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Quality Improvement and Shelf-Life Extension of Iced Nile Tilapia Fillets Using Natural Garlic Extract

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Abstract: Fish represent one of the most perishable food groups. Therefore, it is important to find viable alternatives that contribute to the preservation of quality and increase the shelf life of fishery products, and one alternative is to use natural extracts with antimicrobial activity. The objective of this study was to determine the effect of a natural extract prepared with garlic (NGE) on the quality and shelf life of tilapia fillets stored on ice for 18 days. For this purpose, NGE was prepared by homogenizing peeled garlic cloves with distilled water, which were then centrifuged to obtain the extract (NGE); then, the fish fillets were immersed in the extract and were coated in NGE. The fillets with NGE were packed in high-density polyethylene bags and stored in crushed ice for 18 days. The adenosine 5'-triphosphate (ATP) and degradation products, K-value, color, texture, water holding capacity, pH, total mesophilic count, and total volatile bases (TVB-N) were monitored during storage. The ATP content, K-value, pH, total microbial count, and TVB-N changed with respect to ice storage time, and the results between fillets with NGE and control fillets differed. In conclusion, the application of NGE increased the shelf life of fillets stored on ice by 6 days, obtaining a shelf life of 18 days on ice, which shows its potential to improve the utilization of the species.

Keywords: extract; garlic; tilapia; freshness; shelf life; quality

Key Contribution: One of the main problems involving seafood is its short shelf life during ice storage. For this reason, food technologists are studying different ways to increase its shelf life. In order to solve this problem, this study looks at the effect of a natural extract prepared with garlic on the quality and shelf life of tilapia fillets stored on ice for 18 days.

1. Introduction

Fisheries and aquaculture represent an important source of income and employment, and their food products are greatly appreciated by consumers worldwide because of their health benefits, flavor, texture, protein content, types, quality of fatty acids (polyunsaturated), and the variety of vitamins and minerals they provide. However, these products

represent one of the most perishable foods [1], and thus preservation methods should be applied to avoid economic losses and possible negative effects on consumers' health [2–5].

Some authors indicate that the freshness and quality of fishery products decrease immediately after capture and death due to the development of a series of post mortem biochemical changes. These changes can be endogenous or exogenous; the former involves food enzymes, while the latter involves the response to microorganisms' growth and metabolism, as well as storage and handling conditions [6,7].

To reduce spoilage and preserve the shelf life of fishery products, various technologies have been used. Currently, in the fishing industry, several traditional and emerging technologies are being used to preserve fishery products. Traditional technologies include refrigeration, freezing, the use of modified and controlled atmospheres, canning, drying, dehydrating, salting, and smoking [7–9]. Moreover, in the case of new technologies, high pressures, irradiation, bio-preservation, active and intelligent packaging [3,10], and antimicrobial compounds [11], among others, can be used.

Nowadays, there is a growing demand from consumers to purchase fishery products with greater freshness and quality. Thus, several technologies have been developed. For example, the use of natural compounds from plants, animals, and microorganisms are some of the most innovative [12] practices, involving natural extracts, essential oils, or derived compounds [13]. These natural compounds may contain secondary metabolites that slow down or inhibit the development of bacteria, yeasts, and molds, and they contain aldehydes, aliphatic alcohols, phenolic compounds, acids, terpenes, ketones, and isoflavonoids, as well as their precursors, including thymol, carvacrol, eugenol, geraniol, and citral, among others. Until now, antimicrobial compounds have been obtained from rosemary, oregano, onion, green tea, tomato plants, and garlic; however, very few studies have evaluated their usefulness in preserving the quality and extending the shelf life of fish products [8,11,14].

Garlic (*Allium sativum*) is a vegetable with medicinal properties that belongs to the Liliaceae family, and it has biological activity due to its anticarcinogenic effect. Garlic stimulates the immune system, reduces cardiovascular diseases, and has antioxidant and antimicrobial properties [14]. Regarding its antimicrobial effect, garlic extract has been reported to have broad antibacterial activity on *Staphylococcus*, *Klebsiella*, *Clostridium*, *Escherichia*, *Proteus*, *Salmonella*, *Mycobacterium*, and *Helicobacter* [15]. In garlic, the compound that provides the antimicrobial activity is allicin, which is a volatile sulfur-containing molecule with a distinctive odor [16]. Additionally, allicin has been found to act as an antiparasitic, antiviral, and antifungal agent. The literature indicates that the antimicrobial activity of garlic extract is also caused by the presence of hydrophobic compounds such as diallyl polysulfide, ajoene, and vinylidithiin [17]. In the case of tilapia, which is one of the main species cultivated worldwide, to our knowledge no reports such as the one proposed in this study are available. Therefore, the objective of this study is to evaluate the effect of a natural extract made from garlic on the freshness, quality, and shelf life of tilapia (*Oreochromis niloticus*) fillets on ice over 18 days of storage.

2. Materials and Methods

2.1. Obtaining Tilapia Specimens

The *Oreochromis niloticus* fillets used in this study were purchased commercially from a fishery product retailer in Hermosillo, Sonora, Mexico (average weight and size of 101.87 ± 14.66 g and 16.0 ± 0.41 cm, respectively). The tilapia fillets were shipped to the Food Research Laboratory at the Universidad de Sonora (UNISON) in Hermosillo, where they were washed with distilled water and ice.

2.2. Preparation and Application of the Natural Garlic Extract (NGE)

The natural garlic extract (NGE) preparation process was carried out as follows: 200 g of peeled garlic was homogenized with 800 mL of distilled water in a Molimex (CDMX, México) blender for 2 min. Subsequently, the NGE obtained was centrifuged in a refrigerated centrifuge (SIGMA, Pasadena, CA, USA) at $9000 \times g$ and 4°C for 30 min. The

supernatant was then collected and cooled to refrigeration temperature (2 °C) prior to its use as a preserving agent. The NGE concentration was of 87.5 g of dry garlic/L of water, and 20 mL were used per kg of fillets (1.75 g of dry garlic per kg of fillets). Its application was carried out as follows: first, the tilapia fillets were washed, drained, and immersed in the NGE for 2 min. Then, they were drained for 5 min, packed in high-density polyethylene bags, and stored in crushed ice for 18 days. At the same time, the tilapia fillets (without NGE) were washed with distilled water, drained, and packed directly into the bags as described above to be used as the control group.

2.3. Ice Storage Study

The fillets—with and without NGE—packed in polyethylene bags were subjected to an 18-day ice storage study that consisted of storing the fillets described above in an airtight cooler in alternating beds of ice–fillet–ice. During this time, the determinations of ATP and degradation products, K-value, color, pH, texture, WHC, as well as the total mesophilic count and TVB-N were evaluated at 0, 3, 6, 9, 12, 15, and 18 days of storage. It is important to note that ice was replaced daily and four fillets per sampling day were analyzed for the NGE and control batches, respectively. The analyses performed are described below.

2.4. Quality Determinations

2.4.1. Adenosine 5'-Triphosphate (ATP) and Degradation Products

The determination of the ATP and related compound concentration was carried out using high performance liquid chromatography (HPLC) according to the technique described by Ryder [18] that consisted of preparing a homogenate with 3 g of tilapia muscle with 15 mL of 0.6 M perchloric acid at 0 °C. Then, the homogenate was centrifuged for 1 min at 18,000 rpm in an Ultra-Turrax T18 Basic (IKA Works Inc., Wilmington, NC, USA) and centrifuged at 0 °C in a refrigerated Thermo Electron Model IEC-MULTI RF centrifuge (Thermo Fisher Scientific, Asheville, NC, USA) at $5500 \times g$ for 10 min. Subsequently, 7 mL of the supernatant was adjusted to pH 6.5–6.8 with 1 M KOH, and allowed to stand for 30 min at 0 °C to precipitate the potassium perchlorate and remove it by filtration using Whatman No. 4 paper. Finally, the quantity of the supernatant was increased to 15 mL using distilled water and stored frozen (−80 °C) until quantification.

To determine ATP concentration and related products using HPLC, 20 µL of the neutralized and diluted supernatant was injected into an Agilent 1200 series chromatograph (Agilent, Ratingen, Germany) using a 4.6 mm inner diameter $\times$ 250 mm long C18 reverse phase column (ODS Ultrasphere, Beckman Instruments, Inc. Fullerton, CA, USA). A buffer of 0.04 M KH_2PO_4 and 0.06 M K_2HPO_4 was used as the mobile phase at a flow rate of 1 mL/min. Nucleotides, nucleosides and nitrogenous base were detected at 254 nm with a diode array detector (Agilent 1200 Series Infinity, Agilent Scientific Instruments, Santa Clara, CA, USA).

2.4.2. K-Value (Freshness Index)

The concentrations of adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), inosine 5'-monophosphate (IMP), inosine (HxR), and hypoxanthine (Hx) were used to calculate the K-value. For this purpose, the equation reported by Sagedhal et al. [19] was used, as follows:

$$\text{K-index (\%)} = ((\text{HxR} + \text{Hx}) / (\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{HxR} + \text{Hx})) \times 100.$$

2.4.3. pH

The measurement of the muscle pH was performed using the methodology proposed by Woyewoda et al. [20]. Two grams of tilapia muscle were homogenized with 18 mL of distilled water in an Ultra-Turrax T18 Basic homogenizer (Wilmington, NC, USA) for 1 min at 18,000 rpm. To measure the pH, the electrode of a potentiometer (Orion 420 A, Thermo Electron Corporation, Waltham, MA, USA) was introduced into the homogenate and calibrated daily.

2.4.4. Color

The color determination of tilapia fillets from the control lot and with NGE was carried out using tristimulus colorimetry using the CIE $L^*a^*b^*$ color space. For this purpose, a colorimeter (HunterLab MiniScan, Resto, VA, USA) was used, where L^* , a^* , and b^* corresponded to lightness, red-green hue, and yellow-blue hue, respectively. The colorimeter was set in reflectance mode with a reading port opening of 0.5 cm and calibrated according to the manufacturer's specifications.

2.4.5. Texture

A texturometer (Shimadzu Model EZ-S, Tokyo, Japan) was used to determine muscle texture. We used a Warner–Bratzler shear cell for this purpose. The maximum shear force (N) required to cut the samples used (dimensions $10 \times 10 \times 20$ mm) was recorded. Thus, the force applied was transverse to the muscle fibers, using a 50 kg compression cell with a head speed of 20 cm/min for the measurement.

2.4.6. Water Holding Capacity (WHC)

The WHC of tilapia fillets with NGE and the control was calculated using the technique reported by Cheng et al. [21]. Two grams of fillet was taken and placed in 50 mL centrifuge tubes. The sample was centrifuged for 60 min at $7500 \times g$ at 4°C in a refrigerated centrifuge (Thermo Fisher Scientific, Asheville, NC, USA). The WHC was calculated as the amount of water loss with relation to the initial content of the sample. The following equation was used for this determination:

$$\text{WHC (\%)} = 100 - ((W_i - W_f)/W_i \times 100)$$

where W_i = initial weight, and W_f = final weight after decanting the water from the tube and drying the surface of the muscle.

2.4.7. Total Volatile Bases (TVB-N)

The determination of TVB-N was carried out according to the methodology described by Woyewoda et al. [20]. An aliquot of 10 g of each fillet muscle with NGE and the control was transferred to a 1 L ball flask, to which 2 g of magnesium oxide (MgO) and 30 mL of distilled H_2O were added. The mixture was homogenized for 1 min in an Ultra-Turrax (Wilmington, NC, USA). Next, 20 drops of 1-2-3[®] brand edible vegetable oil were added for defoaming purposes. The TVB-N were distilled for 25 min and collected in 15 mL of 2% boric acid to be later titrated with 0.03 N H_2SO_4 ; similarly, a blank was distilled. Finally, the TVB-N obtained were reported as mg N/100 g of the sample.

2.4.8. Aerobic Mesophilic Count

The evaluation of aerobic mesophiles present in tilapia fillets was carried out using the technique described in the Mexican Official Standard “aerobic microorganism plate count” [22]. Ten grams of each fillet with NGE and the control were taken and mixed with 90 mL of 1% (w/v) sterile peptonized water using a previously sterilized conventional blender. Subsequently, five serial dilutions were prepared with 1 mL of the largest dilution and mixed with 9 mL of sterile 1% (w/v) peptone water. Subsequently, 1 mL of each dilution was manually shaken and placed in a sterile Petri dish, and 20 mL of agar plate count (APC) was poured into the dish. The agar and inoculum were homogenized using the technique described by NOM-092-SSA1-1994 [22]. The plates were incubated in an inverted position at $35 \pm 2^\circ\text{C}$ for 48 h. Each of the dilutions was inoculated in triplicate following the same methodology. Finally, between 25 and 250 colony forming units (CFUs) were counted on the dilution. The results were reported as log CFU/g of muscle.

2.4.9. Statistical Analysis

The data obtained were analyzed with classical statistical tools such as the mean and standard deviation. Likewise, an analysis of variance and Tukey's multiple range test were

performed. For this purpose, the statistical program NCSS (Version 6.0.22) (Kaysville, UT, USA) [23] was used. For each determination, four replicates were carried out, except for the microbiological determinations, where only two replicates were used. A significance level of 5% was used to analyze the data.

3. Results

3.1. Effect of NGE on the Post Mortem Biochemistry of Tilapia Muscle

3.1.1. Nucleotide Catabolism

In the muscle of aquatic organisms, ATP degradation represents an important post mortem biochemical change in freshness and quality, where its degradation to Hx has been widely used to monitor freshness in fishery products [24]. Figure 1 shows the average values of ATP concentration and related compounds in tilapia fillets treated with NGE and the control stored for 18 days on ice. The initial ATP values (0.840 ± 0.957 and 1.156 ± 0.794 $\mu\text{mol/g}$) shown in Figure 1 for the fillet with NGE (a) and the control (b) were slightly higher than the 0.26, 0.15, and 0.10 $\mu\text{mol/g}$ of fillet reported by Montoya-Camacho et al. [25], Ocaño-Higuera et al. [26], and Ocaño-Higuera et al. [27] for tilapia (*Oreochromis niloticus*), cazon fish (*Mustelus lunulatus*), and ray fish (*Dayastis brevis*), respectively. However, they were lower than those found in fillets from freshly slaughtered fish (6–12 $\mu\text{mol/g}$), and this can be attributed to the energy expenditure of the organism during collection and slaughter, as well as the time elapsed between slaughter, filleting, and sampling for analysis. Likewise, Figure 1 shows that IMP was the nucleotide with the highest concentration in tilapia muscle, showing initial values of 4.992 ± 0.762 and 4.914 ± 0.627 $\mu\text{mol/g}$ for fillets with NGE and the control, respectively. However, the IMP values obtained in this study were lower than the concentrations of 7.0 and 11.3 $\mu\text{mol/g}$ obtained in red tail shad (*Brycon cephalus*) and sierra (*Scomberomorus sierra*) muscles by Batista et al. [28] and Castillo-Yáñez et al. [29], respectively. In the case of nucleotides (ADP and AMP), nucleosides (HxR), and the nitrogenous base (Hx), the initial values were less than 1 $\mu\text{mol/g}$ for both the fillets treated with NGE and the control.

With respect to storage time, only IMP and Hx concentrations showed significant changes ($p < 0.05$), where IMP was reduced to 0.811 ± 0.119 and 0.175 ± 0.172 $\mu\text{mol/g}$ for fillets with NGE and the control, respectively, at day 18 of ice storage, while Hx increased ($p < 0.05$) from 0.612 ± 0.173 and 0.548 ± 0.0730 to values of 3.229 ± 0.173 and 4.802 ± 0.768 $\mu\text{mol/g}$ for fillets with NGE and the control, respectively, also at day 18 of ice storage. This behavior is what normally occurs in fish species during ice storage. The literature indicates that AMP and IMP are the compounds responsible for a sweet taste, which is considered a characteristic of fresh fish [24,30], while the formation and accumulation of HxR and Hx are related to a bitter taste, and consequently to a loss of freshness [31]. In this study, Hx production in tilapia fillets was indicative of the onset of autolytic and bacterial spoilage [32]. In this regard, and with respect to the taste and odor of tilapia fillets with NGE, it is important to highlight that an evaluation carried out by the members of the working group, and not by a sensory panel, of fresh and cooked fillets with NGE detected a slight garlic odor and flavor, which rather than generating negative effects on the perception and acceptance of consumers, provided a better odor and flavor. Therefore, it is considered that NGE has potential for use without affecting consumer acceptance.

Regarding the comparison between NGE and the control, significant differences ($p < 0.05$) were detected in Hx concentrations during the 18 days of ice storage, when the concentration of this nucleoside was lower at the end of storage in fillets with NGE compared to the control. As described above, this result can be related to the onset of microbial growth, and this was further corroborated in the determinations of the K-value, TVB-N, and aerobic mesophilic count.