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Validation of cleaning procedures and sanitization of equipment in the fish industry by microbiological analysis

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Abstract. Data by the Pan-American Health Organization show that the death of about two million people a year and over 200 types of diseases are associated with increasing food contamination. Several types of technology and extensive legislation have been developed and targeted to this issue to reduce food microbiological load to safer levels and meet the expectations of increasingly demanding consumers. Current research investigates the process of sanitization of equipment and utensils in the fish industry by microbiological analyses. The study was conducted in a fish industry, installed in São Roque, State of São Paulo, Brazil. Swab technique was employed prior to cleaning and after sanitizing. Microbiological analysis comprised total counts of coliforms, fecal coliforms, psychrotrophic bacteria, mesophylls and *Pseudomonas*. In present study, microbiological analysis available in compendium of methods for the microbiological examinations of foods were useful for establish possible contamination by microorganisms. Correct sanitization throughout the manufacturing process has been targeted to maintain the quality and safety of the final product. Satisfactory results of present study may contribute to the discussion about standardization of sanitary procedures for fishing industry. Further research on the fishery industry should be undertaken to establish reliable standards to be employed nationwide.

Keywords: Coliforms; Food-borne diseases; Food quality; Pathological microorganism; *Pseudomonas*.

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Introduction

Contemporary society is made up of consumers highly interested in choosing and preparing their own meals. Human concentration in towns and cities increased food demands and the distance between food-producing and consumer zones. Concomitant to changes in consumers' demands, technological progress of food-processing industries and increase in international commerce in food products are in the limelight (Germano and Germano, 2015). Abundant supply and demand for industrialized food have triggered several diseases and even increased mortality rates due to food-borne diseases (FBDs). FBDs are clinical occurrences caused by the ingestion of food contaminated by pathological microorganisms, chemical compounds, harming objects or naturally toxic objects. They are diseases caused by the ingestion of biologically, chemically or physically dangerous objects in food (Silva-Jr., 2014). The most common symptom caused by FBDs is acute or chronic diarrhea, dependent on the pathogenicity of the microorganism involved. However, FBDs may not be restricted to the gastrointestinal tract but may cause disorders in the nervous system, blood system, genital apparatus, liver and others (Franco and Landgraf, 1996).

The consumption of fish has increased significantly in recent years due to conscience-raising on its relevance to health, to its great variety and to economic accessibility. Fish may be commercialized either fresh or processed. Recently-caught fish may be kept under refrigeration or not, and may be bought raw by the consumer. Industrialization involves a more elaborated type of handling as, for instance, in the preparation of fish fillets Ogawa and Maia (1999). All animal-originated food is always prone to health risks. Proteins and water cause the product´s fast deterioration and the survival and multiplication of numberless pathogenic microorganisms. According to Ogawa and Maia (1999), the muscles and body liquids of fresh fish are naturally sterile, but skin, scales and gills are not. They are contaminated tissues due to their direct contact with the environment. The skin of sea fish may be contaminated by 10^2-10^4 bacteria/cm^2 of the genera *Pseudomonas* and others.

According to Silva-Jr. (2014) standard plate counts of mesophylls and psychrotrophic bacteria are hygiene indexes of processing. Total count of aerobic mesophylls in plates fails to differentiate types of bacteria. They are employed to obtain general information on the product's quality, processing practices, prime matter employed, manufacturing conditions and shelf-life. To Silva (2010) it is not a safety indicator since counts are not directly related to the presence of pathogens or toxins. They may be useful for the assessment of quality since high bacteria populations indicate defects in hygiene or faults in processing or ingredient control.

Microorganisms may be classified in function of temperature. Psycrotrophic bacteria are a sub-group of mesophylls; they multiply in refrigerated food but grow better at temperatures within the mesophyll range. The species *Alteromonas*, *Photobacterium* and *Vibrio* are agents causing fish deterioration (Silva, 2010). Other important pathogens as causes of food toxins and infections are *Pseudomonas aeruginosa.* According to Silva-Jr. (2014), sanitary indicators are microorganisms that may cause diseases

in humans. Counting of thermotolerant coliforms, belonging to total coliforms, are one of these indicators. Also known as fecal coliforms or coliforms 45 °C, they pinpoint the presence of fecal material, denoting pathogens, contaminated handling, and FBDs if counts are over 10^5/g. The occurrence of *Pseudomonas* in food, associated with the deterioration of meat and derivates, milk and derivates, fish and seafood, eggs and vegetables, is also highly common (Silva, 2010).

Food may be contaminated by contact with utensils, dirty surfaces and badly cleaned equipment. It should be underscored that pathogenic microorganisms may survive in food particles or in water on utensils which had not been properly washed (Silva-Jr, 2014). Consequently, fish industries should have extreme care in handling, storing, conservation, transport and commercialization since the quality of the final product depends on the quality of prime matter, quality and quantity of ice used in the conservation of fish and hygiene-sanitary conditions prior to and during the process (Lima, 2012)

Hygienization eliminates or reduces contamination. It decreases the probability of transmitting disease-causing agents and occurs in two different stages: cleaning comes first: procedure involves the simple removal of dirtiness or microscopic residues of organic or inorganic origin; sanitization comes second: the procedure eliminates or reduces pathogenic microorganisms to the lowest rates to avoid any risk to health (Germano; Germano, 2015). Data retrieved from the Epidemiological Sanitary Surveillance for 1985-1988, in Curitiba, State of Paraná, Brazil, published by Silva-Jr. (2014), on factors that contributed towards FBDs outbreaks, revealed that lack of hygiene in equipment accounted for almost 10% of the causes.

The most important parameters that determine food quality are those that define its microbiological characteristics. The assessment of a product's microbiological quality provides data that evaluates processing, storage and distribution conditions, and life span and health risks for society (Franco; Landgraf, 1996). Current analysis validates the cleaning method and the sanitization of equipment and utensils in a fish factory by quantifying microorganisms to warrant the best quality and safety of the final product.

Materials and methods

Current study was undertaken in a fish industry in São Roque, State of São Paulo, Brazil, in April 2016. The swab (measuring 10 cm x 10 cm) technique was employed for the collection of material from the following sites: filleting table, filleting block, handling table, floor under the filleting table and drain close to the filleting table. Collections were undertaken prior to and during hygienization, divided into two stages: manual cleaning with neutral detergent and brush, and sanitization with ammonia 5%, kept for 10 min before rinsing. Samples were conditioned in isothermal boxes with ice and sent to a commercial laboratory in Campinas, State of São Paulo, Brazil, in April 2016.

Normative Instruction nº 62/2011 (Brasil, 2011) was the methodology used to count thermotolerant coliforms at 45 °C. Instructions of ISO 4833-1:2013 (ISO, 2013) and ISO 13720:2010 (ISO, 2010) were, respectively, followed to count viable aerobic mesophyll microorganisms at 30 °C and *Pseudomonas*. Total coliforms and psycrotrophic bacteria were counted according to Salfinger and Tortorello (2015).

Results

Table 1 gives results of the analyses by which numbers less than 5 cannot be measured. In this case, the microorganism is technically absent. There were no *Pseudomonas* and

thermotolerant coliforms prior to and after hygienization, at all sites. Excepting the drains prior to sanitization, with 1.2×10^3 UFC/cm^2, total coliforms were absent at all sites. Filleting table and drain featured significant mesophyll rates after hygienization, but they were absent in all the other sites. Packaging table and floor failed to show any psycrotrophic bacteria either prior or after hygienization, whilst a decreasing number could be registered on the filleting table and in the drain.

Table 1. Counts of total coliforms, thermotolerant coliforms at 45 °C, viable aerobic mesophylls at 30 °C; psycrotrophic bacteria and *Pseudomonas* on filleting table, filleting block, handling table, floor and drain, prior to and after hygienization.

Collection site	Total coliforms	Thermotolerant coliforms	Aerobic mesophylls	Psycrotrophic bacteria	*Pseudomonas*
Filleting table (before)	< 5 CFU/cm^2 estimate	< 5 CFU/cm^2 estimate	> 3.0 x 10^3 CFU/cm^2	5.2 x 10^2 CFU/cm^2	< 5 CFU/cm^2
Filleting table (after)	< 5 CFU/cm^2 estimate	< 5 CFU/cm^2 estimate	2.2 x 10^1 CFU/cm^2	6.0 CFU/cm^2 estimate	< 5 CFU/cm^2
Filleting block (before)	< 5 CFU/cm^2 estimate	< 5 CFU/cm^2 estimate	2.2 x 10^3 CFU/cm^2	< 5 CFU/cm^2 estimate	< 5 CFU/cm^2
Filleting block (after)	< 5 CFU/cm^2 estimate	< 5 CFU/cm^2 estimate	< 5 CFU/cm^2	< 5 CFU/cm^2 estimate	< 5 CFU/cm^2
Handling table (before)	< 5 CFU/cm^2 estimate	< 5 CFU/cm^2 estimate	1.9 x 10^3 CFU/cm^2	6.0 x 10^2 CFU/cm^2	< 5 CFU/cm^2
Handling table (after)	< 5 CFU/cm^2 estimate	< 5 CFU/cm^2 estimate	< 5 CFU/cm^2	< 5 CFU/cm^2 estimate	< 5 CFU/cm^2
Floor (before)	< 5 CFU/cm^2 estimate	< 5 CFU/cm^2 estimate	> 3.0x10^3 CFU/cm^2	> 3.0 x 10^3 CFU/cm^2 estimate	< 5 CFU/cm^2
Floor (after)	< 5 CFU/cm^2 estimate	< 5 CFU/cm^2 estimate	< 5 CFU/cm^2	< 5 CFU/cm^2 estimate	< 5 CFU/cm^2
Drain (before)	< 1.2 x 10^3 CFU/cm^2	< 5 CFU/cm^2 estimate	> 3.0x10^3 CFU/cm^2	> 3.0 x 10^3 CFU/cm^2	< 5 CFU/cm^2
Drain (after)	< 5 CFU/cm^2 estimate	< 5 CFU/cm^2 estimate	1.2 x 10^3 CFU/cm^2	6.8 x 10^1 CFU/cm^2	< 5 CFU/cm^2

Discussion

As found in present results, microbiological analysis available in compendium of methods for the microbiological examination of foods (Salfinger and Tortorello, 2015) were useful for stablish possible contamination by microorganisms. However, Brazilian legislation does not determine an acceptable standard of the above-mentioned microorganisms in the product under analysis. The Brazilian Agency for Sanitary Surveillance (Anvisa) published Resolution No. 12/2001 (Brasil, 2001) tolerating 5×10^3/g positive *Staphylococcus* coagulase and the absence of *Salmonella* sp. in 25 g of fish and seafood samples. Nevertheless, details for other bacteria, surfaces and utensils used in the fishing industry are lacking. Although much research work has been undertaken to quantify mesophylls, psycrotrophic bacteria, *Pseudomonas* and coliforms in fresh or frozen fish, the Brazilian literature on the subject fails to mention specific environmental counts.

According to Silva-Jr. (2014), international criteria indicate reference

rates for standard counts for aerobic mesophylls in cm^2 of equipment and utensils. The American Public Health Association (APHA) considers satisfactory counts less than or equal to 2; counts above 2 are unsatisfactory (Salfinger and Tortorello, 2015). The Pan-American Health Organization states that parameters ranging between 0 and 10 are very good; between 11 and 29 are good; between 30 and 49 are fair; between 50 and 99 are bad; over 100 very bad (Silva-Jr, 2014).

In a study, a number less than or equal to five was satisfactory; between 5 and 25 utensils should be cleaned again; higher than 25 ranks unsatisfactory. The absence of coliforms in 100 cm^2 of the sample was recommended (Silva-Jr., 2014).

Silva-Jr. (2014) reports on results from analyses undertaken in industrial kitchens in São Paulo and recommends rates higher than 50 should be considered unsatisfactory; less than or equal to 50 should be considered satisfactory as reference for experimental analyses on equipment and utensils. The author also recommends the absence of fecal coliforms and *Pseudomonas aeruginosa* in 50 cm^2 of sample.

Filleting table and drain were the only sites featuring mesophylls and psycrotrophic bacteria after hygienization. Due to previous high contamination, decrease in the number of microorganisms in the drain may be considered satisfactory. Taking into consideration recommendations by Salfinger and Tortorello (2015), cleanliness and sanitization of the filleting table were not efficient in present study and further hygienization was required. The lack of detailed legislation on the theme jeopardizes final considerations since a basis for debates is at fault. On the other hand, satisfactory results, based on food industries, exist, even though results on the fishing industry are not totally satisfactory.

Conclusion

Results of present study may contribute to the discussion about standardization of sanitary procedures for fishing industry. Further research on the fishery industry should be undertaken to establish reliable standards to be employed nationwide.

Conflict of interest statement

Authors declare that they have no conflict of interests.

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