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Microbiological contamination of surfaces in fish industry

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Three hundred and forty (340) samples of surfaces from equipment (skinning machines), utensils (polyethylene cutting boards, polypropylene cases, baskets, and trays, plastic material used to cover the trays, packaging tanks, knives, and stainless steel sorting and packaging tables), and gloves used by handlers in fish industries, collected with swabs in August 2010 and August 2011, were evaluated. In each period, five different collections were made on different days in both the early morning and early afternoon. Counts of aerobic mesophiles and total coliforms were performed and the presence of thermotolerant coliforms was investigated. All samples collected in the afternoon shift, in either collection periods, showed significantly higher contamination by aerobic mesophiles compared to the morning shift ($p<0.05$). 50.0 and 81.8% of the equipment and utensils analyzed in the first and second collection, respectively, were within the recommendations by the Pan American Health Organization (PAHO) regardless of the work shift. The gloves in the first collection period had aerobic mesophile count above 4 log UFC/glove in 76.7% of the samples and in only 21.7% in the second collection. Although surface contamination decreased, corrective measures still must be enforced and the employees must be oriented regarding the importance of hygienization.

Key words: Hygienization, mesophiles, equipment, utensils, handlers.

INTRODUCTION

Since fish is a food with high nutritional value with pH close to neutral and high water activity, it is very susceptible to spoilage. Besides its autochthonous microbiota, located mainly in the intestines, gills, and surface mucus, they may also be contaminated by spoilage and pathogenic bacteria coming not only from the aquatic environment, but also from inappropriate processing and storage (Ghaly et al., 2010; Mol and Tosun, 2011).

Poor hygienization processes of surfaces that make contact with fish during all production stages are also a crucial factor for the quality of the final product (Kusumaningrum et al., 2003; Temelli et al., 2006; Mol and Tosun, 2011). Dirt particles and microorganisms that fail to be removed by correct hygienization procedures may start adhesion processes and lead to the formation of biofilms (Andrade, 2008; Salustiano et al., 2010).

Microorganism contamination of equipment and utensils is a risk factor in the food industry, therefore the choice of material they are made of must be based on their mechanical and anti-corrosive properties and on the ease of hygienization (Silva et al., 2003; Fuster-Valls et

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Table 1. Surfaces analyzed number of samples, type of collection and the total area sampled for microbiological evaluation in fish industry.

Parameter	Analyzed surfaces	n	Collection	Total sampled area (cm^2)
Equipment	Skinning machine 1 (inox)	5	Unit	100
	Skinning machine 2 (inox)	5	Unit	100
Utensils	Baskets	5	Lot*	500
	PVC films	5	Lot	500
	Cutting boards	5	Lot	500
	Trays	5	Lot	500
	Cases	5	Lot	500
	Sorting tables (inox)	5	Unit	200
	Packaging tables (inox)	5	Unit	200
	Packaging tanks (inox)	5	Unit	200
	Knives (inox)	5	Lot	Surface
Gloves	Latex Gloves	30	Pair	Surface

n, total samples per shift; *one lot is a collection of 5 units.

al., 2008). Some studies have reported high incidence of microorganisms in equipment and utensils in food-processing areas caused by failures in employing correct hygienization techniques, which results in serious public health or economic issues (Temelli et al., 2006; Oliveira et al., 2008, Kahraman et al., 2010).

The Brazilian legislation does not set microbiological parameters for surfaces of equipment and utensils used in food processing, as well as for the handlers' hands. The standards of the American Public Health Association (APHA) consider equipment and utensils clean if they have less than 2 log CFU/utensil or 0.3 log CFU/cm^2 (Evancho et al., 2001).

However, developing countries have difficulties in adapting industries to the American standards, so the Pan American Health Organization (PAHO) recommends counts up to 1.7 log CFU/cm^2 or 2 log CFU/utensil for aerobic mesophiles and absence of thermotolerant coliforms mainly due to the ambient temperatures in these countries (Cardoso et al., 2011).

Another factor that must also be taken into account in the food production chain is the handlers, who must be trained in Good Manufacturing Practices (GMPs) and have adequate personal hygiene (Brasil, 2009). Otherwise, they may carry pathogens, which is often reported as the cause of foodborne diseases (Rosas and Reys, 2008; Dias et al., 2012). Andrade (2008) set two count ranges that could serve as a guideline to define hygienic-sanitary hand conditions: range 1 (up to 3 log) and range 2 (between 3 and 4 log), expressed as CFU/hand for aerobic mesophiles and total coliforms.

Surface cleaning and disinfection procedures, despite being essential for good-quality and safe foods, are often not a priority. Not always is the cost-benefit relation of these practices acknowledged since their results are not easily measured in terms of economic gains (Aarnisalo et al., 2006).

The goal of this study was to evaluate the levels of microbiological contamination of several surfaces (equipment, utensils, and gloves) before they were used in the fish processing plant, aiming to verify the efficiency of the Standard Operating Hygiene Procedures (SOHP) applied and the influence of implementing GMPs in the plant.

MATERIALS AND METHODS

Characteristics of the fish processing plant

The fish processing facility is located in the northeast of the state of Pará, Brazil, and was in the process of implementing GMPs. The industry has 129 employees and can process about 10 t/day of fish. It produces several frozen products such as whole eviscerated fish, fish fillets, and steaks of different species, which are marketed across Brazil and exported to the United States.

Sample collection

Equipment, utensils and non-disposable rubber gloves used in the fish processing were analyzed (Table 1). The sampling of the surfaces was performed in two periods: In August 2010, at the beginning of GMP implementation, however before employee training began, and in August 2011, when the plant was already in the final process of GMP implementation. In each period, five collections were made on different days and in both shifts, early in the morning and early in the afternoon, before the surfaces were used in the processing.

These shifts were established based on the time of hygienization of most surfaces, which was performed twice a day, between 11 a.m. and 13 p.m. (lunch) and after the end of the working hours (6 p.m.). In each collection, the surfaces were analyzed individually (two skinning machines, two tables, one tank, and six pairs of gloves) or in batches of five units per surface (baskets, cutting boards, cases, PVC films, and knives). 340 samples were analyzed in total, 170 for each collection period.

During the lunch break, in both collection periods, the skinning machines and the fixed utensils in the production area such as tables and tanks were only washed with water jets. The use of detergent and sanitizer was conditioned to the absence of raw

material and/or products in the processing line to avoid chemical contamination. The other utensils, despite having specific areas for hygienization, underwent only cleaning in the first collection period. However, in the second collection period, the use of sanitizer was verified, albeit sometimes diluted incorrectly. Only by the end of the working hours, in both collection periods, did all the equipment and utensils underwent the cleaning and sanitization steps. However, most times the equipment was not taken apart.

The samples from equipment and utensils were collected with swabs following the procedure proposed by the American Public Health Association (Evancho et al., 2001). After being dipped in a diluent solution (0.1% sterile peptone water), sterile cotton swabs were rubbed three times on an area not smaller than 100 cm^2 or on all the surface area that touched the food.

On the gloves, the analysis area was the surface of the palm and the edges starting from the wrists. In an angle, the swab was rubbed with circular motion from the lower part of the palm until the tip of the fingers and back to the wrist, a procedure that was repeated three times for each finger. The collection on the edges used a back-and-forth motion, starting from one side of the hand where the wrist begins, going between the fingers, and finishing at the wrist on the other side of the hand (Andrade, 2008).

After this sampling, the swabs were placed in test tubes containing 10 ml of sterile peptone water with 1 of 0.25% sodium thiosulfate. The tubes were then capped, identified, and immediately taken to the laboratory under refrigeration for the analyses.

During the collection, the processing's routine, employee behavior, and the Standard Operating Hygiene Procedures (SOHP) applied were followed. This task was performed through observations at the site, by checking paperwork, and through information provided by the employees and owners.

Microbiological analyses

After appropriate decimal dilutions (down to 10-4) with sterile 0.1% peptone water, the samples were plated in Plate Count Agar (PCA - OXOID CM 325) for the count of total aerobic mesophiles, and in Violet Red Bile Glucose Agar (VRBGA, OXOID CM 485) for the enumeration of total coliforms with later confirmation of the presence or absence of thermotolerant coliforms. All the analyses were performed in triplicate and followed the methodology described in the Compendium of Methods for the Microbiological Examination of Foods (Downes and Ito, 2001).

The results of the Colony-Forming Units (CFU) by cm^2 of the surface, or CFU/surface, were converted into logarithms and compared with the recommendations of the Pan American Health Organization (PAHO) (Cardoso et al., 2011).

Statistical analysis

The values of the average counts of aerobic mesophiles (log CFU/cm^2 or log CFU/surface) underwent analysis of variance (ANOVA) and Tukey's test using the software Statistica® version 7.0 to check whether there was a significant difference ($p \leq 0.05$) between the work shifts and periods analyzed.

RESULTS AND DISCUSSION

Evaluation of the application of the SOHP and microbiological analyses of the fish processing plant's equipment and utensils

The variation in the aerobic mesophile counts in the various collections from the same surface (Table 2) shows that there is no standardization in the hygienization processes in the processing plant. The failure in fully following the SOHPs, at all times of hygienization, may lead to a variation in microbial counts, which may then compromise the hygienic-sanitary quality of foods (Kahraman et al., 2010; Salustiano et al., 2010).

The high microbial load found in some of the equipment and utensils analyzed (Table 2) and the significantly higher levels of aerobic mesophile contamination in all samples collected in the afternoon shift, in both collection periods, are attributed to inefficient cleaning. High microbial counts in food-processing plant surfaces indicate the inefficient application of the SOHPs, risk of cross-contamination, possibility of biofilm formation, and possible presence of pathogens (Aarnisalo et al., 2006; Lequette et al., 2010). Foods in contact with contaminated surfaces may have their microbiological quality compromised, especially if they're consumed raw or if the thermal treatment is not adequate for inactivating vegetative cells or bacterial toxins that might be present (Temelli et al., 2006; Jha et al., 2010).

The contamination of foods by sessile microbial cells has already been show in several studies. Salustiano et al. (2010) assessed post-pasteurization recontamination of milk by *Bacillus cereus* using automated ribotyping. Seven ribogroups were identified and the same ribogroup was isolated from four surfaces and milk samples, suggesting the surfaces are repositories of that species. Ravishankar et al. (2010), while studying the occurrence of cross-contamination, showed that *Salmonella enterica* serovar Newport, present in poultry, was able to contaminate the stainless steel knife and polyethylene cutting board, being then transferred to lettuce leaves.

Out of all the samples from the surfaces of equipment and utensils analyzed, irrespective of the shift, 50.0 and 81.8% were within the limits recommended by the PAHO in the first and second collection periods, respectively. The increase in conformity seen in the second period may be mainly attributed to the GMPs that were being implemented in the fish processing plant and to the training of the handlers, especially in hygienization procedures. Hwang et al. (2011), while evaluating several surfaces of different fish-processing areas in Taiwan, found that the contamination level was lower ($p<0.05$) in the industries that had already implemented quality management tools.

In the first period, 31.8% of the samples from equipment and utensils were out of the PAHO standard for aerobic mesophiles and thermotolerant coliforms, while 18.2% were not within the limit established for aerobic mesophiles. However, in the second period, only 9.1% of the samples had mesophiles as the main microorganisms responsible for contamination and 9.1% were not within the limits for aerobic mesophiles and thermotolerant coliforms.

Among the samples out of the standard, 73.3% were detected in the afternoon shift due to the lack of

Table 2. Mesophile count and survey of thermotolerant coliforms in surfaces of a fish industry from collections in August 2010 and August 2011 in two shifts (morning and afternoon).

Surfaces (equipment and utensils)	August 2010				August 2011			
	Aerobic mesophiles[1] (log CFU/cm^2)		Coliforms[2] (P/A)[3]		Aerobic mesophiles[1] (log CFU/cm^2)		Coliforms[2] (P/A)[3]	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
Skinning machine 1	2.52±0.05[a]	4.14±0.07[b]	A	P	1.35±0.19[a]	2.99±0.05[b]	A	P
Skinning machine 2	2.54±0.06[a]	4.17±0.08[b]	A	P	1.41±0.13[a]	3.01± 0.09[b]	A	P
Baskets	1.49±0.09[a]	5.32±0.07[b]	A	P	1.42±0.12[a]	1.81±0.03[b]	A	A
PVC films	1.56±0.08[a]	4.98±0.02[b]	A	P	1.08±0.22[a]	1.59±0.09[b]	A	A
Cutting boards	1.25±0.13[a]	1.82±0.07[b]	A	A	1.30±0.27[a]	1.67±0.07[b]	A	A
Trays	0.99±0.35[a]	1.76±0.04[b]	A	A	0.95±0.28[a]	1.61±0.06[b]	A	A
Cases	1.25±0.36[a]	1.85±0.15[b]	A	A	0.34±0.23[a]	1.08±0.22[b]	A	A
Sorting tables	0.31±0.33[a]	0.84±0.24[b]	A	A	0.30±0.30[a]	0.85±0.08[b]	A	A
Packaging tables	0.57±0.33[a]	1.35±0.13[b]	A	A	0.46±0.18[a]	1.26±0.15[b]	A	A
Packaging tanks	0.36±0.39[a]	0.99±0.14[b]	A	A	0.33±0.32[a]	0.98±0.10[b]	A	A
Knives[3]	3.65±0.05[a]	3.87±0.03[b]	P	P	1.54±0.06[a]	2.03±0.16[b]	A	A

[1]Average±standard deviation (n=5). [2]Thermotolerant coliforms. [3]P, presence ; A , absence. [4]CFU/utensil. [b]Different small letters in the same line, in each year of collection, means the results were significantly different ($p<0.05$).

or inappropriate sanitization.

Several authors (Sneed et al., 2004; Oliveira et al., 2008; Wang et al., 2010), when assessing equipment and utensils in businesses, detected 100% of samples above the PAHO guidelines and highlighted that the inappropriate hygiene and sanitation conditions of the surfaces analyzed are responsible for an increase in spoilage and pathogenic microorganisms in the final products. According to Aantrekker et al. (2003), when the contribution of air contamination can be quantified, its importance can be determined in an overall risk assessment by comparing air contamination to other sources (initial contamination and other contamination routes).

Among the surfaces with high contamination level, the skinning machines 1 and 2 stood out for having high counts of aerobic mesophiles ranging between 2 and 4 log CFU/cm^2 and from 1 to 3 log CFU/cm^2 in the first and second periods, respectively. However, only in the samples collected in the afternoon shift, in either collection period, was the presence of thermotolerant coliforms detected (Table 2). One of the reasons of this contamination is the inappropriate design of the equipment, which makes cleaning hard and hinders the action of the sanitizing agent that makes it necessary to hygienize the equipment more often using more aggressive chemicals, which does not guarantee the safety in food production (Lelieveld et al., 2003). Thus, the equipment must be designed in a way to make cleaning, sanitization, inspection, and maintenance easy (Aarnisalo et al., 2006).

Kahraman et al. (2010) reported that for appropriate hygienization, the equipment must be disassembled prior to cleaning. Several studies have also linked high levels of contamination by aerobic mesophiles (2 to 5 log CFU/cm^2) and the presence of thermotolerant coliforms found in equipment of different food-processing areas to hardships in carrying out cleaning due to the difficulty in disassembling them, which causes accumulation of residues (Oliveira et al., 2008; Keeratipibul et al., 2009; Cardoso et al., 2011).

Among the utensils analyzed, it can be seen in Table 2 that only the knives had high contamination by aerobic mesophiles (>1.7 log CFU/utensil) in either shift in the first collection period and only in the afternoon shift in the second period. The presence of thermotolerant

coliforms was detected only in both shifts of the first period. In the plant analyzed, each handler is responsible for hygienizing the knife used. The results found, despite the significant ($p<0.05$) reduction found between the two collection periods, suggest that the handlers were still not fully aware of the importance of appropriate hygienization, which prevents contamination sites. Therefore, the plant's GMP trainings must be ongoing. Çetin et al. (2006), when evaluating several utensils used in a meat-processing plant, also found high levels of aerobic mesophilic bacteria (up to 2 log CFU/cm^2) on the knives and highlighted that this result means the knives may pose a real threat if associated to the presence of pathogens.

On the baskets, PVC films, cutting boards, trays, and cases, the levels of aerobic mesophiles were also high (>1.7 log CFU/cm^2) in the afternoon shift of the first collection period (Table 2), likely due to the lack of sanitization. In the second period, these utensils were already being sanitized in the afternoon shift, although the baskets still had high microbial counts perhaps since the holes made hygienization harder. The presence of thermotolerant coliforms was only seen on the baskets and PVC films in the afternoon shift during the first collection period. It is important to note that the plastic sheets used to cover the trays were very worn out, making hygienization harder (Kusumaningrum et al., 2003). That is why these sheets had all been replaced in the second collection period, which must also have contributed to reducing the microbial counts in this period (Table 2).

Low counts of aerobic mesophiles (<1.5 log CFU/cm^2) and no thermotolerant coliforms were detected on the tables and in the tank (Table 2) even in the shifts when they were only washed with water jets. These results may be related to the type of material (stainless steel) and to the pristine conditions of these surfaces, which allowed more appropriate hygienization or cleaning. Materials such as stainless steel allow for more efficient hygienization, especially if appropriate chemicals are used (Fuster-Valls et al., 2008). Cabeça et al. (2006) found a reduction in the number of *L. monocytogenes* cells adhering to the surface of stainless steel after the treatment with different sanitizers (iodine, biguanide, quaternary ammonium compounds, peracetic acid, and sodium hypochlorite).

Microbiological analyses of gloves

On the non-disposable rubber gloves used by all handlers in the processing line, counts of aerobic mesophiles and total coliforms ranging from 3.3 to 6.9 log CFU/glove and 2.2 to 3.8 CFU/glove were found, respectively, in the first collection period. In the second period, the values ranged from 2.1 to 5.9 log CFU/glove and from 1.0 to 3.7 log CFU/glove for mesophiles and coliforms, respectively.

Oliveira et al. (2008), when assessing the hands of handlers working directly with meat grinders in five business facilities, detected aerobic mesophiles ranging from 4.4 to 6.8 log CFU/hand and thermotolerant coliforms from 1.2 to 3.7 CFU/hand. According to those authors, these results suggest inappropriate hygienization and may be a source of meat contamination after grinding and handling.

Rosas and Reys (2008) while observing the personal hygiene practices in a fish processing plant noticed that the handlers often did not wash their hand before beginning work in the production area. Thus, they claim that training is crucial to improve handler hygiene practices and that supervision must be constant to assure the correct application of the cleaning and sanitization procedures so as to avoid cross-contamination.

Between the collection periods, only in the first period was a significant difference ($p<0.05$) found between the mesophile counts. Nevertheless, the number of mesophiles and coliforms, regardless of the shift, has a significant reduction ($p<0.05$) between the two periods (Figure 1), once again showing the importance of implementing GMPs. Dias et al. (2012) related the decrease in coliform count, from 5.8 to 1.2 log CFU/hand, in handlers' hands in a cheese processing industry to the implementtation of GMPs. According to those authors, the changes carried out were appropriate to improve the hygiene practices adopted in the industry, which will certainly influence the production of better quality and safer cheese.

According to criterion suggested by Andrade (2008), in the first collection period only 23.3% of the glove samples had counts of aerobic mesophiles within range 2, which is the acceptable limit for microbial count. However, that represents a warning that the hygienization procedure must be controlled; the other 76.7% of samples were above 4 log CFU/glove, indicating a poor hygienization process. In the second period, 20% of the samples were within range 1, 58.3% were within range 2 and 21.7% were above 4 log CFU/glove. The coliform count in both periods was below 4 log CFU/glove. Microbial counts above 4 log CFU/hand highlight the importance of handlers as potential food contamination agents (Dias et al., 2012).

Despite the reduction in contamination level found, glove hygienization, which is a responsibility of the handlers themselves in the industry analyzed, still needs to be improved since they may be sources of spoilage and pathogenic microorganism contamination (Rosas and Reys, 2008; Dias et al., 2012). Such contamination must be reduced or eliminated in order to prevent its introduction in the foods and, consequently, impairing its commercial life or posing potential risks to consumers.

According to Aycicek et al., (2006), seeing apparently clean surfaces may lead to error and give a false feeling of safety. The microbiological trials do not prevent bacteria from entering the industry, but allow keeping an

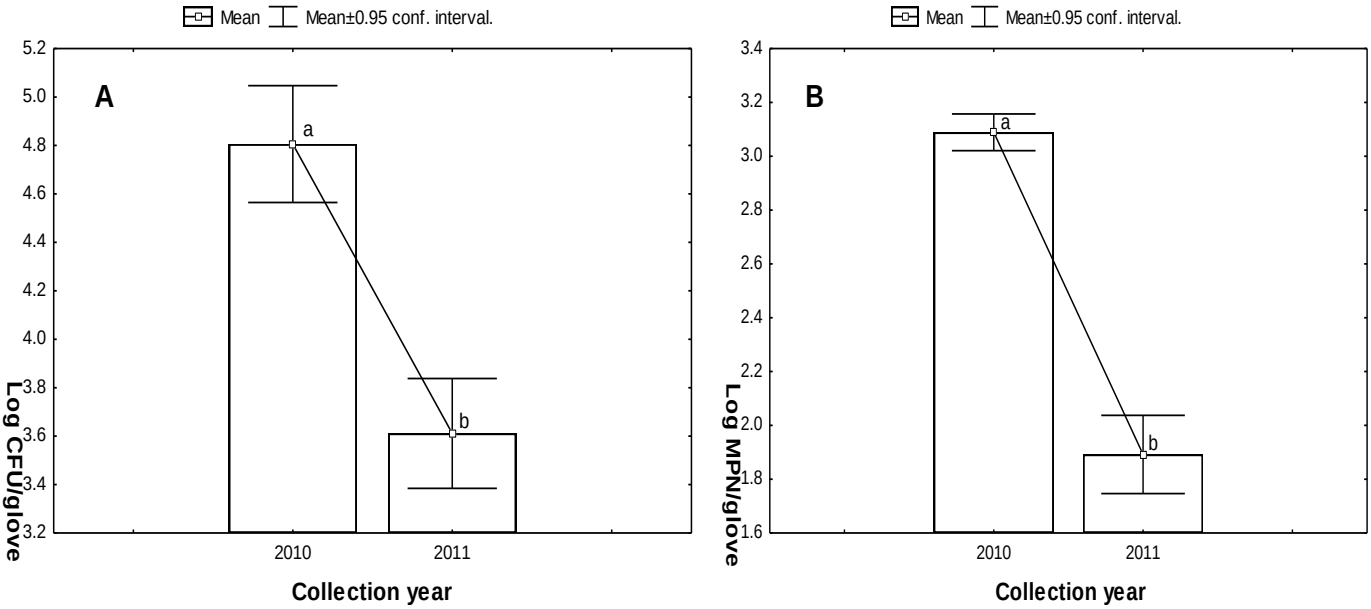

Figure 1. Comparison between the average counts of aerobic mesophiles (A) and total coliforms (B) on handlers' gloves in a fish industry collected in August 2010 and August 2011, regardless of the shift of collection. Means with different letters are significantly different ($p<0.05$).

eye on bacterial hazards and serve as warnings to maintain hygienization in the production areas.

It is crucial that all food production be organized and that the hygienization procedures, often left to second thought, be carried out effectively and uninterruptedly. The repetitive nature of the tasks and the lack of incentive favor a gradual reduction in quality, which increase the risk of pathogenic microorganism contamination. Therefore, it is important that those responsible for food companies acknowledge the value of this activity to obtain quality products from the hygienic-sanitary standpoint.

Conclusions

The results of microbiological analyses from several surfaces indicated inappropriate hygienization, especially in the first sampling period, as a consequence of the incorrect application of the Standard Operating Hygiene Procedures (SOHP). The reduction in microbial surface contamination in the second collection period was directly influenced by the implementation of GMPs in the fish processing plant. Corrective measures must still be continuously employed and the handlers' hygiene habits must be revised, particularly concerning proper rubber glove hygienization.

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