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Thesis for the Degree of Master of Fisheries Science

Hygienic Analysis on Surimi Processing Plant in Busan

By

Yin Pa Pa Tun

KOICA-PKNU International Graduate Program of Fisheries Science

The Graduate School

Pukyong National University

February, 2012

Hygienic Analysis on Surimi Processing Plant in Busan

부산에서 제조된 연육에 관한 위생적 분석 연구

Advisor: Professor Ji-Young Yang

by

Yin Pa Pa Tun

A thesis submitted in partial fulfillment of the requirements for the degree of

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Hygienic Analysis on Surimi Processing Plant in Busan

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Abstract

The hygienic status of the surimi processing environment is a significant factor in the production of microbiologically safe and quality products in the industry. Quality of surimi products and the species type of microbial flora are the main factors for evaluating the hygienic status of surimi processing plant. To analyze the hygienic status of surimi products, the total number of microorganism and its species were investigated. Experimental surimi samples were collected from each place through the production line to

examine the microorganism growth in each stage of production. Swab method was also used to examine the presence of microorganism in the equipment facilities and surrounding environment. The final products of surimi imported from other countries were also examined by collecting the sample randomly from the importing surimi company. To examine the number of microorganism, and their species, microorganisms were cultivated by using 3M Petrifilm for counting the total number of microorganism and pathogenic bacteria species such as *E. coli, Staphylococcus*, and *Listeria monocytogenes*. The total numbers of microorganism found from the processing plant were much more than the limitation standard for all the stages from raw material to final products. Pathogenic bacteria were also found for the stage of mince fish, first flesh wash and pasteurization, but the number was reduced from stage to stage down the processing line. The total of microbial load found from the equipment tools by using swab method was higher than the limitation standard and *E. coli* was also found from a worker's hand but the microbial load was lower than the limitation standard. For the imported surimi product, the total microbial load in some samples was equal to the limitation standard whilst in other samples it was lower than the limitation standard. The microbial load from the imported American surimi product was completely

lower than the limitation standard as well as the coliform bacteria also found in those products.

1. Introduction

Fish and fishery products are primarily the source of animal protein to promote national food security and constitute the international trade and foreign exchange earner of countries in the world. Therefore, maintenance of the quality is of utmost importance in the production and trade of fishery products. Hazard analysis critical control point (HACCP) has been recognized internationally as a logical tool for adapting traditional inspection methods to a modern and science-based food safety system. Based on risk-assessment, HACCP plans allow both industry and government to allocate their resources efficiently in establishing and auditing safe food production practices.

The three major parts of biological, chemical and physical hazards that are connected with fish and fishery products are related to the environment where fish are captured. Moreover fish like any other proteinaceous food may have contamination occurred during processing and pathogen growth may also take place in products. The HACCP concept is used to identify microbiological vulnerable points in the food production process and

production to determine the most appropriate methods of control to be applied. Usually such as improved handling technique, temperature monitoring is more intensive supervision (Edema & Omemu, 2004). The major goal of fish industries is to provide safe, wholesome and acceptable food to consumer and to control microorganism which is essential to meet hygiene analysis (Baggen et al., 2003). A food-borne outbreak is normally defined as the occurrence of the cases of similar illness resulting in the ingestion of insufficiently heat-treated fish or fishery products contaminated during the processing.

Cases of the seafood outbreaks (food borne disease) are generally smaller than food-borne case by poultry, dairy and meat products. However, fish products are very prone to degradation (Liston, 1982). Unlike other animal products, fish quality is often more difficult to be controlled due to variation in species, sex, age, habitats and action of autolytic enzymes as well as hydrolytic enzymes of microorganism on the fish muscle (Venugopal, 2002). Fish has high water content, and also there are some bacteria which are living at low temperature. High enzymatic activity in fish is responsible of susceptibility of fish muscle.

Seafood as vehicle for food-borne disease depends on a number of factors such as diet of population and traditional method of preparing food. Therefore, processors of fish and fishery products might be sources of microbial inoculation, microbial food poison, food intoxication and food spoilage. Hence, the processors may be counterproductive by being responsible for public health hazard and loss of revenue.

2. Objectives

The objectives of study were to identify the contaminating microorganisms through the production line of surimi product, to make comparisons of a number of microorganisms on surimi domestic product with surimi imported products from other countries, and then to give suggestion and recommendation to the surimi companies on critical control point through the production line to fulfill good hygiene practices (GHP), good manufacturing practices (GMP) and hazard analysis critical control point (HACCP).

3. Literature Review

3.1. General introduction of surimi

Fish paste products have long been a part of traditional Asian baking, but surimi has become the popular energetic commodities in Asia seafood industry because of the latest design in production and utilization. The process for the production of surimi was developed in many area of East Asia over 900 years ago. Surimi is an intermediate product from minced fish meat that has been washed, refined, and mixed with cryo-protectants. While fish paste products have been hand-made for centuries, a process for frozen surimi, invented in 1960, has provided the impetus for expanding the industry and surimi markets based on Walleye Pollock and Alaska Pollock resources. The Asian surimi industry was under-going a period of rapid change as the Republic of Korea, Thailand, New Zealand and United States were increasingly challenging Japan's position as the world's leading surimi producer.

The Korean surimi industry shows the greatest potential for independent growth among, the Asian surimi producers, with an output of 60,000 MT in

1989. As a major surimi producer, in Korea does not depend with the Japanese technical assistance as other countries. Thailand and New Zealand industries also show considerable growth potential, but they are at present dependent upon Japanese technical assistance. Quality and adequate raw material resources are the primary obstacles facing these three up and coming surimi producers.

In this study, contaminations at starting point of production, as well as the quality assurance systems in place are used to ensure the safety of products for human consumption. Also, it is needed to apply safety management systems that ensure food safety through the implementation, validation and verification of hazard analysis and critical control point (HACCP) - based systems.

Outbreaks usually occur due to the ingestion of inadequate heat-treated fish or fishery products which are contaminated during their processing. For frozen fish and related products in the seawater, intensive handling, longtime transport or cooking in fish containers on deck contribute to their contamination with microorganisms (Aberoumand, 2010). Temperature and

pH are limiting factors for the survival of bacteria in fishery products. So they can be used during the processes of pasteurization and heat treatment particularly of offal.

Microorganisms differ in their reaction to freezing. Some survive unharmed, while others are sensitive to freezing and freezer storage, but most of microorganisms are killed in the temperature range from 2-10^{o}C (Aberoumand, 2010). Two different toxins are involved in the microorganism of food-borne illnesses: one is a heat-stable peptide that causes nausea and vomiting (emetic type) within 0.5 to 6 hours after consuming the contaminated food; the other type of toxin is a large molecular protein that causes watery diarrhea and abdominal cramps (diarrheal type) within 6 to 15 hours after eating the contaminated food. The emetic type is more severe and acute than the diarrheal type. The latter is mild and of shorter duration, lasting from 6 to 16 hours. The emetic type could last upto 24 hours (Leon et al., 2003).

Contamination is a very important aspect in the method of production as most unwanted microorganisms are transmitted onto seafood and other food

products during production. It is possible that microorganisms can enter into food processing environments through raw material personnel or dynamic equipment such as forklifts, through leakage and openings in buildings or through pests. Some pathogens may grow in the processing plant and from niches where they can survive for long generation of time (Reij et al., 2003). Different kinds of microorganisms grow naturally in aquatic and general environments and they may be infecting seafood before capture, during and after processing. Moreover contamination can occur through dust particles or via aerosols which are formed especially when contaminated surfaces, floors or drains are splashed with high pressure-jets, resulting in formation of droplets that can be suspended in the air (Aantrekker et al., 2003). Water is also a main source for transmission of many agents of diseases (Kirby et al., 2003).

Successful sanitation and hygiene control were critical to avoid the adverse human health and economical loss of food borne illness, food borne injury and food spoilage. Everybody, including farmers and growers, manufacturers and processors, food handlers and consumers, has a responsibility to ensure that food is safe and suitable for consumption.

The general principle of HACCP lays a secure base for ensuring food hygiene and it should be used in conjunction with each precise legislation of hygienic practice, where suitable, and the guidelines on microbiological criteria. The document follows the food chain from initial production to final consumption in order to emphasize the basic control at each stage. HACCP is a key approach wherever possible to develop food safety as specified in hazard analysis critical control Point system and guideline for its aplication.

3.2. Indication on locate and transmit of contamination

3.2.1. Raw material

Many pathogenic bacteria are naturally present in aquatic environments (*Clostridium botulinum* type E, pathogenic *Vibrio sp*., *Aeromonas*) and the general environment (*C.botulinum* type A and B, *Listeria monocytogenes*) (Huss et al., 2000). Other pathogenic microorganisms have their origin in human and animal activity (*Salmonella, Shigella, E. coli* and enteric virus) (Huss et al., 2000).

Different kinds of fish species are used for surimi production from cold

water white fish like Alaska Pollock and tropical species such as threadfin bream (Guenneugues & Morrissey, 2005) to aquaculture species like Chinese carp (Shaviklo, 2000). When marine fish is used as the raw material the quality of fish is not easy to control due to variation in species, sex, age, habitats and action of autolytic enzymes as well as hydrolytic enzymes of microorganism on the fish muscles (Venugopal, 2002). In general, a healthy fish is hygienic as its immune system prevents bacteria to proliferate easily whereas after death the fish immune system collapses allowing easy access of microorganisms into the flesh (Huss, 1995).

The use of decomposed fish as raw material for surimi production, results in bad sensory qualities of finished products. This is because proliferation of spoilage bacteria that cause decomposition of the final products will have a negative effect on the gel-forming ability and denature the salt-soluble protein (CAC/RCP 1-1969, Rev. 4-2003. Recommended International Code of Practice General Principle of Food Hygiene). When raw material of fish is decomposed or tainted, there must be equipment and procedures in place to identify, separate and remove all unacceptable products from the processing environment immediately. Harvesting fish intended for frozen

surimi processing should be preferably kept and also the temperature should be controlled for maintaining its quality. Consideration should be given to the age and condition of fish used for surimi processing. As several factor will affect the final gel strength capability. Instant care should be taken with raw fish received after many hours after harvest. For example, an acceptable period after harvest should be followed, but processing as fast as possible after harvest will better to retain adequate quality of frozen surimi. Presence of decomposition in raw material should not be allowed as it will negatively affect the gel strength capability of final product.

Cold storage period at the processing facility should be reduced with prompt processing in order to minimize protein denaturation and loss of gel strength capability. Unclean, insufficiently or inadequately cleaned processing equipment have been identified as a source of bacterial contamination in processed products (Reij et al., 2003). In addition to the general equipment used to cook, heat treatment, cooling, storage or freezing systems should be designed to achieve the required food temperatures as rapidly as necessary in the interests of food safety and suitability, and to maintain them effectively (CAC/RCP 1-1969, Rev. 4-2003).

3.2.2. Personal hygiene

Personal hygiene facilities should be available to ensure that an appropriate degree of personal hygiene can be maintained to avoid contaminating bacteria into foods. Appropriate facilities should include hygienically washing and drying hands, including washing basins and a supply of hot and cold water. Medical examination of food handlers should be carried out if clinically or epidemiologically indicated. Food handlers need to maintain a high degree of personal cleanliness and to wear suitable protective clothing, head covering and foot wear. All personal should be aware of their role and responsibility in protecting fish or shellfish from contamination and deterioration. Producers should have the necessary knowledge and skill to enable them handles fish or shellfish hygienically. Those who handle strong cleaning chemicals or other potentially hazardous chemical should be instructed in safe handling techniques (CAC/RCP 52-2003).

During the handling and preparation, bacteria are transferred from contaminated hands of food workers to food and subsequently to other surfaces (Montville et al., 2002). Low infection dose of bacteria such as

Shingella and pathogenic *E. coli* has been linked to hands as a source of contamination (Snyder, 1998). Poor handing of hands can lead to damage to fish and fish products that can accelerate the rate of decomposition and increase unnecessary post-harvest losses.

3.2.3. Pest control system

Pests present an important intimidation to the safety and suitability of food. Pest infestation can appear where there are breeding sites and a supply of food. Good hygiene practice might be employed to forestall creating an environment helpful to pests. Good sanitation, inspection of incoming materials and good monitoring can minimize the infestation and limit the need for pesticides. (CAC/RCP 1-1969, Rev. 4-2003).

3.2.4. Processing equipment

Equipment and containers for storing foods should be designed and constructed to ensure that they can be adequately cleaned, disinfected and maintained to avoid the contamination of food. Equipment and container should be made of materials with no toxic effect in intended use. Where

necessary, equipment should be movable or capable of being disassembled to allow for maintenance, cleaning, disinfection, monitoring and to facilitate inspection for pests.

3.2.5. Water

Every processing step should be supplied with manageable water which comes from a source of protection from human or environment contamination because municipal water supply is acceptable without further treatment. The source of water can be exposed to human or environmental contamination such as chlorination or another equivalent treatment. Water is essential for seafood processing establishment that is used for good hygiene, especially personal hygiene, raw material and the production of safe products as in ingredient in order to clean and sanitize conveying or transportation machinery. Water treatment (such as chlorinating system, filtration, etc.) must be maintained and adequate water temperature and pressure are to be provided in processing areas to avoid backing of the soil as in general, higher temperature results in increased inactivation rate (Sobsey, 1989). Water also should be monitored, starting from the source through treatment, distribution and storage within the factory, to ensure that

the water complies with internal or legislative standards (Kirby et al., 2003). Water recirculated for reuse should be treated and maintained in such a condition that no risk to the safety and suitability of food results from its use. The treatment process should be effectively monitored. Recirculated water that has received no further treatment and water recovered from processing of food by evaporation or drying may be used, providing that its use does not constitute a risk to the safety and suitability of food.

3.2.6. Washing technique in surimi process

In surimi processing plant, washing is necessary to remove contaminations from raw fish and it leaves the surface of fish in a suitable condition for the further processing. Fish should be washed as soon as possible after unloading from the trucks to remove the agents which are contaminants from harvesting areas to prevent subsequent loss of the remaining bulk from microbial growth during storage prior to processing (FAO, 1998). In processing as in most of the seafood industry, a lot of evidence indicates that the sanitary conditions of plants correlate well with the microbial quality of finished products (Dunsmore et al., 1980).

In surimi processing plant, the amount of water required for washing will be ten to twenty times from the amount of mince, in shore-based units. The final intermediate dewatering is carried out to keep the moisture content around 90%. Refining of the partially dewatered and leached mince is carried out to remove the connective tissues, skin, scale or any undesirable inclusions using a strainer. Then, refining is done prior to final dewatering.

3.2.7. Meat separation process

In surimi processing, flesh fish is minced using a mechanical separation process. Metal detection equipment is capable of sensing the product which has become contaminated with metal fragment of the size likely to cause human injury. It should be installed at the most appropriate place in the process in order to eliminate the hazard. Separated minced meat should be immediately spread into water and transferred to the washing and dewatering step to prevent blood from congealing and causing loss of gel strength capability (CAC/RCP 52-2003).

3.3. Pasteurization

Pasteurization is a mild or moderate heat treatment with subsequent cooling. The purpose is to eliminate targeted bacterial pathogens and greatly reduce spoilage bacteria in surimi seafood, in order to improve quality of product (Himelbloom et al., 2000). Properly designed pasteurization of seafood should result in a negligible microbial load of vegetative bacteria (FDA 1998). Surimi is a myofibrillar fish protein (mixed with cryoprotectants) manufactured through mincing fillets followed by sequential washings with water to remove impurities. The United States Department of Commerce guidelines for pasteurization of the PUFI (Packed Under Federal Inspection) or FDA's HACCP for vacuum-packed surimi seafood recommends 85°C (internal temperature) for 15 min followed by fast chilling. A water-phase salt level of 2.4% or storage temperature below 3.3°C was also recommended to control *Clostridium botulinum*. Microbiological hazards during surimi seafood processing and storage may include pathogen survival, post-processing pathogen contamination, and growth of *C. botulinum* during storage in vacuum-packed surimi seafood products. Therefore, proper pasteurization, cooling, and storage have been suggested as critical control points for surimi seafood manufacture (Himelbloom et al., 2000).

3.4. Manufacturing of surimi processing

Surimi is minced fish in which all water-soluble protein in fish has been washed out and thus, it contains only 15-16% water-insoluble protein, 75% moisture and 8-9% freezing stabilizers. The water-insoluble proteins are elastic and make it feasible to form surimi in fish cake and crab sticks through further processing. The freezing decreases elasticity, so the condition for stabilization of surimi quality during long periods of frozen storage, should not be more than 1 year (Shaviklo, 2000).

Surimi processing consists of several steps that are described in Fig. 1, because surimi production of raw material should be fresh and kept chilled. Fish flesh is separated from the bones and skin using a fish bone separator machine. This should be done at a low temperature to minimize the deleterious effect of frictional heat on the product. The most important step of surimi processing to ensure maximum gelling, as well as colorless and odorless surimi, is efficient washing. The leaching process involves mixing mince-meat with cold water and removing water by screening and dehydration. This process is usually repeated three times. Before the final dewatering

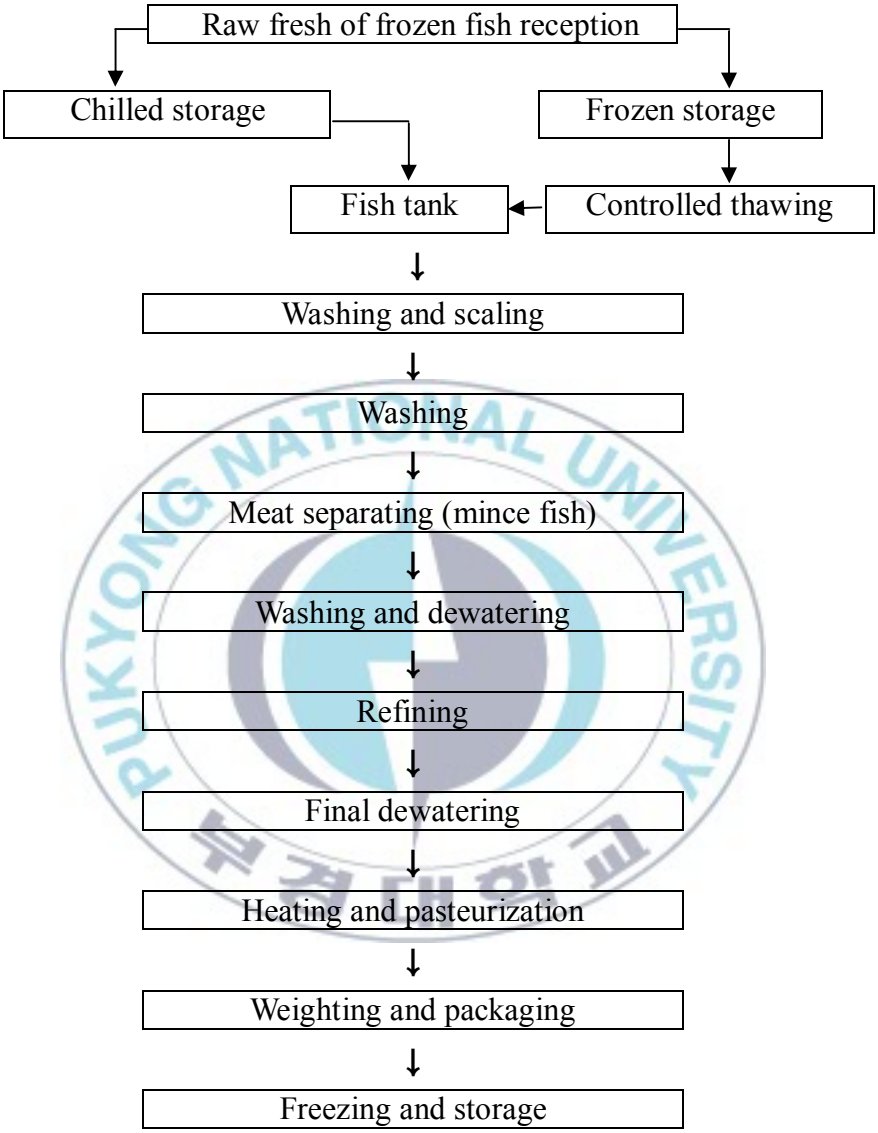

Fig. 1. Flow chart of surimi process.

under a screw press, undesirable material particles such as scales and connective tissue are removed by a refiner.

The number of washing cycles and water volume varies with fish species, freshness of fish, type of washing unit, and the desired quality of the surimi. Finally, the meat is mixed with additives, packed, frozen and stored ultimately to make a surimi product. More specifically, fresh water is used during the repeated washing procedure to remove water-soluble proteins and other materials from the minced meat (Shaviklo, 2006).

3.5. Sanitation and hygiene in processing plant

Fish and fishery products are primarily used for healthful diet, animal protein source of consumptions for the national food security. They constitute the international trade and foreign exchange earner of countries in the world. Each processor should be monitored in view of the condition and practice during processing with sufficient frequency to ensure, at a minimum, similar with the conditions and practice specified in good manufacturing practice (GMP) that are appropriate to plant and food being processed. HACCP principle must be implemented and basic common hygiene requirement must be applied to the production plants. The potential

effects of primary production on the safety and suitability of food should be considered at all times. In particular, this includes identifying any specific points in such activities where a high probability of contamination may exist and take specific measure to minimize that probability. Food hazards can occur through biological, chemical or physical contaminations. A biological hazards of microbial contaminations generally poses the greatest threat to food safety, followed by chemical hazards. In reality, it is difficult to singularly categorize all food-related hazards. The HACCP-based approach may assist in the taking of such measures to control contamination from air, soil, water, feedstuffs, fertilizer, pesticides, veterinary drug or any other agent used in primary production and to protect food sources from fecal coliform and other contamination. HACCP is a systematic approach to identification, assessment and control of hazard during production, processing, manufacturing, preparation and use of food, water or other substances (Kirby et al., 2003).

Factory hygiene as well as personal hygiene and sanitation is for example critical control point in the prevention of contamination of products with microorganism, filth and any other foreign material during processing (Huss,

1994). Limits may then be established such as microbiological criteria or guides at various steps in the production process or in the final products, while monitoring the CCP's points. Monitoring should be the accurate measurement of the chosen factors which control the CCP's. It should be simple, give quick results, and be able to detect deviation from specifications or criteria (Huss, 1994).

3.6. Microbial symptom

A microbial organism is minute, mostly one celled organism which is accomplished of rapid growth under proper growth conditions in the food processing plant. The major concern of microorganisms to the seafood processing industry is spoilage mold, spoilage bacteria and food-borne bacteria. Seafood can be contaminated with *Salmonella* species due to the introduction of fecal material from animals, birds, or through fecal-contaminated water. *Listeria monocytogenes* have been found in seafood processing plants, on the surface of processing equipment and after processing.

Human and environmental sources are the main route for contamination of *Staphylococcus aureus* species on seafood products. *Shigella* species which are main species are mainly spread through humans according to unsanitary handling of seafood by food handlers, but they can also be due to contaminated water. Microbiological criteria for fish and fishery products include quantification of the counts of *Escherichia coli*, thermo-tolerant coliform, mesophilic aerobic bacteria and pathogenic *V. parahaemolyticus* which are performed during production (Aberoumand, 2010). At the end of production stage, the monitored measure is the quantification of count of the *Staphacoccus aureus* and detection of bacteria of *Salmonella* genus because their presence indicates recontamination of final products. Poor fish handling practice and personal hygiene, contaminated water, sorting of fish on contaminated beach and carriers, and inadequate factory sanitation are the result of the presence of *E. coli, Staphylococcus, Salmonella*, and *Vibro-cholera* in fish and fishery products. An abdication of fecal contamination also effects on the quality of final product.

Biological contamination such as bacteria, viruses, fungi, protozoa and helminthes constitutes the major cause of food-borne disease with varying

degrees of severity, ranging from mild indisposition to chronic or life-threatening illness or both. In developing countries, such contaminants are responsible for food-borne disease such as Cholera, Campylobacterious, *E. coli* gastroenteritis, *Salmonellosis*, *Shigellosis*, typhoid fever, brucellosis, amoebiasis and poliomyelitis (Edema et al., 2005). A wide variety of foods including meat, milk, vegetable and fish have been associated with the diarrheal type of food poisoning from *Bacillus* spp. (FDA, 2007).

3.7. Perception of indicator organisms

3.7.1. Petri film technique of aerobic count for coli-form bacteria

Aerobic plate count (APC) indicates the level of microorganisms in products (Maturin & Peeler, 1998) and it can sometimes be used to indicate the quality and spoilage level of the products. This method used for environmental microbiological monitoring can determine how efficient processing equipment has been sanitized. The frequency of sanitation depends on microbes that are located in the environment and can determine different foodborne pathogens. Aerobic plate counts on fish and fishery products generally do not relate to food safety, but it can be useful to indicate quality, shelf life and post heat-processing contamination. Fresh

fish and fishery products often have an APC of 104-105/g, although there is an example of seafood's with an APC of 106-108/g without objectionable quality change (Nickelson & Finne, 1992). 3M Petrifilm, *E. coli* coliform can be used to determine whether fish products are of an acceptable standard or not. It is AOAC- approved method and the results can be obtained from 24 to 48 hours. Also, it is available for enumeration of *Staphylococcus aureus, Listeria*, total aerobic counts of yeasts and molds.

3.7.2. Swab method

Swabs can be used to determine how effective sanitation is done for difficult areas such as drains and conveyor in the fish processing environment. Conventional methods using swabbing are the oldest and most widely used methods for the microbiological examination of surfaces in the food industry, including hospitals and restaurants (Jay, 1992). By use of a sterile swab of cotton-wool, part of the disinfected surface is swabbed and the bacteria now on the swab are transferred to diluents for determination of colony forming units (CFU) on standard agar substrates. Swabs are especially useful in places where other control methods can only be used with difficulty, i.e. pocket, valves etc. (Huss, 2003).

4. Materials and Methods

4.1. Sample collection

4.1.1. Domestic sample collection along the processing line

This study was carried out at Jang Nin surimi factory one of the local surimi processing establishments located in Busan city. The sample was selected in two different ways for checking a processing condition (surimi mince) and for checking a processing environment in the plant (using swabbing). The two ways of sampling were collected from nine different places on the processing line such as raw material before washing, raw material after washing, meat separation (mince fish), the first flesh regulation, the second flesh regulation, pasteurized/dehydrated surimi, recirculated surimi, and the final product. To make sure the samples were taken without being contaminated; the samples were kept in the plastic bags, labeled and stored in a cool box with ice. After arriving in the laboratory, the samples were transferred into the refrigerator at 4^{o}C until use for counting bacteria. The sample for checking the environmental equipment in the plant was collected from before and during processing plant such as hook, conveyor (washing drum), plastic basket, conveyor (transport to mince equipment), mince separation, first dehydration conveyor, dehydration and washing conveyor, conveyor (recirculate surimi), and worker hands by using sterile swab of

cotton-wool which was taken from the surface of 100 cm^2 from each place. According to the undesirable contamination of coliform and fecal coliform entitlement to the processor, the activity was configured to find the sources and routes of contamination on the surface.

4.1.2. Sample collection of imported and domestic final surimi products

Eleven samples of final surimi product were collected from surimi importing companies located in Busan. A total twelve samples were collected randomly from these companies which import surimi products from Vietnam, America and China. One sample was collected from a company producing for the domestic market. Among the imported samples, seven were from Vietnam, three from America and one from China. All samples were kept in the refrigerator until use for analyzing the presence of bacteria and the method of investigation was the same as the process used with the domestic sample collected from the company.

4.2. Preparation for bacteria count

4.2.1. Sample preparation

Ten grams of each surimi sample were weighed by electronic scale, put into a sterile stomacher bag and mixed with 90 ml sterile solution (NaCl 0.5%) in a stomacher machine for 30 seconds.

4.2.2. Medium preparation

The NaCl solution is used to dilute the sample for counting the number of bacteria. The concentration of 25.5 g NaCl was dissolved with 3 liters of distilled water and mixed by a vortex mixer for 15 minutes. Each 9 ml of NaCl solution was kept in a glass test tube of 20 ml volume which was put in an autoclave of 121^{o}C to sterilize for 15 minutes and to cool down to room temperature.

4.2.3. Counting number of bacteria

Quantitative procedure for aerobic bacteria: Decimal dilutions were prepared by aseptically weighing 10 g of sample into 90 ml sterile diluents. The samples were further diluted by 10 dilution factor series for aerobic

counts of each sample. Duplicate experiment counting was diluted from 10 to 7 times of 10 dilution series and kept in incubation for 24 hours at 35^{o}C.

Quantitative procedure for *E. coli*, *Listeria* and *Staphylococcus*:

Decimal dilutions were prepared by aseptically weighing 10 g of sample into 90 ml sterile diluents. The sample is further diluted by 10 dilution factor series for *E. coli*, *Listeria* and *Staphylococcus* for each sample. Duplicate experiment counting was diluted from 10 to 5 times of 10 dilution series and kept in incubation for 24 hours at 35^{o}C except for Listeria at 30^{o}C.

Microbiological guideline: Results for total microbial counts were evaluated according to the Korea Food and Drug Administration guideline. This is the guide present in the manufacture's instruction for Utensil limitation standard. The limitation standard is less than 10,000 CFU/100 cm^2. For personnel hygiene, hands test is less than 10,000 CFU/100 cm^2. A fish and fishery product, limitation standard for raw material of < 1,000,000 CFU/g and fish products is < 100,000 CFU/g (Yang, 2011).

5. Results and Discussion

The amount of microbial load is used to indicate the quality of the final surimi product in Jang Nin surimi industry. Microbial indicator is often employed to assess food safety and sanitation. The total aerobic microbial count before processing (drum conveyor, mince separate conveyor and dehydration conveyor) was below the limitation standard. The total microbial count for the other equipments was greater than the limitation standard. Negative results of high level of microorganisms were recorded during processing time from raw material-used tool including the entire processing establishment.

5.1. Domestic surimi product

5.1.1. Environmental hygienic status in surimi processing plant

The levels of total aerobic microbial counts were collected before processing, showing positive impact (no contamination) at drum conveyor, mince separate conveyor and dehydration conveyor, but for the other equipment, the total microbial counts were greater than the limitation standard (<10,000 CFU/100 cm^2; Yang, 2011). For the sample collected during processing, the total microbial counts were shown to be more than

the limitation standard in the entire processing environment (Table 1). The result also showed that cell numbers of microorganism before processing were much lower than during processing. As for coli-form bacteria as like *E. coli*, only one sample was from a worker's hand showed (Table 2). *Staphylococcus* was also found from conveyor transport to mince separate tool during processing time (Table 3). However, in the others equipments no microorganisms were detected. *Listeria spp*. was also not detected (Table 4).

E. coli was isolated from a sample taken from a worker's hand but the microbial count was found to be below the limitation standard. According to CAC/RCP 52-2003, all working facilities should be maintained at a high degree of personal cleanliness and it should be taken with all necessary precaution to prevent contamination. Most importantly hand washing should be carried out by all working personnel in the surimi processing area. As *Staphylococcus* was found on conveyor transport to mince separate tool, it is possible that contamination may have taken place before the raw material was brought into the factory for processing or from the water source. Therefore, good hygiene practices (GHP) should be implemented. In this study, positive results for fecal coliform were found but were below the limitation standard in the processing area.

Table 1. Cell number of bacteria from surimi device at different days (Unit: CFU/100 cm^2)

Total bacteria	A	B	C	D	E	F	G	H	I
During processing	$1.9x10^8$	$1.4x10^8$	$1.7x10^8$	$8.9x10^7$	$1.3x10^8$	$1.3x10^8$	$3.8x10^6$	$1.1x10^5$	$8.5x10^6$
Before processing	$5.8x10^5$	$2.1x10^5$	$8.4x10^2$	$4.3x10^4$	$1.5x10^4$	$1.0x10^3$	$1.0x10^2$	$3.9x10^4$	$6.4x10^5$

A: Hook, B: Conveyor (Transport raw material to wash) C: Dram conveyor

D: Conveyor (raw material after wash) E: Conveyor transport to mince separate tool

F: Conveyor mince separate tool G: Conveyor (1^{st} dehydration/heating machine)

H: Plastic tray I: Worker's hand

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Table 2. Cell number of *E. coli* from surimi device at different days (Unit: CFU/100 cm^2)

	A	B	C	D	E	F	G	H	I
During processing	-[a)]	-	-	-	-	-	-	-	$4.0x10^1$
Before processing	-	-	-	-	-	-	-	-	-

A: Hook B: Conveyor (Transport raw material to wash) C: Dram conveyor

D: Conveyor (raw material after wash) E: Conveyor transport to mince separate tool

F: Conveyor mince separate tool G: Conveyor (1^{st} dehydration/heating machine)

H: Plastic tray I: Worker's hand a) "–" mean "not detected"

Table 3. Cell number of *Staphylococcus* from surimi device at different days (Unit: CFU/100 cm^2)

	A	B	C	D	E	F	G	H	I
During processing	-[a)]	-	-	-	$1.3x10^2$	-	-	-	-
Before processing	-	-	-	-	-	-	-	-	-

A : Hook B : Conveyor (Transport raw material to wash) C : Dram conveyor

D : Conveyor (raw material after wash) E : Conveyor transport to Mince separate tool

F : Conveyor mince separate tool G : Conveyor (1^{st} dehydration/heating machine)

H : Plastic tray I : Worker's hand a) "–" mean "not detected"

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Table 4. Cell number of *Listeria* from surimi device at different days (Unit: CFU/100 cm^2)

Total	A	B	C	D	E	F	G	H	I
During processing	-[a)]	-	-	-	-	-	-	-	-
Before processing	-	-	-	-	-	-	-	-	-

A : Hook B : Conveyor (Transport raw material to wash) C : Dram conveyor

D : Conveyor (raw material after wash) E : Conveyor transport to mince separate tool

F : Conveyor mince separate tool G : Conveyor (1st dehydration/heating machine)

H : Plastic tray I : Worker's hand a) "–" mean "not detected"

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This suggested that these bacteria may have been transferred from food handlers to the product. The positive result indicated that there was contamination from the food handlers as well as the conveyor surface in the processing environment (Table 5 and Fig. 2). The total number of microorganisms detected with the equipment during processing was quite higher than before processing (Table 6). According to studies by Montville et al. (2002), the contamination of bacteria during handling and preparation may be transferred from contaminated hands of food workers to food and subsequently and to other surface. Other studies done by Reji et al. (2003), attributed poor hygiene, particularly deficiency or absence of hand washing as the causative mode of transmission. However, surimi product is a semi finished-product, and will be cooked before consumption. This will eliminate consume organisms.

5.1.2. Hygienic state in surimi product

The results of the level of microbial counts for harmful bacteria such as *E. coli, Staphylococcus* and *Listeria* by 3M Petri film Aerobic Count from nine different processing sites from raw fish to final surimi products are given as CFU/g in Table 5 and 6. All samples in the surimi processing line were found

Table 5. Checking system of swap from surimi factory (CFU/100 cm^2)

Description			Total bacteria		*E. coli*		*Staphylococcus*		*Listeria*	
			Total	Standard	Total	Effect	Total	Effect	Total	Effect
Utensil	A	DP	190000000	<10000	0	-[a)]	0	-	0	-
		BP	580000	<10000	0	-	0	-	0	-
	B	DP	140000000	<10000	0	-	0	-	0	-
		BP	210000	<10000	0	-	0	-	0	-
	C	DP	170000000	<10000	0	-	0	-	0	-
		BP	840	<10000	0	-	0	-	0	-
	D	DP	89000000	<10000	0	-	0	-	0	-
		BP	43000	<10000	0	-	0	-	0	-
	E	DP	130000000	<10000	0	-	130	detected	0	-
		BP	15000	<10000	0	-	0	-	0	-
	F	DP	130000000	<10000	0	-	0	-	0	-
		BP	1000	<10000	0	-	0	-	0	-
	G	DP	3800000	<10000	0	-	0	-	0	-
		BP	100	<10000	0	-	0	-	0	-
	H	DP	110000	<10000	0	-	0	-	0	-
		BP	39000	<10000	0	-	0	-	0	-
Worker	I	DP	8500000	<10000	40	detected	0	-	0	-
		BP	640000	<10000	0	-	0	-	0	-

DP : During processing, BP : Before processing. a) "–" mean "not detected"

Table 6. Microbiological evaluation of surimi processing plant (CFU/100 cm^2)

Sample place	CFU/100 cm^2					
	Total bacteria count		Coliforms		Pathogenic bacteria	
	Before	During	Before	During	Before	During
Hook	$5.8x10^5$	$1.9x10^8$	-[a)]	-	-	-
Conveyor (transport raw material to wash)	$2.1x10^5$	$1.4x10^8$	-	-	-	-
Dram conveyor	$8.4x10^2$	$1.7x10^8$	-	-	-	-
Conveyor(raw material after wash)	$4.3x10^4$	$8.9x10^7$	-	-	-	-
Conveyor transport to mince separate	$1.5x10^4$	$1.3x10^8$	-	-	-	STA[b)]
Conveyor mince separate tool	$1.0 x10^3$	$1.3x10^8$	-	-	-	-
Conveyor(1^{st} dehydration/he)	$1.0x10^2$	$3.8x10^6$	-	-	-	-
Plastic tray	$3.9x10^4$	$1.1x10^5$	-	-	-	-
Worker's hand	$6.4x10^5$	$8.5x10^6$	-	-	-	*E. coli*

a) "–" mean "not detected" b) STA: *Staphylococcus*

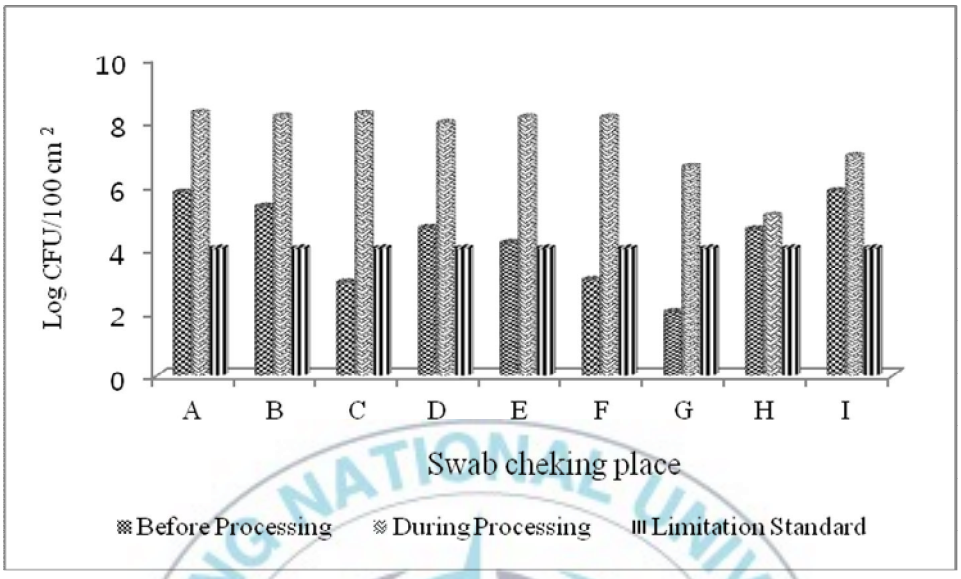

Fig. 2. Comparison of cell numbers counting of bacteria from before, during processing and from processing devices with limitation standard.

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to have high levels of microorganism in each in terms of CFU/g (Table 7) and were completely higher than the limitation standard (Table 11, Fig. 3). For coli form bacteria such as *E. coli*, it was found from raw material fish (Yellow Crocker) (Table 8). *Staphylococcus* was not detected from surimi samples (Table 9). *Listeria* was found from the samples from minced fish, first wash minced fish and pasteurized products (Table 10). Microorganisms were not detected in the other places.

Frozen surimi products are intermediate products that will be further processed into surimi-based products such as kamaboko and crab analogues. Many of the potential food safety hazards need to be checked during subsequent processing. For example, *Listeria monocytogens* and toxin formers that can occur at the end of the products, should be controlled during the cooking or pasteurizing step of final processing. According to Venugopal (2002), fish may be particularly contaminated by pathogens such as *Salmonella* spp., *Staphylococcus aureus*, *Campylobacter jejuni, E. coli* 0157:h7, *Vibrio parahaemolyticus*, *Yersina enterocolitica*, and *Listeria monocytogenes*. Their contamination may occur prior to harvest, during capture, processing, distribution and/or storage.

Table 7. Cell number of bacteria from surimi sample at different days (Unit: CFU/g)

	J	K	L	M	N	O	P	Q	R
1st day	8.3x 10^8	1.2x 10^7	2.5x 10^7	1.5x 10^8	3.6x 10^7	1.1x 10^7	1.4x 10^7	1.9 x10^7	8.8x10^6
2nd day	7.7x 10^6	5.9x 10^7	2.6x 10^7	3.9x 10^7	3.8x 10^6	3.9x 10^6	4.7x 10^6	1.7x10^7	1.8 x10^7

J: Raw material (Yellow Crocker) K: Raw material (Ribbon fish)

L: After washing M: Mince fish N: 1st flesh wash mince fish

O: 2nd wash mince fish P: Pasteurization Q: Recirculation surimi

R: Final product

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Table 8. Cell number of *E. coli* from surimi sample at different days (Unit: CFU/g)

	J	K	L	M	N	O	P	Q	R
1st day	1 x 10^1	-[a)]	-	-	-	-	-	-	-
2nd day	-	-	-	-	-	-	-	-	-

J: Raw material (Yellow Crocker) K: Raw material (Ribbon fish)

L: After washing M: Mince fish N: 1st flesh wash mince fish

O: 2nd wash mince fish P: Pasteurization Q: Recirculation surimi

R: Final product a) "-" mean "not detected"

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Table 9. Cell number of *Staphylococcus* from surimi sample at different days (Unit: CFU/g)

	J	K	L	M	N	O	P	Q	R
1st day	- a)	-	-	-	-	-	-	-	-
2nd day	-	-	-	-	-	-	-	-	-

J: Raw material (Yellow Crocker) K: Raw material (Ribbon fish)

L: After washing M: Mince fish N: 1st flesh wash mince fish

O: 2nd wash mince fish P: Pasteurization Q: Recirculation surimi

R: Final product a) "-" mean "not detected"

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Table 10. Cell number of *Listeria* from surimi sample at different days (Unit: CFU/g)

	J	K	L	M	N	O	P	Q	R
1st day	- [a)]	-	-	3.0×10^2	1.0×10^2	-	1.0×10^1	-	-
2nd day	-	-	-	-	-	-	-	-	-

J: Raw material (Yellow Crocker) K: Raw material (Ribbon fish)

L: After washing M: Mince fish N: 1st flesh wash mince fish

O: 2nd wash mince fish P: Pasteurization Q: Recirculation surimi

R: Final product a) "-" mean "not detected"

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Table 11. Checking system of 3M petrifrim plate count from surimi factory (CFU/g)

Sample		Total bacteria		*E. coli*		*Staphylococcus*		*Listeria*	
		Total	Standard	Total	Effect	Total	Effect	Total	Effect
J	1st day	830000000	<1000000	10	-[a)]	0	-	0	-
	2nd day	7700000	<1000000	0	-	0	-	0	-
K	1st day	12000000	<1000000	0	-	0	-	0	-
	2nd day	5900000	<1000000	0	-	0	-	0	-
L	1st day	25000000	<1000000	0	-	0	-	0	-
	2nd day	26000000	<1000000	0	-	0	-	0	-
M	1st day	150000000	<1000000	0	-	0	-	300	detected
	2nd day	39000000	<1000000	0	-	0	-	0	-
N	1st day	36000000	<1000000	0	-	0	-	100	detected
	2nd day	3800000	<1000000	0	-	0	-	0	-
O	1st day	11000000	<1000000	0	-	0	-	0	-
	2nd day	3900000	<1000000	0	-	0	-	0	-
P	1st day	14000000	<100000	0	-	0	-	10	detected
	2nd day	4700000	<100000	0	-	0	-	0	-
Q	1st day	19000000	<100000	0	-	0	-	0	-
	2nd day	17000000	<100000	0	-	0	-	0	-
R	1st day	8800000	<100000	0	-	0	-	0	-
	2nd day	18000000	<100000	0	-	0	-	0	-

a) "–" mean: "not detected"

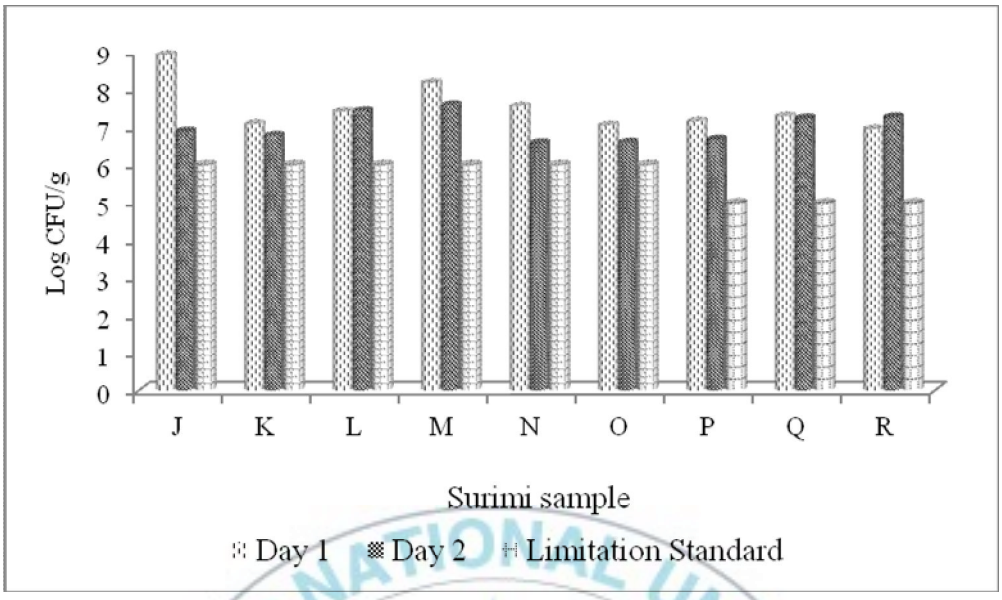

Fig. 3. Comparison of cell number counting of bacteria from surimi sample of two different days with limitation standard.

J: Raw material (Yellow Crocker)
K: Raw material (Ribbon fish)
L: After washing
M: Mince fish
N: 1st flesh washes mince fish
O: 2nd wash mince fish
P: Pasteurization
Q: Recirculation surimi
R: Final product

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Studies done by Vogel et al. (2001), on *L. monocytogenes*, indicated that contamination occurrs along the processing line. Other studies dealing with different processing operations similarly concluded that the plant and processing environment are the source of product contamination rather than the raw material. However, the raw material is initial source of contamination in processing line (Vogel et al., 2001). The contamination of fish products through contaminated surfaces has been observed in many studies. Unclean, insufficiently or inadequately clean processing equipments have been identified as a source of bacterial contamination in processed sea food (Reij et al., 2003). Container, pumps or tanks used for holding or transporting unprocessed raw material have occasionally been used by many processors for processed products without any cleaning and disinfection (Lewellyn et al., 1998).

In this experiment, *Listeria monocytogens* bacteria were found in the sample of minced fish, first flesh wash minced fish and pasteurization sample (Table 12). According to CAC/RCP 52-2003, fish flesh minced meat should be immediately spread into water and transferred to the washing and dewatering step to prevent blood from congealing and causing loss of gel

Table 12. Microbiological evaluation of the surimi product (CFU/g)

Sample place	CFU/g					
	Total bacteria count		Coliforms		Pathogenic bacteria	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Raw material (Yellow Crocker)	8.3×10^8	7.7×10^6	1×10^1	-	-	-
Raw material (Ribbon fish)	1.2×10^7	5.9×10^7	-[a)]	-	-	-
After washing	2.5×10^7	2.6×10^7	-	-	-	-
Mince fish	1.5×10^8	3.9×10^7	-	-	LIS[b)]	-
1st flesh wash mince fish	3.6×10^7	3.8×10^6	-	-	LIS	-
2nd wash mince fish	1.1×10^7	3.9×10^6	-	-	-	-
Pasteurization	1.4×10^7	4.7×10^6	-	-	LIS	-
Recirculation surimi	1.9×10^7	1.7×10^7	-	-	-	-
Final Product	8.8×10^6	1.8×10^7	-	-	-	-

a) "–" mean: "not detected" b) LIS: *Listeria*

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strength capability. The washing and dewatering cycle should be sufficient to achieve separation of water-soluble protein from myofibrillar proteins. If water-soluble protein remains in the product, it will negatively affect the gel forming ability and shelf life during long-term frozen storage. In this view, the surimi processing plant during minced separating state provides the optimum conditions of growth for the coliform and microorganisms. Therefore, the processor must be managed in this stage as a critical control point. The product should be promptly processed to minimize its contamination. The processing temperature of the minced fish flesh in the refining process should be adequately controlled to prevent growth of pathogenic microorganisms.

As for the sanitation and hygiene of fish processing plants for GMP and GHP, an effective sanitation program for equipment and premises must be in place to prevent contamination of products. Each processor should have and implement through a written of sanitation standard operating procedures or similar document which is specific to each location. The conditions during processing should be monitored with sufficient frequency to ensure quality of the products.

Most of the hazards which are related to consumption of fish and fish products can be controlled and applied to GMP, GHP and a well-designed HACCP program.

5.2. Imported surimi products

Depending on the statistics of 2008 and 2009, Korea imported surimi product around 100,000 MT. Although surimi products were imported from China, Vietnam, America, Indonesia, Thailand, India, and others country (Fay & Kim, 2010), the most imported countries were China and Vietnam and the next was America. The imported products were distributed by surimi companies throughout the whole country. In the study of hygienic analysis of sirimi on the contamination of microorganisms, 11 samples of surimi were collected and those samples were imported from Vietnam (7 samples), America (3 samples) and China (1 sample). To evaluate the hygienic status of those surimi products, five tests were carried to determine the microbial of general microorganisms and for specific ones such as coliforms, *Listeria*, *Staphylococcus*, and *E. coli* were cultured and counted.

Table 13 showed the results of microorganism counts from seven samples imported from one of the surimi companies in Vietnam. The total microorganism counts varied from sample to sample; this was because each sample was produced from different raw materials and stored under different conditions. Among the seven samples, only two samples showed the total number of microorganisms which were equal to the limitation standards (10^5) and the other five samples had the number of microorganisms being below the limitation standard. Coliform bacteria were also detected from the culture with only a few numbers of microorganisms (below limitation standard). Table 14 shows the results of microorganism counts three samples from America, one sample from China and one domestic surimi product sample from Busan. The total numbers of microorganism counts from the surimi samples from America were much lower than the surimi samples of other countries. For the surimi sample imported from China, the number of microorganism counts was equal to the limitation standard (Fig. 4). The result also shows the presence of coliform bacteria in all the samples from the importing countries, but the highest number of microorganism was found in the surimi sample imported from China (Table 15).

Table 13. Microbiological evaluation of the surimi product imported from Vietnam (CFU/g)

Country	Vietnam						
Sample	V1	V2	V3	V4	V5	V6	V7
CFU	3.0×10^4	1.5×10^4	1.3×10^4	2.0×10^4	3.0×10^4	1.0×10^5	1.0×10^5
Coliform	1.6×10^1	1.0×10^1	-	2.0×10^1	2.0×10^1	6.6×10^1	2.6×10^1
E. coli	-[a)]	-	-	-	-	-	-
Listeria	-	-	-	-	-	-	-
Staphylococcus	-	-	-	-	-	-	-

V1, V2, …V7: surimi sample imported from Vietman, a) "-" mean: "not detected"

Table 14. Microbiological evaluation of the surimi products of American, Chinese and Korean markets (CFU/g)

Country	USA			China	Korea
Sample	U1	U2	U3	C	K
CFU	3.0×10^2	1.0×10^3	2.0×10^3	1.0×10^5	2.8×10^4
Coliform	2.0×10^1	3.0×10^1	6.0×10^1	9.0×10^1	8.0×10^1
E. coli	-[a)]	-	-	-	-
Listeria	-	-	-	-	-
Staphylococcus	-	-	-	-	-

U1, U2, U3: surimi sample imported from USA C: surimi sample imported from China,

K: Korean domestic surimi a) "-" mean: "not detected"

Table 15. Microbiological evaluation of the surimi products of American, Chinese and Korean markets compared with limitation standard (CFU/g)

Country	Sample	CFU/g					
		Total bacteria count		Coliforms bacteria	Pathogenic bacteria		
		Count	Standard		*E. coli*	*Staphylococcus*	*Listeria*
Vietnam	V1	30000	<100000	16	-[a)]	-	-
	V2	15000	<100000	10	-	-	-
	V3	13000	<100000	-	-	-	-
	V4	20000	<100000	20	-	-	-
	V5	30000	<100000	20	-	-	-
	V6	100000	<100000	66	-	-	-
	V7	100000	<100000	26	-	-	-
USA	U1	300	<100000	20	-	-	-
	U2	1000	<100000	30	-	-	-
	U3	2000	<100000	60	-	-	-
China	C	100000	<100000	90	-	-	-
Korea	K	28000	<100000	80	-	-	-

V1, V2, …V7: surimi sample imported from Vietman, U1, U2, U3: surimi sample imported from USA

C: surimi sample imported from China, K: Korean domestic surimi, a) "-" mean: "not detected"

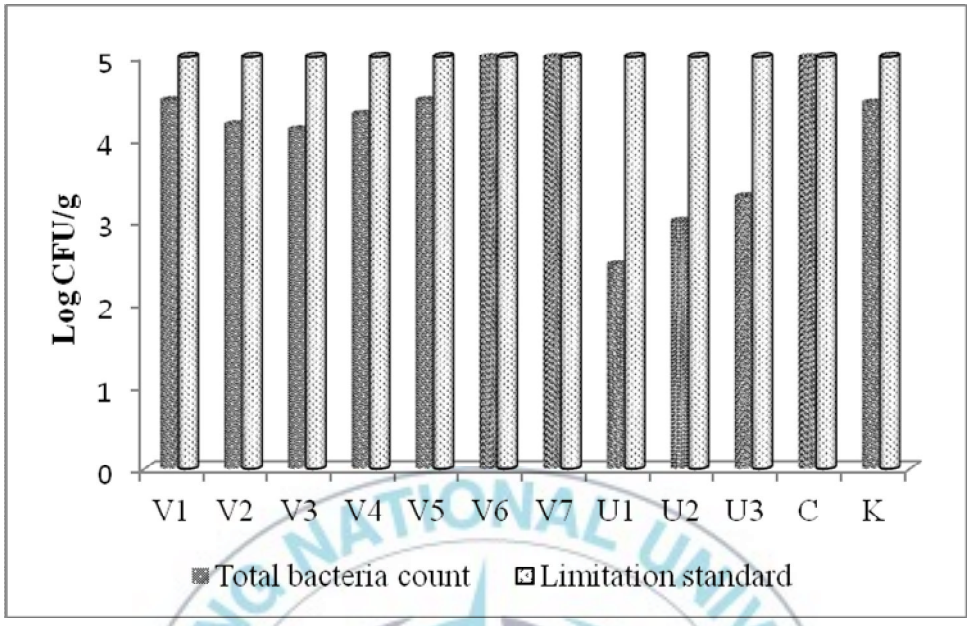

Fig. 4. Comparison of cell number counting of bacteria from surimi samples. of Vietnam, USA, China and Korea with limitation standard

V1, V2, …V7: surimi sample imported from Vietman

U1, U2, U3 : surimi sample imported from USA

C : surimi sample imported from China

K : Korean domestic surimi

Coliform bacteria are an indicator of unsanitary conditions in water and foods. One of the most common applications of coliform bacteria as indicator organisms is in their association with hygienic conditions and overall quality. However, most of coliform bacteria are not associated with food-borne illness and coliforms at normal levels found in foods are killed by most heat processing conditions (example. pasteurization of milk): Therefore, their presence in food should be controlled by adequate heat treatment (Daily Foods Science notes, 2007).

6. Conclusion and Recommendation

The total microorganism counts of surimi product during processing were much more than the limitation standard and this contamination was probably from the raw material or during the mince separating stage. *E. coli* was found on a worker's hand and *Listeria* was detected from different stages of the production line which starts from mince separation stage to pasteurization stage, but it wasn't detected in the final product of the surimi after pasteurization. The contamination by *Listeria* might have been aggravated by the presence blood which provided a conductive growth environment condition for the microorganism. A coliform bacterium was also found in the imported surimi product. The contamination was probably due to improper processing conditions or it can be prolonged storage.

Good manufacturing practice and good hygiene practice should be implemented in the processing environment. The fact that *E. coli* was found on a worker's hand demonstrated that the surimi handler was not observing good personal hygiene. Good hygiene practice is necessary for the processing plant for proper application of hygienic and sanitation procedures. The contamination of *Listeria* was detected from flesh fish

minced stage; and thus the processor must take measures to prevent the contamination of product. Coliform was found on the imported products. To control this, hazard analysis critical control point must be practiced to improve the quality of product and food safety. Surimi is a kind of semi finished-product and the detected microorganism is not a pathogenic microorganism and most of the bacteria can be destroyed during cooking. Therefore, proper care systems and inspection are required to prevent microorganism contamination and ensure good quality products.

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