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The Effect of Muicle–Chitosan Edible Coatings on the Physical, Chemical, and Microbiological Quality of Cazon Fish (*Mustelus lunulatus*) Fillets Stored in Ice

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Abstract: Fishery products are highly perishable; therefore, effective preservation strategies are essential to maintain their freshness, quality, and shelf life. One promising approach involves the use of edible coatings formulated with natural extracts, such as muicle (*Justicia spicigera*). This study evaluated the effect of a muicle–chitosan edible coating on the physical, chemical, and microbiological quality of cazon fish (*Mustelus lunulatus*) fillets stored in ice for 18 days. The muicle extract was obtained by macerating dried leaves for 48 h, and its antibacterial activity was subsequently assessed. A control group (C) and three treatments—muicle extract (ME), chitosan (CH), and a combined muicle–chitosan coating (MECH)—were applied and monitored throughout the storage period. Quality parameters, including pH, colour, water-holding capacity (WHC), texture, total volatile basic nitrogen (TVB-N), and mesophilic microbial counts, were evaluated. The muicle extract exhibited antibacterial activity, with MIC and IC_{50} values of 3.01 ± 0.73 and 204.56 ± 20.23 µg/mL against *Shewanella putrefaciens*, and 0.10 ± 0.07 and 118.09 ± 14.51 µg/mL against *Listeria monocytogenes*, respectively. Treatments of ME, CH, and MECH significantly improved ($p < 0.05$) the quality of fillets by reducing TVB-N, pH, and microbial load compared to the control. In conclusion, the muicle extract demonstrated antibacterial potential and, either alone or in combination with chitosan, effectively preserved the physical, chemical, and microbiological quality of cazon fillets during ice storage.

Keywords: *Justiciera spicigera*; edible coating; cazon fish; quality; antibacterial activity

1. Introduction

Fishery products are among the most perishable food commodities, primarily due to postmortem physical, chemical, and microbiological changes in muscle tissue. These

changes are closely associated with the fish's chemical composition, muscle structure, enzymatic activity, microbial load, and both capture and post-capture handling conditions [1]. It is well established that immediately after death, fish undergo a rapid decline in freshness and quality, initially driven by endogenous enzymatic reactions and subsequently by microbial enzymatic activity [2]. Under optimal harvesting and post-harvest conditions, the shelf life of fish stored in ice typically ranges from 15 to 18 days; however, improper handling practices can significantly shorten this period by accelerating spoilage through temperature abuse and enhanced microbial growth [1,3].

One species of economic importance in Mexico is cazon (*Mustelus lunulatus*), with a reported production of 9808 tons in 2022 [4]. Belonging to the Triakidae family and classified as an elasmobranch, cazon resembles small sharks, reaching lengths of approximately 125 cm and lifespans of up to 10 years [5]. This species is found in the Gulf of California and along the Pacific coast of Mexico [6]. It is typically caught by artisanal fishers using small boats with outboard motors [7], often under suboptimal harvesting and slaughter conditions. Such inadequate handling practices significantly compromise freshness and quality, negatively affecting the fillet's physical, chemical, microbiological, and sensory attributes [1,8] and ultimately posing health risks to consumers and causing economic losses for both producers and processors.

Conventional preservation methods include moisture reduction (drying and salting), temperature control (refrigeration and freezing), and heat treatments (pasteurization, sterilization, and smoking, canning). However, recent advancements have focused on innovative alternatives aimed at preserving freshness and extending shelf life, such as high-pressure processing [9], pulsed electric fields [10], and active or intelligent packaging systems [11]. Of particular interest are natural extracts and essential oils derived from herbs, spices, and plants [12–16], which exhibit antimicrobial and antioxidant properties. These compounds can be applied independently or incorporated into polymer-based edible coatings that serve as physical and biochemical barriers against spoilage [17].

Recent studies have demonstrated the effectiveness of edible coatings in preserving fish quality. For instance, Ramírez-Guerra et al. [18] reported that an edible coating composed of tomato plant extract and chitosan extended the shelf life of sierra (*Scomberomorus sierra*) fillets stored on ice for six days. Similarly, Xiong et al. [19] showed that a coating formulated with salmon bone gelatin, chitosan, gallic acid, and clove oil extended the shelf life of salmon fillets by five days at 4 °C. In another study, Lee et al. [20] found that chitosan–ascorbic acid coating prolonged the shelf life of tilapia fillets by nine days compared to the controls. These findings underscore the potential of natural polymer-based coatings as effective strategies for maintaining quality and extending the shelf life of fishery products.

Among the various plant sources of bioactive compounds, muicle (*Justicia spicigera*) has gained attention due to its reported medicinal properties, including antidiabetic, anti-inflammatory, antiproliferative, antiurolithic, neuroprotective, and hypolipidemic effects [21]. The leaves and flowers of muicle, particularly in ethanolic extracts, are rich in phenolic compounds that exhibit antioxidant, antimicrobial, and antitumor activities [22]. These extracts have demonstrated efficacy against *Shigella flexneri*, *Salmonella typhi*, *Salmonella typhimurium*, *Escherichia coli*, and *Staphylococcus aureus* [21]. Chitosan, a biopolymer primarily derived from crustacean shells via the alkaline deacetylation of chitin [23], is also widely recognized for its antimicrobial and antioxidant properties [24]. Therefore, the aim of this study was to evaluate the effect of an edible coating composed of aqueous muicle extract and chitosan on the physical, chemical, and microbiological quality of cazon fillets during 18 days of ice storage.

2. Materials and Methods

2.1. Specimen Collection

Cazon fish specimens were obtained from artisanal fishermen operating in the Gulf of California, near the coast of Bahía de Kino, Sonora, Mexico. The freshly caught fish, with an average weight of 0.7 kg, were immediately stored in airtight coolers with alternating layers of fish and ice and transported to the Food Research Laboratory (LIA) at the Department of Chemical-Biological Sciences, University of Sonora, in Hermosillo, Sonora, Mexico. The time between capture and arrival at the laboratory did not exceed six hours. Upon arrival, the fish were filleted and rinsed with a chilled mixture of distilled water and ice. The fillets were then randomly assigned to four experimental groups: a control group (C; no treatment) and three treatment groups—the muicle extract (ME), chitosan (CH), and muicle–chitosan combination (MECH) groups.

2.2. Extract Preparation

Muicle extract was prepared following the method described by Burkhard et al. [25]. Muicle plants were acquired in Hermosillo, Sonora, Mexico, and cultivated for four months to allow for leaf growth. The harvested leaves were washed and dehydrated using a convection oven (Thermo Scientific, San Luis Potosí, Mexico) at 45 °C for 8 h. A total of 20 g of dried, crushed leaves were placed into a 1 L Erlenmeyer flask containing 400 mL of distilled water at 60 °C and were allowed to macerate for 48 h at room temperature under dark conditions. The resulting mixture was filtered using Whatman No. 4 paper and a Büchner funnel under vacuum. The filtrate was lyophilized in a freeze dryer (Labconco Corporation, Kansas City, MO, USA) for four days and stored at −20 °C (Torrey, Zapopan, Jalisco, Mexico) until use.

2.3. Evaluation of Antibacterial Activity

2.3.1. Bacterial Activation and Culture Preparation

The antibacterial activity of the muicle extract was evaluated against *S. putrefaciens* (ATCC 8071) and *L. monocytogenes* (ATCC 181115) following the method described by Esparza-Espinoza et al. [26], with slight modifications. Bacterial inocula were prepared by transferring colonies from pure cultures into Mueller–Hinton broth (pH 7.2) and incubating them at 35–37 °C for 18–24 h in a Thermo Electron incubator (LED GmbH, Langenselbold, Germany). The optical density (OD) of the cultures was measured at 600 nm using a double-beam spectrophotometer (Agilent Technologies, Penang, Malaysia) and adjusted to a 0.5 McFarland standard, corresponding to approximately 10^8 CFU/mL. A 1.58 mL aliquot of the adjusted bacterial suspension was subsequently diluted with Mueller–Hinton broth to achieve a final concentration of approximately 10^6 CFU/mL for antibacterial testing.

2.3.2. Determination of Minimum Inhibitory Concentration (MIC) and IC_{50}

MIC values were determined according to Esparza-Espinoza et al. [26]. Five concentrations of muicle extract (14–230 µg/mL) were prepared in distilled water and diluted 1:10 with BHI broth, adjusting the bacterial concentration to 10^6 CFU/mL. A 96-well microplate was loaded with 150 µL of the extract and 50 µL of bacterial suspension (10^8 CFU/mL). After 24 h of incubation at 37 °C, absorbance was read using a Multiskan GO microplate reader (Thermo Scientific, New York, NY, USA). MIC and IC_{50} values were calculated using probit regression analysis in NCSS version 24.0.1.

2.4. Determination of Phytochemical Compounds

2.4.1. Total Phenolic Content

The total phenolic content was determined using the Folin–Ciocalteu method described by Silva-Beltrán et al. [27], with slight modifications. In a microplate, 10 µL of extract was combined with 25 µL of the Folin–Ciocalteu reagent (1 N), 25 µL of 20% Na_2CO_3, and 140 µL of distilled water. After 30 min in the dark, absorbance was measured at 760 nm using a Multiskan GO microplate reader. The results were expressed in mg gallic acid equivalents (mg GAE).

2.4.2. Total Flavonoid Content

Total flavonoids were quantified according to Silva-Beltrán et al. [27], with modifications. In a microplate, 80 µL of the muicle extract was mixed with 80 µL of 2% aluminum trichloride solution. The mixture was incubated for 60 min in darkness, and absorbance was recorded at 415 nm. The results were expressed in mg quercetin equivalents (mg QE).

2.5. Preparation and Application of Treatments and Control

Chitosan solution (CH) was prepared by dissolving 1% (w/v) chitosan in 1% (v/v) acetic acid containing 0.5% (v/v) glycerol as the plasticizer. The mixture was homogenized at 36,000 rpm for 3 min. For MECH (edible coating), the lyophilized muicle extract was added to the chitosan solution at 0.3% (w/v). The ME solution was prepared by dissolving muicle extract in water at 0.3% (w/v). The treatments described above were compared with a control (C) (without CH or ME). Cazon fillets were immersed in each treatment for 2 min, drained for 5 min, and then individually sealed in high-density polyethylene bags for further analysis.

2.6. Ice Storage Study

Fillets from the C, ME, CH, and MECH groups were stored in hermetically sealed coolers with crushed ice for 18 days. Ice was replaced as needed. Samples were collected on days 0, 3, 6, 9, 12, 15, and 18. Evaluations included shear force, pH, colour, WHC, TVB-N, and aerobic mesophilic counts. All analyses were conducted in quadruplicate per sampling point.

2.7. Analytical Procedures

2.7.1. pH

pH was determined following the method of Woyewoda et al. [28]. A 2 g sample of fish muscle was homogenized with 18 mL of distilled water using an Ultra-Turrax T18 Basic homogenizer (IKA Works Inc., Wilmington, NC, USA) at 18,000 rpm for 1 min. pH was measured using a Hanna Instruments HI97107 pH meter calibrated with standard buffers.

2.7.2. Colour

Colour was measured using a MiniScan HunterLab colorimeter (Preston, VA, USA) operating in reflectance mode with a 0.5 cm aperture. L* (lightness), a* (red–green), and b* (yellow–blue) values were recorded from the dorsal region of the fillet.

2.7.3. Texture

Texture was assessed according to Canizales-Rodríguez [29] using a Shimadzu texture analyzer (Model EZS 346-54909-33) fitted with a Warner–Bratzler shear cell. Fillet samples ($10 \times 10 \times 20$ mm) were oriented parallel to the muscle fibres, and maximum shear force (N) was recorded with a 50 kg load cell at a speed of 100 cm/min.

2.7.4. Water-Holding Capacity (WHC)

WHC was measured following the method of Cheng et al. [30]. Two grams of the sample was centrifuged at 7500× *g* for 30 min at 4 °C using a Thermo IEC MULTI-RF centrifuge (Thermo Fisher Scientific, Asheville, NC, USA). WHC was calculated based on water loss relative to the initial sample weight.

2.7.5. Total Volatile Basic Nitrogen (TVB-N)

TVB-N was determined using the protocol of Woyewoda et al. [28]. Two grams of muscle was blended with 2 g of magnesium oxide and 300 mL of distilled water. The mixture was homogenized with an Ultra-Turrax T18 (Ika Works Inc., Wilmington, NC, USA) and defoamed with 20 drops of vegetable oil. Volatile bases were distilled for 25 min and captured in 15 mL of 2% boric acid, then titrated with 0.05 N sulfuric acid. A blank was processed identically. The results were expressed as mg nitrogen per 100 g of the sample.

2.7.6. Aerobic Mesophilic Count

The microbial load was determined according to the Mexican Official Standard [31] for aerobic plate counts. Ten grams of the sample was blended with 90 mL of sterile 1% peptone water. Five serial dilutions were prepared, and 1 mL from each was plated in triplicate on Plate Count Agar (Oxoid Ltd., Basingstoke, UK). Plates were incubated at 35 ± 2 °C for 48 h, and the results were expressed as colony-forming units per gram (CFU/g).

2.8. Statistical Analysis

Data were analyzed using a completely randomized design. Means and standard deviations were calculated. Antibacterial activity, phytochemical content with antioxidant potential in the muicle extract, and microbiological analyses were each conducted in duplicate. In contrast, all other analytical determinations performed during the ice storage period were carried out in quadruplicate, with four replicates per sampling day and per analytical method. One-way ANOVA was applied, and significant differences were analyzed using Tukey's test with a 5% significance level ($p < 0.05$) using NCSS software version 6.0.22 (NCSS, Statistical Sofware, Kaysville, UT, USA).

3. Results

3.1. Evaluation of Antibacterial Activity and Phytochemical Compounds with Antioxidant Potential in Muicle Extract

3.1.1. Minimum Inhibitory Concentration (MIC) and IC_{50} of Muicle Extract

MIC is a widely used indicator to assess the effectiveness of antibacterial compounds, as it defines the minimum concentration required to inhibit bacterial growth and proliferation. In this study, the MIC of an aqueous extract of muicle was evaluated against *S. putrefaciens* and *L. monocytogenes*, which are two bacteria associated with the deterioration of fish product quality.

Figure 1 shows the inhibitory effect of various concentrations of the muicle aqueous extract on the growth of *S. putrefaciens* and *L. monocytogenes*. At the highest concentration tested (230 µg/mL), inhibition rates of 53.82 ± 5.87% and 57.71 ± 3.35% were observed for *S. putrefaciens* and *L. monocytogenes*, respectively. At the lowest concentration (14 µg/mL), inhibition rates decreased to 11.72 ± 2.03% and 23.77 ± 2.61%, respectively. These results indicate that muicle extract is more effective against *L. monocytogenes*. This observation is consistent with previous reports describing the antibacterial activity of the muicle plant, which is attributed to the presence of phenolic compounds and flavonoids. These phytochemicals are known to disrupt bacterial cell membranes and interfere with key metabolic pathways, ultimately inhibiting bacterial growth and survival [32].