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Effect of collagen-lysozyme coating on fresh-salmon fillets preservation

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ABSTRACT

The effect of collagen-lysozyme (CL1, 0.1% lysozyme+4% collagen; CL2, 0.3% lysozyme+4% collagen; CL3, 0.5% lysozyme+4% collagen; CL4, 0.7% lysozyme+4% collagen, w/v) edible coatings on preserving fresh-salmon fillets (*Salmo salar*) were evaluated over a 15 day storage at refrigerated temperature (4 ± 1 °C). Physical, chemical, microbiological, and sensory analyses were performed during storage. Results demonstrated that all treatments significantly improve the preservation quality of fresh-salmon fillets ($P < 0.05$). Especially, CL4 decreased the total volatile basic nitrogen (TVB-N) values and inhibited the growth of bacterial better than other treatments, however presented prejudicial to the overall acceptability of the samples. CL3 had the best effect on reducing the weight loss of the samples. Nevertheless, there were no obvious differences on the color and pH between the control and the treated samples ($P > 0.05$). The results indicated that CL coating was effective for the preservation of fresh-salmon fillets.

1. Introduction

Fresh salmon is a ready to eat seafood product which lacks a thermal microbial inactivation step. They are widespread and consumed around the world due to their high nutrients availability but also be easy to spoil (Lerfall et al., 2015). The spoilage is usually dominated by both microbial activity and chemical changes, such as autoxidation, enzymatic hydrolysis of the lipid fraction, and tissue enzyme activity (Lerfall & Rotabakk, 2016; Leroi et al., 2015; Perera, Abuladze, Li, Woolston, & Sulakvelidze, 2015; Sampels, 2015). Recently some methods have been adopted to inhibit the spoilage and improve the quality of fresh salmon (Kong, Alcicek, & Balaban, 2015; Park et al., 2015).

Among these methods, edible films were highly considered which could exhibit several advantages such as edibility, biodegradability, bio-compatibility and barrier properties (Hui, Liu, Feng, Li, & Gao, 2016; Oh, Roh, & Min, 2016; Padrao et al., 2016). Edible films have proved to enhance the shelf-life and safety of fish, which could retard the decay of fish by delaying volatile loss, decreasing the water vapor permeability as well as other quality deterioration processes (Kester & Fennema, 1986; Krochta & DeMulderJohnston, 1997; McHugh, Avenabustillos, & Krochta, 1993; Miller & Krochta, 1997; Tharanathan, 2003). Proteins, polysaccharides and lipids are the main components of edible films and coatings. Films made from proteins and polysaccharides are excellent barriers to oxygen, because of the tightly packed, ordered structure of their hydrogen-bonded network, films made from lipids have good water vapor barrier properties (Dutta, Tripathi, Mehrotra, & Dutta, 2009; Miller & Krochta, 1997; Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998).

In recent years, collagen has attracted great attention as edible films due to its biodegradability, bio-compatibility, and wide availability (Borges, Silva, Cervi-Bitencourt, Vanin, & Carvalho, 2016; Kuan, Nafchi, Huda, Ariffin, & Karim, 2016; Rath, Hussain, Chauhan, Garg, & Goyal, 2016). Collagen is the main structural protein in the extracellular space in the various connective tissues in animal bodies. It has been widely applied in food, pharmaceutical, medical and photography industries because of its superior gelling, thickening and film-forming properties (Malcor et al., 2016; Tongnuanchan, Benjakul, Prodpran, Pisuchpen, & Osako, 2016).

Lysozyme is a food grade antimicrobial enzyme with bacteriostatic, bacteriolytic and bactericidal activity, particularly against Gram-positive bacteria; and efficient in controlling the growth of a great number of food pathogens. It has been embodied in several

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biodegradable matrices-used to preserve various food types, due to its high stability over a wide range of pH and temperature (Branen & Davidson, 2004; Cagri, Ustunol, & Ryser, 2004; Hughey & Johnson, 1987).

Thus, the present study was aimed to prepare collagen-lysozyme coating and investigate its effect on the quality of fresh-salmon fillets during refrigerated storage (4 ± 1 °C). For achieving this aim, a number of parameters, physical characteristics (weight loss, color, texture), chemical (pH, TVB-N), microbiological (total viable count, TVC) and sensory analyses were measured.

2. Materials and methods

2.1. Materials

Collagen were obtained from Hainan Yuantai Technology Co., Ltd. Lysozyme were from Zhengzhou Mingrui chemical products Co., Ltd. *Salmo salar* were purchased from WalMart supermarket in Shenzhen. Distilled water was used for all sample preparations. All other chemicals used were analytical grade.

2.2. Edible coating solution preparation

Collagen solution (4%, w/v) was prepared by dissolving collagen in distilled water at a controlled temperature (40 °C). Then, lysozyme (0.1 g, 0.3 g, 0.5 g, and 0.7 g) was separately added to the collagen solution to a final concentration (CL1, 0.1%; CL2, 0.3%; CL3, 0.5%; CL4, 0.7%, w/v). Glycerol (1%, w/v) was added as a plasticizer. The collagen-lysozyme (CL) coating was stirred until the mixture became clear, and stored at 4 ± 1 °C then warmed to room temperature for at least 2 h before use.

2.3. Preparation of the fresh-salmon fillets

Fresh-salmon (Atlantic salmon) as purchased from a local supermarket, and the head, bone and skin were removed. They were randomly divided into 93 polyethylene bags. Fifteen over 93 bags were used for weight loss: five treatments (the control, CL1, CL2, CL3 and CL4 treatment) × three replicates. The remaining bags prepared for general quality measurements were divided into the following treatments/replicates: five treatments (the control, CL1, CL2, CL3 and CL4 treatment) × five sampling times (day3, 6, 9, 12 and 15) × three replicates, plus three bags for the initial (day 0) measurements.

Each bag contained approx. 150 g of fresh-salmon, and they were cut into approx.30 fillets. The fillets were immersed in each freshly prepared CL coating (ratio of coating solution to fish samples, 2:1) for 1 min, then removed and allowed to drain at 4 °C for approximately 20 min in order to form the edible coatings. The control samples were dipped in purified water as other coating solutions for 1 min and then drained at 4 °C. Fresh-salmon fillets were then packed in polyethylene bags. Packed fillets with different coatings were stored at 4 ± 1 °C until measurements.

2.4. Physical analyses

2.4.1. Weight loss

To determine weight loss, fresh-salmon fillets in the same bags were weighed at the beginning and during storage with different treatments. It was assumed that weight loss corresponded entirely to water loss. The percentage weight loss relative to the initial weight was calculated by weighing the samples every 3 days in triplicate.

2.4.2. Surface color

Ten fillets were taken from each replicate for the measurement of surface color using a colorimeter (Model CR-300, Minolta Co., New York, NY.). Measurements were taken on both surfaces of the fillets (white and red regions) to obtain the color parameters L*, a*, and b*.

2.4.3. Texture

Firmness and surface breaking force (BF) were assessed using a Warner-Bratzler cell at a speed of 20 cm/min in a Lab Pro texturometer (Food Technology Corp., Sterling, VA), and expressed as Newton. Ten fillets were used per replicate.

2.5. Chemical analyses

2.5.1. Determination of total volatile basic-nitrogen (TVB-N)

The TVB-N value was estimated by the micro-diffusion method. The micro-diffusion method was determined by distillation after the addition of magnesium oxide (MgO) to homogenise fish samples. The distillate was collected in a flask containing a 3% aqueous solution of boric acid and a mixed indicator produced from dissolution of 0.1 g of methyl red and 0.1 g of methylene blue in 100 mL of ethanol. Afterwards, the boric acid solution was titrated with a 0.05 mol L^{-1} sulfuric acid (H_2SO_4) solution. The TVB-N value (mg 100 g^{-1} fish flesh) was determined according to the consumption of sulfuric acid.

2.5.2. pH

10 g fresh-salmon fillets taken from each replicate was dispersed in 100 mL of distilled water and stirred for 30 min, and then the mixture was filtered. pH value of filtrate was measured using a digital pH meter (Hanna Instruments, Italy).

2.6. Microbiological analyses

25 g of fresh-salmon fillets taken from each replicate were mixed with 225 mL of 0.1% peptone water and homogenized in a stomacher for 1 min. In all cases, serial dilutions of the microbial extracts were made in 0.1% peptone water. The mixture was homogenized for 1 min at room temperature. Further serial dilutions were prepared from this homogenate. Appropriate dilutions were used for microbiological analyses. Analysis were carried out at 0, 3, 7, 12 and 15 days. Viable mesophilic bacteria were determined using nutrient agar (Merck, Darmstadt, Germany) after incubation for 48 h at 37 °C. Psychrotrophic bacteria were determined using King agar (Merck) after incubation for 48 h at 21 °C. All counts were expressed as log CFU/g and performed in triplicate.

2.7. Sensory analysis

Measurements were performed by a panel of 7 trained tasters selected. The samples had first been cut into fillets, which they were presented to the panel. The panel assessed the following parameters: color, odor, flavor, moistness, firmness and general acceptability. Points were awarded on a scale of 0–10, and samples scoring less than 4 were rejected.

2.8. Statistical analysis

All data were subjected to post hoc test-ANOVA to test the effects of experimental conditions. The SPSS computer program (SPSS Inc., Chicago, IL, USA) was used. The results were presented as mean ± standard deviation. Differences were considered statistically significant at $P < 0.05$.