

Original Research Paper

Microbiological Analysis of Tilapia Fillet Stored Under Refrigerated Conditions

¹Itzel Yashita Prado-Toledo, ¹Katia Nayely Ramos-Santoyo, ¹Martha Lorena Guzmán-Robles and ²Alejandro De Jesús Cortés-Sánchez

¹Universidad Tecnológica de Nayarit (UT Nayarit). Carretera Federal 200 Km 9. C.P. 63780. Xalisco, Nayarit. México

²Consejo Nacional de Ciencia y Tecnología (CONACYT). Unidad Nayarit del Centro de Investigaciones Biológicas del Noroeste (UNCIBNOR+). Calle Dos No. 23. Cd. del Conocimiento. Av. Emilio M. González. Cd. Industrial. C.P. 63173. Tepic, Nayarit. México

Article history

Received: 04-11-2021

Revised: 07-02-2022

Accepted: 09-02-2022

Corresponding Author:

Alejandro De Jesús Cortés-Sánchez

Consejo Nacional de Ciencia y Tecnología (CONACYT).
Unidad Nayarit del Centro de Investigaciones Biológicas del Noroeste (UNCIBNOR+). Calle Dos No. 23. Cd. del Conocimiento. Av. Emilio M. González. Cd. Industrial. C.P. 63173. Tepic, Nayarit. México
Email: alecortes_1@hotmail.com

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 tilapia 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 *et al.*, 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; Prabu *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 *et al.*, 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 *et al.*, 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 *et al.*, 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 *et al.*, 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 food-borne diseases (da Rocha *et al.*, 2006; Castillo-Jiménez *et al.*, 2017; dos Santos and 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; García 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; Efiuvwevwere *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 (UNCIBNOR+ 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 × 17.5 cm polyethylene bags and finally the bags were introduced into an isothermal polystyrene container with dimensions of 18.5 cm × 12 cm × 16 cm together with 12 cm × 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

The microbiological analysis of the fillets consisted on the determination of different indicators such as aerobic mesophilic bacteria (NOM-092-SSA1-1994; Maturin and Peeler, 2001), aerobic psychrophiles (NOM-092-SSA1-1994; Arias and Chaves, 2012), total coliforms (NOM-113-

SSA1-1994; Pascual *et al.*, 2000), *Pseudomonas* spp. (ISO-13720-2010; Jiménez *et al.*, 2004), mesophyll lactic acid bacteria (ISO-15214-1998), fungi and yeasts (NOM-111-SSA1-1994). The physicochemical analysis of the fillets consisted on the determination of proteins (NMX-F-068-S-1980), humidity (NMX-F-083-1986), ashes (NMX-F-066-S-1978), lipids (NMX-F-089-S-1978), carbohydrates (NOM-086-SSA1-1994), pH (NMX-F-317-S-1978) and Water Activity (AW) through the water activity meter (Aqualab 4TEV).

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.

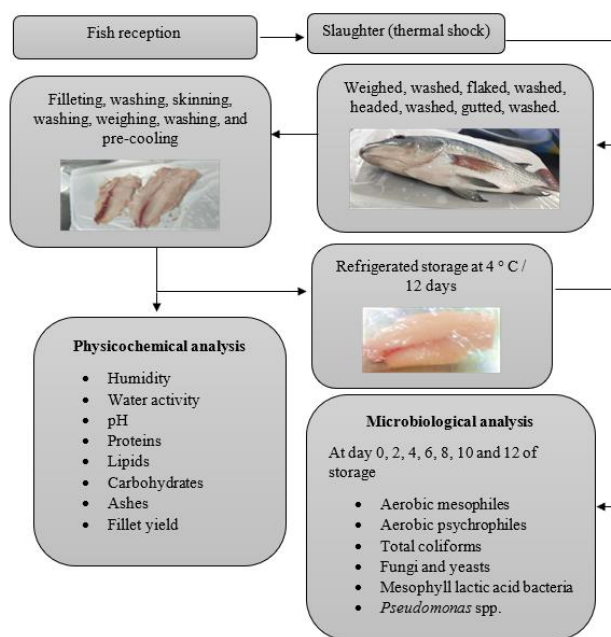


Fig. 1: Flow diagram of the processing of tilapia (*O. niloticus*) to obtain fillet, conservation in refrigeration, physicochemical and microbiological analysis

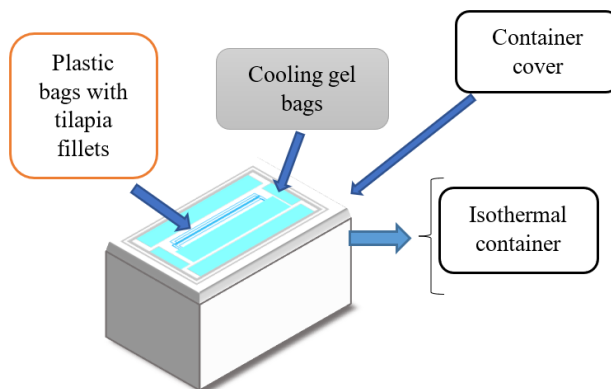


Fig. 2: Isothermal container of tilapia (*O. niloticus*) fillets for their final storage at 4°C for 12 days

Results and Discussion

Physicochemical Fillet Analysis

In the processing of fish, the yield of fresh fillet obtained was $27.7\% \pm 3.6$, 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; Viquez, 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 *et al.*, 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, 2008) 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 *et al.*, 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 *et al.*, 2000; ANMAT, 2014).

Aerobic Psychrophiles

Psychrotrophic microorganisms are those that tolerate and grow at temperatures between 4 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 *et al.*, 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 *et al.*, 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 1×10^5 or 5 Log CFU/g of psychrotrophic aerobes in refrigerated fish fillet (Pascual *et al.*, 2000; da Rocha *et al.*, 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-Jiménez *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 1×10^4 CFU/g and 9×10^4 CFU/g, with a loss of quality and sensory deterioration from the day 6 where it had psychrophile counts between 5.4×10^4 and 1.3×10^5 CFU/g

Álvarez *et al.* (2011) indicated that whole, gutted and fresh fish (*Sphyræna ensis*) collected in the rainy season stored 6 days at 2 and 7°C with initial average counts of psychrophiles of 6.6×10^3 CFU/g in both temperatures; later, on day 3, it had counts of 6×10^4 CFU/g and $>2.3 \times 10^7$ CFU/g respectively and

finally counts $>5.6 \times 10^7$ 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 (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*, *Edwardsiella* and *Citrobacter* (Pascual *et al.*, 2000; 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 *et al.*, 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 10^3 or 3 Log CFU/g (BOE, 1991).

Likewise, researchers have pointed out that counts higher than 10^3 or 3 Log CFU/g are considered the maximum to estimate the moment of onset of deterioration in fishery products (Castillo-Jiménez *et al.*, 2017). In related studies, (Corbo *et al.*, 2005) reported a total coliform count of 2×10^2 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 ± 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 ± 0.85 Log CFU/g for fungi and 7.3 ± 0.85 Log CFU/g for yeasts and reaching a maximum at 12 days with counts for fungi of 9 ± 0.5 Log CFU/g and 9 ± 0.9 Log CFU/g in yeasts (Fig. 4). In related studies, (Centeno and Rodríguez, 2005) reported that in processed and frozen fish (*Scorpaenidae* spp. and *Merluccius* spp.) counts of filamentous fungi and yeasts were found, being 1.9×10^3 CFU/g in *Scorpaenidae* spp. and 2.0×10^2 CFU/g in *Merluccius* 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).

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 *Pseudomonas* count of 2 ± 0.2 Log CFU/g was obtained; subsequently, presenting a constant growth, reaching counts of 5 ± 0.3 Log CFU/g at

eight days of storage, to finally, at 12 days of storage, have its maximum growth point with 7 ± 0.5 Log CFU/g in the fillet. Considering that a count of 1×10^5 CFU/g of psychrotrophic aerobics in refrigerated fish fillet has been established as a microbiological criterion for the quality, acceptability and safety of the product and where *Pseudomonas* spp., are part of this growth indicator microbial group at low temperatures (Pascual *et al.*, 2000; da Rocha *et al.*, 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 *Pseudomonas* spp., in proportions between 4×10^2 and 1×10^2 CFU/g initially; on day 6 between 1.3×10^3 and 5.9×10^3 CFU/g and finally having a maximum of 7.9×10^4 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 *Pseudomonas* spp., between <100 and 1.8×10^3 CFU/g and a maximum count, on day 6, of between 7.2×10^4 and 3×10^7 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.

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). *Pseudomonas* is a bacterial group widely spread in nature and it constitute an abundant part of the normal microbiota of fish (Huss, 1999; Pascual *et al.*, 2000; Britto *et al.*, 2007). Gram negative bacilli under aerobic conditions at refrigeration temperatures, such as *Pseudomonas* spp., *Aeromonas* spp. and *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). *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.

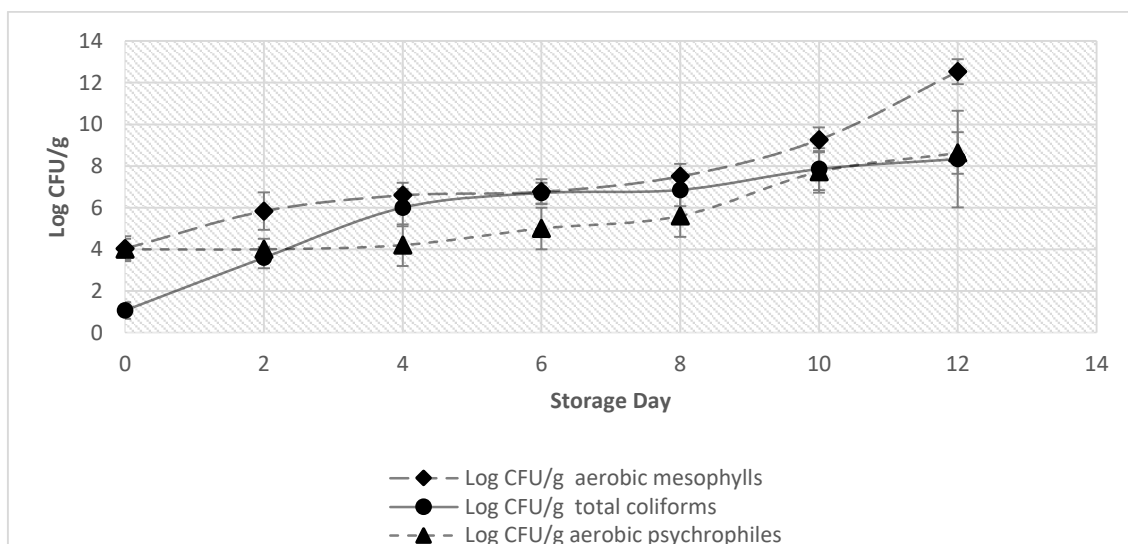


Fig. 3: Analysis of aerobic mesophilic bacteria, aerobic psychrophiles and total coliforms in tilapia (*O. niloticus*) fillet stored at 4°C for 12 days

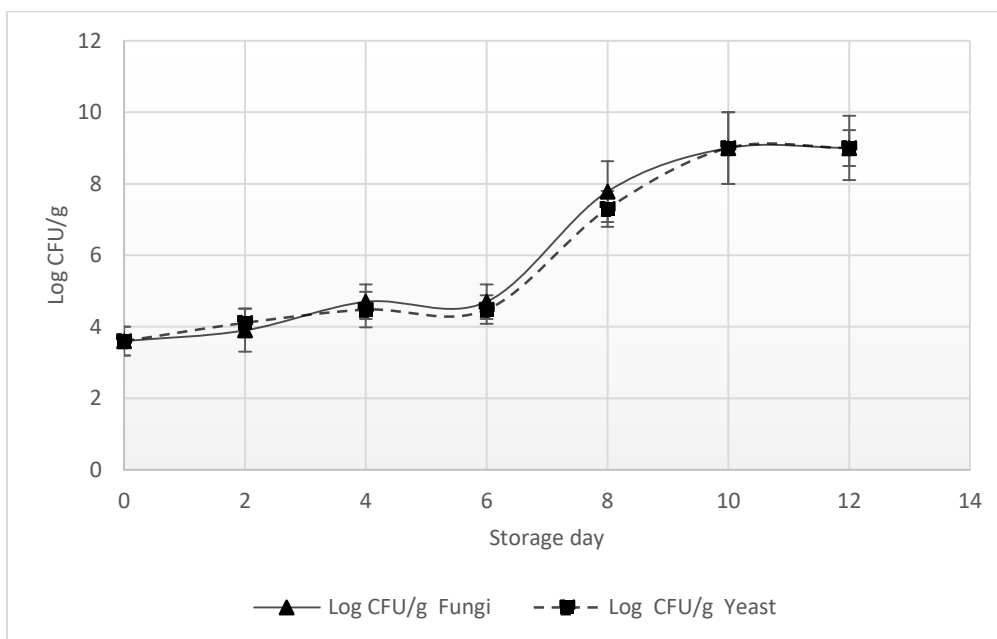


Fig. 4: Count of fungi and yeasts in tilapia fillet (*O. niloticus*) refrigerated at 4°C for 12 days

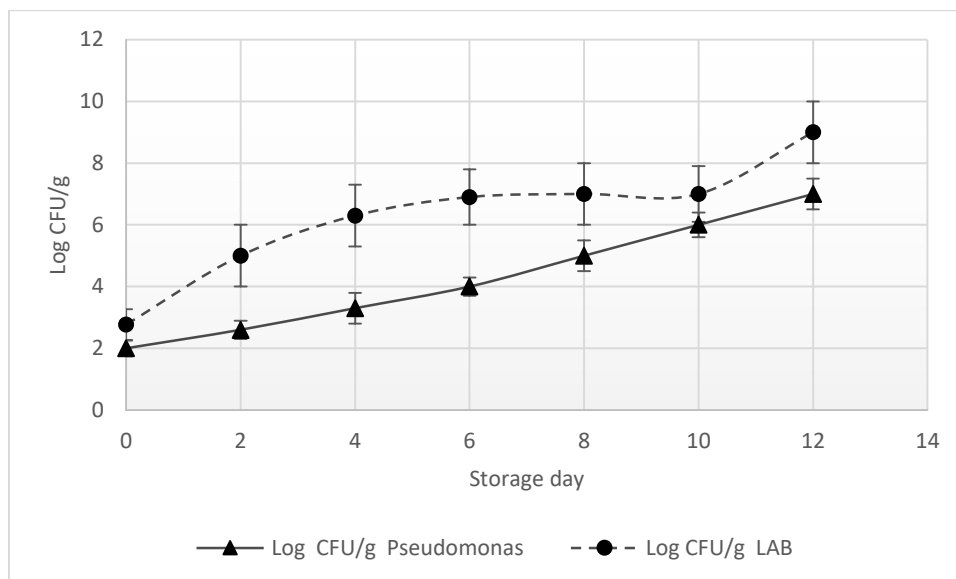


Fig. 5: Count of *Pseudomonas* spp. and mesophilic Lactic Acid Bacteria (LAB) in tilapia fillet (*O. niloticus*) refrigerated at 4°C for 12 days

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

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

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 (40-45°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 *et al.*, 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 1×10^6 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 microbiological 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.

Acknowledgment

To the Nayarit Unit of the Northwest Biological Research Center (UNCIBNOR +) and the Technological University of Nayarit (UT Nayarit), department of engineering in bio-food technologies and corresponding

academic staff for the support and facilities provided for carrying out this study.

Authors Contribution

Itzel Yashita Prado-Toledo: Experimental development, data analysis and writing of manuscript.

Katia Nayely Ramos-Santoyo: Experimental development, data analysis, manuscript writing and materials and equipment management.

Martha Lorena Guzmán-Robles: Experimental development, data analysis, manuscript writing, materials and equipment management.

Alejandro De Jesús Cortés-Sánchez: Conceptualization, experimental design and development, materials and equipment management, data analysis, manuscript writing.

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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