Effect of a chitosan-alginate bilayer coating incorporated with lysozyme on quality of refrigerated turbot fillets

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Abstract. Preservation properties of chitosan (CS) and sodium alginate (ALG) coatings are widely investigated, but their bilayer composite coating application to fish preservation is limited. The aim of present study was to evaluate the quality changes in 4°C turbot fillets treated with a bilayer coating based on lysozyme-CS (LZ-CS) coating layer and ALG coating layer. Changes in total viable counts (TVC), texture profiles, and physiochemical indexes including pH, total volatile basic nitrogen (TVB-N), and K value were examined. The results indicated that bilayer coating was more effective than LZ-CS and ALG monolayer coatings in preservation of turbot fillets. The bilayer coating significantly inhibited the increase of TVC, pH, TVB-N, and K value, and maintained better texture characteristics of turbot fillets during storage. Therefore, the bilayer coating on the turbot is more superior to monolayer coatings, which would have potential applications on fish preservation.

1. Introduction

Edible coatings which are based on biodegradable and edible biopolymers (polysaccharides, proteins and lipids) have been widely used to improve quality of various food products during storage due to its high efficiency, safety and eco-friendly [1]. It is more beneficial to improve the properties of coatings by combining multiple polymers [2]. Therefore, blends and multilayers contained multiple polymers are a priority for coatings application. The multilayer coatings are often prepared by a layer-by-layer approach. In this approach, electrostatic interactions occur among oppositely charged biopolymer in different layers, which gives the coatings some better properties [2]. Studies of multilayer coatings have been conducted in preservation of rainbow trout and many fruits [3-4].

Chitosan is a rare cationic polysaccharide in the nature because a number of amino groups present [5]. Different from chitosan, alginate extracted mainly from brown sea algae is frequently used as a natural anionic polysaccharide for the electrostatic assembly [5]. Although alginate and chitosan both have a lot of advantages in the coating preservation field, they cannot be applied as a composite component coating upon mixing since a homogeneous solution is not formed [4]. Therefore, multilayer coatings for preservation by layer-by-layer approach based on polysaccharides like alginate and chitosan are necessary.

Lysozyme is a kind of natural antibacterial preservative. It was reported that the chitosan coatings incorporating lysozyme can effectively maintain stability and activity of lysozyme and improve antibacterial ability of coatings [6]. In this study, bilayer coatings are prepared based on lysozyme-chitosan (CS-LZ) coating layer and ALG coating layer. The effect of the bilayer coating on qualities of refrigerated turbot fillets was evaluated, and compared with the effect of single-layer coating.
2. Materials and methods

2.1. Materials

Chitosan (CS, deacetylation degree >95%, 200-400 mPa.s), sodium alginate (ALG, 200±20 mPa.s), and lysozyme (LZ, ~70000 U/mg) were purchased from Aladdin Chemical Reagent Co., China, Aladdin Chemical Reagent Co., China and Sigma Aldrich China, respectively. Microbial culture mediums were purchased from Qingdao Haibo Biotechnology Co., Ltd., China. Other regents were commercial sources and were analytically grade. Turbots were obtained from an aquatic product market in Jinzhou (China), and rapidly transported to the laboratory alive for further analysis.

2.2. Preparation of coatings

CS and ALG were dissolved in 1% (v/v) of acetic acid and sterilized deionized water to obtain 15 mg/mL CS solution and 15 mg/mL ALG solution, respectively. Then, 0.3% (v/v) glycerol and 0.3 mg/mL LZ were added to CS solution to obtain LZ-CS coating solution, and 0.3% (v/v) glycerol were added to ALG solution to obtain ALG coating solution. Finally, both solutions were mixed at 55℃ on a magnetic stirrer until complete dissolution and were degassed by ultra-sonication at 53 kHz, 224 W, at 30 ℃ for 15 min before using.

2.3. Preservation effect of coatings on turbot fillets

Fresh turbot dorsal fillets were randomly divided into four groups. The control fillets were uncoated. Fillets in ALG and LZ-CS groups were dipped into ALG and LZ-CS coating solutions for 5 min and were dried in air, respectively. Fillets in bilayer group were dipped in LZ-CS first and then in ALG solution for 5 min then dried in air. At last, fillets were individually packaged into a sterile polyethylene bag and stored in a refrigerator (4℃) before subsequent analysis. Fillets from each group were randomly selected for freshness assessment at 3-day intervals until 21 days.

TVC, pH, TVB-N, and K value were measured following the method of Xu et al. [7]. Fillets cut to size (1.5 × 1.5 × 1.0) cm³ were used for texture profile analysis at room temperature using a TA-XT plus texture analyzer (Stable Micro Systems Ltd., Godalming, UK) with a 5 mm diameter P/50 probe. The sample were compressed twice with 50% compression degree. Test speed was 1 mm/s and recovery time was 2 s. Then, parameters for hardness, cohesiveness, adhesiveness, springiness, chewiness, gumminess, and resilience were obtained.

2.4. Statistical analysis

All tests data in this study were expressed as average with standard deviations (SD) at least in triplicate. SPSS19.0 were used for one-way analysis of variance (ANOVA), using $P < 0.05$ as level of significance. Origin 8.5 was used to produce the graphs.

3. Results and discussion

3.1. TVC

TVC in each group presented a gradual increasing trend (Fig. 1A). In the early days of storage, TVC in LZ-CS and bilayer coating group has barely increased and the phenomenon lasted for six days storage, while TVC in control and ALG group linearly increased, which may due to the antibacterial activities of LZ and CS [6]. The value of TVC in control and ALG reached to the proposed limits (6~7 lg CFU/g) for fresh fish on the 6th day [8], while TVC in LZ-CS and bilayer group had successively exceeded the value of 6 lg CFU/g on day 12 of storage, indicating LZ-CS and bilayer coating can extend the microbiological shelf life. Furthermore, the bilayer group possessed significantly lower ($P < 0.05$) TVC than LZ-CS group, which was maintained until the end of the storage. The results indicated that LZ-CS and bilayer have excellent antibacterial effects and can extend the turbot’s shelf life of 4-6 days. In addition, the effect of bilayer coating is better than LZ-CS, which may due to the better barrier properties of bilayer coating.
3.2. pH, TVB-N, and K value

pH, TVB-N, and K value are all important indicators to evaluate freshness of fish, which reflect chemical change of fish during storage\(^7\). The pH in all groups decreased to minimum value in the first 3 days of storage and subsequently increased (Fig. 1B). The pH in coating groups are all lower than control during the whole storage time, particularly in bilayer coating group, suggesting that bilayer coating was more effective than other coatings to reduce the production of alkaline substances by inhibiting the microbial growth or activity of endogenous enzymes\(^9\). TVB-N, and K value of turbot fillets continually increases during whole storage (Fig. 1C, 1D). TVB-N is produced by protein degradation under the action of enzymes and microorganism during fish storage\(^10\). K value increase with the accumulation of HxR and Hx is an index of ATP degradation\(^11\). The results indicated that the bilayer coating was more effective for slowing down the TVB-N production and ATP degradation than other coatings. The differences of antibacterial activity in each coating may be the main reason. In addition, coatings can reduce water loss of fillets or act as a barrier for oxygen, which also may influence the TVB-N contents and K value of samples.

3.3. Texture analysis.

The texture of fish is related to consumer acceptability\(^12\). The hardness, springiness, chewiness, resilience and cohesiveness of turbot fillets all decrease during the storage (Fig. 2 A-E). The reduction of all the texture indices mainly because of the coagulation and degeneration of proteins in fish and the destruction of skeleton proteins, which weakened the ability of myofibrillar protein to form a spatial network structure\(^7,10\). Moreover, all the texture indices showed slowly decrease in coatings compared with control, and the bilayer coating effectively delayed the reduction of texture quality compared with monolayer coatings. It may be that the bilayer coating ensures that the fish hold enough moisture, while isolating oxygen for fillets and passivating the activity of microorganisms and endogenous hydrolases in the fish meat, ensuring the integrity of myofibrils and collagen substances.

4. Conclusion

The present results strongly suggested that bilayer composite coating based on CS and ALG incorporated with LZ is improved compared with that of monolayer coating in maintaining the quality of turbot fillets during refrigerated storage. The bilayer coating effectively inhibited microbial growth, pH, TVB-N and K value, and maintain better texture profiles quality. The bilayer coating on the turbot is more superior to monolayer coatings, which would have potential applications on fish preservation.

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Fig. 2 Changes in texture profiles of turbot fillets treated with coatings during 4°C storage

References

[1] Dehghani S, Hosseini SV, Regenstein JM. (2018). Edible films and coatings in seafood preservation: A review. Food Chemistry, 240, 505-513.
[2] Zhuang C, Jiang Y, Zhong Y, et al. (2018). Development and characterization of nano-bilayer films composed of polyvinyl alcohol, chitosan and alginate. Food Control, 86, 191-199.
[3] Nowzari F, Shábanpour B, Ojagh SM. (2013). Comparison of chitosan-gelatin composite and bilayer coating and film effect on the quality of refrigerated rainbow trout. Food Chemistry, 141, 1667-1672.
[4] Poverenov E, Danino S, Horev B, et al. (2014). Layer-by-layer electrostatic deposition of edible coating on fresh cut melon model: Anticipated and unexpected effects of alginate-chitosan combination. Food & Bioprocess Technology, 7, 1424-1432.
[5] Cazón P, Velazquez G, Ramírez JA, et al. (2017). Polysaccharide-based films and coatings for food packaging: A review. Food Hydrocolloids, 68, 136-148.
[6] Li X, Tu H, Huang M, et al. (2017). Incorporation of lysozyme-rectorite composites into chitosan films for antibacterial properties enhancement. International Journal of Biological Macromolecules, 102, 789-795.
[7] Xu Y, Yin Y, Li T et al. (2020). Effects of lysozyme combined with cinnamaldehyde on storage quality of olive flounder (Paralichthys olivaceus) fillets. Journal of Food Science, 2020, 85(4), 1037-1044.
[8] Ojagh SM, Rezaei M, Razavi SH, et al. (2010). Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food Chemistry, 120, 193-198.
[9] Yu D, Xu Y, Jiang Q, et al. (2016). Effects of chitosan coating combined with essential oils on quality and antioxidant enzyme activities of grass carp (Ctenopharyngodon idellus) fillets stored at 4°C. International Journal of Food Science & Technology, 52(2), 404-412.
[10] Cai L, Wang Y, Cao A, et al. (2015). Effect of alginate coating enriched with 6-gingerol on the shelf life and quality changes of refrigerated red sea bream (Pagrosomus major) fillets. RSC Advances, 5, 36882-36889.
[11] Hong H, Regenstein JM, Luo Y. (2017). The importance of ATP-related compounds for the freshness and flavor of post-mortem fish and shellfish muscle: A review. Critical Reviews in Food Science and Nutrition, 57(9), 1787-1798.
[12] Wei X, Li Q, Hao H, et al. (2020). Preparation, physicochemical and preservation properties of Ti/ZnO/in situ SiOx chitosan composite coatings. Journal of the Science of Food and Agriculture, 2020, 100(2):570-577.