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EFFICIENCY OF CLEANING AND DISINFECTION ON FISH CONTACT SURFACES

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ABSTRACT

Clean and disinfected fish contact surfaces are of the utmost importance in the fishing industry to control the risk of microbiological contamination in the processing line. This can be obtained by using different cleaning and sanitizing techniques. By sampling surfaces in a fish processing company and testing different washing protocols on three surface materials (stainless steel, electropolished stainless steel and plastic cutting board) the efficiency of cleaning and disinfection on fish contact surfaces has been studied. Surfaces in a factory were sampled during the processing of fish and after cleaning of the factory. The three test surfaces were fouled with juice from minced cod fillets. In order to study the effects of washing practises combined contact times of detergent and sanitizer were used as different washing protocols for the test surfaces. Most bacteria were removed from the surfaces (except from the plastic cutting board) when long contact times were used for the detergent and the sanitizer. The combination of short and long contact time for the detergent and sanitizer cleaned the plastic cutting board better. By testing the different disinfecting procedures on different surface types it was possible to examine how these parameters influence the hygienic results. The results of the study clearly demonstrate the importance of proper washing practises to ensure efficient decontamination of fish processing surfaces.

Keywords: cleaning protocols, surface types, sanitizers, hygiene monitoring, fish processing Factory

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1 INTRODUCTION

Suriname, South America, is a nation with an area of 163,820 km^2 and roughly 520,000 inhabitants. Fisheries is a very important sector because fish and fish products are among the most important export products, with high export value. In 2005 the export of fish was approximately 12000 tonnes (live weight) and in 2007 Suriname had an estimated capture production of 30,000 tonnes (FAO 2008). The fishing industry converts raw materials to end products or semi-manufactured products that are mainly exported. As everywhere else, the hygiene demands are high and well monitored by authorities in order to continue and expand the exports of fishery products to the international market and contribute to the national budget.

A small fish processing plant in Suriname has been dealing with certain hygiene problems. It is a company that does not have lots of machinery compared to common fish factories in Iceland. It produces fresh fish on ice and frozen fish for the international as well as the local market. The main products that are being produced are whole fish, fillets/loins and steak. The species that are mostly processed are yellow fin tuna, mahimahi, wahoo, snappers, weakfishes and butterfishes. The walls, ceilings and doors of the factory are made of easy-to-clean material with the disadvantage that the doors and walls are not corrosive-resistant. The floors are made of concrete. The company is not very advanced and lots of equipment that the company has is not being used. The equipment used for processing are two saws/slicer, two weighing tables, one scale and two vacuum machines. Occasionally a mincer, a mixer and an apparatus for the production of burgers are used to make fish burgers and fish nuggets. In the processing line for tuna there are many tables. Most of them are made of stainless steel and some have polyethylene cutting board surfaces. Utensils that are being used for the processing of fish are knifes, fish tubs, fish containers, fish baskets, ice tubs and spades. The used equipment does not have a complex design and can be easily dismantled for the cleaning procedure. The surfaces of the machinery are smooth. The surfaces of the polyethylene cutting board tables on the other hand are not smooth anymore because of the high usage and the fact that such surfaces easily wear out. After each processing day the plant is cleaned with detergent, chlorine and foaming agents. The foaming agents are products from Johnson Diversy and include chlor substance, sanitizer based on quaternary ammonium and Iodine based detergent and disinfectant. The cleaning and disinfecting of the company is done by the workers after they are finished with the processing of fish. They are supposed to clean/rinse the processing area also before lunch, but this isn't always the case. The company uses only potable water. The water is chlorinated every morning.

The hygiene on the cleaning and disinfecting of the processing company is being monitored monthly by the food authority. This is done using swabs, agar plates pressed directly onto the test surface (RODAC) and adenosine triphosphate (ATP) method on surfaces that come in contact with fish or fish products. The results of these monitoring do not always pass set standards and criteria of hygiene, because sometimes some control points show high microbial counts. This is probably caused by the late working hours of the workers on some days or by not implementing the cleaning and disinfecting method in the right way. Some control points, such as the table in the reception area, plastic baskets and the scale, frequently give bad test results. According to the supplier of the foaming agents other users do get good test results. This issue is a problem since the foaming agents are expensive and the inspecting authority might take strict steps when the results remain bad.

2 AIM OF THE STUDY

The goal of this study was to determine the level of surface contamination in a fish processing factory and to evaluate the efficiency of different disinfecting procedures on different surface types in order to further understand the effectiveness of cleaning procedures to reduce contamination hazards in the fish processing plant in Suriname.

The objectives are to:

a. Test for bacteria on fish contact surfaces in the fish processing factory.
b. Test different contact times of detergent and sanitizer on different surfaces.

3 LITERATURE REVIEW

3.1 Hygiene and the processing industry

The attachment of bacteria with subsequent development of biofilms in food processing environments is a potential source of contamination of finished product that may shorten the shelf life or increase transmission of diseases. Cleaning-in-place (CIP) is the most commonly used practice for cleaning and sanitizing food processing plants. However, even after the use of generally acceptable cleaning processes, soil residues and microorganisms remain on the contact surfaces. These microorganisms can survive, grow, and multiply resulting in formation of invisible films (biofilms). Aformed biofilm is very difficult to remove, because microorganisms within biofilm become resistant to heat and antibacterial agents, including sanitizers (Sharma and Anand 2002).

There are various reasons for inadequate cleaning and disinfection such as not including surfaces in the cleaning programme (usually hand contact surfaces), non-validated cleaning protocol, a work culture that is not aware of the importance of cleaning, failure to monitor cleaning or failure to implement cleaning appropriately (Griffith 2005). In order not to affect the human health negatively it is necessary to ensure the safety and quality of food that is being produced. Regulations and directives on hygiene require a high level of sanitary conditions in food processing plants (Duong 2005). As for quality assurance, methods such as the Good Manufacturing Practices (GMP) / Good Hygiene Practices (GHP) and Hazard Analysis and Critical Control Point (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. This is especially important for the fish processing industry since fish is a product that gets easily contaminated or spoiled. The hygiene in the industry is usually guaranteed by cleaning and disinfecting the processing plant such as the working area, processing equipment and fish contact surfaces. There are different methods for cleaning and disinfecting a processing plant. Assessment of microorganisms on surfaces is important in order to determine the most effective cleaning and sanitizing protocols (Verran and Whitehead 2006a). Although food producers usually use excess levels of disinfectant typically 20% above the recommended dosage, the application of chemical disinfectants in food industries has been shown to be inadequate in terms of total aerobic heterotrophic bacteria counts on the food contact surfaces (Duong 2005). However, the

efficiency of the cleaning process will not only depend on the optimization of the process and the equipment design, but also on the characteristics of the soiled surface.

3.2 Surface characteristics

The presence of bacteria on surfaces is commonplace in the food industry and can be considered an important source of potential contamination for any food, leading to economic and hygienic problems. The type and the degree of surface roughness affects fouling. The wearing of a surface has an obvious effect on its cleanability since attached microorganisms may be protected in grooves and pits created during wear from chemicals and shear forces that are used in cleaning regimes. The area of contact between a microorganism and the surface influences the rate and pattern of cell retention. Thus surface roughness may enhance microbial retention because of the increased surface area available for colonisation by increasing the microorganism/material interface. The surface roughness will therefore affect the total binding energy between the bacterium and the substrate (Verran and Whitehead 2006b). All contact surfaces of food must be easy to clean and must therefore be smooth (Lelieveld *et al.* 2003). A stainless steel surface is preferable for food equipment and is specified in many industry and regulatory design and construction standards (Schmidt 2009). For the electrocleaning or electropolishing (also called chemical machining or reverse plating) of stainless steel, an electric current is used to corrode a minute surface of the steel. By doing so the stainless steel surface gets cleaned of embedded iron, heat tint and the smeared layer that are left by mechanical cleaning operations. This process also smoothens the surface (Tuthill 1994). The following stainless steel surfaces were compared in a study by Arnold *et al.* 2001; glass-beaded, electropolished, acid-dipped, steel-ball burnished, sandblasted or left untreated. These treated surfaces were incubated with bacteria from chicken carcass rinses. It was found that electropolished stainless steel showed fewer bacteria and less biofilm formation than the other surfaces.

3.3 Specific Types of Chemical Sanitizers

There are chemicals that are approved by Food and Drug Administration, FDA, for use as no-rinse, food-contact surface sanitizers. In food-handling operations, these are used as rinses, sprayed onto surfaces, or circulated through equipment in CIP operations. In certain applications the chemicals are foamed on a surface or fogged into the air to reduce airborne contamination. Some of these chemicals are chlorine-based sanitizers, iodine and quaternary ammonium compounds (QACs) (Schmidt 2009).

3.3.1 Chlorine-based Sanitizers

Chlorine, in its various forms, is the most commonly used sanitizer in food processing and handling applications. Commonly used chlorine compounds include liquid chlorine, hypochlorites, inorganic chloramines, and organic chloramines. Chlorine is active at low temperature, is relatively cheap, and leaves minimal residue or film on surfaces. The major disadvantage of chlorine compound is corrosiveness of many metal surfaces (especially at higher temperatures). Health and safety concerns can occur due to skin irritation and mucous membrane damage in confined areas. At low pH (below 4.0), deadly Cl2 (mustard gas) can form. In recent years, concerns have also been raised about the use of chlorine as a drinking water disinfectant and as an antimicrobial with direct food contact (meat, poultry and shellfish). This concern is based upon the involvement of chlorine in the formation of potentially carcinogenic trihalomethanes (THMs) under appropriate conditions. While

chlorine's benefits as a sanitizer far outweigh the risks, its use is under scrutiny (Schmidt 2009).

3.3.2 Iodine

This sanitizer exists in many forms and usually exists with a surfactant as a carrier. These mixtures are termed iodophors. The most active agent is the dissociated free iodine (also less stable). The bactericidal activity of iodine is through cell wall damage and destruction of microbial enzyme activity. Iodophors, like chlorine compounds, have a very broad spectrum, being active against bacteria, viruses, yeasts, moulds, fungi, and protozoans (Lelieveld *et al.* 2003).

3.3.3 Quaternary Ammonium Compounds (QACs)

QACs or QUATS are active and stable over a broad temperature range. Because they are surfactants, they possess some detergency. Thus, they are less affected by light soil than are other sanitizers. However, heavy soil dramatically decreases activity. QACs generally have higher activity at alkaline pH. An advantage of QACs in some applications is that they leave a residual antimicrobial film. This means that they stay active on the cleaned surface for a day or so, which discourages further bacterial growth. However, this would be a disadvantage in operations such as cultured dairy products, cheese, beer, etc. where microbial starter cultures are used (Keener 2005, Torry Research Station 2012).

3.4 Evaluating cleaning effectiveness

There are factors that strongly influence the cleaning and disinfection such as the physiological conditions, types and numbers of the organisms that contaminate the seafood environment, microbiological response to cleaning and disinfection, and the type and amount of soil present. Microbiological sampling and enumeration of bacteria on seafood contact surfaces, non-contact surfaces, and seafood products coupled with an auditing system, is of vital importance for HACCP systems to evaluate and record the microbiological condition of seafood and contact surfaces. The simple small plastic plates filled with general purpose agar media in the form of direct surface plates (RODAC), bioluminometric assay of ATP, and the cotton swab methods are used to assess microbiology of the facilities in the seafood processing plant. The limitations of the impression plate techniques have been extensively emphasized, and this method though readily available to non-microbiologists, provides an indication after 24-72 hours of incubation. In order to have a glimpse and know without glitch that seafood is processed safely, the best indicator is the total viable bacteria count (TVC) of the seafood. Every seafood product, depending on species, has a slightly different count. However, when the total viable count starts to increase over a period of time, there must be a reason for it and it must be found (Marriott and Gravani 2006).

3.4.1 Sampling methods

Swabbing is a widely used sampling method, but it lacks the standardisation required to provide the level of required reproducibility. The efficiency of swabbing is reliant on the efficiency of the individual carrying out the procedure in three areas: the removal of bacteria from the surface, the removal of bacteria from the swab, and cultivation of bacteria. In addition, the properties of the surface (topography, wettability, porosity, etc.), and the presence of organic material on the surface can affect the efficiency of swabbing. It should

perhaps also be routine to check the surface after swabbing for residual microorganisms. Alternative or supplementary methods are many, but all have their limitations. Contact plates, such as RODAC, are more successful if selective culture media are used for particular indicator microorganisms on a surface. If surfaces are rough or wet, then the sampling is inaccurate, or resultant growth on the agar may be confluent (Verran *et al.* 2010). This technique is only applicable on lightly contaminated surfaces, because it is not possible to make dilutions from the plates (Marriott and Gravani 2006). Indicators for surface hygiene include ATP bioluminescence sampling, which requires swabbing, and ultra-violet light irradiation, which enables a simple visual assessment of gross soiling. Neither method discriminates between soil and microorganisms, but the presence of microorganisms raises ATP readings considerably and a consistently low ATP level in the final rinse is no guarantee of absence of residual soil. It is highly likely that in the food engineering plant microorganisms will be present on the surface alongside organic material. This material can affect the efficiency of cleaning and disinfection protocols, and can also provide nutrient, or protection for microorganisms. The most common method relies on removal of the cells from the surface, by swabbing or agitation, plating onto culture media, and counting the number of colonies obtained. However, the surface should always be subsequently examined for residual cells. Low numbers of colonies are deemed indicative of effective cleaning (i.e. few cells on the surface), but they could also indicate that cells have not been removed from the surface. Swabbing efficiency can be affected by moisture at the surface, presence of organic material, surface topography, and presence of antimicrobial compounds. It is important to be aware of the limitations of a given method for assessing the presence of microorganisms on a surface, as well as of the intended antimicrobial property of the surface or agent applied to the surface (Hasting 2005, Verran *et al.* 2010).

4 METHODS

The research consisted of measuring the cleaning efficiency and hygiene of fish contact surfaces in a fish processing factory (Eskja) and of test surfaces in the laboratory. For the laboratory study, surface types that are usually to be found in the fish industry were chosen.

4.1 Hygiene monitoring in a fish processing factory

Samples were collected from various surfaces in the fish processing factory. Swabbing, ATP-bioluminescence and direct contact plates (RODAC) were the monitoring procedures used for checking the hygiene condition on the selected surfaces. Samples were taken from fish contact surfaces in the factory (see Table 1) during the processing of fish and after cleaning (early morning before processing started). The sampling numbers in Table 1 reflect the flow of fish through the processing area.

Of the sampling points, ten samples were analysed by swabbing, 5 by ATP bioluminescence and 15 with RODAC plates (Table 1). For the swab samples, approximately 50 cm^2 of the surface to be tested was swept with a sterile cotton swab that had been moistened in a D/E neutralizer (Difco, Detroit, Michigan, USA). After swabbing the swab heads were broken off into sterile plastic bottles. Five ml of saline buffer (MRD diluent) was added to the cotton swab heads in the bottles. The bottles were shaken by hand in order to release the microbial cells into the buffer. From these solutions inoculations were done on Long and Hammers agar plates (LH) and Plate Count Agar plates (PCA). These types of agar were chosen because PCA is normally used for the general microbiological counts on plates and LH is specifically

used for microbiological analysis in the fish industry. For each sample, tenfold dilutions (10^0 - 10^{-4}) were prepared in order to be able to count the colonies that were going to be formed on the agar plates. The LH and the PCA plates were incubated at 17°C for 5 days. After incubation the generated colonies on these plates were counted. Only the plates from the dilution showing colony numbers 25 – 250 were chosen to be counted, unless all plates had colonies between 0 – 25.

Table 1: Table of control points that were sampled

No.	Control points in Eskja[1]	Area/Equipment	Swabs	ATP	RODAC
1	Fish tub	Cooler	X		X
2	Shovel	Reception/cooler	X	X	X
3	Cutting board	Reception/gutting	X		X
4	Knife	Reception	X	X	X
5	Steel surface	Reception/grader	X		X
6	Steel surface	Raw material insertion into proc. line			X
7	Steel surface – as electropolished	Filleting machine			X
8	Plastic surface	Filleting machine	X		X
9	Steel surface	Liquid ice cooler		X	X
10	Plastic surface	Combined blast cooler (CBC) before		X	X
11	Steel surface	CBC before	X		X
12	Textured steel surface	CBC after			X
13	Normal steel surface	CBC after	X		X
14	Cutting board	Trimming	X	X	X
15	Plastic surface	Grader/Packaging	X		X

[1] Exact sampling position are shown in Figure 1

For the ATP method, surfaces were also swabbed, but with cotton surface test swabs (Ultrasnap TM) specifically designed for the luminometer (Hygiena System SURE II, Hygiena International Limited, UK). After releasing the luciferin/luciferase mixture (attached on top of the swab) into the ATP on the cotton, the swab and the mixture were shaken and then inserted in the luminometer to measure the ATP level on site.

The RODAC method uses RODAC-plates that were pressed directly on the surfaces. These plates were then incubated at 22°C for 3 days and the colonies on these plates were also counted.

4.2 Cleaning various surfaces – laboratory study

The efficiency of different cleaning procedures was examined on 3 types of test surfaces that are usually found in fish processing companies. The surfaces used for this study were untreated stainless steel (SS), electropolished stainless steel (ES) and plastic cutting board (CB) coupons of 13 cm x 2.5 cm (32.5 cm^2). These coupons had undergone some preparation procedures before using them for the tests. They were kept in 0.5 M NaOH for 24 hours and then rinsed with deionised water. Afterwards the stainless steel coupons were kept in acetone for 1 hour while the plastic coupons were kept in ethanol for 30 minutes for the removal of fat. The coupons were rinsed again with deionised water and left to dry in the air. The clean coupons were then placed in sterile glass tubes and further sterilised in the autoclave for 15 minutes at a temperature of 121°C.

To simulate the processing conditions in a fish factory, the coupons were fouled with fish juice of cod (*Gadus morhua*). Cod fillets (*G.morhua*) were minced with a blender (Waring Commercial Laboratory Blender) and the minced meat was stored frozen at -18°C until it was used for the experiment. Fish juice was prepared by mixing the minced fillets with water using the ratio 1:4. Mixing was carried out by putting the mixture in a stomacher (Seward Lab System Stomacher 400) for 30 seconds. Biofilm on the surfaces was formed by pipetting the fish juice into the sterile tubes with coupons and incubate it by agitating for 48 hours at ca 70 rpm (HS 250 basic IKA Labortechnik).

The coupons were hung on a metal rack that was fastened by the faucet at the sink (Figure 1). The rack was divided into two sections (by placing a sheet of alumina foil in the middle) in order to carry out two protocols. The fouled coupons were cleaned and sanitised by rinsing them first with water, cleaned with 5% chlorinated alkaline foam cleaner, TK-Oxogel (Tandur hf.) and sanitised with 1% terminal disinfectant containing quaternary ammonium compounds, TS Sanitizer (Tandur hf.). Cleaning efficiency was tested as a function of contact time of both the detergent and the sanitizer by applying 5 protocols with combination of short (S, 5 min.) and longer recommended (L, 15 min.) contact time (Table 2). In protocol 1 (RW) the surfaces were just rinsed with water and this served as a control or practical reference standard for the dirty surface in this experiment. Protocol 2 (LL) was for the longest contact time of detergent and sanitizer. Protocol 3 (LS) represented a long contact time for the detergent and a short contact time for the sanitizer and vice versa for protocol 4 (SL). The shortest contact time for both detergent and sanitizer were in protocol 5 (SS). The rinsing and cleaning procedures were performed with spray bottles. This was carried out by spraying the replicates 10 times horizontally all together followed by 5 times vertically per coupon with water (rinsing). The replicates were sprayed 15 times horizontally all together with detergent after rinsing. When the contact time of the detergent had been set, the coupons were rinsed with water by following the same procedure for rinsing. Next the coupons were sprayed with sanitizer in the same way as it had been done with the detergent, and the cleaning procedure was finished by rinsing again with water after the contact time for the sanitizer had been set. Surfaces were tested after they were rinsed with water and after they were sanitised according to the different protocols. Swabs were taken from the cleaned surfaces by swabbing the surface area below the insertion hole of the coupon. The swab samples got treated as described in section 4.1 with the difference that inoculation were only done on Long and Hammers agar plates (LH) and colonies of these surfaces were also counted. For the stainless steel test surfaces, 3 replicates were done, but for the plastic coupons only 2 replicates were done due to shortage of these coupons.

5 RESULTS

5.1 Orientation of the fish processing factory

The sampling area for monitoring the hygiene condition of fish contact surfaces in the factory were the cooler, reception area, processing and packaging area. Following the process flow in the fish factory, starting from the cooler to the packaging line, resulted in the sampling order of no. 1 to no. 15 (Figures 2 and 3).

Figure 1: Experiment set up for cleaning and swabbing of test surfaces. From right to left: stopwatch, glass tubes with fouled coupons, spray bottles (water, detergent, sanitizer), rack with to-be-cleaned coupons (triplicate), cleaned coupons to be swabbed and bottles for swab heads.

Table 2: Protocols with two contact times

Protocol	Detergent	Sanitizer	Combination
1	*	*	RW
2	L	L	LL
3	L	S	LS
4	S	L	SL
5	S	S	SS

*= just rinse with water; L = 15 min.; S = 5 min.

The fish tub, shovel, cutting board from the reception area, steel and plastic surface of the filleting machine, textured steel located after the blast cooler, cutting board of the trimming area, and the plastic surface of the grader presented too much bacterial colonies on the RODAC plates, which made them uncountable (Table 3). The RODAC plates for the fish tub and shovel were also uncountable after cleaning. Before cleaning, the steel surface in the reception area and the plastic surface in front of the blast cooler showed higher total viable counts (TVC) than the other 13 control points. The steel surfaces of the liquid ice cooler and of the front of the blast cooler showed the lowest results. Except for the fish tub, shovel and steel surfaces in the reception area, all the control points had a TVC of 0 after cleaning.

ATP-readings were done for five control points, which were selected according to the frequent use of certain tools (shovel and knife) and surfaces that could be related to the test surfaces in the laboratory experiments. Of these control points the shovel had the highest ATP-level, 35 RLU before cleaning and 107 RLU after cleaning (Figure 4). The knife, steel surface of the liquid ice cooler and the plastic surface of the blast cooler had an ATP-reading of 0 RLU after cleaning. For the cutting board in the trimming area this was 1 RLU. These readings (even before cleaning) were below the suggested value of 500 RLU for clean surfaces (Griffith 2005), which means that according to the ATP-readings the five control points were acceptable.

Inoculation of bacteria from the sample points onto PCA plates showed that the fish tub and shovel had the highest TVC (Figure 5). The PCA plates from the fish tub after cleaning were uncountable, because the tub had not been cleaned. This tub was in the cooler and contained fish in ice water. After cleaning, the fish tub, shovel and cutting board (gutting) had high TVC while the rest contained either 0 CFU/cm^2 or 1 CFU/cm^2.

Just as was the case with the measurements from the PCA plates, total viable counts on LH plates also resulted in the fish tub and the shovel having the highest number of colony counts (Figure 6). The LH plates of these control points after cleaning were uncountable because there were too many colonies (>250) on them. After cleaning the cutting board had 15 CFU/cm^2, the plastic surface of the grader had 1 CFU/cm^2 while the other control points had no bacterial colonies on the plates.

When comparing the PCA measurements with the LH measurements (Figure 7) it could be seen that before cleaning there were higher bacterial counts on the fish tub, plastic surface on the filleting machine, the normal steel (CBC after), and the cutting board (trimming) on the LH plates than on the PCA plates. The fish tub that was sampled after 'cleaning' gave uncountable plates for both methods. The knife, cutting board (trimming) and plastic surface (grader) gave a count of zero after cleaning for both methods.

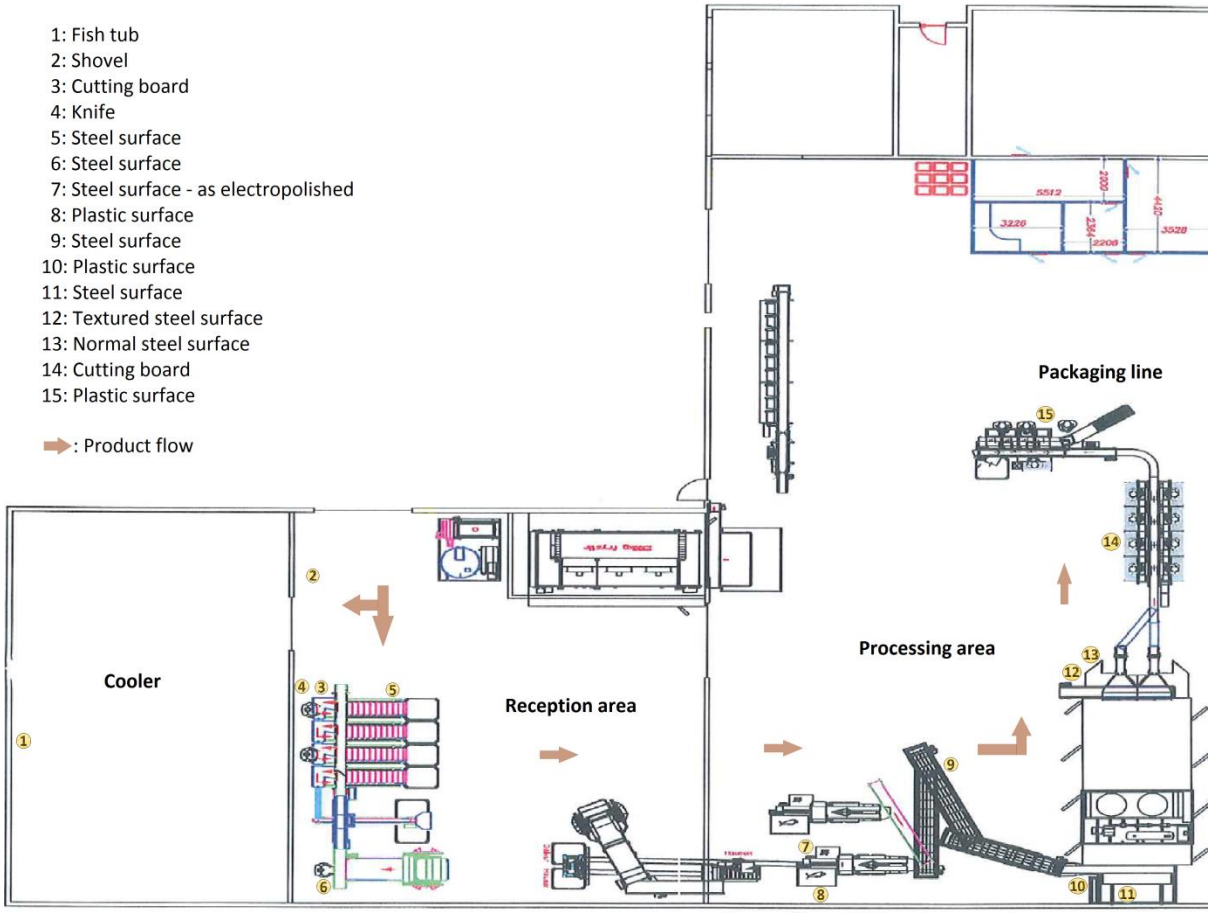

Figure 2: Control points (1-15) that had been monitored at Eskja

5.2 Cleaning efficiency on various surfaces

Cleaning stainless steel surfaces with the longest contact time of detergent and sanitizer (LL) resulted in the least total viable count with an average of 0.48 log 10 CFU/ cm^2. The shortest contact time for detergent and sanitizer (SS) gave the highest number of bacteria left on the surfaces (Figure 8) with an average microbial count of 2.45 log 10 CFU/cm^2. With the different cleaning protocols it was possible to remove most of the bacteria that was present on the stainless steel test surfaces.

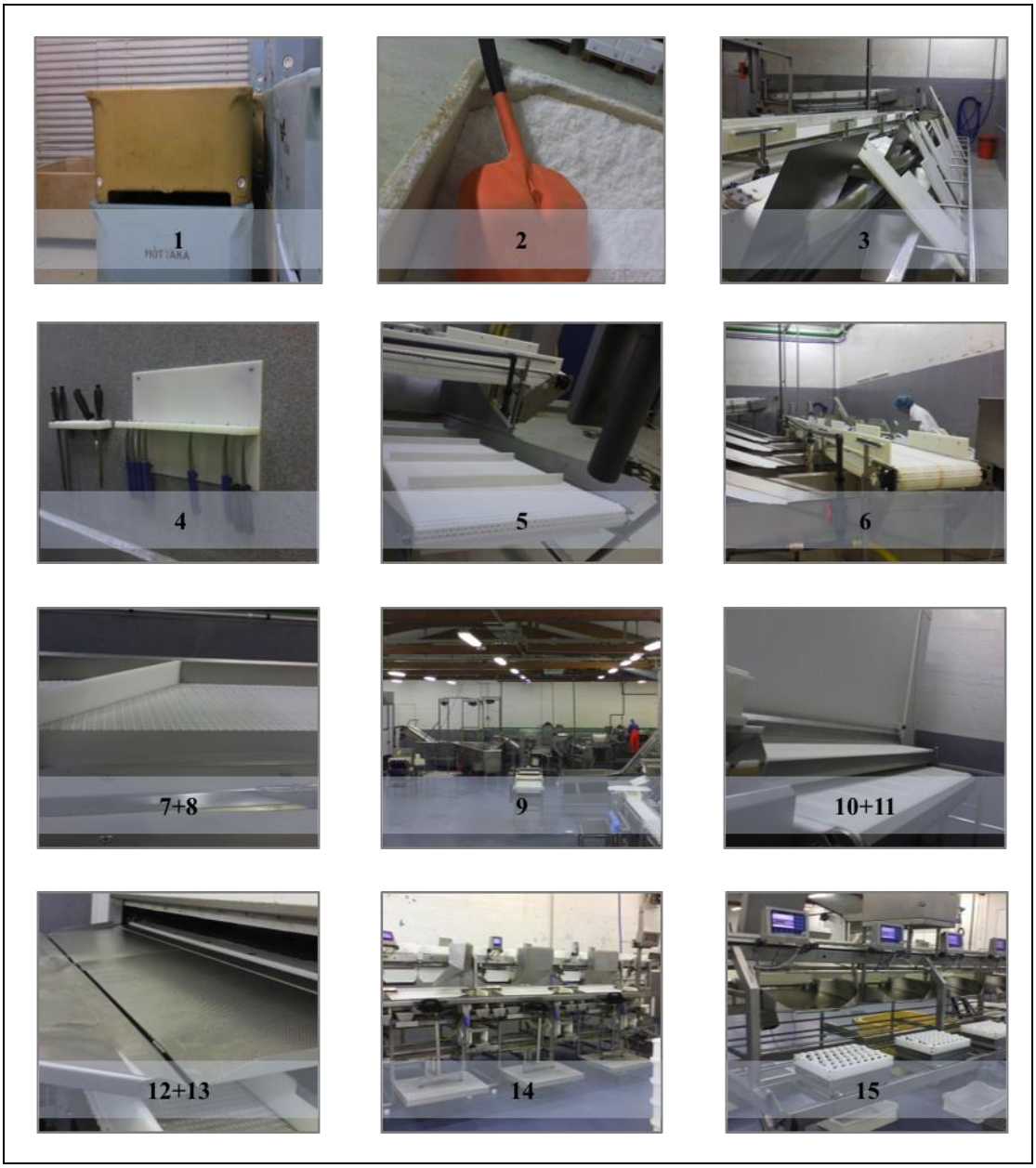

Figure 3: Surfaces of control points (1-15) that had been sampled

The cleaning and sanitizing of electropolished stainless steel coupons with different contact time for detergent and sanitizer showed that the LL protocol had the least bacterial counts of 0.86 log 10 CFU/cm^2 (Figure 9). The protocols LS and SL seemed to leave about the same amount of bacteria on the surfaces, 2.80 log 10 CFU/cm^2 and 2.52 log 10 CFU/cm^2 respectively. Inoculation of bacteria from the SS protocol with a dilution of 10^{-3} resulted in uncountable plates.

Table 3: Total viable counts of RODAC plates from the fish factory before and after cleaning

No.	Control points	TVC_{before} (CFU/plate)	TVC_{after} (CFU/plate)
1	Fish tub	>250	>250
2	Shovel	>250	>250
3	Cutting board (reception area)	>250	0
4	Knife	91	0
5	Steel surface(reception/grader)	54	4
6	Steel surface (reception)	109	9
7	Steel surface - as electropolished	>250	0
8	Plastic surface (filleting mach.)	>250	0
9	Steel surface (liquid ice cooler)	22	0
10	Plastic surface (CBC before)	151	0
11	Steel surface (CBC before)	38	0
12	Textured steel (CBC after)	>250	0
13	Normal steel (CBC after)	51	0
14	Cutting board (trimming)	>250	0
15	Plastic surface (grader)	>250	0

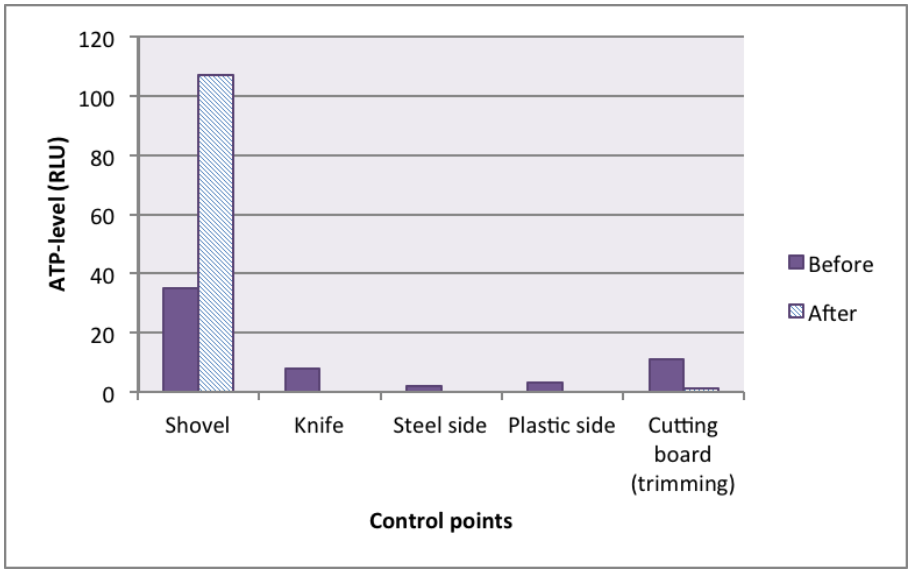

Figure 4: ATP-readings for five control points at the fish factory

Comparing the four cleaning protocols on plastic surfaces (Figure 10) showed that protocol LL was not the most effective cleaning method in this experiment. The cleaning protocols resulted in leaving the least amount of bacteria (1.45 log 10 CFU/cm^2) on the cutting board surfaces for the LS protocol and the most for the SS protocol (4.03 log 10 CFU/cm^2).

Comparing the data from Figure 8 and 9, it turns out that the electropolished stainless steel surfaces had higher microbial counts than the stainless steel surfaces. To confirm this, another experiment was carried out by testing the cleanliness of both surface types at the same time with the LL protocol. Here, there were higher bacterial counts on the electropolished stainless steel surfaces than the stainless steel surfaces before cleaning. After

cleaning about 72% of the bacteria had been removed from the ES while 55% still remained on the SS (Figure 11).

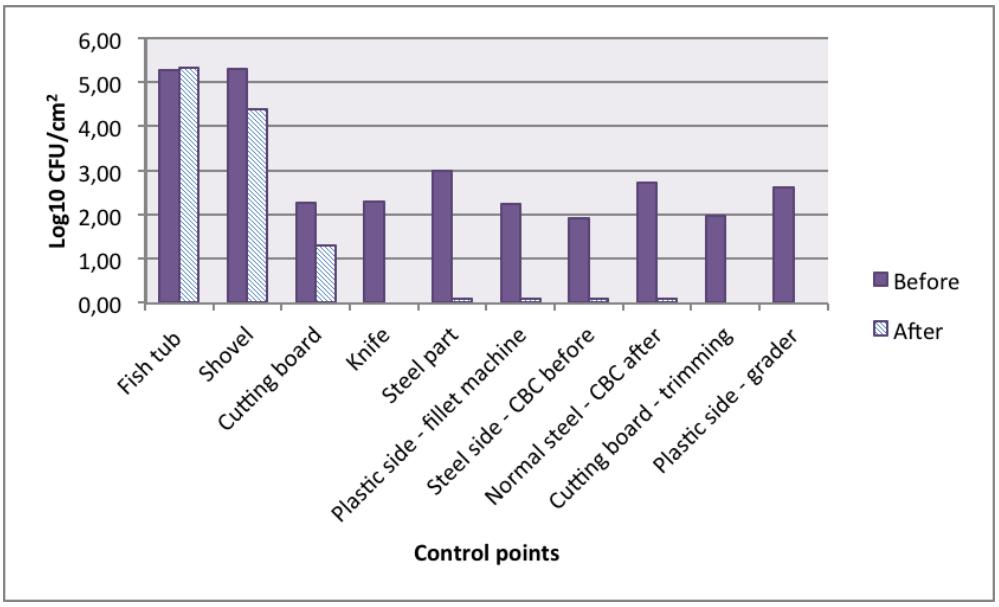

Figure 5: PCA measures from the fish factory

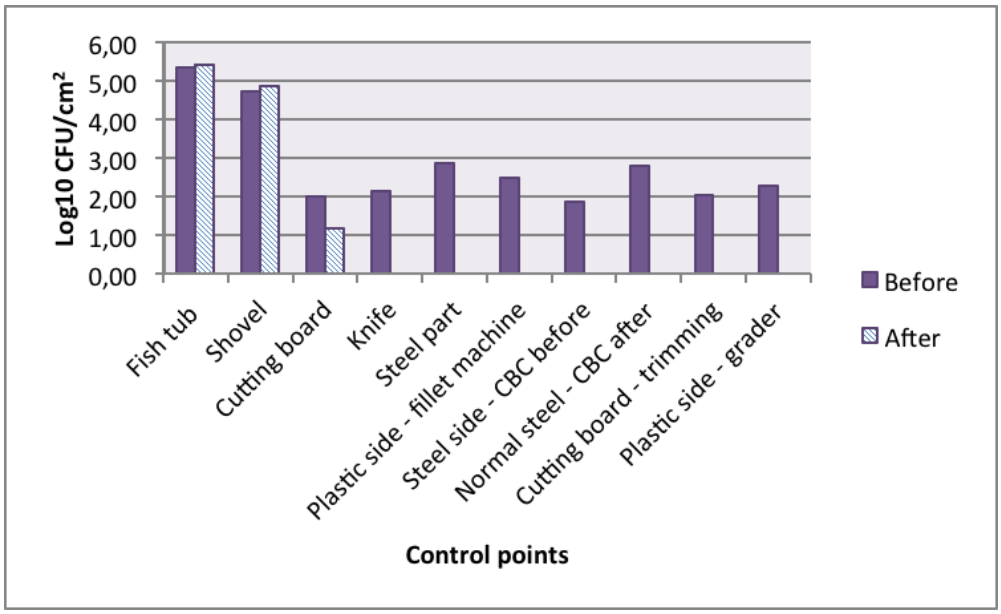

Figure 6: LH measures from the fish factory

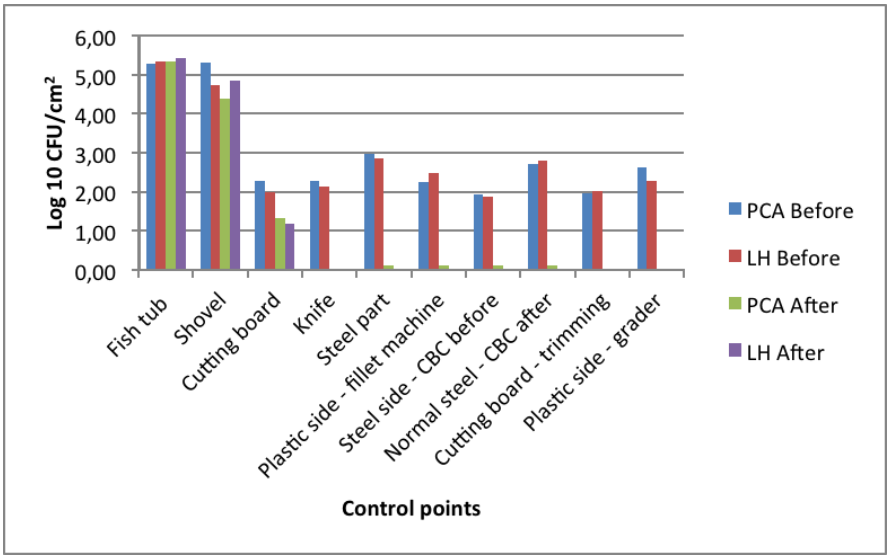

Figure 7: Comparison of the PCA and LH measurements in the fish factory

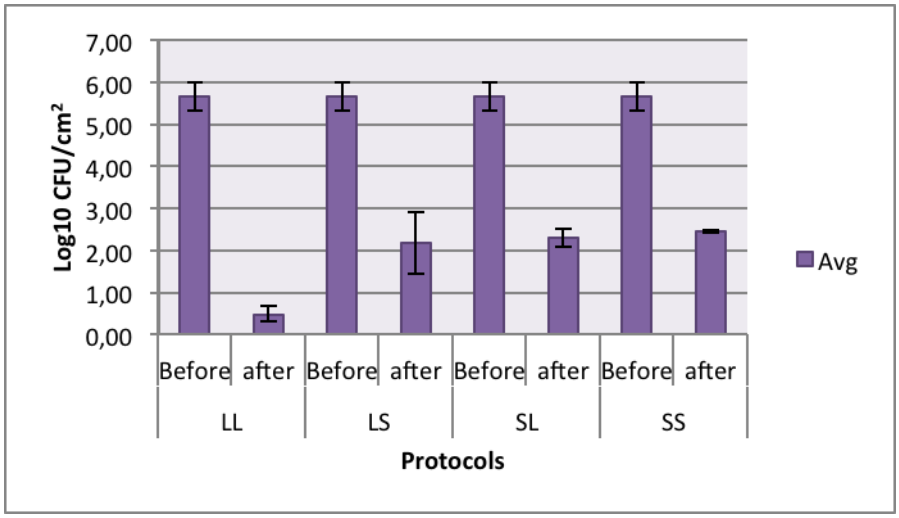

Figure 8: The average total viable count on stainless steel surfaces

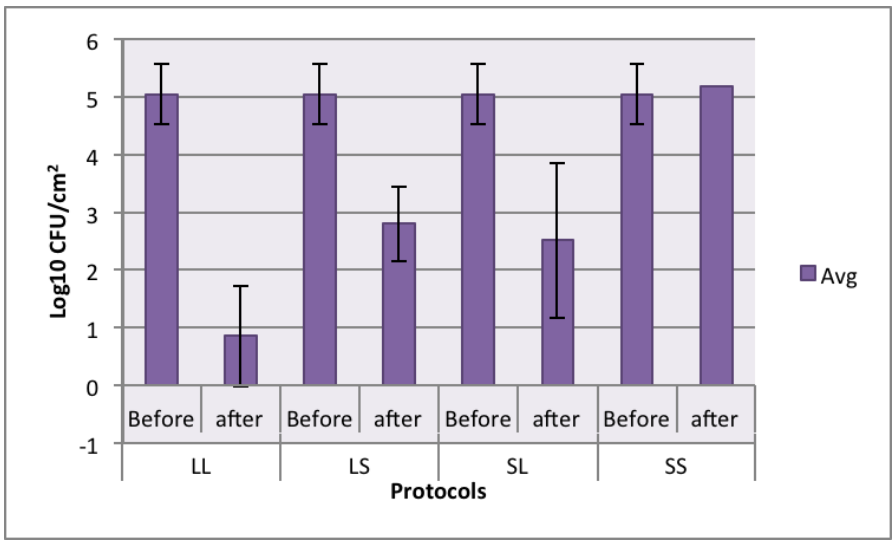

Figure 9: The average total viable count on electropolished stainless steel surfaces

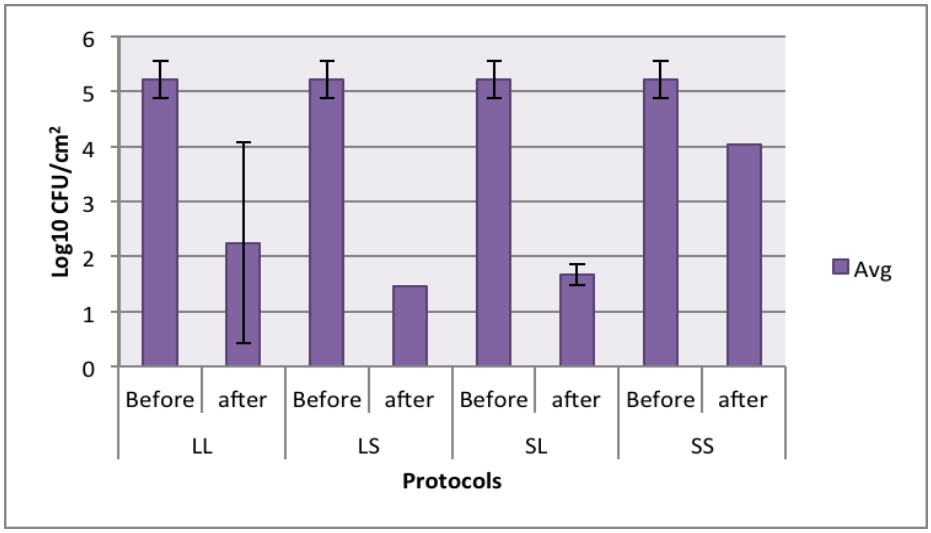

Figure 10: The average total viable count on plastic cutting board surfaces

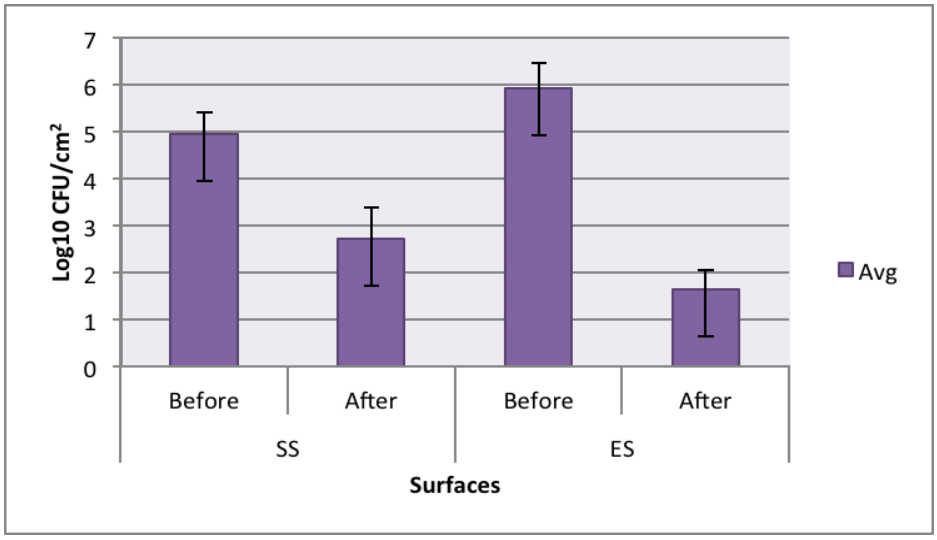

Figure 11: The average total viable count on stainless steel and electropolished stainless steel surfaces from the LL protocol

6 DISCUSSION

6.1 The hygiene condition of surfaces in the fish processing factory

Two control points in the fish factory, fish tub and shovel, cannot be specified as clean, because measurements before and after cleaning seemed to indicated that they were untreated. The fish tub in the cooler still had fish in ice water and residue of fish particles was observed on the shovel. Without cleaning these tools the bacteria that were already there the day before could have multiplied furthermore and in the meantime new ones might have been grown also. The tub and the shovel also looked old and weathered, especially the shovel was a bit discoloured and not very smooth on the side where it had been sampled, which might

have induced the microbiological growth. This caused the incubated colonies on the RODAC, PCA and LH plates after cleaning to be uncountable, except for the PCA measurement for the shovel. According to the guidelines for total bacterial numbers for clean surfaces (Table 4) the RODAC measurements indicate that the surfaces of the control points in the factory were poorly cleaned and unacceptable before the cleaning took place. But after cleaning those contact surfaces could be categorised as acceptable. The steel surfaces that were monitored in the reception area (no. 5 and no. 6) seemed to be fairly good (acceptable), while the fish tub and the shovel remained very poor (unacceptable). As expected, the PCA measurements before cleaning were graded very poor and unacceptable. The PCA measurements after cleaning showed that the cleaned surfaces, except for the fish tub, the shovel and the cutting board from the grader in the reception, could be graded as fairly good (acceptable) to good. The cutting board from the grader in the reception was poorly cleaned. Just as the PCA measurements the LH measurements also showed that the contact surfaces belonged in the grade of being very poor before cleaning, but the cleaned surfaces, except the fish tub, shovel and cutting board, were classified as good.

Table 4: Guidelines for total bacterial numbers for clean surfaces (Matís, 2012)

Grade	**Method**	
	Swab: Counts /cm^2, 22°C	RODAC: No. colonies/plate, 22°C
Good	<1	0
Fairly good; Acceptable	1-4	1-10
Poor	5-50	11-100
Very poor; Unacceptable	>50	>100

6.2 Cleanliness of the test surfaces

Of the four cleaning protocols tested, the LL protocol with longest contact time seemed to remove most of the bacteria from the test surfaces as expected. However, this result did not hold for the plastic cutting board coupons. In this experiment the LS and SL protocols appeared to clean the CB surfaces better than the other two surface types, and also performed better than the LL protocol. The LL protocol in this case showed high degree of variance within the triplicate tested and therefore it must be concluded that the washing protocol failed for some unexplained reason. The SS protocol left most of the adherent microbiological flora on the test surfaces. In the second trial of the experiment between the stainless steel and the electropolished stainless steel surfaces tested with the LL protocol, bacteria seemed to attach more easily to ES, but they also seemed to be more easily decontaminated because there were higher counts on the ES than SS before cleaning and vice versa. It was observed that fish juice got easily rinsed off of the ES coupon than other coupons. In this laboratory study the different outcomes might have been due to the cleaning procedures that had been carried out. The study was conducted in a laboratory sink and washing procedures could not be completely standardized in spite of careful manual washing. For example, the pressure used for spraying the surfaces clean might not always be identical. Another reason might have been that the free metal sticks of the rack that were situated above the hanging surfaces limited to some extent the accessibility to get the coupons fully sprayed. Consequent to this fish juice on the top part of the coupons were not always fully removed after spraying. Even though the top part of the coupons had not been included in the swabbing area, the remaining fish juice from that part dripped down to the area to be swabbed. The accuracy of swabbing on the test surfaces might also play an important part in the results obtained. While swabbing the tested coupon the swab sometimes slipped onto the sterilized surface on which the coupon was placed (aluminum foil). Not using the same or even the right pressure during swabbing,

the length of time of swabbing and full use of the whole swab head might have had an effect on the results. Verran *et al.* (2010) stated that the effectiveness of removing cells from a surface by the swabbing technique is affected by the efficiency of swabbing, whether the cells are dying/drying on the surface, the topography of the surface, and the presence of other material on the surface.

7 CONCLUSIONS AND RECOMMENDATIONS

According to the findings it can be said that the fish processing factory where the fish contact surfaces had been monitored had an overall good cleaning and sanitizing system. Nevertheless, it should be stated that the shovel and tubs could be a source of microbial contamination and therefore need to be cleaned after usage and replaced after reaching a certain life span. Usually the LH plates give higher bacterial counts than the PCA plates because the LH plates are especially used for monitoring fish contact surfaces in the fish industry while PCA plates are regularly used for overall microbial counts. LH further contains salt whereas PCA does not, which usually provides a better growth environment for the marine bacteria. However, in this case the results were almost similar, but no conclusions can be drawn in this case since no microbial identification was performed.

The overall trends from the laboratory experiment showed that with shorter detergent and sanitizer incubation times it is more likely that the bacteria will remain viable. This information is important because it demonstrates that cleaning practises are of high priority and recommended contact times should be respected. Otherwise the risk of persistent microbial contamination gets higher, which can lead to lower product quality and lower product safety. Regarding the cleanliness of the different surface types tested, the data do not allow to draw significant conclusions due to the large variation within replicates. After the first round of experiments it was noted that untreated stainless steel seem to be the most cleanable because the LL protocol returned fewer viable bacteria than the electropolished steel and the plastic surface. In order to confirm these findings the experiment was repeated where stainless steel and electropolished steel were compared directly. In this case the electropolished steel seemed to be more easily cleaned even though it attached higher number of bacteria during the incubation time.

In some cases the protocols gave unexpected or imprecise results. For instance the LS and SL protocol seemed to clean the plastic cutting board coupons better than the LL protocol. The SS protocol had uncountable results for the electropolished stainless steel, while it was expected that compared to the other two surface types this surface would have less bacteria attached. The time did not allow a repetition of these experiments to find out whether these results are reliable.

In the experimental context, it is recommended that these experiments are repeated again to have more reliable results in order to draw any conclusions about the cleaning efficiency on stainless steel, electropolished stainless steel and plastic cutting board by combining different contact times. Care should also be taken on the pressure used for clean spraying the surfaces. A controlled pressure during spraying would be ideal for these experiments e.g. using automated instruments, for example Oran adjusted rack with much less or just enough metal sticks for hanging the coupons might be better for total clean spraying. Fastening the rack in a bit higher place than the sink used for the experiment might also be a solution. It is recommended to master the swabbing technique first before doing the real experiment in

order to carry out the sampling as uniformly as possible. Using more dilutions for samples from short protocols will also give better results. Filling the fish juice a few mm below the insertion hole of the coupon might prevent remaining fish juice on the top part of the coupon dripping down to the area to be swabbed.

In the industrial context, this project clearly shows that washing practises are of high importance for the industry. Fish producers should be concerned about this aspect of their business in order to produce products of higher quality and safety. Lastly, it is likely that workers that have been working long hours during processing and have to end their shift doing the cleaning before going home, are more likely to be tempted to reduce contact times of the cleaning compounds, which ultimately reduces product quality and safety.

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