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ANTIOXIDANT AND ANTIBACTERIAL PROPERTIES OF GELATIN FILMS INCORPORATED WITH CARVACROL

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ABSTRACT

Gelatin films were prepared from gelatin solutions (10% w/v) containing carvacrol (1%, 2%, 3%, 4% and 5% w/w), glycerol (25% w/w) as plasticizer and glutaraldehyde (1% w/w) as cross-linker. The mechanical, water solubility, water swelling, water uptake, water vapor permeability, antioxidant and antibacterial properties of the films were obtained according to American Society for Testing and Materials. Gelatin films exhibited good tensile strength, elongation at break, water solubility, swelling, water uptake and water vapor permeability. Incorporation of carvacrol into the gelatin films caused a significant decrease in tensile strength, increase in elongation at break, increase in water solubility, decrease in swelling and water uptake, and increase in water vapor permeability of the films. Gelatin films exhibited low antioxidant activity while, gelatin films incorporated with carvacrol exhibited excellent antioxidant properties. The films incorporated with carvacrol also exhibited excellent antibacterial properties against both gram-positive and gram-negative bacteria.

PRACTICAL APPLICATION

Development of biodegradable films based on the protein polymer has become a very attractive option and production of protein based packaging with strong antioxidant and antibacterial activities is gradually obtained extensive concern in the world. In this study, the improvement of gelatin film properties incorporated with carvacrol as a potential antibacterial active packaging was investigated. Suitable physico-mechanical properties along with strong antioxidant and antibacterial effects make gelatin films incorporated with carvacrol as suitable food packaging material.

INTRODUCTION

The abundant use of synthetic antibiotics has resulted in the appearance of a number of drug-resistant bacteria, fungi, yeast and parasite in food yields. To conquer the increasing resistance of pathogenic microbes, more effective and safe antimicrobial agents must be developed. The antimicrobials can be directly added into the product formulation, coated on its surface or incorporated into the packaging materials. Incorporation of antimicrobials into foodstuff results in an immediate reduction of microorganisms, while the antimicrobial active food packaging can maintain their activities for a long period of time (Hanusova *et al.* 2009; Lucera *et al.* 2012). Biopolymers (starch, cellulose, chitosan, casein, whey protein, collagen, egg white, soybean protein, zein, gelatin and wheat gluten) have attracted considerable attention as potential for food packaging materials (Marsh and Bugusu 2007; Arora and Padua 2010).

Gelatin is a high molecular weight polypeptide obtained by controlled hydrolysis of collagen presented in the bones and skin and has good biocompatibility and biodegradability. It is a good film-forming material with uses in medicine such as plasma expander, wound dressing, adhesive and controlled drug delivery (Avena-Bustillos *et al.* 2011; Gomez-Guillen *et al.* 2011). Gelatin has been reported to be one of the first materials used as carrier of bioactive

components. Enriching of gelatin films with natural antioxidant and/or antimicrobial substances will extend the functional properties of these biodegradable films and provide active packaging biomaterials. There is growing interest in using plant extracts as natural sources of antioxidant/antibacterial compounds in the formulation of gelatin films (Hanusova *et al.* 2009; Lucera *et al.* 2012; Nunez-Flores *et al.* 2012). In this context, plant essential oils and their main components are gaining a wide interest in health industry for their potentials as antioxidant and antimicrobial agents, as they are generally recognized as safe (Solorzano-Santos and Miranda-Novales 2012). Carvacrol (5-isopropyl-2-methyl phenol) is a phenolic monoterpenes in the essential oils derived from genera *Origanum*, *Thymus*, *Coridithymus*, *Thymbra*, *Satureja*, *Lippia* and *Zataria* that has been used for many generations as food preservative (Baser 2008).

In this study, the gelatin films with antioxidant and antimicrobial activities were prepared from gelatin solutions containing different carvacrol concentrations. The mechanical, water solubility, water swelling and water uptake, water vapor permeability (WVP), antioxidant and antibacterial properties of gelatin/carvacrol films were achieved according to American Society for Testing and Materials (ASTM). In this study, we offered that gelatin films grafted with carvacrol have excellent physical, mechanical and water resistance as well as good antioxidant and antibacterial activity. Flexibility, softness, water resistance and excellent mechanical properties along with strong antibacterial make gelatin films grafted with carvacrol as a suitable antimicrobial active food packaging material.

MATERIALS AND METHODS

Preparation of Gelatin Film-Forming Solutions

To prepare gelatin film-forming solutions, 10 g of bovine gelatin powder (Sigma-Aldrich, St. Louis, MO) dissolved into 80 mL of distilled water at ambient temperature and temperature increased to 45C using a hotplate-stirrer and the mixture was stirred for 30 min at this temperature. After cooling to 37C, carvacrol with different concentration (1%, 2%, 3%, 4% and 5% w/w based on the weight of the gelatin powder = 1, 2, 3, 4 and 5 mg/mL based on the gelatin solutions) as antimicrobial agent, glycerol (25% w/w based on the weight of the gelatin powder = 2.5 mg/mL based on the gelatin solution; Merck, Darmstadt, Germany) as plasticizer and glutaraldehyde (1% w/w based on the weight of the gelatin powder = 1 mg/mL based on the gelatin solution; Merck) as cross-linker were added to gelatin solutions and then distilled water was added to final of 100 mL and homogenized (Bigi *et al.* 2001; Ahmad *et al.* 2012).

Preparation of Gelatin Films

To cast the films, 25 mL of gelatin/carvacrol film-forming solutions containing different carvacrol concentrations were transferred into the polystyrene Petri dish (Farazbin Kimia Co., Tehran, Iran; $120 \times 70 \times 5$ mm) and placed at room temperature until films were dried. Thicknesses of films were measured to the nearest 0.01 mm with a micrometer (The L.S. Starrett Co. Ltd., Jedburgh, Scotland) and the average was taken at 100 ± 5 μm.

Mechanical Properties of Gelatin Films

The mechanical properties of gelatin/carvacrol films were examined according to ASTM D 638-02a in texture analyzer (TA.XT, Stable Micro Systems, Surrey, U.K.). Gelatin/carvacrol films containing different concentrations of carvacrol were transferred to a closed container with relative humidity of 65% and left for equilibrium for 48 h before mechanical testing. Gelatin/carvacrol films were cut into the rectangles with length of 60 mm, width of 10 mm and thickness 0.10 mm. The tensile strength (TS) test was then performed by stretching the film at pretest, test and posttest speeds of 1, 1 and 10 mm/s, respectively. The net length between the jaws for all films was almost constant at about 20 mm. The texture analyzer runs at auto force mode with the trigger force of 5 g (0.049 N). From stress-strain curves, two parameters were calculated: (1) TS was calculated as maximum stress and (2) elongation at break (EAB) where the film is torn (Bigi *et al.* 2001; Ahmad *et al.* 2012):

$$\mathrm{TS}\left(\mathrm{N\ m^{-2}}\right) = \left(\text{breaking force}/\text{cross-sectional area of sample}\right)$$

$$\mathrm{EAB}(\%) = \left(\left[\text{increase in length at breaking point}/\text{initial length}\right)\right] \times 100$$

The area of film used for each experiment was 6×1 cm^2. However, 2 cm of the films were within the jaws, so the initial length of the film was taken as 4 cm^2. All tests were the means of at least three measurements.

Water Solubility of Gelatin Films

For water solubility determination, one piece of film ($20 \times 20 \times 0.10$ mm) placed in an oven at 104C for 24 h and initial dry weight (W_i) was calculated. Then, the dried films were immersed into 100-mL Erlenmeyer flask containing 50 mL of distilled water and placed inside the shaker for 24 h at 25C (incubator with ventilator; Pars Azma Co., Tehran, Iran). Thereafter, the films were taken out and transferred to the oven of 104C for 24 h and the final dry weight (W_f) was calculated. The weight losing or solubility percentage (S%) was determined using the following

formula (Bigi *et al.* 2001; Ahmad *et al.* 2012): S (%) = $([W_i - W_f]/W_i) \times 100$. All tests were the means of at least three measurements.

Swelling of Gelatin Films

The gelatin films were dried in an air-circulating oven at 104C for 24 h until constant weight was reached before the swelling test (W_i). Square films with the dimension of $20 \times 20 \times 0.12$ mm were cut for the swelling experiment. Each sample was immersed into a 100-mL Erlenmeyer flask containing 50 mL of the distilled water. The samples were kept at room temperature for the duration of the swelling experiment (24 h). Each sample was taken out of the flask after 24 h, wiped between filter papers to remove the excess surface water and were weighed (W_f). The weight gaining or swelling percentage (SW%) was calculated using the following equation (Ahmad *et al.* 2012; Nunez-Flores *et al.* 2012): SW (%) = $([W_f - W_i]/W_i) \times 100$. All tests were the means of at least three measurements.

Water Uptake of Gelatin Films

The films ($20 \times 20 \times 0.12$ mm) were dried in desiccators at concentrated H_2SO_4 (relative humidity = 0%) for 3 days until constant weight was reached (W_i). Then, films were transferred into desiccators at 100% relative humidity (sodium sulfate solution) at 37C for 1 week and allowed to absorb water, and then weighed after the equilibrium state reached (W_f). The weight gaining or water uptake percentage was calculated using the following equation (Bigi *et al.* 2001; Ahmad *et al.* 2012): weight gaining or water uptake (%) = $([W_f - W_i]/W_i) \times 100$. All tests are the means of at least three measurements.

WVP of Gelatin Films

The WVP of the films was determined according to the ASTM E96-95 method. The films were conditioned for 24 h at 25C and 75% relative humidity before WVP determination. Film samples were mounted on an aluminum cup (height and diameter of 2.1 and 5.6 cm, respectively). The cup was filled with 20 g of silica gel and covered with a film specimen. The cup was placed at 25C and 75% relative humidity in desiccators. The weight of the cup was measured at 3-h intervals during 1 day. Simple linear regression was used to estimate the slope of mass change versus time plot. The WVP was calculated using the following formula (Bigi *et al.* 2001; Ahmad *et al.* 2012): WVP (g·mm/m²·kPa·h) = $([WVTR \times T])/\Delta P$, where water vapor transmission rate ($WVTR$) is the slope per film area (g/m²·h), T is the film thickness (mm) and ΔP is the partial water vapor pressure difference (kPa) between the two sides of the film (4.2449 kPa at 30C).

Antioxidant Activity of Gelatin Films

Antioxidant activities of the films were determined by the decolorization method with 2, 2′-azino-di (3-ethylbenzthiazoline-6-sulfonate; ABTS, Sigma-Aldrich; Tongnuanchan *et al.* 2012). The method was modified to detect the continuous antioxidant release from the films. The release tests were performed in 24-well plates. Briefly, films cut ($10 \times 10 \times 0.10$ mm) from different parts of the films containing 1%, 2%, 3%, 4% and 4% of the carvacrol 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 carvacrol were used as blank. The program was adjusted to record the absorbance values after shaking the 24-well plates for 30 s using a plate reader (BioTek Elx 808, Winooski, VT). The data recorded up to steady state were reached for each sample. 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 a standard curve.

Antibacterial Activity of the Gelatin Films Using Disk Diffusion

The films were individually tested against two gram-negative bacteria (*Pseudomonas aeruginosa* Persian-type culture collection (PTCC) 1074 (ATCC 9027 and *Escherichia coli* PTCC 1330 [ATCC 8739]) and two gram-positive bacteria (*Staphylococcus aureus* PTCC 1112 [ATCC 6538] and *Bacillus subtilis* PTCC 1023 [ATCC 6633]). All microorganisms were obtained from the PTCC, Tehran, Iran. The antibacterial activity was carried out according to the standard practice for determining resistance of synthetic materials to bacteria (ASTM G22-76; Gomez-Estaca *et al.* 2010). To investigate the antimicrobial activity of the films using disk diffusion, 30-mm-diameter disks (thickness of 0.10 mm) were cut from different parts of the films and sterilized by autoclaving for 30 min at 120C. Bacterial suspensions with a turbidity equivalent to a McFarland 0.5 standard were prepared at 10^8 cfu/mL and then diluted to 10^5 cfu/mL with Luria-Bertani (LB). The adjusted bacterial suspensions (0.1 mL) were spread onto the nutrient agar plates (Farazbin Kimia Co., Tehran, Iran) containing LB. Subsequently, the disks were placed in direct contact with the agar medium. Plates were inverted and incubated at 37C for 24 h (incubator with ventilator, Pars Azma Co.). Films without carvacrol under the same condition were used as control. The diameters of clear inhibition zones, including

the diameter of the disk, were measured using a ruler and were used to evaluate antibacterial potential of films.

Antibacterial Activity of Gelatin Films Using Colony Counting

The bacterial colony-counting assays were carried according to the Clinical and Laboratory Standards Institute and ASTM G22-76 (Gomez-Estaca *et al.* 2010). Bacteria strains were suspended in LB media and the densities were 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 37C for 24 h (Shaking Incubator, Shin Saeng, Fine Tech, Gyeonggi-do, 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.). The plates cultured with the films without Food and Agriculture Organization under the same condition were used as control. All plates were incubated at 37C for 24 h and the numbers of colonies that formed were counted. The antibacterial efficacy of the films was calculated according to the following equation (Maneerung *et al.* 2008): colony reduction (%) = ([*number of colony in test samples* – *number of colony in control*]/*number of colony in test samples*) × 100.

Statistical Analysis

Data are expressed as the means ± standard deviations of at least three independent experiments. The significant differences between treatments were analyzed by one-way analysis of variance and Duncan tests at $P < 0.05$ using the Statistical Package for the Social Sciences (SPSS, Abaus Concepts, Berkeley, CA) and Prism 5 (Graph Pad, San Diego, CA) softwares.

RESULTS

Mechanical Properties of Gelatin Films

TS and EAB are the parameters that relate mechanical properties of films to their chemical structures. The mechanical properties (TS and EAB) of gelatin films are shown in Figs. 1 and 2. TS and EAB of gelatin films were 4.3 ± 0.5 MPa and 128 ± 8%, respectively. Incorporation of carvacrol caused a significant decrease in TS (Fig. 1) and increase in EAB (Fig. 2) dose dependently ($P < 0.05$).

Solubility Determination of Films

The solubility percentages (weight losing) of gelatin films are summarized in Fig. 3. The solubility percentage for gelatin films was 27 ± 1.2%. Incorporation of carvacrol into the films caused a significant increase in the solubility of the gelatin films dose dependently ($P < 0.05$).

Swelling and Water Uptake Capacity of Gelatin Films

The results of swelling and water uptake capacity of gelatin films are summarized in Fig. 4 and 5. The swelling and water uptake percentage for gelatin films were 391 ± 8% and 128 ± 3. Incorporation of carvacrol into the films caused a

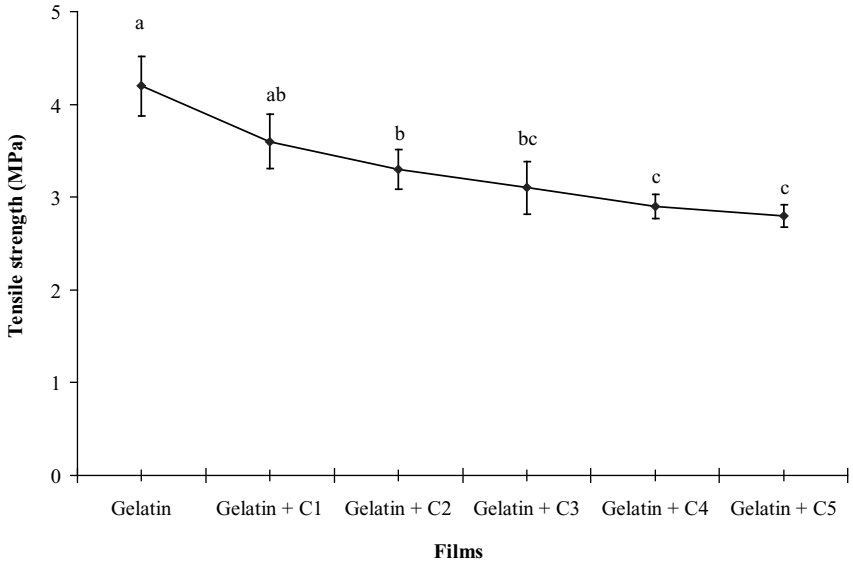

FIG. 1. TENSILE STRENGTH (MPa) OF GELATIN FILMS AS CARVACROL CONCENTRATION
C1, C2, C3, C4 and C5 are 1%, 2%, 3%, 4% and 5% w/w carvacrol based on the gelatin powder. Different letters show significantly difference ($P < 0.05$).

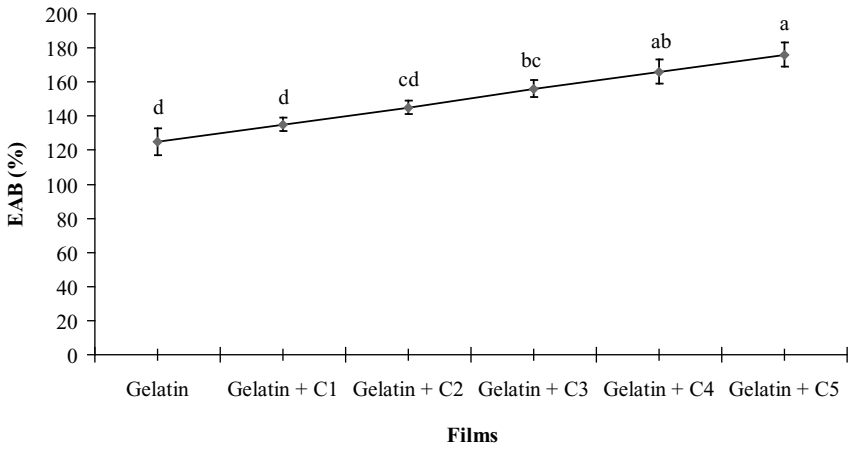

FIG. 2. ELONGATION AT BREAK (%) OF GELATIN FILMS AS CARVACROL CONCENTRATION
C1, C2, C3, C4 and C5 are 1%, 2%, 3%, 4% and 5% w/w carvacrol based on the gelatin powder. Different letters show significantly difference ($P < 0.05$).

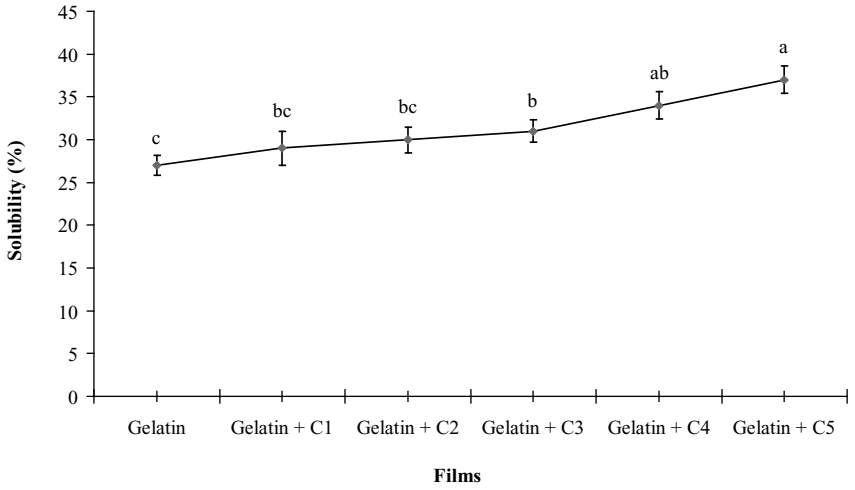

FIG. 3. SOLUBILITY (%) OF GELATIN FILMS AS CARVACROL CONCENTRATION
C1, C2, C3, C4 and C5 are 1%, 2%, 3%, 4% and 5% w/w carvacrol based on the gelatin powder. Different letters show significantly difference ($P < 0.05$).

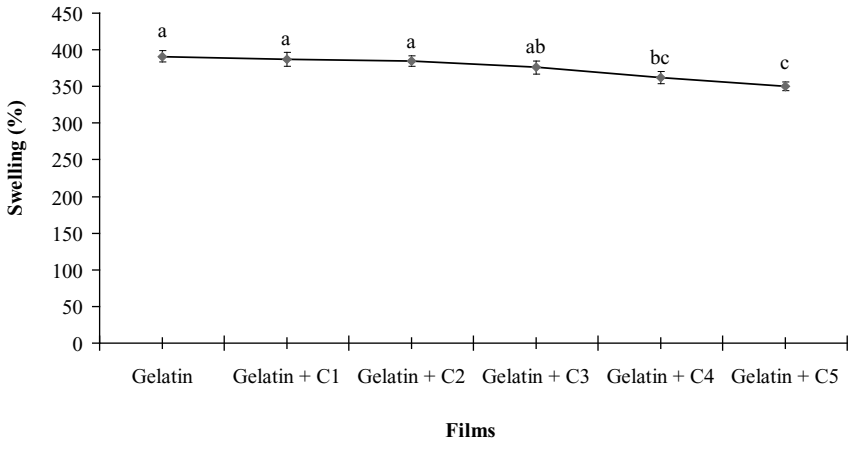

FIG. 4. SWELLING (%) OF GELATIN FILMS AS CARVACROL CONCENTRATION
C1, C2, C3, C4 and C5 are 1%, 2%, 3%, 4% and 5% w/w carvacrol based on the gelatin powder. Different letters show significantly difference ($P < 0.05$).

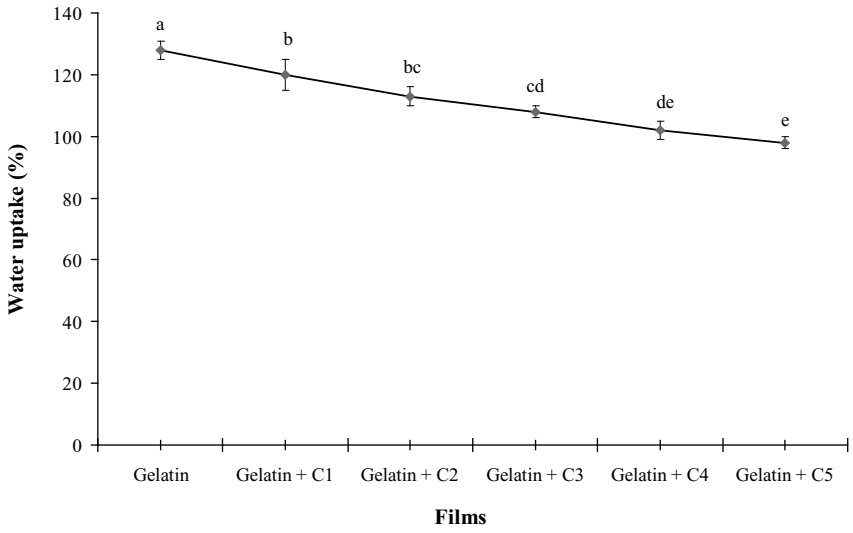

FIG. 5. WATER UPTAKE (%) OF GELATIN FILMS AS CARVACROL CONCENTRATION
C1, C2, C3, C4 and C5 are 1%, 2%, 3%, 4% and 5% w/w carvacrol based on the gelatin powder. Different letters show significantly difference ($P < 0.05$).

significant decrease in the swelling (Fig. 4) and water uptake (Fig. 5) of the films ($P < 0.05$).

WVP of Gelatin Films

The results of WVP of gelatin films are summarized in Fig. 6. The WVP for gelatin films was 0.23 ± 0.018 g·mm/kPa·m^2·h. Incorporation of carvacrol into the films caused a significant increase in WVP of the films dose dependently ($P < 0.05$).

Antioxidant Activity of Gelatin Films

Antioxidant activity of the gelatin films incorporated with different carvacrol concentrations was determined by ABTS decolorization method and expressed as mg ascorbic acid equivalent per gram (AAE/g) of films (Table 1). The gelatin films without carvacrol showed very low activity (0.12 ± 0.03 AAE/g). Incorporation of carvacrol into the films caused a significant increase in their antioxidant activity ($P < 0.05$).

Antibacterial Activity of Gelatin Films

Antibacterial assay of gelatin films incorporated with carvacrol was expressed by disk diffusion method and viable colony-counting assay. The results of disk diffusion are summarized in Table 2. 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 carvacrol showed no activity

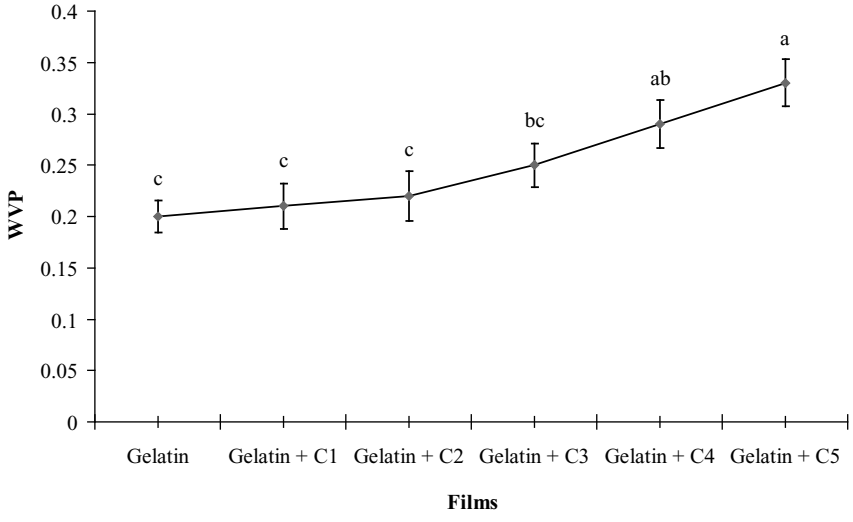

FIG. 6. WATER VAPOR PERMEABILITY (WVP) (g·mm/m^2·kPa·H) OF GELATIN FILMS AS CARVACROL CONCENTRATION
C1, C2, C3, C4 and C5 are 1%, 2%, 3%, 4% and 5% w/w carvacrol based on the gelatin powder. Different letters show significantly difference ($P < 0.05$).

TABLE 1. ANTIOXIDANT ACTIVITY OF GELATIN FILMS INCORPORATED WITH CARVACROL

Gelatin films	Antioxidant activity (mg AAE/g film)
Gelatin + carvacrol 0%	0.12 ± 0.03^{e}
Gelatin + carvacrol 1%	0.86 ± 0.13^{d}
Gelatin + carvacrol 2%	1.86 ± 0.18^{d}
Gelatin + carvacrol 3%	3.46 ± 0.50^{c}
Gelatin + carvacrol 4%	5.86 ± 0.60^{b}
Gelatin + carvacrol 5%	7.22 ± 0.80^{a}

The antioxidant activity was expressed milligram ascorbic acid equivalent (AAE) per gram of films incorporated with different concentrations of carvacrol. Different letters show significantly difference ($P < 0.05$).

against the tested bacteria. The antibacterial activity of gelatin film containing different carvacrol concentrations was greatest against *B. subtilis* and *S. aureus* followed by *E. coli* and then *P. aeruginosa*. The results of colony reduction percentage are summarized in Table 3. According to the results obtained, the antibacterial activity of gelatin film containing different carvacrol concentrations was greatest against *S. aureus* followed by *B. subtilis* followed by *E. coli* and then by *P. aeruginosa*. Thus, gelatin films incorporated with carvacrol 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.

DISCUSSION

The main objectives of this study was the investigation of mechanical, water solubility, water swelling, water uptake, WVP, antioxidant and antibacterial properties of gelatin films incorporated with carvacrol. Gelatin films were mainly stabilized by the weak bond including hydrogen bond and hydrophobic interaction (Bigi *et al*. 2001). Addition of carvacrol possibly resulted in the lowered interaction between gelatin monomers, and may hinder polymer chain-to-chain interactions and consequently, caused a decrease in TS with the simultaneous increase in the EAB of the films (Limpisophon *et al*. 2010). Gelatin films incorporated with citrus oil showed lower TS but higher EAB than the control films without incorporated essential oil, which is similar to our results (Tongnuanchan *et al*. 2012).

Gelatin is water soluble, can easily dissolve partially when coming into contact with an aqueous medium and may lose fibrous structure to high ambient humidity especially for a long period of time. However, cross-linking can stabilize gelatin structure and decrease its solubility in aqueous medium (Bigi *et al*. 2001). Generally, the effects of the additives on the solubility of films depend on the type of compounds and their hyrophilicity and hydrophobicity index (Jang *et al*. 2011). Carvacrol is a hydrophobic material and favorably interacts with the hydrophobic domain

TABLE 2. THE ANTIBACTERIAL ACTIVITY OF GELATIN FILMS INCORPORATED WITH CARVACROL

	Inhibition zone diameter (mm)			
Gelatin films	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*
Gelatin + carvacrol 0%	0 ± 0^{d}	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{b}
Gelatin + carvacrol 1%	31 ± 1.2^{c}	32 ± 0.9^{b}	30 ± 1^{b}	30 ± 0.7^{a}
Gelatin + carvacrol 2%	32 ± 1^{bc}	33 ± 0.8^{ab}	32 ± 0.7^{ab}	30 ± 0.8^{a}
Gelatin + carvacrol 3%	33 ± 1.5^{ab}	34 ± 1.1^{ab}	33 ± 1.6^{a}	31 ± 1.3^{a}
Gelatin + carvacrol 4%	35 ± 1.7^{a}	35 ± 1.3^{a}	34 ± 1.7^{a}	32 ± 1.5^{a}
Gelatin + carvacrol 5%	36 ± 1.3^{a}	36 ± 1.2^{a}	35 ± 1.4^{a}	33 ± 1.6^{a}

Antibacterial activity was expressed as diameter of bacterial growth inhibition zone in the presence of films with different carvacrol concentrations. Mean values with different letters within a column are significantly different by Duncan's multiple range tests at ($P < 0.05$).

TABLE 3. THE ANTIBACTERIAL ACTIVITY OF GELATIN FILMS INCORPORATED WITH CARVACROL

	Colony reduction (%)			
Films	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*
Gelatin + carvacrol 0%	0 ± 0^{d}	0 ± 0^{d}	0 ± 0^{e}	0 ± 0^{e}
Gelatin + carvacrol 1%	58.5 ± 4.0^{c}	54.7 ± 4.2^{c}	34.3 ± 2.6^{d}	33.5 ± 2.5^{d}
Gelatin + carvacrol 2%	79.2 ± 3.4^{b}	76.8 ± 4.7^{b}	66.6 ± 2.2^{c}	61.3 ± 3.5^{c}
Gelatin + carvacrol 3%	94.4 ± 3.3^{a}	90.9 ± 4.3^{a}	89.7 ± 2.8^{b}	78.1 ± 4.2^{b}
Gelatin + carvacrol 4%	97.6 ± 2.3^{a}	92.7 ± 3.6^{a}	94.3 ± 3.8^{b}	86.4 ± 3.2^{b}
Gelatin + carvacrol 5%	100 ± 4.2^{a}	99.5 ± 5.2^{a}	98.8 ± 3.7^{a}	96.5 ± 4.9^{a}

Antibacterial activity was expressed as bacterial growth reduction in the presence of films with different carvacrol concentrations. Mean values with different letters within a column are significantly different by Duncan's multiple range tests at ($P < 0.05$).

of gelatin and may hinder gelatin network formation and consequently, caused an increase in the solubility of films (Hong *et al.* 2009). Gelatin-chitosan films, in the presence of essential oil, showed a significant increase in film solubility, which is similar to our results (Gomez-Estaca *et al.* 2010).

Gelatin as a hydrophilic material absorbs molecules of water, and it shows high swelling and water uptake capacity (Rawdkuen *et al.* 2010; Avena-Bustillos *et al.* 2011). Incorporation of carvacrol could reduce swelling capacity of the films that might be related to the hydrophobic nature of carvacrol. Hydrophobic domains of gelatin can essentially interact with carvacrol through hydrophobic interaction and thereby enhance interfacial interaction between matrix (gelatin) and filler (carvacrol; Zivanovic *et al.* 2005; Hong *et al.* 2009). This event saturates the gelatin network with carvacrol, and water molecules cannot diffuse to the gelatin network, thereby causing a decrease in swelling and water uptake.

Hydrophilic gelatin strongly interacts with water molecules and causes a reduction in the water vapor transmission through the gelatin film (Avena-Bustillos *et al.* 2011; Kim and Min 2012). Incorporation of additive to gelatin films causes a significant change in water vapor transmission through films, while the final WVP capacity is related to the hydrophobicity/hyrophilicity index of all compounds in the films. Hydrophobic domains of gelatin can essentially interact with carvacrol and thereby enhance interfacial interaction between matrix and carvacrol. This phenomenon hinders interactions between gelatin chains and water molecules, thus water molecules freely pass through the films and consequently cause the increase in WVP (Zivanovic *et al.* 2005; Hong *et al.* 2009). The WVP of chitosan films incorporated with thyme oil slightly increased, which is similar to our results (Altiok *et al.* 2010).

Various studies have examined antioxidant properties of peptides derived from gelatin in different sources. These studies have shown that peptides derived from enzymatic hydrolysis of gelatin are lipid peroxidation inhibitors, free radical scavengers and transition metal ion chelators. The antioxidative properties of peptides are related to their amino acid composition, molecular weight, structure and hydrophobicity (Aleman *et al.* 2011; Gomez-Guillen *et al.* 2011). Fish skin gelatin film incorporated with citrus essential oils (Tongnuanchan *et al.* 2012) and chitosan films incorporated with thyme oil (Altiok *et al.* 2010) exhibited strong antioxidant activity, which is similar to our results. The total phenolic content and related total antioxidant capacity of some medicinal plant infusions was analyzed, which indicated that there was a significant linear correlation between total phenol content and antioxidant capacity (Katalinic *et al.* 2006). Thus, the antioxidant activity of gelatin films impregnated with carvacrol could be related to carvacrol content (Huang *et al.* 2011). Carvacrol was gradually released from the films to the ABTS solution and decolorized it.

All the gelatin films grafted with carvacrol showed strong activity 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), chitosan films incorporated with thyme oil (Altiok *et al.* 2010) and soy edible films incorporated with thyme and oregano essential oils (Min and Oh 2009) displayed excellent antibacterial activities, which is similar to our results. The antibacterial capacity of essential oils is related with the attack on the on the phospholipids present in the cell membranes, which causes increased membrane permeability and leakage of cytoplasm, or in their interaction with enzymes located on the cell wall. Thus, the resistance of gram-negative bacteria to the essential oils likely lay in the protective role of their cell wall lipopolysaccharide or outer membranes proteins, which restricts diffusion of hydrophobic compounds through its lipopolysaccharide layer (Burt 2004; Oussalah *et al.* 2007). Essential oils have the ability to disrupt lipid structure of the cell wall of bacteria, leading to destruction of cell membrane, cytoplasmic leakage, cell lysis and ultimately cell death. The decrease in pH that occurs because of cell membrane disruption resulted in a loss of control of cellular process such as ATP biosynthesis, DNA transcription and protein synthesis (Xu *et al.* 2008; Solorzano-Santos and Miranda-Novales 2012). Essential oils also penetrate into mitochondrial membrane, leading to the greater permeability of organelle and the potassium ion leakage process. The leakage of ions especially potassium out of a cell is a clear indication of membrane damage and cell death (Paparella *et al.* 2008). Carvacrol was gradually released from films to the solution and penetrates to the cell membranes and disrupts membrane structure and finally cell death.

Considering all these findings, the incorporation of carvacrol into gelatin films caused a significant decrease in TS, increase in the EAB, increase in water solubility, decrease in swelling and water uptake, and increase in WVP of the films. Gelatin films exhibited very low antioxidant activity, while gelatin films incorporated with carvacrol exhibited excellent antioxidant properties. The films incorporated with carvacrol also exhibited excellent antibacterial properties greatest against both gram-positive and gram-negative bacteria. Thus, excellent mechanical properties, water resistance and water barrier properties along with strong antioxidant and antibacterial effects make gelatin films incorporated with carvacrol as suitable food packaging.

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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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