Development of poly(vinyl alcohol) /starch/ethyl lauroyl arginate blend films with enhanced antimicrobial and physical properties for active packaging

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Abstract: Active packaging films have emerged as alternatives to replace petroleum-based packaging materials. In this work, a blend of poly(vinyl alcohol) (PVA) /starch/ethyl lauroyl arginate (LAE) film was prepared to form a polymer composite structure that possesses enhanced properties. Scanning electron microscopy (SEM) showed that PVA and starch were compatible, the concentrations of LAE greatly affected the structural integrity of the films. Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction showed that the inclusion of LAE did not significantly affect the intermolecular interactions and crystal structures of the polymer matrix. With an increase of the LAE content, the tensile strength (TS) was slightly decreased due to the altered microstructures, the elongation at break (EB) significantly increased ascribed to the synergistic effect of acetic acid, glycerol and LAE. The values of TS and

23 EB were 17.25 MPa and 586.08% when LAE was 10%. Active films showed good

24 barrier properties from UV while retaining the transmittance in the visible light region.

25 The films containing 1% of LAE exhibited antibacterial activity against *Escherichia*

26 *coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*), the inhibition zone of bacterial

27 growth gradually expanded with increasing LAE content. This study demonstrates the

28 potential of using LAE as the antibacterial agent for synthesizing natural-based

29 polymeric films for active packaging applications.

30

31 **Keywords:** Poly(vinyl alcohol)　(PVA), cassava starch, ethyl lauroyl arginate (LAE)

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33 **1. Introduction**

34 　　In recent years, food packaging materials based on petroleum-based polymers

35 have caused serious environmental issues, such as natural resource depletion, waste

36 production and energy crisis contribution [1]. In response to the demand of

37 environmental sustainability and the market trends, a lot of research has been devoted

38 to the development of novel packaging materials based on biopolymers that are

39 biodegradable and eco-friendly [2-4].

40 　　Poly(vinyl alcohol) (PVA) is one of the most common types of synthetic

41 biopolymers that has been widely used in the food packaging industry. PVA is non-toxic

42 and biodegradable, it was approved in 2003 by the Joint FAO/WHO Expert Committee

43 on Food Additives and permitted to be used as food packaging material in the United

44 States [1,5-8]. However, compared with conventional synthetic plastics, PVA has a

2

45 relatively high price. Considerable efforts have been made to blend PVA with other

46 renewable materials to fabricate composite materials with lower manufacturing cost

47 [2,6]. Furthermore, novel PVA composites often possess superior performance and

48 multiple functions to meet the new requirements of customers [9,10].

49   Starch is one of the most abundant polysaccharides on earth, and plays an

50 important role in the food packaging industry because of its low cost, non-toxicity and

51 biodegradability [1,7,11,12]. Moreover, gelatinized starch has good film-forming

52 ability [13]. The cast starch films are usually tasteless, odorless and transparent,

53 showing good oxygen barrier properties for food preservation [14]. Among starches

54 from various botanical origins, cassava starch is suitable to prepare packaging films by

55 its high paste clarity, high gel stability and low gelatinization temperature [15]. The

56 primary deficiencies of starch-based films are their poor mechanical properties and

57 inferior water barrier properties [16]. Blending starch with other biopolymers can form

58 a new class of composite materials with enhanced physicochemical properties [6,17,18].

59   It has been known that blending PVA with starch can result in a high performance

60 PVA/starch composite [6,7,19,20]. PVA and starch are compatible since both have

61 adequate hydroxyl groups in their molecular chains to form strong intermolecular

62 hydrogen bonds. Therefore, the obtained PVA/starch composite films can achieve

63 homogeneous structures and improved properties [21]. Cano et al. [6] prepared

64 composite films by blending PVA with pea starch, in which the tensile strength and

65 elastic modulus of the obtained films were 32.30 MPa and 689 MPa, respectively,

66 showing better mechanical properties than both pure PVA and pure starch films. Negim

67 et al. [7] blended PVA with corn starch to fabricate films, and the thermal stability and

68 biodegradability of the obtained films were superior to those of pure PVA films.

69   Antimicrobial packaging is an important part of active packaging systems. The

70 incorporation of antimicrobial agents into the packaging films can reduce the microbial

71 contamination, which helps to extend the shelf life, guarantee the safety and maintain

72 the quality of packaging food products [22,23,24]. LAE is an antibacterial agent derived

73 from natural compounds, including lauric acid, L-arginine and ethanol [25,26]. It can

74 effectively inhibit the growth of a wide range of spoilage microorganisms and food

75 pathogens [27]. It is a surfactant that is capable of damaging the cell membrane with a

76 low dose, subsequently altering the cell permeability and inhibiting the growth of

77 microorganisms [28,29]. The Food and Drug Administration (FDA) classified LAE as

78 generally recognized as safe (GRAS) in 2005, and the European Food Safety Authority

79 (EFSA) approved its application as a food preservative in 2007 [28]. Moreover, LAE is

80 flavorless, colorless, resistant to high temperature and stable at pH 3-7. These

81 characteristics make it a promising candidate for the development of new antimicrobial

82 packaging films. The physicochemical properties and antibacterial performances of

83 active packaging films incorporated with LAE has been investigated in previous studies

84 [26,30,31]. Theinsathid et al. [4] fabricated PLA films and coated them with LAE, and

85 found that a small amount of LAE coating (0.07%, w/w) efficiently inhibited the growth

86 of *Listeria monocytogenes* and *Salmonella typhimurium*. However, while increasing the

87 amount of LAE coating could improve the antibacterial activity, it compromised the

88 transparency of the films. Haghighi et al. [32] prepared chitosan / PVA / LAE films and

89   found that the addition of LAE increased the water vapor permeability and water

90   solubility of the films while decreasing their mechanical properties and transparency.

91   All films containing different concentrations of LAE (1%, 2.5%, 5% and 10%) showed

92   antibacterial ability to against *Campylobacter jejuni*, *Escherichia coli*, *Listeria*

93   *monocytogenes* and *Salmonella typhimurium*.

94   LAE has been previously introduced as the antimicrobial agent into various food

95   packaging materials [33-37]. However, the effects of LAE on the properties of

96   PVA/starch blend films are not fully understood. As both PVA and starch are not able

97   to inhibit the growth of pathogens and microorganisms in food, in this research, we

98   investigate the effects of incorporated LAE on mechanical and antimicrobial properties

99   of PVA/starch blend films.

100   In this work, a film based on PVA/starch blends enriched with different

101   concentrations of LAE was fabricated by a solution casting process. This blend film is

102   designed for fresh food packaging such as meat and seafood to prolong their shelf life.

103   It is safer and has longer-lasting protective abilities than conventional packaging

104   systems because the LAE content in the polymer matrix is progressively released to the

105   surface of packaged foods rather than directly added to the food formulation. The

106   microstructure of the obtained blend films was characterized and the mechanical,

107   optical and water barrier properties were evaluated. Moreover, the antimicrobial

108   performances of blend films against two major food pathogens, *E. coli* and *S. aureus*

109   were assessed in vitro. This study aims to develop a novel blend film with effective

110   antimicrobial properties and enhanced physical properties, which can be potentially

111　　used in active food packaging applications.

2. Materials and methods

2.1 Materials

　　Cassava starch (food grade, the amylose-amylopectin ratio was 19:81 determined by the iodine method) was obtained from Guangxi Hongfeng Starch Co., Ltd. (Nanning, China). PVA (polymerization degree 1750±50) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). LAE was obtained from Shanghai Bide Pharmatech Co., Ltd. (Shanghai, China). Glycerol (≥99%) and Glacial acetic acid (AR) were purchased from Guangdong Guanghua Sci-Tech Co., Ltd. (Guangzhou, China). *E.coil* (CMCC(B)26003) and *S. aureus* (ATCC25922) were obtained from Shanghai Luwei Technology Co., Ltd. (Shanghai, China). Casein soya bean digest agar (TSA) and Casein soya bean digest broth (TSB) were purchased from Guangdong Huankai Microbial Sci-Tech Co., Ltd. (Guangzhou, China).

2.2 Preparation of blend films

　　A starch solution (10%, w/w) was prepared by dissolving the cassava starch granules in distilled water. Then, the starch solution was gelatinized by continuous stirring at 80°C for 60 min (430 rpm). Similarly, the PVA solution (10%, w/w) was prepared by adding PVA powder to distilled water, and continuously stirred at 80°C for 90 minutes (430 rpm). Afterwards, gelatinized starch was mixed with PVA solution at a ratio of 3:7 (w/w). This ratio was pre-determined by a preliminary experiment, in which five different starch/PVA ratios (1:9, 3:7, 5:5, 7:3 and 9:1) were prepared. The sample with ratio 3:7 demonstrated better clarity and potential for further study.

133   Glycerin (20% of starch and PVA, w/w) and glacial acetic acid (20% of starch and PVA,

134   w/w) were then added to the mixture and stirred at 80°C for 30 min (430 rpm) to obtain

135   the film-forming solution (FFS). Subsequently, different contents of LAE (1%, 2.5%,

136   5% and 10% of starch and PVA, w/w) were added to the FFS and stirred at 80°C for

137   additional 30 min (430 rpm) to reach complete dissolution. After cooling and

138   eliminating the bubbles in the solution at room temperature, 20 g FFS was cast on a

139   polymethyl methacrylate plate (120 × 120 mm) and dried at 25°C in an oven for 24 h

140   to obtain the blend films. Control films were prepared following the same procedure

141   without adding LAE. All the film samples were stored in a desiccator at room

142   temperature for at least 2 days before the measurement, and the relative humidity (RH)

143   of the desiccator was adjusted to 53% by using saturated $Mg(NO_3)_2$.

144   **2.3 Morphology and structural characterizations**

145   The morphology and microstructure of the obtained blend films were

146   characterized by scanning electron microscopy (SEM), Fourier transform infrared

147   spectroscopy (FTIR) and X-ray diffraction (XRD).

148   The morphology of a cross-section of the blend film was observed by a scanning

149   electron microscope (Supra 55, Zeiss, Oberkochen, Germany) operated at an

150   acceleration voltage of 2 kV and 2000× magnification. Fractured samples were

151   collected from the tensile test, fixed with a double-sided adhesive and sputter-coated

152   with gold for the observation.

153   A Fourier transform infrared spectroscopy (Nicolette Magna 550II, GMI, Ramsey,

154   MN, USA) was used to characterize the interactions between cassava starch, PVA and

155 LAE in the blend films. Samples were ground and mixed with KBr powder, then the

156 mixtures were compressed to form the testing disc. Spectra were recorded in the 4000-

157 400 cm^{-1} wavenumber range, with a spectral resolution of 8 cm^{-1}.

158   The X-ray diffraction patterns were collected by an X-ray diffractometer

159 (MiniFlex 600, Rigaku, Tokyo, Japan) equipped with a Kα Cu radiation source operated

160 at 45 kV and 15 mA. The films were cut into squares (20 × 20 mm) and fixed on a

161 sample stage (glass plate). Samples were scanned between 2θ=3° to 40° with a step size

162 of 0.02°. The crystallinity index (CI) of blend films was calculated by Equation (1) [38].

163 CI (%) = A_c / (A_c + A_a) × 100%   (1)

164 where A_c represents the crystalline area, and A_a represents the amorphous area.

165 **2.4 Thickness and mechanical properties**

166   The film thickness was measured using a spiral micrometer (211-101, Jingyou

167 Mould Hardware Co., Ltd. Dongguan, China). Six random positions were selected and

168 measured on the films, one at the center and the other five at the edges. The thickness

169 values were obtained based on the means of these six positions [39].

170   The tensile strength (TS) and elongation at break (EB) of blend films were

171 determined by a universal testing machine (JDL-1000N, Tianfa Instruments Co., Ltd.

172 Yangzhou, China) following the method used by Liu [40] with modifications.

173 Rectangular strips (20 × 60 mm) were cut out from the sample film. Initial grip

174 separation and overhead speed were set as 50 mm and 10 mm/min, respectively. TS was

175 expressed in MPa and calculated by dividing the maximum load by the cross-sectional

176 area of the sample. EB was expressed in percentage (%) and calculated by dividing the

film elongation at break by the initial distance of the grips. The calculations were undertaken by the built-in software. All tests were repeated at least five times for each sample.

2.5 UV barrier properties and opacity

UV barrier properties and the opacity of blend films were investigated by an UV-VIS spectrophotometer (UV-2600, Shimadzu, Kyoto, Japan). The film samples were cut into strips (10 × 40 mm) for the measurement. Their optical properties were evaluated at the UV wavelength range from 200 to 400 nm, and the visible light wavelength range from 400 to 800 nm. The opacity values were calculated according to Equation (2) [32].

$$\text{Opacity value} = (-\log T_{600}) / H \qquad (2)$$

where T_{600} is the fractional transmittance of the blend film at a wavelength of 600 nm, and H is the thickness of the blend film. All measurements of optical properties were performed in triplicate.

2.6 Moisture absorption (MA) and water vapor permeability (WVP)

MA of the blend films was measured by using Abral's method with some modifications [41]. The square film strips (20 × 20 mm) were cut from the film samples and dried in a vacuum oven at 50°C for 24 h to eliminate moisture. Afterwards, the dried film strips were stored in an airtight desiccator with 75% RH (using saturated NaCl) for 24 h to reach equilibrium and taken out for weighting. MA values were calculated using Equation (3) and averaged based on three replicates.

$$\text{MA (\%)} = (M_f - M_i) / M_i \times 100\% \qquad (3)$$

199   where M_f represents the final weight (g) and M_i represents the initial weight (g) of film

200   strips. All measurements of moisture absorption were performed in triplicate.

201   WVP of the blend films was examined using the method in ASTM E96-00 with

202   some modifications [41]. A plastic cup (50 mm diameter) containing distilled water (30

203   mL, 100% RH) was covered by the sample film (60 mm) and completely sealed with

204   Vaseline for avoiding moisture leakage. Then, the initial weight of the cup was weighed

205   and placed into an airtight desiccator containing 1000g silica. An incubator was used to

206   maintain the desiccator at 25 °C. The cup was weighed every 3 h until its weight

207   stabilized. WVP was determined by the relation between the weight loss and storage

208   time and calculated using Equation (4).

209   WVP $(g \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}) = (dW \times H) / (dt \times dP \times S)$ (4)

210   where dW represents the weight loss of the plastic cup system (g), H represents the

211   mean film thickness (m), dt represents the time change (s) under the partial water vapor

212   pressure gradient (dP = 2533 Pa), S represents the permeation area of the film sample

213   (m^2). Tests were repeated four times and averaged to obtain WVP values.

214   **2.7 In vitro antimicrobial activity (Agar diffusion assay)**

215   In vitro antibacterial activity of the control films and active films with different

216   concentrations of LAE against *E.coil* and *S. aureus* was assessed according to the agar

217   diffusion method used by Theinsathid et al., with some modifications [4]. The films

218   were cut into a disc shape with a diameter of 8 mm. Subsequently, the disk shape sample

219   and the TSA plate were each sterilized with UV for 2 h. Then the TSA agar plates were

220   streaked with 0.1 mL TSB inoculant containing 10^8 CFU/mL of the tested bacteria (*E.*

221   *coli* and *S. aureus*). Finally, the sample was placed on the surface of the TSA agar plate

222   and incubated at 37°C for 24 h. The antibacterial activity was evaluated by the average

223   diameter (mm) of the inhibition zone around the disc shape sample. All tests were

224   repeated three times.

2.8 Statistical analysis

226   The experimental data were expressed as the mean ± SD (standard deviation).

227   Analysis of variance (ANOVA) was performed using SPSS statistical analysis software

228   (SPSS Statistics 26 for Windows, IBM, New York, USA). Differences between the

229   mean values of the properties of blend films were detected by Tukey's multiple range

230   test ($p \leq 0.05$).

3. Results and Discussions

3.1 Morphology
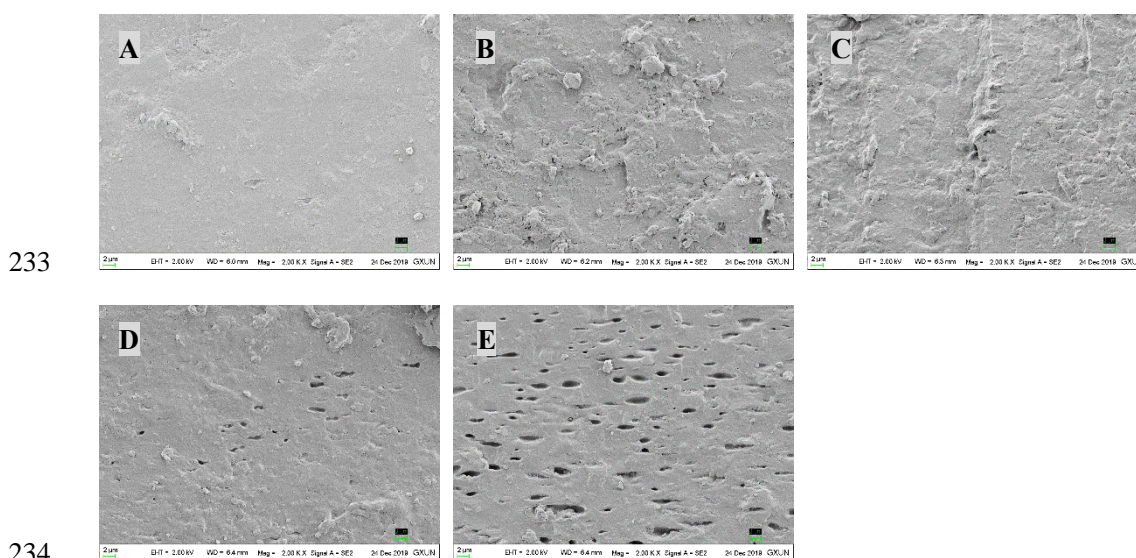
Figure 1. SEM images of films with different LAE content. (A without LAE, B-E LAE content

236   was 1%, 2.5%, 5% and 10% of polymer matrix, w/w)

237   The cross-section images of the control film (LAE-0%) and the active films with

238   different concentrations of LAE are shown in Figure 1. It can be seen that the addition

239   of LAE greatly affected the surface morphology of the blend films. The cross-section

240   of the control film without LAE (Figure 1 A) is relatively smooth and homogeneous as

241   compared with other LAE containing samples. It indicates that PVA and cassava starch

242   have good compatibility in blending, which could be due to the formation of the

243   hydrogen bonding between the PVA and cassava starch [21]. Small aggregations and

244   rough surface were observed at the cross-section of active films incorporated with LAE

245   (Figure 1B-1E), when the concentrations of LAE were 1% and 2.5%. It might be due

246   to the aggregation of LAE in the film matrix, and the interaction between PVA and

247   starch being disrupted by LAE, leading to the destruction of the compact structure of

248   the polymer blends [30,32,42]. When the concentration of LAE was 5%, pores appeared

249   on the film cross-section, and when the concentration of LAE was 10%, the number of

250   pores clearly increased. This can be explained by the enhanced surfactant effect of LAE.

251   Due to the high surface tension, small air bubbles were generated and sustained during

252   the blending process in the film-forming solution, and the high viscosity of the mixture

253   solution made it difficult to eliminate them, thereby causing the formation of pores in

254   the film matrix [30].

255   **3.2 FTIR**
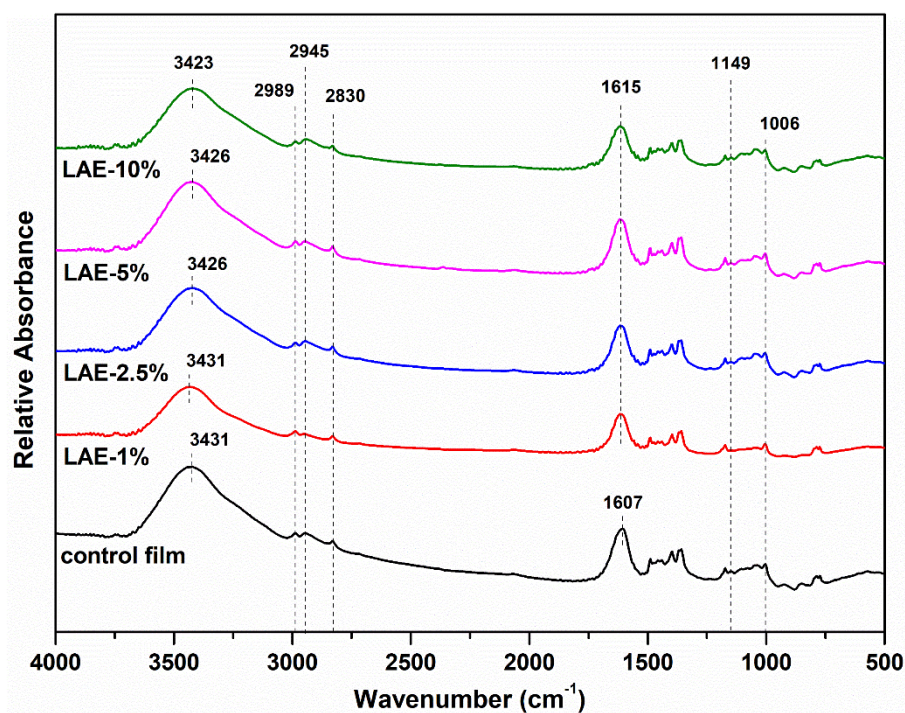
Figure 2. FITR spectra of films with different LAE content (0, 1%, 2.5%, 5% and 10% of polymer matrix, w/w).

Figure 2 shows the FTIR spectra of the control film and active films incorporated with different concentrations of LAE. The broad adsorption band between 3200 and 3600 cm^{-1} is attributed to the O-H stretching vibrations, indicating the intermolecular hydrogen bonds form between PVA and cassava starch [7]. It is noted that there is a slightly peak shift of the O-H stretching vibrations of different PVA/starch blends appeared at this band. With the increase of LAE content, the peak shifted to a lower wavenumber, which could be due to the hydrogen bond interaction between PVA and starch. The characteristics peaks at 2945 cm^{-1}, 2830 cm^{-1} and 2989 cm^{-1} are related to the C-H stretching, symmetric and antisymmetric -CH$_2$ stretching, respectively [7,32]. The peak observed around 1615 cm^{-1} is associated to the starch with bound water molecules, and the peak at 1006cm^{-1} is assigned to the C-O stretching vibrations of C-O-C groups in the glucose ring of starch. The C-O stretching of acetate groups of PVA

13

271 is located at 1149 cm^{-1}. The shapes of these five curves are very similar, suggesting the

272 addition of LAE has no significant influence on the molecular structure and the

273 intermolecular interactions of the blend films.

274 **3.3 XRD**
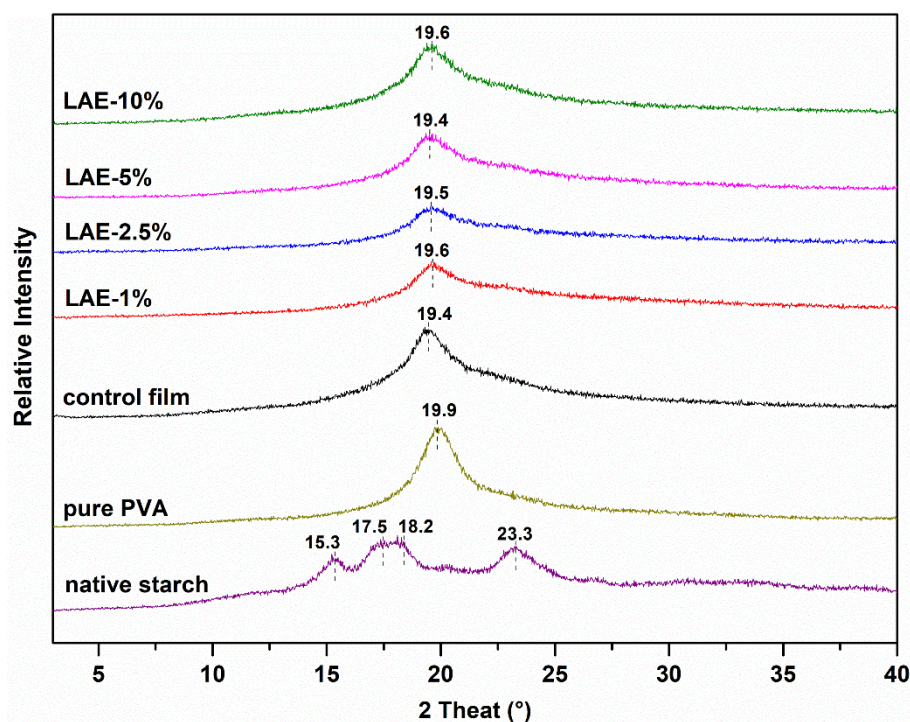
275

276 Figure 3. XRD patterns of starch, PVA and films with different LAE content (0, 1%, 2.5%, 5%

277 and 10% of polymer matrix, w/w).

278 The XRD patterns of the starch, PVA, PVA/starch control film and active films

279 incorporated with different concentrations of LAE are presented in Figure 3. The

280 diffraction peaks of starch granules were located at 2θ=15.3°, 17.5°, 18.2° and 23.3°

281 [43], the diffraction peak of PVA were located at 19.9°. After blending starch with PVA,

282 the peaks of the starch disappeared and only the main diffraction peak at 19.4° to 19.6°

283 of PVA remained, indicating the addition of starch did not affect the crystalline structure

284 of PVA. The reason might be that the starch was gelatinized and the crystalline

structures were destroyed during the blending process, resulting in the diffraction patterns of the blends were being dominated by the crystalline structures of PVA. Furthermore, there were no major changes of the diffraction patterns of the blend films in the presence of LAE. The CI of the control film and blend films with different additions (1%, 2.5%, 5%, 10%) of LAE were 12.09%, 11.87%, 12.37%, 12.45% and 11.44%, respectively. The CI values were close, indicating the crystalline structure of the blends were not significantly affected by the addition of LAE contents.

3.4 Thickness and mechanical properties

Thickness is one of the crucial parameters to determine the mechanical, optical and barrier properties of blend films. Table 1 lists the thickness values of the control and active films. It was found that thickness values ranged from 0.131 mm to 0.141 mm, and the thickness of the blend films was not significantly affected by the inclusion of LAE. This was in agreement with the results of Rubilar et al. [44]. In their work, LAE had a high solubility in the PVA/starch film matrix. In contrast, Haghighi et al. [32] reported that LAE might loosen the microstructure in the chitosan/PVA films, leading to an increase in the film thickness when using high concentrations of LAE. This inconsistency in different studies suggests that the fabrication process might strongly affect the thickness of blend films, despite the type of film matrix.
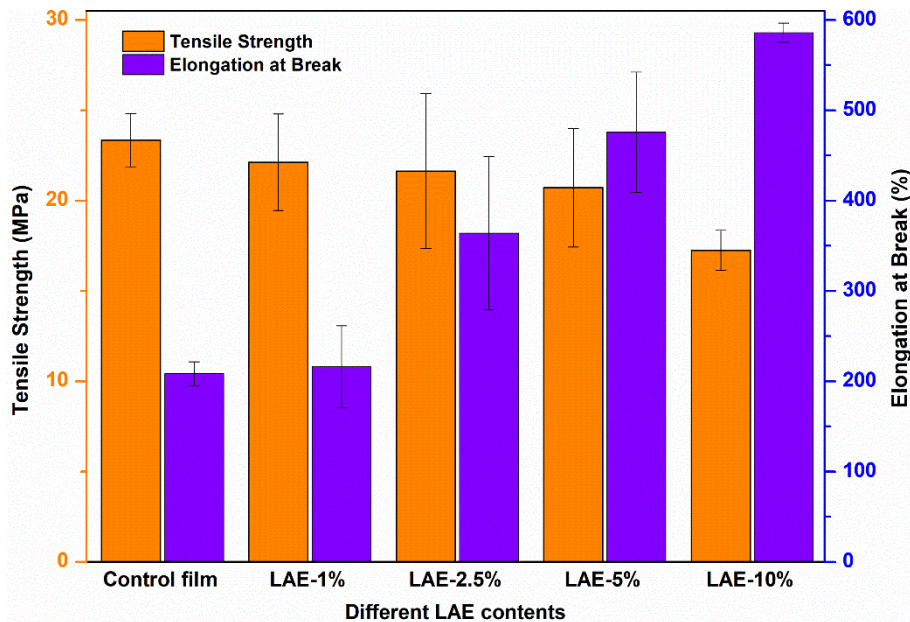
Table 1. Thickness of films based on control film and films with different LAE content (1%, 2.5%, 5% and 10% of polymer matrix, w/w).

Film sample	Control film	LAE-1%	LAE-2.5%	LAE-5%	LAE-10%

Thickness (mm)	0.14±0.03	0.14±0.02	0.14±0.01	0.13±0.02	0.14±0.02

306 Values are shown as mean ± SD (n=3), and there is no statistical difference.

307

308

309 Figure 4. Light transmittance of the blend films with different LAE content (0, 1%, 2.5%, 5%

310 and 10% of polymer matrix, w/w).

311   Mechanical properties are important for evaluating the applicability of blend films

312 as packaging materials. In addition, adequate tensile strength and extensibility are

313 necessary for most food packaging applications [39]. The TS and EB of blend films

314 with different LAE contents are shown in Figure 4. It is evident that the inclusion of

315 LAE strongly influences the mechanical properties of blend films, with an increase in

316 the concentration of LAE, while the TS ($p \leq 0.05$) of the film decreased while the EB

317 ($p \leq 0.05$) increased.

318   The presence of LAE slightly weakened the tensile strength of the blend films.

319 The control film had the highest TS value, and decreased from 23.34 MPa to 17.25 MPa

320 when incorporating 10% of LAE. This tendency of the tensile properties was consistent

16

with the SEM analysis. The integrity and continuity of film matrix were destroyed by the incorporation of LAE, and small aggregates and pores emerged within the structure, thereby resulting in a decrease in the tensile strength [45]. Rubilar et al. [44] also reported that the inclusion of LAE reduced the tensile performances of chitosan-PVA films.

The introduction of LAE significantly improved the extensibility of blend films. The EB value increased from 208.45% to 586.08% when adding up to 10% LAE in this work. It might be ascribed to the synergistic effect of acetic acid, glycerol and LAE. It has been reported that the amylose and amylopectin chains in starch molecules can be hydrolyzed by the acetic acid, leading to the higher solubility of starch and reduced viscosity of the suspension. Therefore, both film-forming abilities and cohesion of the gel structure can be improved. [46,47]. Furthermore, the hydrogen bonds generated among glycerol, LAE and polymers substituted the intermolecular bonds in the polymer matrix, which promoted the sliding of long polymer chains during extension, and thus led to better flexibility of blend films [48]. The elongation properties of our active films were superior to some previous studies with similar formulas. The highest EB value of chitosan/PVA/LAE films prepared by Haghighi et al. was 39% [32], and the highest EB value of starch/LAE films prepared by Motta et al. was 119.63% [48]. It was also noted that the mechanical properties of our active films with LAE were comparable with the high density polyethylene (HDPE, 22 MPa, 500 %) films widely used in the market.

In previous studies, Haghighi et al. [39] reported that low content of LAE (0.1%, v/v) did not influence the mechanical properties of chitosan–gelatin active films.

343 Rubilar et al. [44] found that the inclusion of LAE clearly increased both the TS and

344 EB values of chitosan films. Moreno et al. [45] observed that the addition of LAE

345 decreased the TS values and notably reduced the EB values when preparing films by

346 compress molding. These controversial results in the literature indicate that the

347 mechanical properties of active films containing LAE are determined by multiple

348 factors, including the type of polymer matrix, the amount and conditions of LAE and

349 other additives, as well as the fabrication process.

350 **3.5 UV barrier properties and opacity**
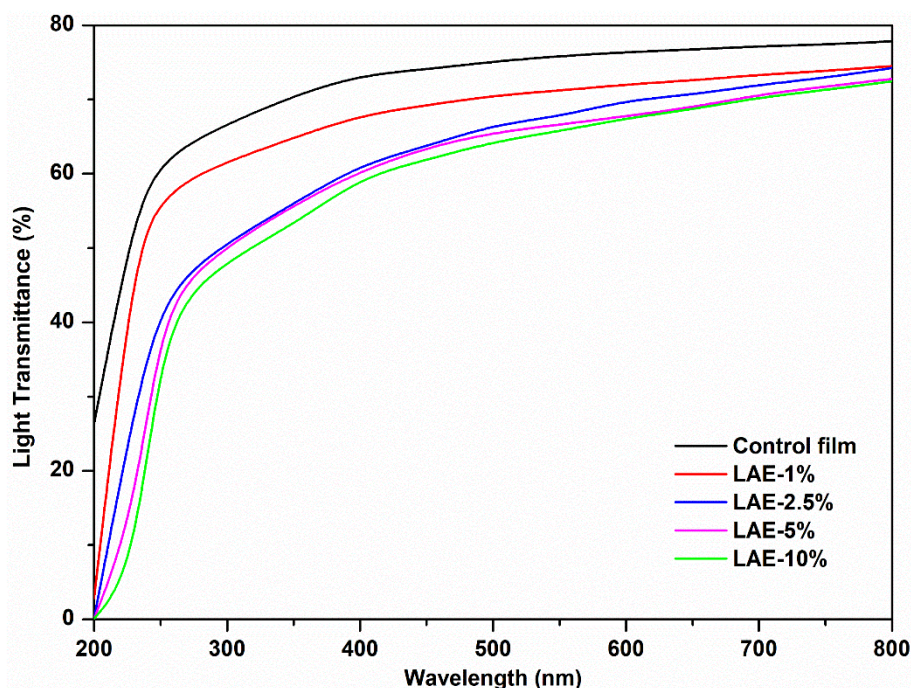
351

352 Figure 5. Light transmittance of the blend films with different LAE content (0, 1%, 2.5%, 5%

353 and 10% of polymer matrix, w/w).

354   The light transmittances of the control and active films with different

355 concentrations of LAE in the wavelength range between 200 and 800 nm are presented

356 in Figure 5. It was found that the active films possess lower UV light transmittances

357 (200-400 nm) as compared to the control film. At a wavelength of 200 nm, the light

transmittance of active films was close to 0%, which means that active films performed

as effective UV barriers. In food packaging applications, the excellent UV barrier

properties of active films can help to prolong the shelf life of food products, and can

reduce UV-induced oxidations, and preserve the nutrition, flavors and colors of foods

[49,50].

In the VIS region (400-800 nm), the light transmittances of active films were

slightly lower than that of the control film. At a wavelength of 800nm, the light

transmittance of the control film was 77.84%, and the light transmittances of active

films (containing 1%, 2.5%, 5% and 10% LAE) were 74.53%, 74.25%, 72.80% and

72.45%, respectively. At the wavelength of 600 nm, the opacity value of our active

films was 0.99, 1.10, 1.19 and 1.21, respectively, similar to the values of

chitosan/PVA/LAE films and chitosan/gelatin/LAE films reported in previous studies

[39].

Packaging materials were often used for wrapping different colors of food

products. To evaluate the transparency of active films on different colors, color charts

were printed on a white paper, pictures were taken when the color charts were covered

by an active film, no film and a control film, respectively. Figure 6 shows that all three

color charts were clear, the clarity was slightly decreased when the charts were covered

by the films. There was no significant difference of color, and the difference between

color charts covered by the active film (a) and the control film (c) in the picture was

difficult to tell. This phenomena observed in the picture was in agreement with

transmittance results shown in Figure 5.
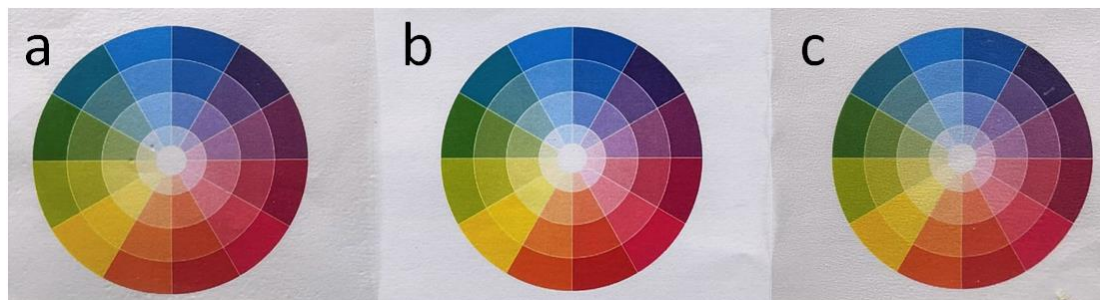
Figure 6. To evaluate the transparency and clarity of the blend films, a color charts covered by (a) LAE-10% blend film, (b) no film (b) and (c) control film (i.e. LAE-0%), respectively.

In general, with an increase in the LAE concentration, light transmittances of active films slightly decrease [39], likely due to the aggregation of LAE. Larger aggregates formed by more LAE inhibits light transmission. Transparency also plays an important role in food packaging, showing the original appearance of food to the consumers. Since the maximum transmittance of all active films in the VIS are higher than 70%, and their opacity values of them at 600 nm are less than 5, as shown in Table 2, all active films obtained in our study can be considered as transparent [32].

Table 2. Opacity value of films based on control film and films with different LAE content (1%, 2.5%, 5% and 10% of polymer matrix, w/w).

| Film sample	Opacity value* (600 nm)
Control film	0.84±0.01 [a]
LAE-1%	0.99±0.05 [b]
LAE-2.5	1.10±0.07 [bc]
LAE-5%	1.19±0.02 [c]
LAE-10%	1.21±0.05 [c]

394   Values are shown as mean ± SD (n=3).

395   *The mean values in the same column of the table with different superscript letters (a, b, c, d)

396   indicate significant differences by Turkey's test at $p \leqslant 0.05$.

397

398   **3.6 Moisture adsorption (MA) and water vapor permeability (WVP)**
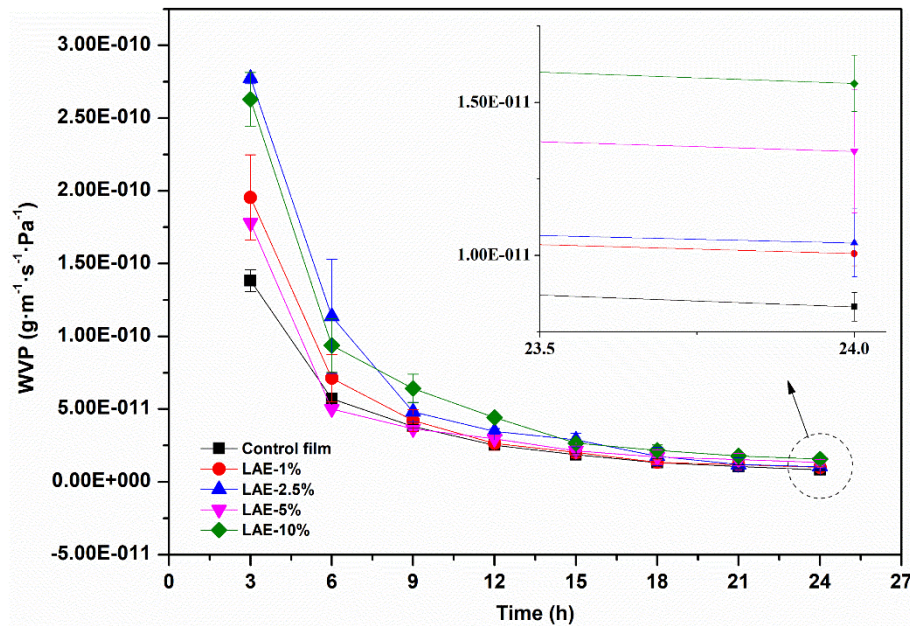
399

400   Figure 7. WVP of films with different LAE content (0, 1%, 2.5%, 5% and 10% of polymer

401   matrix, w/w).

402   Moisture adsorption and water vapor permeability are two significant factors that

403   affect the performances of food packaging materials [44,51], so it is crucial to

404   investigate the water barrier property of active films.

405

406   Table 3. MA and WVP of films based on control film and films with different LAE content (1%,

407   2.5%, 5% and 10% of polymer matrix, w/w).

Film sample	MA* (%)	WVP* ($g \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$)

Control film	17.54±1.15 [a]	8.32×10^{-12}±4.70×10^{-13} [a]
LAE-1%	22.92±0.77 [b]	1.01×10^{-11}±4.07×10^{-13} [b]
LAE-2.5	23.44±0.94 [b]	1.04×10^{-11}±1.11×10^{-12} [b]
LAE-5%	24.49±0.87 [c]	1.34×10^{-11}±2.02×10^{-12} [c]
LAE-10%	25.46±1.12 [d]	1.56×10^{-11}±9.21×10^{-13} [d]

408   Values are shown as mean ± SD (n=3).

409   *The mean values in the same column of the table with different superscript letters (a, b, c, d)

410   indicate significant differences by Turkey's test at p≤0.05.

411

412   Table 3 presents the MA values of the control film and active films with different

413   concentrations of LAE. The addition of LAE led to an increase in MA values. The MA

414   value of the control film was 17.54%, and that of the active film with 10% LAE content

415   reached to 25.46%. This might be due to the high water affinity of LAE. The oil-water

416   equilibrium partition coefficient of LAE was low (less than 0.1), which made it easy to

417   capture the water molecules [26,44].

418   Figure 7 shows the WVP of control films and active films with different

419   concentrations of LAE. The incorporation of LAE led to higher WVP values. After 24

420   h, the WVP value of the control film was 8.32×10^{-12} g·m^{-1}·s^{-1}·Pa^{-1}, while that of the

421   active film with 10% LAE content was 1.56×10^{-11} g·m^{-1}·s^{-1}·Pa^{-1}. Besides the

422   hydrophilic properties of LAE, the water absorbed by starch and PVA during the

423   swelling might break the hydrogen bonding and destroy the crystal structure of PVA

424   matrix, thereby accelerating the movement of the polymer chains, resulting in an

425   increase in the passage of water molecules [32,44]. Moreover, porous structures

emerging in the active films with high LAE content might also promote the transfer of water molecules. We have compared the LAE-10% sample with reported composite based packaging materials in the literature (Table 4).

Table 4. Comparison of physical properties with recently reported packaging films.

Composition	TS (MPa)	EB (%)	MA (%)	WVP	Opacity value	Ref.
Chitosan/ polyvinyl alcohol/ 2.5% LAE	34.5±2.4	39.0±2.6	--	21±0.02 (g mm/ kPa day m²)	1.6±0.2	[1]
Modified starches/ 0.05% LAE	2.65±0.33	119.63±0.21	--	425.56±13.05 (g water m^{-2} / day)	7.98±0.09	[2]
Chitosan/ gelatin/ 0.1% LAE	31.86±1.66	28.69±0.54	--	0.018±0.00031 (g mm/ kPa day m²)	1.43±0.22	[3]
PVA/ starch/ coconut shell extract/ sepiolite clay	32.77±4.98	15.57±1.46	--	0.90±0.06 (g mm/ m² day kPa)	--	[4]
PVA/ starch/ ZnO	7.32±0.45	210.3±12.75	5.49±0.11	--	--	[5]
PVA/starch/TiO$_2$	8.85±0.85	221.7±15.63	7.78±0.19	--	--	[5]
PVA/starch/gelatin protein hydrolysate	4.27±0.33	208.5±17.62	6.52±0.23			[5]
Starch/ PVA/ cinnamon essential oil	52.6±4.1	241.8±22.9	--	0.25±0.02 (g mm kPa^{-1} h^{-1} m^{-2})	23.8±0.5 (AUmm)	[6]
Pineapple peel extract/ PVA/ starch	29.09±3.53	33.39±2.59	--	0.75 (mm/m² day kPa)	--	[7]
PVA/ starch	42.41	209.33	193	--	--	[8]
PVA /starch/10%LAE	17.25	586.08	25.46±1.12	1.56×10^{-11}±9.21×10^{-13} (g·m^{-1}·s^{-1}·Pa^{-1})	1.21±0.05 (@ 600 nm)	This work

3.7 In vitro antimicrobial activity

The antimicrobial properties of the control and active films with different concentrations of LAE against *E. coli* and *S. aureus* were assessed by disk diffusion assay. As shown in Table 5, the control film did not exhibit inhibitory effects against the tested bacteria. No inhibition zone appeared in the TSA plate of the control film

overgrown with *E. coli* and *S. aureus*, indicating that starch and PVA are not able to

inhibit the growth of bacteria, as has been reported previously [52,53]. All active films

containing LAE show antimicrobial effects. Moreover, the inhibition zones of tested

bacteria gradually expanded with an increase in the LAE concentration. When the LAE

concentration increased from 1% to 10%, the diameter of the inhibition zone increased

from 12.2 mm to 29.07 mm for *E. coli*, and from 19.23 mm to 43.37 mm for *S. aureus*,

respectively. The high antimicrobial efficiency of LAE could be ascribed to its role as

an effective cationic surfactant on the cell membrane. Even at a low concentration, LAE

can change the membrane potential and prevent the microorganisms from growing.

When the concentration of LAE increased, the membrane structures were seriously

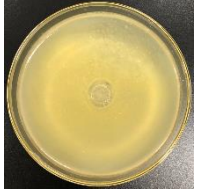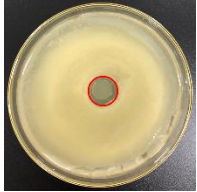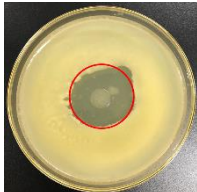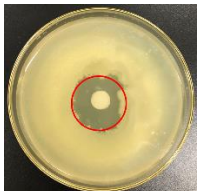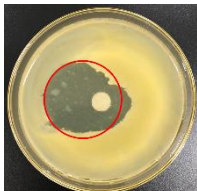destroyed, resulting in rupturing the entire cellular structures [28,29].
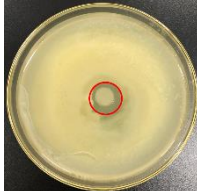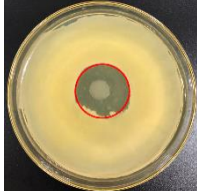
It was also found that *E. coli* showed better resistance to LAE than *S. aureus* in

our study, which can be explained by the structural differences between these two

bacteria. *E. coli* is a gram-negative bacteria with a thick layer lipopolysaccharide

membrane outside of the cell, while *S. aureus* is a gram-positive bacteria only

possessing a single peptidoglycan layer cell structure. It was more difficult for LAE to

damage the membrane of *E. coli* [54]. The antibacterial performances of the active films

in this work were superior to those previous studies using a hydrophobic polymer

matrix [4,32], since the hydrophilicity of our film matrix can promote the diffusion of

LAE in TSA plates [55,56].

Table 5. The inhibition zone of films with different LAE contents (0, 1%, 2.5%, 5% and 10%

of polymer matrix, w/w) on *E. coli* and *S. aureus*.

Film sample	inhibition zone of *E. coli** (mm)		inhibition zone of *S. aureus** (mm)	
Control film		no inhibition zone		no inhibition zone
LAE-1%		12.20±0.10 ᵃ		19.23±0.21 ᵃ
LAE-2.5%		16.33±0.25 ᵇ		29.10±0.20 ᵇ
LAE-5%		20.57±0.21 ᶜ		37.30±0.20 ᶜ
LAE-10%		29.07±0.25 ᵈ		43.37±0.21 ᵈ

*The mean values in the same column of the table with different superscript letters (a, b, c, d) indicate significant differences by Turkey's test at p≤0.05.

4. Conclusions

In this study, blend films based on PVA/starch enriched with different concentrations of LAE (1%, 2.5%, 5% and 10%, w/w) were prepared by a casting process. SEM results showed that PVA and cassava starch were compatible, the addition

of LAE changed the film morphology, and high content of LAE (5% and 10%) led to the generation of pores on the cross-section due to the surfactant effects. The molecular interaction, crystalline structure and film thickness were not significantly affected by the introduction of LAE. Incorporating LAE slightly decreased TS but significantly increased EB of the blend films, which might be ascribed to the aggregation and synergistic plasticizing effects of acetic acid, glycerol and LAE, respectively. The light transmittance values of active films with LAE were lower than those of control films in both the UV and visible light regions, suggesting that the active films had better UV and light barrier properties, which could help to prevent food products from degradation and oxidation. The incorporation of LAE resulted in higher MA and WVP values because of its hydrophilic nature and the increasing mobility of polymer chains. The active films showed antibacterial activities against both *E. coli* and *S. aureus* when including only 1% LAE, and they became more effective when the concentrations of LAE increased. The PVA/starch films containing LAE demonstrated potential as an active packaging material to effectively maintain the food quality and extend the shelf life. They are a promising alternative to replace synthetic plastics in certain food applications.

489

References

491   [1] X. Tang, S. Alavi. Structure and Physical Properties of Starch/Poly Vinyl

492   Alcohol/Laponite RD Nanocomposite Films. Journal of Agricultural and Food

493   Chemistry. 60 (2012) 1954-1962.

494   [2] W. Du, T. Jiang, M. Shi, Z. Yang. Structure and Properties of Starch/Poly (vinyl

495   alcohol) Film Modificated by Different Inorganic Salts. Chemistryselect. 4 (2019) 600-

496   607.

497   [3] S. Quintavalla, L. Vicini. Antimicrobial food packaging in meat industry. Meat

498   Science. 62 (2002) 373-380.

499   [4] P. Theinsathid, W. Visessanguan, J. Kruenate, Y. Kingcha, S. Keeratipibul.

500   Antimicrobial activity of lauric arginate-coated polylactic acid films against Listeria

501   monocytogenes and Salmonella typhimurium on cooked sliced ham. Journal of Food

502   Science. 77 (2012) 142-149.

503   [5] L. Chen, S. H. Imam, S. H. Gordon, R. V. Green. Starch- polyvinyl alcohol

504   crosslinked film— performance and biodegradation. Journal of Environmental Polymer

505   Degradation. 5 (1997) 111-117.

506   [6] A. I. Cano, M. Cháfer, A. Chiralt, C. González-Martínez. Physical and

507   microstructural properties of biodegradable films based on pea starch and PVA. Journal

508   of Food Engineering. 167 (2015) 59-64.

509   [7] E. Negim, R. Rakhmetullayeva, G. Yeligbayeva, P. Urkimbaeva, S. Primzharova, D.

Kaldybekov, J. Khatib, G. Mun, C. D. Williams. Improving biodegradability of polyvinyl alcohol/starch blend films for packaging applications. International Journal of Basic & Applied Sciences. 3 (2014) 263-273.

[8] C. C. Demerlis, D. R. Schoneker. Review of the oral toxicity of polyvinyl alcohol (PVA). Food & Chemical Toxicology. 41 (2003) 319-326.

[9] P. Cazón, M. Vázquez, G. Velazquez. Composite films of regenerate cellulose with chitosan and polyvinyl alcohol: Evaluation of water adsorption, mechanical and optical properties. International Journal of Biological Macromolecules. 117 (2018) 235-246.

[10] E. D. S. Costa-Júnior, M. M. Pereira, H. S. Mansur. Properties and biocompatibility of chitosan films modified by blending with PVA and chemically crosslinked. Journal of Materials Science Materials in Medicine. 20 (2009) 553-561.

[11] R. Jayasekara, I. Harding, I. Bowater, G. B. Y. Christie, G. T. Lonergan. Preparation, surface modification and characterisation of solution cast starch PVA blended films. Polymer Testing. 23 (2004) 17-27.

[12] A. Cano, E. Fortunati, M. Cháfer, J. M. Kenny, A. Chiralt, C. González-Martínez. Properties and ageing behaviour of pea starch films as affected by blend with poly(vinyl alcohol). Food Hydrocolloids. 48 (2015) 84-93.

[13] A. Jiménez, M. J. Fabra, P. Talens, A. Chiralt. Edible and biodegradable starch films: a review. Food and Bioprocess Technology. 5 (2012) 2058-2076.

[14] S. Acosta, A. Jiménez, M. Cháfer, C. González-Martínez, A. Chiralt. Physical properties and stability of starch-gelatin based films as affected by the addition of esters of fatty acids. Food Hydrocolloids. 49 (2015) 135-143.

532 [15] D. Piñeros-Hernandez, C. Medina-Jaramillo, A. López-Córdoba, S. Goyanes.

533 Edible cassava starch films carrying rosemary antioxidant extracts for potential use as

534 active food packaging. Food Hydrocolloids. 63 (2017) 488-495.

535 [16] H. Abral, M. H. Dalimunthe, J. Hartono, R. P. Efendi. Characterization of tapioca

536 starch biopolymer composites reinforced with micro scale water hyacinth fibers. Starch

537 - Strke. 70 (2018) 1700287.

538 [17] B. Priya, V. K. Gupta, D. Pathania, A. S. Singha. Synthesis, characterization and

539 antibacterial activity of biodegradable starch/PVA composite films reinforced with

540 cellulosic fibre. Carbohydrate Polymers. 109 (2014) 171-179.

541 [18] S. Momeni, E. R. Ghomi, M. Shakiba, S. Shafiei-Navid, M. Abdouss, A. Bigham,

542 F. Khosravi, Z. Ahmadi, M. Faraji, H. Abdouss, S. ramakrishna. The Effect of poly

543 (ethylene glycol) emulation on the degradation of PLA/starch composites. Polymers.

544 13 (2021) 1019.

545 [19] P. Zhou, Y. Luo, Z. Lv, X. Sun, Y. Tian, X. Zhang. Melt-processed poly (vinyl

546 alcohol)/corn starch/nanocellulose composites with improved mechanical properties.

547 International Journal of Biological Macromolecules. 183 (2021) 1903-1910.

548 [20] Tanwar R, Gupta V, Kumar P, Kumar A, Singh S, Gaikwad KK. Development and

549 characterization of PVA-starch incorporated with coconut shell extract and sepiolite

550 clay as an antioxidant film for active food packaging applications. International Journal

551 of Biological Macromolecules. 185 (2021) 451-461.

552 [21] Z. W. Abdullah, Y. Dong, I. J. Davies, S. Barbhuiya. PVA, PVA Blends, and Their

553 Nanocomposites for Biodegradable Packaging Application. Polymer-Plastics

554   Technology and Engineering. 56 (2017) 1307-1344.

555   [22] P. Appendini, J. H. Hotchkiss. Review of antimicrobial food packaging. Innovative

556   Food Science and Emerging Technologies. 3 (2002) 113-126.

557   [23] D. Chung, S. E. Papadakis, K. L. Yam. Release of propyl paraben from a polymer

558   coating into water and food simulating solvents for antimicrobial packaging

559   applications. Journal of Food Processing and Preservation. 25 (2001) 71-87.

560   [24] C. Vasile, M. Baican. Progresses in food packaging, food quality, and safety—

561   controlled-release antioxidant and/or antimicrobial packaging. Molecules. 26 (2021)

562   1263.

563   [25] J. F. Rubilar, D. Candia, A. Cobos, O. Díaz, F. Pedreschi. Effect of nanoclay and

564   ethyl-Nα-dodecanoyl-l-arginate hydrochloride (LAE) on physico-mechanical

565   properties of chitosan films. LWT Food Science and Technology. 72 (2016) 206-214.

566   [26] L. Higueras, G. López-Carballo, P. Hernández-Muñoz, R. Gavara, M. Rollini.

567   Development of a novel antimicrobial film based on chitosan with LAE (ethyl-Nα-

568   dodecanoyl-l-arginate) and its application to fresh chicken. International Journal of

569   Food Microbiology. 165 (2013) 339-345.

570   [27] R. Becerril, S. Manso, C. Nerin, R. Gómez-Lus. Antimicrobial activity of Lauroyl

571   Arginate Ethyl (LAE), against selected food-borne bacteria. Food Control. 32 (2013)

572   404-408.

573   [28] C. Nerin, R. Becerril, S. Manso, F. Silva. Chapter 23 - Ethyl lauroyl arginate (LAE):

574   Antimicrobial activity and applications in food systems, Antimicrobial Food Packaging.

575   305 (2016) 305-312.

576 [29] E. Rodríguez, J. Seguer, X. Rocabayera, A. Manresa. Cellular effects of

577 monohydrochloride of L-arginine, N-lauroyl ethylester (LAE) on exposure to

578 Salmonella typhimurium and Staphylococcus aureus. Journal of Applied Microbiology.

579 96 (2004) 903-912.

580 [30] O. Moreno, J. Cárdenas, L. Atarés, A. Chiralt. Influence of starch oxidation on the

581 functionality of starch-gelatin based active films. Carbohydrate Polymers. 178 (2017)

582 147-158.

583 [31] V. Muriel-Galet, G. López-Carballo, P. Hernández-Muñoz, R. Gavara.

584 Characterization of ethylene-vinyl alcohol copolymer containing lauril arginate (LAE)

585 as material for active antimicrobial food packaging. Food Packaging and Shelf Life. 1

586 (2014) 10-18.

587 [32] H. Haghighi, S. K. Leugoue, F. Pfeifer, H. W. Siesler, F. Licciardello, P. Fava, A.

588 Pulvirenti. Development of antimicrobial films based on chitosan-polyvinyl alcohol

589 blend enriched with ethyl lauroyl arginate (LAE) for food packaging applications. Food

590 Hydrocolloids. 100 (2020) 105419.

591 [33] G. Saldaña, E. Puértolas, S. Monfort, J. Raso, I. Álvarez. Defining treatment

592 conditions for pulsed electric field pasteurization of apple juice. International journal

593 of food microbiology. 151 (2011) 29-35.

594 [34] N. H. Woodcock, B. H. Hammond, R. D. Ralyea, K. J. Boor. Short communication:

595 Nalpha-lauroyl-L-arginine ethylester monohydrochloride reduces bacterial growth in

596 pasteurized milk. Journal of Dairy Science. 92 (2009) 4207-4210.

597 [35] Q. Ma, P. M. Davidson, Q. Zhong. Antimicrobial properties of lauric arginate alone

598   or in combination with essential oils in tryptic soy broth and 2% reduced fat milk.

599   International Journal of Food Microbiology. 166 (2013) 77-84.

600   [36] D. V. T. Nair, R. Nannapaneni, A. Kiess, B. Mahmoud, C. S. Sharma. Antimicrobial

601   efficacy of lauric arginate against Campylobacter jejuni and spoilage organisms on

602   chicken breast fillets. Poultry Science. 93 (2014) 2636-2640.

603   [37] T. Li, Y. Liu, Q. Qin, L. Zhao, Y. Wang, X. Wu, X. Liao. Development of

604   electrospun films enriched with ethyl lauroyl arginate as novel antimicrobial food

605   packaging materials for fresh strawberry preservation. Food Control. 130 (2021)

606   108371.

607   [38] R. F. M. J. Cleven, C. V. D. Berg, L. V. D. Plas. Crystal Structure of Hydrated

608   Potato Starch. Starch - Starke. 30 (1978) 223-228.

609   [39] H. Haghighi, R. D. Leo, E. Bedin, F. Pfeifer, H. W. Siesler, A. Pulvirenti.

610   Comparative analysis of blend and bilayer films based on chitosan and gelatin enriched

611   with LAE (lauroyl arginate ethyl) with antimicrobial activity for food packaging

612   applications. Food Packaging and Shelf Life. 19 (2019) 31-39

613   [40] P. Liu, R. Wang, X. Kang, B. Cui, B. Yu. Effects of ultrasonic treatment on

614   amylose-lipid complex formation and properties of sweet potato starch-based films.

615   Ultrasonics Sonochemistry. 44 (2018) 215-222.

616   [41] H. Abral, A. Basri, F. Muhammad, Y. Fernando, F. Hafizulhaq, M. Mahardika, E.

617   Sugiarti, S. M. Sapuan, R. A. Ilyas, I. Stephane. A simple method for improving the

618   properties of the sago starch films prepared by using ultrasonication treatment. Food

619   Hydrocolloids. 93 (2019) 276-283.

[42] K. K. Gaikwad, S. M. Lee, J. S. Lee, Y. S. Lee. Development of antimicrobial polyolefin films containing lauroyl arginate and their use in the packaging of strawberries. Journal of Food Measurement and Characterization. 11 (2017) 1706-1716.

[43] R. Lin, Y. Fan, T. Liu, H. Yang, L. Ma, X. Huang, Y. Liu. Structural Characterization of Controlled Decrystallization of Cassava Starch. Starch - Starke. 72 (2019) 1900049.

[44] J. F. Rubilar, D. Candia, A. Cobos, O. Díaz, F. Pedreschi. Effect of nanoclay and ethyl-Nα-dodecanoyl-l-arginate hydrochloride (LAE) on physico-mechanical properties of chitosan films. LWT Food Science and Technology. 72 (2016) 206-214.

[45] O. Moreno, R. Díaz, L. Atarés, A. Chiralt. Influence of the processing method and antimicrobial agents on properties of starch-gelatin biodegradable films. Polymer International. 65 (2016) 905-914.

[46] M. Majzoobi, Z. Kaveh, A. Farahnaky. Effect of acetic acid on physical properties of pregelatinized wheat and corn starch gels. Food Chemistry. 196 (2016) 720-725.

[47] F. Wu, Z. Zhou, M. Liang, L. Zhong, F. Xie. Ultrasonication improves the structures and physicochemical properties of cassava starch films containing acetic acid. Starch - Starke. 73 (2020) 2000094

[48] J. F. G. Motta, A. R. D. Souza, S. M. Gonçalves, D. K. S. F. Madella, C. W. P. D. Carvalho, L. Vitorazi, N. R. D. Melo. Development of active films based on modified starches incorporating the antimicrobial agent lauroyl arginate (LAE) for the food industry. Food and Bioprocess Technology. 13 (2020) 2082-2093.

[49] S. Hajji, A. Chaker, M. Jridi, H. Maalej, K. Jellouli, S. Boufi, M. Nasri. Structural

analysis, and antioxidant and antibacterial properties of chitosan-poly (vinyl alcohol) biodegradable films. Environmental Science and Pollution Research. 23 (2016) 15310-15320.

[50] J. Wu, X. Sun, X. Guo, Q. Zhang. Physicochemical properties, antimicrobial activity and oil release of fish gelatin films incorporated with cinnamon essential oil - ScienceDirect. Aquaculture and Fisheries. 2 (2017) 185-192.

[51] R. Y. Aguirre-Loredo, A. I. Rodríguez-Hernández, E. Morales-Sánchez, C. A. Gómez-Aldapa. Effect of equilibrium moisture content on barrier, mechanical and thermal properties of chitosan films. Food Chemistry. 196 (2016) 560-566.

[52] S. Tripathi, G. K. Mehrotra, P. K. Dutta. Physicochemical and bioactivity of cross-linked chitosan-PVA film for food packaging applications. International Journal of Biological Macromolecules. 45 (2009) 372-376.

[53] Z. Ziaee, H. Xiao, Y. Guan, P. Fatehi. Coating PHGH-modified starch on papers to induce antimicrobial properties. BioResources. 9 (2014) 3632-3641.

[54] Y. Zhang, X. Liu, Y. Wang, P. Jiang, S. Quek. Antibacterial activity and mechanism of cinnamon essential oil against Escherichia coli and Staphylococcus aureus. Food Control. 59 (2016) 282-289.

[55] L. S. Liu, V. L. Finkenstadt, C. Liu, T. Jin. Preparation of poly(lactic acid) and pectin composite films intended for applications in antimicrobial packaging. Journal of Applied Polymer Science. 106 (2007) 801-810.

[56] I. Leceta, P. Guerrero, I. Ibarburu, M. T. Dueñas, K. D. L. Caba. Characterization and antimicrobial analysis of chitosan-based films. Journal of Food Engineering. 116

664   (2013) 889-899.