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PHYSICO-CHEMICAL AND MICROBIOLOGICAL CHANGES IN COMMERCIAL TILAPIA (Oreochromis niloticus) DURING COLD STORAGE

CAMBIOS FISICO-QUÍMICOS Y MICROBIOLÓGICOS EN TILAPIA COMERCIAL (Oreochromis niloticus) DURANTE ALMACENAMIENTO REFRIGERADO

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ABSTRACT

Background: The microbiological and chemical processes are the main responsible for deterioration of fresh fish. Therefore, it is essential to avoid these processes by applying good manufacturing practices during fish handling, distribution and storage. Objective: The aim of this paper was to evaluate the physico-chemical and microbial changes in commercial tilapia (Oreochromis niloticus) during cold storage in order to establish the shelf life since its arrival at the supermarket. Methods: 27 aquacultured tilapia specimens were analyzed at 0, 2, 4, and 7 days of storage at 4°C. Measurements of texture, color, water holding capacity, total volatile basic nitrogen (TVB-N), thiobarbituric acid index, ATP-related compounds, as well as microbial analyses were carried out. **Results:** The TVB-N content was high at the beginning of the study, remaining stable during the storage. Lipid oxidation of samples was minimum, so this process did not contribute to the fish spoilage. It was observed the breakdown of inosine-5'- monophosphate (IMP) into Ino (inosine), and Ino into Hx (hypoxantine). The texture parameters and colour underwent changes as a consequence of the fish spoilage. Low microbial counts were observed at day 0, but Enterobacteriaceae and mesophilic counts gradually increased throughout storage. **Conclusions:** The K₁-value showed the progressive spoilage of the fish during the cold storage. The decrease of hardness and firmness confirmed the loss of quality throughout the time of study. The low microbial counts at the beginning of the study demonstrated the good quality of the tilapia; however, the increase of the mesophilic counts at the end of the studied period showed that tilapia was not fit for human consumption at day 7.

Keywords: Tilapia, quality, shelf-life, cold storage.

RESUMEN

Antecedentes: Los procesos microbiológicos y químicos son los principales responsables del deterioro del pescado fresco. Por tanto, es esencial evitar estos procesos aplicando buenas prácticas de fabricación durante la manipulación, distribución y almacenamiento del pescado. **Objetivo:** El objetivo de este trabajo fue evaluar los cambios físico-químicos y microbiológicos en tilapia comercial (*Oreochromis niloticus*) du-

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Métodos: 27 especímenes de tilapia fueron analizados a día 0, 2, 4 y 7 de almacenamiento a 4 °C. Se llevaron a cabo medidas de textura, color, capacidad de retención de agua, nitrógeno básico volátil total (N-BVT), índice del ácido tiobarbitúrico, compuestos relacionados con el ATP, así como análisis microbianos. **Resultados:** El contenido en N-BVT fue alto al principio del estudio, siendo estable durante el almacenamiento. La oxidación lipídica de las muestras fue mínima, por lo que este proceso no contribuyó al deterioro del pescado. Se observó la degradación de inosina-5'- monofosfato (IMP) a Ino (inosina), y de Ino a Hx (hipoxantina). Los parámetros de textura y color sufrieron cambios como consecuencia del deterioro del pescado. Se observaron bajos recuentos microbianos a día 0, pero los recuentos de *Enterobacteriaceae* y de mesófilos aumentaron gradualmente durante el almacenamiento. **Conclusiones:** El valor K₁ mostró el deterioro progresivo del pescado durante el almacenamiento en refrigeración. La disminución de dureza y firmeza confirmó la pérdida de calidad a lo largo del tiempo de estudio. Los bajos recuentos microbianos al principio del estudio demostraron la buena calidad de la tilapia; sin embargo, el aumento de los recuentos de mesófilos al final del periodo estudiado mostraron que la tilapia no era adecuada para el consumo humado a día 7.

Palabras clave: Tilapia, calidad, vida útil, almacenamiento refrigerado.

INTRODUCTION

Aquaculture is expanding in all the continents as far as new areas and species are concerned, and is also intensifying and diversifying the product range in species and product forms to meet consumer requirements. Freshwater fish production has increased dramatically in the last two decades, and has been driven chiefly by the rapid development of tilapia and other species. Tilapia is the third most cultured fish, after carps and salmonids, and the global production of this fish rose to 3.5 million of tons in 2010 (1). Tilapia offers adequate biologic and ecologic features, such as adaptability, fast growth, disease resistance and efficient feed conversion capacity (2), and is one of the fish species with the highest production and distribution worldwide. Aquaculture tilapia production is located mainly in Asia, with 72% of world production, followed by Africa (19%) and America (9%) (1). It is believed that imports of tilapia to Europe will increase significantly in the near future because, owing to environmental concerns, more consumers are looking for suitable alternatives to white fish fillets. The main markets in the European Union (EU) are big cities where large communities of African, Chinese and Asian citizens live. However, tilapia consumption in non-ethnic markets has also increased recently. China is the leading supplier of European countries, and Poland, Spain and Germany are the largest markets in the EU (3).

Freshness is the most important attribute when evaluating fish quality. When fish die, several postmortem changes take place. They are related to the breakdown of the cellular structure and biochemistry, and also to the growth of microorganisms that are either naturally associated with fish or form part of the flora due to contamination during handling (4). These modifications, which affect fish quality and shelf life, are changes in pH, texture, water holding capacity and color, protein and ATP degradation, lipid oxidation, as well as production of undesirable compounds such as molecular low weight volatile bases (TVB-N), which are produced by bacterial or enzymatic action.

The quality of fish products found in supermarkets is highly related to handling, transportation and distribution conditions. Scientific shelf life studies have been carried out to determine changes during chilled storage (5, 6, 7). Some of these studies start at the time of slaughter or capture; however, the fish that usually arrive at retailers do not always present the same freshness. Nowadays, consumers are far away from fish production areas. Although distribution channels have improved in the last few decades, the long distance between producers and consumers means that fish arrive at the supermarkets with different grades of freshness.

The aim of this work was to evaluate the physico-chemical and microbial changes in commercial tilapia (*Oreochromis niloticus*) during cold storage in order to establish the shelf life from its arrival at supermarket.

MATERIALS AND METHODS

Materials

In this work, 27 aquacultured tilapia (*Oreochomis niloticus*) specimens were used. Fish were purchased from a local supermarket in Valencia (Spain) and were transported to the laboratory in polystyrene boxes with ice. There was no information available about the background of the fish samples (feed composition, handling or transport conditions) or slaughter dates. Fish were wrapped in plastic film to minimize dehydration and any contact with oxygen during cold storage. Samples were analyzed at 0, 2, 4, and 7 days of storage at 4°C.

Before the analyses, fish were headed, gutted and filleted, and two fillets per fish were obtained. Samples were taken from the dorsal muscle of the two fillets, except for the compression test, which was the analysis performed on the whole fish.

Analytical Methods

Proximate composition: Moisture, lipid, protein and ash contents were assayed by AOAC methods 950.46, 991.36, 928.08, and 920.153, respectively (8).

Physico-chemical parameters

Measurements of pH were carried out according the method described by Rizo et al. (2015) (9). Water holding capacity (WHC) was determined according to the technique described by García et al. (2006) (10). For this determination, a portion of 0.3 g of sample was placed between two dry filter papers and two acrylic plates on which a 10 kg pressure was applied for 15 min. The sample was weighed before and after being compressed, as well as the dry and wet filter papers. WHC was expressed as g of water held per 100 g of total water in the fish sample. The total volatile basic nitrogen (TVB-N) content was determined by steam distillation following the method described by Malle and Tao (1987) (11), and was expressed as mg N/100 g of muscle.

The TBA index was determined by a spectrophotometric technique according to the method described by Vyncke (1970) (12) and the results were expressed as mg malonaldehyde (MDA)/kg of fish muscle.

The ATP-related compounds, consisting of inosine-5'- monophosphate (IMP), inosine (Ino), and hypoxantine (Hx), were determined by HPLC

according to the method described by Barat et al. (2008) (13), with minor modifications. The analysis was conducted in a Hitachi LaChrom Elite liquid chromatography (Hitachi Ltd., Tokyo, Japan®) with a pump (model L-2130), an auto-sampler (model L-2200) and a UV detector (model L-2400). Data acquisition was performed with the EZChrom Elite software (Agillent Technologies, Palo Alto, CA, USA[®]). Separations were done on a reverse-phase Ultrabase C18 250 x 4.6 mm, internal particle diameter of 5 mm (Análisis Vínicos, S.L., Tomelloso, Spain®). Compounds were identified using retention time comparison of unknowns with those of standards and by standard addition or "spiking". IMP, Ino, and Hx were quantified according to the external standard method, using calibration curves of the peak area of compound vs concentration of compound under identical chromatographic conditions. K₁-values were calculated according to Equation (1):

$$K_1(\%) = \frac{[Ino] + [Hx]}{[IMP] + [Ino] + [Hx]} \times 100$$
 Equation1

Where IMP is inosine 5'-monophosphate; Ino, inosine; Hx, hypoxanthine. All chemical reagents were provided by Sigma-Aldrich (St. Louis, MO, USA®).

Texture profile analysis (TPA) and compression test were performed on tilapia using a Texture Analyser TA.XT2® (Stable Micro Systems, Surrey, UK) equipped with a load cell of 250 N. For the TPA analysis, samples were obtained by cutting parallelepiped pieces (40 × 30 mm) from the dorsal muscle of the fillet. In this test, a flatended cylindrical plunger (7.5 mm diameter) was employed. The plunger was pressed into the sample at a constant speed of 0.8 mm/s until 50% of the sample height was achieved. Force-distance curves were processed to obtain seven texture parameters: hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience.

For the compression study, the texture analyzer was equipped with a 24.5 mm-diameter flat-ended cylindrical plunger. The plunger was pressed down at a constant speed of 5 mm/s into the sample until compressing 7 mm (14). This analysis was performed on the whole fish behind the dorsal fin.

Instrumental color analyses were performed with a Minolta Chroma Meter CM-3600d

(Minolta, Osaka, Japan®) with a D65 light source and a 10° observer. Data were expressed using the CIE L*a*b* system to represent lightness (L*), redness (a*), and yellowness (b*). Furthermore, the values of chroma (C*ab), which defines saturation of color, and the angle of hue (h*ab) were obtained.

Microbial analyses. Mesophilic counts were done according to the method provided in standard UNE-EN ISO 4833:2003 (15). *Enterobacteriaceae* were enumerated according to the method described by Pascual and Calderón (2000) (16). All the culture media were provided by Scharlau Chemie, S.A. (Barcelona, Spain). All the analyses were performed in duplicate and the results were expressed as log CFU/g.

Statistical analysis. Statistical treatment of the data was performed using the Statgraphics Centurion (Statpoint Technologies, Inc., Warrenton, VA, USA®). An analysis of variance (One-Way ANOVA), in which storage time was the factor, was conducted for each parameter evaluated to test whether there were significant differences throughout cold storage. The LSD procedure (least significant difference) was used to test for differences between averages at the 5% significance level.

RESULTS

Proximate composition. The proximate composition of fresh tilapia is shown in Table 1. These

values agree with the data presented by Yanar *et al.* (2006) (17). Regarding the fat content, our results agree with the data reported by Suloma *et al.* (2008) (18); however, lipid content was higher, and subsequently moisture was lower, than those provided in other studies. The protein content data are in accordance with those obtained by other authors for the same fish species (17, 19, 20). The crude protein content in fish muscle can range from 11 to 24% (wet weight), depending on specie, state of nutrition, the reproductive cycle of the animals, etc. (20).

Table 1. Proximate composition of tilapia (means and standard deviations, n = 9).

Moisture g/100g	74.6 ± 1.3
Protein g/100g	19.1 ± 1.0
Lipid g/100g	2.8 ± 0.9
Ash g/100g	1.5 ± 0.4

Table 2 shows the values of moisture content, pH, WHC, TVB-N and TBA index of tilapia during cold storage. The initial value of pH was 6.06, increasing progressively during the storage time. These results are in accordance with the results obtained by Tome *et al.* (2000) (21) and Khalafalla *et al.* (2015) (22) for the same fish species. Fresh fish pH oscillates between 6.0 and 6.5 (23), depending on different factors such as fish species, season, diet, level of activity or stress during capture, and storage conditions (24).

Table 2. Physico-chemical parameters of tilapia during cold storage (Mean \pm Sd. n = 6).

Day	0	2	4	7
Moisture (g/100 g)	73.12 ± 1.32^{a}	73.90 ± 1.77^{ab}	75.59 ± 1.01^{bc}	$75.75 \pm 1.03^{\circ}$
рН	6.06 ± 0.09^{a}	6.12 ± 0.06^{ab}	6.27 ± 0.05^{ab}	6.25 ± 0.06^{a}
WHC (g water held/100 g total water)	61.93 ± 0.03^{a}	$56.58 \pm 1.85^{\text{b}}$	$54.40 \pm 1.61^{\circ}$	52.28 ± 1.23^{d}
TVBN (g N/100 g fish)	28.75 ± 1.69^{a}	28.08 ± 3.84^{a}	27.41 ± 1.41 ^a	31.14 ± 0.35^{a}
TBA (mg MDA/Kg fish)	0.36 ± 0.02^{a}	0.33 ± 0.04^{a}	0.43 ± 0.04^{b}	0.42 ± 0.01^{b}

WHC: water holding capacity; TVB-N: total volatile basic nitrogen; TBA: thiobarbituric acid index. Same letters in the same row indicate homogeneous group membership (p < 0.05)

The WHC values significantly decreased during storage (Table 2), which agree with other studies (10, 25). The decrease in the WHC can be due to a gradual denaturation of proteins because of the microbial activity and pH changes during the storage.

The TVB-N content was high at the beginning of the study (Table 2). The changes of this parame-

ter throughout the storage were non-significant. The TVB-N r is commonly considered a quality index for unprocessed fish products as its increase is related to the activity of spoilage bacteria and endogenous enzymes (26, 27, 28). The action of such enzymes results in the formation of compounds, including ammonia, monoethylamine,

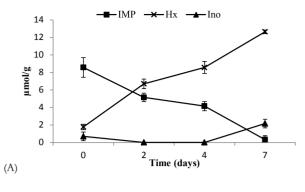
dimethylamine, etc. which give fish a characteristic off-flavour (29).

The TBA index remained low and constant throughout the study, ranging between 0.33 and 0.43 mg MDA/kg (Table 2), which is in accordance with other studies with tilapia (17). Different limit of acceptability values have been reported for this index. According to Connell (1995) (30), TBA values of 1-2 mg MDA/kg of fish flesh are usually regarded as the limit beyond which fish normally develop an objectionable odor. Ruiz-Capillas and Moral (2001) (31) established that the minimum TBA index value detectable by panellists was 1.44 mg MDA/kg.

ATP-related compounds. Figure 1 depicts the changes of inosine 5'-monophosphate (IMP), inosine (Ino), hypoxanthine (Hx) and the K_1 -value during cold storage. The evolution of major adenine nucleotides and their related compounds can provide information about biochemical changes during storage. The ATP catabolism to IMP has been reported to be essentially caused by endogenous enzymes. Nevertheless, the hydrolysis of Ino and Hx formation may also result from bacterial enzymes (32, 33). In addition, several authors have found that loss of sensory quality correlates with increases in the Hx level during storage (34).

In this study, ATP, ADP and AMP were not analyzed because the conversion of ATP into IMP is usually completed in 1 day, is presumed totally autolytic (35), and the main changes during storage occur in IMP, Ino and Hx (9).

The IMP levels decreased from 9.5 to 0.5 μ mol/g. The Ino contents remained low during the 7 days of study with a maximum value of 2.0 μ mol/g at the end of storage. The initial Hx contents were low but increased during the storage period to reach 12.3 μ mol/g.



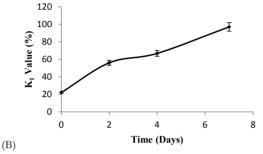


Figure 1. (A) Inosine 5'-monophosphate (IMP), inosine (Ino), and hypoxanthine (Hx) (μ mol/g), and (B) K₁-value of fresh tilapia (day 0) and samples in cold storage for 2, 4 and 7 days (Mean and Sd, n=6).

Ehira and Uchiyama (1987) (4) have demonstrated that the K_1 -value, which measures the extension of IMP degradation, is a good freshness index for a large number of fish species. In this study the initial values of K_1 were over 20%, increasing during the storage period.

Texture. Firmness, hardness, adhesiveness and gumminess significantly changed during storage (Table 3). It is important to note the decrease of firmness and hardness observed throughout the studied period, which indicates changes in the structure of fish.

Table 3. Textural parameters evolution of tilapia during cold storage (Mean \pm Sd., n = 6).

Time (Days)	0	2	4	7			
Compression test (whole fish)							
Firmness (N)	23.85 ± 0.46^{a}	19.38 ± 2.24^{b}	17.37 ± 2.01°	16.60 ± 1.75°			
TPA (fish fillet)							
Hardness (N)	40.70 ± 2.76^{a}	30.76 ± 6.16^{ab}	26.02 ± 2.62^{ab}	19.44± 6.20 ^b			
Adhesiveness (g·s)	-0.58 ± 0.03^{a}	-0.27 ± 0.03^{b}	-0.33 ± 0.03^{ab}	-0.45 ± 0.14^{ab}			
Springiness (ratio)	0.51 ± 0.16^{a}	0.46 ± 0.03^{a}	0.54 ± 0.05^{a}	0.42 ± 0.01^{a}			
Cohesiveness (ratio)	0.36 ± 0.02^{a}	0.33 ± 0.04^{a}	0.43 ± 0.04^{a}	0.42 ± 0.01^{a}			
Gumminess (N)	14.85 ± 1.72^{a}	10.19 ± 0.88^{ab}	11.33 ± 2.27^{ab}	7.67 ± 1.23^{b}			
Chewiness (N)	7.67 ± 3.22^{a}	4.70 ± 0.38^{a}	6.16 ± 1.79^{a}	3.19 ± 0.41^{a}			
Resilence (ratio)	0.13 ± 0.02^{a}	0.12 ± 0.02^{a}	0.13 ± 0.01^{a}	0.12 ± 0.03^{a}			

Color. Lightness of tilapia muscle slightly decreased with storage time, while a* and b* parameters progressively increased (p<0.05) (Table 4). The mechanism which leads to color changes in tilapia is unclear, although some authors have reported that one or the causes is the oxidation of muscle myoglobin (36).

Table 4. Color parameters evolution of tilapia during cold storage.(Mean \pm Sd. n = 6).

Time (Days)	0	2	4	7
L*	44.42 ± 0.42^{a}	44.77 ± 1.47 ^a	43.68 ± 0.35^{ab}	$42.69 \pm 0.34^{\rm b}$
a*	-3.46 ± 0.24^{a}	-3.25 ± 0.16^{a}	-2.33 ± 0.14^{b}	-2.13 ± 0.26^{b}
b*	3.59 ± 1.39^{a}	4.29 ± 0.57^{a}	6.15 ± 0.60^{b}	5.99 ± 0.32^{b}
C* _{ab}	5.05 ± 1.09^{a}	5.39 ± 0.49 ab	6.57 ± 0.61^{b}	6.36 ± 0.31^{b}
h _{ab}	-0.77 ± 0.19^{a}	-0.91 ± 0.06^{a}	-1.20 ± 0.02	-1.22 ± 0.04 ^b
ΔΕ	-	2.02 ± 0.98	3.15 ± 1.19	1.38 ± 0.36

Same letters in the same row indicate homogeneous group membership (p<0.05)

Microbial analyses. The evolution of mesophilic bacteria and *Enterobacteriaceae* counts are shown in Figure 2. Mesophilic counts gradually increased throughout storage. Limits of 6-7 log CFU/g for mesophilic bacteria have been established for fresh water and marine species fit for human consumption (16). Samples reached this limit of acceptability at day 7 of the study. No *Enterobacteriaceae* were found on days 0 and 2 of storage, but they increased to 1.6 log CFU/g at the end of storage.

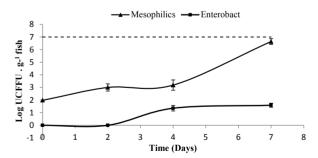


Figure 2. Counts of mesophilic bacteria and *Enterobacteriaceae* (log CFU/g) of fresh tilapia (day 0) and samples in cold storage for 2, 4 and 7 days (Mean and Sd, n=6). Upper areas of the horizontal lines are unacceptable (dashed line for mesophilic).

DISCUSSION

Proximate composition. The chemical composition of tilapia is highly affected by: season,

environmental conditions, water quality, state of maturity, feeding conditions or sex (37, 38). Regarding the fat content, Puwasatien et al. (1999) (19) reported that tilapia muscle contained 1.8% of fat. Visentainer et al. (2005) (39) and Chaijan (2011) (20) reported even lower lipid contents (1.09 and 1.10%, respectively), Younis et al. (2015) (38) reported values lower than 1% of fat. According to these results, tilapia can be classified as a lean fish because its fat content is below 2 g fat/100 g. However, in the present study tilapia showed a lipid content of 2.8% and should, therefore, be classified as low-fat fish (total lipid content from 2% to 4%) (40). The protein content found in this study (19.1%) was higher than the values reported in other studies on tilapia (38). However, the moisture content in this work was lower than those found by Younis et al. (2015) (38) and Fonseca et al., (2013) (41), which could explain the differences found in in lipid and protein contents.

Physico-chemical parameters. The behavior of the TVB-N (high initial value with no significant increase throughout the storage) could not be related to the quality changes of tilapia during the study. Several works have shown a poor correlation of TVB-N with storage time and sensory fish quality (42). Variation in TVB-N of a particular fish species depends on the fish non-protein nitrogen content, which in turn depends on the type of fish feeding, catching season, fish size, and also on other environmental factors (29).

The TBA index remained between 0.33 and 0.43 mg MDA/kg, which can be considered low in comparison with other studies on tilapia, where values about 1 mg MDA/kg were reached at 6 days of storage. Therefore, it can be concluded that in this study lipid oxidation of samples was minimum, so this parameter did not contribute to the fish spoilage.

The changes observed in the IMP, Ino and Hx contents throughout the storage indicate the breakdown of IMP into Ino, and Ino into Hx. Therefore, the K₁-value increased during the storage period. Ehira and Uchiyama (1987) (4) established that in recently captured fish K₁-value should not exceed 10%, a fish of excellent quality should not exceed 20%, and values between 20% and 45% indicate fish of acceptable quality, while values greater than 60% indicate spoiled fish. Based on these categories, at the beginning of the study tilapia fish could be considered moderately fresh and spoiled from day 4 of cold storage.

Fish death triggers autolytic and microbiological processes that make muscle softer and less elastic. Hernández et al., (2009) (43) found that hardness values highly correlated with storage time and microbial counts. In this study, noticeable changes were observed especially in hardness and firmness, which underwent an important decrease during the storage time, which agree with other studies on tilapia (44). The decrease in the hardness and firmness could be due to the protein denaturation.

Regarding color changes, it is important to highlight the variability of data found in different studies on tilapia. Oliveira *et al.*, (2014) (44) reported higher values of L* and b*, while Fonseca *et al.* (2013) (41) found lower values of L* and higher of a*, compared with this study. The differences in the color could be due to the different environment and different diets, which directly affect the color muscle.

Microbial analyses. Enterobacteriaceae was not reached the acceptability limit of 3 log CFU/g established in other studies (26). Enterobacteriaceae in the flora of fish appears when fish are obtained from polluted water, or if there is a delay in chilling after capture. Furthermore, Enterobacteriaceae may occur due to cross contaminations during post-processing; e.g., filleting. In this sense, aquaculture fish are expected to be of good hygienic quality since they live in a controlled environment and are slaughtered through good practices. Moreover handling, distribution and storage conditions are usually more controlled than in wild fish.

Mesophilic bacteria counts reached the acceptability limit (6-7 log CFU/g) at day 7 of the study. Taking into account this limit, the shelf life of tilapia used in this study would be lower than 7 days. In other studies on tilapia (38, 41, 44), this limit has been reached after a longer period of time. This is due to that in this work the day 0 of study was not the slaughter day, but the day of purchase in the supermarket.

In order to establish properly the shelf life period, a sensory analysis is needed to confirm the results obtained by physico-chemical and microbial analyses.

CONCLUSIONS

The results of the physico-chemical and microbial analyses showed the spoilage of tilapia during cold storage. TVB-N was not a good indicator of the fish spoilage. No oxidation could be observed in the tilapia during the 7 days of storage. The ATP-related compounds and K₁-value showed the progressive spoilage of the fish during the cold storage. The decrease of hardness and firmness confirmed the loss of quality throughout the time of study. The low microbial counts at the beginning of the study demonstrated the good quality of the tilapia; however, the increase of the mesophilic counts at the end of the studied period showed that tilapia was not fit for human consumption at day 7.

It is important to consider that the distance between producers and consumers decisively determines degree of fish freshness in the destination market, for this reason it is essential to apply good manufacturing practices to ensure a good quality of fish when reaching consumers.

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