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Antimicrobial Olive Leaf Gelatin films for enhancing the quality of cold-smoked Salmon

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ABSTRACT

Olive leaf was evaluated as antimicrobial/antioxidant ingredient in edible films intended for cold-smoked fish preservation. Olive leaf powder (OLP) and its water/ethanol extract (OLE) were previously tested against *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella enterica*. Antimicrobial and antioxidant capacity, color and water vapor permeability (WVP) of OLE-gelatin films (0, 1.88, 3.75 and 5.63% w/w) were determined.

OLP and OLE showed antibacterial activity against *L. monocytogenes* in agar diffusion tests, and non-effect was observed on *E. coli* and *S. enterica*. Antimicrobial and antioxidant activities of the films increased with increasing OLE concentration in their formulations. Film lightness was not significantly affected by OLE. In contrast, a^* decreased and b^* increased with increasing OLE concentration. Addition of 3.75 and 5.63% of OLE also increased WVP of the films.

A film formulation with 5.63% OLE was considered optimal for further tests against *L. monocytogenes* in inoculated cold-smoked salmon. The films significantly reduced the growth of this pathogen on the fish over storage.

1. Introduction

Fresh fish makes an important nutritional contribution to the diet, providing proteins, fatty acids and vitamins and antioxidants, such as tocopherols and carotenoids. However, it is a perishable commodity highly suceptible to spoilage and oxidation. The activity of microorganism is the main factor limiting the shelf life in fresh fish (Gram & Dalgaard, 2002; Ólafsdóttir et al., 1997). Also, seafood-associated foodborne pathogen outbreaks are a major concern. Seafood is commonly contaminated with several pathogenic microorganisms, including *Listeria monocytogenes, Escherichia coli* and *Vibrio parahaemolyticus* (Vogel, 2009). This incidence is of special concern in ready-to-eat (RTE) products. Furthermore, marine lipids are relatively more susceptible to oxidation, compared to other food lipids, because of their high content of polyunsaturated fatty acids (Kolakowska, Olley, & Dunstan, 2003). Lipid deterioration in seafood is directly related to the production of off-flavours and odors (Harris & Tall, 1994) as well as a number of other reactions that reduce shelf life and nutritional value of seafood. Consequently, preservation technologies are required to maintain fish quality.

Traditionally, temperature-based preservation techniques had been used in fish, such as cooling, super chilling and freezing (Sampels, 2015). Improved packaging systems, specially modified atmospheres, have been successfully applied to fish products (Randell, Hattula, & Ahvenainen, 1997; Özogul, Taylor, Quantick, & Özogul, 2000; Lyhs, Lahtinen, & Schelvis-Smit, 2007). On the other hand, novel technologies may present limitations in their use on fish, such as oxidation and color modifications, for instance, high pressure treatment (Medina-Meza, Barnaba, & Barbosa-Cánovas, 2014) irradiation (Sant'Ana & Mancini-Filho, 2000) and cold plasma (Albertos et al., 2016). Extensive research has investigated the application of natural antioxidants in fish preservation (Vareltzis, Koufidis, Gavriilidou, Papavergou, & Vasiliadou, 1997; Pazos, Alonso, Fernández-Bolaños, Torres, & Medina, 2006; Medina, Gallardo, González, Lois, & Hedges, 2007; Farvin, Grejsen, & Jacobsen, 2012; Albertos, Jaime, María Diez, González-Arnaiz, & Rico, 2015). Most of these natural compounds may adversely affect palatability. The incorporation of these natural antioxidants into edible films appears to be a good strategy to reduce the

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amounts and consequently minimize astringency and bitterness of these compounds. Edible films with natural antioxidants produce a gradual liberation of the additive to the food throughout its shelf life (Campos, Gerchenson, & Flores, 2011). Another advantage is that edible films are based on environmentally friendly biopolymers (Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2011).

Food processing industry generates large quantities of wastes. Nowadays, efforts are focussed on re-utilized, valorized by-products and reduced the plastic as packaging material. Namely, olive leaves are an important by-product of the olive oil industry. Olive leaves are generated during the pruning of olive trees, and also are an industrial residue from olive oil processing. Specifically, 10% of the total weight of olives arriving to the mills is represented by this by-product (Herrero et al., 2011). Olive leaf has demonstrated antioxidant (Pereira et al., 2007; Mylonaki, Kiassos, Makris, & Kefalas, 2008; Lee & Lee, 2010; Apostolakis, Grigorakis, & Makris, 2014) and antimicrobial (Markin, Duek, & Berdicevsky, 2003; Pereira et al., 2007) activities. Scarce reports on olive leaf formulated in films exist (Erdohan, Çam, & Turhan, 2013; Khalil, Ismail, El-Baghdady, & Mohamed, 2013; Marcos et al., 2014). On the other hand, edible films containing olive leaf extract have not been applied on food products to date. Olive leaf extract was incorporated into polylactic acid (PLA) films, showing antimicrobial activity against *Staphylococcus aureus* (Erdohan et al., 2013). Khalil et al. (2013) studied the antibacterial activity of silver nanoparticles synthesized using olive leaf extract. Antioxidant properties of biodegradable films (Ecoflex® and Ecoflex®-polylactic acid PLA) with α-tocopherol and OLE were studied by Marcos et al. (2014).

The aim of this study was to test antimicrobial, antioxidant and physical properties of olive leaf-extract gelatin films at different concentrations and evaluate the effectiveness of the optimum OLP/OLE concentration on cold smoked salmon.

2. Materials and methods

2.1. Materials

Fish gelatin was kindly donated by Mr. Richard Norland (Norland Products Inc., Cranbury, NJ). Folin-Ciocalteau reagent, Trolox and 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, MO). Methanol, ethanol and glycerol were purchased from Fisher Scientific Ltd. (Fair Lawn, NJ). Trypticase Soy Agar (TSA), Trypticase Soy Broth (TSB), Oxford Medium Base (OMB) and peptone were purchased from Becton, Dickinson and Company (Sparks, MD).

2.2. Preparation of olive leaf extract (OLE)

Manually detached Mission olive leaves from an orchard in Pleasanton, CA were washed in a Kenmore series 400 triple action agitator washer (Sears, Roebuck and Co., Chicago, IL) with cold tap water in a 40 min normal express washing cycle. Drained leaves were blanched for 10 s with boiling water in a Groen TDB7-40 steam kettle (Groen, Jackson, MI) at a ratio of 0.012 kg of leaves per L of water. Blanched leaves were dried in a steam-heated cabinet hot–air drier (Procter & Schwartz, Inc., Horsham, PA) with air down circulation at 80 °C for 35 min over perforated stainless steel trays. Olive leaf powder (OLP) was produced by pre-grinding dry olive leaves in a Cuisinart blender and milling through a 0.25 mm S.S. screen in a model 3010-014 cyclone sample mill (UDY Corp., Fort Collins, CO). OLP was packaged in metallized flat pouches with nitrogen flushing before hot-sealing and stored at 2 °C until being used for extraction. To prepare OLE fifty grams of OLP were mixed with 250 mL of 60% ethanol using a PC-351 Corning hot plate stirrer (Corning Glass Works, Corning, NY) at 50% speed for 72 h, at ambient temperature, in a 500 mL erlenmeyer flask covered with a cork stopper and wrapped with Parafilm. The final mixtures were centrifuged in a Sorvall RC 5C Plus with a SA-600 rotor at 29.000*g* for 10 min at 4 °C (Kendro Laboratory Products, Newtown, CT). Supernatant was evaporated in a Büchi rotavapor RE rotary evaporator (Brinkmann Instruments, Inc., Cranberry Township, PA) at 40 °C. Final samples were then lyophilized in a VirTis Ultra 25EL freeze-drier, packaged in metallized flat pouches with nitrogen flushing before hot-sealing and stored at 4 °C until further analysis.

2.3. Preparation of fish gelatin films (FGF) with OLE

FGF with OLE were prepared by mixing 75 g/L of fish gelatin aqueous solution with glycerol (0.67 g/kg biopolymer, as plasticizer). Afterwards, 0, 1.88, 3.75 and 5.63% (w/w) OLE was added to gelatin solutions. The solutions were mixed for 5 min at 500 rpm in a PMC series 730 DataPlate digital hot plate and magnetic stirrer (Barnstead Thermolyne Corp., Hampton, NH) and heated in a Cole-Parmer mod. 12504-50 water bath (Cole-Parmer Instruments Co., Chicago, IL) at 60 °C for 15 min. The solutions were mixed again for 5 min. These steps were repeated 4 times before the gelatin solution was degassed under vacuum to avoid presence of micro bubbles in films. Films were cast on 29 × 29 cm glass plates using a 35 mil (1 mil = 0.0254 mm) gap draw down aluminum bar to spread the filmogenic formulations onto a flat Mylar sheet to facilitate the removal of films after overnight drying at room temperature (25 °C). Films were separated from the Mylar after drying and stored on layers of aluminum foil in zip-locked plastic bags at 4 °C and 65% RH until physical, chemical, and antimicrobial properties were evaluated. Unless otherwise mentioned all analysis on the films was performed in triplicate.

2.4. Screening for effective antimicrobial activity of OLP and OLE against pathogenic bacteria

The sources of bacteria used in the present study had previously been described by Friedman, Henika, and Mandrell (2002). *E. coli* O157:H7, *S. enterica* and *L. monocytogenes* were streaked on TSA and incubated overnight at 37 °C. One isolated colony was picked from each TSA plate and inoculated into a tube with 5-mL TSB at 37 °C for 24 h with agitation. Each inoculum was prepared by serially diluting (10×) in 0.1% peptone water.

Overlay diffusion tests were used for antimicrobial assays. To test antimicrobial effect of OLP and OLE against three pathogenic microorganisms, 100 μL of 10^5 colony-forming units (CFU/mL) of each inoculum was uniformly spread onto TSA plates and left to dry for 5 min at room temperature. A 10 mm diameter sterile filter paper disk was placed at the center of each plate, 20 μL of OLP/OLE solutions (diluted with water, 50/50, v/v) was placed on top of each disk. The plates were incubated at 37 °C for 48 h. The inhibition diameter of colony-free perimeter (including the disk) was measured in triplicate with a digital caliper (Neiko Tools, Ontario, CA, USA).

2.5. Screening for different concentration of OLE in FGF

2.5.1. Antimicrobial activity against L. monocytogenes of FGF with different level of OLE

Antimicrobial activity of FGF with different level of OLE (0, 1.88, 3.75 and 5.63 w/w %) against *L. monocytogenes* was measured using overlay tests. Overlay diffusion test is a direct contact method using solid medium to measure antimicrobial activity in edible films. For overlay diffusion test, edible films were aseptically cut into 12-mm diameter disc and then deposited over the agar plate inoculated with tested bacteria. The procedure was similar to that explained in 2.4., but replacing the filter paper disc with different FGF discs (12 mm diameter). FGF discs were placed over the agar with the filḿs shiny side down.

2.5.2. Total soluble phenolic compounds (TSP) and antioxidant capacity of OLP, OLE and FGF at different level of OLE

The procedure for TSP analysis was adapted from Swain and Hillis