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ORIGINAL ARTICLE

Active Packaging Film Based on Lysozyme/Polyvinyl Alcohol / Alyssum Homalocarpum Seeds Gum

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KEYWORDS

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Mechanical properties

ABSTRACT: The active films composed of polyvinyl alcohol/Alyssum homalocarpum seeds gum (PVA-AHSG) in corporating lysozyme (LY) are developed and characterized. The LY affects the thickness, solubility, and water vapor permeability, mechanical, microstructural, and optical properties of PVA-AHSG films and also the antimicrobial activities of obtained films are evaluated. The LY content increased the thickness of composite films. The LY addition led to an increase in water solubility, moisture contents, and contact angle, and decreased density. The control films without LY had the lower opacity and acted as a weak barrier against the water vapor. The LY reduced elongation at break and slightly increased tensile strength and elastic modulus. The electron micrographs showed that the control films had a smoother structure, and the addition of LY formed films with a rough surface. Fourier Transform-Infrared Spectra indicated some interactions between PVA and AHSG chains and LY. Functional activities were found against Gram-positive *Listeria monocytogenes* and *Staphylococcus aureus.*

INTRODUCTION

A serious concern for public health, export, import, and economic development is food safety. As the main categoryin this field, food borne pathogens threat the safety of public health [1-4]. There are several ways for controlling microbial contaminations. One strategy that canenhance the food safety is the utilization of antimicrobial agentson the surface of foods[5]. In this approach, active compounds canbepartly inactivated overtime when diffused into the food matrix rapidly and interact with food ingredients. In order to overcome this limitation and improve the efficacy, the usage of antimicrobials agents in food packaging is anexcellentchoice [6].

Moreover, post-processing contamination is an important phenomenon in food industry. It can be the cause of food borne illness and haveeconomic burden on people showing a significant effect on public health. The incorporation of antimicrobial agents into package materials has introduced a new approach in the packaging industry. The active packaging is an effective method for improving safety and

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quality of food and refers to the incorporation of antimicrobial agents into the packaging matrix for controlling microbial growth on foods and prolonging theirshelf life[7-10].The growth of pathogenic and / or spoilage organisms has been deleted or limited by antimicrobial packaging in order to increase safety and quality of food [11, 12].

Natural compounds have been used to treat a variety of diseases at differentconditions[13-18]. Natural antimicrobial agents including compounds isolated from plants, animals and microorganisms have been used for the preservation of processed food products [19, 20]. Enzymes, bacteriocins, essential oils and phenolic are natural antimicrobial agents which can be used in film matrices to prevent the microbial contaminations [21-23].

Lysozyme (LY) is a protein with antibacterial activity which is widely find in egg white, human tears and saliva[24].LY is Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA) and as a food additive by the European Union (E 1105)with good stability in food and edible films[25, 26]. LY (muramidase, EC.3.2.1.17) is an enzyme with hydrophilic properties which hydrolyses the cell walls of Gram-positive bacteria through the bonds between N-acetylmuramic acid and N-acetylglucosaminesplitting inside the peptidoglycan chain. In addition, LY can also act as antiviral, anti-inflammatory, anticancer and immunomodulator agent with in a wide range of pH [27-30].

Alyssum homalocarpum(Fisch.&CA.Mey.)Boiss.is an annual plant of the *Brassicaceae* family producing aseed gum (AHSG) composed of a galactan-type polysaccharide. Notably,AHSG is composed of galactose (82.97%), glucose (5.7%), rhamnose (5.04%), xylose (2.72%), mannose (3.04%) and arabinose (0.53%);its average molecular weight is 3.66×10^5 g/mol[31]. As a food hydrocolloid, AHSG can be used in a wide range of food applications, namely as thickening/gelling agent, fat replacer stabilizeragent and film forming materials [32-37]. Our objects in previous work indicated the film based on AHSG had aproper oxygen barrier and good appearance but weak mechanical properties [38, 39]. also blending the AHSG with polyvinyl alcohol (PVA) result in more strength and flexibility in produced film[38].To the best of our knowledge, papersdealing with the usage of this hydrocolloid as active packaging component are scarce. Furthermore, there is no report studying the effects of antimicrobial agents on PVA-AHSG composite film properties. Therefore, this study has been carried out to evaluate the effects of different concentrations of LY on physico-chemical, optical, mechanical and antimicrobial properties of PVA-AHSG composite films.

MATERIALS AND METHODS

Materials

Alyssum homolocarpum seeds (AHS) were obtained from a local market (Mashhad, Iran) and PVA was supplied by Sigma-Aldrich Company (Steinheim, Germany). Anhydrous calcium chloride, saturated potassium sulfate and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Ethanol was purchased from Zakaria Alcohol Company (Jahrom, Iran). Lysozyme was purchased from the Sigma Aldrich (Saint Louis, Missouri, USA).

Preparation of AHSG powder

AHSG powder was produced according Monjazeb Marvdashti, et al. [38]. In brief, AHSwas macerated in distilled water (1:40) and the pH was adjusted at 4 by adding NaOH (10 M) then mixed totally (40°C for 1 h). Using ethanol (96%)the gum was precipitated and extracted. final extracted gum was freeze dried (Martin Christ Freeze Dryer, Osterode am Harz, Germany), and after millingthe powder was sieved to defined particle size and stored at 4°C for next analysis.

Preparation of PVA-AHSG composite films

The composite films were prepared usingthe method of Marvdashti, et al. [36]. Briefly, 1.5% (w/w) of AHSG powder was added todistilled water and stirred (LABINCO L-81, Amsterdam, and The Netherlands) for 20 min at 40°C. PVA (1.5% w/w) was suspended in distilled water and stirred for 60 min at 95°C. Afterwards, the PVA and

AHSG solutions were cooled down to 25°C using cool water and mixed at 60:40 ratio PVA to AHSG (w/w) to reach the final volume of 75 ml. The glycerol and LY powder (30, 50 and 70 mg) were added up to 50% w/w total solid content. The prepared solutions were stirred for 30 min at 160 rpm and centrifuged (7000 g for 10 min).Film forming solutions were spread in petri dishes. After drying overnight (40°C for 48 h) the films were removed from the surface of plate and placed in plastic bags, sealed and kept in a dry cool place.

physical properties

Thel thickness of films was determined randomly at ten pointsusing a micrometer (QLR digital-IP54, China). The samples of film (3 cm × 3 cm) put in a desiccators containingmagnesium nitrate (0.53 % RH) for 1 week at 25°C. Film density was determined using Equation (1):

$$\rho = \frac{\mathrm{m}}{\mathrm{A} \times \mathrm{d}} \qquad (1)$$

where, ρ is the density of film (g/cm^3), m is the film weight (g), A is the film area (9 cm^2) and d is the film thickness (cm).

Moisture content (MC) measurement performed on films after preconditioning at 53% RH and 25°C, and drying(110°C). MC (%) was calculated using the following equation:

$$\mathrm{MC} = (\mathrm{m_i} - \mathrm{m_d})/\mathrm{m_i} \times 100 \qquad (2)$$

Where m_i and m_d are the weight of samples before and after drying, respectively.

The water solubility of films was determined according toGontard and Guilbert [40]. The slices of film (2 cm × 2 cm) were kept in dry calcium sulfate for 5 days. The films were immersed in distilled water (50 ml) overnight then driedat 103°C. The water solubility was calculated using Equation (3):

$$\text{solubility in water (\%)} = \left(\frac{\text{initial dry weight} - \text{final dry weight}}{\text{initial dry weight}}\right) \times 100$$

Water vapor permeability

The water vapor permeability (WVP) were measured gravimetrically according ASTM E96-00 [41]. The films were used to seal the filled small cups containing 3 g of anhydrous calcium chloride (0% RH). The sealed cells were placed in saturated potassium sulfate solution (97% RH) at 25°C and after that were weighted each 2 h during 72 h. The WVP (g mm/m^2 d kPa) was calculated using the Equation (5):

$$WVP = (\Delta m \times X)/(\Delta P \times \Delta t \times A) \qquad (5)$$

where$\Delta m/\Delta t$ is the weight of moisture gain per unit of time (g/d), X is the film thickness (mm), Δp is the difference of partial pressure (kPa) and A is the area of exposed film surface (m^2).

Water contact angle

according optical method the contact angle was measured [42]. The photos were prepared from 20µL of distillated water on the film surface and was processed using the Image J Software.The average values were calculated of sixtest replicate.

Mechanical properties

A TA-XT PlusTM, Texture Analyzer (Stable Micro Systems, England) wasused to determine mechanical properties. The film pieces(1cm × 10cm)were put at RH 50% for 7 days. The film samples were subjected to grips at 60 mmdistance and pulled (10 mm min^{-1}) at 25°C. Elongation at break (EB), Tensile strength (TS) and Young modulus (YM) were calculated as described in ASTM D882 (2000) ineight replicates.

Optical properties

The absorbance (at 500 nm) of films was reported in an UV–vis spectrophotometer (Shimadzu UV-160A). The opacity of the films was calculated using the method of Gontard et al. (1994) method using the Equation (6)[40].

$$\text{Opacity} = \text{absorbance at } 500 \text{ nm} \times \text{film thickness (nm)} \qquad (6)$$

Microstructure of films

Using a scanning electron microscopy (SEM) (LEO 1450 VP, Germany) the cross and surface sections of the films were analyzed.The fixed film samples on copper stubs were coated by a layer of gold-palladium (SC 7620, England). High vacuum condition (10^{-6}torr) along with an accelerating voltage (35kV) were used.

The magnification of × 5000 was used for cross-sectional microstructure of films.

Fourier transform infrared spectroscopy (FT-IR)

Fourier-transform infrared (FTIR) spectra of the films were studied using an AVATAR 370 FTIR (Thermo Nicolet, USA). The FTIR spectra of films were recorded in the range of 400–4000 cm−1 from an average of 15–16 scans at 2 cm-1 resolution.

Antimicrobial activity

Listeria innocua ATCC 33090, *Staphylococcus aureus*ATCC 25923and *Escherichia coli* ATCC 4388were obtained from theIranian Research Organization for Science and Technology (Tehran, Iran). A loop of stocks was inoculated onto modified Oxford agar (MOX: Difco, Becton Dickinson, Sparks, Md., U.S.A.) then incubated at 37°C for 24 h. A 0.5 McFarland standard suspension of each isolate was prepared. 0.1% (v/v) of prepared suspension was added to a Muller Hilton agar medium kept at 50°C (in liquid state) and then poured into a petri dish. The discs of active film (7 mm diameter)were put on agar medium. The petri dishes were incubated at 30°C for 24 h. The clear zones surrounding discs were measured [43, 44].

Statistical analysis

Analysis of obtained data was done using SAS software. All the tests were carried out at three or more replicates. Analysis of variance (ANOVA), and Duncan’s test was also used.

RESULTS AND DISCUSSION

Physical properties of film

Thickness is one of the most important factors that directly affects the properties of films such asdensity, water vapor permeability, mechanical and optical properties. The preparation method and drying conditions have a significant effect on the final thickness [45].

The effect of LY concentrations on the thickness of PVA-AHSG composite films is presented in Table 1. The thickness of composite films increased significant (p<0.05) after adding LY. Results also indicated that the increase of LY concentration from 30to 70mg increased the final thickness of films. Notably, the highest thickness was reached with70 mg of LY (0.126 mm). A significant difference respect to that of the control (p<0.05) was observed. Bonomo et al. reported that the addition of LY to Jackfruit Starch films formed a stronger and thicker film due to the increase of dry matter[46]. This finding does not agree with the result of Park et al. who observed that incorporation of LY into the Chitosan film, had no significant effects on film thickness[47].

The effects of the addition of different LY amounts on the density of PVA-AHSG composite films are shown in Table 1. The composite film without LY showed a higher density compared with others. The film density significantly decreased from 0.126 to 0.112 g/cm^3 upon LY addition content (from 30 to 70 mg of LY). This is probably due to the differences in molecular weight between film polymers (AHSG and PVA polymers) and LY[31, 48, 49]. Density is a parameter that reflects information about molecular weight of polymers [50]. The LY has lower molecular weight than PVA and AHSG and therefore can lower the density of the composite films by decreasing the compactness in the microstructure of film network. These findings agree with those reported by Park et al., who reported a decrease in the density of chitosan film after the LY incorporation[47].

Moisture content of film indicates how the interaction between PVA, AHSG and LY polymer chains could affect the water affinity of the film. The moisture content for composite films with and without LY is given in Table 1. Results pointed out that the PVA-AHSG films had 39.5% water solubility which increased to 40.2, 42.1 and 44.7% in samples containing 30, 50 and 70mgLY, respectively. The results revealed that addition of LY to composite films had no significant effect on average moisture content of the active films ($p<0.05$).

Film water solubility provides information about the stand of film to maintain its integrity in a high moisture content condition[51]. The solubility of PVA-AHSG composite films is presented in Table1. The PVA-AHSG films without LY had the lowest water solubility (41.4%) that was in accordance with the values reported by Monjazeb Marvdashti, et al. [38].

Water solubility of the composite films was significantly increased ($p< 0.05$) from 60.7 to 75.3% after addition of30 and 70 mg of LY to PVA-AHSG matrices, respectively. This behavior can be explained by the penetration of LY into the film matrix taking place between PVA and AHSG polymers with a decrease of interactions between polymers. However, LY decreased the density of the films (Table 1). With the increase of free volumes in the matrix, penetration of water molecules accelerated with a consequent increase in film solubility. A similar trend was reported by Bonomo et al. for jackfruit starch film when LY was added to it [46].

Table 1. Physical properties of PVA-AHSG composite films.

PVA-AHSG film	Thickness (mm)	Density (g/cm^3)	Moisture content (%)	Solubility (%)	Opacity (AU.nm)	Water Contact Angle	WVP (g mm /m^2 h kPa)
Control	0.098 ± 0.002^{c}	1.29 ± 0.02^{a}	39.5 ± 4.0^{a}	41.4 ± 2.5^{c}	0.02± 0.00^{c}	38.1± 1.1^{d}	66.3± 1.4^{a}
30 mg LY	0.117 ± 0.004^{b}	1.26 ± 0.05^{ab}	40.2 ± 3.4^{a}	61.0 ± 3.0^{b}	2.87± 0.30^{b}	67.5± 1.1^{c}	0.3± 0.0^{b}
50 mg LY	0.120 ± 0.003^{b}	1.20 ± 0.02^{b}	42.1 ± 3.1^{a}	62.0 ± 2.0^{b}	3.01± 0.21^{b}	72.6± 1.0^{b}	0.3± 0.0^{b}
70 mg LY	0.126 ± 0.002^{a}	1.12 ± 0.03^{c}	42.0 ± 2.2^{a}	75.3 ± 3.1^{a}	6.74± 0.30^{a}	81.3± 4.9^{a}	0.1± 0.0^{b}

Values within each column with different letters are significantly different ($p<0.05$).

Water vapor permeability (WVP)

WVP is a main factor in selecting packaging materials for their potential applications. Indeed, one of the most important functions of a good packaging system is preventing or minimizing moisture transfer across it. Hence, the most appropriate WVP in food packaging is the lowest one[52]. Many factors affect WVP, such as mobility of polymer chains, polymer interactions, film integrity, hydrophilic-hydrophobic ratio and crystalline-amorphous zones ratio [53]. The WVP of PVA-AHSG composite films decreased with increasing LY concentrations (Table 1). Water vapor barrier properties of the film enhanced more than 100 times with addition of LY at 30 mg level. It seems be related to intermolecular interactions between PVA and AHSG polymer chains (Figure 1) and/or crystallinity ratio in the film matrix. The PVA-AHSG film without LY had tight and compact structure (Figure 2).However, with addition of LY, the density of the films was decreased. It seems that the increasing thickness of the films produced by the addition of LY could be the main reason for this result. On the other hand, these results are in good agreement with those achieved by water contact angle. Likewise, hydrophobicity had greater values in active films. Therefore, increasing the hydrophobicity could minimize the transmittance of water molecules across the film which results in lower WVP.

It seems that hydrogen interaction between the polymers lead to a reduced availability of the hydroxyl groups to water molecules, and thus to a decrease of WVP of the composite films [54]. Manab et al. reported similar results for films based on whey protein and claimed that the addition of LY improved the water resistance of films. They stated that the interaction between whey protein and LY molecules increased with increasing LY concentration[55].

Results showed that the films without LY were passible to water vapor, but LY could decrease the WVP. Therefore, the resistance to water vapor of the active films was better than the pectin, methylcellulose[56] and salepglucomannan[57]films.

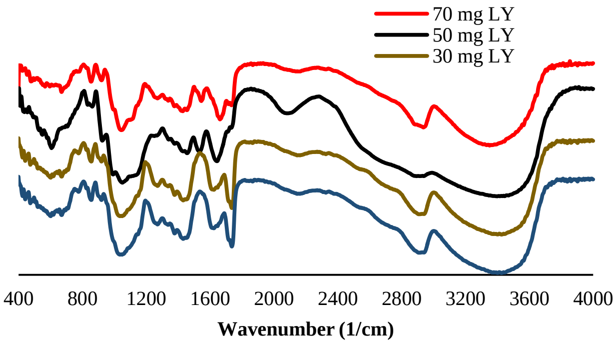

Figure 1. FTIR spectra of PVA-AHSG composite films.

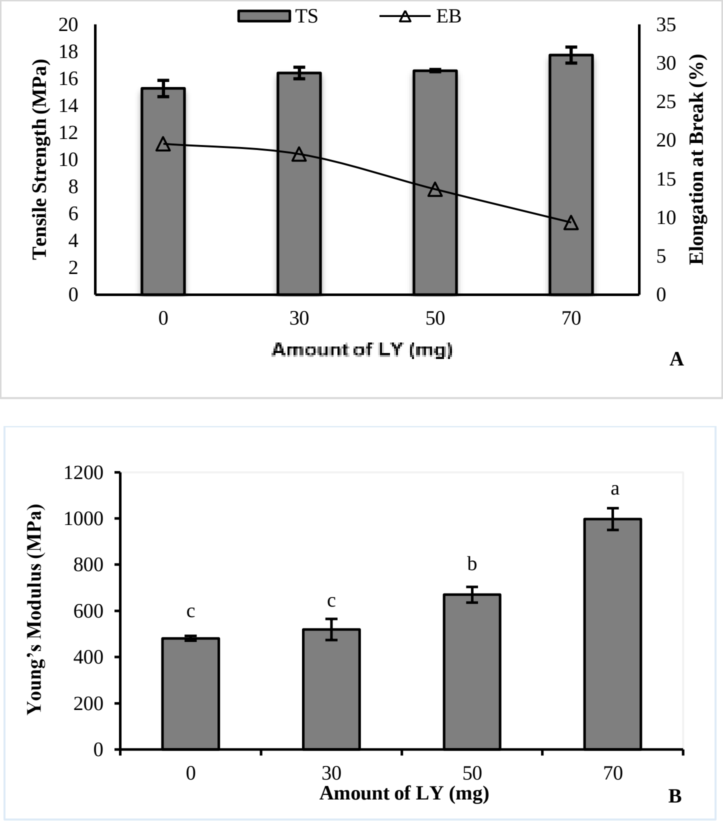

Figure 2. Mechanical properties of PVA-AHSG composite films.a) Elongation at break and tensile strength and b) Young's modulus.

Contact angle

The surface hydrophilicity of films is evaluated with the water contact angle parameter [58]. To show how LY concentration changes the hydrophilicity and wet ability of the film, the contact angle of PVA-AHSG films was examined (Table 1). In general, low ($\theta < 20°$) and high ($\theta > 70°$) contact angles characterize hydrophilic and hydrophobic surfaces, respectively [59]. The results showed that when LY was added to the film matrix, the contact angle of the films increased significantly (p<0.05). The PVA-AHSG composite films without LY showed the lowest hydrophobicity in the surface, while the films with 70 mg of LY had the highest surface hydrophobicity. Therefore, LY could decrease the wet ability of films due to its high affinity to aggregate on the surface of film [60]. The finding were also certified according SEM micrographs (Figure 2) in which the LY addition increased the roughness of film surface thus changing the water contact angle to higher amounts. Thus, hydrophilicity of the active films decreased with increasing LY concentrations. Ozer et al. reported similar results for whey protein isolate and chitosan films when LY was added[23].The reason for this behavior is explained by the hydrophobic amino acid side chains inside the structure of LY. Moreover, Li et al. (2017) also evaluated the influence of LY incorporation onthe surface hydrophobicity of chitosan film and found that the extent of contact angle from an initial valueof 75.3° increased to 87.3° after addition of LY[61].

Opacity

One of the most notable features of a film at packaging aspect is transparency in which food products through the films could more attract consumers. Opacity is associated with transparency inversely[62]. Generally, the opacity is affected by several factors including thickness, color and internal structure [53, 63]. The opacity value of PVA-AHSG composite films was significantly increased by addition of LY (Table 1). Further increase in LY concentration enhanced the opacity up to 7.39 AU/nm. As a result, the LY addition gave a lower light transmittance so that the composite films were more resistant against UV light and food oxidations.

Fourier transform infrared spectroscopy (FT-IR)

Infrared spectroscopy as an efficient technique could use for identifying the type of chemical bonds and also miscibility of polymers. the intensity and position of the peaks affected by molecular interactions of polymers[64]. The PVA-AHSG composite film without LY had some band groups at 2800–3000 and 1000–1500 1/cm, as methylene groups stretch and deformation vibrations of (Figure 1). The peaks at around 3397.97 and 2912 1/cm are afliatedto the −OH and the symmetric stretching vibration of the axial −CH groups to saturated hydrocarbons, respectively. In 1500 and 1050 1/cm the Additional peaks were objected seemsbelonged to C–O from carboxyl groupsstretching vibrations . in the range of 1300–1500 1/cm several peaks founded may related to C–H in CH_3 groups [38].

LY exhibited a significant peak between 1655-1645 1/cmin spectra, which belonged to C=O stretching vibration and is considered as amide-I [65, 66]. As can be understood from FTIR spectra, a new peak appeared around 1550-1530 cm^{-1} which was represented by the bending vibrations out of plane of N-H groups of bound amide II [67]. These peaks could be observed in the spectra of PVA-AHSG-LY films so, the existence of characteristic groups of LY were successfully detected inside the composite films.

With increasing LY concentration in the PVA-AHSG the OH bond area were seen at lower wave numbers (Figure 1).that might be due to hydrogen interactions between PVA and AHSG [68].

Taken together, LY molecules could interact with functional groups of the film component. By increasing the LY concentration the number of hydrogen bonds decreased. Zhang et al. studied the FTIR spectra of chitosan-LY films and found that the existence of characteristic groups of LY was successfully proved after its introduction into CS films. As the concentrations of LY was increased the free volume beside the chains mobility of polymer matrices as well as the WVP increased.

Mechanical properties

The determination of mechanical properties of packaging materials is very important in terms of technological and practical aspects [69]. The mechanical properties of the antimicrobial PVA-AHSG composite films including Young Modulus (YM), Tensile Strength (TS) and Elongation at Break percent(EB%) were studied by tensile tests and results are presented in Figure 2. The results indicated that the rigidity of the films slightly increased after LY incorporation. With increasing LY concentration, EM and TS were increased. A conversely trend was obtained for EB% with lower flexibility and extensibility in active films. In general, the mechanical properties of composite films were affected by the type and degree of interactions between the components[70]. This increase of TS could be related to the increase in crystallinity of the film structure. Stronger intermolecular interactions occurred in the structures ofLY, PVA and AHSG molecules, in comparison with the hydrogen bonds formed in the PVA–AHSG complex. Another factor is the increase of film thickness which might be related to the increase in TS. A thicker film shows a lower evaporation rate in which the mobility of the polymer chainsacceleratesso creation of intermolecular interactions along with film crystallinity increase consequently.

The rate of EB% was slightly decreased by the addition of LY, so that the lowest percentage of elongation (5.34%) was observed for films containing the highest amount of LY. Zhang et al. reported similar results for the incorporation of LY into chitosan films and demonstrated that LY decreased EB% and increases the YM of the chitosan films. In another study, the incorporation of LY into films based on low-methoxylpect in had a negligible effect on the tensile strength, but a significant increase in YM and EB% was observed [71].

Micro structural properties

SEM observations were used to study how the microstructure of the films affect homogeneity, water vapor transmission, mechanical and optical properties [45, 72]. SEM micrographs of the PVA-AHSG composite films with different concentrations of LY are presented in Figure3.

As can be seen, the PVA-AHSG composite film had smooth and homogenous surface without micro-cracks and gaps. Incorporating LY into the film generated arough structure, which could be attributed to the ability of LY molecules to aggregate onto the film surface. Li et al. observed a reduction in smoothness surface micrograph of chitosan films after adding LY and formation of active films. They stated that the addition of LY produced some tiny particles on the surface of the chitosan active films, resulting in a little roughness on the active films surface[61].Güçbilmez et al. studied microstructure of the zein films incorporated with lysozyme and found that the LY caused the formation of globular protein aggregates at the cross sectional micrograph of the films[73].

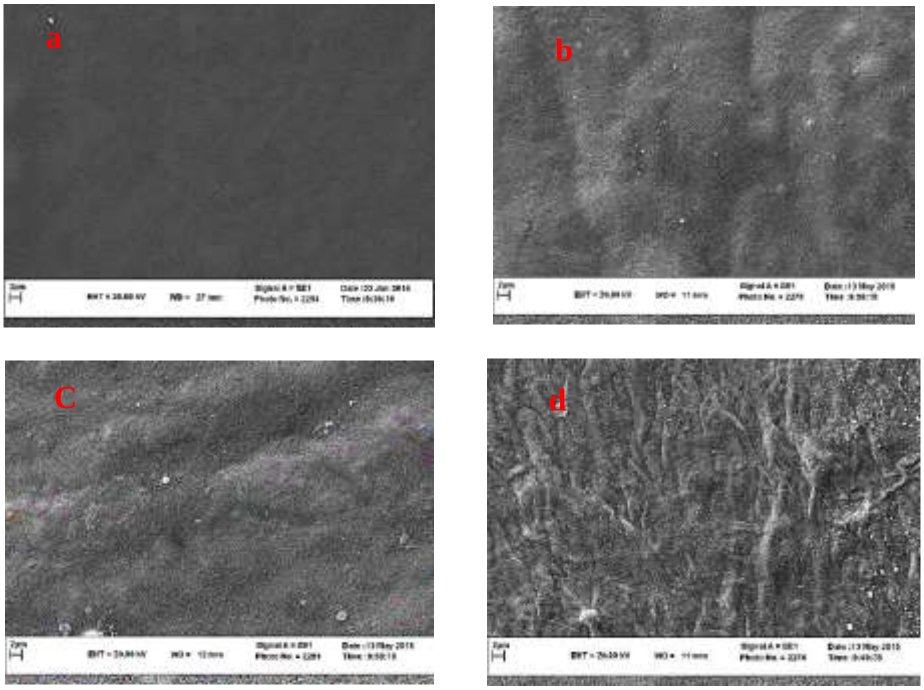

Figure 3. SEM micrographs of PVA-AHSG composite films as a function of LY concentration. a) control; b) 30 mg; c) 50 mg and d) 70 mg of LY.

Antimicrobial activity

The antimicrobial activity of PVA-AHSG composite films incorporated with different concentrations of LY was determined by measuring the growth of bacteria on agar plates. The inhibition zone surrounding film samples deposed to bacteriais displayed in Table 2. Lysozyme could affect the Gram-positive bacteria at peptidoglycan polymer layer otherwise Gram-negative bacteria not affected by lysozyme. We used a gram negative bacteria as negative control in the study. The results also showed that incorporation of LY into PVA-AHSG composite film caused inhibitory effects on L. innocua and S. aureus butnone on E. coli. With increase of LY concentration in the films, the diameter of the inhibition zone against the sensitive species increased significantly. In general, the antimicrobial effect of the active films was species-specific. The higher concentrations of LYcould enhance the antimicrobial activity of PVA-AHSG films reflecting the higher release of LY in agar gel. Composite films with 70 mg of LYproduced the highest inhibition zone (13.1 mm diameter) in*S. aureus*petri-dishes showing that the most sensitivity to LY among the tested strains. The results were consistent with those ofBonomo et al. who added LY to Jackfruit starch films[46], and to those ofFabra et al. and Kaewprachu et al. for isolate pea protein and starch based films and for gelatin film, respectively[74, 75]. It is clear that active films incorporating LY had no inhibition effects against *E. coli.* However, a reverse trend for gelatin/carboxymethyl cellulose films incorporated with LY was observed by Dekina et al. which could inhibit the growth of the gram-negative type of bacteria such as *E. coli* and *P. aeruginosa* [76].

Table 2. Antimicrobial activities of LY incorporated PVA-AHSG composite films using Agar diffusion test.

Film samples	Inhibition Zone (mm)		
	L. innocua	*S. aureus*	*E. coli*
Control	-	-	-
30 mg LY	6.5±0.4[c]	8.3±0.1[c]	-
50 mg LY	7.3±0.0[b]	11.5±0.04[b]	-
70 mg LY	12.3±0.3[a]	17.1±0.1[a]	-

[a]: Data reported are average values ± standard deviations. Values within each column with different letters are significantly different ($p< 0.05$).-: No inhibition zone around the film discs.

CONCLUSIONS

The development of PVA-AHSG composite films containing LY was assessed as an active antimicrobial packaging film, which might be enhance the shelf life of foods and pharmaceuticals.

The texture softening caused by inclusion of LY to PVA-AHSG films are important characteristics. Results showed that thickness, moisture content, water solubility and opacity of PVA-AHSG composite films containing 30, 50 and 70 mg of LY were increased. Micrographs obtained via a SEM analysis demonstrated that control film have a relatively smoother, cleaner and more compact surface compared with films with LY. FTIR analysis and SEM images have shown that LY is properly compatible with PVA and AHSG polymers. In addition, the FT-IR results verified that the hydroxyl group of PVA and AHSG had reacted with the chains of LY. Increasing concentrations of LY resulted in a decrease of WVP. Mechanical analyses showed that LY can improve TS and YM but led to a decrease of EB%. The hydrophobic properties of the films were increased by the addition of LY. The film with lysozyme was effective in inhibiting the growth of *L. innocua* and *S. aureus*, while no inhibition was observed on *E.coli*. The most powerful effect was observed on the growth of *S. aureus* with active film containing 70 mg of LY. Accordingly, further studies seem be needed to investigate information about the controlled release of LY from PVA-AHSG composite films in order to improve the release time/duration also shelf life of foods. Accordingly the PVA-AHSG film could potentially applied as edible films for food packaging.

Study limitations

The most important thing about the film is its solubility. The solubility of the film is high. Therefore the application of this film as active packaging is limited.

ETHICAL CONSIDRATION

The study project had approved by the research ethics committee of Semnan University of Medical Sciences (approval ID: IR.SEMUMS.REC.1399.127).

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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