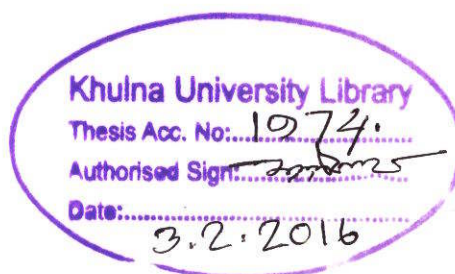


**Organoleptic, biochemical and microbiological
qualities of Shrimp (*Penaeus monodon*) at each
point of marketing channel in Satkhira district of
Bangladesh.**



Fisheries and Marine Resource Technology Discipline

Khulna University

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By
Examination Roll no: MS 010624

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**THIS PROJECT THESIS IS SUBMITTED TO THE FISHERIES AND
MARINE RESOURCE TECHNOLOGY DISCIPLINE IN PARTIAL
FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
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TECHNOLOGY.**

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DECLARATION

The results submitted in this thesis are entirely of the candidate's own investigations and no part of the results has been accepted for any degree, nor it is being concurrently submitted for any degree

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ABSTRACT

Investigation was carried out on the quality aspects of shrimp (*Penaeus monodon*) in the distribution channel of Satkhira district in Bangladesh during September to November in 2004. Organoleptic, biochemical and microbiological (SPC, TC, FC, *Salmonella sp* and *Vibrio sp*) qualities were assessed at each major point of distribution channel (Gher, Agent, Depot, and Processing Plant). Quality changes were studied during normal practice ((NP) and controlled study (CS). For normal practice no ice was used but in controlled study 1:1 icing ratio was maintained during preservation and transportation of the shrimp. In both cases shrimp was acceptable organoleptically at all distribution points. The average organoleptic score ranged between 6.27 to 8.92 in NP and 7.61 to 8.97 in CS. The TVB-N content varied between 2.56 mgN/100g to 6.21 mgN/100g in NP and 2.32 mgN/100g to 5.87 mgN/100g in CS. Lowest amount of TVB-N was found in Gher and highest in Processing Plant. TMA-N value increased slightly from Gher to Processing plant and the value ranged between 2.43 to 3.62 mgN/100gm in NP and 2.65 to 3.73 in CS. The highest SPC was found in processing plant in both practices which were 5.93 cfu/gm (log₁₀ value) in NP and 5.91 cfu/gm (log₁₀ value) in CS. Total coliform counts varied between 27 to 53 MPN/gm for shrimp collected in normal practice while relatively lower Total Coliform counts were observed in the shrimp collected for controlled study (15 to 35 MPN /gm). Faecal Coliform was present at each point of distribution channel. Average Faecal Coliform counts varied between 4.1 to 7.4 in NP and 4.8 to 6.3 in CS. It was absent in Processing plant as observed in controlled study. However the counts were within the acceptable limit of 10 MPN/ gm. *Salmonella* and *Vibrio sp* were not detected at any point of distribution channel in both cases.

CONTENTS



<u>Title</u>	<u>Page No</u>
ACKNOWLEDGEMENT	
ABSTRACT	1
LIST OF TABLES	4
LIST OF FIGURES	5
ABBREVIATIONS	6
LIST OF FLOWCHARTS	7
1. CHAPTER ONE : INTRODUCTION	8-10
2. Chapter Two: Review Of Literature	11-13
2.1 Organoleptic Evaluation	11
2.2 Biochemical Assessment	11
2.3 Microbiological Analysis	13
3. Chapter Three: Materials and Methods	14-31
3.1 Selection of Species	14
3.2 Study Area	14
3.3 Sample Collection	14
3.4 Sampling Method	14
3.5 Techniques for Analysis of Samples	14
3.5.1 Organoleptic Assessment	15
3.5.2 Biochemical Assessment	17
3.5.3 Microbiological Assessment	22
4. Chapter Four: Results	32-40
4.1 Quality Changes of Shrimp during Normal Practice	32-33
4.1.1 Organoleptic Quality	32
4.1.2 Biochemical Quality	32
4.1.3 Microbiological Quality	33
4.2 Quality Changes of Shrimp during Controlled Study	35-37
4.2.1 Organoleptic Quality	35
4.2.2 Biochemical Quality	35
4.2.3 Microbiological Quality	35
4.3 Comparative Study of Quality Changes Between Normal Practice and Controlled Study	38-39
4.3.1 Organoleptic	38

4.3.2 Biochemical	38
4.3.3 Microbiological	39
5. Chapter Five: Discussion	41-48
5.1 Normal Practice and Controlled Study	41
5.1.1 Organoleptic Changes	41
5.1.2 Biochemical Changes	42
5.1.3 Microbiological Changes	44
Conclusion	48
6. Chapter Six: References	49-54
7. Chapter Seven: Appendices	55-59

LIST OF TABLES

Title	PageNo.
Table 1 : Organoleptic score sheet for fresh fish (Shewan and Ehrenberg, 1977)	15
Table 2 : Modified organoleptic score sheet for Shrimp (<i>Penaeus monodon</i>)...	16

LIST OF FIGURES

<u>Figure</u>	<u>Name of the Figure</u>	<u>Page #</u>
01	Organoleptic score of shrimp during NP	34
02	TVB-N content of shrimp at different distribution point during NP	34
03	TMA-N content of shrimp at different distribution point during NP	34
04	SPC content of shrimp at different distribution point during NP	34
05	TC of Shrimp at different distribution point during NP	34
06	FC of Shrimp at different distribution point during NP	34
07	Organoleptic score of shrimp during CS	37
08	TVB-N content of shrimp during CS	37
09	TMA-N content of shrimp during CS	37
10	TMA-N content of shrimp during CS	37
11	TC of shrimp during CS	37
12	FC of Shrimp during CS	37
13	Comparative study of organoleptic score between NP and CS ...	40
14	Comparative study of TVB-N between NP and CS	40
15	Comparative study of TMA-N between NP and CS	40
16	Comparative study of SPC between NP and CS	40
17	Comparative study of TC between NP and CS	40
18	Comparative study of FC between NP and CS	40

ABBREVIATIONS

NP	= Normal Practice
CS	= Controlled Study
BGA	= Brilliant Green Agar
BGLB	= Brilliant Green Lactose Bile Broth
BSA	= Bismuth Sulphate Agar
EMB	= Eosin Methylene Blue Agar
EU	= European Union
FC	= Faecal Coliform
HACCP	= Hazard Analysis Critical Control Point
HEA	= Hekton Enteric Agar
KIA	= Kligler's Iron Agar
LSTB	= Lauryl Sulfate Tryptose Broth
P.Plant	= Processing Plant
SPC	= Standard Plate Count
TC	= Total Coliform
TCA	= Trichloro Acetic Acid
TCBSA	= Triosulphate Citrate Bile Salts Sucrose Agar
TMA	= Trimethyl Amine
TMA-N	= Trimethyl Amine Nitrogen
TMAO	= Trimethyl Amine Oxygen
TVB	= Total Volatile Bases
TVB-N	= Total Volatile Base Nitrogen
XLD	= Xylose Lysine Deoxycholate

LIST OF FLOWCHARTS

<u>Serial #</u>	<u>Title</u>	<u>Page No</u>
1	TVB-N and TMA-N determination by Conway's Microdiffusion Technique	21
2	The enumeration of Standard Plate Count (SPC)	23
3	The quantification Total Coliform (TC)	25
4	The quantification Faecal Coliform (FC)	27
5	The isolation and identification of <i>Salmonella</i> sp.	29
6	The isolation and detection of <i>Vibrio cholerae</i>	31

1. INTRODUCTION

Fisheries sector provides 63% of national animal protein consumption and 5.33% of its GDP, which is about 20 % of the total agriculture sector. The role of fishery in earning foreign exchange is also commendable. It contributes 5.1% to foreign exchange earnings through export. But the lion portion of this export comes from frozen shrimp. Frozen shrimp contributed above 80% of total foreign earnings in fisheries sector in fiscal year 2003-2004. In 2002-2003 out of total 2041.6 crore Tk. export earning in fisheries sector, 1719.9 crore Tk. (88.58% of total) was earned by exporting frozen shrimp (DoF, 2004).

Specially the southern part of Bangladesh plays a vital role in production of shrimp. In 2003-2004 fiscal year greater Khulna region (Satkhira, Khulna and Bagerhat) provided 70.53% of total foreign earnings in fish and shrimp products. Though Satkhira, Khulna and Bagerhat are the main region for shrimp culture yet Satkhira district plays the main role in this regard. Only Satkhira district has 47,566 ha of shrimp farming area from which about 13,726 MT shrimp was harvested in 2003-2004 (DoF, 2005). The main cultivated species in this region are *Penaeus monodon*, more commonly referred to as Bagda and *Macrobrachium rosenbergii*, commonly known as Galda.

Bagda (*Penaeus monodon*) 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 ($20^{\circ} - 32^{\circ} \text{C}$) fast growing individuals which reach marketable size in about 4 to 5 months, has high nutritional value and has carnivorous feeding habit thus being an excellent species for polyculture. *Penaeus monodon* has high demand in domestic and international market as well.

At present, shrimp is being exported to foreign countries as a frozen product. But to reach the final product, it maintains a distribution channel from the harvesting place to processing plant i.e. the post harvest trade flow from farmer to depot to agent to processing plant. The depot holders purchase the shrimp from the farm which is locally known as Gher. The depot holder sell the shrimp to agents and finally the agents sell it to the processing plant. In the processing plant it is processed in several steps, viz. sorting, grading, freezing, packing etc. and thus become ready for export. Quality of shrimp depends largely at every points of distribution channel. But the major areas of quality

control are gher, depot, agent and processing plant. Every year Bangladesh experiences a huge quantitative and qualitative loss due to unscientific handling in these stages.

Shrimp farmers in Bangladesh do not get proper returns of their catch because of qualitative and quantitative loss. Quantitative losses include shrimp which are disposed due to low commercial value while qualitative losses occur through spoilage. Quantitative losses accounted to 6 % of the total marine catch and 2 % of the inland catch (Coulter and Disney, 1988).

In the distribution channel from Gher to processing plant fairly long period of time is elapsed. Thus preservation of shrimp in appropriate storage condition must be available to keep the quality better. Quality means the state of excellence of a particular product in terms of appearance, shape, colour, taste and competitiveness in price . Quality control is a critical element in shrimp distribution channel. Hazard Analysis Critical Control Point (HACCP) system is introduced in Bangladesh to ensure better quality of frozen food.

In Satkhira region, the shrimp farmers usually use bamboo basket, wooden box insulated with hoglamat or similar materials for carrying shrimp . In some cases they use various kinds of aquatic weeds like water hyacinth, banana leaf where ice is not available. Normally insufficient amount of ice is used in the basket to preserve the shrimp. If proper icing ratio is maintained the quality would be improved. In most cases unhygienic condition exist in the farm(Gher) or Depot . Quality of shrimp losses due to unhygienic handling procedure and handling materials.(DoF, 2005).

In the international fish market Bangladesh is sharing and earning a substantial amount of foreign exchange. It is estimated that about Tk. 10,000 crore (US\$1.5b) foreign currency per annum is possible from shrimp by the year 2008 if all the potential shrimp area are taken under scientific shrimp culture and management (BFFEA, 2004). It proves that there is more potential for shrimp culture. But to reach the target, only culture is not enough, quality control is one of the main things in this context.

Quality control of the fish and fish products are like two sides of a coin. One can not go without other. Quality, in general, means the wholesomeness of or the state of excellence of a particular product in term of its appearance, shape, colour, taste and competitiveness

in price of the buyers. Concisely, quality means the fulfillment of the customer's requirements.

To achieve good quality shrimp technical information on each stage of distribution channel is necessary. In Bangladesh less works have been done on the quality of shrimp at different points of distribution channel. Alam (2004) and Naher (2004) made two investigations on the quality changes of shrimp (*Penaeus monodon*) at different points of distribution channel in Khulna and Bagerhat district. But no work of such type was done in Satkhira district though it is the main area of shrimp culture. Considering this, the present study was aimed at assessing the quality of shrimp at different distribution points (Gher, Agent, Depot and Processing Plant) in Satkhira district. Quality changes were studied during normal practice and controlled study. For normal practice no ice was used but in controlled study 1:1 icing ratio was maintained during preservation and transportation of the shrimp. However, the specific objectives of the study were:

- To study the organoleptic quality of shrimp (*Penaeus monodon*) at different points of distribution channel in Satkhira during normal practice and controlled study.
- To study the biochemical quality changes of shrimp (*Penaeus monodon*) at different points of distribution channel in Satkhira during normal practice and controlled study.
- To study the microbiological quality changes of shrimp (*Penaeus monodon*) at different points of distribution channel in Satkhira during normal practice and controlled study.

2. LITERATURE REVIEW

Research works on quality changes in organoleptic, biochemical & microbiological aspects associated with fish and shrimp during normal temperature and controlled condition were reviewed. A good amount of experimental works have been carried out on spoilage of temperate and cold-water marine species of commercial importance in different parts of the world. Published papers on iced storage experiments have been reviewed and attempts have been made to draw general conclusions about the quality changes in the storage lives of fish in different broad categories (Curran *et. al.*, 1986). These reviews confirmed that there is wide variation between species and the same species can have the different quality changes under different condition.

There are several same reports on the storage stability of shrimp in ice (Shamsad *et. al.*, 1990; Yamagata and Low, 1995). It is generally agreed that at 0°C fish and shrimp become unacceptable organoleptically, biochemically & microbiologically within two weeks (Cobb *et. al.*, 1976).

2.1 Organoleptic assessment

Organoleptic methods are universally accepted for estimating the quality of fish (Shewan and Ehrenberg, 1977). The basic method of evaluating the quality is by sensory tasting which is based on the characterization and differentiation of the various sensory perceptible factors such as appearance and odour of the raw fish and odour, flavor, texture etc. of the cooked fish, which affect the sensory judgement of the buyers and consumers.

At the Torry Research Station, Aberdeen, UK, a good technique has been evolved for organoleptic evaluation of fish and fishery products. This method is consisting of using a trained taste panel, to assess the main organoleptic characters (Shewan and Ehrenberg, 1977). An useful method for assessing overall acceptability of food Products was successfully developed by Paryam and Pilgrim (1975).

2.2 Biochemical Tests

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 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 (Botta *et. al.* 1989).

Certain chemical changes in spoiling shrimp appear to run parallel with changes in odour , 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 (Sato, 1960) 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.

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 (Connell and Howgate, 1986).

TVB and TMA measurement is probably the oldest chemical method to assess fish spoilage used as an index of spoilage (Shewan and Ehrenberg, 1977). TVB and TMA-N is the indicator of the Nitrogen content of shrimp.

The TMA could be used as an index of freshness. During spoilage TMAO is reduced by bacteria to TMA (Connel, 1975) reported that TMA could serve as an index of spoilage for marine fish. TMA contents fluctuates with seasons as well as within a single species.

Different workers have suggested a number of method for determining TVB . Among the commonly used methods, the Conway micro diffusion technique is the most suitable and applied in this work for the determination of TVB 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,1971).

2.3 Microbiological Tests

For the bacterial spoilage the most commonly used method is total viable count. Rahman (1980) has found that bacterial counts were valuable as a measure of degree of freshness of fish. Bacterial counting using total viable count is satisfactorily going in research laboratories through out the world. With some unavoidable difficulties this method is more or less suitable for routine testing of the quality of fish (Wittfozel, 1960). The bacterial flora and its relation to fish spoilage has been studied Liston(1980).

Since bacteria are the main agents of spoilage, techniques have been developed to use their numbers as an index of Shrimp quality (Lerke *et. al.*, 1967) were the investigators to study the methods. They developed a technique for the direct counting of bacteria, being able to differentiate between spoiled and unspoiled shrimp on number alone. More precise methods estimating bacterial numbers have been developed; yet these methods tend to be time consuming expensive.

Microbiological quality indices, namely standard plate count, Presence of *Vibrio cholera*, *Salmonella species* and faecal coliforms , were determined at specific points in the preparations of frozen shrimp(Christofer, 1978) .

The location of harvesting (Huss,1988; Love,1988) , time of year (Love, 1988) , method of catching (Botta *et. al.*, 1987) and the manner of handling seriously affect the degree of excellence. The bacteriological loads of farms raised prawns at the time of harvest and at different points during processing were determined(Lobrerria *et. al.*,1990) .

Large numbers of *Vibrio cholera* must usually be ingested to cause cholera, thus problems often occur when poor handling and inadequate refrigeration have allowed the organisms to multiply (Young, 1992) .Inorder to compete in the international market shrimp exporters must meet microbiological standards set by importing countries (Dangla, 1984) . Problems faced by Asian shrimp producers have been reviewed (Summer, *et. al.*, 1982).



3. MATERIALS AND METHODS

3.1 Selection of species

Shrimp (*Penaeus monodon*) was selected as the test specimen. It is the main export item in fisheries sector. Moreover, it is cultured most abundantly in Satkhira district. The size ranging from 20 to 30 grade was used for quality assessment.

3.2 Study Area

The study was conducted at Munsigong thana in Satkhira district of Bangladesh. It is the southern part of Satkhira district and very near to Sundarbans Mangrove Forest. The region being very close to the Bay of Bengal, saline water is available here to support shrimp farming in a large scale.

3.3 Sample Collection

The shrimp (*Penaeus monodon*) was collected from four distribution points i.e. Gher, Depot, Agent and Processing Plant in September, 2004. The shrimp were caught from the Gher (enclosed shrimp farm) situated in Munshigong, Satkhira. The next set of shrimp were collected from Depot, Agent and Processing Plant.

3.4 Sampling Method

Total Two Kg shrimp of 20 to 30 grade were purchased from the harvesting point (Gher). All the shrimp were not of same grades. Most of the shrimp had roughly 20 grades. At this point shrimp were placed in two polythene packets, one was for normal practice and another was iced at a ratio of 1:1. Equal amount of shrimp were taken for normal practice and controlled study. Five shrimp from each basket were separately kept into two polythene bags for microbiological and biochemical analysis. Following this method shrimp was taken to each point of distribution point (depots, agents and processing plants) where the usual practices were done and the samples were collected in separate packets. From each point of distribution channel shrimp were brought to the Quality Control laboratory of Fisheries & Marine Resource Technology Discipline, Khulna University, Khulna.

3.5 Sample Analysis

Organoleptic, Biochemical (TVB-N, TMA-N) and Microbiological (SPC, FC, TC, *Salmonella* & *Vibrio*) quality were assessed in the laboratory. Both samples (normal

practices and controlled study) were analyzed. Quality assessment for each step was carried out in triplicate.

3.5.1 Organoleptic Quality Assessment

For both practices the test specimen was presented directly to the taste panel of 5 members. Score sheet, specially prepared for this purpose was supplied to each of the panel members. A panel of five members performed details organoleptic tests. For both sample, organoleptic evaluations was carried out according to procedure of Shewan and Ehrenberg (1977) with slight modification for the shrimp (Table-2). Five quality factors were considered to assess the organoleptic quality.

Table-1 Organoleptic score sheet for fresh fin fish (Shewan and Ehrenberg, 1977)

<i>Eye</i>	Score	<i>Pupil</i>	Score
Characteristics		Characteristics	
Bright, clear and dull	10	Slight and transparent	10
Slight dull	9	Slight dull/ misty	8
Moderately dull	8	Dull/slight milky white	7
Dull	7	Watery colour/whitish	6
Slightly sunken	6	Slightly misty/milky white	4
Moderately sunken	4	Pale white	2
Sunken	2	Extremely white/more white	0
Completely sunken	0		
Gill	Score	Body surface	Score
Characteristics		Characteristics	
Bright red	10	Shining bright	10
Slight dull red / slight pale	9	Slight loss of brightness	8
Slight pale/pale	8	Loss of brightness	6
Moderately pale/yellowish	7	Slight dull	4
Pale /whitish	6	Dull and slight reddish of tail end	2
Brownish / greyish	4	Completely dull	0
Dark Colour	2		
Blackish	0		

Belly wall		Texture	
Characteristics	Score	Characteristics	Score
Normal fresh	10	Firm and Elastic	10
Slightly discoloration	8	Rigor stage	8
More discoloration	6	Just post rigor stage	6
Slightly digested	4	Slightly soft	4
More digested	2	Moderately soft	2
Extremely digested	0	Soft and loose	0
Odour		Overall Acceptability	
Characteristics	Score	Characteristics	Score
Fresh odour	10		
Sweetly odour	8	Highly acceptable (HA)	10
Slightly spoilage odour	7	Acceptable (A)	9
Moderately spoilage odour	6	Moderately acceptable (MA)	8
Spoilage odour	5	Just acceptable (JA)	6
Slightly off odour	4	Just Unacceptable (JU)	5
Moderately off odour	3	Unacceptable (U)	3
Off odour	2	More unacceptable (MU)	1
Extremely off odour	0	Extremely unacceptable	0

Table-2 Organoleptic score sheet for shrimp modified from Shewan and Ehrenberg (1977).

Odour characteristics	Score	Carapace color characteristics	Score
Fresh Odour	10	Blackish (Fresh)	10
Sweetly Odour	8	Moderately Greenish	8
Slightly Spoilage Odour	7	Slightly Greenish	6
Moderately Spoilage	6	Slightly Darken	4
Spoilage Odour	5	Moderately Darken	2
Slightly Off Odour	4	Darken	0
Moderately Off Odour	3		
Off Odour	2		
Extremely Off Odour	0		

Carapace texture characteristics	Score	Eye characteristics	Score
Hard	10	Bright & Transparent	10
Slightly Soft	7	Slightly Dull	7
Moderately Soft	5	Moderately Dull	4
Very Soft	3	Dull & Opaque	2
Very Soft	0	Fully Dull & Opaque	0

Shell color characteristics	Score	Overall acceptability characteristics	Score range
Yellowish (no spot)	10	Highly Acceptable	8.5-10.0
Slight Loss of Brightness	7	Acceptable	6.5-8.5
Loss of Brightness & Opaque	5	Moderately Acceptable	4.5-6.4
Slightly redish	3	Just Acceptable	3.6-4.5
Redish (spotted)	0	Just Unacceptable	2.6-3.5
		Unacceptable	1.5-2.5
		More Unacceptable	0.5-1.4

3.5.2 Biochemical Assessment

TVB-N and TMA-N were determined according to procedure stated in the manual of Siang and Kim (1992).

Solution and Reagents

Inner ring solution -1 % boric solution containing indicator. 10 g boric acid were weighed into a one litre 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

Bromcresol green (BCG 0.01 gm) and methyl red 0.02gm were dissolved into 10ml of ethanol.

0.02 Hydrochloric Acid, HCl

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

Standard Potassium Carbonate Solution

60gm of K_2CO_3 were weighed and then 50ml of distilled water was added. It was boiled gently for 10 mins. 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 gm Of TCA were dissolved in 1 L of distilled water.

Neutralized 10% formaldehyde Solution

10 gm of $MgCO_3$ were added to 100ml of formalin (35% formaldehyde Solution) and shaken in order to neutralize the activity of formalin. Then diluted the filtrate 3 times with distilled water.

EXTRACT PREPARATION

The extract of shrimp was prepared by mixing 2gm 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 mins at ambient temperature with occasional grinding. Then, it was filtered through filter paper (whatman no. 1) The filtered solution was kept in Mackrerty bottle and was labelled. 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)

3.5.2.1 TVB-N Determination

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 pipetted into the inner ring of each unit. Into the outer ring of each unit, was pipetted 1 ml of the sample extract. 1 ml of Saturated K_2CO_3 solution was carefully pipetted 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°C for 45 mins. 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 (50ml) 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.

3.5.2.2 Determination of Trimethyl Amine Nitrogen (TMA-N)

Trimethyl Amine 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

TMA-N or TVB-N (mg/100g) = Amount of HCl used in titration × Amount of ammonium nitrogen equivalent to 1 ml of 0.02N HCl × Ratio of the amount of sample used to 100g muscle

$$= \frac{(V_s - V_B) \times (N_{HCl} \times A_N) \times \{ (W_s \times M/100) + V_E \}}{W_s} \times 100$$

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 × F, Factor of HCl)

A_N = Atomic weight of Nitrogen (14)

W_s = Weight of muscle sample (g)

M = Percentage moisture of muscle sample

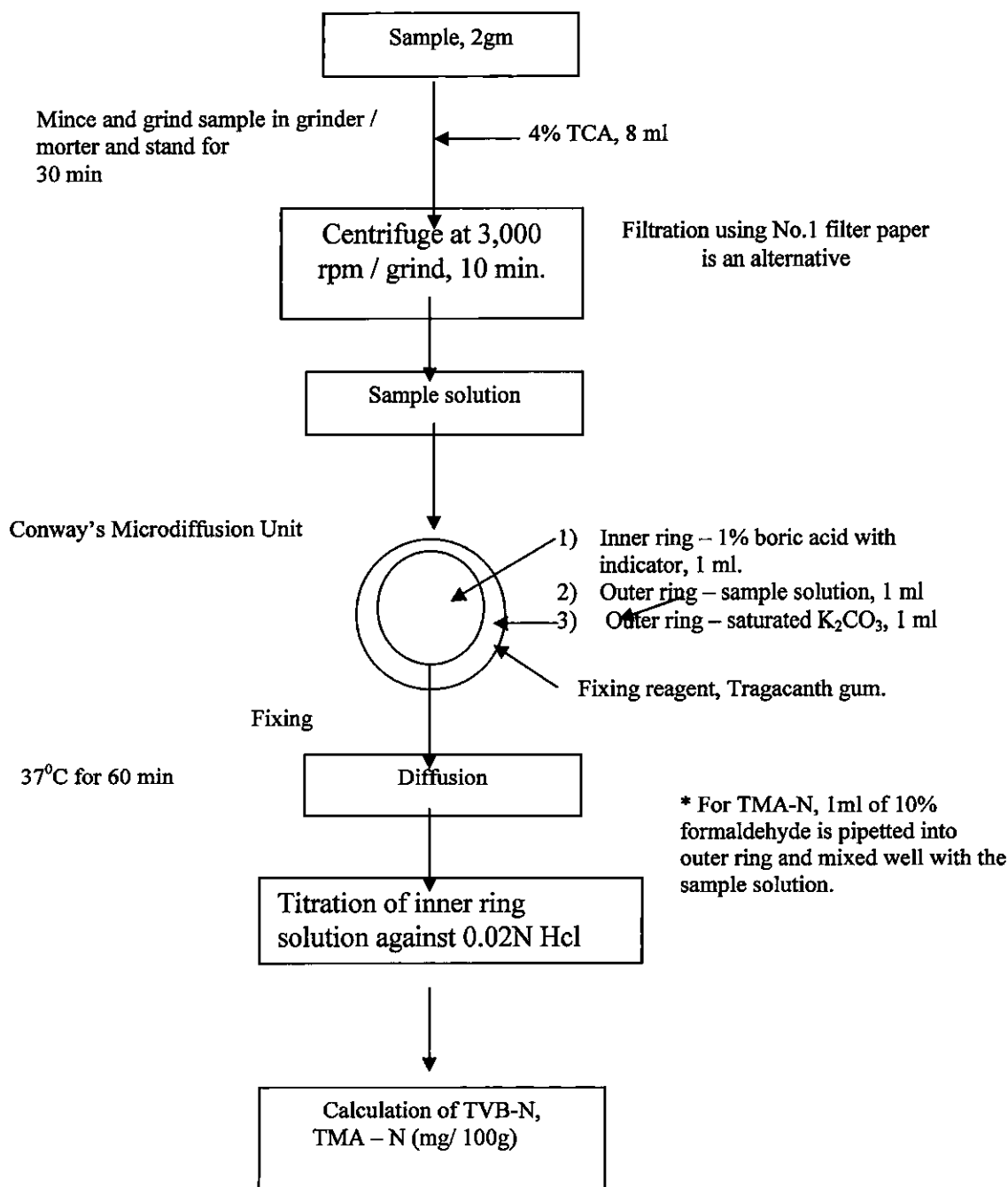
V_E = Volume of 4% TCA used in extraction

Moisture

Procedure

About 5 gm of macerated shrimp sample was taken in porcelain basin of known weight. The sample was weighed accurately by using an electric balance and dried in an oven at 105°C for 24 hours. Drying, cooling, (in a desiccator) 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 1: TVB-N and TMA-N determination by Conway's Microdiffusion Technique**

3.5.3 Microbiological Analysis

Microbial analyses followed the procedures of International Commission on Microbiological Specifications for Foods (ICMSF,1988).

3.5.3.1 Standard Plate Count (SPC)

Culture Media

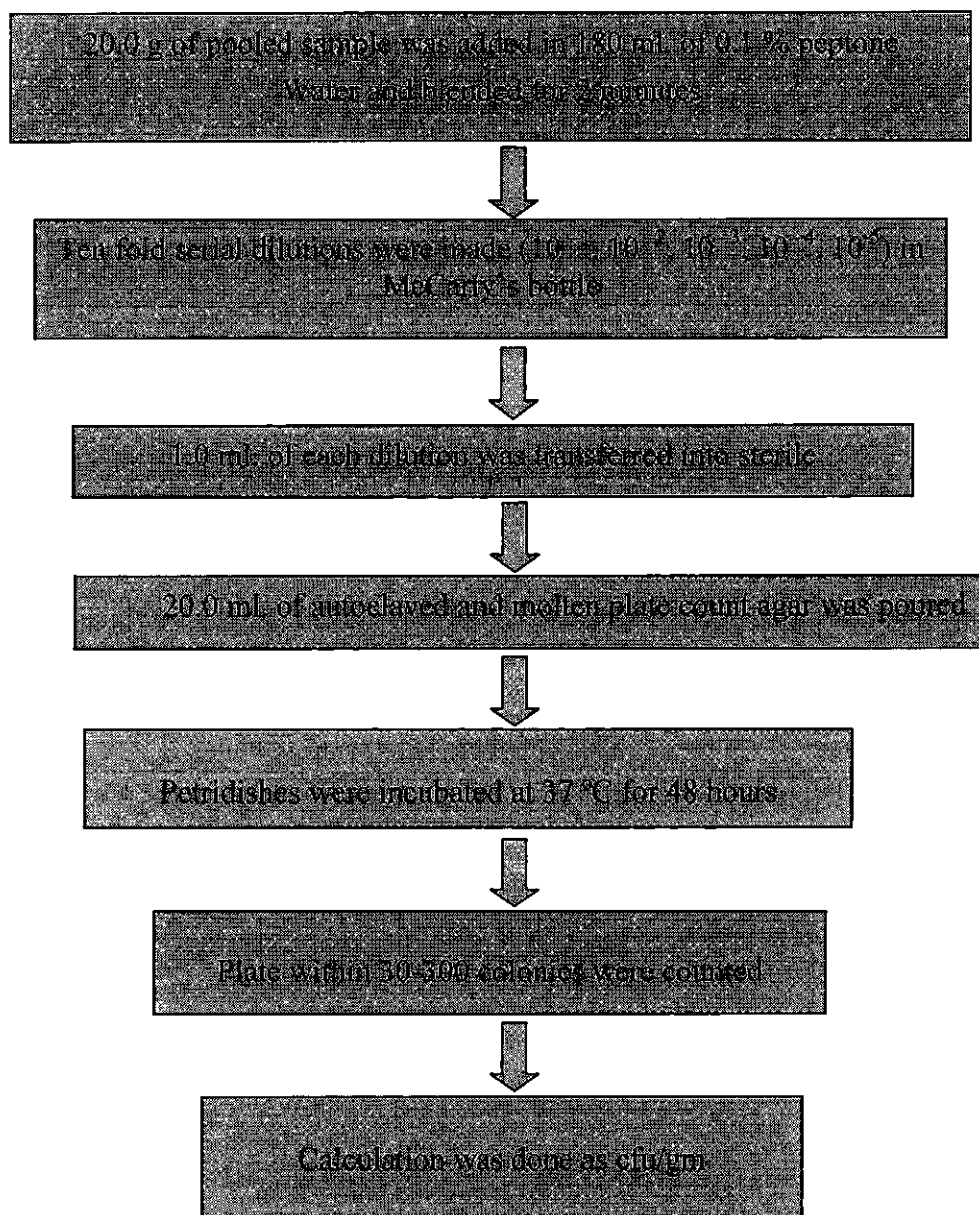
Plate count Agar (PCA)

2.35 grams of plate count agar was first added in 100ml of distilled water and boiled to dissolve the medium completely. The sulphite medium was then sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 minutes.

Procedure:

20.0 gm of pooled sample was weighed and taken into a sterile warring blender jar for blending. The sample was added into a flask containing 180 ml 0.1 % sterile peptone water .The mixture was homogenised for two minutes. This provided a dilution of 10^{-1} . 1.0 ml. of this suspension was transferred into McCarty's bottles containing 9 ml of 0.1 % peptone water to give a dilution of 10^{-2} . The process was repeated for the preparation of 10^{-3} , 10^{-4} and 10^{-5} dilutions respectively.

The appropriate dilutions were selected and for every dilution 1.0 ml aliquot were transferred into a sterile petridishes. 20.0 ml portion of molten Plate Count Agar (PCA) was poured into each of these sterile Petridishes. The plates were then rotated 5 times clockwise, 5 times anticlockwise, 5 times back and forward. Care was taken not to splash agar on the lid of the dish. Plates were left to solidify the plates were inverted and incubated at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 48 hours. Plates with 30-300 colonies on the surface were only counted.



Flowchart 2: The enumeration of Standard Plate Count (SPC)

3.5.3.2 Total Coliform (TC)

Culture Media

Lauryl Sulphate Tryptose Broth (LSTB):

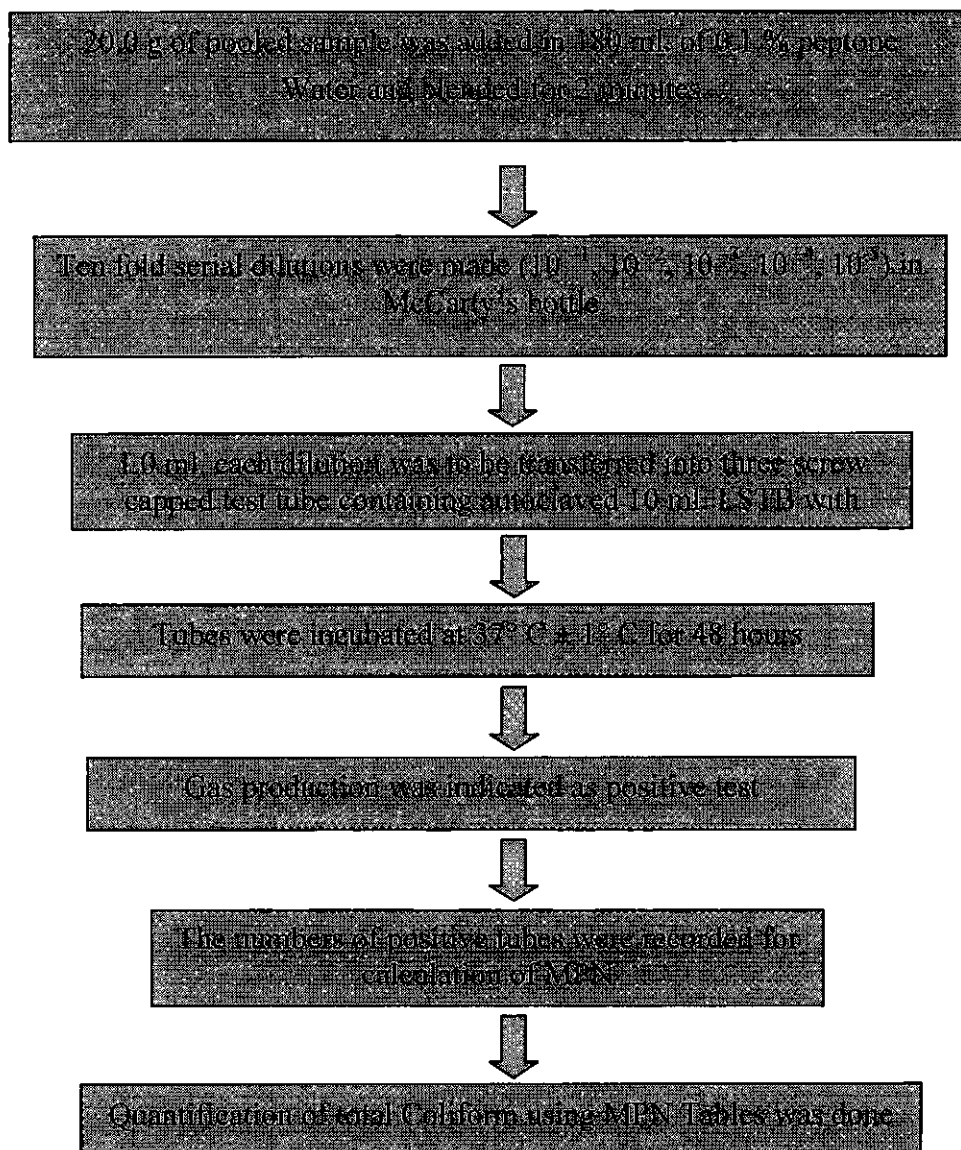
3.56 grams of LSTB was dissolved in 100ml of distilled water and then was distribute into test tubes with inserting Durham tubes and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minute.

Procedure

20.0 grams pooled sample (dried fish) was weighed and aseptically taken into a sterile warming blender jar and then blended. After blending, the sample was added into conical flask containing 180 ml. of 0.1 % peptone water. Ten fold serial dilutions were made with this suspension in McCarty's bottles containing 9.0 ml. of 0.1 % peptone water. 1.0 ml. of each dilution was transferred into three screw-capped test tubes containing autoclaved, Lauryl Sulphate Tryptose Broth (LSTB) (10.0 ml) with Durham's tubes. The tubes were inverted to ensure that Durham tubes did not contain gas bubbles. These tubes were incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 48 hours. The formation of gas within 24 or 48 hours was considered as evidence for the presumptive positive test for Coliform organisms.

A loopful of suspension from all positive LST broth tube was transferred into 10.0 ml volumes of Brilliant Green Lactose Bile (BGLB) broth. These tubes were incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 48 hours.

Gas production in BGLB tubes considered as sufficient evidence for the confirmatory test for Total Coliform organisms. Number of positive tubes out of 3 was recorded for each dilution. Using the MPN charts, the MPN of Total Coliform (TC) was calculated based on the portion of confirmed LSTB tubes (with gas production) for 3 consecutive dilutions in BGLB broth at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$.



Flowchart 3: The quantification Total Coliform (TC)

3.5.3.3 Faecal Coliform (FC)

Culture Media

Brilliant Green Bile Broth (BGBB)

4 grams was suspended in 100 ml distilled water. It was mixed well and later it was distributed in fermentation tubes and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

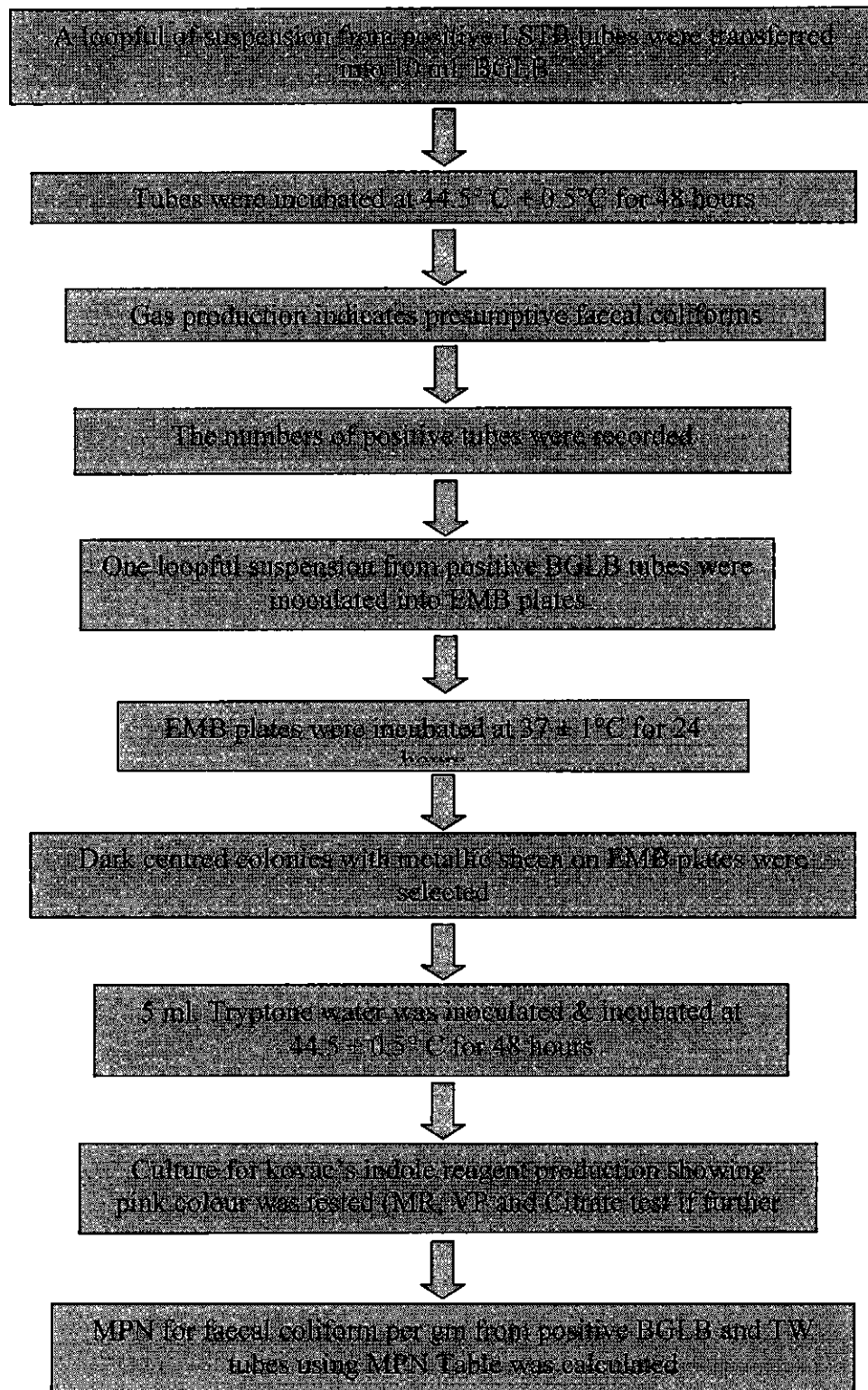
Tryptone Water

1 gram was suspended in 100 ml distilled water. Then it was heated to boiling to dissolve the medium completely. Sterilize by autoclaving for 15 minutes at 15 lbs pressure (121°C).

Procedure

Those tubes of Lauryl Sulphate Tryptose Broth (LSTB), which were positive for gas production in Total Coliform (TC) estimation tests, were considered. Subcultures were made from all positive tubes of Lauryl Sulphate Tryptose Broth (LSTB) into a 10 ml volume of Brilliant Green Bile Broth (BGLB) and incubated at $44.5 \pm 0.5^{\circ}\text{C}$ up to 48 hours. Any tube showing gas production was considered positive for the presence of presumptive Faecal Coliforms (FC).

All positive tubes were sub-cultured by streaking onto plates of EMB agar. Then these plates were incubated at 37°C for 18 - 24 hours. After incubation, the plates were examined for suspicious Faecal Coliform colonies i.e. black or dark centred with the greenish metallic sheen.

**Flowchart 4: The quantification Faecal Coliform (FC)**

3.5.3.4 *Salmonella* sp.

Culture media:

Selenine Cystine Broth

Part A:

Tryptone - 5gm/litre

Lactose - 4 gm/litre

Sodium - 10gm/litre

Part B:

Sodium Acid selenine - 4 gm litre

0.2g of part A and 1.33 gms of part B of selenite broth was suspended in 100ml distilled water. It was warmed to mix well. It was dispensed and sterilized in a boiling water bath or in free flowing steam for 10 minutes. It was not done in autoclave because excessive heating is detrimental.

Tetrathionate Brilliant Green Broth

9.15 grams of broth was suspended in 100ml distilled water. It was heated to dissolve and kept in free flowing steam for 30 minutes. It was kept at 45°C and added 4 ml iodine solution containing 0.5 grams iodine and 0.8 grams potassium iodine. It was mixed well and dispensed as desired.

Xylose Lysine Deoxycholate (XLD) Agar

2.5 grams of XLD agar was suspended in 50 ml distilled water and was heated with frequent agitation until the medium boiled and then transferred to a water bath at 60°C for 15 minutes. After cooling, the medium was poured into sterile petridishes.

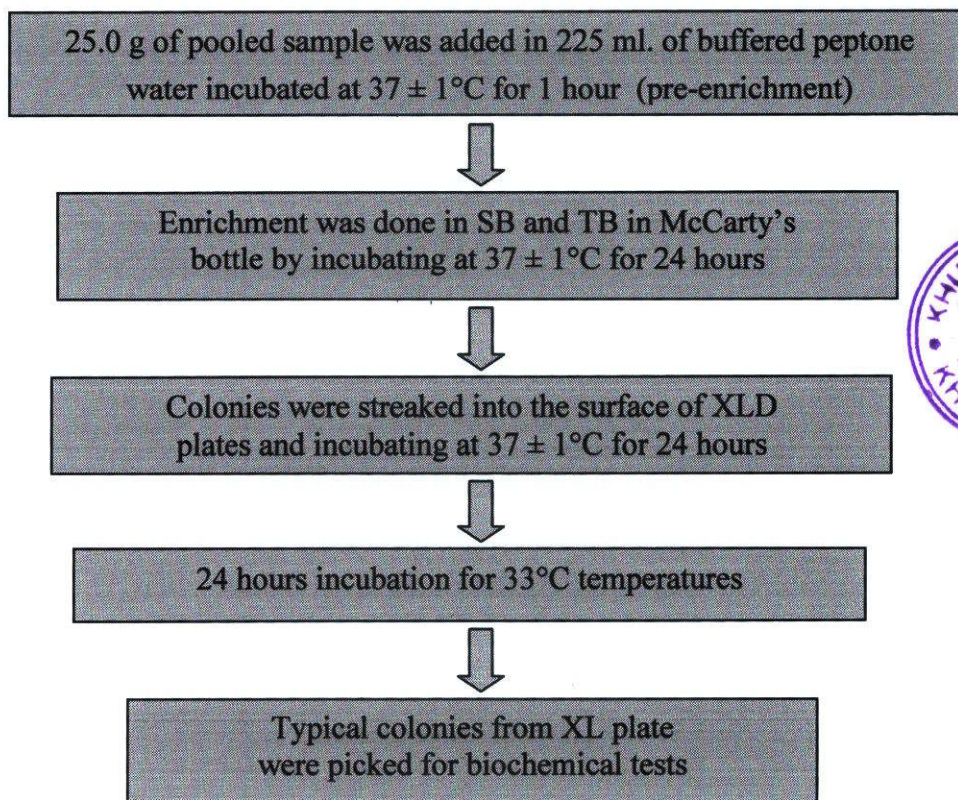
Brilliant Green Agar

5.8 grams of BGA was suspended in 50 ml of distilled water and boiled to dissolve completely and then sterilized by autoclaving at 15lbs pressure for 15 minutes.

Procedure:

25.0 gm of sample (dried fish) was weighed and taken into a waring blender flask. 225 ml. of buffered peptone water (BPW) was added into the sample and then homogenized for 1 minute. Sample was incubated at $37^{\circ} \pm 1^{\circ} \text{C}$ for 24 hours.

The resuscitated culture was gently mixed. 1.0 ml. of the mixture was added each to 10 ml of Selenite broth (SB) and 10 ml of Tetrathionate broth (TB). The selective enrichment broth was then incubated at $37^{\circ} \pm 1^{\circ} \text{C}$ for 24 hours. After incubation, a loopful of each of the two selective enrichment broth were transferred to the surface of the selective agar media, i.e. Xylose Lysine Deoxycholate (XLD) agar. The plates were then incubated at $37^{\circ} \pm 1^{\circ} \text{C}$ for 24 hours and observed for characteristics colonies for *Salmonella sp.* After 24-48 hours, *Salmonella* sps. shows pink colonies with black centre of H_2S on XLD.



Flowchart 5: The isolation and identification of *Salmonella* sp.

3.5.3.5 *Vibrio cholerae*

Culture Media

Alkaline Peptone Water (APW)

4.5 grams of Alkaline Peptone Water was suspended in 225 ml distilled water and slightly heated to dissolved completely and sterilized by autoclaving at 151b pressure at 20 minutes.

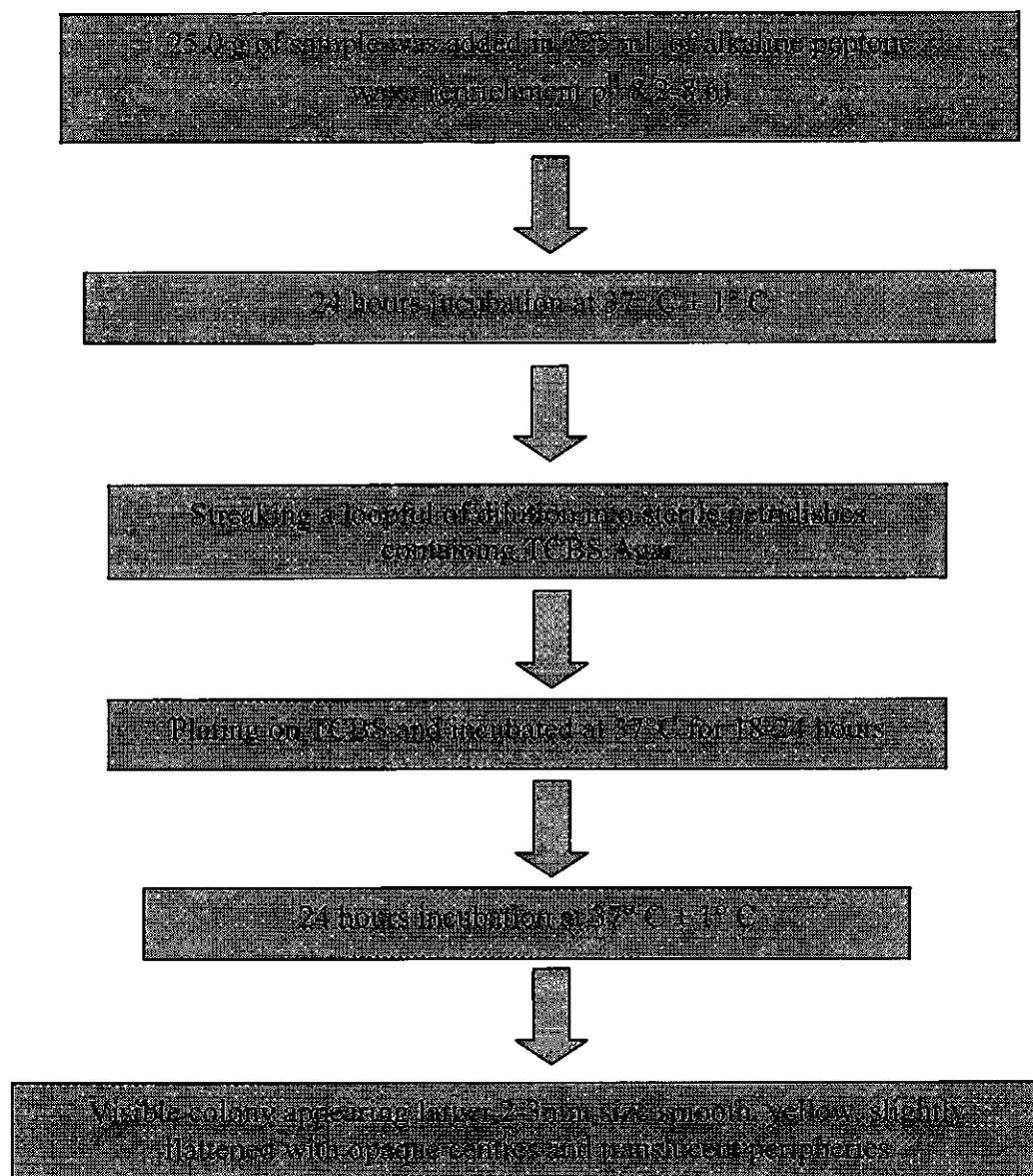
Thio-sulphate Citrate Bile Salts Sucrose Agar (TCBSA)

4.45 grams of TCBS agar was first boiled with 50 ml of distilled water to dissolve and then boiled for 1 minute. Then the media was dried to 60°C for 15 minutes and was poured into Petri dishes and allowed to solidify.

Procedure:

25 g of the sample was added with approximately 225 ml. of alkaline peptone water in a " Warring" blender flask . Then the sample containing alkaline peptone water was blended for 1 min. The sample was incubated at $37 \pm 1^{\circ} \text{C}$ for 6-8 hrs.

At the end of the incubation period, a loopful of the alkaline peptone water was streaked onto Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar plates. The plates were then incubated at $37^{\circ}\text{C} \pm 1^{\circ} \text{C}$ for 24 hrs. At the end of incubation, plates were checked for the characteristic colony of *Vibrio cholerae*. On TCBS agar colonies appeared as large (2-3 mm.), smooth and yellow, slightly flattened, with opaque centre and translucent peripheries. In the test there was no colony suspected for *Vibrio cholerae*. For this, further biochemical tests were not needed.



Flowchart 6: The isolation and detection of *Vibrio cholerae*

4. RESULTS

4.1 Quality Changes of Shrimp during Normal Practice (NP)

4.1.1 Organoleptic Aspect of Quality

Organoleptic quality changes of shrimp at different points of distribution channel during Normal Practice (NP) are presented in Fig-1. On the basis of overall sensory assessment the chart clearly shows that the scores decreased gradually over time. At the harvesting point (Gher) the shrimp was of highest quality having overall acceptance as " Highly Acceptable (HA)" with average organoleptic score of 8.92. As judged by the panellists, shrimp, was " Acceptable (A)" at Depot and Agent levels with an average scores of 7.84 and 6.75 respectively. At the processing plant the sample was 'Moderately Acceptable (MA)' having score 6.27.

4.1.2 Biochemical Aspects of Quality

4.1.2.1 Total volatile Base Nitrogen (TVB-N)

TVB-N contents of shrimp at different points of distribution channel are shown in Fig-2. At the harvesting point TVB-N of the samples was relatively smaller having an average count of 5.3 mgN/100g, which later increased up to 5.39 mgN/100g at Depot. However, TVB-N content increased to 7.89 mgN/100g at the point of agent. As time elapsed , in the Processing Plant the TVB-N content reached at 8.12 mgN/100g (Fig-2).

4.1.2.2 Trimethyl Amine Nitrogen (TMA-N)

The value of TMA-N was very low at the harvesting point (Gher) and it was 10.6 mgN/100g. It increased mildly at other distribution points of Depot, Agent and Processing Plant giving the count as 10.78, 10.89, 11.52 mgN/100gm respectively (Fig-3). At Depot and Agent the shrimp was organoleptically acceptable and it was moderately acceptable (MA) at Processing Plant.

4.1.3 Microbiological Aspects of Quality

4.1.3.1 Standard Plate Count (SPC)

The result of SPC is shown in Fig-4. Initially the SPC was the highest ($\log_{10}$ value 5.99 cfu/gm) which decreased very slightly at another three points and the values were recorded as 5.27, 5.79 and 5.88 cfu/gm at Depot, Agent and Processing Plant respectively.

4.1.3.2 Total Coliform (TC)

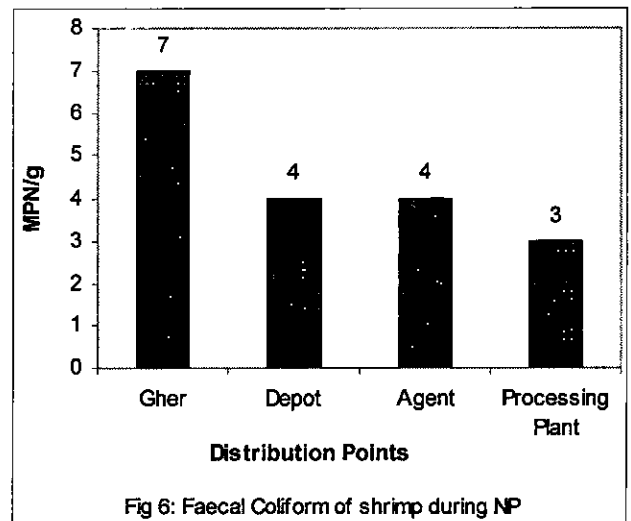
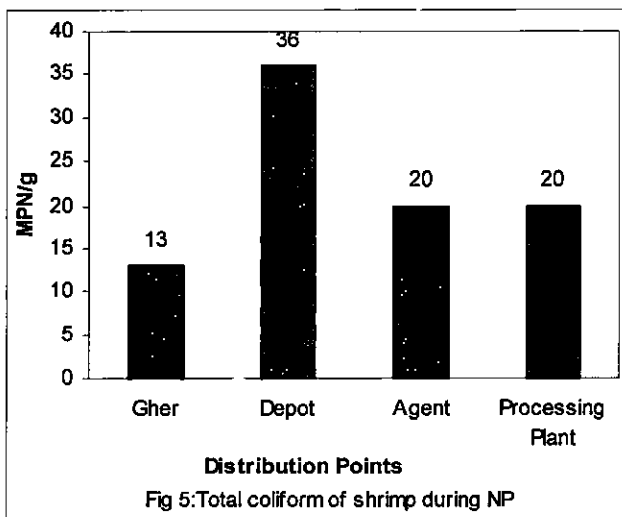
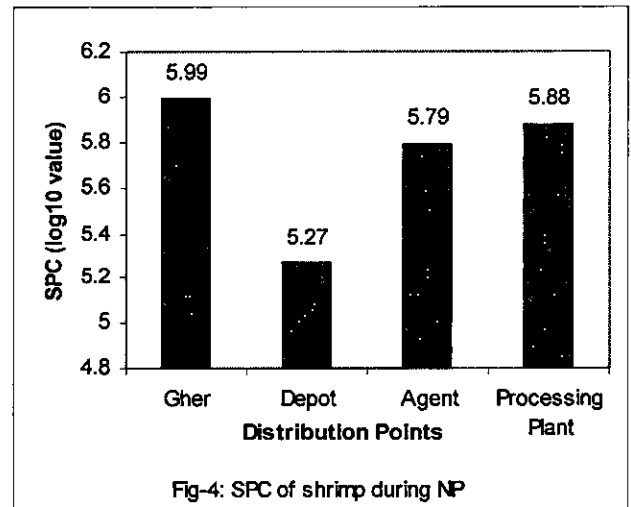
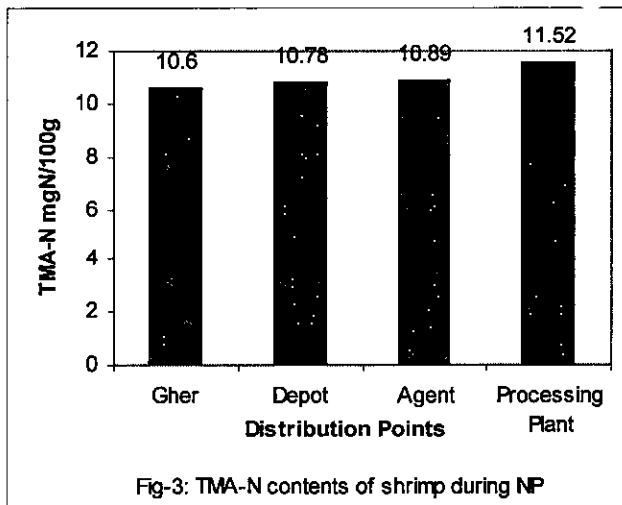
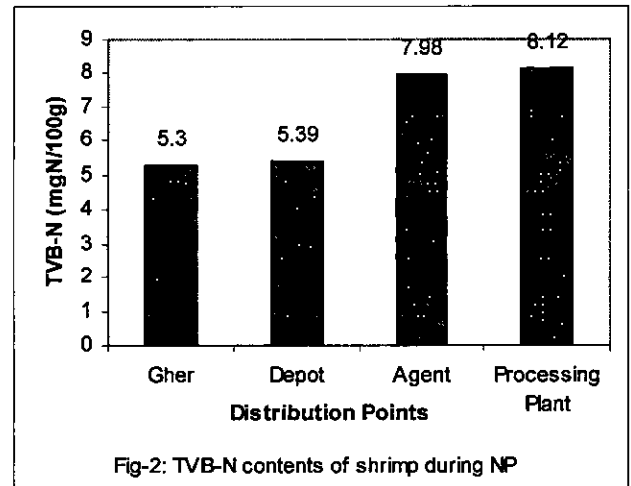
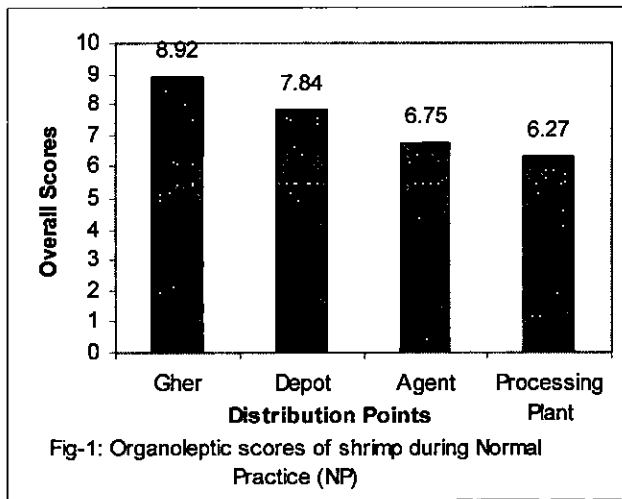
Fig-5 illustrates the result of Total Coliforms (TC) found throughout the study. The count of TC at harvesting point (Gher) was 13, which increased to 36 at Depot. The number showed a slight decrease at Agent where the average MPN was 20. The MPN remained unchanged at Processing Plant (20).

4.1.3.3 Faecal Coliform (TC)

The result of FC is shown in Fig-6. It is evident from the figure that at the harvesting point (Gher) the average most probable number (MPN) was 7/gm. This number decreased at the Depot which was 4/gm. The MPN decreased to the agent and P. Plant where the average count was 4 and 3 respectively. The lowest count was found in the Processing Plant (3).

4.1.3.4 *Salmonella* sp. and *Vibrio cholerae*

Salmonella sp. and *Vibrio cholerae* were not detected in 25 gm sample collected from each point of distribution Channel in Satkhira (Appendix 7 and 8).



4.2 Quality Changes of Shrimp during Controlled Study (CS)

4.2.1 Organoleptic Aspects of Quality

The results of organoleptic changes of the samples during controlled study at different points of distribution channel are illustrated in Fig-7. The figure shows that in the first stage of distribution channel (Gher) the organoleptic score was 8.97. This score gradually decreased at another three points of distribution channel as time elapsed. The organoleptic scores were 8.31, 7.84, 7.61 at Depot, Agent and P. Plant respectively. At Gher and Depot the shrimp was Highly Acceptable (HA) and at other two points it was Acceptable (A).

4.2.2 Biochemical Aspects of Quality

4.2.2.1 Total Volatile Base Nitrogen (TVB-N)

The results of TVB-N content of the sample at the four points of distribution channel in controlled study are shown in Fig-8. The figure depicts that TVB-N content of shrimp ranged from 3 to 8 mgN/100g showing an upward trend. The highest amount of TVB-N was found in the P.Plant (8 mgN/100gm).

4.2.2.2 Trimethylamine Nitrogen (TMA-N)

Fig-9 illustrates the results of TMA-N content of shrimp at different points of distribution channel. The result shows that initially the value of TMA-N was relatively low (5.35 mgN/100gm) which increased slowly as time elapsed. At Depot the value was 7.78 mgN/100gm which increased to 11.4 mgN/100gm at Agent. The highest value was recorded at P.Plant (11.4 mgN/100gm).

4.2.3 Microbiological Aspects of Quality

4.2.3.1 Standard Plate Count

The results of SPC of shrimp are shown in Fig-10. The figure presents that the SPC at harvesting point (Gher) was 5.96 cfu/gm ($\log_{10}$ value). The value showed fluctuation at another three points of distribution channel. The highest value was found at P.Plant (11.4 cfu/gm) and lowest was at Depot level (5.12 cfu/gm).

4.2.3.2 Total Coliform (TC)

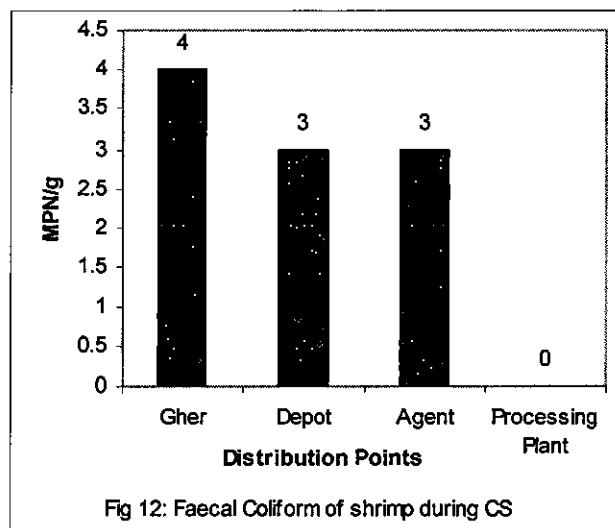
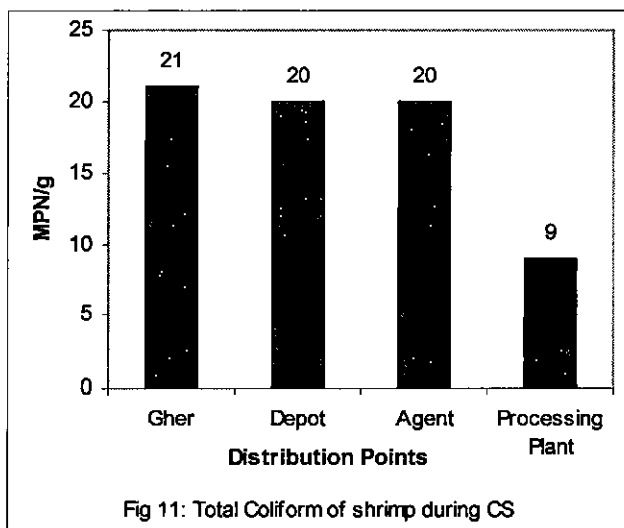
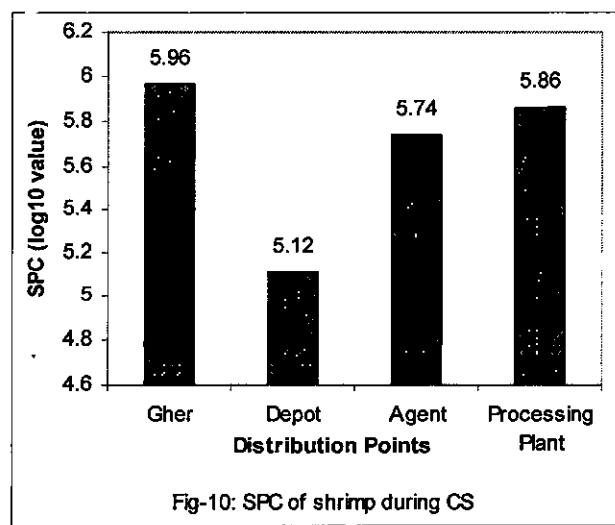
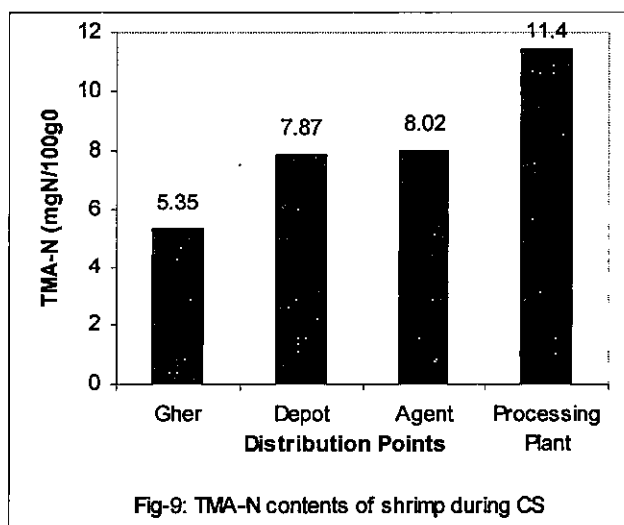
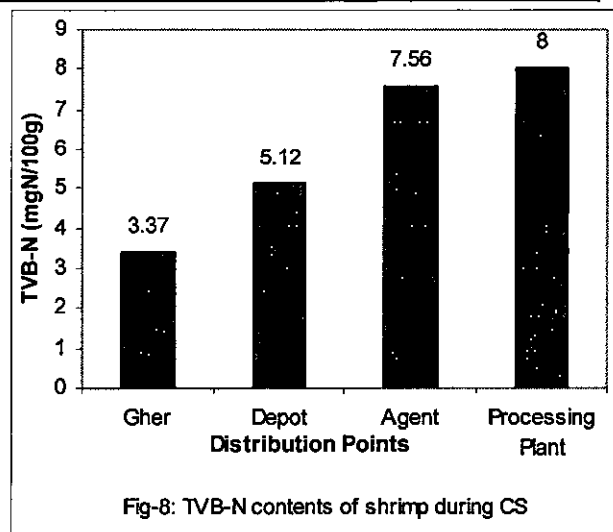
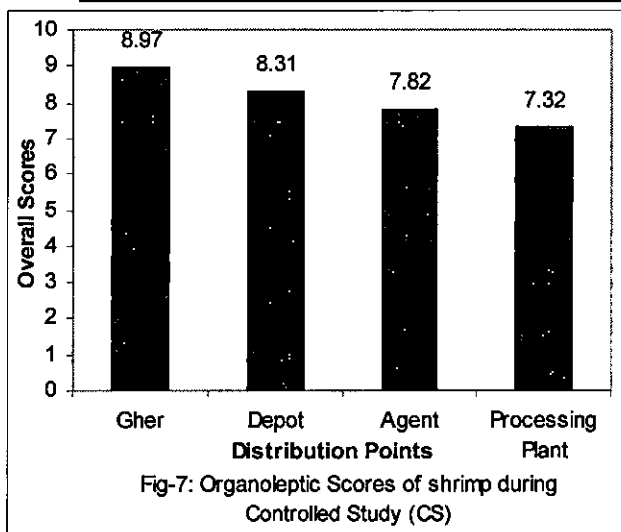
The results of TC is shown in Fig-11 The number fluctuated at different point of distribution channel. Initially at the point of harvesting (Gher) the number was 21/gm which decreased to 20 /gm at the point of Depot. After this point the number decreased to 20/gm at Agent and 03/gm in P.Plant.

4.2.3.3 Faecal Coliform (FC)

The results of FC of shrimp at different points of distribution channel are shown in Fig-12. The figure shows a fluctuation of FC at different point. Initially at Gher the FC was 5.2/g. But at Depot there was an average count of 6.3/gm which was slightly decreased at the point of Agent (4.8/g). FC was totally absent at the point of P.Plant.

4.2.3.4 *Salmonella* sp, and *Vibrio cholera*

Salmonella .SP. and *Vibrio cholera* were not detected in 25 gm sample collected from each point of distribution Channel in Satkhira(Appendix 3 and 4).



4.3. Comparative study of Quality Between Normal practice and Controlled study

4.3.1 Organoleptic Quality

Fig-13 shows the comparative organoleptic changes of shrimp at different point of distribution channel during Normal practice (NP) and Controlled study (CS). The average score of shrimp at Gher during NP and CS were 8.92 and 8.97 having "Highly Acceptable (HA)" quality. These score decreased slowly in the same pattern at another three points of distribution channel. In case of NP the average score decrease from 8.92 to 6.27 while it decreased from 8.97 to 7.61 in CS. At the last point of distribution channel (P.Plant) the shrimp was Moderately acceptable (MA) in NP and Acceptable (A) in CS. The controlled study showed a better organoleptic quality.

4.3.2 Biochemical Quality

4.3.2.1 Total Volatile Base Nitrogen (TVB-N)

Fig-14 shows a comparative picture of quality changes of shrimp in relation to TVB-N content between Normal practice (NP) and Controlled study (CS). The TVB-N content of shrimp between these two different experiments did not differ a lot at the point of Gher. In both cases the TVB-N content increased from the starting point (Gher) to the ending point (P.Plant). At the point of Depot the TVB-N content was 5.39 mg-N/100gm (in NP) and 5.12 mg-N/100gm (in CS) respectively. At the point of P. Plant TVB-N contents in both study were more or less same. But at the point of Agent the difference was slight more (7.98 mg-N/100gm and 7.56 mgN/ 100gm in NP and CS respectively).

4.3.2.2 Trimethylamine Nitrogen (TMA-N)

The comparative changes of TMA-N of shrimp in NP and CS at different points of distribution channel are illustrated in Fig-15. The figure shows that there was no notable difference of TMA-N contents in NP and CS. In case of Normal practice the content TMAN ranged from 10.6 mg-N/100gm to 11.52 mg-N/100gm while in case of Controlled study it ranged from 5.35 mg-N/100gm to 11.4 mg-N/ 100gm.



4.3.3 Microbiological Quality

4.3.3.1 Standard Plate Count (SPC)

Fig -16 shows the changes of SPC of shrimp during NP and CS at different point of distribution channel. In both cases value of SPC at different point increased slightly. But in CS the SPC was more static. In case of Normal practice the SPC ranged between 5.99 cfu/gm to 5.88 cfu/gm ($\log_{10}$ value) while at Controlled study it ranged 5.96 to 5.86 cfu/gm ($\log_{10}$ value).

4.3.3.2 Total Coliform (TC)

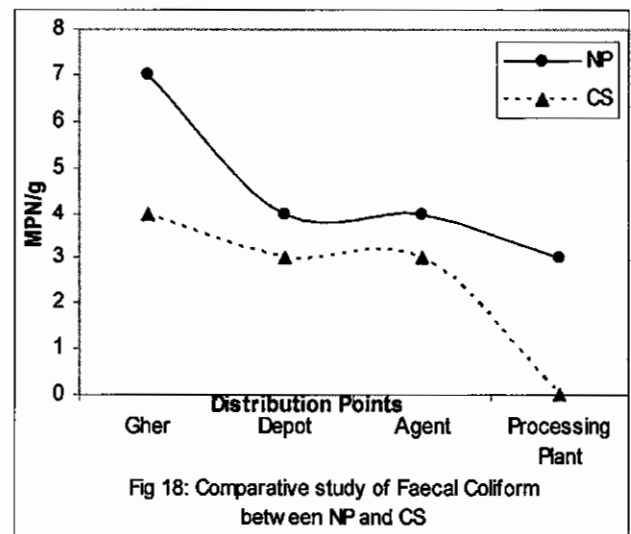
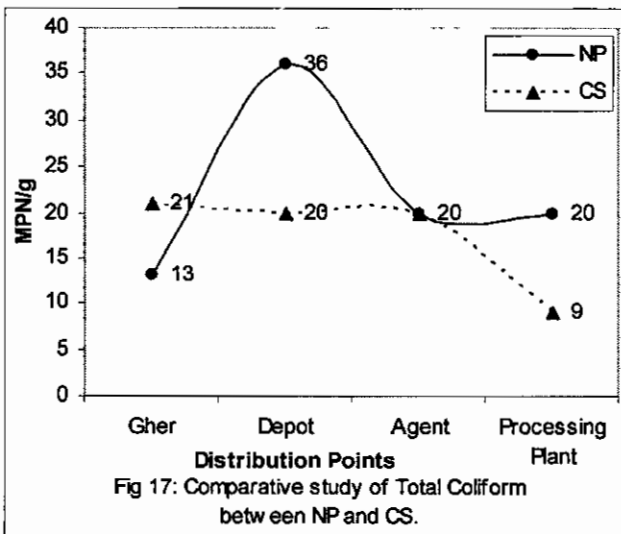
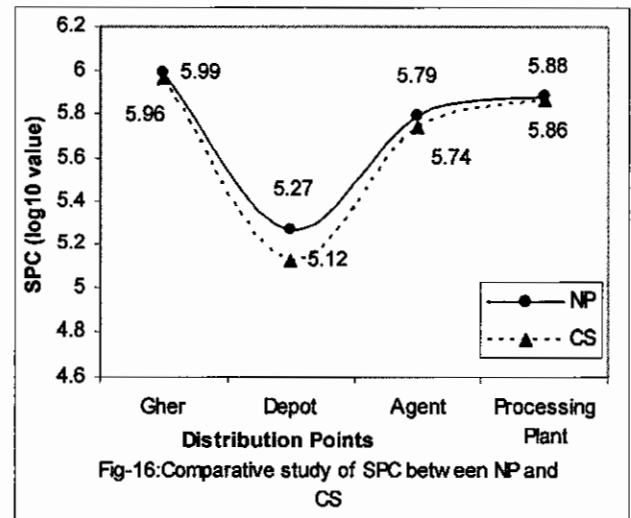
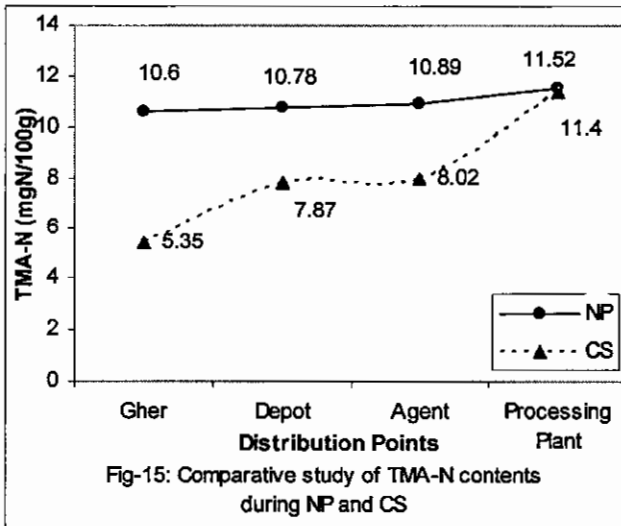
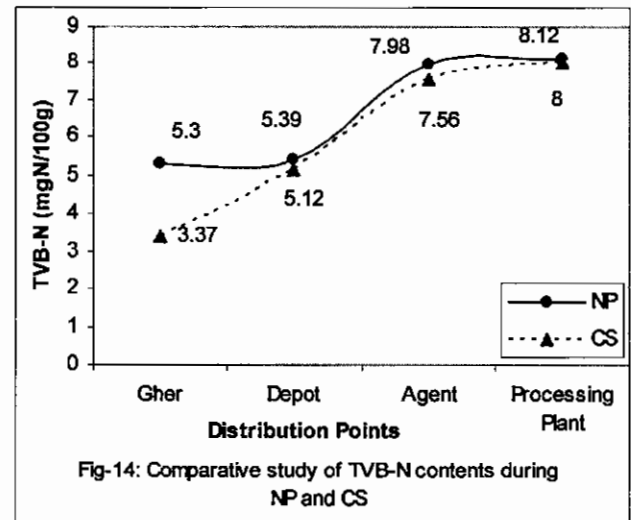
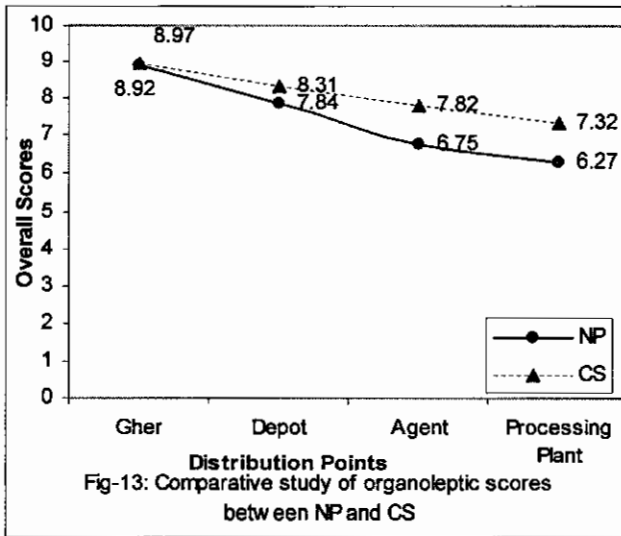
The comparative MPN numbers of Total Coliform of shrimp in NP and CS are presented in Fig-17 . In both case TC count oscillated remarkably. The results clearly show that TC counts were more in all point in NP than those in CS. In case of Normal practice highest number of TC was found at the point of Depot while in case of controlled study the highest count was in Gher.

4.3.3.3 Faecal Coliform (FC)

Fig-18 illustrates the comparative Faecal Coliform counts of shrimp in NP and CS. In both cases highest MPN of FC was recorded at Gher level. FC was relatively less in number at all points of distribution channel in Control study. FC was totally absent at Processing plant in Control study.

4.3.3.4 *Salmonella* sp. and *Vibrio cholera*

Salmonella SP. and *Vibrio cholera* were not detected in 25 gm sample collected from each point of distribution Channel in Satkhira (Appendix 3 and 4).



5. DISCUSSION

5.1 Quality of Shrimp during NP and CS

5.1.1 Organoleptic Aspect of Quality

The wide spread means of evaluating the acceptability and edibility of fish are the sense, smell and sight supplemented by the taste and touch. The reasons for the preferential use of sensory test are obvious: no special laboratory equipment's is needed; the fish can be examined whatever they happened to be, the test can be carried out quickly and many samples can be evaluated in a relatively short time (Farber, 1965). In spite of certain limitation, organoleptic methods are universally accepted for assessing the quality of fish (Shewan,1976) .

The storage quality of shrimp at different temperature and period have been studied by many workers (Naher, 2004; Alam, 2004; Foda., 1984). The most prevalent method of preserving shrimp in the fresh form is icing. The present study always showed better organoleptic quality of shrimp in controlled study (using ice) in comparison to normal practice.

Naher (2004) reported the organoleptic scores of shrimp as 8.8, 5.47, 6.67 and 6.33 at Gher, Depot, Agent and Processing Plant respectively in an experiment on quality of shrimp (*E. monodon*) in normal practice at different distribution channel in Bagerhat district.

The organoleptic scores of shrimp were 8.80, 7.73, 6.93 and 6.66 at Gher, Depot, Agent and Processing Plant respectively during a study on organoleptic, biochemical and microbiological changes in shrimp (*P. monodon*) at each point of distribution channel in Khulna district (Alam, 2004).

At the beginning of the present study, shrimps were considered of High Acceptable (HA). The eyes were bright and transparent, carapace textures were hard, shell color were yellowish, and the odours were fresh. The eyes maintained their clarity up to the P. Plant. At the point of Depot and Agent slightly change was observed in carapace texture, shell color and carapace color. In case of Agent the odour was sweet and

carapace color was moderately blackish. Here the shell color lost its brightness slightly. Over the last three-distribution points the shrimp was in acceptable position.

The present study in controlled condition showed better overall acceptability scores of shrimp than those in normal practice at four points of distribution channel of Satkhira region (Gher, Depot, Agent and P.Plant). The highest score was found at the point of Gher and the lowest score was found to the f. Plant. The organoleptic scores of the study proves that the shrimp gradually losses its quality at each distribution point of the channel.

5.1.2 Biochemical Aspects of Quality

5.1.2.1 Total Volatile Base Nitrogen (TVB-N)

Total Volatile Base Nitrogen test is quite generally applicable; it usually gives reasonable good correlation with organoleptic freshness. Volatile bases are produced in increasing concentration during spoilage of fish. TVB-N value cannot be used to estimate the degree of freshness of fish in the early stages but only the degree of spoilage in the later stages (Shewan and Ehrenberg, 1977).

The TVB-N content of shrimp at the four distribution points were 5.3, 5.39, 7.98 and 8.12 mg-N/100g respectively in Normal Practice (NP). In Controlled Practice (CP) these values were 3.37, 5.12, 7.56 and 8 mgN/100g receptively(Fig-14). According to the result it was low in the initial point and it was high in the last point of P.Plant. At another two points it also increased slightly. The low value of TVB-N initially is an indication of quality of fresh fish while the high value of TVB-N may be due to action of autolytic enzymes and spoilage bacteria which might have passed their lag phase (Adebona, 1982). However, in all cases of Controlled practice (CP) the TVB-N values were slightly lower than in Normal practice. It indicates that the shrimp were in better quality in CP.

Experiments suggested 30mg /1000m `YVB-N as tile limit indicated of fresh shrimp. The present study confirms to the level of 30mg/100gm TVB-N for fresh shrimp as suggested. It changes with the changes of organoleptic score changes. In the present study all the values of TVB-N were within acceptable limit given by Connell (1975) indicated that the shrimp were of good quality through out the distribution Channel.

Connel (1975) stated that TVB-N counted in fresh fish has highly positive and highly negative correlation with storage time indicating that TVB-N is a good indicator of spoilages . "The result of the present study is in absolute agreement with this findings.

According to Connel (1976), the limit of acceptability of TVB-N is 25-30mb/100g. In the present investigation, all the values of TVB-N were within this acceptable limit indicating that shrimps were of good quality throughout the distribution channel.

5.1.2.2 Trimethyl Amine Nitrogen (TMA-N)

The acceptable limit of TMA-N for shrimp is 10-15mgN/100g (Connel, 1976). The TMA-N values found in the present investigation was much lower than this recommended level. The TMA-N contents of shrimp ranged from 10.6 mg-N/100gm to 11.52 mg-N/100gm in NP and 5.35 mg-N/100gm to 11.4 mg-N/100gm in CP at different points of distribution channel (Fig-15 and Appendix 1& 3)

Reilly et. al. (1985) observed that the level of 5mg/100g was never reached during ice storage of shrimps. 'These findings differ from the present investigation in both cases of NP and CP. However', the results of the present study co-relate with the results observed by Naher (2004).

Trimethyl amine is a volatile compound that could serve as an index of spoilage offish. Fresh water fish can not be judged by only TMA values because they do not contain the precursor.

TMA content of shrimp (*Penaeus monodon*) fluctuated during storage at normal temperature and were regarded as poor indices of quality (Reilly et. al., 1985) . The value of TMA was found to be questionable use as indices of spoilage for mackeral (Barile et. al., 1985) . The findings differed with the result of the present investigation as TMA-N value did not fluctuate and showed good correlation with storage time and organoleptic score. Probably, the value of TMA-N at four different distribution part increased slowly due to autolytic and bacterial action (Azam et. al., 1997).

TMA-N is an excellent indicator for the onset of spoilage and for the different stages of spoilage in all shrimp and fish species. The fishy odour is produced when TMA-N reacts with fat in the muscle of shrimp (Davis and Gill,1936) . In the cause of spoilage.

many off odours are produced by bacteria, indicating the onset and development of spoilage. More TMA-N is produced from TAMO by bacterial action than by fish tissue enzymes. TMA-N produced by both these two actions is responsible for the fish odour during spoilages (Jones, 1954)

The results obtained from the present investigation support that there is an increase of TMAN content of shrimp at the distribution channel . At the point of Gher the values were 10.6 and 5.35mg/100g in NP and CP. But at P. Plant it was recorded as 11.52 and 11.4mg/100 g in NP and CS respectively (Fig-15)

As TMA-N is produced by bacteria , and bacterial activity is directly affected by temperature. So temperature gradients in individual fish can affect TMA-N level (Horner ,1995) . Likewise, in the present study the variations in TMA-N contents in NP and CP may be attributed to temperature difference.

5.1.3 Microbiological Aspects of Quality

5.1.3.1 Standard Plate Count (SPC)

The international accepted limit of SPC is 10^6 cfu,/g (ICMSF, 1988). All the results found in the present study were within this acceptable limit. The SPC of shrimp ranged between 5.99 to 5.88 (log10 value) in NP and 5.96 to 5.86 (log 10 value) in CP (Fig-16). These results also agree the results observed by Alam (2004).

Barile *et. al.* (1985) had carried out an experiment on the spoilage pattern of shrimp at ambient temperature and reported that when the shrimp was rejected by the taste panel, the bacterial load was 10^7 / gm . However in the present investigation for shrimp found the bacterial Count (log 10 value) 4.84, 5.72. 5.84, 5.93 respectively at Gher, Depot, Agent and P.Plant in ambient temperature (NP).

For the bacterial spoilage the most commonly used method is total viable count (Rahman ,1980). Castell *et. al.* (1956) have found that bacterial counts were valuable as a measure of degree of freshness of fish.

The initial and rejection of bacterial count of whiting (*Sillago maculata*) and tilapia at normal temperature were within the range reported for temperate species of 10^3 to

10^8 / gm (Shewan, 1976; Groninger , 1959) reported the total bacterial count of the wet fish at ranging from 10^3 to 10^8 / gm when stored at normal temperature . At ambient temperature, there was a rapid increase in bacterial number from 10^4 to 10^8 / gm with 16 hours and bacterial counts were within the range 10^6 to 10^9 / gm on rejection (Reilly, *et.al.*, 1985).

Shrimp collected from three district (Khulna, Bagerhat. Satkhira) and from all distribution points (Gher, Agent, Depot, P.Plant) indicated SPC value within 10^5 /gm (Azam,2004). It was in acceptable limit when practiced normally. The mean $\log_{10}$ Value agreed with limit recommended by Paul (1994).

The mean bacterial count of freshly caught pond-cultured shrimp was 3.2×10^5 and were lower than the counts reported for marine shrimp 3.9×10^6 (Fonsaka.1990) The lower counts in the present study can be attributed mainly to the fact that immediate Win- of cultured shrimps after harvesting limits bacterial multiplication. In the present investigation the bacterial count from SPC varied with the increase of time. At Gher , Depot, Agent and P. Plant the $\log_{10}$ values were 5.99, 5.27, 5.79 and 5.88 receptively during NP while it was 5.96. 5.12, 5.74 and 5.86 in CP (Fig-16). The count increased slowly according to the increase of time.

5.1.3.2 Total Coliform (TC)

The acceptability limit of TC is <100 /g (ICMSF 1988). TC in the present study ranged from I S to 53 at different points of distribution channel (Fig-17 and Appendix 1 & 3). All the results found in the present investigation within this recommended level.

According to Fonseka (1990) 50% of the shrimp samples had coliforms at less than 20 MPN/gm while the rest of samples carried coliforms below 75 MPN/gm. In the present investigation average number of coliform was 13, 36, 20 and 20 MPN/gm respectively at Gher, Depot, Agent and P.Plant during NP while it was 21, 20, 20 and 09 during CP (Fig17). These numbers were lower than the described numbers of Fonseka (1990) . 'File average highest number of TC was found in Agent and lowest number of TC was found in P.Plant. All the numbers of TC at different points of distribution channel in the present study agrees with Fonseka's Statement and results found by Naher (2004).

Jayaweera and Subasinghe (1990) stated the coliform counts of raw materials ranged from < 3 /gm to 100/gm with 50% of the counts in the 3-10³/gm range. Shrimp caught in deeper water generally do not carry coliform, but those caught close to the shore and from inshore water have been found to be contaminated frequently with coliform (Cann, 1977). In the present investigation of controlled study the TC was 21, 20, 20 and 09 MPN/g at Gher, Depot, Agent and Processing Plant respectively (Fig-17). The highest count was found in Depot and the lowest count was found in processing plant. The changes in the bacterial flora of prawns have been studied previously, but not as extensively as that of fish.

From the present investigated result it is confirmed that in controlled study (CS) the number of TC was lower than that in the Normal practice (NP).

5.1.3.3 Faecal Coliform(FC)

The acceptable limit of Faecal Coliform is 10 MPN/g (Paul, 1994). All the counts of FC in the present investigation were within this limit.

In the present study during normal practice (NP) average number of FC ranged between 4 to >3 MPN/g and in case of controlled study (CS) it varied from 7 to 3 MPN/g (Fig-18). Alam (2004) reported the FC of shrimp between 3.33 to 5.66 in normal practice and 0 to 3.66 in controlled study.

In the present investigation highest number of FC was found in Depot and lowest number was found in the P.Plant in both cases of NP and CS. In the Depot deheading was done and here the contamination was directed by hand of workers. It exists to the another point of distribution channel.

FC were not detected in 82.5% sample and counts of FC in Pond water were also found to be less than those in shrimp (Cooseka, 1990). Cann (1977) reported that freshly caught penaeid shrimps do not carry FC but smaller at sea were contaminated with FC.

Jayaweera and Subasinghe (1990) described that FC counts in the raw materials ranged from <3 to 10³/g but were reduced to between (66.7%) and 3 to 10²/g (33.3%). The FC counts do not vary significantly during the processing stage of

beheading and grading but the counts declines slightly during next stage , just before storage in ice and is significantly reduced after lowering the temperature.

Surendran and Gopakumar (1981) reported that *Moraxella* and *Acinobacter* comprised 74%, of the spoilage flora of the shrimp . Reilly *et. al* (1986) working on the spoilage of shrimp at different temperature , found the initial microflora to be mesophilic. The rate of survival of different types of fecal organisms like *E. Coli* occurring along with normal flora of iced Fish at subsequent storage at - 23.3°C had been studied by Pilly *et. al.* (1969) . However in the present investigation in the controlled study resulted in decreased number of bacterial load.

In the present investigation the FC was totally absent P. Plant during CS. But in case of NP fecal coliform was found in all stages of distribution channel, Fecal coliform was found in small number in the Agent and P. Plant. The highest average was found in Depots. The probable cause of contamination through direct handling of water and their unhygienic handling. The result of present investigation agreed with the results observed by Azam (2004).

5.1.3.4 *Salmonella sp*

In the present investigation *Salmonella sp* was not detected in any of the point of shrimp distribution channel in Satkhira during NP and CS. Alam (2004) also reported absence *Salmonella* in the shrimp at different points of distribution channel in Khulna district of Bangladesh.

Rose *et. al.* (1990) stated that , *Salmonella* is not found in the marine shrimp naturally and any salmonella in the marine shrimp is probably due to contamination during handling, transportation or processing . The present investigation also agreed with Rose *et. al.*(1990).

The present investigation agrees with Azam (2004) and Dangla (1984). *Salmonella* were not detected in the sample of shrimp in the distribution channel of Greater Khulna region (Azam, 2004). *Salmonella* were not detected in prawn samples in the storage stability of brackish water prawns during processing, for export (Dangla , 1984).

5.1.3.5 *Vibrio cholerae*

In the present investigation *Vibrio cholerae* was not detected at any point of distribution channel in the study area during NP and CS. According to Rose et. al. (1990) *Vibrio cholerae* was detected twice through out the pond and plant survey. In a investigation of microbial flora of pond cultured shrimp, only two samples were positive for *Vibrio* sp (Fonseka ,1990) The present investigation did not agree with Rose et. al. (1990) and Fonseka (1990) but agreed with the results observed by Azam (2004).

CONCLUSION

Quality of Shrimp largely depends on handling procedure and methods of preservation. Different amount of ice used in depot, agent and processing plant for preserving shrimp also set variation in its quality. But few works have been on the quality aspects of shrimp at different points of marketing channel in Bangladesh. The present work is relatively new in its area. But the duration of the study was short and the size of samples was also small due to shortage of fund and facilities. Nevertheless, it is hoped that the present study will provide important information regarding shrimp quality at different points of its marketing routes. However, further detailed research is needed to set a basic information guideline for better quality maintenance of shrimp.

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Appendix-1: Organoleptic, biochemical and microbiological changes in Shrimp
(*Penaeus monodon*) during Normal Practice (NP).

Distribution Points	Organoleptic Scores	Acceptability	TVB-N (mgN/100g)	TMA-N (mgN/100g)	SPC (log ₁₀ value)	TC (MPN/g)	FC (MPN/g)
Gher	8.92	HA	5.3	10.6	5.99	13	7
Depot	7.84	A	5.39	10.78	5.27	36	4
Agent	6.75	A	7.98	10.89	5.79	20	4
Processing Plant	6.27	MA	8.12	11.52	5.88	20	3

Appendix-2: Organoleptic, biochemical and microbiological changes in Shrimp
(*Penaeus monodon*) during Controlled Study (CS).

Distribution Points	Organoleptic Scores	Acceptability	TVB-N (mgN/100g)	TMA-N (mgN/100g)	SPC (log ₁₀ value)	TC (MPN/g)	FC (MPN/g)
Gher	8.97	HA	3.37	5.35	5.96	21	4
Depot	8.31	A	5.12	7.87	5.12	20	3
Agent	7.82	A	7.56	8.02	5.74	20	3
Processing Plant	7.32	A	8	11.4	5.86	9	>3

Appendix-3: Isolation and identification of *Salmonella* sp.**FOR GHER**

Normal Practice				Controlled Study			
Agar Base	Broth Base			Agar Base	Broth Base		
	Tetrathionate Broth	Selenite Cystine	RVM		Tetrathionate Broth	Selenite Cystine	RVM
XLDA	YES/NO✓	YES/NO✓	YES/NO✓	XLDA	YES/NO✓	YES/NO✓	YES/NO✓
BGA	YES/NO✓	YES/NO✓	YES/NO✓	BGA	YES/NO✓	YES/NO✓	YES/NO✓
HEA	YES/NO✓	YES/NO✓	YES/NO✓	HEA	YES/NO✓	YES/NO✓	YES/NO✓
BSA	YES/NO✓	YES/NO✓	YES/NO✓	BSA	YES/NO✓	YES/NO✓	YES/NO✓
RESULT	<i>Salmonella</i> sp. (Absent ✓ /Present)/25g			RESULT	<i>Salmonella</i> sp. (Absent✓ /Present)/25g		

FOR DEPOT

Normal Practice				Controlled Study			
Agar Base	Broth Base			Agar Base	Broth Base		
	Tetrathionate Broth	Selenite Cystine	RVM		Tetrathionate Broth	Selenite Cystine	RVM
XLDA	YES/NO✓	YES/NO✓	YES/NO✓	XLDA	YES/NO✓	YES/NO✓	YES/NO✓
BGA	YES/NO✓	YES/NO✓	YES/NO✓	BGA	YES/NO✓	YES/NO✓	YES/NO✓
HEA	YES/NO✓	YES/NO✓	YES/NO✓	HEA	YES/NO✓	YES/NO✓	YES/NO✓
BSA	YES/NO✓	YES/NO✓	YES/NO✓	BSA	YES/NO✓	YES/NO✓	YES/NO✓
RESULT	<i>Salmonella</i> sp. (Absent ✓ /Present)/25g			RESULT	<i>Salmonella</i> sp. (Absent✓ /Present)/25g		



FOR AGENT

Normal Practice				Controlled Study			
Agar Base	Broth Base			Agar Base	Broth Base		
	Tetrathionate Broth	Selenite Cystine	RVM		Tetrathionate Broth	Selenite Cystine	RVM
XLDA	YES/NO✓	YES/NO✓	YES/NO✓	XLDA	YES/NO✓	YES/NO✓	YES/NO✓
BGA	YES/NO✓	YES/NO✓	YES/NO✓	BGA	YES/NO✓	YES/NO✓	YES/NO✓
HEA	YES/NO✓	YES/NO✓	YES/NO✓	HEA	YES/NO✓	YES/NO✓	YES/NO✓
BSA	YES/NO✓	YES/NO✓	YES/NO✓	BSA	YES/NO✓	YES/NO✓	YES/NO✓
RESULT	<i>Salmonella sp.</i> (Absent ✓ /Present)/25g			RESULT	<i>Salmonella sp.</i> (Absent✓ /Present)/25g		

FOR PROCESSING PLANT

Normal Practice				Controlled Study			
Agar Base	Broth Base			Agar Base	Broth Base		
	Tetrathionate Broth	Selenite Cystine	RVM		Tetrathionate Broth	Selenite Cystine	RVM
XLDA	YES/NO✓	YES/NO✓	YES/NO✓	XLDA	YES/NO✓	YES/NO✓	YES/NO✓
BGA	YES/NO✓	YES/NO✓	YES/NO✓	BGA	YES/NO✓	YES/NO✓	YES/NO✓
HEA	YES/NO✓	YES/NO✓	YES/NO✓	HEA	YES/NO✓	YES/NO✓	YES/NO✓
BSA	YES/NO✓	YES/NO✓	YES/NO✓	BSA	YES/NO✓	YES/NO✓	YES/NO✓
RESULT	<i>Salmonella sp.</i> (Absent ✓ /Present)/25g			RESULT	<i>Salmonella sp.</i> (Absent✓ /Present)/25g		

Appendix-4: Isolation and identification of *Vibrio cholerae***FOR GHER**

Normal Practice		Controlled Study	
Agar Base	Colony	Agar Base	Colony
TCBSA	YES/NO✓	XLDA	YES/NO✓
TTTGA	YES/NO✓	BGA	YES/NO✓
RESULT	<i>Vibrio sp.</i> (Absent ✓ /Suspected)/25g	RESULT	<i>Vibrio sp.</i> (Absent✓ /Suspected)/25g

For Depot

Normal Practice (DEPOT)		Controlled Study (DEPOT)	
Agar Base	Colony	Agar Base	Colony
TCBSA	YES/NO✓	XLDA	YES/NO✓
TTTGA	YES/NO✓	BGA	YES/NO✓
RESULT	<i>Vibrio sp.</i> (Absent ✓ /Suspected)/25g	RESULT	<i>Vibrio sp.</i> (Absent✓ /Suspected)/25g

FOR AGENT

Normal Practice		Controlled Study	
Agar Base	Colony	Agar Base	Colony
TCBSA	YES/NO✓	XLDA	YES/NO✓
TTTGA	YES/NO✓	BGA	YES/NO✓
RESULT	<i>Vibrio sp.</i> (Absent ✓ /Suspected)/25g	RESULT	<i>Vibrio sp.</i> (Absent✓ /Suspected)/25g