

HYGIENE INDICATORS IN A FISH PROCESSING ESTABLISHMENT-A CASE STUDY IN A WHITE FISH PROCESSING ESTABLISHMENT.

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ABSTRACT

In this study bacterial indicators were investigated in a fish processing plant in order to identify their sources and routes of transmission so that they could be reduced or eliminated. Swabs on food contact surfaces as well as fish samples were taken and tested for coliforms and faecal coliforms. Present/absent tests were carried out for swabs and for fish samples. The Most Probable Number (MPN) technique as well as plate counts to isolate and enumerate coliforms and faecal coliforms were used. Coliforms were present at most steps in the processing environment, with few exceptions, whereas positive results for faecal coliforms were only obtained in the trimming area, suggesting that they may have originated from food handlers. The presence of these microorganisms, although in numbers within company specifications, shows that foodhandlers were not adhering to hygiene and sanitation procedures. Knowledge of contaminations is very critical in the Good Hygienic Practices programme and effective hygiene as well sanitary procedures in food processing plants are necessary, should contamination by coliform and faecal coliform bacteria persist.

Keywords: *Hygiene Indicators; Swab; MPN; foodhandlers; contamination; GMP; Fish processing establishment.*

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1 INTRODUCTION

Fishery products constitute an important part of international trade, currently worth more than US\$ 50 billion, indicating increasing consumer interest in the commodity. Unlike other animal products, quality of fish is often more difficult to control due to variations in species, sex, age, habitats and action of autolytic enzymes as well as hydrolytic enzymes of microorganisms on the fish muscle (Venugopal 2002).

A major goal for the food processing industries is to provide safe, wholesome and acceptable food to the consumer and control of microorganisms is essential to meet this objective (Baggen-Ravn *et al.* 2003). However, this can be very difficult as contamination of products in a food processing environment may take place at all stages, during production (both pre and post-harvesting) and processing (Beuchat 1995, De Rover 1999). Scientific research shows quite clearly that Icelandic seafood is wholesome and nutritious (IMF 2001). Specifications for quality standards are often agreed upon between the processors and the customers. In Iceland, the Directorate of Fisheries, through two private inspection bodies, ensures that the quality standards of seafood produced by the fish processors comply with laws and regulations pertaining to the proper and safe handling of seafood (IMF 2001).

Although seafood may be considered safe, wholesome and nutritious, microorganism may be present indicating the possible presence of pathogens whose presence in given numbers points to inadequate processing for safety (Mossel *et al.* 1995). In general, indicator microorganisms are most often used to assess food sanitation (Jay 1992). As fish and other free-swimming marine animals do not usually carry those organisms, particularly of mammalian microflora, including *Escherichia coli* and faecal coliform, their presence on processed seafood is a clear evidence of contamination from terrigenous source (ICMSF 1986). Thus, the presence of these organisms may not only indicate the hygienic condition under which the processing establishment operates but also the presence of potential microorganisms that may be harmful to the consumer.

The ÚA Seafood Group produces high quality seafood for demanding customers all over the world. The quality of the raw material is a key issue in this context and when combining the purity of fish products from the Icelandic fishing grounds to carefully controlled, state of the art production techniques as well as processing according to certified standards, the result is bound to be sound and wholesome products (ÚA 1999). However, the company has been sporadically experiencing an unacceptable number in hygiene indicators in a final product (fish mince). The company aims to eliminating completely or at least to fall within company specifications.

2 OBJECTIVES

The main purpose of the present study is to identify contamination routes during production and processing of fresh cod (*Gadus morhua*). The aim of the study was to understand how coliforms and faecal coliforms are transferred onto seafood during processing. Also, the quality assurance systems such as Good Manufacturing Practice (GMP)/Good Hygienic Practice (GHP) and Hazard Analysis Critical Control Point (HACCP) will be looked at including the current methods in monitoring hygiene.

Further, recommendations will be given to the processing establishment on measures to reduce the occurrence of coliform and faecal coliform bacteria.

3 LITERATURE REVIEW

3.1 General introduction

In this part of the report, various aspects will be reviewed, including contamination sources and routes, as well as how the quality assurance systems in general are used to ensure safe, wholesome and nutritious food. Also the current methods used to monitor hygiene in food processing establishments will be briefly reviewed.

Contamination is a very important aspect as this is the mode that most unwanted microorganisms may be transmitted onto seafood and other food products. Unwanted microorganisms may access food processing environments through raw material, personnel or mobile equipment such as forklifts, through leakage and openings in buildings, or through pests and some pathogens may even become established in the processing plant and form niches where they can survive for long periods of time (Reij *et al.* 2003). Many of these microorganisms occur naturally in aquatic and general environments, and may be transmitted onto seafood before capture, during and after processing. Also, contamination via air can occur through dust particles or via aerosols which are formed especially when contaminated surfaces, floors or drains are sprayed with high pressure-jets, resulting in formation of droplets that can be suspended in the air (Den Aantrekker *et al.* 2003). Water is also a vehicle for transmission of many agents of diseases (Kirby *et al.* 2003).

As for quality assurance, methods such as the GMP/GHP and HACCP are recommended by the Codex Alimentarius Commission for use by any food processing establishment to ensure safe, wholesome and nutritious food for human consumption. Lastly to be discussed in this part of the report are a few of the current methods used to identify microorganisms in seafood. Many of these involve isolating, identifying and enumerating indicator microorganisms on food contact surfaces and raw fish.

3.2 Overview of sources and routes 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 microorganisms are of the animal/human reservoir (*Salmonella*, *Shigella*, *E.coli*, enteric virus) (Huss *et al.* 2000). Thus, there is always a possibility that these microorganisms may be passed on to the raw material during production and processing.

In general, when a healthy fish is caught, the flesh is sterile as its immune system prevents bacteria to proliferate easily whereas after death the fish's immune system collapses allowing easy access of microorganisms into the flesh (Huss 1995). Some microorganisms have been found on the entire outer surface (skin and gills) and in the

intestines of live and newly healthy caught fish (Huss 1995). Liston (1980) estimated the total number of microorganisms to vary enormously from a normal range of 10^5 - 10^7 cfu (colony forming units)/cm² on the skin surface, whereas counts between 10^3 - 10^9 cfu/g on the gills and intestines were found by Shewan (1962).

3.2.2 Processing equipments

Contamination of fish products through contaminated surfaces has also been observed in many cases (Reij *et al.* 2003). Unclean, insufficiently or inadequately cleaned processing equipment have been identified as a source of bacterial contamination in processed seafood (Reij *et al.* 2003). Containers, pumps or tanks used for holding or transporting unprocessed raw materials, have occasionally been used for processed products without any cleaning and disinfection (Morgan *et al.*1993, Evans *et al.*1996, Hennessy *et al.*1996, Llewellyn *et al.*1998). It is therefore necessary that equipment in the processing establishment, coming in contact with food, be constructed in such a way as to ensure adequate cleaning, disinfection and proper maintenance to avoid the contamination (CAC 1997a).

3.2.3 Personnel

Transfer of microorganisms by personnel particularly from hands, is of vital importance (Chen *et al.* 2001, Montville *et al.* 2001, Bloomfield 2003). During handling and preparation, bacteria are transferred from contaminated hands of food workers to food and subsequently to other surfaces (Montville *et al.* 2002). Low infectious doses of organisms such as *Shigella* and pathogenic *Escherichia coli* have been linked to hands as a source of contamination (Snyder 1998). Poor hygiene, particularly deficient or absence of hand washing has been identified as the causative mode of transmission (Reij *et al.* 2003). Proper hand washing and disinfection has been recognized as one of the most effective measures to control the spread of pathogens, especially when considered along with the restriction of ill workers (Adler 1999, Montville *et al.* 2001).

3.2.4 Pests

Insects, birds and rodents have been recognized as important carries of pathogens and other microorganisms (Olsen and Hammack 2000, Urban and Broce 2000). In one interesting case a *Salmonella* outbreak was traced back to amphibians, which had accidentally entered the production facility (Parish 1998). Beveridge (1988) and Fenlon (1983) demonstrated that some aquatic birds spread for example *Salmonella* and other human pathogens in the environment. GHP should be employed to avoid creating an environment conducive to pests (CAC 1997b).

3.2.5 Water

Water, like food, is a vehicle for the transmission of many agents of disease and continues to cause significant outbreaks of disease in developed and developing countries world-wide (Kirby *et al.* 2003). In Canada, an outbreak of *E.coli* was reported (Kondro 2000) and in the USA *Cryptosporidium* affected approximately 400,000 consumers and caused 45 deaths in 1993 due to consumption of contaminated water (Kramer *et al.*1996, Hoxie *et al.* 1997). A cholera epidemic in Jerusalem in 1970 was traced back to the consumption of salad vegetables irrigated with raw waste

water (Shuval *et al.* 1986). It is therefore important that potable water is used throughout the production process, for cleaning equipment, washing food, as well as ice making. Also there should be a monitoring program 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).

3.3 Quality assurance system

The production of safe food is based on the implementation and application of general preventative measures such as GMP (Reij *et al.* 2003). GMP is the overall management (organising, implementing and adhering) of procedures, processes, control and other precautions that exclude, prevent, minimize, and inhibit product failures, and consistently yield safe, suitable foods of uniform quality, according to their intended use. GHP is part of GMP concerned with general hygiene, microbial safety and product spoilage (Heggum 2001). While it is not possible to achieve zero risk under GMP, the development and use of other approaches, such as HACCP, to ensuring safe food, cannot not be omitted (Jay 1992). 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). However, the approach by itself is not enough to secure fish products to be free of the pathogens. Thus, good hygiene, cleaning and sanitation are necessary to secure low levels of microorganism on the on the final product (Huss 1997). In practice, however, this can be very difficult and (Garland 1995) demonstrated that a very low level of *Listeria monocytogenes* in final products can be obtained in products produced under hygienic conditions, but it has also been claimed as a practical experience by industry in other countries that “the more you clean the more you have” (Huss 1997). Strict hygiene during manufacture of fish products may therefore decrease the risk from some pathogens and increase the risk from others (Huss 1997). Thus, in no case is the application of good hygiene sufficient to secure safety and a second line of defence (prevention of growth) must be established (Huss 1997).

On the other hand, significant specific hazards are addressed by applying the HACCP system. Factory hygiene as well as personnel hygiene and sanitation are for example CCP's 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 product while monitoring the CCP's points. Monitoring should measure accurately the chosen factors which control the CCP's, should be simple, give quick results, and be able to detect deviations from specifications or criteria (Huss 1994). When there is a failure, corrective actions may be taken for the CCP that is not under control, followed by verification as well as documentation concerning all procedures and records according to the HACCP principles and their application (CAC 1997a).

Before applying the HACCP system, any food establishment should operate according to the Codex general principles of food hygiene, the appropriate Codex Codes of practice, and appropriate food safety legislation to achieve the goal of ensuring food safety and suitability for human consumption (CAC 1997a). The system has taken on a global perspective in the production of fish and fishery products (Lima dos Santos

and Sophonphong 1998). Many countries have adopted or decided to introduce HACCP in seafood production. These include, USA, Canada, Australia, New Zealand, Iceland, Thailand, Ireland, Brazil, Mexico, Venezuela, Argentina, Peru, Cuba, Morocco, Norway, Sri Lanka, Vietnam, Bangladesh, Madagascar, Tunisia, Portugal and Honduras as well as member countries of the European Union (Italy, Germany and France) while in many other countries including most African states the status of seafood HACCP is unclear (Cato 1998). Countries aiming at exporting to countries such as the EU and/or USA, must meet the requirements set up by those countries.

The application of HACCP in many manufacturing or treatment processes has led to a more efficient prevention of adverse health effects associated with the consumption or use of the products (Kirby *et al.* 2003). For example the implementation of an industry wide HACCP programme for seafood processors in the US is thought to have averted 20-60% of the normal number of seafood borne illnesses (Birley and Lock 1998). A similar program for the prevention of food-borne listeriosis in the US reduced the incidence and mortality by 44% and 46% respectively over a period of four years (Billy 1997). Thus, hazards related to contamination, recontamination or survival of biological hazard and the growth of pathogens, during processing can be controlled by applying GMP and a well designed HACCP programme (Huss *et al.* 2000).

3.4 Bacterial indicators

Various bacteria are found in the digestive tracts and faeces of animals and humans. Some of these bacteria, i.e. faecal coliforms, *E.coli* (the predominant group of the faecal coliform group), and *Enterococcus* spp., are used as hygiene indicators (Frahm and Obst 2003). Indicator microorganisms are microorganisms or a group of microorganisms indicative for the possible presence of pathogens whose presence in given numbers points to inadequate safety in processing (Mossel *et al.* 1995). In general, they are most often used to assess food sanitation (Jay 1992).

There is no universal agreement on which indicator microorganism(s) is most useful, nor are there federal regulations mandating a single standard for bacterial indicators. Thus, different indicators and different indicator levels identified as standards are used in different states, countries, and regions. Today, the most commonly measured bacterial indicators are total coliforms (TC), faecal coliforms (FC), and enterococci (EC). More recently, *E.coli* (a subset of the FC group) and EC were established as preferred indicators (Noble *et al.* 2003).

3.5 Detection of indicator organisms

Food plants and many other institutions require sanitary conditions in order to prevent microbial contamination. The continuous evaluation of these environments is particularly important in order to assure the safety and quality of products, and the number of microbial cells contaminating food surfaces must be determined for this assessment (Yamaguchi N *et al.* 2003). Many methods have been developed to detect microorganisms, and although some methods of analysis are better than others, every method has certain inherent limitations associated with its use (Jay 1992). Below is a brief description of some of the methods that are currently in use.

3.5.1 *Most Probable Number (MPN) technique for coliform bacteria*

The method consists of inoculating a series of tubes with appropriate decimal dilutions of the sample. Production of gas, acid formation or abundant growth in the test tube after a certain period of time incubation at 35°C constitutes a positive presumptive reaction. Both lactose and Lauryl Tryptose broths can be used as presumptive media. All tubes with positive presumptive reaction are subsequently subjected to a confirmation test. The formation of gas in a Brilliant Green Lactose Bile (BGLB) broth fermentation tube at any time within 48 hours at 35°C constitutes a positive confirmation test. The faecal test (using an EC medium) can be applied to determine TC that are FC (APHA 1992): the production of gas after 24 hours of incubation at 44.5°C in an EC broth medium is considered a positive result. The results are expressed as MPN (most probable number) of microorganisms present. This number is a statistical estimate of the mean number of coliforms in a sample. As a consequence, this technique offers a semi-quantitative enumeration of coliforms. The method is easy to implement but can become very tedious and laboratory intensive and necessitates a subculture stage for confirmation which can take up to a further 48 hours (Rompré *et al.* 2002).

3.5.2 *Membrane filter technique*

In the membrane filter method, membranes with a pore size that will retain bacteria (0.45 µm) but allow water or diluents to pass through are used. Following the collection of bacteria upon filtering a given volume, the membrane is placed on an agar plate or an absorbent pad saturated with culture medium of choice, and incubated appropriately. After growth, colonies are enumerated (Jay 1992). The method also offers quantitative enumeration comparatively to the semi-quantitative information given by the Multiple Tube Fermentation (MTF) technique (Rompré *et al.* 2002). Nevertheless, since this method is not sufficiently specific, a confirmation stage is needed, which could take further 24 hours after the first incubation period on selective media (Rompré *et al.* 2002).

3.5.3 *Swabbing*

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 a diluent for determination of colony forming units (CFU) on standard agar substrates (Huss 2003). Swabs are especially useful in places where other control methods can only be used with difficulty i.e. pockets, valves etc.(Huss 2003).

3.5.4 *Contact plate*

In the contact plate method, direct surface contact plates, petri dishes or contact slides with selective or general purpose agar media are applied to the surface to be examined, followed by incubation and counting of colony forming units (Huss 2003). This technique can only be applied to flat surfaces, which is a limiting factor (Huss 2003). The RODAC plate has been shown to be the method of choice when the

surfaces to be examined are smooth, firm, and nonporous while it is not suitable for heavily contaminated surfaces (Jay 1992).

3.5.5 Bioluminometric assay of ATP

The bioluminometric assay of ATP is almost a real time method giving the answer within minutes or less than an hour. It is very sensitive and can be combined with swabbing for collection of microorganisms from surfaces. The method is rather non-specific, and it may not be able to distinguish between microorganisms and food residues. However, if applied under defined conditions it may prove useful and superior to the conventional methods because it provides the answer in minutes (Huss 2003).

4 PRACTICAL WORK

Due to the unacceptable contamination of coliform and faecal coliform according to the processor, the project was designed to find the sources and routes of contamination. Swab samples were taken from surfaces and raw materials at different processing stages and analysed in the laboratory to establish where the main point of contamination is. Recommendations were made to the processors on the necessary steps to reduce or eliminate coliforms and faecal coliforms.

5 MATERIALS AND METHODS

5.1 Sampling sites

The project was carried out in the north of Iceland (Akureyri) at one of the local fish processing establishments. The layout of the processing plant is in Figure 1 and the sampling sites are shown by green spots. For swabs, spots were sampled twice. As for raw cod, spots were sampled once starting from the filleting area.

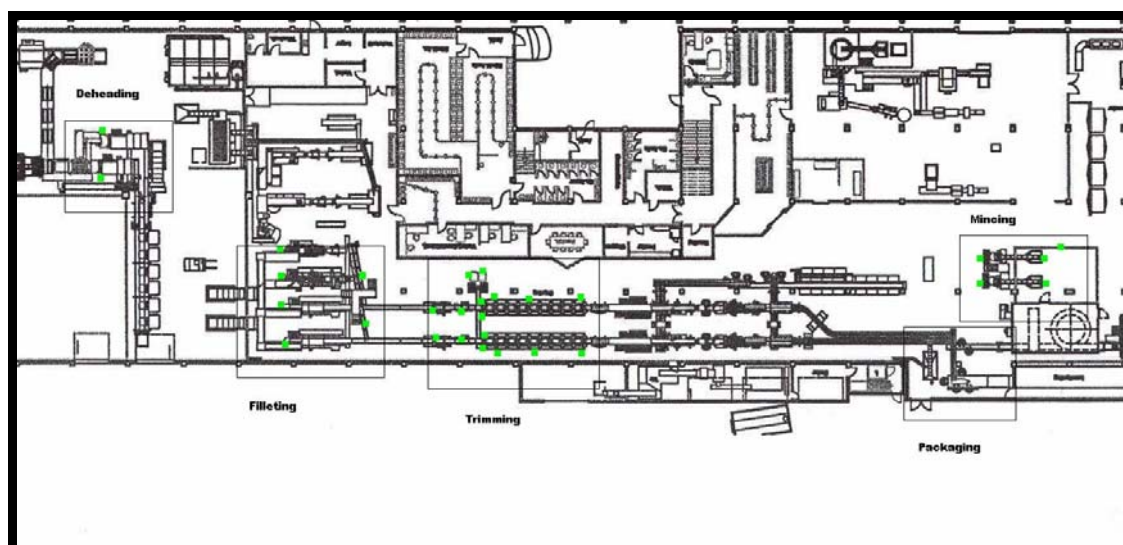


Figure 1: Schematic diagram of the processing establishment showing all sampling sites (small green spots) (ÚA 1999).

5.2 Sampling Procedure

Swabs and fish samples were collected on several occasions. Swabs were taken first, followed by fish samples on different days. It was decided that swabs and fish be taken at points where contamination of the raw material was likely to occur due to contact with hands of fish handlers and/or surfaces of equipment operated by fish handlers during different steps in the production process.

Samples were taken at the end of the first shift during the day, before cleaning and disinfection for the next shift in order to increase the chance of finding contamination. In addition, studies of the flow chart and observations during the production process were also done.

5.3 Bacteriological analyses

5.3.1 Swabbing

Sixty four samples were taken by swabbing on two occasions, 32 in each visit. A number of surfaces (equipment surfaces, conveyor belts, knives etc.) were swabbed thoroughly with moistened (moisturized with D/E Neutralizing agar) sterile cotton swabs. The swabs were placed each in 10 ml of Lauryl Sulphate Tryptose (LST-DIFCO™) broth and incubated at 35 °C for 48 hours. To detect the presence or absence (positive or negative) of coliform and faecal coliform, a loopful of suspension from each of the previously incubated LST samples was transferred into a test tube with Brilliant Green Lactose Bile broth (BGLB- DIFCO™) and (*Escherichia coli* broth (EC-DIFCO™), and incubated at 35°C for 48 hours and 45.5°C for 24 hours, respectively. The positive test tubes were gently agitated and examined for gas formation or effervescence. The results were recorded as positive or negative for coliform and faecal coliform.

5.3.2 Fish samples

A total number of 11 samples of raw cod (*Gadus morhua*) from different areas within the production process were taken, two from the filleting machines, four from the conveyor belts under the candling/trimming table, two from the candling/trimming table and two from the mincing area. To make sure that samples were taken without being contaminated, inverted plastic bags were used to collect them. The inner surface of the bag was used to touch nothing else but the sample. Samples were labelled and transported to the laboratory same day for analysis.

Fish samples were minced and 25 g of each sample was weighed, and put into a sterile stomacher bag, homogenizing it with 225 ml sterile peptone water (NaCl 0.5%) in a stomacher for 60 seconds. A number of 10-fold serial dilutions (1 , 10^{-1} and 10^{-2}) were prepared and transferred with pipettes into test tubes containing the BGLB and EC broths, and incubated accordingly at 35°C for 48 hours and 45.5°C for 24 hours respectively. The Most Probable Number (MPN) was calculated on the basis of the proportion of confirmed gassing in the BGLB and EC broth tubes for three consecutive dilutions using tables from Blodgett (2001). Also, from the dilution 1, 0.5 and 0.1 ml were transferred with pipettes onto Petri dish with the Violet Red Bile Agar (VRBA- DIFCO™).

6 RESULTS

6.1 Swabs

Most swabs throughout the processing establishment were positive for coliforms. Only six samples out of 64 were negative (Figure 2). The negatives swabs were from the beheading machines and the rest were from the processing area.

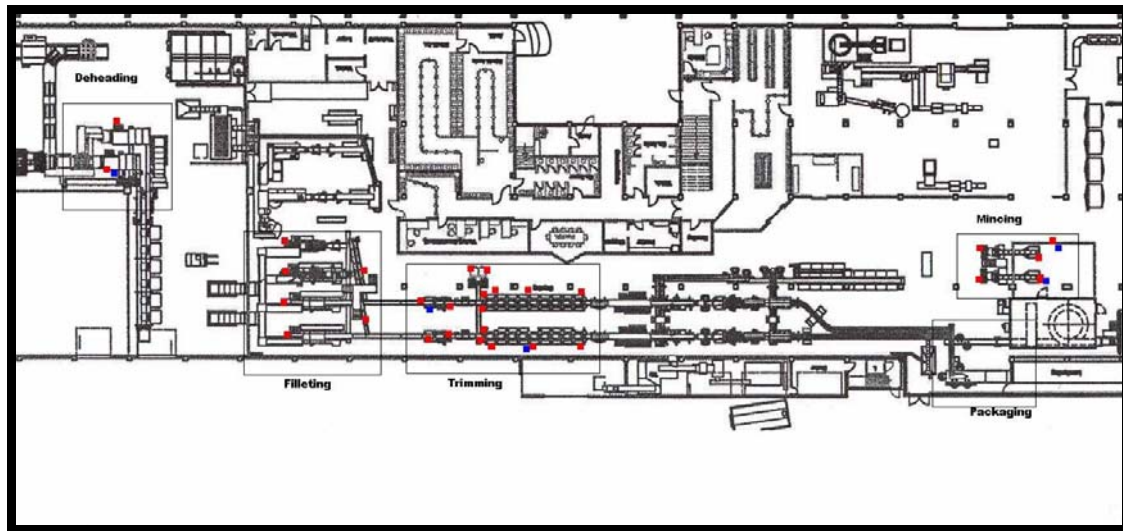


Figure 2: Schematic diagram of the processing establishment showing results for swabs incubated in BGLB; positives (red) and negatives (blue).

As for faecal coliform, only a few swabs (five samples) were positive (Figure 3). Most of these are from the processing area; filleting machine, candling/trimming tables as well as the conveyors both underneath and before the candling table.

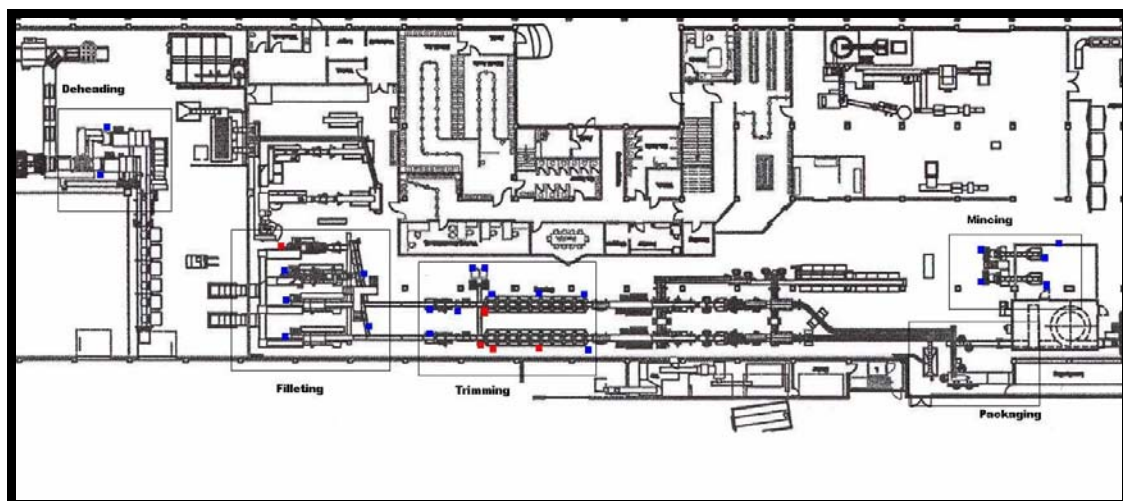


Figure 3: Schematic diagram of the processing establishment showing results for swabs incubated in EC; positives (red) and negatives (blue) (Source ÚA 1999).

6.2 Fish Samples

The results for fish sample tests for coliform and faecal coliform by LST, BGLB and EC broths respectively for different sites in the processing establishment are given as MPN/g (Table 1).

Table 1: MPN/g results for positive samples from the processing establishment.

Processing stage	coliform (BGLB-broth)	faecal coliform (EC-broth)
Filleting	<0.3	<0.3
	<0.3	<0.3
Conveyor belts	24	1.5
	24	0.36
	2.3	<0.3
	46	<0.3
Candling tables	1.5	<0.3
	2.3	<0.3
Container	46	<0.3
Conveyor belt	24	0.36
Finished	21	0.36

It was found that samples in the filleting area (first and second filleting machine) all had less <0.3 MPN/g for both coliform and faecal coliform. Samples from the trimming area were higher in terms of MPN/g for coliform than in the filleting area. However, three samples were found to have <0.3MPN/g for faecal coliforms. Coliform and faecal coliforms in the mincing area were all in the same range as those in the trimming area. As for the Violet Red Bile Agar (VRBA) method, the CFU/g seems to increase at each stage of production. The filleting area had a very low number of presumptive coliforms in terms of CFU/g. Sample taken from the first machine was 0 CFU/g whereas the second machine had 14 and 1 CFU/g for the 0.5 ml and 0.1 ml of aliquots respectively (Table 2).

Table 2: Results for presumptive coliform.

Processing stage	CFU/g (0.5ml)	CFU/g (0.1 ml)
Filleting machine	0	0
	14	1
Conveyor belts	39	2
	28	16
	100	13
	70	12
Candling tables	1	5
	3	21
Container	20	14
Conveyor belt	70	8
Finished product	400	23

In the trimming area, coliform showed an increase up to ca.100 CFU/g and ca. 21 CFU/g for the 0.5ml and 0.1ml aliquots respectively (Table 2). The highest recorded value was from the conveyor belts that take the fish for mincing; ca. 28 to ca.100 CFU/g. The highest value for presumptive coliform was recorded in the mincing area particularly the final product or mince; ca. 400 CFU/g for the 0.5 ml aliquot (Table 2).

7 DISCUSION

A number of microbial tests of fish and fish products are used by the industry for contractual and internal purposes and by the authorities to check that the microbiological status is satisfactory. Microbial indicators are often employed to assess food safety and sanitation. In the historical use of safety indicators, however, the pathogens of concern were assumed to be of intestinal origin, resulting from either direct or indirect faecal contamination. Thus, sanitary indicators (such as coliforms) were used historically to detect faecal contamination of water and this practice was extended to foods (Jay 1992).

Analysis of swabs and fish samples, shows that in general, coliforms were already present at the first sampling point (de-heading) including all other sample steps in the processing establishment, with few exceptions (Figures 2). It is possible that contamination may have taken place before the fish was brought into the factory for processing. On the other hand, one could also argue that contamination of coliforms may have taken place in the processing establishment since some processing equipment also tested positive for coliforms.

That said, the results do not give a clear picture of where exactly before the receiving area or perhaps in the processing area contamination of coliforms took place or was likely to have taken place. Also, lack of data for coliform tests especially before the receiving area, makes it difficult to be certain as to whether contamination took place before or after the raw material was brought into the receiving area. However, it can be assumed that contamination took place before the raw material was brought into

the receiving and subsequently contaminating food contact surfaces of equipments especially those that tested positive for coliforms. In addition, one could also assume that contamination did take place in the processing area, as this is supported by other studies. According to Venugopal (2002) contamination of fish particularly by pathogens such as *Salmonella* sp., *Staphylococcus aureus*, *Campylobacter jejuni*, *Escherichia coli* 0157:H7, *Vibrio parahaemolyticus*, *Yersinia enterocolitica*, and *Listeria monocytogenes*, may occur prior to harvest, during capture, processing, distribution and/or storage. Huss *et al.* (2000) have pointed out that some pathogenic bacteria are naturally present in the aquatic (*Clostridium botulinum* type E, pathogenic *Vibrio* sp., *Aeromonas*) and the general environment (*C. botulinum*, type A and B, *Listeria monocytogenes*) and may therefore be found on live or raw fish. Studies done by Vogel *et al.* (2001) on *L. monocytogenes*, indicated that contamination occurred along the processing line. Other studies dealing with different processing operations have similarly concluded that the plant and processing environment is the source of product contamination rather than the raw material. However, this does not exclude the possibility that the raw fish or material is an important initial source for contaminating processing equipment and environment (Vogel *et al.* 2001). Also, water, like food, is a vehicle for the transmission of many agents of diseases (Kirby *et al.* 2003). However, the water used in Iceland is considered to be potable therefore the possibility that water may have acted as a vehicle of transmission can be ruled out. In this view, although other studies did not involve mainly testing for coliforms, the same sources and routes of contamination presented in the literature review can be linked to the presence of coliforms in the raw material and food contact surfaces. In this case, the presence of coliforms is most likely to have been contributed by transmission from foodhandlers, be it in the vessels, auction or in the processing establishment, including ice and tubs.

As for faecal coliforms the results, according to the microbiological criteria established between the processor and the customers, lie within specified limits (Table 1 and Appendix 3). Faecal coliform is a group that include *Citrobacter* sp., *Enterobacter* sp., *Klebsiella* sp., as well as *E.coli*. Some of these bacteria particularly *E.coli* are associated with faecal contamination.

In this study positive results for faecal coliforms were found in the processing area suggesting that these bacteria may have originated from foodhandlers and not from other warmblooded animals such as seabirds as they were absent. Beveridge (1988) and Fenlon (1983) demonstrated that some seabirds spread for example *Salmonella* and other human pathogens in the environment. That could be another possibility of transferring faecal coliforms to the raw material. The fact that tests for coliforms were positive in the trimming area rules out the possibility of faecal coliforms to have originated from seabirds. Thus, it is safe to say that positive results as well as low numbers of faecal coliforms found on fish samples and food contact surfaces in processing environment; filleting machine and conveyor belts before and underneath the trimming/candling table, were transferred to the fish by food handlers. It is possible that faecal coliforms were transmitted onto the raw material and subsequently to food contact surfaces and/or vice versa by hands. Many studies such as the one done by Montville *et al.* (2002) have similarly concluded that, during handling and preparation, bacteria may be transferred from contaminated hands of food workers to food and subsequently to other surfaces (including food contact surfaces). Snyder (1998) also found that low infectious doses from organisms such as *Shigella* and the

pathogen *E.coli* were linked to hands as a source of contamination. Other studies such as done by Reij *et al.* (2003), attributed poor hygiene, particularly deficient or absence of hand washing as the causative mode of transmission. Also, contamination of fish products through contaminated surfaces has been observed in many cases and unclean, insufficiently or inadequately cleaned processing equipment have been identified as a source of bacterial contamination in processed seafood (Reij *et al.* 2003). Containers, 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 (Morgan *et al.*1993, Evans *et al.*1996, Hennessy *et al.*1996, Llewellyn *et al.*1998). Also according to observations that were done, foodhandlers in the trimming area had placed their feet very close to the conveyor belts under the trimming tables making the transmission of faecal coliform possible. All the above may be possibilities, and there are always bacteria on the hands of foodhandlers, these bacteria may be transferred onto fish and subsequently to processing equipment through touching the raw material.

As for the quality system, particularly the GMP/GHP, which deals with matters pertaining to hygiene and sanitation, the processor has such a system in place as recommended by many organisations such as the Codex Alimentarius. The whole quality system has been summarised in what is termed as the own check system of the company which is a much simpler way of quality checking (Appendix 5). In this system there are a number of parameters which are controlled and monitored, including contamination which is the main subject of this study, by responsible personnel. It is believed that this is a good system and it is expected to work accordingly, by maintaining the level of microbial contamination at a minimum or within specifications established by the processor. Having such a system in place, problems such as unacceptable microbial level may arise if procedures laid out in the manuals for hygiene and sanitation are not adhered to from time to time by personnel. According to the results, microorganisms are being transferred onto the raw material and the food contact surfaces. The only way to avoid or eliminate these microorganisms is by applying proper cleaning and sanitation procedures. It is therefore safe to say that foodhandlers are not practicing proper hygiene and sanitation or perhaps they are ignorant. The quality system may be good but if personnel do not adhere to what is laid out in the quality manual, then problems of this nature are likely to take place. Also, if necessary, the knowledge of personnel with regard to contamination should be increased through training.

It may be very difficult to get rid of these microorganisms from fish products and processing equipments when they originate from foodhandlers (humans) who handle the fish from capture to packaging of finished products. For a processor to maintain the number below or within exactly specified limits a GMP/GHP needs to be applied properly to get good results.

8 CONCLUSION

The results, particularly for coliform, don't give a clear picture of where exactly before the receiving area and/or in the processing area the contamination of coliforms is likely to take place. However the most likely source of contamination is the foodhandlers as these are the only sources of coliform in contact with the raw

material. One can conclude that these bacteria were transferred by personnel to the raw material and subsequently to the food contact surfaces and/or vice versa. The same can be said about faecal coliform as to have originated from foodhandlers and subsequently transferred to the raw material.

The processor has a very good quality system in place. This system should work as expected and if not then this is because the personnel don't adhere to hygienic and sanitation procedures.

9 RECOMENDATIONS

Coliforms and faecal coliforms may be transferred onto the raw material as described in the literature review, however foodhandlers could be the most important source for contaminating the raw material and processing equipment. Since the presence of these microorganisms may give an impression of the hygienic conditions under which the plant is operating, to reduce or eliminate these microorganisms there is a need for proper application of hygiene and sanitation procedures. Also, if necessary, the knowledge of personnel with regard to contamination should be increased through training. However, it may be very difficult to consistently produce fish products with a number of microorganisms within the limits specified by the processor.

The processing establishment should not worry much about the problem of coliform and faecal coliform as these microorganisms are not hazards. In addition to that, normal cooking procedures will eliminate the risks from these organisms particularly pathogens leaving the safety concern to the consumption of raw shellfish or raw fish dishes such as sushi (Huss *et al.* 2000).

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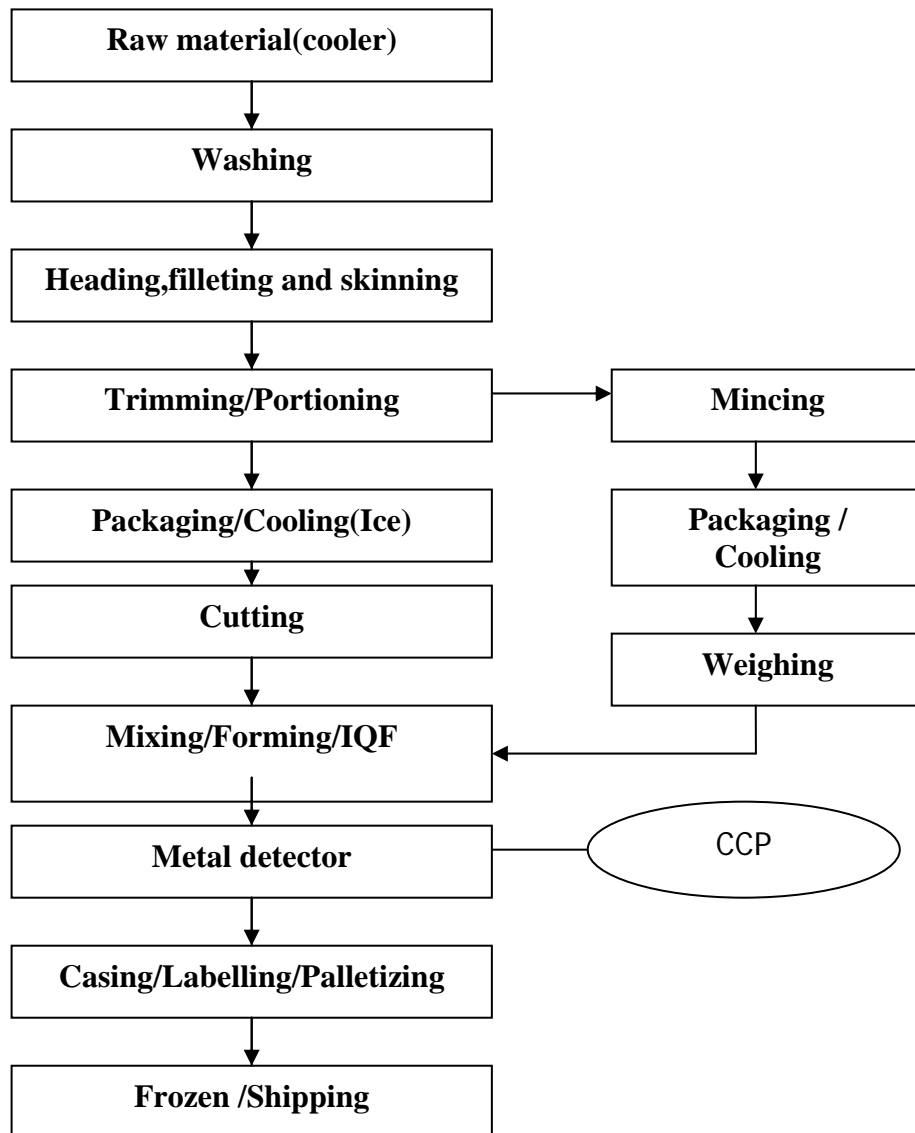
APPENDIX 1: A BRIEF DESCRIPTION OF PRODUCT MANUFACTURING AT ÚA.

The company obtains its raw material from fish trawlers as well as auctions. The fish is bled, gutted, washed properly and iced before transport to the factory. At the factory, the fish is kept in a cooler in the receiving area at 0-4°C in plastic boxes and totes. Before heading and size grading, the fish is emptied into a hopper with an incline steel conveyor belt and washed with a steady overflow of potable water.

The head cutting, filleting and removal of skin are done by commercial machines. The fillets are checked on a candling table for number of defects. All bones except pin bones, visible parasites, blood spots, bruises, skin spots as well as black membrane including any foreign objects are removed from fillets. The trimmed fillets are portioned either manually or automatically before they are quick frozen at -24°C. They are also sprayed with potable water for glazing percentage when necessary. The fish and fish products are weighed and packed directly into plastic bags, cartons and boxes according to specifications, in an area separate from where they are processed. Some of the fish are packed into plastic envelopes and eventually into cartons while blocks (fillets/piece/mince) are packed directly into block cartons. All finished products are labelled according to specifications and frozen in plate freezers for about 2.5 -3 hours or until the core temperature has reached a minimum temperature of -24°C. After freezing, the cartons are depanned and packed.

A complete cleaning and disinfecting procedure is carried out on all equipment, machines and conveyor belts at the end of each production session, fulfilling the requirements for Good Manufacturing Practices. Sodium hypochlorite is used as a sanitizing agent. Palletized products are kept in frozen storage at a minimum temperature of -24°C. They are taken for shipping directly from frozen storage to freezer container at -24°C.

APPENDIX 2: FLOW DIAGRAM FOR PROCESSING OF COD (*GADUS MORHUA*) (ÚA 1999).



**APPENDIX 3: MICROBIOLOGICAL GUIDELINE FOR RAW MATERIAL
(*GADUS MORHUA*) AND POTABLE WATER (ÚA 1999).**

	Good	Defective	Reject
Raw material			
TPC,30°C	<150,000	150,000 - 350,000	>350,000
Coliforms, MPN/g	<100	100 – 200	>200
<i>E.coli</i> , MPN/g	<0,3	0,3 – 4	>4
Staphylococcus / g	<10	10 – 100	>100
Listeria in 25 g	Absent		Present
Potable water			
Coliforms, MPN/100 ml	Absent		Present
<i>E.coli</i> ,MPN/100 ml	Absent		Present

APPENDIX 4: RESULTS FOR SWABS (1ST AND 2ND SAMPLING) TAKEN THROUGHOUT THE PROCESS.

Processing Stage	Coliform (BGLB-35°C)		Faecal Coliform (EC-45.5°C)	
	1 st sampling	2 nd sampling	1 st sampling	2 nd sampling
<i>De-heading</i>	1	+	-	-
	2	-	-	-
<i>Filleting machine</i>	1	+	-	-
	2	+	-	-
	3	+	*	-
	4	+	+	+
<i>Conveyor</i>	1	+	-	-
	2	+	-	-
<i>Before cutting</i>	1	+	-	-
	2	-	+	-
<i>Candling table</i>	1	+	-	-
	2	-	+	-
	3	+	+	-
	4	+	+	-
	5	+	+	-
	6	+	+	-
<i>Conveyor</i>	1	+	-	-
	2	+	-	-
	3	+	-	-
	4	+	+	+
<i>Collection</i>	1	+	-	-
	2	+	-	*
<i>Container</i>	1	-	-	-
<i>Knives</i>	1	+	-	-
	2	+	-	-
<i>Plastic door</i>	1	-	-	-
<i>Container</i>	1	-	-	-
	2	+	-	-
<i>Working table</i>	1	+	-	-
	2	+	-	-

* The LST broth leaked and therefore samples could not be incubated further.

APPENDIX 5: OWN CHECK SYSTEM OF THE COMPANY ÚA (ÚA 1999).

Production Stage	Monitoring frequency	Control measures	Control limits	Actions	Reference	Responsible person
Receiving area Overall quality of raw material	Temperature record. Use of ice, sensory evaluation (each lot)	Chilling of raw material on board vessels, General rules of handling on board.	Raw material assessed as acceptable or better. Ice visible. Temp. of raw material (0-4°C) no contamination.	Raw material chilled in iced water, decomposed and contaminated raw material destroyed.	Raw material purchasing rules.	Forman in reception, production manager and quality manager
Check labelling (traceability)	All containers labelled	Correct labelling of catch board vessel	None	Unlabelled raw material evaluated carefully.	Raw material purchasing rules.	Forman in reception, production manager
Chiller	Temperature of chiller monitored continuously	Raw material iced, chilled door closed all time, sanitation according to plan	Temp in raw material (0-4°C)	Raw material chilled in ice water, sensory evaluation, decomposed raw material destroyed.	Quality manual.	Forman in reception , production manager
Heading/Filleting/Skinning	Evaluation of 10 whole fish and 20 fillets	Handling and good conditions	Score of 3 or higher on a 5 point scale	Defects reported to the quality manager/production manager.	Specifications.	Quality controller, production manager/quality manager

APPENDIX 5: OWN CHECK SYSTEM OF THE COMPANY ÚA (ÚA 1999) CONTINUED.

Production Stage	Monitoring frequency	Control measures	Control limits	Actions	Reference	Responsible person
Trimming	Temp. of fillets checked every two hours.	Sanitation according to plan.	Temp. of raw material not above 4°C.	Defects reported to the quality manager/production manager	Code of practice.	production manager and quality manager
Trimming	Continuous check.	Tanning of staff.	According to specifications	Increase inspection, re-processing of raw material	Specifications.	quality controller, production manager and quality manager
Cutting/forming	Continuous monitoring of nuggets	Training staff. Making sure that enough forming material is in machine.	No misshapen nuggets. should not lie together.	Remove misshapen and pieces which lie together.	Quality manual.	quality manager and quality controller foreman in breading area
Batter and breading	Pick-up, viscosity checked every hour, breading defects 15 min	Training of staff, Adjust machinery. Sanitaion according to plan.	According to specifications.	Notify foreman in breading area who will adjust the machinery.	Quality manual.	quality controller, foreman in breading area

APPENDIX 5: OWN CHECK SYSTEM OF THE COMPANY ÚA (ÚA 1999) CONTINUED.

Production Stage	Monitoring frequency	Control measures	Control limits	Actions	Reference	Responsible person
Prefrying	Temperature and time, every hour. Color of product every 15 min.	Check oil level, circulation and temperature before using the fryer.	Temp 195-200 °C, golden colour	Notify foreman in breading area to adjust machine.	Specifications.	Quality controller, foreman in breading area.
IQF	Continuous monitoring of IQF/Every two hours.	Keep door closed	Temp < -18°C	Slow down IQF, increase cooling.	Quality manual.	Quality controller, foreman in breading area
Glazing	Everyday for each production number	Training of staff check temperature of water used	According to inspection	Notify foreman to adjust temp. of water	Quality manual.	Quality controller, foreman in freezing area
Packing	Every hour	Time in freezer correct	Temp. in product not higher than -18°C when placed in cold store	Adjust temp of flow and temp. of freezer.	Quality manual.	Quality controller
Weighing	Bags/case/block and case closure(Every hour)	Training staff Grader correctly adjusted	According to specifications	Calibrate scale	Specification.	Quality controller production manager and quality manager
Metal detector	Continuously	Calibrated three times/day with >2mm silver metal	>2 mm in length	Report to the foreman or quality manager	Quality manual.	Quality controller

APPENDIX 5: OWN CHECK SYSTEM OF THE COMPANY ÚA (ÚA 1999) CONTINUED.

Production Stage	Monitoring frequency	Control measures	Control limits	Actions	Reference	Responsible person
Labelling	Labels inspected on each product number.	Adjust printer, check the info on label and compare them to inspectec and ISI production almanac	None	Labelling not correct then re-label all packs.	Specification.	Quality controller.
Cold store	Continuous recording of temp. in cold store.	Temp. <-25 °C Limit time doors are opened. De-ice regularly.	Temp <-18°C	Adjust temp. in cold store.	Quality manual.	Foreman at cold store.
Transport		Temp <-25 °C limit time doors are open. Ice regularly.	Temp of product <-20 °C	Evaluate the fitness of product for transport with transport company	Quality manual, Transport company code of practice	Foreman at cold store, Production manager.