QUALITY ASSESSMENT OF BLACK TIGER SHRIMP (*Penaeus monodon*) FROM DIFFERENT CULTURE SYSTEMS OF COASTAL WEST BENGAL

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MASTER OF FISHERY SCIENCE

in

Fish Processing Technology

By

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CERTIFICATE

This is to certify that the work recorded in the thesis entitled **"QUALITY ASSESSMENT OF BLACK TIGER SHRIMP (*Penaeus monodon*) FROM DIFFERENT CULTURE SYSTEMS OF COASTAL WEST BENGAL"** submitted by **Mr. R. Renganathan** in partial fulfilment of the requirement for the degree of **Master of Fishery Science (Fish Processing Technology)** in the Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, is the faithful and *confide* research work carried out under my supervision and guidance. The results of the investigation reported in this thesis have not so far been submitted for any other degree or Diploma.

The assistance and help received during the course of investigation have been duly acknowledged.

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APPROVAL SHEET

APPROVAL OF EXAMINERS FOR THE AWARD OF THE DEGREE OF MASTER OF FISHERY SCIENCE (FISH PROCESSING TECHNOLOGY)

We, the undersigned, having been satisfied with the performance of Mr. **R. Renganathan** in the viva-voce examination, conducted today, the .....2013, recommended that the thesis be accepted for the award of the degree.

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
AOAC	Association of analytical chemist
APC	Aerobic plate count
BGA	Brilliant green agar
BPA	Baird parker agar
BSA	Bismuth sulphite agar
FAO	Food and Agriculture Organization
ICMSF	International commission on microbiological specifications for foods
IMP	Inosine monophosphate
ISO	International Organization for Standardization
MPEDA	Marine products export development authority
MPN	Most probable number
NA	Nutrient agar
RSW	Refrigerated sea water
TCBS	Thiosulphate citrate bilesalt sucrose
TPC	Total plate count
SC	Scientific cultured shrimp
SPC	Standard plate count
T-7	Tergitol 7

TC	Traditional cultured shrimp
TVB-N	Total volatile basic nitrogen
TMA	Trimethylamine
TVC	Total viable counts
XLD	Xylose lysine deoxycholate

CHAPTER I

INTRODUCTION

1. INTRODUCTION

The Food and Agricultural Organization (FAO) predicts that this century, world consumption of aquatic proteins will increase to 150-160 million tonnes (Sahu *et al.* 2012). Shrimp is an important item in the world aquaculture scenario. Shrimps are very rich in protein and free amino acids. World production of shrimp, both captured and cultured, is around six million tonnes of which about 60 percent enters to the world market (Gillet, 2008). Shrimp is now the most important internationally traded fishery commodity in terms of value. Presently Taiwan, Indonesia, Thailand and India are known as global leaders in shrimp production. Since the late 1970s, shrimp production has expanded dramatically in many tropical countries (Martinez-Alier, 2001). International markets appear to have become almost saturated since global production has reached 6,00,000 tonnes/year (FAO, 2005). The black tiger shrimp (*Penaeus monodon*) is the most commercially important cultured shrimp species in the world, accounting 46% of all cultured shrimp (Hanpongkittikun, 1995).

The Indian Shrimp Industry plays an important role in the Indian economy. During the financial year 2012-13, exports of marine products reached an all-time high of Rs. 18,856 crores. Marine product export, crossed all previous records in quantity, rupee value and US $ terms. Exports aggregated to 9,28,215 tonnes valued at Rs. 18856.26 crores and USD 3511.67 million. Compared to the previous year, seafood exports recorded a growth of 7.68% in quantity, 13.61% in value and 0.1% growth in US$ earnings respectively. Frozen shrimp is the major export value item accounting a share of 51.35% of the total US $ earnings. Shrimp exports during the period (2012-13) has increased by 20.88%, 18.73% and 3.56% in quantity, rupee value and US $ value respectively (MPEDA, 2013).

India is now the world's fourth largest producer of shrimp. As huge rise in demand for shrimp at international market exists, there is a proposal to increase the shrimp cultivation area by Government of India. Sundarban's mangrove in West Bengal is considered to be the largest potential area for shrimp aquaculture, because mangroves are considered ecologically important due to their role as breeding grounds and nursery habitats for offshore fisheries. Black tiger shrimp (locally known as *Bagda*) is the most

preferred and practiced species during last two decades in Sundarban's area for brackishwater shrimp aquaculture as it fetches high price in international market.

Among the Indian maritime states, West Bengal ranks second and third in terms of area and production under shrimp culture respectively. The state has a coastal line of 150 km along its three coastal districts namely South Purba Midnapur, South 24 Parganas and North 24 Parganas (Rao *et al.* 2013). Production of shrimp in West Bengal has increased from 28,785 metric tonnes in 1990-91 to as high as 99,977 metric tonnes in 2010-11 (DFAARFH, 2011).

Shrimp is a perishable product. Its shelf life and wholesomeness during storage and shipment is greatly influenced by both enzymatic and microbiological changes. Postmortem autolytic changes occur faster in shrimps because the gut is usually not removed immediately after capture. The chemical composition of shellfish tissue is different and it contains a lot of non-protein nitrogenous compounds that encourage more rapid spoilage (Aitken *et al.* 1982).

The quality deterioration of raw shrimp is usually dominated by microbial activity. This deterioration is highly temperature dependent and can be reduced by lowering storage temperature. Raw shrimp deterioration has two forms: microbiological and non-microbiological. Non-microbial deteriorations, both enzymatic and non-enzymatic also contribute to the spoilage changes. The moment the shrimp is caught, the deterioration process starts which affects its quality. The influencing factors for the shelf life of shrimps are temperature fluctuation in raw material, mishandling after harvest, long transportation time to processing plants and manual processing where shrimp are kept for several hours under each stakeholder supply chain. The above factors influence in deciding the shelf life of shrimps.

Black tiger shrimp (*P.monodon*) which are locally cultured and captured in the various regions of the state are sold in the Kolkata fish retail market. Generally, the traditionally cultured/wild captured shrimp fetches much higher price than cultured shrimp. However, there is a growing concern over the quality of the shrimp sold in Kolkata market. The sanitary condition of fish market in Kolkata is grossly unhygienic. The facilities for preservation of fishes are not up to the mark. The floors of the fish market are filthy with no proper drainage system and there is no proper disposal system

of dressed fish waste. There are no proper facilities of washing, storage or chilling of fish. In addition, there is also no supply of clean water and ice.

Food quality refers to the wholesomeness or the state of excellence of a particular product in terms of its appearance, shape, color, taste and competitiveness in price to the buyer. At present, importing countries are becoming more conscious of quality as a characteristic of the end product and of the process by which the product is produced, and are imposing stringent quality standards for imported products.

Over the past few years, safety has become very topical subject eliciting a great deal of public concern particularly in the developed countries, where food safety offences are now regarded at Government level. Selling, possessing and/or advertising for sale of food that does not comply with food safety requirements are now offences as per food safety requirements. Enforcement Officers have been given very detailed and powerful new provisions for dealing with the process, premises and equipments that contravene the legislation or pose a threat to the health of the consumer. As technology advance and public awareness grows, consumers are becoming increasingly demanding in terms of the choice, quality, freshness, nutritional value and microbiological safety of food.

The term "quality" is defined by ISO (1992) as the 'Collection of features and characteristics of a product or service that confer its ability to satisfy stated or implied needs'. Freshness is the most important attribute when assessing the quality of shrimp. Microbiological, biochemical and sensory methods have been used to assess the freshness and quality of shrimp.

Kolkata market is far away from major shrimp producing farms of West Bengal. Therefore, the shrimp takes several hours to reach the market. Hence, the quality of shrimp is assumed to be degraded unless enough care is taken during transportation. It is desirable to determine the microbial and nutritional quality of such shrimp reaching the market in order to assure good health and hygiene of consumers. In this backdrop, the present study proposes to systematically study the sensory, microbiological and biochemical quality of black tiger shrimp (*P.monodon*) from different culture system (traditional and scientific) of coastal West Bengal region.

The objectives of the present study were:

a) To study the microbiological quality of black tiger shrimp (*P.monodon*) marketed at Kolkata (West Bengal).

b) To study the spoilage status through analyzing the biochemical indices of black tiger shrimp (*P.monodon*) marketed at Kolkata (West Bengal).

c) To analyze the state of freshness of black tiger shrimp (*P.monodon*) through sensory studies.

d) To study the proximate composition of black tiger shrimp (*P.monodon*) marketed at Kolkata (West Bengal).

CHAPTER II

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Quality deterioration of Shrimp

Most important factors in raw seafood are freshness and quality. After harvest, there are pronounced changes in the appearance, texture, chemistry, and redox potential of the muscle. In postmortem muscle, the degradation of ATP to ADP, AMP and IMP usually takes place within 24 hr or less. These changes are thought to be totally autolytic since, in most instances, inordinate delay in processing allows the proliferation of spoilage microorganisms. Several factors can affect the rate of IMP accumulation, including temperature, species, and handling. The initial loss of the attributes characterising freshness in seafood results primarily from catabolic changes in nucleotides and carbohydrates, which are rapidly followed by degradative reactions of nitrogenous compounds as well as hydrolysis and peroxidation of lipids. These reactions are catalyzed mainly by endogenous enzymes during further chilling of the catch and bacterial activity contributes to the quality deterioration (Norman and Benjamin, 2000). It is desirable to determine the microbial and nutritional quality of shrimp reaching the market in order to assure good health and hygiene to the consumers (Lilabati and Vishwanath, 1996).

Quality of shrimp is usually dependent on species and storage temperature, although researchers have focused on temperature as the main determinant of quality. Higher storage temperature results in quicker spoilage (Nguyen and Gaillardin, 1997) and reduces shelf life (Matches, 1982). The most important means of preserving fresh shrimp is by chilling to about 0-4°C. The most common chilling media are wet ice, a mixture of ice and seawater or refrigerated seawater (Sikorski and Sun Pan, 1994). There are several studies on the storage stability of shrimp in ice (Yamagata and Low, 1995). Reilly *et al.* (1985) reported that the storage life for black tiger shrimp (*Penaeus monodon*) reduced by approximately 1 day for every hour delay in icing.

2.2. Chilling and Storage

Icing of fish for transportation is a common practice in India. It is reported that most iced fish in the major marketing centers' of India are of substandard quality (Nair *et al.* 1974; Govindan, 1985). Iced storage of fish results in a decrease of total nitrogen (TN), non-protein nitrogen (NPN) (Reddy and Shrikar, 1991a) and denaturation of myofibrillar proteins (Fredrick & Thomas, 1985).

After sorting and washing, the raw whole shrimp are drained and packed in ice in shallow boxes; the time between catching and chilling must be short. A delay of an hour or so on a warm day can cause considerable spoilage.

The box should be not more than about 200 mm deep to avoid crushing the bottom shrimp. A layer of flake ice or finely crushed block ice should be placed in the bottom of the box. A layer of shrimp not more than 50 mm deep should be laid on the ice and covered with more ice. Successive layers of shrimp and ice are then added until the box is full. Boxes must not be overfilled, or else shrimp get crushed when boxes are stacked. The shrimp should still be well covered with ice when they are landed; if they are not, then insufficient ice has been used. As much as 1 kg ice to 1 kg shrimp may be more ideal in an uninsulated fish room in a tropical country like India. The fish room temperature should preferably be kept at 1-3°C so that the ice melts slowly; melt water should be free to drain out from the bottom of a box.

Refrigerated sea water (RSW) can be used as an alternative to ice for the storage of raw whole shrimp at sea. The shrimp will keep in good condition for up to 4 days in RSW, but for best results they should be processed on shore within 2 days of capture. A suitable stowage rate is 2 kg shrimp to 1 litre of sea water; the sea water can be refrigerated mechanically or chilled by the addition of ice.

Deepwater shrimp stored in RSW have a generally more attractive appearance than iced shrimp of the same age; the raw whole shrimp look cleaner and have a better pink colour, and the cooked meat are again pinker than their iced counterparts. There is some uptake of salt; raw RSW shrimp contain about 2 per cent by weight of salt after 2-3 days storage, which is normally an acceptable concentration.

Chilled or iced preservation during storage, distribution and retailing are necessary to prevent browning in shrimp. This is based on the idea that refrigerated temperature is effective in reducing enzymic activity. The rate of enzyme-catalyzed reactions is controlled to a great extent by lowering the storage temperature. It has been found experimentally that increasing the temperature from 0 to 10 ^{o}C at least doubles the rate of spoilage in fish and the controlling of temperature and time is of prime importance in reducing deterioration of raw material (Norman and Benjamin, 2000).

Preservation of seafood in ice is a common method of retarding spoilage in India and in other tropical countries (Surendran *et al.* 1989). The rate of deterioration during ice storage of seafood varies with species and depends on the concentration of substrates and metabolites in the tissue, microbial contamination and condition of storage after harvest (Pacheco- Aguilar *et al.* 2000).

Liquid ice is a new super chilling technique for seafood that requires less time to chill products and acts more uniformly than other types of traditional ice. Liquid ice is composed of millions of microscopic spherical ice crystals suspended in seawater or brine (Optimar, 2003). These structure characteristics provide the ice with a superior ability to chill fish due to its better heat exchange power and to prevent marking or physical damage to the fish (Huidobro *et al.* 2001). The practical advantage of liquid ice is that it can be pumped through conventional pipes and can be stored in all types of tanks or containers. Moreover, on account of the microscopic size of the ice crystals, the main benefit of liquid ice is its ability for rapid chilling so as to help in storing fish at a lower temperature.

2.3. Quality changes of Fish and Shrimp

2.3.1. Microbiological changes

According to Rajadurai (1985), the time interval between the landings of shrimps and their arrival at the processing plants is very important. The activity of microorganism is the main factor limiting the shelf life of raw seafood. Microorganisms are found on all the outer surfaces and in the intestines of live and newly caught fish. The total number of organisms varies enormously and Liston (1980) states a normal range of 10^2-10^7 cfu (colony forming units)/cm^2 on the skin surface. The gills and the intestines both contain between 10^3 and 10^9 cfu/g.

Bacterial counts were valuable as a measure of degree of freshness of fish. For the bacterial enumeration and isolation, the most common method used is the total viable count or standard plate count (Rahman, 1980). An estimation of the total viable counts (TVC) is usually used as an acceptability index in standards, guidelines and specifications (Olafsdottir *et al.* 1997).

Okpala *et al.* (2013) reported that the initial APC (aerobic plate count) value of *Litopenaeus vannamei* was 4.45±0.09 log cfu/g. The aerobic bacterial spoilage

increases when the shrimp is dead. This is because in the live seafood products bacterial load is under control of the immune system. However, when the fish or shrimp dies the immune system collapses and bacteria proliferate quickly.

Ghosh *et al.* (2013) reported that in semi-intensive management condition of fresh water prawn samples, the total coliform range were 9-23 MPN/g and faecal coliform range were 3-7 MPN/g; In improved traditional management condition total coliform range were 53-160 MPN/g and faecal coliform range were 7-15 MPN/g and in traditional management condition total coliform range were 120-616 MPN/g and faecal coliform range were 14-28 MPN/g. The highest number of total coliform 616 MPN/g and faecal coliform 28 MPN/g were enumerated in traditional or extensive management system of farm. On the other hand, the lowest number of total coliform 9 MPN/g and faecal coliform 3 MPN/g found in the semi- intensive management system and *Salmonella* species found in the sample of traditional culture.

Khan *et al.* (2012) reported that the aerobic plate counts (APCs) of raw freshwater prawn ranged between 3.89×10^5 cfu/g to 5.0×10^5 cfu/g. Total coliforms of raw freshwater prawn among three samples were 64, 28 and 21 MPN/g. Faecal coliforms of raw freshwater prawn of three samples were 6.1, 3.6 and <3 MPN/g.

Nilla *et al.* (2012) reported that the total bacterial count of iced white shrimp (*Fenneropenaeus indicus*) ranged from $4.2 \pm 0.45 \times 10^6$ cfu/g to $1.3 \pm 0.50 \times 10^8$ cfu/g. The total coliform count ranged between $2.8 \pm 0.30 \times 10^3$ cfu/g and $7.8 \pm 0.50 \times 10^5$ cfu/g. The *Salmonella-Shigella* count ranged from $0.7\pm0.0\times10^2$ cfu/g to $2.1\pm0.25\times10^2$cfu/g. Highest load of *E.coli* was $3.1\pm0.25\times10^3$cfu/g. The lowest value was $0.4\pm0.50\times10^2$cfu/g.

Nilla *et al.* (2012) further reported that the *Salmonella - Shigella* (SS) count ranged from $0.5\pm 0.0\times10^2$ cfu/g to $2.1\pm0.25\times10^2$cfu/g for the shrimps. The highest load of *Vibrio* spp. was $2.2 \pm 0.25 \times 10^3$ cfu/g and the lowest density was $2.2 \pm 0.25 \times 10^3$ cfu/g. The highest load of *Staphylococcus* spp. was $6.7 \pm 0.30 \times 10^5$ cfu/g and the lowest was $2.1 \pm 0.25 \times 10^2$ cfu/g.

Siddiqui *et al.* (2011) reported that the initial $\log_{10}$ value of SPC (standard plate count) was nearly same in all storage conditions which were $\log_{10}$ 3.10 ± 0.47 cfu/g, $\log_{10}$ 3.30 ± 0.36 cfu/g and $\log_{10}$ 3.45 ± 0.28 cfu/g in immediate icing (highly acceptable),

delayed icing (highly acceptable), ambient temperature (highly acceptable) respectively. With the increasing of time these values increased to $\log_{10}$ 4.2±0.35 cfu/g (acceptable), $\log_{10}$ 4.4±0.35 cfu/g (acceptable) and $\log_{10}$ 5.1±0.20 cfu/g (moderately acceptable) at 12 hours. After 24 hours it further increased to $\log_{10}$ 5.2±0.32 cfu/g (acceptable), $\log_{10}$ 5.9±0.25 cfu/g (acceptable) and $\log_{10}$ 7.2±0.60 cfu/g (more unacceptable).

Ibrahim (2011) observed that the total counts for all the processed shrimp samples were generally high exceeding the limit of $1.0x10^2$cfu/mL. The counts ranged between $2.7x10^7$ and $7.6x10^7$cfu/mL. The coliforms count was ranged between $7.0x10^6$ and $2.32x10^8$cfu/mL. The Salmonella-Shigella count was ranged from $2.7x10^7$ to $1.10x10^8$cfu/mL.

Hossain *et al.* (2010) observed that the highest bacterial load (8.13± 0.47× 10^4 cfu/g) was detected in raw block frozen shrimp. The lowest bacterial load observed in the sample of cooked IQF shrimp was 1.30± 0.29 × 10^3 cfu/g. Hossain *et al.* (2010) also observed that the mean MPN count per gram for coliform of raw block frozen shrimp, cooked IQF shrimp and raw IQF shrimp was 21.00 ±6.25, <3 ±0.00 and 4.20 ±1.20 respectively. *Salmonella* was detected in 8 of 20 samples of shrimp from departmental shop and local fish market in Dhaka city, Bangladesh.

Ozyurt *et al.* (2009) assessed the microbiological quality of red mullet (*Mullus barbatus*) and goldband goatfish (*Upeneus moluccensis*) during storage in ice. The total viable count (TVC) exceeded 7 log cfu g^{-1} after 8 days of storage in goldband goatfish, and 11 days of storage in red mullet. Hernandez *et al.* (2009) reported that the total aerobic mesophilic count of aquacultured meagre (*Argyrosomus regius*) was 7 log cfu/g after 9 days of storage.

Yousuf *et al.* (2008) reported that the total bacterial counts ranged from $2.04x10^2$ cfu/g to $4.5x10^5$ cfu/g and $1.08x10^2$ cfu/g to $1.2x10^3$ cfu/g in shrimp (*Penaeus monodon*) and prawn (*Macrobrachium rosenbergii*) respectively. The coliform counts ranged between $5.4x10^2$ cfu/g to $8.5x10^5$ cfu/g in shrimp (*Penaeus monodon*) and $5x10^2$ cfu/g to $4.4x10^4$ cfu/g in prawn (*Macrobrachium rosenbergii*).

Yousuf *et al.* (2008) further reported that the *Salmonella-Shigella* (SS) count ranged between $0.26x10^2$ cfu/g and $0.96x10^4$ cfu/g in prawn (*Macrobrachium rosenbergii*) and $0.15x10^2$ to $1.1x10^4$ cfu/g in shrimp (*Penaeus monodon*). The bacterial

pathogens like *Vibrio* spp. and *Salmonella* were isolated from almost all the shrimp and prawn samples.

Ali *et al.* (2008) reported that the value of SPC (standard plate count) ranged between $\log_{10}$ 3.82 ± 0.29436 to $\log_{10}$ 5.11 ± 0.16453 cfu/g for a storage period of 14 hours. It was seen that SPC contents in plastic basket were $\log_{10}$ 3.82± 0.29436, $\log_{10}$ 4.71± 0.166013 and $\log_{10}$ 5.11 ± 0.16453 cfu/g at 0 hr (highly acceptable), 11 hours (just acceptable) and 14 hours (just unacceptable) respectively. The value of SPC varied between $\log_{10}$ 3.78 ± 0.3629 to $\log_{10}$ 4.98 ± 0.6226 cfu/g for a storage period of 15 hours. SPC contents in bamboo basket were $\log_{10}$ 3.78± 0.0.3629, $\log_{10}$ 4.58 ± 0.19562 and $\log_{10}$ 4.98 ± 0.6226 cfu/g at 0 hr (highly acceptable), 12 hours (just acceptable) and 15 hours (just unacceptable) respectively.

Manna (2008) examined the microbiological quality of fresh and ice-preserved *Labeo rohita, Catla catla, Cirrhinus mrigala, Oreochromis mossambica, Heteropneustes fossilis, Clarias batrachus* and *Penaeus monodon* of Kolkata (India) and found that the total plate count of bacteria was within the acceptable or marginally acceptable limits in most samples while fishes were contaminated with coliforms, including *E. coli*, indicating poor hygiene and sanitary conditions.

Abu Bakar *et al.* (2008b) reported that the mesophilic aerobic counts of fresh water prawn showed uptrend profiles throughout the storage period with values reaching log 6 + cfu/g after 20 days of iced storage.

Quintoil *et al.* (2007) reported that of the total of 59 marine samples comprising 40 fin fishes and 19 shrimps, 17 (28.8%) were found to be positive for *vibrio parahaemolyticus* i.e. 10 from fin fishes and 7 from shellfishes. Forty two samples comprising 25 fin fish and 17 prawns of freshwater origin yielded 6 positive samples (14.3%). The break up was 3 each for fin fishes and prawns. Analysis of brackish water fin fishes (20), shrimps (21) and crabs (20) yielded 15 *Vibrio parahaemolyticus* positive samples, 4 from fin fishes, 5 from shrimps and 6 from crabs. The highest incidence of *Vibrio parahaemolyticus* was from marine shrimps (36.8 %), followed by brackish water crabs (30%) marine fishes (25%), brackish water shrimps (23.8%), brackish water fin fishes (20%), freshwater prawns (17.6%) and freshwater fin fishes (12%).

Mahmud *et al.* (2007) reported that the SPC (standard plate count) of black tiger shrimp was $\log_{10}$ 3.93± 0.12cfu/g, $\log_{10}$ 4.22 ± 0.53 cfu/g and $\log_{10}$ 4.33 ± 0.21 cfu/g at 0 hour, 12 hours and 24 hours respectively. Lalitha and Surendran (2006) reported that the total aerobic plate counts at 37 and 20°C on newly caught fresh water prawn *Macrobrachium rosenbergii* was in the range of 4 to 5 $\log_{10}$ cfu g^{-1}.

Kilinc *et al.* (2007) studied the effect of slurry ice and flake ice pretreatments on the quality of aquacultured sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). They observed that the mesophilic counts in sea bass exceeded the level of acceptability after 13 days of storage. Nuray and Ozkan (2007) reported that the mesophilic aerobic bacterial count of ungutted sardine samples was higher than gutted sardine samples.

Jeyasekaran *et al.* (2006) reported that the fresh shrimp (*Fenneropenaeus indicus*) had an initial bacterial load of 10^6cfu g^{-1}. Antony *et al.* (2002) reported that the TPC of the shrimps from the processing plants were within the range of 10^4 and 10^5 cfu/g. Total coliforms were detectable in shrimps collected from all the plants and their MPN counts ranged from 12 to 1600/g.

Nuray and Ozkan (2006) reported that the initial decomposition of sea bass (*Dicentrarchus labrax*) occurred after 7 days of iced storage when bacterial counts were > 4 log cfu/g. Microbial counts showed significant increase in whole and gutted sea bass during storage.

Jeyasekaran *et al.* (2005) reported that the total bacterial load in immediately iced (II) reef cod (*Epinephelus merra*) was 10^6 cfu /g after 17 days of storage.

Ozogul *et al.* (2005) reported that the total viable count of sea bass (*Dicentrarchus labrax*) was 6.4 $\log_{10}$ cfu ml^{-1}.

Chytiri *et al.* (2004) reported that initial mesophilic viable counts of whole ungutted and filleted rainbow trout were 2.5 and 3.8 log cfu/cm^2 respectively. Mesophilic counts reached 7.0 log cfu/cm^2 after 18 days of iced storage in whole ungutted trout.

Jeyasekaran and Ayyappan (2003) studied the microbiological quality of farm-reared rohu (*Labeo rohita*) stored at two different temperatures. The initial total bacterial load of the fish fillet was found to be 4.60 x 10^3cfu/ g and the initial level of

lactics, staphylococci, aeromonads, anaerobes and molds in the fish fillet was only about 10^1/ g. The pathogens such as Vibrios and *E. coli* were absent in fish fillet, but *Salmonella* was present. During ambient temperature storage, the total bacterial load in the fish fillet gradually increased and reached a level of 10^6/g in 10 hours at which the fish was found to be organoleptically unacceptable.

Papadopoulos *et al.* (2003) studied the microbiological properties of gutted sea bass (*Dicentrarchus labrax*) stored in ice. They found that bacterial counts of whole ungutted sea bass contained always higher than gutted sea bass samples. Mesophilic counts in gutted and ungutted fish exceeded 7 log cfu g^{-1} after 9 and 15 days of ice storage, respectively.

Grigorakis *et al.* (2003) studied the seasonal patterns of spoilage during 15 days of ice storage of cultured gilthead sea bream (*Sparus aurata*). They found that winter fish reached higher levels of microbial populations (10^9 vs 10^7 in summer fish) at the end of the storage period in iced condition.

Jeyasekaran and Ayyappan (2002) reported that the initial bacterial load in fresh water prawn (*Macrobrachium rosenbergii*) muscle was found to be 6.5×10^4 cfu/g. The incidence of Staphylococci, Aeromonas, *E. coli*, anaerobes and molds in the prawn muscle was at 10^1 cfu/g, while Lactics and Vibrios were in a higher range of 10^2 cfu/g. *Salmonella* and *Vibrio cholerae* were also present in fresh prawn muscle.

Kumari *et al.* (2001) studied the microbiological quality of rohu (*Labeo rohita*) marketed in Patna and isolated *E. coli, Pseudomonas* sp, *Staphylococcus* sp and *Klebsiella* sp. Leitao and Rios (2000) reported that the total psychrotrophic counts of freshwater prawn were relatively high, with an average of 5.2 log cfu/g.

Surendran (2000) reported that the bacterial population on freshly caught prawns naran (*Penaeus indicus*), kazhanthan (*Metapenaeus affinis*), poovalan (*M. dobsoni*), cultured tiger prawn (*Penaeus monodon*) and cultured giant fresh water prawn (*Macrobrachium rosenbergii*) were 10^3-10^5 cfu/g, 10^2-10^6 cfu/g, 10^3-10^6 cfu/g, 10^4-10^6 cfu/g and 10^4-10^6 cfu/g respectively.

Surendran (2000) further reported that 86% of gram negative bacteria were present in Naran (*Penaeus indicus*) includes *Vibrio, Pseudomonas, Moraxella, Acinetobacter* and *Aeromonas,* 14% of gram positive bacteria includes *Arthrobacter,*

Micrococcus and *Bacillus*. In tiger prawn (*Penaeus monodon*) 61% of gram negative bacteria were present includes *Vibrio, Pseudomonas, Moraxella, Acinetobacter* and *Aeromonas,* 32% gram positive bacteria includes *Arthrobacter, Micrococcus* and *Bacillus*.

Lilabati and Vishwanath (1998) reported that during the iced storage of freshwater fish, *Notopterus chitala*, the total bacterial load ranged between 10^5 to 10^6cfu/g. It has been reported that the effect of freezing gradually reduces the viable counts.

Ali *et al.* (1992) studied the bacteriological changes during iced storage of the tropical fresh water carp (*Labeo rohita*). They reported that the fish remained in acceptable condition in ice for up to 12 days. Microflora of freshly harvested fish consisted of *Staphylococcus, Acinetobacter, Micrococcus*, Enterobacteriaceae, *Aeromonas, Moraxella, Pseudomonas* and *Moraxella*. At the time of spoilage, the flora was dominated by *Aeromonas* (48%) followed by *Pseudomonas* (34%).

Surendran *et al.* (1989) studied the spoilage of oil sardine (*Sardinella longiceps*), Indian mackerel (*Rastrelliger kanagurta*), pearl spot (*Etroplus suratensis*), milk fish (*Chanos chanos*) and tilapia (*Oreochromis mosambica*) during the ice storage. They reported that oil sardine and Indian mackerel had an acceptable iced storage shelf life of nearly 1 week and pearl spot, milk fish and tilapia for nearly 2 weeks. In all these fishes, the spoilage flora were composed mainly by *Pseudomonas sp*.

Heinsz *et al.* (1988) reported that the average bacterial counts of brown shrimp (*Penaeus aztecus*) was $2{\cdot}0 \times 10^5$ cfu/g on the day of purchase.

2.3.2. Biochemical changes

Several chemical tests to determine the freshness of fish such as determination of amines, particularly trimethylamine (TMA), and determination of hypoxanthine have been used (Aitken *et al.* 1982). The former is related to bacterial activity while the latter is a measure of enzymic change. These two methods complement each other and have different ranges of applicability and usefulness. A chemical test does not measure freshness directly but the two are associated because the concentration of chemicals measured is dependent on storage time and temperature. The use of TVB-N as an index of spoilage was first proposed by Shewan and Ehrenberg (1957). Reilly *et al.* (1985)

stated that TVB-N is not reliable as indices of quality. Boee *et al.* (1982) working on the storage of shrimp has observed that TVB-N increased evenly. Matches (1982) working on shrimp stored at 5 different temperatures, found that TVB-N increased both with increase in time and temperature. Cann (1974) has found the increase in TVB-N to be low during the initial period of storage, with a rapid increase noted afterwards. The TVB-N value of ≤ 20 mg N/100g sample was considered fresh, ≤ 30 mg N/100g sample was acceptable and >40 mg N/100g sample was not suitable for consumption (TIS; 1986).

Ali *et al.* (2013b) reported that the TVB-N contents in black tiger shrimp (*Penaeus monodon*) ranged from 15.30±0.04 mg/100g to 19.91±1.18 mg/100g at farm level, 17.98± 3.77 mg/100g to 22.81±0.61mg/100g at faria level, 19.35±0.56 mg/100g to 24.04±2.23 mg/100g at depot level and 22.85±0.46 mg/100g to 25.46±0.99 mg/100g at factory receiving level.

Okpala *et al.* (2013) reported that at the beginning of storage period, the TVB-N value of (*Litopenaeus vannamei*) was 9.94± 0.86 mgN/100g. Begum *et al.* (2012) reported that the TVN value increased fairly from 18 to 30mg/100g in head off shrimp and 20 to 35mg/100g head on shrimp with the increase of storage time.

Siddiqui *et al.* (2011) reported that the TVB-N value of *Macrobrachium rosenbergii* was 2.80mg ± 0.05mg/100g, 4.20±0.15mg/100g and 6.50±0.25mg/100g immediately after icing at 0, 12 and 24 hours respectively.

Ali *et al.* (2008) reported that the value of TVB-N of black tiger shrimp (*Penaeus monodon*) ranged between 2.68± 0.19296 mg /100g to 12.46 ± 0.3396 mg/100g for a storage period of 15 hours. It was seen that TVB-N values in bamboo basket were 2.68 ± 0.19296, 9.78 ± 0.16 and 12.46 ± 0.3396 mg/100g at 0 hr (highly acceptable), 12 hours (just acceptable) and 15 hours (just unacceptable) respectively. The TVB-N content was found to vary between 2.49± 0.96176 mg/100g to 12.51 ± 0.94256 mg/100g for a storage period of 14 hours. Values of TVB-N in plastic basket were 2.49 ± 0.96176, 9.25 ± 0.63542 and 12.51 ± 0.94256 mg/100g at 0 hr (highly acceptable), 11hours (just acceptable) and 14 hours (just unacceptable) respectively.

Abu Bakar *et al.* (2008a) reported that the initial level of TVBs in fresh water prawn was 17.0 mg/100g. Mahmud *et al.* (2007) reported that the value of TVB-N for

24 hours storage of bagda (*Penaeus monodon)* stored in bamboo basket ranged between 2.56 ± 0.32 mg N/100g to 5.21 ± 0.61 mg N/100g whereas in the plastic basket, it was 2.58 ± 0.36 mgN/100g to 5.37 ± 0.37 mgN/100g. After 12 hours of storage, the value was 3.64 ± 0.66 mgN/100g and 3.73 ± 0.58 mgN/100g in the bamboo and plastic basket respectively. Boonsumrej *et al.* (2007) reported that the TVB values of all freeze-thawed black tiger shrimp (*Penaeus monodon*) were found to be 10.2-14.6 mg N/100g sample.

Nuray and Ozkan (2007) reported that the total volatile basic nitrogen (TVB-N) value of gutted sardine increased very slowly, where as whole ungutted sardine obtained a value of 15.03–29.23 mg/100g after 9 days of storage. Nuray and Ozkan (2006) studied the influence of ice storage on the quality and shelf life of gutted and un-gutted sea bass (*Dicentrarchus labrax*) and found that the acceptable quality of both whole and gutted sea bass was 11 days. Total volatile basic nitrogen (TVB-N) values showed no significant increases in whole and gutted sea bass during storage.

Pedro *et al.* (2006) observed the total volatile basic nitrogen contents (TVB-N) to assess the freshness of european sea bass (*Dicentrarchus labrax*) during 21 days of ice storage. They found that no change in the level of TVB-N during the edible storage life of the fish but there were significant increase of volatile bases after 20–22 days of storage.

Kilinc *et al.* (2007) reported that the TVB-N values of sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) reached the legal limits (35 mg/100 g) for consumption after 13 days of storage.

Zeng *et al.* (2005) reported that the TVB-N value measured was 33.5 mg/100g in northern Shrimp (*Pandalus borealis*) at the beginning of storage which increased to more than 70 mg/100 g on the 4th day of storage. Ozogul *et al.* (2005) reported that the TVBN value of sea bass (*Dicentrarchus labrax*) was 24.82 mg/100 gm after 23 days of ice storage. Chytiri *et al.* (2004) reported that the total volatile basic nitrogen (TVB-N) values showed no significant increase in whole ungutted trout after 18 days of storage.

Papadopoulos *et al.* (2003) reported that the TVB-N value of sea bass (*Dicentrarchus labrax*) was 27.7 mg N $100\ g^{-1}$ and 36.9 mg N $100\ g^{-1}$ in whole ungutted and gutted fish respectively.

Leitao and Rios (2000) reported that the TVB of fresh water prawn showed an increase, particularly at 5°C. At this temperature, value above 30 mgN/100g is considered the maximum allowable limit reached after 5 days of storage.

Yamagata and Low (1995) reported that the TVB-N value of banana shrimp (*Penaeus merguiensis*) was 3.83 mg/100g, 8.36 mg/100g, 12.11 mg/100g, 14.48 mg/100g and 15.49 mg/100g after 0, 2, 4, 6 and 8 days of storage respectively.

The use of TMA-N, as an index of fish freshness, was first proposed by Gibbon and Labire (1937). Connell (1975) recommended acceptable level of 10–15 mg/100g TMA-N for human consumption. Ali *et al.* (2013b) reported that the TMA-N contents in black tiger shrimp (*Penaeus monodon*) ranged from 12.75±0.2 mg/100g to 13.67±1.18 mg/100g at farm level, 16.39±2.31 mg/100g to 16.53±0.88 at faria level, 16.28±0.56 mg/100g to 18.53±2.56 mg/100g at depot level and 18.10±1.75 mg/100g to 19.26±0.22 mg/100g at factory level.

Okpala *et al.* (2013) reported that at the beginning of storage period, the TMA-N value of (*Litopenaeus vannamei*) was 0.33±0.12 mg N/100g.

Siddiqui *et al.* (2011) reported that the TMA-N value of *Macrobrachium rosenbergii* was 0.84±0.0141mg/100g when the prawn was fresh. After 12 hours it was 2.5±0.025 mg/100g, 1.70±0.032 mg/100g and 1.40±0.018 mg/100g at ambient temperature (more acceptable), delayed icing (acceptable) and immediate icing (acceptable) respectively. After 24 hours of storage, the value was 5.75±0.0071 mg/100g, 3.80±0.045 mg/100g and 2.50±0.025 mg/100g in ambient temperature (more unacceptable), delayed icing (moderately acceptable) and immediate icing (acceptable) respectively.

Ali *et al.* (2008) reported that the amount of TMA-N of black tiger shrimp (*Penaeus monodon*) ranged between 4.35 ± 0.2089 mg/100g to 11.78 ± 0.141774 for a storage period of 15 hours. It was seen that TMA-N values in bamboo basket were 4.35 ± 0.2089, 8.53± 0.49521 and 11.78 ± 0.141774 mg/100g at 0 hr (highly acceptable), 12 hours (just acceptable) and 15 hours (just unacceptable) respectively. The value of TMA-N obtained ranged between 4.06± 0.1963 mg/100g to 10.45 ± 0.687564 for a storage period of 14 hours. It was seen that TMA-N values in plastic basket were 4.06±

0.1963, 7.88 ± 0.852247 and 10.45 ± 0.687564 at 0 hr (highly acceptable), 11 hours (just acceptable) and 14 hours (just unacceptable) respectively.

Mahmud *et al.* (2007) reported that the TMA-N value of *Penaeus monodon* at 0 hour was 2.68 ± 0.21 mgN/100g, 12 hours it was 3.56 ± 0.53 mgN/100g and after 24 hours of the experiment it was 5.02 ± 0.41 mgN/100g for the bamboo basket whereas in the plastic basket it was 2.72 ± 0.23 mgN/100g, 3.63 ± 0.53 mgN/100g and 5.16 ± 0.47 mg-N/100g respectively at 0 hr, 12 hours and 24 hours respectively.

Chytiri *et al.* (2004) reported that the highest TMA-N value for whole ungutted rainbow trout samples was recorded after 18 days of ice storage. Yamagata and Low (1995) reported that the TMA-N of banana shrimp (*Penaeus merguiensis*) increased from 0.49 mg/100g on 0 day to 0.69 mg/l00g after 4 days and continued to increase to 1.51 mg/l00g after 8 days of iced storage.

Zeng *et al.* (2005) reported that the initial pH of the shrimp (*Pandalus borealis*) was 7.41 upon arrival. The increases of pH value were rapid in the sample stored in ice at 1.5 °C and reached a final pH of 8.26. Changes in pH were slowest in samples stored in liquid ice at –1.5 °C and reached a value of 7.98 at the end of storage.

Leitao and Rios (2000) reported that the initial pH value of *Macrobrachium rosenbergii* was 7.62 at 0°C. The value reached upto 8.54 after 10 days of storage. Leitao and Rios (2000) further reported that the initial pH value of *Macrobrachium rosenbergii* was 7.62 at 5°C. The value reached upto 8.60 after 10 days of storage.

Begum *et al.* (2012) reported that the initial pH value of *Macrobrachium rosenbergii* was 7.2 and the value reached upto 8.4 after 14 days of storage. Siddiqui *et al.* (2011) reported that the pH value of *Macrobrachium rosenbergii* ranged from 7.40 to 7.70z. Abu bakar *et al.* (2008a) reported that the initial pH value of *Macrobrachium rosenbergii* was 6.58. The value increased upto 7.5 after 10 days of storage at 10°C.

2.3.3. Proximate composition changes

Expressible moisture usually reflects the extent of denaturation of the protein and water content of fish muscle under investigation (Suvanich *et al.* 2000). This phenomenon leads to reduction of flavour agents and nutrition value (Reddy and Shrikar, 1991b).

Dayal *et al.* (2013) reported that the moisture (%) content of shrimp was 76.3 ± 0.57.

Bindu *et al.* (2013) reported that the initial pH value of *Fenneropenaeus indicus* was 6.58 and the value reached upto 7.18 after 30 days of storage.

Shalini *et al.* (2013) reported that the protein (%), fat (%) and carbohydrate (%) value of *L.vannamei* ranged from 18.07±0.02% to 25.01±0.02%, 0.01±0.03% to 1.770±0.025% and 3.030±0.023% to 6.23± 0.02% respectively. Shalini *et al.* (2013) further reported that the protein, fat and carbohydrate value of *F. indicus* ranged from 17.14±0.02 to 22.17±0.02%, 1.21±0.02 to 2.13±0.02% and 3.39±0.59 to 6.10±0.02% respectively.

Abdel-Salam (2013) reported that the carbohydrate (%), protein (%) and fat (%) content value of *Penaeus indicus* was 1.89±0.02, 42.88±1.11 and 8.57±0.24 respectively. Puga-lopez *et al.* (2013) reported that the protein (%), ash (%), crude lipid (%) value of *L. vannamei* ranged from 19.99±0.74 to 20.10±0.52, 2.10±1.05 to 2.27±0.45 and 1.27±0.36 to 1.34±0.18 respectively.

Dayal *et al.* (2013) reported that the protein (%) and lipid (%) content of shrimp was 19.4 ± 0.56 and 1.15 ± 0.19 respectively. Ehigiator and Nwangwu (2011) reported that the ash (%) and crude protein (%) value of *Macrobrachiun vollenhovenii* was 20±4.58 and 53.85±5.65 respectively.

Puga-lopez *et al.* (2013) reported that moisture (%) content of *L. vannamei* ranged from 73.14±1.23 to 73.90±0.78.

Ali *et al.* (2013a) reported that the protein content (%) in black tiger shrimp (*Penaeus monodon*) ranged from 17.77±0.36 to 23.43±.64. Ali *et al.* (2013b) reported that the protein (%) value of *Penaeus monodon* ranged from 17.43±.99% to 23.38±0.21% in wet weight method.

Begum *et al.* (2012) reported that the ash content value of *Macrobrachium rosenbergii* was 1.22%. Ehigiator and Nwangwu (2011) further reported that the ash (%) and crude protein (%) value of *M. macrobrachion* was 21±2.65 and 58.92±4.49 respectively.

Oksuz *et al.* (2009) reported that the moisture (%) content value of *Parapenaeus longirostris* was 78.7±0.17. Oksuz *et al.* (2009) further reported that the moisture content value of *Plesionika martia* was 82.2±0.6.

Zeng *et al.* (2005) reported that the initial moisture content of the shrimp (*Pandalus borealis*) was 81.1%.

Ravichandran *et al.* (2009) reported that the crude protein, crude carbohydrate, crude lipid and total ash content of *Penaeus indicus* (% dry weight) was 41.3±0.3, 2.4±0.6, 7.6±0.7 and 18.5±0.6 respectively.

Oksuz *et al.* (2009) reported that the crude protein (%), crude lipid (%) and ash (%) content value of *Parapenaeus longirostris* was 20±0.3, 1.1±0.24 and 1.6±0.03. Oksuz *et al.* (2009) further reported that the crude protein (%), crude lipid (%) and ash (%) content value of *Plesionika martia* was 14.2±1.3, 2.6±0.9 and 1.01±0.1.

Zeng *et al.* (2005) reported that the protein and fat content of the shrimp (*Pandalus borealis*) was 17.4% and 0.4% respectively.

Shekhar *et al.* (2004) studied the changes in muscle biochemical composition of *Labeo rohita* in relation to season and found that, protein, carbohydrate and total ash content were higher in summer season in comparison to winter.

2.3.4. Sensory changes

The sensory/organoleptic evaluation is the most satisfactory and acceptable method in fish-inspection services in assessing the freshness and quality of fish and shellfish (Connell 1995; Hyldig and Peterson, 2004). The organoleptic method offers immediate measurement of perceived attributes and provides information that may be of help in better understanding of consumer responses (Hyldig and Peterson, 2004). Sensory evaluation can be applied to all species of fish. The evaluation is quick and non-destructive unless the sample is being cooked, and moreover, the results often reflect the criteria the consumer uses in evaluating acceptability (Connell, 1990). The sensory characteristics of fish are clearly visible to the consumer and are essential for consumer satisfaction (Reineccius, 1990). Sensory methods are fast, simple and provide immediate quality information (Connell, 1975).

The organoleptic method may be influenced by the physiological and psychological state of the judges. Considerable variations are often observed in the sensitivity and the test of the panel of judges at different times and the different circumstances under which the examinations are performed (Farber, 1965).

Okpala *et al.* (2013) reported that the maximum initial colour score was awarded to shrimp chilled with flake ice where as lower score was awarded to that of liquid ice batch. The colour of shrimp chilled onboard with liquid ice obtained significantly lower score than the control lot chilled with flaked ice after 4 days of storage. Sensory assessors found the colour quality to worsen due to increased whiteness and resultant loss of typical pink/orange colour.

Begum *et al.* (2012) reported that the overall acceptability score (OA) of raw and cooked shrimp dropped from initial score point 9 of fresh to 5 at the border line of acceptability, which was noted at a definite time interval by the trained panel of judges. On the basis of these scores, the mean values had been compared with other quality indices and it indicated that the quality of the ice-stored shrimp crossed the borderline of acceptability limit after 12 days of storage (OA 5), both for the head on and head off shrimp. The stored samples became completely unacceptable after 14 days of storage in ice while the OA dropped down to less than 5. Rejection of raw samples by the panelists were mainly due to gradual changes from bright fresh appearance through slightly reddish to dark reddish dis- coloration of flesh and slimy feeling by touch on the surface of the fish. It was rated below the acceptable limit after 14 days of iced storage.

Azam *et al.* (2010) reported that the overall organoleptic score of shrimp at the farm under both conditions was almost the same 8.80-NP (Normal practice = without ice); 8.87-EC (experimental condition = with ice). Ozyurt *et al.* (2009) reported that the sensory acceptability limit was 8 days for goldband goatfish (*Upeneus moluccensis*) and 11 days for red mullet (*Mullus barbatus*) during storage in ice.

Ali *et al.* (2008) observed that at the time of harvest of shrimp treated as 0 hour, the average score was 10. The initial average score after 2 hour was 9 both in plastic and bamboo basket. This score gradually decreased over the range of time. It was apparent that the quality of shrimp was under highly acceptable limit up to 3 hours both in plastic and bamboo basket. Then the quality was found within the acceptable and

moderately acceptable range after 8 and 9 hours in both baskets respectively. Beside, the quality appeared at just acceptable range in bamboo basket after 12 hours but in plastic basket it was after 11 hours. The organoleptic quality appeared at just unacceptable, unacceptable and more unacceptable after 15, 19 and 21 hours in bamboo basket and 14, 17 and 19 hours in plastic basket respectively.

Mahmud *et al.* (2007) reported that the quality of shrimp was under highly acceptable (HA) limit till 10 hours of observation and the quality were drastically changed soon after 10 hours. It was 8.4 in the bamboo basket and 8.2 in the plastic basket.

Kilinc *et al.* (2007) reported that the quality of sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*), were within the acceptable limits after 13 days of storage, but they were no longer acceptable after 15 days of storage.

Tzikas *et al.* (2007) studied the quality changes of Mediterranean horse mackerel (*Trachurus mediterraneus*) and blue jack mackerel (*Trachurus picturatus*) during ice storage. Sensory results indicated that whole Mediterranean horse mackerel and blue jack mackerel had a shelf-life of 10 and 7 days, respectively. Nuray and Ozkan (2007) assessed the quality of whole and gutted sardines (*Sardina pilchardus*) stored in ice. The sensory analysis showed that both gutted and ungutted sardines were acceptable upto 7 days of storage in ice.

Erickson *et al.* (2007) observed that in terms of appearance, fresh shrimps were glossier than frozen shrimp for both the raw and cooked products. The most notable change in appearance for raw product, however, was the loss in tail iridescence.

Jeyasekaran *et al.* (2006) reported that fresh shrimp (*Fenneropenaeus indicus)* used in their experiment exhibited fresh sea weedy odor characteristic of the species, slight greenish color and firm texture with a sensory score of 9.9. The slight greenish color imparted in the freshly caught shrimp was due to the fact that the shrimp were captured from the fishing area, where there was a thick growth of algae during that season.

Ozogul *et al.* (2006) assessed the freshness of wild turbot (*Scophtalmus maximus*) under chilled storage. The quality of turbot gradually decreased and found

unacceptable after 15 days of storage. Zeng *et al.* (2005) observed that the lowest sensory scores were given to shrimp stored in ice at 1.5 °C and in brine mixed with ice at –1.5 °C. The shrimp stored in liquid ice at –1.5 °C received the highest sensory scores, indicating better quality.

Chytiri *et al.* (2004) reported that after 15 days of ice storage, the trout became unfit for consumption. Acceptability scores of odor, taste and texture of cooked ungutted and filleted trout decreased during storage. Papadopoulos *et al.* (2003) reported that after 11 days of storage in ice the sea bass became unfit for consumption.

CHAPTER III

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Raw materials

Black tiger shrimp (*P. monodon*) originating from traditional and scientific culture system were collected at random sampling method from Kolkata wholesale fish market according to the harvesting time. It includes crop I (September to December), crop II (January to April) and crop III (May to August). The sample was collected in aseptic condition by sterilized plastic bags and carried immediately to the laboratory of Department of fish processing technology, Faculty of fishery sciences, Kolkata in ice boxes in properly insulated condition.

3.1.2. Chemicals and Glasswares

All chemicals used in the present study are "Analytical grade" and supplied Hi-Media, India. Glasswares used are of Borosil make. For microbiological studies specific media for the organisms were used as given in Table 1.

Table: 1

Media	Organism
Nutrient agar (NA)	Total plate count
Baird parker agar (BPA)	*Staphylococcus aureus*
Tergitol 7 (T-7)	*Escherichia coli*
Xylose lysine deoxycholate(XLD) Brilliant green agar (BGA) Bismuth sulphite agar (BSA)	*Salmonella*
Thiosulphate citrate bilesalt sucrose (TCBS)	*Vibrio* spp.

3.1.3. Equipments

a. Hot air oven
b. Bacteriological incubator
c. Sterilizer/Autoclave
d. Electronic balance
e. Laminar flow

f. Homogenizer
g. Kjeldahl digestion system
h. Kjeldahl distillation unit

3.2 Methods

After reaching the laboratory the sensory analysis of the shrimp was carried out and samples for microbiological and bio chemical analysis were taken from the shrimp muscle in aseptic condition. Sampling was done for the different parameters which include biochemical, microbiological and sensory studies at fortnight intervals for a period of six months.

3.2.1. Microbiological assessment

Microbiological analysis carried out in this study included Total plate count (TPC) and quantitative enumeration of *Staphylococcus aureus, Escherichia coli, Salmonella* and *Vibrio* spp.

3.2.1.1. Total Plate count

TPC of muscle was done by following the standard method given by APHA, (2001) with some modifications. In brief, 10 g of shrimp sample was taken and it was homogenized with 90 ml of physiological saline. Serial decimal dilutions of 10^{-2}, 10^{-3}, 10^{-4} etc were prepared using 9 ml of 0.85% physiological saline. 0.1 ml of inoculum from each of the dilutions were spread plated onto Nutrient agar (NA) plates using a sterile glass spreader. The petriplates were incubated at 37°C for 24 hours. The plates containing 25-250 colonies were counted. The results were expressed as number of colony forming units per gram by using the following formula:

Count per gram = Number of colonies counted × reciprocal of dilution × reciprocal of dilution volume plated

3.2.1.2. *Staphylococcus* count

Staphylococcus count was done by following the method given by Varma, (2002). In brief, 10 g of shrimp sample was homogenized with 90 ml of 0.85% physiological saline. Serial decimal dilutions of 10^{-2}, 10^{-3}, 10^{-4} etc were prepared using 9 ml physiological saline. 0.1 ml of inoculum was spread plated on Baird parker agar

(BPA) plates using a sterile glass spreader. The petriplates were incubated at 37°C for 48 hours. *Staphylococcus aureus* colonies were black, convex 1-1.5 mm in diameter with narrow white entire margin and surrounded by a mark of clearing 2-5 mm in width. The plates containing 25-250 colonies were counted. The results were expressed as number of colony forming units per gram by using the following formula:

Count per gram = Number of colonies counted × reciprocal of dilution × reciprocal of dilution volume plated

The colonies were purified onto Tryptose soya agar (TSA) plates and further subjected to following biochemical tests by the standard methods given by Khanna (2002).

1. Coagulase test by slide technique
2. Coagulase test by tube technique

3.2.1.3. *Escherichia coli* count

E. coli count was done by following the method given by Varma, (2002). In brief, 10 g of shrimp sample was homogenized with 90 ml of 0.85% physiological saline. Serial decimal dilutions of 10^{-2}, 10^{-3}, 10^{-4} etc were prepared using 9 ml physiological saline. 0.1 ml of inoculum was spread plated on Tergitol-7 (T-7) agar plates using a sterile glass spreader. The petriplates were incubated at 37°C for 48 hours. *E. coli* colonies on T-7 agar plates appeared as circular, non-mucoid, flat, and yellow with pinkish tinge. The plates containing 25-250 colonies were counted. The results were expressed as number of colony forming units per gram by using the following formula:

Count per gram = Number of colonies counted × reciprocal of dilution ×reciprocal of dilution volume plated.

The colonies were purified onto Tryptose soya agar (TSA) plates and further subjected to following biochemical tests by the standard method given by Khanna (2002).

1. Gram staining
2. Eijkman test
3. Indole test

4. Methyl red (MR)
5. Voges Proskaure (VP) tests
6. Cytrate utilization test

3.2.1.4. *Salmonella* count

Salmonella sp. count was done by following the method described by Manik (1992) with some modification. In brief, 10 g of shrimp muscle was homogenized with 90 ml of 0.85% physiological saline. Serial decimal dilutions of 10^{-2}, 10^{-3}, 10^{-4} etc were prepared using 9 ml physiological saline. 0.1 ml of inoculum was spread plated on Xylose lysine deoxycholate (XLD) agar plates, Bismuth sulphite agar (BSA) and Brilliant green agar (BGA) plates by using a sterile glass spreader. The petriplates were incubated at 37°C for 48 hours. The *Salmonella* colonies on XLD agar plates were pink or red with or without black centre. On BSA plates colonies were brown grey or black, sometimes with a metallic sheen. The surrounding medium was usually brown at first turning black with increasing incubation time. On BGA plates colonies were colourless pink to fuchsia, translucent to opaque with surrounding medium pink to red, some *Salmonella* were appeared as transparent green colonies. The colonies containing the mentioned peculiar characteristics in respective plates were counted and results were expressed as number of colony forming units per gram by using following formula:

Count per gram = Number of colonies counted × reciprocal of dilution × reciprocal of dilution volume plated

3.2.1.5. *Vibrio* count

Vibrio count was done by following the method described by Manik (1992) with some modification. In brief, 10 g of shrimp sample was homogenized with 90 ml of 0.85% physiological saline. Serial decimal dilutions of 10^{-2}, 10^{-3}, 10^{-4} etc were prepared using 9 ml physiological saline. 0.1 ml of inoculum was spread plated onto Thiosulphate citrate bilesalt sucrose (TCBS) agar plates using a sterile glass spreader. The plates were incubated at 37°C for 48 hours. The *Vibrio cholarae* colonies appeared yellow and *V.parahaemolyticus* colonies appeared green on TCBS agar. The colonies were counted and results were expressed as number of colony forming units per gram by using the following formula:

Count per gram = Number of colonies counted × reciprocal of dilution × reciprocal of dilution volume plated.

3.2.2. Biochemical assessment

3.2.2.1. Estimation of total volatile basic nitrogen (TVB-N)

TVB-N concentration was estimated by using the method given by Conway (1947). In brief, Conway cups and lids were washed and dried. Paraffin wax and vaseline in the ratio of 1:2 was melted and cooled. This was applied on the rims of cups. 1 ml of 0.02(N) H_2SO_4 was added into the inner chamber of each cup. Lid was placed over the Conway cup covering the part of outer chamber and complete inner chamber. 1 ml of TCA extract was taken in the outer chamber followed by 1 ml of K_2CO_3 solution. The unit was lidded and the contents were mixed by rotating the unit gently and then the unit was left overnight for reaction. The excess acid left in inner chamber was titrated against 0.02(N) NaOH using a drop of Toshiro's indicator. A reagent blank was done simultaneously. Total TVBN content was calculated with the formula given bellow:

1 ml of 0.02(N) H_2SO_4 = 0.28 mg of TVB-nitrogen

3.2.2.2. Estimation of Trimethylamine (TMA)

TMA concentration was estimated by using the method given by Conway (1947). In brief, Conway cups and lids were washed and dried. Paraffin wax and vaseline in the ratio of 1:2 was melted and cooled. This was applied on the rims of cups. 1 ml of 0.02 H_2SO_4 was added into the inner chamber of each cup. Lid was placed over the Conway cup covering the part of outer chamber and complete inner chamber. 1 ml of TCA extract was taken in the outer chamber followed by 0.5 ml of HCHO. The unit is rotated to ensure mixing and then 1 ml of K_2CO_3 is added to outer chamber. The unit was lidded and the contents were mixed by rotating the unit gently and then the unit was left overnight for reaction. The excess acid left in inner chamber was titrated against 0.02(N) NaOH using a drop of Toshiro's indicator. A reagent blank was done simultaneously. Total TMA content was calculated with the formula given bellow:

1 ml of 0.02 H_2SO_4 = 0.28 mg of TMA nitrogen

3.2.2.3. Determination of pH

pH value was determined by following the method given by AOAC, (1995). In brief, 10 g sample of the fish flesh blended well with 10 ml of distilled water was taken. The pH was measured by using a digital pH meter (Metler Toledo). The pH meter was calibrated to 4.0 and 7.0 before the measurement.

3.2.3. Proximate composition of black tiger shrimp

3.2.3.1. Estimation of moisture content

The estimation of moisture content was done by following the method given by AOAC, (1995). In brief, 5 g of shrimp sample was weighed accurately in a pre weighed aluminium dish. The dish containing the sample was placed in a hot air oven without lid. The temperature of the oven was fixed at 100±5°C and the samples were kept overnight (16 hours) for drying. The dish was taken out from the oven and cooled in dessicators at room temperature. Total moisture content was estimated with the formula given bellow:

$$\text{Moisture (by \% weight)} = \frac{100\,(W_1-W_2)}{W_1-W}$$

W = weight of the empty dish in grams.

W_1= weight of the dish with the material before drying in grams.

W_2= weight of the dish with the material after drying in grams.

3.2.3.2. Estimation of ash content

Estimation of ash content was done by following the method given by AOAC, (1995). In brief, 5 g of sample was weighed accurately in a porcelain crucible and dried in a hot air oven overnight at temperature of 100±5°C. Sample was char dried completely by heating over a burner and incinerated in a muffle furnace at a temperature of 550±50°C with adequate air supply until it became completely white. After that the crucible was taken out and cooled in a desiccator at room temperature. Total ash content was estimated using the formula given bellow:

$$\text{Total Ash (\% by weight)} = \frac{W_2-W}{W_1-W} \times 100$$

W= weight in grams of empty dish.

W_1= weight in grams of dish with the material before incinerating.

W_2= weight in grams the dish with the material after incinerating.

3.2.3.3. Estimation of protein content

Estimation of protein content of the shrimp sample was done by using Kjeldahl method (AOAC, 1995). In brief 1 g sample weighed in a dry Kjeldahl flask and about 5 g of digestion mixture and 20 ml of concentrated H_2SO_4 was added to the digestion flask. Few glass beads were also added to avoid bumping. Mixtures was digested by heating, first slowly and then vigorously for 4-6 hours until the sample became clear and colourless to ensure complete conversion of nitrogen in the sample to ammonium sulphate. The flask was then cooled and the volume was made up to 250 ml by distilled water. 10 ml of 2% boric acid with 2-3 drops of Toshiro's indicator was taken in a 100 ml conical flask and placed in such a way that the tip of the outlet of the condenser of distillation unit remained dipped into the boric acid solution. 5 ml of the digested sample was added to the previously cleaned the distillation chamber and about 10 ml of 40% NaOH was added followed by rinsing with distilled water. The steam distillation continued till about 30 ml distillate was collected (7-8 min) in the receiving flask. At the end the receiving flask lowered and hold for about 1 min at about 1 cm below the tip of the condenser. The condenser outlet washed into the receiving flask with distilled water. The boric acid turned green when ammonia was absorbed. A blank was also done through all the steps by taking distilled water in place of sample. The collected distillate was titrated against 0.02(N) H_2SO_4 until the original red colour was obtained. Total protein content was estimated with the formula given below:

1 ml of 0.02 (N) H_2SO_4 = 0.00028 g of nitrogen

$$\% \text{ Total nitrogen} = \frac{0.00028 \times \text{titre value} \times 250 \times 100}{5 \times \text{weight of sample}}$$

1 g nitrogen = 100/16 = 6.25 (conversion factor)

Hence;

% Protein = % nitrogen × 6.25 (conversion factor)

3.2.3.4. Estimation of total fat content

The fat content was estimated by Soxhlet method (AOAC, 1995). In brief, 5 g of shrimp sample was weighed in a thimble and plugged with cotton. The thimble was then fixed in to the apparatus. The fat was extracted with anhydrous ether (BP 40-60°C) for about 16 hours. After the extraction, the thimble was removed and the solvent from the receiving flask was collected out by distilling it off, before it returned into the flask by siphoning. Thus, maximum possible amount of solvent was restored. Finally, the traces of the solvent were removed from the flask by overnight drying it in oven at 100°C. After cooling the flask in the desiccators, the weight and the value was recorded. Final fat content estimated by using the formula given bellow:

$$\text{Fat content (g per 100g of sample)} = \frac{\text{Weight of fat in the sample}}{\text{Original weight of the sample}} \times 100$$

3.2.4. Sensory analysis

Sensory analysis was done by following the score sheet given by Ali *et al.* (2008). Sensory studies were carried out by expert panel of 5 judges. The judges were requested to give the scores through filling up of a standard questionnaire that include odour, carapace colour, carapace texture, eye and shell colour characteristic.

3.2.5. Statistical analysis

Statistical analysis of data was performed by using Microsoft Excel 2007. Graphs were also made in Microsoft Excel 2007. Results were presented as mean ± standard error. Comparisons of mean values were determined by t-test. A probability level of 0.05 was used to find out the significance in all cases.

CHAPTER IV

EXPERIMENTAL RESULTS

4. EXPERIMENTAL RESULTS

Shrimp samples used in the present study were analyzed for microbiological, biochemical, proximate and sensory characteristics. The maximum and the minimum weight of the traditional and scientific cultured shrimp were 25±5 to 35±5g and 35±5 to 45±5g respectively with an average value of 30g. The results of all these analyses are given in the following sub-sections.

4.1. Microbiological assessment

Microbiological analysis carried out in this study included Total plate count (TPC) and quantitative enumeration of *Staphylococcus aureus, Escherichia coli, Salmonella* and *Vibrio* spp.

4.1.1. Total plate count (TPC)

The total plate count of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 6.23±0.05 to 7.13±0.08, 4.67±0.11 to 5.12±0.11 respectively (Table 2 & Fig 1). The TPC of TC sample was higher in crop III samples than crop I and II. The statistical analysis (Table 3) revealed that there was significantly ($p<0.05$) higher count in TC than SC.

Table 2: Total plate count of raw black tiger shrimp

Sampling time	Sampling nos.	TC	SC
Crop I	1	6.52±0.04	5.12±0.11
	2	6.23±0.05	4.91±0.05
	3	6.32±0.03	4.93±0.08
	4	6.35±0.03	4.98±0.21
Crop II	1	6.79±0.11	4.95±0.08
	2	6.51±0.05	4.75±0.06
	3	6.58±0.08	4.67±0.11
	4	6.67±0.06	4.77±0.44
Crop III	1	6.88±0.11	4.97±0.04
	2	6.41±0.05	4.77±0.05
	3	7.06±0.21	4.69±0.03
	4	7.13±0.08	5.08±0.22

Fig 1: Total plate count (log cfu/g) in raw black tiger shrimp

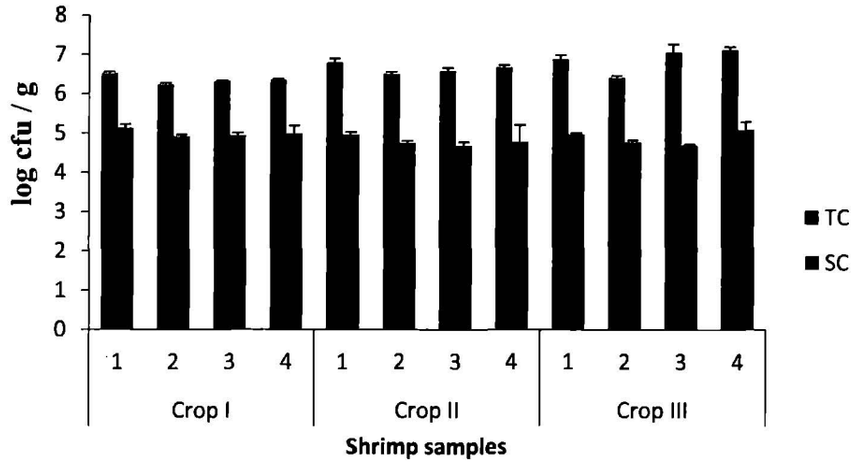


Table 3: t-Test: Two-sample assuming equal variances

	TC	SC
Mean	6.620833333	4.8825
Variance	0.085044697	0.0222932
Observations	12	12
Pooled Variance	0.053668939	
Hypothesized Mean Difference	0	
df	22	
t Stat	18.38007067	
P(T<=t) one-tail	3.86289E-15	
t Critical one-tail	1.717144335	
P(T<=t) two-tail	7.72577E-15	
t Critical two-tail	2.073873058	

4.1.2. *Vibrio* spp. count

The *Vibrio* spp. count of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 4.52±0.18 to 6.65±0.18, 3.37±0.14 to 3.88±0.15 respectively (Table 4 & Fig 2). The statistical analysis (Table 5) revealed that there was significantly ($p<0.05$) higher count in TC than SC. The *Vibrio* spp. count of both TC and SC sample was higher in crop III samples than crop I and II.

Table 4: Total *Vibrio* spp. count of raw black tiger shrimp

Sampling time	Sampling nos.	TC	SC
Crop I	1	4.78±0.12	3.78±0.08
	2	4.52±0.18	3.40±0.05
	3	4.73±0.08	3.54±0.12
	4	4.61±0.10	3.37±0.14
Crop II	1	5.16±0.12	3.70±0.22
	2	5.14±0.14	3.69±0.12
	3	4.97±0.22	3.51±0.14
	4	5.09±0.22	3.60±0.05
Crop III	1	6.65±0.18	3.83±0.12
	2	6.20±0.10	3.70±0.08
	3	6.42±0.05	3.88±0.15
	4	6.25±0.12	3.78±0.12

Fig 2: Abundance (log cfu/g) of *Vibrio* spp.

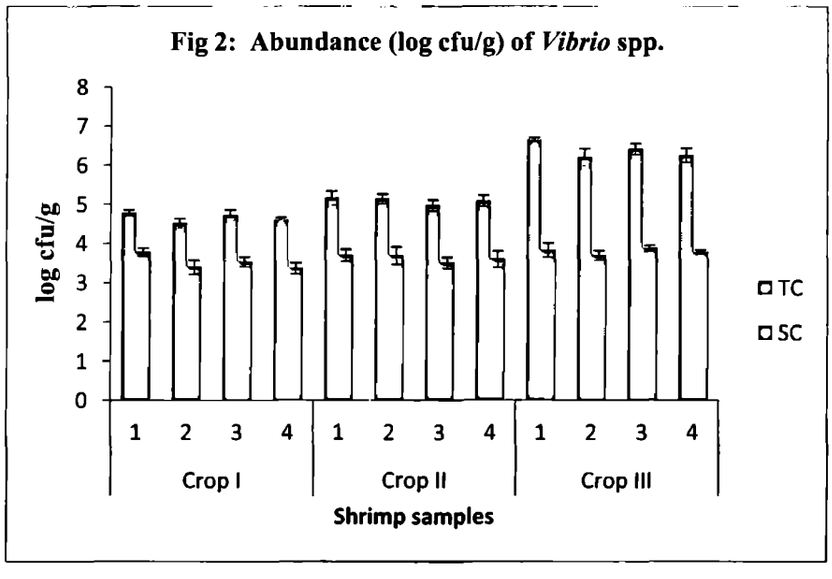


Table 5: Total *Vibrio* spp. count: t-Test: Two-sample assuming equal variances

	TC	SC
Mean	5.3766667	3.6483333
Variance	0.5997152	0.0273424
Observations	12	12
Pooled Variance	0.3135288	
Hypothesized Mean Difference	0	
df	22	
t Stat	7.560742	
P(T<=t) one-tail	7.439E-08	
t Critical one-tail	1.7171443	
P(T<=t) two-tail	1.488E-07	
t Critical two-tail	2.0738731	

4.1.3. *Escherichia coli* count

The *E. coli* count of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 4.27±0.18 to 5.49±0.08, 3.56±0.14 to 4.31±0.08 respectively (Table 6 & Fig 3). The statistical analysis (Table 7) revealed that there was significantly ($p<0.05$) higher count in TC than SC. The *E. coli* count of both TC and SC sample was higher in crop III samples.

Table 6: Total *E. coli* count of raw black tiger shrimp

Sampling time	Sampling nos.	TC	SC
Crop I	1	4.45±0.14	3.90±0.08
	2	4.27±0.18	3.72±0.12
	3	4.32±0.12	3.76±0.14
	4	4.54±0.14	3.84±0.05
Crop II	1	4.96±0.12	3.84±0.18
	2	4.88±0.14	3.65±0.12
	3	4.54±0.08	3.56±0.14
	4	5.04±0.14	4.04±0.05
Crop III	1	5.40±0.22	4.28±0.14
	2	5.32±0.05	4.31±0.08
	3	5.42±0.12	4.15±0.14
	4	5.49±0.08	4.23±0.22

Fig 3: Abundance (log cfu/g) of *E. coli*

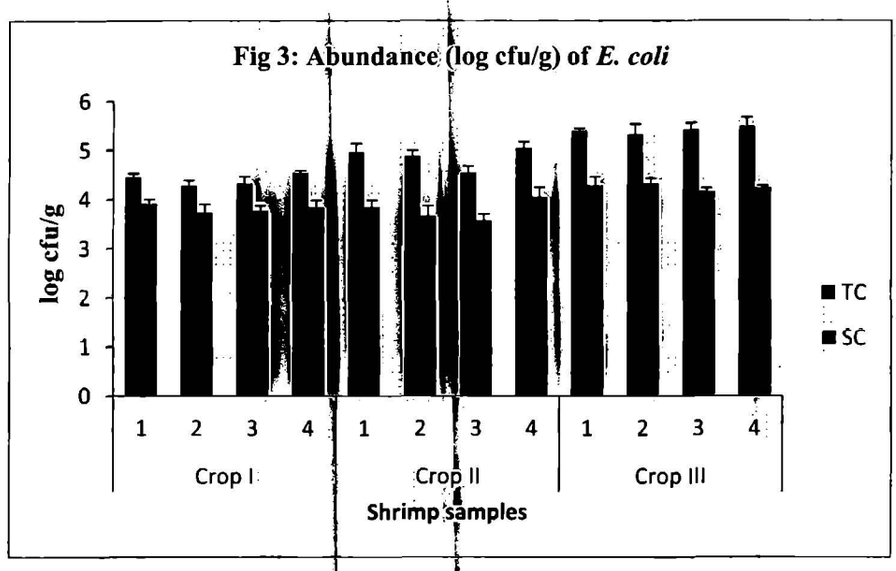


Table 7: Total *Escherichia coli* count: t-Test: Two-sample assuming equal variances

	TC	SC
Mean	4.8858333	3.94
Variance	0.2055538	0.0656
Observations	12	12
Pooled Variance	0.1355769	
Hypothesized Mean Difference	0	
df	22	
t Stat	6.2921259	
P(T<=t) one-tail	1.239E-06	
t Critical one-tail	1.7171443	
P(T<=t) two-tail	2.478E-06	
t Critical two-tail	2.0738731	

4.1.4. *Staphylococcus aureus* count

The *Staphylococcus aureus* count of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 3.76±0.11 to 5.04±0.05, 3.39±0.08 to 4.42±0.15 respectively (Table 8 & Fig 4). It is observed that the count of *Staphylococcus aureus* of TC was higher than SC. The statistical analysis (Table 9) revealed that there was significantly ($p<0.05$) higher count in TC than SC.

Table 8: Total *Staphylococcus aureus* count of raw black tiger shrimp

Sampling time	Sampling nos.	TC	SC
Crop I	1	4.04±0.08	4.30±0.10
	2	3.76±0.11	4.18±0.18
	3	3.83±0.14	3.69±0.12
	4	4.27±0.04	4.13±0.14
Crop II	1	4.58±0.16	4.42±0.15
	2	4.31±0.12	3.69±0.22
	3	4.63±0.14	3.68±0.14
	4	4.98±0.14	3.81±0.21
Crop III	1	5.04±0.05	3.77±0.18
	2	4.98±0.22	3.72±0.12
	3	4.89±0.14	3.39±0.08
	4	4.48±0.18	3.57±0.05

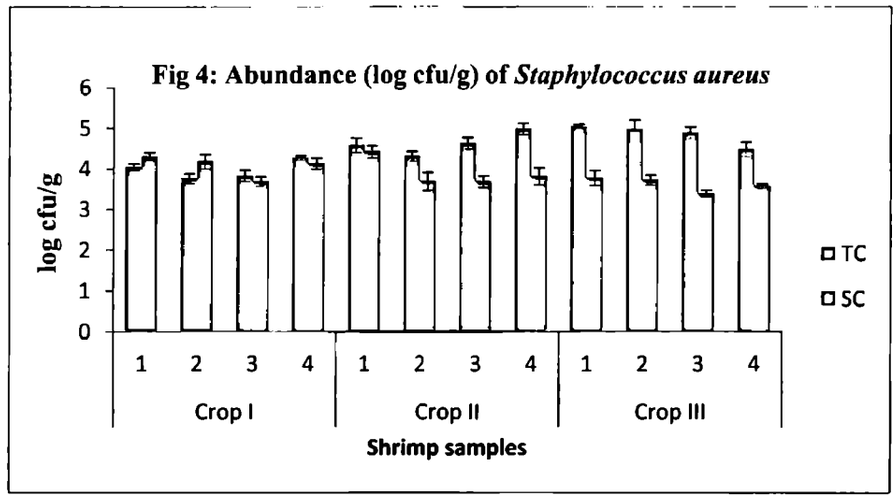


Fig 4: Abundance (log cfu/g) of *Staphylococcus aureus*

Table 9: *Staphylococcus aureus* count: t-Test: Two-sample assuming equal variances

	TC	SC
Mean	4.4825	3.8625
Variance	0.2019659	0.1007114
Observations	12	12
Pooled Variance	0.1513386	
Hypothesized Mean Difference	0	
df	22	
t Stat	3.9038436	
P(T<=t) one-tail	0.0003811	
t Critical one-tail	1.7171443	
P(T<=t) two-tail	0.0007623	
t Critical two-tail	2.0738731	

4.1.5. *Salmonella* count

The *Salmonella* was absent in both traditional and scientific cultured shrimp.

Table 11: TVB-N content: t-Test: Two-sample assuming equal variances

	TC	SC
Mean	1.72	1.3341667
Variance	0.010690909	0.0016992
Observations	12	12
Pooled Variance	0.006195076	
Hypothesized Mean Difference	0	
df	22	
t Stat	12.00748518	
P(T<=t) one-tail	1.96414E-11	
t Critical one-tail	1.717144335	
P(T<=t) two-tail	3.92827E-11	
t Critical two-tail	2.073873058	

4.2.2. Trymethylamine (TMA-N)

The TMA-N value of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 0.94±0.05 to 1.17±0.03, 0.92±0.03 to 1.00±0.04 respectively (Table 12 & Fig 6). The statistical analysis (Table 13) revealed that there was significantly ($p<0.05$) higher value in TC than SC.

Table 12: TMA-N contents (mgN/100g) in black tiger shrimp

Sampling time	Sampling nos.	TC	SC
Crop I	1	1.00±0.02	0.96±0.03
	2	0.94±0.05	0.92±0.01
	3	0.98±0.02	0.97±0.02
	4	1.05±0.01	0.98±0.05
Crop II	1	1.04±0.02	1.00±0.04
	2	1.01±0.02	0.92±0.03
	3	1.00±0.05	0.91±0.08
	4	1.02±0.04	0.93±0.06
Crop III	1	1.17±0.03	0.97±0.01
	2	1.08±0.01	0.93±0.02
	3	0.96±0.05	0.92±0.05
	4	1.06±0.01	0.99±0.02

Fig 6: TMA-N content (mgN/100g) in black tiger shrimp

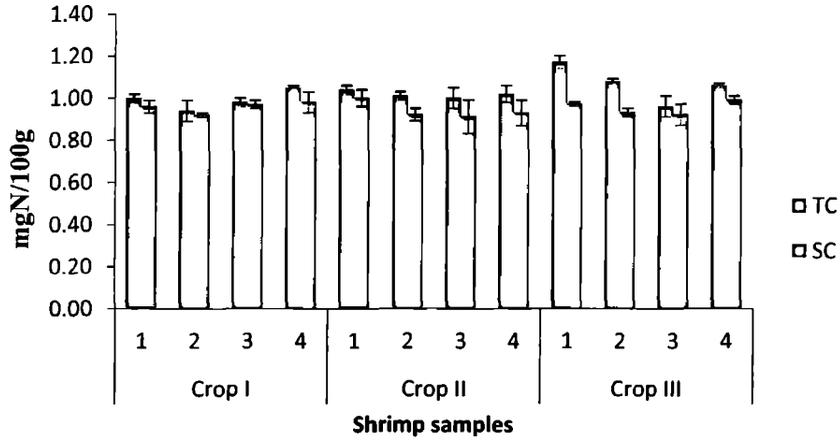


Table 13: TMA-N content: t-Test: Two-sample assuming equal variances

	TC	SC
Mean	1.025833333	0.95
Variance	0.003735606	0.001
Observations	12	12
Pooled Variance	0.002367803	
Hypothesized Mean Difference	0	
df	22	
t Stat	3.817358831	
P(T<=t) one-tail	0.000470304	
t Critical one-tail	1.717144335	
P(T<=t) two-tail	0.000940609	
t Critical two-tail	2.073873058	

4.2.3. pH

The pH value of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 7.02±0.04 to 7.25±0.04, 6.95±0.06 to 7.12±0.04 respectively (Table 14 & Fig 7). The statistical analysis (Table 15) revealed that there was significantly ($p<0.05$) higher value in TC than SC.

Table 14: pH value of black tiger shrimp

Sampling time	Sampling nos.	TC	SC
Crop I	1	7.02±0.04	7.05±0.04
	2	7.12±0.03	7.01±0.04
	3	7.15±0.06	7.03±0.03
	4	7.07±0.05	6.98±0.02
Crop II	1	7.17±0.05	7.12±0.04
	2	7.23±0.04	7.07±0.05
	3	7.21±0.02	7.08±0.01
	4	7.25±0.04	7.11±0.02
Crop III	1	7.21±0.04	7.10±0.05
	2	7.19±0.05	7.04±0.05
	3	7.13±0.06	7.03±0.04
	4	7.10±0.03	6.95±0.06

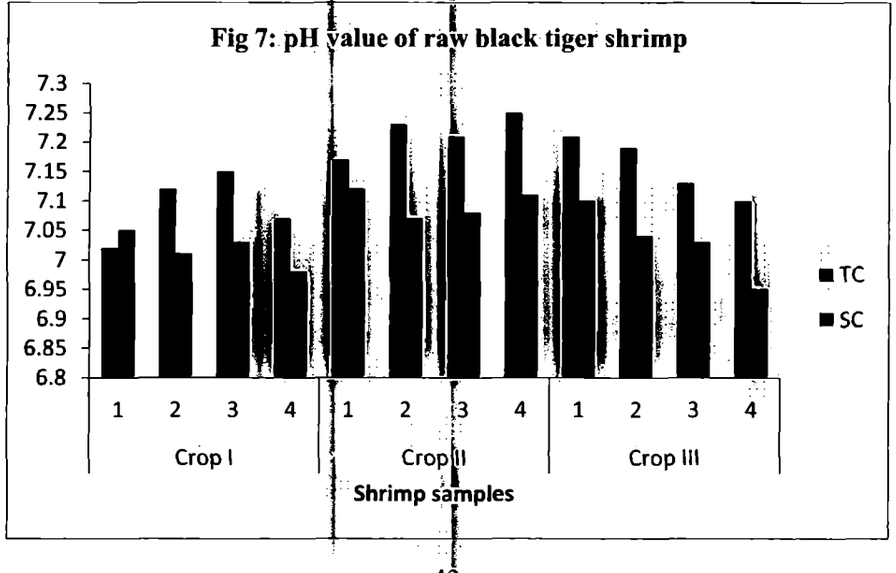


Fig 7: pH value of raw black tiger shrimp

Table 15: pH value: t-Test: Two-sample assuming equal variances

	TC	SC
Mean	7.154167	7.0475
Variance	0.004772	0.002693
Observations	12	12
Pooled Variance	0.003733	
Hypothesized Mean Difference	0	
df	22	
t Stat	4.276614	
P(T<=t) one-tail	0.000154	
t Critical one-tail	1.717144	
P(T<=t) two-tail	0.000307	
t Critical two-tail	2.073873	

4.3. Proximate composition of shrimp

4.3.1. Moisture content

The moisture content value of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 70.27±1.08 to 71.98±1.46, 70.92±0.93 to 72.19±1.23 respectively (Table 16 & Fig 8). The statistical analysis (Table 17) revealed that there was no significant ($p>0.05$) difference between TC and SC.

Table 16: Moisture content (%) in black tiger shrimp

Sampling time	Sampling nos.	TC	SC
Crop I	1	71.05±2.08	72.01±2.13
	2	70.27±1.08	71.05±2.02
	3	71.15±2.14	72.04±1.78
	4	71.56±2.01	71.89±1.27
Crop II	1	71.32±1.10	71.78±1.65
	2	70.98±0.98	70.98±0.98
	3	71.97±0.88	71.08±1.43
	4	71.98±1.46	72.10±2.13
Crop III	1	72.05±1.76	71.45±1.92
	2	71.36±1.20	70.92±0.93
	3	70.64±0.76	72.19±1.23
	4	72.19±2.02	71.78±1.34

Fig 8: Moisture content of of raw black tiger shrimp

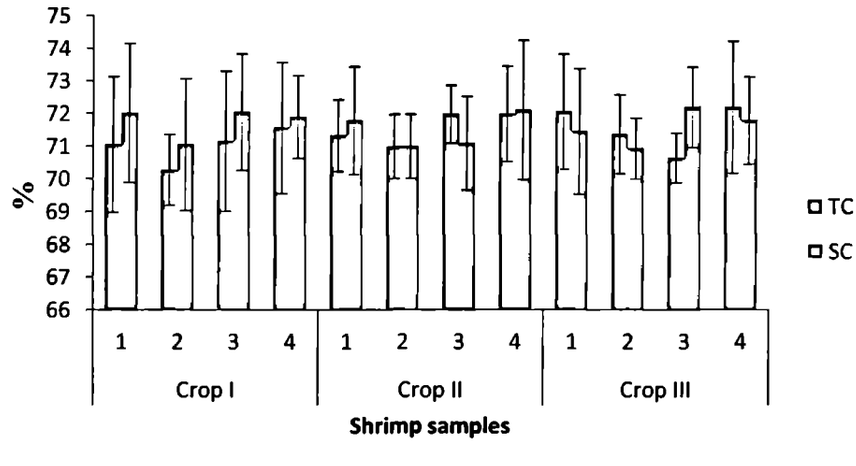


Table 17: Moisture content: t-Test: Two-sample assuming equal variances

	TC	SC
Mean	71.37667	71.60583
Variance	0.35917	0.231863
Observations	12	12
Pooled Variance	0.295516	
Hypothesized Mean Difference	0	
df	22	
t Stat	-1.03261	
P(T<=t) one-tail	0.156503	
t Critical one-tail	1.717144	
P(T<=t) two-tail	0.313005	
t Critical two-tail	2.073873	

4.3.2. Ash content

The ash content of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 0.61±0.02 to 1.04±0.02, 0.67±0.04 to 0.93±0.67 respectively (Table 18 & Fig 9). The statistical analysis (Table 19) revealed that there was no significant ($p>0.05$) difference between TC and SC.

Table 18: Ash content (%) in black tiger shrimp

Sampling time	Sampling nos.	TC	SC
Crop I	1	1.04±0.02	0.93±0.03
	2	1.02±0.04	0.71±0.04
	3	0.95±0.05	0.67±0.04
	4	0.72±0.02	0.93±0.01
Crop II	1	0.84±0.03	0.84±0.03
	2	0.62±0.04	0.78±0.04
	3	0.74±0.07	0.76±0.05
	4	0.68±0.02	0.91±0.02
Crop III	1	0.92±0.05	0.86±0.06
	2	0.61±0.05	0.78±0.04
	3	0.80±0.04	0.89±0.02
	4	0.67±0.03	0.73±0.03

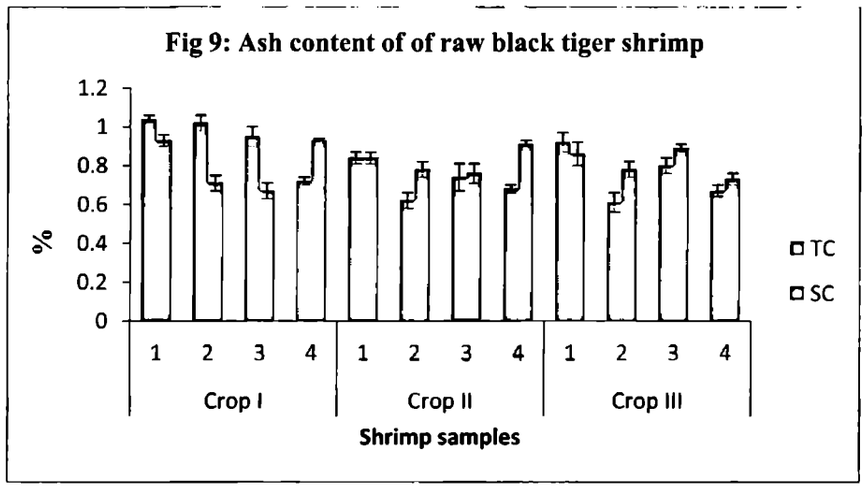


Table 19: Ash content: t-Test: Two-sample assuming equal variances

	TC	SC
Mean	0.800833	0.815833
Variance	0.023117	0.008045
Observations	12	12
Pooled Variance	0.015581	
Hypothesized Mean Difference	0	
df	22	
t Stat	-0.29435	
P(T<=t) one-tail	0.385624	
t Critical one-tail	1.717144	
P(T<=t) two-tail	0.771248	
t Critical two-tail	2.073873	

4.3.3. Protein content

The protein content of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 23.0±0.08 to 24.47±0.06, 23.12±0.04 to 24.34±0.01 respectively (Table 20 & Fig 10). The statistical analysis (Table 21) revealed that there was no significant ($p>0.05$) difference between TC and SC.

Table 20: Protein content (%) in black tiger shrimp

Sampling time	Sampling nos.	TC	SC
Crop I	1	24.20±0.02	23.12±0.04
	2	23.42±0.03	24.02±0.03
	3	23.10±0.08	23.92±0.01
	4	23.56±0.08	24.01±0.02
Crop II	1	24.36±0.07	23.78±0.03
	2	24.15±0.09	24.03±0.02
	3	23.58±0.10	23.42±0.04
	4	23.93±0.12	23.56±0.05
Crop III	1	24.12±0.02	24.01±0.02
	2	24.47±0.06	24.34±0.01
	3	24.38±0.07	23.51±0.03
	4	23.91±0.05	23.92±0.02

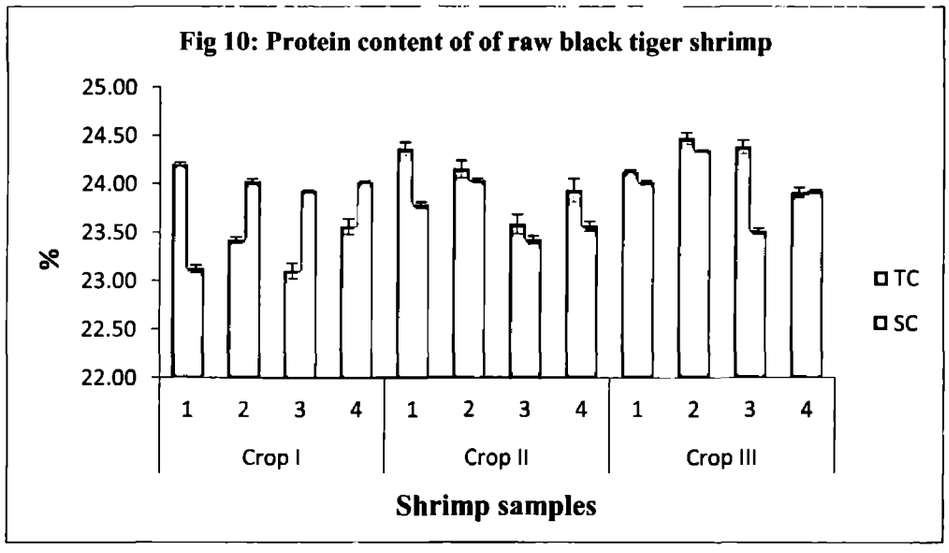


Table 21: Protein content: t-Test: Two-sample assuming equal variances

	TC	SC
Mean	23.93148	23.80333
Variance	0.186075	0.114424
Observations	12	12
Pooled Variance	0.150249	
Hypothesized Mean Difference	0	
df	22	
t Stat	0.809808	
P(T<=t) one-tail	0.213362	
t Critical one-tail	1.717144	
P(T<=t) two-tail	0.426723	
t Critical two-tail	2.073873	

4.3.4. Fat content

The fat content of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 0.52±0.01 to 1.82±0.04, 0.92±0.03 to 1.32±0.02 respectively (Table 22 & Fig 11). The statistical analysis (Table 23) revealed that there was no significant ($p>0.05$) difference between TC and SC. It is observed that the fat content of TC was very low during crop II season.

Table 22: Fat content (%) in black tiger shrimp

Sampling time	Sampling nos.	TC	SC
Crop I	1	0.82±0.03	0.92±0.03
	2	1.82±0.04	1.12±0.03
	3	1.03±0.03	1.10±0.02
	4	0.98±0.02	1.01±0.04
Crop II	1	0.66±0.05	0.98±0.05
	2	0.82±0.02	1.32±0.02
	3	0.77±0.04	1.03±0.01
	4	0.52±0.01	0.92±0.04
Crop III	1	0.71±0.05	0.94±0.05
	2	1.20±0.04	1.23±0.03
	3	1.87±0.04	1.10±0.04
	4	1.28±0.03	0.97±0.02

Fig 11. Fat content value of raw black tiger shrimp

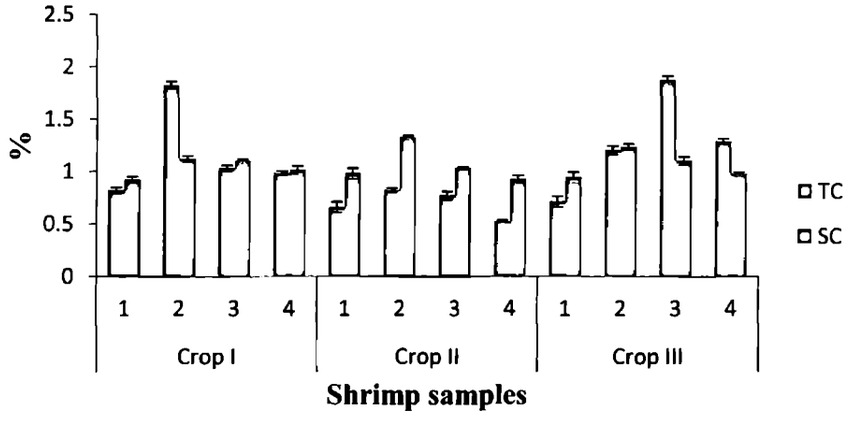


Table 23: Fat content: t-Test: Two-sample assuming equal variances

	TC	SC
Mean	1.04	1.053333
Variance	0.188873	0.015842
Observations	12	12
Pooled Variance	0.102358	
Hypothesized Mean Difference	0	
df	22	
t Stat	-0.10208	
P(T<=t) one-tail	0.459808	
t Critical one-tail	1.717144	
P(T<=t) two-tail	0.919615	
t Critical two-tail	2.073873	

4.3. Sensory analysis

The quality attributes of TC and SC samples were assessed on the basis of organoleptic characteristics given in Appendix I. The characteristics are general odour, carapace colour, carapace texture, eye and shell colour. The scores of different sensory parameters obtained by TC were in the range of 4.8±0.45 to 7.0±0.71 whereas in case of SC, the range was 8.2±0.45 to 9.8±0.45 out of total score of 10 for each parameter (Table 24). The statistical analysis (Table 25) revealed that there was significant ($p<0.05$) difference between TC and SC.

Table 24: Sensory scores for raw black tiger shrimp

Sampling	Sampling nos.	Odor		Color		Texture		Eye		Shell color		OA	
		TC	SC	TC	SC	TC	SC	TC	SC	TC	SC	TC	SC
Crop I	1	7	10	4	10	7	10	7	9	7	10	6.4±1.32	9.8±0.45
	2	8	9	6	10	7	9	7	9	7	9	7.0±0.71	9.2±0.45
	3	9	8	6	8	7	9	5	9	7	9	6.8±1.48	8.6±0.55
	4	5	8	4	8	5	9	5	8	5	9	4.8±0.45	8.4±0.55
Crop II	1	5	9	6	8	5	9	5	8	5	8	5.2±0.45	8.4±0.55
	2	8	9	4	8	7	8	5	8	7	8	6.2±1.64	8.2±0.45
	3	5	9	6	8	7	10	7	8	7	9	6.4±0.89	8.8±0.84
	4	5	10	8	8	7	10	5	8	5	10	6.0±1.41	9.2±1.09
Crop III	1	7	8	6	10	5	9	5	9	7	9	6.0±1.00	9.0±0.71
	2	5	9	6	10	5	9	7	9	5	9	5.6±0.89	9.2±0.45
	3	7	9	8	10	5	8	7	9	7	9	6.8±1.09	9.0±0.71
	4	5	8	6	8	7	8	5	9	7	8	6.0±1.00	8.2±0.45

*Results are mean of five determinations with s.d.

Fig 12: Odour scores for raw black tiger shrimp

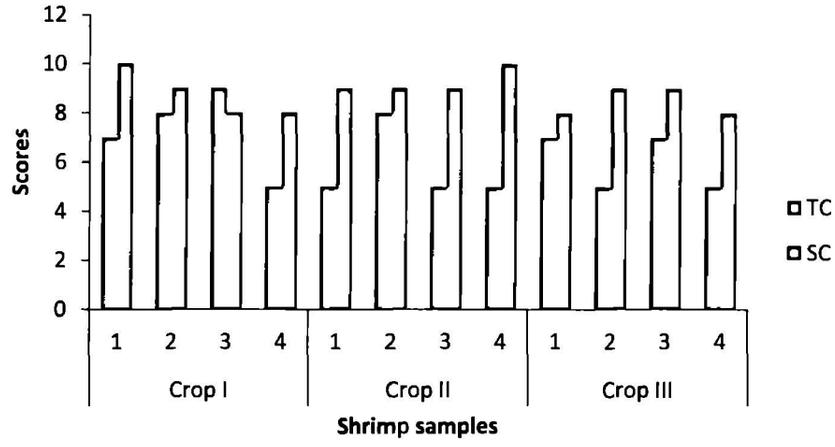


Fig 13: Color scores for raw black tiger shrimp

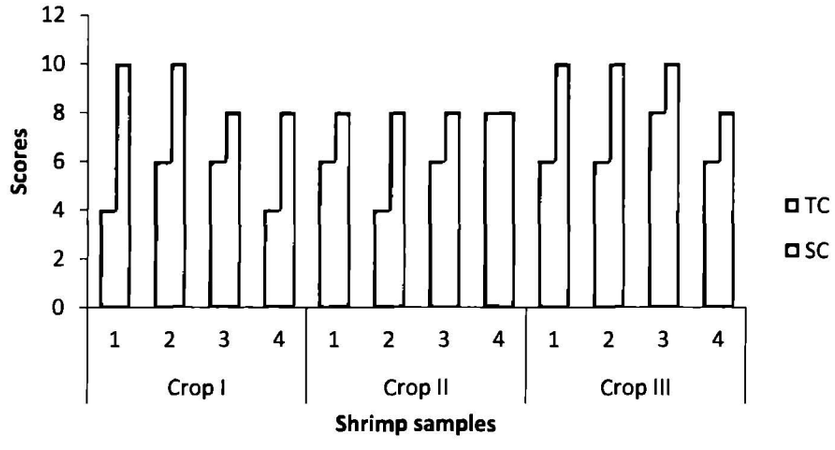
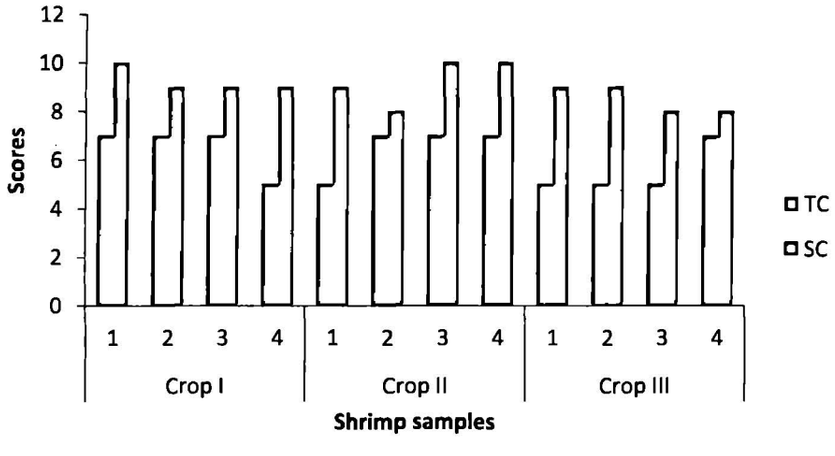


Fig 14: Texture scores for raw black tiger shrimp



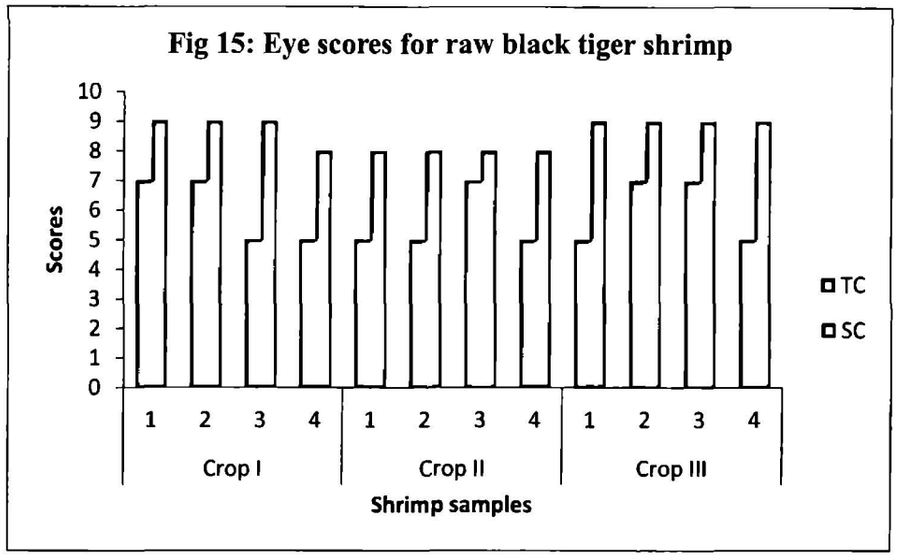


Fig 15: Eye scores for raw black tiger shrimp
Scores
10
9
8
7
6
5
4
3
2
1
0
1 2 3 4 1 2 3 4 1 2 3 4
Crop I
Crop II
Crop III
Shrimp samples
TC
SC

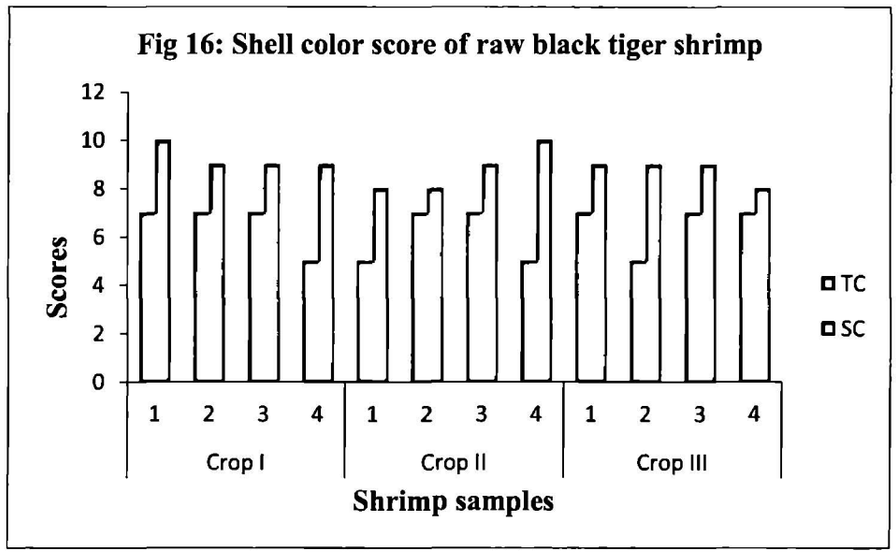


Fig 16: Shell color score of raw black tiger shrimp
Scores
12
10
8
6
4
2
0
1 2 3 4 1 2 3 4 1 2 3 4
Crop I
Crop II
Crop III
Shrimp samples
TC
SC

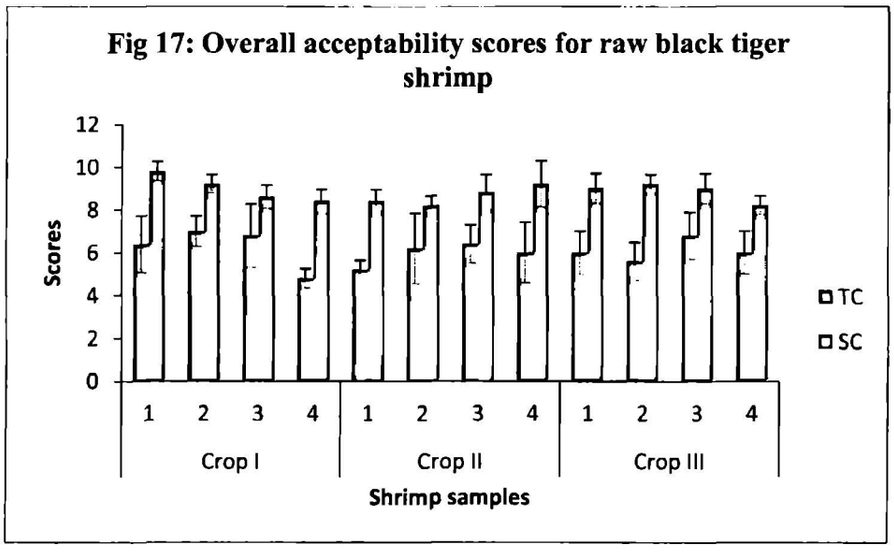


Fig 17: Overall acceptability scores for raw black tiger shrimp
Scores
12
10
8
6
4
2
0
1 2 3 4 1 2 3 4 1 2 3 4
Crop I
Crop II
Crop III
Shrimp samples
TC
SC

Table 25: Sensory analysis: t-Test: Two-sample assuming equal variances

	TC	SC
Mean	6.1	8.833333
Variance	0.432727	0.238788
Observations	12	12
Pooled Variance	0.335758	
Hypothesized Mean Difference	0	
df	22	
t Stat	11.5546	
P(T<=t) one-tail	4.1E-11	
t Critical one-tail	1.717144	
P(T<=t) two-tail	8.19E-11	
t Critical two-tail	2.073873	

CHAPTER V

DISCUSSION

5. DISCUSSION

The microbiological, biochemical and sensory quality of iced black tiger shrimp (*Penaeus monodon*) brought from traditional cultured shrimp (TC) was assessed and compared with scientific cultured shrimp (SC).

5.1. Microbiological assessment

Bacterial counts were valuable as a measure of degree of freshness of fish. For the bacterial spoilage, the most common used method is the standard plate count (Rahman, 1980). The acceptable limit of SPC is 10^6cfu/g (ICMSF, 1988). Counts reported from the tropical countries also ranged from 10^3 to 10^6cfu/g (Varma *et al.* 1982). A report by De Silva (1985), however, indicated counts as high as 10^8cfu/g. The presence of microorganisms in higher number is an indicative of contamination and unhygienic handling. Similarly, the presence of certain species of bacteria like *Escherichia coli* and *Staphylococcus* sp. indicates contamination by faecal matter and poor personnel hygiene respectively.

5.1.1 Total plate count (TPC)

The total plate count of TC is significantly ($p<0.05$) higher than SC (Table 3 & Fig 1). Total plate counts were found to be in the range of 10^6 to 10^7 in all the shrimps brought from traditional culture system. In case of scientific culture system, the TPC ranged from 10^4 to 10^5cfu/g. According to ICMSF (1986), the recommended limit of TPC for good quality fish is 5×10^5cfu g^{-1} and counts at or above 10^7cfu g^{-1} is regarded as unacceptable quality. In the present study, TPC was in the upper level of acceptable limit in TC. This observation corroborates with the findings of Bordoloi *et al.* (2012) who worked on rohu fish collected from Agartala fish market (Tripura).

5.1.2. *Vibrio* spp count

The *Vibrio* spp count of TC is significantly higher ($p<0.05$) than the SC (Table 5 & Fig 2). The *Vibrio* spp counts were found to be in the range of 10^4 to 10^6 in all the shrimps brought from traditional culture system. The probable reason for this could be due to *Vibrios* are predominant bacteria of estuarine water (Panda and Nayak, 2001). In case of scientific culture system, the count was 10^4cfu/g. Karunasagar *et al.* (1985) have reported that freshly caught prawns from mangalore coast were contaminated with *Vibrio parahaemolyticus* in the range of 10^2 to 10^4cfu/g, which is almost similar to the results of the present study.

5.1.3. *Escherichia coli* count

The *E. coli* count of TC is significantly higher ($p<0.05$) than the SC (Table 7 & Fig 3). The *E. coli* counts were found to be in the range of 10^4 to 10^5 in all the shrimps brought from TC. In case of scientific culture system, the count was 10^4cfu/g. This observation corroborates with the findings of Bordoloi *et al.* (2012) who worked on rohu fish collected from Agartala fish market (Tripura). Pathogenic strains of *E. coli* are transferred to seafood through sewage pollution of the coastal environment or by contamination after harvest (Ward *et al.* 1997). Use of poor quality ice and water during handling and transportation may be the reason for these higher counts. In addition, the Kolkata fish market is quite unhygienic. The presence of coliforms, especially *E. coli*, is an indication of faecal pollution and is considered as an indication of the presence of other pathogenic bacteria.

It has been observed that the occurrence of *E. coli* is very high when the shrimp captured during the monsoon season (crop II) and post monsoon season (crop III). In the Kolkata region, the rainy season generally starts by the end of May month, resulting to heavy runoff and carries away domestic sewage from the land to the sea. Iyer *et al.* (1970) also stated that season plays a role in controlling the bacterial quality of fresh shrimps and observed that the bacterial counts were higher in certain specific seasons. They recorded a high incidence of *E. coli* in raw shrimps during the rainy season, which is probably due to the high degree of faecal pollution of the water during that period which also justifies with the present findings of the work.

5.1.4. *Staphylococcus aureus* count

In the present study, *Staphylococcus aureus* was found in high numbers in both TC and SC (Table 8 & Fig 4). The bacteria occurred in the range of 10^4-10^5cfug^{-1} in both the samples. Krishnamurthy and Karunasagar (1986) also reported that *Staphylococcus* was present in significant numbers in raw shrimps. This observation exactly corroborates with the findings of Bordoloi *et al.* (2012) who worked on rohu fish collected from Agartala fish market (Tripura). These results are also matching with the observation of Nilla *et al.* (2012) who worked on Indian white shrimp. The natural habited for *Staphylococcus* sp is the skin and mucus membranes of animal and human. Some strains of *Staphylococcus* produce enterotoxins. *Staphylococcus* does not multiply in fish.

The presence of *Staphylococcus* in fish is an indication of post harvest contamination (Huss, 1994). The incidence of *Staphylococcus aureus* in the fishery products within limits is not a serious problem. However, careless handling during processing results in the multiplication of the organism, which may lead to food poisoning (Lilabati and Vishwanath, 1999). Higher counts of *S. aureus* observed in raw shrimps collected from TC system show that personnel hygiene was not given much importance during handling and transportation of raw shrimps. The lower occurrence of *S. aureus* in SC system might have been due to the proper care taken by the shrimp farmer, because the harvesting method in SC system is done under controlled condition.

5.1.5. *Salmonella* count

Salmonella was not detected in both TC and SC system. The recommended limits for *Salmonella* spp. by ICMSF (1986) is 0 (zero). *Salmonella* spp. was absent in both traditional and scientific cultured shrimps and therefore both the samples are considered acceptable from the consumers point of view. Chen *et al.* (1990) and Antony *et al.* (2002) analyzed the bacteriological quality of *P. monodon* and also observed similar results. Dalsgaard *et al.* (1995) reported that *Salmonella* was not recovered from shrimps tested in Thailand.

5.2. Biochemical assessment

5.2.1. Total volatile basic nitrogen (TVB-N)

TVB-N is widely used as an indicator for meat degradation (Olafsdottir *et al.* 1997). The level of 30-35 mg TVB-N $100g^{-1}$ of fish muscle are generally regarded as the limit of acceptability (Lakshmanan, 2000). The TVBN level of TC and SC ranged from 1.60±0.04 to 1.96±0.03, 1.28±0.03 to 1.40±0.08 respectively (Table 10 & Fig 5). It shows that TVB-N concentration was significantly ($p<0.05$) higher in TC than SC (Table 11). This may be due to prolonged storage time of shrimps in ice. However, TVB-N concentration was within the acceptable limit in both TC and SC.

The level of total volatile nitrogenous bases increases after spoilage begins and thus can be used as an index of spoilage. Using TVB-N as such an index of spoilage does not distinguish the origin or component of these volatile compounds, hence its use is more general. The use of TVB-N as an index of spoilage was first proposed by Shewan (1957). The low value of TVB-N is an indication of quality of fresh shrimp. The high TVN value may also be explained because too little ice had been used

(Cobb *et al.* 1976). The time and method of storage plays an important role in the biochemical changes.

5.2.2. Trymethylamine (TMA)

Bacteria growing on the surface of the fish tissue produce volatile amines. One such volatile base is trimethylamine (TMA), a reduction product of the component trimethylamine oxide. TMA has been used as an indicator of general fish spoilage. Other volatile amines produced include ammonia and small amounts of monomethylamine and dimethylamine. While the fish is still in rigor dimethylamine begins to form followed by trimethylamine with the progress in time. Although not universal, the TMA determination has become one of the established procedures for determining fish quality. It has been proposed that TMA levels between 5 and 10 mg/100 g tissue should be considered the maximum allowable levels in international trading. It should be observed that TMA, as would be expected of the bacterial product, is not useful in determining quality deterioration, which occurs during frozen storage. It should also be observed that the TMA value are dependent upon the storage temperature of the fish and will vary accordingly (FAO, 1994).

The TMA-N level of TC and SC ranged from 0.94±0.05 to 1.17±0.03, 0.92±0.03 to 1.00±0.04 respectively (Table 12 & Fig 6). It shows that TMA concentration was significantly ($p<0.05$) higher in TC than SC (Table 13). But both the samples are within the acceptable limit. These values are found to be similar with Yamagata *et al.* (1995) who worked on banana shrimp with the values of 0.49 mg TMA-N/100g on 0 day to 0.69 mg TMA-N/100g after 4 days of storage. The TMA-N levels were comparable to those of *Pandalopsis japonica*, *Pandolopsis hypsinotus*, and *Metapenaeus barbata* which ranged from 0.1 -2.08 mg/100g (Takagi *et al.*1967; Harada *et al.* 1972).

5.2.3. pH

The pH value of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 7.02±0.04 to 7.25±0.04, 6.95±0.06 to 7.12±0.04 respectively (Table 14 & Fig 7). These values are found to be similar with Puga-lopez *et al.* (2013) who worked on *Litopenaeus vannamei.* The pH value of TC was higher, probably due to metabolism of microorganisms producing alkaline compounds like amines formed by deamination of amino acids (Huss, 1988, Jackson *et al.* 1997). The pH changes, showed

good correlation with sensory and microbiological results. The increment of pH value can be attributed to compounds accumulated from endogenous and microbial enzymatic reactions (Seabra *et al.* 2011). The pH changes also reflected TVB-N and TMA-N accumulation and indicated the spoilage progress. The initial post mortem pH varies with species, catching ground and season.

5.3. Proximate composition of shrimp

5.3.1. Moisture content

The moisture content value of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 70.27±1.08 to 71.98±1.46, 70.92±0.93 to 72.19±1.23 respectively (Table 16 & Fig 8). These values are found to be similar with Puga-lopez *et al.* (2013) who worked on *Litopenaeus vannamei* with the values of 73.11±1.06 and Shalini *et al.* (2013) who worked on *L. vannamei* with the values of 71.00±0.02.

5.3.2. Ash content

The ash content of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 0.61±0.02 to 1.04±0.02, 0.67±0.04 to 0.93±0.67 respectively (Table 18 & Fig 9). The statistical analysis (Table 19) revealed that there was no significant ($p>0.05$) difference between TC and SC.

5.3.3. Protein content

The protein content of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 23.0±0.08 to 24.47±0.06, 23.12±0.04 to 24.34±0.01 respectively (Table 20 & Fig 10). This observation exactly corroborates with the findings of Ali *et al.* (2013a) who worked on *Penaeus monodon* and and Shalini *et al.* (2013) who worked on *L. vannamei.* The lower values of protein content might be due to dripping or leaching out of water soluble protein fraction from muscle along with melt water (Solanki and Venkataraman, 1978). Protein content varies with size, age, sex of the shrimp along with elapse of time, handling and transportation as well as other biochemical reaction (especially decomposition) in shrimp body (Ali *et al.* 2013a).

5.3.4. Fat content

The fat content of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 0.52±0.01 to 1.82±0.04, 0.92±0.03 to 1.32±0.02 respectively

(Table 22 & Fig 11). This observation exactly corroborates with the findings of Nisa and sultana (2010) who worked on *Fenneropenaeus penicillatus*, Puga-lopez *et al.* (2013) who worked on *Litopenaeus vannamei,* Begum *et al.* (2011) who worked on *Macrobrachium rosenbergii* and Shalini *et al.* (2013) who worked on *L. vannamei.* Crop II season (monsoon) was the breeding time of black tiger shrimp. So the fat energy will be utilized by shrimp for breeding. This could be the probable reason for low fat content in that particular season. SC fat content was higher than TC, because artificial feeds are given to those shrimps. Artificial feeds contain high amount of cholesterol than natural feeds. This could be the reason for the variation of fat content between TC and SC.

5.4. Sensory evaluation

The results of sensory analysis reflect the scores obtained by different parameters i.e; general odour, carapace colour, carapace texture, eye and shell colour by all the samples of TC and SC were within the acceptable limit (Table 24). Scores obtained by SC was significantly higher ($p<0.05$) than TC (Table 25) which could be due to longer duration of ice storage of TC (Chytiri *et al.* 2004). According to the score sheet parameters, the samples under SC were found to be of better quality over the TC which could be because of harvesting pattern. All the samples of SC were found to be above acceptable limits. TC samples were found to be either of moderately acceptable or acceptable limits.

Reilly *et al.* (1985) have reported that prawn (*P. monodon*) held at normal temperature were rejected after 16 hours due to discoloration with a pinkish cooked appearance, strong ammonical odours and very soft texture. Prawn lost up to 15% of their body weight after 16 hours. At normal temperature, *Haplochromis* sp. spoiled after 20 hours, *Protopterus pictuss* after 3 days and lates fillets after 2 days. In case of *Haplochtomic* sp. when the fish was found totally spoiled, it gave off a strong odour and showed soft texture (Mlay *et al.* 1985). Farooqui (1978) reported that shrimp in ice maintained good quality for 0-2 days as judged by organoleptic quality was acceptable up to 7 days and rejected after 9 days.

CHAPTER VI

SUMMARY AND CONCLUSION

6. SUMMARY AND CONCLUSIONS

6.1. Summary

The objective of the present study was to assess the microbiological, biochemical and sensory quality of black tiger shrimp (*Penaeus monodon*) brought from traditional cultured shrimp (TC) was assessed and compared with scientific cultured shrimp (SC). The samples were collected from Kolkata wholesale fish market and taken to the Department of Fish Processing Technology, Faculty of Fishery Sciences by using sterilized plastic bags (iced) in aseptic condition

The total plate count (TPC) of traditional cultured shrimp is significantly higher ($p<0.05$) than the scientific cultured shrimp (Table 3). Total plate counts were found to be in the range of 10^6 to 10^7cfu/g in the sample brought from traditional culture system. In case of scientific culture system, the TPC ranged from 10^4 to 10^5cfu/g.

The *Vibrio* spp. count of traditional cultured shrimp is significantly higher ($p<0.05$) than the scientific cultured shrimp (Table 5). The *Vibrio* spp. counts were found to be in the range of 10^4 to 10^6cfu/g in the sample brought from traditional culture system. In case of scientific culture system, the count was 10^4cfu/g.

The *E. coli* count of traditional cultured shrimp is significantly higher ($p<0.05$) than the scientific cultured shrimp (Table 7). The *E. coli* counts were found to be in the range of 10^4 to 10^5 in the sample brought from TC. In case of scientific culture system, the count was 10^4cfu/g.

The statistical analysis revealed that *Staphylococcus aureus* count was significantly ($p<0.05$) higher count in TC than SC (Table 9). However, the bacteria occurred in the range of 10^4-10^5cfug^{-1} in both the samples. *Salmonella* was not detected in both TC and SC system.

The TVBN level of TC and SC ranged from 1.60±0.04 to 1.96±0.03, 1.28±0.03 to 1.40±0.08 respectively. It shows that TVBN concentration was significantly higher ($p<0.05$) in TC than SC (Table 11). However, TVBN concentration was within the acceptable limit in both TC and SC.

The TMA level of TC and SC ranged from 0.94±0.05 to 1.17±0.03, 0.92±0.03 to 1.00±0.04 respectively. It shows that TMA concentration was significantly higher

($p<0.05$) in TC than SC (Table 13). But both the samples were again within the acceptable limit.

The pH value of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 7.02±0.04 to 7.25±0.04, 6.95±0.06 to 7.12±0.04 respectively (Table 14 & Fig 7). The statistical analysis (Table 15) revealed that there was significantly ($p<0.05$) higher value in TC than SC.

The moisture content value of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 70.27±1.08 to 71.98±1.46, 70.92±0.93 to 72.19±1.23 respectively (Table 16 & Fig 8). The statistical analysis (Table 17) revealed that there was no significant ($p>0.05$) difference between TC and SC.

The ash content of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 0.61±0.02 to 1.04±0.02, 0.67±0.04 to 0.93±0.67 respectively (Table 18 & Fig 9). The statistical analysis (Table 19) revealed that there was no significant ($p>0.05$) difference between TC and SC.

The protein content of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 23.0±0.08 to 24.47±0.06, 23.12±0.04 to 24.34±0.01 respectively (Table 20 & Fig 10). The statistical analysis (Table 21) revealed that there was no significant ($p>0.05$) difference between TC and SC.

The fat content of traditional cultured shrimp (TC) and scientific cultured shrimp (SC) ranged from 0.52±0.01 to 1.82±0.04, 0.92±0.03 to 1.32±0.02 respectively (Table 22 & Fig 11). The statistical analysis (Table 23) revealed that there was no significant ($p>0.05$) difference between TC and SC. It is observed that the fat content of TC was very low during crop II season.

The results of sensory analysis reflect the scores obtained by different parameters i.e; general odour, carapace colour, carapace texture, eye and shell colour. Both the samples of TC and SC were within the acceptable limit. Scores obtained by SC was significantly higher ($p<0.05$) than TC (Table 25). According to the score sheet, quality parameters in SC got a better score over the TC samples, which could be because of the better harvesting pattern in SC samples.

The present study revealed that that the microbiological, biochemical and sensory quality of TC is comparatively poor than SC. The results of microbiological analysis shows that TPC was much higher and also pathogenic bacteria of public health significance were present in very high numbers which was beyond the acceptable limit in both TC and SC but in all cases the counts were comparatively higher in TC than SC. However, shrimps are cooked prior to consumption which could be considered as safe to the consumers. These products therefore can cause negligible health problems to the consumers except for gross contamination in kitchens and elsewhere. Among biochemical quality parameters, both TVB-N and TMA concentration was found to be within the limits. Sensory scores of both TC and SC were also within the acceptable limits. However, the SC samples had a better acceptable value over the TC samples.

6.2. Conclusion

In the present study, we could record some important and interesting observations such as;

1. The total plate counts of all the shrimp samples were near the upper limit of the acceptable range.
2. The count of *Staphylococcus* sp. was much above the acceptable limit, indicating poor personal hygiene of the fish handlers.
3. The high count of *E. coli* in all the samples indicated the contamination with faecal matters either in ice or in washing water or both.
4. The Total volatile basic nitrogen (TVB-N) concentration was significantly higher in TC indicated more microbial spoilage in TC.
5. In sensory analysis, the scores obtained by SC was significantly higher than TC.

Suggestive management measures

Based on the findings of the present study, certain management measures can be suggested for maintaining better keeping quality of shrimps.

1. Proper packaging with recommended proportions of ice and shrimp 1:2 (shrimp to ice).

2. Construction of proper drainage system in the auction centers and retail markets.
3. Organization of awareness camps amongst the fish handlers for maintaining good personal hygiene.
4. Government should impose strict rules and regulations for adopting sound measures for maintaining personal hygiene for the fish handlers.
5. Construction of proper infrastructure facilities and chlorinated water supply system in the fish market places with foot dips before entering the fish markets.

CHAPTER VII

REFERENCES

7. RERERENCES

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APPENDIX

APPENDIX I

Sensory analysis of Black tiger shrimp (*Penaeus monodon*)

I.F.Sc Thesis work on "Quality assessment of Black tiger shrimp (*Penaeus monodon*) from different culture systems of coastal West Bengal".

A. Eye Characteristic (Maximum score 10):

a.	Bright and transparent	10
b.	Moderately transparent	9
c.	Slightly transparent	8
d.	Slightly dull	7
e.	Moderately dull	5
f.	Dull and opaque	3
g.	Fully dull and opaque	0

B. Odour (Maximum score 10):

a.	Fresh shrimp odour.	10
b.	Moderately fresh odour	9
c.	Sweetly odour.	8
d.	Slightly off odour.	7
e.	Moderately off odour	5
F.	Spoilage odour	3
g.	Extremely spoilage odour	0

C. Carapace texture (Maximum score 10):

a	Hard	10
b.	Slightly hard	9
c.	Moderately hard	8
d.	Slightly soft	7
e.	Moderately soft	5
f.	Soft	3
g.	Very soft	0

D. Carapace colour (Maximum score 10):

a.	Greenish	10
B.	Moderately greenish	8
c.	Slightly greenish	6
d.	Slightly darken	4
e.	Moderately darken	2
f.	Darken	0

E. Shell colour (Maximum score 10):

a.	Bluish white	10
B.	Moderately bluish	9
c.	Slightly bluish	8
d.	Slightly loss of brightness	7
e.	Loss of brightness and opaque	5
f.	Slightly reddish	3
g.	Reddish and spotted	0

Overall acceptability	**Score range**
Highly acceptable (HA)	10-8.5
Acceptable (A)	6.5-8.4
Moderately acceptable (MA)	4.5-6.4
Just acceptable (JA)	3.6-4.4
Just unacceptable (JU)	2.6-3.5
Unacceptable (U)	1.5-2.5
More unacceptable (MU)	0-1.4

Date:

Name of the panelist: **Signature**