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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 $27 \pm 0.8\%$, $391 \pm 11\%$, $135 \pm 5\%$, 0.22 ± 0.014 g mm/m^2 kPa h, 4.4 ± 0.4 MPa, $125 \pm 7\%$ 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

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(N/m^2) = (Breaking force/Cross-sectional area of sample). 2) Elongation at break (EAB) where the film is torn, i.e., EAB (%) = [(Increase in length at breaking point/Initial length)] × 100. 3) Young's modulus – the initial slope of the stress–stain curve at the linear part (Bigi, Cojazzi, Panzavolta, Rubini, & Roveri, 2001).

2.8. Light absorption and opacity

The light absorbance of the film samples (1 cm × 6 cm × 0.1 mm) was measured at wavelengths ranging from 200 to 700 nm using UV–vis spectrophotometer (Pharmacia, Uppsala, Sweden). The opacity of the films was calculated by the following equation: opacity (nm/mm) = Abs420/film thickness. Abs420 is the value of absorbance at 420 nm (Nunez-Flores et al., 2012).

2.9. Antioxidant activity test

Antioxidant activities of the film samples were determined by decolorization method with 2,2′-azino-di (3-ethylbenzthiazoline-6-sulfonate) (ABTS, Sigma, Germany) (Tongnuanchan et al., 2012). Briefly, films cuts (10 mm × 10 mm × 0.1 mm, 10 mg) from different parts of the films containing 2, 4, 6, and 8% of the ZMO were added to 2.0 mL of diluted ABTS radical solution (7 mM ABTS and 2.54 mM potassium persulfate, A734 = 1 ± 0.1). Films without ZMO were used as blank. The light absorbance was recorded after 120 s using a plate reader (BioTek Elx 808, Winooski, VT 05403, USA). A standard curve of ascorbic acid ranging from 0.44 to 15.76 mg/mL was prepared. Antioxidant activity was expressed as mg ascorbic acid equivalents per gram of films using standard curve.

2.10. Antibacterial activity test using disc diffusion

All microorganisms used in this study were obtained from the Persian type culture collection (PTCC), Tehran, Iran. The film samples were individually tested against two Gram-negative bacteria [*P. aeruginosa* PTCC 1074 and *E. coli* PTCC 1330] and two Gram-positive bacteria [*S. aureus* PTCC 1112 and *B. subtilis* PTCC 1023]. To investigate the antimicrobial activity of the films using disc diffusion, 30 mm diameter discs (with a thickness of 0.1 mm) were cut from different parts of the films and sterilized by autoclaving for 30 min at 120 °C (Bauer, Kirby, Sherris, & Turck, 1996; Gomez-Estaca et al., 2010). Bacterial suspensions with a turbidity equivalent to a McFarland 0.5 standard were prepared (10^8 CFU/mL) and then diluted to 10^5 CFU/mL with Luria–Bertani (LB, Merck, Darmstadt, Germany). The adjusted bacterial suspensions (0.1 mL) spread onto the nutrient agar (Merck, Darmstadt, Germany) plates (Farazbin Kimia Co., Teharan, Iran) containing LB. Subsequently, the discs were placed in direct contact with the agar medium. Plates inverted and incubated at 37 °C for 24 h. Films without ZMO under the same condition were used as control. The diameters of clear inhibition zones, including the diameter of the disc, measured using a ruler and were used to evaluate antibacterial potential of the films.

2.11. Antibacterial activity test using colony counting

Bacteria strains suspended in LB media and the densities adjusted to 0.5 McFarland standards at 640 nm (10^8 CFU/mL) and then diluted to 10^5 CFU/mL with LB. A sample film with 30 mm diameter was placed in a 10 mL liquid culture containing 10 μL microbe cultures. Then, the sample was incubated at 37 °C for 24 h (Shaking Incubator, Shin Saeng, Fine Tech, South Korea). From the incubated samples, a 100 μL solution was taken and diluted with the appropriate dilution factor and the final diluted microbe solution was plated and distributed onto nutrient agar plate (Farazbin Kimia Co., Tehran, Iran). The plates cultured with the films without ZMO under the same condition were used as control. All plates were incubated at 37 °C for 24 h and the numbers of colonies that formed were counted. The antibacterial efficacy of the film samples was calculated according to the following equation (Maneerung, Tokura, & Rujiravanit, 2008): Colony reduction (%) = [(The number of colonies in the presence of pure gelatin – The number of colonies in the presence of gelatin/ZMO)/Number of colonies in the presence of pure gelatin] × 100.

2.12. Statistical analysis

All data are representative of at least three independent experiments and expressed as the mean values plus standard deviations. The significant differences between treatments were analyzed by one-way analysis of variance (ANOVA) and Duncan tests at $P < 0.05$ using statistical package for the social sciences (SPSS, Abaus Concepts, Berkeley, CA, USA) and Prism 5 (Graph Pad, San Diego, USA) software's.

3. Results and discussion

3.1. Film morphology

SEM pictures of the films are presented in (Fig. 1). The control film (pure gelatin) had compact, smooth, transparent, colorless and homogeneous surface structure, which is indicate of ordered matrix surface that is due to the results of excellent film forming properties of gelatin. The addition of ZMO increased roughness, opaqueness and whiteness of the film. The ZMO incorporated films had bubble like structures which homogeneously distributed in film matrix. The number of bubbles increased with the increase in the essential oil concentration, thus this bubbles caused by ZMO (Bigi et al., 2001; Nunez-Flores et al., 2012). This homogenous dispersion of ZMO in the film matrix could improve functional properties of the film, including water binding, light barrier and tensile property.

3.2. Solubility determination of films

The water solubility percentages (weight loss) of the gelatin films are summarized in Table 1. The solubility percentage for the gelatin films was 27 ± 0.8%. Incorporation of ZMO (6% and 8% w/w of gelatin) into the films caused a significant increase in the solubility, dose-dependently ($p < 0.05$). Gelatin is a water-soluble material and can dissolve partially when coming into contact with an aqueous medium and lose their fibrous structure. However, cross-linking by glutaraldehyde can stabilize gelatin structure and decrease its solubility in aqueous medium (Bigi et al., 2001; Gomes, Rodrigues, Martins, Henriques, & Silva, 2013). ZMO is hydrophobic material and favorably interacts with hydrophobic domain of gelatin and may hinder polymer chain-to-chain interactions and consequently, causes an increase in the solubility of the films (Hong, Lim, & Song, 2009; Rhim, Gennadios, Handa, Weller, & Hanna, 2000). Gomez-Estaca et al. (2010) reported that gelatin–chitosan films in the presence of essential oil, show a significant increase in the film solubility which is in accordance to our experimental results.

3.3. Swelling and water uptake capacity

Swelling and water uptake percentage for the gelatin film were 391 ± 11% and 135 ± 5%, respectively. Incorporation of ZMO (4%, 6% and 8% w/w of gelatin) into the gelatin films caused a significant

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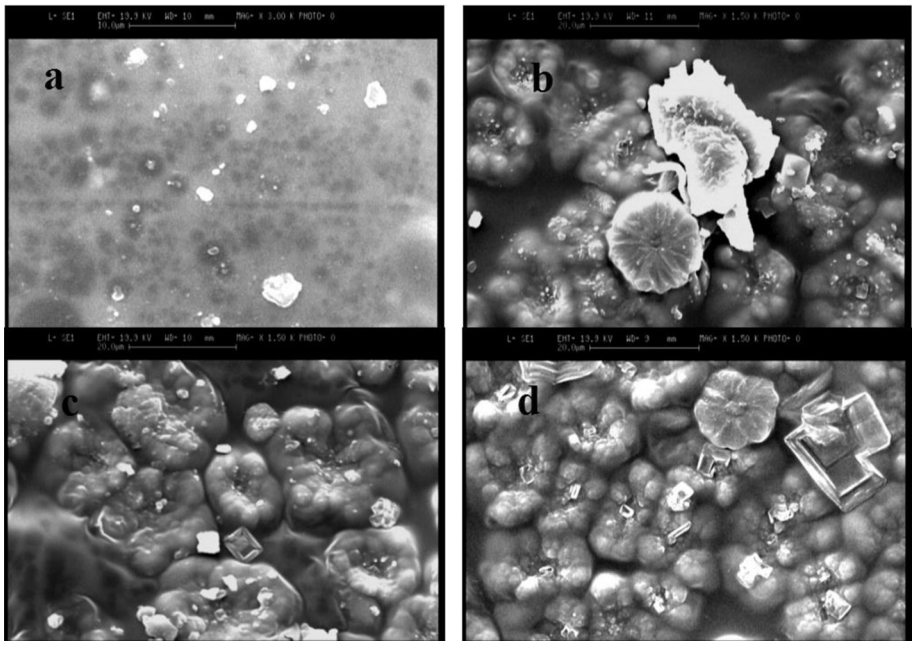

Fig. 1. Scanning electron microscopy images of gelatin film. a, Pure gelatin film. b, gelatin film incorporated with 4% ZMO. c, gelatin film incorporated with 6% ZMO. d, gelatin film incorporated with 8% ZMO.

decrease in the swelling (Table 1) and water uptake (Table 1) of the films ($p < 0.05$). Gelatin is a hydrophilic material that is expected to absorb water molecules. The porous gelatin films showed higher swelling capacity because the porosity in their network structures that allows more water to enter inside the film. Incorporation of ZMO could reduce swelling capacity of the gelatin films which, might be related to hydrophobic nature of ZMO. Hydrophobic domains of gelatin can essentially interact with ZMO through hydrophobic interaction and thereby enhance interfacial interaction between matrix (gelatin) and filler (ZMO) (Hong et al., 2009; Rhim et al., 2000). This event saturates gelatin network with ZMO thus, water molecules cannot diffuse to gelatin network thereby swelling is decreased. These results suggest that gelatin films incorporated with ZMO could be promising candidates for liquid absorbing packaging materials.

3.4. Water vapor permeability

Water vapor permeability (WVP) for gelatin film was 0.22 ± 0.014 g mm/kPa m^2 h. Incorporation of ZMO (4%, 6% and 8% w/w of gelatin) into the gelatin films caused a significant increase in WVP (Table 1). Gelatin is a hydrophilic material so strongly interact with water molecules and cause a reduction in the water vapor transmission through gelatin film (Avena-Bustillos et al., 2011). Incorporation of additive to gelatin films cause a significant change in water vapor transmission through films, while the final WVP capacity is related to hydrophobicity/hydrophilicity index of all compounds in the films. Hydrophobic domains of gelatin can essentially interact with ZMO through hydrophobic interaction and thereby enhance interfacial interaction between matrix and ZMO. This phenomenon hinders interactions between gelatin chains and water molecules thus, cause the increase in WVP (Rhim et al., 2000). Our experimental results showed that the WVP of gelatin films incorporated with ZMO slightly increases. This is in accordance with the results reported by Altiok et al. (2010).

3.5. Mechanical properties

Tensile strength, elongation at break and Young's modulus of the gelatin film cross-linked with glutaraldehyde were 4.4 ± 0.4 MPa, $125 \pm 7\%$ and 8.8 ± 0.4 MPa, respectively (Table 2). Incorporation of ZMO into the gelatin films caused a significant decrease in tensile strength, increase in elongation at break and decrease in Young's modulus of the films ($p < 0.05$). Gelatin films were mainly stabilized by weak bonds including hydrogen bond and hydrophobic interaction. Cross-linking with glutaraldehyde by inserting covalent bond between gelatin strands leads to a significant increase in tensile strength of the films. Change in color

Table 1
Water solubility, swelling, water uptake and water vapor permeability (WVP) of gelatin films incorporated with *Zataria multiflora* essential oil (ZMO).

ZMO	Solubility (%)	Swelling (%)	Water uptake (%)	WVP (g mm/kPa m^2 h)
0% of Gelatin	27 ± 1.2^{c}	391 ± 11^{a}	135 ± 5^{a}	0.22 ± 0.014^{b}
2% of Gelatin	28 ± 1.1^{c}	373 ± 7^{ab}	126 ± 4^{ab}	0.23 ± 0.024^{b}
4% of Gelatin	29 ± 1.3^{bc}	363 ± 9^{bc}	122 ± 3^{b}	0.25 ± 0.022^{ab}
6% of Gelatin	31 ± 1.5^{ab}	350 ± 8^{c}	116 ± 2^{bc}	0.28 ± 0.025^{a}
8% of Gelatin	33 ± 1.2^{a}	326 ± 7^{d}	113 ± 3^{c}	0.31 ± 0.026^{a}

[a–d] Mean values with different letters within a column show significant difference ($p < 0.05$).

Table 2
Mechanical properties and opacity of gelatin films incorporated with *Zataria multiflora* essential oil (ZMO).

ZMO	Tensile strength (MPa)	Elongation (%)	Young's modulus (MPa)	Opacity (nm/mm)
0% of Gelatin	4.4 ± 0.26^{a}	125 ± 7^{d}	8.8 ± 0.4^{a}	7.4 ± 1.2^{c}
2% of Gelatin	3.7 ± 0.21^{ab}	140 ± 5^{cd}	7.5 ± 0.25^{b}	11.7 ± 1.3^{b}
4% of Gelatin	3.4 ± 0.23^{bc}	144 ± 6^{bc}	6.7 ± 0.26^{c}	14.5 ± 2^{ab}
6% of Gelatin	2.9 ± 0.17^{cd}	167 ± 7^{a}	6.2 ± 0.31^{cd}	16 ± 1.8^{a}
8% of Gelatin	2.7 ± 0.18^{d}	172 ± 8^{a}	5.7 ± 0.24^{d}	17.6 ± 2.3^{a}

[a–d] Mean values with different letters within a column show significant difference ($p < 0.05$).

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parameters from white to yellow is a clear cross-linking between gelatin networks. The change in color of the films upon cross-linking with glutaraldehyde is caused by the formation of aldimine linkages between the free amino groups of lysine or hydroxylysine amino acid residues of the protein and the aldehyde groups of glutaraldehyde (Bigi et al., 2001). Addition of ZMO possibly resulted in the lowered interaction between gelatin strands, and may hinder gelatin chain-to-chain interactions and consequently, caused a significant decrease in the tensile strength of the films (Avena-Bustillos et al., 2011; Limpisophon, Tanaka, & Osako, 2010). Tongnuanchan et al. (2012) reported that gelatin films incorporated with citrus oil also showed a lower tensile strength but higher elongation at break rather than the control films without incorporated citrus oil which is in accordance to our experimental results.

3.6. Light absorption and opacity

Pure gelatin films showed light absorbance in the range between 280 and 480 nm, while maximum absorbance was at 420 nm. Incorporation of ZMO into the gelatin films caused a significant increase in the light absorbance and opacity of the films at 420 nm (Table 2). Protein-based films are considered to have high light barrier properties, owing to their high content of aromatic amino acids which absorb UV light (Ahmad et al., 2012). The addition of ZMO increased greatly the opacity of gelatin films. Thus, the gelatin films lost their typical transparent and colorless appearance. However, the resulting gelatin/ZMO films gained in light barrier properties, which could be interesting in certain food applications for preventing UV-induced lipid peroxidation (Altiok et al., 2010; Tongnuanchan et al., 2012). The increase in the light absorbance more likely depended on the distribution of ZMO in the gelatin matrix as well the interaction between ZMO and gelatin. This effect led to differences in film matrix morphology with different light absorbance. ZMO droplets that were localized in the gelatin matrix increased the opacity of the gelatin film, more likely due to the light scattering effect (Tongnuanchan et al., 2012). Ahmad et al. (2012) reported that gelatin films incorporated with bergamot and lemongrass oils also showed a higher light absorbance in the visible range compared to gelatin films without the oils which is in accordance to our experimental results.

3.7. Antioxidant activity

Antioxidant activity of the gelatin films incorporated with different ZMO concentrations was determined by ABTS decolorization method and expressed as mg ascorbic acid equivalent per gram of the films (Table 3). The gelatin films without ZMO showed very low activity against the ABTS decolorization. Aleman et al. showed that the peptides derived from enzymatic hydrolysis of gelatin are lipid peroxidation inhibitors, free radical scavengers and transition metal ion chelators. These antioxidative properties of peptides are related to their amino acid composition, molecular weight, structure and hydrophobicity (Aleman, Gimenez, Montero, & Gomez-Guillen, 2011). The gelatin films containing different ZMO concentrations decolorize ABTS, dose-dependently. Chitosan films incorporated with thyme oil (Altiok et al., 2010) and fish skin gelatin film incorporated with citrus essential oils (Tongnuanchan et al., 2012) were exhibited strong antioxidant activity. The total phenolic content and related total antioxidant capacity of some medicinal plant infusions were analyzed, which indicated that there was a significant linear correlation between total phenol content and antioxidant capacity (Katalinic, Milos, Kulisic, & Jukic, 2006). ZMO possesses nitric oxide (NO) and malondialdehyde scavenging properties and thus could prevent oxidative and nitrative stress and lipid peroxidation (Kavoosi et al., 2012). ZMO gradually released from the films to the ABTS solution and decolorized it. These results suggested that gelatin films with excellent antioxidant activity could be promising candidates for safe radical scavenger material.

Table 3
Antioxidant activity of gelatin films incorporated with *Zataria multiflora* essential oil (ZMO).

ZMO	Milligram ascorbic acid equivalent (AAE)/gram of films
0% of Gelatin	0.84 ± 0.11^{d}
2% of Gelatin	2.6 ± 0.38^{c}
4% of Gelatin	3.5 ± 0.67^{bc}
6% of Gelatin	4.4 ± 0.60^{ab}
8% of Gelatin	5.5 ± 0.51^{a}

[a–d] Mean values with different letters within a column show significant difference ($p < 0.05$).

Table 4
The antibacterial activity of gelatin films incorporated with *Zataria multiflora* essential oil (ZMO).

ZMO	Inhibition zone diameter (mm)			
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
0% of Gelatin	0 ± 0^{e}	0 ± 0^{e}	0 ± 0^{d}	0 ± 0^{d}
2% of Gelatin	32 ± 1.2^{d}	31 ± 0.9^{d}	30 ± 1^{c}	30 ± 09^{c}
4% of Gelatin	35 ± 1^{c}	34 ± 0.9^{c}	31 ± 1.1^{c}	30 ± 0.9^{c}
6% of Gelatin	40 ± 1.5^{b}	41 ± 1.1^{b}	34 ± 1^{b}	33 ± 1.1^{b}
8% of Gelatin	45 ± 1.7^{a}	46 ± 1.3^{a}	38 ± 1.8^{a}	36 ± 1.8^{a}

[a–e] Mean values with different letters within a column show significant difference ($p < 0.05$).

3.8. Antibacterial activity

Antibacterial activity of gelatin films incorporated with ZMO expressed by disc diffusion method and viable colony counting assay. The results of disc diffusion and colony reduction percentage are summarized in Tables 4 and 5, respectively. The initial diameter of all films was fixed at 30 mm. The diameters of clear inhibition zones, including the diameter of the disk, were used for antibacterial activity analysis. According to the results obtained, all the gelatin films without ZMO showed no activity against the tested bacteria. However, gelatin hydrolysates exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria. Several factors such as amino acid composition, sequence, molecular weight and hydrophobicity index as well as type of tested bacteria can affect antibacterial activity of gelatin derived peptides (Di Bernardini et al., 2011; Rojas-Grau et al., 2007). Gelatin/ZMO films are effective against both Gram-positive and Gram-negative bacteria while they are more effective to Gram-positive bacteria rather than to Gram-negative bacteria. Gelatin film from the skin of unicorn leatherjacket incorporated with essential oils (Ahmad et al., 2012) and chitosan films incorporated with thyme oil

Table 5
The antibacterial activity of gelatin films incorporated with *Zataria multiflora* essential oil (ZMO).

ZMO	Colony reduction (%)			
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
0% of Gelatin	0 ± 0^{e}	0 ± 0^{e}	0 ± 0^{e}	0 ± 0^{e}
2% of Gelatin	33 ± 1.2^{d}	35 ± 1.4^{d}	26 ± 1.6^{d}	22 ± 2^{d}
4% of Gelatin	56 ± 2.2^{c}	59 ± 2.2^{c}	45 ± 2.3^{c}	38 ± 3^{c}
6% of Gelatin	78 ± 3.4^{b}	80 ± 3.3^{b}	65 ± 3.2^{b}	49 ± 3^{b}
8% of Gelatin	100 ± 2.7^{a}	96 ± 4.3^{a}	87 ± 3.5^{a}	66 ± 4^{a}

[a–e] Mean values with different letters within a column show significant difference ($p < 0.05$).

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(Altiok et al., 2010) displayed excellent antibacterial activities, which are confirmed our experimental results. These antibacterial activities of essential oils is related with the attack on the phospholipids present in the cell membranes, which causes increased permeability and leakage of cytoplasm, or in their interaction with enzymes located on the cell wall (Paparella et al., 2008). ZMO is a good source of phenolic monoterpenes (thymol and carvacrol), with a significant antimicrobial activity against both gram-positive and gram-negative bacteria (Mohammadi Purfard & Kavoosi, 2012; Saei-Dehkordi, Tajik, Moradi, & Khalighi-Sigaroodi, 2010). ZMO gradually releases from films to the solution and penetrates to the cell membranes and disrupts membrane structure and finally causes cell death. Accordingly, these results recommended that gelatin films incorporated with ZMO could be promising candidates for safe antimicrobial materials.

4. Conclusion

Based on the findings of the current study, incorporation of ZMO into the gelatin films caused a significant decrease in swelling and water uptake and increase in solubility and water vapor permeability of the films, dose-dependently. Incorporation of ZMO into gelatin films caused a significant decrease in tensile strength, increase in elongation at break, decrease in Young's modulus of the films, dose-dependently. SEM observations indicate that ZMO were well dispersed in the film matrix and good adhesion between them was obtained which lead to decrease in the tensile strength and increase in water vapor transmission. The gelatin/ZMO films also exhibited excellent antioxidant and antibacterial properties against both Gram-positive and Gram-negative bacteria. Thus, acceptable physico-mechanical properties and water binding capacity with strong antioxidant and antibacterial effects make gelatin films grafted with ZMO as a suitable antioxidative/antimicrobial active packaging material.

Conflict of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content of this manuscript.

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