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QUALITY MANAGEMENT PROGRAMME BASED ON HACCP IN A COOKED SHRIMP PROCESSING PLANT

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ABSTRACT

The quality management system based on HACCP applied in reality to an Icelandic cooked shrimp processing factory and sampling methods and tests to check hygienic conditions of the processing environment has been studied. The results of the study showed that the quality management program based on HACCP is a flexible system. Though HACCP is intended for the control of safety, it's principles can be applied to non - safety hazards such as the prevention of economic fraud or other aspects of food quality. Cooked, peeled shrimp is considered to be a high-risk product. In order to process this product, the hygienic condition of the factory plays an important role. An HACCP program can not be effectively applied if the hygienic condition is not in place. The microbial analysis can be considered as a tool to help the food processors find the reasons for unhygienic conditions in their factory. Microbial analysis can show whether the sanitation program is working to keep food products safe and equipment, utensils, floors and walls clean. This study will provide useful information for Vietnamese seafood processors.

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1. INTRODUCTION

For decades, fisheries have been an economically important industry to Vietnam. 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 one of the top five leading exported agricultural commodities in Vietnam.

The export of Vietnam fisheries in the first six months of 2001 was 180,400 tons and USD 832 million in value. The seafood products from Vietnam are exported to more than 64 countries, where the main markets are Japan, United State of America (USA), China and European Union countries (EU). Some of the common seafood products exported overseas are frozen shrimp, fish, cephalopod, bivalve mollusc, dried fish and squid, among which, frozen shrimp is the leading item. Frozen shrimp has been the main export product with total volume of 37,635 tons and value of USD 328 million or 39% of the total export value in the first six months of 2001 (Fistenet 2001). 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 black tiger, pink, cat tiger, white and yellow shrimp. The volume of frozen cooked shrimp has been small because its hygiene requirement is high and only a few establishments can process cooked shrimp meeting the requirement of the market.

With the aim of seafood safety assurance for both export and domestic consumption and with expanding markets for seafood exports, the Ministry of Fisheries in Vietnam has conducted concrete activities in order to speed up the changes in seafood quality management since 1995. The quality management system based on Hazard Analysis Critical Control Point (HACCP) has gradually been replacing the checking of the final products.

In 1997, Vietnamese Ministry of Fisheries promulgated regulations according to the regulation of EU and USA on mandatory HACCP application in fish processing plants that are registered for export to EU and USA markets. By the year 2001, The Ministry of Fisheries had issued four regulations and 11 sectional standards related to management of quality, hygiene and safety of fishery products (MOFI 2000), in which:

- 28 TCN 129:1998, sectional standard on fish processing establishments - HACCP based programme for quality and safety assurance.
- 28 TCN 130:1998, sectional standard on fish processing establishment - General condition for food safety.
- Decision No 694/200/QD_BTS promulgates the Regulation on inspection and approval for fishery establishments which meet the requirements on assurance of food safety dated 4 August 2000.

In Vietnam many seafood processing establishments are implementing HACCP to meet the requirements of the markets and comply with regulation and standards of the Ministry of Fisheries. There are now about 264 seafood processing factories, most of them being freezing factories (201 freezing factories), the rest are factories producing dried fish, canned fish and fish sauce. Of these 264 factories, 110 have implemented HACCP effectively.

During the development and implementation of HACCP, some problems have arisen which have limited the full implementation of HACCP. These problems are mainly due to:

- Lack of experience in the implementation of HACCP among processors;
- Hygienic condition / plan layout as prerequisite for HACCP implementation is not in place;

In order to introduce HACCP concepts and to speed up the progress of HACCP application in the country, the Ministry of Fisheries considered HACCP training as a main key for success. Recently, the Ministry of Fisheries has been organising training courses on quality, hygiene and safety assurance of fishery products for fishery processors and fishermen.

1.1 Objective of the study

In the project the quality management system based on HACCP in a cooked shrimp processing factory will be studied. It will cover following aspects of the operation that can impact the safety of the final products:

1. Studying HACCP programme and prerequisites such as: cleaning and disinfecting systems, personal hygienic standard; pest control; water quality.
2. Evaluation of cleaning and disinfecting methods on the food contact surfaces and non-food contact surfaces. It consists of:
   - Samples taken from food contact surfaces and non-food contact surfaces to evaluate the hygienic condition by Replicate Organism Detection and Counting plate (RODAC plate), *Listeria* and also Adenosine triphosphate (ATP) measurement.
   - Samples taken from raw material, semi-finished products, final products to analyse micro-organisms such as total plate count (TPC), *Listeria*, total coliforms and faecal coliform.

Results of this project will give useful information for the fish quality management training courses for processors in Vietnam.

2. LITERATURE REVIEW

2.1 Quality management system based on HACCP

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).

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 programmes in seafood processing establishments. These quality control programmes 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 programme 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).

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 programmes.

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 programme 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 programmes for their fish processing industry in line with international trends. HACCP programmes comply with the regulations of the importing countries especially the EU and USA (Eong and Ngei 2000).

2.2 Hazard Analysis Critical Control Point system

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.

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 colour 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 decision tree is shown in Appendix 1.

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).

2.3 Prerequisites

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 programmes. Prerequisite programmes may impact on the safety of food; they also are concerned with ensuring that foods are wholesome and suitable for consumption. Formal prerequisite program 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 programmes may cover (NACMCF 1997):

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

In the prerequisite programmes, 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).

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).

2.4 Hygienic evaluation of seafood plant

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.

2.4.1 Total plate count (TPC)

This 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.

2.4.2 Faecal coliform group

Faecal coliforms belong to the family *Enterobacteriaceae* whose natural habitat are 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). The growth condition of *Enterobacteriaceae* are shown in Table 1

Table 1:Growth condition of *Enterobacteriaceae* (Huss 1994)

Bacteria	Temp. (°C)	Temp. (°C) optimum	pH minimum	Water activity minimum	NaCl (%) maximum
Salmonella sp.	5-47	37	4.0	0.92	4-5
Shigella sp.	7-46	37	5.5		4-5
Escherichia coli	5-48	37	4.4	0.92	6

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).

Shigella sp. is the cause of shigellosis. Symptoms vary from asymptomatic infection or mild diarrhea to dysentery, characterized by bloody stools, mucus secretion, dehydration, high fever and severe abdominal pain (Huss 1994). Prevention and control requires either that infected persons are not permitted to handle foods or that they practice good personal hygiene. Education of food handlers, with emphasis on good personal hygiene, is the best preventive measure (Lee 1992).

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.

2.4.3 Listeria monocytogenes

The pathogen that causes most problems in dairy products is *Listeria monocytogenes*. This pathogen grows at refrigerator temperatures, so good sanitation is especially important. *Listeria* has been isolated in a variety of seafood, such as shrimp (raw and cooked), cooked crab, cooked lobster, smoked fish, surimi based products and molluskan shellfish (Bonnell 1994).

Listeria monocytogenes is widespread in nature. It can be isolated from faecal specimens of healthy animals and man, as well as from sewage, fertilizer, soil and

vegetation (Bonnell 1994). *Listeria monocytogenes* can survive in aerobic and anaerobic environments, so it can live in many different types of food (Marriott 1997).

This microbe grows best at 37°C, but it can grow at temperature between 0°C and 45°C, at pH of 5.0 - 9.6, at water activity of 0.92 or higher and in high salt concentration (perhaps greater than 10%). It is usually destroyed at temperatures above 61.5°C (Huss 1994). *Listeria* can build up biofilm that is very hard to remove during cleaning (Marriott 1997).

Listeriosis is most common in newborn babies, the elderly, people with a weakened immune system or people with other diseases. Of the people who get listeriosis, 25% die (Marriott 1997).

In the food processing factories, *Listeria monocytogenes* is often found in wet areas such as floors, drains, wash area, ceiling condensation, mops and sponges, brine chillers and at peeler stations. Refrigeration at 4 to 5°C does not stop this pathogen from growing. Excellent sanitation is essential to control this pathogen (Marriott 1997). Cooked, ready-to-eat products such as cooked shrimp are considered to be high-risk products for which a *Listeria* control program should be established. Enviromental samples from processing plants must be evaluated carefully. Management of Listeria through good manufacturing practices and identification of critical control points will allow seafood processors to control but not eliminate *Listeria* (Vanderzant and Splittstoesser 1992).

Currently the USA FDA requires that *Listeria monocytogenes* be absent in ready-to-eat seafood products such as cooked shrimp, crab meat or smoked fish. This restriction does not apply to raw products that will be cooked before eating (Huss 1994).

2.4.4 Adenosine triphosphate monitoring - ATP

Adenosine triphosphate is found in all living or dead cells. The method of monitoring the ATP level via bio-luminescence where concentration is measured in light unit is used to evaluate cleaning procedure. This method gives an indication of the total level of soiling on a particular surface and can be a useful tool in determining hygiene standards and cleaning efficiency (Chesworth 1997). The ATP method can be used for the following purposes (Lundin 1999):

- Routine control after cleaning/disinfection. In food industry ATP testing performed by the cleaning staff has already become part of many HACCP programs.
- Rapid method for finding sources of contamination.
- Evaluation of new cleaning methods and materials.
- Education in hygiene and cleaning.
- Audition of hygiene in production and distribution

3. METHODS

3.1 Study of the product quality management system based on HACCP

Study of the implementation of an HACCP programme was carried out in a cooked shrimp processing factory. A quality manager introduced the operation of the factory. The quality management documents such as quality manual, cleaning sanitation manual, pest control manual were examined. The document of the HACCP plan including some critical control points (CCP), information related to control of raw material quality, finished products, quality of processing water were collected based on the questionnaire shown in Appendix 2.

After looking through the quality documents, an observation of the production was carried out. The following items and processes were observed:
- The process from the reception of the raw material to the final product storage.
- The personnel and solid waste routes.
- The separation between high risk areas and low risk areas.

3.2 Evaluation of the hygiene and sanitation in processing environment

In order to estimate the sanitary quality of the food contact surfaces and non-food contact surfaces in the factory, visual inspection, ATP measurement and microbiological tests were done. The microbial analysis was conducted in terms of *Listeria* and RODAC plate. Samples were taken in an aseptic manner to avoid contamination. Sampling was carried out for ATP measurement, RODAC plate and *Listeria* as below:

- RODAC plate: the cover was removed from RODAC plate. The agar surface was carefully pressed to the surface being sampled. 16 samples were taken after cleaning. Plates were incubated at 22°C for 72 hours and colonies counted.

- ATP measurement: Portable Luminometer was used. The pre-wetted swab was removed from the holder of swab tube and surface areas of 10 cm^2 were sampled. The swab was replaced into the swab tube. 16 samples were taken after cleaning.

- *Listeria*: To identify *Listeria* on the food contact surfaces and non-food contact surfaces, the cotton swab was dipped in the D/E Neutralising broth and then rolled over the surface of equipment, floor, drain and etc. The swab placed in a sterile bottle. 54 samples for *Listeria* isolation were taken after cleaning and during processing.

The sampling plan is presented in Appendix 3.

During processing, samples were taken from raw material, semi-finished products, shell of shrimp after peeling and final products to carry out microbiological tests that consisted of TPC, *Listeria*, total coliforms and faecal coliforms. Samples were taken based on the sampling plans shown in Appendix 3.

Temperature of the raw material, semi-finished product, final product and processing environment was measured. The types of material of the food contact surfaces were also documented.

The procedures for ATP measurement and microbiological tests like TPC, *Listeria*, total coliforms, faecal coliforms and RODAC plates are shown in Appendix 4.

4. RESULTS

4.1 Food quality management system of the cooked shrimp factory

In the cooked shrimp processing factory, the types of documents used in the product quality management system include quality manual, pest control manual, training manual, and cleaning and disinfecting manual.

4.1.1 Quality manual

The quality manual plays an important role in the product quality management system. It describes management responsibility of key staff and provides consistent information about the organisation's quality management system. Its contents are shown in table 2.

Table 2: Contents of the quality manual

1. Management responsibility
2. Quality policy and objectives
3. Design and extent of the quality system
4. Production description
5. Purchase of packing material and additives - Certificates
6. Purchase of raw material
7. Layout of production
8. Flow chart of production
9. Production quality control system
10. Plant quality control system
11. Hygiene
12. Control of foreign matters - Glass control
13. Pest control
14. Temperature in production areas
15. Control of test equipment
16. Control of chemicals
17. Rule of conduct - Employees and guests
18. Training of new employees
19. Traceability and recalling of product
20. Rules of sampling and microbiological standards - Control of non conforming product
21. Criteria standards for quality inspection and method description

The quality objective is to process high quality and safe products to meet the requirements of the customers and to expand export markets.

4.1.2 Pest control manual

Besides the quality manual, the factory established a pest control manual. Contents of that manual include:

- Requirements on pest control
- Responsibilities of staff for pest control
- Layout of building
- List of numbered traps and map showing their location
- Methods killing pest (fly traps, bait traps)
- Handling of toxic substances
- Control sheets

4.1.3 Cleaning and disinfecting manual

The factory has established a cleaning and disinfecting manual consisting of the following items:

- Monitoring system
- Cleaning and disinfecting agents
- Cleaning and disinfecting procedures
- Control sheets

In the food processing factory, cleaning and disinfection plays an important role to ensure that the risk of contamination is kept to a minimum. Cleaning staff is properly trained in the use of chemicals and safety precautions required.

a) Monitoring system
Monitoring of food contact surfaces and non-food contact surfaces typically involves a combination of visual checks and microbiological tests.

Visual inspection is carried out based on control sheet after cleaning. A hygienic controller inspects hygienic condition of processing equipment, floors, walls, drains and other items in production areas such as reception area, cooking area, peeling area, inspecting belt area, freezing and packaging area. Results are documented. If fault is found, it will be documented and the foremen and the cleaners in charge of that area informed. Faulted area has to be cleaned again until it meets the requirements.

The factory has established a sampling plan to carry out tests for *Listeria* and ATP measurements. The accredited laboratory carries out sampling and microbiological tests.

Sampling places for ATP include food contact surfaces of processing equipment like defrosting units, grading equipment, cooking equipment, peeling equipment, hand peeling and grading equipment after freezing. There are about 10-12 sampling places taken every two weeks based on a sampling plan.

Sampling places for *Listeria* comprise surfaces of floors, drains, forklifts, tubs containing raw material and surfaces of processing equipment such as defrosting units, cooking units, peeling equipment, inspection belts, in-feeding to flow freezer,

ice glaze and conveyer belt and scales. Sampling is carried out once every month based on the sampling plan. If *Listeria* is found in any place, the factory has to implement following:

- The final product processed in that day will be sampled to check *Listeria*.
- Places will be immediately cleaned again and samples taken to check *Listeria*.

b) Cleaning and disinfecting agents

All cleaning and disinfecting compounds have certificates to confirm that they are allowed for use in the food processing industry. The Environmental and Food Agency (EFA) have approved them. The factory uses potable water for cleaning and disinfecting.

Cleaning and disinfecting agents include strong alkaline (sodium hydroxide alkaline) with 3% concentration, chlorine based alkaline 1/10, quaternary ammonium compounds and acid (phosphoric acid). The characteristic of the cleaning and disinfecting compounds are shown in Appendix 5.

c) Cleaning and disinfecting procedures

The frequency and the type of cleaning and disinfecting is according to a written sanitation plan and follows instructions from manufacturers of the cleaning and disinfecting agents. In the factory, there is a cleaning station where cleaning and disinfecting agents are dissolved in water. The pipe system for cleaning and disinfecting compounds connects the cleaning station with processing areas.

Cleaning and disinfecting typically involves six main steps: preparatory work, dry clean, pre-rinse, detergent application, post rinse and sanitising application.

- Preparatory work: Solutions of cleaning compounds are prepared according to the requirement in the cleaning station. After that the detergent solutions are delivered to the production areas by pipes. Machines and other mechanical parts such as conveyor belt etc. are dismantled so that all locations, where micro-organisms can accumulate, become accessible for cleaning and disinfection. Electrical installations are protected against water and chemicals.

- Dry cleaning: Before using the cleaning agent, food debris and soil are swept by broom, brush or squeegee.

- Pre-rinsing: Small particles, missed in the dry cleaning step are removed by water. This step prepares wet surfaces for detergent application.

- Detergent application: The factory applies strong alkaline (3%) to remove fat and protein. The pressure of detergent solution at outlet is about 20 bar. The surfaces are cleaned for 15 to 30 minutes. Chlorine based alkaline (1/10) is used for 30 - 60 minutes twice every week on conveyer belt and on difficult dirt as needed. Acid is used once every week.

- Post rinse: After the appropriate contact time of the detergents all parts are rinsed thoroughly with cold water to completely remove all cleaning agents.

- Disinfection application: Quaternary ammonium compounds are used to disinfect contact surfaces overnight. Concentration of quaternary ammonium compound is 300 ppm.

Before the equipment is used, it is rinsed with water.

The cleaning program is developed for each area of the plant. A special cleaning is needed if problems regarding *Listeria* arise. Then the concentration of cleaning agents is higher (10%) and the time is longer (40 min).

4.1.4 The water quality

The factory uses potable water for processing and other operations. Samples are taken for bacteriological analyses once every 12 months. Bacteriological parameters are:
- Total count at 37°C <50/ml
- Total count at 22°C <100/ml
- Total coliforms <1/100ml
- Faecal coliforms <1/100ml

All results of water sample analysis have fulfilled the requirements of Icelandic authorities for quality of water intended for human consumption. The requirements are laid down in Regulation 319/1995.

4.1.5 Personal hygienic standards

Signs that prohibit smoking, spitting, eating and drinking are displayed in a prominent position at every entrance into processing, storage and support areas.

Staff wears clean working clothes and head gear that completely encloses the hair during processing. The clothes of staff working in low risk areas are distinguished from the clothes of workers working in high risk areas by colour (the clothes of staff working in low risk areas are green colour and those working in high risk areas are white colour). All working clothes are changed every working day. They are collected in one area and transported to laundry room. The disposable gloves are clean and waterproof. Sampling of working clothes is carried out twice a year to check TPC.

The use of jewellery and watches in food handling areas is banned. The wearing of wedding ring without stones is allowed, but staffs have to wash their hand carefully and ensure that the skin under the ring is also sanitised. Nail varnish has no place in the factory. The use of strong perfumes in the food handling areas is banned.

Signs guiding staff how to wash their hands are shown in prominent places at every entrance into processing areas. Staff must wash hands at least:
- Before starting work after each break
- Immediately after visiting the toilet
- Immediately after handling any contaminated material or surface

The workers have to give the quality manager their medical certificates before starting working for the factory. Employee health condition that could result in the microbiological contamination of food, food - packaging materials and food contact

surfaces is checked in the factory. The staff is instructed to report any health condition that might result in food contamination to the immediate supervisor. Any visitors that enter the factory have to be asked questions such as their names and address, whether they had or have any infectious diseases such as tuberculosis.

The factory has a training manual on hygiene and HACCP program for the employee. All staff working in the food handling area are fully trained in food hygiene, including all engineers and cleaning staff. The aim of training courses is to ensure that the staff fully understands it responsibilities and learns to take and follow written instructions and procedures.

4.1.6 Quality control of raw material and final product

In the factory, there is no laboratory to implement microbiological tests. Therefore the sampling and quality check of raw material and final product is carried out by accredited laboratory. It is a demand from the buyers that a third party monitor the control system.

a) Quality control of raw material
Sampling is randomly carried out in each lot of raw material of each fishing vessel. One sample unit/case is taken from every 10,000 kg of raw material.

The following parameters of raw material are identified:
- Volume of case
- Volume of raw material in a case
- Volume of water in a case
- Volume of by-catch products
- Volume of raw material that is under size
- Identification of name of fishing vessel, case numbers and code.

The price of raw material depends on the value of above parameters. The factory does not pay attention to the microbiological parameters of raw material.

b) Quality control of final product
Fishery products, especially ready to eat products for human consumption should be safe and uncontaminated. The factory is carrying out microbiological checks on the production at regular intervals, complying with the standards that the customers require. The checks are carried out during processing and before products are placed on the market. Where the acceptability limit is exceeded, the processor investigates the cause thereof and establishes corrective action in order to prevent any further deviation. The microbiological criteria used for evaluation of product safety for consumption are TPC, *Listeria sp.*, total coliforms, faecal coliforms, *Salmonella* and *Staphylococcus aureus*. Besides, the salt concentration of product is checked to fulfil the requirement of the customers. Five samples are taken every week (one sample/day). In the first 47 weeks of 2001, the results of analyses are shown in Appendix 6.

TPC in all samples was less than 1000/g. All checked samples were reported as negative for *Listeria* and *Salmonella. Staphylococcus aureus* was less than 10 in all cases (not detected). Total coliforms in most of the final product were less than 0.3

MPN/g (not detected). Faecal coliforms were less than 0.3 MPN/g (not detected) in all samples. Salt concentration of final product was in the range of 1.7 to 2.2%. The analysis of samples taking from the final product showed that cooked peeled shrimp is safe for consumption. The quality of final product met the requirements of the customer.

Besides microbiological analyses of final product, quality controllers carry out sensory evaluation. The sensory evaluation is based on the Quality Index Method (QIM) shown in Appendix 7. The samples are taken as one unit/bag every hour.

4.1.7 HACCP system

When establishing documents of a HACCP program, the factory carried out duties that comply with the five preliminary tasks and the seven principles of HACCP considered in section 2.2. Those document covers following items:

- Management roles and the responsibilities
- Product description
- Processing flow chart of cooked peeled shrimp
- Production layout
- Hazard Analysis
- Determination of critical control points
- HACCP plan

a) Description of product

In order to prepare a systematic evaluation of the hazards and associated risks in a specific food and its ingredients or components, the factory describes the product, the method of distribution, the intended customer and consumer use of the product. The product description is shown in table 3.

Table 3: Product description

1	Product name	Large Single Frozen Shrimp
2	Source of raw material	Sea area in Canada, Norway, Iceland (North Atlantic). Shrimp was frozen on the fishing vessels
3	Important final product characteristics	Temperature <-18°C
4	Ingredient	Cooked and peeled shrimps, salt and water
5	Packaging	Polyethylene bags 400g / 2000g
6	How the end product is to be used	Product is thawed and normally consumed without further cooking. It is perfect for salads, shrimp cocktail, curries or an indulgent sandwich with mayonnaise.
7	Shelf life	12 months after packaging
8	Where the products will be sold	England
9	Special labelling instructions	As per Fish Inspection Regulation, Food and Drug Regulations and International specifications Keep Frozen
10	Special distribution control shelf life	Store at <-18°C

b) Flow diagram and description of the cooked shrimp processing

To assist the facility in developing a HACCP plan, a flow diagram depicting the operational steps of how shrimp is handled throughout the facility is made. The

diagram shows the steps in numerical order from when the firm takes control of the product until the firm releases control of the product.

Receiving raw material: Raw material is frozen block of whole shrimp. Most of it is frozen on the fishing vessels. Shrimp usually is caught from territorial waters of Canada, Norway and Iceland. After receiving, shrimp lots are numbered and transported to freezer store. Temperature of freezer store is -24°C ±2°C.

Defrosting: The blocks of frozen whole shrimp go through defrosting equipment using potable water. The temperature of water is 10 to 18°C. After that, the shrimp goes to grading equipment by conveyer belt.

Pre-grading: In the grading equipment, shrimp is sized into three groups such as 200-300 bits/kg, 300- 400 bits/kg, 400-500 bits/kg. After that, shrimp flow to tubs with ice and water. Temperature of water is about 1-4°C. Those tubs are transported to cooking room by forklifts.

Cooking: The shrimp falls into the flumes and flows to the steaming equipment. Core temperature of shrimp rises to 79°C and it is maintained for about 3.8 seconds. Then shrimp goes to peeling equipment. The speed of conveyer belts is adjusted depending on the size of shrimp.

Peeling and cooling: Cooked shrimp goes through peeling equipment. At the same time, shrimp is cooled by cold water. Temperature of shrimp decreases to below 8°C.

Laser grading: Peeled cooked shrimp goes to laser grader by conveyer belts. In this step semi-finished products are checked whether shell is completely removed. If shrimp has shell, it will fall down to the conveyer belt and go to the re-peeler. If the shell has been removed, it will go to conveyer belt to hand peeling belt.

Hand peeling belt and brine: Workers standing along the conveyer belt remove small bits of shrimp - shell. Then, shrimp falls down to the cold brine flume. Temperature of brine is from -1°C to 4°C with 1.5% - 2.2% of brine concentration. Semi-finished products and brine are pumped to tubs.

Freezing: From the tubs, semi-finished product goes to IQF (individual quick freezer) by in-feeding conveyer belt. Temperature of shrimp declines to below - 18°C. Capacity of IQF is about 1,200 kg per hour.

Glazing and re-freezing: After freezing, products go to the glazing equipment. In the glazing equipment there are adjusting taps spraying water to product. A layer of thin ice covers the product. Then, the product goes to re-freezer by conveyer belt. Core temperature of the product decreases to at least -18°C.

Weighing and packaging: After leaving re-freezer, the product is checked whether it clumps together. If that happens, it will be removed. Then, the product is divided into two groups depending on the type of package like 400g - 2,000g of shrimp per bag and 10 kg -12kg of shrimp in bag.

The product that will be packaged in small bags goes to automatic weighing equipment. Volume of product in each bag is controlled by a computer system. The product is automatically packed into labelled bags. After packing, bags of the product are checked for quantity by electronic scale. If it is less or more than stipulated volume, quality controller will take it out of the processing line, open bag and put shrimp into conveyer belt before going to the weighing equipment.

The product that will be packaged in big bags goes to the weighing equipment. It is packaged by hand.

Product bags are put into carton boxes. Boxes are labelled with production dates and code of lot.

Cold store: All final products are placed into frozen storage without delay. Final product is kept in the cold store at - 27 ± 2°C.

c) Hazard analysis

As reviewed by the factory, the hazards include safety, wholesomeness or economic fraud. Categories of hazards are biological, chemical and physical. Also of concern are net weight and sensory assessment. For each processing step identified on the flow chart, the factory has carried out hazard identification and hazard evaluation. Hazard identification has resulted in a list of potential hazards at each processing step from the reception of raw material to the release of the finished product.

The factory has organised a hazard analysis worksheet. In the worksheet, there are 5 columns shown in table 4. All potentially significant hazards were considered.

Table 4: Hazard analysis worksheet

Ingredient/ processing step	Potential hazard introduced or controlled	Is the potential hazard significant	Justification for inclusion or exclusion as a significant hazard	Preventative measures of the significant hazards
1	2	3	4	5

Factory has noted a significant hazard at receiving step, defrosting step, cooking step, cooling and peeling step, hand peeling step, brining step, freezing step, ice glazing step, weighing and packaging step, metal detecting step, frozen storage and temperature in processing area. But information was only collected of some processing steps such as cooking, brining step and temperature in processing areas.

At the cooking step, where there is most concern about the sensory quality of product and the survival of pathogens that may contaminate the finished product, the factory has determined three measures that are important in controlling this hazard. First, an adequate cooking time and temperature has been established that ensures the destruction of bacterial pathogens and avoidance of overcooking. Second, cooking time and temperature is monitored to ensure that they meet the requirements of the established process. Third, cooker personnel are trained to operate all cooking equipment, including monitoring devices (times and temperature recorded).

At the brining step, where there is concern about pathogen growth, the factory has determined a preventive measure. Process speed has been controlled. The factory has to ensure that no processing delays occur.

At any processing step, pathogen growth and toxin formation as a result of temperature abuse has been considered a significant hazard. Preventive measures have included control of temperature of ambient, brine, water and products.

d) Determination of critical control point (CCP)
The factory has applied a decision tree shown in Appendix 1 to determinate CCP in the HACCP system. At each processing step, a significant hazard has been determined whether it is a CCP or not. The factory has made a table including 7 columns to present answers of questions in the decision tree (Table 5).

Table 5: Determination of CCPs (see also Appendix 1)

Processing step	Significant hazards	Q. 1	Q. 2	Q.3	Q.4	CCP Yes or No
1	2	3	4	5	6	7

The factory has identified CCPs in the following processing steps: receiving step, defrosting step, cooking step, cooling and peeling step, hand peeling step, brining step, freezing step, ice glazing step, weighing and packaging step, metal detecting step, frozen storage and temperature in processing area. In the production process, there are 12 CCPs.

e) HACCP plan
After determination of CCPs, the factory implemented following steps 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 were presented in a HACCP plan. When I visited the factory, I received an HACCP plan of four CCP in the cooking step, brining step, freezing step and for temperature in processing areas. The HACCP plan is shown in Appendix 8.

4.2 Hygienic survey

4.2.1 Environment

a) Visual inspection
Evaluation of cleaning based on three levels as below:
- 1: Good - no remarks
- 2: Fair - minor remarks
- 3: Poor - too many remarks

The results of visual inspection are shown in table 6. Almost all the equipment was cleaned carefully except the defrosting unit and grading equipment. Some debris of shrimp was found on the conveyer belt made from polyethylene (PE) of the defrosting unit and stainless steel surface of the grading equipment.

Table 6: Results of visual inspection

N°	Equipment	Areas	Surface material	Visual inspection
1	Defrosting unit	Receiving	Polyethylene	2
2	Defrosting unit		Stainless steel	1
3	Grader		Polyethylene	1
4	Grader		Stainless steel	2
5	Cooking equipment 2	Cooking	Acetal	1
6	Cooking equipment 2		Aluminum	1
7	Cooking equipment 4		Acetal	1
8	Cooking equipment 4		Stainless steel	1
9	Peeling machine (roller) 2	Peeling	Rubber with nylon	1
10	Peeling machine (flumes) 2		Stainless steel	1
11	Peeling machine (roller) 4		Rubber with nylon	1
12	Peeling machine (flumes) 4		Stainless steel	1
13	Hand peeling - belt	Hand peeling	Polyproban	1
14	Hand peeling flumes		Stainless steel	1
15	Grader after freezing	Freezing	Polyethylene	1
16	Grader after freezing		Stainless steel	1

b) Adenosine Triphosphate (ATP)

The results from ATP measurements of food contact surfaces of processing equipment are presented in Table 7.

Evaluation of cleaning is carried out as below:

- If the level of ATP is less than 100 RLU/10cm^2, surface of equipment is considered clean,
- If the level of ATP is more than 100 RLU/10cm^2, surface of equipment is considered unclean.

Level of ATP on 10 cm^2 of the swabbed equipment surfaces in areas processing products after cooking were lower than level of ATP of equipment in areas processing products before cooking. Levels of ATP on the conveyer belt surface of the defrosting unit and the conveyer belt surfaces of the cooking equipment 4 are high.

Table 7: Results from ATP measurements

N°	Sample location	Areas	Surface material	ATP (RLU/10cm^2)
1	Defrosting unit	Receiving	Polyethylene	607
2	Defrosting unit		Stainless steel	69
3	Grader		Polyethylene	13
4	Grader		Stainless steel	316
5	Cooking equipment 2	Cooking	Acetal	291
6	Cooking equipment 2		Aluminum	205
7	Cooking equipment 4		Acetal	806
8	Cooking equipment 4		Stainless steel	283
9	Peeling machine - roller 2	Peeling	Rubber with nylon	6
10	Peeling machine - flumes 2		Stainless steel	28
11	Peeling machine - roller 4		Rubber with nylon	11
12	Peeling machine - flumes 4		Stainless steel	75
13	Hand peeling - belt	Hand peeling	Polyproban	11
14	Hand peeling flumes		Stainless steel	24
15	Grader after freezing	Freezing	Polyethylene	81
16	Grader after freezing		Stainless steel	71

c) RODAC plate

Samples were taken from 16 places of eight equipments. The results from RODAC plate counts are shown in Table 8. There were 46 colonies on the plate impressed on the surface of defrosting unit. Furthermore, seven colonies were found in the plate of sample seven taken from surface of cooking equipment 4. A colony was found in the plate of sample nine taken surface of the peeling machine - roller 2. A colony of mould was found in the plate of sample 16 taken from surface of grading machine.

Table 8: Results from RODAC plate counts

N°	Sample location	Areas	Surface material	RODAC (CFU/plate)
1	Defrosting unit	Receiving	Polyethylene	46
2	Defrosting unit		Stainless steel	0
3	Grader		Polyethylene	0
4	Grader		Stainless steel	0
5	Cooking equipment 2	Cooking	Acetal	0
6	Cooking equipment 2		Aluminum	0
7	Cooking equipment 4		Acetal	7
8	Cooking equipment 4		Stainless steel	0
9	Peeling machine - roller 2	Peeling	Rubber with nylon	1
10	Peeling machine - flumes 2		Stainless steel	0
11	Peeling machine - roller 4		Rubber with nylon	0
12	Peeling machine - flumes 4		Stainless steel	0
13	Hand peeling - belt	Hand peeling	Polyproban	0
14	Hand peeling flumes		Stainless steel	0
15	Grader after freezing	Freezing	Polyethylene	0
16	Grader after freezing		Stainless steel	1 (mould)

Cleanliness of equipment surfaces is evaluated as below:

- Surfaces of equipment are considered clean, if number of colonies is less than 10 CFU/plate
- Surfaces of equipment are considered unclean, if number of colonies is more than 10 CFU/plate

These results indicate that the food contact surfaces of almost all equipment were cleaned and disinfected well except surface made of plastic of the defrosting unit.

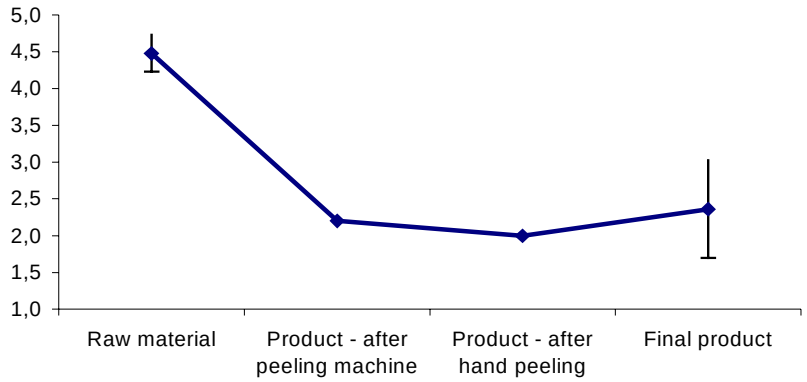
d) Listeria
Total samples taken for *Listeria* isolation were 54. The results are shown in Appendix 9. *Listeria* was isolated from 5 samples taken from outside surfaces, peeling equipment 2 and lubricant and bearing (on a wall between cooking area and peeling area, in the cooking room) after cleaning and surfaces of peeling equipment 2 and 3 in processing. The sampling places on the surfaces of peeling equipment included food contact surfaces like flumes, rollers and non-food contact surfaces.

If *Listeria* is present on the surfaces of the equipment, it indicates that the equipment is unsanitary.

4.2.2 Product samples

Product samples included raw material, semi-finished products after peeling and hand peeling and final product. Microbiological tests of product samples consisted of TPC, total coliforms, faecal coliforms and *Listeria*.

a) Total plate count
Number of bacteria in the samples is presented in the Figure 1. Bacteria in product samples decreased sharply from $3.2x10^4$ cfu/g to $15x10^1$ cfu/g from grading stage to peeling stage. Bacteria in product samples slightly decreased to $8x10^1$ cfu/g after hand



peeling. There was a slight increase of bacteria found in sample of final product.
Figure 1: Number of bacteria during processing

The total number of bacteria present on semi-finished products after cooking and final product is well under the guidelines issued by IFL (Appendix 10).

b) Total coliforms and faecal coliforms
The results from analysis of total coliforms and faecal coliforms are shown in table 9. Average number of total coliforms present on samples of raw material was 24.8 MPN/g. Average number of faecal coliforms on samples of raw material was 1.0MPN/g. Total coliforms and faecal coliforms in samples taken from semi-finished products after cooking and final products were less than 0.3MPN/g (not detected).

Table 9: Results from analysis of total coliforms and faecal coliforms

No	Samples	Total coliforms	Faecal coliforms
1	Raw material 1	46	2.3
2	Raw material 2	46	0.9
3	Raw material 3	24	0.4
4	Raw material 4	3.9	<0.3
5	Raw material 5	4.3	1.5
6	Shrimp shell 1-2	<0.3	<0.3
7	Shrimp shell 3-5	2.3	<0.3
8	After peeling machine	<0.3	<0.3
9	After hand peeling	<0.3	<0.3
10	Final product 1	<0.3	<0.3
11	Final product 2	<0.3	<0.3
12	Final product 3	<0.3	<0.3
13	Final product 4	<0.3	<0.3
14	Final product 5	<0.3	<0.3

c) Listeria

The results from analysis of samples for *Listeria* are shown in Table 10. All checked samples are reported as negative for *Listeria*.

Table 10: Results from analysis of samples for *Listeria*

No	Samples	Listeria/25g
1	Raw material 1	Negative
2	Raw material 2	Negative
3	Raw material 3	Negative
4	Raw material 4	Negative
5	Raw material 5	Negative
6	Shrimp shell 1-2	Negative
7	Shrimp shell 3-5	Negative
8	After peeling machine	Negative
9	After hand peeling	Negative
10	Final product 1	Negative
11	Final product 2	Negative
12	Final product 3	Negative
13	Final product 4	Negative
14	Final product 5	Negative

d) Temperature of products and processing areas

Temperature of samples is shown in Table 11. Raw material after defrosting was kept at 0.2 to 3.4 °C. After peeling, average temperature of samples of semi-finished products was about 16°C. Before falling to brine flumes, temperature of samples of semi-finished product was about 8°C.

Table 11: Temperature of samples

Code	Samples	T°C
1	Raw material 1	1.1
2	Raw material 2	0.2
3	Raw material 3	3.4
4	Raw material 4	0.2
5	Raw material 5	0.4
6	Shrimp shell 1-2	14.6
7	Shrimp shell 3-5	17.4
8	After peeling machine 1-2	15.5
9	After peeling machine 3-5	16.4
10	After hand peeling	8.0

The temperature of processing environment was kept below the critical limits given in the HACCP plan, i.e. that ambient temperature in hand - peeling area must be less than 18°C and temperature of the freezing, glazing, and packaging area must be less than 16°C (Table 12).

Table 12: Temperature of the processing areas after cleaning and during processing

Processing areas	Temperature after cleaning (°C)	Temperature in processing (°C)
Defrosting, grading	9	11.5
Cooking	9	10.5
Peeling	13-15	10.1
Hand - peeling	16	14.4
Freezing, glazing, packaging	16	10.3

5. DISCUSSION

5.1 The HACCP system of factory

- The HACCP program of the factory has operated really effectively because the significant hazards were identified properly and the CCPs have been under constant control by staff and machines and the performance of the control step has been monitored and documented. The factory has modified and improved their HACCP plan many times. In the beginning, they paid attention to analyse hazard only related to food safety for consumption. But their product had not met the requirements of the customer such as lack of weight and sensory quality of all product. Therefore the hazard concept was extended. Hazard relates not only to safety, but also to economic fraud and wholesomeness. After improving the HACCP plan, the product of the factory has met the requirements of the customers on safety, wholesomeness and other commercial aspects.

- The results from microbiological analyses of samples taken from final product (appendix 7) met the requirements of the guidelines issued by IFL. They showed that cooked product was safe for consumption, because the significant hazards of product were controlled and reduced to an acceptable level by the HACCP system. The HACCP program has been effectively applied in the factory.

5.2 Hygienic survey

The HACCP implementation depends on the cleaning and sanitation program in the factory. Use of ATP bioluminescence assey and microbiological tests to assess effective sanitation plays an important role. Those tests can show whether the processing equipment, utensil etc. have been kept in good hygienic condition and whether HACCP system has been operated well.

1. In my study, a comparison of results from RODAC plate count, *Listeria*, ATP measurements and visual inspection presents that:

a) In the areas after cooking, almost all equipment were considered clean since the food contact surfaces of equipment had been cleaned and disinfected properly and complied with requirements of cleaning and disinfecting procedures. But some non-food contact surfaces of the processing equipment were in unsanitary condition, possibly due to poor hygienic design of equipment.

b) In the areas before cooking, the conveyer belt surfaces of the defrosting unit and cooking equipment 4 were considered unclean. The reason was that many small gaps in the conveyer belts which are difficult to clean. This equipment was not cleaned and disinfected properly and did not comply with the requirement of the cleaning and disinfecting procedure.

c) As seen from the results shown in section 4.2.1.d. *Listeria* was found in some places in the processing equipment. When *Listeria* is present on the food contact surfaces, they indicate an unsanitary condition. The presence of *Listeria* on the food contact surfaces in the areas processing ready-to-eat products after cooking is unacceptable. Therefore, samples have been taken again from the equipment surfaces that *Listeria* was isolated from by the staff working in accredited laboratory. Sampling was conducted as follow:
   - Seven samples taken from different places on the surfaces of peeling equipment 2
   - Seven samples taken from different places on the surfaces of peeling equipment 3
   - Two samples taken from surfaces of lubricant and bearing 2 and 3 located on the wall that separates cooking room and peeling room.

   Results showed that *Listeria* was found in three places: on surface of lubricant and bearings 2 and 3 and crossbeam and cogwheel of peeling equipment 2. Those places are non-food contact surfaces. Although those places were cleaned and disinfected, *Listeria* was still found. So the factory decided to replace the type of material of crossbeam and cogwheel of the peeling equipment 2, lubricant and bearing from aluminum to stainless steel. After that, samples were taken one more time. All checked samples were reported as negative for *Listeria*. Surface of equipment that is made from aluminum was not smooth enough. Equipment was designed in an unhygienic manner. Therefore, when *Listeria* sticks to surfaces of the equipment, it forms a biofilm that is hard to remove during cleaning.

2. According to the results from microbiological analyses of sample taken from product presented in section 4.2.2 show that:

- Raw material has been contaminated with faeces of warm-blooded animals in some manner, either directly or indirectly. After cooking, number of bacteria, total coliforms and faecal coliforms had reduced sharply to an acceptable level according to the guidelines issued by IFL. At the cooking stage, vegetative cells of pathogens and other bacteria were eliminated or reduced by heat.

- There was a slight increase of bacteria found in sample from hand peeling step to packaging step. In the freezing equipment, bacteria can not grow and the product will not be contaminated by bacteria from environment if equipment is cleaned properly. So bacterial growth can be due to one of the reasons: bacteria in product multiply; bacteria can be contaminated to product from air, glazing water, brine and food contact surfaces.

- Total number of bacteria, total coliforms and faecal coliforms presented on semi-finished products after cooking and final product were well under the guidelines issued by IFL. *Listeria* was not found in the product samples. The product was kept in good hygienic condition during processing after cooking and it was strictly controlled by the HACCP program.

In Vietnam, shrimp products contribute a significant quantity of total volume of the exported seafood products. Shrimp species are black tiger, cat tiger, pink and white shrimp. The cat tiger and pink shrimp are assessed as suitable for processing cooked shrimp because of color, size and flavor. Recently, most of small shrimp have been processed as uncooked IQF and block frozen peeled shrimp. In my opinion, there will be a tendency of processors to process high value added products like cooked peeled shrimp to get more profit. In order to process this product, processors have to improve hygienic condition of infrastructure, employee, facility, equipment etc. in their factories. The implementation of HACCP and prerequisite program is necessary. In Vietnam, there are now 110 factories that have been applying HACCP effectively. Almost all those factories have identified hazards related to food safety. The results of the study of the quality management system based on HACCP in Icelandic cooked shrimp processing factory show that HACCP principles can also be applied to non-safety hazards like the prevention of economic fraud or other aspect of food quality. In any HACCP system it is very important to be able to separate hazards regarding safety from non-safety hazards like quality, net weight etc. I hope this document can provide some useful information for Vietnamese processors to know how to control hygienic condition in the factory.

6. CONCLUSIONS

- The HACCP program for the cooked shrimp has been applied effectively in the factory. The quality management program based on HACCP is a flexible system. Though HACCP is intended for the control of safety, its principles can be applied to non - safety hazards such as the prevention of economic fraud in relation to labelling, grading, weight, etc., or other aspects of food quality. In any HACCP system it is very important to be able to separate hazards regarding safety from non-safety hazards.

- Although microbial analysis may not give exact results, they can show whether the sanitation program is working to keep food products safe and equipment, utensils, floors, walls, and other item clean. The microbial analysis can be considered as a tool to help the food processors to find the reasons for unhygienic conditions in their factory.

- Cooked peeled shrimp is considered to be a high-risk product. In order to process this product, hygienic condition of the factory plays an important role. HACCP program can not be effectively applied if the hygienic condition is not in place. The factory should use ATP bioluminescence assey and microbiological tests to control and ensure effective plant sanitation practices.

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LIST OF REFERENCES

Bonnell, A.D. 1994. *Quality assurance in Seafood Processing - A practical Guide.* New York: Chapman & Hall, Inc.

Canada. Canadian Food Inspection Agency (CFIA) 2001. *Fish, seafood and production, Quality Management Program.* [7.01.2002] http://www.inspection.gc.ca/english/anima/fispoi/qmp/qmppgqe.shtml

Chesworth, N. 1997. *Food Hygiene Auditing*. London: Chapman & Hall.
Codex Alimentarius Commission - Joint FAO/WHO Food Standards Programme 1997. *Hazards analysis and critical control point (HACCP) system and guidelines for its application.* Annex to CAC/RCP 1-1969, Rev. 3 1997.

Dillon. M and Griffith. C, 1996. *How to HACCP*. 2nd ed. UK: M.D. Associates
European Union. Community Legislation in Force 1991. *Council Directive 91/493/EEC of 22 July 1991 laying down the health conditions for the production and placing on the market of fisheries products.* [16/12/2001] http://europa.eu.int/eur-lex/en/lif/dat/1991/en_391L0493.html

Eong, Y. S. and Ngei, K. T. 2000. *Proceedings of the 1st Regional Workshop on the Application of HACCP in the fish processing industry in Southeast Asia*. Singapore: Marine Fisheries Research Department.

Huss, H. H. 1994. *Assurance of seafood quality*. FAO Fisheries Technical paper 334. Rome:FAO.

Huss, H. H, Reilly, A., Embarek, P. K. B. 2000. Prevention and control of Hazards in seafood. *Food control* 11: 149 – 156.

Marriott, N., G. 1997. *Essentials of food sanitation.* New York: Chapman & Hall.

Lee, J. S. and Hilderbrand, K. S. 1992. *Hazard Analysis & Critical Control Point Application to Seafood Industry.* USA: Sea Grant Communications - Oregon State University. [12.1.2002] http://nsgd.gso.uri.edu/source/oresuh92001.pdf

Lundin, A. 1999. ATP detection of biological contamination. *30th R3 - Nordic contamination control symposium.*

Limpus, L. G. 1997. *An introduction to HACCP for fish processors.*. Singapore: Marine Fisheries Research Department.

Olsen, S. J., Mackinon, L. C., Goulding, J. S., Bean, N. H., Slutsker, L. 2000. Surveillance for Food-borne Disease Outbreaks - United States, 1993-1997. *Surveillance Summaries* 49 (SS01), 1-51. [07.01.2001] http://www.cdc.gov/mmwr/preview/nmwrhtml /ss4901a1.htm

Pierson, M. D. and Stern, N. J. 1986. *Foodborn Microorganisms and Their Toxins Developing Methodology*. United States of America: Marcel Dekker, INC.

Pierson, M. D and Corlett, D. A. 1992. *HACCP principles and Application*. New York: Van Nostrand Reihold.

United States. Food and Drug Administration (FDA) 1998. *Fish and fishery product hazards and control guide.* Part 123. Fish and fishery products, Sec. 123.12. [12/12/2000] http://seafood.ucdavis.edu/HACCP

---. National Advisory Committee on Microbiological Criteria for Foods (NACMCF) 1997. *Hazard analysis and critical control point principles and application guidelines (adopted August 14, 1997).* [12.1.2002] http://seafood.ucdavis.edu/Guidelines/nacmcf1.htm.

---. National Seafood HACCP Alliance (NSHA) 1997. *HACCP: Hazard Analysis and Critical Control Point training Curriculum*. NC State University, Raleigh: North Carolina Sea Grant. [13.1.2002] http://nsgd.gso.uri.edu/ncu/ncue98001.pdf

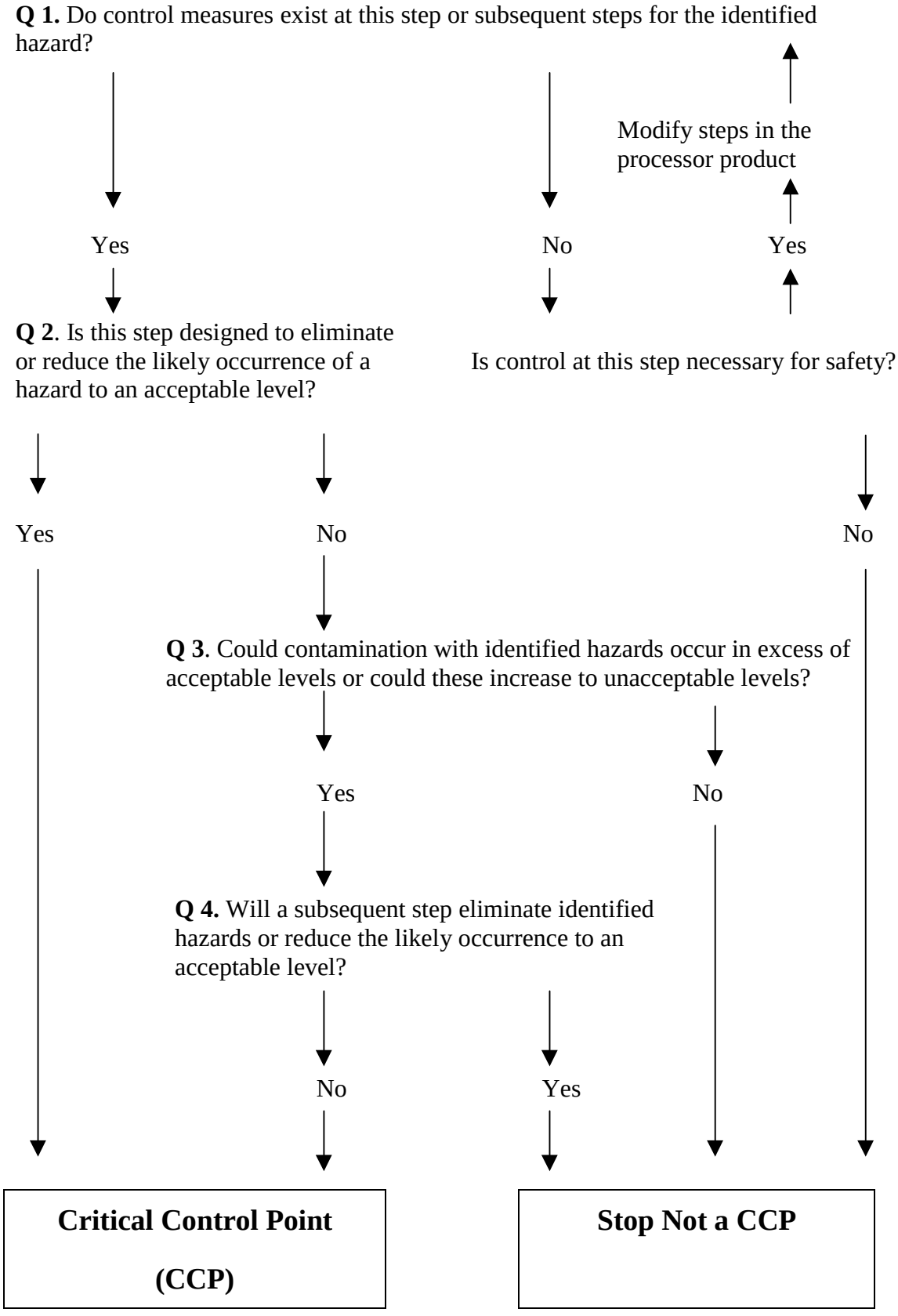
Vanderzant, C. and Splittstoesser, D. F. 1992. *Compendium of methods for the microbiological examination of food.* 3rd ed. Washington: American Public Health Association.

Vietnam. Fisheries Scientific Technological Economic Information (Fistenet) 2001. *Vietnam's fisheries export in the first 6 months of 2001*. [16.12.01] http://asemconnectvietnam.gov.vn/

---. Ministry of Fisheries (MOFI) Department of Science and Technology 2000. *Report on overview the quality and safety assurance in the Period 1996 - 2000 and the plan for the next period of 2001 - 2005*.

Wallace, C. and William, T. 2001. Pre-requisites: a help or a hindrance to HACCP? *Food control* 12: 235 – 240.

APPENDIX 1: Example of decision tree to identify CCPs



APPENDIX 2: Questionnaire

A. General information

- Main products:
- Annual volume of products:
- Main Markets:
- Total employees:
- Total workers:
- Number of shifts per day:
- Total quality controller:

B. Quality management system

1. General

- Quality policy of the factory:
- Quality manual:
- Training of staff:
- ISO standards that factory applies:
- Who inspects health condition in the factory? How often ?
- Requirements related to the products:
- Sampling finished products:
  - ✓ Criteria:
  - ✓ How many % samples of total finished products volume is taken to check:
  - ✓ Who takes samples to analysis:
- Evaluation of raw material quality:

2. Water quality monitored system

Monitoring procedure include:
- Sampling plan of water:
- Frequency of water sampling:
- Water treatment system:
- Results of analyses of water:
  - ✓ Total count at 37°C:
  - ✓ Total count at 22°C:
  - ✓ Total Coliforms:
  - ✓ Faecal Coliforms:
- Distinguishing piping for potable water from piping for non-potable water:

3. Personal hygienic standard

- Washing working clothes:
- Training on hygiene for worker:
- How are they controlled ?:
- How often is health condition of workers checked (records) ?:

APPENDIX 2 (cont.)

4. Pest control

- The documented plan for extermination of insects:
- The location and contents of rodent traps:
- Fly trap:

5. Cleaning and disinfecting system

- Cleaning and disinfecting schedules:
- Cleaning and disinfecting methods:
- Monitoring procedures (microbiological testing):
- Cleaning and disinfecting chemicals:

6. HACCP programme

- Description of the products:
- The flow- chart for cooked shrimp:
- Hazard analysis table:
- HACCP plan:

APPENDIX 3: Sampling plan

1. Products sample

No	*Samples*	*T°C*	*Listeria*	*TVC*	*Total coliforms*	*Faecal coliforms*
1	Raw material 1					
2	Raw material 2					
3	Raw material 3					
4	Raw material 4					
5	Raw material 5					
6	Shrimp shell 1-2					
7	Shrimp shell 3-5					
8	After peeling machine					
9	After hand peeling					
10	Final product 1					
11	Final product 2					
12	Final product 3					
13	Final product 4					
14	Final product 5					

2. Checking of hygienic condition of food contact surfaces

N°	*Sample location*	*Areas*	*Surface material*	*Visual inspection*	*ATP*	*RODAC*
1	Defrosting unit	Receiving				
2	Defrosting unit					
3	Grader					
4	Grader					
5	Cooking equipment 2	Cooking				
6	Cooking equipment 2					
7	Cooking equipment 4					
8	Cooking equipment 4					
9	Peeling machine - roller 2	Peeling				
10	Peeling machine - flumes 2					
11	Peeling machine - roller 4					
12	Peeling machine - flumes 4					
13	Hand peeling - belt	Hand peeling				
14	Hand peeling flumes					
15	Grader after freezing	Freezing				
16	Grader after freezing					

3. Checking of hygienic condition of the food contact surface and non-food contact surface after cleaning and in processing

No	*Sample location*	*Areas*	*Listeria*	*T°C*
1	Outer sample			
2	Outdoor tubs			
3	Wooden pallets			
4	Floor / drain (outer)			
5	Forklift			
6	Defrosting / grader			
7	Indoor tubs			

APPENDIX 3 (cont.)

No	*Sample location*	*Areas*	*Listeria*	*T°C*
8	Floor / drain (inner)			
9	Forklift			
10	Changing room			
11	Cooking equipment 1	Cooking		
12	Cooking equipment 2			
13	Cooking equipment 3			
14	Cooking equipment 4			
15	Cooking equipment 5			
16	Floor			
17	Peeling equipment 1	Peeling		
18	Peeling equipment 2			
19	Peeling equipment 3			
20	Peeling equipment 4			
21	Peeling equipment 5			
22	Floor			
23	Flumes from peeler #1 - #5 Thrasher # 1 Thrasher # 2 Conveyor belt # 1 to cleaner Conveyor belt # 2 to cleaner Cleaner Flume In-feeding conveyor to pulsar separator Pulsar separators 1	Peeling 3		
24	Conveyor belt to pump Pump to after peeler After peeler Conveyor belt to pulsar separator	Peeling 4		
25	Pulsar separator 2 After peeler from pulsar 2 Pump to pulsar 2 Flume to blow separators Blow separator # 1 Blow separator # 2	Peeling 5		
26	Drain, inspection Drain, packing Drain, freezing	2nd floor 6		
27	Inspection belt # 1 Inspection belt #2 Brine flume Brine pump Funnel In-feeding to flow freezer	2nd floor 7		
28	Ice glaze + conveyer belt Grader + conveyor belt 5 conveyors-belts from grader Scale after grader Conveyor-belt to stairway Stairway to heaven Scale	Grading 8		
29	Conveyor-belt to ice glaze Ice glaze Conveyor-belt from ice glaze Conveyor-belt to flow freezer	Repackaging 9		

APPENDIX 4: Methods for ATP measurement and the microbiological tests

1. Replicate Organism Detection and Counting Plate - RODAC Plate

(Vanderzant *et al.* 1992)

1.1. Introduction

The RODAC plate method (agar contact) provides a simple, valuable agar contact technique for estimating the sanitary quality of surfaces. The method is recommended particularly when quantitative data is sought from flat, impervious surfaces. Ideally, the RODAC plate method should be used on previously cleaned sanitised surfaces. If accurate colony counts are desired, the plates should have fewer than 200 colonies. A sufficient number of sites should be sampled to yield representative data. Normally, plate count agar or D/E neutralising agar are used for aerobic plate counts.

1.2. Procedure of test

Disposable plastic RODAC plates are filled with test medium in the laboratory. When prepared in the laboratory, the plates should be filled with 15.5 to 16.5 ml of appropriate medium. The meniscus of the agar should rise above the rim of the plate to give a slightly convex surface to be sampled. Following preparation, the plates are kept at room temperature for 18 to 24 hours as a sterility check. They should be used within 12 hr after preparation unless wrapped and refrigerated.

Remove the cover from the RODAC plate and carefully press the agar surface to the surface being sampled. The places are incubated at 22°C, for 48-72 hours. Colonies are counted using a Quebec colony counters and recorded as the number of colonies per RODAC plate.

2. ATP - Adenosine triphosphate

2.1. Introduction

Adenosine triphosphate is present in all living cells. ATP is the energy pack for all animal, vegetable, bacteria and mould cells (Chesworth 1997). Addition of the sample to an ATP reagent containing firefly luciferase results in light emission. ATP is measured by bioluminescence. Bioluminescence occurs when ATP is combined with luciferase and an enzyme derived from fireflies. The light is measured in a luminometer. The firefly ATP reaction can be summarised in the following reaction (Pierson *et al.* 1986):

- Luciferase + Luciferin + ATP $\rightarrow$ Luciferin-Luciferase-AMP + Pyrophoshate

- Luciferin-Luciferase-AMP + O_2 $\rightarrow$ Oxyluciferin + CO_2 +Luciferase + AMP + LIGHT

The amount of light output is proportional to the amount of ATP. The amount of ATP correlates to the amount of the food residue on the production surface.

To measure amount of light from firefly ATP reaction, portable Luminometer is used.

2.2. Procedure of test

Holding the bulb, remove the pre-wetted swab from the holder and swab test area. Surface areas of 10 cm^2 are sampled. Replace the swab into the swab tube until you are ready to test the sample (up to 4 hours).

Holding the swab tube, invert the device and use the thumb and forefinger of your other hand to break the snap valve by bending the bulb forward and backward. Carefully remove the swab from the swab tube. Place the swab shaft down to the bottom of a clean cuvette.

Squeeze the bulb twice to expel all the liquid. Rotate the swab in the liquid for 10 seconds, to allow any residue to be released, and remove the swab. Apply a cap and place the cuvette in a portable Luminometer and read the light output.

3. Total Plate Count (TPC) (Vanderzant *et al.* 1992)

3.1. Introduction

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.

3.2. Procedure of Pour plate method

25 g of ground sample is mixed with 225 g of dilution buffer to make a 1/10 dilution. Two Petri plates are often used for each dilution. 2x1 ml are then inoculated into the first pair of plates (1/10) and 2x0.1 ml on the next pair (1/100). Higher dilutions might be required. After inoculation melted Plate count Agar (45°C) with 0.5% NaCl is poured on the plates and content mixed. All plates are incubated inverted at 30±0.5°C for 48± 3hrs (or any other temperature required). Do not put more than 4 plates on top of each other.

3.4. Counting the plates

Colonies are usually counted over light with a double magnification in a Quebec Colony Counter. A hand tally is used. Colonies can also be counted by inverting the plates to put the plates in a right dilution order to check whether there is a normal tenfold difference between dilutions.

The following guidelines should be used for selecting plates and calculating the CFU/g, as applicable:

- Plates from the dilution showing colony numbers 25 to 250 are chosen for counting. If two plates are used per dilution count colonies on both plates, find the mean and multiply with the corresponding dilution factor.

Example: Plate pair 1/10.000, Plate 1: 220 colonies, Plate 2: 232 colonies

220+ 232 = 452: 2 = 226 x 10.000 = 2,260,000 (≈ 2,300,000) microbes per 1 g of sample.

- If no plates contain more than 25 colonies, count the number of colonies in the lowest dilution used. If no colonies are found in the lowest dilution (1/10) report results as TPC< 10cfu/g.
- If the number of colonies exceeds 250 in the highest dilution an area of 10 cm^2 is counted, divided by 10 and multiplied with the total area of the plate (56cm^2 for ordinary plastic plates).

4. Microbiological methods used for the isolation and identification of *Listeria* (Vanderzant *et al.* 1992)

4.1. Introduction

Characteristics of *Listeria* are the abilities to grow at refrigeration temperatures and to display resistance to many antibiotics. Most methods use one or more enrichment steps followed by plating onto a selective agar and is the basis for U.S. Department of Agriculture (USDA) isolation methods.

The sample is usually mixed with an enrichment broth and incubated at 30°C for 24 hrs. After incubation, a portion of the enrichment mixture is again mixed with an enrichment broth and then plated onto the final isolation agar. Enrichment broth is usually nutritious liquid media that employ various anti-microbial agents to which *Listeria monocytogenes* is resistant. The most common anti-microbial agents include nalidixic acid, acriflavin, and cycloheximide. Isolation agars include those used for direct plating, although less selective agars have also been used successfully.

4.2. Reagents

All media used were from Difco.

a) Media and reagents:

- *D/E neutralising broth*: When swabbing cleaned surfaces, the swabs are dipped in this broth in order to neutralise possible remains of chemicals from the disinfecting process.

- *University of Vermont (UVM) broth*: Primary enrichment

This medium differs from the original formula in that it contains one - haft the amount of naladixic acid.

– Proteose Peptone	5 g	- Lab Lemco Powder	5 g
– Tryptone	5 g	- Yeast Extract	5 g
– NaCL	20 g	- KH_2PO_4	1.35 g
– Na_2HPO_4	12 g	- Esculin	1 g
– Acriflavin	12 mg	- Distilled Water	1 L
– Naladixic acid (2% in 0.1 M NaOH)	1 ml		

Sterilise at 121°C, 15 minutes. Do not overheat, cool at once after removal from the steriliser. If the media blackens or darkens, it has been overheated and must be discarded. Store in the refrigerator.

- Fraser broth - secondary enrichment: This broth is identical in formula to that above except for increased acriflavin to aid in selection and the addition of lithium chloride and ferric ammonium citrate to produce a visual blackening of tubes containing esculin hydrolysing bacteria. All *Listeria* species and other bacteria that hydrolyze esculin darken or blacken this medium.
  - Proteose Peptone 5 g - Tryptone 5 g
  - Lab lemce Powder 5 g - Yeast extract 5 g
  - NaCL 20 g - KH_2PO_4 1.35 g
  - Na_2HPO_4 12 - Esculine 1 g
  - Lithium Chloride 3 g - Distilled water 1L
  - Naladixic Acid (2% in 0.1M NaOH) 1 ml

Mix well to resuspend the media and dispense 10 ml into 20x150 mm test tubes. Sterilise at 121°C, 15 minutes. Do not overheat, cool at once after removal from the steriliser. Store in the refrigerator. Just before use, add 0.1 ml of 2.5 mg/ml of filter sterilised acriflavin (Sigma) in distilled water to each 10ml tube.

- Modified Oxford medium (MOX): MOX agar is a slight modification of Oxford *Listeria* selective medium developed by Curtis et al (4). MOX Agar base:
  - Agar 2 g/l - Esculin 1 g/l
  - 1% Colistin Solution 1 ml - Distilled water 1 L
  - Ferric ammonium Citrate: 0.5 g/l
  - Lithium Chlorine (sigma L0505) 15 g/l
  - Columbia Blood Agar Base 39 - 44 g/l (depending on brand)

Re-hydrate with constant stirring with a magnetic mixer and adjust pH to 7.2 if necessary. Autoclave at 121°C for 10 minutes, and cool rapidly to 46°C in the water bath. Add 2 ml of 1% filter sterilised Moxalactam Solution to make the complete MOX medium, and pour 12 ml in each place.

4.3. Procedure of test

- To identify *Listeria* on the food contact surfaces and non-food contact surfaces, the cotton swabs are dipped in the D/E Neutralising broth and then rolled over the area. The swab is broken into the bottle. 10 ml of primary enrichment broth is poured in this bottle. After that, bottle is kept in incubator at 30°C for 24 hours.

- To identify *Listeria* in the raw material, semi-finished products and products, sample is taken in process. 25 grams of ground sample are weighed into a sterile stomacher bag and 225 ml of primary enrichment broth (UVM) is added to the bag. The mixture is stomached for 2 min and closed with a wire twist - tie with some air trapped in the bag. To guard against leakage, the sample bag is placed inside another bag or breaker during incubation at 30°C for 24 hrs.

- 0.1 ml of the UVM culture is pipetted into 10ml of Fraser´s secondary enrichment broth in tube, and those tubes are incubated at 35°C for 40 hrs. Culture tubes that remain the original straw colour are to be reported as negative for *Listeria*. If the culture tubes darkened or blacken, they will be streaked for isolation.

- Dip a sterile swab into the Fraser broth positive tubes and swab 1/2 of a MOX. With loop, streak the remainder of the MOX agar plate at a 90° angel twice as shown in Figure 2. MOX agar is incubated at 35°C for 48 hours. If there are no black colonies in the plate, they will be reported as negative for *Listeria*. Confirmation tests are made on black colonies from MOX agar. These include Gram - staining, catalase and motility.

- Species identification includes haemolysis on blood agar and testing on API *Listeria* (system for the identification of *Listeria*, bio Merieux SA/France)

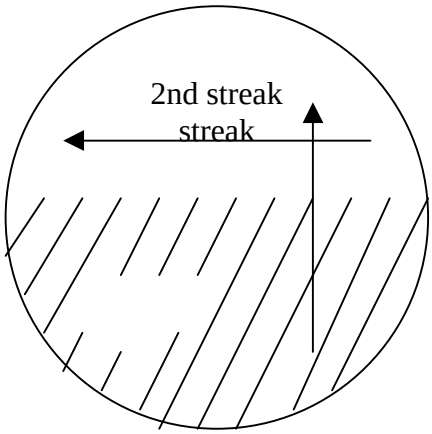


Figure 2: MOX agar plate

5. Total coliform, faecal coliforms (Vanderzant *et al.* 1992)

Methods for the examination of coliform bacteria in solid and liquid samples by the Most Probable Number (MPN) technique.

5.1. Introduction

Coliforms are those *Enterobacteriaceae* that ferment lactose and produce from it acid and gas. The methods for the examination of coliforms are based on this characteristic. Pre-enrichment is done in Lauryl Sulfate Tryptose (LST) broth, confirmed test for total coliforms in Brilliant Green Lactose Bile (BGLB) broth and confirmed test for faecal coliforms in EC broth. The selective media BGLB and EC contain bile salts and they should inhibit the growth of most other bacteria except *Enterobacteriaceae*. All media contain lactose. In all broth tubes are inverted Durham tubes and gas collects in them if lactose is fermented. Ec broth is incubated at a higher temperature than other media in order to find thermotolerant coliforms but *Escherichia coli* is by far the most common of these.

5.2. Procedure of test

a) Pre-enrichment (presumptive test):

When examining solid samples mincing and mixing 25 g of mince with 225 g of dilution buffer makes a 1/10 dilution. Three tubes are used for each dilution. Usually 3 dilutions are used, i. e. 9 tubes altogether. Ten ml of the 1/10 dilution are inoculated into 10 ml of double strength LST (equals 1 g of sample), 1ml in single strength LST (1/10 dilution, 0.1 g of sample) and finally 0.1 in single strength LST (1/100 dilution, 0.01g of sample).

After inoculation the LST tubes are incubated at 35±0.5°C. The presumptive test is considered positive if gas is formed within 48 ± 3 hrs. After 24±2 hrs the tubes are examined with regard to gas formation. Those tubes, which show at that time no sign of gas production, are incubated for another 24 hrs. Positive tubes (gas) are then used for confirmed test for total coliforms and for confirmed test for faecal coliforms. If no gas formation is observed after 48±3 hrs incubation in LST the test is considered negative, i.e. no coliforms were present in the sample. It is highly recommended that + and - controls are used for each set of samples. Then pure cultures of *Escherichia coli* (+= gas in BGLB and EC) and *Enterobacteraerogenes* (+= gas in BGLB, - in EC) are inoculated into LST and then in BGLB and EC broths.

b) Confirmed test for total coliforms.
Inoculate one loopful from all LST tubes showing sign of gas formation after 24 hrs into 10ml of BGLB broth with a loop. If appears after additional 24 hrs incubation inoculate also from these into BGLB broth. The BGLB tubes are incubated at 35±0.5°C for 48±3 hrs. If gas is produced then it has been confirmed that total coliforms were present in the sample.

c) Confirmed test for faecal coliforms.
This test is run parallel to the total coliform test. Inoculate from all LST tubes showing sign of gas formation after 24 hrs into 10ml of EC broth with loop. The EC tubes are incubated at 44.5±0.2°C in a water bath for 24±2 hrs. If gas is produced then it has been confirmed that faecal coliforms were present in the sample. Check that the water level in the bath is always above the highest level of medium.

5.3. Reading of results
Prior to examining the tubes with regard to gas formation it is in some cases necessary to lightly tap the tubes in order to detect "trapped" gas. The results are written down in appropriate lab book. The number of positive tubes within each dilution is recorded. Usually, 3 dilutions are used. Thus "triplet" is obtained and a comparable triplet is found in MPN-tables.

If the test is negative for solid foods report the result as MPN/g =<0.3 (three tube method, 1g, 0.1g, 0.01 g).

If the test is negative for liquids report the results as MPN/100ml=<2 (five tube method, 10ml, 1ml, 0.1ml).

Further information on the coliforms MPN method can be found in the following references.

APPENDIX 5: Cleaning and disinfecting agents

	Advantages	Disadvantages
Strong Alkaline (sodium hydroxide)	- they destroy microbes - they dissolve protein and fat - they disperse and emulsify soil	- they are very corrosive - It is difficult to remove by rinse - If they come in contact with skin they can cause burns, ulcers and scarring.
Chlorine based alkaline	- they are more aggressive in loosening stubborn protein-based soils or for surfaces that are difficult to clean due to their shape or size	- They are very corrosive
Quaternary Ammonium Compounds	- they do not corrosive metals; - they are stable and do not react with organic matter - they can be applied as form for visual control; - they are effective against *Listeria monocytogenes*, reduce mould growth; - they are effective for odour control;	- they do not work so well against certain bacteria - they do not kill spores but can inhibit their growth - they react with anionic - type synthetic detergents. - they form films on food - handling and food processing equipment
Phosphoric acid	- it dissolves mineral scale, they are especially good at removing mineral deposits formed by alkaline cleaning compounds. Remove material that are dried on or encrusted on surfaces. - it clean and brighten certain metal.	- concentrated phosphoric acid corrodes skin and eyes quickly

APPENDIX 6: Results from analysis of final products in 47 weeks of 2001

Weeks	TVC (CFU/g)	Total cliforms (MPN/g)	Faecal coliforms (MPN/g)	*Staph*	*Listeria*	*Salmonella*	Salt (%)
1	45	< 0.3	< 0.3	< 10	neg	neg	1.7
2	114	< 0.3	< 0.3	< 10	neg	neg	1.9
3	190	< 0.3	< 0.3	< 10	neg	neg	1.8
4	210	< 0.3	< 0.3	< 10	neg	neg	1.9
5	130	< 0.3	< 0.3	< 10	neg	neg	2.0
6	140	< 0.3	< 0.3	< 10	neg	neg	1.8
7	420	< 0.3	< 0.3	< 10	neg	neg	2.0
8	80	< 0.3	< 0.3	< 10	neg	neg	2.0
9	120	< 0.3	< 0.3	< 10	neg	neg	1.9
10	170	< 0.3	< 0.3	< 10	neg	neg	2.0
11	300	< 0.3	< 0.3	< 10	neg	neg	1.8
12	200	< 0.3	< 0.3	< 10	neg	neg	1.9
13	112	< 0.3	< 0.3	< 10	neg	neg	2.0
14	150	0.4	< 0.3	< 10	neg	neg	2.0
15	260	< 0.3	< 0.3	< 10	neg	neg	1.9
16	200	< 0.3	< 0.3	< 10	neg	neg	1.9
17	100	< 0.3	< 0.3	< 10	neg	neg	1.9
18	100	< 0.3	< 0.3	< 10	neg	neg	2.1
19	220	< 0.3	< 0.3	< 10	neg	neg	2.2
20	150	< 0.3	< 0.3	< 10	neg	neg	2.2
21	330	< 0.3	< 0.3	< 10	neg	neg	2.2
22	240	< 0.3	< 0.3	< 10	neg	neg	1.8
23	400	< 0.3	< 0.3	< 10	neg	neg	1.9
24	380	< 0.3	< 0.3	< 10	neg	neg	1.9
25	360	< 0.3	< 0.3	< 10	neg	neg	1.8
26	370	< 0.3	< 0.3	< 10	neg	neg	1.9
27	680	< 0.3	< 0.3	< 10	neg	neg	1.9
28	440	< 0.3	< 0.3	< 10	neg	neg	2.0
29	494	< 0.3	< 0.3	< 10	neg	neg	2.0
30	598	< 0.3	< 0.3	< 10	neg	neg	2.0
31	555	< 0.3	< 0.3	< 10	neg	neg	1.9
32	0	0	0	0	0	0	0
33	266	< 0.3	< 0.3	< 10	neg	neg	2.1
34	254	< 0.3	< 0.3	< 10	neg	neg	1.7
35	490	< 0.3	< 0.3	< 10	neg	neg	1.9
36	420	< 0.3	< 0.3	< 10	neg	neg	1.9
37	458	< 0.3	< 0.3	< 10	neg	neg	1.9
38	638	< 0.3	< 0.3	< 10	neg	neg	2.4
39	448	< 0.3	< 0.3	< 10	neg	neg	1.9
40	604	< 0.3	< 0.3	< 10	neg	neg	2.1
41	478	< 0.3	< 0.3	< 10	neg	neg	2.0
42	464	< 0.3	< 0.3	< 10	neg	neg	1.9
43	528	< 0.3	< 0.3	< 10	neg	neg	1.9
44	654	< 0.3	< 0.3	< 10	neg	neg	2.0
45	670	< 0.3	< 0.3	< 10	neg	neg	2.0
46	380	< 0.3	< 0.3	< 10	neg	neg	2.0
47	338	< 0.3	< 0.3	< 10	neg	neg	1.9

APPENDIX 7: Quality Index method (QIM) scheme for peeled shrimp

Quality parameter		Description	Score
Odour	***Odour of peeled shrimp***	Fresh, sea	0
		None	1
		Hint of ammonia	2
		Strong ammonia	3
Colour	***Colour of peeled shrimp***	Pink/red stripes	0
		Pink	1
		Yellowish	2
Flavour	***Flavour of peeled shrimp***	Sweet fresh shrimp flavour	0
		Faint shrimp flavour, neutral	1
		Hint of spoilage, bitter aftertaste	2
		Obvious spoilage, bitter aftertaste	3
Texture	***Springiness***	Springy	0
		Not springy	1
	Juiciness	Juicy	0
		Not juicy	1
	Crumbleness	Does not crumble when chewed	0
		crumbles	1
	Toughness	Tough	0
		Tender	1
	Chewiness	Meaty	0
		Not meaty	1
Quality Index			**0-13**

APPENDIX 8: HACCP plan

Standard	Control measure	Monitoring procedure				Corrective action
		What	How	Frequency	Who	
Law and regulations on production of seafood Own check system quality manual "Code of practice" Specification	Premises/ equipment/ inspection system	Premises Equipment Quality system	Inspection	2 times / year	Directorate of Fisheries	Document and conclude with action plan including date
	Calibration - Icelandic bureau of Metrology Calibration	Scale according to regulation scale. thermometer	General audit by official body Acc. To written description	At least 1 time /18 months Daily	Weights / measure QA	
	Cleaning schedules/ - checks	Inspection, premises / equipment	Visual inspection Swabs: listeria Product: specification	At start of production 1 time/ month Daily/weekly	QA Icelandic fisheries laboratory	
	Hygiene / personal conduct	General rules	General observation	Continuously	Foremen in each area, QA	
	Glass / inspection policy	Glass and brittle plastic	Visual inspection	Daily	QA	
	Training	All general staff management	Basic hygiene training specialise training	Within 12 months from start At start of work	Production manager QA Production manager	
	Medical screening	All staff All staff and guest	Inspection Check list	At start of work Return from work after holiday (1 time / year) guests	Doctor Production manager	
	Pest control	Pest control prem. Traps/ insects.	Inspection Inspection	Premises 2/ year Baits2 /month	Pest controller QA Directorate and QA	
	Air condition	System sieves	Replace	Agreement with supplier	QA/ engineer	
	Cold stores/ chillers	Temperature	Documentation	2 time/day Continuously	QA Engineer	

APPENDIX 8 (cont.): CCP at the Cooking - peeling step

CCP	Risk	Risk Prevention	Limits	Control	Corrective actions	Documentation
Cooking Peeling	+ Contamination comes through with product because of under cooking + Over cooked products - poor sensory score	+ Raw material is cooked according to standards. + Only experienced or good training staff member controls the peeling machine and cooking machine. + Constant contact between the staff member working at peeling area and cooking area + Constant evaluation on cooked shrimp and peeled shrimp + Machinist taking care of steamer. When steam pressure drops, bell will ring	+ The following correlation of shrimp core temperature of and cooking time supposes to kill 1000 *Listeria*/g of shrimp: 32.5 seconds - 72°C 23.8 seconds - 73°C 17.5 seconds - 74°C 12.9 seconds - 75°C 9.5 seconds - 76°C 6.9 seconds - 77°C 5.2 seconds - 78°C 3.8 seconds - 79°C 2.8 seconds - 80°C 2.0 seconds - 81°C 1.5 seconds - 82°C The aim is to reach core temperature of shrimp 79°C and maintain for 3.8 seconds + It should be taken care so that the shrimp is not over cooked + Steaming temperature is at 100°C. + The speed of conveyer belt running through the steamer depends on the shrimp size. The steam pressure should be at least 5 bar in the steam pipes. Bell will ring if pressure is less than 4.5 bar	+ The core temperature is measured with small thermometer into the biggest shrimp. It goes through cooker. Control of shrimp core temperature is carried out work starts and repeated it 3 time over the day. + Thus graph is obtained that shows how the temperature increase with time and how long the core temperature of shrimp is maintained at 79°C. + The core temperature of the biggest shrimp shall also be measured when shrimp falls from cooker to peeler at the same time as core temperature measurement is done. This is done to confirm that core temperature measurement during cooking it correct. + Steam temperature, pressure and the speed of conveyer belt though cooker is checked and documented. These things are adjusted according to size of shrimp 1/hour at the same time of core temperature is measured. + Control on the steam boiler, team pressure is documented 3/day	+ If the core temperature of shrimp is too low or the time is too short, the speed of conveyer belt though cooker will be slowed down. Then the core temperature measurement will be repeated to confirm that the speed of conveyor belt is correct. + If shrimp is uncooked, cooking process will be stopped and gone over and adjusted. Uncooked shrimp will be destroyed. Machine will be cleaned before operating again. + If correlation between the core temperature of shrimp and cooking time show that shrimp is over cooked, the speed of the conveyor belt will be increased and core temperature will be measured again. + Unsafe steamer will be documented and checked by foreman	10. Measurement of core temperature of shrimp at the end of cooking time. Person responsible for peeler 11. Core temperature measurement in cooker. Person responsible of cooker Cooker measurements - foreman + Registration in machine book - machinist is in charge of it 11. Core temperature measurement in cooker Person responsible for cooker

APPENDIX 8 (cont.) CCP at the Freezing step

CCP	Risk	Risk Prevention	Limits	Control	Corrective actions	Documentation
IQF	+ Wrong temperature + Too much products entering to freezer	+ Quantity of shrimp though freezer is in accordance with freezer capacity. +Constant control of temperature in freezer. If temperature is outside limit, bell will ring + IQF is maintained with regular checks	- Air temperature < -22°C core temperature of shrimp <-18°C - Check 1/week - Capacity 1,200 kg/hour	- Temperature in IQF is measured 1/hour. - Core temperature of shrimp after freezing is measured 1/hour. - Maintainer checks the condition of the IQF and informs foremen immediately about some suspicious that IQF is not working properly. - Good connection between persons being in charge of cooker and peeler, quality controller and foreman in order to maintain right balance each step in the process.	- If fault is found in IQF, workmen being responsible for IQF have to call foreman to slow down production process - If fault is found, temperature of shrimp <-16°C, the workmen being responsible for IQF have to call a repairman to stop processing until IQF is in order. - Slow down processing	04. Temperature measurement in processing line - Quality controller and foreman + Confirmed in maintenance book - machinist.

APPENDIX 8 (cont.) CCP for Temperature in production areas

CCP	Risk	Risk Prevention	Limits	Control	Corrective actions	Documentation
Tempe rature In produc -tion areas	Production temperature is too high , product core temperature is too high	+ Control ambient temperature. + Control brine temperature. + Control core temperature of products + Control temperature of cold water using for glazing.	+ Ambient temperature: - Hand peeling area < 18°C - Packaging area < 16°C - IQF < -24°C + Brine: - In order -1°C to 2°C - Warning: 2°C to 4°C - Critical point: >4°C + Core temperature in the shrimp: - Before brining : <8°C - After brining : < 4°C - After IQF. < -18°C - After glazing : < -16°C - After sorting/grading: <-18°C + Max: 4°C	+ Check ambient temperature in the following areas: hand-peeling area, packaging areas 1, 2, IQF. + Check brine temperature 1/hour in two places: - Hand-peeling, - Brine container + Core temperature of shrimp is measures in process as the following places: - Hand - peeling - After inspection belt - Belt before freezing - After freezing - After glazing - Sorting/grading + Check 1/hour	+ Inform foreman if ambient temperature is too high. He will adjust air condition if ambient temperature exceed limit. + If brine temperature is more than 2°C, the brine cooling equipment will be adjusted to decrease brine temperature. If temperature can not decrease, speed of production line will be slowed down. - If brine temperature is still more than 2°C, the brine cooling equipment will be checked. - If brine temperature is more than 4°C, production line will be stopped immediately and equipment will be repaired. + Check for results for high core temperature how to improve it. + Machinist informs to correct immediately	04. Temperature measurement in processing line. Quality controller

APPENDIX 8 (cont.) CCP at the brining step

CCP	Risk	Risk Prevention	Limits	Control	Corrective actions	Documentation
Brine tubs	+ Incorrect salt tolerance	+ Exact description how to make brine solution.	+ 1.5% - 2.2% brine concentration	+ Control 1/hour with brine concentration or salt concentration in products.	+ Brine concentration corrected immediately. Most recent products are taken aside and re-evaluated with regard to salt percent.	05. Salt measurement - quality controller is responsible Test report on microbiological condition and salt content.
	+ Insufficient refrigeration	+ Brine tubs with cooling element. Keep brine cool enough. Regular control on the cooling of brine by thermometer	+ In order : -1 °C to 2°C - Warning : 2°C to 4°C - Critical point : >4°C	+ Check brine temperature 1/hour	+ If brine temperature is more than 2°C inform foremen to look for the reason. + If brine temperature is more than 4°C processing line is stopped and equipment is repaired.	04. Temperature measurement in processing line. Quality controller

APPENDIX 9: Results from analysis of samples to identify *Listeria*

1. Results from analysis of sample taken from the surfaces of the processing equipment and some place after cleaning

No	*Sample location*	*Areas*	*Listeria*	*T°C*
1	**Outside surface**		**Positive**	
2	Outdoor tubs		Negative	
3	Wooden pallets		Negative	
4	Floor / drain (outer)		Negative	
5	Forklift		Negative	
6	Defrosting / grader		Negative	
7	Indoor tubs		Negative	
8	Floor / drain (inner)		Negative	9°C
9	Forklift		Negative	
10	Changing room		Negative	
11	Cooking equipment 1	Cooking	Negative	
12	Cooking equipment 2		Negative	
13	Cooking equipment 3		Negative	
14	Cooking equipment 4		Negative	
15	Cooking equipment 5		Negative	
16	Floor		Negative	
17	Peeling equipment 1	Peeling	Negative	13-15°C
18	**Peeling equipment 2**		**Positive**	
19	Peeling equipment 3		Negative	
20	Peeling equipment 4		Negative	
21	Peeling equipment 5		Negative	
22	Floor		Negative	
23	Flumes from peeler 1 - 5 Thrasher 1 and 2 Conveyor belt 1 and 2 to cleaner Conveyor belt # 2 to cleaner Cleaner, flume In-feeding conveyor to pulsar separator Pulsar separators 1	Peeling 3	Negative	
24	Conveyor belt to pump Pump to after peeler , after peeler Conveyor belt to pulsar separator	Peeling 4	Negative	
25	Pulsar separator 2 After peeler from pulsar 2 Pump to pulsar 2 Flume to blow separators Blow separator 1 and 2	Peeling 5		
26	Drain in inspection, freezing and packing areas	2nd floor 6	Negative	
27	Inspection belt # 1 and #2 Brine flume, Brine pump Funnel In-feeding to flow freezer	2nd floor 7	Negative	16°C
28	Ice glaze + conveyer belt Grader + conveyor belt 5 conveyors-belts from grader Scale after grader Conveyor-belt to stairway Stairway to heaven and Scale	Grading 8	Negative	16°C
29	Conveyor-belt to ice glaze, ice glaze Conveyor-belt from ice glaze Conveyor-belt to flow freezer	Repackaging 9	Negative	

APPENDIX 9 (cont.)

2. Results from analysis of sample taken from the surface of the equipment and other place in processing

No	*Sample location*	*Areas*	*Listeria*	*T°C*
30	Floor / drain (outer)		Negative	11.5
31	Forklift		Negative	
32	Defrosting		Negative	
33	Grader		Negative	
34	Floor / drain (inner)		Negative	11.5
35	Forklift		Negative	
36	Cooking equipment 1	Cooking	Negative	10.5
37	Cooking equipment 2		Negative	
38	Cooking equipment 3		Negative	
39	Cooking equipment 4		Negative	
40	Cooking equipment 5		Negative	
41	Floor		Negative	
42	Peeling equipment 1	Peeling	Negative	10.1
43	**Peeling equipment 2**		**Positive**	
44	**Peeling equipment 3**		**Positive**	
45	Peeling equipment 4		Negative	
46	Peeling equipment 5		Negative	
47	Flumes from peeler #1 - #5 Thrasher # 1 and # 2 Conveyor belt 1and 2 to cleaner Cleaner, flume In-feeding conveyor to pulsar separator, pulsar separators 1	Peeling 3	Negative	
48	Conveyor belt to pump Pump to after peeler After peeler Conveyor belt to pulsar separator	Peeling 4	Negative	10.1
49	Pulsar separator 2 After peeler from pulsar 2 Pump to pulsar 2 Flume to blow separators Blow separator 1 and 2	Peeling 5	Negative	
50	Drain, inspection Drain, packing Drain, freezing	2nd floor 6	Negative	14.4
51	Inspection belt 1 and 2 Brine flume, brine pump and funnel In-feeding to flow freezer	2nd floor 7	Negative	
52	Ice glaze + conveyer belt Grader + conveyor belt 5 conveyors-belts from grader Scale after grader Conveyor-belt to stairway Stairway to heaven Scale	Grading 8	Negative	10.3
53	Conveyor-belt to ice glaze Ice glaze Conveyor-belt from ice glaze Conveyor-belt to flow freezer	Repackaging 9	Negative	
54	**Lubricant and bearing**	**Cooking**	**Positive**	**10.5**

APPENDIX 10: Microbiological guidelines for cooked, frozen shrimp and frozen scallops issued by IFL

The guidelines used at our institute are as follows:

	Good	**Fair**	**Poor**
Plate count/g, 35°C	<20.000	20.000-50.000	>50.000
Plate count/g, 30°C	<50.000	50.000-100.000	>100.000
Plate count/g, 22°C	<100.000	100.000-250.000	>250.000
Total coliforms, MPN/g	<10	10 - 100	>100
Faecal coliforms, MPN/g	<0.3	0.3 - 1	>1
Staphylococcus aureus/g	<10	10 - 50	>50
Listeria in 25 g	Absent		Present
Salmonella in 25 g	Absent		Present