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EFFECT OF MOLECULAR WEIGHT OF CHITOSAN IN THE COATING SOLUTIONS ON THE SHELF LIFE OF TUNA FISH FILLETS

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Abstract

Tuna, a valuable Indonesian marine commodity, is known for its highly valued dietary and nutritional sources. However, this red meat fish is particularly prone to deterioration and spoilage due to its inherent properties. Liquid smoking, a popular fish preservation method in Indonesia, has garnered significant consumer interest but remains insufficient to prevent fish decay entirely. Therefore, this study aimed to enhance tuna fish fillet (TFF) shelf life by developing a novel coating solution. This solution contains liquid smoke seasoning and chitosan with varying molecular weights (MW), as this property affects chitosan's chemical, physical, and biological characteristics. In this research, fresh tuna were divided into four groups: a control group with no coating, a group with liquid smoke coating only (LS), a group coated using chitosan solution (Ch) with variations of MW (Low: 50-190 kDa, Medium: 190-310 kDa, and High: 210-375 kDa), and a group coated using liquid smoke seasoning with the same chitosan MW variation (Ch + LS). During 15 days of storage in the freezer, their chemical properties (pH, and Total Volatile Base Nitrogen - TVB-N) were analyzed. The finding showed that combining high MW of chitosan and liquid smoke as a coating solution improved TFF appearance, maintaining a desirable uniformly cherry red color. Moreover, this treatment showed a slower increase in pH (5.5 – 5.7) and TVB-N level (22 – 28 mg TVB-N/100 gram), indicating deterioration and spoilage delay during 15 days of storage compared to other treatments. This study underscores the efficacy of a preservation strategy in inhibiting deterioration and spoilage in tuna fish fillets during storage, thereby enhancing product quality and ensuring consumer safety.

INTRODUCTION

Tuna constitutes a significant portion of the global seafood market [1], contributing approximately 9.7% to the total export value of all marine products [2]. As the second-largest tuna producer in the ASEAN region, after Thailand, Indonesia plays a pivotal role in meeting global demand [3]. Tuna is known for its highly valued dietary and nutritional sources. This red meat fish provides essential nutrients, including protein, unsaturated fatty acids (omega-3 fatty acids), and key micronutrients like iron, zinc, and vitamin B12 [4]. However, like other fish products, tuna is highly susceptible to deterioration and spoilage due to their inherent properties, such as water-rich content (65-80%), post-mortem pH of 6-7, and abundance of protein and non-protein nitrogen (9-18%) [5], [6]. These characteristics lead to the rapid decay of visual and nutritional properties [7]. Common preservation methods for fresh fish during transportation and distribution include icing and refrigeration. However, this practice only limits their shelf life to 5-10 days [8].

Smoking, another widely practiced fish preservation method, has gained significant consumer interest worldwide, including in Indonesia [9], [10]. A modern technique utilizing smoke components in liquid form is called liquid smoking [11]. This technique can be applied to fish products through immersion, spraying, or misting [9]. Beyond eliminating harmful compounds produced during traditional wood burning [11], liquid smoke offers several advantages, such as easy use, precise concentration control, consistent product quality, and being environmentally friendly [12]. Regardless of these benefits, smoked fish remain perishable [13]. Therefore, further innovation is necessary to enhance shelf life and maintain product quality.

Recently, edible coating has been the most cost-effective method without the requirement of sophisticated machines for fish preservation [14]. Among the promising materials for these coatings is chitosan. This polysaccharide consists of β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine structures [15] and is derived from the deacetylation of chitin, the primary component of

crustaceans and fungi [16]. This compound also aligned with US Food and Drug Administration (FDA) safety standards due to its non-toxic, biodegradable, and biocompatible attributes [17]. Chitosan coatings protect food products by creating a transparent, invisible, and adhesive barrier [18]. These barrier properties extend the shelf life of food products by inhibiting microbial growth, maintaining their water content [19], and controlling moisture and gas exchange [20].

Chitosan has demonstrated its effectiveness as a coating agent to extend the shelf life of various marine products. Studies have demonstrated its antimicrobial activity in hake fish (*Merluccius merluccius*), sole fish (*Solea solea*) [5], vannamei shrimp (*Litopenaeus vannamei*) [21], Atlantic salmon (*Salmo solar*) [22], and catfish fillets [23]. The antibacterial behavior of chitosan is strongly influenced by the degree of deacetylation and molecular weight (MW). It has been explored that the molecular weight variation of chitosan affects its chemical, physical, and biological caharacteristics [24]. Studies have shown varying impacts of molecular weight on shelf-life prolongation across different types of fish. High MW of chitosan (800 kDa) was reported to prolong the shelf life of channel catfish (*Ictalurus punctatus*) fillets kept at 4 °C by hindering chemical and microbial deterioration up to the 10th day [25]. Conversely, 185 kDa of chitosan was found effective in preserving ice-stored houndfish (*Tylosurus crocodilus*), extending their shelf life up to 20 days via reducing lipid oxidation, protein degradation, and microbial growth [26]. Interestingly, studies on salmon (*Salmo salar*) have indicated that lower molecular weight chitosan (30 kDa) exhibited superior antimicrobial and antioxidant properties compared to higher molecular weight chitosan (90 kDa and 120 kDa) [27].

Given the critical role of molecular weight (MW) in tailoring chitosan's functionality as a coating agent in marine products, this research aims to study the influence of chitosan's molecular weight (MW) as a coating agent on the preservation of tuna fish fillets, both alone and in combination with liquid smoke. The appearance, pH, and total volatile basic nitrogen (TVB-N) are evaluated to assess the quality of the treated tuna fish fillets. The findings provide insights into the optimal preservation strategies for tuna fillets, contributing to enhancing product quality and ensuring consumer safety.

EXPERIMENT

Material

Tuna fish was derived from a local farmer in Jember, while liquid smoke seasoning was from CV. Medipety in Jember. Some chemicals, including chitosan with low (50-190 kDa), medium (190-310 kDa), and high (210-375 kDa) molecular weights, acetic acid glacial (CH_3COOH), sodium hydroxide (NaOH), hydrochloric acid (HCl), and phenolphthalein indicator were purchased from Sigma Aldrich.

Procedure

Preparation of Coating Solution

The liquid smoke coating solution (5% v/v) was prepared by dissolving liquid smoke seasoning into distilled water. Meanwhile, chitosan coating solution (1% v/v) was composed by mixing low (50-190 kDa), medium (190-310 kDa), and high (210-375 kDa) MW of chitosan with 2% acetic acid. Finally, a chitosan-liquid smoke coating solution was carried out by adding liquid smoke seasoning into 1% of each chitosan solution until a final concentration of 5% v/v was obtained.

Coating on Tuna Fish Fillet (TFF)

Fresh tuna fish were obtained from a local farmer and immediately delivered to the laboratory in a cold storage box. The fish were manually deboned and skinned, then cut into 100-gram portions. These portions were randomly assigned to four groups: a control group with no coating, a group with liquid smoke coating only (LS), and two groups dipped with 100 mL of chitosan solution for 3 minutes. One chitosan group used a variation of MW (Ch (L): 50-190 kDa, Ch (M): 190-310 kDa, Ch (H): 210-375 kDa). The other combined these chitosan solutions with liquid smoke seasoning (Ch (L)+ LS; Ch (M)+ LS; Ch (H)+ LS). Following the dipping process, all coated fillets were drained briefly and air-dried. The samples were stored for subsequent analysis.

Analysis of pH Changes in Tuna Fish Fillet (TFF)

pH measurements were conducted following the methods described by previous research [28]. Each sample was homogenized and then diluted 1:10 with distilled water. The diluted samples were filtered using filter paper, and the filtrate was measured using the pH meter (Hanna Instrument

HI-9813-5). Prior to sample measurements, the pH meter was calibrated with buffer solutions at pH 4.01 and 6.86.

Analysis of Total Volatile Basic Nitrogen (TVB-N)

Total Volatile Base Nitrogen (TVB-N) content was determined according to the method described by A.E. Goulas and A.G. Kontominas [29]. Briefly, a 10-gram sample was homogenized using a blender and dissolved in 100 mL of distilled water. The mixture was allowed to stand for 30 minutes, followed by 10 minutes of continuous stirring. After that, the solution was filtered through filter paper. A 10 mL aliquot of the filtrate was transferred to a 500 mL round-bottom distillation flask and mixed with 5 mL of MgO solution. A 250 mL Erlenmeyer flask containing 10 mL of 2% H_3BO_3 and 5 drops of Tashiro's indicator (methyl red/methylene blue) was prepared as the receiving flask. The Kjeldahl distillation was then conducted for 5 minutes. The distillate was subsequently titrated with 0.01 N HCl solution until a stable purple endpoint was reached. The TVB-N content was calculated based on the volume of HCl consumed and expressed as milligrams of TVB-N per 100 grams of sample (mg TVB-N/100 g).

RESULT AND DISCUSSION

The Physical Appearance of Coated Tuna Fish Fillet

The freshness of fish fillets is often visually assessed by their color. The red color is considered to be the most important characteristic that influences consumer acceptance and preference [14]. Most consumers tend to prefer brightly cherry-red fish flesh [4]. The physical appearance of the tuna fish fillets (TFF) after treatment with the coating solutions is illustrated in **Figure 1**. Generally, the coating treatment employing high MW of chitosan resulted in an evenly distributed bright red color in the TFF. Conversely, a darker color was observed in the central dorsal region of TFF treated with low and medium MW of chitosan.

The redness of fish meat originates from the presence of myoglobin, a chromoprotein residing within their muscle fibers. Myoglobin exists in three distinct forms: oxymyoglobin, deoxy myoglobin, and metmyoglobin [8], responsible for the bright cherry-red, purplish-red, and brownish-red coloration, respectively [4]. While oxymyoglobin is the preferred color, it is highly susceptible to lipid oxidation [1], [8]. Processes like prolonged low-temperature storage disrupt muscle membranes, exposing lipids to oxygen and accelerating oxidative damage [30]. This oxidation leads to metmyoglobin formation, causing discoloration in fish meat [8]. According to prior research, tuna is considered dark-fleshed meat, characterized by a high concentration of dark-muscle fibers [31]. As dark-muscle fish is richer in lipids than light-muscle fish [2], it will exhibit greater vulnerability to lipid oxidation processes, ultimately contributing to accelerated color deterioration.

Results demonstrated that chitosan coatings effectively inhibit lipid oxidation during storage. Chitosan solutions with high MW exhibited superior antioxidant properties compared to lower or medium MW solutions. This was evident in the color deterioration observed in tuna fish fillets coated with the latter solutions (**Figure 1**). Chitosan and its derivatives possess antioxidant activity. This activity stems from their ability to capture free radicals through their hydroxyl (-OH) and amino ($-NH_2$) groups [24]. The amine group can react with hydrogen ions from the solution to form ammonium (NH_3^+), contributing to free radical scavenging [27].

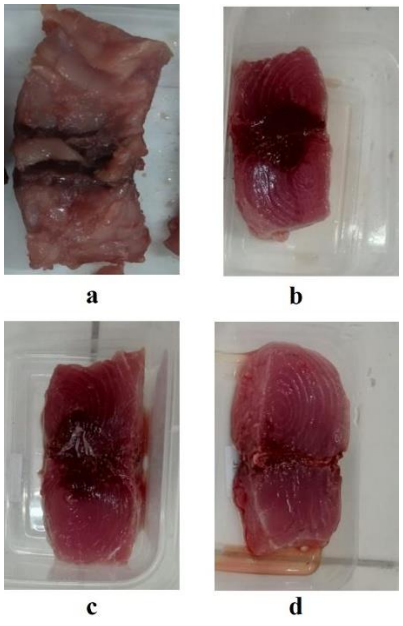

Figure 1. Physical appearance of tuna fish fillets (TFF) without coating (a) and after coating treatment using liquid smoke in combination with chitosan (b) low MW (c) medium MW (d) high MW. All images were captured 3 days after the treatment.

The connection between chitosan's molecular weight and its antioxidant activity has been studied by earlier research. It has been stated that the higher molecular weight chitosan generally exhibits reduced antioxidant activity. This trend is attributed to increased inter- and intramolecular

hydrogen bonds within the polymer chain, leading to a more compact structure. This compact structure limits the accessibility of the hydroxyl and amine groups to free radicals, thereby hindering their antioxidant action [24], [32]. However, research conducted by F.M.S Santos *et al* [11] reported that the antioxidant mechanism of chitosan is through forming a barrier layer on the product's surface to inhibit oxygen penetration and thus prevent oxygen-mediated oxidation reactions. Therefore, although higher molecular weight chitosan can potentially lead to more solid structures, this density may inadvertently limit oxygen entry, thus reducing lipid oxidation and preserving TFF freshness, as observed in this study.

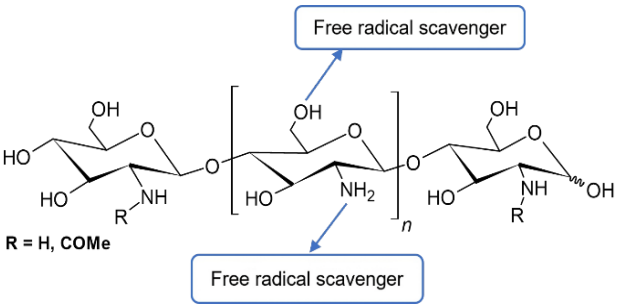

Figure 2. Molecular structure of chitosan [24].

Assesment of pH Changes in Tuna Fish Fillet

The spoilage level of fish can be assessed by measuring its pH value [16]. In this study, the initial pH of all TFF samples (**Figure 3**) was between 5.4 and 5.8. This observation is consistent with prior research by E.J. Moon *et al* [33], which established that the typical initial pH of fresh tuna lies between 5.2 and 6.1. As storage time increased, the pH of all TFF samples rose. These phenomena are likely due to the collective effects of proteolytic-bacterial growth and natural lysosomal protease enzymes present in the fish muscle in post-mortem time. These factors contribute to the production of alkaline compounds, such as ammonia and various amines, which ultimately increase pH [6], [34], [35], [36].

This experiment investigated the pH changes in uncoated, liquid smoke-coated, chitosan-coated, and liquid smoke + chitosan-coated TFF samples. The rate of pH increase varied among treatments, with the uncoated samples exhibited the most rapid pH increase. Meanwhile, chitosan coatings induced a minimal increase in pH. Moreover, the final pH of all chitosan-coated samples remained within the acceptable range for fish (6.8-7.0) as defined by F. Siddiqui *et al* [16], while the uncoated samples exceeded this limit, with the final pH of 8. Among all coated samples, the sample coated with low MW chitosan showed the highest pH increase (5.6–6.1). In contrast, samples coated with high MW chitosan and liquid smoke consistently maintained the lowest pH throughout the storage period (5.5–5.7).

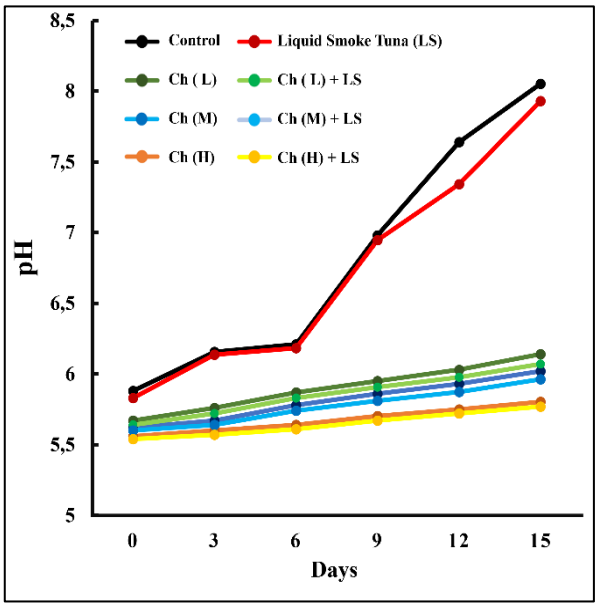

Figure 3. pH changes of tuna fish fillet (TFF) samples during 15-days storage at 4 °C.

Those findings align with previous research demonstrating the pH-lowering effect of high MW of chitosan in coating applications. F. Bonilla *et al* [23] reported lower pH values in catfish fillets coated with 338 kDa chitosan than those coated with distilled water. Similarly, B. Karsli *et al* [25] observed a 12-day decrease in pH in catfish fillets coated with 800 kDa chitosan and acetic acid compared to uncoated controls. Furthermore, F.M.S. Santos *et al* [11] observed slower pH increases in *Nile tilapia* fillets coated with chitosan and liquid smoke than uncoated ones. This effect can be attributed to the formation of layer system from the high MW of chitosan. This compact barrier prevents oxygen transfer and water absorption, inhibiting microbial growth and endogenous protease activity [15].

Additionally, the acidic nature of chitosan [23], [25] and the presence of acids in liquid smoke [11] itself likely contribute to the reduction in pH. However, as shown in **Figure 3**, the pH values for samples coated with chitosan and chitosan–liquid smoke were not significantly different. This data suggests that chitosan had a more profound effect on pH than liquid smoke. Chitosan exerts its antimicrobial action through its amino groups. These functional groups can depolarize cellular membranes and disrupt cell wall integrity, ultimately inhibiting microbial activity [16], [36].

Assesment of TVB-N Changes in Tuna Fish Fillet

Besides its appearance and pH, Total Volatile Basic Nitrogen (TVB-N) serves as a crucial indicator of fish fillet quality and freshness [20]. Elevated TVB-N values also signify both endogenous protease enzyme and proteolytic microbial proliferation (*Chromobacterium, Proteus, Pseudomonas, Halobacterium, Micrococcus*, and Gram-negative bacteria) [6] that breakdown the protein and nonprotein nitrogen-containing compounds within the fish [35], [37]. This degradation process thereafter results in the formation of ammonia and various amines, including monoethylamine, diethylamine, and triethylamine [34], [35], [36]. Monitoring TVB-N levels is crucial for assessing quality and predicting spoilage in fish fillets, particularly during the later storage stages [6].

Figure 4 illustrates the TVB-N changes in TFF during storage. Initial TVB-N levels in all samples ranged from 16 to 21 mg TVB-N/100 g. This finding is consistent with previous research that stated the TVBN level for freshly caught fish is around 5 – 20 mg TVB-N/100 g) [34]. As expected, TVB-N levels raised with storage time, suggesting the initiation of deterioration. However, the chitosan-uncoated samples exhibited a significant increase in TVB-N after 6 days of storage, reaching 59.9 to 64 mg TVB-N/100 g. In contrast, chitosan-coated samples effectively maintained TVB-N within the safe range for spoilage, even after 15 days of storage (22 – 28 TVB-N/100 gram). According to the European Commission's guideline, TVB-N levels exceeding 30 – 35 mg TVB-N/100 g indicate the onset of spoilage [20], [38]. Furthermore, samples coated with high MW chitosan in combination with liquid smoke demonstrated superior preservation, consistently keeping TVB-N levels within the acceptable range of 16 – 22 mg TVB-N/100 g throughout the storage period. Those findings were consistent with the observed pH values throughout the storage time. It was expected since both phenomena derived from the accumulation action of proteolytic microorganisms and protease enzymes that leads to the alkaline or nitrogen-rich compounds production. These compounds will be detected as TVB-N and also contribute to the observed increase in pH [34], [35], [36].

Existing research indicates that each molecular weight of chitosan has different antimicrobial impacts. Low MW chitosan can penetrate cell walls to destroy microorganisms, while high MW chitosan exerts its effect through another mechanism. The higher MW of chitosan serves more intra- and intermolecular interactions, leading to the formation of a thicker film on the product surface and preventing microbial contamination [24]. As mentioned earlier, the increase in TVB-N values in fish fillets suggests the proliferation of Gram-negative proteolytic microorganisms, such as *Chromobacterium, Proteus, Pseudomonas, and Halobacterium* [35], [37]. According to Malekkolaei, *et al.,* (2025) G-negative bacteria can display a double glycoprotein layer to restrict the penetration of antimicrobial agents [39]. Taken together, the antimicrobial mechanism provided by high MW chitosan gives better suited to prevent spoilage in fish products through coating. The layer prevents microbial contamination and protein or other nitrogenous compound degradation in the sample upon storage, thereby lowering the levels of TVB-N during the 15-day storage period of TFF.

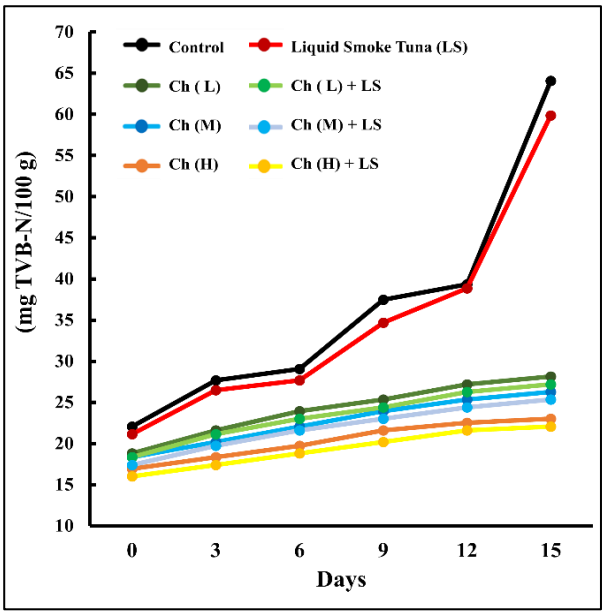

Figure 4. TVB-N changes of tuna fish fillet (TFF) samples during 15-days storage at 4 °C.

CONCLUSION

In order to preserve tuna fish fillets (TFF), this study has effectively created a coating solution containing different MW of chitosan and liquid smoke. Among the evaluated treatments, a coating solution containing liquid smoke and high MW chitosan (210 – 375 kDa) demonstrated superior performance in keeping TFF quality for 15 days of storage period. This was evidenced by the evenly distributed cherry red color in TFF samples under that treatment. Furthermore, this coating solution showed a minimal increase in pH (5.5 – 5.7) and

TVB-N level (22 – 28 mg TVB-N/100 gram), indicating deterioration and microbial spoilage inhibition throughout storage time compared to other treatments. The antimicrobial effect is likely derived from a dense barrier formation by the high MW of chitosan that restricts oxygen penetration and inhibits microbial proliferation. Overall, these findings highlight the potential of a chitosan-based coating solution as a promising strategy for preserving tuna fish quality during storage period.

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