Aquaculture 433 (2014) 196–200

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Microbiological quality of whole and filleted shelf-tilapia

Pedro Gatti Junior [a,b,*], Argos W.A. Assunção [a], Juliana C. Baldin [c], Luiz A. Amaral [c]

[a] Aquaculture Centre of São Paulo State University, UNESP — Univ Estadual Paulista, 14870-000, Jaboticabal, Sao Paulo, Brazil
[b] International Institute of Ecology, 13560-660, Sao Carlos, Sao Paulo, Brazil
[c] Department of Preventive Veterinary Medicine, UNESP — Univ Estadual Paulista, 14870-000, Jaboticabal, Sao Paulo, Brazil

ARTICLE INFO

Article history:
Received 8 August 2013
Received in revised form 16 June 2014
Accepted 17 June 2014
Available online 24 June 2014

Keywords:
Fish
Fillet
Tilapia
Self-contamination
Coagulase-positive *Staphylococcus*
Thermotolerant coliforms

ABSTRACT

Despite fish being a rich source of animal nutrients and having numerous associated health benefits, it is an extremely perishable food, prone to a wide range of hazards. The bacterial load associated with shelf-whole-fish organs (e.g. digestive tracts and skin) or mishandling of fish may be a vehicle of infection and become a risk to public health. The objective of this paper is to evaluate the microbiological quality of whole ungutted and filleted shelf-tilapia, as well as assess the safety for human consumption. For this purpose, in order to investigate the distribution and occurrence of bacterial populations, the count of total and thermotolerant coliforms, coagulase-positive *Staphylococcus* and presence of *Salmonella* spp. was determined. This paper shows that all fish organs were contaminated with thermotolerant coliform. Skin and fillet show higher populations and occurrence of all microorganisms analyzed. Lower bacterial populations were recovered from the gut and muscles of whole tilapia. Two samples of fillet were contaminated with coagulase-positive *Staphylococcus*. It can be concluded that the skin and filleted tilapia are important carriers of food-borne pathogens. In addition, fish might become an important cross and self-contamination source.

1. Introduction

Fish can contribute to a higher level of food safety and security by providing high quality animal protein, essential fatty acids, vitamins and minerals. It also plays an important role in the economy of many countries by increasing employment opportunities and reducing poverty. Global production of fish from aquaculture reached 52.5 million tonnes in 2008 and is currently the animal food-producing sector that has grown most, responsible for 45.6% of the world's food fish consumption (FAO, 2012).

Furthermore, fish is eaten in many ways including smoked, cooked and raw.

However, it has been shown that fish can be a source of food-borne illnesses, causing outbreaks (Fagan et al., 2011; Jain et al., 2008; Piérard et al., 1999; Shao et al., 2011) This has made consumers more aware, and has therefore become an important public health issue, which in many cases is neglected (Ayulo et al., 1994; EFSA, 2010; Zarei et al., 2012). Moreover, food-borne outbreaks have been related to fish fillet (Chen et al., 2010; Kawai et al., 2012) and uneviscerated fish (French et al., 1992; Telzak et al., 1990), showing that processing or non-processing of fish does not necessarily ensure its safety.

Human food pathogens, such as *Vibrio* spp., *Salmonella* spp., *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Clostridium perfringens*, *Listeria monocytogenes*, and *Shigella* spp. can be found to be associated with fish tissue as a consequence of contamination during the food production chain (Roberts et al., 2005). These food-borne pathogens can be found in the environment and survive in fish tissue until the fish is ready to consume (Pillay, 1992; Suhalim et al., 2008). Contaminated fish ponds (e.g. high bacterial load due to fertilization and human and animal wastewater) enable the penetration of pathogens into fish tissue (i.e. digestive tract, gills, muscles, kidneys and liver) and may be responsible for causing food-borne diseases (Mara and Cairncross, 1989; Pillay, 1992; Suhalim et al., 2007). Several studies showed that the concentration of bacteria in water, such as *E. coli*, *Salmonella* spp. and *S. aureus* is proportional to the concentration of bacteria recovered from fish organs and tissue (Buras et al., 1987; El-Shafai et al., 2004; Geldreich and Clarke, 1966; Guzmán et al., 2004; Pal and Gupta, 1992). These organisms are not natural microbiota of fish and their presence can be related to the fish food web, host–microbiota interactions and environment determinants (Kostic et al., 2013). For example, common food-borne pathogens such as *Salmonella* and *S. aureus*, which have been reported as causes of food-borne disease, are not typical environmental contaminants, but usually contaminate the food during processing or food-service operations (Kasai et al., 2010; Vollaard et al., 2004). Mishandling such as improper cooling, inadequate fish handling, and cross contamination to other food may occur when the fish is prepared and cleaned for consumption (Beatty et al., 2009; Leon-Velarde et al., 2004; Mor-Mur and Yuste, 2010; Sofos, 2008; Yan et al., 2005). Moreover, cleaning and filleting decrease the fish's shelf-life. Whole ungutted fish has a greater shelf life than gutted

* Corresponding author at: Instituto Internacional de Ecologia, 13560-660, Rua Bento Carlos, no. 750, Centro, São Carlos, São Paulo, Brazil. Tel.: +55 16 33625400; +55 16 9177 7703.
E-mail address: pgattijunior@gmail.com (P. Gatti Junior).

http://dx.doi.org/10.1016/j.aquaculture.2014.06.015
0044-8486/

fish (Cakli et al., 2006; Papadopoulos et al., 2003) and filleted fish (Chytiri et al., 2004). Therefore, the whole ungutted fish should be safer for human consumption. On the other hand, these studies do not evaluate the population of microorganisms in other organs (e.g. skin and gut) of whole fish. Since these organisms inhabit the organs, they can be a strong source of cross-contamination and represent potential hazards to public health.

The purpose of this paper is to (1) evaluate if the whole fish and its organs can be a source of cross-contamination. (2) compare bacteria populations from fish organs (skin, gut and muscle) with the filleted tilapia populations (3) verify if there is a potential hazard in the consumption of whole fish.

The study was developed to evaluate: (i) quantities of coliforms, thermotolerant coliforms, coagulase-positive *Staphylococcus* and the presence of *Salmonella* ssp. in the muscles, gut, skin and filleted tilapia; (ii) the occurrence, relationships and percentage distribution of coliforms and staphylococci among the samples.

2. Material and methods

Samples were collected from six cities (Ribeirão Preto, São Carlos, Araraquara, São José do Rio Preto, Catanduva and Barretos) at ten supermarkets located in the Northwest region of São Paulo state, Brazil. For the sample collection, the supermarkets had to sell both whole ungutted tilapia (*Oreochromis niloticus*) and fillets stored in ice. In each supermarket, ten fish (five whole and five packets of fish fillets) were placed in individual sterile plastic bags and stored on ice packs and then placed in polystyrene cooler boxes until they reached the laboratory. All whole fish had a seal stating they were originating from captivity (piscicultures). In the end, 40 whole fish and 50 packets of fish fillets were analyzed.

2.1. Sample preparation and dissection

To determine skin contamination, 200 ml of 0.1% peptone-water was added in the sterile plastic bag and massaged (for 60 s) to recover the bacterial load. Afterwards, the skin was rinsed with 70% ethanol, and removed aseptically. Samples of 25 g of muscle (flesh of the anterior–dorsal region) for each whole fish or fillet was taken and diluted in a 500 ml glass flask containing 225 ml of peptone-water (0.1%). Finally, 15 g of the intestinal tract was removed, including its contents, and transferred to a glass flask (135 ml of 0.1% peptone-water) to obtain ten-fold dilution. To recover the organisms, all samples were homogenized (60 s) in a stomacher and further ten-fold dilutions (10^2, 10^3) were prepared. The equipment used for dissection and solutions were sterilized in an autoclave before each use.

2.2. Microbiological analyses

Bacteriological examinations were conducted as soon as possible after the fish were sampled. The methods used to recover the bacterial load were: the most probable number (MPN) with the presumptive test in lauryl sulfate tryptose at 35 °C for 24–48 h and confirmation in brilliant green bile broth 2% (35 °C, 24–48 h), for coliform bacteria as recommended by APHA (2005), and for thermotolerant coliform, confirmation in E.C. broth and incubation at 44.5 °C (24 h) (APHA, 2005).

For coagulase-positive *Staphylococcus*, 0.1 ml samples of serial dilutions of fish tissue homogenates were spread on the surface of Baird Parker Agar at a temperature of 35–37 °C and incubated for 24–48 h. Suspected colonies were identified by the coagulase test (APHA, 2001).

For *Salmonella* spp., pre-enrichment was conducted incubating the tissues sample dilutions at 37 °C for 24 h. Thereafter, 1.0 ml and 0.1 ml samples were inoculated in Selenite Cysteine and Rappaport–Vassiliadis broth respectively, both containing 1.0 ml novobiocin (0.4%) and left for incubation (37 °C; 24 h). Then, the enrichment was spread in both Brilliant Green and MacConkey agar followed by incubation (37 °C; 24 h). Suspected colonies were inoculated in TSI agar and TSA for serological testing (APHA, 2001).

2.3. Statistics

For statistical analysis, concentrations of indicator bacteria in fish tissues were transformed by taking the logarithm of the number per g. Data were subjected to analysis of variance (ANOVA) using a significance level of $p < 0.05$ by Tukey's honestly significant difference test. Analyses were performed in SAS 9.1 (SAS Institute, Cary NC).

3. Results

Fecal contamination and coagulase-positive *Staphylococcus* were found in muscles and fillet respectively. The values for total and thermotolerant coliforms fluctuated between <3 and >1100 MPN/g and for *Staphylococcus* sp. $<1.0 \times 10^2$–1.2×10^6 CFU/g in the whole tilapia. Table 1 shows the distribution of the fish tissues according to their total coliform counts.

A considerable percentage of the skin (22%) presented values higher than 100 MPN/g and 10% showed coliform counts above 1100 MPN/g. Thermotolerant coliforms were present, but in 45% of the samples, they could not be detected. Therefore, 55% of the samples had thermotolerant coliforms counts over 3.0 MPN/g, with 5% showing counts higher than 1100 MPN/g. Furthermore, the skin had a high number of samples with presence of coliform (Fig. 1). Total counts and number of thermotolerant coliforms were present in fewer samples of muscle (17.5 and 5%) and gut (25 and 12.5%), respectively, with a lower number of microorganisms in both organs (e.g. gut; 210 MPN/g; muscle; 93 MPN/g). Table 2 shows a statistical comparison among logarithmic means of total coliforms, thermotolerant coliforms and *Staphylococcus* sp. count in individual fish organs.

The means of coliforms and *Staphylococcus* sp. were significantly higher in the tilapia's skin ($p < 0.05$), while gut and muscle did not show significant differences between them ($p > 0.05$) (Table 2).

From the fillet analysis, results show that coagulase-positive *Staphylococcus* were present in two samples and the count was 1.0×10^2 CFU/g and 2.3×10^3 CFU/g. Moreover, the count of total coliforms ranged from <3.0 to >1100 MPN/g; thermotolerant coliforms from <3.0 to 460 MPN/g; and *Staphylococcus* sp. from $<1.0 \times 10^2$ to 9.4×10^3 CFU/g. A significantly higher count of coliform and *Staphylococcus* sp. ($p < 0.05$) were found in fillet compared to muscles as shown in Table 3. In addition, the occurrence of coliforms in fillet samples was greater (Fig. 1). Here, the bacterial count in tilapia organs declined in the following order: skin > fillet > muscle and gut. Bacteria from the genus *Salmonella* were not found.

4. Discussion

All fish organs were contaminated with thermotolerant coliforms. Generally, fecal contamination is not recovered in the whole fish muscle, although it may occur when the concentration of bacteria in the water is high (Buras et al., 1985, 1987; Guzmán et al., 2004). The skin and intestine contain lymphocytes, granulocytes and macrophages which eliminate antigens (Alexander and Ingram, 1992; Trust, 1986) when the fish is alive, however microorganisms might break the immunological barriers, penetrating and accumulating in different organs (Buras et al., 1985; Fattal et al., 1992; Suhalim et al., 2008). Considering that the muscle remained intact because of the skin cover, the results indicate that thermotolerant coliforms recovery in the whole fish muscles is associated with the pisciculture water quality.

In this study, the gut shows lower bacterial population than skin. Usually the enteric bacteria present in water are recovered from the digestive tract of fish (Buras et al., 1985, 1987; El-Shafai et al., 2004; Geldreich and Clarke, 1966; Guzmán et al., 2004; Hejkal et al., 1983).