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Development of a chicken feather protein film containing clove oil and its application in smoked salmon packaging

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ABSTRACT

Chicken feathers, a by-product of the poultry industry, were utilized as a film base material after extraction of chicken feather protein (CFP). Composite films of CFP and gelatin were prepared, and their mechanical properties were investigated. The tensile strength and elongation at break of the CFP/gelatin composite film significantly ($p < 0.05$) increased as the gelatin content in the film increased. As a cross-linking agent, 0.5% cinnamaldehyde further improved the film's mechanical properties. Incorporation of clove oil into the composite film resulted in strong inhibition zones against *Escherichia coli* O157:H7 and *Listeria monocytogenes* compared with the film without clove oil. Packaging smoked salmon with the composite film containing 1.5% clove oil resulted in a decrease in the populations of *E. coli* O157:H7 and *L. monocytogenes* by 1.41 and 1.34 log CFU/g, respectively, compared with the control during storage at 4 °C for 12 days. Furthermore, the peroxide value and thiobarbituric acid reactive substances value decreased by 28 and 36%, respectively, in the smoked salmon packaged with the composite film containing 1.5% clove oil compared with the control during storage. These results suggest that a CFP/gelatin composite film with 1.5% clove oil can be used as an active packaging material for smoked salmon.

1. Introduction

Biodegradable packaging is one of few alternative strategies that can be considered to reduce the use of environmentally harmful synthetic polymers in the food packaging industry. Biodegradable packaging materials can be degraded into water, carbon dioxide, and inorganic substances without producing any toxic components (Siracusa, Rocculi, Romani, & Rosa, 2008). Moreover, biodegradable packaging films are suitable for food preservation because they can act as a barrier to moisture, oxygen, and volatile substances (Kim & Ustunol, 2001). For the development of biodegradable packaging, protein-based materials are considered attractive sources because of their excellent film-forming properties (Cao, Fu, & He, 2007; Souza, Cerqueira, Teixeira, & Vicente, 2010). A number of protein sources, such as soy protein, sunflower protein, chicken feather protein, barley bran protein, and whey protein, have been studied recently for the manufacture of edible packaging films (Guerrero, Stefani, Ruseckaite, & de La Caba, 2011; Salgado, Molina Ortiz, Petruccelli, & Mauri, 2010; Song, Shin, & Song, 2012; Song et al., 2013). Chicken feathers are one of the potential sources of edible film material because they are abundant, cheap, and a rich source of protein (Martelli, Moore, & Laurindo, 2006).

Chicken feathers are a by-product of poultry industry and are mostly disposed of without any pretreatment, causing severe environmental pollution (Moore, Martelli, Gandolfo, Sobral, & Laurindo, 2006). The major components of chicken feathers are proteins (91%), lipids (1%), and water (8%) (Kock, 2006). The protein portion of chicken feathers is mainly composed of a structural protein called keratin (Schrooyen, Dijstra, Oberthur, Bantjes, & Feijen, 2001). Keratin has high amounts of cysteine and half-cysteine, which can be distinguished from collagen or other structural proteins (Tonin et al., 2007). Reportedly, the cysteine residues present in keratin can be oxidized to form intra- and intermolecular disulfide bonds through cross-linking, resulting in water insolubility and improved mechanical properties for edible films (Schrooyen et al., 2001). Thus, as an abundant source of keratin, chicken feather protein (CFP) can be utilized as a potential edible film material. In general, CFP films are too fragile, and a plasticizer is needed to increase the strength and flexibility of the film (Yamauchi, Yamauchi, Kuisnoki, Kohda, & Konishi, 1996). Recently, Song et al. (2013) reported that the use of plasticizers in CFP film preparation resulted in an improvement in the film's mechanical properties.

Gelatin has been incorporated into edible films to improve the mechanical properties of the films. Gelatin, which is obtained

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through the partial degradation of collagen, has excellent film-forming properties because of its unique thermo-reversibility at a melting point close to body temperature, which is a crucial factor in edible film application (Achet & He, 1995). Although the high cost of gelatin inhibits its application as a base material for edible film preparation, previous experimental results have revealed that the incorporation of gelatin into soy protein isolate and barley bran protein films remarkably improved the mechanical properties of the films (Cao et al., 2007; Song et al., 2012).

Cold smoked salmon is a ready-to-eat food, which is generally consumed without cooking and may provide serious health threats to consumers due to its contamination with pathogenic bacteria, such as *Escherichia coli* and *Listeria monocytogenes* (Rotariu, Thomas, Goodburn, Hutchison, & Strachan, 2014). In addition, the poly-unsaturated fatty acids present in salmon are easily oxidized and lead to spoilage of the salmon during storage (Bell et al., 2001). Therefore, preservation of smoked salmon with an edible film containing antimicrobials and antioxidant substances may be an effective strategy.

Clove oil is a potent antimicrobial substance that has been proven to be very effective against *E. coli* and *L. monocytogenes* (Cressy, Jerrett, Osborne, & Bremer, 2003; Mytle, Anderson, Doyle, & Smith, 2006). In addition, clove oil has nonpolar phenolic compounds with antioxidant properties (Atarés, Bonilla, & Chiralt, 2010). Moreover, a recent study demonstrated that a sunflower seed meal protein film containing clove oil had a very high level of antimicrobial and antioxidant properties in the preservation of fish patties (Salgado, López-Caballero, Gómez-Guillén, Mauri, & Montero, 2013). Therefore, the aim of the present study was to develop a composite film from CFP and gelatin and to apply the CFP/gelatin composite film containing clove oil in the smoked salmon preservation.

2. Materials and methods

2.1. Materials

The white chicken feathers used in this study were supplied by a local company (Harim Corporation, Iksan, Jeonbuk, Republic of Korea). Sorbitol, gelatin from the pork, and cinnamaldehyde were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Clove oil was purchased from The Certification Academy for Holistic Aromatherapy (Seoul, Republic of Korea). Smoked salmon (salmon, 97%) was obtained from a local company (Daejeon, Republic of Korea).

2.2. Extraction of chicken feather protein

Chicken feathers were cleaned and dried at room temperature for 72 h. The feathers were cut into small filaments of 75–700 μm. CFP was extracted according to the method of Nomura et al. (2006) with a modification. The chicken feathers (500 g) were immersed in water (1:1, w/w) and blended for 1 h. After blending, 300 mL of a 1 M NaOH solution was added to the mixture, and the mixture was homogenized at room temperature for 24 h. The mixture was centrifuged at 10,000 × g for 1 h, followed by filtering through a cheesecloth and neutralization with 1 N HCl. Dialysis of the filtrate was conducted using Spectra/Por dialysis membranes of regenerated cellulose (MWCO, 3.5 kDa, Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) for 72 h, and then the samples were freeze-dried.

2.3. Preparation of the CFP/gelatin composite film

Five grams of CFP was dispersed in 100 mL of distilled water. Two grams of sorbitol was chosen as a plasticizer. To prepare the CFP/gelatin composite films, various amounts of gelatin (0.5, 1, 1.5, and 2 g) were dispersed with the sorbitol (2 g) into the CFP solution and stirred for 1 h, followed by ultra-sonication for 8 min. The solution was then heated using a hot water bath at 75 °C for 30 min. In addition, clove oil (0.5, 1.0, and 1.5 g) and cinnamaldehyde (0.5, 1.0, and 1.5 g) were incorporated into the film-forming solution with 0.25 g of Tween 20, and the samples were then cooled to 40 °C for 20 min. After straining through the cheesecloth, each film-forming solution was poured onto a flat Teflon-coated glass plate (24 × 30 cm) and dried at 25 °C for 24 h. The uniformity of the films' thickness was maintained by pouring a constant amount of solution onto each plate. The films were removed intact from the casting surfaces and stored for analyses.

2.4. Film conditioning and film thickness

For proper testing, the film samples were conditioned in an environmentally controlled chamber (25 °C and 50% RH) for 48 h. The thickness of the films was measured at four random points of each film using a digital micrometer (Mitutoyo Co., Tokyo, Japan), and the average thickness was calculated.

2.5. Tensile strength and elongation at break

The tensile strength (TS) and elongation at break (E) values of the films were determined using an Instron Universal Testing Machine (Model 4484, Instron Co., Canton, MA, USA). Rectangular strips (2.54 × 10 cm) were cut and fixed in the self-aligning grips of the device. The grip distance was 5 cm, and the film stretching speed was 50 cm/min. TS was calculated as the maximum stress the film endured before breaking, and E was expressed as the percentage of change of the initial length of a specimen at the point of breaking. Five replicates of each film were tested.

2.6. Moisture content

The moisture content of film pieces (2 × 2 cm) was determined by measuring the weight losses of the films upon drying in an oven at 110 °C. Three replicates of each film were tested. The moisture content (%) was calculated as follows:

$$\text{Moisture content}\ (\%) = ((\text{initial sample weight} - \text{dry sample weight})/\text{initial sample weight}) \times 100$$

2.7. Film water solubility

The film water solubility (FWS) was determined according to the method of Shen, Wu, Chen, and Zhao (2010). Film pieces (2 × 2 cm) were dried to a constant weight at 105 °C. Each sample was placed into a 50-mL beaker containing 20 mL of distilled water and was shaken at room temperature (25 °C) for 24 h. After collecting the undissolved film, its dry weight was determined after drying in an oven at 105 °C for 24 h. The FWS of each film was calculated as follows: