

**Study on Quality Changes of Fresh Water Prawn (Galda) in the Shrimp
Change Value**

by



Anupam Chakraborty

A thesis submitted in partial fulfillment of the requirement for the degree of
Master of Philosophy
in the Department of Chemistry



Khulna University of Engineering & Technology

Khulna 9203, Bangladesh

December 2012

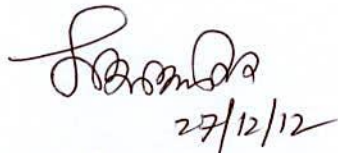
Dedicated
to
my beloved friend

Late Md. Nazmul Huda Mithu

Declaration

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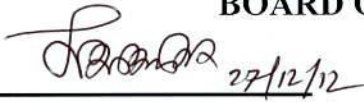
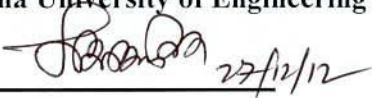
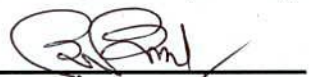


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Approval

This is to certify that the thesis work submitted by Anupam Chakraborty entitled “Study on Quality Changes of Fresh Water Prawn (Galda) in the Shrimp Change Value” has been approved by the board of examiners for the partial fulfillment of the requirement for the degree of Master of Philosophy in the department of Chemistry, Khulna University of Engineering & Technology, Khulna, Bangladesh in December 2012.

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Abstract

The present study was conducted to assess the quality loss of shrimp (*Macrobracium rosenbergii*) known as Galda in the value chain of Bagerhat region. The investigation was carried out in December 2011 to February 2012. The study was undertaken in twelve selected shrimp farms, four faria, four depot and five factory receiving point of different locations, viz. Fakirhat, Bagerhat Sadar, Mollahat and Sharankholla in Bagerhat region. Quality assessment included proximate analysis of Protein parameters and Biochemical {Total Volatile Base Nitrogen (TVB-N) and Trimethylamine Nitrogen (TMA)}. The TVB-N and TMA were determined by using Conway's Micro-diffusion Technique and Protein was determined by proximate analysis (kjeldhal method in wet way). Protein contents of forty eight samples were found in the range of 17.68% to 24.05% in wet weight method. At the farm level, the protein content was 23.17%, 23.80%, 23.01% and 23.16% in Fakirhat, Bagerhat Sadar, Mollahat and Sharankholla station in Bagerhat region respectively. At factory receiving point, protein content was 18.55%, 18.91%, 18.17% and 18.82% in Fakirhat, Bagerhat Sadar, Mollahat and Sharankholla station in Bagerhat region respectively. Protein loss of Shrimp was being 19.94%, 20.55%, 20.03% and 18.74% respectively in Fakirhat, Bagerhat Sadar, Mollahat and Sharankholla from farm to factory receiving point. From farm to factory receiving point, grand protein loss was 19.82%. The TVB-N value was found from 10.14 mg/100g to 19.54 mg/100g. The TMA value was found from 7.37 mg/100g to 16.75 mg/100g. The results obtained from this research will help the shrimp's exporter to export good quality shrimps.

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CHAPTER I

Introduction

1.1 General:

Shrimps are one of the most economically important species in aquaculture due to their high world-wide demand. In Bangladesh, shrimp is the most expanding sector among the agro-based products including tea, raw jute, vegetables, fruits etc. Shrimp is one of main exporting product in Bangladesh. The demand of shrimp is increasing day by day in international market, at same time the culture practice of shrimp is increasing in coastal region area. Area of shrimp farm is increasing from 64000 ha to 1,40,000 ha in few years. The production of those shrimp farm is 1,57,000 MT in financial year of 2009-2010 which increased from 62,000 MT in financial year of 1999-2000. The frozen shrimp industries earned the second largest export sector which reported a total of export earning of US\$ 412.173 million or 2885.212 crore taka in 2009-10 financial year. About 51,599.15 MT shrimp was exported in 2009-10 financial year. From July to November in 2010-2011 financial year, exporting earning of shrimp was US\$ 223.532 million or 1,564.726 crore taka by 25,166.67 MT of shrimp exporting [1]. Especially the southern part of Bangladesh plays a vital role in production of shrimp. Greater Khulna region (Satkhira, Khulna and Bagerhat) provided maximum amount of total foreign earnings in fish and shrimp. Though Satkhira, Khulna and Bagerhat are the main region for shrimp culture yet Bagerhat district plays the main role in this regard. The main cultivated species in this region are *Macrobracium rosenbergii*, more commonly referred to as Galda.

Galda (*Macrobracium rosenbergii*) has been recognized in the last few years as one of the most important aquatic resources of Bangladesh. This species has a number of advantages in comparison to other crustaceans. It adapts to wide range of temperature (20⁰C –32⁰C) fast growing individuals which reach marketable size in about 8 to 10 months, has high nutritional value and has carnivorous feeding habit thus being an excellent species for polyculture. *Macrobracium rosenbergii* has high demand in domestic and international market as well.

Bagerhat district have a great contribute about shrimp export. Exportable shrimp requires special care to retain as much as practicable its original physical appearance, odor and organoleptic conditions. It must be free from dirt, filth, pathogenic organisms, uncertified chemicals and any antibiotics even in the minutest quantity. The importing countries, particularly the EU, USA and Japan are highly conscious about food hygiene and safety. These countries will not accept any food item with doubtful quality in respect of its freshness, hygiene and safety for human health.

There is now being asked a question about the quality of processed shrimp in the international market. Due to having dissatisfaction of the international buyer of Bangladeshi frozen food products about the quality loss from shipment to taking by the importer, an intensive and comprehensive research study is a crying need in this regard. As a result Bangladeshi frozen shrimp exporters will get more currency if we can identify the correct cause and the solution. Ultimately Bangladesh will earn more foreign currency from exporting it with maintaining proper quality control.

Fish are recognized as being highly perishable, having a relatively short shelf life, which is defined as the length of time from the day of catch that fresh fish can be in the marketplace

unspoiled [2]. Therefore fish requires proper handling and preservation to increase its shelf life and retain its quality and nutritional attributes. Quality is defined as the aesthetic appearance and freshness or degree of spoilage which the fish has undergone [3]. Immediately as fish is caught, it loses its natural resistance to attack by microorganisms and also starts to undergo both physical and chemical changes that in return bring changes in appearance, taste, smell and texture.

Fish meat has a high a_w (percent of water) and pH 5.5 to 7.0. Moreover meat is rich in protein, vitamins, and minerals and low in carbohydrates (< 1%) [4] [5]. This means that meat is an ideal substrate for microbial growth.

Bacteria are able to decompose proteins, other nitrogen containing compounds to ammonia, hydrogen sulphide, which produce an unpleasant and disgusting flavour [6]. Trimethyl amine oxide (TMAO), mostly found in marine fish, is broken down to trimethyl amine (TMA), dimethylamine (DMA) and ammonia (NH_3), which are responsible for off-odors in fish undergoing spoilage. Spoilage of fish is for 95% microbial and remaining 5% for other.

Live fish is normally considered to be sterile, but microorganisms are found on all the outer surfaces (skin and gills) and in the alimentary tract of live and newly caught fish in varying numbers. A normal range of 10^2 - 10^7 cfu (colony forming units)/ cm^2 on the skin and between 10^3 and 10^9 cfu/g in the gills and intestines has been observed [7]. When fish dies, its entire body resistance mechanisms breakdown, giving way to microorganisms or the enzymes they secrete to invade or diffuse into the flesh where they react with the complex mixture of natural substances present. During storage a characteristic flora develops, but only a part of this flora, known as the specific spoilage organisms (SSO),



contribute to spoilage. The SSO counts reach a minimal spoilage level where the fish is sensorial rejected. Temperate fish have psychotropic (cold-tolerant) bacteria of the genera *Pseudomonas*, *Moraxella*, *Acinobacter*, *Shewanella*, *Flavobacterium*, *Vibrio*, *Photobacterium* and *Aeromonas* as part of their natural flora whereas tropical fish normally have non-psychotropic (mesophilic) spoilage bacteria that make tropical fish spoil much faster than temperate water fish in the absence of ice.

Chemical spoilage processes are changes taking place in the lipid fraction of the fish. Lipids are oxidized to peroxides, aldehydes, ketones and lower aliphatic acids. The hydro-peroxides are tasteless but can cause brown and yellow discolouration of the fish tissue. The degradation of hydro-peroxides gives rise to the formation of aldehydes and ketones that result in rancid off-flavors. All the chemical by-products eventually reach a level where the fish is rejected.

High temperatures are partly responsible for the speed of the oxidation processes. In addition, direct sunlight, wind, heat, light (especially UV-light) and several organic and inorganic substances may also accelerate oxidative processes.

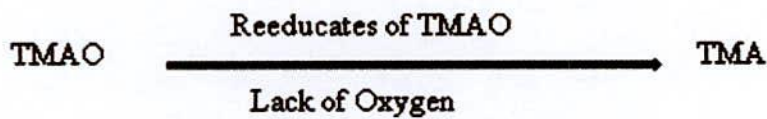
Living cells in fish have enzymatic protection mechanisms against lipid oxidation by having an enzyme, glutathione peroxidase, which acts by reducing hydro-peroxides in cellular membranes to corresponding hydroxyl-compounds. This reaction requires a supply of the enzyme in a reduced form and thus the reaction stops when the fish die.

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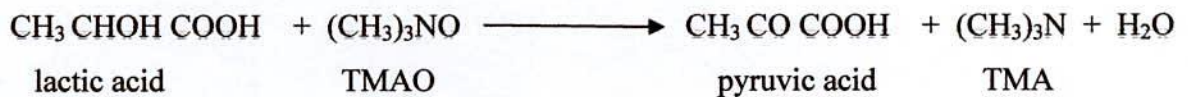
Living cells in fish have enzymatic protection mechanisms against lipid oxidation by having an enzyme, glutathione peroxidase, which acts by reducing hydro-peroxides in cellular membranes to corresponding hydroxyl-compounds. This reaction requires a supply of the enzyme in a reduced form and thus the reaction stops when the fish die.

Shrimp contain TMAO as osmoregulator.

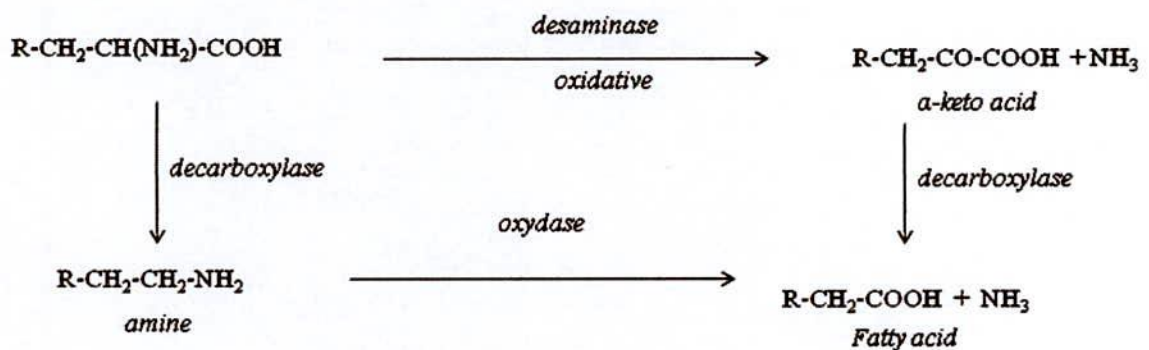
Break down of TMAO:



Reaction occurs as follow:



Break down of Protein:



The samples were collected during the month of December 2011 to February 2012. At first the sample was collected from Fakirhat Upazilla. There are twelve farms to be selected in Fakirhat, Bagerhat Sadar, Mollahat and Sharankhola Station in Bagerhat district. Those farm are situated at Lockpur, Bigha and Moubhog in Fakirhat upazilla, at Sugandhi, Baliadanga and Rakhalgachi in Bagerhat Sadar upazilla, at Joidhiki, Nasukhali and Nagorkandi in Mollahat upazilla, at Kalibari, Nalbunia and Razapur in Sharankholla upazilla . We are facing four faria in the station. Names of the faria are Taposh Biwash in Fakirhat, Alamgir Hawladar in Bagerhat Sadar, Bulu Molla in Mollahat and Babul fakir in Sharankholla. We are collected shrimp from faria after 1-2 hour later of farm's shrimp collection. There are four depots from collection of shrimp. There are Jatrapur and Lockpur Fish and Tina Fish in Faltita, Vai Vai Fish, C & B Bazar in Bagerhat Sadar, Monir Fish Traders, Amragachi Bazar in Sharankholla. After one day later of farm collection, we collect shrimp from Depot/Agent. We are collected sample from factory receiving point namely- Modern Sea Food Ind. Ltd., Bright Sea food Ltd. and Rupali Sea Food Ind. Ltd. in Rupsa, Khulna, Bagerhat Sea Food Ind. Ltd. in Nawapara, Fakirhat, Bagerhat and Rupsa Fish and Alide Ind. Ltd. in Paikpara, Bagerhat. The shrimp products are collecting in the evening period.

The study was conducted by selecting the four distinct part of Bagerhat district namely Fakirhat, Bagerhat Sadar, Mollahat and Sharankholla upazilla. Each station have three farm sample, three faria sample, three depot sample and three factory receiving point sample. Totally twelve sample were collected from each station. We collected an icebox and carried all shrimp inside the ice box with adequate amount of ice to minimize the temperature stress as well as the biochemical degradation.

Studied on value chain and marketing Channels of Shrimp/Prawn Sector of Gher Farming System in Bangladesh. Shrimp/prawn industry plays an important role in value chain in Bangladesh. Shrimp/prawn is the second largest exporting industry followed by garment industry in Bangladesh. The present study aims to explore the detail summary of the value chain, and marketing channels and systems in shrimp/prawn industry of Bangladesh. Various agents are involved in the shrimp/prawn industry from production to final consumption stage as well as the exporting of international markets. The findings of the study shows that a large numbers value chain activity involves in shrimp/prawn industry from production stage of shrimp/prawn to final exporting stage to aboard. Even though the farmers bear the all production risks, they did not get the profit like other agents of the marketing channels of shrimp/prawn industry. All agents of marketing channels gain more financial benefits than the producers of shrimp/prawn of Bangladesh. The farmer sell their shrimp to the faria, the faria sell their shrimp to depot and depot owner sell their shrimp to the factory through the factory enlisted agent. This is the value chain (distribution channel) of shrimp of Bagerhat district. We were collected the same shrimp sample from the factory gate those were caught from the same farm and depot and faria.

All samples were properly iced in the ice box and brought to Fish Nutrition Laboratory of Fisheries and Marine Resource Technology discipline of Khulna University. Taking the information from various the depot owner and agent in Notun Bazar, Rupsa, Khulna and shrimp factory/processing plant namely- Modern Sea Food Ind. Ltd., Bright Sea food Ltd. and Rupali Sea Food Ind. Ltd. in Rupsa, Khulna, Bagerhat Sea Food Ind. Ltd. in Nawapara, Fakirhat, Bagerhat and Rupsa Fish and Alide Ind. Ltd. in Paikpara, Bagerhat.

Overall major problems of quality deterioration in shrimp value chain were use of unclean pond water for washing, longer duration of harvesting, exposure of shrimp at high ambient temperature (delayed icing), contamination, lack of general hygiene, lack of personal hygiene, dipping of shrimp in excess water for long time, packed under pressure, piled up shrimp on a dirty floor, body crushed due to heavy pressure, rough handling, use of dirty utensils, contamination with pets and vermin's at depot level, resulting in considerable loss of shrimp quality.

Shrimps are an extremely good source of protein, yet are very low in fat and calories making them a very healthy choice of food. Shrimp has great importance in food consumed by human and other organisms. It is valuable in the diet, because apart from supply of good quality proteins and vitamins, it also contains several dietary mineral such as calcium, Iron etc, which are beneficial to human and other organisms. Proximate parameters in flesh were generally more concentrated than shell. The flesh of *Macrobracium rosenbergii*, that high level of protein, carbohydrate and moisture content was reported in the flesh tissues. The level of protein in the flesh was of good comparison with the shell of *Macrobracium rosenbergii*. But the flesh contained low content of crude fiber than shell.

The complex chemical composition of proteins makes them quite difficult to characterize by simple chemical or physical procedures. However, their component amino acids may be conveniently detected by various specific chemical tests. The standard method for determining nitrogen in inorganic compounds is the Kjeldahl method.

Until comparatively recent times, the protein content of foods could be estimated from the organic nitrogen by Kjeldahl procedure. In consequence it is included in official and

statutory methods and approved by international organizations. Furthermore, the results obtained by Kjeldahl are used to calibrate physical and automatic methods.

The Kjeldahl method is based on the wet combustion of the sample by heating with concentrated sulphuric acid in the presence of metallic and other catalysts to reduce of organic nitrogen in the sample to ammonia, which is retained in solution as ammonium sulphate. The digest, having been made alkaline, is distilled or steam distilled to release the ammonia which is trapped and titrated. Mercury, as mercuric oxide is generally agreed to be the most affective catalyst, with selenium almost as effective, but both have toxic hazard and waste disposal problems. Moreover, mercury forms ammonia complexes in the digest requiring the addition of sodium thiosulphate to break the complex and to release the ammonia. The use of a mixture of copper (II) sulphate and titanium dioxide is recommended. However, considered this mixture to be at least as effective.

TMA is the compound produced from TMAO by bacterial enzymatic process. TMA has been considered as useful index in quality determination for some species. TVB-N is used as alternative to measuring TMA content. Seafood, when closed to spoilage, contains several bases that are volatile. Volatile bases in TVB-N mainly contain TMA and Ammonia. Changes in TVB-N content during spoilage are very similar to those of TMA in the same species except that the initial value of TVB-N is much higher [8].

TVB-N does not increase much in the early stages of spoilages but rises rapidly with bacterial activity. The variance of TVB-N of a sample of fish of the same origins and subjected to the same treatment is high compared to the average changes over the acceptability. So, it is not sensitive indicator of freshness, until the fish is spoiled rapidly [9].

The TMA could be used as an index of freshness. During spoilage TMAO is reduced by bacteria to TMA. Connel [10] reported that TMA could serve as an index of spoilage for marine fish. TMA contents fluctuate with seasons as well as within a single species.

Different workers have suggested a number of methods for determining TVB-N. Among the commonly used methods, the Conway micro diffusion technique is the most suitable and applied in this work for the determination of TVB-N and TMA.

TMA as an index of bacterial spoilage are similar to those of TVB-N. The index has widespread usefulness and correlates reasonably adequately with sensory changes during spoilage or deterioration e.g. in about 75% of cases, the taste panel and TMA results agree, Ruiter. A [11].

As of late, all personnel involved in shrimp farming and processing are trying to upgrade quality of shrimp products following factors related with good quality. More research support is needed to improve the cultural and management practices of the various shrimp culture systems in Bagerhat. Measures should be taken by the government to improve the collection centers or depots by creating adequate facilities for quality management of raw materials. It is recommended that farmers and depots owners should be provided financial support in the form of loan on easy term for modification of their existing management and infrastructure facilities, installation of ice plants, procurements of refrigerated van and developing other required facilities.

1.2 Objectives of the study:

The investigation was undertaken aiming at assessing the variation of Protein, TVB-N and TMA-N of shrimp at different stages of handling process. The specific objectives were given below.

- To observe the protein loss in the value chain of shrimp (*Macrobracium rosenbergii*) collected from different sources in Bagerhat region.
- To observe the variation of TVB-N contents of shrimp (*Macrobracium rosenbergii*) collected from different sources in Bagerhat region.
- To observe the variation of TMA-N contents of shrimp (*Macrobracium rosenbergii*) collected from different sources in Bagerhat region.

CHAPTER II

Literature Review

Botta et al. [8] studied on TMA, is the compound produced from TMAO by bacterial enzymatic process. TMA has been considered as useful index in quality determination for some species. TVB-N is used as alternative to measuring TMA content. Seafood, when closed to spoilage, contains several bases that are volatile. Volatile bases in TVB mainly contain TMA and Ammonia. Changes in TVB content during spoilage are very similar to those of TMA in the same species except that the initial value of TVB is much higher.

Sato [12] studied on certain chemical changes in spoiling shrimp appear to run parallel with changes in odor, texture, appearance etc. Various attempts have been made to measure freshness by estimating the quantities of same end product as a result of enzymatic and bacterial activity. Chemical tests of assessing the quality of shrimp and other fishery products are based on estimating the products of spoilage either as individuals or as groups.

Connell and Howgate [9] studied on TVB does not increase much in the early stages of spoilages but rises rapidly with bacterial activity. The variance of TVB of a sample of fish of the same origins and subjected to the same treatment is high compared to the average changes over the acceptability. So, it is not sensitive indicator of freshness, until the fish is spoiled rapidly.



Shewan and Ehrenberg [13] studied on TVB and TMA measurement is probably the oldest chemical method to assess fish spoilage used as an index of spoilage. TVB and TMA-N is the indicator of the Nitrogen content of shrimp.

Connel [10] studied on The TMA could be used as an index of freshness. During spoilage TMAO is reduced by bacteria to TMA reported that TMA could serve as an index of spoilage for marine fish. TMA contents fluctuate with seasons as well as within a single species. Different workers have suggested a number of methods for determining TVB-N. Among the commonly used methods, the Conway micro diffusion technique is the most suitable and applied in this work for the determination of TVB-N and TMA-N.

Ruiter [11] studied on TMA as an index of bacterial spoilage is similar to those of TVB-N. The index has widespread usefulness and correlates reasonably adequately with sensory changes during spoilage or deterioration e.g. in about 75% of cases, the taste panel and TMA results agree.

Azam K. [14] studied on Quality of Shrimp in the Distribution Channel, Malle and Poumeyrol [15] studied on Total Volatile Bases Nitrogen (TVB-N) is one of the most widely used methods today to estimate the degree of decomposition of fish. It includes the measurement of trimethylamine (produced by spoilage bacteria), dimethylamine (produced by autolytic enzymes during frozen storage), ammonia (produced by the diminution of amino-acids and nucleotide catabolites) and other volatile nitrogenous compounds associated with seafood spoilage.

Aitken et al. [16] studied on Trimethylamine (TMA), is formed in spoiling fish by the action of certain species of bacteria on the substance trimethylamineoxide, TMAO.

Therefore determination of TMA content is a measure of bacterial activity and spoilage. Increase in TMA during iced storage is similar to the increase in bacterial numbers. TMAO is not only an important compound for maintenance of physiological functions in fish and shellfish but it is also a key substance in the spoilage of raw or processed seafood. The TMAO content in the muscle of crustaceans is 9-28 (mole/kg wet weight). The measurement of total volatile basic nitrogen (TVB-N) is often used as an alternative to measuring TMA content because the TVB-N value includes mainly the content of ammonia, Trimethylamine, and dimethylamine. Therefore, changes in TVB-N content during spoilage are very similar to those of TMA except that the initial value is much higher.

Malle and Poumeyrol [15] studied on ammonia and Trimethylamine (TMA). The suitability of using TMA content itself as chemical method of evaluating freshness quality of seafood has been investigated extensively. Depending on the species (ground fish, pelagic species, and shellfish), it has been observed to be a useful measure of freshness quality (particularly flavor and odour aspects) of a variety of seafood, but this usefulness depends upon the time of the year and the location of catching, stage of spoilage, type of processing and storage, and method of analysis.

Egan et al. [17] studied on the level of TVB-N for white fish is generally considered to be fresh if the TVB-N is less 20 mg N/100 g sample. If the TVB-N reaches 30 mg N/100 g most authorities consider the fish to be stale, whilst at level of 40 mg N/100 g the fish is regarded as unfit for consumption.

The protein content of shrimp could be estimated from the organic nitrogen by Kjeldahl procedure, Nowadays there are several alternative chemical and physical methods available, some of which have been automated or semi- automated. Although it has been subject to modification over the years, the basic Kjeldahl procedure still maintains its position as the most reliable technique for the determination of organic nitrogen. In consequence it is included in official and statutory methods and approved by international organizations. Furthermore, the results obtained by Kjeldahl are used to calibrate physical and automatic methods.

Zeng Qingzhu, [18] studied on Total volatile basic nitrogen (TVB-N) value of 33.5 mg/100g whole shrimp was found at the beginning of storage. The high initial value of TVB-N is most likely because not enough ice was present to maintain constant temperature during the delayed transport and the temperature of the raw material had reached 4°C when it arrived at the laboratory. The high temperature encourages the growth of spoilage bacteria (initial count of 2.4×10^5 cfu/g). The microbial degradation of TMAO and diminution of amino acids resulting in the formation of TMA and ammonia, respectively, is evidenced by high initial values of TVB-N. A comparison of the rates of TMA formation during 6 days of storage revealed in which TMA-N values exceeded 10 mgN/100g, spoiled earlier than other two groups where TMA-N level remained below 10 mgN/100g until day four of storage. The extent of increase in TVB-N and TMA of shrimp stored in liquid ice at -1.5°C were considerably smaller than for sample groups stored under other conditions.

Ali et al. [19] studied on the quality of shrimp (*Penaeus monodon*) at ambient temperature stored in plastic and bamboo basket. Quality assessment included organoleptic, biochemical (TVB-N and TMA-N) and microbiological (SPC) parameters. TVB-N values were 2.68 ± 0.19296 , 9.78 ± 0.16 and 12.46 ± 0.3396 mg/100g at 0th (HA), 12th (JA) and 15th (JU) hour in bamboo basket and 2.49 ± 0.96176 , 9.25 ± 0.63542 and 12.51 ± 0.94256 mg/100g at 0th (HA), 11th (JA) and 14th (JU) hour in plastic basket respectively. While TMA-N values were 4.35 ± 0.2089 , 8.53 ± 0.49521 and 11.78 ± 0.141774 mg/100g at 0th (HA), 12th (JA) and 15th (JU) hour and 4.06 ± 0.1963 , 7.88 ± 0.852247 and 10.45 ± 0.687564 at 0th (HA), 11th (JA) and 14th (JU) hour in bamboo and plastic basket respectively.

Kher-un-Nisa and Razia Sultana [20] studied on Variation in the proximate composition of shrimp, *fenneropenaeus penicillatus* at different stages of maturity. The variation in the proximate chemical composition of the mid gut gland, ovary and muscle at different maturity stages of *F. penicillatus* have been determined on wet weight basis to elucidate the relationship between biochemical composition and the ovarian maturation. Protein content shrimp was determined from 17.2 % (stage I) to 18.0 % (stage IV) showing no significant variation throughout the maturation process.

Paul et al. [21] studied on culture practices and quality loss of shrimp and prawn at different stages of handling and transportation in Bangladesh. Studies were conducted to investigate the quality problems in giant tiger (*Penaeus monodon*) and giant freshwater (*Macrobrachium rosenbergii*) prawn at different stages of value chain in Bangladesh. Thirty nine *P. monodon* farms and 20 *M. rosenbergii* farms located in Cox's Bazar,

Bagerhat, Satkhira, Khulna and Mymensingh were visited and information were collected using prescribed questionnaires from cross-section of people working at farms, depot level and processing industries.

Ahmed et al. [22] studied on identification of cause of shrimp quality loss due to farm operation and post harvesting handling at depot and market of Bangladesh. The major causes identification were poor quality of non chlorinated water, non-maintenance of personal hygiene, poor quality of ice and poor ice ration, long time transportation and unscientific packet materials. About 8-25% quality was loss of shrimp due to improper handling, iceing and transportation.

CHAPTER III

Procedure / Methodology

3.1 Profile of the study area

The study was conducted at Fakirhat, Bagerhat Sadar, Mollahat and Sharankhola Station in Bagerhat district. Those farm are situated at Lockpur, Bigha and Moubhog in Fakirhat upazilla, at Sugandhi, Baliadanga and Rakhalgachi in Bagerhat Sadar upazilla, at Joidhiki, Nasukhali and Nagorkandi in Mollahat upazilla, at Kalibari, Nalbunia and Razapur in Sharankholla upazilla in Bagerhat district. It is the southern part of greater Khulna region and very near to Sundarbans Mangrove Forest. The region being very close to the Bay of Bengal, fresh water is available here to support shrimp farming (Galda) in a large scale.

3.2 Selection of species

Shrimp (*Macrobracium rosenbergii*) known as Galda was selected as the test specimen. It is the main export item in fisheries sector. Moreover, it is cultured most abundantly in Bagerhat district. The size ranging from 10-12 grades was used for quality assessment.

3.3 Sample Collection

The shrimp (*Macrobracium rosenbergii*) was collected from four distribution points in the shrimp value chain i.e. Farm, Faria, Depot/Agent and Factory gate/Processing plant in December, 2011 to February, 2012. The shrimp was caught from the Gher (enclosed

shrimp farm) and Faria situated in proposed area. The next sets of shrimp were collected from Depot/Agent and Processing Plant.

There were twelve farms to be selected in Fakirhat, Bagerhat Sadar, Mollahat and Sharankhola Station. 36 shrimp sample were collected from those farms.

We were facing four faria in the station collected shrimp from faria after 1-2 hour later of farm's shrimp collection.

There were four depots for collection of shrimp. After one day later of farm collection, we collected shrimp from Depot/Agent.

Then we were collected sample from factory receiving point in the evening period.

3.4 Sampling Method

Total six Kg shrimp of 10-12 grades will be purchased from the harvesting point (Gher). All the shrimp were not of same grades. Equal amount of shrimp will be taken for normal practice and controlled study. Three shrimp from each basket were separately kept into two ice box biochemical analysis. Following this method shrimp will be taken to each point of distribution point (depots, agents and Factory Gates) where the usual practices were done and the sample were collected in separate box. From each point of distribution channel shrimp will be brought to the Fish Nutrition Laboratory of Fisheries & Marine Resource Technology Discipline, Khulna University, Khulna.

3.5 Sample Analysis

The Experiment was conducted the Fish Nutrition Laboratory of Fisheries & Marine Resource Technology Discipline, Khulna University, Khulna. The study period was from

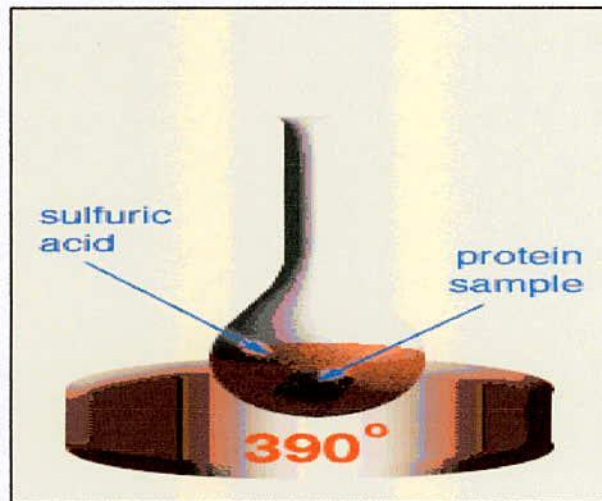
December, 2011 to February, 2012. Organoleptic, Biochemical (TVB-N, TMA-N) quality and proximate analysis (Protein) will be assessed in the laboratory. Both samples (normal practices and controlled study) were analyzed.

3.6 Determination of Protein

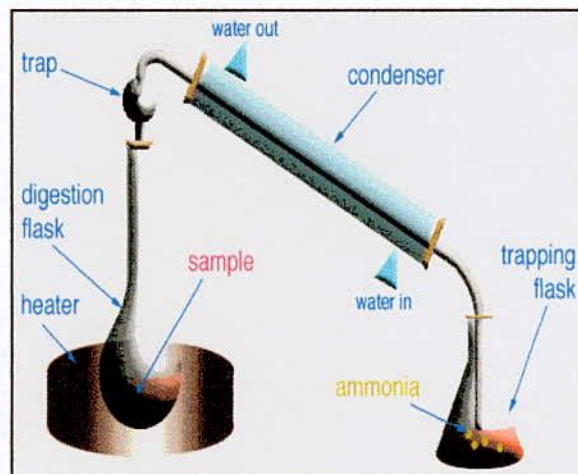
Protein content of shrimp was determined according to kjeldahl procedure.

The kjeldahl method consists of three steps:

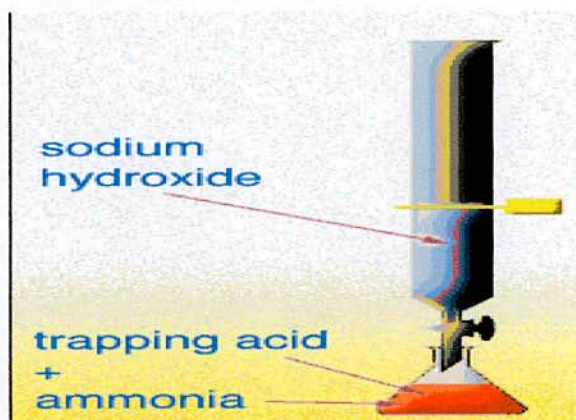
Step One: Digestion of the Sample



Step Two: Distillation



Step Three: Titration



Apparatus:

1. Electronic balance
2. Mortar
3. Kjeldahl flask
4. Kjeldahl nitrogen digestion apparatus (NDG-50E)
5. Kjeldahl nitrogen distillation apparatus (OSK-8416A)
6. Burette
7. Conical flask
8. Blender
9. Beaker (250ml)
10. Oven (N40C)
11. Porcelain crucible
12. Desiccators
13. Filter paper
14. Separations funnel etc

Chemicals:

1. Resolvent:

Potassium sulphate and mercuric oxide in the ratio of 10:0.7 were mixed and crushed properly and preserved in colored bottle.

2. Concentrated sulphuric acid.

3. 2% Boric acid:

20 g Boric acid crystal was added with 1 litter distilled water.

4. Tashiro's indicator:

80 g methyl red and 20 g methyl blue were dissolved in 95% ethanol to make up to 100mL with 95% ethanol.

5. Sandy zinc or zinc powder.

6. 33-40% caustic soda:

400 g NaOH and 10 gm potassium sulfide was added with 1 litter distilled water.

7. 1% K₂S:

Sometimes it is added with the 40% NaOH solution to increase the absorption of NH₃ during distillation.

8. 0.1(N) HCl acid:

8.48 mL HCl was added with 1 litter distilled water.

9. Acetone:

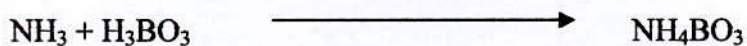
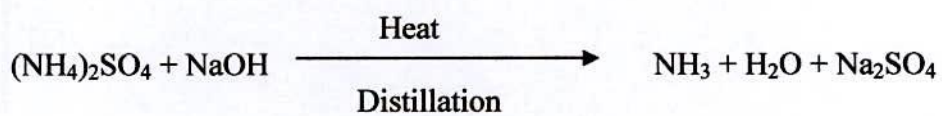
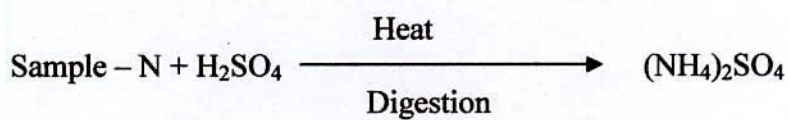
Methnol and Chloroform are mixed at a ratio of 1:2.

Working process:

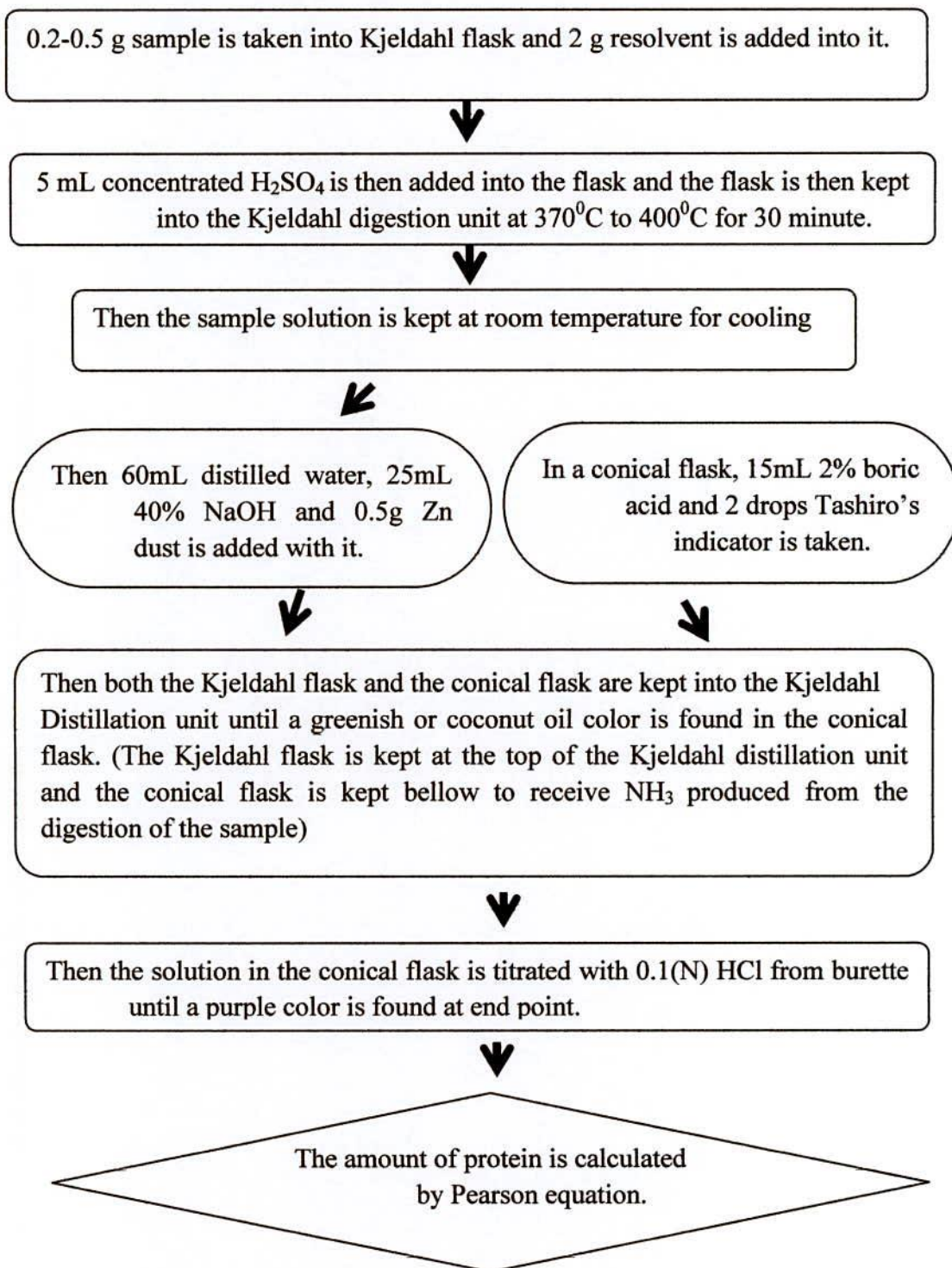
Principle:

The process involves two steps:

- a) Digestion of the sample to convert the sample-N to ammonium sulphate.
- b) Determination of nitrogen in the digest sample through distillation process.



General procedure:



Flowchart 3.1: Working module for determination of amount of protein in a sample.



Calculation:

The amount of crude protein is calculated from the following equations...

$$\% \text{ Total nitrogen (\% N)} = \frac{\text{Titrant (mL)} \times \text{Strength of titrant} \times 0.014}{\text{Weight of sample (g)}} \times 100 \quad \text{----- (1)}$$

$$\% \text{ Protein} = \% \text{ N} \times 6.25 \quad \text{----- (2)}$$

3.7 Biochemical Assessment

TVB-N and TMA-N were determined according to procedure stated in the manual of Siang and Kim [23].

Solution and Reagents

Inner ring solution -1 % boric solution containing indicator. 10 g boric acid were weighed into a one liter volumetric flask then added to 200mL of ethanol. After dissolving the boric acid, 10mL of mixed indicator solution was added, then made up to 1 liter with distilled water.

Mixed indicator

Bromocresol green (BCG) 0.02 g and methyl red 0.01g were dissolved into 10 mL of ethanol.

0.02 Hydrochloric Acid, HCl

20 mL of 1(N) HCl standard solution were diluted with distilled water made up to 1000 mL.

Standard Potassium Carbonate Solution

60g of K_2CO_3 were weighed and then 50mL of distilled water was added. It was boiled gently for 10 minutes. After cooling down, it was then filtered through filter paper

50% Potassium Carbonate Solution

Saturated K_2CO_3 solution was diluted to twice its volume with distilled water.

4% Trichloroacetic Acid (TCA, CCl_3COOH) Solution

40 g of TCA were dissolved in 1 L of distilled water.

Neutralized 10% formaldehyde Solution

10 g of $MgCO_3$ were added to 100 mL of formalin (35% formaldehyde Solution) and shake in order to neutralize the activity of formalin. Then diluted the filtrate three times with distilled water.

Extract Preparation

The extract of shrimp was prepared by mixing 2g of the ground muscle with 8mL of 4% Trichloroacetic Acid in a 50 mL Mackerty bottle and was homogenized well. It was left for 30 minutes at ambient temperature with occasional grinding. Then, it was filtered through filter paper (whatman no. 1). The filtered solution was kept in Mackerty bottle and was labeled. The filtered solution was also stored in a refrigerator at 0 -4°C (to prevent any further chemical, bacterial, enzymic break down of the muscle)

3.7.1 Determination of TVB-N

Three Conway's units were taken which had been thoroughly cleaned with a neutral detergent to remove any containment. To the edge of the outer ring of each unit was applied the gum. Using a micropipette 1 mL of inner ring solution was pipette into the inner ring of each unit. Into the outer ring of each unit, was pipette 1 mL of the sample extract. 1 mL of Saturated K_2CO_3 solution was carefully pipette into the outer ring of each unit, carefully to prevent the entering the inner ring and immediately the units were covered and closed with clip. The solution of the units was then mixed gently, to prevent any solution mixing from one ring to other. After then the units were placed in an incubator at $45^\circ C$ for 45 minutes. After this the units covers were removed and the inner ring solution, now a green color was titrated with 0.02N HCl using a burette (50 mL) until green color solution turned to pink. An average titrated volume of HCl was found from the result of three titration for each muscle sample. For each volume the TVB-N volumes were calculated. A blank test was also carried out using 1 mL of 1% TCA, instead of sample extract.

3.7.2 Determination of Trimethylamine Nitrogen (TMA-N)

Trimethylamine in shrimp muscle was determined by the Conway technique. Prior to addition of K_2CO_3 , 1 mL of 10% neutralized formalin was added to the extract to react with ammonia and thus allowed only the TMA to diffuse over the unit. The calculation was done by the same formula as used in the Conway micro-diffusion technique for TVB-N. The calculation was done by the following formula:

Calculation

$$\text{TMA-N or TVB-N (mg/100g)} = \frac{\text{Amount of HCl used in titration} \times \text{Amount of ammonium nitrogen equivalent to 1 mL of 0.02N HCl} \times \text{Ratio of the amount of sample used to 100g muscle}}{W_s} \times 100$$
$$= \frac{(V_s - V_B) \times (N_{\text{HCl}} \times A_N) \times \{ (W_s \times M/100) + V_E \}}{W_s} \times 100$$

Where,

V_s = Titration volume of 0.02N HCl for sample extract (mL)

V_B = Titration volume of 0.02 N HCl for blank (mL)

N_{HCl} = Normality of HCl (= 0.02N x F, Factor of HCl)

A_N = Atomic weight of Nitrogen (14)

W_s = Weight of muscle sample (g)

M = Percentage moisture of muscle sample

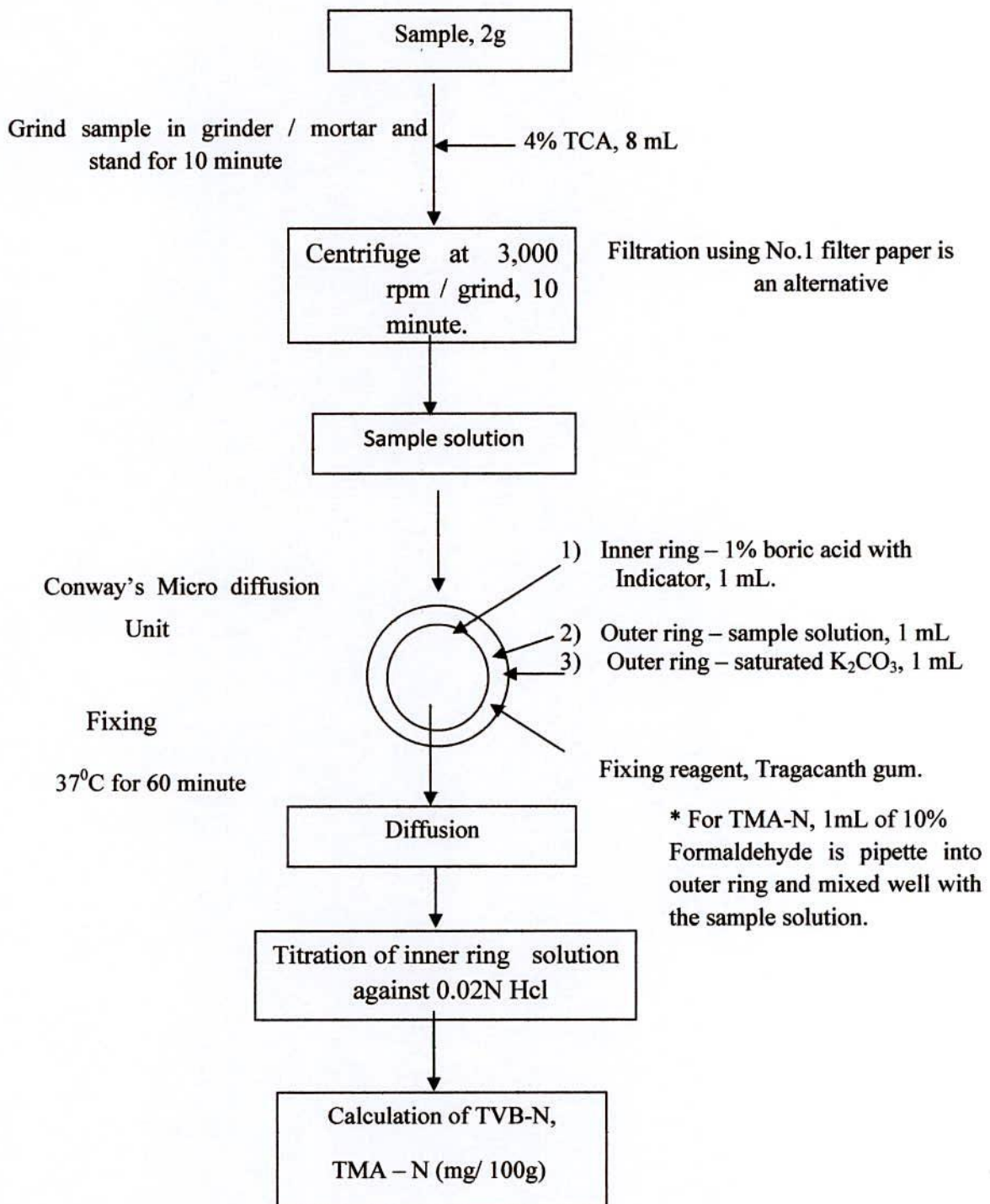
V_E = Volume of 4% TCA used in extraction.

3.7.3 Determination of Moisture

About 5 g of macerated shrimp sample was taken in porcelain basis of known weight. The sample was weighted accurately by using an electric balance and dried in an oven at 105°C for 24 hours. Drying, cooling, (in desiccators) and weighing were continued for a constant

final weight. The percentage of moisture content was calculated by using following equation:

$$\% \text{ of moisture} = \frac{\text{Weight (gm) of the sample after drying}}{\text{Weight (gm) of the sample before drying}}$$



Flowchart 4.2: TVB-N and TMA-N determination by Conway's Micro diffusion Technique.

CHAPTER IV

Results and Discussion

4.1 Results

4.1.1 Protein (%)

The protein content and protein loss of shrimp (*Macrobracium rosenbergii*) at different stages of value chain in Bagerhat District is presented in figure 4.1 to 4.9. The figure shows that protein content was decreased gradually from farm to factory receiving point in Bagerhat region. Grand protein loss was recorded at 19.82% from farm to factory receiving point in Bagerhat region (table 4.1).

At Fakirhat upazilla, protein contents were found 22.41%, 20.19%, 19.08% and 17.68% in value chain 01; 24.03%, 20.52%, 19.33%, and 18.79% in value chain 02; 23.07%, 21.44%, 20.32% and 19.17% in value chain 03 respectively in farm, faria, depot and factory receiving point (figure 4.1).

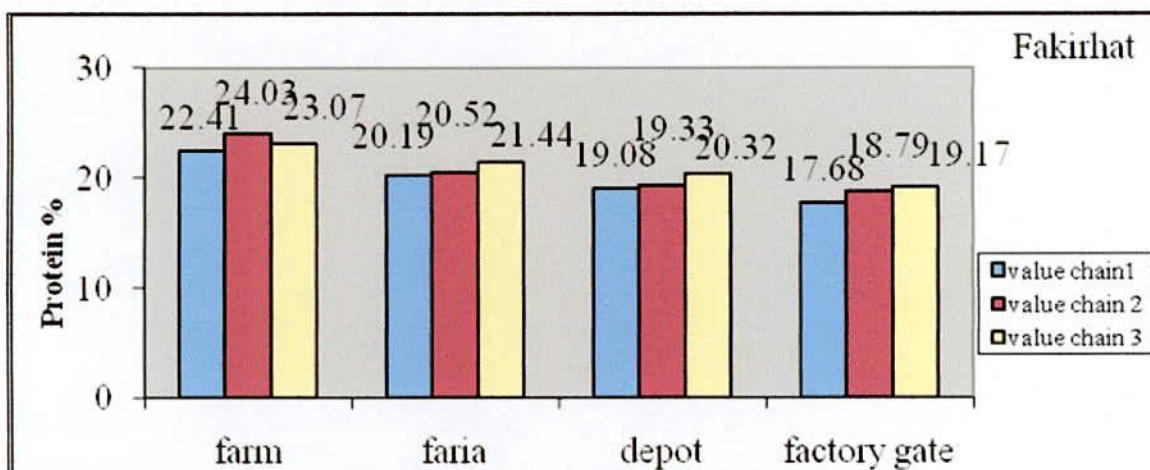


Figure 4.1: Protein contents at different stages of value chain in Fakirhat upazilla.

Protein losses of shrimp were observed at Fakirhat upazilla 9.91%, 5.50% and 7.34% in value chain 01; 14.16%, 5.80% and 2.79% in value chain 02; 7.07%, 5.22% and 5.66% in value chain 03; from farm – faria, faria - depot, depot - factory receiving point respectively (figure 4.2).

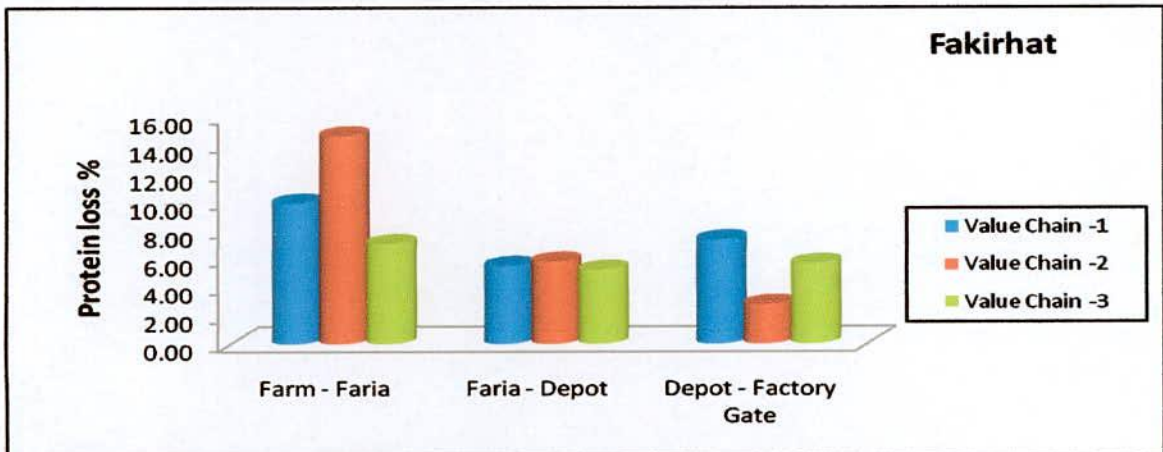


Figure 4.2: Protein loss at different stages of value chain in Fakirhat upazilla

At Bagerhat Sadar upazilla, protein contents were found 24.05%, 23.13%, 20.71% and 18.79% in value chain 01; 23.9%, 22.96%, 21.04%, and 19.34% in value chain 02; 23.44%, 22.02% 20.06% and 18.6% in value chain 03 respectively in farm, faria, depot and factory receiving point (figure 4.3).

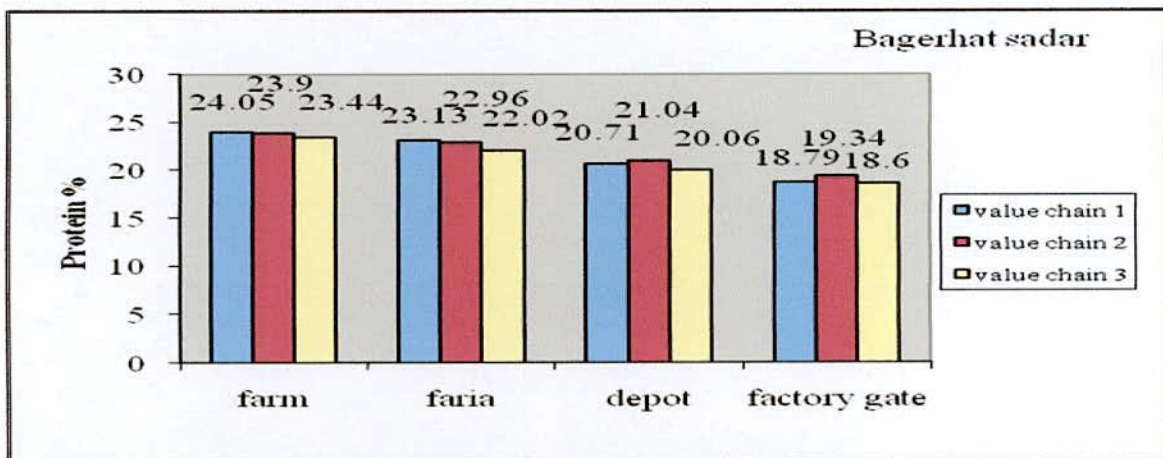


Figure 4.3: Protein contents at different stages of value chain in Bagerhat Sadar upazilla.

Protein losses of shrimp were observed at Bagerhat Sadar upazilla 3.83%, 10.46% and 9.27% in value chain 01; 3.93%, 8.36% and 8.08% in value chain 02; 6.06%, 8.90% and 7.28% in value chain 03; from farm – faria, faria - depot, depot - factory receiving point respectively (figure 4.4).

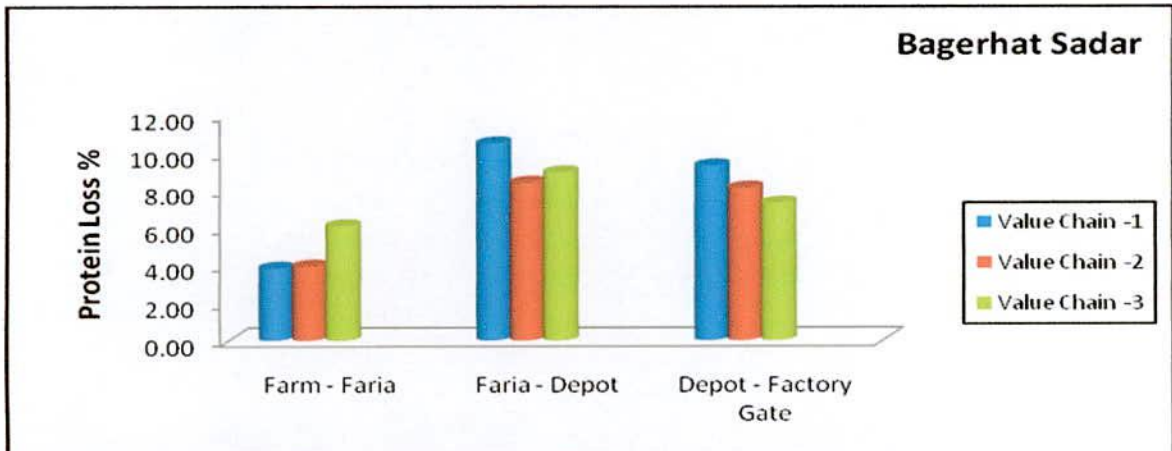


Figure 4.4: Protein loss at different stages of value chain in Bagerhat Sadar upazilla.

At Mollahat upazilla, protein contents were found 22.9%, 21.12%, 19.41% and 18.09% in value chain 01; 22.8%, 20.56%, 19.3%, and 18.09% in value chain 02; 23.33%, 21.17%, 19.86% and 18.06% in value chain 03 respectively in farm, faria, depot and factory receiving point (figure 4.5).

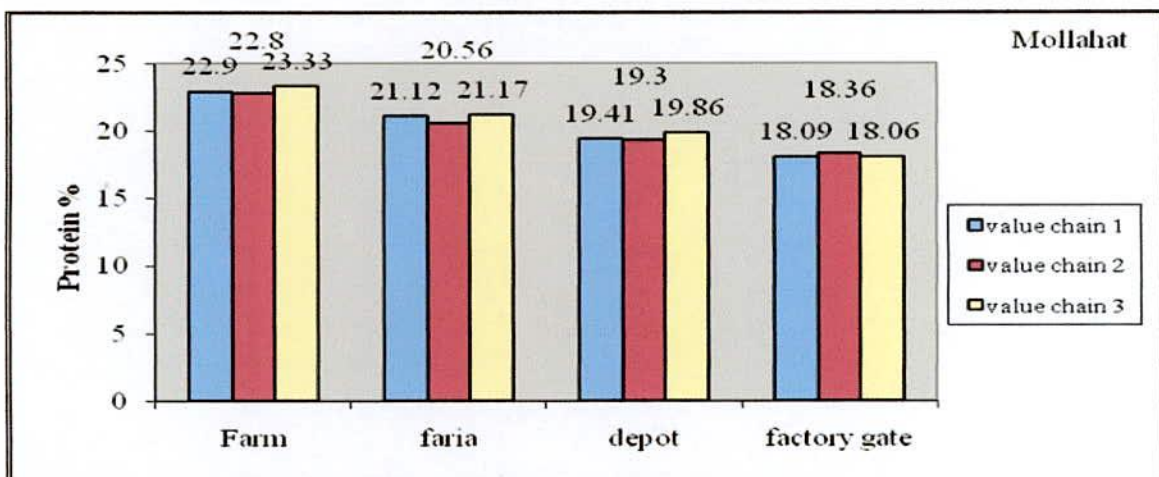


Figure 4.5: Protein contents at different stages of value chain in Mollahat upazilla.

Protein losses of shrimp were observed at Mollahat upazilla 7.77%, 8.10% and 6.80% in value chain 01; 9.82%, 6.13% and 4.87% in value chain 02; 9.26%, 6.19% and 9.06% in value chain 03; from farm – faria, faria - depot, depot - factory receiving point respectively (figure 4.6).

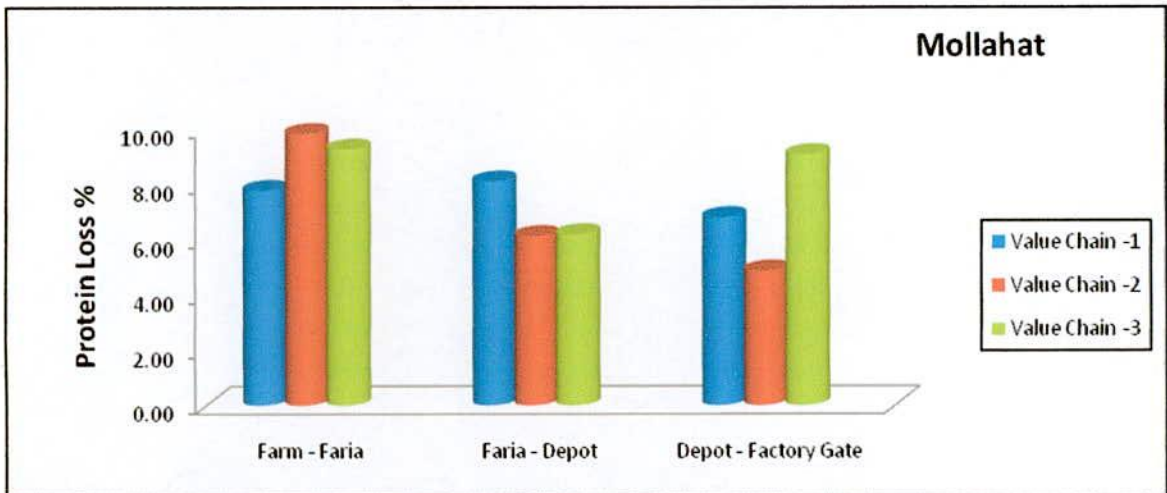


Figure 4.6: Protein loss at different stages of value chain in Mollahat upazilla.

At Sharankholla upazilla, protein contents were found 22.46%, 20.24%, 18.9% and 17.86% in value chain 01; 23.78%, 22.02%, 21.41%, and 18.99% in value chain 02; 23.25%, 22.07% 20.05% and 19.61% in value chain 03 respectively in farm, faria, depot and factory receiving point (figure 4.7).

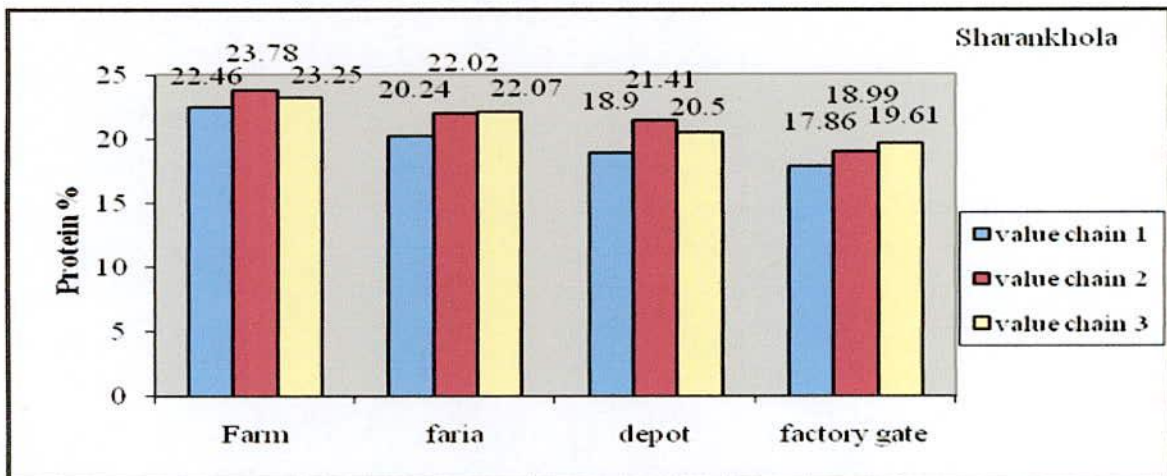


Figure 4.7: Protein contents at different stages of value chain in Sharankholla upazilla

Protein losses of shrimp were observed at Sharankholla upazilla 9.88%, 6.62% and 5.50% in value chain 01; 7.40%, 7.77% and 11.30% in value chain 02; 5.08%, 7.11% and 4.34% in value chain 03; from farm – faria, faria - depot, depot - factory receiving point respectively (figure 4.8).

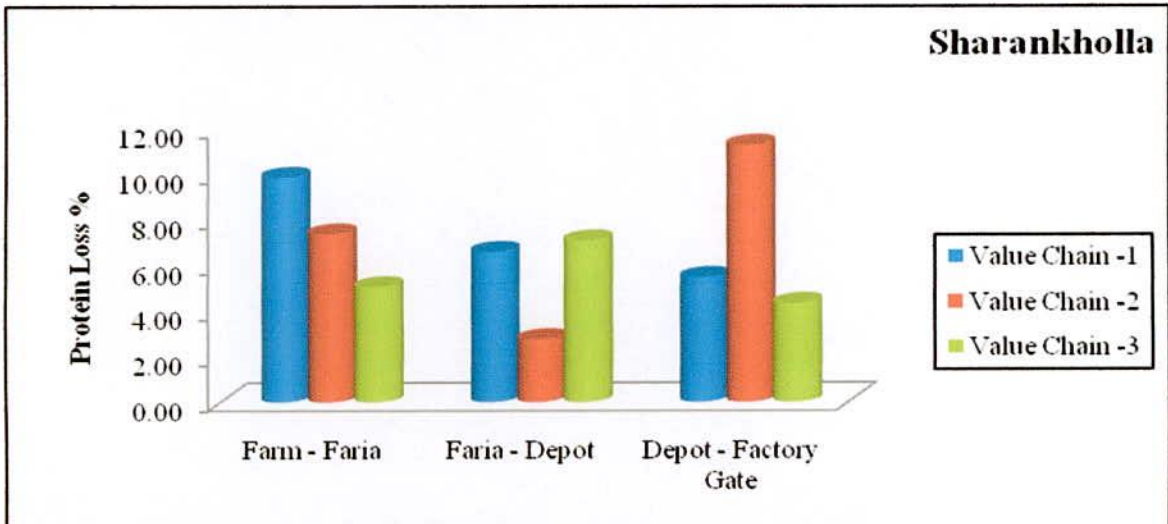


Figure 4.8: Protein loss at different stages of value chain in Sharankholla upazilla

Protein losses of shrimp were observed at Fakirhat upazilla 21.11%, 21.81% and 16.91% in value chain 01, value chain 02 and value chain 03 respectively from farm - factory receiving point. At Bagerhat Sadar upazilla Protein losses were 21.87%, 21.08% and 20.65% in value chain 01, value chain 02 and value chain 03 respectively from farm - factory receiving point. At Mollahat upazilla Protein losses were 21%, 19.47% and 22.59% in value chain 01, value chain 02 and value chain 03 respectively from farm - factory receiving point. At Sharankholla upazilla Protein losses were 20.48%, 20.14% and 15.66% in value chain 01, value chain 02 and value chain 03 respectively from farm - factory receiving point (Figure 4.9).



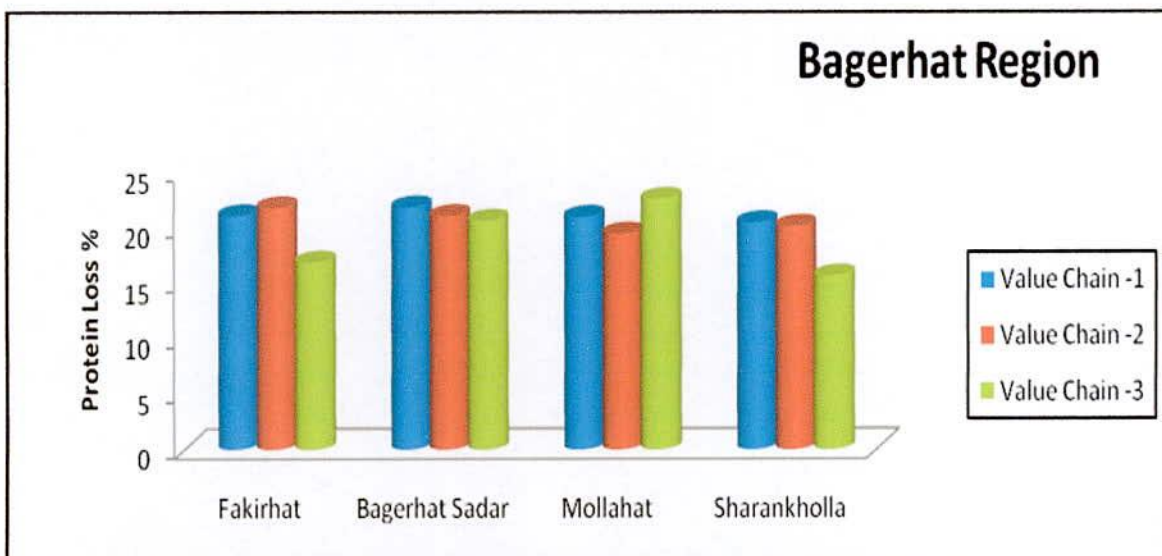


Figure 4.9: Protein loss at different value chain in Bagerhat Region

The average Protein loss of Shrimp were observed 19.94%, 20.55%, 20.03% and 18.74% respectively in Fakirhat upazilla, Bagerhat Sadar upazilla, Mollahat upazilla, and Sharankholla upazilla station from farm to factory receiving point. From farm to factory receiving point in Bagerhat region, Grand protein loss was recorded 19.82% (Table 4.1).

Table 4.1: Protein loss of Shrimp at different value chain in Bagerhat District.

Station	Protein (%) (Farm)	Protein (%) (Factory gate)	Protein Loss (%)	Grand Protein Loss (%)
Fakirhat	23.17	18.55	19.94	19.82
Bagerhat sadar	23.80	18.91	20.55	
Mollahat	23.01	18.17	20.03	
Sharankholla	23.16	18.82	18.74	

However there was no published data on protein loss of shrimp (*Macrobracium rosenbergii*) in the value chain of Bangladesh. In the same way no data was found internationally on protein loss of shrimp (*Macrobracium rosenbergii*).

All those results were presented within the acceptable limit in concurrence with result of others investigation. Kher-Un-Nisa and Razia Sultana [20], Begum et al. [24].

4.1.2 Total Volatile Base Nitrogen (TVB-N) in Shrimp (*Macrobracium rosenbergii*)

The results of TVB-N contents of shrimp (*Macrobracium rosenbergii*) at different stages of value chain in Bagerhat region are given in figure 4.10 to 4.13. The figure shows that TVB-N contents were increased gradually from farm to factory receiving point in Bagerhat region.

At the farm, when shrimp were fresh, TVB-N contents in shrimp were recorded 11 mg/100g, 11.15 mg/100g and 10.84 mg/100g. At Faria, The TVB-N value was observed 13.66 mg/100g, 12.93 mg/100g & 13.94 mg/100g. At depot, The TVB-N value was observed 15.94 mg/100g, 15.56 mg/100g & 15.98 mg/100g. At Factory receiving point, The TVB-N value was found 18.75 mg/100g, 19.34 mg/100g & 19.26 mg/100g respectively three different value chain in Fakirhat upazilla (Figure 4.10).

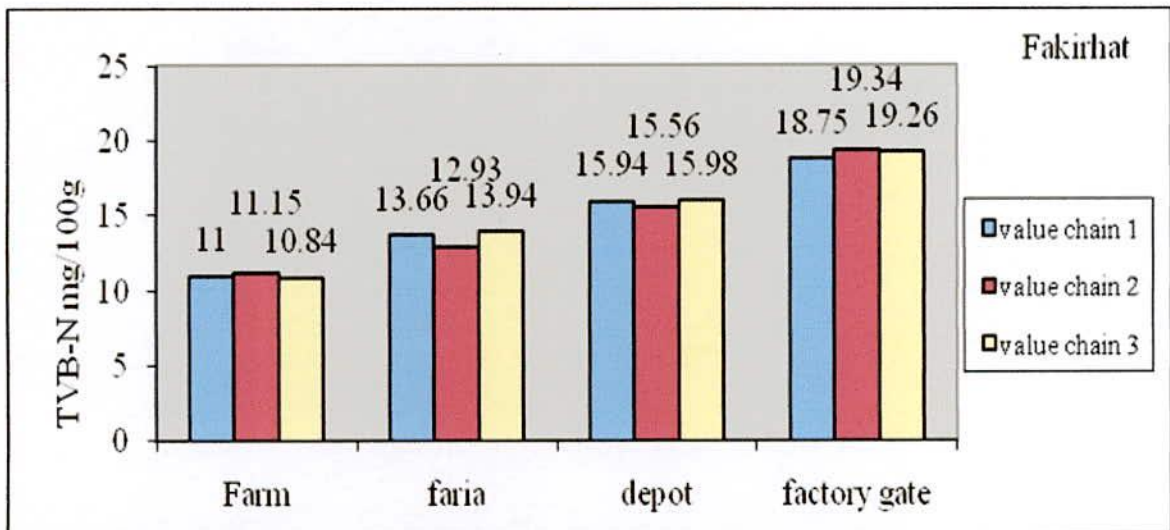


Figure 4.10: TVB-N contents at different stages of value chain in Fakirhat upazilla.

At the farm when shrimp were fresh, TVB-N contents in shrimp were recorded 11.1 mg/100g, 10.14 mg/100g and 10.89 mg/100g. At Faria, The TVB-N value was observed 13.66 mg/100g, 13.88 mg/100g & 13.53 mg/100g. At depot, The TVB-N value was observed 16.65 mg/100g, 16.59 mg/100g & 16.54 mg/100g. At Factory receiving point, The TVB-N value was found 18.72 mg/100g, 19.43 mg/100g & 19.29 mg/100g respectively three different value chain in Bagerhat Sadar upazilla (figure 4.11).

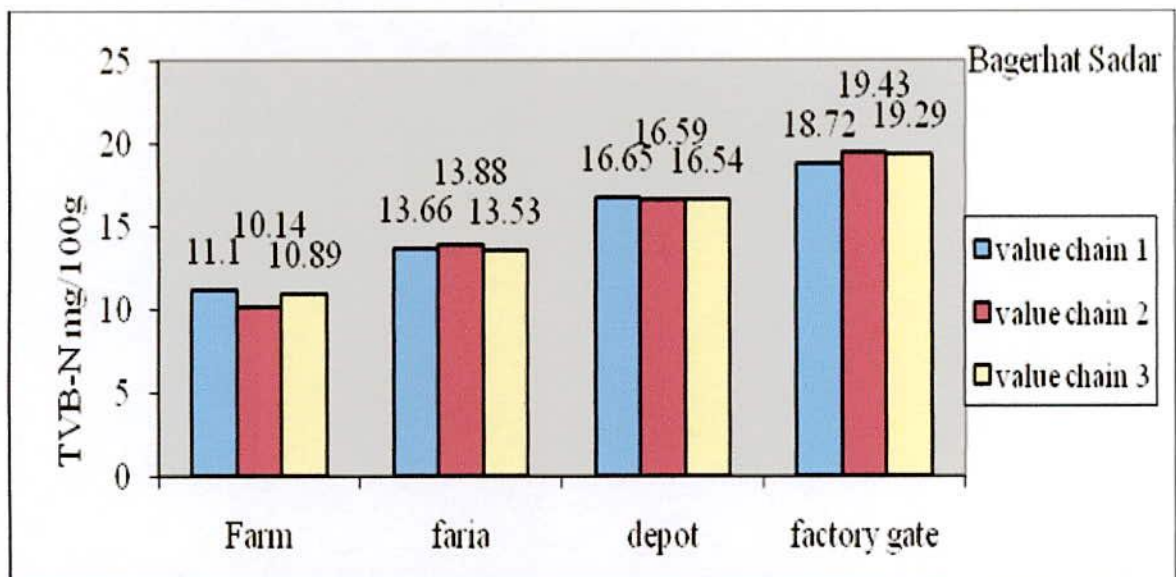


Figure 4.11: TVB-N contents at different stages of value chain in Bagerhat Sadar upazilla.

At the farm when shrimp were fresh, TVB-N contents in shrimp were recorded 11.14 mg/100g, 11.07 mg/100g and 10.61 mg/100g. At Faria, The TVB-N value was observed 13.65 mg/100g, 13.79 mg/100g & 12.13 mg/100g. At depot, The TVB-N value was observed 16.73 mg/100g, 16.04 mg/100g & 15.12 mg/100g. At Factory receiving point, The TVB-N value was found 18.45 mg/100g, 18.52 mg/100g & 16.77 mg/100g respectively three different value chain in Mollahat upazilla (figure 4.12).

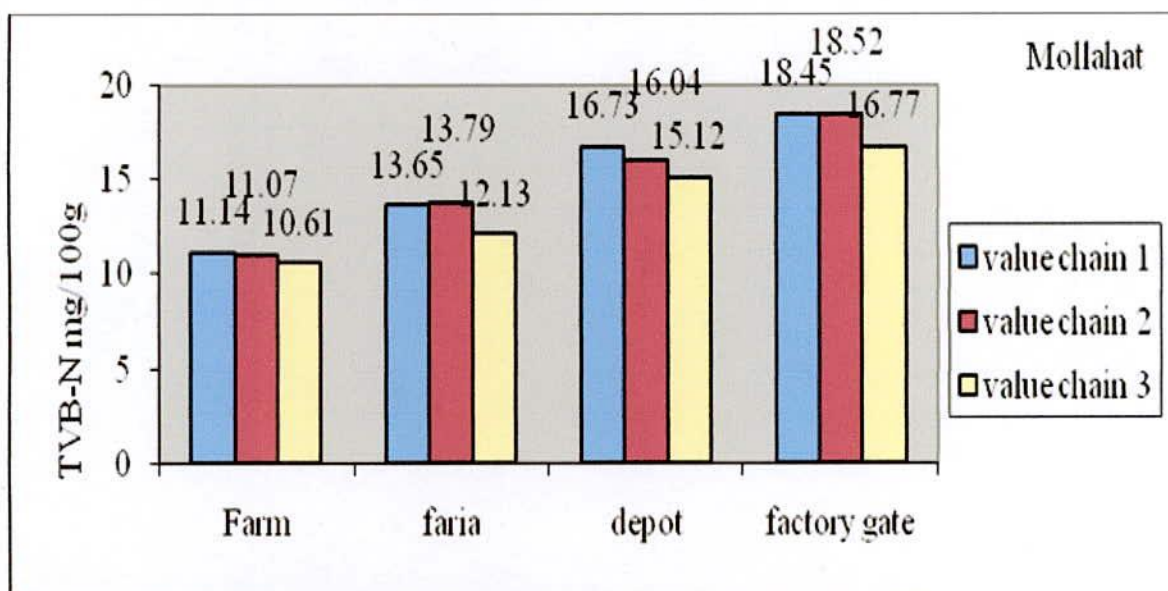


Figure 4.12: TVB-N contents at different stages of value chain in Mollahat upazilla.

At the farm when shrimp were fresh, TVB-N contents in shrimp were recorded 11.12 mg/100g, 10.63 mg/100g and 11.13 mg/100g. At Faria, The TVB-N value was observed 13.39 mg/100g, 12.93 mg/100g & 13.95 mg/100g. At depot, The TVB-N value was observed 16.71 mg/100g, 15.85 mg/100g & 16.69 mg/100g. At Factory receiving point, The TVB-N value was found 19.51 mg/100g, 18.51 mg/100g & 19.54 mg/100g respectively three different value chain in Sharankholla upazilla (Figure 4.13).

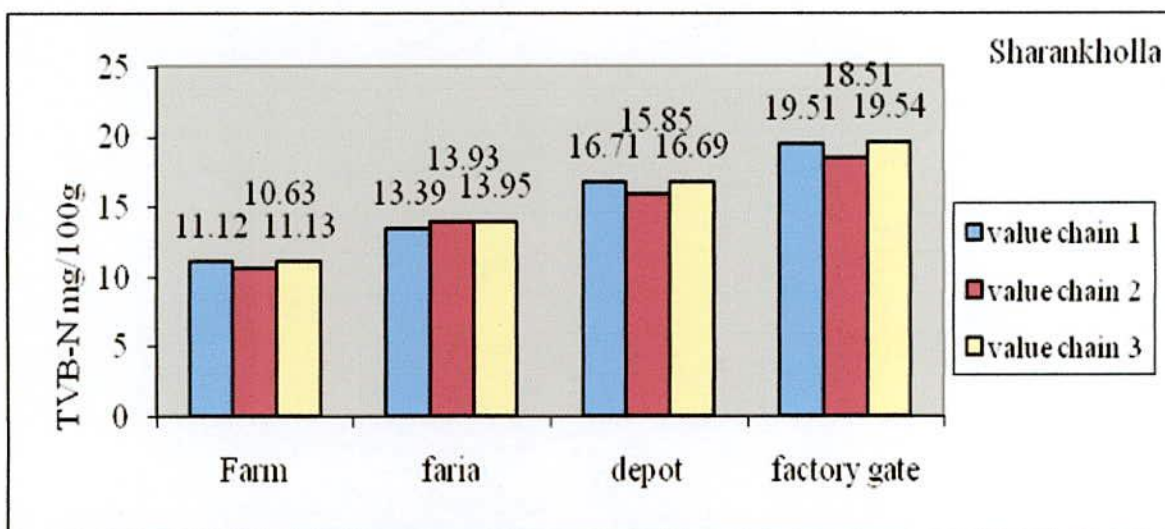


Figure 4.13: TVB-N contents at different stages of value chain in Sharankholla upazilla.

All those results were presented within the acceptable limit in concurrence with result of others investigation. Pushparajan N and P. Soundarapandian [25], Jayasinghe P.S et al. [26], Siang N. C and Kim L. L [23], Ali. M et al. [27].

The value of TVB-N was increasing in every value chain over passing of time. The low value of TVB-N initially was an indication quality of fresh shrimp while the high value may be due to action of autolysis enzymes and spoilage bacteria. Shewan and Ehenberg [13].

4.1.3 Trimethylamine nitrogen (TMA-N) in Shrimp (*Macrobracium rosenbergii*)

The results of TMA contents of shrimp (*Macrobracium rosenbergii*) at different stages of value chain in Bagerhat region are given in figure 4.14 to 4.17 the figure shows that TMA contents were increased gradually from farm to factory receiving point in Bagerhat region.

At the farm when shrimp were fresh, TMA contents in shrimp were recorded 8.25 mg/100g, 8.36 mg/100g and 8.13 mg/100g. At Faria, The TMA value was observed 10.93 mg/100g, 10.34 mg/100g & 11.16 mg/100g. At depot, The TMA value was observed 13.28 mg/100g, 12.96 mg/100g & 13.32 mg/100g. At Factory receiving point, The TMA value

was found 16.06 mg/100g, 15.44 mg/100g & 16.51 mg/100g respectively three different value chain in Fakirhat upazilla (Figure 4.14).

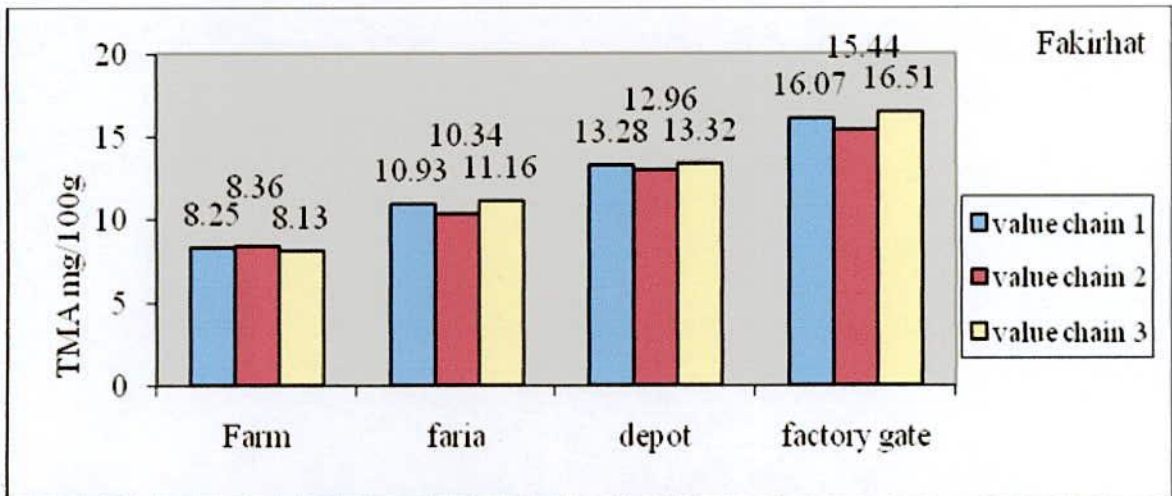


Figure 4.14 : TMA contents at different stages of value chain in Fakirhat upazilla.

At the farm when shrimp were fresh, TMA contents in shrimp were recorded 8.33mg/100g, 7.61 mg/100g and 8.17 mg/100g. At Faria, The TMA value was observed 10.93 mg/100g, 11.1 mg/100g & 10.82 mg/100g. At depot, The TMA value was observed 13.88 mg/100g, 13.83 mg/100g & 13.79 mg/100g. At Factory receiving point, The TMA value was found 16.05 mg/100g, 16.65 mg/100g & 16.34 mg/100g respectively three different value chain in Bagerhat Sadar upazilla (figure 4.15).

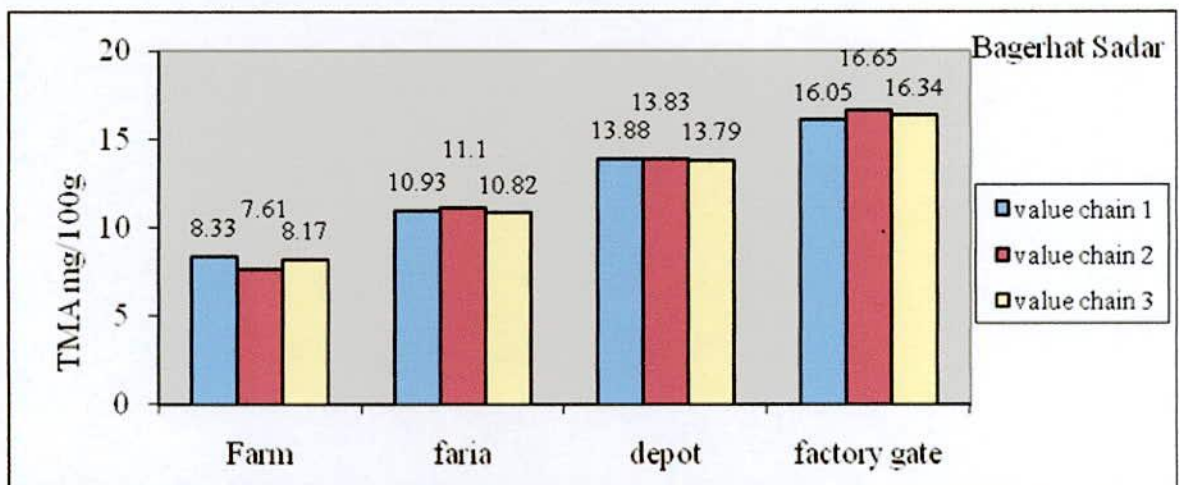


Figure 4.15: TMA contents at different stages of value chain in Bagerhat Sadar upazilla.

At the farm when shrimp were fresh, TMA contents in shrimp were recorded 8.35mg/100g, 8.31 mg/100g and 07.96 mg/100g. At Faria, The TMA value was observed 10.92 mg/100g, 11.03 mg/100g & 9.7 mg/100g. At depot, The TMA value was observed 13.94 mg/100g, 13.37 mg/100g & 12.6 mg/100g. At Factory receiving point, The TMA value was found 15.1 mg/100g, 15.87 mg/100g & 14.37 mg/100g respectively three different value chain in Mollahat upazilla (figure 4.16).

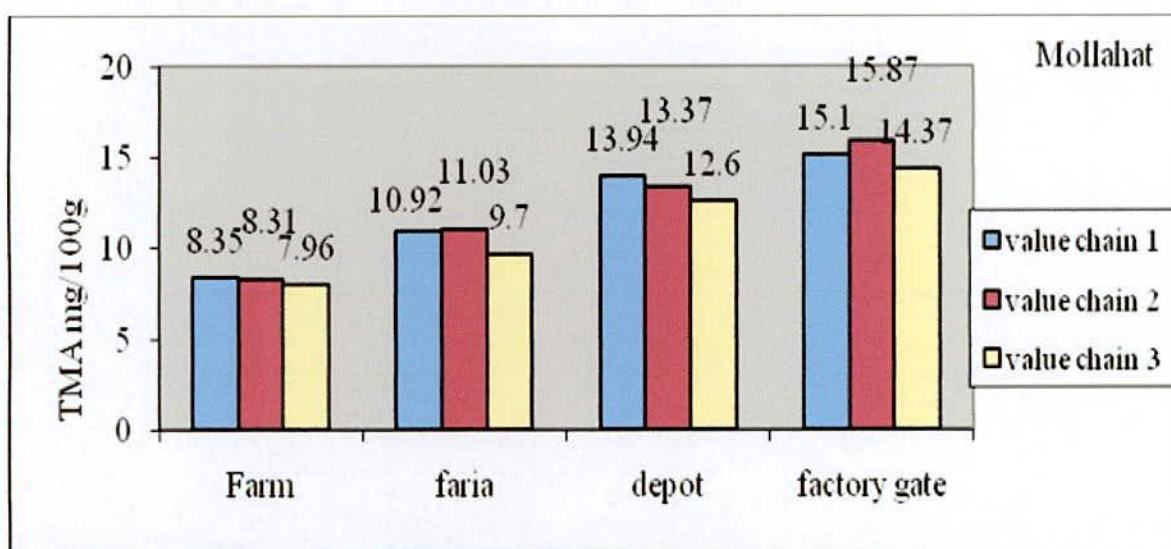


Figure 4.16: TMA contents at different stages of value chain in Mollahat upazilla.

At the farm when shrimp were fresh, TMA contents in shrimp were recorded 8.34 mg/100g, 7.37 mg/100g and 8.35 mg/100g. At Faria, The TMA value was observed 10.71 mg/100g, 11.15 mg/100g & 11.16 mg/100g. At depot, The TMA value was observed 13.93 mg/100g, 13.21 mg/100g & 13.91 mg/100g. At Factory receiving point, The TMA value was found 16.73 mg/100g, 14.5 mg/100g & 16.75 mg/100g respectively three different value chain in Sharankholla upazilla (figure 4.17).

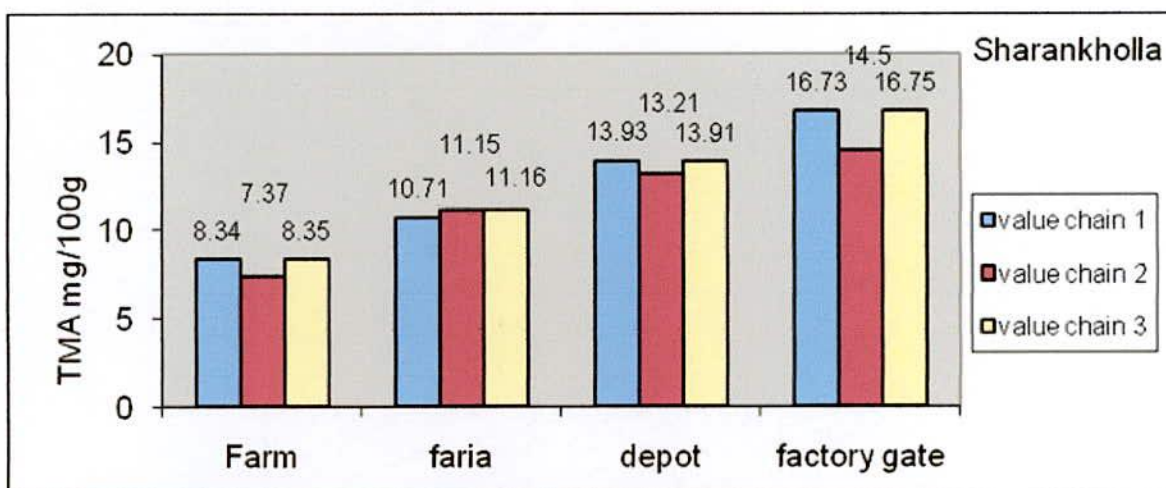


Figure 4.17: TMA contents at different stages of value chain in Sharankholla upazilla.

All those results were presented within the acceptable limit in concurrence with result of others investigation Pushparajan, N. and P. Soundarapandian. [25], Jayasinghe P.S. et al. [26], Siang N. C. and Kim L. L [23], Ali, M. et al. [27].

4.2 Discussion

4.2.1 Protein (%) of shrimp (*Macrobracium rosenbergii*)

From this present study it was found that in fresher sample of farm, the protein value is higher than others of value chain. The value of protein content of shrimp (*Penaeus penicillatus*) was 15.5% to 21% in wet weight method, Kher-un-Nisa and Razia Sultana [16] which is as similarity of present study.

Kher-un-Nisa and Razia Sultana [20] reported that protein increased slightly from 17.2 % (stage I) to 18.0 % (stage IV) showing no variation in the proximate composition during maturation, significant variation throughout the maturation process. Showing a significant increasing trend during maturation whereas, protein increased from initial level of 15.90 % (stage I) to 18.40 % (stage IV). The increase in the protein content was not so significant in

the midgut gland as that of the ovary during different stages of sexual maturation, this increase in the protein in the ovary may be attributed to several biochemical processes.

The proximate analysis of raw and smoked catfish was presented Protein (17.8-18.6%), Fat (3.9 – 4.30%), and Moisture contents (78.2 - 79.4%) of fresh samples respectively subjected to different value chain, Omojowo et al. [28].

The proximate composition of both non-added and formalin added prawn samples are presented. The initial moisture, protein, lipid and ash contents of non-added prawn were 78.67%, 18.53%, 1.34% and 0.94% respectively, Begum et al. [24].

Ali et al. [27] reported that *that* Mean values of various body constituents in different fish species collected from a brackish water pond, Protein (wet wt.) amount of Mori-Rahu Hybrid (21.007%), *Labeo rohita* (18.490%) *Cyprinus carpio* (24.690%), *Thalla- Rahu* Hybrid (19.573%) *Cirrhinus mrigala* (18.975%), *H. molitrix* (20.217%) *Catla catla* (19.000%) were found. This 18.57±1.87% of grand protein loss occurred due to 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. To minimize the protein loss to ensure good icing, good handling, maintain the temperature and better transportation.

However there was no published data on protein loss on shrimp in the value chain of Bangladesh. In the same way no data was found internationally on protein loss of shrimp (*Macrobracium rosenbergii*).

4.2.2 Total Volatile Base Nitrogen (TVB-N)

From this present study it was found that the value of TVB-N was increased by passing the time. At the farm when shrimp were fresh, TVB-N contents in shrimp were recorded 11 mg/100g, 10.71 mg/100g, 10.94 mg/100g and 10.96 mg/100g respectively in Fakirhat, Bagerhat Sadar, Mollahat & Sharankholla station. After passing of time at factory receiving point, the value of TVB-N was found 19.12 mg/100g, 19.15 mg/100g, 17.91 mg/100g and 19.19 mg/100g respectively in Fakirhat, Bagerhat Sadar, Mollahat & Sharankholla station. This result is an agreement with previous study, Pushparajan N and P. Soundarapandian [25], Jayasinghe P.S et al. [26], Siang N. C. and Kim L. L [23], Ali et al. [27].

The level of total volatile nitrogenous bases increases after spoilage begins, both enzymically and bacterially, 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. J. M [13]. The low value of TVB-N initially is an indication of quality of fresh shrimp or fish while the high value may be due to action of autolytic enzymes and spoilage bacteria which might have passed their lag phase, Adebona [29]. The volatile bases, in determination method, are aerated or distilled off from a midley alkaline fish extract, collected in standard acid and measured by titration.

During iced storage, the TVB-N content in Bele (*Glossogobius giuris*) and puti (*Puntius stigma*) were 11.45 mg/100g and 17.84 mg/100g respectively as fish were treated as highly acceptable. Rubbi et al. K.Sakkaravarthi, G. Sankar, A. Elavarasi and K. Ramamoorthy

[30] recommended TVB-N levels of 10 mg/100g or less for fresh fish, 20 – 30 mg/100g for beginning of spoilage and over 30 mg/100g for spoiled fish. Azam. K, et.al, [14] recorded a very high TVB-N value (17.03 mg-N) initially for tilapia. Similarly Mlae P. et.al. [15] also observed very high value of TVB-N (51.8 mg). However, the TVB-N value of the present study agrees with the recommendation of K.Sakkaravarthi, G. Sankar, A. Elavarasi and K. Ramamoorthy [30].

4.2.3 Trimethylamine Nitrogen (TMA)

From this present study it was found that the value of TMA was increased by passing the time. TMA was found at small amount which indicate that Shrimp be freshness. In the value chain, passing of time value of TMA was increased rapidly by activities of bacteria and enzyme. At the farm when shrimp were fresh, TMA contents in shrimp were recorded 8.25 mg/100g, 8.04 mg/100g, 8.21 mg/100g and 8.02 mg/100g respectively in Fakirhat, Bagerhat Sadar, Mollahat & Sharankholla station. After passing of time at factory receiving point, the value of TMA was found 16.01 mg/100g, 16.35 mg/100g, 15.11 mg/100g and 15.99 mg/100g respectively in Fakirhat, Bagerhat Sadar, Mollahat and Sharankholla station. This result is an agreement with previous study Pushparajan N and P. Soundarapandian [25], Jayasinghe P.S. et al. [26], Siang N. C and Kim L. L [23], Ali M.Y et al. [19], [33], Azam. K [34].

TMA is, because of its universal production in all shrimp and fish species, an excellent indicator for the onset of spoilage and for the different stages of spoilage. The fishy odor is produced when TMA reacts with fat in the muscle of shrimp/fish, Davies and Gill [31]. In

the course of spoilage, many off-odours are produced by bacteria, indicating the onset and development of spoilage. More TMA is produced from TMAO by bacterial action than by fish tissue enzymes, TMA produced by both these two actions is responsible for the 'fish odor' during spoilage, Jones [32]. In the bacterial reduction of TMAO to TMA, the participation of cytochromes as electron carriers together with the enzyme TMAO reductase has been suggested. At least 94% of TMA in spoiling fish originates from TMAO.

Beatty and Gibbons (1937) suggested a TMA content of 4–6 mg/100g as the critical value for the edibility of fish, while Connell [10] recommended 10 – 25 mg/100g for human consumption. There is also wide variation in critical values suggested for individual species, like 5 – 7 mg/100g for herring and 1 –5 mg/100g for haddock, Castell and Triggs, [35]. Though, on the rejection, the level of TMA studied in the present investigation was below the limits suggested by Connell [10]. The suggestion appears to be applicable to fish studied. However, the TMA value determined does not seem to be useful as an index of freshness.

As TMA is produced by bacteria, and bacterial activity is directly affected by temperature so temperature gradients in individual fish can affect TMA levels. Horner [36], Horie and Sekine [37] also found a sudden increase in TMA (> 10 mg %) to be concurrent with the onset of bacterial purification.

CHAPTER V

Conclusion

The thesis address the feasibility studies of quality changes of value chain of shrimp cultivated in different area of Bagerhat region. The investigation involves measurement of Protein, TMA & TVB-N.

- ★ The samples were collected from forty eight point of different area of Bagerhat district. At Fakirhat upazilla, protein contents were found 22.41%, 20.19%, 19.08% and 17.68% in value chain 01; 24.03%, 20.52%, 19.33% and 18.79% in value chain 02; 23.07%, 21.44% 20.32% and 19.17% in value chain 03 respectively in farm, faria, depot and factory receiving point. Protein losses of shrimp were observed at Fakirhat upazilla 9.91%, 5.50% and 7.34% in value chain 01; 14.16%, 5.80% and 2.79% in value chain 02; 7.07%, 5.22% and 5.66% in value chain 03; from farm to faria, faria to depot, depot to factory receiving point respectively.
- ★ At Bagerhat Sadar upazilla, protein contents were found 24.05%, 23.13%, 20.71% and 18.79% in value chain 01; 23.9%, 22.96%, 21.04%, and 19.34% in value chain 02; 23.44%, 22.02% 20.06% and 18.6% in value chain 03 respectively in farm, faria, depot and factory receiving point. Protein losses of shrimp were observed at Bagerhat Sadar upazilla 3.83%, 10.46% and 9.27% in value chain 01; 3.93%, 8.36% and 8.08% in value chain 02; 6.06%, 8.90% and 7.28% in value chain 03; from farm to faria, faria to depot, depot to factory receiving point respectively.

★ At Mollahat upazilla, protein contents were found 22.9%, 21.12%, 19.41% and 18.09% in value chain 01; 22.8%, 20.56%, 19.3%, and 18.09% in value chain 02; 23.33%, 21.17% 19.86% and 18.06% in value chain 03 respectively in farm, faria, depot and factory receiving point. Protein losses of shrimp were observed at Mollahat upazilla 7.77%, 8.10% and 6.80% in value chain 01; 9.82%, 6.13% and 4.87% in value chain 02; 9.26%, 6.19% and 9.06% in value chain 03; from farm to faria, faria to depot, depot to factory receiving point respectively

★ At Sharankholla upazilla, protein contents were found 22.46%, 20.24%, 18.9% and 17.86% in value chain 01; 23.78%, 22.02%, 21.41% and 18.99% in value chain 02; 23.25%, 22.07% 20.05% and 19.61% in value chain 03 respectively in farm, faria, depot and factory receiving point. Protein losses of shrimp were observed at Sharankholla upazilla 9.88%, 6.62% and 5.50% in value chain 01; 7.40%, 7.77% and 11.30% in value chain 02; 5.08%, 7.11% and 4.34% in value chain 03; from farm to faria, faria to depot, depot to factory receiving point respectively.

★ Protein losses of shrimp were observed at Fakirhat upazilla 21.11%, 21.81% and 16.91% in value chain 01, value chain 02 and value chain 03 respectively from farm - factory receiving point. At Bagerhat Sadar upazilla Protein losses were 21.87%, 21.08% and 20.65% in value chain 01, value chain 02 and value chain 03 respectively from farm to factory receiving point. At Mollahat upazilla Protein losses were 21%, 19.47% and 22.59% in value chain 01, value chain 02 and value chain 03 respectively from farm to factory receiving point. At Sharankholla upazilla Protein losses were 20.48%, 20.14% and 15.66% in value chain 01, value chain 02 and value chain 03 respectively from farm to factory receiving point.

★ The average Protein loss of shrimp were observed 19.94%, 20.55%, 20.03% and 18.74% respectively in Fakirhat upazilla, Bagerhat Sadar upazilla, Mollahat upazilla and Sharankholla upazilla station from farm to factory receiving point. From farm to factory receiving point in Bagerhat region, Grand protein loss was recorded 19.82%.

★ The values of TVB-N content in deferent value chain of shrimp were found in the range of 10.84 mg/100g to 19.34 mg/100g in Fakirhat upazilla, 10.14 mg/100g to 19.43 mg/100g in Bagerhat Sadar upazilla, 10.61 mg/100g to 18.52 mg/100g in Mollahat upazilla, 10.63 mg/100g to 19.54 mg/100g in Sharankholla upazilla. The values of TVB –N content of shrimp were found within standard limit.

★ The values of TMA content in deferent value chain of shrimp were found in the range of 8.13 mg/100g to 16.51 mg/100g in Fakirhat upazilla, 7.61 mg/100g to 16.65 mg/100g in Bagerhat Sadar upazilla, 7.96 mg/100g to 15.87 mg/100g in Mollahat upazilla, 7.37 mg/100g to 16.75 mg/100g in Sharankholla upazilla. The values of TMA content of shrimp were found within standard limit.

The results obtained from this investigation will immense to help the shrimp's exporters to export improved quality shrimps. The country will be able to export safe and quality products and earned more foreign currency. It will be helpful to develop our economy and the products will be made a room in the global market.

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APPENDIX

Appendix-1: Protein (%), TVB-N and TMA-N contents of Galda (*Macrobracium rosenbergii*) at different stage of value chain of Fakirhat Upazilla in Bagerhat region.

Station	Value chain	Sample No	Protein (%)	TVB-N mg/100g	TMA-N mg/100g
Fakirhat Upazilla	Farm	01	22.41	11	8.25
		02	24.03	11.15	8.36
		03	23.07	10.84	8.13
	Faria	01	20.19	13.66	10.93
		02	20.52	12.93	10.34
		03	21.44	13.94	11.16
	Depot/Agent	01	19.08	15.94	13.28
		02	19.33	15.56	12.96
		03	20.32	15.98	13.32
	Factory receiving point	01	17.68	18.75	16.07
		02	18.79	19.34	15.44
		03	19.17	19.26	16.51

Appendix-02: Protein (%), TVB-N and TMA-N contents of Galda (*Macrobracium rosenbergii*) at different stage of value chain of Bagerhat Sadar Upazilla in Bagerhat region.

Station	Value chain	Sample No	Protein (%)	TVB-N mg/100g	TMA-N mg/100g
Bagerhat Sadar Upazilla	Farm	01	24.05	11.1	8.33
		02	23.9	10.14	7.61
		03	23.44	10.89	8.17
	Faria	01	23.13	13.66	10.93
		02	22.96	13.88	11.1
		03	22.02	13.53	10.82
	Depot/Agent	01	20.71	16.65	13.88
		02	21.04	16.59	13.83
		03	20.06	16.54	13.79
	Factory receiving point	01	18.79	18.72	16.05
		02	19.34	19.43	16.65
		03	18.6	19.29	16.34

Appendix-3: Protein (%), TVB-N and TMA-N contents of Galda (*Macrobracium rosenbergii*) at different stage of value chain of Mollahat Upazilla in Bagerhat region.

Station	Value chain	Sample No	Protein (%)	TVB-N mg/100g	TMA-N mg/100g
Mollahat Upazilla	Farm	01	22.9	11.14	8.35
		02	22.8	11.07	8.31
		03	23.33	10.61	7.96
	Faria	01	21.12	13.65	10.92
		02	20.56	13.79	11.03
		03	21.17	12.13	9.7
	Depot/Agent	01	19.41	16.73	13.94
		02	19.3	16.04	13.37
		03	19.86	15.12	12.6
	Factory receiving point	01	18.09	18.45	15.1
		02	18.36	18.52	15.87
		03	18.06	16.77	14.37

Appendix-04: Protein (%), TVB-N and TMA-N contents of Galda (*Macrobracium rosenbergii*) at different stage of value chain of Sharankholla Upazilla in Bagerhat region.

Station	Value chain	Sample No	Protein (%)	TVB-N mg/100g	TMA-N mg/100g
Sharankholla Upazilla	Farm	01	22.46	11.12	8.34
		02	23.78	10.63	7.37
		03	23.25	11.13	8.35
	Faria	01	20.24	13.39	10.71
		02	22.02	13.93	11.15
		03	22.07	13.95	11.16
	Depot/Agent	01	18.9	16.71	13.93
		02	21.41	15.85	13.21
		03	20.5	16.69	13.91
	Factory receiving point	01	17.86	19.51	16.73
		02	18.99	18.51	14.5
		03	19.61	19.54	16.75