Bioactive edible packaging for feed and food application

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State of the art

Edible films based on renewable natural biopolymers has become a hot topic in recent years in the food packaging industry. They have emerged as alternatives to synthetic films to reduce environmental pollutions due to their biodegradable and environmental friendly properties. Furthermore, they can improve food shelf life and quality by providing a barrier to reduce moisture loss and functioning as effective carriers of active compounds (i.e.: antimicrobial). In this context, the aim of the present work was to investigate mechanical, barrier and physical properties of edible film based on chitosan enriched with ethyl lauroyl arginate (LAE). Furthermore, antimicrobial efficacy was assessed against chicken fillets pathogenic bacteria.

Material and Methods

Chitosan solution (2%, w/V) was prepared by dissolving chitosan in acetic acid solution (1%, V/V) . Glycerol was added as plasticizer (0.45% w/w of chitosan). The antimicrobial compound ethyl lauroyl arginate (LAE) was provided as MIRENAT-G (1 mg/mL). Solution without LAE was prepared to use as control films. Films were prepared by casting 20 gr of film forming solution to petri dishes (14 cm of diameter) under controlled environmental conditions (24 h, 20 ± 2 °C and 40 ± 2% RH) [2].

Mechanical properties: The tensile stress (TS) and elongation at break percentage (EAB%) and elastic modulus (EM) of the films were determinate at room temperature (20 ± 2 °C and 40 ± 2% RH) using a dynamometer (Z1.0, Zwirk-Roell, Italy) according to ASTM standard method D882.

Water vapour transmission rate: The water vapor transmission rate (WVTR) test was performed using a modified E96-95 ASTM standard method at 40 °C ±2 and 80 ±2% RH. Film samples were sealed over the circular opening of a bottle containing $CaCl_2$. Bottles were placed inside the desiccator containing $BaCl_2$. The weight gain of the bottles were monitored every 24 h for 5 days. The slope of the linear regression of weight gain versus time was used for the analysis.

Light barrier properties: Film transmittance was measured by a V – 550 UV/Vis Spectrophotometer (Jascow Corporation, Tokyo, Japan). The absorbance measured in the range between 240-750 nm [2].

***In vivo* microbiological test:** Skinless chicken breast fillet was purchased from a local store at Reggio Emilia. Chicken filets (ca. 25 g and 60 cm^2 of surface) were wrapped with chitosan or chitosan-LAE films to achieve intimate contact between film and meat and externally wrapped with polyethylene film. A negative control sample for each time was also prepared without wrapping. Samples were stored at 4 °C. At appropriate time points (0, 1, 2, 3, 4 and 6 days), samples were transferred into a sterile stomacher bag, diluted with 225 mL sterile NaCl solution (0.9%) and homogenized in stomacher for 2 min. Ten-fold dilution series were made and plated on the brain heart infusion agar (BHIA). After 24 h of incubation at 30 °C, the bacterial colonies were counted to determine the CFU/g. All experiments were run in triplicate [1].

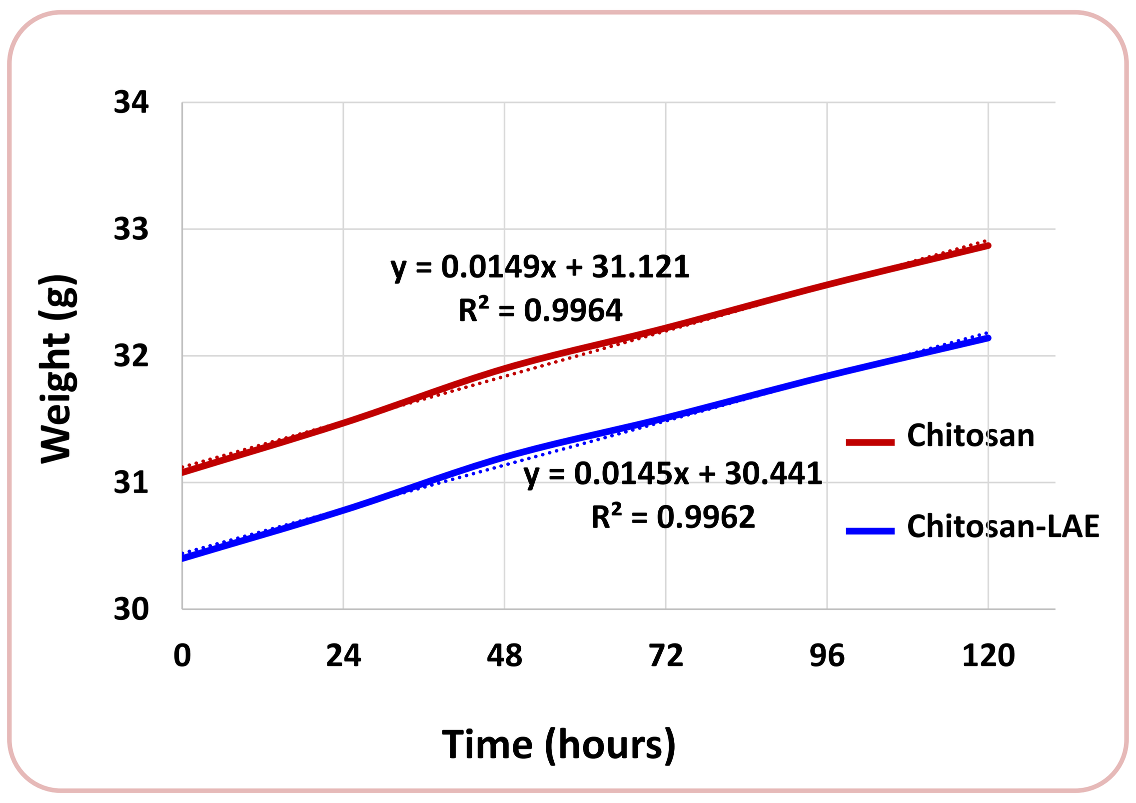
Mechanical properties

Table 1: Thickness, tensile strength (TS), elongation at break percentage (EAB%) and elastic modulus (EM) of chitosan and chitosan-LAE films equilibrated at 20 ± 2 °C and 40 ± 2% RH. Values are given as mean ± SD (n=3).

Film	Thickness (mm)	TS (MPa)	EAB%	EM (MPa)
Chitosan	0.031±0.004	27.89±2.45	29.82±2.61	597.58±46.19
Chitosan-LAE	0.034±0.006	22.77±2.01	32.60±2.89	544.44±34.24

The incorporation of LAE into the chitosan film led to a slight increase in thickness of the film. The tensile stress (TS) and elastic modulus of chitosan-LAE film decreased and the elongation at break percentage (EAB%) increased leading to a softer and more flexible film compared to the pure chitosan film. The molecular network in the films was destroyed by addition of LAE, which explains the drop in TS while increasing the volume of spaces between macromolecules chains and providing the greater extensibility.

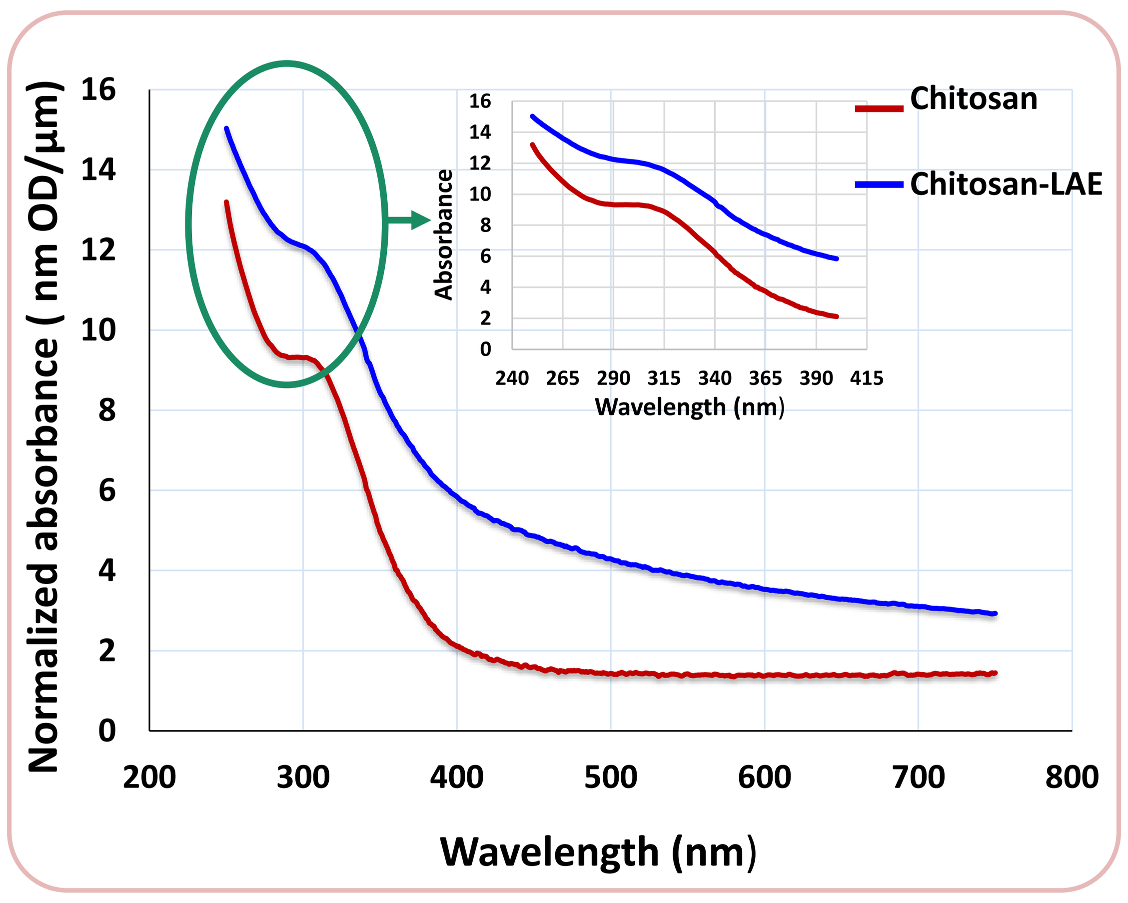
Water vapour transmission rate



Chitosan-LAE film showed lower water vapour transmission rate (WVTR) compare to the chitosan film. This could be due to the presence of the hydrophobic lauroyl group in LAE. Low WVTR of edible films is a desirable property with respect to the film usage and performance.

Figure 1: Water vapour transmission rate of the chitosan and chitosan-LAE films at 40 ± 2 °C and 80 ± 2% RH.

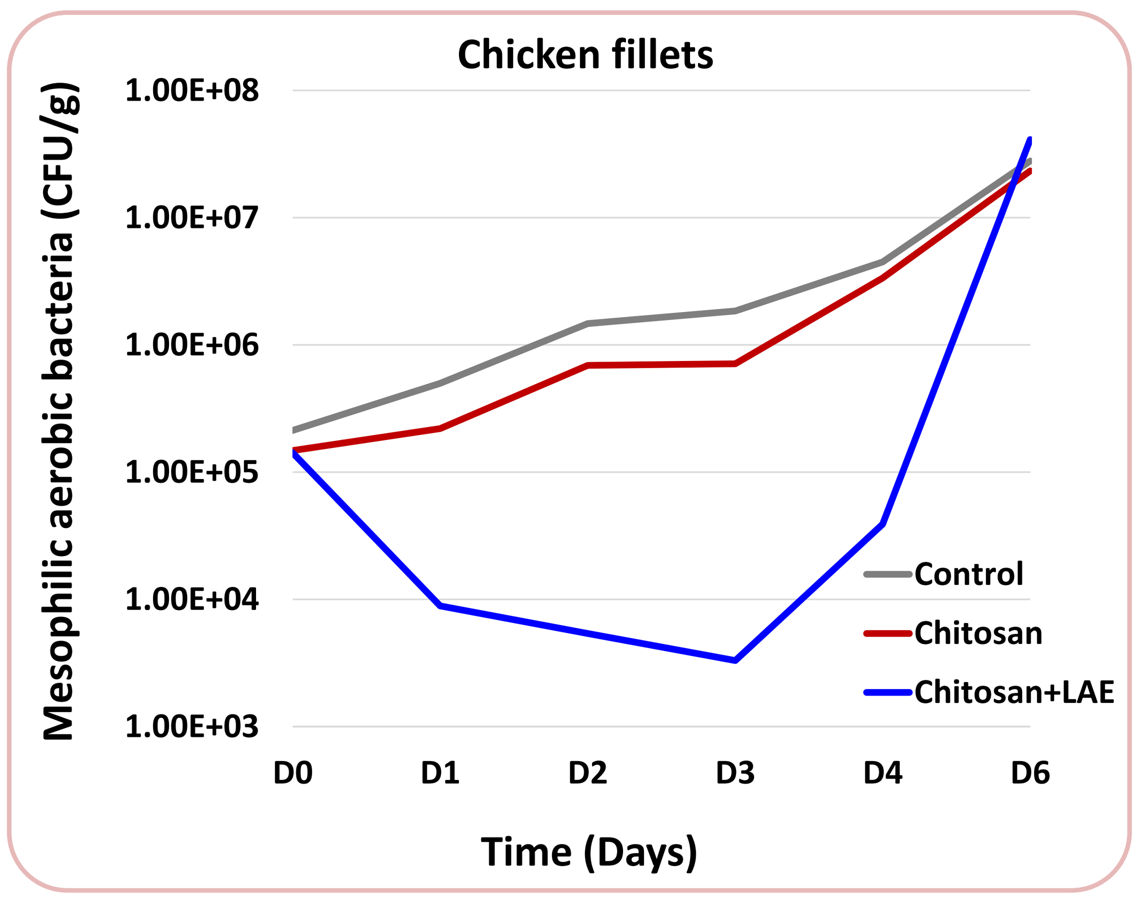
Light barrier properties



Chitosan film showed high transparency in the visible range (400-700 nm) while chitosan-LAE film showed absorbance at the yellow wavelength due to the presence of LAE at film forming solution. Both chitosan and chitosan-LAE films demonstrated good UV protection ability due to the absorption in the UV (240-400 nm) ranges.

Figure 2: UV-Vis spectra of the chitosan and chitosan-LAE films between 250 and 750 nm, with an enlargement of the UV region (near UV-C: 240-280 nm; UV-B: 280-320 nm; UV-A: 320-400 nm).

In vivo microbiological test



Number of mesophilic aerobic bacteria in the control and chitosan group showed similar trend which was increased along the storage time while samples treated with chitosan-LAE showed 2 log reduction after 3 days of storage. This number was acceptable at day 4 of storage and was lower than 1.00E+06 CFU/gr.

Figure 3: Mesophilic aerobic bacteria count in chicken fillets wrapped with chitosan and chitosan-LAE films during 6 days of storage at 4°C. Non wrapped chicken fillets used as control.

Conclusion

This study showed that the enrichment of chitosan film with LAE could change the mechanical, physical and barrier properties of the film. Incorporation of LAE in chitosan film increased extensibility of the film, while it reduced the mechanical strength resulted in the formation of a film network with lower rigidity. The presence of LAE in chitosan film decreased the water vapour transmission rate (WVTR). Considerable absorption in the UV range (240-400 nm) reflecting better UV protection ability of chitosan-LAE film. Regarding the use of the active packaging, wrapping chicken fillets with chitosan-LAE film showed high antimicrobial activity of the LAE against mesophilic aerobic bacteria. These results suggest a potential application of chitosan film enriched with LAE in the development of natural biopolymer-based packaging material with an additional bioactive function.

References

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2. Yao Y, Ding D, Shao H, Peng Q and Y. Huang (2017) Antibacterial activity and physical properties of fish gelatin-chitosan edible films supplemented with D-Limonene. *International Journal of Polymer Science.* 2017, 1-9.