

The Impact of Different Molecular Weight Chitosan Dip-coating on the Quality of *Monopterus albus* during Cold Storage

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Abstract: In this research, the impacts of chitosan with different molecular weights (30, 150, 300 kDa) on the quality of *Monopterus albus* during refrigeration storage were investigated in terms of total colony count (TCC), pH, TVB-N, water loss rate, and cooking loss rate. The results indicated that the 150 kDa chitosan dip-coating demonstrated superior performance in extending the shelf life of *Monopterus albus* by preserving tissue structure, reducing cellular damage, and moisture loss. This research would supply new insights into widening chitosan as natural preservative in aquatic product preservation.

1. Introduction

Monopterus albus, a nutrient-rich freshwater fish, is widely popular among consumers globally due to its tender meat, palatable taste, and various dietary and therapeutic benefits, including nourishing qi and blood, enhancing immunity, protecting vision, and regulating blood lipids. However, it is susceptible to spoilage, leading to quality deterioration during transportation and storage. Consequently, the development of effective preservation techniques for *Monopterus albus* has garnered significant attention in recent years. Dip-coating with antioxidants and bacteriostatic agents has been demonstrated to be effective. Synthetic agents including butylated hydroxyanisole, dibutyl hydroxytoluene, sodium sorbate and sodium dehydroacetate are extensively utilized across the food sector on account of their cost advantages and scalable production characteristics. Nevertheless, their application is limited by potential toxic side effects, including risks of carcinogenesis, teratogenesis, and mutagenesis. Therefore, the exploitation of natural coating-type preservatives is urgently needed to ensure the quality and safety of *Monopterus albus*.

Chitosan, a naturally cationic polysaccharide, is derived from chitin through a deacetylation process^[1]. Owing to its intrinsic properties, such as antibacterial activity, film-forming capability, and biodegradability, chitosan has been widely utilized as a versatile preservative in the food industry. It is broadly employed in preserving fruits and vegetables, aquatic products, meat, and grains/oils^[2]. Numerous studies have demonstrated its ability to delay food quality deterioration, extend shelf life, and maintain the inherent characteristics of products. However, the preservative effects of chitosan on the quality of *Monopterus albus* have been scarcely

investigated. Furthermore, the molecular weight of chitosan has been identified as a critical factor influencing its preservative efficacy^[3]. Therefore, further research is warranted to explore the impact of chitosan dip-coatings with varying molecular weights on the quality of *Monopterus albus* during storage.

This study seeks to enhance the understanding of the impact of chitosan with varying molecular weights on the quality of *Monopterus albus*, specifically focusing on water retention capacity, microbial inhibition efficacy, and structural changes in tissue during refrigeration. The findings of this research would offer theoretical support for the further optimization of preservation techniques for *Monopterus albus*.

2. Materials and Methods

2.1 Materials

Chitosan were kindly supplied by Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Tryptone and yeast extract paste were bought from Sangon Biotech (Shanghai) Co., Ltd. Agar was bought from Shanghai Lanji Technology Development Co., Ltd. (Shanghai, China). Except for the specially designated reagents of specific purity, all other chemical agents were of at least analytical grade.

2.2 Preparation of Chitosan Dip-coating Solution

1.0% dip-coating chitosan solutions were prepared by dissolving weighted amount of chitosan (30, 150, 300 kDa) into 1.0% (w/v) acetic acid solution, followed by magnetic stirring for 4 h.

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2.3 Preparation of *Monopterus albus* Samples

Monopterus albus samples were prepared following the methodology reported by Yu et al.^[4]. Briefly, fresh *Monopterus albus*, after descaling and evisceration, was cut into uniform 4 cm segments and immersed in chitosan solutions or deionised water (control group). After two hours, the samples were retrieved, drained, and stored in polyethylene bags at 4°C for subsequent experimental analysis.

2.4 Determination of Total Colony Count (TCC)

The total colony count (TCC) in the samples during storage was assessed using the pure plate method^[5]. Briefly, samples were collected at predetermined time intervals and homogenized under aseptic conditions. A 25.0 g of the homogenate was combined with 225 mL of sterile physiological saline, homogenized, and serially diluted to achieve 3–5 appropriate concentrations. Subsequently, 1 mL of each dilution was transferred to sterile petri dishes and incubated at 30 °C for 72 h. Microbial growth was monitored and documented every 4 h. The number of colonies at the end of the incubation period was calculated and expressed as log₁₀ CFU·g⁻¹.

2.5 Determination of pH Value

A 5.0 g sample of *Monopterus albus* homogenate (prepared as Section 2.4) was combined with 25 mL of distilled water and subjected to 1 min of homogenization, after which the pH of the resulting supernatant was assayed with an electronic pH meter (PHSJ-5, INESA, China).^[6]

2.6 Total Volatile Basic Nitrogen (TVB-N)

The TVB-N content of the samples was determined using the Kjeldahl method^[7]. 10.000 g sample was added into a vessel containing 75 mL of ultrapure water, thoroughly shaken, and then transferred to the Kjeldahl apparatus. Volatile basic nitrogen in samples was evaporate by heating, and the residual non-volatile nitrogen was quantified by titration. The TVB-N was calculated by subtracting the amount of non-volatile nitrogen from the total nitrogen.

2.7 Determination of Water Loss Rate

The water loss rate was determined according to the Hultmann method with a minor modification^[7]. The total mass of the freshly prepared packaged samples (W_1) and the mass of the packaging bag (W_3) were accurately measured. At predetermined storage time intervals, the *Monopterus albus* meat was removed from the packaging bag, and the total mass of the packaging bag along with residual juices was recorded (W_2). The water loss rate of the sample was calculated using the following formula.

$$\text{Water Loss Rate(\%)} = \frac{W_2 - W_3}{W_1 - W_3} \times 100\% \quad (1)$$

2.8 Determination of Cooking Loss

Steaming loss rate was assayed following the method of Shen et al.^[8]. *Monopterus albus* specimens were sectioned into cubes measuring 20 mm × 20 mm × 20 mm. Surface moisture was carefully removed using filter paper, and the initial mass was precisely measured and documented as m_1 . Subsequently, the *Monopterus albus* samples were placed in a steamer, subjected to steaming at 100°C for a duration of 15–30 minutes, after which they were left to cool to ambient temperature. Residual surface moisture was blotted gently using filter paper before the post-cooking mass of the samples was re-measured and recorded as m_2 . The cooking loss rate was derived by means of formula (2).

$$\text{Cooking Loss(\%)} = \frac{m_1 - m_2}{m_1} \times 100\% \quad (2)$$

2.9 Statistical Analysis

Every experimental procedure was replicated a minimum of three times, and the resulting data were reported in the form of average values and standard deviations. Figures were drawn using Origin 2021 software. The differences between mean values were determined with a significance level of $p < 0.05$.

3. Results and Discussion

3.1 Analysis of Total Colony Count

Microbial activity plays a crucial role in the degradation of fish quality during storage, with TCC values serving as a direct indicator of spoilage levels. As illustrated in Table 1, fresh *Monopterus albus* initially exhibited a TCC of 4.28 log₁₀ CFU·g⁻¹. Following a storage period of two days, the TCC increased to 4.83 log₁₀ CFU·g⁻¹ in the control group, whereas the chitosan-treated samples demonstrated varying degrees of reduction in TCC, ranging from 0.08 to 0.55 log₁₀ CFU·g⁻¹. This suggests that chitosan dip-coating effectively mitigated the initial bacterial load in *Monopterus albus* samples. As the storage period extended to four days, a steady increase in TCC was observed across all samples. However, after four days, a marked acceleration in microbial proliferation was noted, indicating significant microbial growth. According to the standards set by the ICMSF, a TCC exceeding 7 log₁₀ CFU·g⁻¹ renders the food unacceptable. It was obvious that the TCC in the control group had reached 8.22 log₁₀ CFU·g⁻¹ by the sixth day of storage, significantly surpassing the spoilage threshold. In contrast, the chitosan-coated groups exhibited a significant TCC reduction relative to the control group, yet remained within the acceptable threshold. This finding confirms that chitosan dip-coating effectively inhibits microbial growth during the storage of *Monopterus albus*. This inhibitory effect stems from the antibacterial activity of chitosan, which is tightly linked to its cationic character, allowing it to interact with the cellular components of microorganisms^[9]. Furthermore, among the various

groups, the samples coated with 150 kDa chitosan exhibited the lowest TCC levels throughout the entire storage period, demonstrating its superior antimicrobial activity.

Table 1. Effect of chitosan dip-coatings of different molecular weights on microbial enumeration of *Monopterus albus* during refrigeration.

Item	Group	Storage time/d						
		0	2	4	6	8	10	12
Total colony count	30 kD	4.28	4.12	4.80	6.80	7.10	9.21	12.30
	150 kD	4.28	4.20	4.69	6.28	6.83	8.83	11.83
	300 kD	4.28	3.99	4.80	6.56	6.90	9.09	12.19
	Control group	4.28	4.83	5.26	8.22	7.84	9.84	12.84

3.2 pH Measurement Results

As illustrated in Figure 1(A), the pH levels of all samples demonstrated a similar dynamic pattern, characterized by an initial decline followed by an increase. This observation result is consistent with the conclusion

presented in Section 3.1. Notably, the control group reached its lowest pH on the second day of storage, after which it rapidly rebounded, surpassing 7.0 by the sixth day. This variation in pH may be associated with the post-mortem processes and degeneration of muscle [10]. The decrease in pH can be attributed to anaerobic glycolysis, which produces lactic acid and ATP, thereby lowering the pH. Conversely, the subsequent elevation in pH may result from microbial metabolism, which generates alkaline nitrogenous compounds [11]. Furthermore, the three coated groups exhibited a more pronounced reduction in pH compared to the control group during the initial storage. This may be due to the inherently weak acidity of the chitosan solution. From the fourth day of storage onwards, the pH of the chitosan coated fillets demonstrated a gradual upward trend. However, this increase was not pronounced, and there were no significant differences observed across the various chitosan groups. However, in comparison to the control group, the pH levels in the chitosan-coated groups were lower. This distinction may be attributed to the ability of chitosan to inhibit the production of alkaline nitrogenous compounds resulting from microbial metabolism.

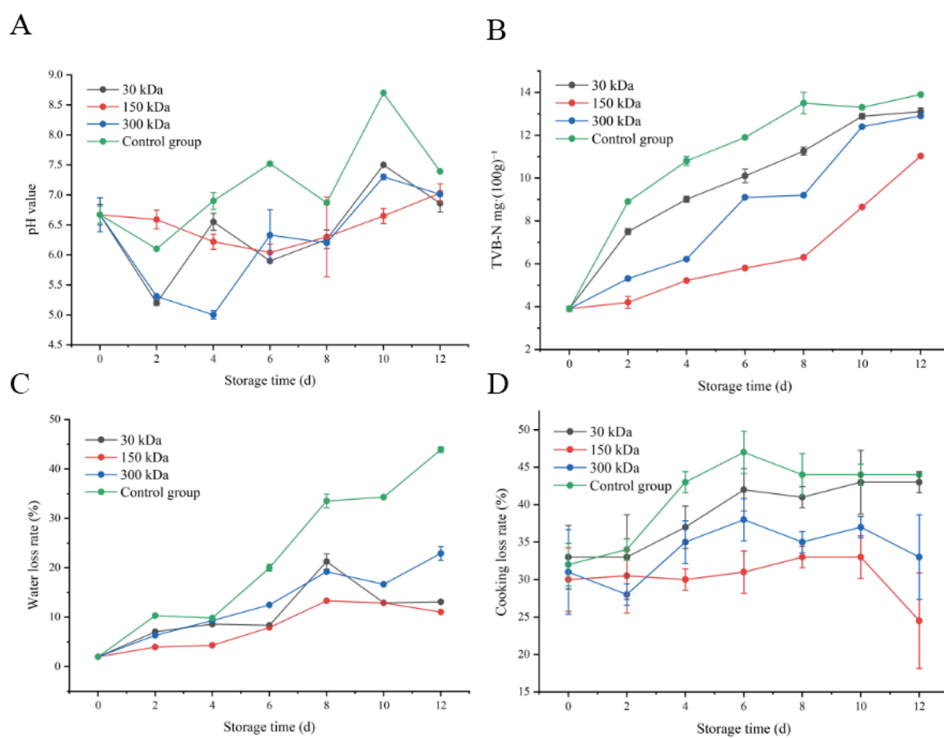


Figure 1. Effects of chitosan dip-coatings with different molecular weights on the pH (A), TVB-N (B), water loss rate (C) and cooking loss rate (D) of *Monopterus albus* during refrigeration.

3.3 Determination of TVB-N

TVB-N, a key index in fish freshness assessment, denotes volatile substances produced by the microbial degradation of proteins as well as non-protein nitrogenous compounds. Figure 1(B) illustrates the changes in *Monopterus albus* during refrigerated storage. Fresh samples exhibited a TVB-N content of 3.90 mg·100g⁻¹. After 12 days of storage, the TVB-N content in the control group reached

13.90 mg·100g⁻¹, representing a threefold increase from the initial value. In contrast, the rate of TVB-N accumulation was significantly reduced in all chitosan-coated groups. Specially, the TVB-N levels in the experimental groups appeared to significantly lower than those observed in the control group since the second day. This finding suggests that chitosan dip-coating effectively retards the accumulation of TVB-N in *Monopterus albus* fillets. The phenomenon may be attributed to chitosan's ability to inhibit spoilage bacteria. According to Yu et al's

report that the 12–15 mg·100g⁻¹ is the spoilage threshold. Obviously, the control group reached a level of 13.50 mg·100g⁻¹ by the eighth day, whereas groups treated with 30 kDa and 300 kDa chitosan surpassed this threshold shoule by the tenth day^[12]. Notably, the 150 kDa chitosan group exhibited a TVB-N of 11.03 mg·100g⁻¹ at 12 days, indicating a reduction of about 15% less than the control group. This findings demonstrated its good effective inhibition in degradation, corroborating the microbial count results.

3.4 Results of Water Loss Rate Measurement

Water loss rate, serving as a critical indicator, could effectively reflect the loss of fish juices during storage^[13]. Excessive water loss means deterioration in fish quality, dull colouration, and nutrient depletion, thereby significantly diminishing the product's commercial value. Figure 1(C) illustrates that, although all groups followed similar trends, the rate of water loss in *Monopterus albus* slices was generally lower in all chitosan-coated groups compared to the control group under refrigeration at 4 °C. During the initial 2 days of storage, the control group exhibited significantly higher water loss rates than the chitosan-coated groups. However, in the subsequent storage period, the increase in water loss rate was less pronounced in the coated groups. By the eighth day of storage, the water loss rates for the 30 kDa, 150 kDa, and 300 kDa chitosan-treated groups were 63.5%, 39.7%, and 57.3% of the control group, respectively. The juice loss from *Monopterus albus* slices was primarily due to water evaporation and a decrease in muscle water retention capacity, attributed to alterations in protein structure. The water-retaining effect of the chitosan dip-coating can be attributed partly to its intrinsic water-holding properties and partly to its potential to suppress the breakdown of muscle structural proteins, thus enhancing the water retention capacity of the *Monopterus albus*^[14]. Additionally, the results suggest that, although no significant differences were observe compared to the other two molecular weight chitosan-treated groups, the 150 kDa chitosan-treated group demonstrated optimal efficacy in inhibiting juice loss ($p < 0.05$).

3.5 Results of Cooking Loss Rate Measurement

The cooking loss rate serves as a key indicator characterising the loss of moisture and nutrients during food preparation, directly impacting both nutritional value and edible quality^[15]. Figure 1(D) demonstrates that the cooking loss rate of *Monopterus albus* slices coated with chitosan was generally lower than that of the control group. The 300 kDa chitosan group exhibited a significantly lower loss rate than other groups during the first two days. Subsequently, the loss rates of all groups rose steadily over the course of storage, with the chitosan-coated groups consistently maintaining lower rates than the control group. Cooking losses in *Monopterus albus* primarily stem from water evaporation and reduced water-holding capacity due to altered protein structure. Chitosan dip-coatings, with their excellent water retention

properties and inhibitory effect on muscle structural protein degradation, significantly mitigate moisture and nutrient loss during cooking^[16]. Results indicate that 150 kDa and 300 kDa chitosan treatments demonstrated superior performance in reducing cooking losses, with the 150 kDa chitosan exhibiting the best pronounced effect.

4. Conclusion

With the analysis of the above-mentioned results and the corresponding discussions, the following conclusions are reached:

- (1) Chitosan dip-coatings may reduce the impact of moisture and oxygen on *Monopterus albus* by forming a barrier layer, thereby extending their shelf life.
- (2) The amino and hydroxyl functional groups of chitosan may inhibiting microbial growth from in *Monopterus albus*.
- (3) Chitosan dip-coatings of varying molecular weights demonstrate positive effects in extending *Monopterus albus* shelf life, with the 150kDa chitosan dip-coating proving superior effective in controlling microbial growth, maintaining *Monopterus albus* sensory quality, and prolonging freshness.

Acknowledgments

This work was funded by the Natural Science Foundation of Jiangxi Province (20232BAB205083 and 20252BAC250074), Postgraduate Innovation Special Fund Project of Jiangxi Provinc (YC2024-X40) and the National Natural Science Funds of China (32560586).

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