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Effects of essential oil on the water binding capacity, physico-mechanical properties, antioxidant and antibacterial activity of gelatin films

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ABSTRACT

Gelatin films were prepared from gelatin solutions (10% w/v) containing *Zataria multiflora* essential oil (ZMO, 2, 4, 6 and 8% w/w of gelatin). Scanning electron microscopy observations indicate that ZMO droplets were well dispersed in the film matrix. Water solubility, water swelling, water uptake, water vapor permeability, tensile strength, elongation at break and Young's modulus for gelatin films were 27 ± 0.8%, 391 ± 11%, 135 ± 5%, 0.22 ± 0.014 g mm/m^2 kPa h, 4.4 ± 0.4 MPa, 125 ± 7% and 8.8 ± 0.4 MPa, respectively. Incorporation of ZMO into gelatin films caused a significant decrease in swelling and water uptake and increase in solubility and water vapor permeability, a significant decrease in tensile strength, increase in elongation at break, decrease in Young's modulus of the films, dose-dependently. Gelatin/ZMO showed UV–visible light absorbance/transmission ranging from 280 to 480 nm with maximum absorbance at 420 nm. Gelatin films exhibited very low antioxidant activity while, gelatin/ZMO films exhibited excellent antioxidant properties. The gelatin/ZMO films also exhibited excellent antibacterial properties against both Gram-positive and Gram-negative bacteria. Our results suggested that the gelatin/ZMO films could be used as an active film due to its excellent antioxidant and antimicrobial properties for food packaging applications.

1. Introduction

Gelatin is a soluble protein obtained by partial hydrolysis of collagen, the main insoluble fibrous protein constituent of bones, cartilages and skins with high potential applications in food and pharmaceutical industries (Gan, Zhang, Liu, & Wu, 2012; Gomez-Guillen, Gimenez, Lopez-Caballero, & Montero, 2011; Rawdkuen, Sai-Ut, & Benjakul, 2010). The pharmaceutical applications of gelatin are based mainly on the gel/film-forming properties. Recently, an increasing number of new applications have been found for gelatin in products, such as emulsifiers, foaming agents, colloid stabilizer, hydrogels, packaging materials, wound dressing and micro-encapsulating agents (Arora & Padua, 2010; Boateng, Matthews, Stevens, & Eccleston, 2008; Sorrentino, Gorrasi, & Vittoria, 2007). Gelatin has also been reported to be one of the first materials used as carrier of bioactive components (Gomez-Guillen et al., 2011). There is growing interest in using plant extracts as natural sources of antioxidant/antibacterial compounds in the formulation of gelatin films (Appendini & Hotchkiss, 2002; Lucera, Costa, Conte, & Del Nobile, 2012). In this context, plant essential oils and their main components are gaining a wide interest in health industry for their potential as antioxidant and antimicrobial agents, as they are generally recognized as safe (Solorzano-Santos & Miranda-Novales, 2012). Lemongrass and bergamot oils (Ahmad, Benjakul, Prodpran, & Agustini, 2012), thyme and oregano oils (Altiok, Altiok, & Tihminlioglu, 2010), citrus oil (Tongnuanchan, Benjakul, & Prodpron, 2012), garlic oil (Pranoto, Salokhe, & Rakshit, 2005) and some other essential oils from medicinal plants have been used to improve antioxidant and antibacterial capacity to gelatin films (Gomez-Estaca, Lopez de Lacey, Lopez-Caballero, Gomez-Guillen, & Montero, 2010).

To our knowledge there is no report on the antioxidant and antimicrobial activity of gelatin films incorporated with *Zataria multiflora* (ZM). ZM is a thyme-like plant belonging to the Lamiaceae family that grows only in Iran, Pakistan and Afghanistan. This plant has played an important role in Iranian traditional medicine.

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It has several traditional uses as an antiseptic, carminative, stimulant, diaphoretic, diuretic, anesthetic, antispasmodic and analgesic. In the modern pharmacological and clinical investigations, ZM is a valuable medicinal plant that has antimicrobial, antioxidative, anti-inflammatory, spasmolytic and anti-nociceptive properties (Sajed, Sahebkar, & Iranshahi, 2013).

In this study the gelatin films with antioxidant and antimicrobial activities were prepared from gelatin solutions containing different ZM essential oil (ZMO) concentrations. The water solubility, water swelling, water uptake, water vapor permeability, light absorbance and mechanical properties of gelatin/ZMO films were examined. Antioxidant activities of the gelatin/ZMO films were examined using 2′-azino-di (3-ethylbenzthiazoline-6-sulfonate) (ABTS) decolorization. The gelatin/ZMO films individually tested against two Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) and two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) commonly found in human pathogenesis.

2. Materials and experimental details

2.1. Preparation of gelatin solutions and film casting

Bovine gelatin powder (10% w/v, Merck, Germany) was dissolved into 80 mL of distilled water at ambient temperature. The mixture was stirred for 30 min at 45 °C using a Hotplate-stirrer. Then after cooling to 37 °C, ZMO (Kavoosi, Teixeira da Silva, & Saharkhiz, 2012) with different concentration (2, 4, 6, and 8% w/w based on the weight of the gelatin powder = 2, 4, 6, and 8 mg/mL based on the gelatin solutions) was added as the antimicrobial agent and mixed carefully. Glycerol (25% w/w based on the weight of the gelatin powder = 250 mg/mL based on the gelatin solution) (Merck, Germany) as plasticizer and glutaraldehyde (0.2% w/w based on the weight of the gelatin powder = 2 mg/mL based on the gelatin solution) were added to gelatin solutions. The solution was diluted to a final volume of 100 mL with distilled water and the solutions were stirred for 10 min (Ahmad et al., 2012). To cast the films, 10 mL of gelatin solutions containing different ZMO concentrations were transferred into a polystyrene Petri dish and placed at room temperature until films were dried. The dried films were peeled off and stored at 4 °C until analysis. The films thicknesses were measured to the nearest 0.01 mm with a digital micrometer (The L.S. Starrett Co. LTD, Great Britain, UK) and the average was taken (in five spots of three films) 97 ± 5 μm.

2.2. Scanning electron microscopy

Scanning electron microscopy (SEM) of the film samples was performed using a Hitachi 570 SEM (FESEM Hitachi S4160, Japan) in the School of Metallurgy and Materials Engineering University of Tehran, Tehran Iran. The film samples (10 mm × 10 mm × 0.1 mm) were immersed in liquid nitrogen and cryo-fractured by hand. SEM pictures with 1500× magnification were taken with an accelerating voltage of 20 kV.

2.3. Water solubility of the films

The film samples (20 mm × 20 mm × 0.1 mm) were placed in an oven at 104 °C for 24 h and were then weighed. This was considered as the initial weight (W_i). Then, the dried films were immersed into a 100 mL Erlenmeyer flask containing 50 mL of distilled water. The flask was placed inside the shaker for 24 h at 25 °C. Thereafter, the film samples were taken out, transferred to the oven at 104 °C for 24 h and were then weighed. This was taken as the final weight (W_f). The occurrence of weight loss or solubility percentage (S%) was determined by using the following formula (Ahmad et al., 2012): $S\ (\%) = [(W_i - W_f)/W_i] \times 100$. The reported results are the average of at least three measurements.

2.4. Swelling test

The films samples (20 mm × 20 mm × 0.1 mm) were dried in an air-circulating oven at 104 °C for 24 h until they reached a constant weight. The weight at this condition was taken as initial weight (W_i). The film samples were immersed into a 100 mL Erlenmeyer flask containing 50 mL of the distilled water for 24 h at room temperature. Then, each samples were taken out of the flask, wiped between filter papers to remove the excess surface water and were weighed. The weights at this condition were used as the final weight (W_f). The weight gaining or swelling percentage (SW%) was calculated using the following equation (Altiok et al., 2010): $SW\ (\%) = [(W_f - W_i)/W_i] \times 100$. All tests are the means of at least three measurements.

2.5. Water uptake test

The film samples (20 mm × 20 mm × 0.1 mm) dried in desiccators at concentrated H_2SO_4 (relative humidity = 0%) for three days to reach a constant weight. The weight at this condition was taken as the initial weight (W_i). Then, the film samples were transferred into desiccators at 100% relative humidity (sodium sulfate solution) at 37 °C for one week and allowed to absorb water, and then weighed after reached to the equilibrium state. The weight at this condition was used as final weight (W_f). The weight gaining or water uptake percentage calculated using the following equation (Tongnuanchan et al., 2012): $\text{Water uptake}\ (\%) = [(W_f - W_i)/W_i] \times 100$. All tests are the means of at least three measurements.

2.6. Water vapor permeability test

The film samples (7 cm diameter) were conditioned for 24 h at 25 °C and 75% relative humidity. Water vapor permeability (WVP) of the film samples was examined using aluminum cups (height and diameter of 2.1 and 5.6 cm, respectively) filled with 20 g silica. The cups were covered with film samples and placed at 25 °C and 75% relative humidity in desiccator. The weight of the cups was measured at 3 h intervals during one day. A graph was plotted to demonstrate the mass change against time (h). Water vapor transmission rates (WVTR) of the films were calculated from the slope of the mentioned plots per film's area (m^2) and expressed as g/m^2 h. The WVP was calculated using the following formula: $\text{WVP}\ (\text{g mm}/\text{m}^2\ \text{kPa h}) = [(\text{WVTR} \times T)]/\Delta P$. Here T is the film thickness (mm) and ΔP the partial water vapor pressure difference (kPa) between the two sides of the film (4.2449 kPa at 30 °C) (Pranoto et al., 2005).

2.7. Mechanical test

The films samples (60 mm × 10 mm × 0.1 mm) were placed in a closed container with relative humidity of 65% (saturated sodium nitrite vapor) for equilibrium for 48 h. The tensile strength test was performed by stretching the film at pretest, test and posttest speeds of 1, 1 and 10 mm/s, respectively in texture analyzer (TA.XT Stable Micro System, UK). The area of the film used for each experiment was 6 cm × 1 cm. As 2 cm of the film were placed between the jaws, then the effective free-standing film area was 4 cm^2. The texture analyzer runs at auto force mode with the trigger force of 5 g (0.049 N). From stress–strain curves, three parameters were calculated: 1) Tensile strength (TS) at the maximum stress, i.e., TS