogen and fat contents were extremely low. In all the indices studied distinct difference was noticed between samples with egg and without egg, lesser values in crabs carrying egg. This may be due to the fact that nutrients were utilised for egg formation.

Table 2 Proximate composition of crab (Scylla serrata) meat (1978)

Chemical constituents	With egg	Without egg
1. Moisture %	78.020	77.200
2. Size ratio	1.385	1.316
3. Meat weight %	21.940	26.460
4. Egg %	12.300	Nil
5. Protein (TN x 6.25) %	19.160	20.920
6. Water extractable nitrogen mg %	1387	1666
7. Non Protein nitrogen mg %	896	812
8. Free alpha amino nitrogen mg %	378	291.3
9. Phosphrous (in) mg %	180	160
10. Fat g %	0.43	0.70
11. Glycogen mg %	870	1345

TN = Total nitrogen

Note: All samples belonged to same size group and were females

2. Results obtained relate to average values of 10 samples of crab.

3. Values are the average of 3 estimations.

Crab meat is assuming great importance in human diet owing to its superior nutritional quality and easy digestibility. Radhakrishnan & Natarajan (1979) had reported the proximate composition (water, protein, fat and carbohydrate) in different size groups of crab - Podophthalmus vigil. The presence of free amino acids in prawns and other crustaceans in concentrations about ten times as high as that in fishes has been reported by Velankar & Govindan (1958). The free amino acids contribute to the flavour of the meat and probably serve as substrate for bacteria in the initial stages of spoilage.

Water, protein, fat and carbohydrates are the main constituents of fish and shellfish with non-protein nitrogenous constituents and salts in small measures.

Tables 3 (a-d) represent the results of biochemical studies of body meat and claw meat of crab and claw liquor. Body meat and claw meat of crab differ in flavour, proximate composition, protein fractions, minerals and free amino acids. Yield is more in claw (42-47.3%) compared to crab body (23.6-36.0%). Water holding capacity is more in claw meat and much variation is not observed in protein, lipid or ash contents in both. Body meat gives higher values for glycogen and phosphorus but the pentose sugar ribose content is slightly higher in claw meat than the body meat. The

Table 3a Composition of body meat and claw meat of crab (Scylla serrata)

	Body meat	Claw meat
1. Meat content g %	23.6-36.0	42.0-47.3
2. Moisture g %	80.19	82.94
3. Protein TN x 6.25 g %	16.80	16.28
4. Fat g %	1.07	1.00
5. Ash DWB g %	5.09	5.11
6. Acid insoluble ash DWB g %	0.18	0.06
7. Glycogen mg %	656	496
8a. Phosphorus total mg %	411	384
8b. Phosphorus (in) mg %	184	131
9. Ribose mg %	168	176
10. Potassium mg %	207.6	180.2
11. Sodium mg %	390.4	515.3
12. Calcium mg %	165.7	161.1
13. Pigments O.D. of acetone extract at 470nm	0.10	0.59

Note: 1. Results obtained relate to average values of a composite sample from 10 crabs.

2. Values are average of 3 estimations.

Table 3b Composition of claw juice

Yield on the basis of whole weight	42 - 47.3 %
Juice volume	20.8 ml % on the basis of whole weight or 41.7 ml % on meat wt. basis
Protein	1.634 g for 100 g meat
Alpha amino nitrogen	46.7 mg for 100 g meat
Glycogen	3.84 mg for 100 g meat
Phosphorus (in)	9.0 mg for 100 g meat
Ribose	11.0 mg for 100 g meat

Table 3c Protein fractions in body meat and claw meat of crab

Protein fractions	Body meat	Claw meat
1. Total nitrogen g %	2.688	2.680
2. Soluble protein in Dyer's buffer as percent of TN	92.70	90.60
3. Non-protein nitrogen as percent of TN	27.34	26.71
4. Sarcoplasmic protein as percent of protein	33.33	28.22
5. Myofibrillar protein as percent of protein	56.63	58.98
6. Nucleoprotein as percent of protein	2.55	1.87
7. Denatured protein as percent of protein	7.00	10.60
8. Stroma or connective tissue as percent of protein	0.39	0.21

Note: 1. Results obtained relate to average values of a composite sample from 10 crabs.

2. Values are average of 3 estimations.

Table 3d Amino acids in crab (Scylla serrata)

Free alpha amino nitrogen (by Pope & Stevens method)	Body meat 283.5 mg %	Claw meat 275.8 mg %
1. Aspartic acid	3.09 %	0.774 %
2. Threonine	0.849 %	0.820 %
3. Serine	0.427 %	0.409 %
4. Glutamic acid	0.724 %	0.469 %
5. Proline	0.452 %	
6. Glycine + Alanine	82.416 %	40.98 + 11.64 %
7. Cystine	0.679 %	0.348 %
8. Valine	1.086 %	5.06 %
9. Methionine	0.335 %	1.82 %
10. Isoleucine	0.4596 %	1.36 %
11. Leucine	1.03 %	3.59 %
12. Phenylalanine	0.554 %	3.415 %
13. Tyrosine	0.253 %	1.475 %
14. Histidine	2.282 %	4.335 %
15. Lysine	4.12 %	19.12 %
16. Arginine	1.20 %	0.32 %
17. Tryptophan	0.17 %	0.198 %

Note: 1. Results obtained relate to average values of a composite sample from 10 crabs.

2. Values are average of 3 estimations.

minerals potassium and calcium contents are slightly higher in body meat but sodium content is extremely higher in claw meat.

The shell cavity of claw is filled with muscle and free liquid. It is a characteristic feature of all shellfishes to retain some liquid in their body to survive unfavourable conditions caused by floods or by the temporary presence of toxic or irritating substances in the water. Various metabolic products accumulate in the shell liquor and also mucous and blood cells.

Table 3(b) gives the analytical data of claw shell liquor. About 21% of the whole weight of claw is liquid. 1.63 gm protein, 46.7 mg alpha amino nitrogen 3.34 mg glycogen, 9.0 mg phosphorus (in) and 11.00 mg ribose on the basis of 100 gm muscle are present in claw liquor.

Table 3(c) represents the protein fractions of body meat and claw meat of crab. The extractability of nitrogenous constituents in Dyer's buffer are 92.70 and 90.60% and non-protein nitrogen 27.34 and 26.71% respectively in body meat and claw meat. Sarcoplasmic protein content is more in body meat (33.33%) but the myofibrillar protein in claw meat (58.98%). Nucleoprotein and connective tissue are slightly higher in body meat. The denatured protein

content is more in claw meat (10.60%). This gives an indication of the denaturation of the native sarcoplasmic or myofibrillar proteins occurred during the extraction process.

Table 3(d) gives the free amino acid pattern of body meat and claw meat of crab. In both body meat and claw meat glycine-alanine constitute the major amino acid pool (82.4 and 52.5% respectively). Other major amino acids in body meat are lysine (4.1%) aspartic acid (3.1%) and histidine (2.28%). In claw meat lysine concentration is 19.1%, valine 5.0%, histidine 4.3%, leucine and phenylalanine 3.5% each.

Both body meat and claw meat contain all the essential amino acids recommended by FAO/WHO (1973). The peculiar flavour characteristics in both can be attributed to the differences in the amino acid pattern, sugar content and mineral salts. Body meat is more juicy and sweet probably due to the presence of more glycine, glycogen and sarcoplasmic proteins. The granular texture in claw meat is due to the presence of more myofibrillar proteins in it. The higher concentration of sodium in claw meat also support the peculiar salty taste in that.

The abnormal concentration of pigments in claw meat is due to the thick membrane covering the muscle inside the claw shell, on the other hand the body meat is not fully

covered with membranes but packed in between thin shells.

The lesser quantity of connective tissue in both muscles shows the easy digestibility of the muscle.

4.1.3 Seasonal variation in chemical constituents in crab (*Scylla serrata*)

There is indication that fish and shell fish undergo changes in chemical characteristics according to season. Tables 4(a,b,c) give the results of the studies on seasonal changes in the chemical characteristics of one of the important commercial species of crab (*Scylla serrata*) caught from Indian waters.

To exclude the variations in composition due to difference in size, sex, maturity, fishing grounds and the stage of rigor mortis, only fully grown live female crabs from the same fishing ground and of nearly identical size (12 ± 1 cm length) were used. They were collected on new moon and full moon days of every month, dissected after taking the size-weight measurements. In almost every month of the year egg was noticed and meat content depended upon the quantity of egg present (Table 4c).

Fig.6 represents the percentage of moisture and protein contents during full moon time. Maximum moisture (83.1%) and minimum protein (14.0%) contents are recorded

Table 4a Changes due to the seasonal variation in the biochemical properties of crab (Scylla serrata) (1979)

Month	N.M.	Size ratio	Weight ratio	Egg	Moisture	Protein WWB	Protein DWB	Water soluble nitrogen	Non-protein nitrogen	Free amino nitrogen	Glycogen	Fat DWB	Fat WWB
				%	%	%	%	%	%	mg%	%	%	%
Jan.	"	1.35	0.18	20.89	77.67	19.17	83.43	1.53	0.63	388.6	0.84	0.68	0.153
Feb.	"	1.41	0.14	31.63	76.32	18.81	79.43	1.50	0.910	342.1	2.08	0.80	0.189
Mar.	"	1.41	0.19	-	77.11	20.37	89.00	1.53	0.917	328.9	0.56	1.25	0.286
Apr.	"	1.33	0.17	9.83	77.52	19.18	80.46	1.43	0.858	332.5	1.354	0.81	0.182
May	"	1.34	0.23	-	77.75	17.76	79.82	1.37	0.723	347.2	1.199	1.18	0.262
June	"	1.31	0.23	-	74.16	19.93	76.86	1.28	-	219.5	1.826	0.86	0.219
July	"	1.40	0.27	-	75.71	20.03	70.71	1.50	0.778	242.5	1.703	1.59	0.385
Aug.	"	1.34	0.24	25.00	78.07	18.23	78.70	1.37	0.724	284.5	1.383	1.13	0.247
Sept.	"	1.33	0.18	20.00	75.28	20.43	74.91	1.41	0.710	256.2	1.384	1.28	0.316
Oct.	"	1.35	0.21	21.00	80.42	18.17	92.81	1.31	0.724	278.4	1.499	0.86	0.169
Nov.	"	1.34	0.27	17.46	77.72	20.07	83.83	1.52	0.796	315.1	2.382	1.20	0.266
Dec.	"	1.35	0.19	21.60	74.82	20.91	81.17	1.77	0.747	293.6	1.444	1.23	0.318

N.M. = New moon

Note: 1. No. of crab samples taken every month of size (same) varied from 8 to 12

2. Results represent average of two estimations.

Table 4 b Changes due to seasonal variation in the biochemical properties of crab (Scylla serrata)

Month	F.M.	Size ratio	Weight ratio	Egg %	Moisture %	Protein WWB %	Protein DWB %	Water soluble nitrogen %	Non protein nitrogen %	Free amino nitrogen mg %	Glycogen %	Fat DWB	Fat WWB
Jan.	"	1.32	0.17	21.71	74.24	20.71	81.37	1.64	0.892	357.1	2.97	0.774	0.199
Feb.	"	1.35	0.24	-	81.87	-	-	1.16	0.928	362.7	0.56	-	-
Mar.	"	1.36	0.16	27.67	75.93	20.64	81.55	1.54	0.980	388.6	1.48	0.797	0.192
Apr.	"	1.29	0.16	-	73.98	21.22	81.58	-	0.489	330.5	-	1.156	0.300
May	"	1.36	0.20	-	83.16	14.05	73.82	1.04	0.649	240.5	0.25	1.963	0.331
June	"	1.35	0.24	11.04	81.06	15.63	79.5	1.13	-	279.6	0.24	1.976	0.374
July	"	1.32	0.24	24.22	78.70	17.64	79.29	1.44	0.727	198.2	0.92	1.166	0.248
Aug.	"	1.41	0.23	-	80.46	16.09	82.33	1.23	-	258.0	1.26	1.455	0.284
Sept.	"	1.40	0.24	18.00	77.45	18.67	77.69	1.76	0.728	254.9	1.48	1.138	0.257
Oct.	"	1.32	0.16	17.30	76.25	19.64	79.49	1.49	0.823	362.1	1.16	2.846	0.676
Nov.	"	1.36	0.21	20.72	76.50	19.63	77.16	1.44	0.788	323.0	1.76	1.824	0.429
Dec.	"	1.40	0.19	-	74.37	22.74	88.74	1.65	0.879	257.1	1.35	1.017	0.261
Dec.	"	1.36	0.21	19.88	76.43	22.31	84.50	1.72	0.910	351.8	1.97	0.993	0.234

F.M. = Full moon

Note: For details refer table 4(a)

Table 4c Relation between egg and meat content in Crab

Length (cm)	Egg %	Meat %
9.5	Nil	21.78
9.5	32.53	14.45
10.1	Nil	29.57
10.1	20.0	18.40
11.5	Nil	25.25
11.5	12.3	21.94

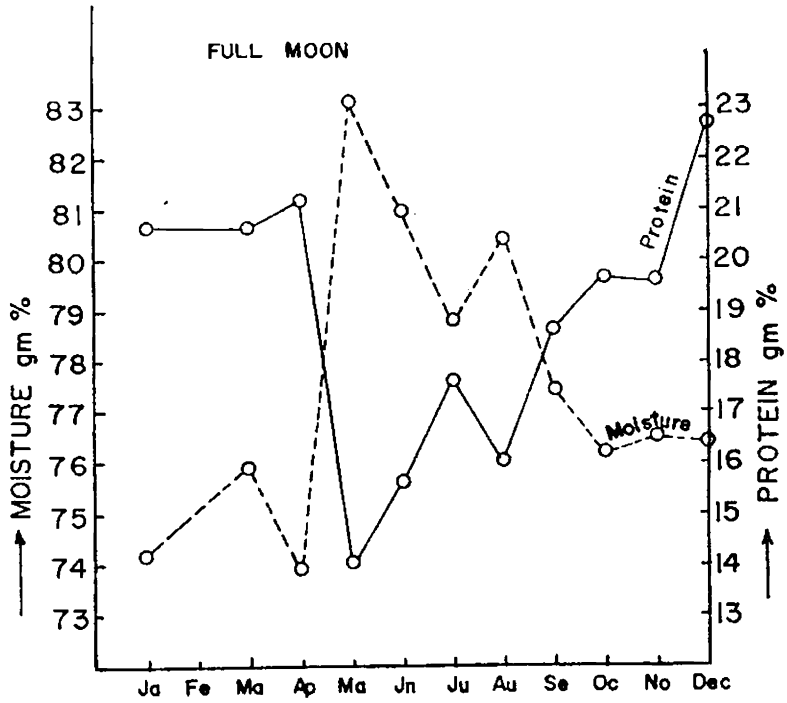


Fig. 6. SEASONAL VARIATION IN MOISTURE AND PROTEIN IN CRAB

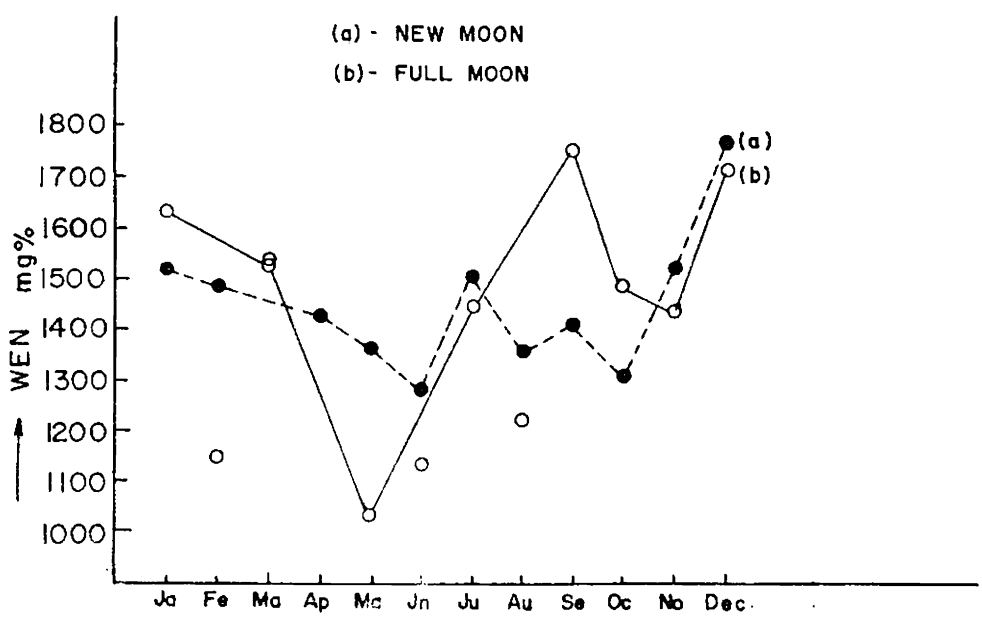


Fig. 7. SEASONAL VARIATION IN WATER EXTRACTABLE NITROGEN IN CRAB

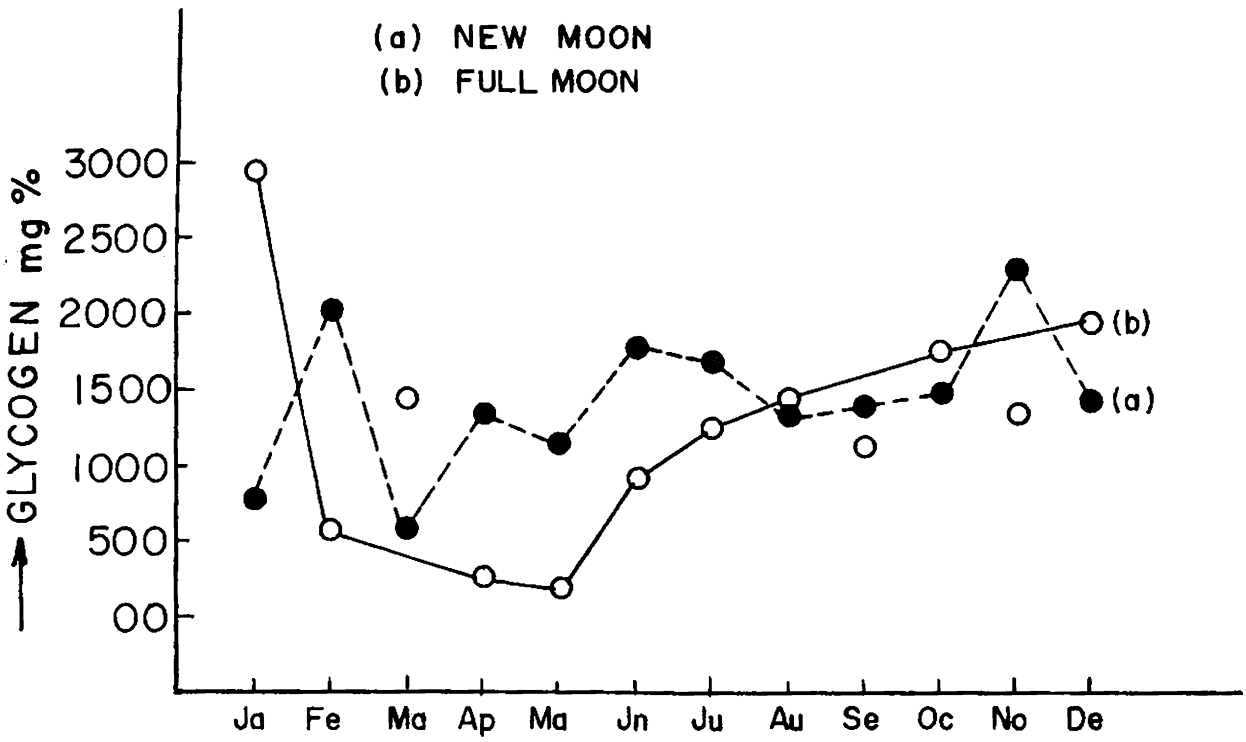


Fig. 10. SEASONAL VARIATION IN GLYCOGEN OF CRAB

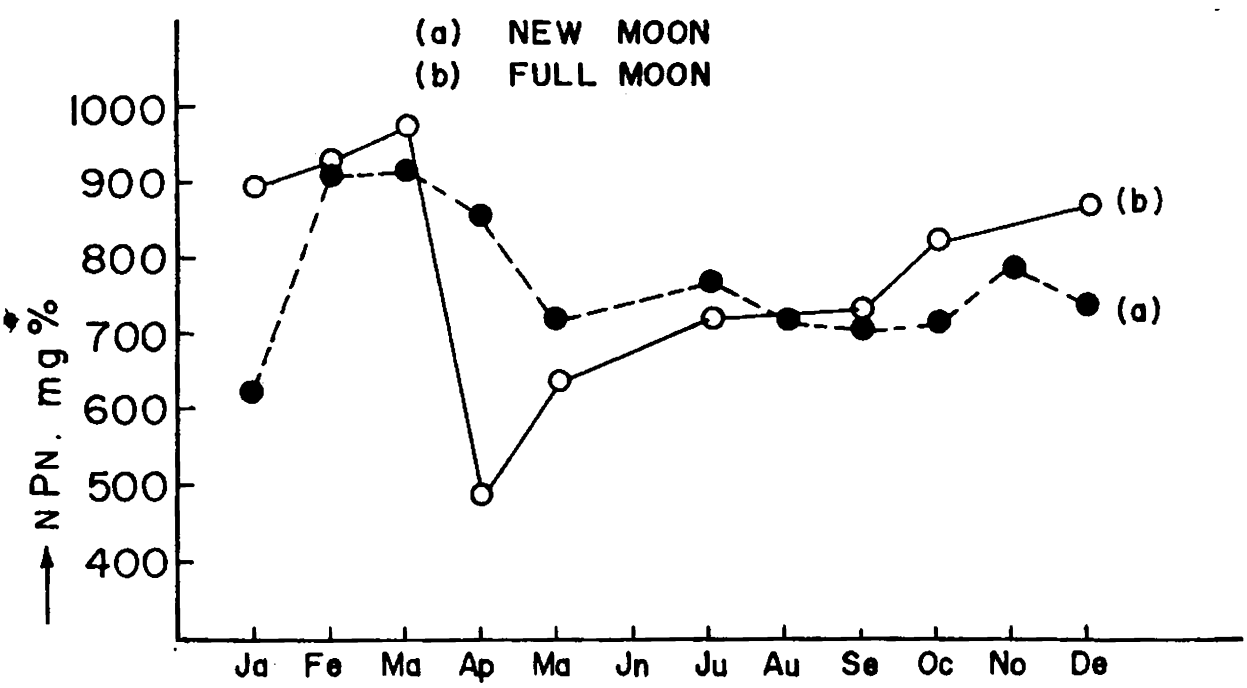


Fig. 8. SEASONAL VARIATION IN NON-PROTEIN NITROGEN IN CRAB

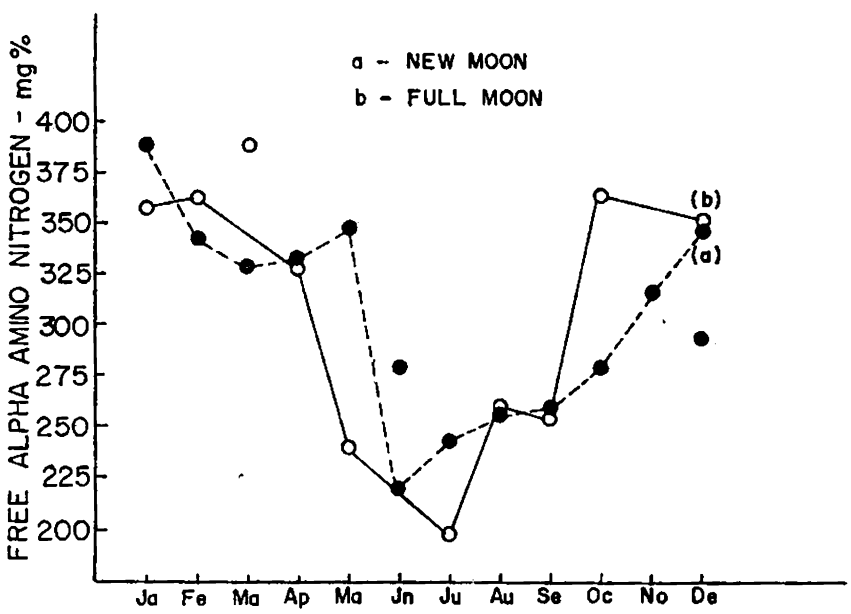


Fig. 9. SEASONAL VARIATION IN ALPHA AMINO NITROGEN IN CRAB (Scylla Serrata)

in the month of May and minimum moisture and maximum protein are recorded during October to April. Galtsoff (1964) while studying the seasonal variation in American oyster, observed a decrease in protein content in May and an increase of solids from October to March corresponding to a fall in water contents. Dambergs (1964) reported that the seasonal variations in water of cod fillets are opposite to those for protein. According to them the major environmental factor affecting chemical composition is salinity of water and the variation in chemical composition is closely linked to the physiological processes taking place in the animals body during the reproductive activities. The feeding condition of the animal play a part in the variation in chemical constituents. Fluctuation in the moisture content due to absorption of water and loss of solids are the most significant features of changes in chemical composition. Goncalves Ferreira (1951) and Del Reigo (1948) observed variations in lipids and protein contents in Atlantic sardines. Fraga (1956) reported that the growth of oysters and mussels varies with the quantity of available feed (plankton).

Figs. 7 and 8 depict the seasonal variations in percentage composition of water extractable nitrogen (WEN) and non-protein nitrogen (NPN) of crab meat. WEN is maximum during September and January and minimum during May and June in both full moon and new moon times. NPN during full moon

time is minimum in April and maximum during November to March and during new moon time the maximum is during February to March and minimum during January. The variation in WEN and NPN correlates with that of protein.

From Fig.9 the free alpha amino nitrogen content during new moon time is maximum during November to May and minimum during June to October and during full moon time it is maximum during October to April and minimum during May - September. The variation in alpha amino nitrogen content also correlates with that of protein. Jones (1955) observed that values for glycine, glutamic acid and taurine showed marked seasonal variations in lemon sole. From the studies of Dachateau & Florkin (1955) on the effects of environment on the amino acid pools of crab (Eriochir senensis) observed that definite changes in amino acids were produced by altering the temperature and very large changes by altering the salinity of water. But Hughes (1959) could not discover any correlation between stage of sexual maturity, sex or age (length) of herring fish and the total or individual amino acid content. Raymond Jacquot (1961) observed seasonal variations in free amino nitrogen in fish.

Fig. 10 represents the glycogen reserve of the muscle. It shows wide variation over 12 months period. Analysis during new moon time shows maximum in November (2.38%) and

minimum in March (0.56%) and during full moon time maximum is in January (2.9%) and minimum in May (0.2%).

Depletion of muscle glycogen during exercise in fish muscle has been studied by Miller et al. (1959) and Stevens & Black (1966). Amano et al. (1953) found different levels of glycogen content between different portions of the muscle and in all fish examined, the glycogen content decreased and lactic acid increased very markedly depending on the method and rapidity of killing after capture. Environmental factors such as lack of food also could have played a part in the depletion of muscle glycogen. A sharp decrease in glycogen content is responsible for oysters becoming thin and watery in late summer and early autmn Feiger et al. (1952).

Compared to other shell fishes fat content is very low in crab muscle. It is maximum during full moon time in October and minimum during January to March and during new moon time the maximum value is in July and minimum in January. Venkataraman et al. (1969) while studying the variation of fat due to season in black pomfret repprted that the rise in fat content coincided with the onset of summer. Kordyl (1953) and Lovern et al. (1959) could not detect any definite seasonal trend in the muscular fat of fish muscle. Maximum values of protein, WEN, NPN and glycogen are found during October to April when the moisture

content of the muscle is at a lower level. No such correlation could be assigned to fat content.

Season is known to affect the qualitative and quantitative nature of the microflora associated with fish and fishery products. Raj & Liston (1963) observed high total bacterial count during the warmer months of the year, Iyer et al. (1970) observed high incidence of total bacterial count during April-August while high faecal streptococci and E. coli are encountered during April-June and May-July respectively in fresh prawn. They attributed this to be due to multiplication of bacteria during warmer months and due to high degree of faecal pollution of water during rainy season.

4.1.4 Biochemical changes in crab body meat stored at different temperatures:

Tables 5, 6, 7 and 8 report the results of studies on bacteriological and biochemical changes in quality of crab meat (Scylla serrata) stored at 37°C, ambient temperature (25-28°C) refrigerator temperature (6.5-7.5°C) and 0°C.

At a higher temperature than atmospheric, the spoilage rate was rapid and the material reached unacceptable stage in 6 hours. Appreciable change was not found in the moisture content; the total bacterial count increased from 7.2×10^5 to 6.0×10^7 in 8 hours time. Slight increase in water

Table 5. Changes in quality in medium sized Scylla serrata (shelloff condition) kept at 37°C

Time hours	Moisture gm %	Bact. count SPC/gm	WSN gm%	NPN gm%	F.A.- NH_2 nitrogen mg%	TVN mg%	TMA mg%	Glycogen mg%	Lactic acid mg%	In phosphorus mg%	Org. quality 0-10 scale*
0	80.54	7.2×10^5	1.26	0.76	282.8	7.92	0.90	310	309	161.7	9
2	80.78	-	1.29	0.95	283.4	8.80	1.10	290	307	164.0	9
3	81.32	6.5×10^6	1.38	1.17	264.5	12.25	3.90	278	313	189.0	8
4	80.98	9.1×10^6	1.42	1.18	277.7	18.5	4.8	265	323	178.0	7
6	81.98	2.2×10^7	1.48	1.21	280.5	22.25	6.6	207	336	162.5	4
8	81.18	6.0×10^7	1.52	1.32	272.1	28.5	7.7	197	396	182.0	3

* Hedonic scale

Note: Results represent average of two estimations.

Table 6. Changes in quality of medium sized Scylla serrata-shelloff condition kept at ambient temperature 25-28°C

Time	Bact. count SPC/gm	WSN gm%	NPN gm%	Fα-NH_2 nitrogen mg%	TVN mg%	TMA mg%	Glycogen mg%	Lactic acid mg%	In phosphorus mg%	Organoleptic score 0-10 scale
0	7.2×10^5	1.06	0.75	251.8	6.5	1.07	450.7	196.6	176	9
3	-	1.10	0.83	257.1	9.7	2.10	332.0	240.0	188	8
6	1.23×10^7	1.16	-	265.1	13.6	3.86	289.0	386.0	190	6
7	-	-	0.85	274.9	16.8	6.50	140.0	520.0	220	5.5
10	8.0×10^7	1.16	0.91	283.9	20.7	9.70	81.2	542.0	211	3
13	4.28×10^8	1.26	0.97	272.8	30.7	17.40	54.7	558.0	230	2

WSN = Water soluble nitrogen; NPN = Non protein nitrogen; Fα-NH_2N = free alpha amino nitrogen; TVN = total volatile nitrogen; TMA = trimethyl amine

Note: Values are average of two determinations.

Table 7. Changes in quality of Scylla serrata (medium sized) in the shelloff condition kept at 6.5 - 7.5°C

Days of storage	Bact. count SPC/gm	WSN	NPN	F-α-NH_2 N	TVN	TMA	Glyco-gen	Lactic acid	In phos-phorus	Organole-ptic score 0-10 scale*
		gm %	gm%	mg %	mg %	mg %	mg %	mg %	mg %	
0	3.4×10^4	1.19	0.91	283.4	4.11	0.87	510	317	192.3	9
1	9.7×10^5	1.21	0.93	297.0	6.18	1.11	470	310	187.6	8
2	4.8×10^6	1.26	0.95	310.9	8.11	1.25	436	342	186.9	8
3	5.1×10^7	1.32	0.97	315.9	12.05	2.70	431	362	193.8	7
4	2.0×10^7	1.35	1.02	335.7	14.17	3.54	424	352	188.8	6
5	4.2×10^7	1.38	1.11	318.6	15.30	4.30	385	411	196.6	6
6	3.2×10^7	1.40	0. 996	326.0	17.26	4.50	345	420	189.9	5
7	3.1×10^7	1.51	1.03	333.4	28.17	5.85	295	485	193.5	3
8	3.6×10^7	1.57	1.09	351.0	39.66	6.74	224	531	199.6	2

* Hedonic scale

Note: Results are average of two estimations.

Table 8. Changes in quality of Crab Scylla serrata (medium sized) in the shellon condition kept under ice (0°C) (in polythelene bags)

Days of storage	Bact. count SPC/gm	Moisture gm %	WSN gm %	NPN gm %	F.C-NH_2 N gm %	TVN mg %	TMA mg %	Glycogen mg %	Lactic acid mg %	Phosphorus (in) mg %	Organoleptic score 0-10 scale
0	5.4×10^5	79.8	1.37	0.74	259.5	5.41	1.62	825	248.4	192.7	10
1	1.01×10^6	80.52	1.11	0.64	251.1	8.24	2.75	735	273.2	174.5	10
3	1.32×10^6	81.10	1.26	-	261.6	6.94	2.67	626	328.7	161.2	9
5	5.58×10^7	82.69	1.26	0.64	265.0	10.43	-	394	330.9	166.4	8
8	3.14×10^7	82.96	0.88	0.61	272.7	12.47	3.72	239	346.9	173.1	6
11	5.9×10^7	82.64	0.96	0.58	274.5	16.40	4.57	-	369.4	169.5	5
18	2.0×10^7	80.11	1.06	0.89	277.7	22.70	10.39	186	383.0	178.9	3

Note: Results are average of 2 estimations.

extractable nitrogen (1.26-1.52 gm%) and appreciable increase in non-protein nitrogen values (0.76-1.32%) (Fig.11) were observed; the amino nitrogen values remained more or less constant probably due to assimilation by the bacteria in the muscle. The formation of total volatile nitrogen (Fig.12) and trimethylamine nitrogen gave an indication of the spoilage rate of the muscle. There was a steady decrease in glycogen value (Fig.13) and an increase in lactic acid content. Appreciable change was not found in inorganic phosphorus values.

A similar spoilage pattern was observed in crab muscle at ambient temperature (Figs. 14-16). The depletion of muscle glycogen and formation of lactic acid was more than that at 37°C (Fig.16). The phosphorus (in) values showed a steady increase giving the indication that it was liberated during room temperature storage. The free alpha amino nitrogen values also showed a steady increase (Fig. 14). The standard plate count increased from 7.2×10^5 - 4.28×10^8 in 13 hours time (table 6) corresponding to a fall in organoleptic score from 9 to 2.

At refrigerated temperature of 6.5 - 7.5°C the shelf-life was much enhanced and for 6 days the material can be preserved for human consumption. The bacterial count reached maximum value in 3 days time after that it remained more or less constant. Water soluble nitrogen, non-protein nitrogen, (table 7)

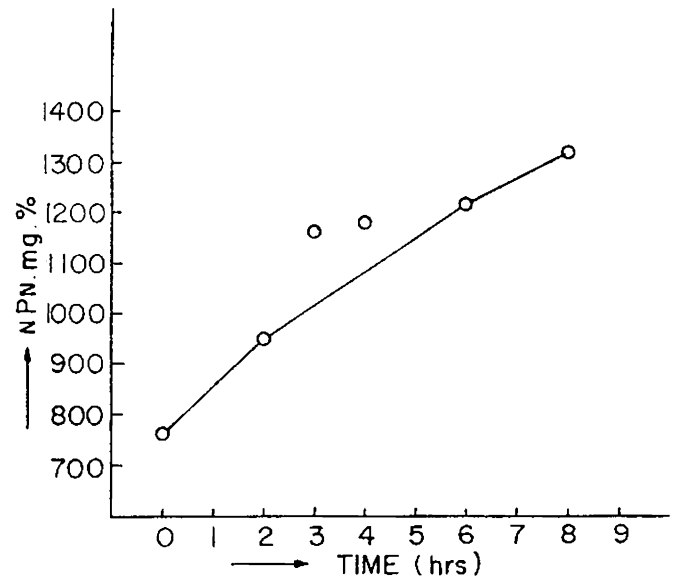


Fig. 11. CHANGES IN NPN CONTENT IN CRAB HELD AT 37°C.

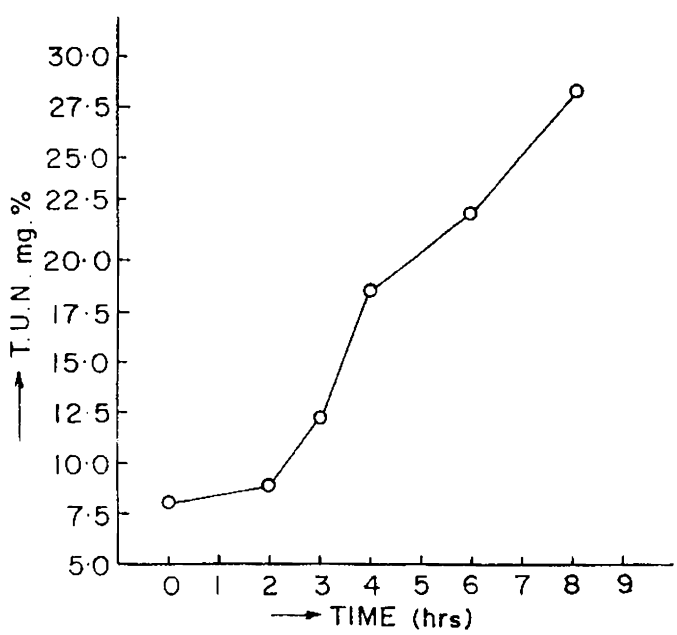


Fig. 12. CHANGES IN TUN OF CRAB HELD AT 37°C.

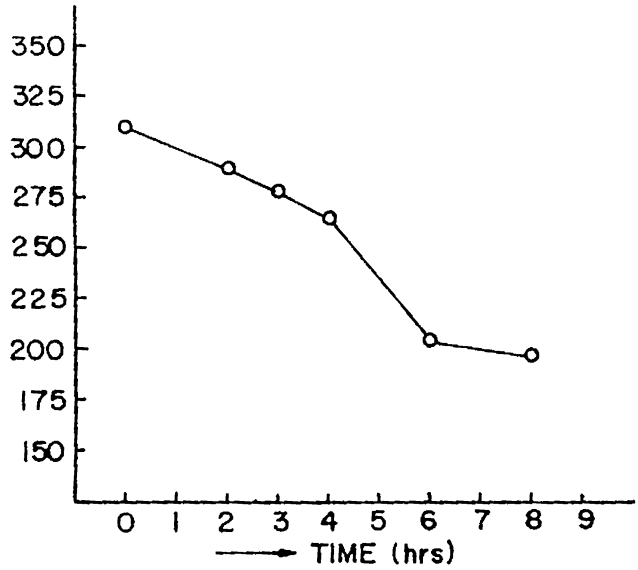


Fig. 13. CHANGES IN GLYCOGEN CONTENT IN CRAB STORED AT 37°C.

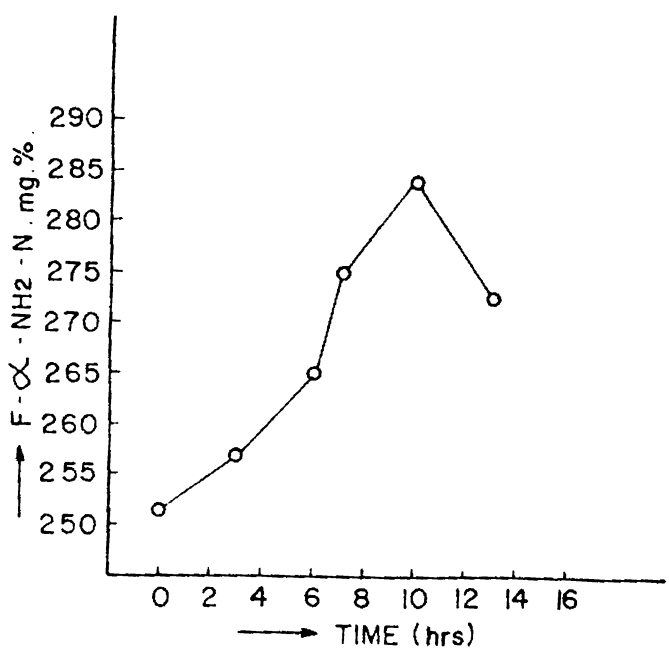


Fig. 14. CHANGES IN F. α. NH_2-N IN CRAB HELD AT 25-28°C

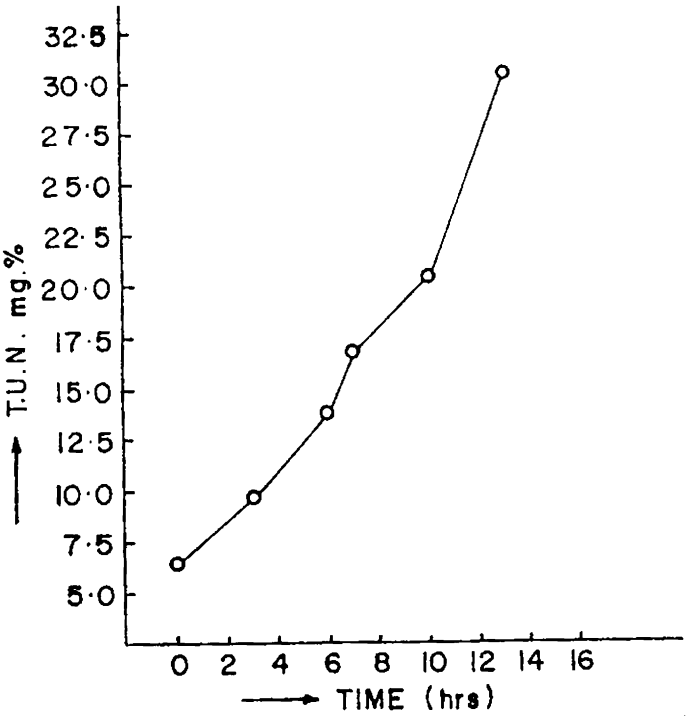


Fig. 15. CHANGES IN LEVELS OF T.U.N IN CRAB HELD AT 25-28°C.

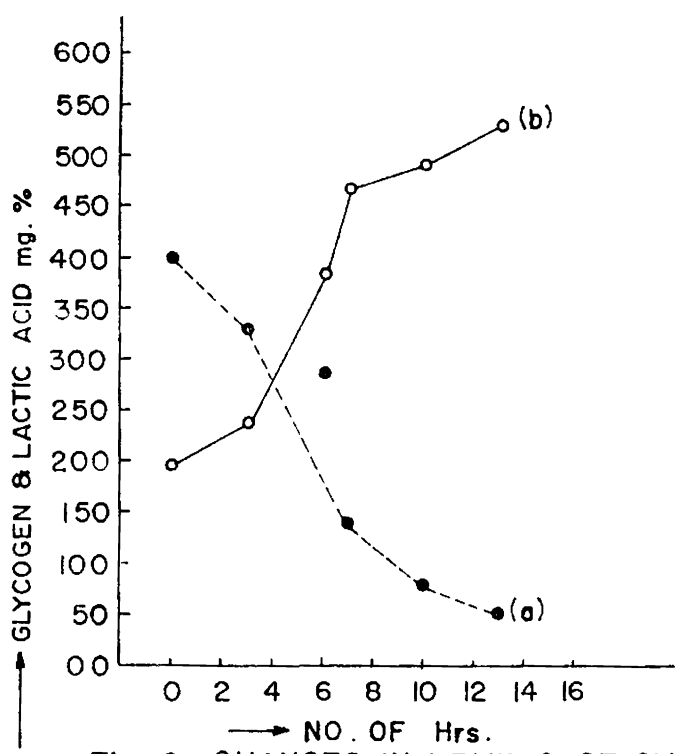


Fig. 16. CHANGES IN LEVELS OF GLYCOGEN (a) & LACTIC ACID (b) IN CRAB HELD AT 25-28°C

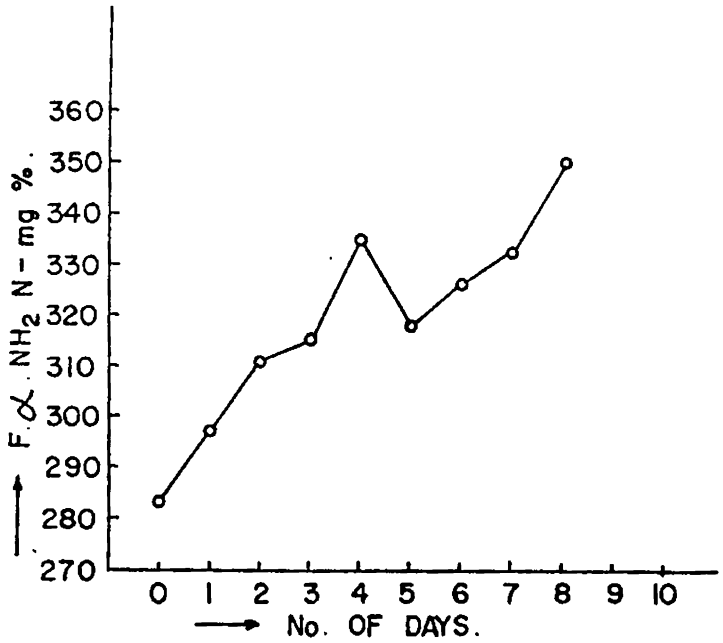


Fig. 17. CHANGES IN F.α.NH2.N IN CRAB AT 6·5 - 7·5°C.

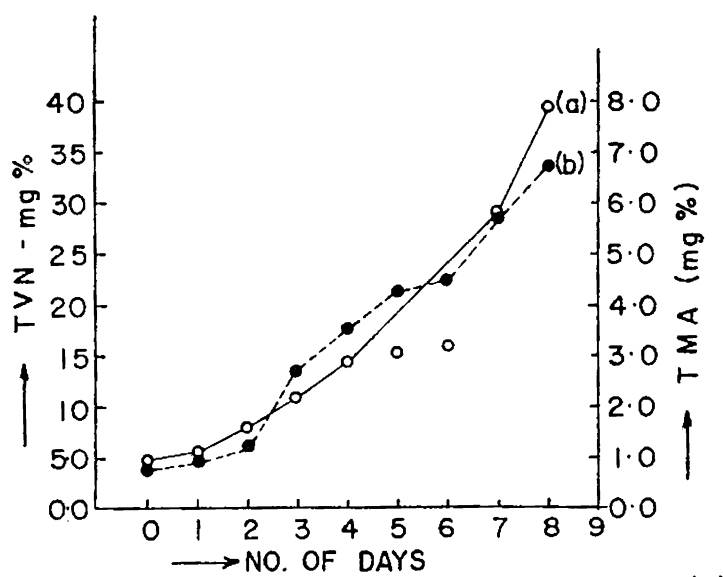


Fig. 18. CHANGES IN LEVELS OF T.V.N(a) & TMA (b) IN CRAB STORED AT 6·5 - 7·5°C.

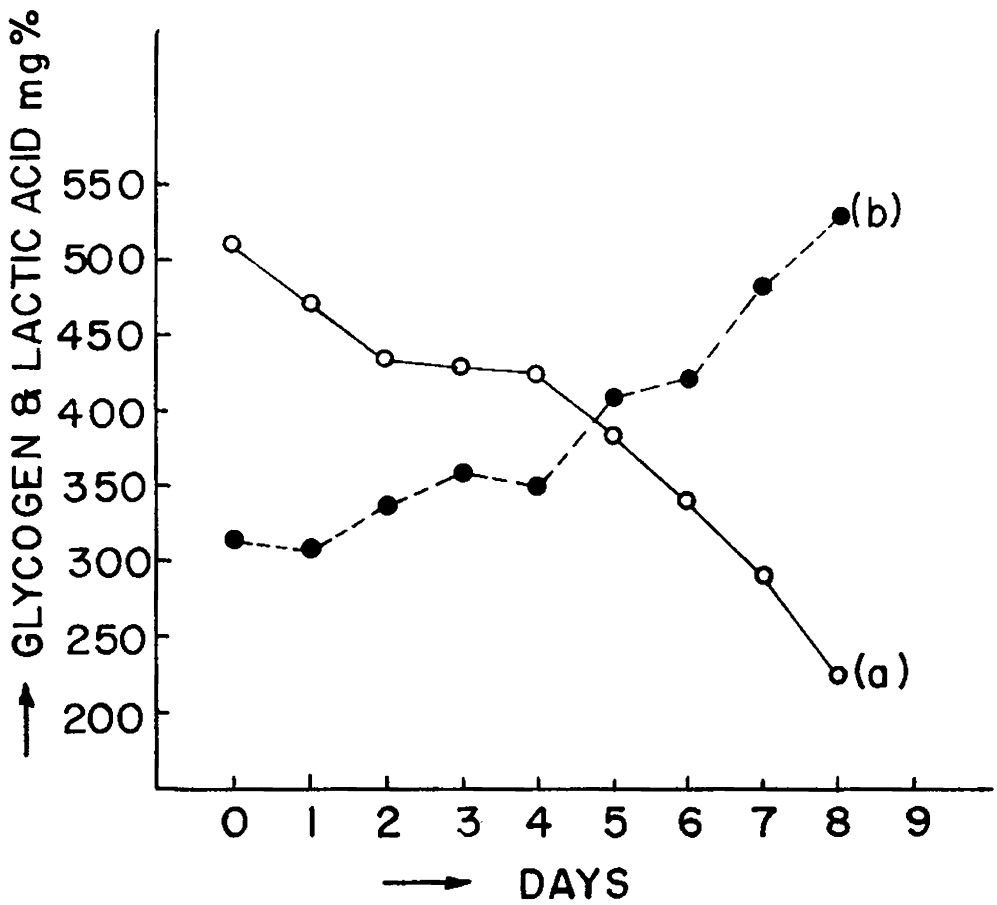


Fig. 19. CHANGES IN LEVELS OF GLYCOGEN (a) AND LACTIC ACID (b) IN CRAB STORED AT 6·5 - 7·5°C.

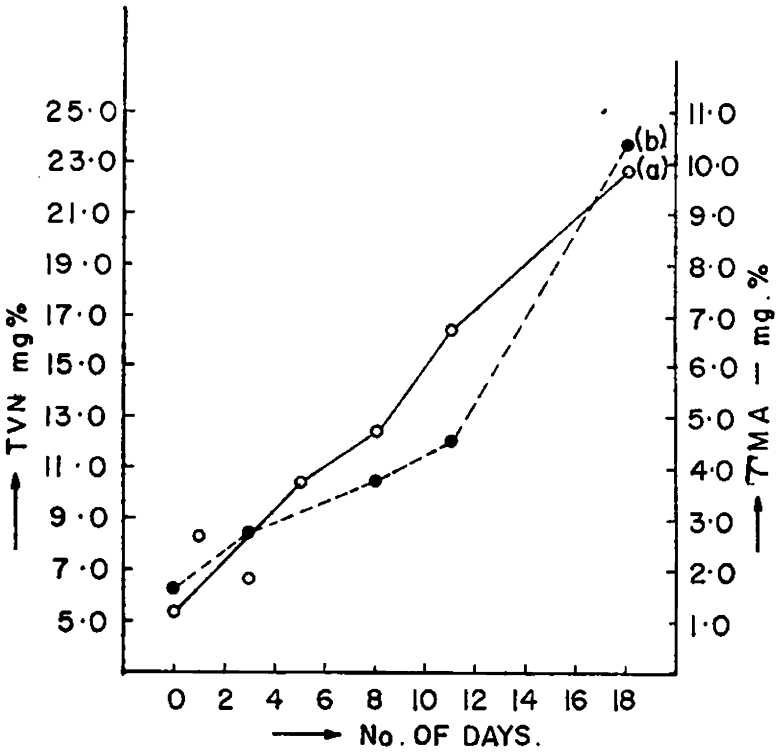


Fig. 20. CHANGES IN T.V.N (a) & TMA (b) IN CRAB STORED AT 0° C

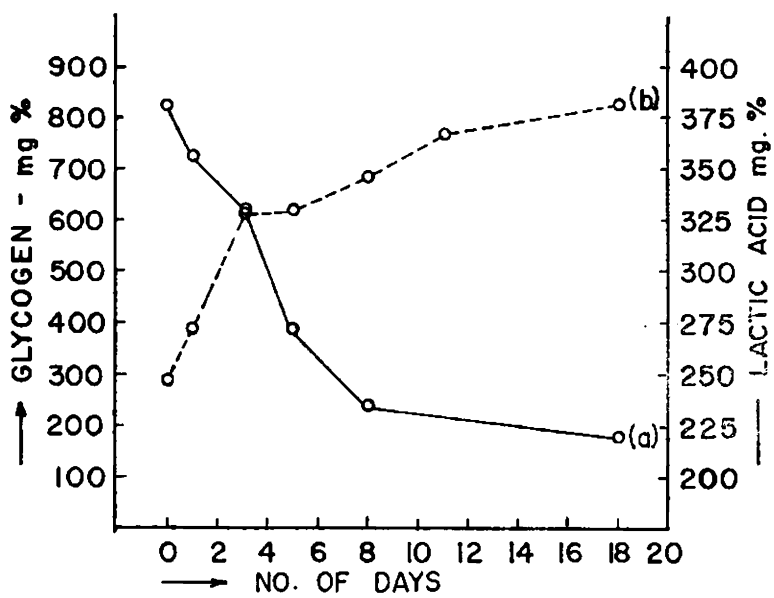


Fig. 21. CHANGES IN LEVELS OF GLYCOGEN (a) & LACTIC ACID (b) IN CRAB STORED AT 0°C

free alpha amino nitrogen (Fig.17), total volatile nitrogen (Fig.18), trimethylamine nitrogen (Fig.18) and lactic acid values (Fig.19) recorded a steady increase during the storage period, the phosphorus (in) content remained more or less constant and a gradual fall in glycogen values was observed.

The material stored at 0°C (in ice) was in acceptable condition for 11 days. The spoilage pattern was more or less similar to that at 6.5-7.5°C (Figs.20 & 21). There was chance for absorption of moisture by the muscle and slight leaching of water soluble constituents. The bacterial count recorded gradual increase up to 5 days, there after remained more or less constant (table 8). Much change was not found in water extractable nitrogen, non-protein nitrogen, free alpha amino nitrogen and phosphorus (in), but the values of total volatile nitrogen and trimethylamine nitrogen recorded steady increase during storage. Muscle glycogen was reduced from 825-186 mg% and lactic acid values increased to 383 mg% from 248 mg%.

The detection of spoilage should provide an accurate measure of the degree of spoilage and should be based on the most characteristic change occurring in the material as spoilage progresses. The major single factor that causes rapid spoilage in fishery products is bacterial spoilage. The rates at which the biochemical and bacteriological

changes takes place depended on the temperature of storage. From the studies it was evident that the higher the temperature the more rapid the changes occurring, and the faster the bacteria living in the fish multiply. According to Tarr (1954) the growth of micro organisms in fish results in the decomposition of proteins, lipids and carbohydrates. Velankar et al. (1961) observed an increase in the bacterial count showing a sharp rise after 4 hours storage, in prawns kept at room temperature. Shaikhmahamud & Magar (1965) also observed a gradual increase in the bacterial count of prawns kept at 0°C. Among the factors responsible for quality loss in fishery products the bacterial action is the most important.

The increase in the values of water soluble nitrogen, non-protein nitrogen and free alpha amino nitrogen in crab meat stored at 4 different temperatures revealed the fact that proteolytic enzymes (cathepsins and peptidases) were active in crab meat. Fish muscle proteins were degraded by these enzymes to simpler compounds like peptides and amino acids. Siebert (1961) studied the proteolytic enzyme activity in fish muscle. Tarr (1954) suggested that the bacterial action, not autolysis was responsible for most of the breakdown of proteins. Raymound Jacquot (1961) suggested that the water soluble protein in fish muscle was derived from the sarcoplasm and interstitial fluid and contains the

enzymes of the respiratory system. The water soluble nitrogen constitutes soluble protein and non-protein nitrogen including amino nitrogen. Susamma Jacob et al. (1964) observed that the total alpha amino nitrogen content increased in prawns by nearly three fold during the period of 26 days in ice (out of contact).

Trimethylamine nitrogen (TMAN) and total volatile nitrogen (TVN) contents were negligible in the fresh condition in crab meat, these are increased gradually as the spoilage advanced in all four series of experiments; in agreement with previous findings by Nazir & Magar (1963), Beatty & Gibbons (1937), Velankar et al. (1961) and Shaikhmahamud & Magar (1965). The peculiar smell of spoiled crab meat was due to the formation of TMA and was formed by the bacterial action on trimethyl amine oxide (TMAO) an odourless compound (Mac Leod & Eva Onofrey). Burnett James (1966) used ammonia as an index of decomposition of crab meat. Mac Leod & Eva Onofrey also suggested the production of ammonia as a result of the bacterial action on amino acids.

The products of postmortem degradation of glycogen contribute to both the flavour and texture of fish (Tarr, 1966). The accumulation of lactic acid in the muscle was positively correlated with toughness (Murray, Jones & Burt, 1964), Lactic acid is produced by anaerobic glycolysis

(Buttkus & Tomlinson, 1966, H.O. Triebold and L.W. Aurand & Tarr, 1966). Manohar (1970) and Tarr (1966) observed that there was no stoichiometric relation between glycogen lost and lactic acid formed in white muscle or white sucker and northern pike, which is in agreement with the present observation. According to Partman (1965), postmortem glycolysis will continue until the glycogen was completely depleted or until pH of 5.4-5.5 was reached in the muscle, at which point the enzyme system producing lactic acid was inactivated and some residual glycogen may be found. In all the experiments conducted some residual glycogen was detected confirming the earlier finding of Nazir & Magar (1963).

The slight increase in P (in) observed in crab muscle during ambient temperature storage suggest the presence of phospho mono esterase enzymes capable of releasing ortho-phosphates from both hexose and pentose phosphate esters (Tomlinson et al., 1960).

4.1.5 Spoilage during ice storage in crab muscle

Ice storage of shell fishes probably constitutes in all cases the first important technological factor in the utilization of shell fishes for food. The quality or freshness of the material issuing from ice storage will determine, to a large extent, regardless of subsequent processing, the quality of the material reaching the consumer.

Table 9a Loss in weight of crab (Scylla serrata) held under ice storage

Days in ice	Weight loss %
0	-
1	0.51
5	2.51
9	3.09
10	4.12
12	5.15
14	5.15

Note: 1. Number of crab samples studied 50

2. Number of samples examined/day 6 to 7

Table 9b. Spoilage pattern of crab (Scylla serrata) in ice (open system)

Days in ice	Moisture	WSN	NPN	F_{α}-NH_2 N	TVN	TMA	Glycogen	Lactic acid	Phosphorus (inorganic)	Cooked characteristics			
										Colour	Odour	Texture	Flavour
	gm %	mg%	mg%	mg%	mg%	mg%	mg%	mg%	mg%				
0	79.91	1675	719	342	7.49	1.73	1345	368	129	Characteristic white	Good	Soft & firm	Good
1	79.80	1580	720	315	11.37	1.91	1250	471	130.5	"	"	"	"
2	79.97	1457	714	269	14.02	2.27	1183	506	131	"	"	"	"
4	80.87	1336	646	210	18.75	3.09	862	639	133	"	"	"	"
6	81.77	1096	650	195	20.02	4.05	646	742	127	Almost white	G - F	Soft	F
8	83.86	1270	626	200	26.67	5.66	480	773	114	Faded colour	F	Soft but granular	F
11	85.96	1016	619	187	22.01	4.00	388	973	86	Slight pink	F - P	Soft & pasty	F - P
15	86.46	1020	602	175	32.38	6.77	357	968	81	Slight pink	F - P	"	Poor

Note: 1. No. of samples of crab stored 40-50 per series.
2. No. of series studied 3 (1978, 1979, 1981).
3. Results are average calculated from 3 series.

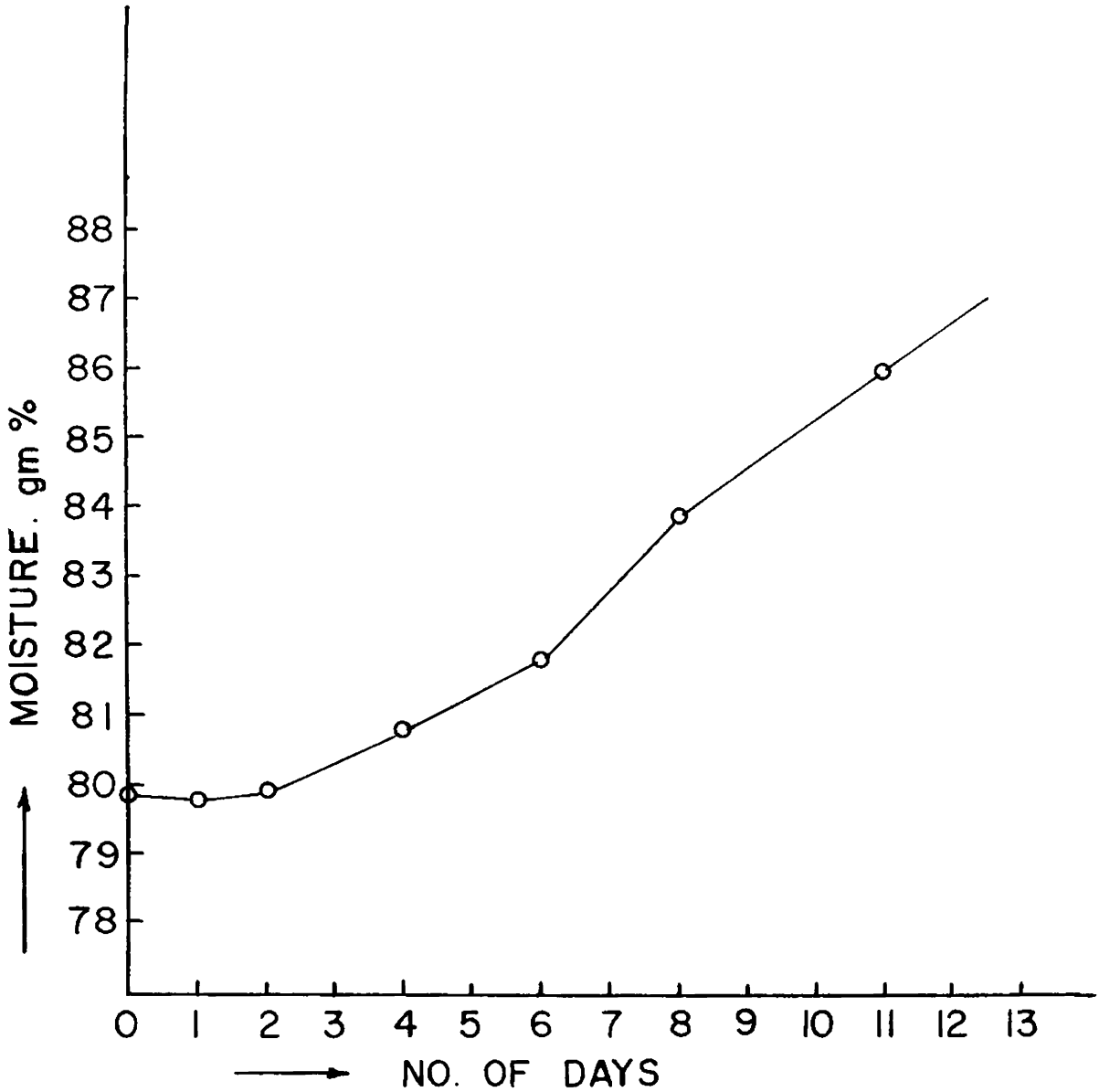


Fig. 22. CHANGES IN MOISTURE VS NUMBER OF DAYS OF ICE STORAGE OF CRAB MEAT

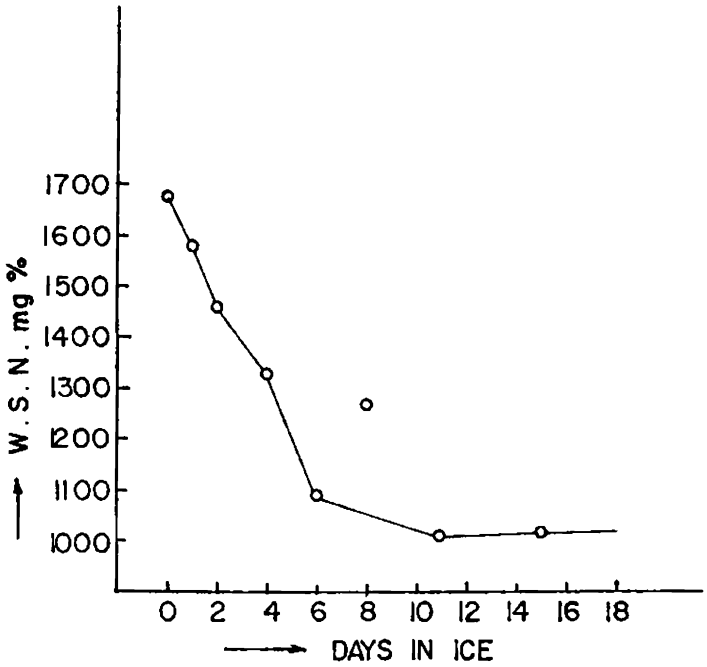


Fig. 23. CHANGES IN WATER SOLUBLE NITROGEN DURING ICE STORAGE OF CRAB.

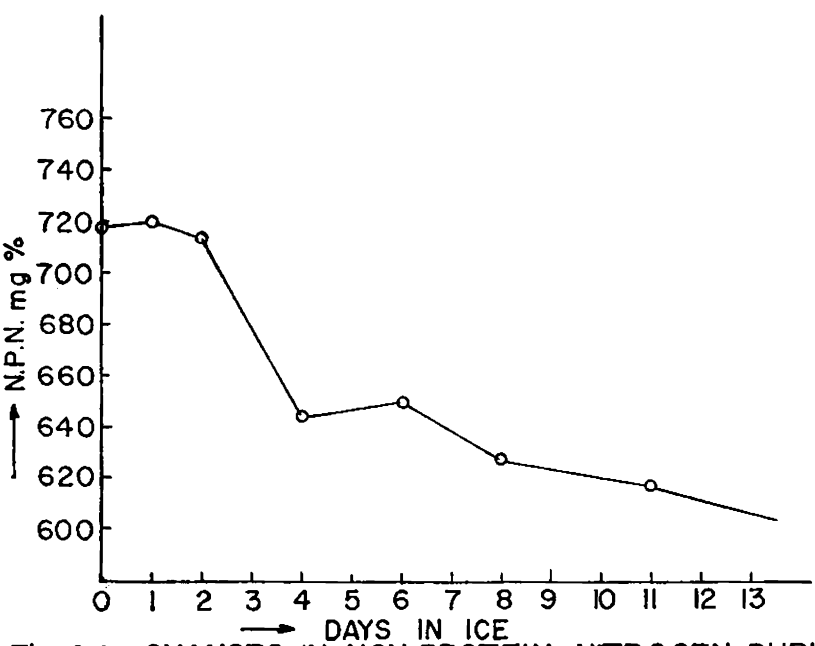


Fig. 24. CHANGES IN NON-PROTEIN NITROGEN DURING ICE STORAGE OF CRAB

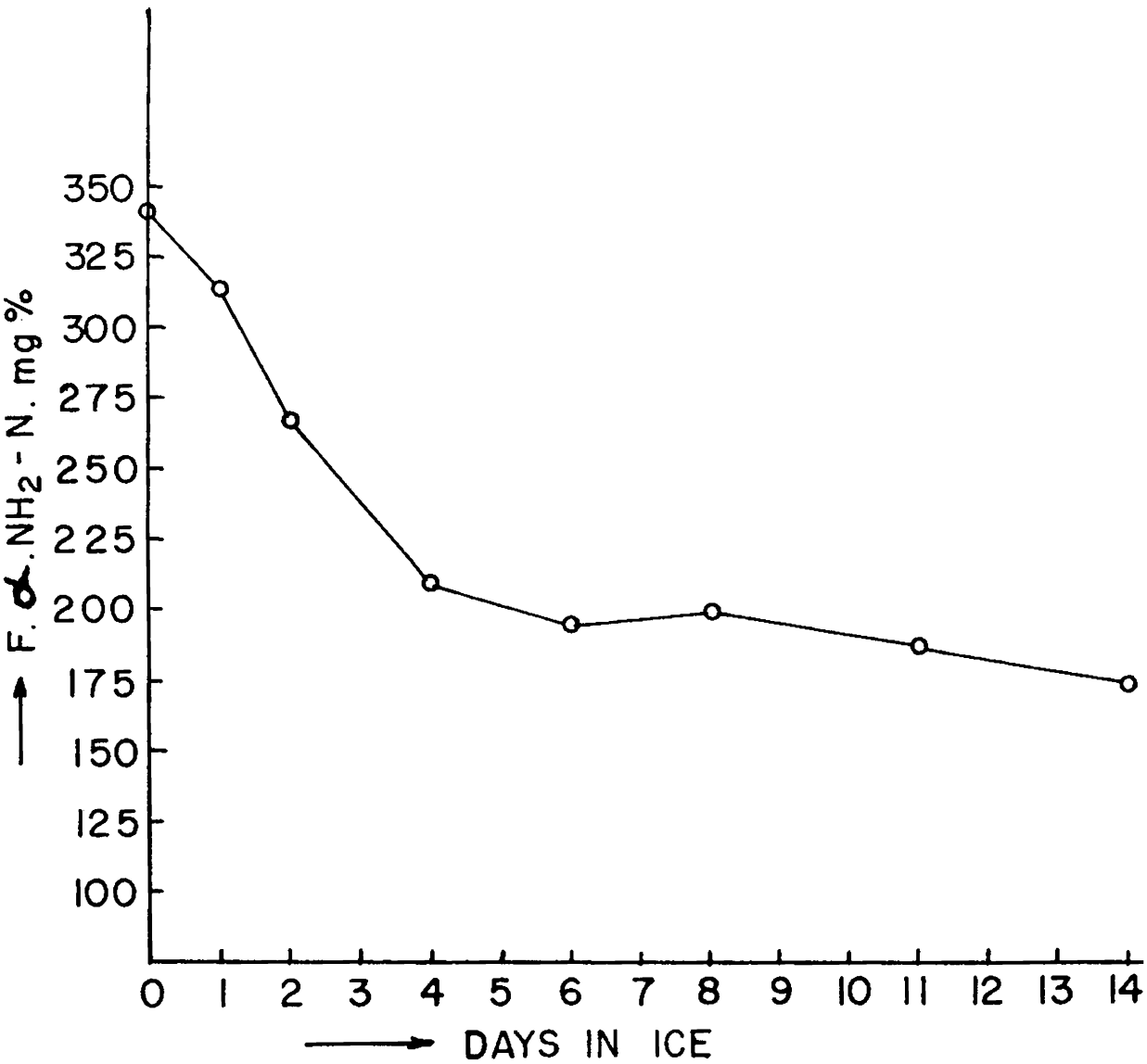


Fig. 25. CHANGES IN F.α.NH2.N DURING ICING OF CRAB.

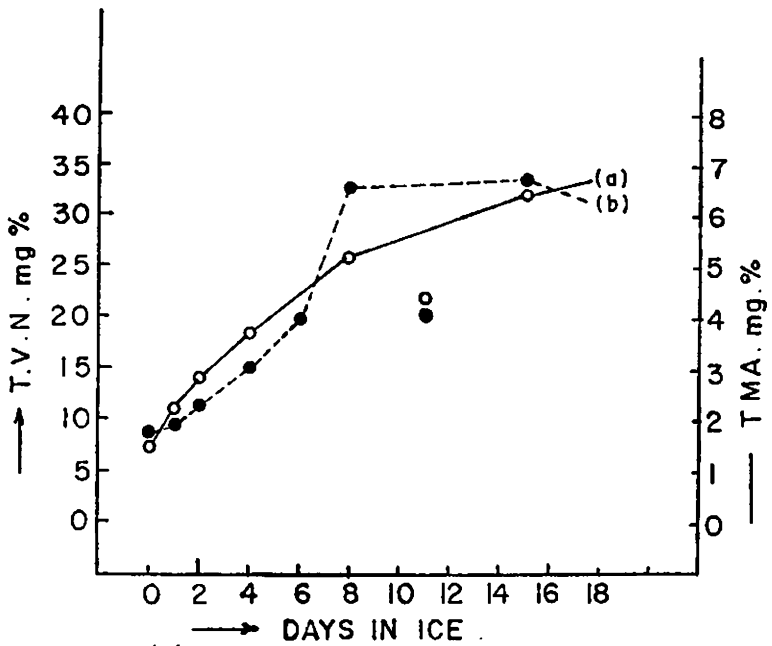


Fig. 26. (a) CHANGES IN TOTAL VOLATILE NITROGEN & (b) CHANGES IN TRIMETHYL AMINE NITROGEN DURING ICING OF CRAB.

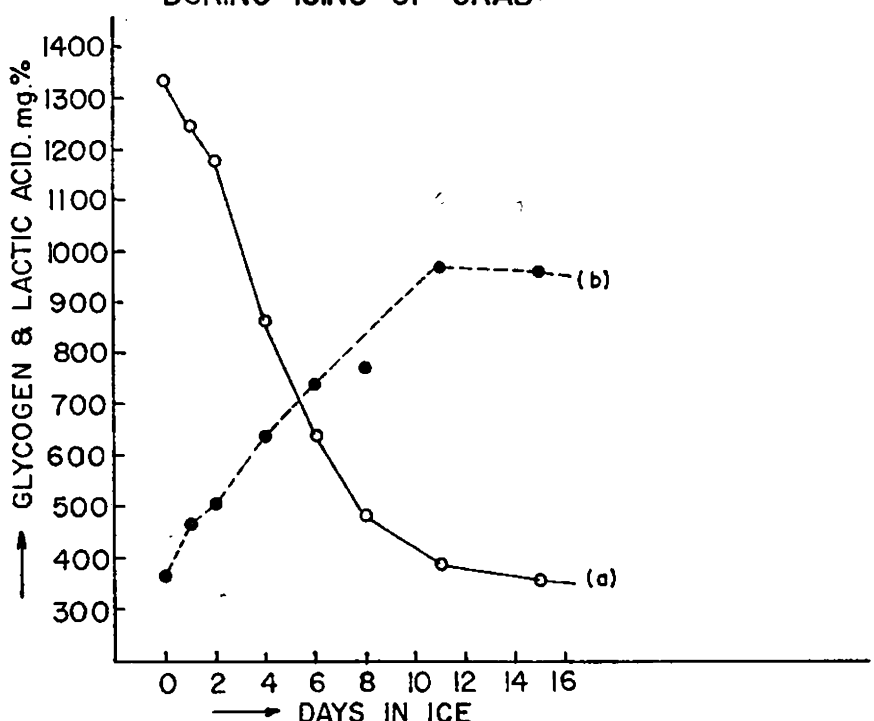


Fig. 27. (a) CHANGES IN GLYCOGEN & (b) CHANGES IN LACTIC ACID DURING ICE STORAGE OF CRAB.

In 14 days of ice storage, 5.15% of weight loss was observed in whole crabs (Table 9a). In live crabs the flesh is held to the shell by a membrane making it difficult to remove the meat. If it is stored in ice for 18-24 hours, the membrane breaks down and the flesh can be easily taken out as flakes getting more yield.

Table 9(b) indicates the biochemical and organoleptic changes occurred in whole crabs (claws and legs cut off) stored in direct contact with ice. The increase in moisture content (Fig.22) during 13 days of ice storage was 6% and total volatile nitrogen, trimethylamine nitrogen (Fig.26) and lactic acid (Fig.27) values showed gradual increase; there was slight increase in phosphorus (in) values during the first 4 days, after that showed gradual decrease (table 9b). The water soluble nitrogen (Fig.23), non-protein nitrogen (Fig.24), free alpha amino nitrogen (Fig.25) and glycogen (Fig.27) values showed steady decrease during the storage period. Upto 8 days the material remained in good condition after that discolouration and softening of muscle tissue with loss of characteristic flavour occurred. Upto 11 days the material remained in edible condition.

There was good correlation between organoleptic quality and the amounts of water soluble nitrogen, free amino nitrogen, glycogen and phosphorus (in) retained in the muscle.

The higher rate of spoilage in shell fishes may be due to the higher level of free alpha amino nitrogen. In crab muscle the free alpha amino nitrogen content is about 50% of the total non-protein nitrogen and the higher percentage of glycine-alanine (83%) may probably be responsible for its sweet flavour.

The production of total volatile nitrogen and trimethylamine during ice storage provided a fair measure of the degree of spoilage, in agreement with the findings of Liston et al. (1961) that total volatile acid and total volatile base agreed with spoilage of fish and Lahiry et al. (1963) observed the increase in pH value due to the production of amines and ammonia in fresh water fish stored in ice. According to Cutting (1953), the bacterial reduction of trimethylamine oxide (TMAO) to trimethylamine (TMA) is a factor that changes the buffering power of the fish muscle. Shewan & Jones (1956) claimed that the changes occurring in chilled fish were due to autolytic and bacterial actions and considerable leaching losses occurred during ice storage.

Govindan et al. (1962, 1964) and Lekshmy et al. (1962) observed loss of water soluble constituents such as water soluble nitrogen (WSN) and non-protein nitrogen (NPN) in ice stored prawns and increase in moisture content of the muscle and attributed these to be due to leaching by ice melt

water and absorption of water by the muscle. Fritz A.L. Bramstedt (1962) and Lahiry et al. (1963) observed a decrease in the free amino acid in fish muscle during ice storage which is in harmony with the present observation.

The initial increase in phosphorus (in) values revealed the liberation of orthophosphates from sugar phosphates, phospholipids, ribo nucleic acid or deoxyribo nucleic acid fractions by enzymes in the muscle; In the later stages, due to inactivation of muscle enzymes, utilization of phosphate by bacteria and leaching losses are the probable factors for the decreasing trend in phosphorus (in) observed in crab muscle. The observed loss of glycogen may be attributed to glycolysis producing lactic acid and loss due to leaching.

In the case of crabs, the cut surfaces of the legs and claws provided sufficient area for leaching even though the thick carapace was covering the body. The loss of water soluble flavour bearing compounds by leaching and accumulation of products of autolytic anc bacterial actions were responsible for the quality loss in ice stored crabs.

4.1.6 Factors influencing the keeping quality of frozen stored crab muscle

Table 10(a) & Figs. 28 & 29 represent the changes in protein fractions and adenosine triphosphatase (ATP-ase)

Table 10a. Protein changes of crab meat stored at -23°C

Weeks of storage	Sarcoplasmic P.N. as % T.P.N.	Myofibrillar P.N. as % T.P.N. in Kcl phosphate buffer	Soluble N as % T. N. in Dyer's buffer	Actomyosin as % TPN	ATPase activity units/ min	T.N. in drip mg %
RM*	22.57	47.9	85.81	48.83	24.76	..
0	22.26	48.9	85.41	45.14	18.46	174.8
4	22.06	45.2	81.50	45.40	15.60	156.5
8	22.52	43.5	77.82	45.66	14.20	..
12	20.44	42.2	77.49	44.80	13.60	262.8
16	20.27	38.2	77.50	43.10	9.40	241.9
20	19.28	36.4	76.68	39.30	9.76	297.5
28	18.49	35.1	76.08	36.50	2.99	304.7
36	16.91	32.8	74.40	34.79	0.99	..
44	15.51	23.95	61.02	24.20	0.33	340.5
48	15.30	17.48	60.10	22.30	nil	355.1

*RM = raw material

Note: Sample of meat was kept as block frozen (2 kg). One block was analysed/ week. Two estimations were carried per sample.

Table 10b. Changes in biochemical characteristics of frozen crab meat stored at -23°C

Storage time weeks	Moisture gm %	T.N. mg %	N.P.N. mg %	Weight loss gm %	T.N. in drip mg/100 gm meat
Raw material	79.86	2972	686	-	-
0	79.80	2863	693	Nil	121.3
8	79.50	2800	686	2.00	162.9
16	80.65	2894	847	2.75	197.3
20	80.00	2754	721	3.75	222.2
28	79.91	2737	679	4.00	269.5
36	79.32	2662	742	6.98	306.8
44	79.18	2685	742	6.00	284.9
48	80.41	2632	679	11.50	289.2

Table 10c. Changes in organoleptic characteristics of frozen crab meat stored at -23°C

Storage time weeks	Colour	Texture	Flavour
0	Creamy white	Soft but firm	Good
8	"	"	"
14	"	"	"
20	White	Granular	G - F
28	"	"	Fair
36	"	"	"
44	Slight brownish	"	"
48	"	Slight tough	F - P
52	"	"	F - P

Note: For details of samples ref. table 10a.

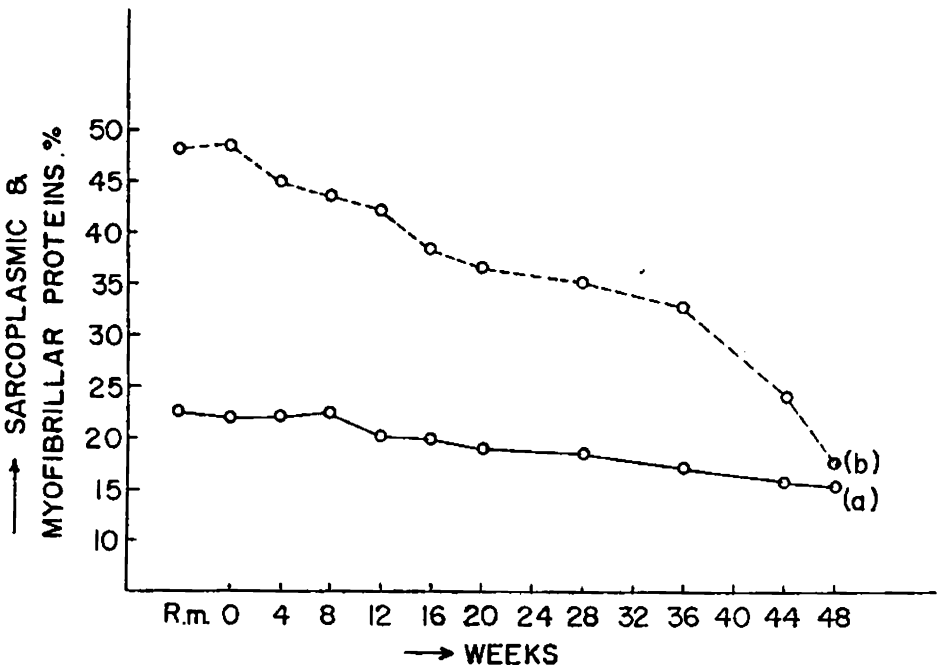


Fig. 28. CHANGES IN SARCOPLASMIC (a) AND MYOFIBRILLAR PROTEIN NITROGEN (b) IN CRAB MEAT STORED AT -23°C.

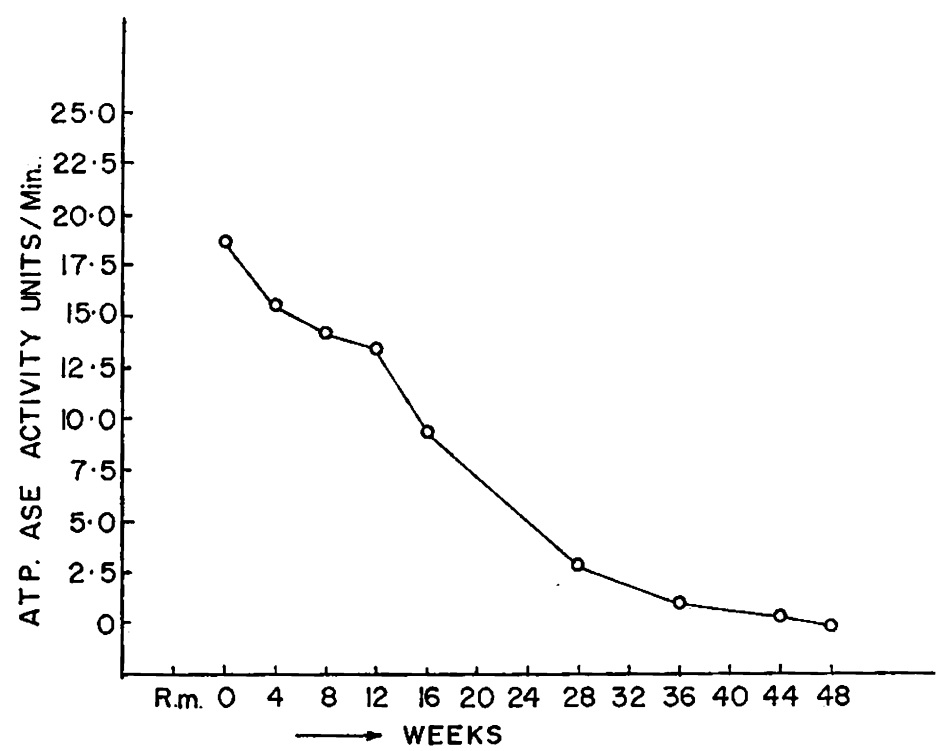


Fig. 29. CHANGES IN ATP - ASE ACTIVITY OF ACTOMYOSIN OF CRAB MEAT AT - 23°C.

activity of actomyosin during freezing and subsequent cold storage of crab meat at -23°C. There was a slow but gradual decrease in the sarcoplasmic protein fractions, that is 22.5 - 15.3%, but the extractability of myofibrillar protein diminished at a faster rate i.e 48-17.5% in 48 weeks time. By that time the nitrogen extracted with Dyer's buffer decreased from 85.8 to 60.0% and actomyosin precipitated by ten fold dilution of the salt extract from 49.0-22.0%. The adenosine triphosphatase activity dropped from 24.3 to 0.098 μ g Pi/mg protein/minute during a period of 48 weeks. A gradual increase in the drip nitrogenous constituents was also observed.

The development of tough texture during frozen storage of fishery products can be correlated to the protein denaturation, leading to loss of water holding capacity of the muscle. According to Banks et al. (1977) protein damage may be due to a number of different inter-related physical and chemical changes that vary with species and methods of processing, storage and handling.

Experimentally the denaturation of proteins was studied best by changes in solubility characteristics of proteins (Dyer et al., 1950). Batesmith (1934 & 1937) and Reay (1933, 1934 & 1935) observed that during frozen storage of fish there was progressive loss in solubility of its muscle proteins, especially the globulins. Connell (1960) and

Awad et al. (1969) observed practically negligible change in sarcoplasmic protein in frozen stored cod and white fish muscles, in agreement with the present findings. According to the previous workers the only protein actually involved in cold storage denaturation was actomyosin (Love, 1962; Connell, 1960; 1962; King, 1966; Awad et al., 1969).

According to Love & Ironside (1958), the change in salt concentration in liquid solution in the frozen fish was the agent causing changes in solubility in Dyer's buffer and Love et al. (1965) suggested that changes in protein extractability were the consequence of binding together of structural protein molecules.

The enzyme activity with respect to hydrolysis of adenosine triphosphate (ATP) is the most important biochemical characteristics of myosin or actomyosin. Baily (1942) and Perry (1960) observed substantial differences in the ATP-ase activity of myosins extracted from the skeletal muscle of various species. Connell (1962) observed loss of enzymic activity (ATP-ase) of myosin in cod stored at -14°C. Buttkus (1966) suggested that the loss of ATP-ase activity of myosin proceeds with positive entrophy changes and can be looked upon as an opening or unfolding of the secondary and tertiary structure of the myosin molecule.

Anon (1968), Ken-ichi Arai & Reiji Takashi (1973) and

Takashi Muruzuka (1976) reported loss of ATP-ase activity during frozen storage of carp and big eye tuna muscle which is in agreement with the loss of ATP-ase activity of actomyosin of crab muscle in the present study.

The observed increase in drip nitrogenous constituents agreed with the findings of Empey & Howard (1954).

Table 10(b) gives an account of the changes in biochemical characteristics of quick frozen crab meat stored at -23°C. Appreciable change was not observed in moisture content giving the indication that the water holding capacity of the muscle was not reduced or dehydration loss was minimised because of proper glazing and packaging of the product. Pawar & Magar (1966) observed less moisture loss in glazed fish. The total nitrogen content in meat registered a steady fall with a corresponding increase in nitrogenous constituents in drip (the free fluid that exuded while thawing of the product).

Gangal & Magar (1963); Empey & Howard (1954); Anon (1966); Bethea & Ambrose (1961); Jun-ichi-Nishimoto (1962); Radhakrishnan et al. (1973) and Dyer et al. (1968) reported that the drip volume and its characteristics such as pH, colour and soluble constituents change during prolonged storage of the frozen product due to the condition of the fish at the time of freezing, nature of freezing, temperature and humidity of the cold storage.

The non-protein nitrogen values in muscle remained more or less constant throughout the storage period. Weight loss due to frozen storage increased from 2 - 11.5% after 48 weeks of storage. Similar trends of results were observed by Sawant & Magar (1961) on different types of fishes of Maharashtra coast.

The organoleptic and textural changes in fresh frozen crab muscle during storage at -23°C is given in Table 10(c). After 36 weeks of storage, slight changes in colour and texture were noticed, up to 52 weeks the material remained in acceptable condition. A close correlation was noticed between taste panel results and the loss of solubility of structural proteins and loss of actomyosin ATP-ase activity.

A storage period of 12 months for quick frozen crab meat was proposed at -20°F (Anon, 1966) and Strasser & King (1971) observed a storage period of 8 months for frozen crab meat. Melvin E. Waters (1964) suggested that sensory evaluations were the most reliable means for detecting spoilage in scallops. Loss of characteristic flavour, appearance of stringy texture and yellowing of the pigment in frozen stored crab meat was observed (Anon, 1966).

The changes taking place during freezing and storage of raw materials having different pre-process ice storage periods are represented in Table 10(d) and Figs.30 to 33 and histogram 1.

During ice storage the moisture content increased from 79.47 - 81.85% and about 2% reduction in moisture was observed in all the iced and frozen samples during 40 weeks of storage. The loss of moisture was accounted for the loss in water holding capacity due to protein denaturation. Gangal & Magar (1963) observed a decrease in moisture content in crab meat during frozen storage. They attributed this loss to be to desiccation and drip due to protein denaturation. In the present work chance for dessication was minimised by giving glaze and proper packing.

The denaturation of protein during freeze storage as measured by the decrease in soluble protein is shown in Table 10(d) and Fig.31. A steady fall in values was found during ice storage as evident from the table; during freezing appreciable change was not found but on subsequent storage a gradual fall in values was noticed as observed by Reay (1933, 1934, 1935); Bate-smith (1934, 1937); Dyer et al. (1950); Snow (1950b); Dyer (1951); Nikkila & Linko (1954 & 1956); Dyer & Morton (1956); Dyer et al. (1956); Ironside & Love (1956); Love (1956); Luijpen (1957); Love & Ironside (1958); Sawant & Magar (1960); Connell (1960, 1962) ; Kaoru Tamoto et al. (1964); Love et al. (1965); King (1966) and Awad et al. (1969).

Connell (1962) stated that the consequence of protein denaturation was due to the formation of increased numbers

Table 10d. Biochemical changes in frozen crab meat stored at -23°C

Weeks of frozen storage	Days of ice storage 1	4	7	14
1. Moisture gm %				
0	79.47	79.95	81.16	81.85
8	79.17	79.47	80.63	80.93
12	78.50	78.70	80.15	80.63
20	78.29	79.23	80.04	80.55
28	77.39	79.02	79.84	80.15
40	77.31	78.96	79.41	80.04
2. Salt extractable protein nitrogen (mg %)				
0	1756	1620	1036	1071
8	1694	1575	1029	994
12	1665	1589	966	980
20	1638	1442	840	966
28	1603	1176	819	959
40	1596	1155	784	883
3. Inorganic phosphorus mg %				
0	140.4	145.5	126.9	117.5
8	153.4	149.6	128.1	122.0
12	162.0	154.7	132.0	134.4
20	169.2	160.0	137.5	149.6
28	184.2	171.1	141.6	158.2
40	184.9	182.0	156.0	164.3
4. Total nitrogen in drip (mg/100 gm meat)				
0	402	323	188	166
8	398	327	187	193
12	412	364	198	197
20	475	380	199	258
28	557	409	290	256
40	720	458	277	273

Note: Results are average of 3 series studied.

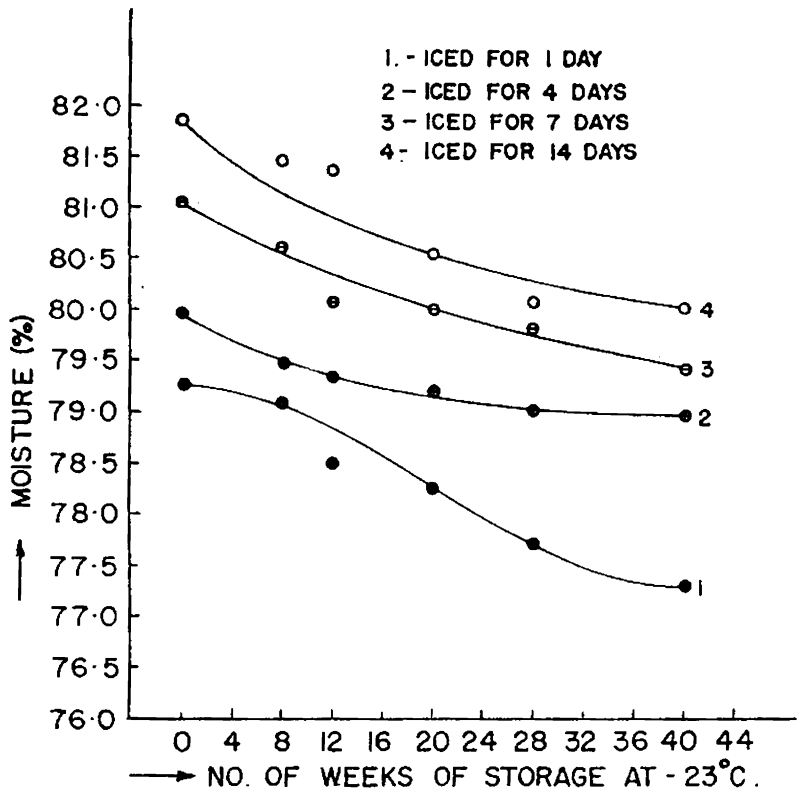
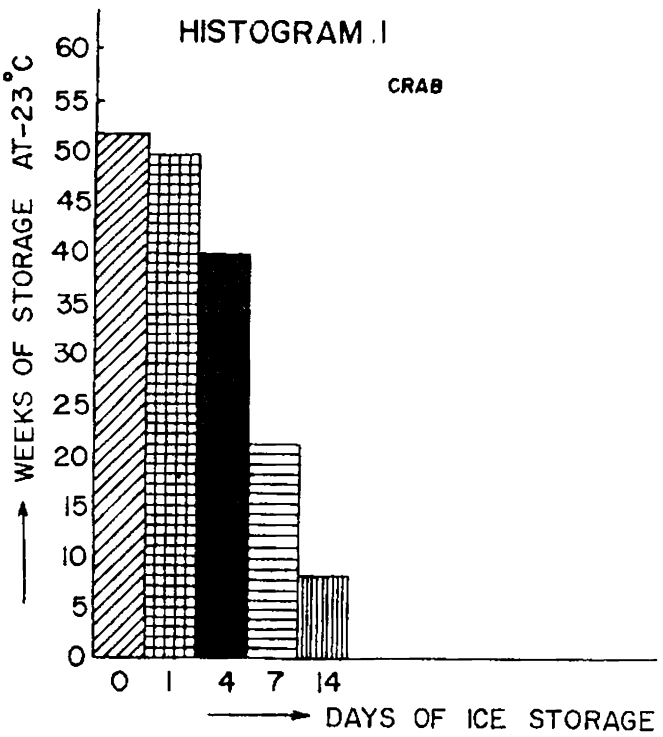


Fig. 30. EFFCT OF ICE STORAGE PERIOD ON MOISTURE CHANGES IN FROZEN STORED CRAB MEAT.



EFFECT OF ICE STORAGE PERIOD ON SHELF-LIFE WITH RESPECT TO ORGANOLEPTIC RATING.

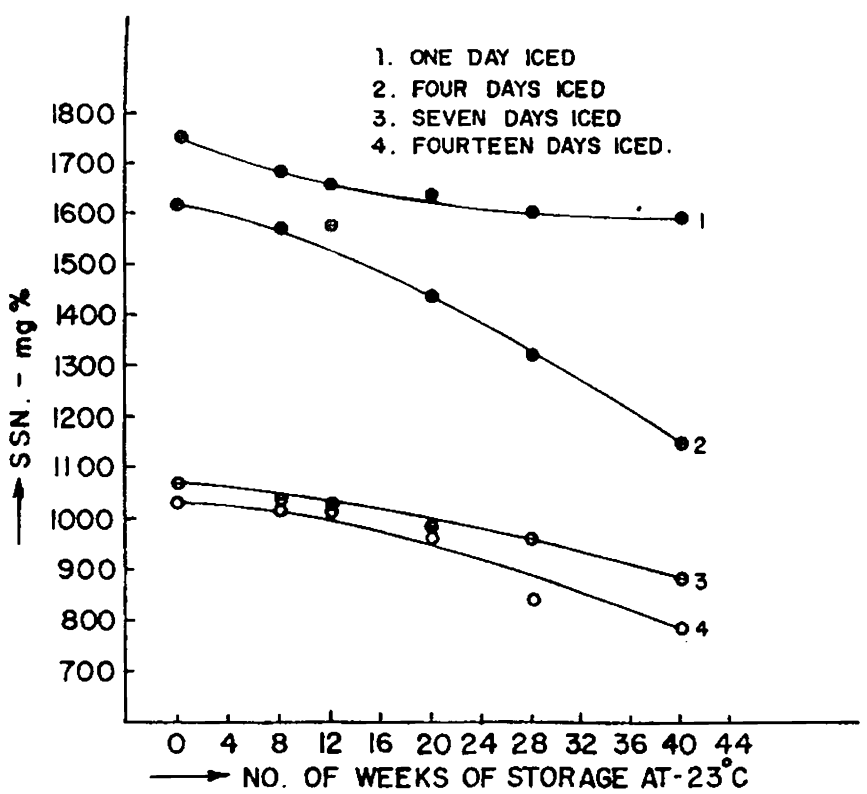


Fig. 31. EFFECT OF PREPROCESS ICE STORAGE ON CHANGES IN SALT SOLUBLE PROTEIN NITROGEN OF FROZEN CRAB STORED AT −23°C.

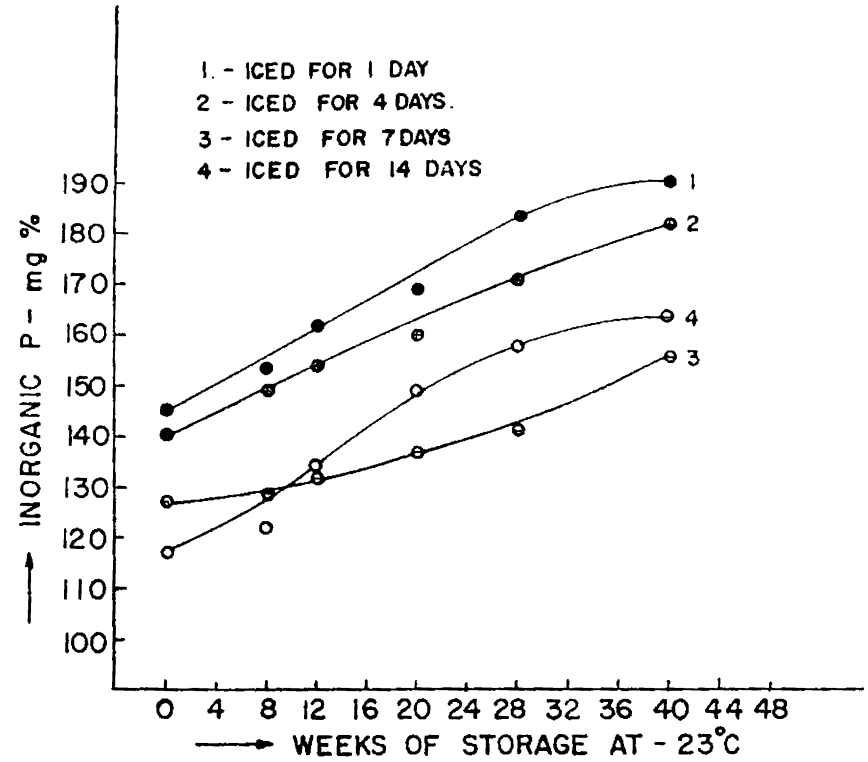


Fig. 32. EFFECT OF PREPROCESS ICE STORAGE ON CHANGES IN INORGANIC PHOSPHORUS IN CRAB MEAT HELD AT - 23°C.

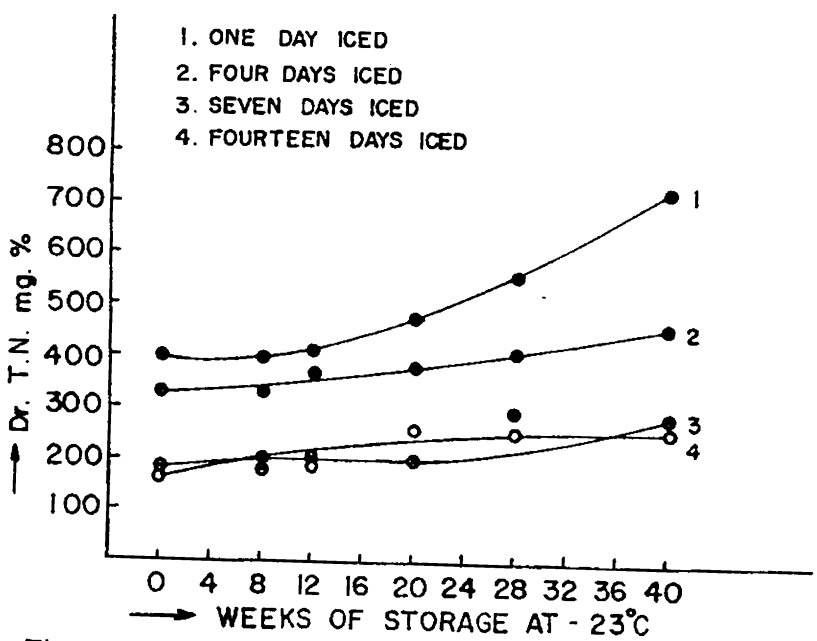


Fig. 33. EFFECT OF PREPROCESS ICE STORAGE ON NITROGENOUS CONSTITUENTS IN DRIP OF FROZEN CRAB MEAT HELD AT - 23°C

or increased strength of bonds between the constituent myofibrillar proteins.

Love (1970) stated that in frozen stored fish the properties of the myofibrillar proteins gradually changed, an uncoiling of the molecular helix leading to cross linking between the adjacent parallel molecules occurred.

Another possibility of actomyosin insolubilisation in fish held in frozen storage was due to free fatty acid accumulation in the muscle (Dyer & Fraser, 1959; Olley & Lovern, 1960; Olley et al., 1962; Boyd et al., 1967). In crab muscle, due to lesser amount of lipid content, this possibility cannot be fully applied.

It was proposed by Moritoshi Oguni et al. (1975) that during frozen storage actomyosin filaments form net-worked masses through aggregation and entangling and become increasingly insoluble with the growth in net work and particle size of masses.

There was a progressive increase in phosphorus (in) in all the samples during frozen storage. The decreasing trend observed during ice storage can be accounted for leaching loss by ice melt water. Sharp (1934 & 1935) reported that glycolysis can proceed in frozen fish muscle with the formation of lactic acid and freezing increases the rate of breakdown of high energy phosphate compounds notably adenosine triphosphate (ATP) and creatine phosphate (CP)

(Nowlan & Dyer, 1974). Partman (1963) found that ATP splitting in several species of freshwater fish was accelerated between -1 and -2°C. The present observation clearly indicated the liberation of inorganic phosphorus in frozen crab muscle during storage and this clearly agrees with the previous observations.

Nitrogenous constituents in drip of frozen stored samples having different pre-freezing ice storage period (Fig.33) showed that immediately after freezing the values recorded a steady fall in samples with respect to the length of ice storage period due to leaching of soluble nitrogenous constituents but during subsequent frozen storage a gradual increase in values was observed in all the samples. This observation was in agreement with the previous findings (Bethea & Ambrose, 1961; Gangal & Magar, 1963; Empey & Howard, 1954; Anon, 1966; Jun-ichi Nishimoto, 1962; Radhakrishnan et al., 1973 and Dyer et al., 1968).

The organoleptic rating as judged by taste panel assessment is given histographically (histogram 1). As could be seen, frozen storage quality has a direct bearing on the pre-freezing ice storage. One day iced material remained in good condition for about 50 weeks while 7 days iced material had a shelf-life of only about 21 weeks and 14 days iced material about 8 weeks.

4.1.7 Comparative efficiency of different glazes in the preservation of frozen crab meat

Glazes may be defined as a continuous thin film or coating that adheres closely to the product. The most widely practiced method to overcome or retard the deleterious changes due to frozen storage has been to give the product an ice glaze by dipping or spraying the frozen fish in cold water. Ice glaze still remains to be the only procedure of commercial importance inspite of its susceptibility to cracking, its brittleness and high vapour pressure. Although water is used generally as glaze, because of its cheapness and ease of availability, glazing by harmless chemical solutions have been reported to be distinctly advantageous in prolonging storage life of fish (Pawar & Magar, 1966) and shellfish (Strasser & King, 1971; Tarr et al., 1950; Feiger et al., 1956).

Table 11 gives the analytical data on crab meat frozen with different chemical glazes such as water, a 1% solution of ascorbic acid, citric acid, ascorbic-citric acid mixture (1:4), sodium bisulphite, disodium salt of ethylene diamine tetra-acetic acid (EDTA) and glycine and stored at -23°C.

Solubility of protein in Dyer's buffer was followed even though the pH of the muscle changed according to the nature of the chemical glaze. The values indicated a

decreasing trend in all the samples but the rate of fall was less in ascorbic-citric acids and glycine glazed samples.

The thawed yield (Fig.34) and nitrogenous constituents in drip did not show appreciable difference among samples initially, but on prolonged storage marked changes were found to occur. Ascorbic-citric acids and glycine glazes are effective for reducing the drip loss during cold storage.

Fig.35 depicts the change in meat colour during frozen storage. Only in ascorbic-citric acid glazed sample the characteristic colour was retained up to 420 days (60 weeks), in others brown discolouration and in some samples blackening developed by this time.

Histogram (2) gives an account of the shelf-life with respect to flavour - maximum shelf-life was obtained in the case of ascorbic-citric acid glazed samples and the next was glycine glazed.

Greig et al. (1967) observed that ascorbic acid was found to be more effective than propylgallate, monosodium glutamate or sodium tripolyphosphate. The effectiveness of ascorbic acid or ascorbic-citric acids mixture as glaze or dipping solution was proved for preventing discolouration in fish, black spot formation in shrimp, inhibition of growth of the natural mixture flora at ambient temperature (28°C) and at -18°C and for improving the general quality

Table 11. Effect of glazes on frozen shelf-life of crab meat at -23°C

Weeks of storage	Control	Ascorbic acid	Citric acid	Ascorbic + citric acid	Sod. bisulphite	EDTA	Glycine
1. Solubility of protein as % T.N.							
0	86.9	87.2	86.8	86.8	87.2	87.2	86.9
8	84.2	85.8	84.6	87.0	84.8	85.1	86.4
24	76.8	81.0	81.7	81.7	78.0	78.2	82.8
40	66.0	71.0	79.6	79.5	65.6	74.8	78.3
50	61.8	65.8	66.6	70.0	63.8	69.5	73.6
60	56.0	62.3	61.0	67.8	62.1	59.4	66.9
2. Thawed yield							
0	99.0	99.0	99.0	100.0	99.0	99.0	100.0
8	98.0	99.0	99.0	100.0	98.0	98.0	100.0
24	94.0	94.0	97.0	99.0	96.0	96.0	99.0
40	86.0	91.0	95.0	97.0	93.0	92.0	96.0
50	79.0	88.0	92.0	93.0	89.0	88.0	93.0
60	76.0	81.0	82.0	85.0	79.0	79.0	85.0
3. Nitrogenous constituents in drip mg %							
0	9.0	7.0	12.0	9.0	14.0	14.0	9.0
8	42.6	14.0	18.0	10.7	28.7	33.0	13.7
15	93.9	28.0	31.0	32.0	50.0	50.0	39.0
25	212.0	82.0	62.0	54.0	112.0	112.0	42.0
35	307.0	94.0	92.0	78.0	198.0	202.0	57.0
40	357.0	142.0	214.0	112.0	262.0	260.0	119.0
50	413.0	262.0	282.0	218.0	302.0	313.0	289.0
60	511.0	333.0	331.0	301.0	389.0	398.0	319.0

Note: Samples were kept as 2 kg frozen blocks. One block was analysed per sampling.

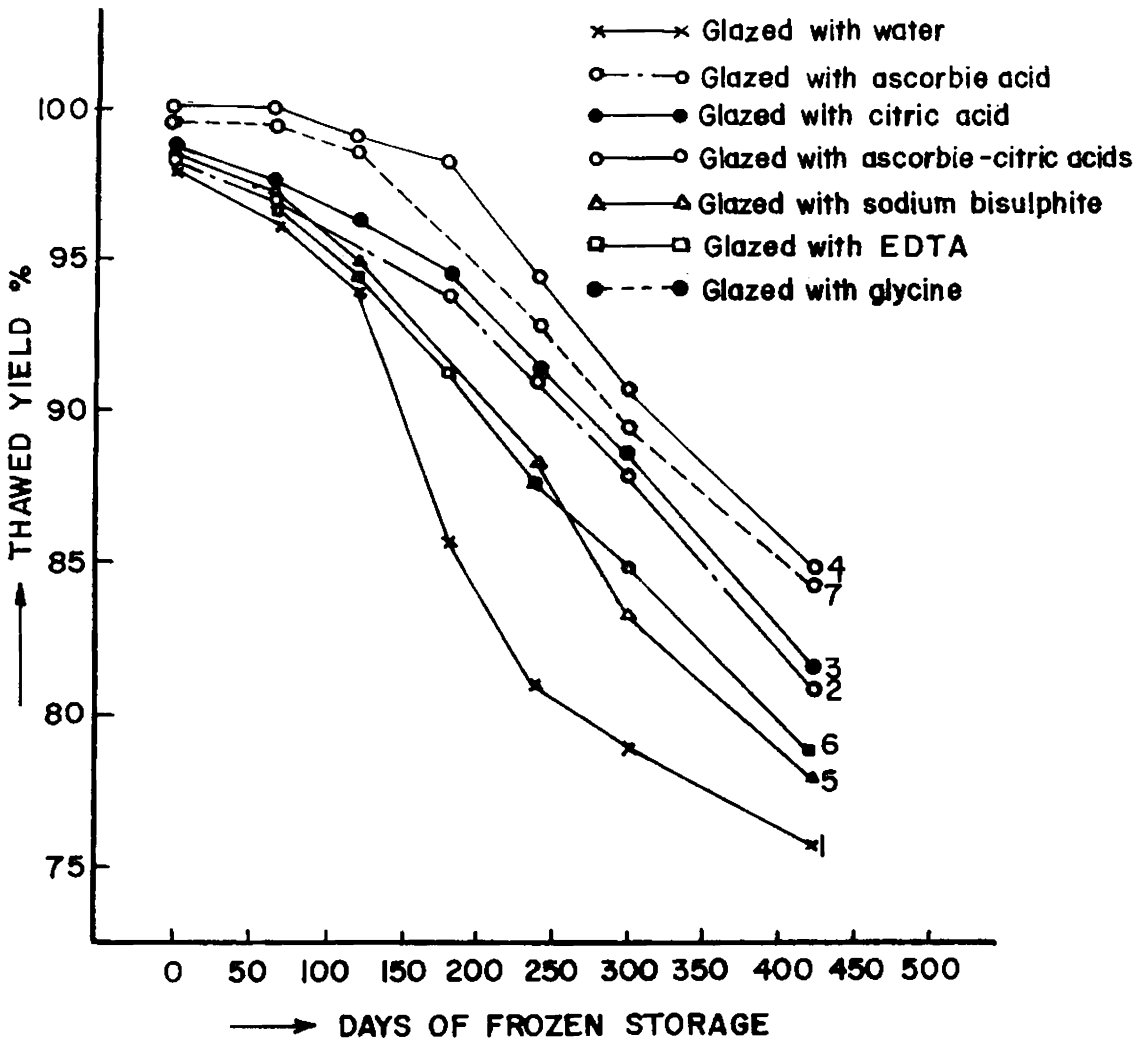


Fig. 34. THAWED YIELD

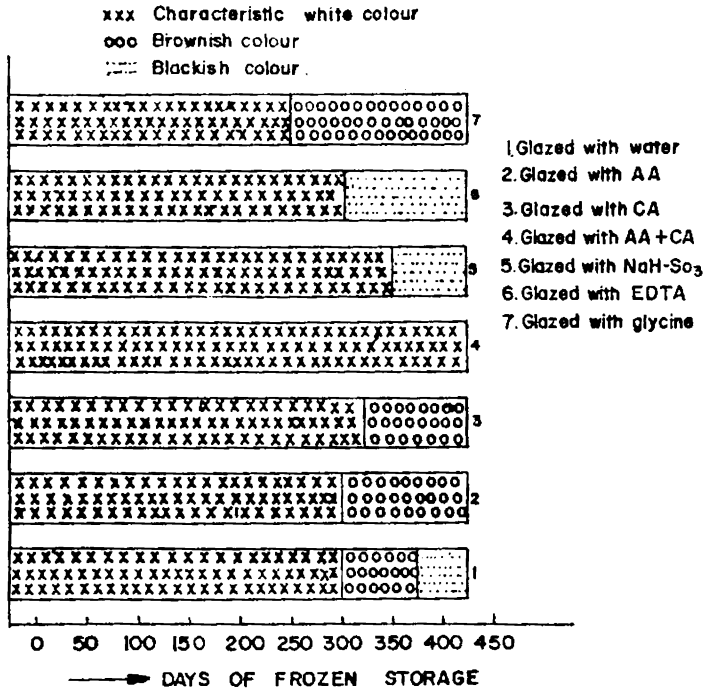
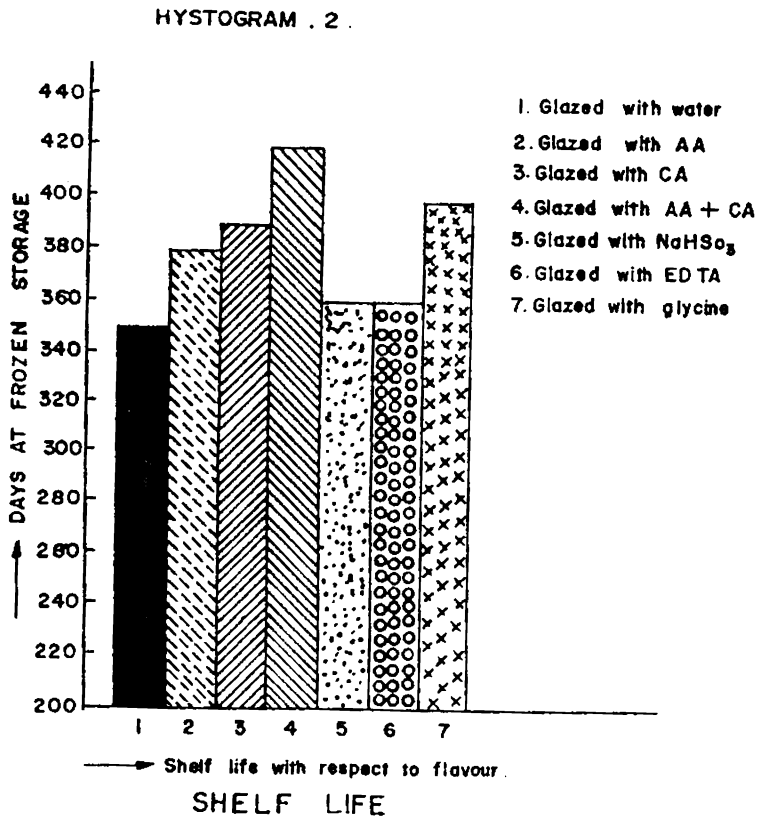


Fig. 35. CHANGE IN MEAT COLOUR



SHELF LIFE

of frozen stored fishery products (Jadav & Magar, 1970; Bailey & Fieger, 1954; Shaikhmahamud & Magar, 1965; Fieger et al., 1956; Tressler, 1957; Tanikawa, 1971 and Sawant & Magar, 1961). Sodium bisulphite was found to be effective for retarding melanosis up to 10 days in prawns kept at 5 and -1°C but it was not as effective as antibiotics or ascorbic-citric acid mixture in arresting the bacterial growth (Shaikhmahmud, 1965).

Previous work on the use of ethylene diamine tetra acetic acid (EDTA) or glycine as glazes for frozen fishery products was scanty.

The use of different glazes was distinctly advantageous in extending the shelf-life of frozen crab meat. Blackwood et al. (1969) disagreed to the use of acids (citric) as dip to crab meat prior to freezing since it adversely affects products flavour, texture and drained weight.

4.1.8 Studies on cooked frozen crab meat

Generally, the consumers prefer cooked meat to raw material in the shell because the procedure involved in preparing and cooking the latter is troublesome. Further more, if the crabs are not cooked, shelling is difficult and this results in lower yields. The present study was aimed at working out the processing conditions for the preparation of cooked frozen crab meat and further the storage

Table 12 Effect of cooking on yield of crab meat (Scylla serrata)

	Cooking time min	Cooking loss gm%	Percentage yield gm%	Appearance	Thawed yield gm%
Crab body	10	10.01	20.00	Under cooked	
	15	13.15	18.41	Properly cooked	91.8
Average:			16-18%		
	20	14.35	14.38	Over cooked	
Crab claw	15	22.51	19.72	Properly cooked	92.6

Table 13(a) Effect of icing and cooking on frozen storage characteristics (organoleptical) of crab meat (Scylla serrata)

Frozen storage weeks	Ice storage days							
	Series A				Series B			
	1	3	7	9	1	3	7	9
1	10	9	8	8	10	9	8	7
4	10	9	8	7	10	9	7	6
12	9	8	6	5	10	8	5	5
18	9	8	5	5	9	8	5	5
24	7	5	3	2	8	7	4	4
34	6	4	2	1	7	5	2	2
42	5	2	0	0	6	4	1	1
46	-	-	-	-	5	3	1	0

Series A = cooked and iced; Series B = iced and cooked

Score = 0 to 10 scale

Note: Table 12. Values represent average of 6 series.

13. Average of 2 series (1978, 1979).

Table 13b. Loss of nitrogenous constituents and thawed weight in the cooked frozen crab (Scylla serrata) due to freezing and subsequent storage

Ice storage	Frozen storage	Water extractable nitrogen (g %)		Non-protein nitrogen (g %)		F.A.-amino nitrogen (g %)		Weight (g %)	
Days	Weeks	A	B	A	B	A	B	A	B
1	1	24.5	20.8	18.4	19.1	19.7	17.0	11.4	8.8.
	4	25.2	22.0	20.7	20.9	22.8	19.0	15.0	10.0
	12	25.8	30.0	26.2	25.3	28.3	22.0	19.4	15.0
	24	29.6	30.7	26.6	24.5	28.7	28.1	20.3	17.5
	42	30.4	33.5	31.0	31.1	30.7	30.5	23.9	19.3
3	1	25.1	24.1	19.8	24.2	23.7	20.5	14.5	9.8
	4	25.6	28.3	25.7	24.0	28.0	21.7	19.4	12.0
	12	28.2	34.5	25.1	29.7	28.4	26.7	23.0	14.8
	24	30.2	32.6	27.3	29.9	30.5	31.9	25.2	18.5
	42	29.2	33.8	29.1	31.9	33.4	29.8	26.0	19.3
6	1	25.8	26.1	25.0	24.9	24.1	23.5	16.9	11.1
	4	28.4	28.7	25.6	25.8	28.5	26.7	20.0	13.9
	12	33.4	31.0	33.4	30.2	30.5	31.9	23.0	17.5
	24	33.9	32.2	33.9	28.4	36.3	32.8	25.0	19.1
	42	32.8	35.1	34.5	31.9	37.6	34.5	30.4	22.7
9	1	26.5	32.5	26.5	34.5	27.5	29.5	17.9	13.9
	4	29.2	37.7	26.0	36.0	30.2	32.8	20.4	15.3
	12	30.3	40.8	28.5	39.7	35.3	39.1	25.2	17.5
	24	31.8	42.0	34.0	39.9	41.4	43.8	30.4	26.2
	42	34.0	41.6	41.2	40.7	43.5	43.5	33.5	28.7

A = cooked and iced; B = iced and cooked

Note: For details of sampling refer table 13(a).

characteristics and nutritive value of the frozen products processed from raw material previously subjected to preservation by icing.

Large scale trials carried out on crabs showed that the optimum cooking time was 15 minutes in boiling water or steam at atmospheric pressure. The yield of body meat was 16-18% and that of claw meat about 20%. Average Thawing loss of frozen body meat was 6-12% and that of claw meat 6-8% (Table 12).

Table 13(a) depicts the organoleptic ratings of cooked frozen crab meat during storage. Shelf-life in the case of crab iced for one day prior to freezing is 42 weeks for series A samples (cooked and iced prior to freezing) and 46 weeks for series B samples (iced then cooked). Discolouration and hardening of texture are the limiting factors in the organoleptic acceptability. Shelf-life was having a bearing on pre-process ice storage; as the length of ice storage advanced there was progressive decrease in frozen storage shelf-life.

It is a known fact that ice stored material does not have the flavour characteristics of fresh material due to leaching out of important flavour constituents and accumulation of the products of bacterial spoilage.

Anon (1966) recommended cooking time for crab as 13-15

minutes in steam at 212°F and Backwood et al. (1969) suggested optimum pre-cook time for queen crab as 7 minutes in boiling freshwater.

Gangal & Magar (1963) observed more rapid loss of nutrients in cooked frozen crab meat (Scylla serrata) compared to raw frozen sample and Blackwood et al. (1969) reported that cooked brine frozen and glazed sections can be held for 10 months at -10 to -15°F storage without detectable loss in quality.

Discolouration was quite negligible in iced and cooked lot; in cooked and iced lot even during ice storage browning and blackening developed. Gangal & Magar (1963) observed toughness and loss of nutritive value in cooked frozen crab. The probable causes of discolouration are sugar-amino reactions, oxidation of pigments, copper-ammonia complex, formation of copper sulphide (CuS) or iron sulphide (FeS), and melanin (Oshima 1932; Groninger 1964; Melvin E.Waters 1964; Eiichi Tanikawa 1971; Tarr 1954, 1966). But Bailey, Fieger & Novak (1960) indicated that melanin was probably not a cause for blueing in heat processed blue crab since they found that tyrosinase activity was absent from the blood of blue crab and Groninger & Dassow (1964) found that king crab meat discoloured blue had similar properties to those of copper proteins and biuret complexes.

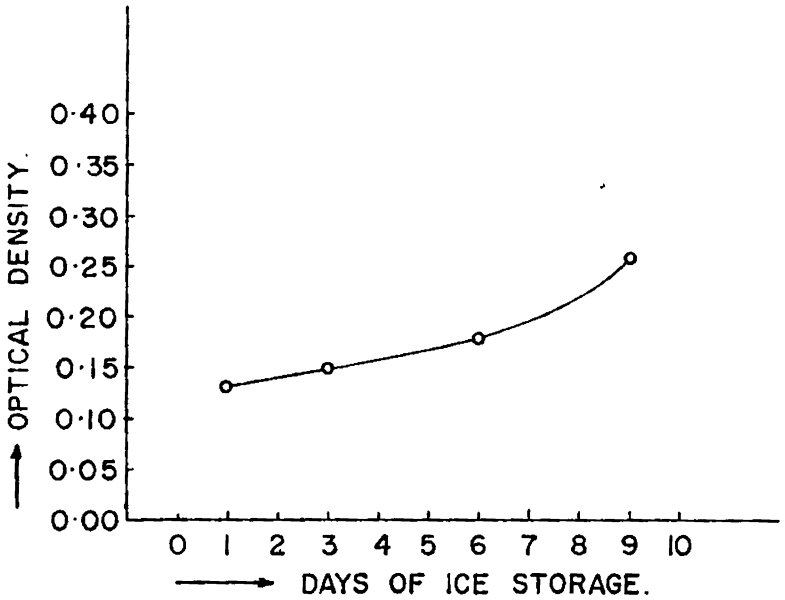


Fig. 36 COLOUR INTENSITY IN MEAT OF COOKED AND ICED CRAB AFTER 24 WEEKS OF COLD STORAGE.

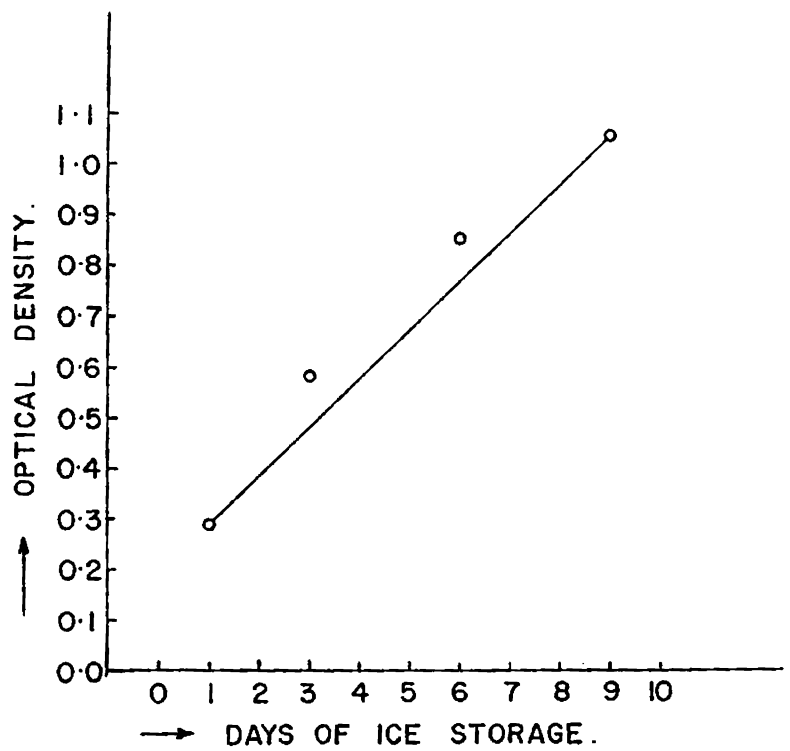
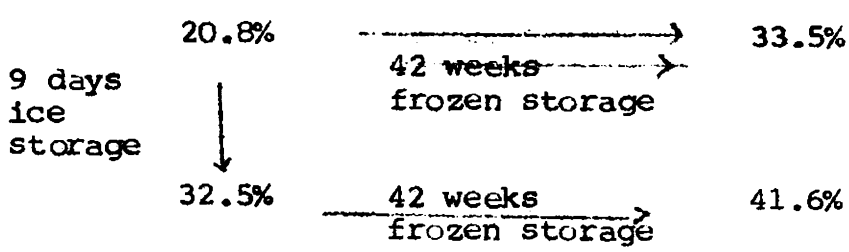


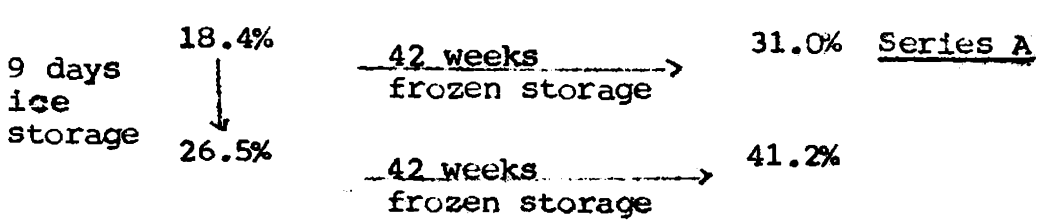
Fig. 37. COLOUR INTENSITY IN DRIP OF COOKED AND ICED CRAB AFTER 24 WEEKS OF COLD STORAGE.

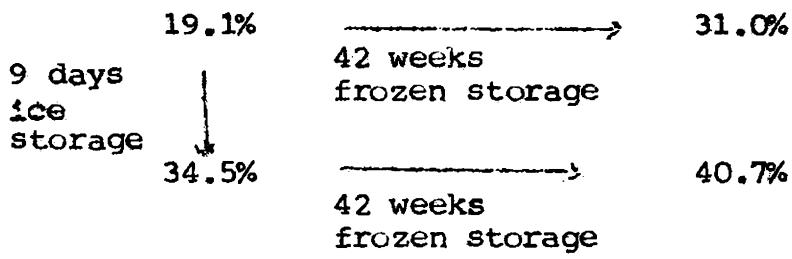
Figures 36 & 37 show the optical densities of the acetone extracts of meat and drip of crab after 24 weeks of cold storage. It is clear that the discolouration shows an increasing trend with progressive pre-freezing ice storage and change is marked in the case of meat after 7 days of ice storage.

Table 13(b) gives an account of the loss of water extractable nitrogen (WSN), non-protein nitrogen (NPN), free alpha-amino nitrogen (α-NH_2N) and thawed weight of cooked frozen crab during freezing and subsequent storage up to 42 weeks. The percentage loss of WSN, NPN, α-NH_2N and thawed weight showed steady increase in both series with comparatively less loss in series B. Loss of WSN varied from 24.5 to 26.5% in one day iced material and that iced up to 9 days initially, it increased to 30.4% and 34.0% after 42 weeks of frozen storage (Series A). The corresponding values in the case of B Series were



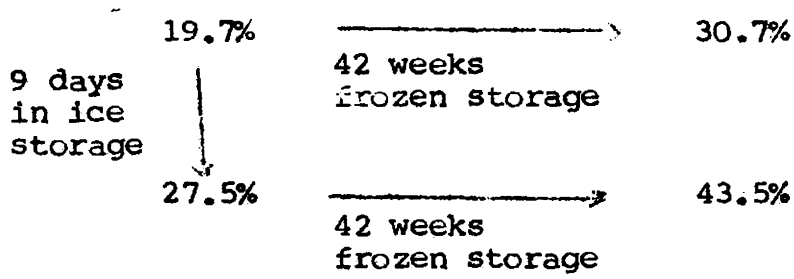
The loss of NPN in both series was as shown:



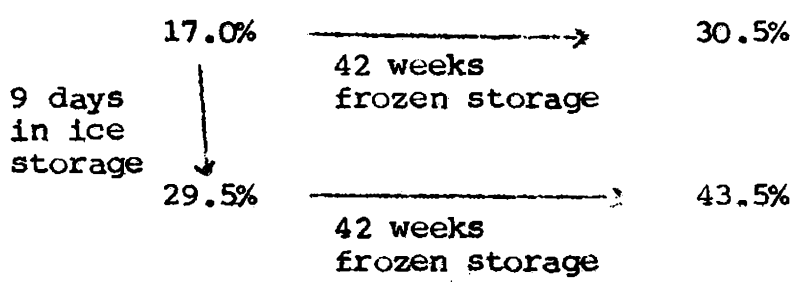


Series B

The loss of free alpha amino nitrogen in the two series was more or less same and it was as shown:



Series A



Series B

The loss of characteristic flavour may be attributed to the loss of water soluble flavour bearing constituents. There was a steady increase in weight loss of crabs of both series and the loss was more in the case of series A samples as shown in the table.

Iyer et al. (1969) observed in the studies on cooked

frozen prawns that the loss of constituents such as WSN, NPN & α-NH_2N during ice storage for 12 days was more than the loss taking place during frozen storage of the material for 6 months at -20°C.

Comparative studies on changes in quality during freezing (slow, with water glaze and packaging) and storage in crab both raw and precooked, frozen as meat and with inner shell on and claw with shell on were undertaken and the results are represented in Tables 14(a) and (b).

Cooking loss was found to be 10% in whole crabs, 16% in outer shell (carrapace) removed lots and 22.5% in claw. Yield was found to be 26.4% in raw crab, 19.7% in pre-cooked crab, 51.6% in raw claw and 44.5% in pre-cooked claw.

The solubility of protein in Dyer's buffer was found to be less in pre-cooked samples because of heat denaturation. Negligible change occurred in the soluble protein nitrogen values in cooked samples during storage, on the other hand in raw frozen samples the values showed considerable reduction during storage i.e 2252-1470; 2253-1500 and 1726-1100 mg nitrogen % in raw shellon, raw meat and raw claw respectively.

Free alpha amino nitrogen values showed wide variation between raw and pre-cooked samples; the loss can be accounted for loss due to cooking. During frozen storage in both raw and pre-cooked samples gradual reduction was found in

Table 14a. Frozen storage (-18°C) characteristics (Biochemical) of raw and cooked crab and claw

Frozen storage weeks		Moisture g %	Protein g %	SEN mg%	F$\ddot{\text{A}}$-NH_2N mg%	Thawed yield g%	TN in drip mg %	Drip colour
0	1	82.60	15.50	2252	285.4	96.80	161	Grey
	2	80.98	17.30	650	138.5	92.51	280	Cloudy white
	3	81.50	16.16	2253	309.0	93.85	210	Grey
	4	80.70	17.68	670	181.5	86.50	397	Cloudy white
	5	81.30	15.40	1726	286.4	96.80	38	Grey
	6	79.50	17.96	690	224.5	97.50	21	Pink
10	1	83.05	15.05	2218	283.6	94.06	244	Grey
	2	80.78	17.24	546	136.6	90.93	392	Cloudy white
	3	80.56	16.36	2253	301.0	90.84	297	Grey
	4	80.07	17.42	616	171.5	80.81	508	Cloudy white
	5	80.81	15.23	1526	276.6	93.20	41	Grey
	6	78.27	17.76	658	220.6	94.64	25	Pink
20	1	82.28	15.05	1952	246.4	94.62	256	Grey
	2	81.28	16.27	854	120.4	90.20	323	Cloudy white
	3	80.95	15.75	1960	260.4	95.98	192.5	Grey
	4	79.46	17.15	742	100.8	75.08	613	Cloudy white
	5	82.33	14.43	1232	212.8	87.54	132	Black
	6	78.72	18.46	686	198.8	95.01	25	Pink
30	1	82.10	15.16	1652	244.1	93.65	270	Grey
	2	81.05	16.72	840	124.5	91.10	365	Cloudy white
	3	80.60	15.96	1700	259.0	90.00	230	Grey
	4	78.80	17.85	792	102.0	76.80	670	Cloudy white
	5	81.80	15.10	1142	208.0	88.10	152	Black
	6	78.40	18.96	672	196.2	93.50	31	Pink
40	1	81.90	15.76	1470	240.5	93.10	291	Grey
	2	80.70	17.05	810	116.5	90.08	404	Cloudy white
	3	80.50	16.10	1500	254.5	89.50	284	Grey
	4	78.50	17.50	790	99.8	75.40	690	Cloudy white
	5							
	5	81.40	15.90	1100	206.5	87.00	171	Black
	6	78.60	18.70	650	198.5	93.10	51	Pink

1 - raw shellon crab; 2- cooked shellon crab; 3 - raw crab meat, 4 - cooked meat; 5 - raw claw; 6 - cooked claw

Note: Frozen samples as 2 kg blocks.

Table 14b. Organoleptic characteristics of frozen crab and claw (Scylla serrata) both raw and cooked

Frozen storage weeks		Colour	Texture	Flavour
0	1	Good	soft and firm	Good
	2	Good	"	G - F
	3	Good	"	Good
	4	Good	"	Good
	5	Characteristics grey	Firm	Good
	6	Pinkish	Firm	Good
10	1	Good	Soft & firm	Good
	2	Good	"	G-F slight loss of sweetness
	3	Good	"	G-F slight loss of characteristic sweet flavour
	4	Good	Firm	G-F characteristic flavour
	5	Good	Firm	F slight loss of characteristic flavour
	6	Good	Firm	"
20	1	G - F	Soft & firm	G - F
	2	F	Slight hard	F
	3	F - white	Soft	G - F
	4	Slight yellow	Fibrous	F
	5	Slight black	Firm	G - F
	6	Pinkish	Slight tough	G - F
30	1	G - F	Soft & firm	G - F
	2	F	Slight hard	F
	3	F - white	Soft	G - F
	4	Slight brownish	Fibrous	F
	5	Black	Granular	F
	6	Pink	Granular	F
40	1	Fair	Soft	G - F
	2	F - P	Slight hard	F
	3	F	Soft	F
	4	FP sl. brown	Fibrous	F - P
	5	Black	Granular	F
	6	Pink	Slight tough	F - P

1 - raw shellon crab; 2 - cooked shellon crab; 3 - raw crab meat; 4 - cooked crab meat; 5 - raw claw; 6 - cooked claw; G-F = good to fair; F = fair

amino nitrogen values, but the rate was more in pre-cooked samples. The nitrogen content in drip of all samples recorded a steady increase during storage. Drip loss was minimum in claw owing to the protection by the shell and maximum in pre-cooked crab meat. The rate of decrease in thawed yield also was more in pre-cooked crab meat. In 20 weeks time the drip colour of raw claw was changed to black, in others much discolouration was not observed.

The organoleptic characteristics (Table 14b) showed that the raw frozen samples retained much of the flavour bearing constituents; in raw claw the characteristic colour of the pigment was changed to slight black after 20 weeks storage, corresponding to the change in the drip colour. Gangal & Magar (1963) observed more loss of nutrients in cooked frozen crab meat than fresh muscle and solubility of protein was reduced considerably in cooked frozen samples. This finding was in confirmity with the present observation. According to Sayre & Briskey (1963) the muscle protein solubility appeared to be one of the major factors affecting the juice retaining properties of the muscle.

The sensory methods likely to remain the sensitive way of measuring freshness, the chemical tests studied such as, protein solubility, thawed yield and drip loss also had a role. In 40 weeks time the shell on crab remained as good to fair; and the cooked meat was graded as fair to poor (border

line of acceptability). In raw claw, even though the pigment colour was changed from grey to black, the characteristic flavour was retained more than that of cooked claw.

In cooked crab meat, slight browning was observed during storage probably due to sugar-amino reactions. Tarr & Bisset (1954) and Jones (1962) reported about the possibility of sugar-amino reactions in processed fishery products leading to unpleasant taste and smell.

4.1.9 Studies on canning of crab meat

There is a good world market for canned crab meat, but India's contribution to International markets for this commodity is insignificant owing partly to lack of technological know-how. This study was aimed at working out the processing conditions for the preparation of good quality canned product.

Gangal & Magar (1967) observed that the technique of preparing and handling the crab meat affects the quality of the canned product considerably. Each stage of operation under canning process has to be done with great care. Even the small pieces of cartilages are to be removed in order to protect the delicious taste of the meat. Canning conditions are to be standardised, blanching and sterilization - in order to get the finished product with good organoleptic quality and correct drained weight. Blanching removes gases, saturates the tissues with water, inactivates the enzymes

and minimises discolouration. Excess or less blanching results in short weight of the finished product.

Since sterilisation implies complete destruction of all living organisms and this condition may not be attained in some processed foods, the term commercial sterilization has been introduced in the canning industry. Prolonged heating at high canning temperatures almost invariably cause greater losses of colour, flavour, texture and vitamins and other nutritive value properties of canned foods than do relatively high temperature short time methods. For practical purposes a pH of 4.5 is taken as the point above which food products should be processed under steam pressure to a degree sufficient to destroy all food poisoning bacteria, i.e 10 lbs pressure for 20 minutes.

Table 15(a) represents the hot blanching of crab carcasses in different concentrations of brine and at different time intervals and found that 7 or 8 minutes in 7% brine, 6 minutes in 8% brine, 5 minutes in 9% brine, or 4 minutes in 10% brine and subsequent heat sterilization of the picked meat in 2.5% brine containing 0.1% citric acid will give a product ~~with~~ bauing minimum loss in drained weight, ~~with~~ acceptable organoleptic qualities and negligible ~~celudiness~~ cloudiness in the filling medium.

The main defects in the crab canning industry are the

Table 15a. Effect of salt concentration and blanching times on canning of crab meat (Scylla serrata)

Blanching condition		Salt concentration in brine		Moisture content		Difference in packed and drained weight	Nature of brine	Organoleptic quality of the product
Concn. of brine (%)	Time of blanching min.	before sterilization g%	after sterilization g%	before blanching g%	after blanching g%	g%		
5	8		1.870		79.09	5.9	Sl. colloidal	Good
	9	2.5	1.872	84.35	77.52	4.68	"	"
	10		1.931		77.30	4.00	"	"
	12		1.989		75.26	3.20	"	"
7	5	2.5	2.018	84.35	80.56	5.1	"	"
	6		2.135		80.13	3.4	"	"
	7		2.341		79.16	0.39	Clear	"
	8		2.341		79.16	0.40	"	"
8	6	2.5	2.390	83.80	79.66	0.20	"	"
9	5	2.5	2.410	84.05	79.29	0.10	"	"
10	3	2.5	2.340	84.35	78.57	2.80	"	"
	4		2.420		78.54	0.0	"	"
	5		2.486		77.76	+4.00	"	"

Note: Outer shell removed. Crab segment taken and blanched. Number of samples blanched 50 pieces/each concentration of brine.

discolouration and flavour changes occuring in the canned product on storage (Groninger & Dassow, 1964; Blackwood et al., 1969; Varga et al., 1969; Melvin E. Waters, 1970; Edwards & Early, 1972; Gangal & Magar, 1967; Farber, 1953; and Tanikawa, 1971). These changes according to Fellers & Harries (1940) result largely from the breakdown of the rather unstable proteins under the influence of heat. Ammonia and volatile sulphur compounds liberated in the meat during canning mightreact with copper and iron present in sufficient quantity in the meat to produce blue or black complexes, producing a discolouration.

Blackwood et al. (1969) and Dewberry (1970) recommended fractional cooking to remove the blood and hence the formation of blueing can be minimised. Farber (1953) suggested 4 factors to be followed in order to get canned crab meat with characteristic organoleptic qualities. Complete removal of blood and dipping the picked meat in a slight acid solution (citric, lactic, acetic or tartaric) was recommended for the prevention of blueing. Dewberry (1959) and Deberry E.B. (1970) suggested that successful crab meat canning depends on the use of live, healthy, vigorous crabs, prompt butchering, cleaning, processing in a hygienic condition and cooling and use of stainless steel, aluminium or monel metal for handling of the meat.

Many crab meat processors suggested rinsing the meat

Table 15(b) Analytical data of canned crab meat (Scylla serrata)

Can exterior	Good
Can interior	Good
Nature of brine	clear, some claw pigment is dissolved in brine
Organoleptic quality of the canned product	characteristic crab meat flavour, still claw meat is better
Protein meat (g %)	21.17
brine (g %)	3.8
Free alpha amino nitrogen (mg%) in brine	121.65
Glycogen (mg%) in brine	46.44
Phosphorus (mg%) in brine	42.8
Rebose (mg%) in brine	89.0

Note: No. of cans analysed 5.

with an acid solution to prevent discolouration, but Gangal & Magar (1967) did not agree with this because of the leaching of soluble flavour bearing constituents. Use of lacquered cans or vegetable parchment lining to the cans to avoid contact of the meat are widely recommended.

In the present studies also the parchment lining was found to be quite essential to maintain the colour and flavour of the product.

Table 15(b) gives the analytical data of canned crab meat. It is seen that loss of nutrients in brine to the extent of protein (3.8%); amino nitrogen (121 mg%); glycogen (46 mg%); phosphorus (43 mg%) and ribose (89 mg%) occured in the canned product.

4.2 Mussels (Perna viridis)

4.2.1 The morphometric and weight measurements

Table 16 represents the size weight measurements of mussel Perna viridis. The result was statistically analysed.

For the Perna viridis mussels, two categories were tested namely (a) wild and (b) cultured. The height of the wild variety ranged from 7.8 to 11.1 cms whereas the cultured variety was in the range 3.1 to 9.1 cms.

Table 16 Size to weight ratio of mussels (Perna viridis)

(a) Wild

	Height (cm)	Length (cm)	Ratio	Whole wt. (gm)	Wt. of meat (gm)	Ratio
1	10.8	5.0	2.16	61	19.0	0.31
2	11.1	4.6	2.41	76	16.0	0.21
3	10.9	4.9	2.22	74	19.5	0.26
4	9.2	4.0	2.30	47	12.0	0.25
5	9.0	4.0	2.22	38	10.0	0.26
6	8.90	3.8	2.37	36	9.0	0.25
7	8.45	3.8	2.22	39	12.0	0.30
8	8.10	3.5	2.28	27	9.0	0.33
9	7.80	3.8	2.05	31	10.5	0.33

(b) Cultured

	Height (cm)	AV	Length (cm)	Ratio	Whole weight (g)	AV	Meat wt. (g)	AV	Ratio
1	9.1		4.1	2.21	135		58		0.429
	9.1		4.0	2.27					
	9.1	9.1	4.0	2.27		45		19.3	
2	8.0		3.7	2.16	109		41.5		0.38
	8.1		3.7	2.19					
	8.1	8.1	3.6	2.25		36.3		13.8	
3	7.6		3.8	2.00	85		36.5		0.429
	7.2		3.3	2.18					
	7.6	7.6	3.5	2.17		28.3		12.2	
4	6.8		3.1	2.19	97		40.5		0.417
	7.1		3.3	2.15					
	7.2		3.1	2.32					
	7.0	7.1	3.3	2.12		24.3		10.1	
5	6.3		2.8	2.25	69		36.0		0.521
	6.9		3.3	2.09					
	6.6		3.0	2.20					
	6.3	6.5	3.0	2.10		17.3		9.0	
6	6.1				56		28.0		0.50
	6.0								
	6.0								
	6.2	6.1				14.00		7.0	

Table contd.

Table 16 contd.

Height (cm)		AV	Whole weight (g)	AV	Wt of meat (g)	AV	Ratio
7	5.7		153	12.8	78	6.5	0.509
	5.9						
	5.9						
	5.7						
	5.7						
	5.7						
	5.6						
	5.7	5.7					
	5.6						
	5.6						
	5.6						
	5.6						
8	5.5		151.5	10.8	78	5.6	0.514
	5.5						
	5.5						
	5.4						
	5.4						
	5.4						
	5.5						
	5.5						
	5.5						
	5.2	5.4					
	5.5						
	5.4						
	5.3						
	5.3						
9	5.1		55	9.2	23.5	3.9	0.427
	5.1						
	5.1	5.0					
	4.9						
	4.8						
	4.9						
10	4.5		33.5	6.7	11.5	2.3	0.343
	4.6						
	4.1	4.4					
	4.5						
	4.2						
11	3.5		27.0	3.0	9	1.0	0.341
	3.4						
	3.0						
	3.5						
	3.1						
	3.1						
	3.2						
	2.9						
	2.0	3.1					

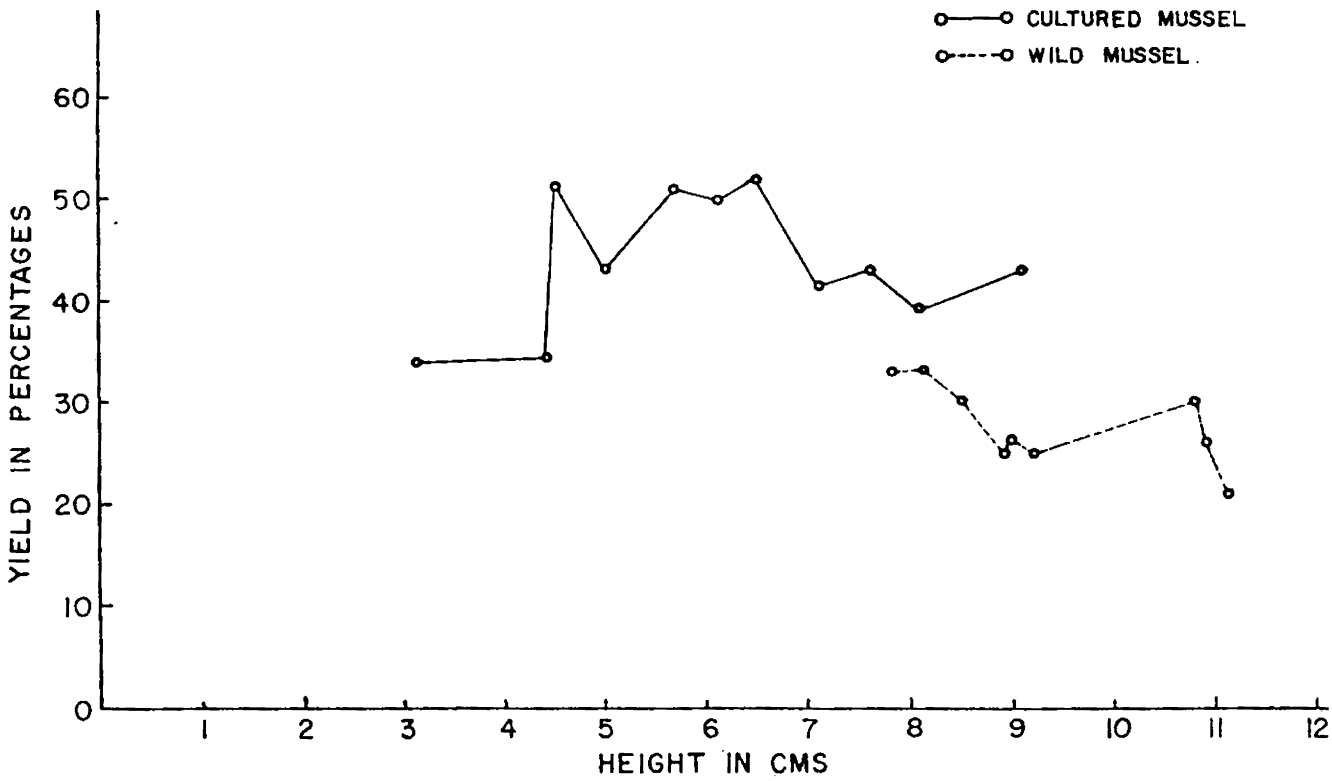


Fig. 38. YIELD OF PERNA VIRIDIS

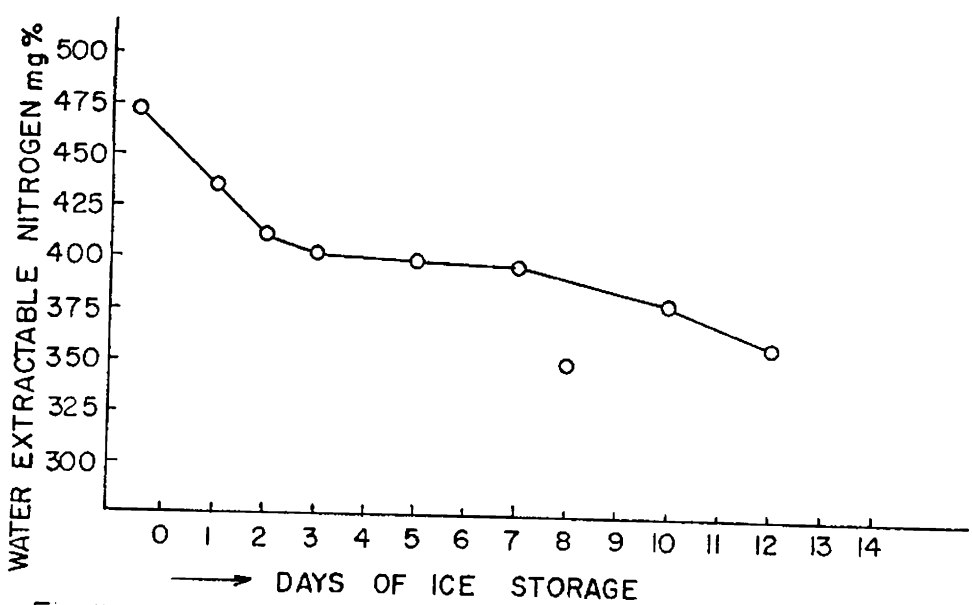


Fig. 39. WATER EXTRACTABLE NITROGEN IN MUSSELS HELD IN ICE

Given any height the corresponding meat weight can be obtained by the regression equation:

$\log W = -0.8178+1.9769 \log H$ for wild variety (1) and

$\log W = -1.3049+2.8385 \log H$ for cultured variety (2)

where W is the meat weight (gm) and H is the height (cms) of the mussel. The correlation coefficients were found to be $r = 0.87$ and $r = 0.78$ respectively and were highly significant ($P \leq 0.01$).

From Table 16 & Fig.38 it is observed that the yield percentage (i.e $\frac{\text{meat weight} \times 100}{\text{whole weight}}$) was generally in the range (25; 33) for the wild. There was a marked increase in yield in the case of cultured mussel compared to the wild. This yield percentage was noted bo be in the range (34, 53). Also the mussels of medium height (about 4.5 to 6.5 cms) were observed to have the maximum yield of about 50%.

Length-Height relationship

For Perna viridis mussel, length-height relationship was worked out for wild and cultured categories. The correlation coefficients were found to be $r = 0.94$, $r = 0.99$ for wild and cultured respectively. r values were highly significant ($P \leq 0.01$) indicating a linear relationship between height and length. The regression equations were:

1. $Y = 0.405+0.401\ X$ for wild

2. $Y = 0.141+0.435\ X$ for cultured

where Y is the length (cm) and X is the height (cms).

The relation between linear dimensions, weight and volume of shell in Meretrix meretrix and the probable influence of environmental factors were studied by Hamai (1934). He found that the growth of M. meretrix at different localities and also under different substrata is influenced by temperature, salinity and other parameters of the environment. Durve & Dharmaraja (1965) also noticed the influence of environmental conditions prevailing at the locality influencing the morphometry of M. casta (chemnitz). Galtsoff (1931) assigned variations in growth ratios of the pearl oyster Pinetada sp. to differences in their origin. Rao & Nayar (1956) studied the rate of growth in the backwater oyster Crassostrea madrasensis (Preston) from Adayar estuary. Richards (1946) studied the comparative growth rate in the green mussel Mytilus californianus and M. edulis from California and Woods Hole respectively.

4.2.2 Studies on biochemical characteristics of mussel meat

Table 17(a) gives the proximate chemical composition of mussel meat; 78.27% moisture, 12.80% protein, 2.24% fat and 2.72% ash are reported. Glycogen content is very high (8.53%) and less phosphorus (42 mg%). Protein and glycogen are the main solids in mussel meat.

Table 17a. Proximate chemical composition of Mussels (Perna viridis)

Chemical constituents	
1. Moisture (g %)	78.27
2. Protein N x 6.25 (g %)	12.80
3. Water soluble nitrogen (mg %)	560
4. Non protein nitrogen (mg %)	420
5. Free alpha amino nitrogen (mg %)	184
6. Total volatile nitrogen (mg %)	8.3
7. Glycogen (g %)	8.53
8. Ribose (mg %)	199
9. Total phosphorus (mg %)	42.3
10. Fat (g %)	2.24
11. Ash (g %) D.W.B.	2.72
12. Standard plate count S.P.C. colonies/gm	1.34×10^5
13. Escherichis coli/gm	76
14. Faecal streptococci/gm	279

Table 17b. Protein fractions of Mussel (Perna viridis)

Total nitrogen (TN) mg %	2291.9
Protein nitrogen (PN) mg %	2033.3
Non protein nitrogen as percent of TN	11.28
Sarcoplasmic protein as percent of PN	21.66
Myofibrillar protein as percent of PN	52.91
Nucleoprotein or protamines as percent of PN	1.48
Denatured protein as present of PN	22.80
Stroma protein as percent of PN	1.14

Note: Results are average of 2 estimations of 100 g comminuted meat.

The studies on commercial fishes from the Medeterranean sea and the Red sea by Herzberg & Rachel Pasteur (1969) revealed that these are high in protein and low in oil. Pandit & Magar (1972) reported that Sepia orientalis and Loligo vulgaris could be classed as high protein and low fat food and according to Anon (1959) halibut meat is low in oil and high in protein. From the results mussel meat is considered as medium protein and medium fat food.

According to Banks & House (1958) and Waterman (1969) seasonal variation influences the quality of the mussel meat considerably and are best in the autumn and winter months. Waterman (1969) reported the composition of edible portion of mussel meat as water 80%, protein 9-13%, fiat 0-2% and glycogen 1-7% in agreement with the present findings. The seasonal influence on quality or biochemical characteristics was not covered in this thesis. Sebastian (1970) reported the composition of the meat of Mytilus edulis as protein 10.18%, fat 1.64%, total carbohydrates 1.74%, water 83.27% and ash 1.99%. Here the carbohydrate content was less than that reported by other workers. Suryanarayanan & Alexander (1972) also reported lesser quantity of glycogen in mussel meat. Reay et al. (1943) had given the composition of mussel meat as water 84.1-84.4%, fat 0.8-2.3% and protein 8.9-11.9%.

Moorjani et al. (1962) studied the proximate composition of freshwater fishes, all are high protein and low fat

fishes. The composition of the white and red meat of tuna by C.George (1975) revealed that both contain higher percentage of protein (20.8 & 19.7%) and medium fat (1.23 & 2.22%) and moisture content comparatively less (72 & 71% respectively).

The composition of Chanos chanos by Jose Joseph et al. (1980) showed very high percentage of protein (24.06%) and medium fat (3.5%) and composition of oil sardine by Chinnamma George et al. (1982) showed high percentage of fat in it and fat content varies according to season.

According to Mannan et al. (1961) the factors which influence the composition of any particular species of fish were the abundance of food, spawning cycle, spawning migrations and age of the species. They reported the composition of halibut, mackerel, tuna and sword fish.

Table 17(b) represents the protein fractions of mussel meat. On the basis of total nitrogen (TN) 11.28% is non-protein nitrogen, and sarcoplasmic, myofibrillar, nucleo-protein, denatured proteins and stroma proteins are 21.66, 52.91, 1.48, 22.80% and 1.14% respectively on the basis of total protein.

Paul (1966); Baliga et al. (1962, 1969); Sayre (1968); Carpenter & Saffle (1965); Connell (1962); Sayre & Briskey (1963) and Yuji Maruyama & Taneko Suzuki (1968) isolated

individual or all the protein fractions from different fishes of sea water or freshwater and studied their properties.

The results obtained for mussel meat agreed fully with the results reported by Raymond Jacquot (1961).

Fraser et al. (1961) fractionated the albumin or myogen fractions of Canadian Atlantic fishes and found that the myogen fraction was constant (20-23%) in all the species except tuna where it was 31% of the protein nitrogen.

Reay et al. (1943) fractionated the proteins of haddock muscle. 85% of the protein was soluble in 7% lithium chloride solution, 18% myogen and 67% myosin were reported.

Moorjani et al. (1962) fractionated the proteins of 5 species of freshwater fishes. The sarcoplasmic proteins range from 29-35.7% and fibrillar protein from 65.0-59.7% and stroma protein from 6-4.6%. The non-protein nitrogen was found to be 10% of the total nitrogen in all the species studied.

Table 17(c) gives the free amino acid pattern of mussel meat, glycine, histidine, leucine, arginine, phenylalanine, glutamic acid, serine, methionine, proline, lysine and tyrosine constitute the major amino acid pool with threonine, cystine, valine in lesser concentrations and iso leucine and tryptophan in trace quantities. Mussel meat contains all the essential amino acids.

Table 17c. Free amino acids in mussel meat (Perna viridis)

Free alpha amino nitrogen (by Pope and Stevens method)	119.1 mg%
Amino acids	
1. Glycine	18.42 %
2. Proline	6.17 %
3. Serine	6.82 %
4. Phenylalanine	7.73 %
5. Arginine	8.60 %
6. Valine	0.746 %
7. Tryptophan	0.10 %
8. Methionine	6.25 %
9. Lysine	5.87 %
10. Histidine	12.15 %
11. Cystine	0.82 %
12. Leucine	11.04 %
13. Iso leucine	0.194 %
14. Tyrosine	5.84 %
15. Glutamic acid	6.94 %
16. Threonine	2.15 %

Table 17d. Composition of mussel juice

Days in ice	Volume per 100 gm meat	T.N. mg/100 gm	NPN mg/100 gm	Free alpha amino nitrogen mg/ 100 gm
1	29.78	221	112.0	26.0
2	20.96	213	151.5	45.5
4	19.35	189	138.7	42.4
6	19.82	255	99.9	37.53

TN = total nitrogen; NPN = non protein nitrogen

Note: Details of samples analysed ref. table 17a.

Suryanarayanan & Alexander (1972) studied the free amino acid pattern of mussel meat (Mytilus edulis) and reported the presence of phenylalanine, glycine, cystine, tyrosine, histidine, valine, lysine, methionine, glutamic acid, isoleucine, leucine, serine, tryptophan and prolinein agreement with the present observation.

The peculiar sweet flavour of mussel meat can be attributed to the glycogen content (8%) and free amino acid - glycine (18% of the total amino acids) and sarcoplasmic proteins (22%).

The nutrients lost per 100 gm mussel meat in the liquid collected in the mussel shell with progressive ice storage is depicted in Table 17(d). 221 mg% of total nitrogen (TN) 112 mg% of non-protein nitrogen (NPN) and 26 mg% of free alpha amino nitrogen (α-NH_2-N) were lost in the mussel liquor of one day iced sample. A clear pattern of change was not observed in any of the parameters studied during ice storage probably due to the leaching effect of ice melt water.

Table 17 (e) gives the relation between age (size) and chemical constituents in mussel meat. 11 size grades ranging from 2.0 to 9.1 cm height were subjected for the studies. Appreciable reduction in moisture content (5.4%) and slight increase in protein (2.4%) was observed as the size of the animal increases. A marked increase in glycogen

Table 17e Relation between age (size) and chemical constituents in Perna viridis - cultured

Height	Moisture	Protein Nx6.25	Alpha amino nitrogen	Glycogen	Ribose	P(in)	Fat
cm	gm %	gm %	mg%	gm %	mg %	mg %	gm%
2.0-3.5		13.48	96.2	0.758	188	27.7	
4.1-4.6		13.48	113.8	1.100	190	30.9	
4.7-5.1	82.64	-	126.9	1.32	214	30.5	1.27
5.2-5.5		12.95	148.8	1.138	256	34.8	
5.6-5.9	80.04	13.74	135.7	1.175	252	33.0	1.37
6.0-6.2		13.82	122.6	1.64C	200	32.0	
6.3-6.8		14.70	135.7	1.670	252	32.0	
6.9-7.2	78.84	14.70	122.6	4.32	350	30.5	1.37
7.2-7.6		15.23	131.3	4.09	330	30.5	
8.0-8.1	77.27	15.92	153.2	6.98	470	30.2	1.55
9.1		15.92	162.0	7.13	490	29.5	

Note: Results relate to 100 g composite meat sample per each size.

reserve in bigger sized mussels (0.75-7.13 gm%) and in ribose values also appreciable increase was noticed (i.e 188-490 mg%). Free alpha amino nitrogen also recorded an increasing trend in values. A systematic pattern was not seen in phosphorus (in) values. Fat content showed slight increase in bigger sized mussels.

Relation between size and chemical constituents were subjected to statistical analysis. The results proved the existence of highly significant correlation between height and alpha amino nitrogen, glycogen and ribose.

$r = 0.80$, $P \leq 0.01$ for height and alpha amino nitrogen

$r = 0.87$, $P \leq 0.01$ for height and glycogen

$r = 0.88$, $P \leq 0.01$ for height and ribose

correlation between height and protein (N x 6.25) and height and phosphorus were however non significant ($P \leq 0.05$). A highly significant ($P \leq 0.01$) positive correlation between glycogen and ribose also was noted ($r = 0.98$). These relations can be expressed by the following regression equations.

$Y = 80.58+8.35\ X$, for height X and alpha amino nitrogen Y

$Y = -4.23+1.16\ X$ for height X and glycogen Y

$Y = -33.10+52.80\ X$ for height X and ribose Y

$Y = 165.40+43.82\ X$ for glycogen X and ribose Y

Protein (N x 6.25) was observed to be in the range (12.95, 15.90%) and phosphorus (in) in the range (27.73, 34.3 mg%).

Khawaja (1966) observed more percentage of protein, less fat and more water content in juveniles than adults of 18 different species of freshwater fishes. In the present studies the protein content showed steady increase with respect to the decrease in moisture content as the animal grows.

4.2.3 Studies on ice storage characteristics of mussel

Loss of weight in mussels in the shell-on condition during ice storage is depicted in Table 18(a). 20% reduction in weight occurred in the first day itself and in 14 days time the weight loss increased to 25.1%. There was possibility to drain off the shell liquor on opening of the shell as the animal dies.

Ice storage retards the spoilage of shellfishes considerably. Icing is the preferred method of preservation both on board and for transportation over long distance to the interior parts of the country as icing delays rigor mortis.

Mussels (Perna viridis) stored in ice in the shell-on condition were analysed at intervals and the results are tabulated in Table 18(b) & (c). Total bacterial count which was 7.25×10^5 initially decreased to 2.86×10^4 on the first day and up to 8th day the count remained more or less constant. Again on 10th day the count increased to 2.4×10^5. Coliforms,

Table 18a. Weight loss in mussel (Perna viridis) (shellon) stored in ice

Days in ice	% loss of weight
1	20.0
2	20.0
3	20.0
4	20.0
5	22.3
7	22.3
9	22.5
10	23.1
12	24.3
14	25.1

Note: Sample weight held under ice 10 kg.

Table 18b. Changes during ice storage (with shellon in ice) in Mussels (Perna viridis)

Days	SPC/gm	Coliforms/ gm	E. coli/ gm	Faecal streptococci/ gm	Organoleptic Rating	Organoleptic Score 0-10 scale
0	7.25×10^5	1451	1161	4184	Good	9
1	2.86×10^4	253	243	1096	Good	8.5
2	4.60×10^4	304	177	365	Good	8.0
3	2.48×10^4	199	40	266	Fair	7.5
5	2.09×10^4	119	36	43	Fair	7.5
7	1.60×10^4	43	nil	23	F - P	6.0
8	1.27×10^4	11	nil	22	F - P	5.0
10	2.40×10^5	23	nil	-	F - P	4.0
12	2.10×10^5	9	nil	46	Poor	3.0

Note: Details of sample analysed ref. table 18a.

Table 18c Changes in chemical constituents during ice storage of Mussels (P. viridis) with shellon

Days	Moisture	Protein (Nx6.25)	WEN	NPN	F_{α}-NH_2-N	TVN	TMA	Glycogen	Lactic acid	Phosphorus	Phorphorus (in)	Fat
	gm %	gm %	mg%	mg%	mg%	mg%	mg%	gm%	mg%	mg%	mg%	gm%
0	76.25	15.03	472	349	119	6.6	3.07	8.4	33.5	64.2	30.6	2.46
1	77.20	14.45	435	308	126	9.3	4.05	5.7	38.1	66.2	30.9	2.40
2	77.54	14.44	412	344	100	7.9	4.99	5.99	56.2	60.3	33.8	2.46
3	77.69	13.82	402	332	89	9.61	6.93	5.54	46.1	57.8	28.2	2.30
5	77.58	13.63	401	348	89	10.35	7.31	4.7	45.0	36.4	27.1	-
7	78.30	13.81	399	289	60	10.35	7.21	4.35	48.9	38.8	24.6	2.2
8	79.90	13.20	351	265	67	10.08	7.21	4.32	56.2	42.1	23.8	-
10	79.79	13.19	379	252	62	11.06	7.25	4.19	65.2	32.6	20.8	-
12	79.97	13.02	359	263	50	18.82	8.96	4.08	67.5	29.8	17.2	2.26

Note: Details of sample analysed ref. table 18a.

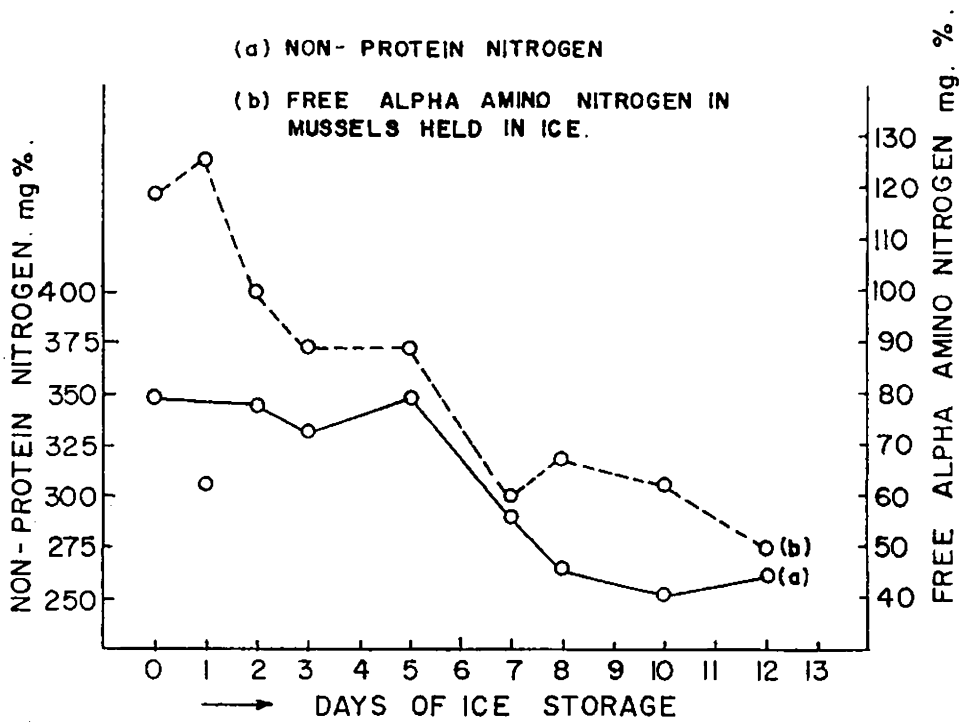


Fig. 40. (a) NON - PROTEIN NITROGEN (b) FREE ALPHA AMIND NITROGEN IN MUSSELS HELD IN ICE

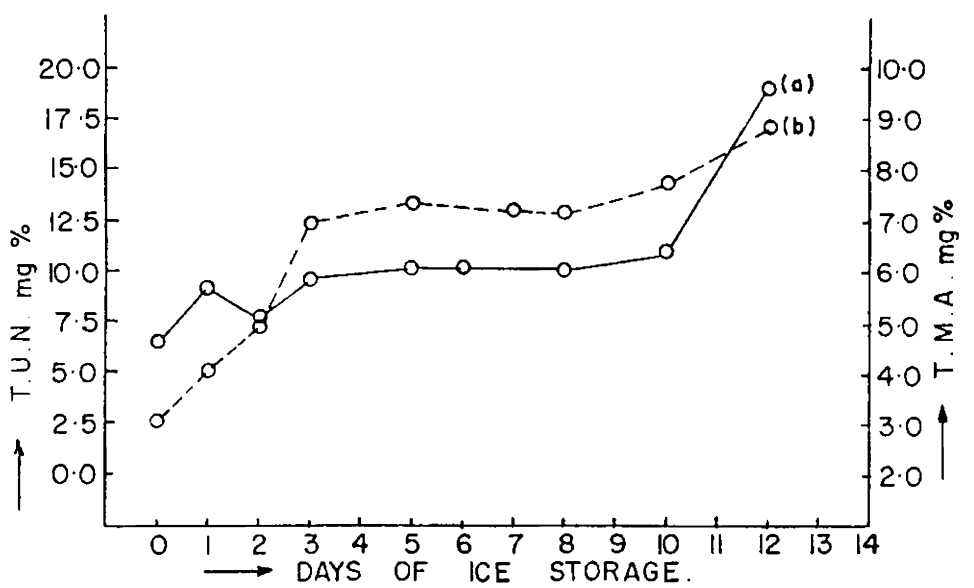


Fig. 41. TOTAL VOLATILE NITROGEN (T.V.N)(a) & TRIMETHYL AMINE (T.M.A) (b) IN MUSSELS HELD IN ICE.

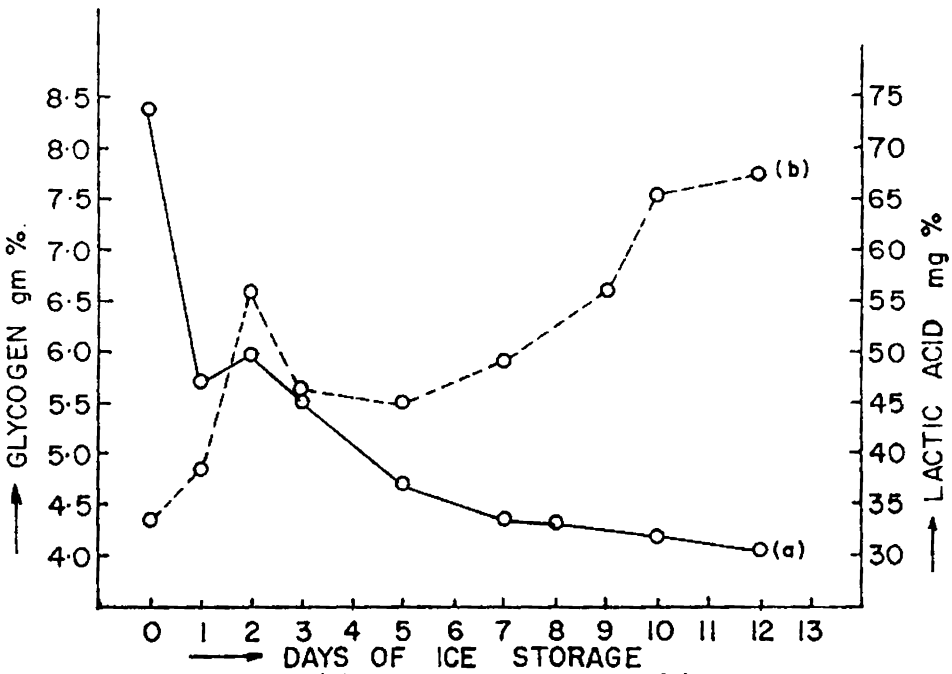
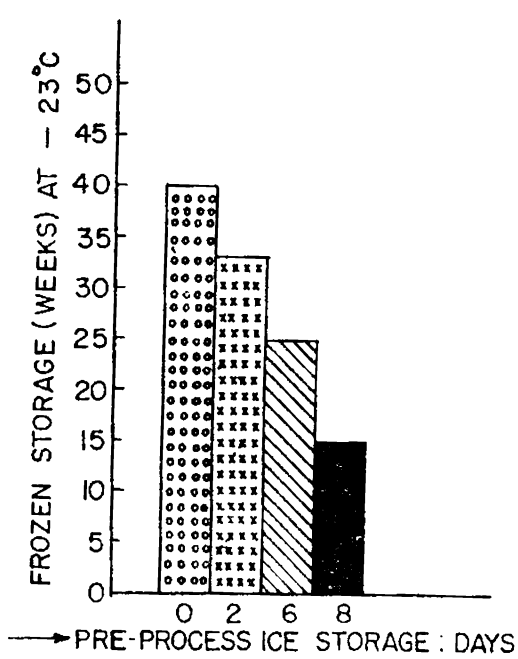


Fig. 42. GLYCOGEN (a) and LACTIC ACID (b) IN MUSSELS HELD IN ICE

HYSTOGRAM. 3.



SHELF-LIFE OF FROZEN MUSSELS

E. coli and Faecal streptococci also showed gradual decrease as the days of ice storage advanced. E. coli was absent from 7th day onwards. The fall in bacterial count during the early stages of ice storage may be due to the washing out of surface slime together with bacteria by the ice melt water. Gradual increase towards the end of ice storage may be due to the proliferation of psychrophilic bacterial flora.

The moisture content of the stored fish increased from 76.25-79.97% in 12 days time and protein content decreased from an initial value of 15.03% to 13.02%. Water extractable nitrogen (Fig.39), non-protein nitrogen (Fig.40) and free alpha amino nitrogen (Fig.40) showed a decreasing pattern i.e 472 to 359 mg%, 349 to 263 mg% and 119 to 50 mg% respectively. Glycogen (Fig.42) and phosphorus both total and inorganic (Table 18 c) also decreased during ice storage i.e 8.4 to 4.08 gm%, 64.2 to 29.8 mg% and 30.6 to 17.3 mg%. Only negligible change in fat content towards the end of the storage period was seen (2.46-2.26%). The values of total volatile nitrogen (Fig.41), trimethyl amine (Fig.41) and lactic acid (Fig.41) increased steadily (6.6-18.82 mg%, 3.07 to 8.96 mg% and 33.5 to 67.5 mg% respectively) during ice storage. The organoleptic score also correlated with the chemical indices studied. In 10 days time the material reached the stage of fair to poor (score 4), with slight loss of characteristic flavour and slight yellowish discolouration.

Solanki et al. (1977) and Jose Joseph et al. (1977) observed gradual increase in total bacterial count during ice storage of perch and squid; on the other hand Garg & Jose Stephen (1982) and Lekshmy et al. (1962) reported gradual fall in total bacterial count during the initial period of ice storage of Kati (Pellona sp.) and prawns and increasing trend towards the end of the storage period in harmony with the present findings. The change in the pattern of pathogenic organisms in the present work agreed with the previous observations (Lekshmy et al., 1962; Solanki et al., 1977; Jose Joseph et al., 1977 and Garg & Jose Stephen, 1982).

The trend in moisture changes and protein content during ice storage of mussels agreed well with the results of previous workers (Garg & Stephen, 1982; Solanki et al., 1977 and Govindan, 1962)

The spoilage pattern of mussels during ice storage was found to be similar to that of crab (Chinnamma George repored in this thesis elsewhere); Prawns (Govindan, 1962 & 1964 Lekshmy et al., 1962); Fish of sea water and freshwater origin (Bramstedt, 1962; Lahiry et al., 1963; Liston, 1961; Shewan & Jones, 1956 and Cutting, 1953).

The quality loss in mussel stored in crushed ice can be attributed to leaching losses by ice melt water and accumulation of products of bacterial and autolytic actions.

4.2.4 Freezing of mussel meat

Freezing as a method of preservation comes closer to preserving shellfish in its natural state than other methods of preservation. The condition of the material at the time of freezing, type of freezing and the temperature and humidity of the cold storage are the factors that affect the keeping quality of the frozen product (Love, 1962 a,b; Anon 1965; Dyer et al., 1968 and John A.Peters, 1970).

Tables 19 (a & b) and Fig. 43 and histogram 3 represent the biochemical, bacteriological and organoleptic changes in mussels (Perna viridis) having different pre-freezing ice storage life, during freezing and further storage. In fresh and iced samples the moisture content was reduced during cold storage. The reduction in moisture content may be due to the loss in water holding capacity of the muscle due to protein denaturation. Similarly the solubility of protein also was reduced (Fig.43) from 64.2% to 52.2% in uniced sample and 58.0 to 45.2% in 8 days iced sample.

Gangal & Magar (1963) and Chinnamma George (given in this thesis elsewhere) observed reduction in moisture content in frozen stored crab meat. The reduction in solubility of protein due to protein denaturation may be attributed to the extent of ice crystal formation and consequent increase in the concentration of tissue salts (Love, 1958, 1962); binding

Table 19a. Biochemical, bacteriological and organoleptic changes in Mussels (Perna viridis) during freezing and subsequent storage at -23°C

Frozen storage weeks	Moisture gm%				Salt soluble protein as percentage of total protein				Free alpha amino nitrogen mg%			
	Pre-process ice storage days											
	0	2	6	8	0	2	6	8	0	2	6	8
RM	79.0	77.9	78.1	77.8	64.2	62.5	60.6	58.0	112.6	117.6	119.2	92.4
2	76.0	75.3	76.2	76.0	62.5	54.0	53.1	53.0	95.2	95.2	86.8	84.0
12	76.6	75.5	76.0	76.5	60.6	54.8	48.79	52.2	86.8	95.2	81.2	78.4
22	74.8	74.2	75.9	75.4	58.0	53.1	49.90	49.9	84.0	92.4	78.4	72.8
32	73.9	74.3	75.9	75.1	56.1	44.8	46.20	46.3	84.0	92.4	75.4	70.0
44	73.9	73.4	75.9	75.1	52.2	46.2	43.2	45.2	86.8	90.8	72.8	70.0

	Lactic acid mg%				Phosphorus (in) mg%				Organoleptic score (0-10 scale)			
	0	2	6	8	0	2	6	8	0	2	6	8
RM	45.9	62.7	88.7	92.7	20.3	21.5	17.2	16.8	9.0	8.0	6.5	5.5
2	42.9	65.3	74.6	90.6	22.6	23.4	20.8	23.8	8.5	8.0	6.0	5.0
12	42.6	67.5	82.7	97.0	27.9	27.2	32.5	29.5	7.5	6.5	5.5	4.5
22	56.6	68.9	98.8	99.5	30.7	30.7	31.6	31.6	6.5	5.5	5.0	4.0
32	58.3	67.7	101.2	103.2	33.1	32.6	31.1	31.5	6.0	4.5	4.0	3.0
44	56.1	80.1	105.2	122.8	34.9	33.9	34.7	34.4	5.0	3.5	2.5	2.0

RM = Raw material without icing

Note: Sample of meat was kept as block frozen (2 kg). One block from each lot was analysed. Two estimations were carried per each sample.

Table 19(b) contd

Frozen storage weeks	Standard plate count nos/gm				Escherichia coli nos/gm				Faecal streptococci nos/gm			
	Pre-process ice storage days											
	0	2	6	8	0	2	6	8	0	2	6	8
RM	3.8×10^5	6.5×10^5	3.5×10^6	1.7×10^7	330	370	175	111	280	211	168	178
2	4.57×10^4	8.1×10^4	6.9×10^5	2.2×10^6	44	25	11	14	125	85	72	58
12	1.7×10^4	4.4×10^4	1.9×10^5	8.0×10^5	57	14	nil	nil	93	71	78	44
22	7.0×10^3	1.5×10^4	6.5×10^4	4.9×10^5	nil	nil	nil	nil	29	13	8	16
32	2.4×10^3	7.7×10^3	3.1×10^4	3.7×10^5	nil	nil	nil	nil	15	21	nil	8
44	1.6×10^3	6.0×10^3	2.2×10^4	1.7×10^5	nil	nil	nil	nil	nil	nil	nil	nil

Drip constituents

	Free alpha amino nitrogen mg%				Lactic acid mg%				Phosphorus (in) mg%			
	0	2	6	8	0	2	6	8	0	2	6	8
2	28.3	29.78	32.4	30.0	15.5	20.3	38.4	34.2	3.0	2.8	4.0	3.8
12	36.1	35.20	41.9	34.0	16.1	22.3	35.9	32.7	8.0	2.1	10.2	10.7
22	44.1	36.10	40.9	34.2	14.3	20.3	38.5	36.8	12.8	9.5	15.0	13.3
32	37.7	36.80	36.0	33.1	16.6	26.1	43.1	41.3	11.0	9.6	13.5	14.2
44	48.7	39.50	37.8	36.1	22.9	27.6	44.4	46.9	13.3	11.8	16.4	16.4

RM = Raw material without icing

Note: Details of sampling same as that of table 19(a).

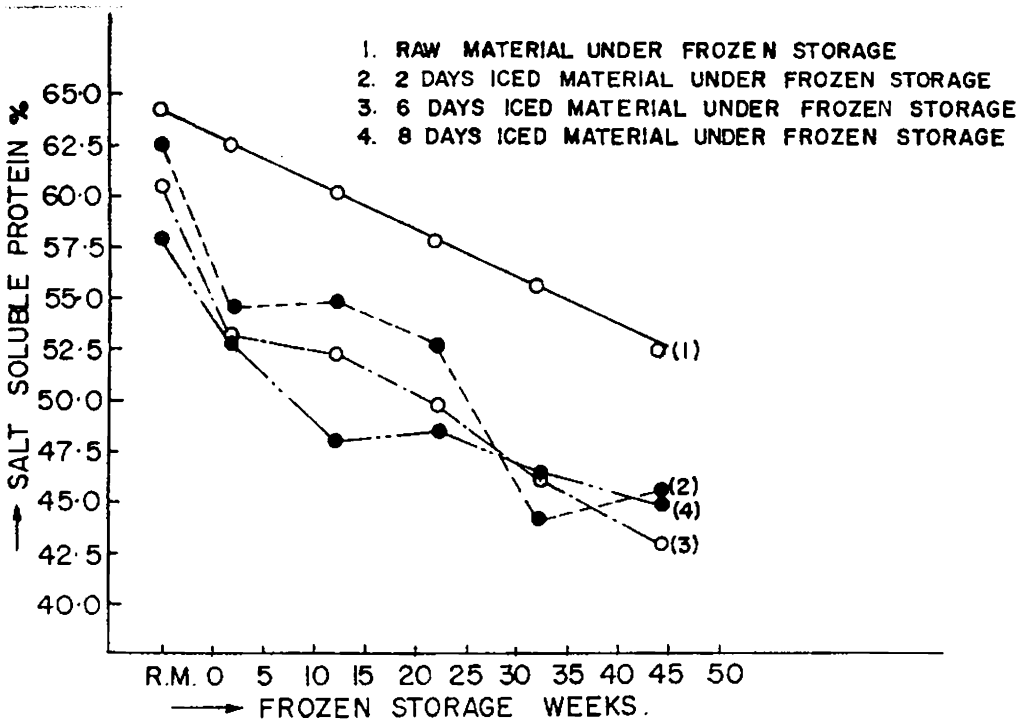


Fig. 43. SALT SOLUBLE PROTEIN AS PERCENT OF TOTAL PROTEIN IN MUSSELS

together of structural protein molecules (Love, 1965); change in properties of myofibrillar proteins: an uncoiling of the molecular helix leading to cross linking between the adjacent parallel molecules (Love, 1970); free fatty acid accumulation in the muscle as a result of lipid hydrolysis (Dyer & Fraser, 1959; Olley & Lovern, 1960; Olley et al., 1962; Boyd et al., 1967) and during frozen storage, the actomyosin filaments form net worked masses through aggregation and entangling and become increasingly insoluble with the growth in net work (Moritoshi Oguni et al., 1975).

Several workers observed decrease in extractability of salt soluble proteins during frozen storage (Dyer et al., 1950; Bate Smith, 1934 and 1937; Reay, 1933, 1934 and 1935; Love & Ironside, 1958; Love et al., 1965; Love , 1970; Connell, 1960 and 1962; Kaoru Tamoto et al., 1964; King, 1966 and Awad et al., 1969).

Free alpha amino nitrogen content in muscle showed gradual fall during storage in fresh and ice stored samples, but in drip the trend was reversed, that is an increase from 28.3 to 48.7 mg% in uniced samples and 30.0 to 36.1 mg% in 8 days iced sample. Sawant & Magar (1961) observed a decrease in total amino nitrogen during frozen storage of fish. Pawar & Magar (1966) also observed very slow decrease in total amino nitrogen in fish; Jun-ichi-Nishimoto (1962) reported that the amount of mono and diamino fraction nitrogen in stored frozen

fishes progressively decreased in early periods of storage.

The rate of formation of lactic acid in muscle and drip showed a gradual increase in fresh and iced samples during frozen storage; minimum increase in uniced material as can be seen from the Table 19 (a) indicating glycolysis during freezing and subsequent storage. Sharp (1934); Tomlinson et al. (1963); Burt (1971) and Nowlan & Dyer (1974) all agreed to the view that glycolysis proceeded in fish muscle stored at subzero temperatures and maximum activity between -3.2°C and -3.5°C and were significant down to -10°C. According to Tomlinson et al. (1963), glycolysis as indicated by lactic acid accumulation clearly, proceeded at temperatures down to and including -20°C and it may proceed at a very slow rate at a temperature of -30°C.

The inorganic phosphorus content in fresh and ice stored mussels and its changes during freezing and subsequent storage at -23°C is depicted in Table 19 (a).
A decreasing trend during ice storage and an increasing trend during frozen storage both in muscle and drip was seen, in uniced sample the value increased from 20.3 to 34.9 mg% and in 8 days iced sample from 16.8 to 34.4 mg% in 44 weeks of storage, indicating leaching losses during ice storage and release of orthophosphates from sugar phosphates and other phosphorus compounds during freezing and subsequent cold storage. Tomlinson et al. (1963) and Nowlan & Dyer (1974)

observed an increase in the values of inorganic phosphates and lactic acid in lingcod muscle and cod muscle during frozen storage. Nowlan & Dyer (1974) suggested that freezing increases the rate of breakdown of high energy phosphate compounds, notably adenosine triphosphate and creatin phosphate.

Under frozen storage the quality of the material was deteriorated gradually. The changes observed were mainly:-

(1) The colour changed to dull white and to brown in spoiled samples.

(2) the firmness and clasticity of the material was replaced by sponginess and in spoiled samples the breaking up of the flesh and resultant oozing out of drip.

(3) the loss of characteristic sweet flavour.

The development of tough texture may be attributed to the denaturation of protein and glycolysis leading to production of lactic acid which decreased the pH of the muscle. The loss of metabolites such as amino acids and glycogen also reduced the flavour of the muscle. In 44 weeks time the organoleptic score was reduced from 9.0 to 5.0 in uniced sample and from 5.5 to 2.0 in 8 days iced sample. The fresh frozen material remained in acceptable condition for 40 weeks and the 8 days iced material only for 15 weeks.

The bacterial load and the count of pathogenic organisms like E. coli and Faecal streptococci showed considerable variation during cold storage. Standard plate count during ice storage showed steady increase, but during freezing and storage after 44 weeks at -23°C, the viable count came down by 99%. Reduction in pathogenic organisms like E. coli and Faecal streptococci was observed during frozen storage and E. coli was completely destroyed towards the end. This is in agreement with the findings of Lekshmy (1964) that faecal indicator organisms underwent significant reduction during freezing and cold storage.

4.2.5 Cooked frozen mussel meat

Consumers prefer cooked meat to raw material in the shell because the procedure in preparing and cooking the latter is troublesome. The influence of preprocess ice storage on the nutritive value and chemical constituents of cooked frozen mussel - Perna viridis available on the west coast of India has been studied. The optimum cooking time was found to be 15 minutes and the yield 14-16%. The thawing loss in the product was 2-4% (Table 20 a). Cooking causes the mussels to open and facilitates separation of the meat. Cooking procedure however, influences the texture of the mussel meat (Banks & House 1958; Rietz & Wanderstock, 1965; Scattergood & Taylor, 1949). Banks & House (1958) found that steaming the mussels just suffi-

Table 20a. Standard processing conditions for Mussel

Cooking time (min)	Cooked yield %	Appearance	Thawed yield %
10	18.20	Under cooked	
15	15.00	Properly cooked	97.7
20	11.50	Over cooked	

Table 20b. Organoleptic score in cooked frozen mussel during storage (rating 0 to 10)

Frozen storage weeks	Ice storage days					
	A			B		
	1	3	5	1	3	5
0	8	8	7	9	8	7
4	8	7	6	9	7	7
16	7	6	5	5	3	5
28	6	5	5	2	2	2
38	5	4	3	1	1	0

A = cooked and iced; B = iced and cooked

Note: Results represent studies of one series, but 3 series were conducted to confirm results.

Table 20c Percentage loss of nitrogenous constituents and thawed weight in the cooked frozen mussel (expressed as percentage of original) due to freezing and subsequent storage

Days in ice	Frozen storage weeks	Water extractable nitrogen (WEN)		Non-protein nitrogen (NPN)		Free alpha amino nitrogen		Weight loss	
		A	B	A	B	A	B	A	B
1	1	9.3	10.8	8.5	9.8	11.3	13.0	2.5	2.9
	4	10.7	12.4	10.8	11.4	13.5	15.5	2.5	3.0
	16	11.3	13.1	8.3	13.7	14.7	15.7	2.5	4.5
	28	12.4	15.8	12.9	12.8	13.6	17.0	5.8	5.0
	38	15.0	17.4	15.9	14.5	15.0	18.5	6.2	5.9
3	1	10.8	11.2	9.8	10.9	13.5	15.0	4.8	4.6
	4	11.3	13.7	11.0	12.8	15.4	17.0	5.4	5.0
	16	11.9	13.7	12.8	14.0	16.7	16.8	6.3	6.6
	28	13.5	15.6	13.5	16.4	16.8	19.7	7.4	8.3
	38	14.9	17.2	17.0	16.8	17.9	19.3	8.3	9.1
5	1	11.0	11.9	10.5	13.1	15.0	16.5	5.1	5.4
	4	11.6	14.3	11.2	16.0	17.9	18.6	6.2	6.6
	16	12.7	15.3	12.7	17.2	18.1	19.8	7.4	8.7
	28	14.7	17.2	15.9	20.6	19.0	19.7	8.5	9.1
	38	16.5	20.3	16.5	26.7	19.8	21.2	9.0	12.0

A = cooked and iced; B = iced and cooked

Note: Details of sample studied ref. table 20a.

ciently to open the shells and cooling the cooked mussels quickly with a water spray was the best method.

Table 20(b) gives the organoleptic ratings of cooked frozen material during storage. Frozen mussel of series A (cooked and iced prior to freezing) was better than that of series B (iced and cooked prior to freezing). Shelf life was 38 weeks for series A sample and only 16 weeks for B series sample iced for one day. Shelf life was related to pre-process ice storage since, as the length of ice storage advanced there was a decrease in frozen shelf life. Discolouration and hardening of texture increased with progressive pre-process ice storage.

Investigations by Banks & House (1958) showed that by choosing the right conditions for ~~of~~ cooking, freezing and cold storage, frozen mussel meat can be kept in a vertually unchanged condition for 8 to 9 months. This is in agreement with the Danish experience (Anon, 1960a) saying that steamed shucked and breaded mussels keep a good texture during cold storage.

Waterman (1969) observed that glazed blocks of cooked frozen mussels stored at -20°F retained the flavour for 8-9 months.

Table 20 (c) shows the loss of water extractable nitrogen (WEN), Non-protein nitrogen (NPN), free alpha

nitrogen (α-NH_2-N) and thawed weight of cooked frozen mussel meat during freezing and subsequent storage up to 38 weeks. Loss of WEN was in the range of 9.3 to 16.5%, NPN 8.5 to 16.5%, α-NH_2N 11.3 to 19.8% and weight 2.5 to 9.0% in series A samples; for series B samples the losses were 10.8 to 20.3%, 9.8 to 26.7%, 13.0 to 21.2% and 2.9 to 12.0% for WEN, NPN, α-NH_2-N and weight respectively. The losses were comparatively more in series B samples and this correlates with the organoleptic score.

The nutritional loss as well as the weight loss of cooked frozen mussel was less during subsequent frozen storage when compared to crab (given in this thesis elsewhere).

4.2.6 Canning of mussel meat

Canning procedure was standardised in order to get good quality product with minimum weight loss and maximum shelf life.

Table 21 gives an account of the analytical data of the canned mussel. The loss in weight during sterilization was only 1.2% and the soluble constituents lost in brine (filling medium) were protein 2.9%, free alpha amino nitrogen 40.5 mg%, phosphorus (in) 22.1 mg%, glycogen 129.7 mg% and ribose 176.3 mg%. This is in agreement with that of canned crab (given in this thesis elsewhere).

Table 21. Examination of canned mussel (Perna viridis) in brine

Can exterior and interior	
Sensory evaluation	Good
a) Colour	Good
b) Smell	Good
c) Texture	soft and firm
d) Flavour	Good
e) Nature of brine	Slightly cloudy
Biochemical parameters	
a) Reduction in weight during sterilisation	1.2 %
b) Moisture	74.66 %
c) Protein in meat TN x 6.25	19.95 %
d) Protein in brine TN x 6.25	2.90 %
e) Alpha amino nitrogen in brine	40.5 mg %
f) Phosphorus (in) in brine)	22.1 mg%
g) Glycogen in brine	129.7 mg %
h) Ribose in meat	116.4 mg %
i) Ribose in brine	176.3 mg %

TN = total nitrogen

Note: Number of samples analysed 5, average value is reported.

4.3 Clams - Villorita cyprinoids

4.3.1 The size-weight measurements of clams

The size weight measurements of clams are depicted in Table 22 and the data was statistically analysed. The yield varied from 8.9 to 13%.

Length-height relationship worked out for clams - Villorita sp. is

$Y = -0.254+0.927\ X$ for length Y and height X. Correlation coefficient $r = 0.96$ which is highly significant ($P \leq 0.01$).

A systematic study was not conducted to examine the age and size of clams. Nair (1955) made detailed studies on length-weight, length-breadth and length thickness studies in the clam - Donax (Latona) Cuneatus (Linnaeus) but observed only proportional variations of length in all size groups. However, Ohba (1959) observed changes in the ratio of dimensional relations of shell during definite stages in Tapes japonia. Hanoka & Shimatzu (1949) also made notable contributions to the morphometry of clams. Allometric relation of height-depth was observed in Mya arenaria by Newcombe & Kessler (1936) and Alagarswami (1966). Talikhedkar et al. (1976) studied the growth rate in the wedge clam Donax cuneatus inhabiting miriya at Ratnagiri. The relation between linear dimensions, weight and volume of the shell in Meretrix meretrix and the probable influence of

Table 22. Size weight measurements of clams Villorita sp.

	Height (cms)	Length (cms)
1	3.5	2.5
2	3.0	2.4
3	3.1	2.6
4	2.9	2.4
5	2.9	2.7
6	3.3	3.0
7	4.9	4.3
8	4.9	4.4
9	4.7	4.2
10	3.8	3.6
11	3.3	2.4
12	3.5	2.8
13	2.0	1.8
14	2.1	1.8

(1) Whole weight of a lot 3.00 kg

Meat weight (free of intestines) 390 gms

Meat content 13%

(2) Whole weight of a lot 4995 gm

Average weight of one clam 10.9 gm

Meat weight 445 g ie 8.9%

(3) Whole weight of a lot 1047.2 gm

Meat weight 97.27 gm ie 9.29%

environmental factors were studied by Hamai (1934). He found that the growth of M. meretrix at different localities and also under different substrata is influenced by temperature, salinity and other parameters of the environment. Durve & Dharmaraja (1965) also noticed the influence of environmental conditions prevailing at the locality influencing the morphometry of Meretrix casta (chementz).

4.3.2 Studies on biochemical characteristics of clam muscle

Table 23 (a) gives the chemical composition and bacterial count of the clam muscle. Moisture content was on the higher side and protein slightly less than that of crab or mussel meat. Wide variation was observed in the chemical constituents during different seasons of the year especially in protein, amino acids, glycogen, ribose and fat contents. Retention of sand in the muscle was occurred even if care was taken to remove the gut portion. The standard plate count was 6.4×10^5 and coliforms and streptococci were 132 and 131 numbers per gm muscle.

Venkataraman and Chari (1951) reported wide variation in chemical composition in clam meat as water varied from 73.18 to 84.02%, protein 5.96 to 12.2% and fat 0.50 to 1.89%. Galtsoff (1964) also observed variation in composition according to season in American oyster. As regarding food value clam meat is slightly inferior to crab or

mussel meats. According to Sebastian (1970) clam meat rich in protein and mineral contents should not be allowed to go to waste especially at a time when the country is facing malnutrition. People eating for the first time clam meat preparations served in hotels may feel a subtle taste of mud in it. This flavour is due to the retention of a small quantity of digested or semi digested matter with mud particles in the winding gut of the clam. To avoid contamination and to keep good flavour it is highly necessary that the digested or semi digested matter inside the winding gut of the clams and the mucus on the body surface especially on the gills are removed thoroughly.

The protein fractions isolated from clam muscle (Table 23b), revealed more percentage of sarcoplasmic protein (34.54%) and lesser quantity of myofibrillar protein (30.2%). Non-protein nitrogen was comparatively more in clam muscle (25.13% of TN). The denatured protein content was slightly higher (34%); probably the myofibrillar protein was denatured during successive extraction. Lesser quantities of nucleoprotein and stroma protein were isolated.

Yuji Maruyama & Taneko Suzuki (1968); Sayre & Briskey (1963); Connell (1962); Carpenter & Saffle (1965); Sayre (1968); Baliga et al. (1962 & 1969) and Paul (1966) extracted individual or all the protein fractions of fish muscle and studied their properties. The present finding agreed

Table 23a. Proximate chemical composition of clam Villorita sp.

1. Moisture gm %	79.5 - 84.6
2. Protein TN x 6.25 gm %	7.6 - 11.0
3. Water extractable nitrogen mg %	420 - 564
4. Non-protein nitrogen mg %	191 - 428
5. Free alpha amino nitrogen mg %	32.3 - 205
6. Glycogen gm %	1.4 - 7.8
7. Phosphorus (in) mg %	22 - 34
8. Ribose mg %	79 - 187
9. Fat gm %	1.1 - 2.1
10. Ash (dry weight basis) gm %	10.02
11. Acid insoluble ash (sand) D.W.B. gm %	2.742
12. Standard plate count	6.4×10^5/gm
13. Coliforms	132/gm
14. Faecal streptococci	131/gm

Table 23b. Protein fractions in clam muscle Villorita sp.

Total nitrogen mg %	1778.2
Protein nitrogen mg %	1331.2
Non-protein nitrogen (percent of T.N)	25.13
Sarcoplasmic protein (percent of PN)	34.54
Myofibrillar protein (percent PN)	30.20
Nucleoprotein or protamines (percent of PN)	0.81%
Denatured protein (percent of PN)	34.0
Stroma protein or connective tissue (percent of PN)	0.44

TN = Total nitrogen; PN = Protein nitrogen

Note: 100 g comminuted minced sample was prepared. Results are average of 2 estimations.

Table 23c. Free amino acids in clam muscle Villorita sp.

Amino acids	%
1. Phenyl alanine	11.83
2. Glycine	6.25
3. Cystine	0.09
4. Tyrosine	8.85
5. Histidine	11.83
6. Valine	0.42
7. Lysine	17.75
8. Methionine	3.55
9. Glutamic acid	6.72
10. Iso leucine	2.65
11. Leucine	14.58
12. Tryptophan	0.05
13. Serine	3.90
14. Proline	11.50

Note: Details of sample analysed ref. table 20a.

with those of the previous observations. The values of protein fractions reported by Raymond Jacquot (1961) for fishes and shellfishes agreed with the present values obtained for clam muscle.

Table 23c gives the free amino acid pattern of clam muscle. 14 amino acids were isolated and estimated. Wide variation in the free amino acid content was observed (32.3 to 205 mg%) during different seasons of the year. Irrespective of crab, lysine (17.7%) and leucine (14.5%) constitute the major amino acid pool in clam muscle with phenylalanine, histidine, proline, tyrosine, glycine, glutamic acid, serine, methionine, and isoleucine content in moderate quantities in decreasing order and cystine, valine and tryptophan in trace quantities.

Literature is scanty on the studies on free amino acids in clam muscle.

4.3.3 Ice storage studies of clams

Icing is the common method to preserve fishery products for a short duration and it is done onboard and for transportation to the distant interior parts of the country to keep the material prime. Ice storage is the first important technological development in the utilization of clams for food.

Table 24. Changes during ice storage (with shellon) in clams (Villorita cyprinoids)

Days in ice	Volume of drip per 100 g muscle	Moisture	Nitrogen in drip	Water extractable nitrogen		Non-protein nitrogen		F. alpha amino nitrogen		Glycogen		Lactic acid		Phosphorus (in)		Ribose		Organoleptic rating 0-10 scale
				M	D	M	D	M	D	M	D	M	D	M	D	M	D	
	ml	gm%	mg%	mg%	mg%	mg%	mg%	mg%	mg%	g%	mg%	mg%	mg%	mg%	mg%	mg%	mg%	
0	29.6	83.9	241	558	117	227	11.5	56.3	1.7	1.31	30.	18	2.2	29	1.2	79	1.9	9.5
1	31.8	84.6	282	541	128	232	12.6	58.2	1.9	1.33	65	41	3.3	38	1.5	86	2.2	9.0
2	32.2	85.9	490	522	121	211	18.5	57.3	5.2	1.10	91	20	4.2	31	3.0	66	7.4	9.0
5	58.6	87.0	650	497	130	232	52.7	52.8	14	1.08	207	29	13.4	35	8.2	98	14.1	8.0
7	109	87.0	1852	426	180	164	84.1	48.0	15	0.80	177	27	19.1	27	11	76	23.7	7.0
9	105	88.7	1278	232	125	179	57.8	37.5	25	0.63	176	32	4.7	29	6	43	14.8	5.0
13	47.3	89.7	787	332	184	164	36.5	48.1	11	0.54	82	14	5.2	21	4.8	24	12.9	3.0

M = muscle; D = drip

Note: Results indicate the values of one series out of 3 series done in 1980, 81 and 82. 25 kg sample was kept under ice/series.

Clams (Villorita cyprinoids) ice stored in the shellon condition was analysed at intervals. It is a general feature of the bivalves to retain sea water in the mantle cavity. The ability of clams to retain liquor in the shell cavity is an adaptation to life in the unfavourable circumstances, with its shell tightly closed. Under ice storage the shell was in closed condition for 7 days, then only started opening.

Live clams stored in ice had a shelf-life of 9 days, By that time the organoleptic score had fallen to 5.0 from 9.5. The liquid collected in the shell with the accumulated metabolic products had drained off from 9th day when the shell was completely opened. There was noticeable increase in liquid volume per 100 gm solid matter from 7th day onwards. Correspondinly the chemical constituents such as total nitrogen non-protein nitrogen, free alpha amino nitrogen, glycogen, lactic acid and phosphorus (in) in the drip recorded rapid increase from 5th day onwards.

When the shell was opened completely there was chance for draining of the clam liquor. The chemical constituents recorded a fall with respect to loss of drip after 9 days of ice storage.

In muscle the moisture content increased from 83.8 to 89.7%; water soluble nitrogen values reduced from 558

to 332 mg%; free alpha amino nitrogen from 56.3 to 48.1 mg% and glycogen from 1.31 to 0.54 mg% in 13 days of time. Phosphorus (in) increased up to 5th day, after that the value fell down. A clear pattern of trend was not seen in ribose and lactic acid values up to 7th day then a sudden fall in ribose value had occurred probably due to leaching effect.

Amano et al. (1953) found different levels of glycogen content in different parts of the muscles and in all the fish examined, a decrease of glycogen content and a corresponding increase in lactic acid level depending upon the method and rapidity of killing after capture. In the present studies with the decrease in glycogen level lactic acid values do not show any corresponding increase. The mechanism of depletion of glycogen level with the corresponding variation in lactic acid is not very clear, which may probably be due to secondary reactions or the method tried is not very specific (Schweiger & Gunther, 1964).

Gangal & Magar (1963) observed a decrease in glycogen and an increase in lactic acid and inorganic phosphorus during rigor mortis in fish muscle. In crabs, the phosphorus (in) values increased during the first 6 days of ice storage, then slowly decreased (given in this thesis eleswhere).

The trend in moisture changes during ice storage of clams agreed well with the results of previous workers (Garg & Stephen, 1982; Solanki et al., 1977; Govindan 1962).

Govindan (1962) and Lekshmy et al. (1962) observed a rapid fall in water soluble nitrogen and non-protein nitrogen in ice stored prawns and Fritz A.L. Bramstedt (1962) and Lahiry et al. (1963) observed a decrease in free amino acid in fish muscle during ice storage.

Jones (1962) claimed that the loss of flavour associated with chill storage of fish derived partly from leaching losses into ice melt water but to a great extent from the actions of autolytic enzymes which cleave flavorous compounds present in the muscle.

It is evident from the table that there is a good correlation between the quality as determined organoleptically and the amounts of WSN, free amino nitrogen, glycogen and phosphorus (in) retained in the muscle.

4.3.4 Freezing characteristics of clam meat

Freezing and cold storage of raw clam meat preserve the material in its natural state than other methods of preservation. The factors that influence the shelf life of the frozen product include, the condition of the material

at the time of freezing, type of freezing, temperature of storage of the frozen product, and the temperature and humidity of the cold storage.

Table 25a represent the changes brought out by quick freezing and subsequent storage at -23°C in clams (Villorita sp.) having different pre-freezing ice storage life.

The moisture content was reduced from 78.23 to 75.45% in fresh uniced and from 82.26 to 79.4% in the material preiced for 8 days. The dehydration losses were minimised by proper glazing and packaging hence the loss in moisture content may be attributed to the loss in water holding capacity due to denaturation of muscle proteins. A similar trend in the extractability of soluble protein in Dyer's buffer was found (Fig.44). The soluble protein was dropped from 63.0 to 55.0% in uniced samples and from 59.4 to 47.3% in samples iced for 8 days (Fig.44). The loss in solubility of muscle protein during cold storage was related to the change in texture, water holding capacity of the muscle and loss of juiciness. Many workers reported loss in protein solubility consequent to protein denaturation in fish and shellfish during frozen storage (Dyer et al., 1950; Reay, 1933, 1934 & 1935; Batesmith, 1934 and 1937; Connell, 1960 and 1962; Awad et al., 1969;

Table 25a Biochemical, bacteriological and organoleptic characteristics of clams (Villorita sp.) during freezing (-40°C) and subsequent storage -23°C

Frozen storages weeks	Moisture gm %				Salt soluble protein as percentage of total protein				Free alpha amino nitrogen mg %			
	Pre-process ice storage (Days)											
	0	4	6	8	0	4	6	8	0	4	6	8
RM	78.23	80.5	81.93	82.26	63.0	61.1	59.6	59.4	212.8	151.2	126.0	114.8
0	76.34	78.8	80.15	80.56	60.9	53.1	57.2	56.2	198.8	151.2	137.2	112.0
8	75.39	78.39	79.39	80.17	57.9	55.3	54.8	53.5	204.5	159.6	126.0	98.0
16	75.80	78.30	79.45	79.43	55.1	55.1	52.8	50.3	196.0	145.6	131.6	106.4
24	76.15	78.00	79.16	79.10	54.5	50.3	50.2	49.1	212.8	162.5	120.4	103.6
36	75.45	77.85	78.87	79.40	55.0	50.8	49.8	47.3	215.2	170.8	125.2	107.5

	Lactic acid mg%				Phosphorus (in) mg%				Free alpha amino nitrogen in drip mg%			
	0	4	6	8	0	4	6	8	0	4	6	8
RM	32.6	41.4	28.4	34.5	46.1	32.9	25.2	28.2	-	-	-	-
0	16.9	17.6	25.7	11.4	42.8	39.3	33.9	30.0	38.1	41.0	49.8	41.0
8	27.0	29.8	31.2	26.8	49.6	42.1	37.4	36.9	53.2	48.2	62.4	-
16	43.6	44.3	32.7	36.6	45.7	38.2	35.6	32.8	63.2	54.8	69.0	88.3
24	68.0	58.1	44.2	42.1	53.3	43.9	41.9	38.6	66.9	64.5	76.0	67.4
36	64.0	60.0	51.8	43.5	57.5	46.9	43.8	38.3	81.0	78.0	84.2	84.4

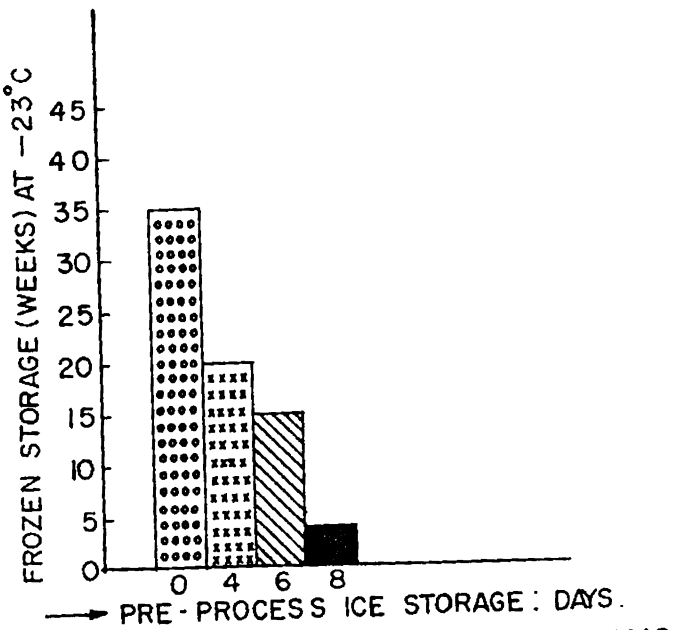
RM = raw material without icing

Note: Samples were kept as 2 kg block frozen with water glaze. Results are values obtained for series 1, out of 3 series studied.

Table 25acontd.

Frozen storage weeks	Lactic acid in drip mg%				Phosphorus (in) drip mg%				Standard plate count/ gm				Escherichia coli count/gm				Faecal streptococci count/gm			
	0	4	6	8	0	4	6	8	0	4	6	8	0	4	6	8	0	4	6	8
RM	-	-	-	-	-	-	-	-	$2.2x_{10^5}$	$9.8x_{10^5}$	$6.7x_{10^5}$	$1.0x_{10^6}$	300	172	151	97	131	91	65	59
0	9.5	12.8	12.8	13.0	7.2	9.3	8.9	8.1	$3.1x_{10^4}$	$1.5x_{10^5}$	$3.0x_{10^5}$	$7.0x_{10^5}$	132	71	25	nil	72	32	13	nil
8	10.8	14.5	14.5	15.5	14.2	11.3	11.8	13.5	$2.5x_{10^4}$	$7.0x_{10^4}$	$9.6x_{10^4}$	$1.0x_{10^5}$	nil	nil	nil	nil	nil	nil	nil	nil
16	13.1	13.9	16.2	20.9	14.6	13.7	14.3	13.7	$1.0x_{10^4}$	$3.7x_{10^4}$	$7.8x_{10^4}$	$3.7x_{10^5}$	nil	nil	nil	nil	nil	nil	nil	nil
24	16.7	24.1	23.1	26.3	16.1	16.7	15.9	17.2	$8.9x_{10^3}$	$8.3x_{10^3}$	$1.6x_{10^4}$	$4.1x_{10^5}$	nil	nil	nil	nil	nil	nil	nil	nil
36	15.5	28.5	24.8	28.3	16.8	18.8	17.1	19.1	$4.3x_{10^3}$	$1.6x_{10^4}$	$3.3x_{10^4}$	$3.6x_{10^4}$	nil	nil	nil	nil	nil	nil	nil	nil

HYSTOGRAM.4.



SHELF-LIFE OF FROZEN CLAMS

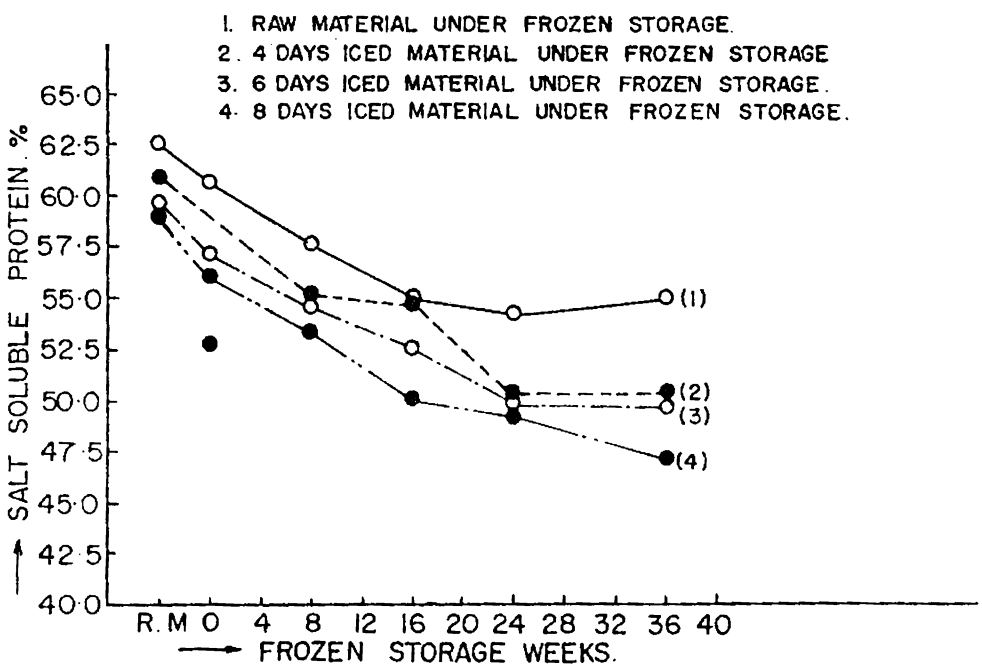


Fig.44. SALT SOLUBLE PROTEIN AS PERCENT OF TOTAL PROTEIN IN CLAMS

Love, 1962; King, 1966; Love & Ironside, 1958; Love et al., 1965)

Unlike frozen stored mussel meat the free alpha amino nitrogen content in clam meat remained more or less constant throughout the storage period, the value dropped from 212.8 to 114.8 mg% during 8 days of ice storage. In drip samples the amino nitrogen values recorded an increasing trend in fresh and ice stored samples. The increasing trend in amino nitrogen values revealed proteolytic activity in clam muscle during cold storage. The loss in amino nitrogen content during ice storage can be accounted for the leaching losses by ice melt water.

The lactic acid content in fresh and ice stored samples showed erratic results, but in drip samples a regular increasing trend was noticed giving the indication of glycolytic activity under frozen storage.

Phosphorus (in) content increased from 46.1 to 57.5 mg% in fresh and from 28.2 to 38.3 mg% in 8 days iced sample. In drip samples a regular increasing trend was seen indicating the liberation of orthophosphates from phosphorus compounds in clam muscle during cold storage.

Bacteriological analysis of frozen stored samples indicated that standard plate count increased from

$2.2x10^5$ to $1.0x10^6$ in 8 days ice storage; the count decreased to $4.3x10^3$ during 36 weeks of frozen storage. A regular decreasing pattern was observed during cold storage in all the samples. The pathogenic organisms like E. coli and faecal streptococci also showed the same trend. By 8 weeks time E. coli and streptococci were completely destoryed. This is in agreement with the previous finding of Lekshmy (1964) that faecal indicator organisms underwent significant reduction during freezing and cold storage in prawns. According to Shewan (1954) the bacteria when frozen some suffered immediate death, irrespective of the rate of freezing or its temperature. Radhakrishnan et al. (1973) observed a gradual decrease of total and pathogenic bacterial counts in Bombay duck as the frozen storage period increased.

The shelf-life with respect to flavour, texture and colour was depicted in histogram 4. The fresh frozen sample had a shelf-life of 35 weeks and that of 8 days preiced sample had only 4 weeks. The changes observed were mainly - colour changed to dull white to brown, the firmness and elasticity of the material was replaced by sponginess and in spoiled samples the breaking up of the flesh and resulting oozing out of the drip and loss of the characteristic flavour.

Tressler & Evers (1957) reported that all clams especially the surf clam are to be well preserved by

freezing. This statement is not in agreement with the contention of Pottinger (1956). According to him clams lose their quality relatively fast, when frozen and become tough and spongy with a considerable loss of drip on being thawed.

The shelf-life for frozen stored clams were less than that of crab meat or mussel meat as evident from histograms 1,3 & 4.

4.3.5 Studies on canning of clam meat

Table 26 represents the analytical data of the canned products prepared from clams iced stored up to 13 days.

The moisture content of the finished product was ranged from 72.44 to 69.08% and loss of weight from 10.96 to 20.63%. Free amino nitrogen content in muscle and brine showed a gradual increasing trend as the days of icing advanced, i.e 24.3 to 60.3 mg% in meat and 26.5 to 52.6 mg% in brine. The organoleptic qualities showed that the products prepared from material iced up to 3 days had fair grade with respect to colour, odour and flavour, after that the colour was changed to dull white to brown, texture was changed to tough with bitter taste.

Table 26 Chemical and organoleptic characteristics of canned product prepared from fresh and ice stored clams

Pre-process ice storage	Moisture	Percent loss during sterili-zation	Free alpha amino nitrogen		Organoleptic qualities			
Days	gm%		Muscle (mg%)	Brine (mg%)	Colour	Texture	Odour	Flavour
0	72.44	10.96	24.3	26.5	Good	Soft & firm	Good	Good
2	72.40	12.50	26.0	19.3	"	"	"	"
3	70.37	17.58	69.9	47.1	Fair	Sl. tough	Fair	Fair
5	69.29	20.66	73.9	65.3	"	"	F - P	F - P
7	70.03	13.40	82.1	65.3	"	"	F - P	Poor
9	69.69	15.00	80.0	72.6	F - P	"	Poor	Bitter
12	70.31	9.65	44.6	43.2	F - P	"	Poor	"
13	69.08	20.63	60.3	52.6	Poor	Very tough pieces are quite small	"	"

4.3.6 Estimation of ribose in mussel and clam muscles

Table 27 represents the results of ribose obtained by the following methods namely;

(1) precipitate formed in TCA extracts during colour development using Mejbaum's method was centrifuged off, then the intensity was measured.

(2) using basic zinc for removal of protein in the muscle extract and then using the standard Mejbaum's method for estimating ribose.

(3) hydrolysis of the TCA extract of the muscle with 2N hydrochloric acid, then according to Mejbaum's method.

(4) development of colour of ribose in TCA extract was restricted at 90°C instead of boiling water (Mejbaum's method).

Table 27 Ribose mg/100 gm muscle

	1	2	3	4
Mussel				
1	199.6	82.4	197.0	196.7
2	202.1	99.0	198.1	197.1
3	179.7	75.2	178.5	178.0
4	198.6	81.7	196.2	195.5
5	231.3	108.0	229.2	228.5
Clams				
1	79.0	12.7	78.1	77.7
2	101.5	52.8	100.9	99.5
3	130.8	60.5	129.7	128.1
4	180.5	79.5	179.1	178.5
5	187.0	94.5	184.2	184.0

In the case of basic zinc a clear extract was obtained and the formation of black particles during colour development was prevented, but the extractability of ribose was reduced considerably. Comparable results were obtained in the other two methods without the precipitation during colour development. So methods 3 and 4 can be applied safely for ribose estimation while analysing mussel and clam meat.

SUMMARY AND CONCLUSIONS

5. SUMMARY AND CONCLUSIONS

Next in importance to fish and prawn is the crab, mussel and clam which have attracted the attention of man probably because of their sedentary habits and easy accessibility. In India there is a steady increase in the utilisation of crustaceans other than prawn. Crab meat is assuming great importance in human diet owing to its superior nutritional quality.

Crab (*Scylla serrata*)

Morphometric and weight measurements

Length to breadth ratio, decreased as the animal grew. 61 observations on length, breadth, whole weight and meat weight were done. Statistical analysis was also done. The

result of the study showed that there is significant correlation between length, breadth, whole weight and meat weight relations. It is also seen that the relation between length and breadth is directly proportional. From the study the most significant observation made is that if the whole length of crab is known the whole weight can be calculated by the equation:

$$\log w = -0.1586 + 2.3300 \log L$$

Similarly from length the meat weight or meat yield can be calculated as

$$\log w = -1.5745 + 3.0148 \log L.$$

Biochemical aspects of body meat and claw meat

Crab meat is very rich in protein 19.16 to 20.9%, high free alpha amino nitrogen and phosphorus. There is distinct difference between sample with egg and without egg; lesser values for crab carrying egg. There is appreciable difference in proximate composition, protein fractions, minerals and free amino acids between body meat and claw meat of crab. Claw yields higher meat content (42-47.3%) than body (23.6-36.0), and claw meat has higher water holding capacity. Claw meat is susceptible to rapid protein denaturation (10.6%) compared to body meat. The

textural difference of the claw meat is due to higher myofibrillar protein (58.98%) compared to body meat (56.63).

Studies on variation in chemical composition according to season in crab meat showed that maximum values of protein, water extractable nitrogen, non-protein nitrogen and glycogen were obtained during October to April when the moisture content of the muscle was at a lower level.

At the higher temperature (37°C) than atmospheric spoilage rate was found to be very high. Bacterial count increased from 7.2×10^5 to 6×10^7 in 8 hours time. At ambient temperature compared to 37°C depletion of muscle glycogen and formation of lactic acid was higher, most probably higher temperature retarded the enzymatic activities of post mortem changes.

At chilled storage temperature (6.5 - 7.5°C) the shelf-life was enhanced, the material remained in edible condition upto 6 days. Increase in bacterial count was observed upto 3 days and thereafter the changes were marginal.

Under ice (0°C) the material remained acceptable upto 11 days. Bacterial count recorded gradual increase upto 5

days, thereafter it remained more or less constant. Muscle glycogen dropped from 825 to 186 mg% and muscle lactic acid from 248 to 383 mg%.

The study provided an accurate measurement of the rate of spoilage. The study also showed that bacterial change was significant.

The study proved that proteolytic enzymes, most probably (Cathepsins and peptidases were very significant and active in crab meat.

An important observation made during this study is that in live crabs the flesh is held to the shell by a membrane making it difficult to remove the meat and if it is kept in ice for 18-24 hours the membrane breaks down and the flesh can be easily taken out as flakes resulting more yield. There was approximately 5% solid loss when whole crab was stored under ice upto 14 days. Organoleptic edibility reached fair after 10-11 days of storage. There was good correlation between organoleptic rating, amount of water soluble nitrogen, free amino acids, glycogen and phosphorus retained in the muscle.

This study thereby shows that any of these parameters be successfully employed for ascertaining quality of crab meat held under ice storage. 50% of total non-protein nitrogen

is contributed by free amino acids dominated by glycine and alanine (83%) responsible for the sweet flavour.

During cold storage of crab meat at -23°C, the sarcoplasmic protein showed slow but gradual decrease, but the extractability of myofibrillar protein diminished at a faster rate. The enzyme myosin ATP-ase activity dropped from 24.3 to 0.098 µ gpi/mg protein/minute during a period of 48 weeks. Appreciable changes were noted in the moisture content where proper glazing was given. Decrease in total nitrogen content of the muscle was noted followed by increase in nitrogen in the drip. At -23°C the frozen meat remained in organoleptically edible condition upto 52 weeks. Much loss in flavour was noted. Drip content decreased as the number of days the material was held under ice increased. Leaching of water solubles has taken place to a maximum in samples held for long in ice.

Glazes

Water, 1% ascorbic acid, citric acid, ascorbic-citric acid mixture, sodium bisulphite, glycine and EDTA were tried. Maximum shelflife was obtained for ascorbic-citric acid glazed sample, next being glycine glazed.

Optimum cooking time for crabs was worked outto be 15 minutes in boiling water or steam without pressure. Cooking loss was found to be 13-15 gm% and yeild of body meat 16-18% and that of claw meat 20%.

In 40 weeks time inner shellon crab remained good to fair at -18°C, but cooked meat was graded only fair to poor.

Canning

Hot blanching for 7 to 8 minutes in 7% brine, 6 minutes in 8% brine, 4 minutes in 10% brine was found to be best. Then canning in 2.5% brine containing 0.1% citric acid will give a product with minimum loss in drained weight and high organoleptic acceptability.

Mussel (*Perna viridis*)

Morphometric and weight measurements were done in this case also and the correlation equations were worked out for length to weight, length to height, whole weight to meat weight etc. From this yeild percentage per body could be mathematically worked out.

Mussel meat contained high protein (12.8%), glycogen (8.3%), alpha amino nitrogen content (184 mg%) and ribose (199 mg%). Most samples collected contained *E. coli* and *F. streptococci*. Among the free amino acids glycine (18.42%) followed by histidine (12.15%) and leucine (11.04%) constitute the major share.

In material held under ice 20% reduction in weight was observed in the first day and after 14 days the weight

loss was about 25%. 221 mg% of total nitrogen was lost from one day icedmussels. In 10 days time the material changed from good to poor stage with progressive loss of all nutrients.

In frozen mussel meat (held at -23°C) the protein solubility was dropped from 64.2 to 52.2% in uniced sample and from 58.0 to 45.2% in 8 days iced sample. During frozen storage the following changes were noticed.

1. Colour changed from dull white to brown.
2. Firmness and elasticity of the material was replaced by sponginess.
3. The loss of characteristic sweet flavour.

In 44 weeks the organoleptic rating was reduced from 9 to 5 in uniced sample and 5.5 to 2 in 8 days iced sample. On the whole the mussel meat had a shelf-life upto 40 weeks at -23°C (uniced) and 8 days iced only 15 weeks.

Optimum cooking time worked out for mussels was 15 minutes in boiling water or steam without pressure.

Weight loss occurred during sterilization of canned mussel was 1.2%.

Clams (Villorita cyprinoides)

Size-weight and length-height measurements were worked out.

Proximate composition of clam meat is as shown:

Protein	5.9 to 12.1%
Glycogen	1.4 to 7.8%
Ribose	79 to 187 mg%
Lysine	17.75% of free amino acids
Leucine	14.5% of free amino acids
Phenylalanine	11.82% of free amino acids

Coliforms and F. streptococci were detected

Under ice the shell remained closed upto 7 days and opened after. Live clams had a shelf-life under ice upto 9 days. Organoleptic score had fallen from 9.5 to 5.0. Chemical constituents in drip showed rapid increase after 5 days.

Fresh frozen clam meat stored at -23°C had a shelf-life of 35 weeks. By that period the colour was changed to brown, with loss of firmness and elasticity.

Canning was done with clam meat picked from clams held under ice upto 13 days. The organoleptic and chemical analysis showed that material held under ice upto 3 days had a fairly good flavour, odour and colour when canned.

Scope for future work

There are a number of investigations which could be carried out further in this line. Some of them can be listed as:

i. Microbial flora of natural materials and their changes during various processing. As the investigator has not much expertise in the field of microbiology these problems were left unsolved.

ii. Microbial standards can well be worked out by future investigations.

iii. Number of enzymes of high proteolytic activities are seen in these crustacea having marked influence on their shelf-life under ice. The characterisation of some of them may open new vistas in the muscle biochemistry of these crustaceans.

Industrial processing of crabs, mussels and clams although taken widely in India has not shown rapid strides as expected in terms of quantity inspite of heavy demands from overseas markets. This is largely due to the lack of precise information on the resources and absence of technical data on the amenability to processing of the different

varieties available. The high labour costs involved in harvesting and separating the muscle from the shell and the subsequent removal of sand from the meat (clam) also hinder their industrial processing economically.

The results of the investigations prove that a number of parameters as reported in this work have to be considered and evaluated both at harvesting and subsequent post-harvesting and processing of these valuable food commodity if it is to be an economically viable proposition industrially.

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