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Antioxidant, Antibacterial, Water Binding Capacity and Mechanical Behavior of Gelatin-Ferula Oil Film as a Wound Dressing Material

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Abstract

Background: Development of biodegradable and biocompatible films based on protein polymer with strong antioxidant and antibacterial activities has gradually obtained extensive concern in the world. In this study, the improvement of gelatin film properties incorporated with Ferula assa-foetida essential oil (FAO) as a potential antioxidant/antibacterial wound dressing film was investigated. **Materials and Methods:** Gelatin films were prepared from gelatin solutions (10% w/v) containing different concentration of FAO. The effect of FAO addition on water solubility, water swelling, water vapor permeability, mechanical behavior, light barrier properties as well as antioxidant and antibacterial activities of the films were examined. **Results:** Water solubility, water swelling and water vapor permeability for pure gelatin films were 29 ± 1.6%, 396 ± 8%, 0.23 ± 0.018 g.mm/m2.h, respectively. Incorporation of FAO into gelatin films caused a significant decrease in swelling and increase in solubility and water vapor permeability. Tensile strength, elastic modulus and elongation at break for pure gelatin films were 4.2 ± 0.4 MPa, 5.8 ± 4.2 MPa, 128 ± 8 %, respectively. Incorporation of FAO into gelatin films caused a significant decrease in tensile strength and elastic modulus and increase in elongation at break of the films. Gelatin film showed UV-visible light absorbance ranging from 280 to 480 nm with maximum absorbance at 420 nm. Gelatin/FAO films also exhibited excellent antioxidant ad antimicrobial activities. **Conclusions:** Our results suggested that gelatin/FAO films could be used as active films due to their excellent antioxidant and antimicrobial features for different biomedical applications including wound-dressing materials.**[GMJ.2015;4(2):103-14]**

Keywords: Gelatin; Wound-dressing; Antioxidant; Antimicrobial; Mechanical Property

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Introduction

Gelatin (a denatured collagen peptide with MW=100 Kilo Dalton) is a soluble protein obtained by partial acid/alkaline hydrolysis of insoluble native collagen. Gelatin derivatives have been reported to have antimicrobial activity [1], antioxidant and radical scavenging capacity [2], lipid peroxidation inhibitor effects [3], radioprotection effects [4], antihypertensive effects [5] and immuno-modulatory effects [6]. A large number of new applications have been found for gelatin in products such as emulsifiers, foaming agents, colloid stabilizer, hydrogels, biodegradable packaging materials, wound-dressing, micro-encapsulating agents as well as biologically active peptides [7].

Gelatin-based materials can be formed into gels, films, fibers, sponges, beads, foams and nanoparticles with high potential applications in food and pharmaceutical industries [8, 9]. Gelatin film has been reported to be one of the useful materials used as carrier of bioactive components. Enriching gelatin films with natural antioxidant and/or antimicrobial substances will extend functional properties of these biodegradable materials. There is a growing interest in using plant extracts as natural sources of antioxidant/antibacterial compounds in the formulation of gelatin films [10]. 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 [11]. Ahmad and his colleagueses suggested the antimicrobial activity and physico-mechanical properties of gelatin films incorporated with bergamot and lemongrass essential oils [12]. Tongnuanchan et al. reported antioxidant activities and mechanical properties of gelatin films incorporated with citrus essential oils [13]. Some other essential oils including clove oil, fennel oil, cypress oil, lavender oil, and thyme oil have been used to improve antioxidant and antibacterial properties to gelatin films [14]. However, to our knowledge, there is no report on the antioxidant and antimicrobial activities of gelatin films incorporated with Ferula assa-foetida (FA) essential oil (FAO). FA is a useful plant in the Apiaceous family. In traditional medicine, this plant is used for the treatment of different diseases such as asthma, epilepsy, stomachache, flatulence, intestinal parasites, weak digestion and influenza [15].

The aim of this work was to develop gelatin films incorporated with different concentrations of FAO in order to obtain improvements in water solubility, water swelling, water vapor permeability, tensile strength, elastic modulus, elongation at break, light barrier, antioxidant and antimicrobial properties of the film. It is expected that incorporation of FAO into gelatin films can improve antioxidant and antimicrobial properties which are important for the production of active films as a potential wound-dressing material.

Materials and Methods

Materials

Bovine gelatin (type A) was purchased from Sigma-Aldrich (Saito Louis Mo, USA). All other chemicals including 2, 2′-azino-di (3-ethylbenzthiazoline-6-sulfonate) (ABTS), glycerol and glutaraldehyde used in this work, were analytical grade and Sigma chemical Co. (Saito Louis Mo, USA). FAO was prepared from the latex of Ferula through hydro-distillation using an all-glass Clevenger apparatus. Obtained FAO was dehydrated over anhydrous sodium sulphate and then was dissolved in one volume of pure ethanol to emulsify.

Preparation of Film-forming Solutions and Film Casting

To prepare gelatin film-forming solutions, different concentrations of FAO (2, 4 and 8% w/w based on the weight of gelatin powder) were mixed with 10% (w/v) gelatin in distilled water and stirred for 12 h at 40 °C. Glycerol (25% w/w based on the weight of gelatin powder) as plasticizer and glutaraldehyde (0.2% w/w based on the weight of gelatin powder) as cross-linker were added to film-forming solutions. Gelatin/FAO solutions were mixed and sonicated using a sonication bath (Bandlin, Germany) at 70 W for 30 min at 40 °C. The homogenous mixture was poured onto a Polystyrene Petri dish (Farazbin Kimia Co., Teharan, Iran) for film casting. The mixtures

were cast onto flat and leveled trays to cast. Once set, trays were held overnight at 50 °C in a vacuum oven (Fan Azma Gostar, Tehran, Iran) to yield a uniform thickness in all cases and then cooled to room temperature before peeling film off the plate [17]. The obtained films were stored in plastic bags and held in desiccators at 60 ± 5% relative humidity for further testing. Thicknesses of films were measured with a micrometer (L.S. Starrett Co. LTD. Great Britain, U.K.) and the average was reported (90 ± 5 µm).

Scanning Electron Microscopy (SEM)

The morphology of film samples was examined through SEM. The magnified surface pictures of the films were taken with a Hitachi 570 SEM (FESEM Hitachi S4160 Japan) in the School of Metallurgy and Materials Engineering University of Tehran, Tehran, Iran. The samples were fixed on the sample holder and then coated with gold. SEM pictures with 700X magnification were taken with an accelerating voltage of 20 kV [12].

Water Solubility

Film samples were cut into 20 mm radius disc and then dried in a vacuum oven (Fan Azma Gostar) at 70 °C for 24 h and then weighed to determine the initial dry mass (W0). Then, dried films were immersed into 50 mL capped falcon tubes containing 25 mL of distilled water and placed inside the shaker oven (Jal Tajhiz Labtech. co. Ltd. Tehran, Iran) for 24 h at 25 °C. Thereafter, the solution was filtered thought Whatman filter paper (no. 1) to recover the remaining un-dissolved film. The remaining film pieces were placed in a vacuum oven (Fan Azma Gostar) of 70 °C for 24 h and then were weighed to determine the final dry mass of film (Wf). The percentage of weight losing was taken as water solubility (S) and was calculated by using the following equation: S% = [(W0-Wf)/W0] × 100 [16]. At least four tests were performed and the average values were reported.

Swelling Test

Film samples were cut into 20 mm radius disc and then dried in a vacuum oven (Fan Azma Gostar) at 70 °C for 24 h and then weighed to determine initial dry mass (W0). Film samples were immersed into a 50 mL falcon tube containing 25 mL of distilled water. Samples were kept at room temperature for 2 h. Each sample was taken out of tubes after 2 h, wiped between filter papers to remove excessive surface water and were weighed to determine the final weight of swollen film (Ws). The percentage of weight gaining was taken as water swelling (SW) percentage and calculated by using the following equation: SW (%) = [(Ws – W0) / W0] × 100 [17]. At least four tests were performed and the average values were reported.

Water Vapor Permeability Test

Film cuts were conditioned at 27°C and 60 ± 5% relative humidity by placing them in desiccators over saturated solution of Mg(NO3)2.6H2O for three days. Water vapor permeability (WVP) of film samples was examined using aluminum cups filled with 20 g silica gel to produce 0% relative humidity under these films [18]. After taking the initial weight of test cups, they were placed in a box over saturated solution of Mg(NO3)2.6H2O. Cups weight gain was measured at 3 h intervals during one day using electronic balance (Acculab, Sartorius group, Germany). A plot of weight gain (g) against time (h) for each cup was prepared. The slope of linear portion of this plot showed the amount of water vapor diffusion through film per time unit and expressed as gram unit per time unit (g/h). Water vapor transmission rates (WVTR) of the films were calculated from the slope of linear portion of this plot per square meter (m2) and expressed as g/m2. h. WVP was calculated by multiplying WVTR by the film thickness (mm) and expressed as g.mm/m2.h [19]. Tests were done in at least triplicate and average values were provided.

Mechanical Test

Tensile strength, elastic modulus and elongation at break of the films were measured using an Instron testing machine (Santam, Tehran, Iran). Films were cut to 60 mm×10 mm. The thickness of films was measured at different points with micrometer and the average was taken. The film cuts were condi-

tioned at 27°C and 60 ± 5% relative humidity by placing them in desiccators over saturated solution of Mg(NO3)2.6H2O for two days [13, 20]. The tensile strength test was then performed by stretching the film cut at speed of 10 mm/min. The nominal stress-strain curves were obtained and tensile strength, elastic modulus and elongation at break values were determined. Tensile strength was calculated by dividing maximum stress by the initial cross-sectional area and expressed as MPa. Elastic modulus was the initial slope of stress-stain curves at the linear part and was expressed as MPa. Elongation at break was calculated by dividing extension length where the film is torn by the initial length of the film cut and multiplying it by 100. The area of film used for each experiment was 60 mm×10 mm. However, 20 mm of these films were within the jaws, so the initial length of the film was taken as 40 mm. The initial cross-sectional area of film samples was 10 mm×0.09 mm [21]. All mechanical tests were carried out at room temperature. A minimum of four tests were performed and averages were reported.

Light Absorption and Opacity

Film samples were cut to 1 cm × 8 cm and directly placed against two sides of empty spectrophotometer covets. The thickness of films was measured at different points with micrometer and the average was taken. UV-visible absorption spectrum of film cuts was measured over a wavelength range from 200 to 700 nm using Unico 2100 spectrophotometer (New Jersey, USA). The opacity of films was calculated by dividing light absorbance at 420 nm by film thickness [19]. A minimum of four tests were performed and the averages were reported.

Antioxidant Activity

Antioxidant activities of film samples were determined by decolorization of ABTS radical [22]. Films cuts (20 mm radius disc from different parts of films approximately 100 mg) were added to 2.0 mL of diluted ABTS radical solution. Films without FAO were used as control. Light absorbance was recorded after 10 min 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 (AAE) per gram of films using standard curve.

Antibacterial Activity Assay Using Disc Diffusion

All microorganisms were obtained from Persian culture collection (PTCC), Tehran, Iran. Films were individually tested against two Gram-negative bacteria [P. aeruginosa PTCC 1074 (ATCC 9027 and E. coli PTCC 1330 (ATCC 8739)] and two Gram-positive bacteria [S. aureus PTCC 1112 (ATCC 6538) and B. subtilis PTCC 1023 (ATCC 6633)]. To investigate antimicrobial activity of the films using disc diffusion, 20 mm diameter discs were cut from different parts of films and sterilized by autoclaving for 30 min at 120°C [12, 19]. Bacterial suspensions with a turbidity equivalent to a McFarland 0.5 standard were prepared (108 colony-forming unit (CFU)/mL) and then diluted to 105 CFU/mL with Luria-Bertani (LB). The adjusted bacterial suspensions (0.1 mL) were spread onto nutrient agar plates (Farazbin Kimia Co.) containing LB. Subsequently, discs were placed in direct contact with agar medium. Plates were inverted and incubated at 37°C for 24 h (Incubator with inventilator, Pars Azma Co. Tehran, Iran). Films without FAO under the same condition were used as control. The diameters of clear inhibition zones including diameter of discs were measured using a ruler and were used to evaluate antibacterial potential of films.

Antibacterial Activity Assay Using Colony Counting

Bacterial strains were suspended in LB media, densities adjusted to 0.5 McFarland standards at 640 nm (108 CFU/mL) and then diluted to 10^5 CFU/mL with LB. Film samples with 20 mm diameter were placed in a 10 mL liquid culture containing 10 µL microbe cultures. Then, sample was incubated at 37 ºC for 24 h (Shaking Incubator, Jal Tajhiz Labtech. co. Ltd.). From the incubated samples, a 100 µL solution was taken and diluted with appropriate dilution factor and the final diluted mi-

crobe solution was plated and distributed onto nutrient agar plate (Farazbin Kimia Co.). The plates cultured with films without FAO under the same condition were used as control. All plates were incubated at 37 ºC for 24 h and a number of formed colonies were counted. The antibacterial efficacy of films was calculated according to the following equation [16]: Colony reduction(%)=[(number of colonies in the presence of pure gelatin - The number of colonies in the presence of gelatin/FAO) / Number of colonies in the presence of pure gelatin] × 100.

Statistical Analysis

All data are representative of at least three independent experiments and expressed as mean values plus standard deviations. Significant differences between treatments were analyzed by Duncan test at $P<0.05$ using statistical package for social sciences (SPSS 14, Abaus Concepts, Berkeley, CA) software.

Results

Plant Materials

GC-MS analysis of FAO indicated main components as (Z)-1-propenyl sec-butyl disulfide (27.7%), (E)-1-propenyl sec-butyl disulfide (20.3%), α-pinene (10.7%), β-pinene (10.2%), (Z)-β-ocimene (7.8%), 10-epi-γ-eudesmol (5.3%), (E)-β-ocimene (2.9%) and β-dihydroagarofuran (1.8%).

Film Morphology

SEM pictures of film samples are presented in (Fig. 1). Pure gelatin film had compact, smooth, transparent, colorless and homogeneous surface structure which is indicative of ordered matrix. Incorporation of FAO increased roughness, opaqueness and whiteness of the film and introduced bubble like structures in film matrix.

Film Solubility

Pure gelatin film showed a low solubility value of 29 ± 1.6%. Addition of 2% to 8% FAO to gelatin film caused a significant ($P<0.05$) increase in water solubility of films from 30 ± 1.3% to 37 ± 1.6%, dose-dependently (Table 1).

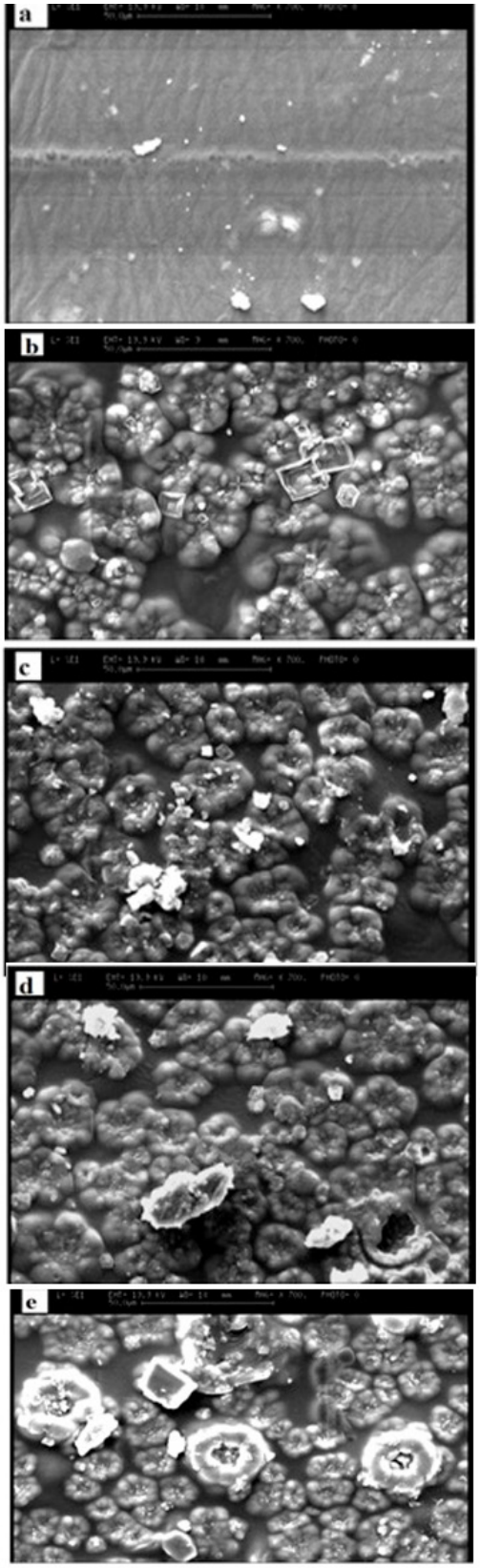

Figure 1. Scanning Electron Microscopy Images of Pure Gelatin Film (A), Gelatin Film with 2% W/w Ferula Oil (B), Gelatin Film with 4% W/w Ferula Oil (C), and Gelatin Film with 8% W/w Ferula Oil (D).

Film Swelling

Pure gelatin film showed a high swelling value of 396 ± 8%. Addition of 2% to 8% FAO to gelatin film caused a significant ($p < 0.05$) decrease in the swelling of films from 381 ± 9% to 344 ± 5%, dose-dependently (Table 1).

Water Vapor Permeability

Pure gelatin film showed a low water vapor permeability (WVP) of 0.23 ± 0.018 g.mm/m2.h. Addition of 2% to 8% FAO to gelatin film caused a significant ($p < 0.05$) increase in WVP of films from 0.24 ± 0.012 to 0.32 ± 0.015 g.mm/m2.h, dose-dependently (Table 1).

Mechanical Properties

Tensile strength, elastic modulus and elongation at break of pure gelatin film were 4.2 ± 0.4 MPa, 5.8 ± 0.4 MPa, 128 ± 7%, respectively. Incorporation of 2 to 8% FAO caused a significant ($P < 0.05$) decrease in tensile strength (from 3.7 ± 0.21 to 2.6 ± 0.13 MPa), decrease ($P < 0.05$) in elastic modulus (from 5.2 ± 0.25 to 3.6 ± 0.22 MPa) and increase ($p < 0.05$) in elongation at break (from 139 ± 6 to 165 ± 7%)(Table 1).

Light Absorption and Opacity

Pure gelatin films showed light absorbance in a range between 280 to 480 nm, while maximum absorbance was at 420 nm. Pure gelatin film showed a low opacity value of 15 ± 1.5 nm/mm. Addition of 2% to 8% FAO caused a significant ($p < 0.05$) increase in the opacity of films from 19 ± 2.2 to 26 ± 3.3, dose-dependently (Table1).

Antioxidant Activity

Antioxidant activity of gelatin films was determined by ABTS decolorization method and expressed as mg ascorbic acid equivalent per gram of the films (Table 1). Gelatin films without FAO showed low antioxidant activity. Addition of FAO into gelatin films caused a significant increase in antioxidant activity. With the increase in essential oil, the antioxidant activity also increased.

Antibacterial Activity

Antibacterial activity assay of gelatin films incorporated with FAO was expressed through disc diffusion method and viable colony counting assay. The results of disc diffusion and colony reduction percentage are summarized in Tables 2 and 3, respectively. According to the results obtained, gelatin films without FAO showed no activity against tested bacteria. By addition of FAO into gelatin films, the antibacterial activities positively increased. Gelatin films incorporated with FAO are effective against both Gram-positive and Gram-negative bacteria tested.

Table 1. Solubility, Swelling, Water Vapor Permeability (WVP), Tensile Strength (TS), Elastic Modulus (EM), Elongation at Break (EAB), Opacity and Antioxidant Properties of Gelatin Films Incorporated with Ferula Oil (FAO).

Properties	Gelatin	Gelatin-FAO 2%	Gelatin-FAO 4%	Gelatin-FAO 8%
Solubility (%)	29 ± 1.6^{c}	30 ± 1.3^{c}	33 ± 1.2^{b}	37 ± 1.6^{a}
Swelling (%)	396 ± 8^{a}	381 ± 9^{ab}	368 ± 6^{b}	344 ± 5^{c}
WVP (g.mm/m².h)	0.23 ± 0.01^{d}	0.24 ± 0.01^{c}	0.26 ± 0.01^{b}	0.32 ± 0.01^{a}
TS (MPa)	4.2 ± 0.42^{a}	3.7 ± 0.21^{ab}	3.2 ± 0.23^{b}	2.6 ± 0.13^{c}
EM (MPa)	5.8 ± 0.42^{a}	5.2 ± 0.25^{ab}	5.2 ± 0.25^{b}	3.6 ± 0.22^{c}
EAB (%)	128 ± 7^{c}	139 ± 6^{bc}	145 ± 8^{b}	165 ± 7^{a}
Opacity	15 ± 1.5^{c}	19 ± 2.2^{bc}	21 ± 2.6^{ab}	26 ± 3.3^{a}
Antioxidant activity	0.42 ± 0.05^{c}	0.70 ± 0.1^{c}	2.06 ± 0.2^{b}	3.86 ± 0.5^{a}

Mean values with different small letters (a, b, c or d) within each one raw are significantly different by Duncan test at $P < 0.05$. The antioxidant activity was expressed milligram ascorbic acid equivalent (AAE) per gram of film incorporating different concentrations of Ferula oil.

Table 2. Antibacterial Activity of Gelatin Film Incorporated with Ferula Oil (FAO).

	Inhibition zone diameter (mm)			
Films	**S. aureus**	**B. Subtilis**	**E. coli**	**P. aeroginosa**
Gelatin	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{b}	0 ± 0^{b}
Gelatin-FAO 2%	30 ± 1.2^{b}	30 ± 0.9^{b}	30 ± 1^{a}	30 ± 0.7^{a}
Gelatin-FAO 4%	31 ± 1^{ab}	31 ± 0.8^{ab}	30 ± 1.2^{a}	30 ± 0.8^{a}
Gelatin-FAO 8%	34 ± 1.7^{a}	33 ± 1.3^{a}	32 ± 1.7^{a}	31 ± 1.5^{a}

Antibacterial activity was expressed as diameter of bacterial growth inhibition zone in the presence of films with different Ferula oil concentration. Mean values with different small letters (a, b or c) within each one column are significantly different by Duncan test at $P< 0.05$.

Table 3. The Antibacterial Activity of Gelatin Film Incorporated with Ferula Oil (FAO).

	Colony reduction (%)			
Films	**S. aureus**	**B. Subtilis**	**E. coli**	**P. aeroginosa**
Gelatin	0 ± 0^{d}	0 ± 0^{d}	0 ± 0^{d}	0 ± 0^{d}
Gelatin-FAO 2%	46.3 ± 2.4^{c}	62.8 ± 3.6^{c}	17.4 ± 2^{c}	1.73 ± 1^{c}
Gelatin-FAO 4%	75.9 ± 3.3^{b}	83.4 ± 3.3^{b}	61 ± 4.2^{b}	31.8 ± 3^{b}
Gelatin-FAO 8%	100 ± 4^{a}	98.5 ± 3.8^{a}	96.6 ± 5^{a}	90.2 ± 4.5^{a}

Antibacterial activity was expressed as bacterial growth reduction in the presence of films with different ferula oil concentration. Mean values with different small letters (a, b, c or d) within each one column are significantly different by duncan test at $p< 0.05$.

Discussion

Plant Materials

GC-MS analysis of FAO indicated the main components were (Z)-1-propenyl sec-butyl disulfide, (E)-1-propenyl sec-butyl disulfide, α-pinene, β-pinene, (Z)-β-ocimene, 10-epi-γ-eudesmol, (E)-β-ocimene and β-dihydroagarofuran. Thus, FAO constituted high levels of acyclic sulfur-containing compounds [(Z)-1-propenyl sec-butyl disulfide and (E)-1-propenyl sec-butyl disulfide], bicyclic monoterpenes [β-pinene and α-pinene] and bicyclic sesquiterpenes [10-epi-γ-eudesmol] which had roughly same components with other previously analyzed FAO [23] however, showed important differences in their quality and quantity of the components.

Film Morphology

SEM pictures showed that pure gelatin film had compact, smooth, transparent, colorless and homogeneous surface structure. Addition of FAO increased roughness, opaqueness and whiteness of the film. FAO-incorporated films had bubble like structures. The number of bubbles increased with increase in essential oil concentration, thus these bubbles are caused by FAO. Furthermore, an increase in FAO resulted in more bubbles which homogeneously distributed in film structure [12, 13]. This homogenous dispersion of FAO in film matrix could affect functional property of the film including water binding, light barrier and tensile properties.

Films Solubility

Water resistance is an important property of biodegradable films for biomedical applications where water activity is high or when film must be in contact with water. Pure gelatin film showed a low solubility, while addition of FAO caused a significant increase in water solubility of the films. Gelatin is naturally water soluble. However, cross-linking by glutaraldehyde can stabilize gelatin structure and decrease its solubility in aqueous medium. This water resistance is confirmed when film cuts maintain their integrity (did not dissolve) in water after 24 h. This indicates that

gelatin network remained intact in water and only gelatin monomers and other non-binding materials were soluble and the scaffold of gelatin network remained insoluble [8]. Incorporation of FAO into films caused a significant increase in solubility. This increase in water solubility might be attributed to the establishment of protein-monoterpenes compound interactions weakening interactions (mainly hydrogen binding) that stabilize the gelatin network [24]. FAO contain monoterpenic compound and favorably interacts with hydrophobic domain of gelatin and may hinder polymer chain-to-chain interactions and consequently causing an increase in the solubility of the films [25]. Gomez-Estaca and coworker [14] reported that gelatin-chitosan films in the presence of essential oil, show a significant increase in film solubility which is in accordance with our experimental results.

Film Swelling

The swelling capacity of edible films plays an important role in biomedical applications and successful release of antioxidant/antibacterial and other compounds to the contact surface. Pure gelatin film showed a high swelling capacity, while addition of FAO caused a significant decrease in the swelling of films. Although gelatin has poor solubility in water (more water resistance), it can absorb large number of water molecules probably due to hydrophilic amino acids [8, 17]. Incorporation of FAO could reduce swelling capacity of films which might be related to hydrophobic nature of FAO. Hydrophobic domains of gelatin can essentially interact with FAO through hydrophobic interaction and thereby enhance interfacial interaction between gelatin matrix and FAO [26]. This event saturates gelatin network with FAO, thus water molecules cannot diffuse to gelatin network thereby swelling is decreased.

Water Vapor Permeability

Pure gelatin film showed low water vapor permeability (WVP) while addition of FAO caused a significant increase in the WVP of films. WVP is assumed to be dependent on water vapor pressure gradient applied across the film, hydrophilicity/hydrophobicity of film material, film structure and porosity of the given film. Pure gelatin is a hydrophilic material (due to hydrophilic amino acid), so strong interaction between these polar groups and water molecules causes a reduction in water vapor transmission through the film [12, 19]. Generally, incorporation of additives into films causes a significant change in water vapor transmission through films, while the final WVP capacity is related to hydrophobicity index of all compounds in films and porous structure of the film. Hydrophobic domains of gelatin can essentially interact with FAO through hydrophobic interaction and thereby enhance interfacial interaction between matrix and FAO [26]. This phenomenon impedes interactions between gelatin chains and water molecules; thus, causing an increase in water vapor transmission across films [19]. Our experimental results show that the WVP of gelatin films incorporated with FAO slightly increases. This effect could be explained by the formation of porous structure with FAO addition that is in accordance with the results reported by Ahmad and co-workers [12].

Mechanical Properties

Tensile strength is the maximum tensile stress sustained by the films during tension test. The edible film must withstand the normal stress encountered during its application to maintain its integrity. High tensile strength in required, but deformation values must be adjusted according to intended film applications. Incorporation of FAO caused a significant decrease in tensile strength, decrease in elastic modulus and increase in elongation at break. Our results demonstrated that, the tensile strength of gelatin film decreased with the addition of FAO. Gelatin films were mainly stabilized by weak bond including hydrogen bond (between hydroxyl and amino groups of amino acid side chain) and hydrophobic interaction (between proline and hydroxlyproline). Addition of FAO possibly resulted in lowered interaction between gelatin strands that may hinder gelatin chain-to-chain interactions and consequently, cause a significant decrease in the tensile strength of films [19]. Elastic modulus is a measure of stiffness (hardness) of film. A stiff material has high elastic modu-

lus and changes its shape only slightly under elastic loads. A flexible material has low elastic modulus and changes its shape considerably. By the incorporation of FAO into film, elastic modulus reduced significantly thus film structure becomes much stiffer. Elongation at break is an indication of flexibility and extensibility of film prior to breakage. In the presence of FAO, flexibility of gelatin film increased which might be due to increase in pore sizes and porosity of the films and decrease in cross-linking density with added FAO that is in accordance with the results reported by Ahmad et al [16]. Ahmad and others [12] reported that gelatin films incorporated with bergamot oil also showed a lower tensile strength and elastic modulus but higher elongation at break rather than control films which are in accordance with our experimental results. Tongnuanchan and his co-workers [13] reported that gelatin films incorporated with citrus oil also showed a lower tensile strength but higher elongation at break rather than control films which are in accordance with our experimental results.

Light Absorption and Opacity

Pure gelatin films showed light absorbance in the ranges between 280 to 480 nm, while maximum absorbance was at 420 nm. Addition of FAO caused a significant increase in light absorbance of the films. Protein-based films are considered to have high light barrier properties owing to their high content of aromatic amino acids absorbing UV light [12]. Incorporation of FAO into gelatin films caused a significant increase in light absorbance and opacity. Thus, gelatin films lost their typical transparent and colorless appearance. However, resulting gelatin/FAO films gained light barrier properties, which could be interesting in certain applications for preventing UV-induced lipid peroxidation [17]. The increase in light absorbance more likely depends on the distribution of FAO in gelatin matrix as well as interaction between FAO and gelatin. This effect led to differences in film matrix morphology with different light absorbance as confirmed by SEM analysis. FAO droplets which were localized in gelatin matrix increased the opacity of gelatin film, more likely due to light scattering effect [17]. Ahmad and co-workers [12] reported that gelatin films incorporated with bergamot and lemongrass oils also showed a higher light absorbance in the visible range compared to films without oils which is in accordance with our experimental results.

Antioxidant Activity

Gelatin films without FAO showed low antioxidant activity. Aleman et al. reported the antioxidant properties of peptides derived from gelatin in different sources. Their studies have shown peptides derived from enzymatic hydrolysis of gelatin as lipid peroxidation inhibitors, free radical scavengers and transition metal ion chelators [2]. The antioxidative properties of peptides are related to their amino acid composition, molecular weight, structure and hydrophobicity [9]. Addition of FAO into gelatin films caused a significant increase in antioxidant activity. With increase in essential oil, antioxidant activity also increased. Fish skin gelatin film incorporated with citrus essential oil [13] exhibited strong antioxidant activity which is in accordance with our experimental results. Generally, essential oils have been reported as excellent sources of natural antioxidant. These antioxidant activities may be attributed, at least in part, to the presence of phenol, flavonoid, sesquiterpene and sulfur-containing compounds in these essential oils [27]. FAO is a good source of phenolic monoterpenes and sulfur-bearing compounds with a significant antioxidant activity. Thus, the higher antioxidant activity of gelatin/FAO films could be related to its FAO content. FAO is reported as having antioxidant activity as measured by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ABTS assay [28]. Kavoosi and Rowshan have also shown that FAO possesses nitric oxide (NO) and malondialdehyde scavenging properties and thus could prevent nitrative stress and lipid peroxidation [23]. These results demonstrated that FAO synergistically increased antioxidant activity of gelatin and gelatin films incorporated with FAO could be a promising candidate for safe radical scavenger wound dressing material.

Antibacterial Activity

Based on the results obtained, gelatin films without FAO showed no activity against tested bacteria. It is reported that 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 [1, 17]. By addition of FAO into gelatin films, antibacterial activities positively increased. Gelatin films incorporated with FAO are effective against both Gram-positive and Gram-negative bacteria tested. Gelatin film from the skin of unicorn leatherjacket incorporated with essential oils [12] and catfish gelatin films incorporated with oregano oil [29] displayed excellent antibacterial activity which is pursuant to our experimental results. These antibacterial activities of essential oils would be related to hydrophobicity of essential oils due to hydrophobic monoterpenic compounds [30]. The antibacterial activities of essential oils are related to the attack on phospholipids present in cell membranes causing increased permeability and leakage of cytoplasm, or in their interaction with enzymes located on cell wall [30]. FAO is a good source of monoterpenes, with a significant antimicrobial activity against both Gram-positive and Gram-negative bacteria [15]. These results recommended that FAO synergistically increased antibacterial activity of gelatin and gelatin films incorporated with FAO which could be a promising candidate for active antibacterial packaging material.

Conclusion

Gelatin/FAO films were prepared by dissolving FAO in gelatin solutions. Gelatin/FAO films showed increase in water solubility and water vapor permeability and decrease in water swelling as compared to pure gelatin films. FAO increased solubility and decreased swelling capacity probably by hydrophobic interaction between FAO and gelatin. Incorporation of FAO also caused a significant decrease in tensile strength and elastic modulus and increase in elongation at break of the films. FAO droplets that were localized in film matrix increased the opacity of films, more likely due to the light scattering effect of the essential oil compounds. SEM observations indicate that FAO was well dispersed in film matrix and good adhesion between them was obtained leading to decrease in tensile strength and increase in water vapor transmission. Pure gelatin films exhibited low antioxidant and antimicrobial activities while, gelatin/FAO films exhibited excellent antioxidant and antimicrobial properties. Thus, although the bioactivity of gelatin films was increased by FAO incorporation however, the mechanical properties and water binding capacity of the films decreased slightly. Further study is needed to improve both bioactivity and physico-mechanical of such films. In future studies, we will focus on the improvement of gelatin film properties and on the applications of such films to ex vivo and in vivo systems in order to investigate biocompatibility of films as a wound-dressing material.

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Conflict of Interest

Authors declare no conflicts of interest. They, alone, are responsible for the content of this manuscript.

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