Effectiveness of Low-Deacetylation-Degree Chitosan as an Edible Coating for Apples, Tofu, and Tilapia Fillets

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Abstract. Food products like fish, fruits, and tofu are highly perishable, with quality degradation being a significant concern during storage. Chitosan, derived from shrimp waste, has potential as an edible coating to enhance shelf life. The degree of deacetylation (DD) is a key factor in chitosan's effectiveness, with high-DD chitosan (>80%) often requiring substantial resources for production. This study explores the use of low-DD chitosan (50%) as an edible coating for Nile tilapia fillets, apple slices, and tofu. Coatings were applied at intervals of 0, 6, 12, 18, and 24 hours, and samples were stored at room temperature (25 ± 2°C). Results show that low-DD chitosan significantly preserved the texture of Nile tilapia and tofu for up to 5 days ($p<0.05$). However, chitosan-coated apple slices exhibited increased browning, although oxidation was delayed compared to uncoated controls. These findings indicate that low-cost, low-DD chitosan could be an effective alternative for extending the shelf life of certain perishable foods without the need for high-purity chitosan.

1 Introduction

Foods such as fish, fruits, and fermented products are highly prone to damage during storage, resulting in a shorter shelf life and increased susceptibility to microbial attack, oxidation, and browning [1]. Preservation of the quality and extension of the shelf life of products can be achieved through post-harvest technologies. The quality of food products, in terms of their

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sensory evaluation, physicochemical, and sensory properties, is maintained and protected against various external damages, including chemical, physical, and biological factors [1, 2]. Edible coatings, a form of post-harvest technology, are used in safe packaging to minimize moisture loss, regulate water and gas exchange, and improve product texture [3]. The ease of consumption, non-toxicity, and cost-effectiveness of edible coatings compared to other synthetic coatings are considered advantageous [4]. Proteins, polysaccharides, lipids, plasticizers, and emulsifiers, all of which are environmentally friendly, are used to produce these coatings [1, 5].

Chitosan is a natural polymer obtained from chitin derived from shrimp shells, crabs, and other insects. Chitosan exhibits antimicrobial, antioxidant, and film-forming properties and can be utilized as a protective layer for fruits, thereby extending their shelf life and preserving their quality. Chitosan is one of the most effective antimicrobials used in food packaging [6]. The antimicrobial mechanism of chitosan involves the binding of its positive charge (NH_2) to the peptidoglycan layer of Gram-positive bacterial cells, causing the cytoplasmic contents to leak out owing to osmotic differences, leading to the destruction and rupture of the bacterial cell wall. In Gram-negative bacteria, the mechanism of chitosan works by blocking nutrient flow into bacterial cells, depriving them of the nutrients necessary for survival, ultimately causing cell death [7].

The application of chitosan as a coating has been shown to extend the shelf life of tomatoes and lychees [8]. Chitosan concentrations of 0.5% and 1% have been found to extend the storage life of snake head fruits for up to 15 days [9]. Additionally, chitosan has been used as a natural preservative to extend the shelf life of whole fish, fish fillets, shrimp, and chicken, serving as both a coating and an antibacterial agent. The ability of chitosan to inhibit the growth of bacteria and molds is due to its positively charged polycation properties [10].

Chitosan is of various types based on the degree of deacetylation (DD). A higher DD value in chitosan corresponds to a higher cost, whereas chitosan with a lower DD value is less expensive. This is because the production of chitosan with a higher DD value requires a longer processing time, more chemicals, and additional time.

To the best of our knowledge, no studies have explored the use of low-degree deacetylation (DD) chitosan as an edible coating. In contrast, numerous reports have focused on the application of purified chitosan as an antimicrobial agent. Thus, based on these hypotheses, there is still ample room to utilize this quality of chitosan for low-cost applications in the food industry. This research contributes to the growing focus on sustainability through the application of bioproducts. Specifically, it explores the effects of low-degree deacetylation (DD) chitosan as an edible coating on various food products, including apple slices, tofu, and fillets of Nile tilapia (*Oreochromis niloticus*). In addition, the study systematically assesses the degree of deacetylation (DD) throughout the processing stages—shrimp shell, chitin, and chitosan—and conducts a comprehensive comparison of the extracted chitosan's quality against the standards established by the Indonesian National Standard (SNI).

2 Materials and Methods

2.1 Materials

The equipment used in this study included a magnetic stirrer, beaker (Pyrex), analytical balance (HAR-205A), plastic containers, a knife, and a smartphone camera. The materials used were chitosan extracted from Bio Chitosan Indonesia; shrimp carapace and chitin as the standard, and apples; Nile tilapia and tofu from Dramaga Market, Bogor Regency; and 99.8% glacial acetic acid. The apple samples were first cleaned to remove any dirt by washing and

draining. The apples were cut into six sections. The fish samples used were fillets, each weighing approximately ±60 g per piece. Before filleting, gutting and descaling were performed to remove innards and scales. The tofu samples were washed with clean water to remove any dirt, then cut into 6 pieces.

2.2 Coating chitosan

The edible coating was prepared using a 2% (w/v) concentration [11]. A total of 2 g of chitosan was dissolved in 100 mL of 1% acetic acid and stirred with a magnetic stirrer for 60 min. The preparation of the edible coating was repeated three times. The coating process involved immersing the samples in the chitosan edible coating solution, ensuring that the entire surface of each sample was submerged. The immersion process was performed for 15 min, after which the samples were drained and placed into storage containers. The storage process was conducted at room temperature with the containers left uncovered.

2.3 The evaluation of edible coating chitosan

2.3.1 Degree of deacetylation

The degree of deacetylation of chitosan was determined by Fourier Transform Infrared (FTIR) spectroscopy [12]. The degree of deacetylation of chitosan was measured based on the curve produced by the spectrophotometer. The highest (P0) and lowest (P) peaks were recorded and measured using a selected baseline.

2.3.2 Sensory evaluation

The evaluation criteria varied depending on the sample type [13]. For Nile tilapia fillets, the parameters assessed included slime formation, flesh condition, odor, and texture. In contrast, for apple and tofu samples, the evaluation focused on color, texture, and odor. A sensory evaluation was performed to assess the quality of the samples during storage at time intervals of 0, 6, 12, 18, and 24 hours. The sensory score used was a standard 1-9 scale, where 1 represents "strongly unacceptable" and 9 represents "strongly acceptable". Each panelist assigned a quality score to the samples, with a total of nine panelists participating in the study. The data collected from this evaluation will be utilized to examine the changes in sample quality over the specified storage duration.

2.3.3 Colour profile

The pr color was obtained by photographing each sample at 0, 6, 12, 18, and 24 h. The photos of each sample were uploaded to https://html-color-codes.info/colors-from-image/ to obtain the color code, which was then entered into https://serennu.com/colour/hsltorgb.php to generate the RGB code. The RGB code was then converted to CIE-L*a*b* using http://colormine.org/convert/rgb-to-lab. The CIE-L*a*b* values were calculated using the following formula to determine the color difference during storage:

$$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2} \quad (1)$$

2.4 Data Analysis

Sensory evaluation and color profile analyses were conducted using Factorial Completely Randomized Design. The data were processed using Microsoft Excel 2021. If

the data analysis indicated a significant effect ($p<0.05$), further testing was conducted using Duncan's Multiple Range Test, with a confidence interval of 95%.

3 Results and Discussions

3.1 Characterization raw materials

Characterization was performed on the raw materials, chitin, and chitosan, obtained from the Chitosan Industry. This section aims to evaluate the degree of deacetylation (DD) across different stages of processing, including shrimp carapace, chitin, and chitosan, and to rigorously compare the quality of the extracted chitosan with the specifications outlined in the Indonesian National Standard (SNI). Shrimp carapace was used as the raw material in this study, from which chitosan was extracted. The samples used in this study are shown in Fig. 1.

Fig. 1. (A) shrimp shell; (B) chitin; and (C) chitosan

Based on shrimp shell samples, chitin and chitosan were analyzed to assess key quality parameters, including odor, color, texture, and solubility. These properties were evaluated to ensure compliance with the standards outlined in SNI No. 7949-2013, which specifies that chitosan should be odorless, white in powdered form, and soluble in 1% acetic acid. The results of this comprehensive characterization are summarized in Table 1**,** providing a detailed comparison of the chitin, shrimp shell, and chitosan samples to the established quality benchmarks.

Table 1. Characterization of shrimp carapace, generated chitin and chitosan.

Parameters	Results	SNI No. 7949-2013
Odor	No	No
Colour	White	White
Form	Powder	Powder
Solubility in acetic acid 1%	Soluble	Soluble
Degree deacetylation sample A	3%	-
Degree deacetylation sample B	37%	≤75%
Degree deacetylation sample C	50%	≥75%

The degree of deacetylation (DD), a critical parameter reflecting the efficiency of the deacetylation process in converting chitin into chitosan, was determined by analyzing the FTIR spectra. The DD is calculated based on the removal of acetyl groups from the amide linkages, and the results obtained from the FTIR spectra are summarized in Fig 2**.** The findings from this study indicate that Sample A exhibited a DD of 3%, Sample B showed a DD of 37%, and Sample C reached a DD of 50%. According to the classification by Kumari and Kishor [14], chitosan with a DD value of 47%-53% is categorized as low-deacetylation chitosan. These results confirm that Sample C falls within this range, whereas Samples A and B fall below the threshold for low-deacetylation chitosan. The analysis of shrimp carapace and industrially produced chitin was conducted to establish a reference standard and demonstrate that the sample under investigation showed a low degree of deacetylation (DD), but surpasses the values observed in the two established standards.

Result from analyst FTIR can see in the Fig. 2. The FTIR spectrum can be used to characterize compounds through the analysis of peaks corresponding to the functional groups characteristic of chitin-chitosan. Typical characteristics of chitosan are its amide and hydroxyl groups. The absorption of the amine group is located at a wavenumber range of 1650-1310 cm^{-1}, while the hydroxyl group is located at a wavenumber range of 3550-3300 cm^{-1}. The FTIR spectrum of the synthesized chitosan obtained is shown in Fig 2. Based on the FTIR spectrum in Fig. 2, an absorption band can be seen in the wavenumber region of 3450 cm^{-1}, indicating the presence of hydroxyl groups, and absorption in the region of 1654 cm^{-1}, indicating the presence of amide groups.

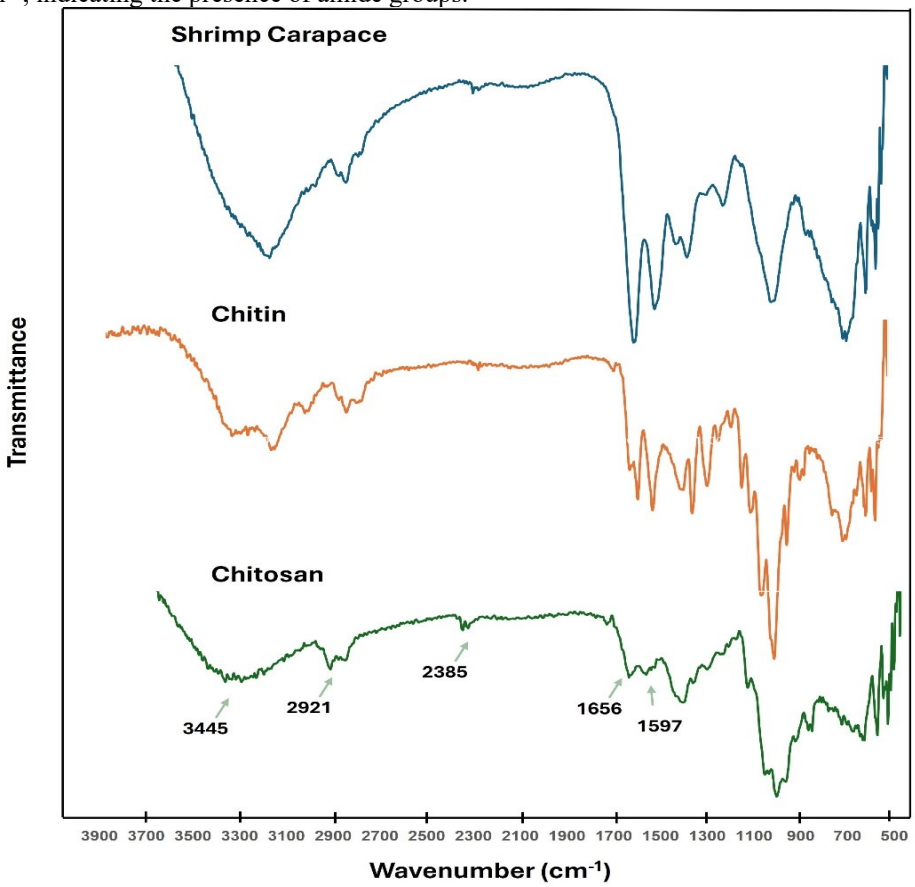


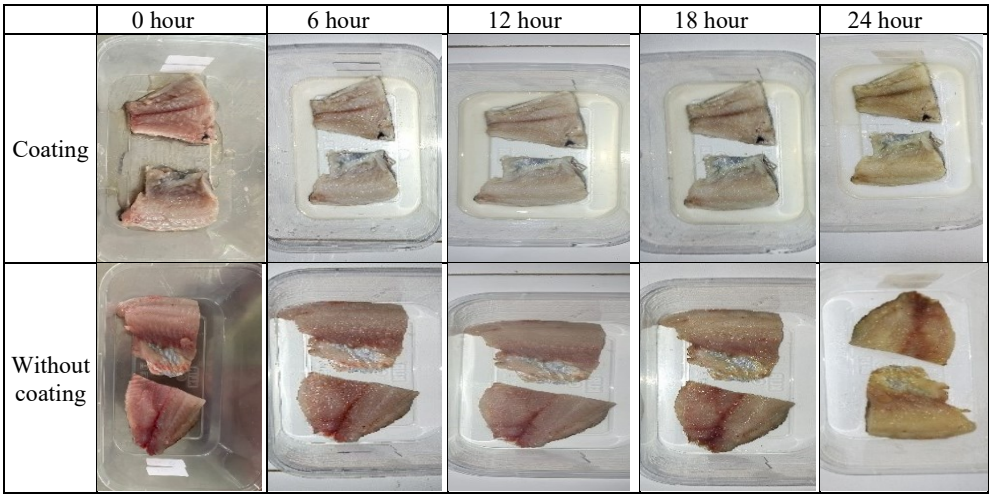
Fig. 2. Spectra FTIR shrimp shell; chitin and chitosan

The FTIR spectra results of the sample (a) from shrimp shell material show absorption peaks at 3266 and 2925 cm^{-1} (stretching vibrations of N-H and O-H from chitosan), absorption at 2356 cm^{-1} (aromatic C-H stretching vibration), 1637 and 1398 cm^{-1} (amide I and amide III), 1542 cm^{-1} (bending vibration of NH_2, likely overlapping with amide II), 1238 cm^{-1} (C-O-C bridge stretching vibration), and 1022 cm^{-1} (C-O stretching vibration). The sample (b), chitin, shows absorption peaks at 3259 and 3108 cm^{-1} (stretching vibrations of N-H and O-H from chitosan), 2923 cm^{-1} (aliphatic C-H stretching vibration), 1617, 1374, and 1309 cm^{-1} (amide I, II, and amide III), 1552 cm^{-1} (NH_2 bending vibration), 1413 cm^{-1} (C-N amine bending vibration), 1066 cm^{-1} (C-O stretching vibration), and a peak at 892 cm^{-1} (β-1,4-glycosidic stretching vibration).

Sample C, exhibits absorption peaks at 3363 and 3295 cm^{-1} (stretching vibrations of N-H and O-H from chitosan), 2917 and 2356 cm^{-1} (symmetric stretching vibrations of aliphatic and aromatic C-H), 1745 cm^{-1} (C-O anhydride stretching vibration), 1652 and 1579 cm^{-1} (stretching vibrations of C=O in secondary amide and its protonation), 1423 cm^{-1} (C-H stretching vibration), 1147 cm^{-1} (symmetric C-O stretching vibration), 1025 cm^{-1} (C-O-C stretching vibration), and 875 cm^{-1} (β-1,4-glycosidic stretching vibration). The formation of chitosan is indicated by the reduction of acetyl groups, marked by the decrease in absorption intensity in the wavenumber region of 1800-1600 cm^{-1}, particularly at the absorption peak at 1617 cm^{-1} (C=O stretching vibration) and 1552 cm^{-1} (N-H stretching vibration in acetamide), where the intensity in the chitin spectrum remains sharp [15].

3.2 Application of low-dd-chitosan as the coating agent.

The prepared low-deacetylation-degree (Low-DD) chitosan was subsequently applied as an edible coating in three different food models: apple slices, tofu, and Nile tilapia (*Oreochromis niloticus*) fillets. These foods were chosen for their varying textures and compositions, which allowed for a comprehensive evaluation of the effectiveness of the coating across diverse food matrices. The sensory attributes and color profiles of the coated samples were monitored and evaluated over periods of 0, 6, 12, 18, and 24 h to assess the impact of the low-DD chitosan coating on their quality. Coating concentrations of 2% were applied following the standard edible coating procedures. The application of edible coatings in the samples is shown in Fig. 3.



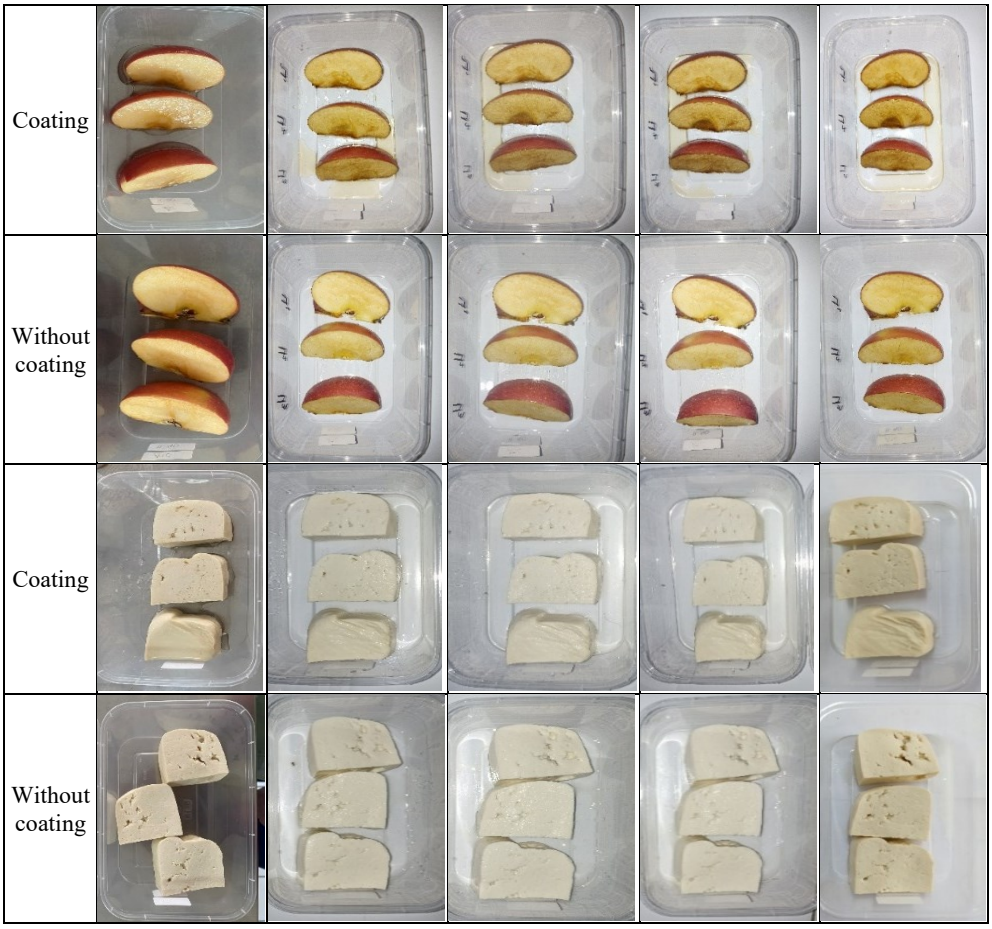


Fig. 3. Sample change with and without chitosan coating during room temperature storage

3.2.1 Sensory Evaluation

Sensory evaluation was performed by scoring tests between the coated and uncoated samples using the existing parameters. The sensory evaluation parameters of Nile tilapia fillets were flesh condition content, flesh, smell, and texture. The parameters for apple slices and tofu were color, smell, and texture.

Nile tilapia fillets

Sensory evaluation of Nile tilapia fillets was conducted by observing changes in the slime, flesh, odor, and texture of Nile tilapia fillets with and without chitosan coating. The sensory evaluation of Nile tilapia fillets with and without chitosan coating is shown in Fig. 4.

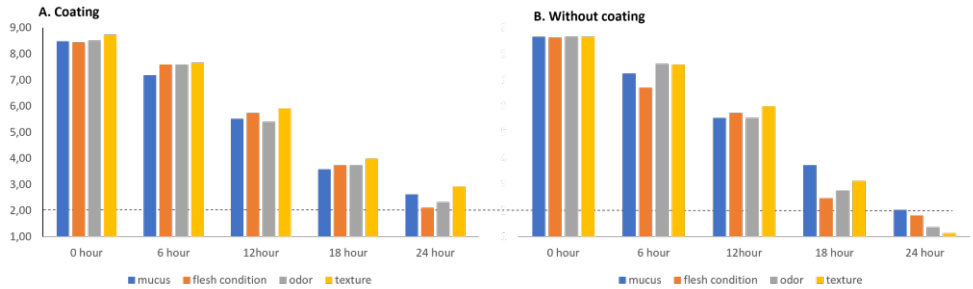


Fig. 4. Evaluation sensory coating and without coating Nile tilapia fillets

The results of the sensory evaluation showed that chitosan-coated Nile tilapia fillets had a significant effect compared to those without chitosan coating. The lowest average score during the 24-hour storage period was 2.62 (slightly thick slime, beginning to change color), while the lowest average score for Nile tilapia fillets without coating was 2.03 after 24 hours. This indicated the onset of spoilage during storage. The spoilage process in fish is characterized by thickening and discoloration of the mucus. Fish undergoing quality deterioration will become slimy and exhibit a grayish color [16]. This is because chitosan can function as an antimicrobial agent that helps inhibit spoilage in fish. This finding is consistent with the study by Mohan [17] which showed that chitosan coating on catfish extended the shelf life of the fish during storage.

Chitosan coating on Nile tilapia fillets also had a significant impact compared to uncoated fish. Fish coated with chitosan had an average score of 2.33 after 24 hours of storage, while the uncoated fish had an average score of 1.37. This difference may be due to chitosan's ability to inhibit bacterial growth, thereby preventing the fish from developing a foul odor [17]. A decrease in fish quality with respect to texture also showed a significant effect. Fish coated with chitosan had an average texture score of 2.92 after 24 h of storage, while the uncoated fillet scored 1.14 after 24 h of storage. The texture of the fish fillet, which was firm, compact, and very elastic at the beginning of storage, became less elastic and soft by the end of storage. Utami [18] reported that cold-stored catfish fillets experienced a decline in texture value, with the flesh becoming watery by the 14th day of storage. Changes in texture occur during the storage period owing to chemical and enzymatic reactions, as well as increased water activity [19].

Apple slices

Sensory evaluation of the apple slices was conducted by observing changes in the color, texture, and odor of apples with and without chitosan coating. The sensory evaluation of apple slices with and without chitosan coating is shown in Fig. 5.

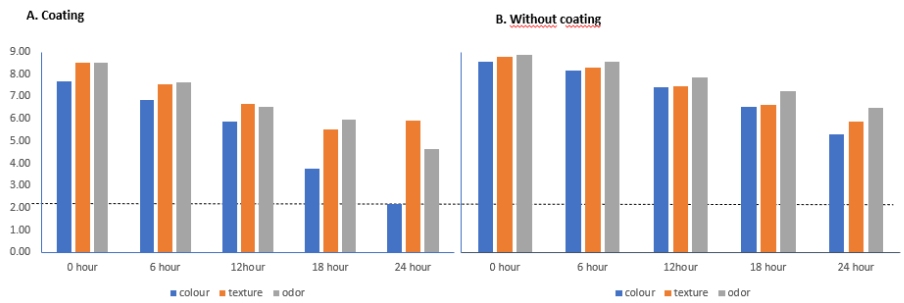


Fig. 5. Evaluation sensory of coating and without coating apple slices

The test results showed that after 24 h of storage, apple slices without coating received a score of 5.30, which was higher than that of apple slices with chitosan coating (2.18). This is likely due to the chitosan coating protecting the apple's surface and giving it a shiny appearance, consistent with the statement by Santoso [20], that edible coatings improve the surface structure of materials, making them glossy. A difference was also observed in the texture parameter of coated apple slices during 24-hour storage, where the lowest average score was 3.74, whereas apple slices without chitosan coating received a score of 5.85. A lower respiration rate in coated fruits leads to delayed ripening and reduces texture degradation during storage [21].

The sensory evaluation of tofu was conducted by observing changes in color, texture, and odor with and without chitosan coating. The lowest average sensory evaluation score for color was found in coated tofu after 24 h of storage, with a score of 6.41, while uncoated tofu received a score of 4.44 after 24 h. This is likely because the chitosan solution used was white and clear; therefore, the coating did not significantly alter the color of the tofu. Chitosan coatings can control the transfer of dissolved solids, thereby maintaining the natural color of food products and serving as carriers for additives such as colorants to improve food quality [22].

Tofu

The sensory evaluation of tofu was conducted by observing changes in the color, texture, and odor of tofu with and without chitosan coating. The sensory evaluation of tofu with and without chitosan coating is shown in Fig. 5. Changes in tofu texture with chitosan coating were also observed, with the lowest average sensory evaluation texture score of 6.30 after 24 h of storage, compared to 4.30 for uncoated tofu. According to Novianti [23], texture is largely determined by the water content. Other factors influencing food texture, as stated by Fellows [24], include the processing temperature, fat content, protein, ratio, water content, and water activity. Water, which is a medium for bacterial growth, promotes spoilage activity, and bacterial degradation of chemical compounds that can damage food texture is inhibited by chitosan coating. On the other hand, tofu without coating becomes softer because there is no layer to prevent the transfer of water vapor and other contaminants into the product.

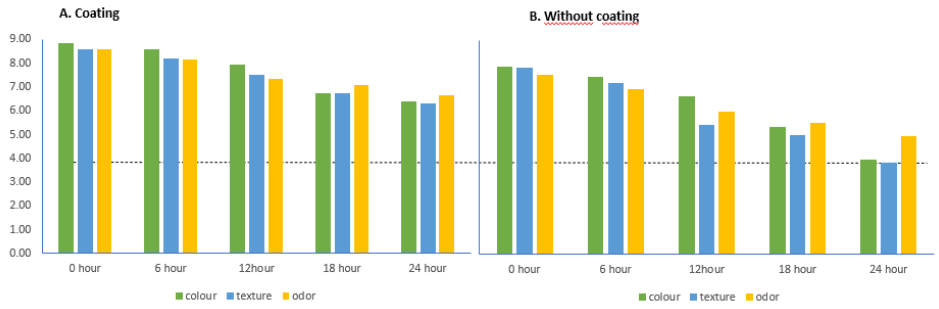


Fig. 6. Evaluation sensory of coating and without coating in tofu samples

The odor parameter in tofu with chitosan coating had the lowest average sensory evaluation score 6.63 after 24 h of storage, while uncoated tofu scored 5.52 after 24 hours. Odor is often used to determine a person's preference for a food product based on the smell emitted. The antimicrobial properties of the chitosan solution can inhibit the growth and activity of spoilage microbes, which can produce a sour odor from ammonia compounds (NH_3) [25].

3.3 Colour profile

The color test is a key parameter for observing changes in Nile tilapia fillets, apple slices, and tofu with and without chitosan coating during storage at room temperature. The color changes were analyzed using CIE L* a* b* coordinates. The CIE Lab values were entered into the calculation formula to obtain the ΔE value. The smaller the ΔE value, the closer the sample color is too white, while a higher ΔE value indicates a darker color of the sample.

Table 2. Color changes in fish, apple slices and tofu with and without chitosan coating during room temperature storage

Treatment	Sample	ΔL	Δa	Δb	ΔE
Coating	Apple slices	-17.75±7.75	3.31±1.4	8.09±2.31	20.27±6.2
	Nile fillets	22.99±24.13	-14.44±5.39	8.48±10.43	32.12±19.64
	Tofu	-4.95±3.52	-3±1.76	0.78±3.41	5.63±4.29
Without Coating	Apple slices	-27.49±9.72	7.11±2.32	19.83±4.47	42.06±7.72
	Nile fillets	-0.71±14.64	-9.32±3.66	7.44±3.77	17.15±3.89
	Tofu	-17.75±7.75	3.31±1.4	8.09±2.31	20.27±6.2

The color index is an important parameter for assessing the quality of food products. The effect of coating food products with chitosan versus not coating them can result in noticeable color differences, which can be analyzed using CIE L*a*b* coordinates. The color test results on apple slices (Table 2) showed varying lightness (L*) values at each hour. Both coated and uncoated apple slices exhibited a gradual decrease in lightness over time, resulting in browning. This is likely due to enzymatic browning in phenolic compounds when cells are exposed to oxygen [26]. After 24 h, the uncoated apple slices were found to have a lighter color than those coated with chitosan. This is consistent with the study by Assis [27], which reported that control apple slices had a lighter color than those coated with chitosan. The color difference (ΔE*) between uncoated and chitosan-coated apple slices ranged from 8.541 to a maximum of 16.955. The color of the apple slices gradually darkened or browned, producing specific color changes that were observed in the CIE Lab values. If the treatment time is extended, the apple slices become darker. These findings suggest that while chitosan coating has a positive effect on preserving mass and firmness, it does not effectively control browning in apple slices during room-temperature storage. This could be due to the interaction of chitosan with water absorption and pH effects.

The color change in Nile tilapia fillets with and without chitosan coating, as indicated by the ΔE* values, decreased over time. The color of the fish at 0 h of storage had a ΔE* value of 23.847, whereas the lowest value after 24 h of storage dropped to 3.983. The longer the fish was stored at room temperature, the more the color deviated from that of the control (0 h). The fish flesh color changed from reddish to yellowish-white, indicating a decline in the quality of tilapia fillets due to autolysis. Autolysis is the destruction of cells or tissues caused by ongoing enzymatic activity and biochemical reactions in fresh fish [28]. This process causes fish, particularly those without a chitosan coating, to spoil more rapidly.

Based on the ΔE* color calculation results for tofu samples with and without chitosan coating, the color changes from 0 to 24 h showed values ranging from 0.416 to 2.961. For tofu samples observed from 0 to 24 h of storage, the ΔE* value increased, indicating slight

color changes over time from white at 0 h to yellowish-white at 24 h. The smaller the ΔE* value, the more similar is the color between the two objects being studied. Conversely, a larger ΔE* value indicates a greater color difference between the two objects [29]. The chitosan-coated tofu treatment did not effectively control the color during storage. However, the coating treatment could control the aroma and firmness of tofu samples during storage.

4 Conclusions

The potential of low-deacetylation-degree (Low-DD) chitosan, derived from shrimp waste, has been highlighted as a viable and cost-effective edible coating for preserving the quality of various perishable food products such as Nile tilapia fillets, apple slices, and tofu. It was demonstrated that Low-DD chitosan effectively maintained texture and inhibited spoilage in fish and tofu, while oxidation in apple slices was delayed. Sensory evaluations confirmed that the application of chitosan coatings significantly enhanced the texture and aroma of the coated products during storage. Although browning was observed in apple slices, the oxidation process was delayed compared to uncoated samples. The use of Low-DD chitosan has been presented as a sustainable alternative to high-DD chitosan, requiring fewer resources and less energy for production. This research has contributed to the development of cost-effective and environmentally friendly food preservation methods, promoting sustainable practices within the food industry. Future research is recommended to focus on optimizing the chitosan formulation to improve browning control and explore its application across a wider range of food products.

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