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Effect of quince seed mucilage edible films incorporated with oregano or thyme essential oil on shelf life extension of refrigerated rainbow trout fillets

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ABSTRACT

The effects of quince seed mucilage film (QSMF) containing oregano (O) or thyme (T) essential oil on shelf life extension of rainbow trout (*Oncorhynchus mykiss*) fillets during refrigerated storage (4 °C) were evaluated over a period of 18 days. Films were prepared in four different concentrations of essential oils, including 0, 1, 1.5 and 2%. The control and the wrapped fillet samples were analyzed periodically for microbiological (aerobic and psychrotrophic count, *Pseudomonas* spp., H_2S-producing bacteria, lactic acid bacteria, and *Enterobacteriaceae*), chemical (TBA, TVB-N, TMA-N), and sensory characteristics. Bacteria grew most quickly in trout fillets stored in air, followed by those wrapped with QSMF and the lowest counts were in wrapped samples with QSMF + 2%T. *Pseudomonas* spp., *Enterobacteriaceae* and LAB counts were significantly lower in samples wrapped with QSMF + 2%T. The lowest TBA value was obtained in fillets wrapped QSMF containing 2% oregano essential oil. The strong antioxidant activity of QSMF + 2%O was related to the composition of oregano essential oil. The GC analysis of essential oil components revealed that carvacrol (81.85%) was the major component of oregano essential oil. TBA value varied for all treatments and remained lower than 2 mg MDA/kg throughout storage. The formation of TVB-N, TMA-N increased with time of storage. TVB-N and TMA-N correlated well with the microbiological data, indicating that along with TVB-N, TMA-N may serve as a useful index for fillets spoilage. QSMF extended the microbial shelf life of rainbow trout fillets by 2 days, whereas the QSMF + 1%O, QSMF + 1.5%O, QSMF + 2%O, QSMF + 1%T, QSMF + 1.5%T and QSMF + 2%T resulted in a significant shelf life extension of the trout fillets by 3, 5, 9, 6, 10 and 11 days, respectively, as compared to the control samples.

1. Introduction

The heightened demands by consumers for better quality and improved freshness of food products have given rise to the development and implementation of edible films (Beverlya et al., 2008). These films can help maintain and improve the quality of fresh, frozen and processed muscle foods by reducing moisture loss, lipid oxidation and color deterioration, and acting as carriers for antimicrobial and antioxidant food additives (Sothornvit and Krotcha, 2005; Chamanara et al., 2012; Krochta and De Mulder-Johnston, 1997; Campos et al., 2011; Jouki et al., 2014a, 2014b). Different spoilage mechanisms reported to be involved in the quality loss of fish during storage include microbial development, endogenous enzyme activity, non-enzymatic lipid oxidation and enzymatic browning (Özogul et al., 2006). These activities lead to a short shelf life in fish and other seafood products (Arashisar et al., 2004). Applications of edible films, such as polysaccharides and proteins, containing essential oils (EOs) may be useful to prolong the shelf life for fish and meats. Ojagh et al. (2010) studied the effects of a chitosan coating enriched with cinnamon oil on quality of rainbow trout during refrigerated storage. They reported that chitosan coating together with cinnamon oil provides a type of active coating that can be utilized as a safe preservative for fish under refrigerated storage. Jeon et al. (2002) demonstrated that chitosan-coated Atlantic cod and herring had reduced moisture loss and lipid oxidation. Souza et al. (2010) reported that chitosan-based coatings may be an alternative for extending the shelf life of salmon fillets during storage at 0 °C. EOs such as oregano and thyme have natural antimicrobial and antioxidant properties with the potential to extend the shelf life of foods (Chouliara et al., 2007). Thymol, carvacrol, p-cymene and γ-terpinene are the most active constituents of oregano and thyme EOs, with a wide spectrum of antimicrobial and antioxidant properties (Ultee and Smid, 2001; Burt, 2004; Rocha-Guzman et al., 2007). Recently we investigated the possibility of producing the edible film from quince seed mucilage (QSM) with glycerol as plasticizer in the different concentrations, and reported the physical, mechanical, barrier, thermal, microstructure and antioxidant properties of QSM-films (Jouki et al., 2013). Antimicrobial activity of QSM-films incorporated with thyme or oregano essential oil was

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evaluated in our previous studies (Jouki et al., 2014a, 2014b). We also reported that the QSM-films containing thyme or oregano inhibited to a varying degree the growth of 11 strains of microorganisms. The present study investigated the effects of quince seed mucilage-based edible films containing oregano or thyme essential oil on the microbiological, chemical and sensory qualities of refrigerated rainbow trout fillets for 18 days.

2. Materials and methods

2.1. Mucilage extraction

Quince seed mucilage was prepared according to the method of our previous work (Jouki et al., 2013). Briefly, aqueous quince seed mucilage was extracted from whole seeds using distilled water (water to seed ratio of 30:1). Then, the swelled seeds were stirred with a rod paddle blender (Rondo-2500, KA702, France) at 1100 rpm, at 45 °C for 15 min to scrape the mucilage layer off the seed surface. The solutions were then filtered with cheese cloth and the obtained mucilage was dried by an oven at 45 °C.

2.2. Film solutions

Film solution was prepared by slowly dissolving 1% mucilage and glycerol as a plasticizer in 35% (w/w) based on QSM weight prepared under constant stirring (750 rpm) at 45 ± 2 °C for 15 min. Then oregano or thyme essential oil (1, 1.5, or 2% v/v) dissolved in the solution with agitation using a magnetic stirrer during 1 h at room temperature (25 °C). Tween 80 was added as a surfactant at concentrations between 0.1 and 0.2% (w/v). The solution was homogenized (IKA T25-Digital Ultra Turrax, Staufen, Germany) at 12,000 rpm for 5 min to obtain an emulsion. To remove air bubbles, the emulsion was placed into collection tubes and centrifuged for 3 min at 3800 ×*g* (6000 rpm) (Jouki et al., 2014b).

2.3. Preparation of QSM-based films

Antimicrobial films were prepared by the methods of Jouki et al. (2014a). Briefly, the film solution (55 mL) was placed on the Teflon coated glass petri dishes (13 cm diameter). The castings were placed in a fume hood, which was maintained at 37 ± 2% RH and 25 ± 2 °C, for 24 h. The dried QSM films were peeled from the petri-dishes.

2.4. Fish samples and preparation

Fresh water rainbow trout (*Oncorhynchus mykiss*, with average weight of 550 ± 70 g) were purchased from a local aquaculture farm located at Savojbolagh (Alborz, Iran). Fish were transferred to the laboratory within 15 min of harvesting, packed in insulated boxes containing ice. The fish were eviscerated, headed and filleted (approximately each fillet of uniform 25 × 10 cm^2 and weight 165 ± 10 g) by hand. Skin and spiny bones were removed by hand from fillets.

2.5. Application of films on the fish fillets

Fillet samples (with average weight of 55 ± 5 g) were randomly divided into eight treatments lots including: treatment groups consisted of (1) unwrapped samples (Control), (2) samples wrapped by QSM-film without addition of essential oils (QSMF), (3) samples wrapped by QSM-film containing 1% oregano essential oil (QSMF + 1%O), (4) samples wrapped by QSM-film containing 1.5% oregano essential oil (QSMF + 1.5%O), (5) samples wrapped by QSM-film containing 2% oregano essential oil (QSMF + 2%O), (6) samples wrapped by QSM-film containing 1% thyme essential oil (QSMF + 1%T), (7) samples wrapped by QSM-film containing 1.5% thyme essential oil (QSMF + 1.5%T), (8) samples wrapped by QSM-film containing 2% thyme essential oil (QSMF + 2%T). Wrapped fillets were subsequently labeled and stored at 4 °C. The sliced rainbow trout fillets were sampled for examination at storage days 0, 3, 6, 9, 12, 15 and 18.

2.6. Determination of chemical composition of essential oils

For the quantification of individual components, the EOs were analyzed using a Hewlett-Packard 6890 series gas chromatograph (Perkin Elmer (PE) Auto System XL, USA), connected to a Hewlett-Packard, model 5973, mass spectrometric detector (Agilent Technologies, Wilmington, DE, USA). A capillary column DB-5MS (60 m × 0.320 mm i.d. and 1 μm, film thickness) was used for the separation of individual components of the EO. Chemical composition of essential oils was determined according to the method described by Karabagias et al. (2011). The oven temperature was programmed at 100 °C for 5 min, increased by 10 °C/min to 130 °C, increased by 7 °C/min to 270 °C and held to 270 °C for 3 min. Helium was used as the carrier gas at a flow rate of 1.5 mL/min. 0.1% solution of EO was prepared in hexane and 1 μL of this solution was injected. The injector was operated in split mode (20:1 split ratio) at a temperature of 270 °C. The mass spectrometer was operated under the following conditions: scan range 30–330, source temperature: 230 °C, quadruple temperature: 150 °C, and electron impact (EI) ionization at 70 eV. Identification of compounds was achieved by comparing the mass spectra of the recorded chromatographic peaks with the Wiley 275 MS database.

2.7. Proximate composition

The moisture content and crude ash were of the fish determined in an oven at 103 °C and 550 °C, respectively, until the weight became constant (AOAC, 2002). Lipid content was determined by the Bligh and Dyer (1959) method. The total crude protein was calculated by converting the nitrogen content determined by Kjeldahl's method (AOAC, 2005). Analyses were conducted in triplicate, and all reagents were of analytical grade.

2.8. Microbiological analysis

Twenty-five grams of fillet samples was drawn aseptically and transferred to 225 mL of sterile 0.1% peptone water solution. The mixture was homogenized for 60 s using a Lab Blender400, stomacher at room temperature (Seward Medical). For microbial enumeration, 0.1 mL samples of serial dilutions (1:10, diluent, 0.1% peptone water) of fish homogenates were spread on plates of various agar materials. Decimal serial dilutions up to six serial decimal dilutions were applied for bacteriological evaluation of fish samples. Enumeration of total viable count (TVC) performed on standard plate count agar (PCA, Merck) with incubation at 30 °C for 48 h; meanwhile at 7 °C for 10 days to enumerate the psychrotrophic count (PTC) on the same medium (Arashisar et al., 2004). Lactic acid bacteria (LAB) were enumerated on de Man Rogosa Sharpe agar (MRS, Oxoid code CM361, Basingstoke, UK) incubated at 25 °C for 5 days under anaerobic conditions (Giatrakou et al., 2008). *Pseudomonas* spp. were enumerated on cetrimide fusidin cephaloridine agar (CFC, Oxoid code CM 559, supplemented with SR 103, Oxoid, Basingstoke, UK) and incubated at 20 °C for 2 days (Mead and Adams, 1977). For *Enterobacteriaceae* and H_2S-producing bacteria (including *Shewanella putrefaciens*) enumeration, a 1.0 mL sample was inoculated into 10 mL of molten (45 °C) violet red bile glucose agar (VRBGA, Oxoid code CM 485) and iron agar (IA, Oxoid code CM 867), respectively. After setting, a 10-mL overlay of molten medium was added. For the former (VRBGA), incubation was carried out at 30 °C for 24 h. The large colonies with purple haloes were counted (Mossel et al., 1979). IA plates were incubated at 20 °C and black colonies formed by the production of H_2S were enumerated after 2 to 3 days. Three replicates were made for each test sample and four appropriate dilutions were used for each replicate. Microbiological data were transformed into logarithms of the number of colony forming units (CFU/g).