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Application of chitosan and gelatin based active packaging films for peeled shrimp preservation: A novel functional wrapping design

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ABSTRACT

The aim of the present study was to evaluate the effects of chitosan (Ch) and gelatin (Ge) films containing *Ziziphora clinopodioides* essential oil (ZEO; 0 and 1%), pomegranate peel extract (PPE; 0 and 1%) and cellulose nanoparticle (CN; 0 and 1%), separately and in combination, on survival of *Listeria monocytogenes* and shelf life extension of fresh shrimp during refrigerated storage. The control and wrapped shrimps were evaluated for chemical (PV and TVB-N), bacterial (mesophilic and psychrotrophic bacteria, *Pseudomonas* spp., *P. fluorescens*, *Shewanella putrefaciens*, lactic acid bacteria and Enterobacteriaceae) and sensory properties (odor, color, texture and acceptability). All wrapped shrimps in Ch and Ge films had lower bacterial counts compared to control ($P < 0.05$). Final population of *L. monocytogenes* was decreased 2–3 log CFU/g in treated samples compared to control ($P < 0.05$). The bacterial population of shrimps wrapped in Ch films were significantly lower than those wrapped in Ge films probability due to the inherent antibacterial property of Ch ($P < 0.05$). The groups treated with CN had insignificantly lower bacterial count compared straight film ($P > 0.05$). The Ch group treated with ZEO 1% + PPE 1% + CN 1% had the best antibacterial effectiveness and also the highest organoleptic scores after 11 days ($P < 0.05$).

1. Introduction

Fresh shrimp is usually vulnerable to microbial, physical and biochemical changes during post-mortem storage due to high water content, free amino acids and other non-protein nitrogenous compounds (Nirmal & Benjakul, 2011). On the other hand, the presence of *Listeria monocytogenes* is one of the most important concerns of the seafood industry since this bacterium can survive and/or grow in a wide range of pH (4.1–9.6) and temperature (0.5–45 °C), low water activity (0.91) and even high salt concentration (up to 20% w/v) (Warriner & Namvar, 2009). There are several published studies with dealing the possibility of using edible/biodegradable films and/or coatings to extend the fresh shrimp shelf life (Arancibia, Giménez, López-Caballero, Gómez-Guillén, & Montero, 2014; Bahrami, Shahbazi, & Nikousefat, 2016; Farajzadeh, Motamedzadegan, Shahidi, & Hamzeh, 2016; Kakaei & Shahbazi, 2016). Regarding these biopolymers, gelatin (Ge) and chitosan (Ch) have good properties such as high compatibility with other incorporated compounds, biodegradability, edibility and serving as carriers of food additives (Volpe et al., 2015).

Recently, incorporation of natural antimicrobial and antioxidant compounds specially plant essential oils (EOs) and extracts into edible films and/or coatings has become popular because consumers are increasingly concern about the potential health risks of synthetic additives (Aghajani et al., 2008; Bekhechi et al., 2007; Schulz, Özkan, Baranska, Krüger, & Özcan, 2005). In this sense, *Ziziphora clinopodioides* is an aromatic and medicinal plant belonging to the Lamiaceae family. Our previous studies have proved that *Z. clinopodioides* essential oil (ZEO) has a strong antibacterial property in foodstuff (Shahbazi, 2015a,b; Shahbazi, Shavisi, & Mohebi, 2016a,b). Moreover, pomegranate is known as a highly perishable fruit containing phytochemicals such as phenolic acid and flavonoids, which have a number of biological activities including antibacterial, antifungal, antioxidant, anti-inflammatory, antitumor and antidiarrheal (Basiri, Shekarforoush, Aminlari, & Akbari, 2015).

In spite of numerous benefits of EOs and extracts, one of the most limitations to use of these compounds as food preservatives is the persistence of the strong aroma affecting the sensory properties of the foodstuffs (Arancibia et al., 2014; Ozturk & Ercisli, 2007; Yuan, Zhang, Tang, & Sun, 2016). In order to overcome the adverse effects of the EOs and extracts, several approaches specially

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incorporation of cellulose nanoparticle (CN) have been explored (El-Wakil, Hassan, Abou-Zeid, & Dufresne, 2015). The CN can be obtained from natural renewable sources such as polysaccharides, proteins and synthetic biodegradable polymers. This particle is very suitable for production of cheap, lightweight and strong bilayer and/or composite films (Dehnad, Mirzaei, Emam-Djomeh, Jafari, & Dadashi, 2014). Films containing CN has been reported to have improved mechanical and physical properties (e.g. high elongation moduli, tensile strength and low density) compared with straight film (Chang, Jian, Zheng, Yu, & Ma, 2010). To our knowledge, there are no published data on the use of pomegranate peel extract (PPE) combined with ZEO and CN in Ch and/or Ge films to extend the fresh shrimp shelf life. Therefore, the aim of the present study was to evaluate the effects of Ch and Ge films containing ZEO, PPE and CN on survival of *L. monocytogenes* and chemical, bacterial and sensory characteristics of fresh peeled shrimp during refrigerated storage.

2. Materials and methods

2.1. Materials

Fresh leave part of *Z. clinopodioides* was collected from the Gilan-e-Gharb, Kermanshah, Iran, in March–July 2015. Authentication of the plant was conducted by a taxonomist and a representative voucher specimen (6816) was deposited in the herbarium of the Research Center of Natural Resources, Tehran, Iran. Wet form of CN gel with 35 nm diameter and certified purity greater than 99.0% was purchased from Nanonovin Polymer Mazandaran Co. Ltd. (Mazandaran, Iran). Ch (medium molecular weight (w450 KDa), 75–85% deacetylated Ch flakes) and fish skin Ge powders were purchased from Sigma-Aldrich Co., UK. Moreover, all media were obtained from Merck Co., Germany.

2.2. Isolation of essential oil

The isolation of the ZEO was performed using a Clevenger-type apparatus according to the standard technique (European pharmacopoeia, 1997). 100 g of the powdered *Z. clinopodioides* was submitted to hydro-distillation for 3.5 h with double-distillated water. The EO floating on top of the condensed water was collected, dried with anhydrous sodium sulfate (0.5 g) and stored at 4 °C for further analysis.

2.3. Gas chromatography–mass spectrometry (GC–MS) analysis of essential oil

The ZEO was analyzed using a Thermo Quest Finningan Gas chromatograph coupled to mass spectrometer (GC-MS) system operating on EI mode. The column was a HP-5MS fused phenylmethylsiloxane capillary column (30 m × 0.25 mm × 0.25 μm film thickness). Helium was used as a carrier gas at a flow rate of 1.2 ml/min. The analysis was performed in following conditions: injector temperature, 290 °C; column temperature, from 50 °C to 265 °C at 2.5 °C/min; injected volume, 1 μl; split ratio, 1:20. MS acquisition parameters were as follows: electron ionization (EI), 70 eV; scan range, 30–550 amu; ion source temperature, 220 °C. The identification of chemical compounds was also performed by Thermo Quest Finningan GC using the same capillary column and analytical conditions as described above.

2.4. Preparation of pomegranate peel extract

Newly ripe pomegranate fruits (*Punica granatum* L.) were obtained from a local market in the Kermanshah, Iran. The fruits were peeled first and then air dried in a dark place at room temperature. 1 g of powdered pomegranate peel was dissolved in 20 ml methanol and extracted with a shaker at room temperature for 24 h. The extract was filtered through Whatman filter paper no.3, concentrated in a rotary evaporator at 40 °C and stored at refrigerated temperature until further use (Basiri et al., 2015).

2.5. Preparation of bacterial strain

L. monocytogenes (ATCC 19118) was obtained from the culture collection of the Iranian Research Organization for Science and Technology (IROST), Tehran, Iran and maintained on slant of Brain Heart Infusion (BHI) agar at 4 °C. Culture for antimicrobial activity test was prepared by transferring a loop of the bacterial colony from the agar slant to a test tube containing 5 ml of BHI broth. The overnight culture was diluted to 5 log CFU/ml in buffered peptone water for the inoculation of peeled shrimps.

2.6. Preparation of chitosan and gelatin films

Preparation of Ch and Ge films was adapted from the work done by Nowzari, Shábanpour, and Ojagh (2013). 2 g of Ch powder was dissolved in 100 ml 0.1 M glacial acetic acid (1% v/v) and stirred on a magnetic stirrer/hot plate at room temperature for approximately 12 h. Moreover, 3 g of fish skin Ge powder was dissolved in distilled water, first being allowed to swell at 7 °C for 15 min and then stirred at 55 °C for 30 min. Then, glycerol as a plasticizer was added into both solutions at the concentration of 75% (w/w) of Ch or Ge. After stirring for 30 min, tween 80 as an emulsifier was incorporated to a level of 0.25 ml/100 ml Ch or Ge emulsion. The ZEO (0 and 1% v/v) alone and in combination with PPE (0 and 1% v/v) and CN (0 and 1% w/v) were added to the mixture and homogenized at 1300 × g for 1 min. The film forming solution (50 ml) was poured on the 12 cm diameter glass petri dish and dried at ambient temperature (25 °C) for approximately 48 h.

2.7. Preparation of shrimp

Fresh shrimp weighing 30–35 shrimp/kg were purchased from a local farm in Kermanshah, Iran. In order to determine the effects of Ch and Ge films during storage, purchased shrimps were peeled aseptically with a sterile surgical scalpel. The shrimp samples of each treatment (100 g) were subdivided into two groups: lot 1: without artificially contaminated pathogenic bacteria to determine the shelf life and lot 2: inoculated with *L. monocytogenes*. In the latter group, shrimp portions were immersed for 5 min at room temperature in the bacterial suspension with agitation by a shaker to ensure even distribution of the organism. Then, the samples were air dried for 15 min to allow bacterial uniformly attachment onto shrimp samples. In the following, both groups were aseptically covered by the films and stored in a refrigerator (4 ± 1 °C) for further bacterial and chemical analyses at 0, 2, 4, 7, 9 and 11 days.

To assess the effects of Ch and Ge films containing ZEO, PPE and CN on survival of *L. monocytogenes* and chemical, bacterial and sensory characteristics of fresh shrimp during refrigerated storage, the experiment was arranged in a factorial design. This design (2 × 2 × 2 × 2 × 1 × 6) included two levels of ZEO (0 and 1%), PPE (0 and 1%) and CN (0 and 1%), two types of film (Ch and Ge), one storage temperature, six periods of storage (0, 2, 4, 7, 9 and 11) and repeated examinations for growth (viable count) in a food model system (peeled shrimp). All experiments were conducted in independent triplicate.