**Microbiological Analysis of Tilapia Fillet Stored Under Refrigerated Conditions**

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**Abstract:** Fish is a food susceptible to deterioration and contamination by microorganisms through the food chain, affecting its quality and safety. Fish is subjected to various processing and preservation methods in order to maintain availability, quality and safety for a longer time. The objective of the study was the microbiological analysis of tilapia fillets in refrigeration for 12 days. The fillet processing was carried out to obtain fillets. The fillets were packed in polyethylene bags and later in an isothermal polystyrene container and kept in refrigeration for 12 days and the microbiological analysis was carried out through quality and safety indicators. The results of the microbiological analysis indicated a regular growth dynamic during storage, reaching maximums for Aerobic Mesophylls (AM) and Aerobic Psychrophiles (AP) of 12.5 and 8.6 Log CFU/g respectively, Fungi and Yeast (FY) 9 Log CFU/g, Lactic Acid Bacteria (LAB) 9 Log CFU/g, Total Coliforms (TC) 8.3 Log CFU/g and Pseudomonas (P) 7 Log CFU/g. According to different microbiological criterion related to deterioration and acceptability in food, the counts obtained in fillet reached maximum values on day 4 of storage for AM, day 6 for AP, day 8 for P, day 2 for TC and after day 2 for LAB. The analysis of microbial growth in fillet under refrigeration through quality and safety indicators, allow estimating conditions and periods of availability of healthy and nutritious food.

**Keywords:** Aquaculture, Food Safety, Microbiological Indicator, Quality, Spoilage

**Introduction**

Fish is any animal that commonly inhabits fresh or salt water and is intended for human consumption (da Rocha, 2006). Fish and products belong to the foods mostly produced and marketed globally in different forms, whether it is live, fresh or refrigerated, frozen, prepared, preserved, dry, salted or cured fish (Castillo-Jiménez et al., 2017; FAO, 2020). The global trend in fish production has been on the rise for several years now, reaching by 2016 approximately 171 million tons via fishing and aquaculture, of which 88% was for direct human consumption, with a per capita of 20.3 Kg (Castillo-Jiménez et al., 2017; FAO, 2018).

Within the fishing and aquaculture activities aimed at the production of food for human consumption, a relevant product is tilapia. Tilapias are freshwater fish native to Africa, they belong to the Cichlid family and consist of three genera according to parental care patterns: *Tilapia, Oreochromis* and *Sarotherodon* (Jácome et al., 2019). Species of the genus *Oreochromis* (*O. niloticus, O. aureus, O. mossambicus*) and interspecific hybrids (red tilapia) are considered very suitable organisms for cultivation as they tolerate high densities, are fast growth, disease resistant, adaptable to captivity, balanced diet, in addition to its meat being of quality and affordable price, thus becoming one of the fish with the highest production globally (approximately 10% of the fish production of fin), commercialization and acceptance for consumption mainly as a complete product or fillet (Arias and Chaves, 2012; FAO, 2018; Prabhu et al., 2019; Jácome et al., 2019).

Fish is considered a highly nutritious food as it is mainly a source of proteins, lipids, vitamins and minerals; however, it is also very susceptible to deterioration that can cause losses and waste at all levels of the fishing and aquaculture value chain (Pascual and Calderón, 2000;
Corbo et al., 2005; Corrales-Ramírez et al., 2011; Castillo-Jiménez et al., 2017; FAO, 2020). It is estimated that chemical and microbial spoilage can cause annual losses in the food industry for up to 25% in gross primary agriculture and fishery products (Alak et al., 2010). The deterioration of the fish is due to endogenous auto lytic activity, chemical (oxidative) activity and post-mortem microbiological activity, being the latter the main responsible for the loss of quality by modifying its nutritional and sensory characteristics (smell, taste, appearance and texture), determining thus its useful life and safety (Pascual and Calderón, 2000; Corbo et al., 2005; Corrales-Ramírez et al., 2011; Fuertes et al., 2014; Castillo -Jiménez et al., 2017; FAO, 2020; Cortés- Sánchez et al., 2021).

As microbial activity is mainly related to the deterioration and safety of the fish, it should be noted that factors such as the time of year, feeding characteristics, geographic area, fish species and capture system influence the load and type of microorganisms present initially, while the processing, handling and storage conditions (temperature and atmosphere), in turn, can condition the altering and pathogenic microbiota (Pascual and Calderón, 2000; Corbo et al., 2005; Sousa, 2006; Álvarez et al., 2011; Corrales-Ramírez et al., 2011; Fuertes et al., 2014; Cortés-Sánchez et al., 2021).

Microbiological contamination can be present in all phases of the food chain and meat is a good means to maintain microbial development, due to its chemical composition with a high protein content, slightly acidic pH and high water activity; therefore, for obtaining this type of food, it is necessary, right after the death of the animal, for the meat to be subjected to low temperatures with the use of ice, refrigeration or freezing for its conservation in an uninterrupted way, until its consumption, in order to avoid or delay the growth rate of the present microbiota and thus the deterioration and safety (Pascual and Calderón, 2000; Flores-Rondon, 2011; Fuertes et al., 2014; Castillo-Jimenez, 2017; Cortés-Sánchez et al., 2021).

For several years now, the consumer has demanded compliance with regulatory measures in aspects of freshness and safety in the available fish, in order to protect health; also, being of global interest the quality of the fish and the implementation, control and inspection of practices in fishing and aquaculture, all of this aimed at protecting public health through the prevention of foodborne diseases (da Rocha et al., 2006; Castillo-Jiménez et al., 2017; dos Santos and e Barros, 2020). To determine the quality of a food, made by the inspection approach in the production and final product of the food, it is made through the physical-chemical and microbiological analysis, with the aim of verifying whether the product complies with regulations of the country of origin or not and those of foreign markets as well (Sousa, 2006).

The analysis of the microbiological quality of food is of vital importance since it allows knowing the possible sources of contamination, evaluating hygiene practices in the preparation and handling of food, as well as detecting the possible presence of risk pathogens for the health of the consumer and to establish at what moment alteration phenomena occur to determine their conservation period and shelf life (Campos et al., 2003; Sousa, 2006; Puig et al., 2014; dos Santos and Barros, 2020). For the above, different microbiological indicators of food quality and hygiene have been established, such as total aerobic mesophilic bacteria, total coliforms, psychrophiles, fecal coliforms, lactic acid bacteria, fungi, yeasts, among others (Sousa, 2006; Rodrigues et al., 2008; Puig et al., 2014; Campuzano et al., 2015; Castillo-Jiménez et al., 2017; Garcia and Gonzalez, 2017; dos Santos and Barros, 2020). These indicators are regularly used to monitor and detect changes in quality, to classify and restrict the use of equipment, to monitor production facilities, water or food, as well as to validate quality and safety systems such as Hazard Analysis and Critical Control Points (HACCP) in the food industry (Sousa, 2006; Rodrigues et al., 2008; Puig et al., 2014; Efuvwewere et al., 2020). Therefore, the purpose of this study is the microbiological analysis of tilapia fillets during their storage and conservation in refrigeration at 4°C for 12 days and the influence of these conditions on the growth of different microbial indicator groups frequently used to determine the quality and food safety.

Materials and Methods

Sample Collection

The study sample consisted of nine adult tilapia specimens (Oreochromis niloticus) with an average weight of 821.5±166.6 g and a total length of 30.6±4.1 cm, which were supplied alive by the Nayarit Unit of the Center for Biological Research of the Northwest (UNICIBNOR+ acronym in Spanish) maintained and transported under such conditions, in two containers of approximately 1 m³, to the meat technology laboratory of the Technological University of Nayarit (UT Nayarit, acronym in Spanish), for further slaughtering and processing to obtain fillet.

Obtaining Fillets

To obtain fillet, the fish was subjected to slaughter through a thermal shock with water-ice, to later manually perform operations of minimal processing (peeling, gutting, washing, heading and filleting) until obtaining a constituent product of skinless fillet, which was subjected to pre-cooling by immersion in ice-water at temperatures of ≤4°C/5 min Subsequently, 100 g portions of fillet were weighed and placed in 16.5 cm x 17.5 cm polyethylene bags and finally the bags were introduced into an isothermal polystyrene container with dimensions of 18.5 cm x 12 cm x 16 cm together with 12 cm x 14 cm cooling
gel packs (Fig. 2). The final container was covered and stored under refrigeration conditions at 4°C for 12 days in an upright Torrey model R14L refrigerator with dimensions: 74 cm wide x 183 cm high and a useful capacity of 340 L. Initially, the physicochemical analysis of the fillets obtained; meanwhile, for the microbiological analysis, the fillet sample was collected at 0, 2, 4, 6, 8, 10 and 12 days of storage at 4°C (Fig 1).

**Microbiological and Physicochemical Analysis**


**Statistical Analysis**

The analysis of results was carried out in spreadsheets of Microsoft Excel Windows 2013 software, expressed as the average of at least three replications.

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**Fig. 1:** Flow diagram of the processing of tilapia (*O. niloticus*) to obtain fillet, conservation in refrigeration, physicochemical and microbiológica analysis.

**Fig. 2:** Isothermal container of tilapia (*O. niloticus*) fillets for their final storage at 4°C for 12 days.
Results and Discussion

Physicochemical Fillet Analysis

In the processing of fish, the yield of fresh fillet obtained was 27.7% ±3.6, finding these values within the range of fillet yield of tilapia (O. niloticus) reported in related research, which ranges between 21 and 41% (Rojas-Runjaic et al., 2011). Table 1 shows the different values of the physicochemical analysis performed on the tilapia fillets, where the pH was 6.33 and water activity was 0.99, which corresponds to the fresh state and characteristics of perishable foods that favor microbial growth and deterioration. Regarding their chemical composition, the fillets presented a higher proportion of water with a 79%, followed by proteins with 17.5% and lipids with 1.65%; therefore, tilapia is a lean species due to the fact that it is in accordance with the fish classification related to the fatty content, lean or white fish are those whose proportion is less than 3%, semi-fatty with values between 3 and 5% and fatty or blue when having values higher than 5% of lipids (Arza et al., 2014) and finally, the fillets had an ash content of 1.36%. The values obtained in the composition of fillets is within the range of the values reported in investigations related to the composition of fish, including tilapia (Huss, 1999; Víquez, 2002) (Table 1). It should be noted that the chemical composition of fish can vary between different species, individuals of the same species, diet, age, sex, environment and even the season of the year (Huss, 1999).

Animal meat is the basis of human nutrition and is considered a nutritious food due to its source of protein but, at the same time, it is a very perishable food due to its chemical composition rich in macro and micronutrients, pH close to neutrality and high water activity, which is favorable for contamination and microbial growth, which contributes to its deterioration, giving rise to sensory changes (smell, color, taste and appearance) that reduce its quality, acceptability and safety, making it a food of sanitary risk (Pascual and Calderón, 2000).

Microbiological Analysis of Fillet

Aerobic Mesophilic Bacteria

The initial total count of viable Aerobic Mesophilic bacteria (AM) in fillet was 4.03±0.6 Log CFU/g showing continuous growth over time in refrigeration, reaching a maximum on day 12 with 12.5±0.6 Log CFU/g (Fig. 3). On day 6, the AM count was 6.5±0.6 Log CFU/g, exceeding the microbiological specifications of sanitary regulation of countries such as Peru with a maximum of 6 Log CFU/g (RM N° 615-2003 SA/DM) and afterwards at on day 8, the count was 7.50±0.6 Log CFU/g, exceeding the specifications of the International Commission on Microbiological Specifications for Foods and Mexican sanitary regulations for these products, which mark a maximum of 7 Log CFU/g for quality and do not represent a risk to health (ICMSF, 1986; NOM-027-SSA1-1993; NMX-FF-002-SCFI-2011). In addition, other researchers point out that total counts higher than 6 Log and 7 Log CFU/g in food tend to be the beginning of decomposition (Pascual and Calderón, 2000; Corbo et al., 2005; Rodríguez Jerez, 2005). And in the case of tilapia fillets, counts of 6 Log CFU/g are considered as an indicator of deterioration (Castillo-Jiménez et al., 2017). In related studies initially reported total bacterial counts between 4 Log and 6 Log CFU/g in tilapia fillet stored on ice at 0°C and maximum counts of 11 Log CFU/g at 26 days of storage, reaching ranges of values frequently above the sanitary regulations mentioned after 6 days of storage, concluding that this microbiological parameter is considered a good indicator of the deterioration of fish.

The purpose of the analysis of the AM indicator is to provide information on the total viable population of bacteria present in the food, which are capable of growing in the presence of oxygen at temperatures optimally between 30°C and 45°C and where the total number of microorganisms can influence the quality and deterioration, reflecting the hygienic conditions and sanitary handling of the product (NOM-092-SSA1-1994; ANMAT, 2014; Campuzano et al., 2015). For fish, the initial microbial load may be a function of the degree of initial bacterial contamination of the fish skin, where in fresh fish this may vary according to the microbial load of the waters from which they come. Within the aerobic mesophilic count, it must be considered that low values in the health context do not imply or ensure the absence of pathogens or toxins, as well as that a high count does not mean the presence of pathogens; on the other hand, in general aspects, high counts in food are not recommended, as it can mean enormous contamination of the raw material, improper handling during the production process, the possibility of having pathogens as the majority are mesophilic and rapid deterioration of the product (Pascual and Calderón, 2000; ANMAT, 2014).

Aerobic Psychrophiles

Psychotrophic microorganisms are those that tolerate and grow at temperatures between 4°C and 20°C including commercial refrigeration temperatures. Within these, it is the group of psychrophiles that have optimal growth at temperatures close to 0°C (Pascual and Calderón, 2000; Herrera, 2001; El-Tawab et al., 2019). The control of psychrophiles in fish and products in
particular fillets, is of importance in studies of the shelf life due to the common use in the conservation of low temperatures, where different microorganisms involved in the deterioration belong to this microbial group and where the initial load of psychrophilic bacteria and their growth dynamics are determining elements in the deterioration of food stored at refrigeration temperatures (Izquierdo et al., 2004; Álvarez et al., 2011; Flores-Rondon, 2011; Castillo-Jimenez et al., 2017).

The usual microbiota of fish depends on the microorganisms existing in the waters, where it comes from and varies according to the habitat of the species, temperature and degree of contamination of the waters, highlighting the presence of Gram negative psychrophiles such as Pseudomonas, Shewanella, Moraxella, Acinetobacter, Flavobacterium and Aeromonadaceae, as well as Gram positives of variable proportions including Bacillus, Micrococcus, Clostridium, Lactobacillus and Corynebacterium, as well as fungi and yeasts (Huss, 1999; Pascual and Calderon, 2000; Herrera, 2001; Arias and Chaves, 2012; El-Tawab et al., 2019).

In this study, the indicator of Aerobic Psychrophiles (AP) in fillets had initial values of 4±0.50 Log CFU/g, maintaining the same proportion on day 2 and having a slight growth until day 4 with 4.4±1 Log CFU/g, for subsequently having a continuous growth at day 6, 8 and 10 with 5±1, 5.6±1 and 7.2±1 Log CFU/g, respectively, having a maximum count at day 12 of 8.63±1 Log CFU/g (Fig. 3). A microbiological criterion of quality, acceptability and safety of the product has been established as a count of 1 x 10^5 or 5 Log CFU/g of psychrotrophic aerobes in refrigerated fish fillet (Pascual and Calderon, 2000; da Rocha, 2006). Therefore, in our study, the proportion in the psychrophile count is reached after six days of storage in refrigeration. On the other hand, in related studies, like (Castillo-Jimenez et al., 2017), it was reported that a product can be acceptable with counts up to 6.1 Log CFU/g in tilapia products such as fillet vacuum packed and stored at refrigeration temperature for 22 days. Likewise, (Britto et al., 2007) reported that fish (Jaraqui Semaprochilodus spp.) was kept on ice for 19 days, presenting initial counts of psychrophiles between 1 x 10⁴ CFU/g and 9 x 10⁴ CFU/g, with a loss of quality and sensory deterioration from the day 6 where it had psychrophile counts between 5.4 x 10⁴ and 1.3 x 10⁵ CFU/g.

Álvarez et al. (2011) indicated that whole, gutted and fresh fish (Sphyraena ensis) collected in the rainy season stored 6 days at 2°C and 7°C with initial average counts of psychrophiles of 6.6 x 10³ CFU/g in both temperatures; later, on day 3, it had counts of 6 x 10⁴ CFU/g and >2.3 x 10⁷ CFU/g respectively and finally counts >5.6 x 10⁷ CFU/g on day 6 at both temperatures. The author and collaborators concluded that, considering the total bacterial counts, the shelf life of the fish in refrigeration is not greater than the average 7 days. In chilled fish, psychrophilic and psychrotrophic bacteria play an important role in the deterioration process of the fish, because they multiply well under these conditions. Fish from tropical regions have a longer lifespan than fish from cold/temperate regions. This is probably because the number of mesophilic microorganisms is greater than the number of psychrophiles in tropical regions and the opposite is registered in temperate/cold regions (Britto et al., 2007).

**Total Coliforms**

This group of microorganisms act as indicators of contamination of water and food (Arcos et al., 2005; Campuzano et al., 2015). Bacteria of this genus are part of the Enterobacteriaceae family; they are Gram negative and are found as saprophytes in the intestine of humans and homeothermic animals, being part of the normal microbiota and eliminated through fecal matter; although they are also widely distributed in the nature, some of the genera that make up the group of total coliforms are Escherichia, Enterobacter, Klebsiella, Serratia, Edwarsiella and Citrobacter (Pascual and Calderon, 2000; Arcos et al., 2005; Sousa, 2006; Puerta-García and Mateos-Rodríguez, 2010; Campuzano et al., 2015). The presence of high levels in food may indicate poor hygienic processing and handling, post-production contamination, or both, as well as inadequate heat treatment leading to a loss of quality, deterioration and a risk to food safety and consumer’s health (Pascual and Calderon, 2000; Corbo et al., 2005; Sousa, 2006).

In the present study, the behavior of these indicator microorganisms in refrigerated fillets is shown in Fig. 3, where initially the fillets had a total coliform count (TC) of 1.07±0.4 Log CFU/g, to later have a sustained growth presenting daily two counts of 3.6±0.5 Log CFU/g; on day 4 being of 6±0.9 Log CFU/g; on day 6 of 6.7±0.5 Log CFU/g, on day 8 of 6.85±0.5 Log CFU/g, until presenting a maximum count at day 12 of 8.33±2.3 Log CFU/g. According to the food legislation of European countries such as Spain, through the Official State Bulletin (BOE acronym in Spanish), it indicates, as microbiological criteria in the field of hygiene and food safety applied to total coliforms or Enterobacteriaceae, maximum counts in refrigerated fishery products of 10³ or 3 Log CFU/g (BOE, 1991; Moraga et al., 2019).

Likewise, researchers have pointed out that counts higher than 10⁵ or 3 Log CFU/g are considered the maximum to estimate the moment of onset of deterioration in fishery products (Castillo-Jimenez et al., 2017). In related studies, (Corbo et al., 2005) reported a total coliform count of 2 x 10⁵ CFU/g as a limit value for the stability and acceptability of fish fillet (Cod) when
stored at 4°C for approximately three days. Therefore, we consider that the product in this study shows stability prior to the start of deterioration and food safety before 2 days of storage in refrigeration at 4°C.

Fungi and Yeasts

The counts obtained in fungi and yeasts in refrigerated fillets were very similar during the refrigerated storage period, where initially counts of \(3.6 \pm 0.4\) Log CFU/g were obtained for fungi and yeasts, with a considerable increase of approximately four (4) logarithmic units, until day 8, being \(7.78 \pm 0.85\) Log CFU/g for fungi and \(7.3 \pm 0.85\) Log CFU/g for yeasts and reaching a maximum at 12 days with counts for fungi of \(9 \pm 0.5\) Log CFU/g and \(9 \pm 0.9\) Log CFU/g in yeasts (Fig. 4). In related studies, (Centeno and Rodríguez, 2005) reported that in processed and frozen fish (Scomberomorus spp. and Merlaccius spp.) counts of filamentous fungi and yeasts were found, being \(1.9 \times 10^4\) CFU/g in Scomberomorus spp. and \(2.0 \times 10^5\) CFU/g in Merlaccius spp., noting that although there are no microbiological limits or criteria for these microorganisms in this type of food products, their presence in these foods can affect the quality and cause deterioration and loss of the nutritional and commercial value of the product.

Fungi are eukaryotic microorganisms, generally aerobic, that can be found widely distributed in nature, even forming part of the normal microbiota of a food (fish), or as pollutants (Huss, 1999; Berenguer, 2000; Campuzano et al., 2015). The importance of the presence of fungi and yeasts in food is due to its potential in spoilage causing alteration of taste, odor and color on the surface of contaminated food, in addition to favoring the growth of pathogenic bacteria, generating harmful metabolites (mycotoxins), infections and allergic reactions in humans (Berenguer, 2000; Campuzano et al., 2015). In addition, it has been pointed out that food preservation conditions, which tend to inhibit the growth mainly of bacteria, can favor the appearance of contaminating fungi and yeasts, causing effects on the sensory parameters of quality in fresh, semi-processed and processed foods (Orberá Ratón 2004). The consideration in the use of fungi and yeasts in food is to act as an indicator of quality of raw materials, inadequate or deficient sanitary practices during the production and storage of food products (Borbolla-Sala et al., 2004; Centeno and Rodríguez, 2005).

\textit{Pseudomonas} spp

In the study of the growth dynamics of these microorganisms in the refrigerated tilapia fillet, it is shown in Fig. 5 an initial \textit{Pseudomonas} count of \(2 \pm 0.2\) Log CFU/g was obtained; subsequently, presenting a constant growth, reaching counts of \(5 \pm 0.3\) Log CFU/g at eight days of storage, to finally, at 12 days of storage, have its maximum growth point with \(7 \pm 0.5\) Log CFU/g in the fillet. Considering that a count of \(1 \times 10^5\) CFU/g of psychrotrophic aerobic in refrigerated fish fillet has been established as a microbiological criterion for the quality, acceptability and safety of the product and where \textit{Pseudomonas} spp. are part of this growth indicator microbial group at low temperatures (Pascual and Calderón, 2000; da Rocha, 2006; Britto et al., 2007; Álvarez et al., 2011; Arias and Chaves, 2012; El-Tawab et al., 2019). In our study, considering the previous criteria, the maximum proportion of acceptable microbial load is reached after eight days of storage in refrigeration at 4°C. In related studies, Britto et al., 2007, during the ice storage of Semaprochilodus spp., during 23 days, they determined the presence of different microorganisms including \textit{Pseudomonas} spp., in proportions between \(4 \times 10^4\) and \(1 \times 10^4\) CFU/g initially; on day 6 between 1.3 \(\times 10^4\) and 5.9 \(\times 10^4\) CFU/g and finally having a maximum of \(7.9 \times 10^4\) CFU/g on day 23, indicating that the fish begins to have a sensory quality lower than class A from approximately day 9 of conservation in ice. Meanwhile, Álvarez et al. (2011) reported that during fish recollection in the rainy season, for processed and stored for 6 days at 7°C, there were initial counts of psychrophiles, including \textit{Pseudomonas} spp., between <100 and \(1.8 \times 10^3\) CFU/g and a maximum count, on day 6, of between \(7.2 \times 10^3\) and \(3 \times 10^4\) CFU/g. Therefore, the author concluded that, considering the total counts, the shelf life of the fish in refrigeration was not longer than 7 days on average.

\textit{Pseudomonas} spp., is a microbial group of interest, not only in food production and conservation but also in food safety and human health (Britto et al., 2007; Maia et al., 2009; Álvarez et al., 2011; Arias and Chaves, 2012). \textit{Pseudomonas} is a bacterial group widely spread in nature and it constitute an abundant part of the normal microbiota of fish (Huss, 1999; Pascual and Calderón, 2000; Britto et al., 2007). Gram negative bacilli under aerobic conditions at refrigeration temperatures, such as \textit{Pseudomonas} spp., Aeromonas spp. and \textit{S. putrefaciens}, have been classified as specific microorganisms for spoilage of stored tropical freshwater and marine fish (Huss, 1999; Centeno and Rodríguez, 2005; Álvarez et al., 2011). \textit{Pseudomonas} is part of the group of psychrotrophic and psychrophilic bacteria responsible for the deterioration of food at low temperatures (Britto et al., 2007; Álvarez et al., 2011; Arias and Chaves, 2012). These microorganisms produce sensory changes in products such as sweet, rotten and sulfurous odor of decomposing fish, due to the metabolism of proteins and peptides present, generation of ketones, esters, aldehydes and ammonia, in addition to reducing Trimethylamine Oxide (OTMA) to Trimethylamine (TMA) (Centeno and Rodríguez, 2005).
Mesophyll Lactic Acid Bacteria

In the study of the growth dynamics of lactic acid bacteria (LAB) in the refrigerated tilapia fillet, it is shown in Fig. 5 counts, initially, for 2.77±0.50 Log CFU/g and subsequently having an exponential growth reaching counts to 6.3±1 Log CFU/g at four days of storage and later stabilization at eight and ten days with 7±0.9 Log CFU/g, to finally rise again and reach its maximum peak of 9±1 Log CFU/g after 12 days of storage in tilapia fillet.

In studies related to the analysis of LAB in fish, reported in Sarda fillets stored at 4°C, under aerobic conditions, initial BAL counts of 3.61 Log CFU/g, reaching in 9 days of storage, approximate values of 5 Log CFU/g, indicating that LAB counts ≥5 Log CFU/g, are indicative of spoilage and unacceptable product.

Fig. 3: Analysis of aerobic mesophilic bacteria, aerobic psychrophiles and total coliforms in tilapia (O. niloticus) fillet stored at 4°C for 12 days

Fig. 4: Count of fungi and yeasts in tilapia fillet (O. niloticus) refrigerated at 4°C for 12 days
Fig. 5: Count of Pseudomonas spp. and mesophilic Lactic Acid Bacteria (LAB) in tilapia fillet (O. niloticus) refrigerated at 4°C for 12 days

Table 1: Physicochemical analysis of tilapia (O. niloticus) fillet.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values obtained in this study</th>
<th>Reported values for fish (Huss, 1999; Leyva et al., 2008)</th>
<th>Reported values for tilapia (O. niloticus) (Viquez, 2002; Rojas-Ruiz et al., 2011)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ashes (%)</td>
<td>1.36±0.2</td>
<td>1.2-1.5</td>
<td>1.12</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>17.5±1.1</td>
<td>16-21</td>
<td>19.9</td>
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<tr>
<td>Humidity (%)</td>
<td>79.09±1</td>
<td>66-81</td>
<td>78.6</td>
</tr>
<tr>
<td>Lipids (%)</td>
<td>1.65±0.1</td>
<td>0.2-25</td>
<td>1.5</td>
</tr>
<tr>
<td>Carbohydrates (%)</td>
<td>0.4±0.1</td>
<td>&lt;5</td>
<td>ND</td>
</tr>
<tr>
<td>Water activity (aw)</td>
<td>0.992±0.001</td>
<td>≥0.98</td>
<td>ND</td>
</tr>
<tr>
<td>pH</td>
<td>6.33±0.1</td>
<td>6.6-6.8</td>
<td>6</td>
</tr>
<tr>
<td>Fillet yield (%)</td>
<td>27.7±3.6</td>
<td>ND</td>
<td>21-41</td>
</tr>
</tbody>
</table>

LAB is a microbial group made up of different genera with common metabolic and morphological characteristics (Huertas, 2010; Ramírez et al., 2011). These microorganisms are widely distributed in nature, they are facultative anaerobes, acid tolerant with various applications in food (preservation and fermentation processes) and have modifying sensory characteristics (Huertas, 2010; Ramírez et al., 2011). Foods such as milk, meat, fish, vegetables and cereals are considered good growth media for these microorganisms. LABs are classified according to the final product of their growth and fermentation where they can generate only lactic acid (homofermentative), or mix with other compounds such as acetate, ethanol, CO₂, among others (heterofermentative). They are also classified according to their optimal growth temperature that can be mesophilic (20-25°C) or thermophilic (40°C-45°C) (Monroy et al., 2009; Huertas, 2010; Ramírez et al., 2011).

Among the representative genus of LABs are Bifidobacterium, Pediococcus, Leuconostoc, Lactococcus, Enterococcus and Lactobacillus (Agurto and Ramos, 2008; Huertas, 2010; Ramírez et al., 2011). This last genus is considered part of the normal Gram-positive microbiota in fish along with Bacillus, Micrococcus, Clostridium and Corynebacterium (Huss, 1999; Pascual and Calderón, 2000; Arias and Chaves, 2012).

LAB and metabolites present a double edge of function in food since they can intervene in conservation processes (Monroy et al., 2009; Vásquez et al., 2009; García and González, 2017). Such is the case of fish (tilapia) in fillet presentation stored in refrigeration and under vacuum, thus increasing its shelf life (Salazar et al., 2011; Montalvo-Rodríguez et al., 2016; Castillo-Jiménez et al., 2017). But they can also intervene in the deterioration of food with undesirable effects such as excessive acidity, structural changes and discolorations; therefore, the LAB group is considered an indicator that are focused on determining the shelf life of fresh, fermented, processed, refrigerated and, mainly, vacuum-packed foods (García and González, 2017).

The case of the duality of mesophilic growth lactic acid bacteria is because, in the preservation function, they
act as protection against temperature abuse. On the other hand, an initial concentration in low temperature conditions and under states of temperature abuse, these microorganisms will grow competitively against others, including pathogens, avoiding health hazards. However, in conditions of temperature abuse, lactic acid mesophylls can act as the main agent of deterioration and preventing the food from being consumed (Vásquez et al., 2009). It is generally estimated that, in an unfermented and minimally processed food, counts of 1 ×106 CFU of BAL or 6 Log are indicative of spoilage. Meanwhile, in foods subjected to heat or acid treatment, counts of a single cell per gram can be indicative of spoilage, poor heat treatment or post-process contamination (García and González, 2017). Considering the above, the LAB counts obtained in the tilapia fillet stored in refrigeration at 4°C after four days with 6.3±1 Log CFU/g indicate a food in a state of deterioration.

Conclusion

The microbiological analysis of tilapia fillets stored under refrigeration indicated the presence and growth of the different microbial groups (aerobic mesophilic, psychrophiles, coliforms, lactic acid bacteria, Pseudomonas, fungi and yeasts) selected and related to the quality, deterioration and safety of the food during the 12 days of storage.

Among the conservation methods generally used in fish is refrigeration for simplicity and economy. This low temperature method reduces but does not stop the activity and growth of microorganisms that lead to deterioration, loss of quality and safety and influence shelf life and availability for consumption. The conditions of refrigeration and microbiological analysis in the present study provide a conservation and storage time of 1 to 8 days according to the microbial groups to be evaluated. This refrigerated storage time obtained according to microbiological analysis is consistent with the period of 1 to 8 days reported and recommended for fish and products by other researchers and food regulators. We consider that the food under these conditions must be submitted jointly with other conservation processes in order to reduce microbial activity and obtain longer storage periods in refrigeration than those obtained, for which more studies will be required in this regard.

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Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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