INVESTIGATIONS ON HEAD DROOPING IN HEAD-ON PROCESSED PRAWNS

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THESIS

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DECLARATION

I hereby declare that this thesis entitled INVESTIGATIONS ON HEAD DROOPING IN HEAD ON PROCESSED PRAWNS is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree diploma association fellowship or any other similar title of any other University or Society

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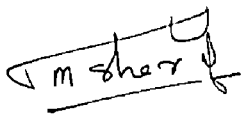
Dr P M Sh r t Ass t Prof (B oc l emistry) D pa tm of Pro sing Te h olo gy C l ge of F sheries Pa a gad Cochi n	CHAIRMAN	
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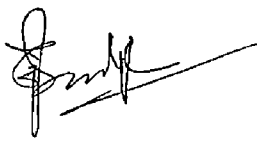
o T M San a an A o Prof and H ad Depa tm nt of Ma agem t s ud es Co g of F sheri s P n ad Co hin	MEMBER	
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INTRODUCTION

INTRODUCTION

The seafood industry and export of frozen seafood items from India started during 1950s The first processing plant in the country was commissioned in the year 1951 with a capacity of only 1 5 tonnes In 1953 the frozen seafood item was exported for the first time from India by M/s Cochin Company Cochin From this humble beginning the seafood export industry started growing steadily Plate freezing was the major freezing method employed for processing marine foods and the major markets for these items were USA and Japan up to the first half of 1980s The products preferred in these markets were headless(H/L) peeled and undeveined(PUD) and peeled and deveined(P&D) and cooked peeled and deveined (CPD) forms of prawns

But a sea change has occurred in the seafood industry during the second half of 1980 s with the emergence of European Economic Community (EEC) providing a potential market for our marine products and with the introduction of Individually Quick Freezing(IQF) machineries

The European market is characterised by its high potential and with member countries differing greatly in product variety quality requirements and demand As a result of this processing of seafood into a number of novel forms such as head on frozen prawns vacuum packed

prawns, modified atmosphere packed (MAP) prawns etc came into existence in the Indian seafood industry

The first IQF machine was installed in India by M/s Gallant Sea Foods Cochin in 1986 Later a number of imported as well as indigenous IQF machines were commissioned Now over three dozens of IQF machines are in operation and many are in the course of installation

Highly competitive professional management groups supported by qualified and trained person from various fisheries organisations hand in hand with the overseas buyers with very strict quality stipulations have already created a quality conscious approach in the industry

In addition the production of prawns through aquaculture in India is in the waking up stage now The most outstanding development in the world prawn industry during the last few years has been the boom in aquaculture With the natural resources of prawns reaching the optimum limits of exploitation production through culture is the only logical answer In the aquaculture sector India is in the most advatageous position with its vast potential area suitable for culture In addition we have a seafood industry with over four decades of experience in the international seafood business Marine products are one of the few items included in the thrust area for development of exports and our government and industry are

committed to the development of prawn culture for augmentation of production It is expected that large additional areas will be brought under prawn culture within a couple of years and our country will be in a position to supply cultured prawn in adequate quantities to meet the growing world demand before long But a sudden flooding of the international market with cultured prawn will cause glut situation and poor price realisation So product diversification and product quality upgradation are inevitable for India to survive and to gain an edge over others in the highly competitive global prawn market

Aquaculture can produce superior quality raw material with certain predictability of catch Such raw materials are best suited for the production of highly value added and premium priced products such as head on prawns for export So the future of Indian seafood industry will be an era of producing value added premium priced products using sophisticated machineries and advanced technologies In such a production sector quality of the final product rather than the quantity will decide the success of any processing firm So to boost up our export product diversification is necessary through research and development activities In this context ~~x~~ ~~x~~ processing of prawns as head on has become a new field of interest with good developmental potential

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Processing of prawns is gaining wide spread attention now a days due to a number of advantages associated with this method of processing Firstly it is one of the most economic ways of processing prawns Here the advantage is that nothing is wasted during processing Yield is almost 100 It is equally competent with headless form in fetching the price Also the problem of waste disposal is eliminated in this way of processing

Quality and reliability of supply are the two major factors that decide the success of any product in the international market with an existing demand The producer of a head on frozen prawn product is in the most advantageous position as he gets 100 yield and a premium price supported by a consistent demand for the product The consumer is also benefitted by purchasing head on frozen prawns as the raw material used for this type of processing is of excellent quality

The development of European market as a potential market for Indian seafood items paved the way for producing head on frozen prawns in India Of the total export from India the quantity of head on prawns contributed 2 0 to 2 5 during the last two years Almost 95 of this was exported to European countries In Japan also there exists some demand for head on frozen prawns

Earlier Indian seafood export concentrated mainly on two markets viz Japanese and U S markets The product forms preferred in these markets included block frozen

peeled and undeveined prawns headless prawns and peeled and deveined prawns Now export to both these markets is declining In Japanese market we are facing stiff competition from the South East Asian countries because of their fast growth in aquaculture and nearness to the target market The export to USA is a risky operation due to the stringent USFDA regulations and other reasons

Head drooping is the major problem one faces during the production of head-on frozen prawns Eventhough there exists a good market for head on frozen prawns which can fetch high economic returns the problem of head drooping makes it a high risk operation

Head drooping is the result of the breakage of the arthrodial membrane which is also called as the neck membrane of the prawn It is this thin and delicate membrane which connects head of the prawn to the abdomen The breakage of this membrane results in the oozing out of the hepatopancreatic juice which the consumer values much Head on frozen prawns are purchased at a premium price for this hepatopancreatic juice which is sweet in taste on cooking and is relished by the consumer As a result of head drooping the much valued and relished hepatopancreas is lost and the product gets rejected by the consumer

The hepatopancreatic juice is present within the outer covering of the gland and the arthrodial membrane gives

further protection to it Hepatopancreas contains the major digestive enzymes of prawns The arthrodial membrane which is responsible for the flexibility of the abdomen is a delicate calcified nonchitinous membrane In addition during the process of moulting it is this arthrodial membrane which breaks first (Cheng 1991)

Most of the Indian packers who received orders for head on frozen prawns failed in their initial attempt mainly due to the problem of head drooping Later some of the processors with technical collaboration with their overseas buyers became successful in exporting good quality head on frozen prawns However many are still facing problems and now only half a dozen firms (Fig 1) in India are involved in the processing of head on prawns Some of these firms pack their products in the brand names of their overseas buyers as a proof of their product quality Those producing head on prawns are still receiving countinuous orders from their overseas buyers

The prawns used for head on processing in India are Penaeus monodon, Penaeus indicus , Metapenaeus affinis Metapenaeus monoceros and Penaeus semisulcatus

As such head on processing is a new field of interest The problem of head drooping is the major obstacle in the development of this industry in India No study has been carried out so far in understanding the cause of head drooping in head on frozen prawns Present study is an attempt to find out the factors causing head drooping in

1. M/S BABY MARINE EXPORTS – 'Indian Star'
2. M/S ABAD FISHERIES – 'Pesca Brava'
3. M/S CHOICE CANNING CO. – 'Choice'
4. M/S BHARATH SEA FOODS – 'Beirafrio'
5. M/S CHEMMEENS EXPORTS – 'Maharaja'
6. M/S KERALA SEAFOODS – 'Venus'

Fig. 1 Firms involved in the processing of head on prawns in India

head - on processed prawns The results of such a study will enable us to suggest remedial measures for head drooping in head-on frozen prawns which in turn will be of great help to the prawn pro essing industry

In view of the above detailed investigations have been undertaken to find out the cause of head drooping in head on frozen prawns

The investigations carried out in this respect include

1 Effect of prawn species on head drooping

2 Effect of prawn size on head drooping

3 The influence of various hauling times on the extent of head drooping species wise and size wise variations

4 The influence of Iced storage duration of prawn before processing on the extent of head droop ng in various size groups of prawn

5 Different methods of freezing and their influence on various size groups in producing head drooping

6 Different durations of cold storage and their effect on head drooping in different size groups of prawns

7 Different methods of thawing and their effect on head drooping in various size groups

8 Thickness of arthrodial membrane specieswise and size wise variations

9 Bacterial load of different species and size groups of prawns

10 The biochemical composition of the arthrodial membrane Species wise and size wise variations

11 The activities of various hydrolytic enzymes of hepatopancreas and their variations among different species and size of prawn

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

Prawns are caught processed and traded by many countries in large quantities and in different attractive styles It is one of the most valuable products in the international market However they are highly and easily perishable To produce premium quality products from such highly perishable raw materials the conditions promoting quality deterioration have to be checked at all stages starting from the operating conditions of the gear used Such a system approach to food quality management is gaining importance in most fields and hence in seafood industry also (Hubbard 1990)

Numerous biochemical reactions continue to take place in the body of the shell fishes even after death Biochemical changes affecting the composition of the prawn meat can be influenced by a number of factors along the total commercial cycle of a commodity To produce finished product of high quality it is important to protect the integrity of the product at every stage of commercial cycle (Pedraja 1970)

Harvesting processing transportation and marketing must operate as a unified system if aquatic products of highest quality are to reach the consumer Aspects of quality maintenance begin with harvesting and are carried

through the production system to the consumer (Wheaton and Lawson 1985) So studies on the variations in quality during individual unit process is gaining importance

Quality maintenance begins with harvest The quality of aquatic products vary based on the type and operating conditions of the gear used Of the various gears available trawling especially bottom trawling is the main harvesting method used for capture of prawn from sea which accounts for the bulk of prawn landing in India

The successful operation of trawler depends mainly on three groups of factors such as operational factors design factors and cost factors of which the most important are the operational factors and includes distance of the fishing grounds economic cruising speed of trawlers trawling speed speed of the trawling winch duration of trawling cycle number of fishing trips per year and number of hauls per trip (Choudhuri 1985)

According to Govindan (1985) in the trawling method of fishing the hauling time should be reduced to a minimum to increase the keeping quality of trawl caught fish The average duration of trawling cycle will comprise of the time required for shooting the net the actual duration of trawling the time required for hauling the net and the time required for emptying the nets This will in turn depend on the richness of the fishing ground nature of

sea bottom and sea condition In India this is found to vary from 30 120 minutes and the corresponding duration of trawling varies from 18 80 minutes (Choudhuri 1985)

Harvesting is a traumatic experience to aquatic animals They are confined in a small area and crowded closely together Oxygen level depletes in the water and they react to this crisis by high activity increased oxygen demand and rapid swimming The prawns strike each other removing some of their protective slime and bruising their flesh (Wheaton and Lawson 1985)

Intense physical activity usually observed during harvesting by trawling causes rigor to start earlier The rigor so produced is less intense and is of short duration The intensity and duration of rigor mortis profoundly influence fish quality and the spoilage bacteria do not attack fish until rigor mortis is completed (Amal her 1961)

Shimuza and Yayuri (1986) studied the influence of death condition of fish on gelling potential of fish muscle He is of the opinion that the struggling killed fishes are not only poor in gelling potential but also more susceptible to quality deterioration compared to instantly killed fishes

LimaDos Santos (1978) is of the opinion that in tropical climates (25 40°C) the chance of spoilage of fish

even before their removal from the net is possible But fish from cold water (10 °C or less) may remain in an acceptable condition for up to 3 days

Tsuchimoto et al (1985) observed that the mean k value for fish by one hour of towing was significantly lower than by 2 or 3 hours of towing Also the mean k values were found to increase with higher occurrence of trash fish per hour and in percentage of total catch A negative correlation was observed between the k values just after catching and body weights of fishes Tsuchimoto et al (1986) studied the freshness of trawl caught fish at fishing ports and retail markets and reported a significant corelation between the mean k values of the fish landed by fishing boats and the number of days in a cruise

Harvesting and on board handling practices should aim to extend rigor and/or maintain quality Good practice can almost double the length of rigor mortis and hence a prolonged shelf life to the product

The typical biochemical composition of prawn makes them more susceptible to quality deterioration As a whole they contain higher amounts of free amino acids than teleosts This largely facilitates bacterial growth and presumably explains their rapid spoilage which is highly evident in most shellfish (Velankar & Govindan, 1957 1958) The studies on bacterial population in

relation to spoilage of seafoods started as early as the begin & of the present century The role of bacteria in spoilage of seafoods was reported by Anderson (1907) and subsequently it was established that bacteria involved in spoilage are those present in fresh condition (Hunter 1920, Harrison 1929)

The microbial population of a frozen product depends upon various factors such as the nature of raw material its pre and post process treatments the sanitary condition of the processing factories the rate and nature of freezing (Tannenbaum 1976 Chen et al 1990 Koburger et al, 1973) the temperature and period of storage (Reilly et al 1986 Pillai et al 1961) the original numbers types and stages of growth of microorganisms present (Hess 1934 Yasuda & Kitao 1980) thawing process and physical protection offered by the food (Chang 1989 Hood and Meyers 1973)

Large amount of data is available with respect to the composition of microflora associated with marine fish The various works on the bacterial flora of Indian marine fishes have shown the presence of Achromobacter Flavobacter Pseudomonas and Vibrio as the main composition of flora and either Achromobacter or Flavobacter dominating in early spoilers whereas Pseudomonas dominating in the late spoilers (Anand &

Setty ,1977 Karthiayani & Iyer 1967)

Studies on the bacterial flora of head on prawns especially tropical species are scanty Shamshad *et al* (1990) studied the bacterial flora of tropical prawn, *Penaeus merguensis* The main bacterial flora in order of predominance were of the genera *Moraxella* ,*Micrococcus* *Pseudomonas* and *Bacillus* The presence of other species such as *Alteromonas* and *Vibrio* depends on the storage time and temperature Studies on fresh Gulf shrimp (Campbell & Williams 1952) revealed *Micrococcus* *Achromobacter*, *Pseudomonas* and *Flavobacterium* as dominant species Carroll *et al* (1968) reported a similar pattern of population changes in White Brown and Pink shrimp But according to Vanderzant *et al* (1970) Coryneforms *Pseudomonas* *Moraxella* and *Micrococcus* are the predominant forms in Gulf shrimp Cobb *et al* (1976) reported the initial predominence of *Vibrio*, *Pseudomonas* and / or *Moraxella*, *Acinetobacter* species in ice stored *Penaeus aztecus* and *Penaeus setiferus* They further observed that after 12 15 hours,*Vibrio* species disappeared and *Pseudomonas* species predominated followed by *Moraxella* and *Acinetobacter* species The predominance of psychotrophic gram negative bacterial flora belonging to the genera *Pseudomonas* and *Alteromonas* were investigated by several workers (Hobbs , 1983 Hobbs and Hodykiss 1982 Lee & Pfeifer 1975, Nickelson & Venderzant 1976, Spreekens 1977)

Pseudomonas and Alteromonas are known to be active spoilers having proteolytic activity Certain strains ofPseudomonas exhibit collagenolytic activity (Hobbs & Hodykiss 1982 Kazanaz 1967)

Yasuda and Kitao (1980) observed in Penaeus japonicus that the dominant microbial flora in the digestive tract of adult prawn is Pseudomonas and remains same for specimens obtained from wild and culture ponds He also observed a definite correlation between the bacterial flora of the adult prawn to its habitat (bottom sediment) In head on fresh tiger prawns obtained from culture ponds Reilly et al (1986) observed the predominance of Enterobacteriacea (20)

The studies of Hood & Meyers (1973) showed that species of bacteria present in the intestinal tract of penaed shrimp are characterised by active production of proteolytic amylolytic lipolytic and chitinolytic extracellular enzymes growth at relatively low pH short generation times (ie 30 minutes) at relatively low temperatures (ie 22 C)

Flick and Lovell (1972) showed that in many studies concerning post mortem biochemical characteristics of fish and shellfish muscles the samples were obtained from commercial fishing sources and the animals were used after allowing for severe exhaustion before death So the

antemortem handling and storage of shrimp before sampling are important in deciding the type and amount of bacterial population To get a true picture of degradation processes, the freshness indicators are to be determined using nonstressed muscles of prawns measured at close intervals from the point of death through an extended storage period Setty (1985) is of the opinion that studies on the bacterial flora of fish collected from uniced fish collected from landing centres and market places as well as using pour plate technique are bound to give a wrong picture of bacterial flora associated with fish

Cobb et al (1976) pointed out that the differences in bacterial flora of prawn can be attributed to differences in shrimp species marine environment handling practice onboard and period and temperature of ice storage In addition to the above mentioned factors differences in bacterial population reported for fresh shrimp may involve differences in composition of plating media and conditions of plate incubation Mathen et al (1979) and subsequent studies of Varma et al (1986) showed that the TPC obtained at 30 °C gives better results eventhough the relevant specifications in the Indian standards prescribe 37°C as incubation temperature Elliot (1963) is of the opinion that microbial standards and specifications should be specified in detail not only as to the number of bacteria but also to the methods and media employed

Pedraja (1970) is of the opinion that uneven levels of bacterial distribution often observed in prawn samples can be explained by the mechanisms affecting the muscle substrata of individual prawns Shewan (1971) and Lee & Pfiefer (1977) concluded that the environment is the major factor that dictates the composition of micro flora in seafoods A low initial bacterial count is supposed to extend the shelf life compared to high bacterial counts Controversial reports are available on the variations of bacterial flora with size of prawn Green (1949) showed that an inverse correlation between the size of the shrimps and bacterial count Larger the size of prawn smaller the bacterial count However Williams et al (1952) could not correlate size to bacterial count They contended that the presence of mud on prawn caused high bacterial counts The influence of mud in affecting the bacterial count obviously depends on the relative numbers of organisms in the mud and prawn

Considerable differences exist in the values of intial bacterial load and predominant microbial flora of fresh prawns Reports on the bacterial load of Penaeus setiferus (Cobb et al 1976) cultured head on Penaeus monodon (Reilly et al 1986) Penaeus merguensis (Shamshad et al 1990), Parapenaeopsis stylifera Metapenaeus dobsoni (Pillai et al ,1961) show that the values of TPC reach

10^6 10^7 in prawns at the stage of reaching spoilage

In the processing plant bacterial load undergoes changes during various unit process as washing ice storage freezing cold storage etc Bacterial load have been used as an index of spoilage and sanitary quality High bacterial counts are unacceptable but do not always indicate extent of quality loss or spoilage This is due to the differences in biochemical activities of individual bacterial species

Pillai et al (1961) showed that almost 90% of the surface bacterial flora of prawn can be removed by washing The observations of Iyer and Choudhuri (1966) show a mean reduction of 14% in surface bacterial count by the washing effect of ice during first and second day of ice storage Significant lowering of aerobic plate count in iced and oxygenated packs of headon Penaeus monodon during 26 hours was observed by Chen et al (1990) In head on Penaeus monodon an increase of standard plate count (SPC) at 20 C was observed by Reilly et al (1986) Shewan (1949) found a 90 reduction of residual bacteria by deep freezing

Observations show that various unit process influence both bacterial load and flora In the ice storage, duration and temperature of storage play a predominant role in the variation of intial bacterial flora A definite succession of bacterial genera during ice

storage of tropical prawns was reported by Surendran et al (1985)

The influence of storage temperature and time on the bacterial population of head on Penaeus merguensis obtained from tropical waters (15 40°C) were studied by Shamshad et al (1990) in storage temperatures ranging from 0 40 ° C The results show that initial bacterial flora were gram positive types but changed to gram negative psychrophiles at lower storage temperatures and to mesophiles at higher storage temperatures A short term exposure of prawns to unfavourable temperatures can significantly augment quality deterioration during further processing Fieger et al (1958) showed that holding fresh shrimp for 2 hours at air temperature of 26 29 ° C before icing resulted in a 2 fold increase in bacteria after 6 days compared to controls Similarly a 6 hours exposure resulted in 5 fold increase in bacterial count

The raw prawns are held in ice during transport and subsequent storage prior to processing Knowledge of the biochemical and bacteriological changes occuring in the ice stored material is of considerable importance in predicting the quality of processed products (Jacob et al ,1962)

Spoilage of prawns in or out of contact with ice and its assessment have been studied by Velankar et al (1961) Velankar and Govindan (1958)

reveal that considerable losses of extracives such as free aminoacids occur during storage of prawns in ice due to leaching

Collins (1961) and Iyengar et al (1960) have also shown that water soluble chemical romponds including those produced by spoilage leach out during storage in ice Velankar et al (1961) studied the chemical and bacteriological changes of prawns during storage in ice when held in 3 forms as Round Headless and Peeled and deveined Based on this they concluded that the prawns held as headless are comparatively of better quality than if they are held as round or peeled and deveined

The studies of Reilly et al (1986) on storage life of head on Penaeus monodon at 0 5 and 10C reported a shelf life based on sensory score of 4 as 16 11 5 and 6 5 days respectively At the point of rejection samples showed soft texture ammoniacal odour and a bitter to strong sulphide taste On the suitability of prawns for premium quality head on product they observed that at 0°C head remained firmly attached for two days only after which prawns lost their value as prime quality head on produce

Quality deterioration of head on fresh water prawn in ice stored or refrigerated condition is charecterised by softening or mushiness This causes loosening and flaking of the cooked prawn tail when touched or rubbed (Nip et

al ,1985 a) This mushy texture develops within 2 3 days in ice storage and is most pronounced in the proximal tail section (adjacent to cephalothorax) It progresses downward to other sections of the tail with prolonged storage in ice One possible cause of this problem may be the activity of proteolytic enzymes released during postmortem (Branowski et al 1984 Nip et al 1985b) Other possible sources of enzymes to cause mushiness may be the psychrophilic bacteria But Premartne et al (1986) have shown that the psychrophilic bacteria producing collagenolytic and proteolytic enzymes do not reach a high percentage of the population until after day 6 of ice storage So a significant bacterial degradation of tissue cannot be expected during the first 2-3 days of ice storage

Velankar et al (1961) carried out studies on prawns held at 0°C out of contact with ice They observed that by the 6 th day the prawn meat at the point where the head was broken off was yellow ,presumably due to autolytic spoilage At this point odour became deep though not markedly offensive But at the same period the percentage incidence of melanosis reaches 100

Eventhough the breakage of head from tail happened within 6 days by autolytic changes in head on prawns the various parameters as TVN (Total Volatile Nitrogen) bacterial count volatile acid number TMA (Tri Methyl Amine) etc did not show significant variation

This points out the unsuitability of using the above parameters in assesing the quality of head on prawns during ice storage The above parameters showed sharp rise only after 6 days Whether this change is due to the psychrophilic bacteria or by the direct contact of hepatopancreatic enzymes is yet to be established

Devadasan and Nair (1970) observed that in prawns myofibrillar proteins got denatured at a rapid rate than sarcoplasmic and stroma proteins during ice storage

Matsumoto & Yamanaka (1990 a) observed drastic variations of the glycogen and lactic acid during the first day of storage at 5 $^{\circ}$ C (in Kuruma prawn muscle) Glycogen content decreased from a maximum of 63 mg/100g to 22 mg/100g Whereas lacticacid increased from 5 6 mg/100gm immediately after death to 49 mg/100g during one day of storage Matsumoto and Yamanaka (1990b) observed in Kuruma prawn muscle that the lactic acid content increased to a level of about 50 mg/100g during storage at low temperatures and that it can be used as a useful decomposition index for freshness of seafoods The increasing rate of lactic acid formation depend on the rise of storage temperature The maximum level of lacticacid during storage at 5 0 and 1 $^{\circ}$ C reached after 1 7 and 9 days respectively

Freezing is an excellent process for preserving the quality of meat and meat products for a long period It functions by a combination of internal dehydration (ice crystal formation) and lowering of temperature (Fennema, 1970) Though freezing controls various biochemical reactions including those of microorganisms such changes will countinue or will be active at a low pace even in lowered temperatures and produce quality deterioration of frozen products

Great many classifications of freezing methods and freezers are available based on various freezing characteristics such as rate of freezing time temperature mode of heat transfer form of final product etc Block freezing (plate freezing) air blast freezing, immerson freezing (using Na Cl / Ca Cl_2 Solution) cryogenic freezing (using liquid N /CO_2) are the major methods in use for the freezing preservation of shell fishes in India

The biological tissue is composed of cells The water of the tissue is contained within the cell (intracellular fluid) and between the cells (intercellular fluid) The concentration of salts and other solubles is higher with in the cell than outside The cell membrane acts as an osmotic barrier and maintains the difference in concentration

When a product is frozen first ice crystals are formed outside the cell since the freezing point of the diluted

fluids there is higher than that inside the cell If the freezing rate is low the cell wall lose water by diffusion through membrane and water will crystalise to ice on the surface of the crystals already formed in the intercellular space As the cell loses water the remaining solution becomes more and more concentrated and results in the collapse of cell membrane Larger crystals formed outside the cell membrane occupy a larger volume than water outside the cell and therefore excert physical pressure on the cell membrane Such pressures are high enough to damage the cell membrane and result in increased drip loss on thawing of the product The slow freezing results in dryness and loss of tenderness of the product (Londahl 1991)

Reay (1931) pointed out loss of water holding and gel forming capacity and salt solubility of brine frozen fish muscle and observed max mum denaturation around 3°C Reay (1934) observed that slow freezing results in rupturing of cells and membranes and disorders the ultra structure of cells Rehbein et al (1978) and Konagaya (1980) observed the excessive softening of muscle occurs at 3°C and is of the opinion that quality deterioration is by activation of cathepsins rather than by denaturation

Pedraja (1970) showed that free moisture influences enzyme activity in frozen foods So the importance of

achieving the lowest possible temperature at the earliest time during the processing and freezing of prawns is important to reduce autolytic enzyme activity Electron microscopic studies revealed a reduction in distance between filaments and myofibrils on cold storage of frozen products (Olley 1980)

The electron microscopic studies of DeMan (1990) showed that increase in the volume of specific weigh of water during freezing causes disruption of the orientation and organization of microorganells of the cell This leads to reduction of distance between myofilaments and favours the formation of cross bridges between them and stiffens the fibres

The dehydration of protein molecules through freezing by the migration of hydration water molecules to form ice crystals would result in the disruption of the hydrogen bonding system as well as the exposure of surface regions of protein molecules leaving them unprotected and vulnerable This induces protein aggregation and consequently denaturation (Shenouda 1980)

The effect of salt concentration on protein denaturation aggregation or dissociation is based on the effects of salts on the secondary forces which help to stabilize the tertiary and quarternary configuration of protein molecules The stability of ionic bonding and other secondary forces is dependant on dielectric constant

pH and ionic strength of the media The increasing ionic strength will result in a mixture of dissociational aggregational and conformational changes The critical salt concentration differs according to the type of salts and type of tissues involved (Shenouda 1980)

The orientation and hydrolysis of lipids in fish and shell fishes during frozen storage causes serious quality deterioration During frozen storage contents of phospholipids along with other lipid classes decreases due to the actions of endogenous lipolytic enzyme systems Leung et al (1990) observed that changes in lipid classes proceed at higher rate in samples stored at 20 ^{o} C than in samples stored at 35 ^{o} C They also showed that the lipid deterioration was low in the samples with enclosed deoxygeniser

The apparent relationship between the fat content of fish and their storage stability noted on various species (Dyer 1951 Dyer and Dingle 1961) have led to the hypothesis that the presence of moderate levels of lipids may protect proteins from denaturation during frozen storage The protective effect was credited to neutral lipids such as triglycrides However cell damage and deformation in micro organells during freezing and frozen storage leading to liberation of lipid protein components may affect the texture quality of muscle tissue (Shenouda 1980)

Also the effects of free fatty acids and lipid deterioration products on protein during frozen storage × × × × may outweigh the protective effect of intact lipids The mechanism of free fatty acid myofibrillar protein interaction has not been fully understood but suspected to be primarly through secondary forces (Hansen & Olley 1965)

It is now well recognised that deteriorative changes take place during frozen storage of fishery products and that major determinants of these changes are the conditions of time and temperature that prevail during several phases of frozen transport and storage involved Shaban et al (1987) indicated that 40 ^{o} C is reasonable to keep quality unchanged for a long term storage though 20 ^{o} C is low enough to keep for a few months Compared to 20 ^{o} C & 30 ^{o} C those prawns stored at 40 ^{o} C showed no significant change in pH expressible drip and tyrosine content It is a well established fact that frozen prawn can be stored for a longer time at lower temperature without detectable loss of quality Riaz & Quandri (1987) proposed that by knowing the time temperature history and quality loss during storage the handlers of frozen prawn can predict shelf life

Hepatopancreas is the main perhaps the only organ of

digestive enzymes in crustacea (Vonk 1960 Lockwood 1968 Gibson & Barker 1979) The organ is involved in digestion storage and resorption of nutrients (Djnamah & Grove 1970) The composition of the organ is dependent on various factors such as body size season and dietary stress (Leung et al 1990) Starvation alone affects the biochemical composition (Cuzon et al 1980) and histological structure (Pappathanassiou & King 1984) The structure and function of this organ in crustacea in relation to biochemical activities formed the subject of various studies (Caeccie et al 1988 Almohanna & Nolt 1986 Djanamah & Grove 1970)

Caeccie et al (1988) studied the ultra structure of hepatopancreas of White shrimp and showed that while the cells in the mammalian digestive system are extremely interdigitated and held together with desmosomes this is not the case in prawns In the hepatopancreas tubules of prawns the lateral boundaries of cells are very straight and parallel and the sloughing off process of the epithelial cells are more prominent This sloughing off process and loss of cells is compensated by the proliferation and differentiation of E cells at the distal ends of the tubule So the new cells are present on the distal part of the tubule Comparing to the mammals the cells are less interdigitated and hence are less resistant

to mechanical stresses In the case of prawns the degree of protection is against mechanical damage might not be warranted as in mammals since prawns possess a screen of setae at the caudal end of the foregut which allows only finest particles of food to enter the lumen of the tubules In general hepatopancreas of prawn is less sturdy and hence is easily susceptible to mechanical damages especially on postmortem autolysis and handling

Most aquatic species including crustaceans contain a number of enzymes capable of acting on the tissues after death Studies on these enzymes in relation to degradation are scanty (Eitenmiller 1974) Pedraja (1970) is of the opinion that biochemical changes taking place after death of shrimp will increase with increase in temperatures due to higher activity of proteolytic enzymes at higher temperatures In addition the autolysing enzymes present in the shrimp muscle will bring about an incraease in the activity of bacterial enzymes The autolising enzymes degrade the muscle proteins and release simple peptides for bacterial growth The release of mitochondrial and lysosomal enzymes during freezing and thawing process is reported to correlate with quality deterioration (Shimomura 1987) The progressive leaching out of hydrolytic enzymes causing autolytic spoilage of fishes krill and prawns were reported by many authors (Warrier et al 1985 Gildberg 1988 Nip

et al 1985 a) Uchiyama et al (1966) reported that in the early stages of deterioration freshness of seafoods is effected by autolysis rather than bacterial action The action of endogenous enzymes and exogenous proteases causing an increase in muscle tyrosine content was reported during the ice storage of Penaeus japonicus as headless prawns (Shaban et al 1987)

The texture deterioration and resulting mushiness formation in freshwater prawn is supposed to be the action of hepatopancreatic enzymes released up on death of prawn (Branowski et al 1984 Nip et al 1985 a) This problem is more pronounced in the proximal section of the tail muscle (adjacent to the hepatopancreas) in whole prawns suggesting the theory related to the action of collagenolytic enzymes released from hepatopancreas during storage The proximal deterioration in head on prawn than headless prawn suggest the role of hepatopancreatic enzymes in postmortem tissue degradation (Papadopoulose et al 1989)

The hepatopancreas of crustaceans produce a variety of carbohydrases proteases peptidases lipases and special enzymes like cellulase lichenase chitobiase chitinase and alginase in certain species (Vonk 1960 Van Weel 1970 Koopmans 1970)

Quantitative enzyme studies of hepatopancreas utlizing

more closely related taxonomic groups have revealed differences in enzyme activities that can be related to feeding habits and/or gut morphology (Sather, 1969 Reid and Rauchert 1972 Brun and Wojtowicz, 1976) Several authors have described differences in the properties and characteristics of invertebrate enzymes as compared to those of vertebrates (Zwilling and Neurath 1969 Gates and Travis 1969 1973) Sather (1969) discovered a higher protease activity in omnivorous than in carnivorous while investigating the amylase and protease of decapods Tsai and Chaung (1986) showed that species of prawns under genus Penaeus contain high levels of digestive serine proteases and the hepatopancreas is found to be the organ having the highest activity

Low or absence of digestive chymotrypsins in shrimps was proposed by Maugle et al (1982) Galgani et al (1984) Lee et al (1980). But later studies by Tsai and Chaung (1986) using specific and synthetic substrates showed abundant activity of both trypsin and chymotrypsin from different parts of the digestive tracts of Penaeus monodon Penaeus japonicus Penaeus pencillatus and a low occurence in Metapenaeus monoceros and Macrobrachium rosenbergii Phylogenetic similarities and differences do exist for the activities of enzymes between closely related species (Ceccaldi et al 1982) Proteolytic enzymes have been elucidated to be involved in the rapid

deterioration of crustacea which even proceeds at low tepmeratures

Honjo et al (1990) is of the opinion that although shrimps are caught and cultured in a large amount all over the world the biochemical data available on enzymes are not enough to evolve useful means for suppression of shrimp deterioration

The enzymes involoved in the autolysis are many However lipases including phospholipases trypsin like enzymes and cathepsins mainly cathepsin D are important The almost neutral pH optimum and high activity of phosphorylase at ambient temperatures as well as subzero temperatures (Nowlan and Dyer 1974 Mukundan and Nair,1977) considerably augment glycolysis or glycogenolysis in muscle leading to the accumulation of lactic acid

Trypsin and trypsin like enzymes have been isolated from different kinds of mammals fishes and invertebrates Recently such enzymes have been elucidated to be concerned with rapid deterioration of crustacea especially Antarctic krill which proceeds even at low temperatures (Sather et al 1987)

Detailed studies of Honjo et al (1990) on the trypsin type enzymes of Penaeus indicus showed that the enzymes were significantly different from any of those reported previously The enzymes showed broad pH optimum (pH

6 5 to 11) and stability (pH 6 12) Similar studies on other species such as Penaeus kerathurus and Penaeus setiferus showed only a narrow range for optimum action

Sather et al (1987) observed during the autolysis of krill a significant accumulation of free aminoacids in thorax This is very high when the digestive tract is not removed on postmortem compared to one without digestive tract

Cathepsins belonging to lysosomal proteases take part in the protein breakdown in animal tissue and also in the post mortem autolysis of muscle tissue (Yamashita et al 1990) Pepsins have not been detected from invertebrates and apparently cathepsin D and other cathepsins act both as digestive and lysosomal enzymes in many of the invertebrates The cathepsin D in invertebrates is supposed to be the ancestor enzyme of aspartic proteinase as pepsin in higher animals (Gildberg,1988)

Although pepsins and cathepsin D have similar specificity and molecular structures they differs in physiological role Cathepsin D is a lysosomal enzyme active in intracellular protein digestion and pepsin is having an extracellular digestive function

Cathepsin D is stable in weak alkaline and acid conditions but denatured at a pH below 2 5 (Barret 1977) The optimum pH is about 3 5 and there is no conclusive evidence for the existence of a zymogen form of this

enzyme The enzyme splits peptide bonds on the amino side of aromatic amino acids with bulky sidechains (Barret 1977)The smallest peptide susceptible to cathepsin D are pentapeptides ATP (Adenosine Triphosphate) found to activate Cathepsin D (Pillai and Zull 1985)

Almy (1926) was the first to show that proteins in muscle tissue were slowly degraded even if the viscera had been removed Similar observations were obtained by Sibert (1958) Cathepsin is now considered as one of the major factors in ripening as well as deterioration of fishery products

The findings of Vonk and Western (1984) confirmed that cathepsins play an important role in digestion in many invertebrates (Vonk and Western 1984)

Occas ionally presence of pepsin like enzymes in aquatic invertebrates have been reported (Dabrowski & Gloyowski 1977 Kawamura et al 1984 Rehbein et al 1986 Lee et al 1980) However no evidence has been presented for the existence of zymogen forms of pepsin in invertebrates (Vonk & Western 1984) and most likely observations of pepsin like activities in such animals is due to the action of cathepsin D

Fish muscle apparently contains about 10 times as such cathepsin D as mammalian tissue It has been suggested that the high enzyme level in fish is a compensation for

reduced activity at low habitat temperature (Sibert,1958 Mukundan et al 1986) However it may also be due to high metabolic activity

Cathepsin D as well as other cathepsins have mainly intracellular function in many invertebrate animals (Vonk & Western 1984) Usually the pH optimum of cathepsin D from fish and invertebrates is in the range of 2 8 4 0 with haemoglobin as substrate (Suzuki et al 1983 Chang et al 1989 Okada& Aikawa 1986 Gildberg , 1987 McLay 1980 Boneto et al 1984) Though the enzyme performed maximal haemoglobin digestion at a pH 4 the optimum pH for muscle protein digestion is pH 5 (Doke et al 1980) At optimum pH and temperature the cathepsins and proteases of fish are observed to digest fish protein in less than one day (Karmas,1978)

The Cathepsin D isolated from squids revealed that they are very unstable at a pH above 7 5 but the mammalian cathepsin D are fairly stable at pH 8 (Barret 1977) The enzyme shows variation in activity according to substrate Study on temperature optimum and thermostability have given qute different results for cathepsin D from different sources A pH dependant change in temperature optimum was also reported by Gildberg (1987)

Lipases including phospholipases are active during struggling as well as postmortem (Mukundan et al 1985)

Most lipases from fish and shellfishes showed lipolysis in the pH range 6 10 and at all temperatures from 20 to 40°C (Patton and Quinn,1972)

The properties of lipases as well as their environment in marine organisms free of inhibitors are extremely favourable for lipid breakdown at ambient as well as low temperatures has been observed by several workers (Gopakumar 1972 Nair et al 1976 Mai & Kinsella 1979 Shenouda 1980) The free fatty acids one of the end product of lipolysis although weak will assist in lowering the pH of the tissue So combined effect of lacticacid and free fattyacids in lowering the pH of tissue on postmortem is important (Gould 1965 Mukundan and Nair 1980)

It has been proposed that the neutral pH of tissues favours carbohydrases and lipases activity (Mukundan et al 1985) producing acid pH favo rable for protease activity

From the review it is evident that very limited research work is available on head on processed prawns Moreover the problem of head drooping in head on frozen prawns has not been investigated so far by any workers

MATERIALS AND METHODS

III MATERIALS AND METHODS

3 1 Procurement of raw materials

The two important species of prawns used for the production of head - on frozen prawns by the Indian seafood industry are *Penaeus indicus* commonly known as Indian white prawn and *Penaeus monodon* commonly known as Tiger prawn In addition they are the two species used for aquaculture in India So the above two species were selected for the present study (Fig 2 and 3)

The prawns were procured from different parts of India viz Cochin Calicut Madras and Visakhapattanam The locations were selected based on the availability of experimental facilities and materials In all studies prawns free of any physical injury were used Care was always taken to keep the temperature of the material below 5°C either by icing or by keeping in a refrigerator immediately on procurement of the material

3 2 Measurement of head drooping

The head drooping in prawns leads to oozing out of the hepatopancreatic juice as a result of breakage of arthrodial membrane (Fig 4) Even a slight damage in the arthrodial membrane can eventually produce a drooping condition in prawns So from the practical and utility point of view prawns having damaged arthrodial membrane irrespective of the magnitude of damage can cause head drooping

Fig. 2

Fig. 3

Fig.2 Penaeus indicus used as the raw material for the study

Fig.3 Penaeus monodon used as the raw material for the study

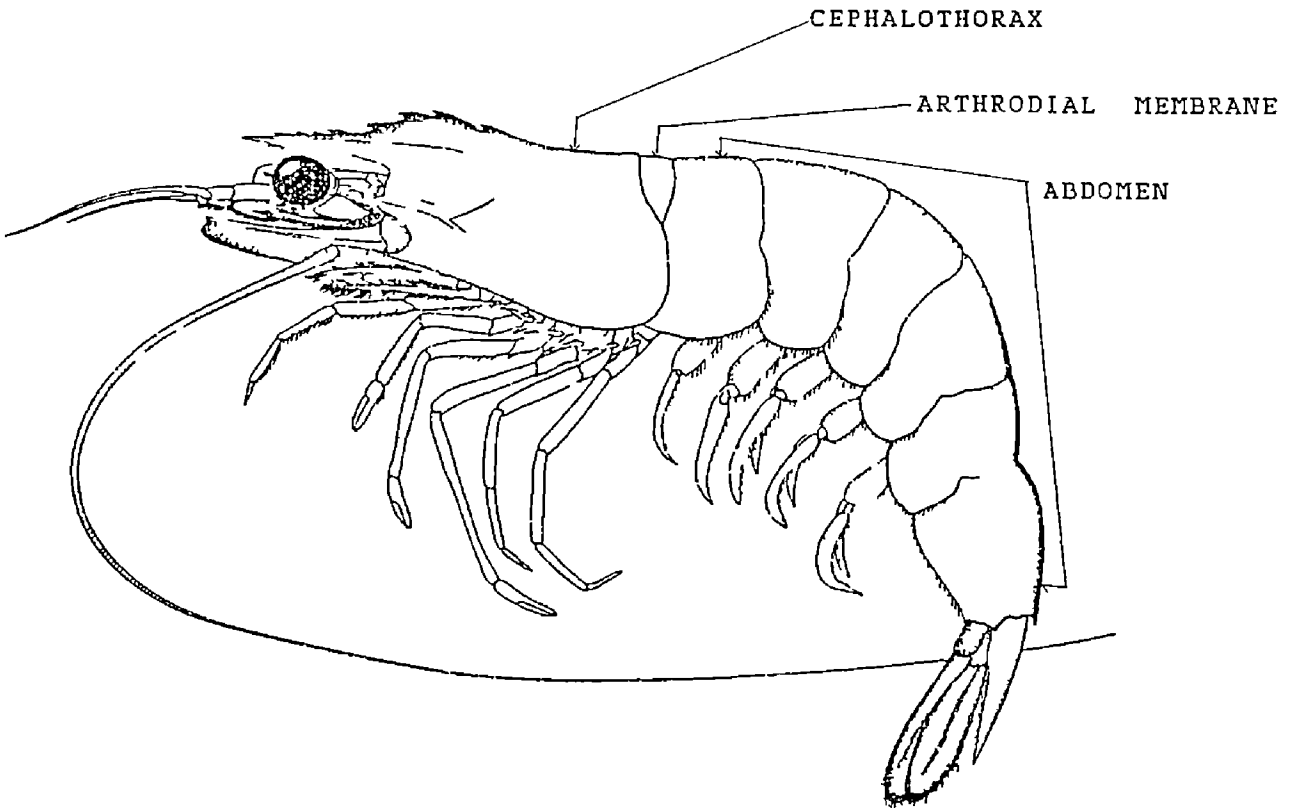


Fig 4 Figure showing the arthrodial membrane which connects cephalothorax to abdomen

In the present study any of the following types of damage to the arthrodial membrane was taken as head drooping

a Arthrodial membrane with a pinhole size damage

b Arthrodial membrane partially broken prawns remain in the normal shape on holding Head is not in a hanging position from the abdomen

c Arthrodial membrane fully broken On holding head remains in a hanging position from the head (Fig 5)

The percentage head drooping was calculated using the formula -

$$\text{The head drooping} = \frac{\text{No of pieces with head drooping}}{\text{Total No of pieces}} \times 100$$

3 3 Studies on the effect of prawn species and size on head drooping

A preliminary study was performed using the two species Penaeus indicus and Penaeus monodon to see whether any size wise or species wise difference exists for head drooping Commercially used size groups such as 30/40 40/60 and 60/80 were used for the study Five replications were kept for each size species combinations

The samples of ½ lbs (1 8 Kg) were layered on the bottom half of an inner duplex carton provided with holes on bottom and sides All the samples were brine immersion frozen and thawed in running water Later percentage head drooping was calculated as in 3 2

A B

Fig. 5A Head drooping in Penaeus indicus due to complete breakage of arthrodial membrane

Fig. 5B Head drooping in Penaeus monodon due to complete breakage of arthrodial membrane

3 4 Studies on hauling time

To study the influence of hauling time of trawl net on head drooping three different durations 60 105 and 150 minutes were selected The catch was segregated immediately on reaching the deck for two species (Penaeus indicus Penaeus monodon) and three size groups (30/40 50/60 and 70/80 pieces per Kg) under each species Now a representative sample of 2Kg was selected from each lot Percentage of head drooping was calculated as in 3 2

3 X 3 X 2 factorial design with 3 replication per cell was used in the experiment (Snedecor and Cochran 1967)

3 5 Ice storage

Prawns of Penaeus indicus obtained in normal hauling time were segregated sizewise Temperature of the material was maintained between 2 4°C during iced storage This was done by icing whenever product temperature rose above the temperature range Samples were drawn at intervals of 12 24 and 36 hours At each interval samples of 1 Kg were taken semi IQF frozen thawed and the percentage head drooping was calculated as in 3 2

3 6 Freezing methods

Various size groups belonging to the species Penaeus indicus were used for the study as in the previous case Immediately after procurement the material was sorted and graded as 30/40 50/60 70/80 groups Prawns free of head drooping only were used for the study

From each of the prescribed size groups 1Kg samples were taken

3 6 1 Plate freezing

Prawns were layered in lengthwise fashion with their head on either side in the lower half of an inner duplex carton (Fig 6 and 7) Glaze water of 250 ml at a temperature of 5°C was added and plate freezing was done in a precooled plate freezer

3 6 2 Semi IQF freezing

The procedure followed were same as in the previous case but no glaze water was addded before freezing (Fig 8 & 9)

3 6 3 IQF Freezing

The prawns were layered as 500 gms in metal trays having holes on bottom and sides After layering the samples to two metal trays with 500 g in each they were subjected to IQF at an air temperature of 40°C The belt speed was adjusted to receive frozen samples at the outlet point This is necessary as the freezing time varied based on the size of the prawn At the outlet point the two halves of each sample were put together

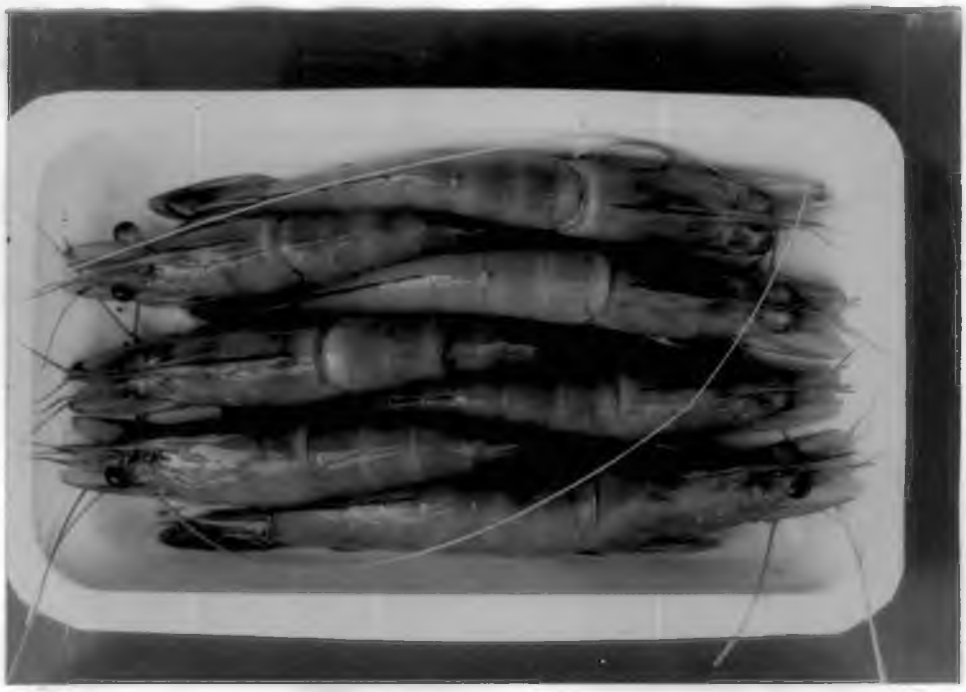


Fig. 6

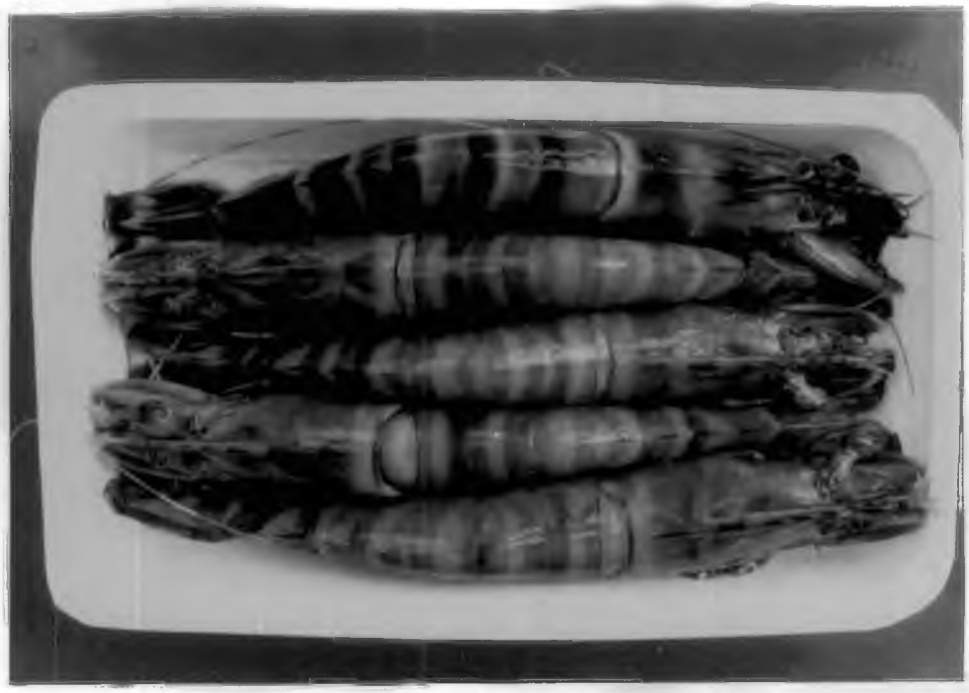


Fig. 7

g. 6 Length wise layering of Penaeus indicus before freezing

g. 7 Length wise layering of Penaeus monodon before freezing

Fig. 8

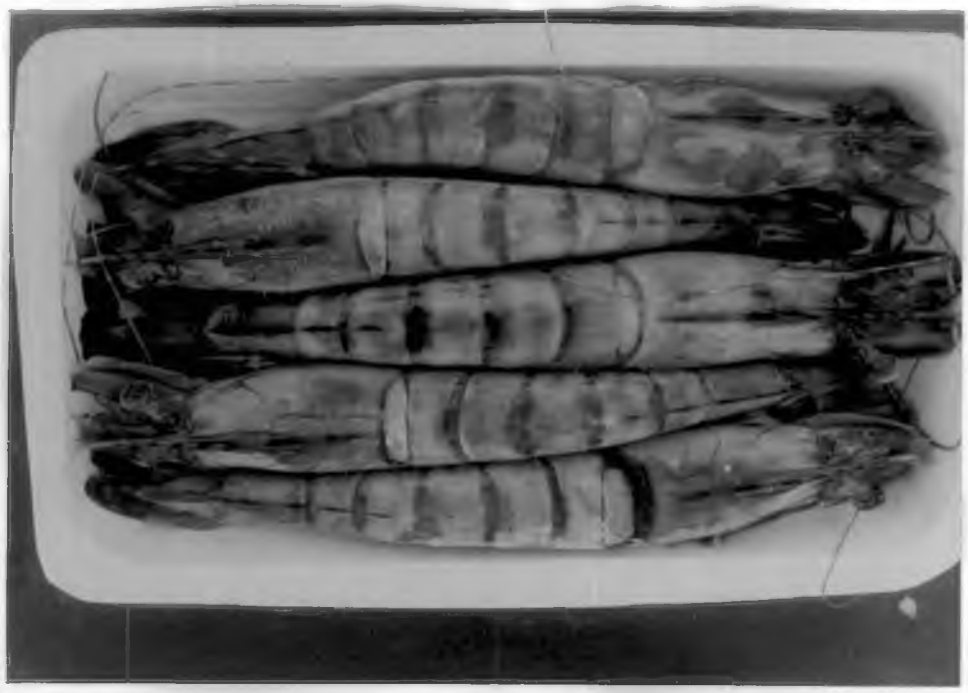


Fig. 9

Fig. 8 Semi - IQF Penaeus indicus

Fig. 9 Semi - IQF Penaeus monodon

After freezing the samples were immediately thawed as described in 3 8 3 and head drooping was determined

The whole experiment was planned using randomized block design with three replications

3 7 Cold storage duration studies

For cold storage duration studies various size groups 30/40 50/60 and 70/80 belonging to Penaeus indicus were used

The prawns obtained were first segregated for various size groups Prawns with drooped heads were discarded The materials were then seperated into lots of 1 8 Kg(4 lbs) The same was then layered in duplex cartons with holes on bottom and sides and lined inside with a polyethylene sheet The holes are provided to increase the heat transfer efficiency during freezing The prawns were layered in legthwise fashion

Later the samples were brine immerson frozen The freezing was stopped once the product core temperature reached 20 C After freezing the samples were kept in duplex cartons (top and bottom type) Such 10 samples were packed in a 5 ply corrugated master carton The master carton was sealed using bi axially oriented polypropylene tapes and kept in a cold storge maintained at 18°C samples were drawn at intervals of 2 4 and 6 months thawed as in 3 8 3 and the percentage head drooping was calculated

The design used for the experiment was randomized block design with 3 replications

3 8 Thawing

For studying the influence of various thawing conditions such as thawing in running water stagnant water and in air at temperatures of 28 ±2 °C on head drooping in *Penaeus indicus* various size groups such as 30/40 50/60 and 70/80 were procured as in the previous case Only prawns without head drooping were used for the study One Kg samples were subjected to semi IQF in a plate freezer The samples were put in polyethylene bags of 400 guage and were subjected to different thawing methods The process of thawing was completed once the product core temperature reached 20°C

3 8 1 Air thawing

Here the product was kept in room temperature of 28±2°C The percentage head drooping was calculated once the process of thawing was over

3 8 2 Stagnant water of 28 ±2°C

The samples were kept in polybags and allowed to undergo thawing in stagnant water in a tank of 1 5m X 1m X 1m dimension The depth of water in the tank was maintained at the level of o 75m through out the process The water in the tank is changed whenever the temperature fall below

25 °C with water of 30°C Thus the temperature was maintained in the range of 26 28°C

3 8 3 Running water thawing

The above procedure was followed with continuous flow of water into the tank and with the removal of same volume from the bottom of the tank

The experiment was laid out using randomized block design and results analysed accordingly

3 9 Thickness of the arthrodial membrane

The thickness of the membrane was determined using a microscope PZO Warszawa SK14 by differential focussing

3 10 Bacterial load

Tryptone glucose beef extract agar was used to determine the viable plate count

Procedure

About 50 g of the samples were collected in a sterile stainless steel dish From the sample collected 10g of the material was transferred to a sterile mortar Mixed with 90 ml of phosphate buffer after grinding using a sterile pestle From this serial dilutions were prepared Later pour plate method was followed using 0 5 ml of the diluted solution Plates were incubated at 37°C for 48 hours Plates containing colonies in the range of 30 200 were taken for determining viable plate count

3 11 Biochemical composition of Arthrodial membrane

Penaeus indicus and Penaeus monodon were procured from Cochin The prawns were immediately sorted into 3 size groups viz 30/40 50/60 and 70/80 (pieces/Kg) Arthrodial membranes from each size groups were collected and pooled The membranes were blotted off to remove any adhering moisture The material thus obtained was subjected to biochemical analysis for the estimation of moisture total protein phospholipid cholesterol and calcium Each estimation was carried out in 5 replicates

3 11 1 Moisture

A known weight of the sample was taken in a preweighed tared crucible and was dried to a constant weight at 60°C in a hot air oven The moisture was then calculated from the loss of weight of the tissue after drying

3 11 2 Total Protein

Total protein was estimated by the method of Wooton(1964) The material after digestion with conc sulphuric acid in presence of a suitable catalyst was neutralised and was treated with Nessler s reagent The colour produced was read in a spectrophotometer

Reagents

1 Nitrogen free conc sulphuric acid

2 Digestion mixture ($CuSO_4$ 5 H2O K_2SO_4 1 49 w/w)

3 Standard Ammonium chloride solution containing 30 micro gram of nitrogen per ml

Procedure

Digested about 100 mg of the membrane with 1 ml of conc sulphuric acid and 100 mg of digestion mixture in a digestion flask The digestion was stopped when the solution became clear The solution was then quantitatively transferred to a 25 ml standard flask and made up to the volume using distilled water An aliquot of this was pipetted into a 50 ml standard flask The solution was neutralised 1 ml of Nessler s reagent was added and colour Made upto 50 ml developed, and optical density was determined at 400 nm using a Specronic 20 spectrophotometer Using a standard ammonium chloride solution a series of concentrations were prepared nesselerised and the absorbance measured A standard curve was drawn to find out the percentage of nitrogen in the sample A factor of 6 25 was used to convert the percentage of nitrogen present in the sample to the percentage of protein

3 11 3 Phospholipid

Phospholipid was determined following the method of Zilversmith and Davis (1950)

Reagents

1 He ane Isopr parol mi ture It w s prepare i mixing volumes of hexane with 2 volumes of isopropanol

2 5 N sulphuri acid

3 4 5% ammon um mo yb late

4 ANSA

For the preparation o 2 g of 1 amino 2 naphthol 4 sulphonic acid was mixed with 1 2 grams of sodium bisulphite and 1 2 grams of sodium sulphite 50 mg of the above was dissolved in 2 ml of distilled water and used

5 Standard Phosphorous solution Using Analar KH_2PO4 a standard solution containing 31 micro gram of phosphorous / ml was prepared

Procedure

About 200 250 mg of the tissue was extracted with hexane isopropanol mixture following the method of Radin(1981) It was filtered and collected in a tube This was then made up to 10 ml 5 ml of the extract was pipetted into a Kjeldahl's flask and the solvent was evaporated to dryness over a boiling water bath 1 ml of 5 N sulphuric acid was added and digested in a digestion rack till it became light brown It was then cooled to room temperature One or two drops of 2 N nitric acid was added and digestion was continued till the solution became colourless The Kjeldahls flask was cooled 1 ml of water was added and heated in a boiling water bath for about 5 minutes 1 ml of 2 5% ammonium molybdate and 0 1 ml of ANSA were added to this This volume was made upto 10 ml with distilled water and absorbance was measured at 660 nm within 10 minutes Using a standard phosphorus solution a series of concentrations were prepared colour was

developed and the absorbance measured From the standard curve the percentage of phosphorous in the sample was determined This was then multiplied by a factor of 25 to get the phospholipid in the sample

3 11 4 Cholesterol

The method of Abell et al (1952) was used for the analysis

Reagents

1 33% KOH solution

2 Ethanolic KOH solution

prepared by adding 6 ml of 33 KOH to 94 ml of ethanol

4 Petroleum ether

boiling point 60 80°C was used

5 Colour Reagent

20 ml of acetic anhydride was taken in a dry conical flask and was chilled in ice To this added 1 ml of concentrated sulphuric acid Mixed the whole solution and allowed it to chill for another 10 minutes in ice To this mixture was added 10 ml of glacial acetic acid and allowed it to attain the room temperature

6 Standard Cholesterol solution

Dissolved 20mg of cholesterol in 50 ml of alcohol in a standard flask

Procedure

The total lipid extract prepared as in the previous case was used for the cholesterol estimation A known

volume of lipid extract was pipetted into a stoppered test tube labelled as test and evaporated to dryness over a boiling water bath Cooled the tube to room temperature and added 5 ml of ethanolic KOH solution After addition of ethanolic KOH solution the tube was stoppered and mixed Another tube containing 5 ml of standard cholesterol solution and o 3 ml of 33 KOH solution labelled as standard was also taken Both tubes were incubated at 37 40°C for 55 minutes After cooling to room temperature 10 ml of petroleum ether was added and mixed To this added 5 ml of water stoppered well and mixed for 1 minute The tubes were kept undisturbed for the seperation of petroleum ether layer After seperation 4 ml of the petroleum ether layer was pipetted from the test into a clean dry test tube

Pipetted different volumes of petroleum ether layer from the standard to test tubes suitably labelled Kept the tubes in a water bath of 60 80 °C till the solvent completely evaporated off Cooled the tubes to room temperature Now to each tube added 6 ml of colour reagent After addition of the colour reagent it was mixed well in a cyclomixer and kept for 30 35 minutes in dark at 25 °C Optical density was measured at 620 nm in a Spectronic 20 spectrophotometer The reagent was used as the blank A standard curve was plotted between optical density and cholesterol content From this the amount of cholesterol present in 100g of the sample was determined

3 11 5 Calcium

The titrimetric method of Clarke and Collip (1925) was used for the determination of calcium in the arthrodial membrane

Reagents

1 25 HCl

2 Conc Nitric acid

3 Methyl red indicator solution

4 50% ammonia solution

5 4 2 ammonium oxalate solution

6 1 50 ammonia solution

7 Sulphuric acid water mixtue 5 ml of conc sulphuric acid was mixed with 125 ml of distilled water

8 0 005 N potassium permangnate

Procedure

Into a clean dry crucible weighed about 1 gm of the membrane It was ignited in a muffle furnace at 550°C for 6 h The ash obtained was dissolved in 25 HCl and transfered quantitatively into a conical flask Added one drop of nitric acid Boiled the solution in a flame cooled to room temperature and transferred the solution carefully to a 25 ml standard flask washing the flask with 25 HCl Made up the solution with acid and mixed well Pipetted 10 ml of this extract into a clean conical flask Added 2

drops of methyl red solution to obtain a pink colour Now added 50 ammonia solution drop by drop till a pale yellow colour was obtained Added one drop of HCl to get back the original pink colour Now added 50 ml of distilled water and boiled over a flame While boiling added 10 ml of 4 2 hot ammonium oxalate solution drop by drop with constant stirring using a glass rod After the complete addition of the ammonium oxalate boiled the solution for one more minute Removed the solution from the flame and cooled overnight Filtered the solution through Whatman No 42 filter paper so that the whole precipitate was transferred to the filter paper Washed the flask with dilute ammonia solution and the solution was transferred to filter paper This was repeated 3 more times

Transfered the funnel along with the filter paper to a clean dry conical flask Dissolved the precipitate in sulphuric acid water mixture(5 125 v/v) Warmed to 60 70° C and titrated against 0 005 N potassium permangnate till a pale pink colour was obtained From the titre value the amount of calcium in 100 gm of tissue was calculated

3 12 Activity of hepatopancreatic enzymes

3 12 1 Cathepsin D (EC 3 4 4 23)

The enzyme activity was estimated using 2 haemoglobin in 0 1 M acetate buffer (pH 4 5) as the substrate and determining the amount of tyrosine by the method of Folin and Ciocalteu (1927)

3 12 1 1 Reagents

1. Triton X 100 (0 1% w/v)
2. 0 2 M acetate buffer of pH 4 5
3. 0 1 M acetate buffer p H 4 5
4. 10 % Trichloro acetic acid solution
5. standard tyrosine solution The standard tyrosine solution was prepared by dissolving 50 mg of tyrosine in 100 ml of distilled water in a standard flask 1 ml of this solution is equivalent to 2 76 micromole of tyrosine
6. Folin's reagent (1 N)
7. 2 8 N sodium carbonate solution
8. 0 1 N sodium hydroxide
9. 2 sodium carbonate solution in 0 1 N NaOH

   Dissolved 2 g of anhydrous sodium carbonate in 100 ml 0 1 N sodium hydroxide
10. 0 5 % Copper sulphate in 1 % potassium sodium tartarate

    Dissolved 1 g of potassium sodium tartarate in 100 ml

distilled water To this 0 5 g of copper sulphate was added and dissolved

11 Alkaline copper reagent

To 50 ml of reagent No 9 1 ml of reagent No 10 was added

12 Standard bovine serum albumin solution Dissolved 10 mg of bovine serum albumin in 50 ml distilled water in a standard flask 1 ml of this solution contains 200 micrograms of protein

3 12 1 2 Preparation of the enzyme extract

Homogenised 1 g of the the tissue with 3 ml of Triton X 100 in a mortar kept cooled in ice Passed the solution through cotton and collected the clean enzyme extract The solution thus obtained was diluted with an equal volume of 0 2 M acetate buffer of pH 4 5

3 12 1 3 Assay of enzyme

Pipetted 0 5 ml of haemoglobin into a clean dry test tube labelled as test and to another tube labelled as blank To the test added 0 5 ml of the diluted enzyme solution Incubated both the tubes at 37 °C for 30 minutes The reaction was stopped by adding 1 ml of 10 Trichloroacetic acid solution to each test tube To the blank immediately added 0 5 ml of enzyme extract The solution was centrifuged and the supernatant was collected and used for the estimation of tyrosine

3 12 1 4 Estimation of Tyrosine

Pipetted 1 ml of distilled water into a test tube labelled as blank To a test tube labelled as standard pipetted 0 1 ml of standard tyrosine solution and 0 9 ml of distilled water To a test tube labelled as test pipetted 0 1 ml of test solution and 0 9 ml of distilled water To each tube now added 5 ml of 2 8 N sodium carbonate followed by 3 ml of Folin s reagent Mixed well and kept for 5 minutes Read the OD at 490 nm From the OD determined the micromoles of tyrosine in 1 ml of test solution The number of micromoles of tyrosine liberated per minute per ml of the enzyme solution was then calculated

3 12 1 5 Specific activity of the enzyme

3 12 1 5 1 Estimation of protein in enzyme extract

Pipetted 0 1 ml of enzyme extract into a centrifuge tube Added 0 9 ml of distilled water Then added 1 ml of 10 TCA solution Mixed and centrifuged Discarded the supernatant and the centrifuge tube was kept inverted to drain off the TCA completly Dissolved the protein residue in 1 ml of 0 1 N sodium hydrxide This solution was used for the estimation of protein by Lawry s method (Lowry et al , 1951)

To the test tube labelled as blank pipetted 1 ml of water. To the test tube as standard added 0 ? ml of

standard protein solution (40 micro gram) and followed by 0 8 ml distilled water To each tube added 5 ml of alkaline copper reagent Mixed and kept for 10 minutes Added 0 5 ml of Folin s reagent Mixed and kept for 30 minutes and read the OD at 670 nm From this number of g of enzyme protein present per ml of the extract was determined

Now specific activity was determined which was defined for the assay as the micromoles of tyrosine liberated per minute per g of enzyme protein

3 12 2 Tryptic Activity

The method of Kunitz (1947) was used for the assay of trypsin like activity in the hepatopancreas of prawn

3 12 2 1 Reagents

1 Casein solution

Dissolved 4 g of fat free casein in about 90 ml of water containing 2 ml of 1 N NaOH The solution was shaken continuously and gently warmed until the casein was completly dissolved Now adjusted the pH to 8 5 with 1 N HCl After this made up the solution to 100 ml

2 Formalin solution

3 Phenolphathelein solution

4 0 1 N NaOH (Standardised)

3 12 2 2 Preparation of the enzyme extract

1 g of the hepatopancreas tissue was homogenised with 3 ml of distilled water and filtered through cotton

3 12 2 3 Assay of enzyme

Two numbers of 25 ml conical flask were taken and labelled one as blank and other as test Pippetted 5 ml of formaldehyde into each flask Added one drop of phenolphathalein solution to each Then added 0 1 N NaOH until the mixture is faintly pink

Two large testtubes were taken and labelled one as blank and other as test Pippetted 10 ml of casein solution to each tube Pipetted 1 ml of enzyme to the test only Incubated both the tubes at 37 ^{0} C for 30 minutes To the blank now added 1 ml enzyme Mixed and transfered the blank solution to the formaldehyde solution in the conical flask labelled as blank and the test solution to the other conical flask Added 10 drops of phenophathelein to each flask Titrated the contents of each flask against 0 1 N NaOH till a pale pink color is obtained

Now activity of the enzyme per ml of the enzyme extract per minute is calculated using the formula

$$\text{Tryptic Activity/ ml/minute} = \frac{(A - B) \times 0.1 \times 10^{6}}{\frac{1000}{30}}$$

(Micromole aminoacid /min / ml of enzyme extract)

Where A is the titre value for the blank and B titre value for test

3 12 2 4 specific activity of the enzyme

Determined the protein content of the enzyme extract

following the procedures described in 3 12 1 5 1 and the specific activity of the Trypsin was calculated It was expressed as micromoles of aminoacid liberated per minute per g enzyme protein

3 12 3 Phospholipase D (EC 3 4 4 4)

The enzyme activity was estimated using soya lecithin as substrate and determining the amount of choline liberated by the method of Appleton et al ,(1953)

3 12 3 1 Reagents

1.Choline chloride To obtain pure choline chloride dissolved 10 g of commercialy available choline chloride in minimum volume of alcohol Added diethyl ether until no further precipitation occurred Filtered and collected the precipitate after washing with ether Dried in vacuum to remove the ether Powdered the sample and dried over P_2O_5 This purified choline chloride was used to prepare a 100 micromole solution

2 Iodine reagent

12 5 g of KI and 9 8 g of iodine were dissolved in water to a total volume of 250 ml Stored below 4 °C in brown bottle

3 Enzyme substrate

Commercially available soya lecithin was used as substrate A 10mg/ ml lecithin solution was prepared in diethyl ether

4 Diethyl ether

3 12 3 2 Preparation of the enzyme extract

Same as in 3 12 2 2

3 12 3 3 Assay of enzyme

Pipetted out 1 ml of ether solution of substrate into a stoppered centrifuge tube Removed the solvert by warming at 55 60 C To the residue added 0 2 ml of enzyme extract and mixed well using a cyclomixer till an emulsion formed Incubated at 25 C for 30 minutes At the end of this period added 4 8 ml ether mixed well and kept stoppered for 45 minutes Centrifuged and removed the ether layer on top The choline in aquous layer was estimated as follows

To the aquous layer added 0 3 ml of distilled water 0 2 ml of iodine reagent Kept the tubes in icebath for 15 minutes Centrifuged and discarded the supernatant The precipitate is dissolved in 10 ml of chloroform and OD was measured at 365 nm

For the estimation of blank values pipetted 1 ml of ether solution of substrate into a test tube and removed the solvent by warming at 55 60 C To the residue added 0 2 ml of heat denatured enzyme extract Then proceeded as in the case of test

The OD for the standard choline was also determined using 0 5 ml of standard choline solution and developing the colour using iodine reagent

3 12 3 4 Specific activity of the enzyme

The enzyme protein was estimated as in the previous experiments and specific activity was determined It was expressed as micromoles of choline liberated per minute per g enzyme protein

RESULTS

IV RESULTS

4 1 Head drooping in various species and size groups of prawns

The results of the preliminary study is shown in Fig 10 The results showed an increased occurrence of head drooping in both Penaeus indicus and Penaeus monodon with decrease in size of the prawn Also under the same size group Penaeus indicus always registered a higher percentage of head drooping than Penaeus monodon

4 2 Head drooping and Hauling Time

The results obtained for the effect of hauling time in the incidence of head drooping in the three size groups of P indicus and P monodon are given in Table 1 The ANOVA table showing the effect of hauling time size species and their interactions on head drooping are given in Table 2 The results show that the contribution of various factors such as time species size and their interactions Viz time size time species time size species combinations towards head drooping are significant while that of size species interaction is non significant

Head drooping is found increasing with decrease in size of the prawn in both species The percentage head drooping showed a significant increase with increase in time of hauling for both species under all size groups considered

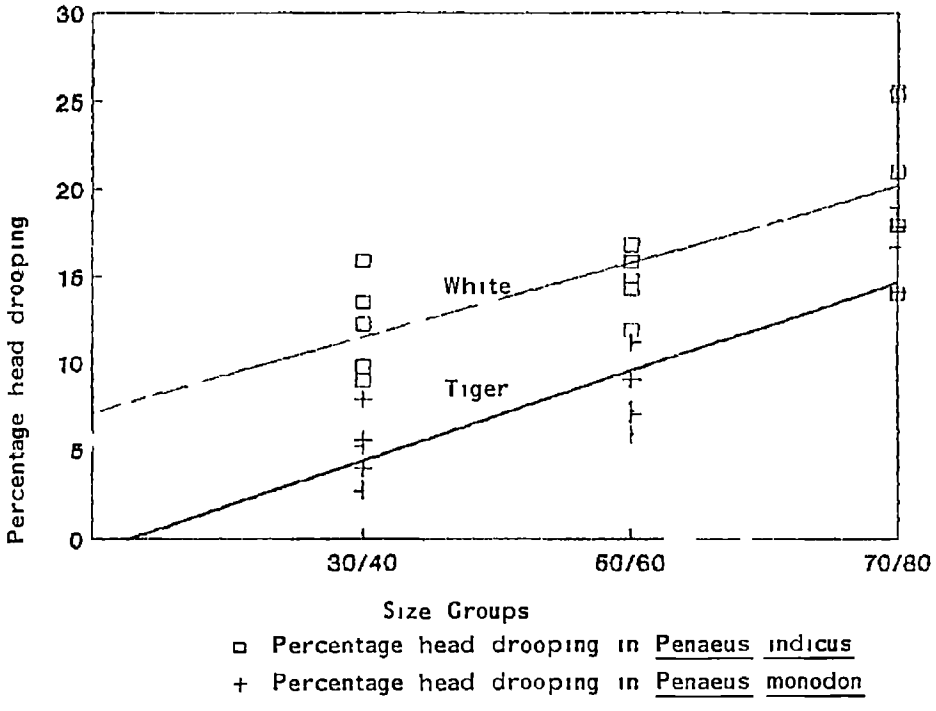


Fig 10 Effect of Size and Species on head drooping

TABLE 1 PERCENTAGE DAD DROOPING IN DIFFERENT SIZF GROUPS OF P_a__ _____AND Pe a u mo o o
UNDER DIFFERENT IAU ING DURATIONS

Spe les	P n us				u o		
Si e	30 0	50 60	70 80	30 40	0/60		0/8
Haul o Time							
60 M u s	3 9 6 18 03(9 6 6 65(8 2	9 (0 19 70(11 6 20 0 1 1)	23 7(5 5) 7 4(18 4) 22 0 14	11 4 11) 9 9 8) 60 2 78	4 82 6 54) 13 4(5 37) 13 44 5 40)		16 95(8 19 6(0 18 30 9 8
105 M ut	4(1 0 74 (7 0 7 0 2 4	6 6 20 00 24 44 7 2 2 0 2 4	0 7 6 18 28 40) 28 2 22 30	7 26) 5 0(7 4 6 8 26	9 0 0 71) 20 0 1 0 9 80(1 47		4 6 (17 24 86 7 24 4(17 0
150 M ut s	5 90 19 10) 30 0 25 1 (3)	34 0 2 7) 34(1 2 4 1 3 48	37 3 06 4 67 4 9) 40 4 (42 0	20 70(2 49) 7 7 3 67 22 0 14 03	23 4 1 80) 4 30 16 93 25 40 8 40		28 63(22 30 0(25 30 33 25

T v lu o a oo g c n o
$ T va u f ead droo g af r a gu r transforma o

TABLE 21 RESULTS OF ANALYSIS OF VARIANCE SHOWING THE EFFECT OF VARIOUS SIZE GROUPS OF *Penaeus indicus* AND *Penaeus monodon* UNDER VARIOUS DURATIONS OF HAULING

ANALYSIS OF VARIANCE

Source	DF	SS	MSS	F RATIO	
Time(T)	2	1420 26	710 13	377 53	*
Size group(G)	2	618 7173	309 36	164 47	*
Species (S)	1	660 591	660 591	351 19	*
T me X Size	4	21 54	5 385	2 86	*
Time X Spec es	2	31 072	15 536	8 26	*
S ze X Species	2	1 5465	0 7733	0 411	@
Time X Size X Spec es	4	22 589	5 647	3 0	*
Error	36	67 727	1 881		
Total	53	2844 04			

Where D F Degrees of freedom SS Sum of squares MSS Mean sum of squares

* S gn f cant at 5 level
@ Not significant at 5 level

Table 2 2 RESULTS OF TIME - SIZE INTERALTIONS

TIME \ SIZE	30/40	50/60	70/80
60 MINUTES			
105 MINUTES			
150 MINUTES			

Table 2 3 RESULTS OF TIME - SPECIES INTERACTIONS

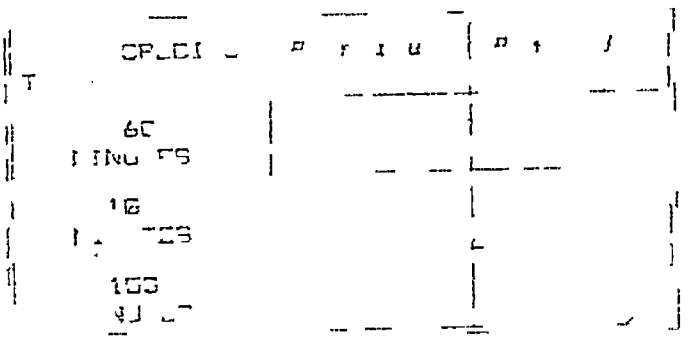
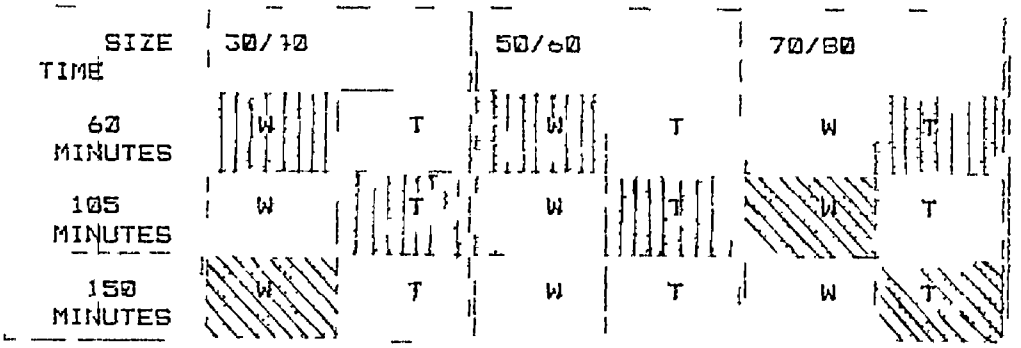


Table 2 4 RESULTS OF TIME - SIZE - SPECIES INTERACTIONS



W - *Peraeus indicus*
T *Peraeu mirador*

* Combinations with similar shades show no significant difference among them

The mean percentage head drooping in Penaeus indicus is higher than that of Penaeus monodon

The results of various interactions such as time size time species and time size species are shown in Tables 2 2 2 3 and 2 4 respectively

4 3 Head drooping and ice storage

The results showing the effect of ice storage durations on Head drooping in three size groups of P indicus along with the results of analysis of variance are given in Table 3 The mean percentage head drooping was found to increase with increase in ice storage duration and decrease in size of prawn

All the size groups and ice storage durations were found to exhibit significant difference among them The results indicated that prolonged ice storage duration promoted head drooping and was more evident in smaller sized prawns

4 4 Head drooping and Freezing Methods

The results showing the effects of different freezing methods on head drooping in the three size groups of P indicus along with the results of the analysis of variance are given in Table 4 The time taken for freezing of samples were 25 125 and 150 minutes approximately for different methods as IQF semi IQF and block freezing The mean value of head drooping produced by block freezing was higher than those produced by semi IQF

TABLE 3 PERCENTAGE EAD DROOPING IN DIFFERENT SIZE GROUPS OF P nd cus UNDER DIFFERENT ICE STORAGE DURATIONS AND THE RESULTS OF ANALYSIS OF VARANCE OF THE DATA

TABLE 3 1 PERCENTAGE HEAD DROOPING

S ze gp / Iced durat on	30/40	50/60	70 80	MEAN
12	16 32 16 32 13 26 $ # (7 8 7 8 5 3)	19 3 17 1 18 9 (10 9 8 6 10 5)	20 2 22 4 20 6 (11 92 14 5 12 4)	18 26
24	18 93 19 2 18 7 (10 5 10 8 0 3	21 6 23 4 18 1 (13 6 15 8 9 7)	23 41 26 27 11 15 8 19 2 20 8	21 83
36	23 4 25 1 23 4 (15 8 18 15 8)	24 5 27 6 27 3 (17 2 21 5 21)	29 3 27 26 5 (23 9 20 6 20)	26 011
MEAN	19 4	21 98	24 72	

TABLE 3 2 ANALYSIS OF VARIANCE

Sourc	D F	SS	MSS	F Rat o
Iced durat on(T)	2	271 079	135 54	53 31
S ze group(S)	2	127 03	63 52	24 98
Interact on	4	10 72	2 68	1 05 @
Between S X T	8	408 83	51 113	
Error	18	45 76	2 54	
Total	26	454 59		

Where D F Degrees of freedom SS Sum of squares MSS Mean um of quares

TABLE 3 3 CR TICAL D FFERENCE ANALYSIS

C cal d ffrence value (t at 5) for ice sto age durat on and s ze compar son 1 5792

Durat ons compared	D fference between means		size groups compa ed	D fferen e between means
12 hrs Vs 24 hrs	3 570		30/40 Vs 50 60	2 58
12 hrs Vs 36 hr	7 753	*	30/40 Vs 70 80	5 316
24 hrs Vs 36 hrs	4 183	*	50/60 Vs 70/80	2 737 *

\# The valu of head droop ng as percentage
$ The value of head droop ng after angular transformat on

* S gn f cant at 5 level
@ Not signif cant at 5% level

TABLE 4 PERCE TAGE HEA DROOPING 'N DIFFERENT SIZE GROUPS OF P_ nd_ us UNDER DIFFERENT FREEZIN METHODS AND THE RESULTS OF ANALYSIS OF VARIANCE

TABLE 4 1 PERCENTAGE EAD DROOPING

s ze gp / method	30 40	50 60	70 80	MEAN
BLOCK	$ 16 54 13 63 13 44 # (8 1 5 55 5 4)	19 3 17 55 20 5 (10 9 9 1 12 3)	23 1 20 4 21 3 15 38 12 15 13 4)	18 42
SEMI IQF	13 26 13 16 3 5 26 5 26 7 9)	20 5 17 2 15 4 12 3 8 76 7	20 3 21 2 22 5 (12 13 1 14 64	17 77
IQF	13 4 16 3 13 06 (5 4 7 9 5 11)	15 4 15 5 18 9 (7 0 7 14 10 52)	17 7 20 16 21 (9 24 11 7 7 8)	16 28
MEAN	14 36	17 80	20 30	

TABLE 4 2 ANALYSIS OF VAR ANCE

Source	D F	SS	MSS	F Rat o
Freez ng methods(F)	2	21 593	10 796	3 332 *
S ze group(S)	2	159 93	79 96	24 679
Intera t on	4	12 63	3 16	0 97 @
Between(F X T)	8	194 15	24 269	
Error	18	58 32	3 24	
Total	26	252 47		

Where D F Degrees of freedom SS Sum of squares MS Mea sum of quares

TABLE 4 3 CRITICAL DIFFERENCE ANALYSIS

Cr t cal d fference value (t at 5) for ce storage d ratio and s ze comparison 1 7828

Freez ng methods compared	D ffer nce betw en means	s ze groups compared	D fference between means
Block Vs Sem IQF	0 65 @	30/40 Vs 50 60	3 44 *
Block Vs IQF	2 13 *	30/40 Vs 70 80	5 940 *
Semi IQF Vs IQF	1 490 @	50 60 Vs 70 80	2 500 *

* S gn f cant at 5 evol
@ Not s gn ficant at level

$ Th e values after angular tran formati on
# The values as percentage head droop g

and IQF freezing of prawns Also the mean values increase with decrease in size of prawn

Analysis of the data showed significant difference among three freezing methods as well as among the size groups with regard to head drooping (Table 4 2) On pairwise comparison the head drooping produced by block and IQF are found significantly different(Table 4 3) Also the head drooping in various size groups were showing significant difference However the interaction between freezing methods and size groups did not show any significant difference (Table 4 2)

4 5 Head drooping and Cold storage duration

The percentage head drooping was found increasing with increase in cold storage duration and decrease in size of prawns The head drooping produced in various size groups shows significant difference among them Also the head drooping produced by various cold storage durations are also found significantly different In addition a significant interaction exists between various size cold storage durations in producing head drooping (Table 5)

4 6 Head drooping and Thawing Methods

Various thawing methods were found to produce significant variations in head drooping (Table 6) The mean time taken for various thawing methods are approximately 25 60 and 125 miniutes respectively for

TABLE 5 PERCENTAGE HEAD DROOPING IN DIFFERENT SIZE GROUPS OF P nd cus UNDER DIFFERENT DURATIONS OF COLDSTORAGE AND THE RESULTS OF ANALYSIS OF VARIANCE

TABLE 5 1 PERCENTAGE HEAD DROOPING

s ze gp / Durat on	30 40	50 60	70/80	MEAN
2 Months	$ 15 60 15 6 18 14 # (7 23 7 23 9 70	17 7 19 57 18 5 (9 24 11 22 10)	22 6 21 09 21 25 (14 8 12 95 13 10)	18 8944
4 Months	22 22 22 4 21 60 (14 27 14 50 13 55	23 8 26 10 24 50 (16 30 19 35 17 20	25 70 22 70 26 70 (18 80 14 89 20 20)	23 9611
6 Mo ths	24 27 24 80 24 20 (16 89 17 60 16 80)	29 66 30 50 25 00 (24 50 25 80 17 90)	34 36 31 76 3 0 (31 90 27 70 33 70)	28 90
MEAN	20 9789	23 9233	26 8533	

TABLE 5 2 ANALYSIS OF VARIANCE

Source	D F	SS	MSS	F Rat o
Duration(D)	2	450 525	225 263	91 271
S e group(S)	2	15 29	77 646	31 276
Interact o	4	37 526	9 382	3 80 *
Between(D X S)	8	643 344	80 418	
Error	18	44 43	2 468	
Total	26	687 7735		

Where D F Degrees of freedom SS Sum of squares MSS Mean sum of squares

TABLE 5 3CRITICAL DIFFERENCE ANALYSIS

Cr t cal d fferenc value (t at 5 for ce storage durat on and s e comparison 1 556

Durat ons compared	D ffer nce between means	size groups compared	D fferen e between means
2Months Vs 4 Months	5 0667	30/40 Vs 50 60	2 9444 *
2 Months Vs 6 Months	10 006	30 40 Vs 70 80	5 8744
4 Mont s Vs 6 Months	4 940	50 60 Vs 70 80	2 930

\# The value of head drooping as percentage
$ The value of head drooping after angular transformat on

* S gn f cant at 5 level
@ Not s gn f cant at 5 level

TABLE.6 PER ENTAGE HEAD DROOPING IN VARIOUS SIZE GROUPS OF Penaeus nd cus UNDER DIFFERENT METHODS OF THAWING AND THE RESULTS OF ANALYSIS OF VARIANCE

TABLE 6 1 PERCENTAGE HEAD DROOPING

s ze gp / Method	30 40	50 60	70 80	MEAN
A r	$ 25 40 23 75 25 70 (18 40 16 22 18 8)	29 09 25 49 30 90 (23 64 18 52 26 40)	36 64 36 50 36 10 (35 60 35 40 34 70)	29 9522
Stagnant water	19 47 19 20 21 27 (11 11 10 82 3 16)	21 10 24 09 22 21 (12 96 16 66 4 29	24 70 23 90 25 00 (17 46 16 41 17 86)	22 3267
Runn n water	17 28 19 47 19 47 (8 82 11 11 11 11)	21 10 22 42 19 11 (12 96 14 29 10 72)	22 50 24 10 9 33 (14 64 16 67 10 96)	20 5311
MEAN	21 2233	23 9456	27 6411	

TABLE 6 2 NALYSIS OF VARIANCE

Source	D F	SS	MSS	F Rat o
Method (M)	2	450 396	225 198	88 4 6 *
S e group(S)	2	186 77	93 39	36 695 *
Intera tio	4	67 356	16 84	6 62 *
Between(M X S	8	704 525	88 07	
Error	18	45 816	2 545	
Total	26	750 341		

Wh re D F Degrees of freedom SS Sum of squares MSS Mean s m of squares

TABLC 6 3CR T CAL DIFFERENCE ANALYSIS

Cr t cal d ffrence value (t at 5) for ce storag durat on and s ze compar son 1 580

Methods compared	D ffere ce between means	size groups compared	D fference between means
A r Vs St water	7 62 *	30/40 Vs 50 60	2 7223
A r Vs Run water	9 42 *	30/40 Vs 70/80	6 4178 *
St water VsRun water	1 7956 *	50 60 Vs 70/80	3 6955 *

\# The value of head droop ng as percentage
$ The value of head droop ng after angular transfo mat on

* S gn f cant at 5 level
@ Not significant at 5 level

running water stagnant water and air thawing The mean head drooping values registered an increasing trend with decrease in size of prawn All size groups show significant difference among them

The mean percentage head drooping is least in running water thawing and highest in air thawing

4 7 Thickness of Arthrodial membrane

The results are shown in Table 7 The Table 8 shows the results of statistical analysis of species wise comparison Table 9 shows ANOVA table for comparison among size groups within the same species

4 7 1 Thickness of the arthrodial membrane and species

The thickness of arthrodial membrane was significantly more in the P monodon when compared to P indicus of the same size group (Table 8)

4 7 2 Membrane thickness and size

Within the same species the thickness of the arthrodial membrane significantly increased as the size of the prawn increased (Table 9) Smaller size prawns have thin arthrodial membrane whereas the larger ones have thick membranes (Table 7)

4 8 Bacterial load

The log mean number of colony forming units (Log CFU/gm) for different size groups of two species of prawns are given in Table 10 Table 11 shows the results

TABLE 7 BIOC EMICAL COMPOSITION AND THICKNESS OF ART RODIAI MEMBRANE IROM DIFFEPENT SIZE GROUPS OF P nd us AND P monodon

		Penaeus nd cus				Penaeus monodon			
		MEAN	LOWEST	H GHEST	SE	MEAN	LOWEST	HIGHEST	SE
MOISTURE( )	A	77 26	75 06	79 48	1 76	77 04	76 4	77 7	0 532
	B	79 2	77 4	81 01	1 53	80 72	9 3	83 1	1 496
	C	81 58	8 1	82 03	0 42	81 77	80 6	83 6	1 186
PHOSPHOLIPID ( )	A	0 746	0 604	0 768	0 09	1 145	1 11	1 16	0 021
	B	0 492	0 425	0 567	0 064	0 972	0 972	0 991	0 014
	C	0 345	0 309	0 403	0 046	0 776	0 69	0 794	0 012
PROTEIN ( )	A	5 767	5 27	6 35	0 495	7 31	7 31	7 31	0
	B	5 962	5 32	6 42	0 442	6 8	6 3	6 36	0 258
	C	5 62	5 10	6 25	0 497	6 68	6 68	6 68	0
CHO E TEROL (mg o/g)	A	85 25	83 17	87 72	2 319	175 5	61	183 95	8 684
	B	77 39	75 66	79 11	1 726	125 2	124 3	26 1	0 895
	C	65 8	63 44	66 91	1 4	108 5	07 2	109 8	1 29
CALCIUM mg)	A	2239 3	2224	2255	10 42	2679	2660	26 8	14 3
	B	867 4	1810	1924	40 36	2454	2401	2498	45
	C	998 4	993	1003	3 56	022	2007	2028	8 96
THICKNESS (M crometer)	A	63 8	57	74	6 7	121 2	103	138	15 3
	B	51 2	48	57	3 56	89	78	98	8 54
	C	40 8	38	45	2 77	68 4	59	78	7 77

SE Standard Error

A 30 40 P ce. Kg
B 50 60 P c es
c 70/80 P e es /Kg

TABLE 8 COMPARISON BETWEEN THE TWO SPECIES FOR BIOCHEMICAL COMPOSITION AND THICKNESS OF ARTHRODIAL MEMBRANE

PARAMETER	SIZE GROUP	t VALUE CALCULATED	LEVEL OF SIGNIFICANCE
MOISTURE	A	0 2721	@
	B	1 537	@
	C	0 3308	@
PHOSPHOLIPID	A	9 12	*
	B	16 38	*
	C	17 61	*
PROTEIN	A	6 188	*
	B	3 72	*
	C	4 113	*
CHOLESTEROL	A	22 46	*
	B	5 73	*
	C	49 82	*
CALCIUM	A	54 77	*
	B	21 70	*
	C	237 6	*
THICKNESS	A	7 692	*
	B	9 13	*
	C	7 484	*

TABLE VALUE OF t 2 571 at 5 level

A 30/40 P eces / Kg
B 50/60 P eces / Kg
C 70/80 Pieces /Kg

* SIGNIFICANT AT 5 LEVEL
@ NOT SIGNIFICANT

TABLE 9 RESULTS OF ANALYSIS OF VARIANCE FOR THE EFFECT OF VARIOUS SIZE GROUPS OF *Penaeus indicus* AND *Penaeus monodon* ON THE BIOCHEMICAL COMPOSITION AND THICKNESS OF ARTHRODIAL MEMBRANE

PARAMETER		*Penaeus indicus*		*Penaeus monodon*	
	SIZE GROUP	F RATIO	RESULTS OF PAIRWISE COMPARISON	F RATIO	RESULTS OF PAIRWISE COMPARISON
MOISTURE( )	A	12 54	a	23 6	a
	B		b		b
	C		c		b
PHOSPHOLIPID ( )	A	30 28	a	691	a
	B		b		b
	C		c		c
PROTEIN)	A	@		24 4	a
	B	0 29			b
	C				b
CHOLESTEROL	A	138 3	a	234	a
	B		b		b
	C		c		c
CALCIUM (mg)	A	3488	a	723	a
	B		b		b
	C		c		c
THICKNESS (Micrometer)	A	30 6	a	29	a
	B		b		b
	C		c		c

A 30 40 Pieces /Kg

B 50 60 Pieces Kg

C 70 80 Pieces Kg

Same letters in the results of pairwise comparison shows no significant difference

Different letters in the results of pairwise comparison shows significant differen

F Ratio is significant at 5 level

@ F Ratio is not significant

TABLE 10 BACTERIAL LOAD IN DIFFERENT SIZE GROUPS OF P nd cus AND P monodon

PARAMETER	SIZE	Penaeus nd cus MEAN	RANGE OF VALUES LOWEST	RANGE OF VALUES HIGHEST	SE	Penaeus monodon MEAN	RANGE OF VALUES LOWEST	RANGE OF VALUES HIGHEST	SE
BACTERIAL LOAD (Log CFU gm)	A	5 429	5 380	5 474	0 036	5 420	5 35	5 474	0 046
	B	5 413	5 350	5 394	0 040	5 394	5 369	5 428	0 029
	C	5 407	5 3 0	407	0 005	5 407	5 358	5 465	0 040

SE Standard Error

A 30 40 P eces Kg
B 50 60 P eces /Kg
c 70/80 P eces /Kg

of statistical analysis of the data on bacterial load between the species Table 12 shows the results of size wise comparison of bacterial load within the same species

4 8 1 Bacterial load and size

In both the species studied the bacterial load did not show any significant variation with size group (Table 12)

4 8 2 Bacterial load and species

The results showed no significant variation in the total bacterial load between two species (Table 1)

4 9 The Biochemical Composition of Arthrodial Membrane

The results are given in Table 7 The Table 8 shows the results of statistical analysis for comparison between the two species The results of analysis of variance on the effect of various size groups of Penaeus indicus and Penaeus monodon on the biochemical composition of the arthrodial membrane are given in Table 9

4 9 1 Biochemical composition of arthrodial membrane and species

Within the same size group the moisture level did not show any significant variation between two species On the other hand within the same size group phospholipid protein cholesterol and calcium levels were significantly higher in P monodon than in P indicus (Table 9)

TABLE 11 COMPARISON BETWEEN THE TWO SPECIES

FOR TOTAL BACTERIAL LOAD

PARAMETER	SIZE GROUP	t VALUE CALCULATED	LEVEL OF SIGNIFICANCE
BACTERIAL LOAD	A	0 352	@
	B	0 890	@
	C	0 0302	@

TABLE VALUE OF t 2 571 at 5 level

A 30/40 P eces / Kg
B 50/60 P eces / Kg
C /0/80 Pieces /Kg

@ NOT SIGNIFICANT

TABLE 1 RESULTS OF ANALYSIS OF VARIANCE FOR THE EFFECT OF VARICUS SIZE GROUPS OF *P indicus* AND *P monodon* ON TOTAL BACTERIAL LOAD

PARAMETER		*Penaeus indicus*		*Penaeus monodon*	
	SIZE GROUP	F RATIO	RESULTS OF PAIRWISE COMPARISON	F RATIO	RESULTS OF PAIRWI COMPARISON
BACTERIAIAL LOAD	A B C	0 60 @		0 56 @	

A 30/40 Piece /Kg
B 50/60 Piece /Kg
C 70/80 Pieces /Kg

@ F Ratio not significant

4 9 2 Biochemical composition of the arthrodial membrane and size

In P indicus except protein all other constituents namely moisture phospholipid cholesterol and calcium showed a significant variation as the size of the prawn varied Moisture content significantly increased as the size of the prawn decreased That is higher the size lower the moisture content of the arthrodial membrane Phospholipid cholesterol and calcium levels in the membrane decreased as the size of the prawn decreased However protein content did not show any variation with size In short moisture level was high in smaller prawns whereas phospholipid cholesterol and calcium levels were low and the protein content remained unchanged (Table 9)

In P monodon moisture content of the arthrodial membrane was significantly low in 30/40 size group when compared to 50/60 and 70/80 size group The latter two size groups did not show any significant difference in moisture content The protein level on the other hand showed a significant higher value for 30/40 size group when compared to the other two size groups which intirn did not show any significant difference The other constituents namely phospholipid cholesterol and calcium significantly decreased as the size decreased

There was significant variation in the biochemical composition of the arthrodial membrane in P indicus and P monodon showing that the b ochemical composition varied with species Also within the same species biochemical composition varied as the size varied

4 10 Activity of Hepatopancreatic enzymes

The results of the activity of the hepatopancreatic enzymes cathepsin D Trypsin and phospholipase D are given in Table 13 Table 14 shows the results of statistical analysis of the data for species wise comparison and Table 15 shows the results of sizewise comparison within the same species In P indicus and Penaeus monodon the values of specific activity increased with increase in size of the prawn for the three enzymes studied

4 10 1 Activity of Cathepsin D

4 10 1 1 Activity of Cathepsin D and species

Within the same size group activity of Cathepsin D was significantly more in P monodon than in P indicus (Table 14)

4 10 1 2 Activity of Cathepsin D and size

The enzyme showed an increase in specific act vity with increase in size of prawn in both species (Table 13) All size groups of both species showed significant differences in enzyme acitivity (Table 15)

TAB E ... OF ... ENZYMES FROM DIFFERENT SIZE GROUPS OF *Penaeus indicus* AND *Penaeus monodon*

PARAMETER	SIZE	*Penaeus indicus* MEAN	RANGE OF VALUES LOWEST	HIGHEST	SE	*Penaeus monodon* MEAN	RANGE OF VALUES LOWEST	HIGHEST	SE
Ch He ... A I I Y S Act v	A	[illegible]	[illegible]	39 00	[illegible]	7 0	0	0 6	3 4
	B	[illegible]	[illegible]	2 30	3 66	C	[illegible]	3 [illegible]	0
	C	[illegible]	4	1 09	0 48	40	30 0	3 1	0 6
P I A TI I ... Activity	A	[illegible] 8	6 3	37	5	1 7	[illegible]	946	5
	B	[illegible]	0	600	0	[illegible]	83	530	0
	C	[illegible]	[illegible]	[illegible]	0	[illegible]	[illegible]	258	0
PHOSPHO ...	A	[illegible]	5	4	0	[illegible]	1 9	609	0 0
	B	0	0	36	0 C0	0	[illegible]	44	0
	C	C 0	0	0	0 03	[illegible]	0 2	0 6	0 0

... an ... o

A 0 ... 3

B 0 ... ce ...

C 70 8 Pie es ...

TABLE 14 COMPARISON BETWEEN THE TWO SPECIES

FOR THE ACTIVITY OF HEPATOPANCREATIC ENZYMES

PARAMETER	SIZE GROUP	t VALUE CALCULATED	LEVEL OF SIGNIFICANCE
CATHEPSIN ACTIVITY	A	20 55	*
	B	11 86	*
	C	46 47	*
TRYPTIC ACTIVITY	A	15 54	*
	B	28 52	*
	C	26 46	*
PHOSPHOLIPASE ACTIVITY	A	2 81	*
	B	3 34	*
	C	2 33	@

TABLE VALUE OF t 2 571 at 5 level

A 30/40 P eces / Kg
B 50/60 Piece / Kg
C 70/80 Pieces /Kg

* SIGNIFICANT AT 5 LEVEL
@ NOT SIGNIFICANT

TABLE 15 RESULTS OF ANALYS S OF VARIANCE FOR THE EFFECT OF VAR OUS S ZE GROUPS OF P. nd cus AND P. monodon ON TH ACTIVITY OF HEPATOPANCREATIC ENZYMES

PARAMETER	P naeus nd cus			Penaeus onodon		
	SIZE GROUP	F RATIO	RESULTS OF PA R SE COMPARISON	F RATIO	RESULTS OF PAIRW E COMPAPISON	
CATHEPSIN ACTIVITY	A	57 54	a	73	a	
	B		b		b	
	C				c	
TRYPTIC ACTIVITY	A	64	a	60	a	
	B		b		b	
	C		b		c	
P O PHOLIPA E ACT VITY	A	22 7	a	30 31	a	
	B		b		b	
	C		b		c	

A 30 40 P eces K

B 50 60 P ces Kg

C 70 80 P eces Kg

Sam l tt s th results of pa rw s ompa son s ows no sign f cant d ffer nc
D ffer nt letters n the results o pa rw se compar son s ows s gn f cant differe

F Rat o s gn f a t at 5 level
@ F Ra o s ot s gn f cant

4 10 2 Tryptic activity

4 10 2 1 Tryptic activity and species

The tryptic activity was found to be high in P monodon than in P indicus The t values showed significant difference between two species in all size groups for tryptic activity (Table 14)

4 10 2 2 Tryptic activity and size

The mean tryptic activity (Table 13) shows an increasing trend with increse in size of prawn In P indicus the tryptic activity on an average ranges from 1389 22 in 70/80 size group to 1816 75 in 30/40 grade The corresponding values of P monodon are 2581 4 and 2915 7 respectively The 30/40 size group of P indicus showed significant difference from 50/60 and 70/80 size groups But no significant variation in activity was observed between the latter two size groups In P monodon all the size groups showed significant differences in their tryptic activity

4 10 3 Phospholipase D

4 10 3 1 Phospholipase D and species

The P monodon registered a higher specific activity for phospholipase than P indicus for the same size group 30 40 and 50/60 But the 70/80 size groups of P indicus and P monodon did not show any significant variation in the enzyme activity (Table 14)

4 10 3 2 Phospholipase D and size

As in tryptic activity the White prawn 30/40 size group showed significant difference from 50/60 and 70/80 size groups But the latter size groups showed no significant variation between them In P monodon all the size groups studied are found significantly different from each other (Table 15) The general trend was a higher activity in larger sized prawns

DISCUSSION

V DISCUSSION

5 1 Head drooping and species of prawn

The results of preliminary studies as well as the studies on the effect of hauling time on head drooping show significant differences in the head drooping in the two species of prawns Penaeus indicus and Penaeus monodon The reasons for this species difference in the occurrence of head drooping can be attributed to the difference in the stability of the arthrodial membrane in Penaeus indicus and Penaeus monodon because damage to the membrane causes head drooping The weaker the membrane more is the chance of it getting damaged during different unit process The stability of the membrane inturn is dependent on its biochemical composition and thickness

The various constituents that stabilize the membraneous structures present in the arthrodial membrane of prawn are protein calcium cholesterol and phospholipid The percentage of each component and its relative proportion s the deciding factor to the membrane stability A significantly high proportion of all these constituents are observed in Penaeus monodon than in Penaeus indicus suggesting a more stable membraneous make up of the former one In addition to the biochemical

constituents thickness of the membrane in Penaeus monodon is higher than that of Penaeus indicus The physical and biochemical properties of arthrodial membrane show that the membrane of Penaeus monodon is more stable and hence more resistant to damage mediated through various physical and biochemical factors This will explain a significant lower percentage of head drooping in Penaeus monodon than in Penaeus indicus

The activities of the hydrolytic enzymes especially cathepsin D and trypsin of hepatopancreas are more in Penaeus monodon than in Penaeus indicus The arthrodial membrane which is in proximity to the hepatopancreas can be damaged during unit process by the action of these enzymes on the membrane This is possible only if these enzymes leach out of hepatopancreas and reach the membrane This leaching out depends on the stability of the membrane covering the hepatopancreas The results show that the head drooping is more in Penaeus indicus though the activities of the enzymes are lower So it may be presumed that the easiness with which the enzymes are released on postmortem is more important than the absolute activity of these enzymes So it may be true that the enzymes are more easily released from the hepatopancreas of Penaeus indicus than from

Penaeus monodon due to less stable structures present in the former

The dietary availability of cholesterol and phospholipid do influence the biochemical composition of prawns The above two are to be supplied through the diet since they are either not produced (as in cholesterol Zandee 1967) / or if produced are not sufficient to meet the requirements (as in phospholipid Chen 1993) Such problems are expected to become worse in crowded conditions So poor quality of feeds especially the finisher feed used in prawn culture can influence head drooping during later periods of storage The same also explains the observations of some skippers of fishing vessels that some fishing grounds with crowded occurrence of prawns are less suitable to process as premium quality head-on produce

5 2 Head drooping and size of prawn

In both species head drooping increases with decrease in size of prawn This may be due to the similar reasons as observed in earlier cases for interspecific differences The various factors such as biochemical constituents (mainly cholesterol calcium and phospholipid) and physical properties vary between size groups and this causes difference in the sugceptability of prawns to head drooping Tho gh the activities of the hepatopancreatic enzymes are lower in smaller size grou s

the leaching out of these enzymes may be more in these groups leading to a higher extent of head drooping

5 3 Head drooping and hauling time

The various factors that influence quality of the catch obtained by trawling are the nature of fishing ground type and composition of catch and the operating condition of the trawl net (Choudhuri 1985) Of the various operating conditions the actual hauling time is the most important single factor that influences quality of the raw material and is within the control of the skipper

In the present study an increase in head drooping is observed with increase in hauling time For a given hauling time the extent of head drooping is more in Penaeus indicus than in Penaeus monolon under the same size group Also within the same species the percentage head drooping increases with decrease in size for a given hauling time The reasor for the difference in head drooping between the two species and among the different size groups have already been dealt with in 5 1 and 5 2

The results of time size interactions show that increase in hauling time or decrease in size of prawn can produce increased head drooping Also interactions of time

species show that comparable head drooping for the two species Penaeus indicus and Penaeus monodon are obtained when the hauling time of Penaeus monodon is enhanced by 45 minutes

The increase in head drooping with increase in hauling time is due to the struggling and exhaustion of prawns in the trawl net as well as the physical damage to the membrane This can inturn cause a decrease in the yield of head on prawn immediately after catch Further the exhaustion during hauling can cause increased head drooping during later unit process

Usually fishermen do operate 2 3 trawling cycles per day The nature of fishing ground composition and abundance of the catch (species wise) become available after the first operation Based on these the hauling parameters can be suitably manipulated to obtain a good quality catch of prawns suitable for the production of premium quality head on produce

The results of the present investigation suggest that the hauling time should be judiciously fixed for different species of prawn For those species prone to higher percentage of head drooping a lower hauling time should be preferred For a fishing ground yielding smaller size prawns the hauling duration should be minimised for good quality head-on prawns

A similar problem can be expected during the harvesting of prawns from the culture ponds The ponds have to be designed to facilitate a quick and easy harvesting operation Also the harvesting methods and practices must limit the exhaustion of prawns The prawns obtained by hand picking especially during the final stage of harvesting are likely to lower the yield of head-on produce as they get excessively exhausted and contaminated with bacteria

5 4 Head drooping and ice storage

Ice storage duration significantly affect the quality of prawns to produce good quality head-on prawns Head drooping in ice stored prawns is more in smaller sized prawns than in larger size groups The factors leading to the formation of head drooping can be attributed to the enzymic hydrolysis similar to the mushiness problem of freshwater prawn (Nip et al 1985 a) or to the rapid hydrolysis of Antartic krill(Gildberg 1988) Since the problem occurs within a short duration of 12 36 hours as observed in the present study it is unlikely to be caused by the xxx spoilage organisms So the hydrolytic enzymes of hepatopancreas leaching out during the ice storage come in contact with the arthrodial membrane and damage the membrane The more fragile arthrodial membranes in smaller size prawns get

easily damaged by the enzymes causing higher percentage of head drooping in lower size groups of prawns The role of enzymes in tissue degradation is supported by the observations of Shaban et al (1987) which show increased accumulation of the protein break down products such as aminoacids (tyrosine) in the cephalothorax region during ice storage of Penaeus japonicus Such breakdown products can again interact with other components of the body and with the microorganisms in a slow manner causing discolouration of the cephalothorax (to rather yellowish colour) as observed by Velankar et al (1961) in raw head on prawns during ice storage Reilly et al (1980) observed that the shelf life of Penaeus monodon (size 16/20 pieces/Kg) stored at 0°C is 2 days for use in producing prime quality head on produce But the method of evaluation of quality is not mentioned in this study In addition the study was conducted for a single species with single size group But the results of the present study show that the shelf life or suitability of ice stored prawns to process as prime quality head on prawns varies based on species and size of prawns So the shelf life of raw material is to be indicated with respect to size and species

During the process of harvesting sorting grading icing etc the smaller size prawns are to be given

priority compared to larger size groups due to their easy susceptibility to head drooping

5 5 Head drooping and freezing

The various freezing methods used in the study differ in their freezing rate The freezing rate is highest for IQF The various methods show significant variations in causing head drooping The percentage of head drooping is the lowest in IQF and highest in block freezing which indicates an inverse relationship between percentage head drooping and rate of freezing This inverse relationship is attributed to the difference in the size of ice crystals formed under different rates of freezing In block freezing as the freezing rate is low the ice crystals formed are larger than those produced in IQF Such ice crystal formation will cause tissue and membrane damage The damage to hepatopancreas by the formation of large ice crystals by slow freezing will result in the release of a number of hydrolytic enzymes which can further damage the arthrodial membrane

Although some enzymes are denatured at subfreezing temperatures most remain quite active after freezing In addition many enzymes exhibit significant activity in partially frozen systems During freezing solute concentration in the unfrozen pools of water increases

The increased concentration of electrolyte and the change in pH that often occurs simultaneously is a major cause of biochemical damage and no doubt affect the enzymic activity of frozen food system Whether the effect is activation stabilization or inhibition depends on the enzyme the nature and concentration of salts the pH the temperature and the types of other substances present Increase in the concentration of solutes can result in the inhibition of some enzymes and activation of others Activation of cathepsins in relation to freezing in the zone of maximum crystallisation is observed by Rehbein et al (1978) In the rapid freezing the zone of maximum crystallisation is passed quickly and the ice crystals formed are smaller in size

In any type of freezing methods employed (Block semi IQF IQF) the percentage of head drooping increases with decrease in size of prawns This can be interpreted in the following ways It has already been shown that in smaller size prawns the concentration of the different constituents like protein phospholipid cholesterol and calcium are low but moisture is high in the arthrodial membrane This higher content of moisture will lead to formation of more ice crystals in the membrane making it more vulnerable to freeze injury

Usually in smaller size prawns the tissue moisture level is higher which can cause the formation of more

ice crystals during freezing leading to more tissue damage as well as release of hydrolytic enzymes especially from the hepatopancreas This inturn affects the stability of the arthrodial membrane

A combination of slow freezing and a higher moisture content will cause more damage to biological membranes than a combination of quick freezing and low moisture content This explains the variation in head drooping observed under different freezing methods in various size groups of prawns

5 6 Head drooping and cold storage

The results indicate that the extent of head drooping increases significantly with increse in cold storage duration The smaller size groups are observed to be more susceptible to head drooping than large ones during cold storage as in the case of ice storage and freezing The various combinations of cold storing durations and size groups showed significant interactions in all cases except for the combination of 4 months cold storage duration with size groups

The degradative process taking place during cold storage depends on a number of factors such as storage conditions and intrinsic biochemical composition of the raw material In addition the various pre storage unit process starting from the harvesting stress and

exhaustion influence the rate of quality loss of the product during frozen storage The biochemical constituents such as proteins and phospholipids undergo hydrolytic/ oxidative degradations The products of these degradative changes can accelerate further spoilage by interacting with other macromolecules and tissue components The changes are further augmented by the action of various enzymes present in the tissues and hepatopancreas of prawns Some of these enzymes eg phospholipase D are active even at low temperatures (Tannenbaum 1976) Enzymes like cathepsin D may leach out from the tissues during cold storage and can damage membranes The results of these activities will cause a marked change in the structural integrity of the product The rate of such changes in a product is mainly influenced by its biochemical composition and storage conditions

All the above degradative changes are applicable to the deterioration of arthrodial membrane as it contains significant amount of proteins phospholipid moisture etc The proximity of the membrane to the hepatopancreas with its hydrolytic enzymes which can leach out during storage makes the problem worse This may also explain the low storage life for head on prawns

A cold storage temperature of 20° C is not sufficient to provide a reasonable storage life for head on

prawns The various degradative process, although proceed at a low pace are sufficient to cause considerable damages to the arthrodial membrane So a cold storage temperature lower than 20°C combined with a short duration of storage before reaching the consumer is preferred in head on frozen prawns

5 7 Head drooping and Thawing methods

The various thawing methods used in the study showed significant difference among them in relation to the occurrence of head drooping in prawns Of the various thawing methods tried, running water thawing produced least and air thawing produced the highest percentage of head drooping The different methods vary in their heat transfer efficiency hence in the rate of thawing The thawing rate is fastest for running water and lowest for air thawing

The factors causing head drooping during freezing can also be attributed for thawing Both process are known to affect the stability of membranes of cells and organelles A slow freezing and thawing of meat products is accompanied with increased release of enzymes The slow thawing combined with unstable arthrodial membranes observed in smaller size prawns make them more vulnerable to head drooping compared to larger sized prawns having a comparatively stable membraneous make up Head drooping can

be minimised during thawing by employing a fast thawing method such as running water thawing

5 8 Head drooping and bacterial load

Results of this study show that there is no interspecies or intraspecies (with respect to size) variation in the bacterial load The result is in agreement with Williams et al (1952) This suggests that bacterial load do not seem to play any significant role in causing head drooping in prawns Whether the individual spoilage type of bacterial population influences the process is not known But such a possibility is less likely since bacterial population of spoilage type is not likely to reach a substantial level within a short period (Nip et al 1985a) of 2 days at 0^{0} C after which the raw prawns become not suitable for production of prime quality head on produce But an excessive load of bacteria on the surface and gut may cause problems which can arise due to poor aquaculture husbandry or improper harvesting methods

5 9 Arthrodial membrane and head drooping

As we have already seen head drooping in head-on processed prawns is a result of damage to the arthrodial membrane The inherent capacity of the membrane to withstand any damage (physical or chemical) depends on

its stability which inturn is determined by its physico chemical structure As has already been discussed the biochemical composition and thickness of the arthrodial membrane is different for Penaeus indicus and Penaeus monodon Also they vary from size to size in a given species The physico chemical structure of arthrodial membrane with respect to its stability in Penaeus monodon is better than that in Penaeus indicus This explains why head drooping is less in Penaeus monodon than in Penaeus indicus Also the study has revealed that the physico chemical structure of the arthrodial membrane in bigger prawns is better and hence they are less susceptible to head drooping when compared to smaller prawns

5 10 Hepatopancreatic enzymes and their role in head drooping

The specific activities of the hepatopancreatic enzymes Cathepsin D Trypsin Phospholipase D are higher in Penaeus monodon than in Penaeus indicus and also they are higher in larger prawns But head drooping is found to be low in Penaeus monodon and in larger prawn Ihis suggests that the total activity of enzymes in the intact hepatopancreas is not important but the rate of release of these enzymes from the hepatopancreas during unit

process x x x x x x is important This rate may be higher in Penaeus indicus than in Penaeus monolon and also in smaller size prawns of a given species So the rate of release of hepatopancreatic enzymes is another factor which influences head drooping in prawns

SUMMARY

V SUMMARY

1 The objective of the present study was to find out the cause of head drooping in headless processed prawns of different species size groups and during various unit process such as trawling ice storage freezing cold storage and thawing

2 Significant differences in the occurrence of head drooping exist among species and are due to the differences in the biochemical composition related to membrane stability and thickness of the arthrodial membrane

3 The various size groups show significant difference in the occurrence of head drooping and the same is due to

(a) differences in the biochemical composition and thickness of the arthrodial membrane in relation to stability

(b) differences in the rate of leaching out of the hydrolytic enzymes of hepatopancreas in various size groups

4 Head drooping in prawns is significantly affected by hauling duration and it increases as the hauling duration increases The effect of hauling time on the occurrence of head drooping is dependent on species and size of prawn

5 The extent of head drooping is significantly affected by ice storage duration Higher occurrence of head drooping is observed with increased ice storage duration

Also smaller sized prawns are to be stored for a shorter duration compared to larger sized prawns In fact prawns are to be ice stored for a shorter duration for use in the production of head on frozen prawns

6 A significant decrease in head drooping with decrease in the duration of freezing is observed Quick freezing is better suited for the production of premium quality head on frozen prawns

7 The factors leading to the occurrence of head drooping in prawns are active during cold storage temperature of 20 °C So the cold storage duration of head on processed prawns is to be as short as possible A temperature much below 20° C is preferred for the frozen storage of head on frozen prawns

8 Thawing time significantly influences the occurrence of head drooping Reduced thawing time favours significant lowering in the occurrence of head drooping

9 No significant correlation is observed between head drooping and bacterial load Also the TPC shows no significant difference among size groups or species

10 The thickness of the arthrodial membrane shows significant difference between species Within the same species thickness of the membrane increases with increase in size of the prawn

11 Biochemical composition of the arthrodial membrane shows significant difference between species and size groups

12 The activities of the hepatopancreatic enzymes show a significant difference between the two species of prawns studied Also the activities decrease with decrease in size of the prawns

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INVESTIGATIONS ON HEAD DROOPING IN HEAD-ON PROCESSED PRAWNS

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ABSTRACT OF A THESIS

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ABSTRACT

Head drooping is the major quality problem associated with processing of head on prawns The head drooping in head on prawns refers to the condition of oozing out of hepatopancreatic juice resulting from the breakage of the arthrodial membrane which connects cephalothorax of prawn to abdomen dorsally

The susceptibility of prawns to head drooping depends on the stability of the arthrodial membrane which in turn is determined by its biochemical composition In this connection various biochemical constituents of the arthrodial membrane such as protein phosrholipid moisture calcium and cholesterol were analysed for two species of prawn Penaeus indicus and Penaeus monodon unde three size groups (30/40 50/60 an 70/80 pieces/kg) The activities of various hydrolytic enzymes of the hepatopancreas were also studied

The various biochemical constituents showed significant difference between species Within the same species a size wise variation in the constituents of the arthrodial membrane and enzyme activity of hepatopancreas was observed

The various unit process such as hauling time ice storage durations freezing methods old storage

durations and thawing methods were studied individually for their influence on head drooping

The study shows that the major determinants of head drooping in prawns are the biochemical composition of the arthrodial membrane and the easiness with which the hydrolytic enzymes of the hepatopancreas are released on postmortem handling and processing