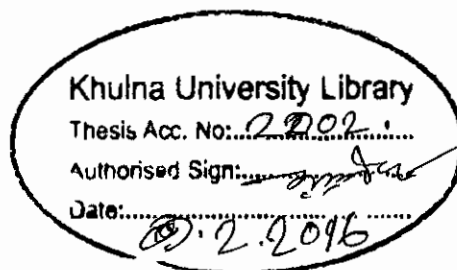


**QUALITY ASPECTS OF FROZEN SHRIMP PRODUCT
IN GEMINI SEAFOOD INDUSTRY BETWEEN NEW
MOON AND FULL MOON**



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January, 2009

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A Project Thesis

By

Md. Baharul Isalm

Examination Roll No: MS-070611

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**A project thesis submitted in partial fulfillment of the requirements
For the Degree of Master of Science in Fisheries and Marine Resource Technology**

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Approved as to style and content

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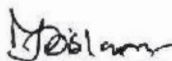
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Declaration

QUALITY ASPECTS OF FROZEN SHRIMP PRODUCT IN GEMINI SEAFOOD INDUSTRY BETWEEN NEW MOON AND FULL MOON

The results presented in the project thesis with the above mentioned title are entirely of the candidate's own investigations and no part of the results has been accepted for any degree nor is it being concurrently submitted for any degree.



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DEDICATION

This project- Thesis is

Dedicated to my

BELOVED PARENTS

Acknowledgement

All praises are to the almighty Allah, whose compassion retains the author on the earth and enables him to continue his education in the FMRT Discipline and to complete the project thesis for the fulfillment of the degree of Master of Science (M.S.) in Fisheries and Marine resource Technology.

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Abstract

The present study was conducted to assess the quality of shrimp in processing industry. The study area was Gemini Seafood Industry and the study period was 6 (six) months. The study included identification of Critical Control Point (CCP) in the processing industry. There are four shrimp process lines in the processing industry. Among four process lines, the CCP of block frozen shrimp, semi IQF shrimp and IQF shrimp were received raw material. The left process line is cooked IQF shrimp and the CCP was received raw material and cooking. Another set of experiment was carried out microbiological test of shrimp (received raw shrimp, export graded shrimp and final product), water and SWAB. The microbiological test was done during full moon and new moon and compared the result. The SPC of shrimp at different sampling points were varied between 0.854×10^5 to 5.74×10^5 cfu/g and 0.614×10^5 to 4.8×10^5 cfu/g during full moon and new moon correspondingly. The TC of shrimp was varied between 7.80 to 40.20 MPN/g and 5.60 to 31.20 MPN/g in full moon and new moon respectively. The FC of shrimp was varied between 00 to 6.2 MPN/g and 00 to 5.4 MPN/g in full moon and new moon respectively. *Salmonella* sp. and *V. cholerae* were not detected in any sample in different sampling points during full moon and new moon. The SPC of water varied between 3-7 cfu/ml and 3-6 cfu/ml in full moon and new moon respectively. The SPC of SWAB (workers hand) before and after working was varied between 20-30/cm² and 20-25/cm² for full Moon and new Moon respectively. The SPC of SWAB (SS table) before and after working was varied between 15-25 cfu/cm² and 15-20 cfu/cm². All the values that were gotten, were within the acceptable limit which proved highly hygienic condition of the industry

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List of Abbreviations and non-English Words

SSOP	Standard Sanitation Operation Procedure
SOP	Standard Operation Procedure
GHP	Good Hygiene Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Point
CCP	Critical Control Point
QC	Quality Control
SPC	Standard Plate Count
TC	Total Coliform
FC	Faecal Coliform
EU	European Union
PCA	Plate Count Agar
ICMSF	International Commission on Microbiological Specification for Foods
LSTB	Lauryl Sulphate Tryptose Broth
BGLB	Brilliant Green Lactose Bile Broth
TW	Tryptone Water
EMB	Eosin Methylene Blue Agar
XLD	Xylose Lysine Deoxycholate
TCBSA	Thio-sulphate Citrate Bile Salts Sucrose Agar
APW	Alkaly Pepton Water
BACS	Bangladesh Center for Advanced Studies
DoF	Directorate of Fisheries
USFDA	United States Food and Drug Administration
BFFEA	Bangladesh Frozen Food Exporters Association
IQF	Individual Quick Freezing

Chapter 1

Introduction



1.1. Background of the study

Bangladesh has limited wealth but have a lot of possibilities to increase wealth. One of the most important potential resources of is its vast area of water bodies with a diversified species of commercially important fish and shellfish. The total production reached 1.78 million metric ton (MT) and per capita consumption was 26g/day. Fish is the main sources of animal protein in the diet of the people of Bangladesh. More than 63% of the animal protein in Bangladesh comes from fish and 1.2 core people are engaged in this sector (DoF, 2001).

For decades, fisheries have been an economically important industry to Bangladesh. It is not only because of its benefit in providing food supply, local employment and the generation of other related industry, but it is also one of the major earners of foreign exchange for the country. Fish products are the second exported materials in Bangladesh.

Shrimp sector plays a vital role in the national economy of Bangladesh and its contribution to export earning is rapidly increasing. In Bangladesh, shrimp industry engaged various stages i.e., Fry, Wild, Hatchery, Agent, Shrimp farms, Transportation, Processing Plants, Exports.

Bangladesh produces 2.5% of the global production of shrimp. In Bangladesh, shrimp sector is the second largest export industry, giving employment to 840,000 people (Frankenberger, 2002). The total contribution of the shrimp sector to the country's Gross Domestic Product (GDP) is estimated at 0.6% but the frozen seafood accounts for a round 6% of the total export value, of which shrimp export constitutes almost 90% (BCAS, 2001 b). The total export of fish and shrimp in percentage during 2006-07 is shown in the figure 1.1.

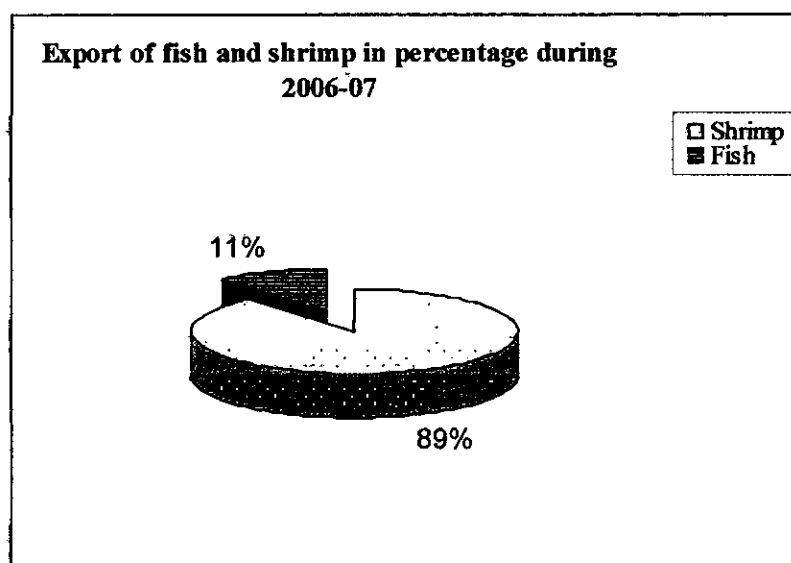


Figure 1.1: Export of fish and shrimp in percentage during 2006-07.

Shrimp export and cultivation in Bangladesh has undergone rapid expansion over the last two decades. Shrimp is a particularly valuable export crop generating substantial revenues and foreign exchange, earning in excess of \$360 million annually and accounting for 4.9 percent of exports in 2004. After the garment industry, shrimp production ranks second in Bangladesh in terms of the sector's ability to earn foreign exchange. Not only does this crop earn valuable foreign exchange, but the sector also employs significant numbers of rural workers and provides a livelihood for households throughout much of Bangladesh. Our study estimates that as many as 1.2 million people may be directly involved in shrimp production with an additional 4.8 million household members supported by the industry.

The export of Bangladesh fisheries in 2006-07 was 73,704 MT and 3,352.89 crore Tk in value. The main exported markets of fisheries products from Bangladesh are Japan, United State of America (USA) and European Union countries (EU). Some of the common fisheries products exported overseas are frozen shrimp and prawn, live fish, frozen fish, dried fish, salted and dehydrated fish etc., among them; frozen shrimp is the leading item. Frozen shrimp has been the main export product with total volume of 53361 MT and value of 2992.33 crore Tk in 2006-07. The export of fish and fisheries product from Bangladesh from 2002 to 07 is shown in figure 1.2.

The export products of frozen shrimp are of various kinds, such as: whole, headless shell on, peeled tail on, breaded, cooked peeled shrimp and other value added products. The shrimp species used are mainly *Penaeus monodon* (tiger shrimp), *Macrobrachium rosenbergii* (gaint freshwater prawn), *Fenneopenacus indicus* (Chaka or Indian white shrimp), *Metapenacus monocetos* (horina/ brown shrimp), *Penaeus semisulcats* (green tiger shrimp) and *Fenneropenacus merguinsis* (banana shrimp).

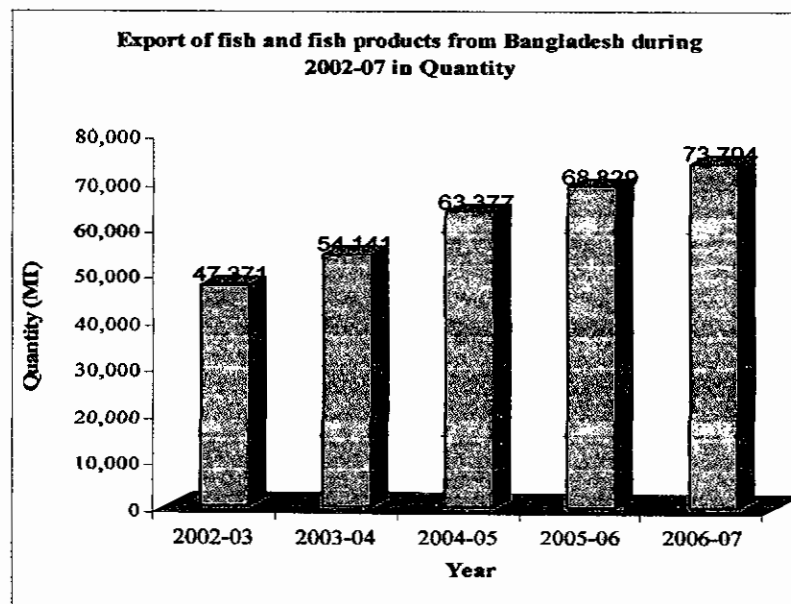


Figure 1.2: Export of fish and fisheries product from Bangladesh during 2002-07.

Export oriented shrimp culture has undergone rapid horizontal expansion in Bangladesh since the mid 1980s. This sector has benefited from a growing global demand for shrimp and a series of stimulus measures to increase Bangladesh exports and diversify the export base.

Currently, shrimp and prawn contribute about 4.9 percent of all exports, more than 80 percent of which is cultivated or caught in inland waterways. As the sector expanded, the number of fish and shrimp processing plants grew concomitantly. In the early 1970s there were 13 processing plants. By the mid 1980s there were 54 plants and currently there are 146. Not all of these plants are currently operational.

Shrimp culture is mainly confined to the district of Khulna, Satkhria and Bagerhat in the Khulna Division and the district of Cox's Bazar, Chittagong and Noakhali in the Chittagong Division.

The main cultivated species is *Penaeus monodon* (tiger shrimp), more commonly referred to as bagda and *Macrobrachium rosenbergii* (giant freshwater prawn) commonly known as golda. In 2001, 25,000 tons of bagda (representing 58% of the total national shrimp production of 42,000 tons) and 12,000 tons of golda (28% of the total production) were produced (Banks, 2001). Whilst production is orchestrated to produce bagda and golda, small amounts of other shrimp species (all salt/ brackish type) are also grown. They are: Chaka or Indian white shrimp, horina/ brown shrimp, green tiger shrimp and banana shrimp. Together they account for about 6,000 tons, equivalent to 14% of the total production (Banks, 2002).

Total area from both bagda and golda farms in 2001 was Tk. 17,169 M (US\$ 301M) (Banks, 2002). Bagda gher outputs account for 80% (Tk. 13,864 M or US\$ 243M) and golda outputs for 20% (Tk. 3,305 or US\$58M).

Once harvested, the flow of shrimps from gher to factory takes place within 12-24 hours. The post harvest flow goes from farmer to depot to agent to factory. All trade ultimately feeds through the agents who buy the entire product to supply to the factory.

Each depot purchase directly from the farm while some large scale farmers are able to supply direct to the depot or even to the agent. There are no direct link between farmers and the processing factories. Processors provide a commission to agents who complete for supplies at the depot. One agent may deal with several factories. The exert influence at both ends of scale. The commission agents largely fix the prices.

Processing includes de-heading and cleaning of the shrimps, freezing and packing. There are 146 processing factories in Bangladesh with a capacity for processing of 2, 65,000 MT per year. Of these, 44 are presently operated in Khulna (mostly located along Rupsha River), 38 are operational in the Chittagong district and 51 plants have either closed down or are awaiting approval for the quality inspection license. Khulna accounts for around for 60-75% of the total production capacity. Only around 20% of the total factory capacity is utilized.

Quality control of fish and fish products are like the two sides of a coin. One cannot do without other. Quality, in general, means the wholesomeness or the state of the excellence

of a particular product in terms of its appearance, shape, color, taste, and competitiveness in price to the buyer. In a nutshell, quality means the fulfillment of the customer's requirements. Quality control is a critical element in the shrimp distribution channel. DOF has recently amended the Fish Inspection and Quality Control Act to incorporate Hazard Analysis Critical Control Point (HACCP) standards and principles and EU requirements. Moreover, the Upazila Fisheries Officers in Bangladesh have been instructed officially to enforce The Fish Inspection and Quality Control Act under which they can visit shrimp depots and take action on defaulters.

Fresh fish and shrimp losses are mainly a result of spoilage, caused principally by lack of chilling, coupled with poor storage, distribution and marketing facilities. The spoilage process in shrimp result in changes related to flavor, texture, appearance and many biochemical components of shrimp muscle. Spoilage is irreversible, and process does not improve the quality of bad raw material. Proper processing technique can only preserve the state of raw material. Thus loss of freshness of shrimp during storage can be evaluated by using many different methods i.e. sensory, biochemical and microbiological parameters. Increasing fresh fish and shrimp stability will not only permit the retail marketing of an acceptable product over a longer period of time, but will also permit market expansion by allowing high quality shrimp to be sent distant inland markets.

USA, Japan and the EU are the main buyers of Bangladeshi shrimp. Over the past 6 years (1995-2000), the value of their total shrimp imports was quite stable at US\$ 10 billion. Worldwide, however, the consumption of shrimp is steadily increasing. The Republic of Korea, Singapore and Hong Kong emerged as new shrimp markets. The average annual price increase of shrimp from 1991 to 1998 was about 13% while the total price increase in 10 years was about 140 %. In 2001-2002, Bangladesh achieved its ever-highest export earnings from fisheries sector (Tk. 18,851M or US\$325M) by exporting almost 30,000 tons of shrimp, which contributed 90% of the frozen fish export. The percentage of export of frozen fish and fish products to different countries during 2006-07 is shown in the figure 1.3.

Bangladesh exports shrimp to three major markets the USA, Japan, and the EU, competing with Thailand, Ecuador, Indonesia, India, Mexico and Vietnam. Despite of the relative importance of shrimp for economic growth, the industry is plagued by inefficiencies

(institutional, production, processing and marketing) and is exposed to important social and environmental risks.

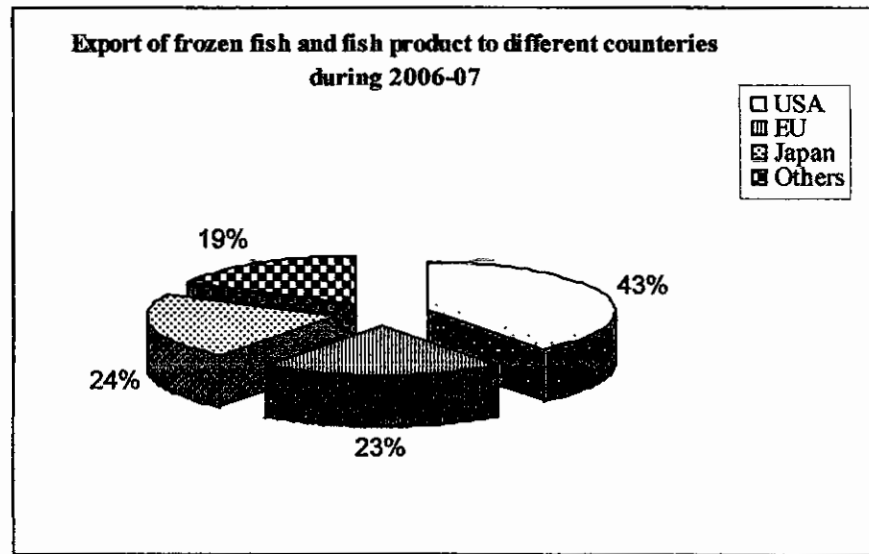


Figure 1.3: Export of frozen fish and fish product from Bangladesh during 2006-07.

Shrimp is one of the most perishable food items, highly susceptible to bacteria and enzymatic spoilage unless handled, processed packed and stored scientifically. Shrimp fishery product is the largest signal foreign exchange earning export item for Bangladesh (Mazid, 2002).

At present importing countries are becoming more and more quality conscious and are imposing stringent quality standards for the products imported by them. Quality concepts have undergone tremendous changes over years. Earlier the quality control was done at the end product stage. However, the quality is the most vital problem in beginning of the process until the end product.

Quality of shrimp is usually dependent mainly on species, storage temperature (0°C , 10°C , 20°C , and 30°C). But researchers have confined the degree of quality to be mainly dependent on temperature. Higher storage temperature results in quicker spoilage of shrimp (Nguyen, 1997). In Bangladesh shrimp are distributed from harvest to the factory /industry with or without ice. There are great variations in the usage of ice (ratio of ice to shrimp) even when it is being used (Azam, 2004). Even when, ice is used for shrimp, usually the ratio varied i.e. 1:0.5, 1:1, 1:2.

Shrimp are grown primarily for the international market and, although Bangladesh is a small player in terms of its share of the international market (i.e. 4.2% of world production of farmed shrimp), it is the seventh largest cultured shrimp producer in the world. The United States of America (USA), Japan and the European Union (EU) are the major destinations of exports. Foreign exchange earnings from Bangladesh's fisheries sector depend, in the main, on shrimp (Kashem, 1996).

To better distribute gains along the chain, increase production, raise productivity, and benefit the poor, this report employs several intersecting and overlapping frameworks to analyze the costs and returns within this sector.

Although shrimp production and cultivation in Bangladesh has proven to be dynamic and growth rates have been positive and sustained, fish and fisheries products are typically a high-risk food export, subject to careful inspection for pathogens, natural toxins, bacteria, and other possible contaminants. Additionally, concern was raised about contamination from banned antibiotics (Nitrofurans, Chloramphenicols) that occur primarily in hatcheries and through the feed supply and grow-out systems.

In 1996, the Food and Agricultural Organization developed a program to provide targeted support and technical assistance to the sector based on the Hazard Analysis Critical Control Point (HACCP) endorsed by the US Food and Drug Administration.

The European Union imposed a ban on shrimp imports from Bangladesh in 1997, in response to inspection failures in a number of processing plants. Between 1997 and the end of 1998, the volume of shrimp exported dropped by 20 percent. By December 1998, shrimp exports had fallen by almost a quarter. The ban deprived Bangladesh of their largest shrimp export market and set in motion a series of changes throughout the sector, spurring investment in technology, hygiene practices, and new processing techniques. Cato and Subasinge report that by 2003 the shrimp industry had invested \$17.6 million in processing plant upgrades. By 2002, of the 65 plants licensed for export, 45 had secured EU approval.

Despite these investments, there is evidence that a number of processing plants have failed to implement adequate changes in securing their supply of shrimp and that the risk

of contamination remains significant. The antibiotic contamination has been the focus of the recent EU inspection and their primary concerns center on inadequate laboratory testing. Unfortunately, in the absence of comprehensive inspection for these banned antibiotics throughout the chain, it is unlikely that improvements in handling in the processing plants will control the problem.

The Frozen Food Export Association (BFFEA) has fix up the target of 1.5 Billion \$ by 2012. So to fulfill the target we should gave much more attention to produce quality and safe food in the shrimp industry.

Recently some of consignments are rejected in EU and USFDA due to presences of micro-organism, decomposition, filth as well as short of weight and count. So this is the high time to address the quality aspects for producing safe shrimp otherwise the country will lose the international market.

1.2. Objectives of the study

The objectives of the study were:

- To determine the Critical Control Point (CCP) where the quality is deteriorate in the processing industry.
- To determine the microbiological load of shrimp (Received raw shrimp, Export graded shrimp and Final product), water and SWAB.

Chapter 2

Literature Review

At a temperature of 0° C merely slows down microbiological activity. An increase in temperature of 1 or 2 will markedly increase the rate of bacterial growth and reduced the shelf life (Matches, 1982).

Microbiology of fresh and spoiled fish has been reviewed extensively (Tarr, 1954; Shewan, 1961; Shewan and Hobbs, 1967; Wood, 1967). Microbial proliferation begins with death of fish, at which time each natural defense mechanisms are destroyed. The rate of growth will depend on the number of types present on the fish and the temperature at which the fish are held.

Microbiological quality indices, namely standard plate count (SPC), presence / count of *V. cholera*, *Salmonella* sp. and faecal coliforms, were determined at specific points in the preparation of frozen prawns (*Penaeus monodon*) (Rose *et. al.*, 1990).

The importance of cool chain food products in the international market had stimulated many studies of the effects of freezing and low temperature storage in the bacteria associated with food. From the public health point of view, particular interest has been shown in reports indicating the survival of bacteria that have been frequently implicated, directly or indirectly with potential food poisoning hazard (Kereluk and Gunderson, 1959; Kereluk *et. al.*, 1961).

In general the effects of reducing temperature on bacteria depends on various factors such as rate and type of freezing (Luyet and Gehinio, 1940), initials numbers types and stages of growth phase of microorganisms involved (Record and Tailor, 1953) thawing processes, number of cyclical defrosting (Hess, 1934) and the physical protection offered by the food itself its components (Keith, 1913).

Microbiological studies are useful in determining the build-up and movement of pathogens and other organisms throughout the facility during processing. Microbiological testing has an indispensable role in the HACCP system. The danger from microorganisms is greater

in tropical countries where the surrounding conditions are favorable for their rapid growth (Cann, 1977).

During processing the raw material may be contaminated by bacterial in various ways. If adequate precautions are not adopted soon after catching to prevent such entry, the fish may become contaminated by heavy bacterial load within a very short period (Valankar, 1961).

It is apparent that traditional quality control methods that are based on the checking of the final product are unable to eliminate food safety problems. In order to improve seafood quality and safety, new management tools have been applied in quality control programs in seafood processing establishments. These quality control programs include Total Quality Management (TQM) in the 1980's; ISO 9000 series of standards; and Hazard Analysis Critical Control Point (HACCP) (Limpus 1997). HACCP is currently regarded as the best preventive system of quality control. HACCP is a systematic approach to be used in food production as a means to ensure food safety (Dillon and Griffith 1996).

In the 1960's, the Pillsbury Company in cooperation with the National Aeronautic and Space Administration (NASA) first constructed HACCP to describe the systematic approach to food safety. The goal of the program was to come as close to 100% assurance as possible that the food produced for space use would not be contaminated with bacterial or viral pathogens, toxins, chemicals or physical hazards that could cause an illness or injury (Pierson and Corlett 1992).

Codex Alimentarius states that the HACCP system, which is science based and systematic, identifies specific hazards and measures for their control to ensure the safety of food. HACCP is a tool to assess hazards and establish control systems by focus on prevention rather than relying mainly on end-product testing (Codex 1997). HACCP systems are designed to prevent and control food-safety hazards from the time a factory receives raw material through production to distribution to the consumer (NSHA 1997). Effective HACCP implementation is very important to avoid the adverse human health and economic consequences of food-borne illness or food borne injury.

In the food industry, sanitation means creating and maintaining hygienic and healthy conditions. Sanitation can reduce the growth of microorganisms in the processing environment. This can reduce contamination of food by microorganisms that cause food-borne illness and food spoilage. Equipment can be free of visible dirt and still be contaminated with microorganisms that can cause illness or food spoilage (Marriott 1997). Cleaning and disinfection are among the most important operations in today's food industry. In order to ensure the microbiological quality of foods, it is important that all factors are addressed when carrying out cleaning and disinfection procedures (Huss 1994). Faecal coliforms belong to the family *Enterobacteriaceae* whose natural habitat is the faeces of man and warm-blooded animals. In this family there are a number of "pathogens", such as *Salmonella sp.*, *Shigella sp.* and *Escherichia coli* (Bonnell 1994).

Salmonella sp. can build up biofilm. Biofilms are very hard to remove during cleaning. Salmonellosis usually causes nausea, vomiting, and diarrhea, because the toxins irritate the walls of intestines. Salmonellosis rarely causes death, but deaths may occur if the patient is infant, elderly, or already sick from other illnesses (Marriott 1997). Seafood can be contaminated directly or through polluted water (Lee and Hilderbrand 1992).

The freshness quality of a particular food refers to the degree of excellence (Botta, 1995). The degree of excellence referred all sensory/ organoleptic variables (appearance, texture, odor and flavor). Freshness quality is considered to be an extremely important factor in determining overall quality (Howgate, 1982). The location of harvest (Huss, 1988), time of the year (Love, 1988), method of catching (Botta *et. al.*, 1987; Huss, 1988) and the manner in which seafood handled (Botta *et. al.*, 1989) seriously affect the degree of excellence. Freshness of seafood has a considerable influence in its quality and is the most important criteria for judging the quality (Botta, 1994)

Shrimps are considered to be among the most perishable of foodstuffs. Fresh shrimp are very susceptible to spoilage and as a consequence may be expected to undergo some degree of deterioration between catching and delivery to the receiving room of the processing plant. Temperature affects the rate at which bacteria multiply (Ronsivalli and Charm, 1975). The most important means of preserving fresh shrimp is by chilling to about 0 to -40°C. The most common chilling media are wet ice, mixture of ice and sea water or refrigerated seawater (Sikorski *et. al.*, 1994).

Sensory/ organoleptic evaluation is the most satisfactory and acceptable method to assess the quality of fish and shellfish (Connell, 1976; Howgate, 1982; Larmond, 1986).

Samples collected from a commercial processing plant had higher initial counts than those processing in the laboratory (Lobrerria *et. al.*, 1990). With proper washing (use of clean, potable, chilled water), the bacterial load of shrimp can be reduced by as much as 65% (Lisac, 1983; De Silvia, 1985; Rajaduraic, 1985).

Studies on farmed brackish water shrimp aimed to reveal whether the microbiological load of tropical shrimps was significantly higher than those from temperate waters and whether salmonella is a natural or commercially contaminant of farmed shrimp (Putro *et. al.*, 1990).

The tropical shrimp have higher bacterial load compared to temperate species (Peranginangin *et. al.*, 1982). Cold water shrimp frequently have bacterial load of 10^3 /g, although the range sometimes vary greatly and on certain occasions has been reported up to 10^7 /g (Liston, 1980). In most cases, warm water shrimp and prawns have counts of 10^6 /g on capture (Vanderzant *et. al.*, 1970; Cann, 1974).

Microbiological evaluation of prawn processing was made once a month, from samples obtained at four processing stages in a factory, over a period of six months (Jayaweera and Subasinghe, 1990).

Large numbers of *V. cholerae* must usually be ingested to cause cholera, thus problems often occur when poor handling and inadequate refrigeration have allowed the organism to multiply. In order to compete in the international market, prawn/ shrimp exporters must meet microbiological standards set by importing countries (Dangla, 1984). Problems faced by Asian prawn/shrimp producers have been reviewed (Sumner *et. al.*, 1982).

In some countries, seafood is a main supply of animal protein for people. However, consumption of fish may also cause diseases due to the presence of biological, chemical and physical hazards (Huss 1994).

The true number of incidences of disease transmitted by food is not known. There are many reasons for this. Only few countries have reported incidence of food-borne diseases. Number of cases in outbreaks of food-borne diseases caused by seafood is generally small when compared to those caused by poultry, dairy and meat products. For example, in the United States in 1993 to 1997 there were about 2,751 food-borne disease outbreaks. These outbreaks caused a reported 86,058 persons to become ill. Of these there were only 188 food-borne outbreaks (7%) related to seafood (Olsen *et al.* 2000).

In 1971, the HACCP concept was first presented at the first National Conference on Food Protection. During the 1970's and 1980's a number of the food companies requested information to help them establish their own HACCP programs.

In 1985, USA National Academy of Sciences (NAS) recommended the HACCP system in the publication *Evaluation of the role of microbiological criteria for food and food ingredients* (Pierson and Corlett 1992). The Advisory Committee on Microbiological Criteria for food developed material elaborated principles of this food safety and quality management system based on NAS recommendation and provided guidance for their application for food processing operations. HACCP was recommended in both food regulator and industry because it was the most effective and efficient means of assuring the safety of the food supply (Limpus 1997).

In 1990 the Codex Alimentarius Commission (CAC) on Food Hygiene started to prepare a draft guideline for the application of HACCP system (Huss 1994). In the last ten years, HACCP has become widely used. It is now a legislative requirement in USA, Canada and EU-countries. Some countries such as Australia, New Zealand, Canada, Japan, Egypt, South Africa, and many others have also adopted or are considering food safety control systems based on HACCP.

In Canada, the Quality Management Program (QMP) was established as a mandatory program for food inspection in February 1992. It was based on HACCP principles. The QMP uses the principles of HACCP for ensuring safe food production, to provide a high level of assurance that fish and seafood products produced in Canada are safe and wholesome to eat (CFIA 2001).

In 1995, The United State Food and Drug Administration (FDA) published final regulations that require processors of fish and fishery products to develop and implement HACCP systems for their operations including imported fish and fishery products. Those regulations became effective on December 18, 1997 (FDA 1998).

The European Union has issued the Directive 91/493/EEC (22/7/1991) and the Directive 94/356/EC (20/5/1994), which requires all seafood processing establishments that export their products to EU market to carry out HACCP system called "Own check".

In the past five years, many Asian countries have implemented national HACCP programs for their fish processing industry in line with international trends. HACCP programs comply with the regulations of the importing countries especially the EU and USA (Eong and Ngei 2000).

Before the application of HACCP principles, five preliminary tasks need to be accomplished. The five preliminary tasks are following (NACMCF 1997):

- Assemble the HACCP team
- Describe the food and its distribution
- Describe the intended use and consumers of the food
- Develop a flow diagram which describes the process
- On-site confirmation of flow diagram

After the five preliminary tasks have been completed, the seven principles of HACCP are applied. As reviewed by Codex Alimentarius, the HACCP system consists of the following seven principles (Codex 1997):

1. Conduct a hazard analysis.
2. Determine the Critical Control Points (CCP).
3. Establish the Critical limit. Critical limit is defined as an established point, which must not be exceeded if a hazard is to be controlled at a CCP.
4. Establish a system to monitor the CCP. Monitoring is the scheduled measurement or observation of a CCP relative to its critical limits. The monitoring must be able to detect loss of control at the CCP.
5. Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.

6. Establish procedures for verification to confirm that the HACCP system is working effectively.
7. Establish documentation concerning all procedures and records appropriate to these principles and their application.

In this food safety and quality management system, potential hazards can be identified in processing of safe food, and where and when they are most likely to occur. Then necessary steps must be taken to prevent them from happening or to correct them if they do occur. The hazard analysis steps are fundamental to the HACCP system. To establish a plan that effectively prevents food safety hazards it is crucial that all significant safety hazards and the measures to control them are identified (NSHA 1997).

As reviewed by the National Advisory Committee on Microbiological Criteria for foods (NACMCF 1997), the hazards are defined as a biological, chemical or physical agent that is reasonably likely to cause illness or injury in the absence of its control. Examples of hazards consist of (Limpus 1997):

- Biological hazards, which include pathogenic microbes (bacteria, viruses, parasites), toxic plants and animals, and products of decomposition (histamine).
- Chemical hazards, which include natural toxins, pesticides, cleaning compounds, veterinary drug residues (antibiotics), heavy metals, and unapproved food and color additives.
- Physical hazard, which include bones, metal fragments, glass, stone that may cut the mouth, break teeth, cause choking, or perforate the alimentary tract.

Determining a critical control point (CCP) plays an important role in a HACCP program. CCP is defined as a step at which control can be applied and is essential to prevent or eliminate a food-safety hazard or reduce it to an acceptable level (NSHA 1997). The CCPs are the points in the process where HACCP control activities will occur. The CCP should be under constant control by humans or by machines and the performance of the control step should be monitored and documented (Lee and Hildibrand 1992). The determination of a CCP in the HACCP system can be applied with a decision tree that can be a useful as a tool to identify CCP, but it is not mandatory element of HACCP (NSHA 1997).

The inspection of plants operating under HACCP plans differs from traditional inspection methods of food safety control. Traditional methods evaluate processing practices on the day or days of inspection. The approach of this food safety and quality management program allows regulators to look at what happens in the plant back in time by examining the firm's monitoring and corrective action records (NSHA 1997).

HACCP can not stand alone; it is a part of a larger system of control procedures. HACCP implementation depends on the competence of people who develop and operate it and the prerequisite programs. Prerequisite programs may impact on the safety of food; they also are concerned with ensuring that foods are wholesome and suitable for consumption. Formal prerequisite programs are increasingly and successfully used to support the implementation of HACCP in food processing (Wallace and William 2001).

Some countries have already identified prerequisites. For example, in North America the US Department of Agriculture Food Safety Inspection Service required not only HACCP, but also Good Manufacturing Practice and Sanitation Standard Operation Procedures (SSOPs) (NSHA 1997). Similarly, the Food and Drug Administration required HACCP and the prerequisite of GMP as a specific requirement for seafood production. The prerequisite programs may cover (NACMCF 1997):

- Facilities
- Supplier control
- Specification
- Production equipment
- Personnel hygiene and training
- Cleaning and sanitation program
- Pest control program
- Traceability and recall
- Chemical control
- Receiving, storage and shipping

In the prerequisite programs, cleaning and sanitation plays an important role. When it is in place, HACCP can be more effective because it can be concentrated on the hazards associated with the food or processing and not on the processing plant environment. In

some situation, it may reduce number of critical control points in HACCP plans (Marriott 1997).

As reviewed by Bonnell (1994), it is common for food industries to use bacteriological indicators to assess, control and ensure effective plant sanitation practices and ensure a food product that is of a quality that is acceptable to the customer.

Total plate count (TPC) is one of the most commonly used microbiological indicators. It provides an assessment of the general sanitation level of plant practices. It serves as an index of the probable shelf life of the product (Bonnell 1994).

High levels of TPC in fish products can be caused by a number of conditions:

- Pre-processing spoilage.
- Poor plant sanitation. This can be due to the unsanitary handling of the products or contact of the product with improperly cleaned equipment.
- Improper temperature control during processing.

Generally speaking, the presence of these bacteria on fish products indicates a failure in sanitary practices of the plant and is usually due to one or more of the following (Bonnell 1994):

- Poor employee hygienic practices
- Unsanitary handling practices
- Poor clean up procedures
- The use of unapproved water.

It has been established that rapid fish spoilage even at cold temperature is preliminarily due to bacterial action (Shewan, 1962).

Smole and Raspor, (1997) carried out rapid detection of food born pathogens and food spoilage microorganisms is becoming increasingly important in modern food production.

Howgate (1985) worked on the handling and processing of fish in Pakistan with special reference to the quality and quality of fish and fish products.

Fresh fish and shellfish are highly perishable food commodities due to their biological composition. The muscle tissue of fish and shellfish spoils fast. This can be explained by the higher water content of shrimp, the higher free amino acid content, and the lower content of connective tissue as compared to other flesh foods. This is good condition for activity of microorganisms (Ranke, 1959).

Pokern (1990) worked on microbiology in the food processing and provides an analysis of the following: microbiological control of food quality, role of microbiologists in conducting the production microbiologic criteria and food legislature, source of microorganisms in food, development of microbial association, in processed article, ecology of microorganisms growth, physiology of food spoilage, inhibition of microorganisms by technologic procedures, thermal processing, freezing, dehydration, preservation with preservatives, strategy of microbiologic control in food industry.

Quality problem are also caused by the particular nature and variability of raw materials due to the influence of environment, feed and season. Quality control or the war against spoilage can be define as 'all methods, processing, techniques and processed employed to inhibit, delay or prevent deteriorative changes in freshly killed fish and in fish products and to prevent contamination or adulteration'. More simply quality control is the application of technology to the handling, processing, manufacture, storage and distribution of fish and fish products in order to meet established or acceptable forms. Dempsey (1971) and Sunarya (1996) studied on improvements in fish handling, processing and quality control.

A great variability exists in the size and extent of handling in fish processing establishments. Accordingly the hygienic requirements and the design in fish handling areas may vary considerably. Quite obviously the requirements to a small establishment which is only repacking fish in ice and catering for a local market are different from the hygienic requirements to a large establishment, processing a variety of sophisticated products including heat treated and composite products and exporting to countries all over the world. However, all the requirements commonly listed in legislation and codes of practice are not equally important. The more important factors include: facilities for water supply, waste disposal and cooling and cold storage facilities and -capacity. Of less

importance are buildings, ventilation, factory location, clothes changing facilities, lightning and roadways (ICMSF 1988).

Pathogen survival through a cook step can cause consumer illness. Cooking is a relatively severe heat treatment, usually performed before the product is placed in the finished product container. Cooking procedures are often established to develop the desirable sensory attributes characteristic of cooked fish and fishery products, not specifically to eliminate pathogens. An important consequence of thorough cooking is the destruction of vegetative cells of pathogens (or reduction to an acceptable level) that may have been introduced in the process by the raw materials or by processing that occurs before the cook step. Cooking processes are not usually designed to eliminate spores of pathogens. (FDA, 2001; Rippen 1998).

Undercooking may allow the survival of pathogens leading to several unintentional but potentially hazardous conditions: 1) direct contamination of a ready-to-eat product with pathogens, 2) elimination of other less heat resistant microorganisms that, if present, may suppress pathogen growth or cause spoilage prior to significant pathogen growth, and 3) thermal conditioning of pathogens and increasing their heat resistance to any subsequent cooking or reheating step. It is also possible for a sublethal heating step to trigger bacterial spores to germinate, producing vegetative cells that are more hazardous than spores, but also far more vulnerable to subsequent reheating (Rippen, 1998).

More recently, FAO has initiated a project in Bangladesh to assist in the development of the seafood industry. The project began in April 1996, and focuses directly on the preparation of a HACCP-based fish safety and quality assurance program³ for shrimp and fish plants in Bangladesh. This project is designed to train key persons in the private and public sectors to design and implement HACCP-based programs. It is also designed to assist the government and shrimp industry in developing the HACCP concept and to inform Bangladesh industry and government personnel about the new sanitary and quality requirements of major importing countries, in particular those that relate to the application of HACCP principles (FAO 1995).

A parallel project carried out by INFOFISH with Common Fund for Commodities (CFC) funding, focuses on the export promotion of value-added products and their sustainable

development. This project began in mid-1996 and includes such activities as market feasibility studies to identify potential exporters, cost analyses, visits to target markets, the development of pilot production programs, assessing consumer acceptability, exporting value-added products and monitoring the export activity, providing production manuals and industry training, and organizing investment seminars. A final objective includes improving the quality and sustainability of the processed products (INFOFISH 1996).

Industry buyers have experienced problems with supply irregularity, including poor delivery times, an inability to execute orders in time, under-sizing, inclusion of mixed sizes, and poor quality (such as black spot and under-weight shipments). Some buyers have reduced their Bangladesh imports, except for those from time-tested reliable suppliers. Buyers in the U.S. indicated that some shipments from Bangladesh were filthy and decomposed and that product sourcing was inconsistent. Bangladesh products also brought lower retail prices than those from some other countries, and insurance costs for the product were higher due to the refusal of some shrimp processors to take back product in quality disputes. Buyers in Japan also reported quality problems with previously imported shrimp from Bangladesh (CFC/INFOFISH 1997).

As part of the value-added INFOFISH project, visits by Bangladesh shrimp processors to Japan, the EU, and the U.S. were sponsored in February and March 1997. Potential buyers in Germany, France, and Belgium were interested in Bangladesh shrimp products, as long as the processors could assure a constant supply and satisfy quality requirements. Successful initial exports of added-value shrimp products resulted by the end of 1997, particularly to Japan (CFC/INFOFISH 1997).



Chapter 3

Materials and Methods

1. Study Area:

There are 44 running shrimp processing plants in Khulna region. Among them 42 are EU approved. One of the EU approved processing plant (Gemini Sea Food Limited) is selected as study area. Gemini Sea Food Limited is a modern 100% export oriented EU approved shrimp processing plant, which is established in the year of 1984. The factory is located on the eastern bank of the river Rupsha and about 4 kilometers on the south of Khulna city. The processing plant is well communicated with Khulna city; shrimp culturing areas like Bagherhat, Satkhira and the seaport Mongla. The factory is established on an area of about 5 acres of land, which provides enough space for easy movement of raw materials and easy disposal of wastes of the factory. The surrounding area of the plant with green vegetation provides an eye catching sylvan shadow. Seaport “Mongla” is about 39 kilometers to the south of the factory and well connected by road. and Northeast side of the Bay of Bengal between 20°34' N to 20°34' N latitudinal and 88°01' E to 92°41' E longitudinal. There are lots of rivers, canals, ponds and other aquatic reservoirs in the country. The coastal area of Bangladesh is abundant with aquatic resources. As such, shrimps and other shrimps are abounded in this region.

2. Study Period: The study period was conducted over six months (June to November 2008) in the study area.

3. Sampling Plan: Table 1.1

<i>Sample name</i>	<i>Sampling point</i>	<i>Quantity of sample / sample unit</i>	<i>Test frequency</i>
1. Receiving raw shrimp	Receiving room	200 gm	Every full Moon and new Moon
2. Export graded shrimp	Export grading table	200 gm	-
3. Final product	Frozen storage lot	200 gm	-
4. Water	1. Overhead tank water	200 ml	-
	2. Receiving tape water	200 ml	-
	3. Beheading tape water	200 ml	-
	4. Processing tape water	200 ml	-
	5. Panning tape water	200 ml	-
6. SWAB	Workers hands (female)	Five female workers	-
	SS-table	Five space of SS table	-

4. Sample Analysis method

4.1: CCP determination

4.1.1: Shrimp Processing Line in Plant:

4.1.1.1. Processing Line-1- Block frozen shrimp

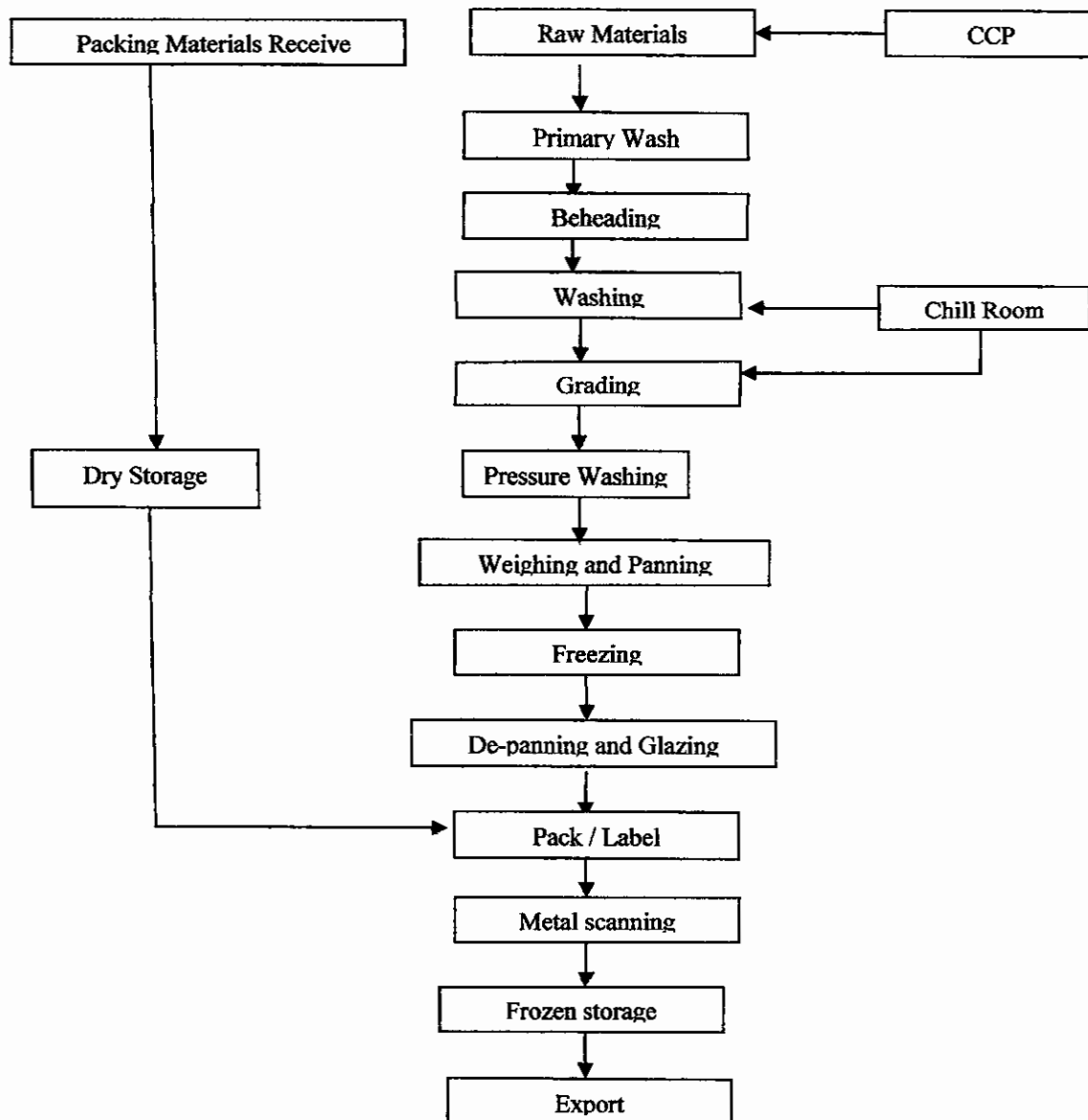
4.1.1.2. Processing Line-2 -Semi-IQF shrimp

4.1.1.3. Processing Line-3 -IQF shrimp

4.1.1.4. Processing Line-4-Cooked IQF shrimp

Process Flow Chart and Narratives in each process line

4.1.1.1. A. Flow Chart- Block frozen shrimp



4.1.1.1. B. Narratives-Block frozen shrimp

1. Raw materials receive: Fresh and high quality head-on raw materials having temperature as low as +5° C with sufficient ice is received after landing, ice sorting, organoleptic check, grading and weighing.

2. Primary wash: It is done with UV- treated chilled running water immediately after receiving.

3. Beheading: Head-on shrimps are beheaded on SS-table with proper ice.

4. Washing: Beheaded shrimps are pressure washed with running UV-treated chilled water.

5. Chill room: If raw materials arrival is too much then, some raw materials are transferred into cold store, where it is kept under +5° C and may have store maximum for 48 hours.

6. Grading: Raw materials are directly carried on to the SS- table of grading section and within shortest time grading is done.

7. Pressure wash: After grading pressure wash is done with running RO and UV-treated chilled water.

8. Weighing and Panning: After pressure wash, the shrimps are weighing as per block sized required and dressed in the SS-tray and kept for load adding cold potable water (around +2° C).

9. Freezing: The shrimp blocks are frozen in the tray for 2 hours.

10. De-panning and glazing: The trays are unloaded and then de-panning and glazing is done with running chilled water.

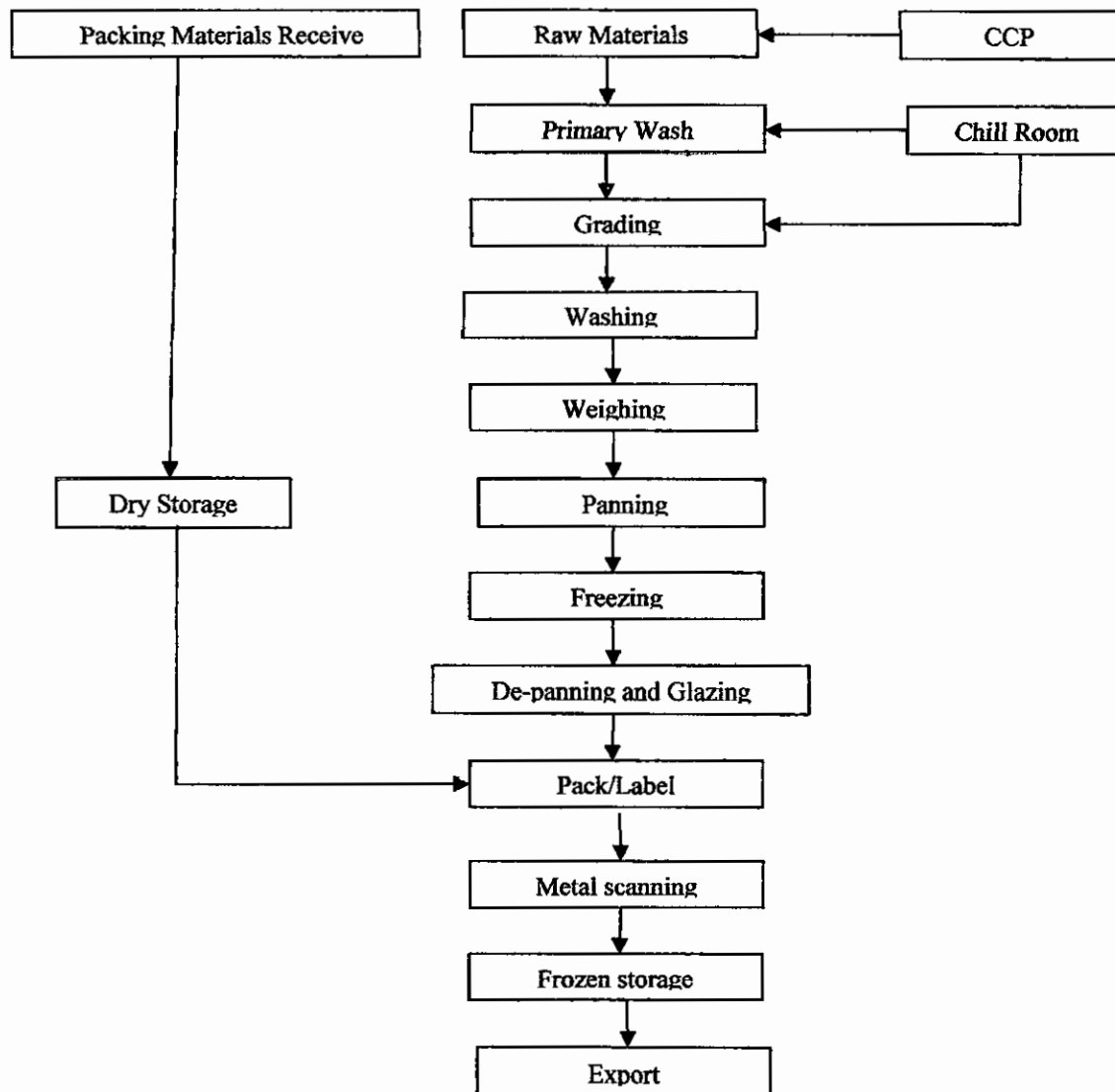
11. Pack/Label: The blocks are packed in small inner cartons and 6 inner cartons are packed within master cartons.

12. Metal Scanning: The master cartoons are passed through metal detector for scanning metallic objects. The detecting sensitivity of the machine is given in the plant facilities.

13. Frozen Storage: The master cartons are carried immediately in to the frozen storage.

14. Export: The finished products are exported from the store following FIFO system within refrigerated van.

4.1.1.2. A. Flow Chart- Semi-IQF shrimp



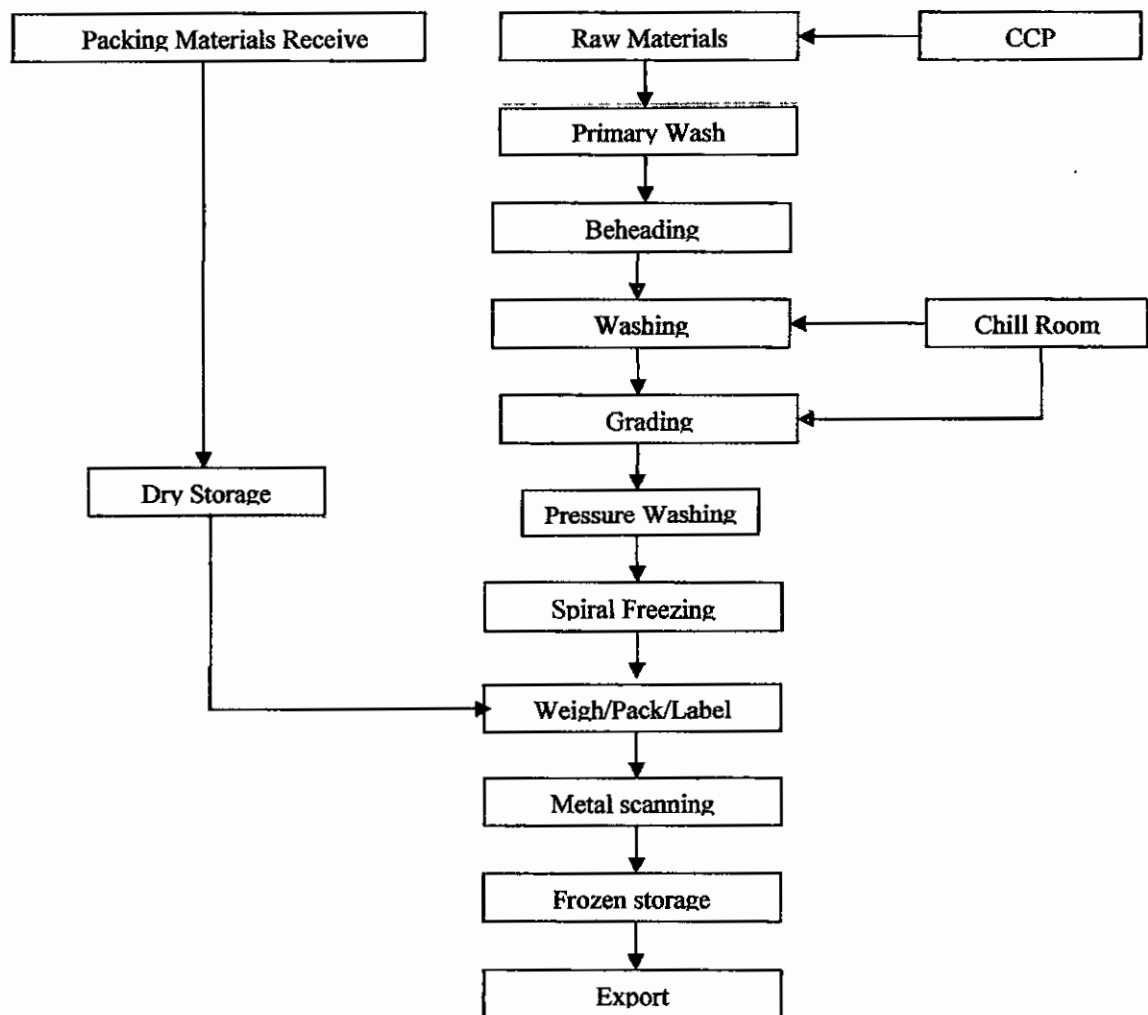
4.1.1.2. B. Narratives- Semi-IQF shrimp

- 1. Raw materials receive:** Fresh and high quality head-on raw materials having temperature as low as +5° C with sufficient ice is received after landing, ice sorting, organoleptic check, grading and weighing.
- 2. Primary wash:** It is done with UV- treated chilled running water immediately after receiving.
- 3. Chill room:** If raw materials arrival is too much then, some raw materials are transferred into cold store (chill store), where it is kept under +5° C and may have store maximum for 48 hours.
- 4. Grading:** Raw materials are directly carried into the grading section from primary wash or from the cold store and within shortest time grading is done. Temperature is maintained enough low.
- 5. Pressure wash:** After grading pressure wash is done with running RO and UV-treated water for removing all types of extraneous materials from shrimp body.
- 6. Weighing:** After pressure wash, shrimps are immediately weighed as per block sized required by the buyer.
- 7. Panning:** Immediately after weighing, shrimps are dressed in SS-tray adding cold potable water (around +2° C) and then loaded for freezing.
- 8. Freezing:** The shrimp blocks are frozen in the paper box/ tray for 2 hours.
- 9. De-panning and glazing:** The trays are unloaded, de-panning and glazing. It is done with running chilled potable water.
- 10. Pack/Label:** The blocks are packed in small inner cartons and inner cartons are packed within master cartons.
- 11. Metal Scanning:** The master cartoons are passed through metal detector for scanning metallic objects. The detecting sensitivity of the machine is given in the plant facilities.

12 Frozen Storage: The master cartons are carried immediately in to the frozen storage.

13. Export: The finished products are exported from the store following FIFO system within refrigerated van.

4.1.1.3. A. Flow Chart-IQF shrimp



4.1.1.3. B. Narratives- IQF shrimp

1. Raw materials receive: Fresh and high quality head-on raw materials having temperature as low as +5° C with sufficient ice is received after landing, ice sorting, organoleptic check, grading and weighing.

2. **Primary wash:** It is done with UV- treated chilled running water immediately after receiving.

3. **Beheading:** Shrimps are beheaded immediately after receiving on SS-table with sufficient ice.

4. **Washing:** Beheaded shrimps are pressure washed with running UV-treated chilled water.

5. **Chill room:** If raw materials arrival is too much then, some raw materials are transferred into cold store, where it is kept under +5° C and may have store maximum for 48 hours.

6. **Grading:** Raw materials are carried on to the SS- table with sufficient ice for controlled temperature in grading section either after washing or from cold store and within shortest time grading is done.

7. **Pressure wash:** After grading pressure wash is done with running RO and UV-treated water for removing all types of extraneous materials from shrimp body.

8. **Spiral Freezing:** The shrimp are loaded on conveyer belt for IQF frizzling.

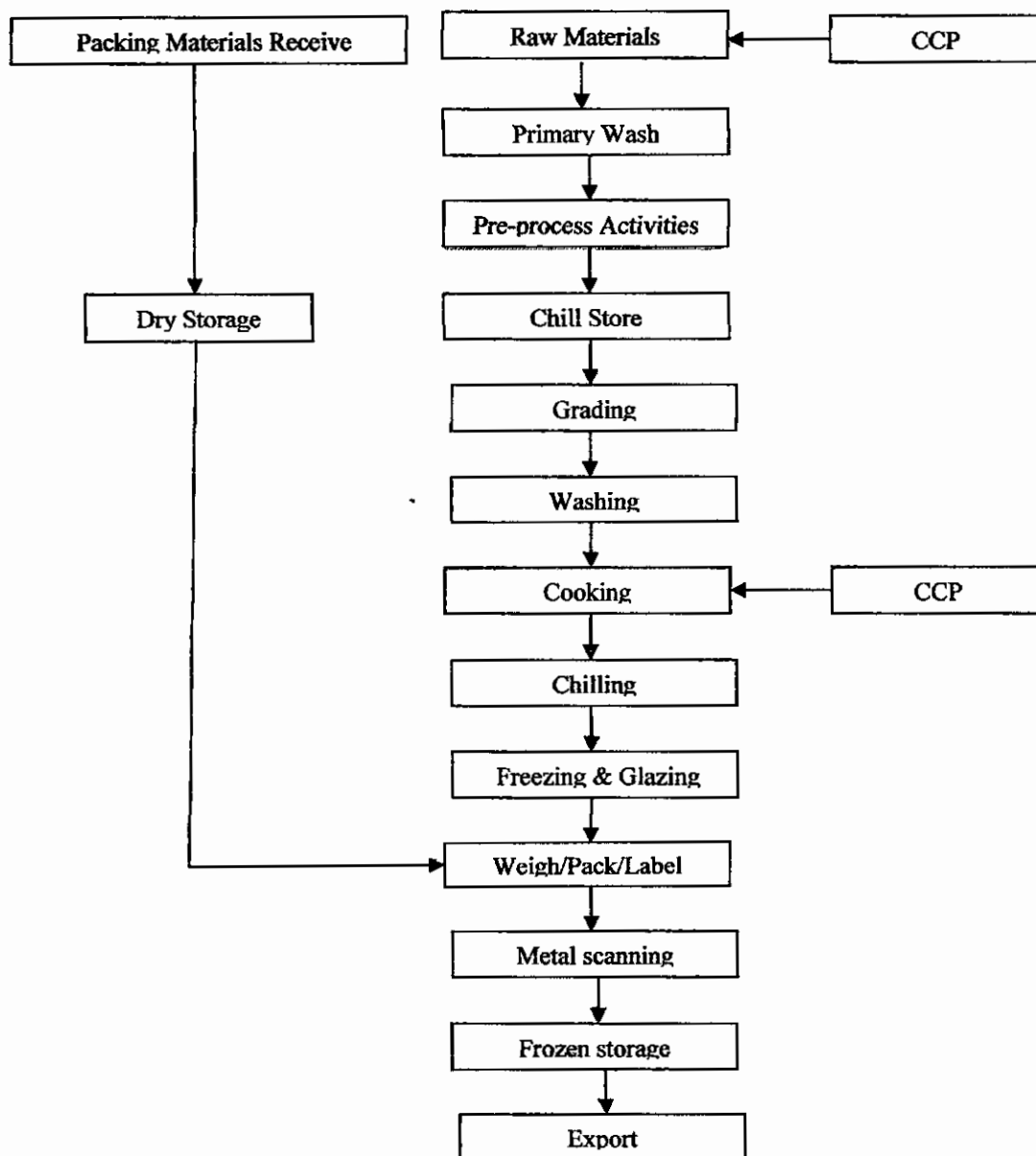
9. **Weigh/Pack/Label:** The frozen IQF shrimps are weighed as per required packing size in printed Polly-bags. Keeping in one master Polly, Inner Polly-bags are kept in one white five –fly master carton.

10. **Metal Scanning:** The master cartoons are passed through metal detector for scanning metallic objects. The detecting sensitivity of the machine is given in the plant facilities.

11. **Frozen Storage:** The master cartons are carried immediately into the frozen storage - 20° C.

12. **Export:** The finished products are exported from the store following FIFO system within refrigerated van.

4.1.1.4. A. Flow Chart-Cooked IQF shrimp



4.1.1.4. B. Narratives-Cooked IQF shrimp

1. Raw materials receive: Fresh and high quality head-on raw materials having temperature as low as +5° C with sufficient ice is received after landing, ice sorting, organoleptic check, grading and weighing.

2. Primary wash: It is done with UV- treated chilled running water immediately after receiving.

- ▶ **3. Beheading:** Shrimps are transferred to beheading section for all required pre-process activities with time and temperature control on SS-table with sufficient ice.
- 4. Chill room:** If raw materials arrival is too much then, some raw materials are transferred into cold store, where it is kept under +5° C and may have store maximum for 48 hours.
- 5. Grading:** Grading is done on SS-table with sufficient ice possibly quickest bringing from primary wash or from chill room.
- 6. Pressure wash:** A second pressure wash is practiced after pre-process activities or chill room hold on with chilled potable water.
- ✦ **7. Cooking:** marinated shrimps are brought to conveyor of system cooker. Cooker is monitored for time and core temperature of shrimp.
- 8. Chilling:** Cooked shrimps are automatically transferred on conveyor of chilling.
- 9. Spiral Freezing & Glazing:** Chilled shrimp are further transferred to freezer with conveyor.
- ✦ **10. Weigh/Pack/Label:** Shrimps are subject to unit weigh, pack and label all require information.
- 11. Metal Scanning:** The master cartoons are passed through metal detector for scanning metallic objects. The detecting sensitivity of the machine is given in the plant facilities.
- 12. Frozen Storage:** Finished products are carried into the frozen store under -20°c temperature. All products are stored count-wise and lot-wise.
- ✦ **13. Export:** The finished products are exported from the store following FIFO system within refrigerated van.

4.1.2. Hazard analysis

There are some steps in each shrimp product manufacturing. The potential hazards of each shrimp product manufacturing were identified through a hazard analysis worksheet with CCP. Through the worksheet whether the hazards were significance or not, justification of hazards and preventive or control measures were identified properly. The worksheet contains 9 columns shown in the Appendix 1. All potentially significant hazards were considerer. The CCP in each shrimp product manufacturing was determining through a decision tree shown in Appendix 3 to determinate CCP in the HACCP system. At each processing step, a significant hazard was determining whether it was a CCP or not. After determining of the CCP, following steps is implementing for each CCP:

- Setting the critical limits,
- Establishing monitoring procedures,
- Establishing corrective action procedures,
- Establishing a record keeping system,
- Establishing verification procedures.

Results of this implementation are presented in the HACCP plan. The HACCP plan form contains CCP, significant hazards, critical limit for each preventive measure, monitoring procedure, corrective active action procedure, record keeping system and verification procedure. The HACCP plan form was shown in Appendix 2.

4.2. Microbiological Test of Shrimp

Microbial analyses followed the procedures of ICMSF (1988).

4.2.1. Standard Plate Count (SPC)

SPC is used to determine the total number of aerobic bacteria present at mesophylic temperature (30°C-37°C). The SPC can be used as general, relative gauge, of the overall load of product. When SPC is used as a part of a shelf life analysis to determine spoilage, the number generated can be compared from test interval to test to verify if the bacteria initially present are capable of growing. When SPC reaches 1,000,000 CFU (Colony Forming Units) per gram or milliliter of product (ICMSF 1986), the product is assumed to be at, or nearing, spoilage. The number may vary greatly and the ultimate shelf life determination should be based upon the physical changes the product is undergoing. The study was carried out to assess the bacterial level in shrimp muscle through out the processing. The result would help creating awareness among the processors and consumers of the potential sources of contamination

Colony count methods provide an estimate of the number of viable microorganisms in the food according to the medium and the time and temperature of incubation. These procedures are based on the assumption that each microbial cell in a sample will form a visible, separate colony when mixed with an agar or other solid medium and permitted to grow.

Culture media

Plate count Agar (PCA)

2.35 g 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 15lbs/inch² pressure (121°C) for 15 minutes.

Procedure

20.0 g of pooled sample was weighed and taken into a sterile warring jar for blending. The sample was added into a flask containing 180 ml 0.1% sterile peptone water. The mixture was homogenized for two minutes. This provided a dilution of 10⁻¹. 1.0 ml of this suspension was transferred into McCart's bottle containing 9 ml of 1.0 % peptone water to give a dilution of 10⁻². This process was repeated for the preparation of 10⁻³ dilutions respectively.

4.2.2. Total Coliform (TC)

Culture media

Lauryl Sulphate Tryptose Broth (LSTB)

3.56 g of LSTB was dissolved in 100 ml of distilled water and then was distribute into test tubes with inserting Durham tubes and sterilized by autoclaving at 15 lbs/inch² pressure (121°C) for 15 minute.

Procedure

20.0 g of pooled sample was weighed and aseptically taken into a sterile warring blender jar and then blended. After blending, the sample was added into a conical flask containing 180 ml 0.1% peptone water. Ten fold serial dilutions were made with this suspension in McCart's bottle containing 9.0 ml of 1.0 % peptone water.

1.0 ml of each dilution was transferred into three screw-caped test tubes containing autoclaved. 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°C ± 1°C for 48 hours. The formation of gas within 48 hours was considered as evidence for the positive test for Coliform organisms.

Number of positive tubes out of 3 was recorded for each dilution. Using the MPN tables, the MPN of TC was calculated based on the portion of confirmed LSTB tubes (with gas production).

Flow chart for the Quantification TC of shrimp is shown in the figure

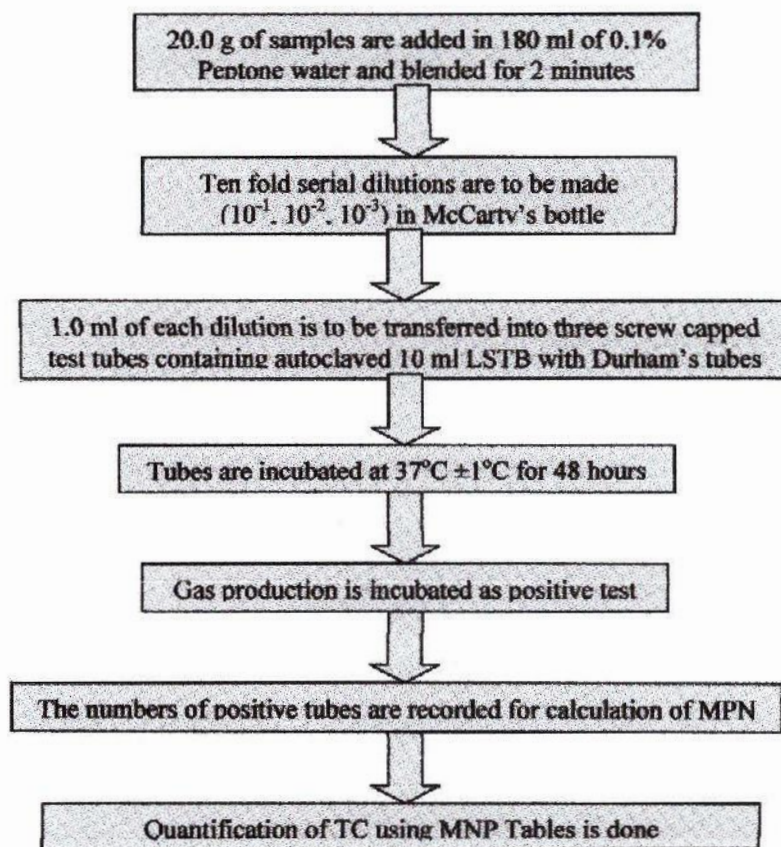


Figure 2.2: Quantification TC



4.2.3. Faecal Coliform (FC)

Because coliform counts are inadequate to differentiate between fecal and nonfecal contamination, a fecal coliform test was developed. Fecal coliforms are coliforms that ferment lactose in EC medium with gas production within 48 h at 45.5°C. With all shellfish isolates, an incubation temperature of 44.5°C (rather than 45.5°C) is used. Fecal coliforms are considered to be more directly associated with fecal contamination from warm-blooded vertebrates than are other members of the coliforms. *E. coli* usually makes up 75-95% of the fecal coliform count in shellfish growing areas, but at times can represent less than 1% of the coliform count (APHA, 1970; Greenberg and Hunt, 1985; Paille *et al.*, 1987).

Culture media

Brilliant Green Lactose Bile Broth (BGLBB)

4 g of was suspended in 100 ml of distilled water. It was mixed well then it was distributed in fermentation tubes and sterilized by autoclaving at 15 lbs/inch² pressure (115°C) for 15 minutes.

Tryptone Water (TW)

1 g was suspended in 100 ml distilled water. Then it was heated to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs/inch² pressure (121°C) for 15 minutes.

Procedure

Those tubes of LSTB were positive for gas productions in TC estimation tests were considered. Subcultures were made from all positive tubes of LSTB into a 10 ml volume of BGLBB and 5 ml TW incubated at $44.5 \pm 0.5^{\circ}\text{C}$ up to 48 hours. BGLBB tubes showing gas production was considered positive for the presence of presumptive 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 FC colonies i.e. black or dark centered with greenish metallic sheen.

Subcultures were made from all positive tubes of LSTB into 5 ml volume of TW Kovac's indole reagent for indole test. Any positive tubes showing pink color was considered for the presence of FC. When both BGLBB tubes showing gas production and TW tubes showing pink color for the same dilutions then this situation was considered for confirmation test of FC.

Flow chart for the Quantification FC of shrimp is shown in the figure

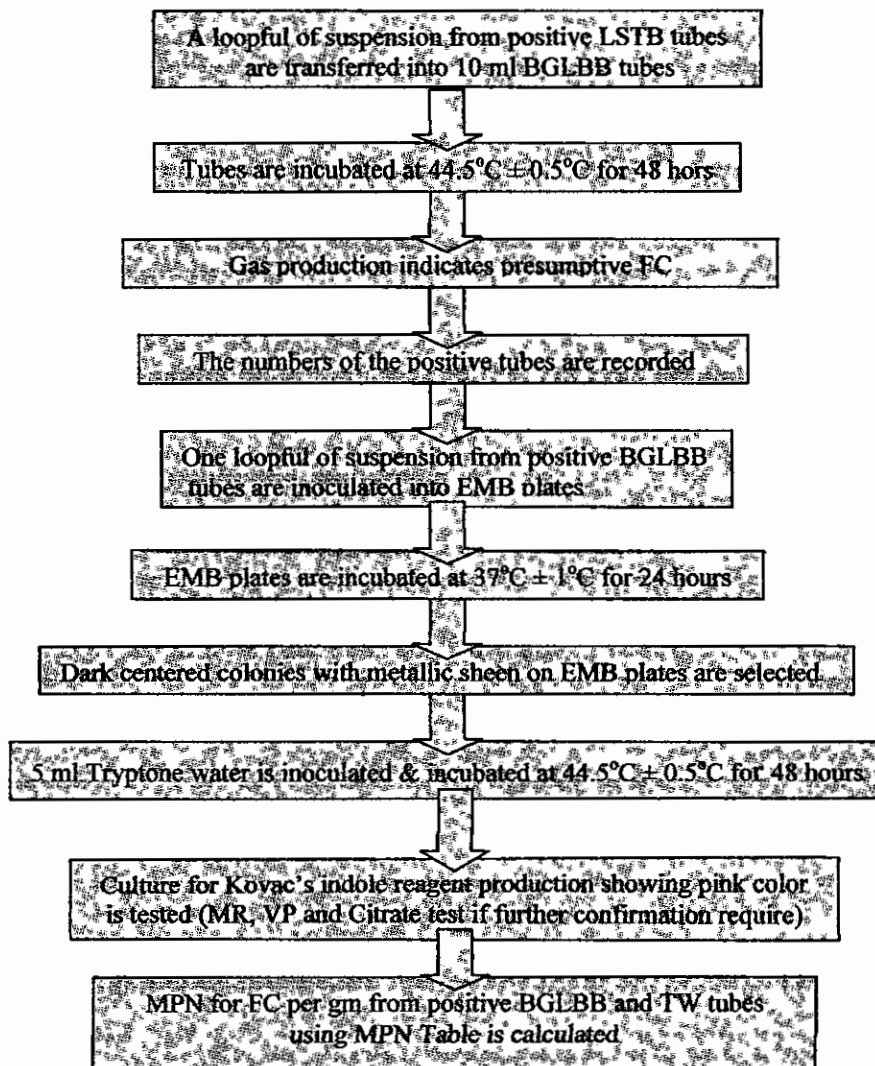


Figure 2.3: Quantification FC

4.2.4. *Salmonella* sp.

Salmonella is a rod-shaped, motile bacterium -non motile exceptions *S. gallinarum* and *S. pullorum*-, non-spore forming and Gram-negative. There is a widespread occurrence in animals, especially in poultry and swine. Environmental sources of the organism include water, soil, insects, factory surfaces, kitchen surfaces, animal feces, raw meats, raw poultry, and raw seafood's, to name only a few.

S. typhi and the paratyphoid bacteria normally cause septicemia and produce typhoid or typhoid-like fever in humans. Other forms of salmonellosis generally produce milder symptoms.

Acute symptoms include nausea, vomiting, abdominal cramps, diarrhea, fever, and headache. Chronic consequences include arthritic symptoms that may follow 3-4 weeks after onset of acute symptoms. Onset of the illness is usually 6-48 h. The infective dose is as few as 15-20 cells; depends upon age and health of host, and strain differences among the members of the genus. Acute symptoms may last for 1 to 2 d or may be prolonged, again depending on host factors, ingested dose, and strain characteristics.

The disease is caused by penetration and passage of *Salmonella* organisms from gut lumen into epithelium of small intestine where inflammation occurs; there is evidence that an enterotoxin may be produced, perhaps within the enterocyte. Foods commonly associated with the disease include raw meats, poultry, eggs, milk and dairy products, fish, shrimp, frog legs, yeast, coconut, sauces and salad dressing, cake mixes, cream-filled desserts and toppings, dried gelatin, peanut butter, cocoa, and chocolate.

Culture media

Selenine Cystine Broth

Part-A

Tryptone –5g/liter

Lactose – 4 g/ liter

Sodium – 10 g /liter

Part- B

Sodium acid Selenine -4 gm/liter

0.2g of part A and 1.33g of part B of selenite broth was suspended in 100 ml distilled water. It was warmed to mix well. It was dispensed and sterilized in 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 g of broth was suspended in 100 ml distilled water. It was heated to dissolve and keep in free flowing system for 30 minutes. It was cooled to 45°C and added 4 ml iodine

solution containing 0.5g iodine and 0.8g potassium iodine. It was mixed well and dispensed as desired.

Xylose Lysine Deoxycholate (XLD) Agar

2.5g 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 roused into sterile petridishes.

Procedure

25.0g of sample was weighed and taken into a warring 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 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 was transferred to the surface of the selective agar media, i.e. 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* sp. shows pink colonies with black centre of H_2S on XLD.

Flow chart of the isolation and identification of *Salmonella* sp in shrimp is shown in the figure

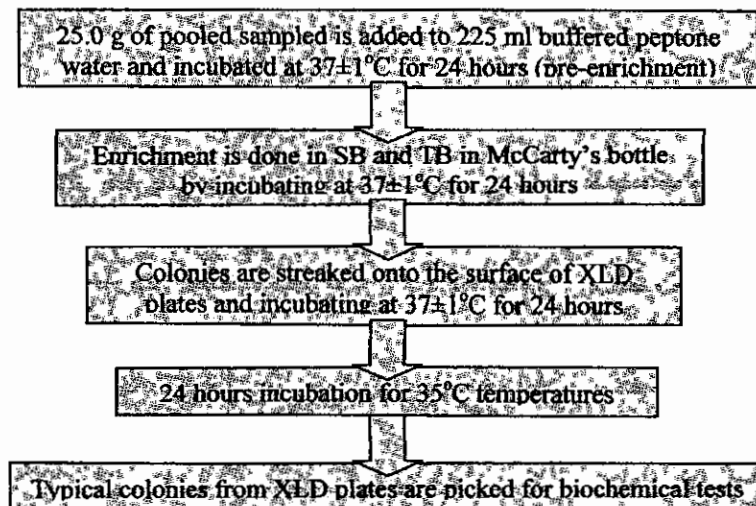


Figure 2.4: Flow chart of the isolation and identification of *Salmonella* sp.

4.2.5. *Vibrio cholerae*

The genus *Vibrio* includes Gram-negative, oxidase-positive (except two species), rod or curved rod-shaped facultative anaerobes. Many *Vibrio* spp. are pathogenic to humans and have been implicated in food-borne disease. *Vibrio* spp. other than *V. cholerae* and *V. mimicus* do not grow in media that lack added sodium chloride, and are referred to as "halophilic" (Elliot *et al.*, 1998)

Culture media

Alkaly Peptone Water (APW)

4.5g of APW was suspended in 225 ml distilled water and slightly heated to dissolved completely and sterilized by autoclaving at 15 lbs/ inch² pressure at 20 minutes.

Thio-sulphate Citrate Bile Salts Sucrose Agar (TCBSA)

4.45g of TCBSA agar was first boiled 50 ml of distilled water to dissolved and boiled for 1 minute. Then the media was dried to 60°C for 15 minutes and was poured into petridishes and allowed to solidify.

Procedure

25.0g of the sample was added with approximately 225 ml of APW in a Warring blender flask. Then the sample containing APW was blended for 1 minute. The sample was incubated at 37°C ± 1°C for 6-8 hours.

At the end of the incubation period, a loopful of the APW was streaked onto TCBSA plates. The plates were then incubated at 37°C ± 1°C for 24 hours. At the end of the incubation, plates were checked for the characteristics of colony of *V. cholerae*. On TCBSA colonies appeared as large (2-3mm.) smooth and yellow, slightly flattened, with opaque centre and translucent peripheries.

4.3. Microbiological Test of Water

4.3.1. Standard Plate Count (SPC)

Culture media

Plate count Agar (PCA)

2.35 g 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 15lbs/inch² pressure (121°C) for 15 minutes.

Procedure

The Petri dishes and test tubes were labeling unambiguously. 1 ml of the sample (10^0) was transferring into the test tube containing 9 ml of peptone saline water to prepare 10^{-1} suspension. 1 ml of the 10^{-1} suspension was transferring into the test tube containing 9 ml peptone saline water (10^{-2}). This process was repeated for the preparation of 10^{-3} dilutions respectively.

The appropriate dilutions were selected and for every dilution 1 ml water sample were transferred into sterile Petri dishes. 15 ml portion of molten PCA was poured into each of these sterile Petri dishes. The plates were then rotated 5 times clockwise, 5time 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. Colonies on the surface were only counted.

Flow chart of the isolation and identification of SPC in water and ice is shown in the figure-

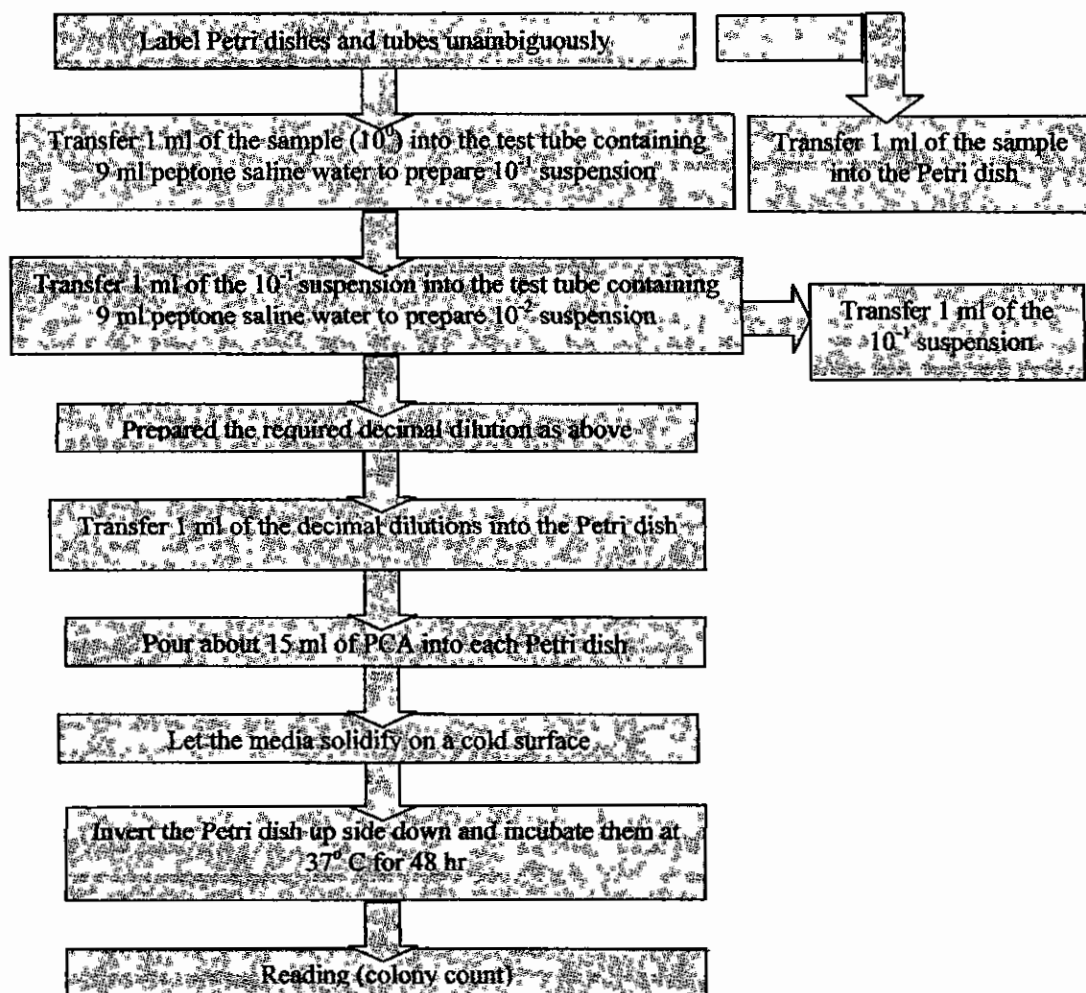


Figure 2.5: Enumeration of SPC of Water

4.3.2. Standard Total Coliform MPN Test (TC)

Culture media

Lauryl Sulphate Tryptose Broth (LSTB)

3.56g of LSTB was dissolved in 100 ml of distilled water and then was distribute into test tubes with inserting Durham tubes and sterilized by autoclaving at 15 lbs/inch² pressure (121°C) for 15 minute.

Procedure

A series of fermentation tubes (primary fermentation tubes) were inoculated with appropriate quantities of the water to be tested.

50 ml water sample in 50 ml (DS) LSTB tube- in 1 tube

10 ml water sample in 10 ml (DS) LSTB tube- in 5 tubes

01 ml water sample in 10 ml (DS) LSTB tube- in 5 tubes

After adding the water sample to the LSTB broth mixed thoroughly in the fermentation tubes. The inoculated fermentation tubes were incubated at 37°C for (24hr+24hr+24hr) totally 72 hr. For gas production in the inverted Durham's tube in the fermentation tubes at the end of 24 hr were observed. Each tube was shaking gently and examined properly whether it produced gas or not. This step was repeated at the end of 48 hr and 72 hr. The presence or absence of gas formation at each examination of the tubes, regardless the amount was recorded. The formation of gas within 72 hr in any amount in the inner fermentation tubes was considered as evidence for positive test for Coliform organisms.

Number of positive tubes out of 3 was recorded for each dilution. Using the MPN tables, the MPN of TC was calculated based on the portion of confirmed LSTB tubes (with gas production).

Flow chart for the Quantification Total Coliform (TC) of water and ice is shown in the figure

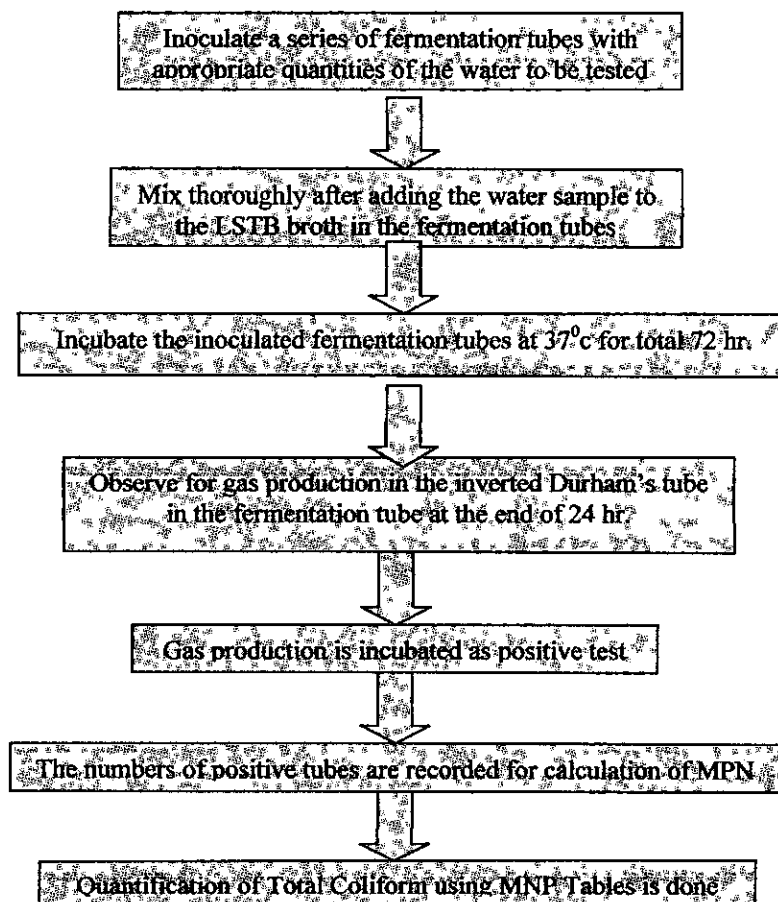


Figure 2.6: Quantification TC of Water

4.3.3. Faecal Coliform MPN Test (FC)

Culture media

Brilliant Green Lactose Bile broth (BGLBB)

4 g BGLBB of was suspended in 100 ml of distilled water. It was mixed well then it was distributed in fermentation tubes and sterilized by autoclaving at 15 lbs/inch² pressure (115° C) for 15 minutes.

Tryptone Water (TW)

1 gm was suspended in 100 ml distilled water. Then it was heated to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs/inch² pressure (121° C) for 15 minutes.

Procedure

Those tubes of Lauryl Sulphate Tryptose Broth (LSTB) were positive for gas productions in TC estimation tests were considered. A loopful of broth from each positive culture into a tube of (2%) BGLBB and a tube of Tryptone Broth (TB) were inoculated. The tubes were incubated at $44 \pm 0.1^\circ\text{C}$. After 24 hr and 48 hr of incubation (2%) BGLBB was observed for gas production in the inverted Durham's tube. After 24 hr of incubation, pipette aseptically 5 ml portion of each TB to a separate tube and were test for the presence of Indole as follows-

0.2-0.3 ml of Indole Reagent to each tube was added and shaking. The tube was left to stand for 10 min and the result was observed. A dark red color in the amyl acetate layer constitutes a positive reaction. An orange color incubates the probable presence of shatole and may be reported as positive reaction. Cultures producing gas in the presence of bile and indole, at 44°C were presumed positive for FC organisms. The MPN of FC organisms per ml of water were determined.

4.4. Microbiological Test of SWAB

4.4.1. Standard Plate Count (SPC)

Culture media

A solution of peptone (1/L) and sodium chloride (8.5g/L) was made up. The solution was distributed in tubes/ bottles and was sterilized for 15 min. at 121°C .

Preparation of SWAB

A SWAB usually consists of a piece of aluminum or iron wire. This rough was made one end for about 1.25 cm. 1 g of absorbent cotton of surgical grade was rolled tightly around this rough end so as to make a SWAB of about 1.9 cm thick and 2.5 cm long. The wire was placed with the SWAB in narrow, thick walled test tube of 18x150 mm size. The end of the test tube was plugged with non-absorbent cotton. Sterilizing was done in the autoclave for 15 lbs for 20 min.

Collection of SWAB

The standard area for swabbing is 25 cm² (5cmx5cm). A steel template of correct size were used which was sterilized by alcohol flaming to out line the area.

Procedure

The sterilized template was placed on the surface from where the sample was collected. A SWAB from the sterile wrapping container was aseptically removed. The tip of the SWAB was placed on the investigated surface and streak an area of 20 cm², rotated the SWAB between thumb and forefinger in two directions at right angle to each other. The SWAB was putted in the tube with dilution liquid and was cutting of the stick aseptically. The contents of the tubes containing SWAB was thoroughly mixed using a mixer for 30s; the speed was adjusting so that the wall of the tube was wetted up to a height of 2 cm to cm below top. This initial suspension was treated as a 1:10 dilution.

SPC analysis: 1ml of the required dilution in the sterile petridishes of 100 ml dia was poured in duplicate. 20 ml of the molted plate count agar (PCA) was added at about 45°C to the incubation plate. The medium and the sample were mixed by tilting and rotting the dish. After solidification of the agar, the plates were inverted and incubated at 37°C ±1°C for 48 hr.

Calculation: The numbers of colony forming unit (CFU) per ml of initial suspension or of its dilution were recorded. The CFU/cm² of the surface investigated was calculated by using the formula-

$$N_s = \frac{N \times F}{A}$$

Where, N is the number of CFU in 1 ml of dilution liquid

F is the amount in milliliters, of dilution fluid in the tube

A is the surface investigated in cm

Chapter 4

Results

1. Hazard analysis and CCP determination

The hazards include safety, wholesomeness or economic fraud. Categories of hazards were biological, chemical and physical. For each processing step identified on the flow chart, the hazard was identified and hazard evaluated. Hazard identification was resulted in a list of potential hazards at each processing step from the reception of raw material to the release of the finished product. After analysis of hazard analysis worksheet the potential steps of hazards were received raw shrimp and cooking.

1.1. Hazard analysis with CCP determination of Block frozen shrimp

In case of block frozen shrimp the potential hazards were received raw shrimp. The sources of hazards in the received raw shrimp were biological and chemical. Through the analysis of hazard analysis worksheet, the biological hazard was caused by pathogenic bacteria. The sources of pathogenic bacteria may be natural source and unhygienic handling by the workers. Chemical hazard caused by pesticide, use of drug or antibiotics and sulfites. Pesticide came with received raw materials from environmental source. Drug or antibiotics came with received raw materials due to use of shrimp feed. And sulfites came from supplier usage during culture period. Incase of block frozen shrimp the identified CCP was the receiving raw materials. Hazard analysis worksheet with CCP of block frozen shrimp showed in Table 2.1.

1.2. Hazard analysis with CCP determination of semi-IQF shrimp

In case of semi-IQF shrimp the potential hazards were receiving raw material. The sources of hazards in the receiving raw material were biological and chemical. After analysis of hazard analysis worksheet, it was found that the biological hazard caused by pathogenic bacteria. The sources of pathogenic bacteria were natural source and unhygienic handling by the workers. Chemical hazard caused by pesticide, use of drug or antibiotics and sulfites. Pesticide came with receiving raw materials from environmental source. Drug or antibiotics came with receiving raw materials due to use of shrimp feed. And sulfites came

from supplier usage during culture time. In case of semi-IQF shrimp the identified CCP was the receiving raw materials. Hazard analysis worksheet with CCP of semi-IQF shrimp showed in Table 2.2.

1.3. Hazard analysis with CCP determination of IQF shrimp

In case of IQF shrimp the potential hazards was receiving raw material. The sources of hazards in the receiving raw material were biological and chemical. After analysis of hazard analysis worksheet, it is found that the biological hazard caused by pathogenic bacteria. The sources of pathogenic bacteria were natural source and unhygienic handling by the workers. Chemical hazard caused by pesticide, use of drug or antibiotics and sulfites. Pesticide came with receiving raw materials from environmental source. Drug or antibiotics came with receiving raw materials due to use of shrimp feed. And sulfites came from supplier usage during culture time. In case of IQF shrimp the identified CCP was the receiving raw material. Hazard analysis worksheet with CCP of IQF shrimp showed in Table 2.3.

1.4. Hazard analysis with CCP determination of cooked IQF shrimp

In case of cooked IQF shrimp the potential hazards was receiving raw material and cooking. The sources of hazards in the receiving raw material were biological and chemical. After analysis of hazard analysis worksheet, it is found that the biological hazard caused by pathogenic bacteria. The sources of pathogenic bacteria were natural source and unhygienic handling by the workers. Chemical hazard caused by pesticide, use of drug or antibiotics and sulfites. Pesticide came with receiving raw materials from environmental source. Drug or antibiotics came with receiving raw materials due to use of shrimp feed. And sulfites came from supplier usage during culture period. Cooking was another potential hazard of cooked IQF shrimp. At the cooking step, where there were most concern about the sensory quality of product and the survival of pathogens that contaminated the finished product. In case of cooked IQF shrimp the identified CCP were the receiving raw material and cooking. Hazard analysis worksheet with CCP of cooked IQF shrimp showed in Table 2.4.

Table 2.1: Hazard analysis of Block frozen shrimp:

Step/ Position	Potential Hazards	Justification of Hazards	Preventive/ Control measures	Q₁ (Yes/ No)	Q₂ (Yes/ No)	Q₃ (Yes/ No)	Q₄ (Yes/ No)	CCP (Yes/ No)
Raw materials receive	Biological: Pathogenic bacteria	i) Natural resources ii) Unhygienic handling	Raw materials' source control i) Proper tagging of basket ii) Suppliers' certificate	Y	Y	-	-	Y
	Chemical: a) Pesticide b) Drugs/ Antibiotics	a) May come with raw materials from environmental source b) May come with raw materials due to usage in shrimp feed	a) i) Suppliers' lot by lot certificate ii) Shrimp flesh test for pesticide residue	Y	Y	-	-	Y
	c) Sulfites	c) May come from supplier' usage. But no evidence of usage.	b) i) Suppliers' lot by lot certificate ii) Shrimp flesh test for antibiotic residue c) i) Suppliers' lot by lot certificate ii) Test for sulfite residue	Y	N	Y	Y	N
	Physical: Extraceous materials	Due to improper way of handling and transport of shrimp	Controlled by proper way of handling and transport of shrimp	Y	N	Y	Y	N
Beheading	Biological: i) Cross contamination ii) Bacterial growth	i) Unhygienic practices by the worker ii) Insufficient temperature of shrimp during monitoring	i) Controlled by hygienic practice ii) Proper temperature of shrimp during monitoring	Y	N	Y	Y	N
	Chemical: None Physical: None							
Washing	Biological: i) Cross contamination ii) Bacterial growth	i) Unhygienic practices by the worker ii) Due to wash in non-potable water	i) Controlled by hygienic practice ii) Properly wash in potable chill water	Y	N	Y	Y	N
	Chemical: None Physical: None							
Cold store	Biological: Bacterial growth	Insufficient temperature of shrimps	Proper time and temperature control	Y	N	Y	Y	N
	Chemical: None Physical: None							
Grading	Biological: Bacterial growth	Unhygienic handling of the product	Controlled by hygienic practice	Y	N	Y	Y	N
	Chemical: None Physical: Mis-grading	Improper way of grading	Controlled proper way of grading	Y	N	Y	Y	N
Weighing	Biological: i) Cross contamination ii) Bacterial growth	i) Unhygienic handling of the product ii) Insufficient temperature of product	i) Controlled by hygienic practice ii) Proper time and temperature	Y	N	Y	Y	N

Table 2.2: Hazard analysis of Semi-IQF shrimp:

Step/ Position	Potential Hazards	Justification of Hazards	Preventive/ Control measures	Q₁ (Y/ N)	Q₂ (Y/ N)	Q₃ (Y/ N)	Q₄ (Y/ N)	CCP (Y/N)
Raw materials receive	Biological; Pathogenic bacteria	i) Natural resources	Raw materials' source control i) Proper tagging of basket ii) Suppliers' certificate	Y	Y	-	-	Y
	Chemical: a) Pesticide	ii) Unhygienic handling	a) i) Suppliers' lot by lot certificate	Y	Y	-	-	Y
	b) Drugs/ Antibiotics	a) May come with raw materials from environmental source	b) i) Shrimp flesh test for pesticide residue	Y	Y	-	-	Y
	c) Sulfites	b) May come with raw materials due to usage in shrimp feed	ii) Shrimp flesh test for antibiotic residue	Y	Y	-	-	Y
Beheading		c) May come from supplier' usage. But no evidence of usage.	c) i) Suppliers' lot by lot certificate	Y	N	Y	Y	N
	Physical: Extraneous materials	Due to improper way of handling and transport of shrimp	ii) Test for sulfite residue	Y	N	Y	Y	N
	Biological: i) Cross contamination	i) Unhygienic practices by the worker	Controlled by proper way of handling and transport of shrimp	Y	N	Y	Y	N
	ii) Bacterial growth	ii) Insufficient temperature of shrimp during monitoring	i) Controlled by hygienic practice	Y	N	Y	Y	N
Washing	Chemical: None		ii) Proper temperature of shrimp during monitoring					
	Physical: None							
	Biological: i) Cross contamination	i) Unhygienic practices by the worker	i) Controlled by hygienic practice	Y	N	Y	Y	N
	ii) Bacterial growth	ii) Due to wash in non-potable water	ii) Properly wash in potable chill water					
Cold store	Chemical: None							
	Physical: None							
	Biological: Bacterial growth	Insufficient temperature of shrimps	Proper time and temperature control	Y	N	Y	Y	N
	Chemical: None							
Grading	Physical: None							
	Biological: Bacterial growth	Unhygienic handling of the product	Controlled by hygienic practice	Y	N	Y	Y	N
	Chemical: None							
	Physical: Mis-grading	Improper way of grading	Controlled proper way of grading	Y	N	Y	Y	N
Weighing	Biological: i) Cross contamination	i) Unhygienic handling of the product	i) Controlled by hygienic practice	Y	N	Y	Y	N
	ii) Bacterial growth	ii) Insufficient temperature of product	ii) Proper time and temperature control					
	Chemical: None							
	Physical: Weight short	Lack of training personnel	Controlled by personnel training	Y	N	Y	Y	N
Panning	Biological: i) Cross contamination	i) Unhygienic handling of the worker	i) Control of time and temperature	Y	N	Y	Y	N
	ii) Bacterial growth	ii) Slowness and without controlling temperature bacteria may be	ii) Proper time and temperature control					

Table 2.3: Hazard analysis of IQF shrimp

Step/ Position	Potential Hazards	Justification of Hazards	Preventive/ Control measures	Q₁ (Y/ N)	Q₂ (Y/ N)	Q₃ (Y/ N)	Q₄ (Y/ N)	CCP (Y/N)
Raw materials receive	Biological: Pathogenic bacteria	i) Natural resources ii) Unhygienic handling	Raw materials' source control i) Proper tagging of basket ii) Suppliers' certificate	Y	Y	-	-	Y
	Chemical: a) Pesticide	a) May come with raw materials from environmental source	a) i) Suppliers' lot by lot certificate ii) Shrimp flesh test for pesticide residue	Y	Y	-	-	Y
	b) Drugs/ Antibiotics	b) May come with raw materials due to usage in shrimp feed	b) i) Suppliers' lot by lot certificate ii) Shrimp flesh test for antibiotic residue	Y	Y	-	-	Y
	c) Sulfitres	c) May come from supplier' usage. But no evidence of usage.	c) i) Suppliers' lot by lot certificate ii) Test for sulfite residue	Y	N	Y	Y	N
	Physical: Extraneous materials	Due to improper way of handling and transport of shrimp	Controlled by proper way of handling and transport of shrimp	Y	N	Y	Y	N
Beheading	Biological: i) Cross contamination ii) Bacterial growth	i) Unhygienic practices by the worker ii) Insufficient temperature of shrimp during monitoring	i) Controlled by hygienic practice ii) Proper temperature of shrimp during monitoring	Y	N	Y	Y	N
	Chemical: None							
	Physical: None							
Washing	Biological: i) Cross contamination ii) Bacterial growth	i) Unhygienic practices by the worker ii) Due to wash in non-potable water	i) Controlled by hygienic practice ii) Properly wash in potable chill water	Y	N	Y	Y	N
	Chemical: None							
	Physical: None							
Cold store	Biological: Bacterial growth	Insufficient temperature of shrimps	Proper time and temperature control	Y	N	Y	Y	N
	Chemical: None							
	Physical: None							
Grading	Biological: Bacterial growth	Unhygienic handling of the product	Controlled by hygienic practice	Y	N	Y	Y	N
	Chemical: None							
	Physical: Mis-grading	Improper way of grading	Controlled proper way of grading	Y	N	Y	Y	N
Weighting	Biological: i) Cross contamination ii) Bacterial growth	i) Unhygienic handling of the worker ii) Slowness and without controlling temperature bacteria may be increased	i) Control of time and temperature ii) Proper time and temperature control	Y	N	Y	Y	N
	Chemical: None							
	Physical: Weight short	Lack of training personnel	Controlled by personnel training	Y	N	Y	Y	N
Panning	Biological: i) Cross contamination	i) Unhygienic handling of the worker	i) Control of time and temperature	Y	N	Y	Y	N

	ii) Bacterial growth	ii) Slowness and without controlling temperature bacteria may be increased	ii) Proper time and temperature control				
Freezing	Chemical: None						
	Physical: None						
	Biological: Bacterial growth	The product is going to be frozen	No possibility at the time of freezing	Y	N	Y	N
Depanning and glazing	Chemical: None						
	Physical: Freezer burn	Due to improper freezing management	Controlled by proper freezing management	Y	N	Y	N
	Biological: i) Cross contamination ii) Bacterial growth	Not likely to occur due to product is frozen	Controlled by proper time and temperature	Y	N	Y	N
Pack/label	Chemical: None						
	Physical: None						
	Biological: i) Cross contamination ii) Bacterial growth	Not likely to occur due to product is frozen	Controlled by hygienic practice	Y	N	Y	N
Metal scanning	Chemical: None						
	Physical: Mislabeled						
	Biological: None	Lack of training personnel	Controlled by personnel training	Y	N	Y	N
Frozen storage	Chemical: None						
	Physical: Metallic objects	Unintentionally added by the worker	Scanned by metal detector	Y	N	Y	N
	Biological: i) Cross contamination ii) Bacterial growth	Not likely to occur due to product is frozen	Controlled by proper time and temperature	Y	N	Y	N
Export	Chemical: Autolytic spoilage	Due to improper storage system	Controlled by proper storage system	Y	N	Y	N
	Physical: Dehydration	i) Time and temperature control ii) Proper store management	Controlled by proper time and temperature	Y	N	Y	N
	Biological: i) Cross contamination ii) Bacterial growth	i) Time and temperature control ii) Proper store management	Controlled by proper time and temperature	Y	N	Y	N
	Chemical: None						
	Physical: None						

Q₁. Do control measures exist at this step or subsequent steps for the identified hazard?

Q₂. Is this step designed to eliminate or reduce the likely occurrence of a hazard to an acceptable level?

Q₃. Could contamination with identified hazards occur in excess of acceptable levels or could these increases to unacceptable levels?

Q₄. Will a subsequent step eliminate identified hazards or reduce the likely occurrence to an acceptable level?

Table 2.4: Hazard analysis of Cooked IQF shrimp:

Step/ Position	Potential Hazards	Justification of Hazards	Preventive/ Control measures	Q ₁ (Y/ N)	Q ₂ (Y/ N)	Q ₃ (Y/ N)	Q ₄ (Y/ N)	CCP (Y/N)
Raw materials receive	Biological: Pathogenic bacteria	i) Natural resources ii) Unhygienic handling	Raw materials' source control i) Proper tagging of basket Suppliers' certificate	Y	Y	-	-	Y
	Chemical: a) Pesticide	a) May come with raw materials from environmental source	a) i) Suppliers' lot by lot certificate ii) Shrimp flesh test for pesticide residue	Y	Y	-	-	Y
	b) Drugs/ Antibiotics	b) May come with raw materials due to usage in shrimp feed	b) i) Suppliers' lot by lot certificate ii) Shrimp flesh test for antibiotic residue	Y	Y	-	-	Y
	c) Sulfites	c) May come from supplier's usage. But no evidence of usage.	c) i) Suppliers' lot by lot certificate ii) Test for sulfite residue	Y	N	Y	Y	N
	d) Fuel/ oil	d) May come from sea/ river transport	d) Hygienic transport	Y	N	Y	Y	N
Primary wash	Physical: Extraneous materials	Due to improper way of handling and transport of shrimp	Controlled by proper way of handling and transport of shrimp	Y	N	Y	Y	N
	Biological: Bacterial growth	i) Unhygienic practices by the worker ii) Due to wash in non-potable water	i) Controlled by hygienic practice ii) Properly wash in potable chill water	Y	N	Y	Y	N
Grading	Chemical: None							
	Physical: None							
Beheading	Biological: i) Cross contamination ii) Bacterial growth	Unhygienic handling of the product	Controlled by hygienic practice	Y	N	Y	Y	N
	Chemical: None							
	Physical: Mis-grading	Improper way of grading	Controlled proper way of grading	Y	N	Y	Y	N
	Biological: i) Cross contamination ii) Bacterial growth	i) Unhygienic practices by the worker ii) Insufficient temperature of shrimp during monitoring	i) Controlled by hygienic practice ii) Proper temperature of shrimp during monitoring	Y	N	Y	Y	N
	Chemical: None							
Chill store	Physical: None							
	Biological: i) Cross contamination ii) Bacterial growth	Insufficient temperature of shrimps	Proper time and temperature control	Y	N	Y	Y	N
Pressure	Chemical: None							
	Physical: None							
Pressure	Biological: Bacterial growth	i) Unhygienic handling of the worker	i) Control of time and temperature	Y	N	Y	Y	N

wash		ii) Slowness and without controlling temperature bacteria may be increased	ii) Proper time and temperature control				
Cooking	Chemical: None						
	Physical: None						
	Biological: i) Cross contamination ii) Bacterial growth	i) Survival of pathogen for time and temperature abuse ii) Unhygienic handling of the workers	Y	N	Y	N	Y
Chilling	Chemical: None						
	Physical: None						
	Biological: i) Cross contamination ii) Bacterial growth	Not likely to occur due to product is frozen	Y	N	Y	Y	N
Spiral Freezing	Chemical: None						
	Physical: None						
	Biological: Bacterial growth	The product is going to be frozen	Y	N	Y	Y	N
Pack/label/weighting	Chemical: None						
	Physical: Freezer burn	Due to improper freezing management	Y	N	Y	Y	N
	Biological: i) Cross contamination ii) Bacterial growth	Not likely to occur due to product is frozen	Y	N	Y	Y	N
Metal scanning	Chemical: None						
	Physical: Mislabeled	Lack of training personnel	Y	N	Y	Y	N
	Biological: None						
Frozen storage	Chemical: None						
	Physical: Metallic objects	Unintentionally added by the worker	Y	N	Y	Y	N
	Biological: i) Cross contamination ii) Bacterial growth	Not likely to occur due to product is frozen	Y	N	Y	Y	N
Export	Chemical: Autolytic spoilage	Due to improper storage system	Y	N	Y	Y	N
	Chemical: Dehydration	i) Time and temperature control ii) Proper store management	Y	N	Y	Y	N
	Biological: i) Cross contamination ii) Bacterial growth	i) Time and temperature control ii) Proper store management	Y	N	Y	Y	N
	Chemical: None						
	Physical: None						

Q1. Do control measures exist at this step or subsequent steps for the identified hazard?

Q2. Is this step designed to eliminate or reduce the likely occurrence of a hazard to an acceptable level?

Q3. Could contamination with identified hazards occur in excess of acceptable levels or could these increase to unacceptable levels?

Q4. Will a subsequent step eliminate identified hazards or reduce the likely occurrence to an acceptable level?

1.5. HACCP plan

According to the HACCP plan form the identified CCP in the factory were in receiving step and in cooking step.

1.5.1 HACCP plan form of Block frozen shrimp, Semi IQF shrimp and IQF shrimp

The CCP of block frozen shrimp, semi IQF shrimp and IQF shrimp were the receiving raw materials and the significant hazard were pathogenic bacteria from harvest area, pesticide (environmental chemical) and drug (antibiotics, growth hormone etc). The HACCP plan form of block frozen shrimp, semi IQF shrimp and IQF shrimp showed in the Table 2.5.

1.5.2. HACCP plan form of Cooked IQF shrimp

The CCP of Cooked IQF shrimp were the receiving raw materials and cooking. The significant hazards were pathogenic bacteria from harvest area, pesticide (environmental chemical) and drug (antibiotics, growth hormone etc). Cooking step was very important in cooked IQF shrimp where there was most concern about the sensory quality of product as well as survival of pathogens that contaminated the finished product. The HACCP plan form of cooked IQF shrimp showed in the Table 2.6.

Table 2.5: HACCP Plan form of Block frozen shrimp, Semi IQF shrimp and IQF shrimp

Critical Control Point (CCP)	Significant Hazards	Critical Limit for each Preventive measure	Monitoring procedure				Corrective actions	Record	Verification
			What	How	Frequency	Who			
Receiving raw materials	Pathogenic Bacteria from harvest area	i) All baskets must bear a tag, that discloses the date, time and place, type and quantity of shrimp and by whom they were harvested. Also the name address and code number of supplier. ii) Supplier's certificate discloses that shrimp is handled, preserved and transported in a hygienic manner.	Basket tag	Visual	Each basket	Quality control staff	i) Improper tag basket rejected.	i) Receiving tag ii) Corrective action record.	i) Daily receive record review. ii) Weekly review of monitoring reports and corrective action records.
			Supplier's certificate	Visual / Analytical	Each lot	Receive In-charge	ii) Improper or without certificate lot rejected.	i) Supplier's certificate copy. ii) Corrective action records.	i) Weekly review of monitoring, corrective action and verification records. ii) Microbiological test of raw shrimp and end product. iii) Visit the farm area twice in a year.
	Pesticide (Environmental chemical)	i) Supplier's lot by lot certificate indicates that shrimps is not harvested from culture farm which is so contaminated or not contaminated by pesticide and the level of pesticide is below the established tolerance, action level and guidance level. ii) Shrimp flesh test shows that no pesticide or level of pesticide is below the established tolerance, action level and guidance level	i) Presence of supplier's certificate	i) Visual	i) Each lot received	Quality control staff	i) Without certificate or Improper certificate lot is rejected.	i) Supplier's certificate copy ii) Corrective action records.	i) Daily receive record review. ii) Weekly review of monitoring and corrective action records
			ii) Level of pesticide residue	i) Test kit method / referred laboratory	i) Each lot received or required	Quality control staff/ Lab technician	ii) Lot is rejected.	i) Analytical results ii) Corrective action records.	i) Yearly or half yearly review of monitoring and corrective action and records .ii) Shrimp, soil and water sample collection from culture farm for analysis annually, half yearly or more .iii) Visit all new supplier's farm and 30% of existing supplier's farm area yearly to review the agricultural practices in farm area.

Drug (Antibiotics, growth hormone, etc.)	i) Supplier's lot by lot certificate indicates that no drug or proper drug is used in all incoming lot and shrimps harvested after approved time of drug usage. ii) No shrimp would be accepted that contains unapproved drug residues.	i) Presence of supplier's certificate ii) Drug residue test	i) Visual ii) Test kit method/ referred laborato ry	i) Each lot received ii) Each lot received or required	Quality control staff Quality control staff / Lab technician	i) Without certificate or improper certificate lot is rejected. ii) Lot is rejected.	i) Supplier's certificate copy ii) Corrective action records. i) Analytical results ii) Corrective action records.	i) Daily receive record review. ii) Weekly review of monitoring and corrective action records i) Yearly or half yearly external analytical repot review. ii) Shrimp, soil and water sample collection from culture farm for analysis annually, half yearly or more. iii) Visit all new supplier's farm and 30% of existing supplier's farm area to review the producer's drug usage method each year.
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Table 2.6: HACCP Plan form of Cooked IQF shrimp

Critical Control Point (CCP)	Significant Hazards	Critical Limit for each Preventive measure	Monitoring procedure				Corrective actions	Record	Verification
			What	How	Frequency	Who			
Receiving raw materials	Pathogenic Bacteria from harvest area	i) All baskets must bear a tag, which discloses the date, time and place, type and quantity of shrimp and by whom they were harvested. Also the name address and code number of supplier.	Basket tag	Visual	Each basket	Quality control staff	i) Improper or without tag basket rejected.	i) Receiving tag ii) Corrective action record.	i) Daily receive record review. ii) Weekly review of monitoring reports and corrective action records.
		ii) Supplier's certificate discloses that shrimp is handled, preserved and transported in a hygienic manner.	Supplier's certificate	Visual / Analytical	Each lot	Receive In-charge	ii) Improper or without certificate lot rejected.	i) Supplier's certificate copy. ii) Corrective action records.	i) Weekly review of monitoring, corrective action and verification records. ii) Microbiological test of raw shrimp and end product. iii) Visit the farm area twice in a year.
	Environmental Chemical contaminants (Pesticide)	i) Supplier's lot by lot certificate indicates that shrimps is not harvested from culture farm which is so contaminated or not contaminated by pesticide and the level of pesticide is below the established tolerance, action level and guidance level. ii) Shrimp flesh test shows that no pesticide or level of pesticide is below the established tolerance, action level and guidance level	i) Presence of supplier's certificate ii) Level of pesticide residue	i) Visual ii) Test kit method / referred laboratory	i) Each lot received ii) Each lot received or required	Quality control staff / Lab technician	i) Without certificate or Improper certificate lot is rejected. ii) Lot is rejected.	i) Supplier's certificate copy ii) Corrective action records. i) Analytical results ii) Corrective action records.	i) Daily receive record review. ii) Weekly review of monitoring and corrective action records i) Weekly review of monitoring and corrective action and records ii) Shrimp, soil and water sample collection from culture farm for analysis annually, half yearly or more. iii) Visit all new supplier's farm and 30% of existing supplier's farm area yearly to review the agricultural practices in farm area.
	Drug /Antibiotics (Like Chloramphenicol,	i) Supplier's lot by lot certificate indicates that no drug or proper drug is used in all incoming lot and shrimps harvested after approved time of drug usage.	i) Presence of supplier's certificate	i) Visual	i) Each lot received	Quality control staff	i) Without certificate or Improper certificate lot is	i) Supplier's certificate copy ii) Corrective action records.	i) Daily receive record review. ii) Weekly review of monitoring and corrective action records

	Tetracycline, Nitrofrin etc.)	ii) No shrimp would be accepted that contains unapproved drug residues.	ii) Drug residue test	i) Test kit method / referred laboratory	i) Each lot received or required	Quality control staff / Lab technician	rejected. ii) Lot is rejected.	i) Analytical results ii) Corrective action records.	i) Yearly or half yearly external analytical report review. ii) Shrimp, soil and water sample collection from culture farm for analysis annually, half yearly or more. iii) Visit all new supplier's farm and 30% of existing supplier's farm area to review the producer's drug usage method each year.
Cooking	Pathogenic Bacteria (<i>Salmonella</i> , <i>Vibrio</i> etc.) survival	i) Cooking time 3 minute for medium grades (21/25) of shrimps and accordingly for other sizes.	Time of cooking	Digital time keeper	Once per day and after any adjustment	Cooker operator / Quality control staff	i) Extend cook time or elevate temperature to rectify deviation from limit and Segregate and hold evaluation	i) Cooking monitoring records ii) Microbiologic al analysis results.	i) Plant documents ii) Weekly review of monitoring reports and corrective action records.
		ii) Cooking temperature 65°C - 70°C for medium grades (21/25) of shrimps and accordingly for other sizes.	Time of cooking	Digital temperature meter	Continuous visual check daily cooking time	Cooker operator / Quality control staff		i) Cooking log. ii) Corrective action records.	i) Weekly cook log review. ii) Weekly lab report review. iii) Yearly calibration records.

2. Microbiological Test of Shrimp

2.1. SPC of Shrimp at Full Moon

SPC was measured at different points in the shrimp processing industry. Shrimp sample were collected from three different points (Received raw shrimp, Exported graded shrimp and Final product). Table- 2.7 showed the microbial test result of shrimp at different sampling point during full Moon. Figure 3.1 illustrates the mean value of SPC at three different points in shrimp processing industry. The $\log_{10}$ values (cfu) of SPC at received raw shrimp were 5.76 while the $\log_{10}$ values (cfu) of SPC of export graded shrimp and final product were 5.48 and 4.93 respectively. The results in the graph indicated clearly a reduced value of SPC at final product. The value of SPC varied between $\log_{10}$ 5.76 to $\log_{10}$ 4.93. Within different points of sample collection, the mean values of SPC were much higher at received raw shrimp ($\log_{10}$ value 5.76), lower at export graded shrimp ($\log_{10}$ value 5.48) and lowest at final product ($\log_{10}$ value 4.93). The acceptable limit of SPC of shrimp is 10×10^5 SPC/gm ($\log_{10}$ value 6). All the values that were found in different sampling point were within the acceptable limit.

2.2. TC of Shrimp at Full Moon period

TC was measured at different points in the shrimp processing industry. Shrimp sample were collected from three different points (Received raw shrimp, Exported graded shrimp and Final product). Table- 2.8 showed the microbial test result of shrimp at different sampling point during full moon. Figure 3.2 illustrates the mean value of TC counts at three different points in shrimp processing industry. The mean values of TC at received raw shrimp were 40.20 MPN/g while the mean values of TC of export graded shrimp and final product were 22.6 MPN/g and 7.8 MPN/g respectively. The results in the graph indicate clearly a reduced value of TC at final product. The value of TC varied between 22.6 MPN/g to 7.8 MPN/g. Within different points of sample collection, the mean values of TC were much higher at received raw shrimp (40.20 MPN/g), lower at export graded shrimp (22.6 MPN/g) and lowest at final product (7.8 MPN/g). The acceptable limit of TC of shrimp is 100/gm. However the counts of TC at all the sampling points in shrimp processing industry were much lower compared to acceptable limit.

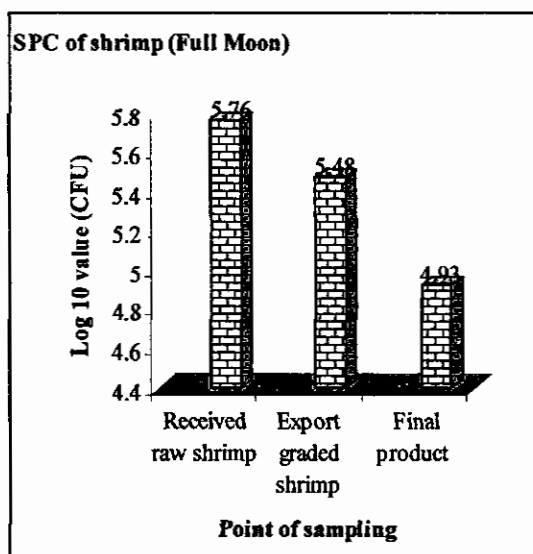


Figure 3.1: SPC of received raw shrimp, export graded shrimp and final product (Full Moon)

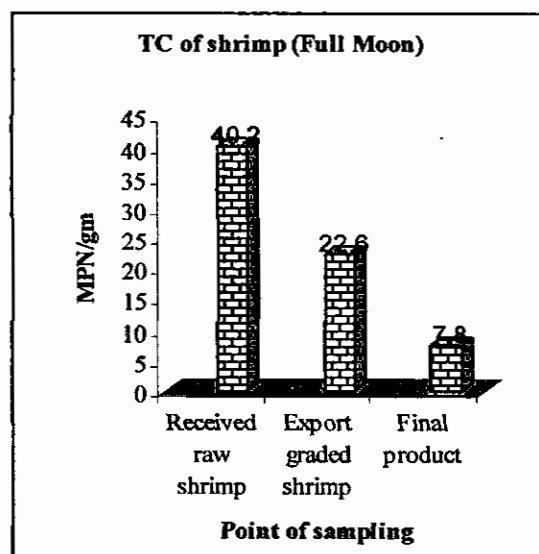


Figure 3.2: TC of received raw shrimp, export graded shrimp and final product (Full Moon)

Table 2.7: Microbial test- SPC of Shrimp (Full Moon):

Test Microbes	Acceptable limit	Test result (Full Moon) SPC/g		
		Received raw shrimp	Export graded shrimp	Final product
SPC /g	10x10 ⁵ /g	5.3 x10 ⁵	2.7 x10 ⁵	0.69 x10 ⁵
		5.5 x10 ⁵	2.8 x10 ⁵	0.80 x10 ⁵
		6.0 x10 ⁵	3.2 x10 ⁵	0.90 x10 ⁵
		5.7 x10 ⁵	3.1 x10 ⁵	0.88 x10 ⁵
		6.2 x10 ⁵	3.4 x10 ⁵	1.0 x10 ⁵
Mean value (<i>X</i>)		5.74 x10 ⁵	3.04x10 ⁵	0.854 x10 ⁵
Log 10 Value (CFU)		5.76	5.48	4.93

Table 2.8: Microbial test- TC of Shrimp (Full Moon):

Test Microbes	Acceptable limit	Test result (Full Moon) TC/g		
		Received raw shrimp	Export graded shrimp	Final product
TC /g	100/g	34	14	4
		53	34	14
		43	28	9
		28	9	3
		43	28	9
Mean value ($\bar{X}$)		40.20	22.6	7.8

2.3. FC of Shrimp at Full Moon period

The result FC count for shrimp at different points during full moon in the shrimp processing industry is shown in Table- 2.9. Shrimp sample were collected from three different points (Received raw shrimp, Exported graded shrimp and Final product). Figure 3.3 illustrates the mean value of FC counts at three different points in shrimp processing industry. The mean values of FC at received raw shrimp were 6.2 MPN/g while the mean values of FC of export graded shrimp and final product were 2.0 MPN/g and 00 MPN/g respectively. The results in the graph indicate clearly that the value of FC at final product is totally absent. The value of FC varied between 6.2 MPN/g to 00 MPN/g. Within different points of sample collection, the mean values of FC were much higher at received raw shrimp (6.2 MPN/g), lower at export graded shrimp (2.0 MPN/g) and lowest at final product (00 MPN/g). The acceptable limit of FC of shrimp is 11/g. However the counts of FC at all the sampling points in shrimp processing industry were much lower compared to acceptable limit.

2.4. SPC of Shrimp at New Moon period

The result SPC for shrimp at different points during new moon in the shrimp processing industry is shown in Table- 2.10. SPC was measured at different points in the shrimp processing industry. Shrimp sample were collected from three different points (Received raw shrimp, Exported graded shrimp and Final product). Figure 3.4 illustrates the mean value of SPC at three different points in shrimp processing industry. The $\log_{10}$ values (cfu) of SPC at received raw shrimp were 5.68 while the $\log_{10}$ values (cfu) of SPC of export graded shrimp and final product were 5.39 and 4.79 respectively. The results in the graph indicate clearly a reduced value of SPC at final product. The value of SPC varied between $\log_{10}$ 4.79 to $\log_{10}$ 5.68. Within different points of sample collection, the mean values of SPC were much higher at received raw shrimp ($\log_{10}$ value 5.68), lower at export graded shrimp ($\log_{10}$ value 5.39) and lowest at final product ($\log_{10}$ value 4.79). The acceptable limit of SPC of shrimp is 10×10^5 SPC/g ($\log_{10}$ value 6). All the values that were found in different sampling point of shrimp processing industry were much lower compared to acceptable limit.

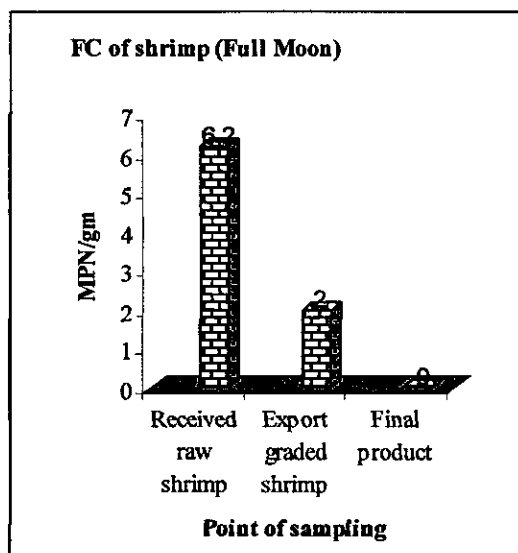


Figure 3.3: FC of received raw shrimp, export graded shrimp and final product (Full Moon)

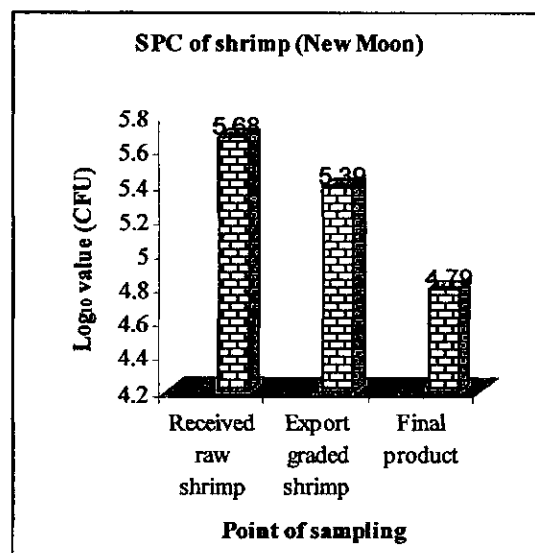


Figure 3.4: SPC of received raw shrimp, export graded shrimp and final product (New Moon)

Table 2.9: Microbial test- FC of Shrimp (Full Moon):

Test Microbes	Acceptable limit	Test result (Full Moon) FC/g		
		Received raw shrimp	Export graded shrimp	Final product
FC/g	11/g	9	4	Absent
		4	Absent	-
		7	3	-
		7	3	-
		4	Absent	-
Mean value ($\bar{X}$)		6.2	2.0	-

Table 2.10: Microbial test- SPC of Shrimp (New Moon):

Test Microbes	Acceptable limit	Test result (New Moon) SPC/g		
		Received raw shrimp	Export graded shrimp	Final product
SPC /g	10x10 ⁵ /g	4.3 x10 ⁵	2.1 x10 ⁵	0.49 x10 ⁵
		5.2 x10 ⁵	2.5 x10 ⁵	0.58 x10 ⁵
		4.7 x10 ⁵	2.4 x10 ⁵	0.60 x10 ⁵
		5.5 x10 ⁵	3.0 x10 ⁵	0.90 x10 ⁵
		4.5 x10 ⁵	2.3 x10 ⁵	0.50 x10 ⁵
Mean value (X)		4.84 x10 ⁵	2.46 x10 ⁵	0.614 x10 ⁵
Log 10 Value (CFU)		5.68	5.39	4.79

2.5. TC of Shrimp at New Moon period

The result TC count for shrimp at different points during new moon in the shrimp processing industry is shown in Table- 2.11. Shrimp sample were collected from three different points (Received raw shrimp, Exported graded shrimp and Final product). Figure 3.5 illustrates the mean value of TC counts at three different points in shrimp processing industry. The mean values of TC at received raw shrimp were 31.2 MPN/g while the mean values of TC of export graded shrimp and final product were 16.6 MPN/g and 5.6 MPN/g respectively. The results in the graph indicate clearly a reduced value of TC at final product. The value of TC varied between 5.6 MPN/g to 31.2 MPN/g. Within different points of sample collection, the mean values of TC were much higher at received raw shrimp (31.2 MPN/g), lower at export graded shrimp (16.6 MPN/g) and lowest at final product (5.6 MPN/g). The acceptable limit of TC of shrimp is 100/g. However the counts of TC at all the sampling points in shrimp processing industry were much lower compared to acceptable limit.

2.6. FC of Shrimp at New Moon period

The result FC count for shrimp at different points during new moon in the shrimp processing industry is shown in Table- 2.12. Shrimp sample were collected from three different points (Received raw shrimp, Exported graded shrimp and Final product). Figure 3.6 illustrates the mean value of FC counts at three different points in shrimp processing industry. The mean values of FC at received raw shrimp were 5.4 MPN/g while the mean values of FC of export graded shrimp and final product were 1.4 MPN/g and 00 MPN/g respectively. The results in the graph indicate clearly that the value of FC at final product is totally absent. The value of FC varied between 00 MPN/g to 5.4 MPN/g. Within different points of sample collection, the mean values of FC were much higher at received raw shrimp (5.4 MPN/g), lower at export graded shrimp (1.4 MPN/g) and lowest at final product (00 MPN/g). The acceptable limit of FC of shrimp is 11/g. However the counts of FC at all the sampling points in shrimp processing industry were much lower compared to acceptable limit.

2.7. *Salmonella* sp. and *V. cholerae* of Shrimp at Full Moon period

Salmonella sp. and *V. cholerae* were measured at different points in the shrimp processing industry. Shrimp sample were collected from three different points (Received raw shrimp, Exported graded shrimp and Final product). Table- 2.13 showed the microbial test result of shrimp at different sampling point during full moon. *Salmonella* sp. and *V. cholerae* were not detected in 25 g samples of shrimp sample collected from each point (Received raw shrimp, Exported graded shrimp and Final product) of shrimp processing plant. The acceptable limit of *Salmonella* sp. and *V. cholerae* of shrimp is absent.

Table 2.13: Microbial test (*Salmonella* sp. and *V. cholerae*) of Shrimp (Full Moon):

Test Microbes	Acceptable limit	Test result (Full Moon)		
		Received raw shrimp	Export graded shrimp	Final product
<i>Salmonella</i> sps. in 25 g	Absent	-	-	-
		-	-	-
		-	-	-
		-	-	-
		-	-	-
<i>V. cholerae</i> in 25 g	Absent	-	-	-
		-	-	-
		-	-	-
		-	-	-
		-	-	-

2.8. *Salmonella* sp. and *V. cholerae* of Shrimp at New Moon period

Salmonella sp. and *V. cholerae* were measured at different points in the shrimp processing industry. Shrimp sample were collected from three different points (Received raw shrimp, Exported graded shrimp and Final product). Table- 2.14 showed the microbial test result of shrimp at different sampling point during new moon. *Salmonella* sp. and *V. cholerae* were not detected in 25 g samples of shrimp sample collected from each point (Received raw shrimp, Exported graded shrimp and Final product) of shrimp processing plant. The acceptable limit of *Salmonella* sp. and *V. cholerae* of shrimp is absent.

Table 2.14: Microbial test (*Salmonella* sp. and *V. cholerae*) of Shrimp (New Moon):

<i>Test Microbes</i>	<i>Acceptable limit</i>	<i>Test result (New Moon)</i>		
		<i>Received raw shrimp</i>	<i>Export graded shrimp</i>	<i>Final product</i>
<i>Salmonella</i> sps. in 25 gm	Absent	-	-	-
		-	-	-
		-	-	-
		-	-	-
		-	-	-
<i>V. cholerae</i> in 25 gm	Absent	-	-	-
		-	-	-
		-	-	-
		-	-	-
		-	-	-

2.9. Comparison of mean value of SPC of shrimp between Full Moon and New Moon

The comparative study of mean value of SPC of shrimp between full moon and new moon at different points of shrimp processing industry is shown in the figure 3.7. SPC of shrimp was measured at three different points (Received raw shrimp, Exported graded shrimp and Final product) of shrimp processing industry during full moon and new moon. Table- 2.7 and Table- 2.10 showed the SPC test result of different points of shrimp processing industry during full moon and new moon. In totally the mean value of SPC during full moon and new moon at different sampling points of shrimp processing industry were lower than the acceptable limit. The figure 3.7 clearly indicates that the mean value of SPC for both the experiment differs significantly. The $\log_{10}$ values (cfu) of SPC of received raw shrimp during full moon were 5.76 while the $\log_{10}$ values (cfu) of SPC during new moon were 5.68. The differences of SPC of $\log_{10}$ values (cfu) between full moon and new moon were 0.08. The $\log_{10}$ values (cfu) of SPC of export graded shrimp during full moon were 5.48 while the $\log_{10}$ values (cfu) of SPC during new moon were 5.39. The differences of SPC of $\log_{10}$ values (cfu) between full moon and new moon were 0.09. The $\log_{10}$ values (cfu) of SPC of final product during full moon were 4.93 while the $\log_{10}$ values (cfu) of SPC during new moon were 4.79. The differences of SPC of $\log_{10}$ values (cfu) between full moon and new moon were 0.14. The table 2.7 and 2.10 and figure 3.7 indicates that though there were significant differences between the $\log_{10}$ values (cfu) of SPC of full moon and new moon at different points of shrimp processing industry, all the values were much lower than the standard recommended limit.

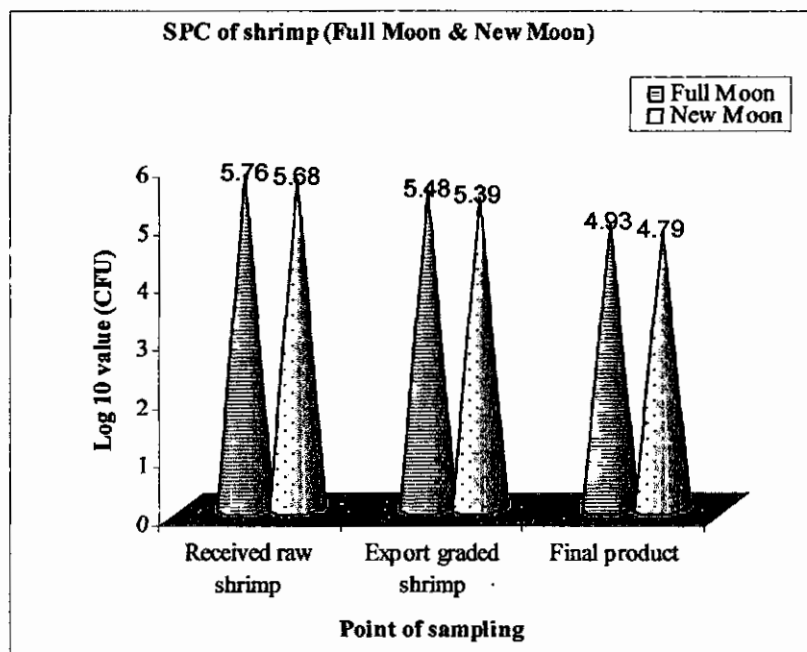


Figure 3.7: Comparison of $\log_{10}$ value of SPC during full Moon and new Moon

2.10. Comparison of mean value of TC of shrimp between Full Moon and New Moon

The comparative study of mean value of TC of shrimp between full moon and new moon at different points of shrimp processing industry is shown in the figure 3.8. TC of shrimp was measured at three different points (Received raw shrimp, Exported graded shrimp and Final product) of shrimp processing industry during full moon and new moon. Table- 2.8 and Table- 2.11 showed the TC test result of different points of shrimp processing industry during full moon and new moon. In totally the mean value of TC during full moon and new moon at different sampling points of shrimp processing industry were lower than the acceptable limit. The figure 3.8 clearly indicates that the mean value of TC for both the experiment differs significantly. The mean values TC of received raw shrimp during full moon were 40.2 MPN/g while the mean values of TC during new moon were 31.2 MPN/g. The differences of mean values of TC of shrimp between full moon and new moon were 9.0 MPN/g. The mean values TC of export graded shrimp during full moon were 22.6 MPN/g while the mean values of TC during new moon were 16.6 MPN/g. The differences of mean values of TC of shrimp between full moon and new moon were 6.0 MPN/g. The mean values TC of final product during full moon were 7.8 MPN/g while the mean values of TC during new moon were 5.6 MPN/g. The differences of mean values of TC of shrimp

between full moon and new moon were 2.2 MPN/g. The table 2.8 and 2.11 and figure 3.8 indicates that though there were significant differences between the mean values of TC of shrimp of full moon and new moon at different points of shrimp processing industry, all the values were much lower than the standard recommended limit.

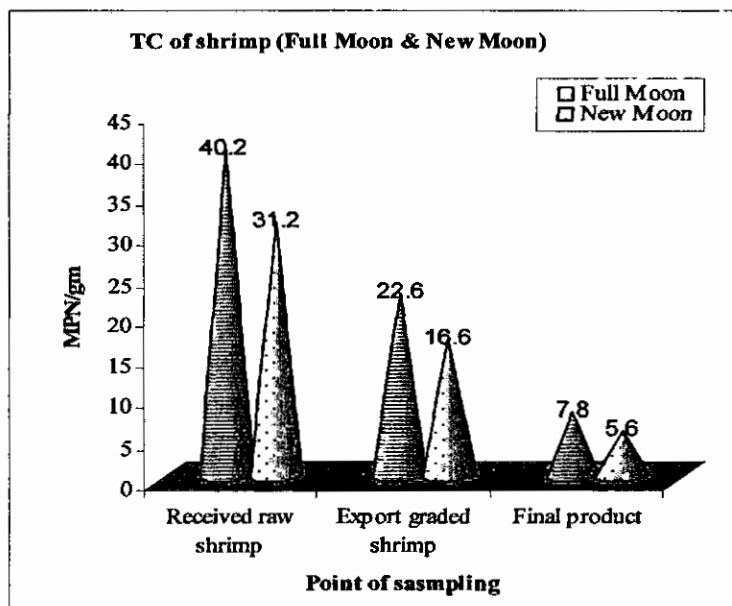


Figure 3.8: Comparison of mean value of TC during full Moon and new Moon

2.11. Comparison of mean value of FC of shrimp between Full Moon and New Moon

The comparative study of mean value of FC of shrimp between full moon and new moon at different points of shrimp processing industry is shown in the figure 3.9. FC of shrimp was measured at three different points (Received raw shrimp, Exported graded shrimp and Final product) of shrimp processing industry during full moon and new moon. Table- 2.9 and Table- 2.12 showed the FC test result of different points of shrimp processing industry during full moon and new moon. In totally the mean value of FC during full moon and new moon at different sampling points of shrimp processing industry were lower than the acceptable limit. The figure 3.9 clearly indicates that the mean value of FC for both the experiment differs significantly. The mean values FC of received raw shrimp during full moon were 6.2 MPN/g while the mean values of FC during new moon were 5.4 MPN/g. The differences of mean values of FC of shrimp between full moon and new Moon were 0.8 MPN/g. The mean values FC of export graded shrimp during full moon were 2.0 MPN/g while the mean values of FC during new moon were 1.4 MPN/g. The differences of mean values of FC of shrimp between full moon and new moon were 0.6 MPN/g. The mean values FC of final product both full moon and new moon were absent. The table 2.9

and 2.12 and figure 3.9 indicates that though there were significant differences between the mean values of FC of shrimp of full moon and new moon at different points of shrimp processing industry, all the values were much lower than the standard recommended limit.

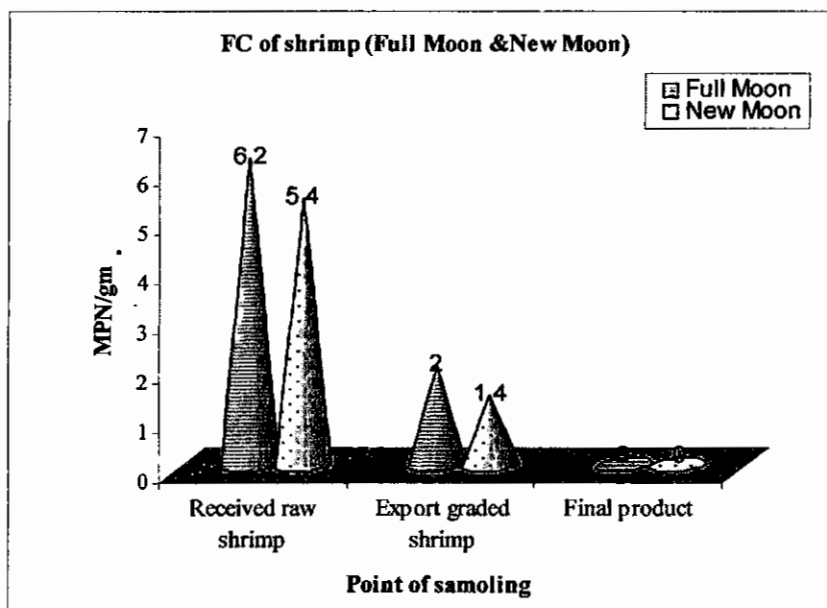


Figure 3.9: Comparison of mean value of FC during full Moon and new Moon

3. Microbiological Test of water

3.1. SPC of Water at Full Moon period

SPC was measured at different points in the shrimp processing industry. Water sample were collected from five different points (Over head tank, Receiving tape, Beheading tape, Processing tape and Panning tape). Table- 2.15 showed the microbial test result of water at different sampling point during full moon. The mean values of SPC at Overhead tank water were 7 cfu/ml while the mean values of Receiving tape, Beheading tape, Processing tape and Panning tape water were 6, 5, 4 and 3 cfu/ml respectively. Within different points of sample collection, the values of SPC were highest at Overhead tank water (7 cfu/ml) and lowest at Panning tape water (3 cfu/ml). The acceptable limit of SPC of water is 10 cfu/ml. All the values that were found in different sampling point were within acceptable limit.

3.2. TC and FC of water at Full Moon

TC and FC were measured at different points in the shrimp processing industry. Water sample were collected from five different points (Overhead tank, Receiving tape, Beheading tape, Processing tape and Panning tape). Table- 2.15 showed the microbial test result of water at different sampling point during full moon. TC and FC were not detected in 100 ml samples of water sample collected from different point of the processing plant. The acceptable limit of TC and FC of water is absent/100 ml.

3.3. SPC of water at New Moon period

SPC was measured at different points in the shrimp processing industry. Water sample were collected from five different points (Overhead tank, Receiving tape, Beheading tape, Processing tape and Panning tape). Table- 216 showed the microbial test result of water at different sampling point during new moon. The mean values of SPC at Overhead tank water, Receiving tape, Beheading tape water were 6 cfu/ml whereas the mean values of Processing tape and Panning tape water were 4 and 3 respectively cfu/ml. Within different points of sample collection, the values of SPC were highest at Overhead tank water, Receiving tape and Beheading tape water (6 cfu/ml) while the lowest at Panning tape water (3 cfu/ml). The acceptable limit of SPC of water is 10 cfu/ml. All the values that were found in different sampling point were within acceptable limit.

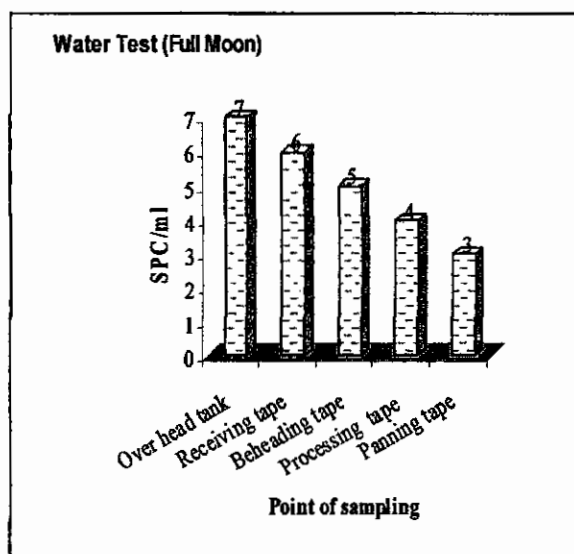
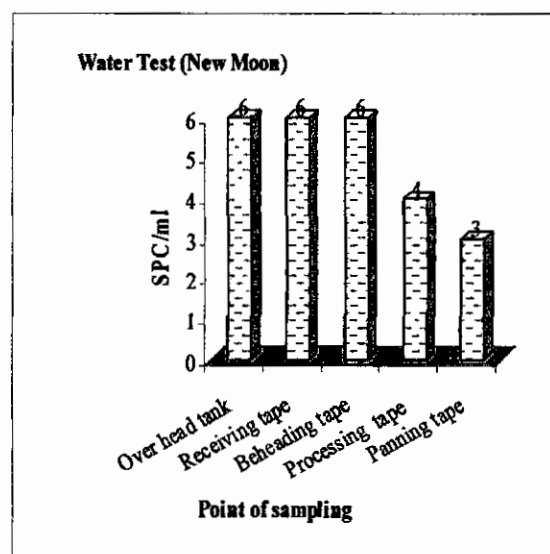


Figure 3.10: Mean value of SPC of water during Full Moon. **Figure 3.11:** Mean value of SPC of water during New Moon.

Table 2.15: Water test (Full Moon)

Sl No.	Test microbes	Maximum limit	Test results of water (Full Moon) SPC/ml				
			Over head Tank	Receiving tape	Beheading tape	Processing tape	Panning tape
1	SPC / ml	10 SPC/ml	7	6	5	4	4
			8	6	4	3	3
			7	5	6	3	2
			6	7	4	5	3
			7	6	6	5	3
			7	6	5	4	3
Mean value ($\bar{X}$)			7	6	5	4	3
2	TC / 100ml	Absent / 100ml	-	-	-	-	-
			-	-	-	-	-
			-	-	-	-	-
			-	-	-	-	-
			-	-	-	-	-
3	FC / 100ml	Absent / 100ml	-	-	-	-	-
			-	-	-	-	-
			-	-	-	-	-
			-	-	-	-	-
			-	-	-	-	-

Table 2.16: Water test (New Moon)

Sl No.	Test microbes	Acceptable limit	Test results of water (New Moon) SPC/ml				
			Overhead Tank	Receiving tape	Beheading tape	Processing tape	Panning tape
1	SPC / ml	10	6	5	7	4	3
			7	7	6	3	2
			6	6	5	5	4
			5	5	6	4	3
			6	7	6	4	3
Mean value ($\bar{X}$)			6	6	6	4	3
2	TC /100ml	Absent / 100ml	-	-	-	-	-
			-	-	-	-	-
			-	-	-	-	-
			-	-	-	-	-
			-	-	-	-	-
3	FC /100ml	Absent / 100ml	-	-	-	-	-
			-	-	-	-	-
			-	-	-	-	-
			-	-	-	-	-
			-	-	-	-	-

3.4. TC and FC of Water at New Moon period

TC and FC were measured at different points in the shrimp processing industry. Water sample were collected from five different points (Over head tank, Receiving tape, Beheading tape, Processing tape and Panning tape). Table- 2.16 showed the microbial test result of water at different sampling point during new moon. TC and FC were not detected

in 100 ml samples of water sample collected from different point of the processing plant. The acceptable limit of TC and FC of water is absent /100 ml.

3.5. Comparison mean values of SPC of water between Full Moon and New Moon

The comparative study of SPC of water during full moon and new moon at different points in the shrimp processing plant are provided in the Figure 3.12. In totally the mean value of SPC at all points were lower than the acceptable limit. The figure 3.12, is clearly indicates that the mean value of SPC for both the experiment did not differ among three points of sampling. These three points are the receiving tape (6 cfu/ml), processing tape (4 cfu/ml) and panning tape (3 cfu/ml). However water collected from overhead tank showed better result at new Moon (6 cfu/ml) than full moon (7 cfu/ml). Incase of beheading tape full moon showed the better result (5 cfu/ml) than the new moon (6 cfu/ml). It is clearly observed from the table 2.15 and 2.16 and the figure 3.12, there is no significant differences between full moon and new moon and all the values at each points of sampling in the processing plant shows better quality as well as within the acceptable limit.

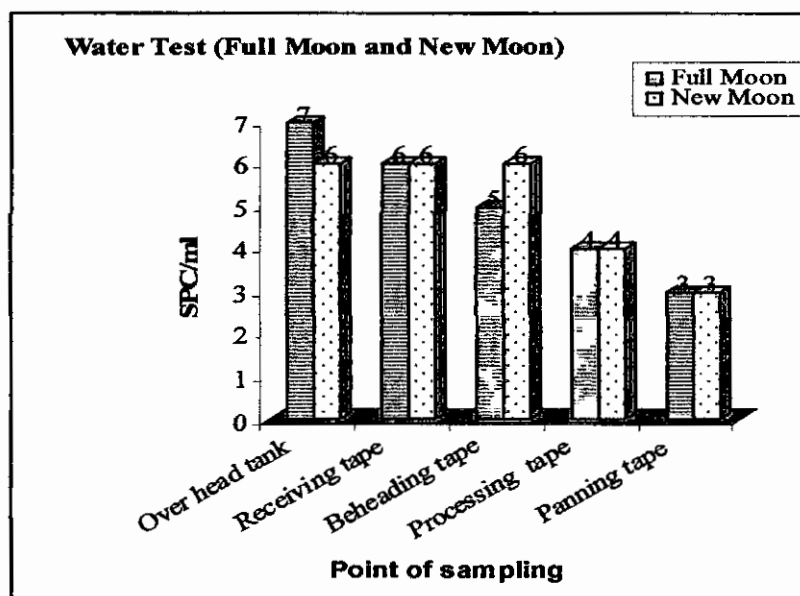


Figure 3.12: Comparison of mean value of SPC of water during Full Moon and New Moon

4. Microbiological Test of SWAB

4.1. SWAB Test –Workers hands at Full Moon

SWAB test of workers hands was done before and after working. Through SWAB test SPC was measured before and after working of the workers and the contact surfaces were the workers hands. Table 2.18 showed the SWAB test result at full moon. The mean values of SPC before working were 20 cfu/cm² while the mean values of SPC after working were 30 cfu/cm². The difference between before and after working was 10 cfu. The standard limit of SPC incase of SWAB was 50 cfu/cm². From Table 2.18 and the figure 3.14 it was clearly observed that the mean values of SPC before and after working were much lower than the standard recommended limit.

4.2. SWAB Test –Workers hands at New Moon

SWAB test of workers hands were done before and after working. Through SWAB test SPC was measured before and after working of the workers and the contact surfaces were the workers hands. Table 2.19 showed the SWAB test result at new moon. The mean values of SPC before working were 20 cfu/cm² while the mean values of SPC after working was 25 cfu/cm². The difference between before and after working was 5 cfu. The standard limit of SPC incase of SWAB was 50 cfu/cm². From Table 2.19 and the figure 3.15 it was clearly observed that the mean values of SPC before and after working were much lower than the standard recommended limit.

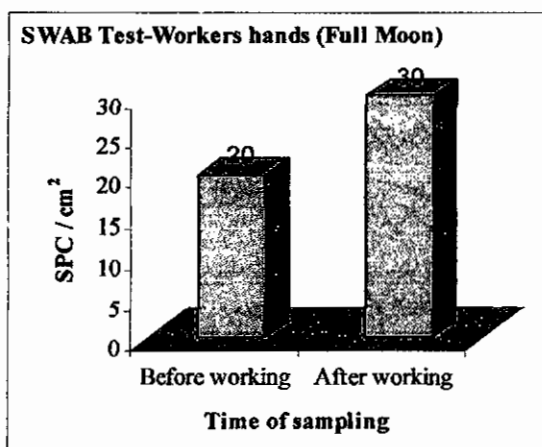


Figure 3.13: SWAB Test- Workers hands at Full Moon

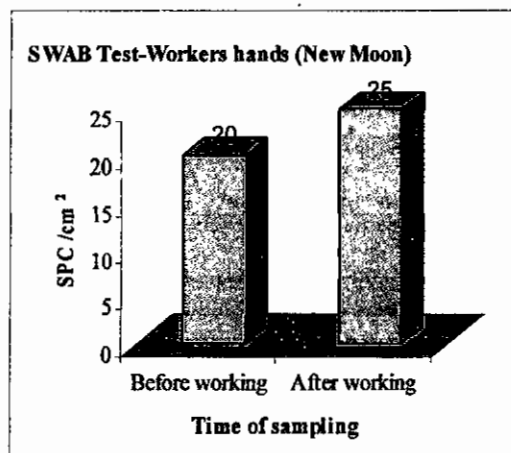


Figure 3.14: SWAB Test- Workers hands at New Moon

Table 2.17: SWAB test - Workers hands (Full Moon)

Sl No.	Contact Surface	Test Microbes	Standard limit	Test result (SPC/ cm ²)		
				Before working	After working	Difference
1	Workers hands (female)	SPC/ cm ²	50/cm ²	20	30	
2			50/cm ²	15	25	
3			50/cm ²	20	30	
4			50/cm ²	25	35	
5			50/cm ²	25	30	
Mean value ($\bar{X}$)				20	30	10

**Table 2.18: SWAB test- Workers hands (New Moon)**

Sl No.	Contact Surface	Test Microbes	Standard limit	Test result (SPC/ cm ²)		
				Before working	After working	Difference
1	Workers hands (female)	SPC/ cm ²	50/cm ²	15	20	
2			50/cm ²	25	30	
3			50/cm ²	15	20	
4			50/cm ²	20	25	
5			50/cm ²	25	30	
Mean value ($\bar{X}$)				20	25	5

4.3. SWAB Test –SS table at Full Moon

SWAB test of SS table was done before and after working. Through SWAB test SPC was measured before and after working of the workers and the contact surfaces were the SS table. Table 2.20 showed the SWAB test result at full moon. The mean values of SPC before working were 15cfu/cm² while the mean values of SPC after working was 25 cfu/cm². The difference between before and after working was 10 cfu. The standard limit of SPC incase of SWAB was 50 cfu/cm². From Table 2.20 and the figure 3.16 it was clearly observed that the mean values of SPC before and after working were much lower than the standard recommended limit.

4.4. SWAB Test –SS table at New Moon

SWAB test of SS table was done before and after working. Through SWAB test SPC was measured before and after working of the workers and the contact surfaces were the SS table. Table 2.21 showed the SWAB test result at new moon. The mean values of SPC before working were 15 cfu/cm² while the mean values of SPC after working was 20 cfu/cm². The difference between before and after working was 5 cfu. The standard limit of

SPC incase of SWAB was 50 cfu/cm^2 . The mean values of SPC before and after working were much lower than the standard recommended limit.

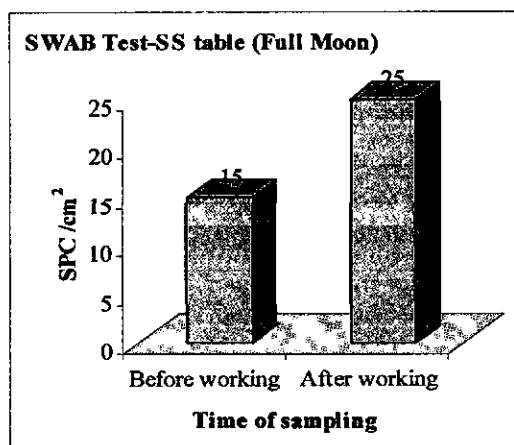


Figure 3.15: SWAB Test- SS table at Full Moon

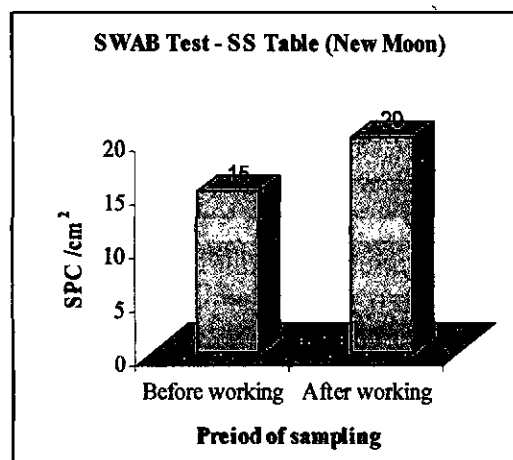


Figure 3.16: SWAB Test- SS table at New Moon

Table 2.19: SWAB test - SS-table (Full Moon)

Sl No.	Contact Surface	Test Microbes	Standard limit	Test result (SPC/ cm ²)		
				Before working	After working	Difference
1	SS-table	SPC/ cm ²	50/cm ²	15	25	
2			50/cm ²	15	25	
3			50/cm ²	10	20	
4			50/cm ²	20	30	
5			50/cm ²	15	25	
Mean value ($\bar{X}$)				15	25	10

Table 2.20: SWAB test- SS-table (New Moon)

Sl No.	Contact Surface	Test Microbes	Standard limit	Test result (SPC/ cm ²)		
				Before working	After working	Difference
1	SS-table	SPC/ cm ²	50/cm ²	15	20	
2			50/cm ²	20	25	
3			50/cm ²	15	20	
4			50/cm ²	15	20	
5			50/cm ²	10	15	
Mean value ($\bar{X}$)				15	20	5

4.5. Comparison of mean value of SWAB Test between Full Moon and New Moon (SS table)

The comparative study of mean value of SWAB Test between full moon and new moon before and after working is shown in the figure 3.18. SWAB test of SS table was done before and after working at full moon and new moon. Through SWAB test SPC was measured before and after working of the workers and the contact surfaces were the SS table. Table 2.22 showed the SWAB test result of SS table at full moon and new moon. In totally the mean value of SPC at full moon and new moon before and after working were lower than the acceptable limit. The figure 3.18 clearly indicates that the mean values of SPC for both the experiment differs significantly before and after working. The mean values of SPC before working were 15 cfu/cm² while the mean values of SPC after working was 25 cfu/cm² at full moon. The differences between before and after working were 10 cfu. On the other hand at new moon the mean values of SPC before working were 15 cfu/cm² while the mean values of SPC after working were 20 cfu/cm². The differences between before and after working were 5 cfu. It is clearly observed from the table 2.22 and figure 3.18, the SPC of both full moon and new moon before working were 15 cfu/cm² but after working SPC increased significantly. The values of SPC after working were 25 cfu/cm² and 20 cfu/cm² for full moon and new moon respectively. However the table 2.22 indicates that though there were significant differences between the values of full moon and new moon before and after working, all the values were much lower than the standard recommended limit.

Table 2.21: SWAB test- SS-table (Full Moon and New Moon)

Sl No.	Contact Surface	Test Microbes	Standard limit	Full Moon (SPC/ cm ²)		New Moon (SPC/ cm ²)	
				Before working	After working	Before working	After working
1	SS-table	SPC/ cm ²	50/cm ²	15	25	15	20
2			50/cm ²	15	25	20	25
3			50/cm ²	10	20	15	20
4			50/cm ²	20	30	15	20
5			50/cm ²	15	25	10	15
Mean value ($\bar{X}$)				15	25	15	20

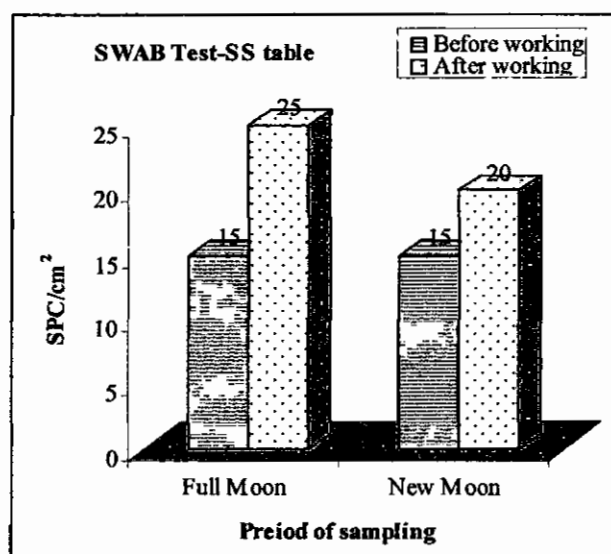


Figure 3.17: Comparison of mean value of SWAB Test-SS table

4.6. Comparison of mean value of SWAB Test between Full Moon and New Moon (Workers hands)

The comparative study of mean value of SWAB Test between full moon and new moon before and after working is shown in the figure 3.19. SWAB test of workers hands were done before and after working at full moon and new moon. Through SWAB test SPC was measured before and after working of the workers and the contact surfaces were workers hands. Table 2.23 showed the SWAB test result of workers hands at full moon and new moon. In totally the mean value of SPC at full moon and new moon before and after working were lower than the acceptable limit. The figure 3.19 clearly indicates that the mean values of SPC for both the experiment differs significantly before and after working. The mean values of SPC before working were 20 cfu/cm² while the mean values of SPC after working were 30 cfu/cm² at full moon. The differences between before and after working were 10 cfu. On the other hand at new moon the mean values of SPC before working were 20 cfu/cm² while the mean values of SPC after working were 25 cfu/cm². The differences between before and after working were 5 cfu. It is clearly observed from the table 2.23 and figure 3.19, the SPC of both full moon and new moon before working were 20 cfu/cm². The values of SPC after working were 30 cfu/cm² and 25 cfu/cm² for full moon and new moon respectively. However the table 2.23 indicates that though there were

significant differences between the values of full moon and new moon before and after working, all the values were much lower than the standard recommended limit.

Table 2.22: SWAB test- Workers hands (Full Moon and New Moon)

Sl No.	Contact Surface	Test Microbes	Standard limit	Full Moon (SPC/ cm ²)		New Moon (SPC/ cm ²)	
				Before working	After working	Before working	After working
1	Workers hands (female)	SPC/ cm ²	50/cm ²	20	30	15	20
2			50/cm ²	15	25	25	30
3			50/cm ²	20	30	15	20
4			50/cm ²	25	35	20	25
5			50/cm ²	25	30	25	30
Mean value ($\bar{X}$)				20	30	20	25

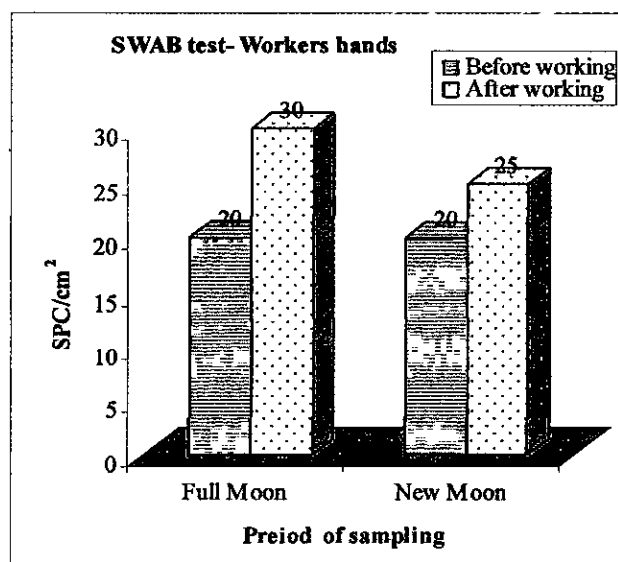


Figure 3.18: Comparison of mean value of SWAB Test- Workers hands

Chapter 5

Discussion

CCP Determination

1. Hazard analysis and CCP determination

The hazards include safety, wholesomeness or economic fraud. Categories of hazards were biological, chemical and physical. For each processing step identified on the flow chart, the hazard was identified and hazard evaluated. Hazard identification was resulted in a list of potential hazards at each processing step from the reception of raw material to the release of the finished product. After analysis of hazard analysis worksheet the potential steps of hazards were receiving step and cooking step.

1.1. Hazard analysis with CCP determination of Block frozen shrimp, semi-IQF shrimp and IQF shrimp

The potential hazard of block frozen shrimp, semi-IQF shrimp and IQF shrimp was receiving raw material. The sources of hazards in the receiving raw material were biological and chemical. The biological hazard was caused by pathogenic bacteria. The sources of pathogenic bacteria may be come from natural source and unhygienic handling of shrimp by the workers. Natural source was the culture farm from where good aquaculture practice was not properly maintained. Culture farm is the main natural source of pathogenic bacteria. After harvesting, if a long time was taken for transporting the shrimp to the processing plant then the pathogenic bacteria increased rapidly and destroy the quality of shrimp. Another source of pathogenic bacteria was unhygienic handling of the workers. In processing plant there was hand dip point with 40-50ppm Cl_2 concentration and there also foot dip point with 200ppm Cl_2 concentration for good hygiene practices. If the workers did not wash their hand and foots properly then the pathogenic bacteria may contaminated the shrimp during handling. Use of unhygienic basket and ice made of non-potable water may also be source of pathogenic bacteria. Chemical hazard caused by pesticide, use of drug or antibiotics and sulfites. Pesticide may come with received raw materials from environmental source. Through different environmental sources such as industry, agricultural farm etc. by heavy rainfall or tidal boar pesticide mixed with water and transfer to the shrimp body by biological process.

Drug or antibiotics may come with received raw materials due to use of shrimp feed. Different types of drug or antibiotics and hormone are mixed with shrimp feed to reduce disease, increase growth and reduce mortality. And sulfites may come from supplier uses during culture period. But there is no evidence of using sulfites.

Through analysis the CCP decision tree the receiving raw material was selected as CCP of block frozen shrimp. CCP was such a point or step after crossing this point or step sufficient hazards may occur to the consumers. Pathogenic bacteria, pesticide drug or antibiotics were the potential hazards of receiving raw material. If we were unable to control pathogenic bacteria, pesticide, drug/antibiotics, hormone etc. at this step then we can not control them in the next steps and ultimately go to the final product. This caused hazards to the consumers. Pathogenic bacteria of received raw material can be control through raw materials' source control, monitoring of proper tagging of basket, suppliers' certificate monitoring visiting the farm area twice in a year etc. Pesticide of received raw shrimp could be control through suppliers' lot by lot certificate monitoring, shrimp flesh test for pesticide residue, collecting the shrimp, soil and water sample from culture farm for analysis annually, half yearly or more, visiting all new supplier's farm and 30% of existing supplier's farm area yearly to review the agricultural practices in farm area. Drug or antibiotics and hormone of received raw shrimp could be control by suppliers' lot by lot certificate monitoring, shrimp flesh test for antibiotic residue, collecting the shrimp, soil and water sample from culture farm for analysis annually, half yearly or more, visiting all new supplier's farm and 30% of existing supplier's farm area to review the producer's drug usage method each year. So from the above discussion it could be said that the selection of received raw material as CCP is correct and appropriate.

1.2. Hazard analysis with CCP determination of cooked IQF shrimp

The potential hazard of cooked IQF shrimp were receiving raw material and cooking. The sources of hazards in the receiving raw material were biological and chemical. The biological hazard was caused by pathogenic bacteria. The sources of pathogenic bacteria might be natural source and unhygienic handling by the workers. Natural source was the culture farm from where the shrimps were harvested. Culture farm was the main natural source of pathogenic bacteria. After harvesting, if a long time was taken for transporting the shrimp to the processing plant then the pathogenic bacteria increased rapidly and

destroy the quality of shrimp. Another source of pathogenic bacteria was unhygienic handling of the workers. In processing plant there was hand dip point with 40-50ppm Cl_2 concentration and there also foot dip point with 200ppm Cl_2 concentration for good hygiene practices. If the workers did not wash their hand and feet properly then the pathogenic bacteria might contaminated the shrimp during handling. Use of unhygienic basket and ice made of non-potable water might also be source of pathogenic bacteria. Chemical hazard caused by pesticide, use of drug or antibiotics and sulfites. Pesticide may come with received raw materials from environmental source. Through different environmental sources such as industry, agricultural farm etc. pesticide mixed with water and transfer to the shrimp body by biological process. Drug or antibiotics may come with received raw materials due to use of shrimp feed. Different types of drug or antibiotics and hormone are mixed with shrimp feed to reduced disease, increased growth and reduced the mortality. And sulfites may come from supplier uses during culture period. But there is no evidence of using sulfites.

The source of hazards in the cooking was biological. The biological hazard was caused by cross contamination and bacterial growth. Cross contamination may occur due to unhygienic handling by the workers. Bacterial growth may occur due to survival of pathogen for time and temperature abuse. If proper cooking time and temperature was not maintained then the pathogens can survive in the cooking product and caused hazard to the consumers. Established cooking time and temperature for cooked shrimp product was 3 min. and 65-72°C.

Through analysis the CCP decision tree the receiving raw material and cooking step was selected as CCP of cook IQF shrimp. CCP was such a point or step after crossing this point or step sufficient hazards may occur to the consumers. Pathogenic bacteria, pesticide drug or antibiotics were the potential hazards of receiving raw material. If we were unable to control pathogenic bacteria, pesticide, drug/antibiotics, hormone etc. at this step then we could not control them in the next steps and ultimately go to the final product. This caused hazards to the consumers. Pathogenic bacteria of received raw material can be control through raw materials' source control, monitoring of proper tagging of basket, suppliers' certificate monitoring, visiting the farm area twice in a year etc. Pesticide of received raw shrimp can be control through suppliers' lot by lot certificate monitoring, shrimp flesh test for pesticide residue, collecting the shrimp, soil and water sample from culture farm for

analysis annually, half yearly or more, visiting all new supplier's farm and 30% of existing supplier's farm area yearly to review the agricultural practices in farm area. Drug or antibiotics and hormone of received raw shrimp can be control by suppliers' lot by lot certificate monitoring, shrimp flesh test for antibiotic residue, collecting the shrimp, soil and water sample from culture farm for analysis annually, half yearly or more, visiting all new supplier's farm and 30% of existing supplier's farm area to review the producer's drug usage method each year.

Another selected CCP of cooked IQF shrimp was cooking. The cooking step was most concern about the sensory quality of product and the survival of pathogens that may contaminate the finished product. As cooked IQF shrimp was ready to eat product so cooking steps was very important and high risk step. If proper cooking time and temperature was not maintained in cooking step then the pathogens can survive in the cooked product and caused hazard to the consumers. Established cooking time and temperature for cooked shrimp product was 3 min. and the core temperature must be maintained at 68-72°C. After this step we can not able to control the survival pathogens. So this step was very important. There were three important measures for controlling this hazard. First, an adequate cooking time and temperature was established that ensures the destruction of bacterial pathogens and avoidance of overcooking. Second, by monitoring cooking time and temperature to ensure that they meet the requirements of the established process. Third, training of the cooker personnel to operate all cooking equipment, including monitoring devices (times and temperature recorded). So from the above discussion it can be says that the selection of received raw material and cooking as CCP was correct and appropriate.

2. Microbiological Test of Shrimp

2.1. SPC of Shrimp at Full Moon and New Moon

The international accepted limit of SPC is 10^6 cfu/g (ICMSF, 1988). All the results showed that the SPC were lower than accepted limit (Table 2.7 & 2.10) for both experiments. SPC of freshly harvested shrimp ranged from 6.8×10^4 to 1.5×10^5 as observed by Lobrerra *et. al.*, 1990. These counts are within the range of reported values (10^3 - 10^5) of shrimp from temperate environments. (Lillard *et. al.*, 1984; Matches and Layrisse, 1985) although Lannelongue *et. al.*, (1982) mentioned counts as high as 10^6 - 10^7 . Counts reported from tropical countries also ranged from 10^3 to 10^6 (Varma *et. al.*, 1982; Surendran *et. al.*, 1985). A report of De Silva (1985), however, indicated counts as high as 10^8 /g. Result of this present study showed that freshly harvested shrimp could meet the existing standards for SPC which is 10^6 /g (ICMSF, 1974).

Bacterial counts on *P. monodon* immediately after harvesting ranged from 2.23 to 4.20 (expressed as $\log_{10}$ cfu/gm) at 20°C and 3.36 to 4.20 at 37°C (Dangla, 1984). The findings of this investigation did not correlate with the findings of Dangla having higher bacterial load. However the results were in convenience with the results reported Cobb *et. al.*, (1976) for *Penaeus* sps. (10^4 - 10^6). Cann (1976) also observed as high as 10^6 - 10^7 . The lower counts of brackish water species were probably due to the method of harvesting and to the avoidance of extensive handling and board storage of marine prawns (Dangla, 1984).

The total bacterial load of shrimp samples gradually declined during processing. A significant drop in bacterial load was noticed when the shrimp arrived in the processing plant. The initial load of bacteria was $\log$ 6.99 to $\log$ 7.55, whereas the frozen shrimp product was between $\log$ 5.98 to $\log$ 6.73 (Putro *et. al.*, 1990). The results of the present investigation are in concurrence with the result of Putro.

There is evidence to support the fact that surface water carries less bacteria compared to bottom mud about 50ft below the surface (Williams *et. al.*, 1952). As shrimp are bottom dwelling animals, the livelihood of their becoming contaminated with bacteria from the muddy substrate is always possibility. Beheading of shrimps has lead to reduction of bacterial counts by 75% and the effect of through washing on the reduction of microbial load of prawns has been documented by Green (1949) and Williams *et. al.*, (1952). These

results demonstrate that a wash with clean water and proper handling techniques will reduce high SPC.

Cann (1977), states that based on the studies in Thailand and USA, the differences in bacterial counts between whole and beheading shrimps was not significant. The beheading of shrimp in the present study was carried out manually. The effect of washing on the bacterial load of the processed product was a majority (58.3%) of counts fell within the 10^5 - 10^6 /g range.

The mean values of SPC of three different points were lower during new moon compared to full moon. During full moon the harvesting of shrimp was increased compared to new moon. So the received raw materials during full moon was comparatively much than new moon. The much amount of shrimp needs more time for processing. As a result a large number of shrimp baskets was wait in the landing center, received room, primary wash room, grading section, beheading section etc. during full moon. With the passes of time, the number of SPC was increased rapidly and the quality of shrimp becomes poor. Due to this high load of SPC, some SPC was still present after processing which was comparatively higher amount than new moon. For this reason the mean values of SPC of three different points were higher during full moon compared to new moon. The mean values of SPC of received raw material were 5.74×10^5 cfu/g and 4.85×10^5 cfu/g (Table 2.7 & 2.10) at full moon and new moon respectively. The mean values of SPC of export graded shrimp of at full moon and new moon were 3.04×10^5 cfu/g and 2.46×10^5 cfu/g respectively. And the mean values of SPC of final product were 0.854×10^5 cfu/g and 0.614×10^5 cfu/g at full moon and new moon respectively. The acceptability limit of SPC is 10×10^5 cfu/g. All the points of sampling of both full moon and new moon the values of SPC were lower and within the acceptable limit. This also indicated a better status of shrimp product.

2.2. TC of Shrimp at Full Moon and New Moon

According to Fonseka (1990), 50% of the shrimp samples had coliform less than 20 MPN/gm while the rest of the samples carried Coliform below 75 MPN/g. Detailed bacteriological evaluation of shrimp from cold waters have been attempt by Harrison and Lee (1969); Cann, (1971); Novak (1973) and Lee and Pfeifer (1977). Such data regarding

tropical shrimp is rather scanty (Cann, 1977; Zuberi *et. al.*, 1983). Jayaweera *et. al.*, (1990) observed coliform counts of raw material ranging from <3 to 1,100/g with 50% of the counts in the $3-10^2$ /g range. Shrimp caught in deeper waters generally do not carry coliform, but those caught close to the shore and inshore waters have been found to be contaminated frequently with coliform (Cann, 1977).

The mean values of TC of three different points were lower during new Moon compared to full moon. During full moon the harvesting of shrimp was increased compared to new moon. So the received raw materials during full moon was comparatively much than new moon. The much amount of shrimp needs more time for processing. As a result a large number of shrimp baskets was wait in the landing center, received room, primary wash room, grading section, beheading section etc. during full moon. With the passes of time, the number of TC was increased rapidly and the quality of shrimp becomes poor. Due to this high load of TC, some TC is still present after processing which was comparatively higher amount than new moon. For this reason the mean values of TC of three different points were higher during full moon compared to new moon. The mean values of TC of received raw material were 40.20 MPN/g and 31.20 MPN/g (Table 2.8 & 2.11) at full moon and new moon respectively. The mean values of TC of export graded shrimp of at full moon and new moon were 22.60 MPN/g and 16.60 MPN/g respectively. And the mean values of TC of final product were 7.80 MPN/g and 5.60 MPN/g at full moon and new moon respectively. The acceptability limit of TC is 100 MPN/g. All the points of sampling of both full moon and new moon the values of TC were lower and within the acceptable limit. This also indicated a better status of shrimp product.

2.3. FC of Shrimp at Full Moon and new Moon

Putro *et. al.*, (1990) could not detect FC either for shrimp or surrounding waters. According to Fonseka, (1990), FC was not detected in 82.5% samples and counts of FC in pond water were also found to be less than those in shrimps. Cann (1977) reported that freshly caught penaeid shrimps did not carry FC but smaller samples were observed water contaminated with FC.

Coliform organisms are primarily used to indicate some degree of potentiality hazardous contamination based on the assumption that the natural habitat of the family (Enterbacteriaceae) to which these bacteria belong is the faeces of man and other

mammals, thereby indicating faecal contamination. Jayaweera *et. al.*, (1990) found FC counts in the raw material ranging from 3 to 10^2 MPN/g. The FC counts did not vary significantly during the processing stage of beheading and grading, but the counts declined slightly during the next stage i.e., just before storage in ice, but significantly reduced after keeping ice for a prolonged period.

The mean values of FC of three different points were lower during new moon compared to full moon. During full moon the harvesting of shrimp was increased compared to new moon. So the received raw materials during full moon was comparatively much than new moon. The much amount of shrimp needs more time for processing. As a result a large number of shrimp baskets was wait in the landing center, received room, primary wash room, grading section, beheading section etc. during full moon. With the passes of time, the number of FC was increased rapidly and the quality of shrimp becomes poor. Due to this high load of FC, some FC was still present after processing which was comparatively higher amount than new moon. For this reason the mean values of FC of three different points were higher during full moon compared to new moon. The mean values of FC of received raw material were 6.20 MPN/g and 5.4 MPN/g (Table 2.9 & 2.12) at full moon and new moon respectively. The mean values of FC of export graded shrimp of at full moon and new moon were 2.0 MPN/g and 1.40 MPN/g respectively. And the mean values of FC of final product at both full moon and new moon were absent. The acceptability limit of FC is 11 MPN/g. All the points of sampling of both full moon and new moon the values of FC were lower and within the acceptable limit. This also indicated a better status of shrimp product.

2.4. *Salmonella* sp. of Shrimp at Full Moon and New Moon

According to Azam, (2004) *Salmonella* were not detected in the sample of shrimps. The present investigation is in absolute agreement with the findings of Azam, (2004). *Salmonella* was not isolated from freshly harvested shrimp (Lobrerria *et. al.*, 1990; Putro *et. al.*, 1990). However *salmonella* contamination was observed in freshly harvested shrimp as found by Rattagool *et. al.*, (1990). In a study of microbial flora of pond cultured shrimp, *salmonella* was not detected (Fonseka, 1990). The results of the microbial parameters in this investigation indicated a similar pattern as observed by Fonseka (1990). The author reported that *salmonella* was not detected from farm shrimp.

The present study indicated no detection of *Salmonella* sp. 25gm samples of shrimp collected from any points of shrimp processing plant. *Salmonella* were detected in prawn samples in the storage stability of brackish water prawn during processing for export (Dangla, 1984) particularly after harvesting but not found during or after processing.

Rose *et. al.*, (1990) found the presence of *salmonella* on the frozen marine prawn immediately after processing. The absence of *Salmonella* in the frozen farmed prawn might have been caused due to absence of these bacteria in the raw material rather than the effect of processing measures. Rattagool *et. al.* (1990) found *Salmonella* sp. Associated with raw peeled shrimp and factory peeled shrimp. Jayaweera *et. al.* (1990) could not detect *Salmonella* in 25gm samples from any stage of processing. The present study is in absolute agreement. Rose *et. al.* (1990) state that *Salmonella* was not found in the marine shrimp naturally and any *Salmonella* in the marine shrimp was probably due to contamination during handling, transportation or processing.

2.5. *V. cholerae* of Shrimp at Full Moon and New Moon

V. cholerae were detected in prawn samples in the storage stability of brackish water prawn during processing for export (Dangla, 1984) particularly after harvesting but was not found during or after processing. In the present investigation no *V. cholerae* were detected in any samples throughout the laboratory experiment for shrimp at any points of shrimp processing plant (Received raw material, Export graded shrimp and Final product). In the study of microbial flora of pond cultured shrimp, only two samples were positive for *Vibrio* sp. (Fonseka, 1990). The presence of *Vibrio* in pond reared shrimps (Christopher *et. al.*, 1978; Vanderzant *et. al.*, 1971) and fish and shellfish from estuarine and marine water is well documented (Durairaj *et. al.*, 1983, Reilly, 1984; Simidu *et. al.*, 1969)

Rosmaewaty *et. al.* (1985) found that *Vibrio parahaemolyticus* being not detected throughout the processing lines. Reilly *et. al.* (1984) working on brackish water prawns reported that *Vibrio parahaemolyticus* was present commonly as part of natural flora, but not isolated after processing. According to Azam, (2004) *Vibrio* sp. were not detected in 25gm samples of shrimp sample collected from any point of distribution channel in Bagerhat, Khulna and Satkhira district. The present investigation did not differ from those observed by Azam, (2004). However *Vibrio cholerae* strains have been reported to be

widely distributed in the environment in Europe, Asia and United States (Karunasagar *et. al.*, 1990). Blake *et. al.* (1979) summarized that *Vibrio cholerae* is an autochthonous species of the estuarine ecosystem in as much as the occurrence of *Vibrio cholerae* was not correlated with other micro-bial indicators of faecal pollution.

Reilly *et. al.* (1986) working on the spoilage of *P. monodon* at different temperatures, found the initial micro-flora to be meso-philic and dominated by several sp. and *V. cholerae* (6.7%). According to Rose *et. al.* (1990), *V. cholerae* was detected twice throughout the pond and plant survey. Reilly (1986) reports isolated of *V. cholerae* in brackish water prawn and suspected that the bottom mud is the source. Iyer *et. al.* (1990) found no presence of *V. cholerae* as observed in the present study

3. Microbiological Test of water

3.1. SPC of Water at Full Moon and New Moon

In totally the mean values of SPC at all sampling points were lower than the acceptable limit. The mean values of SPC for both the experiment did not differ in three points of sampling. These three points were the receiving tape (6 cfu/ml), processing tape (4 cfu/ml) and panning tape (3 cfu/ml). The water collected from overhead tank showed better result at new moon (6 cfu/ml) than full moon (7 cfu/ml). Incase of beheading tape full moon showed the better result (5 cfu/ml) than the new moon (6 cfu/ml). The difference of SPC between full moon and new moon in overhead tank and beheading tape is only 1 cfu. This is not significantly difference. The sources of water are same; water treatment system is also same. So there is no affect of full moon or new moon on water. All the values that were found at each points of sampling in the processing plant showed better quality as well as within the acceptable limit.

3.2. TC and FC of water at Full Moon and New Moon

Water sample were collected from five different points (Over head tank, Receiving tape, Beheading tape, Processing tape and Panning tape). TC and FC were not detected in any samples of different sampling points in the shrimp processing industry.

4. Microbiological Test of SWAB

4.1. SPC of SWAB at Full Moon and New Moon (Workers hands)

SWAB test of workers hands were done before and after working at full moon and new moon. Through SWAB test SPC was measured before and after working of the workers and the contact surfaces were workers hands. In totally the mean value of SPC at full moon and new moon before and after working were lower than the acceptable limit. The mean values of SPC for both the experiment differs significantly before and after working. During full moon and new moon the differences between before and after working were 10 cfu and 5 cfu respectively. After working the SPC was increased due to handling of shrimp. During handling the SPC come to direct contact with the workers hands. For this reason after working the SPC of SWAB test result increased. Although there were significant differences between the values of full moon and new moon and before and after working, all the values were much lower than the standard recommended limit.

4.2. SPC of SWAB at Full Moon and New Moon (SS table)

SWAB test of SS table was done before and after working during full moon and new moon. In the course of SWAB test SPC was measured before and after working of the workers and the contact surfaces were the SS table. In totally the mean value of SPC at full moon and new moon before and after working were lower than the acceptable limit. The mean values of SPC for both the experiment differs significantly before and after working. The differences between before and after working were 10 cfu and 5 cfu correspondingly. After working the SPC was increased due to handling of shrimp. During handling the SPC come to direct contact with the workers hands. For this reason after working the SPC of SWAB test result increased. Although there were significant differences between the values of full moon and new moon and before and after working, all the values were much lower than the standard recommended limit.

Recommendation and Conclusion

Quality of shrimp product (raw shrimp, export graded shrimp and final product), water and SWAB in the processing were assessed through microbiological test during full moon and new moon. The results were then compared with each other. All the value that were gotten, were within the acceptable level which indicate highly hygienic condition of the industry. The microbiological parameters of shrimp, water and SWAB showed variation in numbers at each point of sampling as well as in full moon and new moon but remained well within the recommended range of ICMSF. *Salmonella* and *V. cholerae* were not detected in any sample. The CCP of processing plant were identified. The CCP of block frozen shrimp, semi-IQF shrimp and IQF shrimp were receiving raw material and the CCP of cooked IQF shrimp was cooking.

Shrimp is the mainly for export in as Bangladesh earns sufficient amount of foreign exchange. Though the quality of shrimp in both the experiments was acceptable, yet the country has to cater for the need of the buyers in the international market. The importers are not only too satisfied with quality now. They would like to see that the product has a "Seal of Quality" brand certified by third party which is internationally recognized. The "Seal of Quality" encompasses traceability, food safety, human rights violation, fair trade practices and environment friendly product. The entire person related with shrimp production and export should be aware of "Seal of Quality" in order.

A set of recommendation has been proposed to improve the quality standard as well as to increase the export:

- The overall hygienic conditions of the industry are satisfactory. The industry should have maintained hygienic condition.
- International quality assurance approaches should be followed in the processing.
- Effective quality control measure should be ensured for high quality products from the shrimp industry.

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Appendix

Appendix 1: Hazard analysis worksheet with CCP

<i>Step/ Position</i>	<i>Potential Hazards</i>	<i>Justification of Hazards</i>	<i>Preventive/ Control measures</i>	<i>Q₁ Y/N</i>	<i>Q₂ Y/N</i>	<i>Q₃ Y/N</i>	<i>Q₄ Y/N</i>	<i>CCP (Y/N)</i>
Raw materials receive	Biological:							
	Chemical:							
	Physical:							
Beheadin g	Biological:							
	Chemical:							
	Physical:							
Washing	Biological:							
	Chemical:							
	Physical:							
Cold store	Biological:							
	Chemical:							
	Physical:							
Grading	Biological:							
	Chemical:							
	Physical:							
Weighing	Biological:							
	Chemical:							
	Physical:							

Panning	Biological:								
	Chemical:								
	Physical:								
Freezing	Biological:								
	Chemical:								
	Physical:								
Depannin g and glazing	Biological:								
	Chemical:								
	Physical:								
Pack/ label	Biological:								
	Chemical:								
	Physical:								
Metal scanning	Biological:								
	Chemical:								
	Physical:								
Frozen storage	Biological:								
	Chemical:								
	Physical:								
Export	Biological:								
	Chemical:								
	Physical:								

Appendix 2: HACCP Plan form

Critical Control Point (CCP)	Significant Hazards	Critical Limit for each Preventive measure	Monitoring procedure			Corrective actions	Record	Verification
			What	How	Frequency			

Appendix 3: CCP Decision Tree

Q1. Do control measures exist at this step or subsequent steps for the identified hazard?

