

Article

Effect of Chitosan and Oregano Oil Edible Coatings on the Physicochemical and Microbiological Quality of Bullfrog Legs (*Lithobates catesbeianus*) Stored on Ice

Nathaly Montoya-Camacho ¹, Estefania Guadalupe Valdez-Álvarez ², Víctor Manuel Ocaño-Higuera ³, Enrique Marquez-Rios ¹, Carmen Lizette Del-Toro-Sánchez ¹, José Rogelio Ramos-Enríquez ³, Enrique Wenceslao Coronado-Aceves ⁴, Santiago Valdez-Hurtado ^{5,*} and Dalila Fernanda Canizales-Rodríguez ^{3,*}

¹ Departamento de Investigación y Posgrado en Alimentos, Universidad de Sonora, Blvd. Luis Encinas y Rosales s/n, Hermosillo 83000, Mexico; nathaly.montoya@unison.mx (N.M.-C.); enrique.marquez@unison.mx (E.M.-R.); carmen.deltoro@unison.mx (C.L.D.-T.-S.)

² Ciencia de los Alimentos, Centro de Investigación en Alimentación y Desarrollo, A. C., Hermosillo 83304, Mexico; evaldez225@estudiantes.ciad.mx

³ Departamento de Ciencias Químico Biológicas, Universidad de Sonora, Blvd. Luis Encinas y Rosales s/n, Hermosillo 83000, Mexico; victor.ocano@unison.mx (V.M.O.-H.); rogelio.ramos@unison.mx (J.R.R.-E.)

⁴ Laboratorio de Alta Seguridad (BSL-3), Instituto de Investigaciones Biomédicas (IIBo), Universidad Nacional Autónoma de México, Mexico City 04510, Mexico; wenceslao.coronado@iibiomedicas.unam.mx

⁵ Unidad Académica Navojoa, Universidad Estatal de Sonora, Blvd. Manlio Fabio Beltrones 810, Col. Bugambillas, Navojoa 85875, Mexico

* Correspondence: santiago.valdez@ues.mx (S.V.-H.); dalila.canizales@unison.mx (D.F.C.-R.)

Abstract

The bullfrog (*Lithobates catesbeianus*) is an aquatic species of increasing commercial importance in Latin America; however, its muscles are highly perishable during ice storage. This study evaluated the effect of three edible coating treatments compared to a control (uncoated)—chitosan (CH), oregano oil (OO) and chitosan + oregano oil (CH-OO) on the physicochemical and microbiological quality of bullfrog legs stored on ice for 24 days. The color (CIE L*, a*, b*), texture (N), water-holding capacity (WHC), pH, total volatile basic nitrogen (TVBN), and mesophilic and psychrotrophic bacterial counts were measured every three days. Compared to the control treatment on day 24, the CH-OO treatment most significantly delayed color changes (3.30) and affected the textural properties (21.29 to 12.28 N). The OO treatment moderated the pH increase (6.2 to 6.65) and controlled TVBN accumulation (13.91 to 29.37 mg N/100 g), while the CH treatment impacted the WHC. However, no treatment was effective in inhibiting bacterial growth, except for the OO treatment on day 9 for mesophilic control, although this effect was not sustained. These results demonstrate that chitosan, oregano oil and chitosan + oregano oil represent effective natural preservation strategies to extend the commercial shelf life of ice-stored bullfrog muscle, although under these experimental conditions, they did not work for controlling microbiological contamination.

Keywords: bullfrog; *Lithobates catesbeianus*; quality; chitosan; oregano; edible coating; TVBN; microbiological; WHC; ice storage

Academic Editor: Domingo Martínez-Romero

Received: 8 May 2026

Revised: 23 June 2026

Accepted: 26 June 2026

Published: 28 June 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article

distributed under the terms and

conditions of the [Creative Commons](https://creativecommons.org/licenses/by/4.0/)

[Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

The bullfrog (*Lithobates catesbeianus*) is the most widely farmed amphibian species worldwide, with production primarily concentrated in Brazil, China, Mexico, Cuba, and Colombia [1,2]. Its meat is characterized by a high protein content (15–20 g/100 g), low fat levels (0.3–1.0 g/100 g), abundant essential omega 3 and omega 6 fatty acids, and an amino acid profile comparable to other high-biological-value animal proteins [3,4]. These nutritional attributes, combined with its mild flavor and soft texture, position bullfrog meat as an increasingly attractive alternative protein for both local markets and export [5,6].

However, like aquatic food products, bullfrog muscle is highly susceptible to spoilage during postharvest storage due to its elevated water activity ($a_w > 0.98$), near-neutral pH (6.2–6.5), high content of soluble myofibrillar proteins, and limited connective tissue, which provides minimal physical resistance to surface microbial colonization [7,8]. Under ice storage (0–2 °C), recent studies have reported that bullfrog muscle can reach the microbiological acceptability limit of 7 log CFU/g between days 9 and 15 without additional preservation treatments [8], representing a significant limitation for long-distance fresh distribution.

A recent study evaluated changes in the freshness and biochemical, physical, chemical, and microbiological quality attributes of American bullfrog (*L. catesbeianus*) legs during ice storage, reporting progressive increases in the TVBN, pH, bacterial counts, and texture loss from day 0 to day 24. These authors identified psychrotrophic bacterial proliferation as the primary spoilage mechanism under ice-storage conditions, supporting the development of technological interventions specifically targeting this microbial group [8].

Active edible coatings have emerged as one of the most promising preservation technologies for meat and aquatic food products, combining barrier properties with the controlled release of antimicrobial and antioxidant agents at the food surface [9,10]. Unlike active packaging, edible coatings form an integral part of the product and generate no additional waste, making them particularly attractive from a sustainability perspective and aligned with consumer demand for minimally processed additive free foods [11].

Chitosan, a cationic polysaccharide obtained by the deacetylation of chitin from crustacean shells, is one of the most widely studied biopolymers for antimicrobial edible coatings for animal origin products [12,13]. Its antimicrobial mechanism involves electrostatic interactions between protonated amino groups (NH_3^+) and the anionic phospholipids of bacterial cell membranes, leading to membrane disruption, osmotic imbalance, the leakage of intracellular contents, and eventual cell lysis [14]. Chitosan also acts as a metal ion chelator, inhibiting lipid oxidation and contributing to the preservation of color and oxidative stability in meat products [15].

Oregano (*Origanum vulgare* L.) is among the aromatic plants whose extracts have been most extensively documented for antimicrobial and antioxidant potency. Its major phenolic compounds, carvacrol and thymol, act synergistically on bacterial membranes by disrupting the phospholipid bilayer and dissipating the electrochemical proton gradient [16,17]. Aqueous oregano oils also contain rosmarinic acid, chlorogenic acid, luteolin, and apigenin, which have demonstrated capacity to scavenge free radicals and inhibit lipid peroxidation in meat matrices [18]. The combination of chitosan with plant extracts has been validated as a synergistic preservation strategy in various food matrices, including fish fillets, chicken meat, and beef patties [19–21]. However, its specific application to bullfrog muscle has not been previously reported in the literature.

A comprehensive evaluation of bullfrog muscle quality during storage requires the simultaneous measurement of physicochemical parameters (pH, texture, WHC), chemical spoilage indicators (TVBN), microbiological counts (mesophilic and psychrotrophic bacteria), and colorimetric surface characterization using the CIE $L^*a^*b^*$ system [8]. The

TVBN, whose acceptability limit for aquatic food products is set at 35 mg N/100 g [22], reflects the bacterial catabolic activity on nitrogenous muscle compounds [23].

This study aimed to evaluate the effect of the control, chitosan, oregano oil, and chitosan + oregano oil edible coatings on the physicochemical and microbiological quality of bullfrog legs during ice storage.

2. Materials and Methods

2.1. Bullfrog Collection

The bullfrogs [*L. catesbeianus* (Shaw 1802); $n = 79$] used in the present study were obtained from the NutriFrog farm (Mazatlán, Sinaloa, Mexico 23°16'28.3"N 106°12'55.5"W). The specimens included 37 females and 42 males, with average body weights of 169.70 ± 24.85 and 171.62 ± 22.79 g, respectively, and an average age of 13 months. The bullfrogs were transported (for a duration of 4 h) in a container allowing air circulation to Caseros, S. de R. L. M. I (Navjoa, Sonora, Mexico), where they were maintained with an adequate food supply (The Top Choice® brand pellets, with the following information declared on the packaging: protein, 19.5% to 20%; fat, 9%; fiber maximum, 5%; moisture maximum, 12%) and allowed to rest for a period of 4 days to reduce transport-related stress before their sacrifice. The bullfrogs were sacrificed via desensitization by a dry blow to the anterior dorsal area, followed by evisceration, skin removal, and procurement of their legs [24]. The obtained bullfrog legs were washed with potable water, individually placed in high-density polyethylene bags, and arranged in alternating layers of ground ice and bullfrog legs in a hermetic cooler for transport to the Food Research Laboratory of the University of Sonora (Mexico). The time between the sacrifice of the bullfrogs and the reception of the samples at the laboratory did not exceed 12 h.

2.2. Preparation and Application of Treatments

The chitosan (CAS 9012-76-4) and concentrated oregano oil (CAS 8007-11-2) were obtained commercially (75%–85% deacetylated, MW 50–190 kDa, Sigma-Aldrich, Darmstadt, Germany, and MW 300 kDa, Comercial Zazueta, Hermosillo, Mexico, respectively). The samples were randomly assigned to three experimental groups compared to a control: (1) control (uncoated); (2) chitosan (CH); (3) oregano oil (OO) and (4) chitosan + oregano oil (CH-OO). Chitosan (CH) was dissolved at 1% (w/v) in a 1% (v/v) acetic acid solution containing 0.5% (v/v) glycerol as a plasticizer [12]. The mixture was homogenized at 36,000 rpm for 3 min by Ultra-Turrax T18 Basic (IKA Works Inc., Wilminaton, NC). To prepare the chitosan + oregano oil (CH-OO) formulation, 0.2425 g of oregano oil was added to the chitosan solution and homogenized for 1 h. Another 1% chitosan solution was left unchanged. Oregano oil (OO) was used at a concentration of 1% (w/v). The prepared edible coatings were applied to the bullfrog legs by immersion for 2 min, followed by draining for 5 min [19]. The treatments described above were compared with a control (C) (without CH, CH-OO, or OO).

2.3. Ice Storage

All the samples were stored in hermetically sealed coolers in polystyrene bags on crushed ice. The ice was renewed as needed to maintain a constant storage temperature (0–2 °C). The samples were aseptically withdrawn at each sampling point for analysis. A completely randomized design was used with four biological replicates per treatment at each sampling day. The analyses were performed on days 0, 3, 6, 9, 12, 15, 18, 21, and 24 of storage.

2.4. Analytical Procedures

2.4.1. Color Measurement

The surface color was measured with a portable MiniScan HunterLab colorimeter (Reston, Virginia, VA, USA). Prior to data collection, the colorimeter was calibrated according to the manufacturer's guidelines. Color parameters [L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness)] were taken at evenly distributed surface points on each sample. After that, the ΔE was calculated by using the following formula: $\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$ [25].

2.4.2. Texture

The texture of the bullfrog legs was evaluated using a texture analyzer (EZ-S Shimadzu 346-54909-33) (Shimadzu Corp., Model EZ-S, Kyoto, Japan) equipped with a Warner–Bratzler shear cell. A 50 kg compression load cell was used, applying force at a head speed of 20 cm/min. The force was exerted perpendicular to the muscle fibers (cut samples measuring $10 \times 10 \times 20$ mm³). The shear force was measured and the results were expressed as the maximum shear force (N) following the International System of Units (SI) standards [26].

2.4.3. Water-Holding Capacity (WHC)

The WHC was determined by the centrifugation method [27]. An amount of 2 g of bullfrog leg was placed into a 50 mL centrifuge tube and centrifuged in a refrigerated centrifuge (Thermo IEC-MULTI RF, Thermo Fisher Scientific, Asheville, NC, USA) at 19,600 g for 60 min at 4 °C. The WHC was calculated as the ratio of the final to initial sample weight $\times 100$ and expressed as a percentage.

2.4.4. pH Determination

Bullfrog leg samples (2 g) were homogenized in distilled water (18 mL) using the Ultra-Turrax T18 Basic homogenizer (IKA Works Inc., Wilmington, NC, USA) at 18,000 rpm for 1 min. The pH was measured using a glass electrode pH meter (Orion 420 A, Thermo Electron Corporation, Waltham, MA, USA). The equipment was calibrated using standard pH buffer solutions [28].

2.4.5. Total Volatile Basic Nitrogen (TVBN)

The TVBN was determined by the steam distillation method [28]. Briefly, 10 g of bullfrog was mixed with MgO (2 g) and distilled water (300 mL), and homogenized with an Ultra-Turrax T18 Basic homogenizer (IKA Works, Inc., Wilmington, NC, USA) for 1 min. After that, edible vegetable oil (1-2-3® brand, 20 drops) was added as a defoaming agent. The TVB-N content was obtained by steam distilling (25 mL) into a 2% boric acid solution (15 mL). The distillate was titrated with 0.03 N H₂SO₄. The results are expressed as mg N/100 g muscle.

2.4.6. Microbiological Analyses

The total mesophilic aerobic bacteria in the bullfrog leg samples were enumerated on plate count agar (PCA) incubated at $35\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ for 48 h. Psychrotrophic bacteria were counted on PCA incubated at $5\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ for 7–10 days, following the previously reported methods [29]. Finally, a colony count was performed at the dilution corresponding to the presence of 25–250 colony-forming units (CFU). The results are expressed as log CFU/g of bullfrog leg.

2.4.7. Statistical Analysis

The data are presented as the mean $\pm$ standard deviation of four replicates. Simple random sampling and a chi-square test ($p \geq 0.05$) were performed. A one-way ANOVA was applied for each sampling day, followed by Tukey's multiple range test for *post hoc* comparisons ($p < 0.05$). Statistical analyses were conducted using the Statistica software (8.0; Tulsa, OK, USA).

3. Results

3.1. Color

Color is one of the most important parameters employed for evaluating the quality of aquatic food products, and it especially influences consumer acceptance of these products [27]. The control treatment showed a progressive increase in the color difference relative to day 0. Figure 1 shows the changes in the color (ΔE) of bullfrog legs during storage on ice for 24 days.

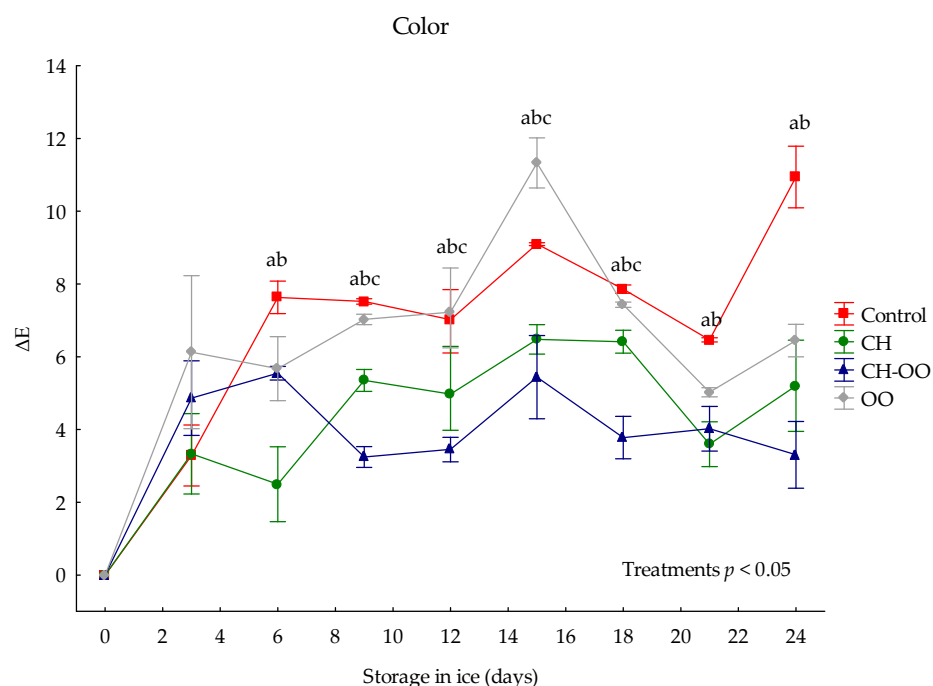


Figure 1. Changes in the color of bullfrog legs (*L. catesbeianus*) during storage on ice for 24 days by three treatments: CH (chitosan); CH-OO (chitosan + oregano oil); and OO (oregano oil). The data points represent the mean values from four samples ($n = 4$) for each day of sampling. Bars represent the standard deviation.

Figure 1 shows the behavior of the treatments on each day of the evaluation. Overall, the treatment that recorded the highest color was the coated OO treatment on day 15 (11.32 ± 0.97) and the one with the lowest was the CH treatment, with 2.49 ± 1.45 on day

6. The ΔE for the uncoated control group increased from 0.00 in the first measurement to 10.94 ± 1.19 on day 24, which was also the highest value recorded. Significant differences ($p < 0.05$) occurred on days 6, 9, 12, 15, 18, 21, and 24. Significant differences ($p < 0.05$) were also observed for the CH treatment on days 9, 12, 15, and 18. Its ΔE reached 5.20 ± 1.77 on day 24, with its maximum value occurring on day 15 (6.48 ± 0.05).

Similar behaviors were observed in the CH-OO- and OO-coated treatments. The CH-OO group reached its maximum value on day 6 (5.55 ± 0.63), reaching 3.30 ± 1.29 on day 24. Significant differences ($p < 0.05$) were observed on days 6 and 12. Finally, the OO treatment showed an increase in ΔE until reaching $6.45 \pm (0.33)$ on day 24, with its maximum value on day 15 (11.33 ± 0.97), showing significant changes ($p < 0.05$) on days 9, 12, 15, 18, 21, and 24. On the other hand, if we consider the overall average of ΔE per treatment during the 24 days—which was 6.65 ± 3.01 , 4.21 ± 2.08 , 3.71 ± 1.66 and 6.35 ± 2.86 for the uncoated control, CH, CH-OO and OO treatments, respectively—it suggests a greater cumulative chromatic alteration in the treatments.

3.2. Texture

Texture, measured as hardness or firmness, is a key quality attribute in aquatic food products, as structural softening resulting from proteolytic activity, moisture redistribution, and myofibrillar degradation is closely associated with freshness loss and reduced consumer acceptability [30]. Figure 2 shows the changes in the texture of bullfrog legs during storage on ice for 24 days.

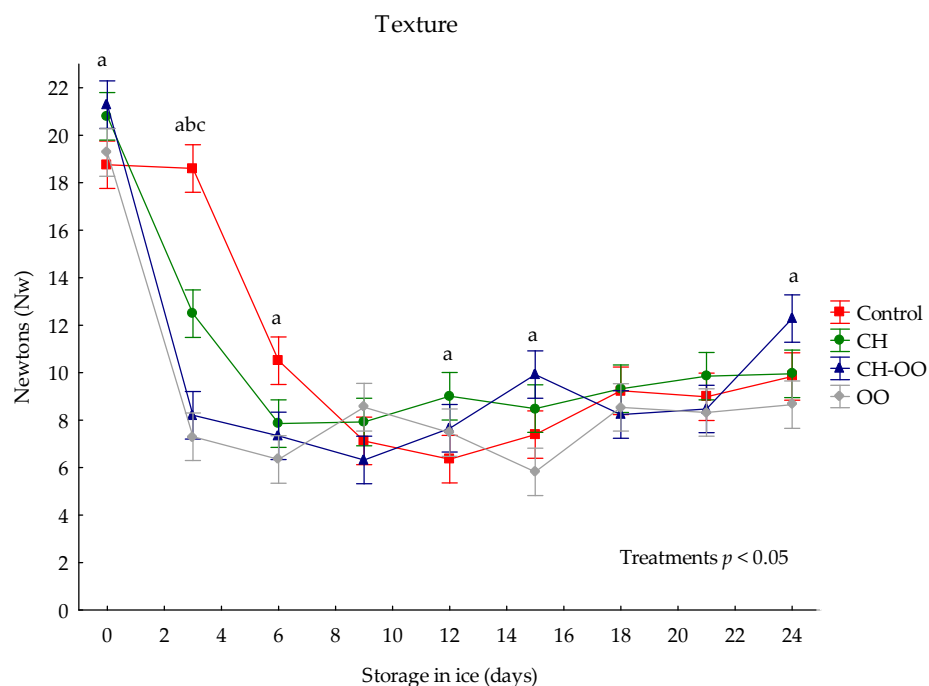


Figure 2. Changes in the texture of the bullfrog legs (*L. catesbeianus*) during storage on ice for 24 days by three treatments: CH (chitosan); CH-OO (chitosan + oregano oil); and OO (oregano oil). The data points represent the mean values from four samples ($n = 4$) for each day of sampling. Bars represent the standard deviation.

It is noteworthy that the dataset shows a sharp drop in the texture parameter between day 0 and day 9, regardless of the treatment. All treatments began with values between 18 and 21 N on day 0 and decreased to a range of 6–10 N (day 9), where they remained relatively stable until the end of the study. At the start of storage (day 0), significant differences ($p < 0.05$) were detected between the control (18.76 ± 0.29 N) and CH-OO ($21.29 \pm$

0.01 N) treatments. By day 3, the control treatment maintained a texture level very similar to that of day 0 (18.60 ± 1.98 N), while the three remaining groups showed a marked decrease: CH (12.49 ± 3.37 N), CH-OO (8.21 ± 1.19 N), and OO (7.30 ± 1.39 N), with significant differences among them. By day 6, only the control treatment showed significant differences. On day 9, no significant differences were detected between the groups, although by day 12, a statistical analysis identified a single comparison [control (6.36 ± 1.53 N) vs. CH (9.01 ± 0.48 N)]. A similar result was observed for day 15 between the CH-OO treatment (9.92 ± 2.19 N) and OO treatment (5.82 ± 0.31 N). No significant differences were observed between the treatments toward the end of storage until day 24, when the CH-OO treatment exhibited significantly greater firmness retention than the OO treatment, with texture values of 12.28 ± 2.75 N and 8.65 ± 0.43 N, respectively.

3.3. Water-Holding Capacity (WHC)

The WHC refers to the ability of proteins to retain water against external forces [31]. Figure 3 shows the changes in the WHC of bullfrog legs during storage on ice for 24 days. On the initial day (day 0), all of the treatments showed high WHC values (96.1%–98.0%), with no significant differences observed. The control group had the highest mean ($98.03 \pm 0.66\%$), followed by the OO, CH, and CH-OO treatments ($97.85 \pm 0.22\%$, $96.59 \pm 0.59\%$, and $96.12 \pm 2.29\%$, respectively). On day 3, the CH-OO treatment showed a significant difference ($p < 0.05$) compared to the other groups ($92.53 \pm 0.98\%$). During day 6, the treatments experienced a decrease in the WHC, with the control treatment registering the lowest value ($89.92 \pm 0.48\%$), while CH-OO had the highest, with no significant differences. As the days of ice storage progressed, significant differences appeared (day 9) between the two combinations with oregano oil, remaining unchanged by day 12. However, on day 15, statistically significant differences again emerged between all the treatments compared to the control group ($88.29 \pm 1.29\%$). At the end of the storage period, between days 18 and 21, the results remained unchanged until the final day (24), when the CH treatment reached its highest value ($97.40 \pm 1.12\%$), with statistically significant differences ($p < 0.05$).

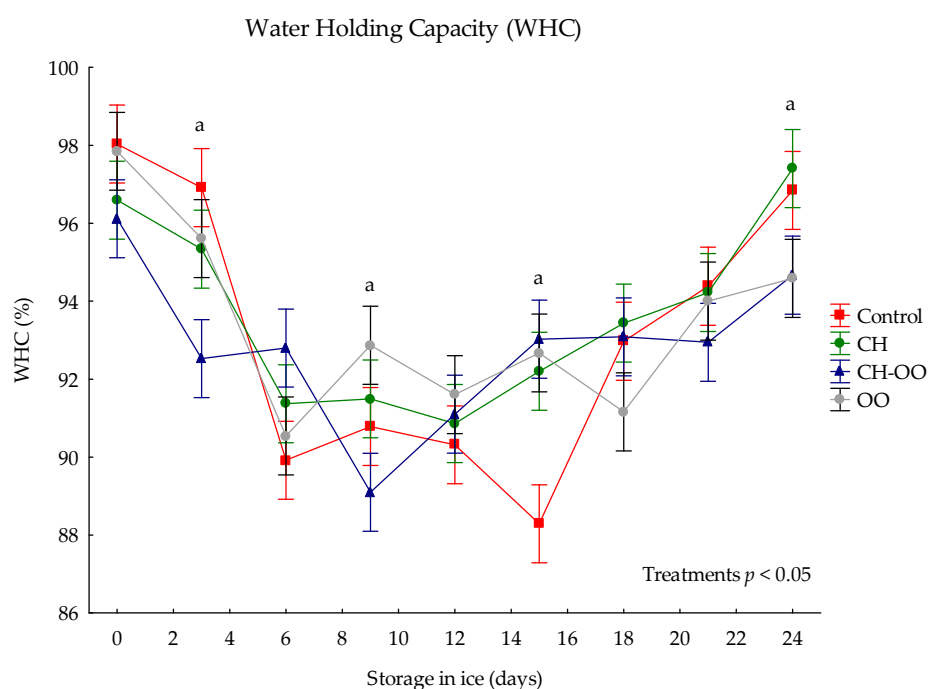


Figure 3. Changes in the water-holding capacity (WHC) of bullfrog legs (*L. catesbeianus*) during storage on ice for 24 days by three treatments: CH (chitosan); CH-OO (chitosan + oregano oil); and OO

(oregano oil). The data points represent the mean values from four samples ($n = 4$) for each day of sampling. Bars represent the standard deviation.

3.4. pH

The pH is a critical indicator of freshness and spoilage progression in muscle food products, as rising pH values reflect the accumulation of basic volatile compounds, produced through the microbial and endogenous enzymatic degradation of proteins and nitrogenous substrates [32].

In this study, the pH of all the treatment groups increased throughout the 24-day storage period (Figure 4). During the first six days, no significant differences were detected between the treatments ($p > 0.05$). The pH values of the three groups ranged between 6.10 and 6.50, reflecting a high initial homogeneity among the samples. The control group displayed the most pronounced and consistent pH increase throughout the 24-day storage period, rising from 6.30 ± 0.08 to 6.90 ± 0.08 (day 0 to 24, respectively). The rise was relatively gradual, but accelerated markedly during the final phase. This acceleration was consistent with the exponential phase of microbial spoilage activity and the corresponding accumulation of alkaline metabolites. The control consistently recorded the highest pH values among all of the treatments, reflecting the absence of an antimicrobial barrier. The CH samples showed a moderate and steady pH increase over the storage period, rising from 6.28 ± 0.05 on day 0 to 6.73 ± 0.05 on day 24. A distinctive feature of the CH treatment was its plateau between days 6 and 9, where the mean pH remained unchanged at 6.425. The CH-OO treatment showed a steady increase, rising from 6.35 ± 0.05 on day 0 to 6.73 ± 0.05 on day 24. The OO treatment showed a steady increase, rising from 6.25 ± 0.05 on day 0 to 6.65 ± 0.05 on day 24. A distinctive feature of the CH treatment was its plateau between days 6 and 9, where the mean pH remained unchanged at 6.425.

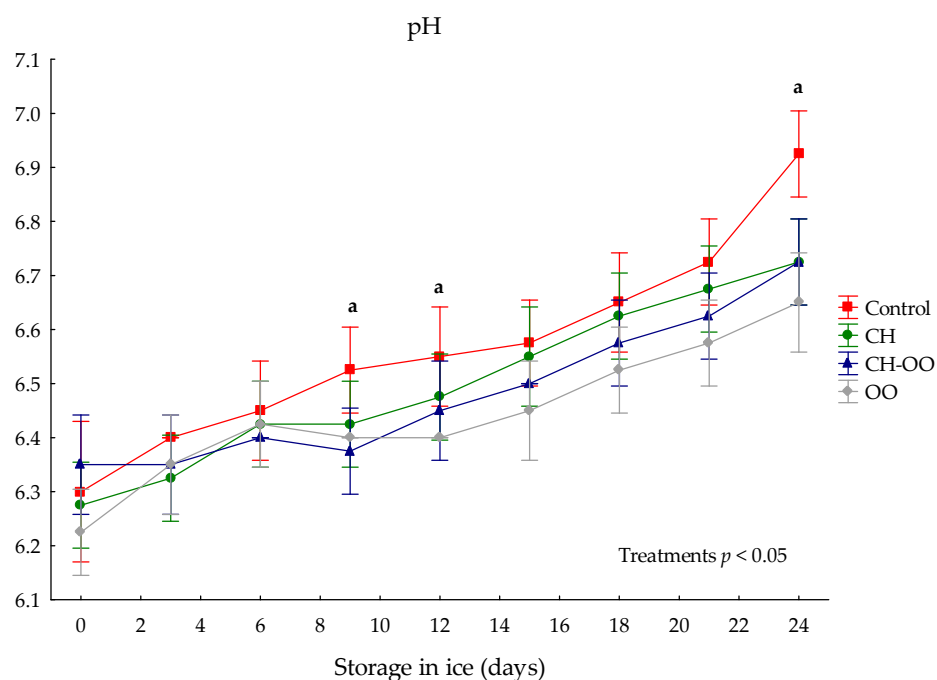


Figure 4. Changes in the pH of bullfrog legs (*L. catesbeianus*) during storage on ice for 24 days by three treatments: CH (chitosan); CH-OO (chitosan + oregano oil); and OO (oregano oil). The data points represent the mean values from four samples ($n = 4$) for each day of sampling. Bars represent the standard deviation.

From day 9 (6.53 ± 0.05), significant differences ($p < 0.05$) began to be observed between the control treatment and the OO group, extending until the next day of analysis (day 12, 6.4 ± 0.00). By day 24, the divergence widened, and the control group (6.90 ± 0.06)

was statistically different from the CH, CH-OO, and OO treatments ($p > 0.05$), with the OO treatment having the lowest pH (6.65 ± 0.06). These characteristics collectively suggest that the combined coating provided the most effective moderation of pH increase among the coated treatments.

3.5. Total Volatile Basic Nitrogen (TVB-N)

The total volatile basic nitrogen (TVBN), expressed in mg N/100 g, is a widely used indicator of protein degradation and freshness loss in muscle foods. TVBN accumulates as a result of microbial and endogenous enzymatic activity that breaks down nitrogenous compounds into volatile bases such as ammonia, trimethylamine, and dimethylamine [33]. Figure 5 shows the behavior of the TVB-N of bullfrog legs stored in ice over 24 days. In all the treatments, a sustained upward trend in the TVBN values was observed over time, consistent with the natural protein decomposition process of the analyzed product. However, the magnitude and trajectory of this increase differed between groups, indicating varying levels of the antimicrobial and antioxidant efficacy of the applied coatings. The analysis showed significant results ($p < 0.05$) on all sampling days except days 0, 3 and 6. The control group starts on day 0 with an average of approximately 13.36 ± 0.29 mg N/100 g and gradually increased to reach values close to 32.99 ± 0.92 mg N/100 g on day 24. The most pronounced increases occurred between days 6 and 9 (17.2 – 20.9 mg N/100 g, respectively) and between days 18 and 21 (26.5 – 29.4 mg N/100 g, respectively).

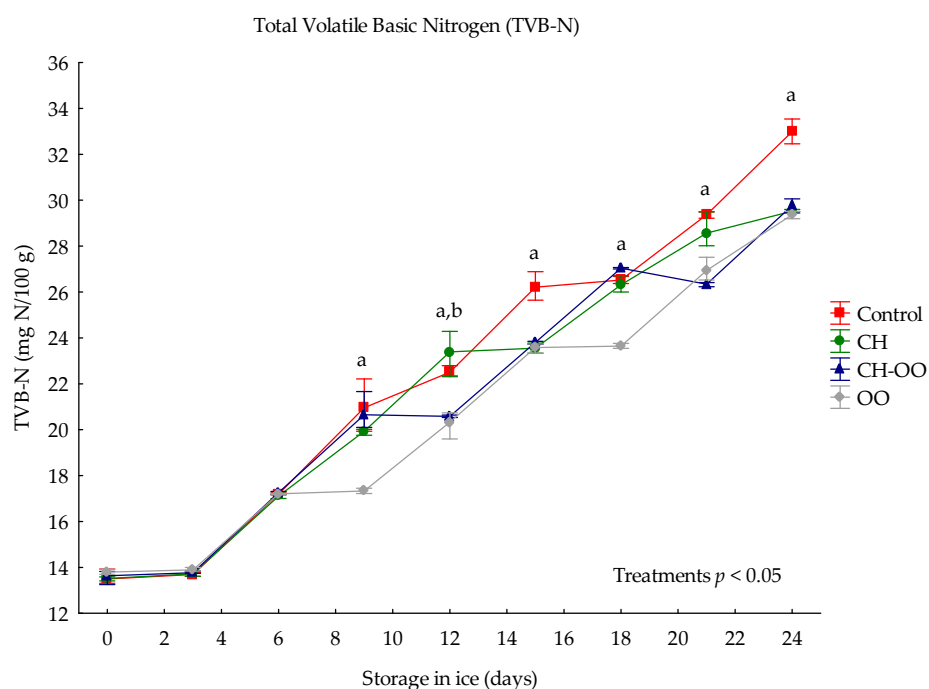


Figure 5. Change in the TVB-N values (mg N/100 g) of bullfrog legs (*L. catesbeianus*) during storage on ice for 24 days by three treatments: CH (chitosan); CH-OO (chitosan + oregano oil); and OO (oregano oil). The data points represent the mean values from four samples ($n = 4$) for each day of sampling. Bars represent the standard deviation.

The CH treatment showed a very similar evolution to the control group during the first stages (days 0–12). From day 15 onwards, it began to diverge, with values slightly lower than the control towards days 21–24, where it reached 28.6 ± 0.17 mg N/100 g compared to 32.99 ± 0.62 mg N/100 g for the control ($p > 0.05$). On the other hand, the CH-OO treatment showed lower values than the control group from day 12 onwards, with the differences becoming progressively more pronounced. On day 15, it reached

approximately 23.8 mg N/100 g, compared to approximately 26.5 mg N/100 g in the control group. Its retarding effect remained constant until day 24, where it registered approximately 29.8 mg N/100 g. This treatment showed significant differences ($p < 0.05$) compared to the control group on days 12, 15, 21, and 24. Finally, the OO treatment showed the greatest divergence from the control, with significant differences every day except days 0, 3, and 6. Day 9 stands out, where OO registered an average of 17.33 ± 0.11 mg N/100 g, considerably lower than the control (20.96 mg N/100 g). Furthermore, from day 12 onward, the OO treatment values remained consistently lower than the other treatments at several points. The results indicate that the evaluated coatings differentially modulate TVBN accumulation. The OO treatment showed the greatest number of time points with significant differences compared to the control, suggesting a sustained inhibitory effect on proteolytic deterioration. The CH-OO treatment also showed significant differences over multiple days, particularly in the second half of the experimental period. In contrast, the CH treatment only showed a significant effect at the end of the storage, indicating a later and smaller effect.

3.6. Total Count of Bacteria

3.6.1. Mesophilic Bacteria

Mesophilic microorganisms, defined by their optimal growth range of approximately 20–45 °C, are important indicators of the overall hygienic quality and general spoilage potential in muscle food products. Although their growth is partially inhibited during cold storage, their enumeration provides a comprehensive index of the total microbial load and the effectiveness of antimicrobial treatments [34]. Figure 6 shows the behavior of the mesophilic bacteria of bullfrog legs during storage on ice for 24 days.

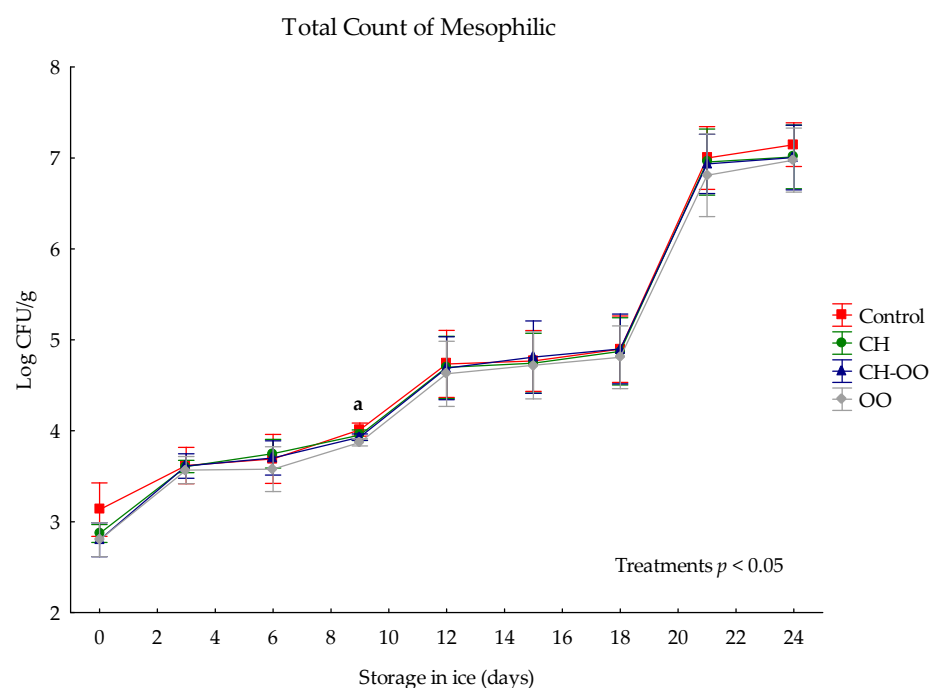


Figure 6. Total count of the mesophilic bacteria (log CFU/g) of bullfrog legs (*L. catesbeianus*) during storage on ice for 24 days by three treatments: CH (chitosan); CH-OO (chitosan + oregano oil); and OO (oregano oil). The data points represent the mean values from four samples ($n = 4$) for each day of sampling. Bars represent the standard deviation.

The total mesophilic bacterial count increased progressively in all three treatments over the 24-day storage period. During the first six days, no significant differences were detected between treatments ($p > 0.05$). The initial counts (2.6–3.0 log CFU/g) reflect the surface-contamination bacterial load typical of fresh meat and were similar across groups.

The absence of differences in this phase indicates that the antimicrobial effect of the coatings was not yet statistically detectable, possibly because the initial bacterial load was low and growth in the initial phase is slow. The treatments with oregano oil (OO, CH-OO) showed values marginally lower than that of the control, but the difference was not sufficient to reach statistical significance. Day 9 was the only storage point at which significant differences were detected between the treatments ($p < 0.05$). The control had a mean count of 4.01 log CFU/g, significantly higher than CH-OO (3.93 log CFU/g) and OO (3.87 log CFU/g). The absolute difference between the treatments was small in magnitude, but highly consistent across replicates with a level of significance ($p < 0.05$). This result indicates that, by day 9, the OO treatment showed significant inhibition of mesophilic growth compared to the control.

Between days 12 and 18, the counts for all treatments stabilized between 4.6 and 4.9 log CFU/g, with no significant differences between groups ($p > 0.05$). The most critical period of storage occurred simultaneously across all treatments between days 18 and 21, with no statistically significant differences among them ($p > 0.05$). On day 21, the counts ranged from 6.36 log CFU/g for the OO treatment to 6.58 log CFU/g for the control, while on day 24, the counts ranged from 6.62 log CFU/g for the OO treatment to 6.85 log CFU/g for the control. The OO and CH-OO treatments tended to have counts that were marginally lower than that of the control at the end of storage. No treatment exceeded the acceptability limit of 7 log CFU/g throughout the entire evaluation period.

3.6.2. Psychrotrophic Bacteria

Psychrotrophic microorganisms are of particular concern in cold-stored aquatic food products, as they are capable of active growth at refrigeration temperatures and are primary contributors to spoilage through proteolytic and lipolytic activity [35].

Figure 7 shows the behavior of the psychrotrophic bacteria of bullfrog legs during storage on ice for 24 days. The statistical analysis did not detect significant differences among the three treatments on any of the days evaluated ($p > 0.05$). The growth curves of the three groups were similar, with all treatments reaching values above 6.7 log CFU/g towards the end of the experiment (days 21–24). However, a numerical trend towards slightly lower counts was observed in the OO and CH-OO treatments in the second half of the period, but these were not sufficient to differentiate them from the control treatment, at least under the experimental conditions of this study.

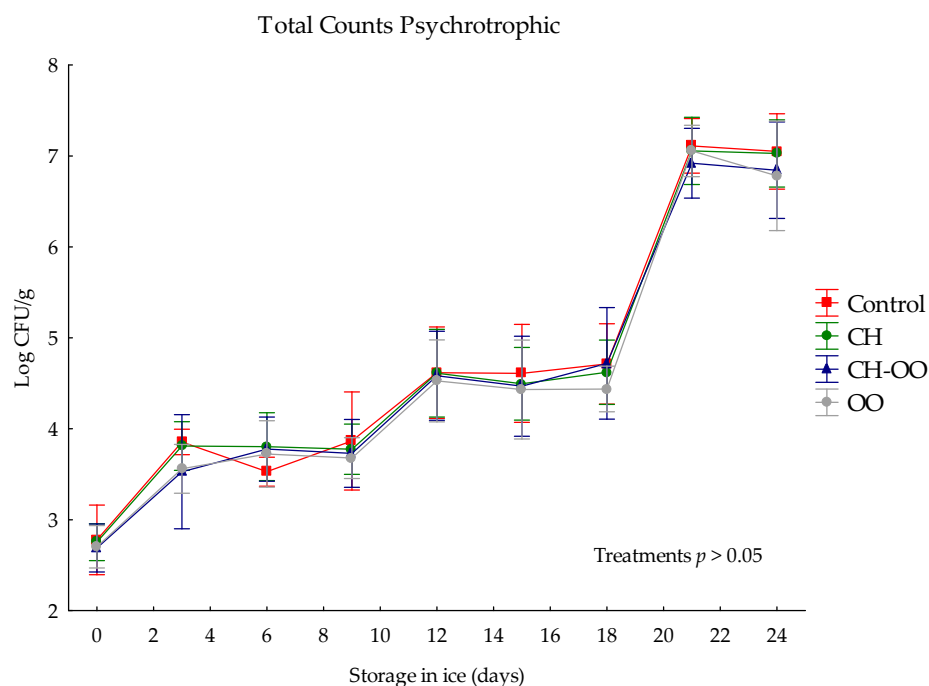


Figure 7. Total count of the psychrotrophic bacteria (log CFU/g) of bullfrog legs (*L. catesbeianus*) during storage on ice for 24 days by three treatments: CH (chitosan); CH-OO (chitosan + oregano oil); and OO (oregano oil). The data points represent the mean values from four samples ($n = 4$) for each day of sampling. Bars represent the standard deviation.

4. Discussion

4.1. Color Measurement

In this study, the uncoated control treatment showed the fastest and most sustained increase in ΔE of all the groups. The first significant differences ($p < 0.05$) appeared from day 6 onward and persisted throughout the evaluation period. This behavior is consistent with reports for muscle from aquatic organisms without surface protection, where direct exposure to oxygen accelerates the oxidation of membrane pigments and lipids, leading to browning and the loss of surface luster from early storage stages. This pattern mirrors observations reported by other studies on bullfrog (*Lithobates catesbeiana*) meat stored in ice [5,8], where untreated control samples underwent the most pronounced sensory and chromatic deterioration over 14 days, linked to the accelerated microbial proliferation of psychrotrophic genera responsible for oxidative pigment modification.

The CH and CH-OO treatments consistently maintained lower ΔE values compared to the control throughout the majority of the storage period. This preservation of color is attributable to the oxygen barrier and polyphenol oxidase (PPO)-inhibiting properties of chitosan, which limit both enzymatic melanosis and non-enzymatic browning reactions at the muscle surface. Similar results were documented for *Litopenaeus vannamei* treated with chitosan nanoliposome coatings [36], where the ΔE values remained significantly lower than those of the controls, a threshold below which discoloration remained perceptible, but commercially tolerable. In the context of fish species, it has been reported that coatings containing oregano oil significantly retarded b^* (yellowness) increases in *Oreochromis niloticus* fillets [37], with uncoated controls showing the highest b^* progression associated with lipid oxidation products, a mechanism equally relevant to frog muscle given its similarly low fat, but high myoglobin, content composition.

The OO treatment showed a behavior similar to the control, with high and significant ΔE values from day 9 onwards, including the second highest value of the experiment on

day 15. This indicates that applying the essential oil without a polymer matrix to retain it on the surface is insufficient to protect the fabric's color. The essential oil is volatile and diffuses or evaporates rapidly, losing its protective effect and leaving the surface exposed to the same oxidative and dehydration processes as the control. This behavior is consistent with findings in *Litopenaeus vannamei* coated with fully deacetylated chitosan combined with clove extract oil [38], where the synergistic formulation most effectively retarded ΔE increases and melanosis development compared to individual treatments or uncoated controls.

4.2. Texture

The initial general decline recorded in all treatments at the start of the study is consistent with the typical post-mortem changes in the muscle of aquatic products during ice storage: the activity of endogenous proteases (calpains, cathepsins) and the formation of ice crystals that damage the myofibrillar structure cause a progressive softening of the tissue, which is usually more intense during the first few days of storage and then attenuates as the substrate available for enzymatic degradation decreases. Previous studies on *Lithobates catesbeiana* [8] reported a progressive decrease in hardness throughout refrigerated storage, while studies on the same species similarly reported data showing that untreated controls deteriorated faster than samples treated with allicin combined with bamboo leaf antioxidants [5], a pattern consistent with the faster early softening observed in the present uncoated control group from day 6 onward.

The significant difference observed on day 0 between the control and CH-OO treatments likely reflects the natural variability among the samples. Day 3 showed the most marked significant differences. In all cases, the control maintained higher texture values than the other treatments. This effect was probably due to the coatings modifying the surface and interface properties of the muscle from the early stages of storage. The lipid components of oregano oil (rich in phenolic compounds such as carvacrol and thymol) can interact with the proteins of the muscle matrix and/or alter the tissue's water retention, producing faster softening in the first hours or days, even though these same compounds exert a protective effect against microbial and oxidative deterioration. This effect trend mirrors findings for *Rana tigrina* meat [39]. The absence of significant differences on days 6 and 9 (between treatments) indicates that, at this stage, the three groups converged toward similar texture values. This is consistent with a scenario in which tissue softening, once the deterioration process has begun, progresses at a comparable rate across all treatments, such that the initial differences related to the coating type become undetectable compared to the biological variability between replicates. This activity has been reported in the degradation of muscle texture in aquatic organisms [40].

On day 12, a higher texture value was observed for the CH treatment, similar to the effects attributed to chitosan coatings in the preservation of aquatic products. However, on day 15, the combined effect of the CH-OO treatment showed higher values, suggesting a possible synergistic effect between chitosan and oregano oil. Conversely, the OO treatment showed the lowest value (which could indicate the homogeneous and advanced degradation of the tissue in the absence of the protective chitosan film). The CH-OO combination showed the joint effect of the physical barrier provided by chitosan and the antimicrobial/antioxidant activity of oregano oil, which together more effectively slowed down the degradation processes than either component separately. This effect seemed to strengthen on day 24. This is consistent with the results reported for *Nemipterus virgatus* fillets [41], where chitosan combined with ϵ -polylysine and carrageenan coatings most effectively maintained the hardness and chewiness over 18 days of refrigerated storage by suppressing microbial proteolysis.

4.3. Water-Holding Capacity (WHC)

The absence of significant differences on day 0 confirms the initial homogeneity of the biological material and the proper standardization of the experiment. However, by day 3, a depressant effect of the CH-OO coating on the WHC was evident, statistically less pronounced than in the other treatments. This behavior could be explained by the action of the phenolic and terpenic components of the essential oil on membrane proteins, altering the structure of myosin and actin in the initial stages of storage, which would temporarily reduce the protein matrix's capacity to retain water [42]. The oregano oil treatment (OO) showed no differences compared to the control or CH on day 3, suggesting that the interaction of the oil with the chitosan matrix is responsible for the effect observed in CH-OO. Chitosan may act as a vehicle for releasing the oil's active compounds, concentrating them on the muscle surface and enhancing their interaction with proteins. The widespread decline observed in all treatments between days 6 and 9 coincides with the logarithmic phase of microbial growth typical of aquatic products stored on ice, where bacterial proteolytic enzymes attack the myofibrillar structure [43]. Despite this overall trend, the coated treatments maintained WHC values comparable to or higher than the control, with the exception of CH-OE on day 9. These findings align closely with those for *Litopenaeus vannamei* [44], which demonstrated that chitosan-based coatings maintained a significantly higher WHC throughout 10 days of refrigerated storage by inhibiting collagenase, cathepsins, and calpain activities, and reducing the formation of TCA-soluble peptides derived from myofibrillar disassembly. On this same day, the behavior of CH-OO suggests that, at this stage, the oregano oil incorporated into the chitosan matrix may have exhausted some of its surface antimicrobial capacity, while the free OO fraction still exerted a more efficient inhibitory effect.

On day 15, the control treatment reached its minimum WHC while CH, CH-OO and OO maintained higher values, corroborating the ability of the three coatings to preserve muscle protein integrity during prolonged storage on ice. From day 18 onward in all treatments, the changes can be interpreted as a tissue water redistribution phenomenon, in which alterations in protein structure generate new, lower-affinity water-binding sites, transiently stabilizing the WRC measured by centrifugation. This recovery pattern has been reported in studies of fish stored on ice and does not necessarily imply a real improvement in protein quality [45]. At the end of the storage period (day 24), the CH treatment reached the highest WHC value, significantly higher than CH-OO and OO. The absence of significant differences between the control and CH treatments at the end of the study further suggests that, in the long term, chitosan not only protects the WHC during critical storage phases, but also allows the muscle to recover water retention levels close to the initial values.

4.4. pH Determination

The progressive increase in pH in all of the treatments was consistent with the physicochemical and microbiological processes typical of food spoilage during storage. This increase can be associated with the production of alkaline compounds derived from proteolysis, amino acid deamination, and microbial activity, processes that generate biogenic amines and ammonia, raising the pH of the food matrix. This progressive alkalization pattern is consistent with what has been described for post mortem aquatic muscle tissue in *Rana esculenta* legs [46], where the pH initially decreases due to post mortem glycolysis and subsequently increases as a consequence of microbial activity and protein degradation, a dynamic that aligns with the gradual increase observed in the present study. The relatively moderate pH values on day 0 are consistent with early post mortem glycolytic acidification in frog muscles. In bullfrog legs [8], an initial similar pH value has been reported, which closely matches the values observed in all three treatment groups at the

start of storage in the present study. The sustained upward trend over 24 days suggests that these mechanisms were progressively activated over time.

The fact that the control group consistently exhibited the highest pH values suggests that the CH, CH-OO, and OO treatments had a moderating effect on pH increase. The OO treatment showed the greatest pH-reducing effect compared to the control, with significant differences maintained compared to days 9, 12 and 24. The CH-OO combination showed intermediate behavior, although without significant differences compared to the control, which could indicate limited synergies or a delay in the release of the active agent. The accumulation of volatile bases such as ammonia, biogenic amines, and trimethylamine, formed during protein hydrolysis and amino acid breakdown by microorganisms or endogenous enzymes, is the most likely cause of the pH rise in stored meat samples. The lower pH values in the chitosan and oregano oil samples are attributable to the antibacterial effect of them [47].

4.5. Total Volatile Basic Nitrogen (TVBN)

Throughout this investigation, all of the experimental treatments presented similar and acceptably low initial TVB-N values on day 0, indicative of fresh, high-quality legs. These values are comparable to those reported for *Lithobates catesbeiana* legs [5] at the start of refrigerated storage at 4 °C, where the TVB-N content increased progressively as a function of microbial proliferation and protein catabolism. By day 6, all three groups in the present study converged to a narrowly similar TVB-N level, suggesting that, during this early phase, neither the coatings nor the extract oil provided a differential protective effect against the early onset of protein degradation, likely because endogenous autolytic enzymes, rather than microbial proteases, dominated this initial TVB-N accumulation.

From day 9 onward, notable divergences emerged among the treatments. The OO treatment maintained significantly lower TVB-N values on days 9 and 12 compared to the control and the CH-OO treatment, suggesting that the direct surface application of oregano oil at this mid-storage phase effectively suppressed the psychrotrophic spoilage bacteria. This is consistent with findings in *Anguilla anguilla* [48], which demonstrated that combined chitosan and oregano oil treatments significantly inhibited bacterial growth and delayed TVB-N increases beyond acceptability limits compared to untreated controls. The control treatment crossed the widely accepted spoilage threshold of 25 mg N/100 g for aquatic organisms on day 15, whereas the CH, CH-OO, and OO groups delayed this threshold exceedance to days 18, 18, and 21, respectively, representing effective shelf life extensions of 3–6 days relative to the control.

At the final sampling point, all treatments exceeded 25 mg N/100 g, with the OO group showing the lowest final TVB-N value, followed by CH-OO and CH. This progressive increase beyond the rejection threshold mirrors the findings in *Litopenaeus vannamei* [36], where the uncoated control TVB-N exceeded 30 mg N/100 g, the generally accepted spoilage limit for marine products, before day 20 of ice storage, while chitosan-nanoliposome-coated samples remained below this threshold for 20 days.

4.6. Microbiological Analyses

The results indicate that, under the evaluated experimental conditions, none of the active coatings achieved sufficiently robust and sustained microbiological inhibition of mesophiles to represent a clear technological advantage over the control. The OO treatment showed potential in the intermediate storage phase (day 9), but this effect was neither sustained nor significant. The chitosan treatment did not result in any significant differences compared to the control on any day of the experiment. There may be several explanations for the lack of effect observed in this study. The coating was applied by immersion, which likely limited its effectiveness to the microbiota on the product's surface,

while the mesophilic bacteria inside the product were not affected by the antimicrobial action [49]. The pH of the system may not have been sufficiently acidic to maintain the protonated amino groups that underpin the antimicrobial activity of chitosan (pKa 6.3–6.5), since at a pH close to neutrality, the polymer loses its positive charge and, consequently, its ability to interact with bacterial membranes [50]. The concentration of chitosan used may have been insufficient to address the microbial load present in the product [51].

The OO was the only treatment that showed a significant reduction in the mesophilic count compared to the control (day 9). The difference in absolute terms was small and has limited practical relevance from a microbiological safety perspective. The antimicrobial activity of OO is primarily attributed to its major phenolic components (carvacrol and thymol), which disrupt the bacterial cytoplasmic membrane, alter the proton gradient and driving force, and inhibit membrane ATPase. One explanation is that oregano oil exerts its maximum inhibitory effect during the logarithmic phase of microbial growth, when bacteria are metabolically most active and, paradoxically, most vulnerable to agents that disrupt membrane integrity [52]. Consequently, the effective concentration of free antimicrobial agents in the system would be lower in the CH-OO treatment than in the pure OO treatment, resulting in a loss of statistical significance.

In contrast, with psychrophilic bacteria, the most notable result was the complete absence of statistically significant differences between the treatments on any sampling day. This finding contrasts with the extensive published evidence on the antimicrobial activity of chitosan and oregano oil against psychrophilic bacteria.

Psychrotrophic microbiota are known for their high resistance to natural antimicrobial agents. Some of them possess multidrug efflux pumps and outer membranes with a low permeability to hydrophobic compounds, which reduces the intracellular penetration of the phenols in oregano oil. In addition, these organisms have an additional barrier of lipopolysaccharides in their outer membrane that hinders the action of carvacrol and thymol [53–55]. The cooling temperature affects the activity of the phenolic compounds in the oils. This effect is a critical factor that is often underestimated in studies of active coatings. At low temperatures, the solubility and diffusion of molecules (such as thymol and carvacrol) decrease considerably at the product/coating interface, reducing the available active concentration on the food surface. Furthermore, the viscosity of the chitosan matrix increases at temperatures near 0–4 °C, which can slow the release of the oil incorporated into the polymer network [56].

5. Conclusions

The application of edible coatings based on oregano oil, alone or in combination with chitosan, partially preserved the physicochemical quality (color, WHC texture, pH, and TVB-N), but not the microbiological quality (mesophiles and psychrophiles) of frog legs during ice storage. In the present work, the different responses among the quality parameters indicate that the edible coatings mainly delayed the physicochemical deterioration (color and pH), while attributes such as the texture, water retention capacity and microbial count were also strongly influenced by intrinsic post mortem changes and biological variability, which limited the detection of significant changes due to the effects of the treatments. Although the antimicrobial activity of both edible coatings has been documented, they did not control microbial growth, at least under the conditions of this experiment.

Author Contributions: N.M.-C., S.V.-H. and D.F.C.-R. designed the study; E.G.V.-Á., J.R.R.-E., and V.M.O.-H. performed formal analyses of the manuscript; and S.V.-H., C.L.D.-T.-S., E.M.-R. and E.W.C.-A. wrote the original draft. All authors contributed to writing, reviewing, and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The authors acknowledge the Universidad Estatal de Sonora for the support under the project number UES-PII-24-UAN-LNH-01, and the Food Research Laboratory of the Department of Chemical Biological Sciences of the University of Sonora, in Hermosillo, Sonora, Mexico, in which the work was developed.

Institutional Review Board Statement: Not applicable

Informed Consent Statement: Not applicable

Data Availability Statement: The data presented in this study are available from the corresponding author upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. FAO. *The State of World Fisheries and Aquaculture 2022: Towards Blue Transformation*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2022. <https://doi.org/10.4060/cc0461en>.
2. FAO. Rana Catesbeiana. In *Cultured Aquatic Species Information Programme*. In *Fisheries and Aquaculture Division*; Flores Nava, A., Ed.; Food and Agriculture Organization of the United Nations: Rome, Italy, 2009.
3. Haddad, L.R.; Tejada-Ortigoza, V.; Martín-del-Campo, S.T.; Balderas-León, I.; Peña, M.; Garcia-Amezquita, L.E.; Welti-Chanes, J. Evaluation of Nutritional Composition and Technological Functionality of Whole American Bullfrog (*Lithobates catesbeianus*), Its Skin, and Its Legs as Potential Food Ingredients. *Food Chem.* **2022**, *372*, 131232. <https://doi.org/10.1016/j.foodchem.2021.131232>.
4. Zhang, C.X.; Huang, K.K.; Lu, L.K.; Wang, L.; Song, K.; Zhang, L.; Li, P. Effects of Different Lipid Sources on Growth Performance, Body Composition and Lipid Metabolism of Bullfrog *Lithobates catesbeiana*. *Aquaculture* **2016**, *457*, 104–108. <https://doi.org/10.1016/j.aquaculture.2016.02.023>.
5. Lan, W.; Zhang, B.; Du, J.; Zhu, S.; Xu, X.; Xie, J. Synergistic Effect of Combined Treatment with Allicin and Antioxidant of Bamboo Leaves and Preservation of Bullfrogs (*Lithobates catesbeiana*) during Refrigeration Storage. *Foods* **2023**, *12*, 3467. <https://doi.org/10.3390/foods12183467>.
6. Du, J.; Lan, W.; Xie, J. Quality Characteristics and Moisture Migration of Refrigerated Bullfrog (*Lithobates catesbeiana*) under Slightly Acidic Electrolyzed Water Combined with Composite Preservative Treatment. *Food Biosci.* **2023**, *55*, 102947. <https://doi.org/10.1016/j.fbio.2023.102947>.
7. Gram, L.; Dalgaard, P. Fish Spoilage Bacteria—Problems and Solutions. *Curr. Opin. Biotechnol.* **2002**, *13*, 262–266. [https://doi.org/10.1016/S0958-1669\(02\)00309-9](https://doi.org/10.1016/S0958-1669(02)00309-9).
8. Jiménez-Ruiz, E.I.; Valdez-Hurtado, S.; Ocaño-Higuera, V.M.; Canizales-Rodríguez, D.F.; Garzón-García, A.M.; Marquez-Rios, E.; Ruiz-Cruz, S.; Del-Toro-Sanchez, C.L.; Valdez-Álvarez, E.G.; Paredes Quijada, G.T. Effect of Ice Storage on Freshness and Biochemical, Physical, Chemical, and Microbiological Quality of Leg Muscle Samples from Bullfrog (*Lithobates catesbeianus*). *Processes* **2025**, *13*, 910. <https://doi.org/10.3390/pr13030910>.
9. Dhall, R.K. Advances in Edible Coatings for Fresh Fruits and Vegetables: A Review. *Crit. Rev. Food Sci. Nutr.* **2013**, *53*, 435–450. <https://doi.org/10.1080/10408398.2010.541568>.
10. Vargas, M.; Pastor, C.; Chiralt, A.; McClements, D.J.; González-Martínez, C. Recent Advances in Edible Coatings for Fresh and Minimally Processed Fruits. *Crit. Rev. Food Sci. Nutr.* **2008**, *48*, 496–511. <https://doi.org/10.1080/10408390701537344>.
11. Sánchez-Ortega, I.; García-Almendárez, B.E.; Santos-López, E.M.; Amaro-Reyes, A.; Barboza-Corona, J.E.; Regalado, C. Antimicrobial Edible Films and Coatings for Meat and Meat Products Preservation. *Sci. World J.* **2014**, *1*, 248935. <https://doi.org/10.1155/2014/248935>.
12. Dutta, P.K.; Tripathi, S.; Mehrotra, G.K.; Dutta, J. Perspectives for Chitosan Based Antimicrobial Films in Food Applications. *Food Chem.* **2009**, *114*, 1173–1182. <https://doi.org/10.1016/j.foodchem.2008.11.047>.
13. Ojagh, S.M.; Rezaei, M.; Razavi, S.H.; Hosseini, S.M.H. Effect of Chitosan Coatings Enriched with Cinnamon Oil on the Quality of Refrigerated Rainbow Trout. *Food Chem.* **2010**, *120*, 193–198. <https://doi.org/10.1016/j.foodchem.2009.10.006>.
14. Rabea, E.I.; Badawy, M.E.T.; Stevens, C.V.; Smagghe, G.; Steurbaut, W. Chitosan as Antimicrobial Agent: Applications and Mode of Action. *Biomacromolecules* **2003**, *4*, 1457–1465. <https://doi.org/10.1021/bm034130m>.

15. Kim, K.W.; Thomas, R.L. Antioxidative Activity of Chitosans with Varying Molecular Weights. *Food Chem.* **2007**, *101*, 308–313. <https://doi.org/10.1016/j.foodchem.2006.01.038>.
16. Burt, S. Essential Oils: Their Antibacterial Properties and Potential Applications in Foods—A Review. *Int. J. Food Microbiol.* **2004**, *94*, 223–253. <https://doi.org/10.1016/j.ijfoodmicro.2004.03.022>.
17. Dorman, H.J.D.; Deans, S.G. Antimicrobial Agents from Plants: Antibacterial Activity of Plant Volatile Oils. *J. Appl. Microbiol.* **2000**, *88*, 308–316. <https://doi.org/10.1046/j.1365-2672.2000.00969.x>.
18. Knez Hrnič, M.; Cör, D.; Simonovska, J.; Knez, Ž.; Kavrakovski, Z.; Rafajlovska, V. Extraction Techniques and Analytical Methods for Characterization of Active Compounds in *Origanum* Species. *Molecules* **2020**, *25*, 4735. <https://doi.org/10.3390/molecules25204735>.
19. Pabast, M.; Shariatifar, N.; Beikzadeh, S.; Jahed, G. Effects of Chitosan Coatings Incorporating with Free or Nano-Encapsulated *Satureja* Plant Essential Oil on Quality Characteristics of Lamb Meat. *Food Control* **2018**, *91*, 185–192. <https://doi.org/10.1016/j.foodcont.2018.03.047>.
20. Petrou, S.; Tsiraki, M.; Giatrakou, V.; Savvaidis, I.N. Chitosan Dipping or Oregano Oil Treatments, Singly or Combined on Modified Atmosphere Packaged Chicken Breast Meat. *Int. J. Food Microbiol.* **2012**, *156*, 264–271. <https://doi.org/10.1016/j.ijfoodmicro.2012.04.002>.
21. Ruiz-Navajas, Y.; Viuda-Martos, M.; Sendra, E.; Perez-Alvarez, J.A.; Fernández-López, J. In Vitro Antibacterial and Anti-Oxidant Properties of Chitosan Edible Films Incorporated with *Thymus moroderi* or *Thymus piperella* Essential Oils. *Food Control* **2013**, *30*, 386–392. <https://doi.org/10.1016/j.foodcont.2012.07.052>.
22. FAOLEX Database. Acuerdo No 280—NSO 67.32.02:08 Sobre Valores Límites de Nitrógeno Básico Volátil Total e Histamina para Productos Pesqueros. Diario Oficial N° 56, 23 de marzo de 2009. Available online: <https://www.fao.org/faolex/results/details/es/c/LEX-FAOC087598/> (accessed on 06 January 2024).
23. Nychas, G.J.E.; Skandamis, P.N.; Tassou, C.C.; Koutsoumanis, K.P. Meat Spoilage during Distribution. *Meat Sci.* **2008**, *78*, 77–89. <https://doi.org/10.1016/j.meatsci.2007.06.020>.
24. SENASICA. Manual de Buenas Prácticas de Producción Acuicola de Rana Toro. Cd. Mexico, Mexico. Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria; SAGARPA. Available online: https://www.gob.mx/cms/uploads/attachment/file/859955/4_Rana_2019.pdf (accessed on 14 January 2024).
25. Kuehni, R.G. Color-Tolerance Data and the Tentative CIE 1976 L* A* B* Formula. *J. Opt. Soc. Am.* **1976**, *66*, 497–500. <https://doi.org/10.1364/JOSA.66.000497>.
26. Canizales-Rodríguez, D.F. Evaluación de la calidad y vida de anaquel del camarón *Litopenaeus stylirostris* Almacenado en Hielo y Caracterización de los Principales Compuestos Relacionados con su sabor y Deterioro. Master's thesis, Universidad de Sonora, Hermosillo, Sonora, Mexico, 2012.
27. Cheng, C.S.; Hamann, O.O.; Webb, N.B.; Sidwell, V. Effects of Species and Storage Time on Minced Fish Gel Texture. *J. Food Sci.* **1979**, *44*, 1087–1092. <https://doi.org/10.1111/j.1365-2621.1979.tb03453.x>.
28. Woyewoda, A.D.; Shaw, S.J.; Ke, P.J.; Burns, B.G. *Recommended Laboratory Methods for Assessment of Fish Quality*; Canadian Technical Report of Fisheries and Aquatic Sciences; Department of Fisheries and Oceans: Halifax, NS, Canada, 1986; Volume 1448.
29. Secretaría de Salud. Norma Oficial Mexicana NOM-092-SSA1-1994, Bienes y Servicios. Método Para La Cuenta de Bacterias Aerobias En Placa; Diario Oficial de la Federación: Cd, Mexico City, Mexico, 1995.
30. Cheng, J.-H.; Sun, D.-W.; Han, Z.; Zeng, X.-A. Texture and Structure Measurements and Analyses for Evaluation of Fish and Fillet Freshness Quality: A Review. *Compr. Rev. Food Sci. Food Saf.* **2014**, *13*, 52–61. <https://doi.org/10.1111/1541-4337.12043>.
31. Tarahi, M.; Abdolizadeh, L.; Hedayati, S. Mung Bean Protein Isolate: Extraction, Structure, Physicochemical Properties, Modifications, and Food Applications. *Food Chem.* **2024**, *444*, 138626. <https://doi.org/10.1016/j.foodchem.2024.138626>.
32. Kim, D.-Y.; Park, S.-W.; Shin, H.-S. Fish Freshness Indicator for Sensing Fish Quality during Storage. *Foods* **2023**, *12*, 1801. <https://doi.org/10.3390/foods12091801>.
33. Shao, H.-H.; Zhou, X.-J.; Xiang, X.-W.; Zhang, J.; Song, L.-L.; Li, R.-X.; Fu, M.-N.; Huang, Y.-Z. Changes in Total Volatile Basic Nitrogen and Biogenic Amines in 4 Kinds of Marine Products during Refrigerated Storage. *J. Food Saf. Qual.* **2022**, *13*, 2794–2801. <https://www.cabidigitallibrary.org/doi/full/10.5555/20220230362>.
34. Mendez, R.; Lema, J.M.; Soto, M. Treatment of Seafood-Processing Wastewaters in Mesophilic and Thermophilic an-Aerobic Filters. *Water Environ. Res.* **1995**, *1995*, 33–45. <https://doi.org/10.2175/106143095X131178>.
35. Wang, J.; Xie, J.; Mei, J. Research Progress Regarding Psychrotrophic *Pseudomonas* in Aquatic Products: Psychrophilic Characteristics, Spoilage Mechanisms, Detection Methods, and Control Strategies. *Foods* **2025**, *14*, 363. <https://doi.org/10.3390/foods14030363>.

36. Kamali, M.; Shabanpour, B.; Pourashouri, P.; Kordjazi, M. Evaluating Shelf Life and Anti-Browning of Shrimp by Chi-Tosan-Coated Nanoliposome Loaded with Licorice Root Extract. *Food Chem. X* **2024**, *23*, 101532. <https://doi.org/10.1016/j.fochx.2024.101532>.
37. Vital, A.C.P.; Guerrero, A.; Ornaghi, M.G.; Kempinski, E.M.B.C.; Sary, C.; Monteschio, J.D.O.; Matumoto-Pintro, P.T.; Ribeiro, R.P.; do Prado, I.N. Quality and Sensory Acceptability of Fish Fillet (*Oreochromis niloticus*) with Alginate-Based Coating Containing Essential Oils. *J. Food Sci. Technol.* **2018**, *55*, 4945–4955. <https://doi.org/10.1007/s13197-018-3429-y>.
38. Liu, X.; Zhang, C.; Liu, S.; Gao, J.; Cui, S.W.; Xia, W. Coating White Shrimp (*Litopenaeus vannamei*) with Edible Fully Deacetylated Chitosan Incorporated with Clove Essential Oil and Kojic Acid Improves Preservation during Cold Storage. *Int. J. Biol. Macromol.* **2020**, *162*, 1276–1282. <https://doi.org/10.1016/j.ijbiomac.2020.06.248>.
39. Chen, D.; Ci, M.; Dai, R.; Chen, R.; Li, T. Changes in the Microbial Communities of Tiger Frog (*Rana tigrina*) Meat during Refrigerated Storage. *J. Food Prot.* **2021**, *84*, 1136–1140. <https://doi.org/10.4315/JFP-20-381>.
40. Jia, S.; Jia, Z.; An, J.; Ding, Y.; Chang, J.; Wang, Y.; Zhou, X. Insights into the Fish Protein Degradation Induced by the Fish-Borne Spoiler *Pseudomonas psychrophila* and *Shewanella putrefaciens*: From Whole Genome Sequencing to Quality Changes. *Int. J. Food Microbiol.* **2024**, *416*, 110675. <https://doi.org/10.1016/j.ijfoodmicro.2024.110675>.
41. Huang, Y.; Nie, Y.; Zhou, F.; Li, B.; Luo, Q.; Zhang, B.; Zeng, Q.; Huang, Y. Effects of Collagen-Based Coating with Chitosan and ϵ -Polylysine on Sensory, Texture, and Biochemical Changes of Refrigerated *Nemipterus virgatus* Fillets. *Food Sci. Nutr.* **2024**, *12*, 2145–2152. <https://doi.org/10.1002/fsn3.3916>.
42. Maqsood, S.; Benjakul, S. Comparative Studies of Four Different Phenolic Compounds on in Vitro Antioxidative Activity and the Preventive Effect on Lipid Oxidation of Fish Oil Emulsion and Fish Mince. *Food Chem.* **2010**, *119*, 123–132. <https://doi.org/10.1016/j.foodchem.2009.06.004>.
43. Gram, L.; Huss, H.H. Microbiological Spoilage of Fish and Fish Products. *Int. J. Food Microbiol.* **1996**, *33*, 121–137. [https://doi.org/10.1016/0168-1605\(96\)01134-8](https://doi.org/10.1016/0168-1605(96)01134-8).
44. Gan, H.; Lv, M.; Lv, C.; Fu, Y.; Ma, H. Inhibitory Effect of Chitosan-Based Coating on the Deterioration of Muscle Quality of Pacific White Shrimp at 4 °C Storage. *J. Food Process. Preserv.* **2021**, *45*, e15167. <https://doi.org/10.1111/jfpp.15167>.
45. Offer, G.; Knight, P.; Jeacocke, R.; Almond, R.; Cousins, T.; Elsey, J.; Parsons, N.; Sharp, A.; Starr, R.; Purslow, P. The Structural Basis of the Water-Holding, Appearance and Toughness of Meat and Meat Products. *Food Structure* **1989**, *8*, 151–170.
46. Baygar, T.; Ozgur, N. Sensory and Chemical Changes in Smoked Frog (*Rana esculanta*) Leg during Cold Storage (4° C $\pm$ 1). *J. Anim. Vet. Adv.* **2010**, *9*, 588–593.
47. Hussain, S.A.; Wani, S.A.; Rafeh, S.; Adil, S.; Sofi, A.H.; Ghamry, H.I.; Wani, M. Enhancing Meat Emulsion Quality and Storage Stability During Refrigeration Using Thyme and Oregano Essential Oil Nanoparticles. *Foods* **2025**, *14*, 1076. <https://doi.org/10.3390/foods14061076>.
48. Pierozan, M.B.; JGD, O.F.; Cappato, L.P.; Costa, A.C.; Egea, M.B. Essential Oils Against Spoilage in Fish and Seafood: Impact on Product Quality and Future Challenges. *Foods* **2024**, *13*, 3903. <https://doi.org/10.3390/foods13233903>.
49. Kamali, M.; Shabanpour, B.; Pourashouri, P.; Kordjazi, M. Effect of Chitosan-Coated Ulva Intestinalis Sulfated Polysaccharide Nanoliposome on Melanosis and Quality of Pacific White Shrimp during Ice Storage. *Int. J. Biol. Macromol.* **2023**, *230*, 123275. <https://doi.org/10.1016/j.ijbiomac.2023.123275>.
50. Wu, K.; Yan, Z.; Wu, Z.; Li, J.; Zhong, W.; Ding, L.; Zhong, T.; Jiang, T. Recent Advances in the Preparation, Antibacterial Mechanisms, and Applications of Chitosan. *J. Funct. Biomater.* **2024**, *15*, 318. <https://doi.org/10.3390/jfb15110318>.
51. Poznanski, P.; Hameed, A.; Orczyk, W. Chitosan and Chitosan Nanoparticles: Parameters Enhancing Antifungal Activity. *Molecules* **2023**, *28*, 2996. <https://doi.org/10.3390/molecules28072996>.
52. Luo, K.; Zhao, P.; He, Y.; Kang, S.; Shen, C.; Wang, S.; Guo, M.; Wang, L.; Shi, C. Antibacterial Effect of Oregano Essential Oil against *Vibrio Vulnificus* and Its Mechanism. *Foods* **2022**, *11*, 403. <https://doi.org/10.3390/foods11030403>.
53. Lu, M.; Wong, K.I.; Li, X.; Wang, F.; Wei, L.; Wang, S.; Wu, M.X. Oregano Oil and Harmless Blue Light to Synergistically Inactivate Multidrug-Resistant *Pseudomonas aeruginosa*. *Front. Microbiol.* **2022**, *13*, 810746. <https://doi.org/10.3389/fmicb.2022.810746>.
54. Munir, F.; Manal; Ahmed, S.; Safdar, W.; Shah, S.A.; Maan, M.K.; Khan, R.; Karim, A. Native Oregano Essential Oil: Phytochemical Composition, Antibacterial and Synergistic Activities with Oxytetracycline against Multi-Drug Resistant (MDR) Bacteria. *Microb. Pathog.* **2025**, *208*, 108034. <https://doi.org/10.1016/j.micpath.2025.108034>.

55. Rodríguez-Melcón, C.; Alonso-Calleja, C.; Capita, R. The One Health Approach in Food Safety: Challenges and Opportunities. *Food Front.* **2024**, *5*, 1837–1865. <https://doi.org/10.1002/fft2.458>.
56. Yousuf, B.; Panesar, P.S. Advances in Lipid Edible Films and Coatings. In *Edible Films and Coatings*; Yousuf, B., Panesar, P.S., Eds.; CRC Press: Boca Raton, FL, USA, 2026; Volume 1, pp. 58–85. <https://doi.org/10.1201/9781003735649>.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.