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Quality assessment of shrimps preserved with orange leaf essential oil incorporated gelatin

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A B S T R A C T

Assessment of edible gelatin coating solution enriched with essential oil obtained from orange (*Citrus sinensis* (L.) Osbeck) leaves on the quality and shelf life of cold stored deep water pink shrimp (*Parapenaeus longirostris* Lucas 1846) was studied. Gelatin (G) and 2% orange leaf essential oil incorporated gelatin (G+OL) solution, which was selected after antimicrobial and antioxidant analysis, were used for coating. Microstructure characterization of the gelatin films was done by Scanning Electron Microscopy. Sensory and melanosis, microbiological [total viable counts (TVC), psychrotrophic bacteria counts (PBC), *Enterobacteriaceae* (EB)], chemical [sulphur dioxide (SO_2), pH, total volatile base nitrogen (TVB-N), trimethylamine nitrogen (TMA-N), thiobarbituric acid (TBA), peroxide value (PV)] and physical (color) analysis were carried out for control and coated samples and shelf-life was determined throughout the storage period of 14 days.

The results indicate that the edible gelatin coating solutions enriched with orange leaf essential oil has noticeable effects on the quality and shelf life of shrimps when compared to control group. It was determined that edible gelatin coating solutions used were effective in all groups particularly on melanosis which may occur in shrimps and edible gelatin coating solutions with orange leaf essential oil preserved the quality of shrimp better.

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1. Introduction

Shrimp is an easily perishable seafood and under cooled storage, its shelf life is limited due to its biochemical metabolism (Haard, 1993; Gram & Dalgaard, 2002). Melanosis (discoloration) caused by the polymerization of phenols into insoluble black pigments, i.e., melanins, is also an important factor that cause observable shrimp spoilage (Nirmal & Benjakul, 2011).

Edible films and coatings, one of the most interesting techniques of active packaging, are mainly used for preserving the food and prolonging its shelf-life. Edible films and coatings that are made from polysaccharides, proteins and lipids can extend the shelf-life of foods by acting as moisture, oxygen, carbon dioxide or vapor barriers and as enhancers of mechanical properties (Ojagh, Rezaei, Razavi, & Hosseini, 2010). Gelatin-based edible film coatings have already been proposed to extend the shelf-life of various meat products (Alparslan, Baygar, Baygar, Hasanhocaoglu, & Metin, 2014). Films including antimicrobial, antioxidant and aromatic agents can enhance the mechanical and biological features of the food (Aşık & Candoğan, 2014). Essential oils are considered generally recognized as safe (GRAS) so they can be used in foods, as long as their maximum protective effects is attained with the minimum change in the sensorial and organoleptic properties of the food (Viuda-Martos, Ruiz-Navajas, Fernandez-López, & Perez-Alvarez, 2008).

Citrus oils are widely used in diverse applications in the flavor, food, cosmetic, pharmaceutical and chemical industries. Citrus oils are mixtures of over a hundred compounds that can be classified into three fractions: terpene hydrocarbons, oxygenated compounds, and non-volatile compounds (Sousa, Raeissi, Aguiar-Ricardo, Duarte, & Peters, 2004). Limonene, a monoterpene, is found in many plant essential oils and is the major aroma constituent of orange essential oil. Antimicrobial and antioxidant activities of orange essential oil were shown by researchers (Arruda, Miguel, Yokoyama-Yasunaka, Katzin, & Uliana, 2009; Gürsoy, Tepe, & Sokmen, 2010; Jin et al., 2008; Marostica & Pastore, 2009). Leaves of

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citrus species have been found to contain higher levels of sabinene than peel and fruits (Kirbaslar, Dulger, Turker, & Tavman, 2012). Sabinene reported to have antifungal (Santoyo et al., 2006), antibacterial (Glišić, Milojević, Dimitrijević, Orlović, & Skala, 2007) and antioxidant (Quiroga, Asensio, & Nepote, 2015) properties.

The aim of the present study is to evaluate the effects of edible gelatin coating solutions containing with essential oil obtained from orange leaf (*Citrus sinensis* (L.) Osbeck) on the quality and shelf life of deep water pink shrimp (*Parapenaeus longirostris* Lucas 1846).

2. Material and methods

2.1. Shrimps

Deep water pink shrimps (*Parapenaeus longirostris* L. 1846; mean length 11.62 ± 0.74 cm and mean weight 7.89 ± 1.33 g) caught around Guzelcamlı Harbour (Soke, Aydın) of Turkey were used as raw material. 30 kg shrimps were obtained from a vessel harvesting shrimps using a trawl and were immediately iced and transferred to the laboratory for analysis. Proximate composition, sensory, physical, chemical and microbiological analyses were done initially and the remaining samples were prepared for coating as analysis groups.

2.2. Extraction and analysis of essential oil

Orange leaves (*Citrus sinensis* (L.) Osbeck) were collected from an orange farm in Koycegız, Mugla, Turkey and brought to Mugla Sıtkı Kocman University, Faculty of Fisheries laboratories. In average of 100 kg orange leaves were used in this study for obtaining an efficient amount of essential oil. Orange leaf essential oil was obtained after it was air-dried in the dark at room temperature and ground. The essential oil was obtained by hydro distillation, using a Clevenger apparatus (Edutek Instrumentation, Haryana, India) with 150 g of dry plant material and 1500 mL of water. The oil was obtained after 3 h of distillation at boiling temperature and stored at (4 ± 1) °C in airtight glass vials covered with aluminum foil.

The gas chromatography-mass spectrophotometer (GC-MS) analysis of the obtained essential oil was conducted at the ARGE-FAR-ÇEG Laboratory of Aegean University (Izmır, Turkey), using an Agilent gas chromatograph model 6890 equipped with an Agilent mass selective detector (MSD) model 5973 (Agilent Technologies, Santa Clara, CA, USA). Identification of components in the essential oil was carried out with the Wiley 275 mass spectral library (NIST, Wiley, New York, NY, USA).

2.3. Preparation of film-forming solution

Preparation of edible films was slightly modified from Gomez-Estaca, Lopez de Lacey, Lopez-Caballero, Gomez-Guillen, & Montero (2009). Food grade gelatin powder (8 g; Doğa Drug and Raw Material Co. Ltd., Ankara, Turkey) was dissolved in 100 mL of distilled water (at room temperature) and the mixture was stirred until the gelatin completely dissolved (approx. 15 min). Glycerol (Merck) (0.15 mL per g of gelatin) and D-sorbitol (Merck) (0.15 g per g of gelatin) were then added to the gelatin coating solution, which was kept at 45 °C for additional 15 min. Orange leaf essential oil (OLEO) in a ratio of 0.5, 1 and 2% (by volume per mass of gelatin) was then added to the coating solution. To stabilize the emulsion at OLEO, Tween-20 was also added to the gelatin solution with a ratio of 0.2% of the OLEO.

One of the gelatin groups was prepared without the addition of OLEO (0%). Then the coating solution with OLEO was homogenized with an Ultraturrax T25 basic blender (21 500 rpm, position 5, for 1 min; IKA-Werke GMBH & Co. KG, Staufen, Germany).

2.4. Antimicrobial activity of gelatin coating solution combined with essential oil

The antimicrobial activity of essential oil combined with gelatin coating solutions was tested using agar well diffusion assay over five food pathogen microorganisms (NCCLS, 1993). The standard strains were obtained from the Ankara Refik Saydam Hıfzısıhha Institutes Culture Collection; *Bacillus subtilis* (ATCC 25922), *Staphylococcus aureus* (ATCC 43300), *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 15442) and *Candida albicans* (ATCC 10231). The above mentioned bacteria were cultured in Nutrient Broth (NB) at appropriate temperatures. Inoculums were prepared by adjusting the turbidity of the medium to match the 0.5 McFarland Standard Dilutions. 20 milliliters of Mueller Hinton Agar (Difco) were sterilized in separated flasks and cooled to 45–50 °C. After injecting the microorganism cultures to sterile plates (1000 μL), media were distributed and mixed homogenously. 20 μL of solutions were injected to the wells of 6 mm in diameter. Three different concentrations of gelatin + orange leaf essential oil combination were evaluated for antimicrobial activity; 0.5%, 1% and 2%. After the proper incubation period for each microorganism, antimicrobial activity was evaluated by measuring the zone of inhibition against the tested microorganisms. Measurements were performed in triplicate.

2.5. Radical-scavenging activity of gelatin coatings

Gelatin coatings (0.1 g) were dissolved in distilled water (2 mL) at 45 °C. Following the addition of ethanol (4 mL), the solutions were centrifuged (NUVE, NF 400R, Turkey) at 4000× g for 10 min at 20 °Cao, Xue, and Liu (2009). The filtrate was analyzed for 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity (Yen & Hsieh, 1995). 500 μL aliquot of ethanol extract was mixed with 5.5 mL of DPPH (0.036 mmol L^{-1} in ethanol) and allowed to stand for 30 min at room temperature in the dark. The absorbance was measured using a spectrophotometer at 517 nm. DPPH radical-scavenging activity was calculated according to the following equation:

$$\text{Radical} - \text{scavenging activity}(\%) = [1 - (A_s - A_0)/A_c] \times 100$$

where A_s is the absorbance of the sample, A_c is the absorbance of the control (ethanol used instead of sample) and A_0 is the absorbance of the mixture of 5.5 mL of ethanol and 500 μL of sample.

2.6. Microstructure of gelatin films added essential oil

Microstructure analysis of the films was carried out by using SEM technique in a JEOL JSM-7600F (Japan) field emission scanning electron microscope (Garcia, Martino, & Zaritzky, 2000). Gelatin films with and without orange leaf essential oil were dried on foam plates. Pieces were cut from films and mounted on copper stubs using double side adhesive tape. Samples were gold coated and observed, using an accelerating voltage of 15 kV.

2.7. Coating treatment and storage

Three different groups were created for quality analysis: non-coated control group (C), gelatin coating without essential oil (G) and gelatin coating with 2% OLEO (G+OL). For non-coated control group, 10 shrimps were put onto sterile foam dishes and vacuum packaged (Culinary, ATM Machinery 7483 BV, Haaksbergen, The Netherlands).

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