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RESEARCH ARTICLE

Bacteriological Assessment of Rainbow Trout (*Oncorhynchus mykiss*) Subjected to Low-Temperature Storage

Román Alejandro Téllez-Cortez[1], Ángel Ruyan García-Hurtado[1], Mayra Diaz-Ramírez[1], Erika Torres-Ochoa[2], Alejandro De Jesús Cortés-Sánchez[3,1*]

[1]*Departamento de Ciencias de la Alimentación, División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana, Unidad Lerma. Av. de las Garzas 10, Col. El Panteón, CP. 52005. Lerma de Villada, Estado de México, México*
[2]*Departamento Académico de Ingeniería en Pesquerías, Universidad Autónoma de Baja California Sur, Carretera al sur Km 5. Colonia el Mezquitito, La Paz CP. 23080, Baja California Sur, México*
[3]*Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI). Av. Insurgentes Sur 1582, Col. Crédito Constructor, Alcaldía Benito Juárez, CP. 03940. Ciudad de México, México*

*Corresponding Author: alecortes_1@hotmail.com

Abstract: Fish is a food with high nutritional value and an essential part of the human diet. However, this food is highly perishable and poses a health risk when contaminated along the food chain, requiring preservation processes at low temperatures. Trout (*Oncorhynchus mykiss*) is one of the most widely produced and marketed fish species for human consumption worldwide, through products such as fresh or chilled fillets. The objective of this study is to assess the bacteriological profile and freshness of trout (*Oncorhynchus mykiss*) fillets intended for human consumption, stored at 5°C ±1. The fish were obtained fresh and processed into fillets, which were then stored for 12 days at 5°C ±1. Every 48 h, the bacteriological profile (total coliforms, aerobic mesophiles, and psychrophiles) and freshness of the fillets were analyzed. It was determined that the trout (*Oncorhynchus mykiss*) fillets were acceptable for consumption in terms of freshness until the eighth day of storage. The bacteriological assessment indicated that the fillets were suitable for human consumption, meeting the microbiological limits established by regulations for total coliforms until day 2, for psychrophiles until day 6, and for aerobic mesophiles until day 10. The results obtained allow us to determine the storage time, degree of freshness, and sensory acceptability (quality grade), as well as the sanitary conditions for consumption of trout fillets stored at low temperatures, helping us to expand our knowledge about the consumption period of this product and reduce the risk of foodborne illnesses.

Keywords: Quality, Fish, Spoilage, Hazard, Food Safety, Shelf Life

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Introduction

Fish is considered a staple food in the human diet due to its nutritional composition, which includes proteins, lipids, minerals, and vitamins [1, 2]. Globally, fish for human consumption is obtained through aquaculture and fishing activities. Only in 2022, these two activities produced 185.4 million tons live weight, with an apparent per capita consumption of 20.7 kg [3] . One of the main species in inland aquaculture is rainbow trout (*Oncorhynchus mykiss*), which contributed 739,500 tons live weight in 2022. Like other live, fresh, or chilled aquatic foods, rainbow trout represent the majority of fisheries and aquaculture production, preferred and used for direct human consumption, followed by prepared, frozen, canned, and cured products [4]. In Mexico, fish and aquaculture have great economic and food security relevance, with fish such as trout being among the main products of fishing and aquaculture, with a total production of 2748 tons in 2023, destined mainly for human consumption, being Veracruz, Tamaulipas, Hidalgo, Puebla, Michoacán, State of Mexico, among others, the states with the highest production [5]. Due to factors such as the chemical composition of the muscle, almost neutral pH, high water activity, among others, fish is considered a food susceptible to contamination and spoilage through autolysis, lipid oxidation, and microbiological activity, compromising its nutritional quality, shelf life, and safety throughout the food chain [1, 2, 6]. To extend the shelf life and microbiological safety of fish and products intended for marketing and consumption, different preservation methods are used, which usually employ temperature control, either at low temperatures (refrigeration or freezing) or at high temperatures (cooking or canning), reducing water activity through drying or salting [2, 7].

Fish contamination and spoilage can be physical, chemical, or biological in nature, the latter involving various microorganisms, primarily bacteria. Some are associated with spoilage, such as Gram-positive and Gram-negative mesophiles and psychrophiles, while others are considered a health risk, including *E. coli, Listeria sp., S. aureus, Salmonella sp., Shigella sp., Vibrio sp., Clostridium botulinum*, Hepatitis A virus, Norwalk virus, Calicivirus, Astrovirus, and/or parasites (*Anisakis simplex, Gnathostoma sp., Diphyllobothrium latum*, *Opisthorchis* sp.) [8, 9]. Therefore, fish is linked to numerous cases or outbreaks of foodborne illnesses worldwide, with bacteria being the usual causative agents [10-13]. In the supply and availability of food, consumers demand compliance with food legislation regarding freshness, diversity, and safety to protect their health. This has led to increased interest in understanding fish production practices and quality, as well as inspection and control methods, to ensure nutritious and safe food and prevent foodborne illnesses [14, 15]. The study of shelf life and microbiological hazards in fish stored at low temperatures is versatile due to the wide variety of species and edible products, the diversity of microbial agents to be evaluated, and the broad range of low temperatures used for product preservation. Bacteriological and shelf-life studies of fish or fish products (fillets) of trout stored at low temperatures are scarce. The study of shelf life and bacteriological hazards in fish or fish products stored at low temperatures is essential to ensure their safety and quality during storage, marketing, and consumption. Refrigeration is one of the commonly used low-temperature preservation methods for these types of products, and although it helps slow microbial growth, it does not stop it completely. Therefore, it becomes necessary to identify pathogenic and spoilage-associated bacteria that can proliferate under these conditions. This analysis allows for determining safe consumption times and compliance with health regulations, thus reducing risks to consumer health. Therefore, the purpose of this study was to assess the bacteriological profile and degree of freshness of trout (*Oncorhynchus mykiss*) fillet stored at low temperature to determine the microbiological hazards during its storage and shelf life for consumption.

Materials and Methods

Sample Collection

The samples were rainbow trout (*Oncorhynchus mykiss*) from fish farms in the municipality of Temoaya, State of Mexico, Mexico. They were grouped into three separate batches of trout with a weight of 355.8±24 g. They were transported in an isothermal container with drinking-quality ice to the laboratory for processing into fillets and subsequent quality grade (freshness) and bacteriological assessment.

Filleting and Storage

The processing to obtain skinless fillets was carried out by washing, scaling, gutting, and manually cutting the muscle along the backbone on both sides of the fish to obtain the fillets. Finally, the skin was removed. The processing and handling were carried out under hygienic conditions and practices. Primary Packaging. For initial or primary packaging, the fillets were pre-cooled by immersion in ice water at temperatures of ≤4°C for 5 min. Subsequently, 50 g portions were weighed and manually placed into a 16.5 cm wide by 17.5 cm long polyethylene bag with a hermetic seal (primary packaging). The fillets were then transferred to secondary packaging, which consisted of placing them inside an expanded polystyrene container with a lid (previously washed, disinfected, and dried), measuring 15 cm wide, 22.5 cm long, and 16 cm high. Ice-substitute refrigerant gel blocks were then added inside the container. The secondary container was finally placed in storage in a refrigeration unit (Imbera, model VR35CBMAD) for 12 days at a temperature of 5°C ±1 and relative humidity of 40% ±2.8, which correspond to typical conditions in the commercial and/or domestic environment (Figure 1).

Quality Grade Analysis

The quality analysis of fillets stored for 12 days at 5°C ±1 was performed on days 0, 2, 4, 6, 8, 10, and 12 of storage at 5°C ±1, using the procedure described in the Standard [16] with the support of ten untrained panelists. The procedure consisted of evaluating different sensory attributes of the fillet (color, odor, skin remnants, scales, bones, among others), assigning a value to each. The total sum of the attribute values, subtracted from a base of 100, determines the final score and freshness of the fillet.

Bacteriological Assessment

The bacteriological assessment of fillets stored for 12 days at 5°C ± 1 was performed on days 0, 2, 4, 6, 8, 10, and 12, considering the analysis of total aerobic mesophiles [17], total psychrophiles [17], and total coliforms [18]. The result was expressed as the logarithm of CFU/g of sample. Microbiological assessment was performed in triplicate on separate batches of fillets.

Results Analysis

The analysis of results was performed by calculating the mean, standard deviation, and conducting one-way and two-way ANOVAs. The differences between the means were established through Tukey's test ($p<0.05$). Microsoft Excel 2019 software was used.

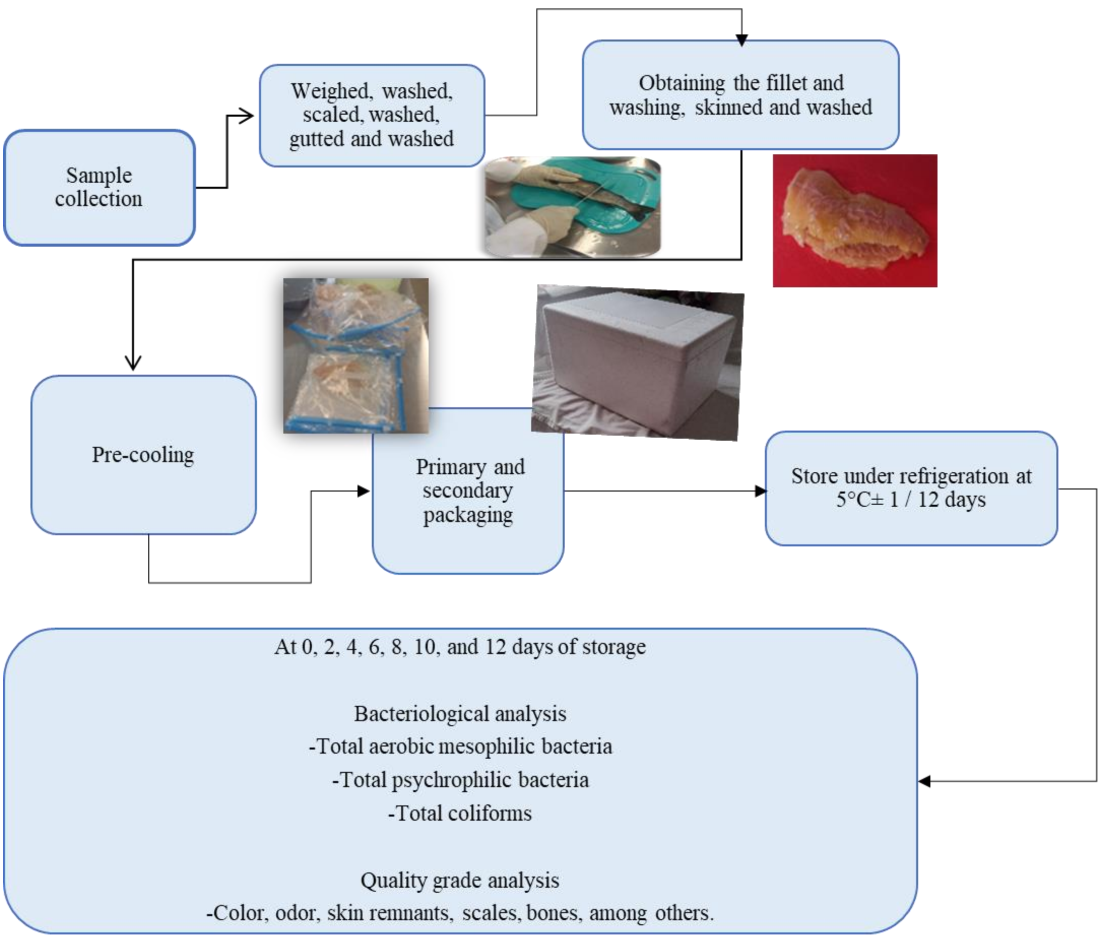

Fig. 1: General diagram of the bacteriological and freshness assessment of trout fillets (O. mykiss) preserved under refrigeration for twelve days

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Results and Discussion

The fillets had an average weight of 130.30 g ±19, with a yield of 36.6% ±3. This yield depends on factors such as species, size, bone structure, visceral volume, head size (an animal with a large head or developed opercular series reduces the edible muscle portion), skill of the personnel performing the processing, among others [19]. Furthermore, in related studies, [20] reported fillet yields of 36 % in tilapia (*Oreochromis* spp.) processing. [19] indicated that they obtained Eleotris picta fish fillet with a yield of 23.1%. Meanwhile, [21] indicated yields of 31 % to 54 % in filleting various fish species (*Salminus brasiliensis, Leporinus obtusidens, Prochilodus lineatus,* and *Luciopimelodus pati*).

Quality Grade

In fish, the attribute of freshness is an indicator that the fish possesses properties similar to those it has while alive or after being caught or harvested [22]. Various physical, chemical, sensory, and microbiological methods have been reported as applied to determine the degree of freshness or spoilage as well as the safety of fish. [6, 23]. Sensory methods are the most frequently used because they provide reliable and valid results, are affordable, practical, and efficient, and are commonly used in the industrial sector, commerce, and sanitary inspections [24, 25]. Figure 2 shows the sensory analysis (Quality grade) score for stored fillets. From day 0 to 8, the fillets scored between 96±2.1 and 86.6±3.9, respectively, and were considered first- and second-quality products suitable for consumption. From day 10 of storage, the sensory characteristics score of the fillets was 52.5±10.5. The standard [16] stipulates that any fillet with a score below 85 is considered a deteriorated product, unfit for consumption and a health risk.

The analysis of variance (ANOVA) performed to evaluate the effect of storage time (12 days at 5±1°C) on the freshness of trout fillets showed significant differences between storage days ($p < 0.05$), indicating that storage time has a significant effect on the freshness of the trout fillets. Subsequently, Tukey's test indicated significant differences in freshness between storage days 0 and 10, 2 and 10, 4 and 10, 6 and 10, and 8 and 10. No significant differences in freshness were found between storage days 0, 2, 4, 6, and 8. Similarly, no significant differences were observed between storage days 10 and 12.

In related studies, [22] reported that tilapia (*Oreochromis* sp.) fillets stored at 4°C for 12 days maintained acceptable freshness for consumption until day 6, after which they were considered spoiled and unsuitable for consumption. [24] indicated that sensory assessment of *Prochilodus lineatus* fillets stored under refrigeration with ice flakes at temperatures of 0°C to 3°C showed a shelf life of 6 days. [26] reported that sensory assessment of tilapia (*Oreochromis* spp.) stored under refrigeration at 0°C indicated a shelf life of 21 days, and 9 days when stored at 10°C. Finally, [21] indicated that fish species (*Salminus brasiliensis, Leporinus obtusidens, Prochilodus lineatus,* and *Luciopimelodus pati*) subjected to storage temperatures of 0°C to 3°C, for 14 days, show signs of decomposition on day 10 of storage, destined for disposal.

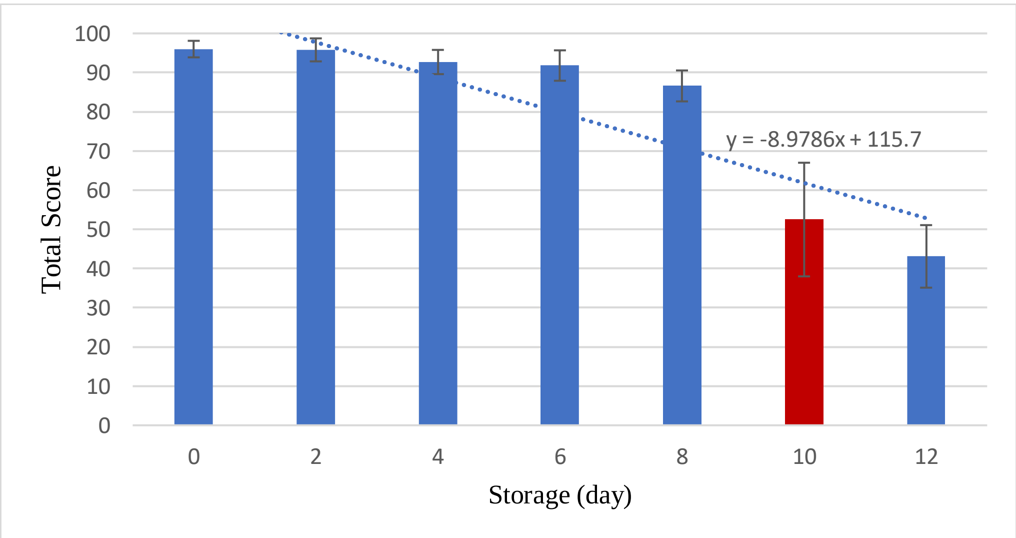

Fig. 2: Sensory assessment (Quality grade) of trout (*Oncorhynchus mykiss*) fillet stored at 5 ± 1°C for 12 days. *Each total score per day represents the mean ± SD of the deductions assigned to the sensory characteristics of the product made by ten evaluators or panelists

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Bacteriological Assessment

The quality and sanitary control of food available and offered to the population is considered a necessary factor in reducing the transmission of disease-causing agents through food and protecting consumer health. Microbiological regulations and criteria for food can provide guidelines for determining shelf life and/or controlling product safety throughout the food chain [27, 28]. Various microorganisms can be used as microbiological criteria to indicate contamination, the presence of pathogens, or the detection of specific toxins. The indicator microorganisms generally used to determine food spoilage and sanitary quality include aerobic mesophiles, molds, yeasts, and coliforms, among others [27-29].

The safety and quality of fish are primarily related to their freshness, microbiological profile, and physicochemical properties. Fish products are highly susceptible to spoilage and rejection compared to other foods of animal origin due to several factors, including microbial activity [25]. Captured fish are prone to spoilage due to the action of autolytic enzymes, lipid oxidation, and microbial growth [30, 31]. The degree of microbial spoilage is related to factors such as the type and quantity of microorganisms present, origin and type of fish, as well as the capture method used [30]. The edible portion of fish is abundant in proteins and non-protein nitrogenous compounds (trimethylamine oxide, creatinine, and free amino acids), has a moisture content of approximately 75–80%, and a pH that is usually between 6 and 7 [30, 31]. This makes fish highly susceptible to spoilage by contaminating bacteria, including Gram-negative bacilli such as coliforms, *Pseudomonas spp., Acinetobacter, Moraxella, Flavobacterium, Shewanella, Aeromonas, Alcaligenes,* and *Vibrio*. Preservation methods that use low temperatures, such as refrigeration, increase the shelf life of fish and products by reducing the growth of microorganisms associated with spoilage; however, psychrotrophs such as Pseudomonas sp. and Shewanella spp. predominate in spoilage processes due to their rapid growth, particularly in aerobic storage at refrigeration and higher temperatures [30]. Gram-negative bacteria, during their growth in fish, produce proteolytic and hydrolytic enzymes, metabolizing nitrogenous compounds and generating various volatile compounds such as ammonia, trimethylamine, aldehydes, ketones, indoles, hydrogen sulfide, mercaptans and histamine (associated with cases of food poisoning) present in the decomposition and putrefaction process [30-32], putrescine, cadaverine, and volatile fatty acids generate unpleasant odors and flavors during spoilage. Furthermore, bacterial proliferation can cause discoloration of the gills and eyes, mucus formation, and loss of flesh texture, leading to softening that compromises the sensory quality and safety of the fish [30, 31].

Figure 3 shows the presence and growth of three bacterial groups (mesophiles, coliforms, and psychrophiles) in trout fillets during twelve days of storage at 5°C ±1. The psychrophiles group showed the greatest growth on day twelve, followed by the mesophiles group, and finally the coliforms. ANOVA showed significant effects of bacterial group and storage time ($p<0.05$), as well as a significant interaction between these two factors, indicating that bacterial growth differed among the bacterial groups throughout the twelve days of storage at 5°C. While the Tukey test revealed significant differences in bacterial growth between the mesophiles and psychrophiles groups, and between the coliform and psychrophilic groups ($p<0.05$), there were no significant differences between the mesophiles and coliform groups ($p>0.05$) during storage for 12 days at 5 ±1°C. The above may be related to the difference in physiological growth temperature conditions for each of the groups. For mesophiles, the growth temperature ranges from 20 to 37°C, for coliforms from 10 to 45°C [33], and for psychrophiles between 0 and 20°C [34]. Furthermore, it should be noted that the mesophilic bacteria and coliforms detected in the fillets stored at a low temperature of 5°C, although different from their growth temperature, continued to proliferate. The above could be due to several factors, such as: Growth at 5°C does not stop, but only slows down, intrinsic conditions (moisture content, neutral pH, nutrient content) of the fish fillet that may favor their growth even at this low temperature, survival adaptations, or the fact that some of the mesophilic and/or coliform bacteria are psychrotolerant, such as E. coli [35-37] or *Pseudomonas* sp., which are considered psychrophiles and mesophiles [38, 39].

Total Aerobic Mesophiles

The mesophilic group includes all bacteria, molds, and yeasts capable of growing at around 35°C. The detection and quantification of mesophiles in food products is because they can indicate:

a) inadequate hygienic handling and storage (time/temperature)

b) hygienic conditions of raw materials, equipment and utensils

c) possible presence of pathogenic microorganisms, since most foodborne pathogenic bacteria are mesophilic and some are opportunistic pathogens, and

d) many bacteria of this type are responsible for organoleptic modifications of food [40, 41]

A high mesophilic count in food can indicate different situations: high contamination of the raw materials, inadequate handling during the processing, possible presence of pathogens due to their mesophilic nature (although a low aerobic mesophilic count does not guarantee the absence of pathogens either), and rapid food spoilage [29, 42]. Aerobic mesophiles, also called Aerobic Plate Count, are used as a quality index in fish and other products due to their relationship with spoilage [43].

In this study, the initial microbial load of total aerobic mesophiles in trout fillets on day 0 was 2.3± 0.6 log CFU/g, reaching 5 ± 1 log CFU/g on day 6 of storage, and finally reaching its highest level of 8.2 ± 1.07 log CFU/g on day 12 (Figure 3). According to the International Commission on Microbiological Specifications for Foods (ICMSF), the maximum limit is 6 log CFU/g of aerobic mesophiles in fishery products for human consumption, while the Standard [44] NOM-027-SSA1-1993 establishes a maximum permissible limit of 7 log CFU/g of aerobic mesophiles for fresh chilled fish. The trout fillets in this study reached a growth of 6.1±1.07 and 7.09 ± 1.04 log CFU/g on days 8 and 10 of storage, respectively, which is considered unfit for consumption. This coincides with the low degree of freshness and quality obtained through the sensory assessment of day 10, where the fillet obtained a score of 52.5±10.5, which considers it unclassifiable and a deteriorating product unfit for human consumption. In related studies, [28] indicated that in *Oreochromis* niloticus fillets stored at 2±1°C for 18 days, the aerobic mesophilic load of the control sample on day 9 exceeded 7 log CFU/g of meat, at which point it was considered a decomposing product and unfit for consumption. Furthermore, [45] reported that tilapia (*O. niloticus*) fillets stored at 4°C for 12 days showed an initial aerobic mesophilic bacterial count of 4.03 log CFU/g, reaching a final count of 12.5 log CFU/g by day 12. By day 8 of storage, the bacterial count in the fillets exceeded the microbiological specification of 7 log CFU/g established by the ICMSF, thus posing a risk to consumer health.

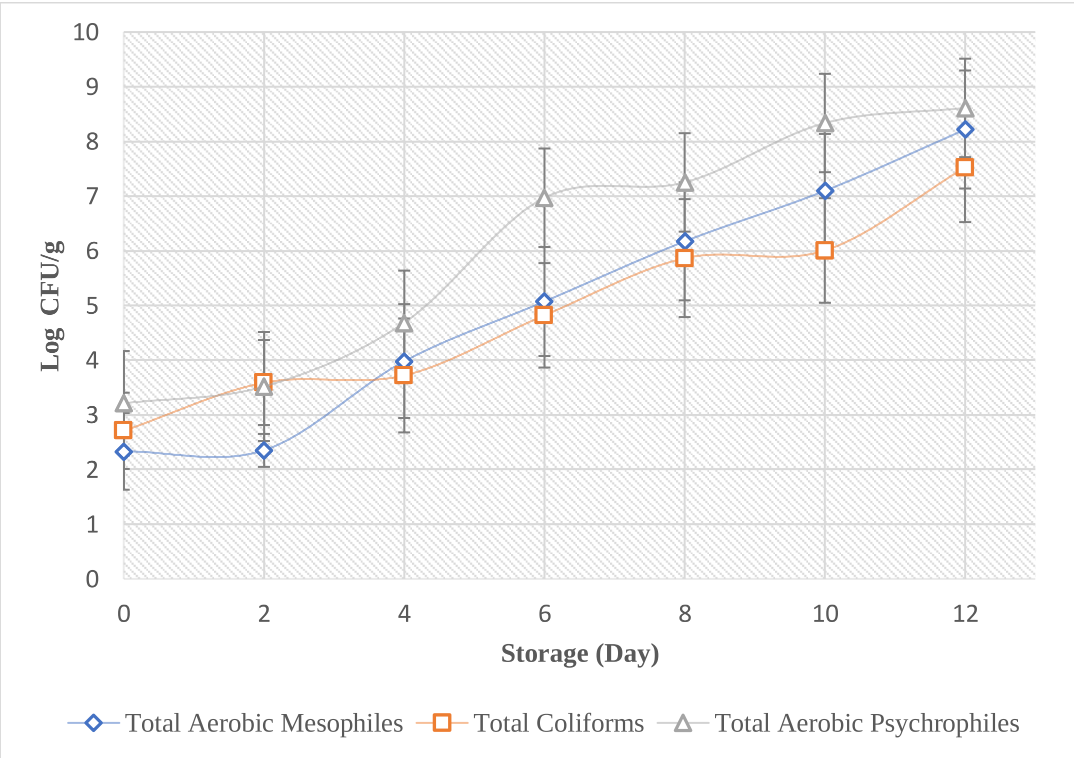

Fig. 3: Bacteriological count (Mesophiles, psychrophiles and total coliforms) in trout (*Oncorhynchus mykiss*) fillets stored at low temperature (5 ± 1°C) for 12 days. Values are presented as mean ± SD in triplicate (n = 3). Colony Forming Units (CFU). In the analysis of growth between the different microbial groups, statistically significant differences were found (p<0.05)

Total Aerobic Psychrophiles

Based on their ability to grow within a specific temperature range, bacteria are classified as psychrophiles due to their growth at low temperatures, and they exhibit minimum growth temperatures below 0°C and maximum temperatures around 20°C [34]. In food, these microorganisms produce highly active proteolytic and lipolytic enzymes that generate changes in

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organoleptic properties and promote spoilage [41]. Some psychrophilic Gram-negative bacterial genera include: *Palaromonas, Polaribacter, Alteromonas, Shewanella, Flavobacterium, Pseudoalteromonas, Pseudomonas, Psychrobacter, Psychroflexus, Moraxella, Moritella*, and *Vibrio*, as well as some Gram-positive bacteria from the genera *Micrococcus*, *Planococcus, Arthrobacter*, and *Bacillus* [34, 43, 46]. *Shewanella, Flavobacterium, Alteromonas*, and *Pseudomonas* have been regularly identified in fish [43]. Psychrophilic microorganisms are associated with food spoilage and health risks associated with low-temperature storage. These microorganisms can be found in various environments, including aquatic habitats and fish species such as trout, and are part of the typical microbiota of cold waters due to their temperature requirements [46]. Fish frequently exhibit contamination with pathogenic psychrophiles such as *L. monocytogenes*, which can grow at low temperatures, either naturally or through improper hygiene practices. Therefore, fish is considered a high-risk food due to the multiple handling processes involved, preparation for direct consumption, and storage at low temperatures for extended periods [47].

In the present study, the psychrophilic group showed the highest proportion in trout fillets compared to the mesophilic and total coliform groups, with an initial load of 3.2±0.95 log CFU/g, increasing to 6.9±0.9 log CFU/g on day 6, and reaching a maximum of 8.6±0.9 log CFU/g on day 12 (Figure 3). This bacterial group may contribute to the spoilage of trout fillets stored at low temperatures. The presence of psychrophilic microorganisms in food during low-temperature storage (refrigeration and freezing) has been reported as a major cause of spoilage, as they act through the hydrolysis and degradation of proteins [48].

The ICMSF specifies that the permissible limit for psychrophiles in food to avoid spoilage is 6 log CFU/g [49]. In this study, on day 6 of storage, the trout fillets exceeded this permissible limit with 6.9 log CFU/g, having until day 4 to avoid being considered a spoiled product and unfit for consumption. In related studies, [49], reported that trout fillets (*Oncorhynchus mykiss*) stored at -5°C for 3 months showed psychrophilic growth at < 41x105 CFU/g in the first month, >1x106 CFU/g in the second month, and >1x106 CFU/g in the third month of storage, failing to meet the microbiological specification of 6 log CFU/g for these products from the second month of storage onwards, considering it a product unfit for consumption. On the other hand, [22] reported that *O. niloticus* fillets stored at 4 °C for 12 days had an initial aerobic psychrophilic bacteria count of 4 log CFU/g, which increased to 5 Log CFU/g on the sixth day and reached a maximum count of 8.63 CFU/g on day 12. They indicated a maximum microbiological limit of acceptability and safety of 5 log CFU/g of aerobic psychrotrophs in refrigerated fish fillets.

Total Coliforms

Coliforms are Gram-negative bacteria of the Enterobacteriaceae family, which includes various genera such as *Escherichia, Klebsiella, Enterobacter, Citrobacter*, among others. They are non-spore-forming, generally motile bacilli capable of fermenting lactose at temperatures around 35°C. They are regularly found in the intestines of humans and animals and are widely distributed in nature [40, 50, 51]. The presence of coliforms in foods such as fish makes them agents that cause gastrointestinal diseases, affecting both humans and animals through consumption [51]. Coliforms are indicators of food safety; their presence indicates cross-contamination and poor handling, processing, and storage practices, causing contamination and posing a risk to consumer health [18, 51]. In this study, the initial coliform load of rainbow trout fillets was 2.7±0.69 log CFU/g, reaching 5.8 ±1.07 log CFU/g by day 8 of storage, and finally reaching a maximum of 7.5±1 log CFU/g by day 12 (Figure 3). The Spanish food safety standard specifies that the maximum limit for total coliforms in fishery products for human consumption is 3 log CFU/g [52]. Therefore, by day 2 of storage, the trout fillets were no longer edible, were spoiled, and presented a health risk.

In related studies, [43] reported that in the shelf-life assessment of *Mugil cephalus* fillets stored at 4°C for 24 days, control samples on day 0 had a coliform count of 2.2 log CFU/g, on day 12 of 6.21 log CFU/g, and finally on day 24 of 8.61 log CFU/g. Sensory assessment, based on sample color, indicated acceptable results up to 12 days of storage. The aforementioned suggests that microbial activity is the main limiting factor for fish quality and that a lower microbial load in the product can extend its shelf life. On the other hand, [53] indicated that in samples of fish fillets (Wallago attu) stored at 4±1°C for 14 days, the total coliform counts were 1.13 log CFU/g on day 7 of storage, 1.4 log CFU/g on day 10, and subsequently 2.1 log CFU/g on day 14, suggesting that it is preferable to consume the fish by the seventh day and up to a maximum of the tenth day of storage to ensure product safety. [22] reported that tilapia fillets stored at 4°C for 12 days had an initial total coliform count of 1.07 log CFU/g, which then increased to 3.6 log CFU/g on day 2, 6 log CFU/g on day 4, reaching a maximum of 8.33 log CFU/g on day 12. They noted that, according to Spanish food legislation, the maximum microbiological criteria for hygiene and food safety in chilled fish products are total coliforms or *Enterobacteriaceae* counts of 3 log CFU/g; this means that, on the second day of storage, the fillet does not meet this microbiological safety criterion.

It should be noted that the bacterial population in captured fish depends on their environment or geographical area, season, species, age, size, feeding, life stage, and anthropogenic influence [54-56]. Bacterial populations in live captured fish are generally found on external surfaces such as the skin, gills, and intestines [6, 56]. The proportion of bacteria varies and is established as a normal range of 10^2 to 10^7 CFU/cm² on the skin surface, while on the intestines and gills it is between 10^3 and 10^9 CFU/g [6, 30, 54].

Freshly caught fish are exposed to a variety of bacteria present in their aquatic environment, from handlers and during processing conditions and surfaces. Gram-negative bacteria, such as *Aeromonas, Pseudomonas, Serratia, Vibrio, and Shewanella*, are often the initial colonizers, and their prevalence and proportion depend on the water microbiota, conditions, and hygiene practices during and after capture [31].

The bacterial population in fish is diverse and can reach very high numbers in fish caught in waters contaminated with a high proportion of *Enterobacteriaceae* [6, 57]. Furthermore, the temperature of the source water can have a selective effect on the presence of bacteria, meaning that cold-water fish primarily contain psychrophiles (*Pseudomonas, Moraxella, Acinetobacter, Shewanella*, and *Flavobacterium*), while warm-water fish primarily contain mesophiles [6, 54]. Likewise, the predominant bacteria in processed fish vary according to the product's storage temperature [53]. Fish and fish products are not only nutritionally important in our diet but also economically important from the perspective of producing countries, in addition to posing a risk to public health [58]. According to quality aspects associated with fish safety and the wide range of diseases transmitted as a result of consuming aquatic foods, this is mainly due to the different pathogenic bacteria present in these foods, which can be divided into autochthonous and allochthonous [8, 54]. Autochthonous bacteria are distributed in aquatic environments, with water temperature being a selective determinant. These include members of the genera *Vibrio sp., Listeria sp., Aeromonas sp*., and *Clostridium sp*., coliforms and other members of the Enterobacteriaceae family, such as Shigella sp., Salmonella sp., Campylobacter sp., and Gram-positive bacteria like *S. aureus*, which are allochthonous representatives that are introduced into the water from infected animals or humans, or by direct contamination during processing stages. They are used as indicators of contamination in aquatic environments and foods [8, 49, 54, 59].

The results obtained in this study regarding the sensory status of the fillets during twelve days of storage and the population of different microbial groups (mesophiles, psychrophiles, and coliforms) provide valuable information and indicate a significant discrepancy between the product's safety status and acceptable sensory properties. On the one hand, the sensory assessment indicates that the stored fillets exhibit acceptable attributes for consumption up to day 8 of storage. However, the coliform profile exceeded the limits established by the sanitary standard on day 2 of storage, while the same occurred for psychrophiles on day 6. The mesophilic group exceeded the limits established by the ICMSF and Mexican standards on days 8 and 10, respectively. Microbiological safety and sensory acceptability of fresh fillets may not coincide during storage. A fillet may have characteristics of freshness that promote consumption but contain dangerous levels of bacteria. This phenomenon poses a significant health risk to consumers who may rely solely on sensory attributes to make purchasing decisions, as sensory perception does not guarantee microbiological safety. While sensory assessment is a common and widely used method for determining the quality and consumption of fish or derived products, relying exclusively on sensory aspects can have health repercussions due to the consumption of fish contaminated by various microbiological hazards. Therefore, sensory assessment studies of fish must be complemented with microbiological controls.

Conclusion

The quality grade analysis of trout (*O. mykiss*) fillets stored at low temperature (5°C ±1) indicated that it was not suitable for consumption after 8 days of storage.

The trout (*O. mykiss*) fillets stored at low temperatures showed microbial contamination (mesophiles, coliforms, and psychrophiles). The growth of the mesophilic, coliform, and psychrophilic groups in trout fillets stored at 5°C±1 for 12 days showed differences between them. Psychrophiles represented the highest microbial load in the fillets, with a count of 8.6 log CFU/g on day 12, making them one of the main bacterial groups responsible for spoilage, followed by mesophiles at 8.2 log CFU/g, and total coliforms at 7.5 log CFU/g.

According to sanitary specifications for aquatic foods, the presence of coliforms made the trout fillets (*O. mykiss*) unfit for consumption until day 2 of storage, as they exceeded the established limit of 3 log CFU/g. On the 6th day of storage, the psychrophilic group exceeded the maximum permitted limit of 6 log CFU/g established by the ICMSF, rendering the fillets unfit for consumption and indicating a spoiled product. Finally, on the eighth day of storage, the mesophilic group exceeded the maximum limit of 6 log CFU/g established by the ICMSF, and on the tenth day of storage, the aerobic mesophilic group exceeded the maximum permitted limit of 7 log CFU/g according to regulation [44], indicating that the product was spoiled and posed a health risk if consumed.

Actions to increase the shelf life and safety of fish begin from the moment of capture or harvesting, and continue immediately with storage and processing at low temperatures, under hygienic conditions and practices at all times, in order to increase the availability of nutritious and safe food for the population.

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Author's Contributions

Román Alejandro Téllez-Cortez and Ángel Ruyan García-Hurtado: Experimental development, results analysis, writing of final manuscript.

Mayra Diaz-Ramírez and Erika Torres-Ochoa: Management of materials, equipment and reagents, results analysis, review of final manuscript.

Alejandro De Jesús Cortés-Sánchez: Study conception, experimental design, results analysis, management of materials, equipment and reagents, review of final manuscript.

Ethics

This document represents a single work. The corresponding author confirms that this has been reviewed and approved in its final version by all co-authors and is free of any ethical or interest conflicts.

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