



Original research

Investigating the mechanical, physical and antimicrobial properties of gelatin films containing *Cinnamomum verum* hydrosol

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ABSTRACT

Hydrosol is a side product of essential oil extraction from plants. In this study, the effect of various ratios (5-100 %v/v) of cinnamon hydrosol replacement in the formulation of edible gelatin films was investigated. Physical properties were evaluated and the antibacterial properties of gelatin films against *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Staphylococcus epidermidis* were assessed. Evaluation of chemical component of cinnamon hydrosol was performed by GC - Mass chromatography. After production control film, modified gelatin films were made by replacement different ratios of cinnamon hydrosol (5, 30, 50, 80 and 100 %v/v) instead of the water in gelatin film formulation. Physical and mechanical properties of resulted films such as water vapor permeability, film thickness, transparency, light transmittance were evaluated. FTIR and SEM were also carried out. Antimicrobial characteristic of gelatin films were evaluated against *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Staphylococcus epidermidis* by measuring inhibition zone diameter. Results showed that the use of cinnamon hydrosol increased barrier property of gelatin films. Tensile strength increased by adding hydrosol in the edible film formulation (from 15.3 ± 1.5 MPa in control sample to 32.3 ± 3.3 MPa in gelatin film incorporated with 100% cinnamon hydrosol), but the elongation of the films, decreased (from $175 \pm 5.5\%$ in control sample to $92 \pm 3\%$ in gelatin film incorporated with 100% cinnamon hydrosol). Furthermore, addition of various hydrosol ratios significantly decreased the WVP values ($p \leq 0.01$). *Staphylococcus aureus* was most sensitive to gelatin films incorporated with cinnamon hydrosol and its inhibition zone at 100% hydrosol was 33 mm.

Keywords: Gelatin film, Hydrosol, Cinnamon, Packaging, Antimicrobial effect

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1. Introduction

Plastic packaging cause environmental issues finding a suitable biodegradable packaging as replacement has become a research context for many researchers (Tongnuanchan et al., 2016). Gelatin is one of the most widely used compound for the preparation of edible films due to its good barrier properties such against gas, volatile compound and UV- radiation (Tongnuanchan et al., 2014). Enhancing antimicrobial activity of edible packaging increases its protective activity of packaging against microbial contamination. Several methods for increase antimicrobial activity to food packaging were proposed (Wu et al., 2017) such as the addition of various herbal essences like cinnamon (Wu et al., 2017; Kim et al., 2018), Indian essential oils and basil (Tongnuanchan et al., 2014; Alparslan et al., 2016). A study on citral essential oil addition to alginate and pectin was carried out by Siracusa et al. (2018). Similarly; Dashipour et al. (2014) used carboxymethyl cellulose edible film cooperated with clove essential oil (Dashipour et al., 2014). Application of chitosan-flaxseed mucilage with

antimicrobial activity has been investigated by Karami et al. (2019). Hydrosol is a by-product of essential oil extraction from aromatic plants. Hydrosol contains water- soluble compounds and a small amount of essential oil (less than 1 g/L) (Labadie et al., 2016). Hydrosol has a high shelf life (more than one year) in suitable condition. Various studies have shown the antimicrobial properties of hydrosol (D'Amato et al., 2018). Hay et al. (2018) reported the antimicrobial effects of *Thymus vulgaris* and *Rosmarinus officinalis* hydrosols against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger* (Hay et al., 2018). Cid- Perez et al. (2019) pointed out the antimicrobial activity of Mexican Oregano (*Poliomintha longiflora*) hydrosol against *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, and *Salmonella Typhimurium* (Cid-Pérez et al., 2019). The anti-fungal (Zatla et al., 2017) and antiviral (Kaewprom et al., 2017) activity of various plant hydrosol have also been reported. *Cinnamomum verum*, the dried pieces of the skin of the cinnamomum tree, is used in foods and in medications. Cinnamon contains cinnamaldehyde, gamma-eugenol camphene, terpiene and

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4-terpene, as well as various phenolic and non-phenolic compounds that have antioxidant properties. These compounds act as radical scavenger and prevent oxidative reactions (Mürfeizi et al., 2014). Cinnamon has therapeutic, antimicrobial, antioxidant, anti-diabetes and anti-viral activity.

Producing an edible film with suitable physical, mechanical and antimicrobial properties for possible application in food industry and extending the shelf life of raw food materials have been investigated by several researchers. Most of these studies have been carried out on the effect of adding essential oils to various edible film formulations, but the effect of adding hydrosol to the formulation of edible films has not yet been investigated. The purpose of this study was to investigate the effect of cinnamon hydrosol addition in gelatin film formulation and assessment the mechanical, physical and antimicrobial properties of the obtained films.

2. Material and Methods

2.1. Hydrosol extraction

Cinnamomum verum was purchased from the local market of Neyshabur. Hydrosol extraction was done as follows: 100 g cinnamon placed in a 2-liter flask containing 1 liter of water (1:10 w/v) and connected to a Clevenger in which steam distillation was carried out for 2 hours. Thereafter, the essential oil was separated and the hydrosol was kept in sterile bottles at 4 °C until further use (D'Amato et al., 2018).

2.2. Preparation of microbial strains

Microbial strains of *Staphylococcus aureus* (PTCC 1112), *Staphylococcus saprophyticus* (PTCC 1440) and *Staphylococcus epidermidis* (PTCC 1435) were purchased from the Institute of Scientific and Industrial Research of Iran (Iran, Tehran). The bacterial vial was broken in sterile conditions and transferred to a suitable culture medium (tryptic soy broth for *Staphylococcus aureus* and nutrient broth for *Staphylococcus saprophyticus* and *Staphylococcus epidermidis*) and incubated for 24 hours at 37 °C. Microbial cells were harvested by centrifugation (ALC4232 model) at 4000 rpm. The bacterial enumeration was carried out by McFarland's method (the optical density at 625 nm wavelength was 0.08-0.13, which is equivalent to the 0.5 McFarland and the population of approximately 1.5×10^8 cfu/mL) (Mohammadi et al., 2016). Then, dilution was performed of sterile physiological serum to reach turbidity equal to 1.5×10^6 cfu/mL (Moradian Eivari et al., 2016).

2.3. GC- Mass spectroscopy of cinnamon hydrosol

In order to determine the chemical composition of cinnamon hydrosol, the gas chromatography-mass spectrometry according to Garneau et al. (2014) was carried out. Agilent Technologies 7890A model, with the 5975CVLMSD absorber and the 7683B (US build) injector equipped with HP-5 (30 m length, 320 µm diameter and thickness of 0.25 µm) was applied.

2.4. Gelatin Film preparation

According to Table 1, 4 g gelatin was dissolved 100 mL distilled water at 45 °C for 30 min. Glycerol was then added to the solution at a ratio of 25 % w/w. Hydrosol was replaced the water at ratios of 5, 30, 50, 80 and 100%. To remove the air bubbles the solution subjected to ultrasonic treatment in an ultrasonic device (Eurosonic 4D) (Italy). Then 10 mL of film solution was casted in a plate (120 × 80 mm) and dried at 22 °C, relative humidity (RH)= 50% for 48 h. The resulted gelatin films were placed inside the desiccator (22 °C, RH = 50%) for 3 days for conditioning (Wu et al., 2017).

Table 1. Sample definition

Sample Code	Description
A ₀	Control
A ₁	Gelatin film incorporated with 5% cinnamon hydrosol
A ₂	Gelatin film incorporated with 30% cinnamon hydrosol
A ₃	Gelatin film incorporated with 50% cinnamon hydrosol
A ₄	Gelatin film incorporated with 80% cinnamon hydrosol
A ₅	Gelatin film incorporated with 100% cinnamon hydrosol

2.5. Study of textural properties of gelatin films

Tensile strength (TS) and elongation at break (EAB) were determined as described by Sazedul Hoque (2011) using the Texture analyzer equipment (TA-XT plus, England). The samples were clamped and deformed under tensile loading using a 100 N load cell with crosshead speed of 1 mm/s until the samples were broken. The maximum load and the final extension at break were used for calculation of TS and EAB, respectively.

2.6. Water Vapor Permeability (WVP)

Water vapor permeability was measured by gravimetric method (Wu et al., 2017). 10 mL of distilled water was added in a 14 mL container and covered with gelatin films with a surface area of 1.5 cm². The capped bottle was first weighed and placed in a silica gel with specific relative humidity and temperature. The weight changes were assessed every 12 h during 3 days. WVP was calculated according to following formula.

$$WVP \text{ (gm}^{-1}\text{Pa}^{-1}\text{s}^{-1}\text{)} = \frac{(W \times X)}{A \times t \times \Delta P} \quad (1)$$

where, W is the difference between the weight of the bottle, X is the gelatin film thickness, A is the gelatin film area, t is the time in seconds, and Δp pressure difference.

2.7. Study of gelatin film thickness

The thickness of the gelatin films were measured using a digital caliper Guanglu (model HB 102-111, China). The eight-point of

film thickness was determined and the mean numbers was considered as the thickness of the films.

Table 2. Chemical composition of *cinnamom verum* hydrosol.

Peak number	Retention time (min)	Chemical composition
1	20.398	<i>Cinnamic acid, methyl ester</i>
2	27.684	<i>Methyl palmitate</i>
3	27.701	<i>Hexadecanoic acid methyl ester</i>
4	25.685	<i>Corymbolone</i>
5	28.814	<i>3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl</i>
6	28.867	<i>Vulgarol</i>
7	28.902	<i>Alloaromadendrene oxide</i>
8	37.843	<i>9-Octadecenoic acid</i>
9	37.895	<i>6-Formyl-3- methyl- 2- oxo- 4- hexenoic acid.</i>
10	39	<i>1-Eicosene</i>
11	39.440	<i>Cyclopropaneoctanal</i>
12	39.644	<i>Emersol</i>
13	39.667	<i>1-Nonadecene</i>

Table 3. Mechanical and physical properties of gelatin films.

Sample	Thickness(mm)	WVP (10×10^{-10} g/m.pa.s)	Elongation (%)	Tensile strength (MPa)
A ₀	0.11 ± 0.02 ^a	1.28 ± 0.02 ^a	175 ± 5.5 ^a	15.3 ± 1.5 ^c
A ₁	0.1 ± 0.02 ^a	1.21 ± 0.02 ^b	164 ± 4.5 ^b	16.9 ± 1 ^{bc}
A ₂	0.095 ± 0.05 ^a	1.13 ± 0.03 ^c	111 ± 3.6 ^c	20.1 ± 2.4 ^{bc}
A ₃	0.076 ± 0.03 ^a	1.02 ± 0.04 ^d	95 ± 3 ^d	22.3 ± 2.3 ^b
A ₄	0.071 ± 0.041 ^a	0.9 ± 0.02 ^c	93 ± 3 ^d	22.6 ± 2.6 ^b
A ₅	0.062 ± 0.02 ^a	0.9 ± 0.03 ^e	92 ± 3 ^d	32.3 ± 3.3 ^a

2.8. Light transmission

The light transmittance of gelatin films was performed by spectrophotometric method using spectrophotometer (Jenway 6305) (US). The light transmittance was determined at 200-800 nm wavelengths. The gelatin films were cut into rectangular shapes and placed directly into the spectrophotometer cell. Blank cell was used as control. The results were recorded as a percentage of light transmission.

2.9. Antimicrobial properties of gelatin films

To evaluate the antimicrobial properties, the method described by Martouky et al. (2015) was applied. Accordingly, 0.1 mL of microorganisms including *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* with a population of approximately 1.5×10^6 cfu/mL were transferred to culture medium including tryptic soy agar and nutrient agar. Gelatin films (12 mm) were cut and placed on the medium. The incubation was carried out at 37 °C and relative humidity of 50% for 24 h. The diameter of the inhibition zone was measured in millimeters. All tests were performed in three replications.

2.10. Infrared Fourier Transform (FTIR) spectrum

The Fourier transform infrared spectrum was recorded by the Spectroma2 Perkin-Elmer device (USA) at a frequency range of 4500-400 cm^{-1} (Sharma et al., 2015).

2.11. Scanning microscopic imaging

In order to investigate the morphology of gelatin films, a phenom proX scanning electron microscope (Netherlands) with a magnification of 1000-10000 was performed.

2.12. Statistical analysis

All experiments were performed trice. Statistical analysis was performed in a completely randomized design using STATISTICA software version 13. The means were compared with Duncan's method at a significance level of 0.01%. Excel 2010 was used to draw charts.

3. Results and Discussion

The chemical composition of the cinnamon hydrosol was analyzed by gas mass chromatography and mass spectrometry method and the results are shown in Table 2. According to these results, some of the most important constituents of cinnamon hydrosol are vulgarol, emersol, cinnamic acid methyl ester, methyl palmitate and oleic acid.

3.1. Physical and mechanical properties

Physical and mechanical properties of gelatin films are shown in Table 3. Results showed that addition of cinnamon hydrosol to gelatin film formulations caused significant changes in the physical and mechanical properties of gelatin films ($p \leq 0.01$). As cinnamon hydrosol concentration increased, Tensile strength increased. Elongation percentage is another mechanical property of gelatin film that affected by addition of hydrosol. The elongation of the gelatin films reduced as hydrosol was added to the formulation.

3.2. Water vapor permeability

The results of WVP of the gelatin films are shown in Table 3. According to the results it is evident that the addition of cinnamon hydrosol to gelatin film formulation led to a lower WVP in comparison to the control sample ($p \leq 0.01$). An explanation to this phenomenon could be that the hydrogen bonds and hydrophobic interactions increased which resulted in a more compact structure of gelatin films containing cinnamon hydrosol (Sazedul Hoque et al., 2011).

3.3. The thickness of gelatin films

Investigations showed that by adding various percentages of cinnamon hydrosol to gelatin films, the thickness does not change and there was no significant difference between resulted gelatin films ($p \leq 0.01$) (Table 3).

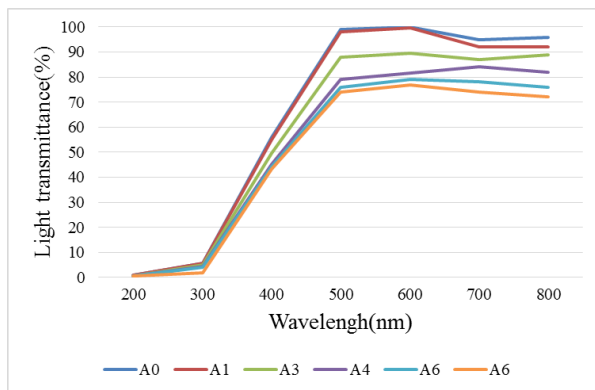


Fig. 1. Light transmittance of gelatin film samples.

3.4. Light transmittance

The results showed that the use of cinnamon hydrosol in gelatin film formulations causes a change in the light transmittance magnitude at different wavelengths (Fig. 1). In the ultraviolet range of 200-300 nm, light transmittance of gelatin films containing cinnamon hydrosol was decreased. This effect could be beneficial in preventing food oxidation since of such food packaging could protect food from UV radiation leading to preventing from photo oxidation of fats. In the visible wavelengths (350-600 nm), the addition of cinnamon hydrosol also cause reduction of light transmittance magnitude of resulted films and led to lower transparency compared to control sample.

3.5. Scanning electron microscopy (SEM)

Fig. 2 shows SEM images of gelatin film with or without cinnamon hydrosol. Observation showed no significant difference between uniformity of modified gelatin film compared to control samples. Cross section images also; approved similarity of the structure in gelatin film samples.

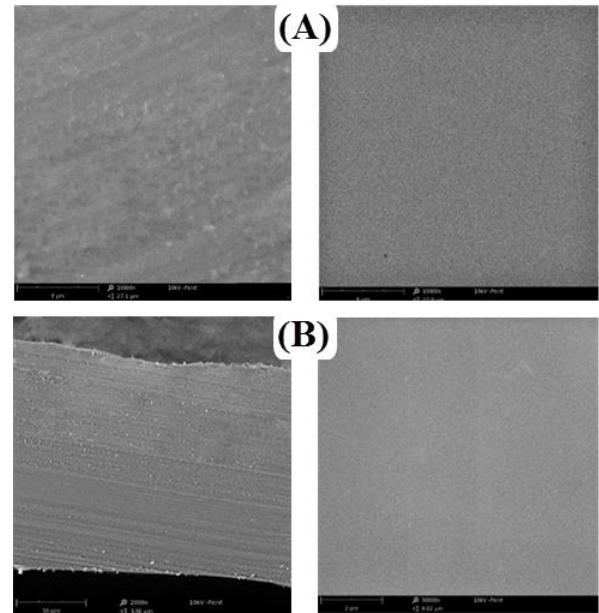


Fig. 2. Scanning electron microscopy images of (A) gelatin control film sample and (B) gelatin film containing 100% cinnamon hydrosol. Right (surface) and left (cross section).

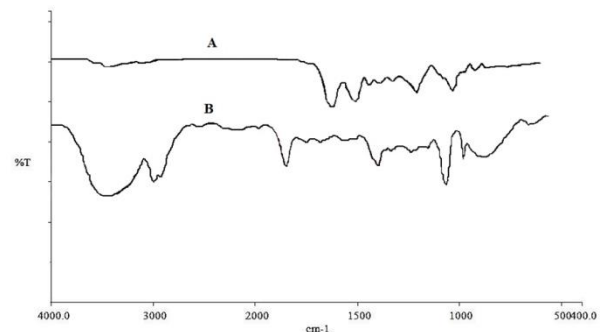


Fig. 3. FTIR spectrum of gelatin films. A: Control sample B: Cinnamon hydrosol included gelatin film.

3.6. Fourier- transform infrared spectroscopy (FTIR)

FTIR is a method for analysing structural interaction of materials in molecular scale. FTIR of gelatin films and gelatin film included 100% cinnamon hydrosol is shown in Fig. 3.

The Fourier infrared spectroscopy of the gelatin control film showed that there is a peak at 11036 cm^{-1} , which is probably related to the presence of glycerol in the gelatin film structure and the presence of a hydroxyl group related to glycerol. The peak generated at the wavelength of 1239 cm^{-1} is related to the primary amine and the C-N bond of the third type amide. A peak at 1452 cm^{-1} is related to the hydroxide group of the alcoholic group of gelatin structure. The peak 1630 cm^{-1} corresponds to the C = C and C = O bonds (Bonilla et al., 2018). Adding hydrosol causes changes in the infrared spectrum of gelatin films. In a gelatin film containing cinnamon hydrosol, the existence of the band at 3432 cm^{-1} was since of O-H stretching of alcohols and phenols. The peak 1648 cm^{-1} was ascending to -C=C- stretching of alkenes and the band 1449 cm^{-1} was C-H alkanes. The absorption band 1330 cm^{-1}

showed the presence of aromatic compounds. The peak at 1261 cm^{-1} indicates C-H bands (Maruthamuthu & Ramanathan, 2016).

The existence of various aromatic compounds has been reported in various plant hydrosols (Labadie et al., 2016).

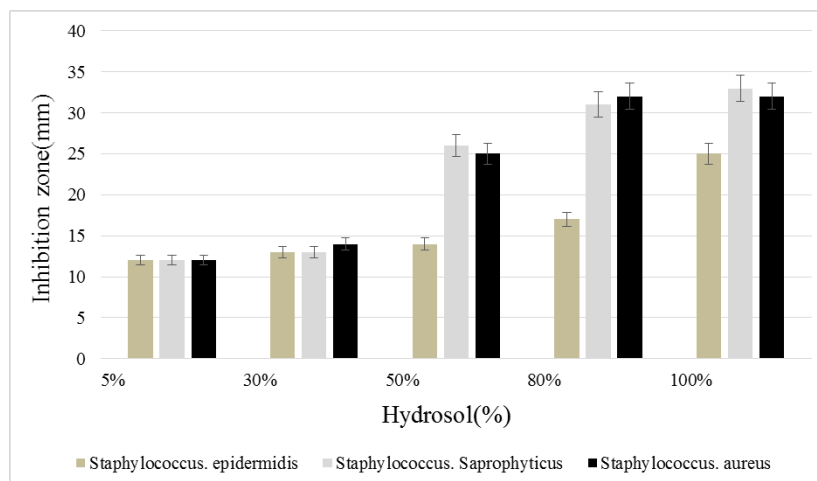


Fig. 4. Inhibition zone of gelatin film incorporated with cinnamon hydrosol against *staphylococcus* strains.

3.7. Evaluation of the antimicrobial properties of gelatin films

The results from the studying of the antimicrobial properties of gelatin films are shown in Fig. 4. Among three studied strains, *staphylococcus aureus* and *staphylococcus saprophyticus* showed the highest sensitivity to gelatin films containing cinnamon hydrosol, but *staphylococcus epidermidis* exhibited higher resistance to cinnamon hydrosol included gelatin films but its susceptibility increased as the hydrosol concentration in gelatin films increased.

The anti-microbial effects of different plant hydrosol have been studied by other researchers. Acheampong et al. (2015) pointed out the antimicrobial activity of hydrosol extracted from leaves of *Cymbopogon nardus*, *Chromolaena odorata*, *Ocimum gratissimum* and *Cymbopogon citrate* and peels of *Citrus sinensis* and *Citrus aurantifolia* against *E. Coli*, *S. aureus*, *C. albicans*, *B. subtilis* and *E. faecalis*. Cid-Perez et al. (2019) also reported the antimicrobial effects of Mexican Oregano (*Poliomintha longiflora*) hydrosol against *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, and *Salmonella Typhimurium*.

4. Conclusion

Including cinnamon hydrosol in gelatin film formulation increased antimicrobial activity to gelatin films against *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Staphylococcus epidermidis* in gelatin films. Incorporation cinnamon hydrosol in gelatin film formulation caused changes in WVP of the resulted film. The mechanical properties of gelatin films are also affected by hydrosol, increasing the tensile strength and decreasing the elongation percentage. Transparency was also decreased by an extent in the hydrosol containing gelatin films while barrier property against UV-radiation was increased. Microscopic observations showed the uniformity of the hydrosol included gelatin films compared to the control sample.

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