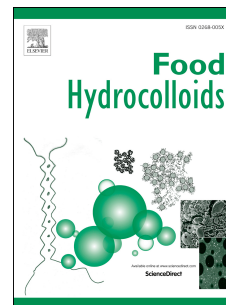


Journal Pre-proof

Development of antimicrobial films based on chitosan-polyvinyl alcohol blend enriched with ethyl lauroyl arginate (LAE) for food packaging applications

Hossein Haghghi, Serge Kameni Leugoue, Frank Pfeifer, Heinz Wilhelm Siesler, Fabio Licciardello, Patrizia Fava, Andrea Pulvirenti



PII: S0268-005X(19)31741-2

DOI: <https://doi.org/10.1016/j.foodhyd.2019.105419>

Reference: FOOHYD 105419

To appear in: *Food Hydrocolloids*

Received Date: 31 July 2019

Revised Date: 2 October 2019

Accepted Date: 2 October 2019

Please cite this article as: Haghghi, H., Leugoue, S.K., Pfeifer, F., Siesler, H.W., Licciardello, F., Fava, P., Pulvirenti, A., Development of antimicrobial films based on chitosan-polyvinyl alcohol blend enriched with ethyl lauroyl arginate (LAE) for food packaging applications, *Food Hydrocolloids* (2019), doi: <https://doi.org/10.1016/j.foodhyd.2019.105419>.

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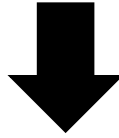


Chitosan (CS)
(1% w/v)

+

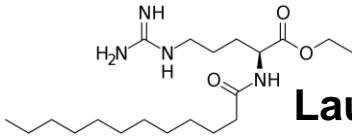


Polyvinyl Alcohol (PVA)
(5% w/v)

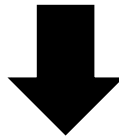


CS-PVA Blend FFS
(1:1)

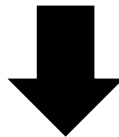
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Lauroyl Arginate Ethyl (LAE)
(1, 2.5, 5 and 10 % w/w)

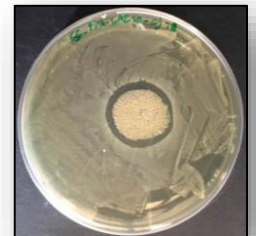
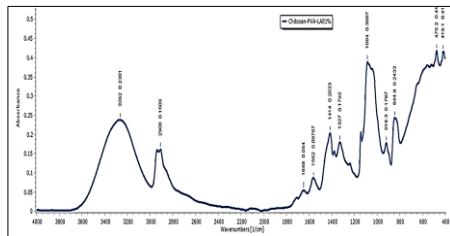
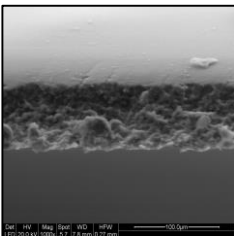


CS-PVA-LAE Film



Characterization for Food Packaging Applications

(Microstructure, FT-IR, Thickness, Mechanical, Optical, Water Barrier and Antimicrobial Properties)



1 **Development of antimicrobial films based on chitosan-polyvinyl alcohol blend**
2 **enriched with ethyl lauroyl arginate (LAE) for food packaging applications**

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12 **Abstract**

13 The main aim of this study was to characterize microstructural, physical, optical, mechanical,
14 water barrier and antimicrobial properties of chitosan-polyvinyl alcohol blend films (CS-PVA)
15 enriched with ethyl lauroyl arginate (LAE) (1-10% w/v) for food packaging applications. The
16 film microstructure was determined by scanning electron microscopy. Active films containing
17 10% LAE showed cracks on the surface with irregular shape in the cross-section indicating a
18 weaker cohesion of the CS-PVA polymer blend at high LAE concentrations. The possible
19 interaction of CS-PVA blend film with incorporated LAE was also investigated using Fourier-
20 transform infrared (FT-IR) spectroscopy in the attenuated total reflection (ATR) mode. FT-
21 IR/ATR spectra showed a low molecular interaction between the CS-PVA and LAE up to
22 2.5% while for films containing 5 and 10% LAE such interactions between the functional
23 groups of the CS-PVA matrix and LAE have been detected. The active films were
24 transparent and showed barrier properties against UV and visible light. The incorporation of
25 LAE into the CS-PVA increased the thickness, water solubility, water vapor permeability, and
26 the b^* and ΔE^* values, while it decreased mechanical properties and transparency ($p < 0.05$).
27 Active films inhibited the growth of four major food bacterial pathogens including
28 *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella typhimurium*.

29 Particularly, films containing 5 and 10% LAE were the most effective ($p < 0.05$). Overall, the
30 characterization of functional properties revealed that CS-PVA blend film incorporated with
31 LAE could be used as an environmentally friendly antimicrobial packaging material to extend
32 the shelf life of food products.

33 **Keywords:** Active food packaging, ATR/FT-IR, Bio-based packaging, Ethyl lauroyl arginate
34 (LAE)

35 1. Introduction

36 The current trend in food packaging is mainly oriented towards the substitution of non-
37 biodegradable petroleum-based polymers by packaging materials that are eco-friendly and
38 also prolong food shelf life (Kanatt, Rao, Chawla, & Sharma, 2012). In this context,
39 considerable research has been conducted involving the fabrication of biodegradable food
40 packaging materials that come from renewable natural resources and agri-food industry
41 wastes (Cazón, Vázquez, & Velazquez, 2018; Sarwar, Niazi, Jahan, Ahmad, & Hussain,
42 2018). Among bio-based natural polymers, chitosan (CS) has received significant attention
43 for its potential to substitute - partially or totally - petroleum-based polymers (Leceta,
44 Guerrero, & De La Caba, 2013). CS is a cationic linear polysaccharide consisting of poly- β -
45 (1-4)-D-glucosamine units obtained by partial deacetylation of chitin, the major component of
46 the insect's exoskeleton and shells of crustacean such as crab, shrimp, and crawfish. CS is
47 the second most abundant polysaccharide after cellulose with unique biological properties
48 such as biocompatibility, biodegradability and non-toxicity. In addition, this amino
49 polysaccharide has high antimicrobial activity against many pathogenic and spoilage
50 microorganisms, including both Gram-positive and Gram-negative bacteria which makes it an
51 excellent candidate for food packaging applications (Rubilar, Candia, Cobos, Díaz, &
52 Pedreschi, 2016). However, there are some limitations which are associated with CS such as
53 low mechanical strength, low thermal stability, rigid crystalline structure and high production
54 cost. A simple and effective alternative to overcome these drawbacks could be blending of
55 CS with synthetic polymers. Films formed by the blending of natural and synthetic polymers
56 represent a new class of material with modified physical and mechanical properties

57 compared to films made of individual components. Blending CS with polyvinyl alcohol (PVA)
58 has been intensively investigated by many researchers to gain biodegradable and
59 antimicrobial films for food packaging applications with new and desired properties (Bonilla,
60 Fortunati, Atarés, Chiralt, & Kenny, 2014; Kanatt et al., 2012; Liu, Wang, & Lan, 2018;
61 Tripathi, Mehrotra, & Dutta, 2009; Parida, Nayak, Binhani, & Nayak, 2011).

62 PVA is a synthetic, low cost, non-toxic and water-soluble polymer commercially obtainable
63 from hydrolysis of polyvinyl acetate with excellent film forming properties. Despite its
64 synthetic character, this polymer was recognized as biodegradable and it shows high tensile
65 strength, flexibility, gas barrier properties and good resistance to acid and alkali media (Aloui
66 et al., 2016). PVA has been evaluated for safety by the Joint FAO/WHO Expert Committee
67 on Food Additives (JECFA) in 2003 at the 61st meeting (Bellelli, Licciardello, Pulvirenti, &
68 Fava, 2018) and it has also been approved for use in packaging meat and poultry products
69 by the USDA (Kanatt et al., 2012). PVA is highly miscible with other hydrophilic polymers
70 such as CS, owing to the formation of intermolecular hydrogen bonds between hydroxyl
71 groups of PVA and hydroxyl and amine groups of CS. Due to the high compatibility of CS
72 and PVA, the resulting films show homogeneous structure. Moreover, blending PVA with CS
73 is a promising strategy to reduce the production cost and improve the mechanical property
74 and stability of CS films.

75 Antimicrobial packaging as a part of active packaging systems is intended to extend the shelf
76 life of food products and assure the safety and quality of packaged foods (Tripathi, Mehrotra,
77 & Dutta, 2008). Ethyl lauroyl arginate (LAE) is considered as an effective antimicrobial
78 substance among novel food additives (Rubilar et al., 2016). LAE remains stable at pH 3-7
79 and is odorless and colorless as well (Kashiri et al., 2016). LAE is a synthetic surfactant
80 consisting of an ethyl esterified arginine head with a lauroyl tail attached to the α -amino
81 group that is highly active against a wide range of food pathogens and spoilage
82 microorganisms including bacteria, yeast and molds with a low-dose application (Becerril,
83 Manso, Nerin, & Gómez-Lus, 2013). This cationic surfactant disrupts the cytoplasmic
84 membrane of microorganisms and inhibits the growth of microorganisms by causing cell

85 deformation and affecting their metabolic process negatively (Muriel-Galet, Carballo,
86 Hernández-Muñoz, & Gavara, 2016). LAE has been considered as GRAS (generally
87 recognized as safe) by the U.S. Food and Drug Administration (FDA, 2005) and has been
88 authorized as food preservative by the European Food Safety Authority (EFSA, 2007).
89 Incorporation of LAE as an antimicrobial compound into antimicrobial packaging to improve
90 food safety and quality has been reported in several studies (De Leo et al., 2018; Haghghi et
91 al., 2019a; Higuera, López-Carballo, Hernández-Muñoz, Gavara, & Rollini, 2013; Kashiri et
92 al., 2016; Moreno, Cárdenas, Atarés, & Chiralt, 2017a; Rubilar et al., 2016). However,
93 literature concerning the effects of LAE on the functional properties of CS-PVA blend film is
94 not available. Therefore, the objective of the present study was to develop biodegradable
95 films based on CS-PVA blend enriched with different concentrations of LAE to evaluate
96 microstructural, physical, optical, mechanical and water barrier properties for food packaging
97 applications. Moreover, the antimicrobial activity of films against four common food bacterial
98 pathogens including *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes* and
99 *Salmonella typhimurium*, was investigated.

100 **2. Material and methods**

101 **2.1 Materials and reagents**

102 Chitosan (CS) with a molecular weight of 100-300 kDa was obtained from Acros Organics™
103 (China). Polyvinyl alcohol (PVA) with molecular weight of 27 kDa and 98% degree of
104 hydrolysis was purchased from Fluka (Steinheim, Germany). Glycerol ($\geq 99.5\%$) was
105 purchased from Merck (Darmstadt, Germany). Acetic acid ($\geq 99.5\%$) was obtained from
106 Brenntag S.p.A (Milan, Italy). Ethyl lauroyl arginate (LAE) was kindly provided as Mirenat-D
107 (69.3% LAE, 30.7% maltodextrin) by Vedeqsa Grupo LAMIRSA (Terrassa, Barcelona,
108 Spain). Brain heart infusion agar (BHIA) was purchased from Biolife (Milan, Italy).

109 **2.2. Preparation of film-forming solutions (FFS) and films**

110 Preparation of films was adapted from the procedures of Higuera et al., (2013) and Kanatt
111 et al., (2012) with slight modification. In this study, four CS-PVA blend films enriched with
112 different concentrations of LAE (1, 2.5, 5 and 10% w/w of biopolymer) were analyzed.

113 Furthermore, a CS-PVA film-forming solution (FFS) without LAE was used to produce a
114 control film. The CS FFS (1%, w/w) was prepared by dissolving CS in an acetic acid solution
115 (1% v/v) under continuous stirring at 55°C for 60 min. The PVA FFS (5% w/w) was prepared
116 by dissolving PVA in distilled water at 80°C for 60 min. Glycerol (0.5% v/v of FFS) was then
117 added as a plasticizer into both FFSs, followed by additional stirring for 60 min. The CS-PVA
118 blend FFS was prepared by mixing CS and PVA FFSs at 1:1 ratio and final plasticizer
119 content was 17g glycerol/ 100 g dry polymer. Different amounts of LAE were added to the
120 CS-PVA blend FFS to obtain active films with 1-10% LAE (g of LAE/100 g of dry CS-PVA
121 blend) considering the LAE concentration (69.3%) in Mirenat-D. All FFSs were degasified
122 with a vacuum pump (70 kPa) for 15 min to remove bubbles. Films were obtained by casting
123 20 mL of the FFS into Petri dishes (14.4 cm in diameter) and drying at 25±2 °C in the
124 chemical hood overnight.

125 **2.3. Scanning electron microscopy (SEM)**

126 The SEM images from the surface and cross-section of the films were obtained with the use
127 of a scanning electron microscope (FEI, Quanta 200, Oregon, USA). Film samples were
128 fixed on a stainless-steel support with a double-sided conductive adhesive. The analysis was
129 conducted in low vacuum (0.6 Torr) at an acceleration voltage of 20 kV.

130 **2.4. Atomic force microscopy (AFM)**

131 The surface morphology of the films was analyzed using an atomic force microscope (Park
132 Scientific Instruments, South Korea). Films were fixed onto AFM specimen metal discs using
133 a double-sided tape and then placed to a magnetic sample holder located on the top of the
134 scanner tube. The images were scanned in no-contact mode under ambient condition. The
135 surface roughness (R_a) of the films was calculated on the basis of the root mean square (R_q)
136 deviation from the average height of peaks after subtracting the background using ProScan
137 software (version 1.51b). All samples were analyzed in triplicate.

138 **2.5 Attenuated Total Reflection (ATR) / Fourier-Transform Infrared (FT-IR)** 139 **Spectroscopy**

140 The infrared spectra of different films were obtained using an ATR/FT-IR spectrometer (type
141 Alpha, Bruker Optik GmbH, Ettlingen, Germany). Spectra were collected from two different
142 locations from the top and bottom side of the same samples in the 4000-400 cm^{-1}
143 wavenumber range by accumulating 64 scans with a spectral resolution of 4 cm^{-1} .

144 **2.6. Thickness and mechanical properties**

145 Film thickness was measured with a digital micrometer (Model IP65, SAMA Tools, Viareggio,
146 Italia) at five different random positions (one at the center and four at the edges). The means
147 of these five separate measurements were recorded.

148 The tensile strength (TS), elongation at break (E%) and elastic modulus (EM) were
149 determined using a dynamometer (Z1.0, Zwick/Roell, Ulm, Germany) according to ASTM
150 standard method D882 (ASTM, 2001a). The films with known thickness were cut into
151 rectangular strips (9.0 x 1.5 cm^2). Initial grip separation and cross-head speed were set at 70
152 mm and 50 mm/min, respectively. Measurements were repeated 10 times from each type of
153 film. The software TestXpert® II (V3.31) (Zwick/Roell, Ulm, Germany) was used to record the
154 TS curves. TS was calculated by dividing the maximum load to break the film by the cross-
155 sectional area of the film and expressed in MPa. The E% was calculated by dividing film
156 elongation at rupture by the initial grip separation expressed in percentage (%). EM was
157 calculated from the initial slope of the stress-strain curve and expressed in MPa.

158 **2.7. UV barrier, light transmittance, opacity value and color**

159 The barrier properties of films against UV and visible light were determined at the UV (200,
160 280 and 350 nm) and visible (400, 500, 600, 700 and 800 nm) wavelengths. These optical
161 characteristics were estimated with a VWR®Double Beam UV-VIS 6300PC
162 spectrophotometer (China) using square film samples (2 x 2 cm^2). The opacity of the films
163 was calculated by Eq. (1):

$$164 \text{ Opacity value} = \frac{-\log T_{600}}{x} \quad (1)$$

165 where T_{600} is the fractional transmittance at 600 nm and x is the film thickness (mm). The
166 greater opacity value represents the lower transparency of the film. For each film, four
167 readings were taken at different positions and average values were determined.

168 The color of films was measured with a CR-400 Minolta colorimeter (Minolta Camera, Co.,
 169 Ltd., Osaka, Japan) at room temperature, with D65 illuminant and 10° observer angle. The
 170 instrument was calibrated with a white standard ($L^* = 99.36$, $a^* = -0.12$, $b^* = -0.07$) before
 171 measurements. Results were expressed as L^* (lightness), a^* (red/green) and b^* (yellow/blue)
 172 parameters. The total color difference (ΔE^*) was calculated using the following Eq. (2):

$$173 \quad \Delta E^* = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]} \quad (2)$$

174 where ΔL^* , Δa^* and Δb^* are the differences between the corresponding color parameter of the
 175 samples and that of a standard white plate used as the film background. For each film, five
 176 readings were taken at different positions and the average values were determined from the
 177 top and bottom sides.

178 **2.8. Moisture content and water solubility**

179 Moisture content (MC) of the films ($2 \times 2 \text{ cm}^2$) was determined as the percentage of weight
 180 loss upon drying to constant weight (M_d) in an oven at $105 \pm 2 \text{ }^\circ\text{C}$ and the initial weight (M_w)
 181 according to the following Eq. (3):

$$182 \quad \text{MC (\%)} = \frac{M_w - M_d}{M_w} \times 100 \quad (3)$$

183 The solubility of films ($2 \times 2 \text{ cm}^2$) in water was determined by drying to constant weight in an
 184 air-circulating oven at $105 \pm 2 \text{ }^\circ\text{C}$ (W_i) and then each film was immersed in 50 mL distilled
 185 water at $25 \text{ }^\circ\text{C}$ following Gontard, Guilbert, & Cuq, (1992) with slight modifications. After 24 h,
 186 the film samples were dripped and dried to constant weight at $105 \pm 2 \text{ }^\circ\text{C}$ (W_f) to determine
 187 the weight of dry matter which was not solubilized in water. The measurement of water
 188 solubility (WS) was determined according to the following Eq. (4):

$$189 \quad \text{WS (\%)} = \frac{W_i - W_f}{W_i} \times 100 \quad (4)$$

190 All measurements for MC and WS were made in triplicate.

191 **2.9. Water vapor transmission rate and water vapor permeability**

192 Water vapor transmission rate (WVTR) of the films was determined gravimetrically in
 193 triplicate according to the ASTM E96 method (ASTM, 2001b) with some modifications. Films
 194 were sealed on top of glass test cups with an internal diameter of 10 mm and a depth of 55

195 mm filled with 2 g anhydrous CaCl_2 (0% RH). The cups were placed in desiccators containing
196 BaCl_2 (90% RH), which were maintained in incubators at 45 °C. WVTR was determined
197 using the weight gain of the cups and was recorded and plotted as a function of time. Cups
198 were weighed daily for 7 days to guarantee the steady state permeation. The slope of the
199 mass gain versus time was obtained by linear regression ($r^2 \geq 0.99$). WVTR (g /day m^2) and
200 WVP (g mm/kPa day m^2) were calculated according to the following Eqs. (5) and (6):

$$201 \quad \text{WVTR} = \frac{\Delta W}{\Delta t \times A} \quad (5)$$

$$202 \quad \text{WVP} = \frac{\text{WVTR} \times L}{\Delta P} \quad (6)$$

203 where $\Delta W/\Delta t$ is the weight gain as a function of time (g/day), A is the area of the exposed
204 film surface (m^2), L is the mean film thickness (mm) and ΔP is the difference of vapor
205 pressure across the film (kPa).

206 **2.10. In vitro antimicrobial activity**

207 **2.10.1. Disk diffusion assay**

208 Antibacterial activity test on films was assessed against four typical food bacterial pathogens
209 including *Listeria monocytogenes* (UNIMORE 19115), *Escherichia coli* (UNIMORE 40522),
210 *Salmonella typhimurium* (UNIMORE 14028) and *Campylobacter jejuni* (UNIMORE 33250)
211 using the disk diffusion assay according to (Haghighi et al., 2019). Films (sterilized with UV
212 light) were cut into a disc shape of 22 mm diameter and placed on the surface of BHIA agar
213 plates, which had been previously streaked with 0.1 mL of inocula containing 10^6 CFU/mL of
214 tested bacteria. The plates were then incubated at 30 °C for 24 h (*C. jejuni* plates were
215 incubated at 37 °C). The diameter of the inhibition zones was measured with a caliper and
216 recorded in millimeters (mm). All tests were performed in triplicates.

217 **2.10.2. Evaluation of antimicrobial activity in liquid medium**

218 Antimicrobial activity of CS-PVA films enriched with LAE (1-10%) evaluated against *L.*
219 *monocytogenes*, *E. coli*, *S. typhimurium* and *C. jejuni* in liquid medium (Kashiri et al., 2016).
220 A loop of each strain was transferred to 10 mL of BHIB and incubated at 30°C (*C. jejuni*
221 plates were incubated at 37 °C) for 24 h to obtain stationary phase (optical density of 0.9 at
222 600 nm). Then, cells were diluted in BHIB and incubated at 30 °C (*C. jejuni* tube was

223 incubated at 37 °C) to obtain exponential phase (optical density of 0.2 at 600 nm). One
224 hundred μL of microorganism in exponential phase was inoculated into tubes with 10 mL of
225 BHIB. A 0.025 g portion of film ($1.5 \times 1.5 \text{ cm}^2$) was added to each tube in sterile conditions.
226 The tubes were incubated at 30°C for 24 h. As a control, CS-PVA film without LAE was used.
227 Depending on the turbidity of each tubes, serial dilutions with NaCL were made and plated
228 on the Petri dishes with BHIA culture medium. Colonies were counted after incubation at 30
229 °C for 24 h.

230 **2.10. Statistical analysis**

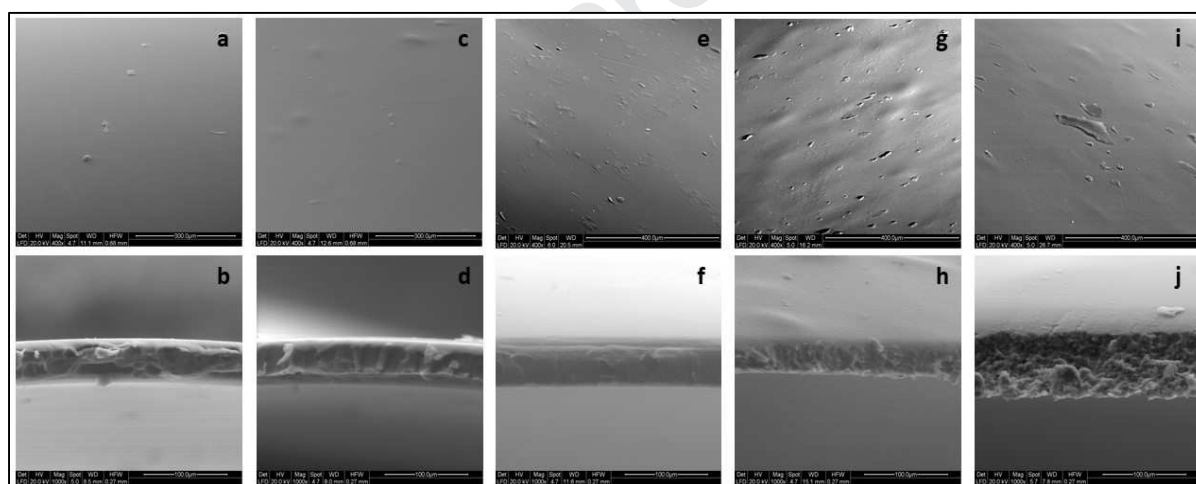
231 The statistical analysis of the data was performed through analysis of variance (ANOVA)
232 using SPSS statistical program (SPSS 20 for Windows, SPSS INC., IBM, New York). The
233 experiment was performed in 3 replicates and the number of repeats varied from one
234 analysis to another and was reported in each subsection. The differences between means
235 were evaluated by Tukey's multiple range test ($p < 0.05$). The data were expressed as the
236 mean \pm SD (standard deviation).

237 **3. Results and discussion**

238 **3.1. Scanning electron microscopy (SEM)**

239 The surface and cross-section images of CS-PVA blend film (control) and CS-PVA film
240 enriched with different concentrations of LAE (1-10%) (active films) are presented in Fig. 1.
241 The film microstructure greatly affects the final physical, mechanical and barrier properties.
242 This is mainly due to the interaction between the film components and LAE. The surface of
243 the control film was smooth and homogenous and did not show pores or cracks indicating
244 good compatibility between CS and PVA to form a blend (Fig. 1a). This could be explained
245 by strong molecular interaction between functional groups of chitosan and PVA. Similar
246 results were reported by Ghaderi, Hosseini, & Gómez-Guillén (2019) and Jahan, Mathad, &
247 Farheen (2016) who noticed that the surface of CS-PVA blend film was homogenous without
248 pores. Addition of LAE up to 1% did not affect the surface morphology of active films (Fig. 1c)
249 indicating LAE was evenly distributed and well dispersed in the film matrix. Small particles
250 and aggregations were observed at the surface of films with increasing concentration of LAE

251 up to 10% (Fig. 1e, 1g and 1i). A compact and continuous structure without irregularities like
 252 air bubbles and pores and any evidence of phase separation can be observed in the cross-
 253 section of the control film (Fig. 1b). The cross-section of active films containing LAE up to
 254 2.5% showed similar results (Fig. 1d and 1f). However, active films containing 5 and 10%
 255 LAE showed irregular and sponge shape structure (Fig. 1h and 1j). This effect was more
 256 obvious in CS-PVA film containing 10% LAE. This might be related to the agglomeration of
 257 LAE in the film matrix at high concentrations which resulted in disrupted structures. The
 258 interaction between the polymer chains was disturbed by interactions with functional groups
 259 of LAE, producing films with less integrity. Gaikwad, Lee, Lee, & Lee (2017) also reported
 260 that the order of low-density polyethylene (LDPE) films was interrupted by the addition of
 261 high amount of LAE powder (5 and 10%) mainly due to the inhomogeneous distribution of
 262 LAE inside the matrix and the low interfacial interaction between the LDPE and LAE powder.



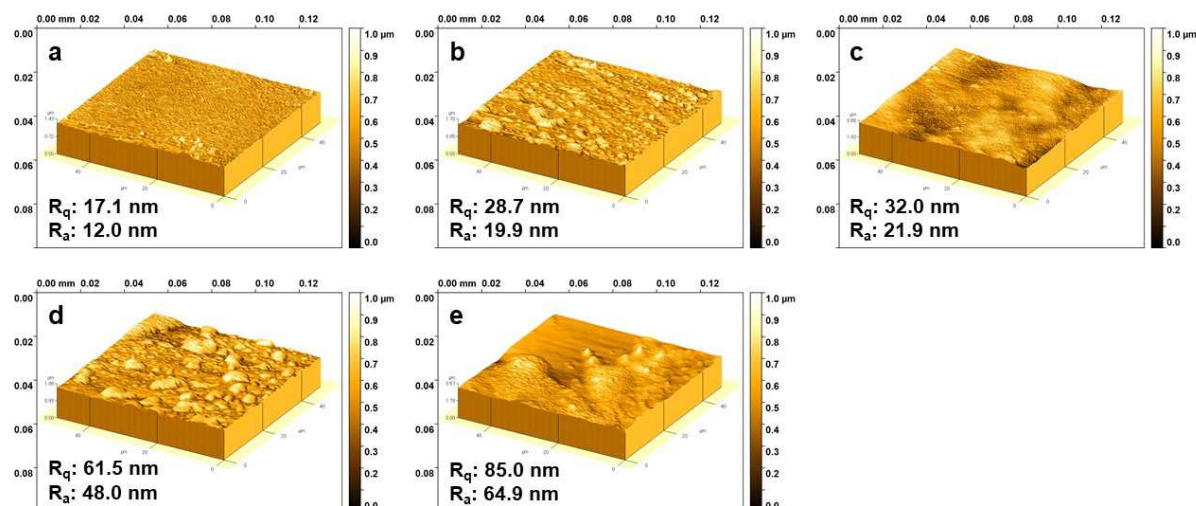
263
 264 **Fig. 1.** Scanning electron microscopy (SEM) images of the surface and cross-section of films
 265 based on a control CS-PVA blend (CS-PVA) (a and b), CS-PVA-LAE1% (c and d), CS-PVA-
 266 LAE2.5% (e and f), CS-PVA-LAE5% (g and h) and CS-PVA-LAE10% (i and j).
 267

268 **3.2. Atomic force microscopy (AFM)**

269 AFM was further performed to characterize the surface morphology of control and active
 270 films. Typical 3D surface topographic AFM images and root mean square (R_q) and
 271 roughness (R_a) values are presented in Fig. 2. The surface of control film was relatively
 272 smooth and homogenous as indicated by lower R_q and R_a values (17.1 and 12.0 nm,
 273 respectively). Increasing concentration of LAE up to 10% led to the increase in the
 274 roughness of the films, as indicated by higher R_q and R_a values. The difference in roughness

275 value between control and active films was in accordance with the film microstructure
 276 observed by SEM analysis.

277



278
 279 **Fig. 2.** 3D AFM images, root mean square (R_q) and roughness (R_a) of films based on a:
 280 control CS-PVA blend, b: CS-PVA-LAE 1%, c: CS-PVA-LAE 2.5%, d: CS-PVA-LAE 5% and
 281 e: CS-PVA-LAE 10%.

282 3.3. Attenuated Total Reflection (ATR) / Fourier-Transform Infrared (FT-IR)

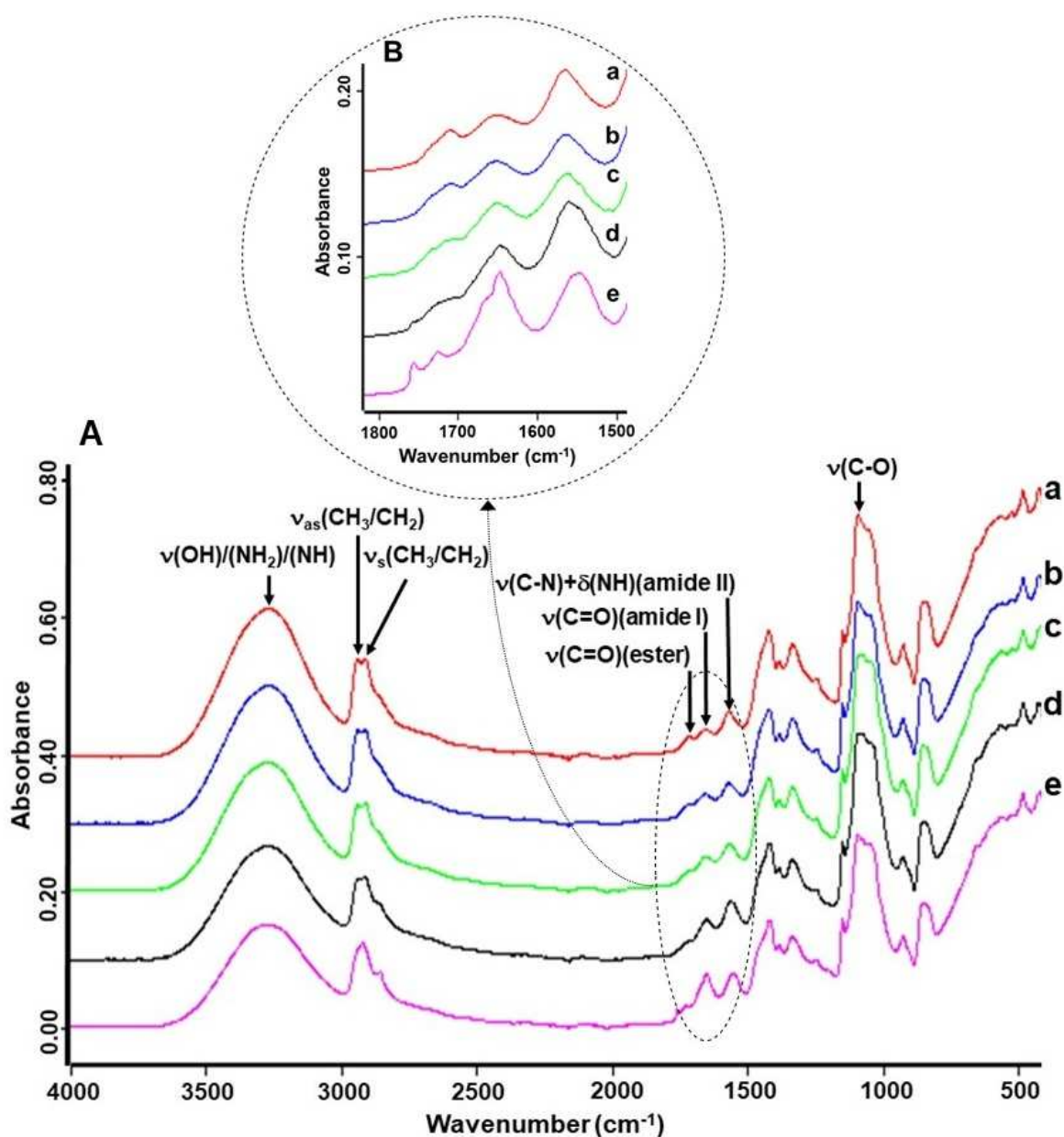
284 Spectroscopy

285 ATR/FT-IR spectroscopy was performed to characterize the structural and spectroscopic
 286 changes due to the incorporation of different amounts of LAE (1-10% w/w) into the CS-PVA
 287 film matrix by measuring the spectra in the wavenumber range of 4000-400 cm^{-1} at a
 288 spectral resolution of 4 cm^{-1} . The spectrum of the control CS-PVA blend (Fig. 3A, a) revealed
 289 the characteristic bands of the two polymer components (CS and PVA) with relative
 290 intensities according to the respective composition. Under the broad and intense absorption
 291 band with the maximum at about 3270 cm^{-1} , the $\nu(\text{OH})$, $\nu(\text{NH})$ and $\nu_{\text{as}}(\text{NH}_2)/\nu_{\text{s}}(\text{NH}_2)$
 292 absorption bands of these inter- and intramolecularly hydrogen bonded functionality of the
 293 CS and PVA blend components are superimposed (Costa-junior, Pereira, & Mansur, 2009;
 294 Kumar, Krishnakumar, Sobral, & Koh, 2019). The neighboring band doublet at about
 295 2937/2908 cm^{-1} can be assigned to antisymmetric and symmetric $\nu_{\text{as}}(\text{CH}_2)/\nu_{\text{s}}(\text{CH}_2)$ and
 296 $\nu(\text{CH})$ stretching vibrations of the corresponding polymer chain functionalities (Liu et al.,
 297 2018). Characteristic absorption bands for CS can be assigned at 1646 cm^{-1} (amide-I) due to

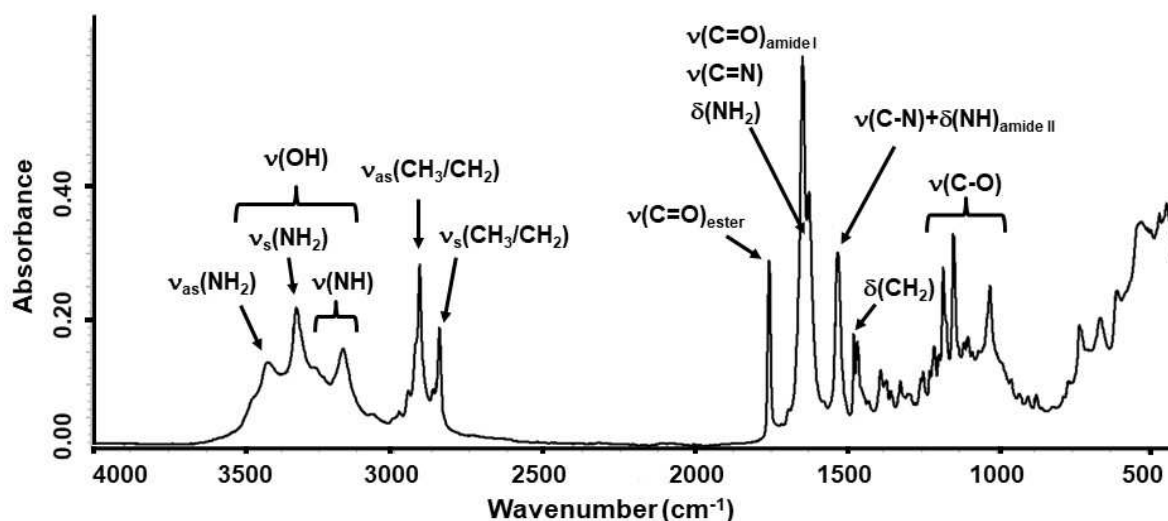
298 the $\nu(\text{C}=\text{O})$ stretching vibration, and at 1561 cm^{-1} (amide II) to a combination band of the
299 $\nu(\text{C}-\text{N})$ stretching and $\delta(\text{N}-\text{H})$ bending vibrations (Costa-junior et al., 2009). Because in this
300 study a PVA with only 98% degree of hydrolysis was used, 2% of acetate groups remained
301 non-hydrolyzed during the manufacturing process (Koosha & Mirzadeh, 2015). Thus, the
302 band at 1708 cm^{-1} belongs to the $\nu(\text{C}=\text{O})$ stretching vibration of residual vinyl acetate units in
303 the PVA backbone (Costa-junior et al., 2009). The very sharp, crystallinity-sensitive band of
304 PVA at 1140 cm^{-1} is also observed in the CS-PVA blend (Kim, Kim, Lee, & Kim, 1992;
305 Tripathi et al., 2009). Furthermore, absorption bands at 1413 cm^{-1} ($\delta(\text{CH}_2)$ bending
306 vibration), between 1085 and 1045 cm^{-1} ($\nu(\text{C}-\text{O})$ stretching vibrations), and 917 cm^{-1} (CH_2
307 rocking vibration) can be observed in the ATR/FT-IR spectrum of the CS-PVA blend
308 (Pavaloiu, Stoica-Guzun, Stroescu, Jinga, & Dobre, 2014; Pereira Jr, de Arruda, & Stefani,
309 2015). For a better visualization and understanding of the – although minor – spectral
310 changes in the wavenumber ranges $2800 - 3000\text{ cm}^{-1}$ and specifically $1500 - 1800\text{ cm}^{-1}$ (see
311 Fig. 3B) upon addition of LAE to the CS-PVA blends, the spectrum of pure Mirenat-D is
312 reproduced in Fig.4. The most important absorption bands of the spectral range between
313 1000 and 3500 cm^{-1} can be readily assigned to the different vibrations of the chemical
314 building blocks of this additive.

315 Up to 2.5% LAE content, the characteristic absorption bands in the ATR/FT-IR spectra of the
316 CS-PVA blends (Fig. 3A, b and c) are wavenumber invariant and do not show intensity
317 changes, thereby suggesting low molecular interaction between the polymer and the LAE.
318 Similar results were reported by Gaikwad, Lee, Lee, & Lee (2017); Rubilar et al. (2016) and
319 Kashiri et al. (2016). However, CS-PVA blend films containing 5% and 10% LAE (Fig. 3A, d
320 and e) revealed new absorption bands at 2918 cm^{-1} and 2851 cm^{-1} ($\nu_{\text{as}}(\text{CH}_2)/\nu_{\text{s}}(\text{CH}_2)$), 1755
321 cm^{-1} ($\nu(\text{C}=\text{O})$), 1724 cm^{-1} ($\nu(\text{C}=\text{O})$) and $1661/1645\text{ cm}^{-1}$ ($\nu(\text{C}=\text{O})$, $\nu(\text{C}=\text{N})$, $\delta(\text{NH}_2)$) originating
322 from the introduction of new CH_2 -segments, ester, amide, NH_2 and imine groups (Gamarra,
323 Missagia, Urpí, Morató, & Muñoz-Guerra, 2018; Moreno et al., 2017a). The evolution of the
324 band doublet ($1661/1645\text{ cm}^{-1}$) from the original 1646 cm^{-1} band and the shift of the 1561 cm^{-1}
325 cm^{-1} band to 1557 cm^{-1} (LAE 5%) and to 1544 cm^{-1} (LAE 10%), respectively (Fig. 3B, d and e), is

326 a clear evidence, that at elevated LAE content the C=O, NH₂ and NH functionalities of this
 327 additive contribute to competitive intermolecular interactions with the hydroxyl, amino, ether
 328 and residual acetate groups of the CS-PVA film network. Furthermore, LAE can also promote
 329 the formation of C=N groups, by reacting with both, residual acetate carbonyls of PVA and
 330 CS amino groups, as revealed by the relative intensity of the peak at 1645 cm⁻¹ for LAE 10%
 331 (Fig 3B, e). Thus, the carbonyl-amino reaction to form C=N groups was enhanced by the
 332 presence of LAE (Moreno et al., 2017a).



333
 334 **Fig. 3.** ATR/FT-IR spectra (A) of films based on a: control CS-PVA blend, b: CS-PVA-LAE
 335 1%, c: CS-PVA-LAE 2.5%, d: CS-PVA-LAE 5% films and e: CS-PVA-LAE 10%. In (B) the
 336 enlarged 1800-1500 cm⁻¹ region is shown to highlight the spectral changes at elevated LAE
 337 content.



338
339 **Fig. 4.** ATR/FT-IR spectra of LAE formulation (Mirenat-D).
340

341 **3.4. Thickness**

342 The thickness value is a crucial parameter for determining final physical, mechanical and
343 barrier properties of biodegradable films. The thickness values of control and active films are
344 reported in Tab. 1. Thickness values ranged from 44.81 to 59.82 μm . The control film
345 showed the lowest value ($p < 0.05$). This is due to the well-organized and dense network
346 structure in the CS-PVA blend film as confirmed by SEM images. The incorporation of LAE
347 into the CS-PVA blend increased the thickness ($p < 0.05$) and the CS-PVA film enriched with
348 10% LAE showed the highest thickness value ($p < 0.05$). In this study, all films were prepared
349 by casting a constant amount of FFS in Petri dishes with the same surface ratio, therefore
350 differences in thickness between control and active films are due to the addition of LAE to the
351 FFS. According to Gaikwad et al. (2017), film thickness is influenced by the solid content of
352 the FFS. Thus, LAE might contribute to loosen film matrix, reduce the homogeneity, and
353 consequently increase the thickness. In contrast, Rubilar et al., (2016) reported that addition
354 of LAE to CS films did not influence the thickness. It is noteworthy that the type of LAE
355 applied in different studies as a powder (Mirenat-D or Mirenat-P) or dissolved in glycerol
356 (Mirenat-G) strongly affects film thickness.

357 **3.5. Mechanical properties**

358 The tensile strength (TS), elongation at break (E%) and elastic modulus (EM) are three
359 important parameters for evaluation of mechanical properties. Generally, adequate
360 mechanical strength and extensibility are required for the development of biodegradable films
361 for food packaging applications. The mechanical properties of control and active films are
362 presented in Tab. 1. The control film showed the highest TS, E% and EM values. The
363 presence of LAE greatly influenced TS and E% ($p < 0.05$). Films containing LAE were less
364 resistant and less stretchable than the control film ($p < 0.05$). Incorporation of LAE up to 10%
365 decreased the tensile strength from 42.48 to 15.70 MPa and E% from 54.25 to 14.31%. The
366 significant deterioration of mechanical properties above 2.5% incorporation of LAE (Tab. 1) is
367 also consistent with the ATR/FT-IR observation, that band shifts and intensity changes occur
368 above this threshold concentration. This tendency could be explained by the fact that active
369 films containing a high concentration of LAE are unable to form a cohesive and continuous
370 matrix as it was confirmed by SEM analysis. This can be attributed to the competitive
371 interaction of the functional groups of LAE and the CS-PVA blend that limit cohesion forces
372 within the polymer in the film matrix and consequently decrease the degree of physical
373 crosslinking by weakening the intermolecular hydrogen bonding, thereby resulting in the
374 reduction of mechanical properties. Despite the reduction of TS after incorporation of LAE, it
375 should be noted that the TS values for the active films containing LAE up to 2.5% were
376 comparable with those of plastic films that are used widely in the market, such as high
377 density polyethylene (22-31 MPa) and polypropylene (31-38 MPa) but slightly lower than that
378 for polystyrene (45-83 MPa) (Theinsathid, Visessanguan, Krueenate, Kingcha, & Keeratipibul,
379 2012). Moreno, Díaz, Atarés, & Chiralt (2016) also reported that the incorporation of LAE into
380 starch-gelatin blend film notably reduced the stiffness and resistance to break compared to
381 the control film. In contrast to these results, Rubilar et al. (2016) reported that the
382 incorporation of LAE (1g/L) to CS films significantly increased TS and E% values ($p < 0.05$)
383 which might be due to the application of Mirenat-G (10% LAE, 90% glycerol) as LAE source.
384 Hence the observed effect could be mainly due to the plasticizing effect of glycerol. Literature
385 data regarding mechanical properties are controversial and are influenced by multiple factors

386 such as the molecular mass of the polymer, deacetylation degree of CS, degree of hydrolysis
 387 of PVA, pH of the FFS, drying conditions and type of LAE.

388 **Table 1**

389 Thickness, tensile strength (TS), elongation at break (E%) and elastic modulus (EM) of the
 390 films based on control CS-PVA blend (CS-PVA) and CS-PVA enriched with LAE (1-10%
 391 w/w).

Film sample	Thickness (μm)	TS (MPa)	E (%)	EM (MPa)
CS-PVA	44.8 ± 1.0^a	42.5 ± 1.6^d	54.2 ± 2.0^d	1570.1 ± 139.0^d
CS-PVA-LAE 1%	45.1 ± 0.5^a	33.0 ± 2.4^c	38.9 ± 3.1^c	1153.1 ± 121.1^{bc}
CS-PVA-LAE 2.5%	49.9 ± 1.6^b	34.5 ± 4.2^c	39.0 ± 2.6^c	1287.3 ± 77.8^c
CS-PVA-LAE 5%	54.9 ± 2.7^c	22.2 ± 0.9^b	25.0 ± 2.8^b	1016.9 ± 90.8^b
CS-PVA-LAE 10%	59.6 ± 0.8^d	15.7 ± 1.2^a	14.3 ± 1.9^a	701.3 ± 53.8^a

392 Values are given as mean \pm SD (n = 3).

393 Different letters in the same column indicate significant differences ($p < 0.05$).

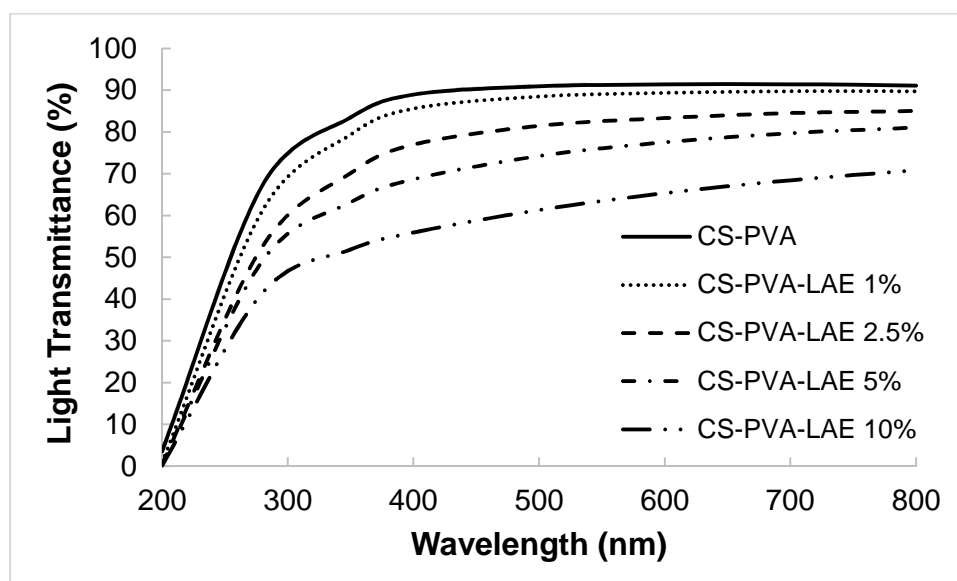
394 **3.6. UV barrier, light transmittance and opacity value**

395 Protecting food from the effect of UV-Vis radiation is one of the desired characteristics of
 396 packaging material due to their influence on product performance and consumer acceptance.

397 The UV-Vis light transmittance of control and active films in the wavelength range of 200–
 398 800 nm is presented in Fig. 5. Control film showed a higher UV light transmittance (200–350
 399 nm) compared to the active films. UV light transmittance was reduced at increasing LAE
 400 concentration and active films behaved as effective UV barriers at 200 nm since the
 401 transmittance value was below 1%. UV barrier property of films is an important parameter for
 402 food packaging applications to minimize UV-induced lipid oxidation, to preserve the
 403 organoleptic properties of the packaged food, to avoid nutrient losses, discoloration and off-
 404 flavors, thereby prolonging food shelf life (Hajji et al., 2016; Wu, Sun, Guo, Ge, & Zhang,
 405 2017).

406 The transmission of visible light (400–800 nm) was higher than 80% for the control film. The
 407 active films containing 1 and 2.5% LAE showed similar results while active films containing 5
 408 and 10% LAE showed lower values. Thus, once a critical LAE concentration is exceeded,
 409 aggregates are formed, that were large enough to scatter the light and thereby interfere with

410 its transmission (Bonnaud, Weiss, & McClements, 2010). The light barrier property is also an
 411 important factor for food preservation to avoid photo-oxidation of organic compounds and
 412 degradation of vitamins and other pigments. In addition, it provides a clear view of the food
 413 content and its condition (Figuroa-Lopez, Andrade-Mahecha, & Torres-Vargas, 2018;
 414 Yadav & Chiu, 2019). In this study, all films can be considered as transparent due to the
 415 opacity value lower than 5 at 600 nm (Tab. 2). The higher value of this parameter represents
 416 the lower transparency of the film.



417
 418 **Fig. 5.** UV-Vis light transmittance of the films based on a control CS-PVA blend (CS-PVA)
 419 and CS-PVA blends enriched with LAE (1-10% w/w).
 420

421 3.7. Color

422 The color values (L^* , a^* and b^*) and total color difference (ΔE^*) of control and active films are
 423 shown in Tab. 2. The L^* , a^* , b^* offer objective evaluation of the appearance of films while
 424 ΔE^* measures the color change of treatment from a reference color. Color is an important
 425 feature of a film for food packaging applications since it evaluates the visual characteristic of
 426 the food product inside the packaging system and affects consumer purchase decision. The
 427 L^* value indicates lightness and represents the apparent proportion of incident light reflected
 428 by an object, varying between 98.22 and 98.99, which means that all the films were almost
 429 clear. No significant differences ($p > 0.05$) for L^* between the control and active films were
 430 found. These results were in agreement with opacity values that showed all films were clear
 431 and transparent. The a^* value, expressing the green-red color component, was negative for

432 all films, which means that films were not truly red (Virginia Muriel-Galet, López-Carballo,
 433 Hernández-Muñoz, & Gavara, 2014). The b^* value measures the blue-yellow color
 434 component. This value increased upon addition of LAE ($p < 0.05$), suggesting a gain of slight
 435 yellow color. ΔE^* was used to compare the color of active films with commercial plastic films
 436 (perceptibility threshold of $\Delta E^* = 1$). The selected value is often used as the smallest color
 437 difference that the human eye can detect (Thakhiew, Devahastin, & Soponronnarit, 2013).
 438 Control film had ΔE^* value of 0.91. The ΔE^* increased upon addition of LAE ($p < 0.05$) and
 439 reached a value of 1.70 in the active film containing 10% LAE. This behavior might be
 440 attributed to the increase in the colorimetric coordinate b^* and increase in film thickness upon
 441 addition of LAE. In summary, the consumer might not be able to detect color difference in
 442 active films, despite the slightly higher values than the threshold. A similar result has been
 443 reported by Rubilar et al. (2016).

444 **Table 2**

445 Color parameters (L^* , a^* and b^*), total color difference (ΔE^*) and opacity values of the films
 446 based on a control CS-PVA blend (CS-PVA) and CS-PVA enriched with LAE (1-10% w/w).

Film sample	Color parameters				Opacity value (600 nm)
	L^*	a^*	b^*	ΔE^*	
CS-PVA	99.0 ± 0.2^a	-0.3 ± 0.03^c	0.7 ± 0.1^a	0.91 ± 0.2^a	0.9 ± 0.01^a
CS-PVA-LAE 1%	98.8 ± 0.1^a	-0.3 ± 0.01^c	0.7 ± 0.1^a	0.99 ± 0.1^a	1.1 ± 0.08^{ab}
CS-PVA-LAE 2.5%	98.8 ± 0.1^a	-0.4 ± 0.02^b	1.0 ± 0.1^b	1.22 ± 0.1^b	1.6 ± 0.31^{bc}
CS-PVA-LAE 5%	98.6 ± 0.1^a	-0.4 ± 0.03^a	1.3 ± 0.2^c	1.63 ± 0.2^c	2.0 ± 0.13^c
CS-PVA-LAE 10%	98.7 ± 0.2^a	-0.5 ± 0.02^a	1.4 ± 0.1^c	1.70 ± 0.1^c	3.1 ± 0.27^d

447 Values are given as mean \pm SD ($n = 3$).

448 Different letters in the same column indicate significant differences ($p < 0.05$).

449 **3.8. Moisture content, water solubility, water vapor transmission rate and water** 450 **vapor permeability**

451 One of the major drawbacks of biodegradable films for food packaging applications is their
 452 sensitivity to water. Due to the hydrophilic nature of CS and PVA, when these films are
 453 exposed to high relative humidity conditions, water molecules are absorbed by the polymeric
 454 chains, exerting a plasticizing effect and resulting in changes of the mechanical and barrier

455 properties (Aguirre-Loredo, Rodríguez-Hernández, Morales-Sánchez, Gómez-Aldapa, &
 456 Velazquez, 2016). Therefore, measuring water sensitivity plays an important role in the
 457 packaging performance for the food products. The moisture content (MC), water solubility
 458 (WS), water vapor transmission rate (WVTR) and water vapor permeability (WVP) of control
 459 and active films are presented in Tab. 3.

460 The MC value ranged from 16.42 to 17.53%. The effect of LAE incorporation on the MC was
 461 not significant ($p < 0.05$). Moreno, Gil, Atarés, & Chiralt (2017b) reported that the enrichment
 462 of starch-gelatin films with different LAE concentrations did not influence the MC. Solubility is
 463 defined as the content of dry matter solubilized after 24 h immersion in water. The control
 464 film showed the lowest WS value and addition of LAE increased the WS value. CS-PVA film
 465 containing 10% LAE showed the highest WS value ($p < 0.05$). The higher solubility values of
 466 active films could be explained by the hydrophilic nature of CS-PVA blend film and low oil-
 467 water equilibrium partition coefficient of LAE ($K_{ow} < 0.1$), which means that LAE has a high
 468 affinity to water molecules (Higuera et al., 2013; Rubilar et al., 2016).

469 The barrier properties of biodegradable films to water play an important role in determining
 470 the shelf life of packed foodstuffs (Del Nobile, Fava, & Piergiovanni, 2002). In this study, the
 471 WVTR was not significantly influenced by the addition of LAE, despite showing higher values
 472 in active films ($p > 0.05$). However, the incorporation of LAE to CS-PVA blend film increased
 473 the WVP value. This might be due to the difference in each film thickness considered in WVP
 474 calculation (Rubilar et al., 2016). Additionally, incorporation of LAE into the CS-PVA film
 475 network may break hydrogen bonding and disrupt the long-range ordering between CS and
 476 PVA molecules, resulting in an increase in WVP value (Ma, Zhang, & Zhong, 2016).

477 **Table 3**

478 Moisture content (MC), water solubility (WS), water vapor transmission rate (WVTR) and
 479 water vapor permeability (WVP) of the films based on a control CS-PVA blend (CS-PVA) and
 480 CS-PVA enriched with LAE (1-10% w/w).

Film sample	MC (%)	WS (%)	WVTR (g /day m ²)	WVP 90:0% RH (g mm/kP day m ²)
CS-PVA	16.4 ± 0.5 ^a	22.1 ± 0.4 ^a	3605.6 ± 450.8 ^a	18 ± 0.02 ^a
CS-PVA-LAE 1%	16.7 ± 0.9 ^a	23.7 ± 2.8 ^{ab}	3608.7 ± 362.1 ^a	19 ± 0.02 ^a

CS-PVA-LAE 2.5%	16.8 ± 0.7 ^a	25.4 ± 3.4 ^{ab}	3729.5 ± 369.3 ^a	21 ± 0.02 ^{ab}
CS-PVA-LAE 5%	17.1 ± 0.6 ^a	27.8 ± 2.1 ^b	3742.1 ± 341.5 ^a	24 ± 0.02 ^b
CS-PVA-LAE 10%	17.5 ± 0.6 ^a	33.2 ± 2.1 ^c	3808.9 ± 337.7 ^a	26 ± 0.02 ^c

481 Values are given as mean ± SD (n = 3).

482 Different letters in the same column indicate significant differences (p<0.05).

483 **3.9. In vitro antimicrobial activity**

484 **3.9.1. Disk diffusion assay**

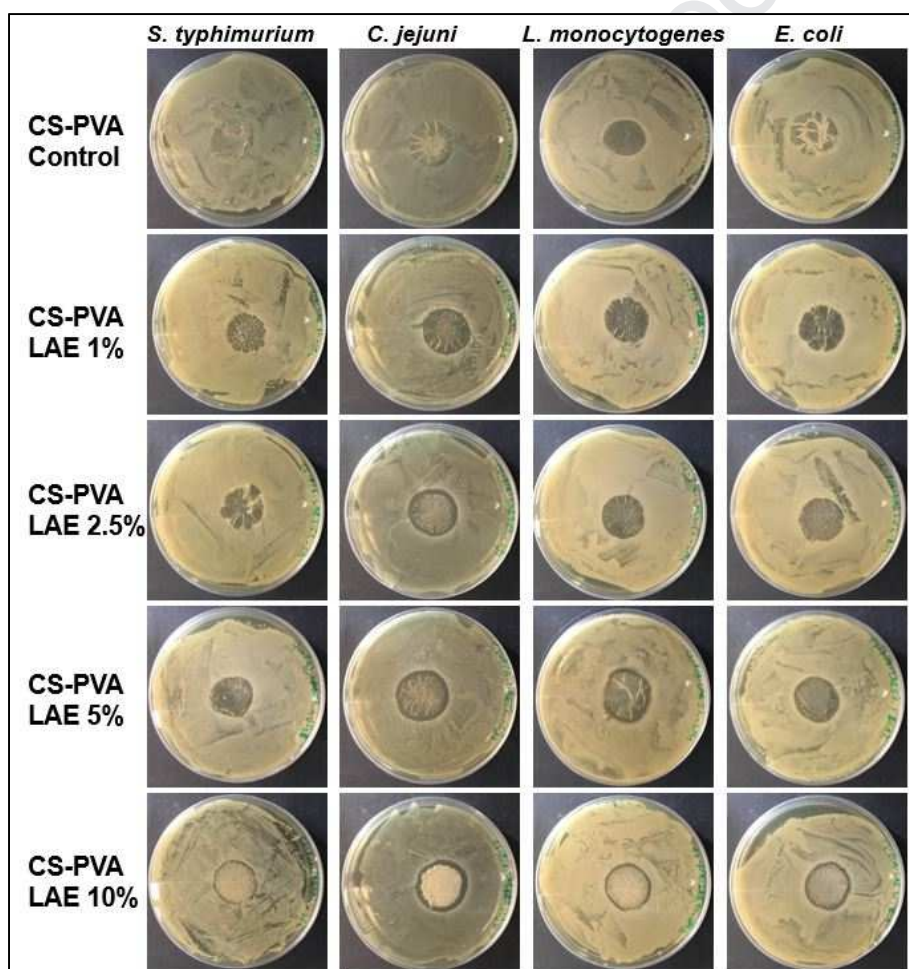
485 The antimicrobial activity of control and active films against common bacterial food
 486 pathogens, namely *C. jejuni*, *E. coli*, *L. monocytogenes* and *S. typhimurium*, was evaluated
 487 by the disk diffusion assay (Fig. 6) and the details are presented in Tab. 4. The control film
 488 did not show an inhibition zone against any of the tested microorganisms. The absence of
 489 inhibition zone could be explained by the limitation of CS to diffuse in agar medium (Leceta,
 490 Guerrero, Ibarburu, Dueñas, & De La Caba, 2013) and incapability of PVA to inhibit bacterial
 491 growth as it has been reported by other authors (Hajji et al., 2016; Tripathi et al., 2009), so
 492 that only microorganisms in direct contact with the active sites of CS in the CS-PVA film
 493 network are inhibited. Active films containing 1% LAE were only effective against *C. jejuni*. In
 494 general, LAE was more effective against *C. jejuni* compared to the other microorganisms
 495 considered, which showed inhibition haloes ranging from 3 to 5-fold wider. No differences
 496 were observed in the inhibition zones produced by CS-PVA films incorporating 5 and 10% of
 497 LAE against all tested microorganisms. Similar results reported by Muriel-Galet, López-
 498 Carballo, Gavara, & Hernández-Muñoz (2015). Pattanayaiying, H-Kittikun, & Cutter (2015)
 499 also found that incorporation of LAE into pullulan film inhibited the growth of foodborne
 500 pathogens such as *Salmonella spp.*, *L. monocytogenes* and *E. coli*. The antimicrobial activity
 501 of LAE is attributed to its action as a cationic surfactant on the cytoplasmic membranes of
 502 microorganisms, causing a disturbance in membrane potential and resulting in cell growth
 503 inhibition and loss of viability (Kashiri et al., 2016; Muriel-Galet, López-Carballo, Gavara, &
 504 Hernández-Muñoz, 2012).

505 **Table 4**

506 Inhibition zone diameters of the film disks (22 mm diameter) based on a control CS-PVA
 507 blend (CS-PVA) and CS-PVA enriched with LAE (1-10% w/w).

Film sample	<i>L. monocytogenes</i>	<i>E. coli</i>	<i>C. jejuni</i>	<i>S. typhimurium</i>
CS-PVA	N. D.	N. D.	N. D.	N. D.
CS-PVA-LAE 1%	N. D.	N. D.	1.8 ± 1.0 ^{aA}	N. D.
CS-PVA-LAE 2.5%	0.5 ± 0.5 ^{aA}	0.6 ± 0.3 ^{aA}	3.7 ± 1.5 ^{bB}	0.6 ± 0.7 ^{aA}
CS-PVA-LAE 5%	1.3 ± 0.7 ^{abA}	1.2 ± 0.9 ^{abA}	4.6 ± 1.8 ^{bB}	1.7 ± 1.1 ^{abA}
CS-PVA-LAE 10%	1.6 ± 0.9 ^{bA}	1.6 ± 1.0 ^{bA}	5.2 ± 1.4 ^{bB}	1.8 ± 1.8 ^{aA}

508 Values are given as mean ± SD (n = 3). N.D: not detected.
 509 Different lowercase letters in the same column indicate significant differences (p<0.05).
 510 Different capital letters in the same row indicate significant differences (p<0.05).
 511



512
 513 **Fig. 6.** Disk diffusion results of films based on a control CS-PVA blend (CS-PVA) and CS-
 514 PVA enriched with LAE (1-10% w/w).

515
 516 **3.9.2. Evaluation of antimicrobial activity in liquid medium**

517 The Antimicrobial activity of control and active films against common bacterial food
 518 pathogens including *C. jejuni*, *E. coli*, *L. monocytogenes* and *S. typhimurium* was also
 519 evaluated in liquid medium and the details are presented in Tab. 5. CS-PVA film without LAE

520 used as a control. Among tested microorganisms *C. jejuni* showed higher log reduction (2)
 521 for CS-PVA-LAE 10% films that is in agreement with the DDA result. The incorporation of
 522 LAE (1-10%) showed a log reduction against all tested microorganisms. Clearly, the higher
 523 the LAE concentration in the film, the greater the antimicrobial efficiency of the CS-PVA film.
 524 Similar result were reported by (V. Muriel-Galet et al., 2015) and (Kashiri et al., 2016).

525 **Table 5**

526 Antimicrobial activity of films based on a control CS-PVA blend (CS-PVA) and CS-PVA
 527 enriched with LAE (1- 10% w/w) expressed as logarithm of colony forming units (log CFU/
 528 mL) and log reduction value (LRV).

Film sample	<i>L. monocytogenes</i>		<i>E. coli</i>		<i>C. jejuni</i>		<i>S. typhimurium</i>	
	log (cfu/mL)	LRV	log (cfu/mL)	LRV	log (cfu/mL)	LRV	log (cfu/mL)	LRV
CS-PVA	9.2 ± 0.2 ^b		9.3 ± 0.1 ^c		9.4 ± 0.1 ^b		9.5 ± 0.1 ^c	
CS-PVA-LAE1%	9.2 ± 0.2 ^b	0	9.1 ± 0.1 ^{bc}	0.2	9.2 ± 0.1 ^b	0.2	9.4 ± 0.1 ^{bc}	0.1
CS-PVA-LAE2.5%	9.0 ± 0.2 ^b	0.2	9.0 ± 0.1 ^{bc}	0.3	9.1 ± 0.3 ^b	0.3	9.3 ± 0.1 ^{bc}	0.2
CS-PVA-LAE5%	8.9 ± 0.2 ^b	0.3	8.8 ± 0.1 ^b	0.5	8.8 ± 0.1 ^b	0.6	9.2 ± 0.1 ^{ab}	0.3
CS-PVA-LAE10%	7.7 ± 0.8 ^a	1.5	8.3 ± 0.4 ^a	1	7.4 ± 0.1 ^a	2	9.1 ± 0.1 ^a	0.4

529 Values are given as mean ± SD (n = 3).

530 Different lowercase letters in the same column indicate significant differences (p<0.05).

531

532 4. Conclusion

533 In this study, biodegradable active films based on CS-PVA blends enriched with LAE at
 534 different concentrations (1-10%, w/w) were developed and their microstructural, physical,
 535 optical, mechanical, barrier and antimicrobial properties were evaluated for food packaging
 536 applications. The results showed that all films containing LAE were transparent. The
 537 incorporation of LAE could improve the UV and light barrier properties of CS-PVA film, which
 538 may be useful to protect food from UV degradation and photo-oxidation. The characteristic
 539 absorption bands in the ATR/FT-IR spectra of the CS-PVA blends did not show significant
 540 band shifts and intensity changes up to 2.5% LAE content, indicating low interactions
 541 between the polymer and the LAE. However, at elevated LAE content the C=O, NH₂ and NH
 542 functionalities of this additive contribute to competitive molecular interactions with the

543 hydroxyl, amino, ether and residual acetate groups of the CS-PVA film network. The
544 presence of LAE greatly influenced TS and E%. Films with LAE were less resistant and less
545 stretchable than the control film and a significant deterioration of mechanical properties
546 occurred above 2.5% incorporation of LAE. The developed active films, especially those
547 including 5 and 10% LAE, were effective against common bacterial food pathogens. The
548 results suggest that the CS-PVA films enriched with different concentrations of LAE could be
549 considered as environmentally friendly packaging material with antimicrobial properties to
550 extend the shelf life of food products and that might be an alternative to synthetic plastics for
551 certain applications.

552 **Declaration of interest**

553 None.

554 **Acknowledgements**

555 The authors would like to thank Dr. Massimo Tonelli and Centro Interdipartimentale Grandi
556 Strumenti (CIGS) of the University of Modena and Reggio Emilia for assistance in the SEM
557 and AFM measurements.

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Highlights:

- LAE was effectively incorporated into chitosan-polyvinyl alcohol films
- High LAE levels negatively affected mechanical and water barrier properties
- Addition of LAE improved UV barrier of chitosan-polyvinyl alcohol blend films
- The developed active films were effective against four food bacterial pathogens

Journal Pre-proof

Conflicts of Interest Statement

Development of antimicrobial films based on chitosan-polyvinyl alcohol
Manuscript title: _____

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

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