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Bio-based composite edible films containing *Origanum vulgare* L. essential oil

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ABSTRACT

Biocomposite films based on fish gelatin and chitosan containing *Origanum vulgare* L. essential oil (OEO) at 0.4, 0.8, and 1.2% (w/v) were prepared by a solvent casting method and their physicochemical and antimicrobial properties were examined. The results showed that the incorporation of OEO caused a significant decrease ($p < 0.05$) in tensile strength (TS) and elastic modulus (EM), although no significant change was observed in elongation-at-break (EAB). Water vapor permeability (WVP) and solubility of films containing OEO increased as the amount of oil increased ($p < 0.05$). Oregano essential oil-loaded films displayed superior barrier capability to ultraviolet light. Fourier transforms infrared (FTIR) spectra indicated that the interaction between polymer matrix and essential oil was occurred. The properties of the films were related with their microstructure, which was observed by scanning electron microscopy (SEM). Atomic force microscopy (AFM) images showed that the surface roughness of the films was influenced by the inclusion of oil. OEO-incorporated films also exhibited higher antimicrobial activity against the gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*), and lower for gram-negative bacteria (*Salmonella enteritidis* and *Escherichia coli*). This study showed that OEO has the potential to prepare active edible films.

1. Introduction

Currently, there is an increasing trend to utilize eco-friendly packaging materials with the goal of replacement non-degradable materials, accordingly reducing the environmental affect resulting from waste accumulation (Desobry and Debeaufort, 2012). So, conventional synthetic polymers, such as polyethylene or polypropylene, are being replaced by biopolymers acquired from natural and sustainable origins.

Bio-based polymers such as polysaccharides, proteins, lipids and their composites are regarded as the most promising candidates for this goal because these materials have the benefits of being biodegradable, abundantly available and renewable (Gontard and Guilbert, 1994). Gelatin is one of the most examined biopolymers for its film-forming capacity (Denavi et al., 2009; Gómez-Estaca et al., 2011; Gómez-Guillén et al., 2009; Liu et al., 2012). In general, most of the commercial gelatins are prepared from mammalian sources (porcine and bovine). Nevertheless, due to the happening of bovine spongiform encephalopathy and the prohibiting of gelatin from pig skin and bone in some areas for religious causes, the search for alternative gelatin raw materials has received increasing attention.

Fish gelatin has obtained increasing consideration in current years as an encouraging alternative to mammalian gelatin, since it can be separated from byproducts of fish processing industry and has no health problems for consumers' safety concerns (Surh et al., 2006). This biopolymer shows good film-forming properties, yielding transparency, more or less colorless and very flexible films (Gómez-Estaca et al., 2009).

Nevertheless, gelatin has approximately poor water barrier and mechanical characteristics, which is the major disadvantages of gelatin films for food packing applications (Chiou et al., 2008). One of the operative approaches, which would preserve biodegradability, is to fabricate bio-composites by combination of fish gelatin with other bio-based polymers with film forming properties and obtained from sustainable reserves.

Chitosan is an interesting biodegradable and cationic polysaccharide, with a good film-forming ability. Chitosan-based films are tenacious, enduring, extensible, and very difficult to cut (Jeon

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et al., 2002). Furthermore, on account of antibacterial activity and non-toxicity, this biopolymer has a high potential as antimicrobial packaging film (Kurek et al., 2013; Ojagh et al., 2010; Shahidi et al., 1999). The blending of fish gelatin with chitosan in films has been relatively considered, primarily to improve the film barrier properties (Hosseini et al., 2013a; Jeya Shakila et al., 2012).

An important cause of food spoilage is microbial growth on food surfaces (Ben Arfa et al., 2007). To protect food surfaces from spoilage, dipping, and spraying of antimicrobial substances are done (Lanciotti et al., 2003). But direct surface applications onto foods have little advantages science the active substances can become deactivated by food components or neutralized on contact, or diffuse quickly from the surface into the bulk food (Ben Arfa et al., 2007). To solve this problem, antimicrobial packaging, in which antimicrobial substances can be gently released from the packaging onto the food surface, has been proposed (Chalier et al., 2009).

Essential oils (EOs) from plant extracts are natural antimicrobial agents (Debiagi et al., 2014). EOs from herbs and spices are shown to have strong antibacterial activities against food borne pathogens (Ben Arfa et al., 2007). EOs can be incorporated into edible films and coatings to change flavor, aroma, and odor, besides to induce antimicrobial characteristics (Desobry and Debeaufort, 2012).

The essential oil of *Origanum vulgare* L. (or commonly known as oregano) is well recognized for its antioxidative and antimicrobial action (Burt, 2004). These activities are mostly attributed to the two phenols, carvacrol, and thymol (major constituents of oregano essential oil) and the monoterpene hydrocarbons *p*-cymene and γ-terpinene which are present at low concentrations (Burt, 2004; Hosseini et al., 2013b). The antimicrobial properties of OEO have been demonstrated in several studies (Benavides et al., 2012; Pelissari et al., 2009; Seydim and Sarikus, 2006).

It is expected that the incorporation of OEO into fish gelatin–chitosan films suggests the probability not only of giving antimicrobial activity, but also enhancing the physicochemical attributes. To our knowledge, no data is accessible about the incorporation of OEO in biocomposite films based on fish gelatin and chitosan.

Hence, this work was undertaken to evaluation of oregano essential oil effect, in different proportions, on the properties of biocomposite films. The characterizations included water barrier ability, mechanical behavior, optical properties, microstructural, and surface morphology. The antimicrobial properties of the films against gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*) and gram-negative bacteria (*Salmonella enteritidis* and *Escherichia coli*) were also assessed.

2. Materials and methods

2.1. Materials

Cold water fish skin gelatin, chitosan (medium molecular weight, 75–85% degree of deacetylation) and tween 80 were obtained from Sigma–Aldrich (St. Louis, MO, USA). Acetic acid and glycerol (analytical grade) were obtained from Merck Chemicals Co. (Darmstadt, Germany). OEO (100% pure) was purchased from New Directions Aromatics Inc. (Hampshire, UK). All chemical reagents were utilized without further purification. *S. aureus* PTCC 1337, *L. monocytogenes* PTCC 1298, *S. enteritidis* PTCC 1709 and *E. coli* PTCC1330 were acquired from the Persian type culture collection (Tehran, Iran). All strains were kept in brain heart infusion broth added with 30% glycerol at −20 °C until utilized.

2.2. Biocomposite films preparation

The films were produced according to the casting method as reported by Gómez-Estaca et al. (2011) with slight modifications. The filmogenic solution was prepared according to the following procedure: firstly, a gelatin film-forming solution (FFS) (2% w/v) was prepared by dissolving 2 g gelatin in 100 ml distilled water for 30 min and then heated at 45 °C for 30 min under continuous stirring. After that, chitosan solution was prepared with 1.5% (w/v) chitosan in 1% (v/v) acetic acid, agitated overnight at ambient temperature and filtered by 1 μm pore size filters to remove insoluble substances. The fish gelatin–chitosan film-forming solutions were prepared by mixing a 2% (w/v) gelatin solution with a 1.5% (w/v) solution of chitosan, in a proportion of 1:1 (v/v). This mixture was heated and stirred at 45 °C for 30 min to obtain a uniform blend. Glycerol (0.3 g/g gelatin plus chitosan) was added as a plasticizer and the solution was again heated and agitated at 45 °C for 15 min. Then, tween 80 at volume of 0.2% (w/w, based on essential oil), was added as an emulsifier to assist essential oil dispersion in FFS. After 30 min of agitating, OEO at 0.4, 0.8 and 1.2% (w/v) ratios was added to the FFS. The mixture was homogenized at 13,000 rpm for 2 min using an IKA T25 digital homogenizer (Ultra-Turrax, Germany). FFS without essential oil (control film) was also prepared. After cooling to room temperature, the FFS was vacuum degassed for 15 min to remove air bubbles. The final film-forming solution (∼100 g) were poured in rectangular plastic dishes ($24 \times 12\,\text{cm}^2$) and dried at ambient temperature (23–25 °C) for 72 h to provide a uniform thickness of 70 ± 12 μm in all samples. The dry films were peeled off and stored at 25 °C with 50 ± 4% RH for 48 h until for further analysis.

2.3. Characterization of the films

2.3.1. Mechanical properties

A universal testing machine (SMT-20, Santam, Tehran, Iran) equipped with a 60 N load-cell was used to determine the tensile strength (TS), elongation-at-break (EAB), and elastic modulus (EM) of the biocomposite films according to the ASTM standard method D 882-09 (2009). Film specimens (rectangular strips of 110 × 20 mm) were conditioned in an environmental chamber for 48 h at 23 ± 2 °C and 53 ± 2% RH before testing. The initial grip separation and mechanical crosshead speed was set at 50 mm and 5 mm/min, respectively. At least five replicates were analyzed for each film and the mean values were reported.

2.3.2. Film solubility

Film solubility was tested according to the method reported in the literature (Gontard et al., 1992). Three pieces ($1 \times 4\,\text{cm}^2$) of film were weighed to the nearest 0.0001 g and afterwards dried in a forced-air oven at 105 °C for 24 h. At this time, films were regained and re-weighed to calculate their initial dry weight (W_i). Then, the samples were submerged in distilled water (30 ml) and mildly shaken (100 rpm) for 24 h at ambient temperature. The samples were then filtered using Whatman No. 1. The filter paper plus undissolved portions was dried in an air-circulating oven (105 °C, 24 h) and weighed (W_f). The solubility of film (FS %) was determined using the Eq. (1):

$$\text{FS\%} = \frac{W_i - W_f}{W_i} \times 100 \tag{1}$$

W_i = Initial dry film weight (g).

W_f = Final dry film weight (g).

2.3.3. Water vapor permeability

The water vapor permeability (WVP) of films was determined gravimetrically using the ASTM E96-05 method (2005) with the adaptations proposed by McHugh et al. (1993) specifically for edible films. Circular glass cups with a diameter of 49 mm and a depth of 1.1 cm were applied. The film samples were sealed onto the cup mouth containing 6 ml distilled water (100% RH; 2.337×10^3 Pa