Active gelatin-mannan film: physicochemical, antifungal and aflatoxin binding properties

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Abstract

Mannoprotein (mannan) extracted from the cell wall of *Saccharomyces cerevisiae* is a bioactive glycoprotein that has many biological effects. In the present work, the preparation of gelatin-based blend films containing different concentrations of mannan (0, 0.5, 1.0, and 1.5%) was investigated. The effects of mannan on moisture content, water solubility, water vapor permeability (WVP), mechanical, optical, and thermal features of films were investigated. Moreover, the antifungal and aflatoxin B_1 (AFB_1) binding traits of the developed films were also studied. The results indicated that the addition of mannan significantly increased the water solubility, elongation at break, and WVP, whereas glass transition temperature and elastic modulus drastically decreased in the blend films. Furthermore, an obvious interaction between mannan and gelatin was concluded from Fourier-Transform Infrared (FTIR) spectra. Scanning electron microscopy assessment of the films revealed a heterogeneous structure. According to antifungal assay, the gelatin-mannan blend films had antifungal effect against *Aspergillus flavus* in disc diffusion evaluation. Similarly, these active films could reduce AFB1 content of pistachio extract agar.

Introduction

Recent innovations in edible biodegradable film production have attracted more attentions as compared to non-biodegradable synthetic ones, due to serious waste disposal and environmental pollution concerns (Khwaldia *et al*., 2010). The tendency towards new packaging such as coating, edible film, and self-standing packaging is increasing in food production. Active packaging is able to extend food shelf-life, enhance safety, improve sensory properties, and maintain food quality (Musso *et al*., 2017). Edible bio-based films are physical barriers against water vapor, gases, aromas and oil; which can also act as binding agents and as glazes (Dutta *et al*., 2009). Among the biopolymer sources of edible films including proteins, carbohydrates, lipids, and their blends, gelatin is widely used due to its good film-forming ability, minimizing water losing as coating, and destructive oxygen / light effect on food stuff (Gómez-Guillén *et al*., 2009). However, blending with other polymer chains can modify the polymer network and improve the functional properties of protein film (Gennadios *et al*., 1994). Recently, attention has been paid to the production of antimicrobial active systems using antimicrobial materials into films / coatings to enhance the shelf-life of foods. Some of the bioactive agents effectively promote the safety of foods (Rawdkuen *et al*., 2012). Various bio-materials have been used in active gelatin-based films such as lysozyme (Bower *et al*., 2006), nisin (Ku and Song, 2007), chitosan (Pereda *et al*., 2011), catechin-lysozyme (Rawdkuen *et al*., 2012), and essential oils (Gómez-Estaca *et al*., 2010; Ahmad *et al*., 2012).

The external layer of *S. cerevisiae* cell wall is composed of about 3 - 14% of β-1,6 glucan and 35 - 40% of mannoproteins (Klis *et al*., 2002). The mannoprotein has 20,000 to 200,000 molecular weight that consists of protein (10 - 30%) and mannan (70 - 90%), where mannose polymer is covalently linked to protein (Nakajima and Ballou, 1974). The *S. cerevisiae* cell wall mannoprotein has shown many health benefits including antioxidant

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(Križková, *et al.*, 2001), emulsifier (Dikit *et al.*, 2010), antimutagenic, antigenotoxic (Kogani *et al.*, 2008), antifungal (Abdolshahi *et al.*, 2016), aflatoxin binder (Abdolshahi *et al.*, 2018), anti-infection, anticancer (Kogan *et al.*, 2002), balancing the enterobacteria, combining with the extrinsic pathogen, and acting as antineoplastic (Liu *et al.*, 2011). In addition to these properties, it is recognized as GRAS (Generally Recognized as Safe) for human consumption. Therefore, the mannoprotein extracted from *S. cerevisiae* cell wall (mannan) could be considered as a bioactive agent for incorporation into edible film / coating structure.

The purpose of the present work was therefore to investigate the film formation ability of mannan blended with gelatin, and assess the physicochemical properties and antifungal efficiency of the developed gelatin-mannan blend films.

Materials and methods

Microorganism and chemicals

Saccharomyces cerevisiae (PTCC 5052) were obtained from the Persian Type Culture Collection (IROST, Iran). Glycerol and bovine gelatin (type B, bloom 150) which were used to prepare film-forming solutions were purchased from Sigma, Aldrich (MO, USA). All reagents and chemicals used in the extraction of mannan were purchased from Merck (Darmstadt, Germany).

Mannan extraction and analysis

S. cerevisiae (PTCC 5052) was cultured in YM agar (incubated at 24°C for 48 h) then sub-cultured into YM broth (incubated at 30°C for 24 h). The mannoprotein was extracted by the modified method of Dikit *et al.* (2010). After 5 - 6 times centrifugation (4,500 g), the cells were suspended in buffer (0.1 M potassium citrate and 0.02 M potassium metabisulphite; pH 7) and heated at 121°C for 120 min. Five volumes of chilled ethanol (containing 1% acetic acid) were added to the supernatant and incubated at 4°C overnight. The obtained precipitate was centrifuged (8,000 g for 10 min at 4°C), dialyzed against distilled water (8 kDa mw cut-off) over night at 4°C, and freeze-dried. Chemical analysis of the extracted mannan showed 87% yield, 98.68% purity, 64% protein, 35.53% carbohydrate, 0% fat and 104 Da molecular weight.

Film preparation

The gelatin powder (5.0, 4.5, 4.0, and 3.5 g) were dissolved in deionized water (100 mL) to obtain film solution. The solutions were incubated at 55°C for 30 min in a water bath with stirring. After cooling the solutions to room temperature, the mannan (0, 0.5, 1.0, and 1.5 g) was added to previous solutions (total solid concentration of 5% w/v). Four gelatin-mannan blend film solutions at ratios of 5:0, 4.5:0.5, 4:1, and 3.5:1.5 were prepared. The glycerol (2 g) was added in all film solution as plasticizer. Final film forming solutions were homogenized (Ultraturrax D125, Janke and Kunkel, Germany), cast onto Teflon plate (50 × 50 mm), and dried in a ventilated oven (25°C for 24 h). The prepared films were peeled manually. The films were conditioned in a chamber over NaCl saturated solution with 75% relative humidity (22°C) before analysis.

Solubility

To measure the water solubility of films, small samples of films (2 cm × 2 cm) were dried at 105°C for 24 h and then weighed. The film samples were immersed in deionized water (100 mL) at 25°C for 24 h. After filtering the samples over filter paper, the paper was dried (105°C for 24 h) and the dry matter was weighed. The solubility of the film was determined using Eq. 1:

$$\% \; S = (m_i - m_d) \; m_i \times 100 \qquad \text{(Eq. 1)}$$

where S = solubility of films, m_i = weight of dried films, and m_d = weight of dried films after immersion (Marvdashti *et al.*, 2017).

Water vapor permeability

The ASTM E96 standard method (ASTM, 2016) was used to determine the WVP of the films. The lids of circular test cups filled with anhydrous calcium chloride (0% RH) were sealed by the films. Then, the cups were placed in a desiccator with a saturated solution of sodium chloride (RH 75%). The WVP of the films was measured using Eq. 2:

$$WVP = \frac{X \times \Delta m}{A \times \Delta t \times \Delta p} \qquad \text{(Eq. 2)}$$

where X = film thickness (mm), $\Delta m / \Delta t$ = weight of moisture gain per unit of time (g/s), A = area of the film surface (m²), and the gradient water vapor pressure across the film sides (Pa). The WVP measurement was replicated three times.

Mechanical properties

Tensile strength (TS), elongation at break (EB), and elastic modulus (EM) of the samples were analyzed using a texture analyzer (TA-XT Plus TM, Stable Micro Systems, UK).

Optical properties

A colorimeter (Minolta CR300 Series, Minolta Camera Co. Ltd., Osaka, Japan) was used to describe the color parameters of the films. The instrument was calibrated with a white standard plate ($L* = 93.49$, $a* = 0.25$, $b* = 0.09$). The total color difference (ΔE) index was quantified using Eq. 3 (Bonilla *et al.*, 2014):

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \qquad \text{(Eq. 3)}$$

where $L*$, $a*$, $b*$ = color parameter of the film on a white standard background, and L, a, b = color parameter of the samples.

Film opacity

The film opacity was determined by spectrophotometric method (Tunç and Duman, 2010), and quantified using Eq. 4:

$$Opacity = Abs\ 600/X \qquad \text{(Eq. 4)}$$

where Abs 600 = absorbance at 600 nm, and X = film thickness (mm).

Scanning electron microscopy (SEM)

The surface and cross-section morphology of gelatin-mannan blend films were analyzed by SEM (VP1450, German). A sputter coater (SC 1620) was used for gold-coating the samples. The films were analyzed at 20 kV under high vacuum condition. The magnification fold was 10 µm.

Fourier-transform infrared spectroscopy (FTIR)

FTIR spectra were studied using an AVATAR 370 FTIR Thermo Nicolet. A total of 20 scans were collected between 400 and 4,000 cm^{-1} resolution.

Differential scanning calorimetry (DSC)

Thermal characterization of gelatin-mannan blend films was evaluated using a differential scanning calorimeter (Shimadzu, DSC 60). The scanning of samples was determined between -100 to 300°C with a heating rate of 10°C/min. The melting point (T_m) and glass transition temperatures (T_g) of samples were reported.

Antifungal activity assay

The antifungal activity of gelatin-mannan blend films against *A. flavus* PTCC 5004 was evaluated by the disk diffusion method. A loopful of spore suspensions containing approximately 10^4 spores of *A. flavus* in Tween 80 (0.01 v/v) was inoculated onto potato dextrose agar (PDA) (Difco™, USA). A disc (5 mm) of each film was placed on inoculated plates and incubated at 25°C for 72 h. The diameter of growth free area surrounding the film disk at the end of incubation was reported as the inhibition zone (mm).

Aflatoxin binding assay

The aflatoxin binding effect of gelatin-mannan blend films was determined in Petri dishes containing pistachio extract agar (PEA). The PEA medium was prepared according to Aldars-García *et al.* (2017) by boiling 60 g of ground pistachio kernels in 1 L of distilled water (30 min) followed by filtration of the extract. Then agar was added to the medium (12 g/L) and autoclaved. Then, 500 ng/g AFB_1 standard solution was spiked into the medium and poured into sterile Petri dishes under aseptic condition. The film samples were cut in size of Petri dishes (90 mm diameter) and were put on PEA medium surface. For each film sample, three plates were prepared. Two plates not spiked with AFB_1 served as control samples. The plates were incubated at 21°C and after five days, the AFB_1 content of medium was analyzed. The AFB_1 determination was performed using high performance liquid chromatography (HPLC) as previously described (Abdolshahi *et al.*, 2018).

Statistical analysis

The results were analyzed by one way analysis of variance (ANOVA) using SPSS version 19, and were expressed as mean ± standard deviation.

Results and discussion

Water solubility (WS)

Film water solubility provides information on the stand of film to maintain its integrity in a high moisture content condition (Stuchell and Krochta, 1994). The solubility of gelatin-mannan blend films is presented in Table 1. The neat gelatin film has the lowest water solubility (56.21%) that was based on the values of films made from fish gelatin reported by Hosseini *et al.* (2013). These values were lower than those calculated for films made from cod skin gelatin (Denavi *et al.*, 2009).

Water solubility of the blend films significantly increased *(p < 0.05)* from 79.37% to 90.34% by

Table 1. Physicochemical and mechanical properties of gelatin-mannan blend films.

Gelatin-mannan (%)	WS (%)	WVP (g/m.s.Pa) ×10⁻⁵	TS (MPa)	EB (%)	EM (MPa)	L^*	a^*	b^*	ΔE	Opacity
5:0	56.21 ± 0.81[c]	0.41 ± 0.03[b]	1.30 ± 0.11[a]	103.00 ± 4.50[c]	11.32 ± 0.32[a]	84.46 ± 0.82[b]	-1.25 ± 0.04[a]	13.09 ± 0.58[c]	17.08 ± 0.41[b]	5.22 ± 0.59[b]
4.5:0.5	79.37 ± 0.19[bc]	0.45 ± 0.06[ab]	1.14 ± 0.06[a]	135.51 ± 3.35[b]	7.39 ± 0.08[b]	85.22 ± 0.29[a]	-1.68 ± 0.01[b]	11.40 ± 0.90[b]	16.16 ± 0.96[c]	4.73 ± 0.37[b]
4:1	83.50 ± 1.01[ab]	0.44 ± 0.04[ab]	1.21 ± 0.10[a]	143.14 ± 6.83[ab]	7.26 ± 0.20[b]	85.17 ± 1.14[a]	-1.91 ± 0.03[c]	12.74 ± 0.73[ab]	17.10 ± 1.41[b]	7.44 ± 0.31[a]
3.5:1.5	90.34 ± 0.79[a]	0.50 ± 0.04[a]	1.24 ± 0.09[a]	154.23 ± 7.91[a]	7.20 ± 0.44[b]	84.72 ± 0.86[ab]	-2.00 ± 0.04[c]	13.98 ± 0.95[a]	18.23 ± 0.87[a]	7.39 ± 0.89[a]

adding mannan to gelatin (0.5 and 1.5% mannan, respectively). Mannan has a glycoprotein structure and has more hydroxyl group as compared to gelatin. The interaction between mannan and gelatin could increase affinity between the films and water molecules. Modifying new network structure may induce differences in moisture content. It appeared that the presence of mannan in the film matrix may change the structure of the blend films. Similar results have been observed by Arda *et al.* (2009) by adding galactomannans to K-carrageenan films. The obtained water solubility values were higher than other blend films such as chitosan-tapioca (Vásconez *et al.*, 2009) and modified starch-carboxymethyl cellulose (Ghanbarzadeh *et al.*, 2010).

Water vapor permeability

WVP is considered the main factor in selecting packaging materials for different applications (Sun *et al.*, 2013). WVP of the neat gelatin film was 0.41 g/m s Pa, and this was significantly affected by blending with mannan. Table 1 shows the WVP of the gelatin-mannan blend films. Since polysaccharides have a hydrophilic structure, they are highly sensitive to water vapor hence are weak barrier to water molecules (Benbettaïeb *et al.*, 2016). The increase in WVP might be caused by higher hydrophilic groups of mannan structure. When 1.5% mannan was added to gelatin, the WVP significantly increased up to 0.50 g/m s Pa. This behavior indicated that there were hydrogen interactions between polymers. This occurrence may increase the availability of hydroxyl groups for water molecules and increase in WVP of the blend films (Wu *et al.*, 2012).

The scanning electron micrographs (SEM) (Figure 1) indicate that the increase in mannan might have changed the morphology of the blend films. Based on cross-sectional micrograph of the blend films, intermolecular interactions between two polymers decreased by adding mannan to gelatin. Therefore, the SEM results confirmed the WVP findings. High hydrophilic groups in polysaccharides structure allow water molecules to easily migrate

across the films, therefore, the films will be sensitive to humidity (Benbettaïeb *et al.*, 2016). The increase in mannan concentration might have also accelerated the migration of water molecules across the films.

Mechanical properties

The mechanical properties of the gelatin-mannan blend films including elastic modulus (EM), tensile strength (TS), and elongation at break (EB) were studied by tensile tests. Generally, the type and degree of interactions between the components affected the mechanical properties of blend films (McHugh *et al.*, 1994). The TS and EB mean values of neat gelatin films were 1.30 ± 0.11 MPa and 103.00 ± 4.50%, respectively. By increasing the mannan content, EB% increased; however, TS values did not significantly change (Table 1). Increased EB value seems to be associated with different amounts of hydrogen bonds between two polymers (Ghanbarzadeh and Oromiehi, 2009), but here in gelatin, it was lower than those of mannan.

Color

Color properties of films could affect appearance and overall acceptability of products (Rao *et al.*, 2010; Ramos *et al.*, 2013). The rectangular coordinates (L^*, a^*, and b^*) and the total color difference (ΔE) of gelatin-mannan blend films are shown in Table 1. By adding mannan to gelatin, the a^* values significantly decreased from -1.25 ± 0.04 to -2.00 ± 0.0.

Also, the presence of mannan in films ranging from 0.5 to 1.0% resulted in significant increase in lightness (expressed as the L^* value). However, at 1.5% (mannan), the L^* value slightly decreased. The blend films containing 0.5% mannan had lower b^* and ΔE values, whereas further increase of mannan content increased these values. These changes may be related to the differences in the matrix, moisture content, and interactions (Muñoz *et al.*, 2012). Similar results were obtained by Pranoto *et al.* (2007) for gellan and carrageenan addition to gelatin film. The obtained L^* values of blend films were higher than those reported for Lepidium perfoliatum seed
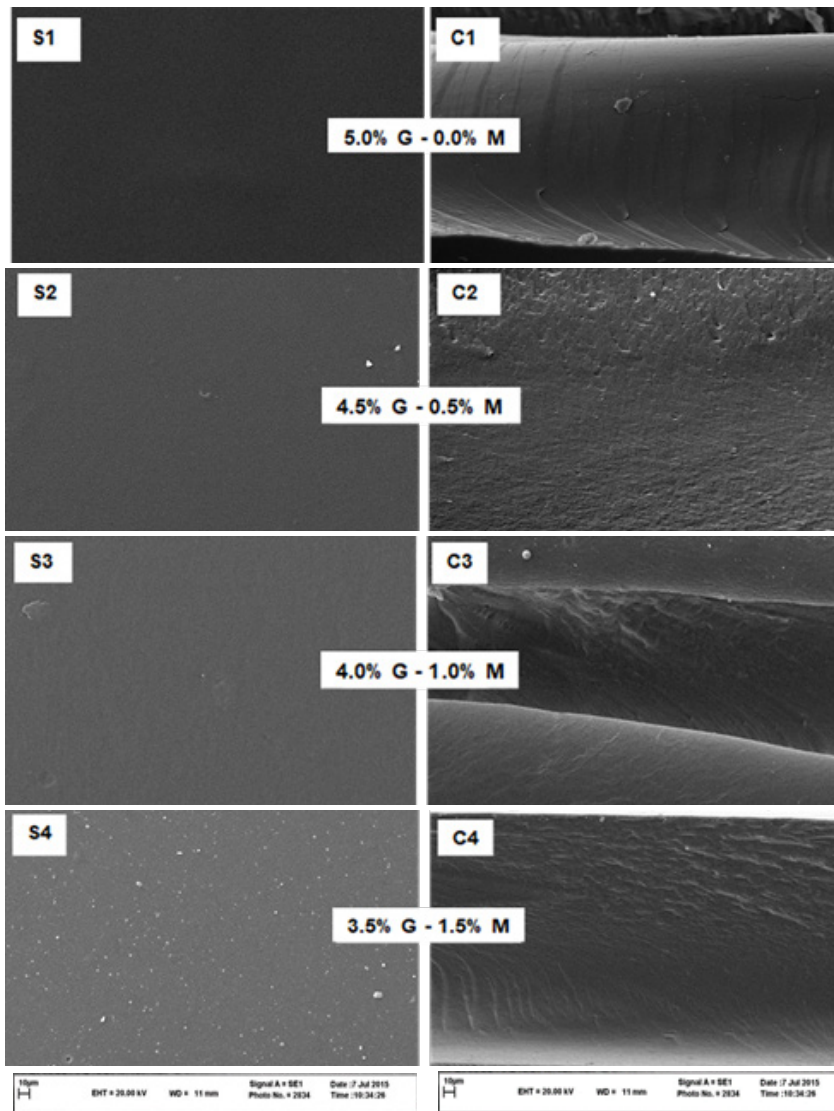
Figure 1. Scanning electron micrographs (10 µm) of surface (S) and cross-section (C) of gelatin film 5.0% G - 0.0% M, and gelatin-mannan blend films of 4.5% G - 0.5% M, 4.0% G - 1.0% M and 3.5% G - 1.5% M.

gum-grass pea (Lathyrus sativus), protein isolate (Ebrahimi *et al*., 2016) and starch-carboxymethyl cellulose (Ghanbarzadeh *et al*., 2010), but lower than Alyssum homolocarpum seeds gum (Marvdashti *et al*., 2016) and pea starch-peanut protein isolate (Sun *et al*., 2013).

Opacity

Transparency is one of the impressive features of a film especially for food packaging, as more transparent films have more consumer acceptability. Transparency is inversely associated with opacity (Pereda *et al*., 2011). The opacity values of gelatin-mannan blend films are shown in Table 1. The opacity of neat gelatin film was 5.22 A/mm, whereas the incorporation of 1.0 - 1.5% mannan in gelatin matrix resulted in significant increase of opacity up to 7.39 A/mm. Different factors including thickness, color, and internal structure can affect the opacity (Villalobos *et*

al., 2005; Sun *et al*., 2013). The higher opacity of film is desirable when the light reactions may deteriorate foods such as lipid oxidation (Gómez-Guillén *et al*., 2007). As a result, the higher mannan content in the matrix led to films with lower light transmittance.

Scanning electron microscopy (SEM)

To assess the effect of microstructure on homogeneity, water vapor transmission, mechanical, and optical properties of the films, SEM was used (Galus and Lenart, 2013; Jouki *et al*., 2013). Figure 1 shows the morphology of neat gelatin and gelatin-mannan blend films containing 0.5, 1.0, and 1.5% mannan. As indicated in Figure 1 (S1, S2, and S3), the surface of all films was homogenous and no micro-cracks or gaps were present in all film samples. On the contrary, the gelatin-mannan blend film containing 1.5% mannan (S4) appeared as a rough structure, where the film integrity was lost. This may

be related to the aggregation of some mannan in film matrix. The cross section in Figure 1 (C1, C2, C3 and C4) of the blend films was smooth, homogeneous, and not obviously different from neat gelatin film. On the other hand, the cross section revealed that increasing mannan content might have resulted in higher compactness of films. Furthermore, a proper interaction between mannan and gelatin indicated good compatibility of two components in film matrix. It was demonstrated that high concentration of mannan in blend film matrix tend to aggregate. Similar results were also found by Sahraee *et al*. (2017) for gelatin-based films containing N-chitin.

FTIR

Fourier-transform infrared spectroscopy has been used to monitor chemical component, interaction, structural changes, and miscibility of polymers in films. The polymers' compatibility can be investigated according to variation of intensity and peaks' position (Xu *et al*., 2007). As shown in Figure 2, the first remarkable peak was in $3,300 - 3,500$ cm^{-1}, which may be referred to O-H and N-H vibration stretches existed in hydrogen bond with carbonyl groups of protein structure (in both gelatin and mannan) (Pranoto *et al*., 2007; Andreuccetti *et al*., 2009). The peak around $2,850 - 2,980$ cm^{-1} was observed in all samples spectra, which could be caused by assigning the amid-B bound to CH and NH2 stretching vibration (Karnnet *et al*., 2005). By

increasing the mannan concentration in blend films, the intensity of peaks at $3,300 - 3,500$ cm^{-1} and 1,044 cm^{-1} also increased. It seems that mannan could interact with gelatin through hydrogen bands by O-H and N-H groups of both molecules in the film matrix.

A significant peak occurred around 1,665 cm^{-1} in all films spectra which could be considered as amide-I attributed to C=O stretching vibration (Guerrero *et al*., 2010). The band at 1,044 cm^{-1} indicated C-O stretching vibrations of the carbon number 1 and 3 in glycerol. The stretching vibration peak of C-O in carbon 2 of glycerol appeared at 1,114 cm^{-1} (Guerrero *et al*., 2010). Another three peaks at 852, 922, and 991 cm^{-1} were observed, which were related to C-C stretching vibration, respectively. As it was demonstrated by FTIR spectra, a new peak appeared around $1,200 - 1,400$ cm^{-1}, which represented the vibrations in plane of C-N and N-H groups of bound amide (amide III) or vibrations of CH$_2$ groups of glycine (Ebrahimi *et al*., 2016).

The miscibility is determined as ability of interaction of blend compounds with each other at molecular level and homogenous mixture fabrication (Wanchoo and Sharma, 2003). The miscibility of mannan with gelatin polymer can be acquired from peak shifting of 3,344 cm^{-1} to higher wave numbers, which indicated that there were more O-H and N-H stretching vibration (Sahraee *et al*., 2017).

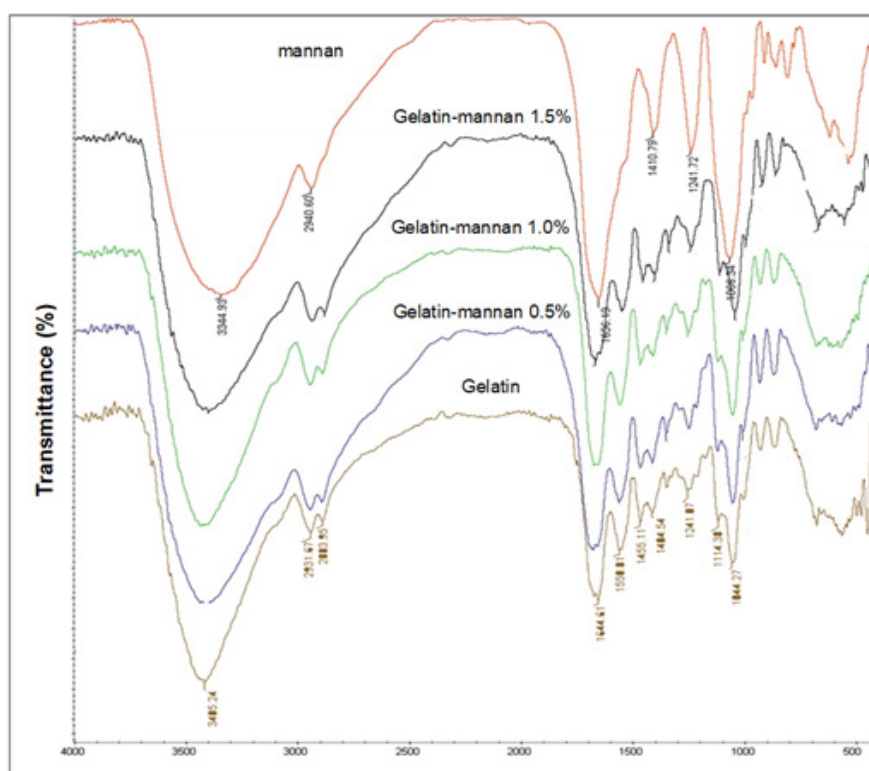In addition, the shifts of C-H groups (at $2,800 - 3,000$ cm^{-1}) to lower wave numbers could be

Figure 2. FTIR spectroscopy of mannan and gelatin-mannan blend films.

clearly observed, which could be associated with the increasing electrostatic and hydrogen interactions between the protein and glycoprotein (Abugoch *et al.*, 2011; Hosseini *et al.*, 2013).

Thermal characterization of films

The DSC thermograms of films containing various concentrations of mannan were considered for determining the thermal properties (Figure 3). Based on the obtained heating scan (from -100 to 200°C), all the films showed a transition peak that was represented as glass transition temperature (T_g), which was followed by an endothermic melting transition (T_m). As illustrated by the thermograms, the thermal stability has been altered by adding mannan to the film solution. The Tm of gelatin-mannan films incorporated with 0, 0.5, 1.0, and 1.5% mannan were 59.8, 55.7, 53.9, and 58.0°C, respectively. The presence of mannan in film formulation resulted in the melting point reduction of gelatin film. Although mannan concentration of 1.5% has enhanced the melting point of blend films, lower concentration indicated inverse impact.

The reduction of melting point in gelatin-mannan blend films could be related to the glycoprotein structure of mannan. In real situation, an increase in mannan could amplify the hydrogen bands, hence, the viscosity of polymer was increased in the film matrix.

Antifungal assay

Antifungal activity of films against *A. flavus* was determined as inhibition zone diameter (mm). All the films had inhibitory effect against *A. flavus* (Figure 4). Increasing the mannan concentration in films increased the growth inhibition area of fungi
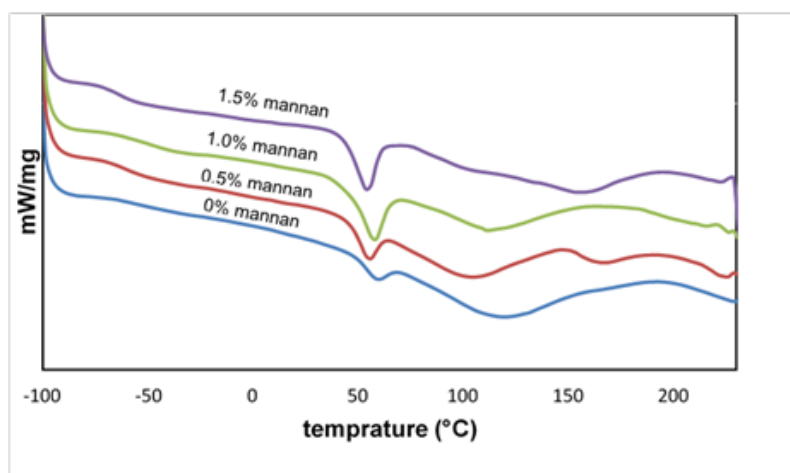

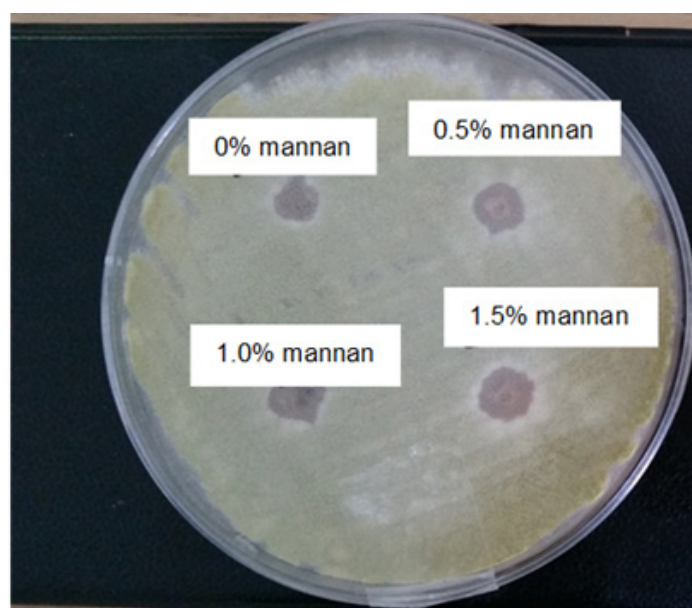Figure 3. DSC thermograms of gelatin-mannan blend films.

Figure 4. Disc diffusion assay of blend films with different ratios of mannan (0, 0.5, 1.0, and 1.5%) on potato dextrose agar (PDA) plates inoculated with *Aspergillus flavus* after five days of incubation at 25°C.

as the inhibition zone for gelatin-mannan of 5:0, 4.5:0.5, 4:1 and, 3.5:1.5 were 5.05 ± 0.30, 7.23 ± 0.06, 8.52 ± 0.11 and, 9.11 ± 0.27 mm, respectively. Our observations indicated that the films containing mannan could inhibit *A. flavus* cells growth at the contact area on the culture medium. It appeared that the mannan could be properly released from the film to the surface of medium which resulted in the inhibition of the fungal growth. Nevertheless, the neat gelatin film with no mannan also had inhibitory effect against *A. flavus*. This needs further study on the impact of gelatin on fungal growth. The results are in agreement with our previous work, which indicated that mannan has antifungal effect against *A. flavus* on PDA. Furthermore, the coating containing mannan was able to postpone the growth of *A. flavus* and its mycelium proliferation in pistachio (Abdolshahi *et al.*, 2016). The obtained disk diffusion results indicated that mannan could be released from film on the PDA surface medium and inhibited the growth of fungi on it. As a matter of fact, the mechanism of antifungal activity of mannan is still not clear. It has been proposed that mannan might affect the fungal cell membrane and the protein synthesis system.

Aflatoxin binding

According to the results of aflatoxin analysis, all films had AFB1 binding effect. The AFB1 binding percentage in gelatin-mannan films 5:0, 4.5:0.5, 4:1 and, 3.5:1.5 were 0, 44.62 ± 0.30, 57.13 ± 0.17 and, 72.02 ± 0.01, respectively. This finding indicated that mannan could be well released from film to medium, thereby binding the spiked AFB1. These findings could be verified by our previous results about aflatoxin reduction in contaminated pistachio (Abdolshahi *et al.*, 2018).

Conclusion

In the present work, gelatin-mannan blend films containing 0, 0.5, 1.0, and 1.5% (based on dry gelatin) of mannan have been prepared and characterized. FTIR analyses and SEM images have indicated that mannan is properly compatible with gelatin polymer. Increasing the mannan concentration resulted in the increase of WVP, opacity, and water solubility. Mechanical analyses have demonstrated that mannan could improve EB; however, it could lead to the decrease of EM while TS did not change. According to DSC thermograms, the blend film containing 1.0% mannan had more thermal stability as compared to gelatin film. Antifungal assay indicated that the films containing mannan had inhibitory effect against *A. flavus* growth. Accordingly, the incorporation of mannan in gelatin film could contribute to the development of a film with good physical and antifungal properties. This edible film is thus recommended to be applied in the food industry.

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