Postmortem Biochemical Changes and Evaluation of the Freshness in the Muscle of Tilapia (Oreochromis niloticus) during the Storage in Ice

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ABSTRACT

Tilapia fish were harvested, filleted and stored in ice. Fillets were analyzed over 18 days to determine biochemical and physical changes and their relation to the eating quality of the muscle. Adenosine-Triphosphate (ATP) content and breakdown products, K-value, pH, total volatile bases, total bacterial count, texture, water-holding capacity and color changes were examined. At the beginning of the study, the muscle contained low concentration of ATP and a high concentration of Inosina-Monophosphate (IMP). Of the measurements of freshness and spoilage, K-value increased exponentially, from an initial value of 11.61% to a final value of 86.46%. Total volatile bases and pH significantly increased during storage in ice. Bacteria counts exceeded 7 log CPU g⁻¹ after 15 days. Texture and water-holding capacity were not affected. Results indicated that the edible quality of tilapia fish muscle was satisfactory for at least 15 days when stored in ice and no spoilage-related odors were detected.

Key words: Tilapia, postmortem changes, K-value, freshness, quality

INTRODUCTION

Tilapia fish is an important species cultured in Mexico which is based on the large production and high price. In 2012, about 77547 metric tons were harvested (SAGARPA., 2012) and commercialized at a domestic market price of US$ 7 per kg. Its high nutritional value, appearance and flavor of the muscle, make this fish attractive to consumers. Despite the importance of this fishing resource, little is known about postmortem biochemical properties and freshness of its muscle. Studies on postmortem biochemistry and shelf-life of species cultured, such as tilapia fish are scarce. The rate of spoilage of species of freshwater, when stored in ice, might differ considerably from marine species. Difference in storage time is related to the microflora initially present in fish at the moment of capture (El-Marraikchi et al., 1990). Loss of freshness and spoilage
in fish markedly vary from species to species. At death, changes result from breakdown of the cellular structure and biochemistry, as well as growth of microorganisms that are either naturally associated with fish or result from contamination during handling (Ehira and Uchiyama, 1987). Postmortem biochemical changes in fish muscle are strongly influenced by post-catch handling practices. Endogenous enzymes cause loss of freshness and normally precede and are independent of decomposition from bacteria. Normally, decomposition follows four stages: Rigor mortis, dissolution of rigor, autolysis (loss of freshness) and bacterial spoilage. The rapidity of each stage depends on species, physiological condition, microbial contamination and temperature. The degree freshness must be determined at the autolysis stage, before spoilage begins (Ehira and Uchiyama, 1987). The changes that directly and strongly affect quality and shelf life are associated with protein degradation, ATP degradation, lower pH, lipid oxidation, production of trimethylamine (TMA-N) and low molecular weight volatile bases (TVB-N). The latter two result from bacterial action. Muscle undergoes changes in texture, water-holding capacity and color (Shahidi et al., 1994; Alasalvar et al., 2002).

Methods for evaluating freshness and quality of different fishery species are based on measurements of postmortem changes associated with sensory, chemical and physical changes and microbiological growth (Gokoglu et al., 1998). Quality indices based on nucleotide degradation for monitoring freshness during handling and processing has received special attention (Ryder, 1985). The concentration of major adenine nucleotides and their related compounds in muscle correlates well with the loss of freshness in many fish. Total molar concentration of ATP and related compounds in muscle, as well as the rates and patterns of changes in these levels during storage are species-dependent and even muscle-dependent. Regardless of the species and muscle type, ATP decreases rapidly in the first 24 h after death. In fish muscle, ATP is metabolized as follows: ATP→ADP→AMP→IMP→HxR (inosine)→Hx (hypoxantine). K-value is calculated from the ATP concentration and its products of degradation and is used to measure how quickly these compounds degrade. K-value shows the relation, expressed in percentage, between the sum of the HxR concentrations and Hx between the sum of the ATP concentrations and related compounds. This index is widely used to determine fish freshness and has a close correlation with storing time (Ocana-Higuera et al., 2006, 2009, 2011; Ozurt et al., 2007; Ozogul et al., 2009).

In Mexico, tilapia fish is important and highly appreciated species; however, studies about postmortem changes of its muscle during handling and storage are limited. This study reports on postmortem changes of tilapia fish muscle under optimal post-harvest handling to establish changes in freshness and spoilage indices. The results obtained will serve to define quality parameters for this species and should help fish farmers and processors establish better processing and marketing strategies for domestic consumption and exportation.

MATERIALS AND METHODS
Collecting and handling: Forty-two live tilapia fish Oreochromis niloticus weighing ~500 g were obtained from a local fish farm company at Hermosillo, Sonora, Mexico. Immediately after the collect, the tilapia fishes were plunged in a container with ice and water. Subsequently, the organisms were placed in ice inside an insulated cooler in alternating layers of ice-organisms-ice and transported to the Laboratorio de Investigacion en Alimentos (Universidad de Sonora, Hermosillo, Sonora) within 2 h after harvest, where they were filleted and packed in polyethylene bags and then stored in an insulated cooler in alternating layers of ice-fillet-ice for 18 days.
Biochemical and chemical tests were made on days 0, 3, 6, 9, 12, 15 and 18, while the physical and microbiological tests were carried out on days 0, 6, 12 and 18. Samples taken for biochemical and chemical analyses were frozen at -85°C and the physical and microbiological changes were analyzed on the sampling day. Day 0 samples were tested immediately after the tilapia fish were received and processed in the laboratory. Six organisms for sampling were taken.

**ATP, related compounds and K-value:** Determinations of nucleotides and related compounds were determined by HPLC (Ryder, 1985). Identification of nucleotides, nucleosides and bases were made by comparing their retention time with those of commercial standards and by adding or spiking of standards. The K-value was calculated as the percent rate of HxR and Hx to the sum of ATP and degradation products, where:

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K(\%) = \frac{[(HxR+Hx)/(ATP+ADP+AMP+IMP+HxR+Hx)]}{100}
\]

**TVB-N and pH:** TVB-N and pH were determined by previously described methods (Woyewoda et al., 1986).

**Microbiological analysis:** Samples (10 g) of tilapia fish muscle were taken aseptically from the fish muscle and homogenized in 90 mL of peptone solution (1 g L⁻¹). Serial dilutions of homogenates were made and Total Viable Count (TVC) were determined by the pour plate method, using Plate Count Agar (Difco, ref. 218071) as described in Mexican Official Norm. Plates were incubated at 35±2°C for 48±2 h and TVC were quantified according to the methodology described by Mexican Official Norm.

**Color:** Changes in color of the muscle were determined with a tri-stimulus colorimeter (Model CR-300, Minolta, New York). Measurements were taken of the surface of the muscle.

**Texture:** To measure texture of the muscle, shear force was measured with a Warner-Bratzler blade in a universal testing machine (Model 1130, Instron, Canton, MA) equipped with a 50 kg weight. Speed was set at 20 cm min⁻¹ and shearing force was transversally applied in the direction of the muscle fibers. Standardized cuts (10×10×20 mm) were used and necessary force to shear the muscle was recorded.

**WHC:** Water-holding capacity was measured by a standard method (Cheng et al., 1979). WHC was expressed as “loss of water”, which was the weight loss by the sample compared to the initial weight (%).

**Statistical analysis:** Descriptive statistics (mean, standard deviation), one-way ANOVA and linear regression analysis were applied. Analyses were performed with the NCSS 2000 statistics software (NCSS, Kaysville, UT). Significance was set at p<0.05.

**RESULTS AND DISCUSSION**
**ATP, related compounds and K-value:** One of the most important postmortem biochemical changes in the muscle is ATP degradation which along has been widely studied and applied to monitor freshness and shelf life of fish muscle (Vazquez-Ortiz et al., 1997). Figure 1 shows the
changes of ATP and related products in tilapia fish muscle stored 18 days in ice. The mean Total Molar Concentration (TMC) for ATP and derivatives was 4.61±0.31 μmol g⁻¹. This value is lower than that reported by Murata and Sakaguchi (1986) (9.30 μmol g⁻¹) and by Massa et al. (2005) (12.5 μmol g⁻¹) for yellowtail (Seriola quinqueradiata) and for flounder (Paralichthys patagonicus), respectively. Variations in TMC have been associated to differences in species, season, physiological condition, feeding, among other variables (Ocaño-Higuera et al., 2006).

With respect to ATP concentration at the beginning of storage, it was found to have an initial value of 0.08±0.01 μmol g⁻¹, similar values were reported by our group (Ocaño-Higuera et al., 2009, 2011) for cazón and ray fish muscle with values of 0.15 and 0.10 μmol g⁻¹, respectively. Literature reports that ATP is depleted within the first 24 h postmortem (Haard, 1992); however, initial low ATP values found in the tilapia fish muscle could be mainly attributed to the energy consumption for the struggle in the net during harvest. Same behavior was previously reported in sierra (Scomberomorus sierra) by Castillo-Yanez et al. (2007). On the other hand, in freshwater fish, Bosworth et al. (2007) reported a higher initial values of ATP (3.9 μmol g⁻¹) for rested channel catfish Ictalurus punctatus, however, they reported a value of 1.10 μmol g⁻¹ of initial ATP when specimens were stressed for 45 min in low-water tanks.

The predominant nucleotide in the tilapia fish muscle after the harvest was the IMP with an initial value of 3.76±0.34 μmol g⁻¹. The high concentration of IMP in this study indicated a rapid degradation of ATP into IMP. Because of the rapid disappearance of ATP in the muscle, levels of ATP, Adenosine-Diphosphate (ADP) and Adenosine-Monophosphate (AMP), did not show significant changes with regard to the storage time (p>0.05). Furthermore, it was found that the IMP concentration decreased in accordance with the equation y = -0.23x+4.73 with a r² value of 0.85 (p<0.05); such degradation is similar to that usually shown by other species where it is linearly presented, correlating quite well with the freshness reduction and the sensorial acceptability (Howgate, 2006). The initial IMP value obtained in the present study is lower to that reported by Ozogul et al. (2009) for catfish (12.6 μmol g⁻¹). In another study Ozyurt et al. (2007) reported 2.4, 4.1 and 16.7 μmol g⁻¹ for pike perch caught by gillnet, longline and harpoon, respectively. Several authors have suggested that the IMP content is an adequate freshness indicator, since is the main responsible for desirable odor and taste in fresh fish (Woyewoda et al., 1986; Huss, 1995).
During the storage in ice the HxR concentrations were not significantly increased (p>0.05). Figure 1 shows that tilapia fish is a slow inosine-producer species, since highest values of 0.84 μmol g⁻¹ were obtained on day 12 of the storage time. On the other hand, unlike HxR, Hx concentrations were significantly increased \([y = 0.21x+0.99, r^2 = 0.85 (p<0.05)]\) with values of 4.95 μmol g⁻¹ until the last day of storage. Ocaño-Higuera et al. (2009) reported values of 2.00 and 1.09 μmol g⁻¹ until the 18th day of storage, for HxR and Hx, respectively in cazon fish muscle. Postmortem Hx formation in fish muscle indicates the onset of autolytic spoilage and of microbiological alterations (Woyewoda et al., 1986) which has been related with the loss of freshness and taste in some species (Cox and Karahadian, 1998).

Sometimes, IMP degradation or HxR and/or Hx accumulation prove to be a good indicator of freshness reduction not only in marine species but also in freshwater fishes. In fact, these metabolites have been reported for monitoring quality and freshness in freshwater fish, such as pike perch (Ozyurt et al., 2007) and catfish (Ozogul et al., 2009).

All above mentioned effects are due to the linearity of how these compounds are degraded or formed. In this study, only IMP degradation and Hx accumulation could be used as freshness indicators due to the good correlation of their formation regarding the storage time.

Quantification of ATP concentration and its degradation products until Hx were used as the basis to calculate the K-value or freshness index which is defined as the ratio (x100) of non-phosphorylated ATP breakdown products to the total ATP breakdown products which has been used as a freshness measure in many species (Ehira and Uchiyama, 1987). Figure 2 shows a significant and linear increase \([y = 4.16x+14.42, r^2 = 0.99 (p<0.05)]\) in K-value of tilapia fish muscle during the storage period, from a value of 11.61% (day 0) to a final value of 86.46% (day 18).

Saito et al. (1959), described to the fishery products with K-values lower than 20% as very fresh ones, less than 50% as moderately fresh and higher than 70% as not fresh. Based on these K-value categories, the tilapia fish muscle under the experimental conditions of this study can be considered moderately until day 9 (K-value = 54.83±3.02%) and borderline for freshness at day 12 (K-value = 63.41±6.44%) of the storage, with a shelf life between 12 and 15 days. This indicator has been extensively used in a variety of freshwater species such as carp (Ieokson et al., 1996), pike perch (Ozyurt et al., 2007), catfish (Ozogul et al., 2009) or marine species as sea bass (Chang et al., 1998), cazon fish, ray fish (Ocaño-Higuera et al., 2009, 2011) and scallops (Ocaño-Higuera et al., 2003; Pacheco-Aguilar et al., 2008). It is important to point out that to use the classification previously described, it is necessary to have in mind that this rate depends on the species, therefore, it must be calculated for each kind of fish.
Fig. 3: Postmortem changes in TVB-N and pH in tilapia fish muscle (*Oreochromis niloticus*) stored in ice for 18 days. Data points are the mean of n = 6 for each sampling day. Bars represent SD.

**TVB-N:** TVB-N analyses have been traditionally used as indicators of quality in fishery products stored in ice. TVB-N is a term that includes measurement of trimethylamine, dimethylamine, ammonia and other compounds associated with seafood spoilage which increases as spoilage progresses. For several fish species, TVB-N values were reported to increase curvilinearly or linearly with time and a level of 30 mg of TVB-N/100 g of muscle has been considered the upper limit above which some fishery products are considered spoiled and unfit for human consumption (Huss, 1988). In this study the average values of TVB-N content significantly increased (p<0.05) from 24.2±1.34 g to 38.37±2.94 mg per 100 g of muscle at day 0 and 18 days of storage in ice, respectively (Fig. 3). This increase may be due to ammonia production in the muscle during storage, due to the fact that TVB-N levels still rise as a result of the NH₃ formation and other volatile amines (Mazorra-Manzano et al., 2000). According to our results we can consider that after 15 days of storage in ice, the tilapia fish muscle was not apt for human consumption since at this time it reached a value of 34.01±1.16 mg of TVB-N/100 g of muscle. These results agree with the total viable counts as discussed later.

The total volatile bases have been used successfully depending on the species and storage conditions as reported in several studies for catfish stored at 4°C (Chomnawang et al., 2007) and for cazon fish and scallops stored in ice (Ocaño-Higuera et al., 2006, 2009; Pacheco-Aguilar et al., 2008). However, has been reported that this indicator did not show a good correlation with the deterioration in some iced stored fish as carp (Scherer et al., 2006), pike perch (Ozyurt et al., 2007) and catfish (Ozogul et al., 2009). They explain that this is due to freshwater fish have a low content of TMAO and the latter product (TMA-N) is quantified into the TBV-N measurements.

**pH:** Figure 3 shows pH changes of the tilapia fish muscle during a period of storage in ice of 18 days. At day 0 pH was 6.61±0.07, a similar value has been reported by Love (1976) for fishery products after being caught which is found between values 6.70 and 7.00. Even more is higher than the 6.43, 6.24 and 6.41 reported by Ocaño-Higuera et al. (2009), Pastoriza and Sampedro (1994) and Chomnawang et al. (2007) for cazon fish, ray fish (*Raja clavata*) and freshwater catfish, respectively at the initial time of storage in ice. However, is lower than the 6.9 reported by Kristoffersen et al. (2006) for atlantic cod (*Gadus morhua* L.) and 6.8 by Ozyurt et al. (2007) for pike perch. Variations among the initial pH values may be due to the
Fig. 4: Changes of bacterial Total Viable Count (TVC) in tilapia fish muscle (*Oreochromis niloticus*) stored in ice for 18 days. Data points are the mean of n = 2 for each sampling day. Bars represent SD.

species, season, diet, level of activity or stress during the catch as well as type of muscle. Bosworth *et al.* (2007) reported differences in initial values of pH in channel catfish depending of catch season and the stress level prior to slaughter.

In the same Fig. 3, it can be noticed that pH showed a significant increased (p<0.05) until day 18, reaching a value of 7.33±0.06. According to this parameter and considering that the value of pH = 7 correlates with the spoilage, we can suggest that the tilapia fish muscle preserved an edible quality at least 12 days stored in ice. These results agree with the TVB-N.

**Microbiological analyses:** In several studies has been reported that the TVC is the best index of quality, as this represents the main spoilage cause of fish stored in ice (Shewan and Ehrenberg, 1977; Gram, 1992; Huss, 1997). In agreement to these studies, the Mexican Official Norm and the International Commission on Microbial Specifications for Foods (ICMSF., 1978) report values of $10^7$ (7 log CFU g$^{-1}$ of muscle) as recommended maximum limits for refrigerated fresh fish. As shown in Fig. 4, in this study the set limited at day 18 was reached, therefore and in spite of the fact that the results of TVB-N and pH indicated at least a shelf life of 12 days, the results of the microbiological analysis established a shelf life of at least 15 days in tilapia fish muscle during the storage in ice.

Previously, the shelf life of freshwater fishery products based on TVC has been reported for iced stored carp and channel catfish, where limit for human consumption of 13-16 and 7 days, respectively was established. In these studies a limit of acceptability for human consumption of 7 log CFU g$^{-1}$ of muscle also as reference was used.

**Determination of color:** Ocañó-Higuera *et al.* (2009) reported that color is one of the most important parameters used to evaluate the quality of fishery products. Haard (1992) reported that the initial color of fishery products changed during storage in ice, affecting the quality. At the same time, it determines the acceptance of the product by the consumer. Surface color parameters for the tilapia fish muscle are shown in Fig. 5, where it can be noted that initial values of “L”, “a” and “b” were 54.59±2.23, 10.11±1.73 and 10.20±1.01, respectively which placed the product within the yellow-red quadrant with a “hue” angle of 45.25, indicating that muscle has an intermediate initial
Fig. 5: Postmortem changes in color parameters in tilapia fish muscle (*Oreochromis niloticus*) stored in ice for 18 days. Data points are the mean of n = 6 for each sampling day. Bars represent SD.

Fig. 5: Postmortem changes in texture (shear force) and Water-Holding Capacity (WHC) in tilapia fish muscle (*Oreochromis niloticus*) stored in ice for 18 days. Data points are the mean of n = 6 for each sampling day. Bars represent SD.

color towards yellow-red. Since "a" and "b" values were low, the product can be considered opaque because is localized in the grey zone of the color sphere. With respect to storage time, the color parameters of tilapia fish muscle did not show significant differences (p>0.05).

**Texture and WHC:** The loss of texture during storage of fishery products has been reported by Sato *et al.* (1991). Whilst several researchers have associated low muscle pH with tough texture and high drip loss (Mattio *et al.*, 2001), others suggest the involvement of several enzymes in texture deterioration during storage (Sato *et al.*, 1991). Several publications have indicated that after the rigor mortis, the muscle tissue progressively loses its firmness (Azam *et al.*, 1989; Montero and Borderias, 1990; Andersen *et al.*, 1997), process mainly related with enzymatic degradation of muscle proteins (Papa *et al.*, 1997). In our study, the initial shear force value was 2.78±0.66 kg and no significant (p>0.05) differences were obtained for texture measurement in the tilapia fish muscle during the 18-days storage period (Fig. 6). Likewise, no significant difference (p>0.05) was obtained for WHC during the storage period. An initial value of 92% was maintained during the study. WHC has been reported to be a good indicator for fish quality evaluation because a decrement in WHC had been shown to result in texture loss (Chen *et al.*, 1990). However, our
results suggested that denaturation (aggregation and/or hydrolysis) of muscle proteins during the storage period was negligible with no effect on muscle, WHC or texture.

CONCLUSION

Postmortem changes in tilapia fish muscle indicated that endogenous and microbial processes could be controlled with appropriate post-capture handling practices. Since K-value was linearly increased with respect to the storage time, this can be used as a good indicator for monitoring the loss of the muscle freshness whilst kept in ice. Furthermore, it was notable that microbial deterioration processes influenced the fish spoilage during iced storage which was supported by TVC results. Regarding texture, WHC and color, no changes during storage time was observed. Overall results indicated that the tilapia fish muscle in iced storage could hold its shelf life at least 15 days under our experimental conditions, hence this procedure is recommended for applying in commercial handling operations.

REFERENCES


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