



Microbiological quality assessment of fresh tilapia marketed in the Federal District and of the ice used for its conservation

Avaliação da qualidade microbiológica de tilápia fresca comercializada no Distrito Federal e do gelo utilizado na sua conservação

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Tilapia (*Oreochromis niloticus*) is the most cultivated and consumed freshwater fish in Brazil. The present study aimed to evaluate the microbiological quality of ice and fresh tilapia samples commercialized in the Federal District. Tilapia samples were tested for counts of mesophilic and psychrotrophic bacteria, determination of total and thermotolerant coliforms, *Staphylococcus aureus* counts and presence of *Salmonella*. Ice samples were analyzed for determination of total coliforms and thermotolerant coliforms and presence of *Escherichia coli*. Of the 20 samples of fresh tilapia analyzed, ten samples (50%) presented *Salmonella* (genetically confirmed through the presence of the *invA* gene) and, therefore, were unfit for consumption. *S. aureus* was found in 11 samples (55%), and one sample of fillet presented *S. aureus* counts (3.15 CFU/g) above the limit allowed by Brazilian legislation (3 log CFU/g). *S. aureus* colonies were confirmed by detection of *CoA* gene in molecular analysis. Of the 14 ice samples analyzed, 12 samples (85.7%) were unfit for use in fish conservation due to the presence of total coliforms and 9 ice samples (64.3%) were also contaminated with thermotolerant coliforms. *E. coli* was isolated from 6 ice samples (42.9%) and confirmed in the molecular analysis through the amplification of the *MalB* gene. In conclusion, the high contamination of tilapia samples with *Salmonella* and of the ice used for its conservation with coliforms and *E. coli* indicates the need for better hygienic practices in the tilapia production chain, to increase its quality and microbiological safety.

Keywords: *Oreochromis niloticus*, freshwater fish, foodborne pathogens.

A tilápia (*Oreochromis niloticus*) é o peixe de água doce mais cultivado e consumido no Brasil. O presente estudo teve como objetivo avaliar a qualidade microbiológica de amostras de gelo e de tilápia fresca comercializadas no Distrito Federal. As amostras de tilápia fresca foram analisadas em relação a contagem de bactérias mesófilas e psicrotróficas, determinação de coliformes totais e termotolerantes, contagem de *Staphylococcus aureus* e presença de *Salmonella*. As amostras de gelo foram analisadas em relação a determinação de coliformes totais e termotolerantes e presença de *Escherichia coli*. Das 20 amostras de tilápia fresca analisadas, 10 (50%) apresentaram *Salmonella* (confirmadas através da presença do gene *invA*) e, portanto, estavam impróprias para o consumo. Bactérias *S. aureus* foram encontradas em 11 amostras (55%), e uma amostra de filé de tilápia apresentou contagem de *S. aureus* (3,15 CFU/g) acima do permitido pela legislação brasileira (3 log UFC/g). As colônias de *S. aureus* foram confirmadas pela detecção do gene *CoA*. Das 14 amostras de gelo analisadas, 12 (85,7%) estavam impróprias para uso na conservação do pescado devido à presença de coliformes totais e 9 amostras (64,3%) também estavam contaminadas com coliformes termotolerantes. Bactérias *E. coli* foram identificadas em 6 amostras de gelo (42,9%) e confirmadas pela amplificação do gene *MalB*. A elevada contaminação das amostras de tilápia com *Salmonella* e do gelo utilizado na sua conservação com coliformes e bactérias *E. coli* indica a necessidade de melhores práticas de higiene na cadeia produtiva da tilápia, para garantir a sua segurança e qualidade microbiológica.

Palavras-chave: *Oreochromis niloticus*, peixe de água doce, patógenos de origem alimentar.

1. INTRODUCTION

Tilapia (*Oreochromis niloticus*) is a popular farmed freshwater fish in the world [1]. A total of 323.700 tons of tilapia was produced in Brazil, corresponding to 61.1% of the total aquaculture

fish production [2]. The advantages of farming tilapia are its rapid growth, relatively resistance to various diseases, and tolerance to changing environmental conditions. It is simple to culture tilapia intensively and economically. Tilapia presents an excellent meat quality and taste, with white-color and the absence of intramuscular bones [3-5].

Fish is an essential source of protein and provides many health benefits; however, it is a very perishable product and possesses a tendency for rapid microbial spoilage [5, 6]. It has been shown that fish can cause foodborne illnesses [3, 7]. In United States it was reported 857 outbreaks associated with the consumption of fish, resulting in 4815 illnesses, 359 hospitalizations and 4 deaths, during the period 1998–2015 [8]. Outbreaks associated with the consumption of fish and fishery products increased markedly in the European Union (EU) (by 80 outbreaks; 101.3% more than in 2018) even if this rise was entirely attributable to France which reported 129 outbreaks (81.1% of total outbreaks in the EU). The fish and fishery products were the food most frequently implicated in strong evidence outbreaks in the EU in 2019 [9]. In Brazil, 12503 cases of outbreaks were reported in the period 2000-2017 and fish consumption was associated with 0.83% of these outbreaks [10].

The fish bacterial flora depends on the water quality of where they live. Penetration and establishment of pathogenic bacteria such as *Escherichia coli* and *Salmonella* spp. in different fish tissues and organs, such as digestive tract, gills, and muscle have been reported in polluted aquatic environments [11-13].

The handling practices observed after the capture until the storage influence the fish shelf life. Inadequate fish handling and cross-contamination can contaminate the fish with foodborne pathogens, such as *E. coli*, *Salmonella*, and *S. aureus* [12, 13]. Temperature is an important factor influencing the freshness of fish. Thus, to slow the growth of bacteria in fish, ice is used as a preservation method. Chilling slows down the deterioration of stored fish [14, 15].

Ice used in fish chilling must be of the same microbiological quality as drinking water [16]. Since almost all known bacterial enteropathogens of the *Enterobacteriaceae*, including *E. coli*, have been found in water, contamination of ice samples with these pathogens is possible. It is necessary to keep the microbiological ice quality to avoid cross-contamination of fish [17, 18].

When improper handling and storage occurs, the fish deteriorates rapidly, and its shelf life decreases. The high levels of microbiological contamination may pose a potential public health risk with the consumption of fish [6, 13]. Hence, this study aimed to evaluate the microbiological quality of ice and fresh tilapia samples commercialized in the Federal District.

2. MATERIALS AND METHODS

2.1. Microbiological analyses of fish

The samples analyzed in this study were collected from different supermarkets in the Federal District, Brazil, from July 2018 to June 2019. Fish samples were represented by whole fresh tilapia (10 samples) and by 200-300 g of fresh tilapia fillets (10 samples), totalizing 20 samples. Note that the filleting process of tilapia is not done in supermarkets, but the fish processing industry. A total of fourteen ice samples were gathered and placed in sterile plastic bags in the form of flakes. The samples were analyzed in triplicate, and their results were expressed as mean.

For microbiological evaluation of tilapia fish, 25 g of each sample was diluted in 225 mL of 0.1% peptone water. The material was homogenized, thus obtaining the first dilution (10^{-1}). From the first dilution the other decimal dilutions were obtained (up to 10^{-5}). Microbiological analyzes included total counts of mesophilic and psychrotrophic bacteria, determination of total and thermotolerant coliforms, research of *Salmonella* spp. and counts of *Staphylococcus aureus* and were performed according to official methods from American Public Health Association –APHA [19].

For mesophilic and psychrotrophic bacteria counts, the dilutions of the samples were surface plated on Plate Count Agar (HiMedia, USA), following incubation at 37°C for 24 h for mesophilic bacteria and at 8°C for 7 days for psychrotrophic bacteria.

Total and thermotolerant coliforms were determined by most probable number technique. For this, the dilutions of the samples were transferred to tubes containing Lauryl Sulfate Tryptose broth (LST) (HiMedia, USA) with Durham tubes in its interior. After 24 h of incubation at 37°C in LST, aliquots from positive cultures (determined by turbidity and gas production) were transferred to tubes containing Brilliant Green Bile Broth 2% (HiMedia, USA) for enumeration of total coliforms (incubated at 37°C for 24 h), and to tubes containing *E. coli* broth (Acumedia, USA) for enumeration of thermotolerant coliforms (incubated at 45°C for 24 h). Tubes with turbidity and gas production were considered positive for both tests.

For the research of *Salmonella* spp., the 10⁻¹ dilution of the samples was incubated at 37°C for 24 h. After incubation, aliquots were transferred to tubes containing 10 mL of Selenite Cystine broth (SC) (HiMedia, USA). A loopful of SC was streaked onto the xylose lysine deoxycholate agar (XLD) (HiMedia, USA) and Salmonella Shigella agar (SS) (HiMedia) and incubated at 37°C for 24 h. Presumptive *Salmonella* colonies in XLD and SS agars were confirmed biochemically using triple sugar iron agar (TSI) (HiMedia, USA) and lysine iron agar (LIA) (HiMedia, USA) slants. These slants were incubated at 37°C for 24 h. The presumptive *Salmonella* isolates that tested positive on TSI and LIA were confirmed by polymerase chain reaction (PCR).

For *S. aureus* counts, the dilutions of the samples were surface plated in Mannitol Salt Agar (HiMedia, USA) and incubated at 37°C for 48 h. The colonies were counted and sub-cultured in Mannitol Salt Agar tubes. The characteristic colonies of *S. aureus* (yellow colonies with yellow zones, mannitol-fermenting) were stained by Gram's Method to confirm Gram-positive cocci. The colonies of *S. aureus* were further confirmed by PCR.

2.2. Microbiological analyses of ice

For microbiological evaluation of ice, the samples were analyzed for total coliforms and thermotolerant coliforms, according to Funasa (2006) [20]. The ice samples were placed and stored at 8°C until completely melted. For detection of total and thermotolerant coliforms, 10 mL, 1 mL and 0.1 mL of melted ice were transferred to three series of five tubes containing Lauryl Sulfate Tryptose with Durham tubes in its interior. Total coliforms were enumerated in Brilliant Green Bile Broth 2%, incubated at 37°C for 24 h and thermotolerant coliforms were determined in *E. coli* broth (EC) incubated at 45°C for 24 h. Tubes with turbidity and gas production were considered positive for both tests. From the EC broth, the phenotypical characteristic colonies of *E. coli* were isolated using MacConkey agar and then confirmed by PCR.

2.3. Molecular analyses

The bacteria *S. aureus*, *Salmonella* and *E. coli* were identified using the technique of polymerase chain reaction (PCR). Table 1 presents the primers CoA forward and reverse specific for the coagulase gene (Coa gene) of *S. aureus*, the primers InvA forward and reverse specific for the invasion A gene (invA gene) of *Salmonella* and the primers MalB forward and reverse specific for the formation of acetaldehyde and ammonia from ethanolamine (MalB gene) for the identification of *E. coli*.

For DNA extraction, the isolates were cultivated overnight in Mueller-Hinton broth and had their DNA extracted employing the NucleoSpin Food kit (Macherey-Nagel, Düren, Germany), as per the manufacturer's instructions. PCR was performed in a 25 µl final volume reaction mixture containing: 2.5 µl of PCR buffer; 0.7 µl of MgCl₂; 1.5 µL of dNTP (2,5 mM); 0.5 µl of Taq DNA polymerase; 1.5 µL of each primer forward and reverse; and 18.3 µl of Milli-Q water. Thermal cycling reactions were conducted with Techne TC-512 thermal cycler (Bibby Scientific Inc., USA), and each PCR run included negative and reagent controls. The reagent control consisted of all PCR components except for the DNA template. The amplified DNA was separated by electrophoresis at 100 V for 50 min in 1.5% (w/v) agarose gel, stained with ethidium bromide and visualized under UV light. A 100 bp DNA ladder was used as a molecular weight marker.

Table 1: Primers sequence of PCR used for identification of *Staphylococcus aureus*, *Salmonella* and *Escherichia coli*.

| Primer | Sequence 5' - 3' | Amplified product | Bacteria |
|--------------|----------------------|-------------------|------------------------------|
| CoA forward | GATCTTCGCGTGATACGTCA | 303 pb | <i>Staphylococcus aureus</i> |
| CoA reverse | GTTCGTGCAATGTTTGTCC | | |
| invA forward | GCTGATGCCGGTGAAATTAT | 103 bp | <i>Salmonella</i> spp. |
| invA reverse | TGTCACCGTGGTCCAGTTTA | | |
| MalB forward | TCTATGGGCTGTGACTGCTG | 113 bp | <i>Escherichia coli</i> |
| MalB reverse | GGCATCCCCATGATGTAGTT | | |

3. RESULTS AND DISCUSSION

3.1. Microbiological analyses of tilapia samples

Table 2 shows the results of microbiological analyses in the samples of tilapia. The *International Commission on Microbiological Specifications for Foods - ICMSF* [21], recommends 7 log CFU/g as the maximum acceptable limit for counting mesophilic and psychrotrophic bacteria in fresh fish.

Table 2: Microbiological analyses of tilapia samples.

| Samples | MM (log CFU/g) | PM (log CFU/g) | TT (log MPN/g) | TC (log MPN/g) | <i>S. aureus</i> (log CFU/g) | <i>Salmonella</i> spp. |
|-----------------------|-------------------|-------------------|-------------------|-------------------|---------------------------------|---------------------------|
| Whole samples | | | | | | |
| 1 | 4.90±0.18 | 5.36±0.05 | 0.95±0.53 | ND | 2.00±0.12 | Negative |
| 2 | 3.71±0.09 | 1.16±2.00 | 2.43±0.05 | ND | 2.00±0.21 | Positive |
| 3 | 1.77±1.66 | 4.23±0.12 | ND | ND | ND | Negative |
| 4 | 1.43±1.25 | 3.46±0.27 | 0.95±0.10 | ND | 1.52±0.23 | Positive |
| 5 | 5.20±0.69 | 6.25±0.62 | 2.68±0.37 | 2.00±0.62 | ND | Negative |
| 6 | 3.92±0.19 | 4.85±0.30 | 1.10±0.17 | ND | ND | Negative |
| 7 | 2.00±0.00 | 5.84±0.20 | 0.20±0.10 | ND | ND | Positive |
| 8 | 3.85±0.20 | 5.08±0.83 | 0.34±0.30 | 0.50±0.05 | ND | Negative |
| 9 | 1.10±0.01 | 2.89±0.19 | 1.62±0.26 | 0.20±0.61 | 2.95±0.49 | Negative |
| 10 | 4.80±0.11 | 3.63±0.11 | 0.76±0.17 | ND | 2.26±0.45 | Positive |
| Fillet samples | | | | | | |
| 11 | 6.31±0.21 | 7.36±0.39 | 3.04±0.10 | 1.11±0.42 | 2.84±0.10 | Negative |
| 12 | 5.90±0.42 | 6.37±0.23 | 2.81±0.30 | 1.49±0.10 | ND | Positive |
| 13 | 4.80±0.10 | 6.73±0.53 | 1.90±0.23 | 1.90±0.10 | 2.00±0.42 | Positive |
| 14 | 5.71±0.09 | 8.00±0.01 | 1.90±0.10 | 0.70±0.53 | 2.65±0.01 | Positive |
| 15 | 5.85±0.42 | 7.54±0.03 | 2.90±0.22 | ND | ND | Negative |
| 16 | 3.09±0.10 | 5.02±0.19 | 1.00±0.55 | ND | 3.15±0.21 | Negative |
| 17 | 4.28±0.12 | 4.90±0.19 | 2.53±0.45 | 0.79±0.24 | ND | Positive |
| 18 | 5.83±0.15 | 6.21±0.01 | 1.91±0.65 | ND | 2.20±0.17 | Negative |
| 19 | 5.59±0.38 | 6.29±0.02 | 1.76±0.26 | 0.56±0.10 | ND | Positive |
| 20 | 4.18±0.17 | 6.08±0.58 | 0.63±0.20 | ND | 2,15±0.21 | Positive |

MM = Mesophilic microorganisms; PM = Psychrotrophic microorganisms; TT = Total coliforms; TC = Thermotolerant coliforms. Samples 1-10: whole fresh tilapia; Samples 11-20: fresh tilapia fillets. Results reported as means ± standard deviation of three measurements; ND = not detected.

In this study, of the 20 samples analyzed, 17 samples (85%) showed acceptable mesophilic and psychrotrophic bacteria counts ($< 7 \log \text{CFU/g}$) while 3 tilapia fillets samples (15%) showed high counts of psychrotrophic bacteria (7.36-8.00 CFU/g). An increase in psychrotrophic bacteria counts to levels above $7 \log \text{CFU/g}$ is usually indicative of long storage at chilling temperatures or temperature abuse prior to chilling [22, 23].

Eltholth et al. (2018) [23] reported similar results for microbiological analyzes of farmed tilapia collected from Egyptian fresh fish markets, and more than 85% of samples tested were within the mesophilic bacteria counts $< 7 \log \text{CFU/g}$. In contrast, Budiati et al. (2015) [22] reported 20 of 32 tilapia samples (62.5%) obtained from wet markets in Malaysia recorded counts of total bacteria higher than $7 \log \text{CFU/g}$. According to Sundarambal et al. (2017) [24], freshwater fish species such as tilapia, rainbow trout, and silver perch reported mesophilic bacteria counts between 4 and $6 \log \text{CFU/g}$, furthermore, the gutting procedure can increase the initial load of microorganisms by exposing the fish flesh to environmental conditions.

According to Soares et al. (2014) [6], the enumeration of total coliforms is a good indicator of the quality and expected shelf life of the product. Most tilapia samples (95%) showed acceptable enumeration of total coliforms ($< 3 \text{MPN/g}$) and just one sample of fillet presented high enumeration of total coliforms (3.04 MPN/g). Gatti Junior et al. (2014) [13] reported higher total coliform enumeration in fillet compared to whole fish muscles. Sundarambal et al. (2017) [24] also reported that the total coliforms count in the gutted samples were higher than the whole samples.

The maximum value allowed for thermotolerant coliforms or *E. coli* in fresh fish is $2.7 \log \text{CFU/g}$ according to Brazilian legislation [25]. In the present study, thermotolerant coliforms were detected in 9 tilapia samples (45%), with values ranging from ND to $2 \log \text{MPN/g}$. Gatti Junior et al. (2014) [13] reported similar results for thermotolerant coliforms (ND to $2 \log \text{MPN/g}$) in farmed tilapia collected from ten supermarkets located in the region of São Paulo, Brazil.

All the studied tilapia samples showed acceptable enumeration of thermotolerant coliforms ($< 2.7 \text{MPN/g}$), though the fillet samples (7 positive samples from 10 analyzed, 70%) were more contaminated with thermotolerant coliforms than whole samples (3 positive samples from 10 analyzed, 30%). The filleting process can increase microbial contamination by exposing fish flesh to improper and unsanitary handling and cross-contamination [13, 24]. The study of Rong et al. (2009) [26] reported changes in the microbial flora of filleted tilapia during iced storage to be higher than those in whole fish. This microbial contamination during fillet processing makes fish fillets difficult to sustain prolonged storage even under correct refrigeration. The storage life of tilapia can be expected to be 12 days for the whole fish and 6 days for the fillets.

The limit value for *S. aureus* in fresh fish is $3 \log \text{CFU/g}$ according to Brazilian legislation [25]. In the present study, *S. aureus* was found in 11 samples (55%), and which one fillet sample presented *S. aureus* counts (3.15 CFU/g) above the limit allowed by Brazilian legislation. These results were confirmed by detection of the CoA gene. According to Gatti Junior et al. (2014) [13], cross-contamination of tilapia samples during the filleting process is an ongoing problem. In humans, *S. aureus* can be in the respiratory tract, nasal mucosa, and skin; and, therefore, inadequate food handling may contaminate food supply [27]. *S. aureus* is particularly harmful as it produces a thermostable toxin, which is one of the most prevalent causes of gastroenteritis [13].

The presence of *Salmonella* in fresh fish is unacceptable according to Brazilian legislation [25]. In this study, of the 20 samples of fresh tilapia analyzed, 10 samples (50.0%) presented *Salmonella* (6 samples were fillets, and 4 samples were whole fish) and were confirmed as such by detection of the *invA* gene. Recently, some studies were carried out on the presence of *Salmonella* in cultivated freshwater fish [28-31]. Lerma-Fierro et al. (2020) [28] detected *Salmonella* spp. in 41.7% (5/12) of fresh Nile tilapia fillets marketed in Tepic Nayarit city, Mexico. Elhadi (2014) [32] studied the proportion of imported frozen fish contaminated with *Salmonella* in Saudi Arabia and reported that of 25 tilapia samples imported from Thailand, 16 samples (64.0%) were positive for *Salmonella* spp. Siala et al. (2017) [33] confirmed the presence of *Salmonella* using the *InvA* gene in 11 of 46 fish samples analyzed (23.9%) in Tunisia.

Salmonella's primary habitat is the intestinal tract of warm-blooded animals, especially birds, and although *Salmonella* has also been found in the gut of different species of tropical fish, its never detected in fish caught in unpolluted waters as this bacterium is not part of the fish's natural

microbiota [12, 27]. Fisheries are particularly concerned by pathogenic bacteria contamination in polluted waters. Their presence in fish may cause a potential risk of causing foodborne illnesses [11, 12].

The use of poultry litter as fertilizer in culture tanks is another crucial factor contributing to *Salmonella* contamination in fish [12]. Elsaïdy et al. (2015) [34] assessed the microbial water and Nile tilapia quality in ponds, using fertilized chicken manure and fermented chicken manure. Bacteriological analysis of water and fish samples of ponds revealed that the bacterial load that received fermented chicken manure was much lower than the bacterial load in ponds that received raw chicken manure. Also, *E. coli* and *Salmonella* were isolated with high incidence in ponds that received chicken manure than in ponds that received fermented chicken manure. According to Elsaïdy et al. (2015) [34] the use of organic matter originating from animal feces, poses a risk to the water environment and represents great public health concern, owing to an increase in the concentrations of pathogenic microorganisms in system units with little exchange of water.

It was observed that from the total of 20 tilapia samples, 11 samples (55%) were unfit for consumption: 4 samples of whole tilapia (20%) and 7 samples of fillet (35%) (Figure 1). This result of more fillet samples than whole samples unfit for consumption was also observed by Gatti Junior et al. (2014) [13]. According to the authors, possible sources of contamination of tilapia fillets may include the various utensils used (cutting board, knives), personnel (workers' hands) and the ice used to store the fish.

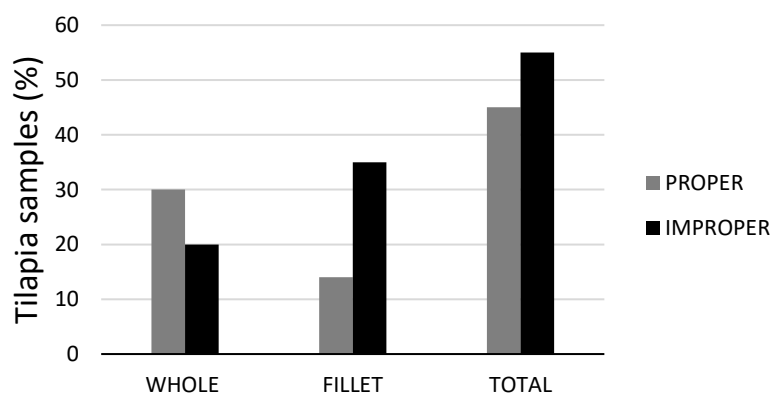


Figure 1: Percentage of tilapia samples proper and improper for consumption.

3.2 Microbiological analyses of ice used in tilapia conservation

To slow the growth of bacteria in fish ice is used as a conservation method. In this context, chilling is defined as the process of cooling fish or fish products to a temperature close to its freezing point by means of heat withdrawal [14, 35]. Ice used in fish chilling must be of the same microbiological quality as drinking water. According to Brazilian legislation, total and thermotolerant coliforms must be absent in 100 mL of ice water, to guarantee the criteria for its potability [16]. Of the 14 ice samples analyzed in this study, 12 samples (85.7%) were unfit for use due to the presence of total coliforms, and 9 (64.3%) were also contaminated with thermotolerant coliforms. *E. coli* represents 90% of the composition of the thermotolerant coliforms group, therefore, of the 9 positive samples for thermotolerant coliforms, *E. coli* was isolated in 6 ice samples (42.9%), and the strains were genetically confirmed by amplification of the MalB gene (Table 3).

Table 3: Microbiological analyses of ice used in tilapia conservation.

| Ice Samples | Total coliforms (log MPN/g) | Thermotolerant coliforms (log MPN/g) | * <i>Escherichia coli</i> |
|-------------|--------------------------------|---|---------------------------|
| 1 | 3.20±0.05 | 1.95±0.20 | |
| 2 | 3.20±0.01 | 2.45±0.30 | MalB |
| 3 | 1.70±0.20 | 1.34±0.05 | MalB |
| 4 | 1.11±0.01 | ND | |
| 5 | 0.60±0.45 | 0.60±0.40 | |
| 6 | 1.41±0.08 | 0.78±0.30 | |
| 7 | ND | ND | |
| 8 | ND | ND | |
| 9 | 1.24±0.10 | 0.60±0.01 | MalB |
| 10 | 1.23±0.07 | 0.95±0.05 | MalB |
| 11 | 1.52±0.40 | 1.32±0.20 | MalB |
| 12 | 0.60±0.07 | ND | |
| 13 | 3.20±0.01 | 2.45±0.05 | MalB |
| 14 | 0.84±0.05 | ND | |

Results are reported as means ± standard deviation of three measurements; ND: not detected; **E. coli* were identified through the amplification of the MalB gene using PCR.

In Brazil, some studies reported a high level of coliforms in the ice used to preserve fish. Ferreira et al. (2014) [17] reported that of the 8 ice samples used for fish conservation collected in the city of Raposa, Maranhão, 6 samples (75%) were contaminated by total and thermotolerant coliforms and 2 samples (25%) by *E. coli*. Lopes et al. (2012) [36] evaluated the microbiological quality of ice samples from 3 factories located in Cedral, Maranhão, and all ice samples presented total coliforms. Dorta et al. (2011) [37] identified total coliforms and *E. coli* in all analyzed ice samples from factories in located the city of Teresina, Piauí.

Other studies reported contamination of ice used to cool drinks and foods. A study in Malaysia revealed the presence of thermotolerant coliforms in 36% of samples of ice cubes from 30 foodservice outlets [38]. Mako et al. (2014) [39] reported that 37% of samples of ice bagged from vending machines in Georgia contained coliforms bacteria.

The probable causes of ice contamination with total coliforms are the use of non-potable water for ice making, cross-contamination of ice and fish, and lack of hygiene during the production and handling of ice. The presence of thermotolerant coliforms and *E. coli* is a strong indication of recent fecal contamination. To avoid ice contamination personnel involved in the production and handling of ice should be trained in relative hygiene matters while ice-machines should be cleaned and disinfected regularly [38-40].

4. CONCLUSIONS

The results of this study showed high contamination of tilapia samples marketed in the Federal District with *Salmonella*. Of the 20 samples of fresh tilapia analyzed, 11 samples (55%) were unfit for human consumption according to the Brazilian legislation, due to presence of *Salmonella* in 10 samples and 1 sample exceeds the limit counts for *S. aureus*. Most of the ice samples analyzed (85.7%) were unfit for use in fish conservation due to the presence of total coliforms; moreover, 64.3% ice samples were also contaminated with thermotolerant coliforms. Thus, there is a need for greater hygienic-sanitary control during the tilapia production, processing, and marketing stages, as the presence of potentially pathogenic microorganisms may pose a potential public health risk among fish consumers. It is also an alert to the authorities to improve the inspection of good practices of storage and handling of fish and ice to guarantee the food safety of the consumer.

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