Comparative analysis of blend and bilayer films based on chitosan and gelatin enriched with Lauroyl Arginate Ethyl for food packaging applications

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Introduction

Environmental concerns as well as consumer demand for healthy food products, rises the attention of food packaging industries on the development of bio-edible active films. In this sense, biopolymers such as chitosan (CS) and gelatin (GL) have been proven to be promising alternative for synthetic plastics. However, their uses are currently limited due to weak mechanical and water barrier properties. These properties can be improved by blending or laminating CS and GL biopolymers to combine the advantages of these two biopolymers as well as to minimize their disadvantages (1).
Lauroyl arginate ethyl (LAE) is a synthetic derivative of lauric acid, L-arginine and ethanol. LAE have been verified to be nontoxic with antimicrobial properties. LAE is classified as GRAS (generally recognized as safe) by the Food and Drug Administration and the European Food Safety Agency (2).

Since, limited information is available concerning the enrichment of CS and GL blend and bilayer films with LAE. The main aim of the present study was to develop blend and bilayer bio-edible active films based on CS and GL enriched with LAE to evaluate some physical, optical, mechanical and antimicrobial properties

Material and Methods

Chitosan/LAE (CS/LAE), gelatin/LAE (GL/LAE), blend/LAE and bilayer/LAE films were developed using solvent casting technique (Fig. 1).

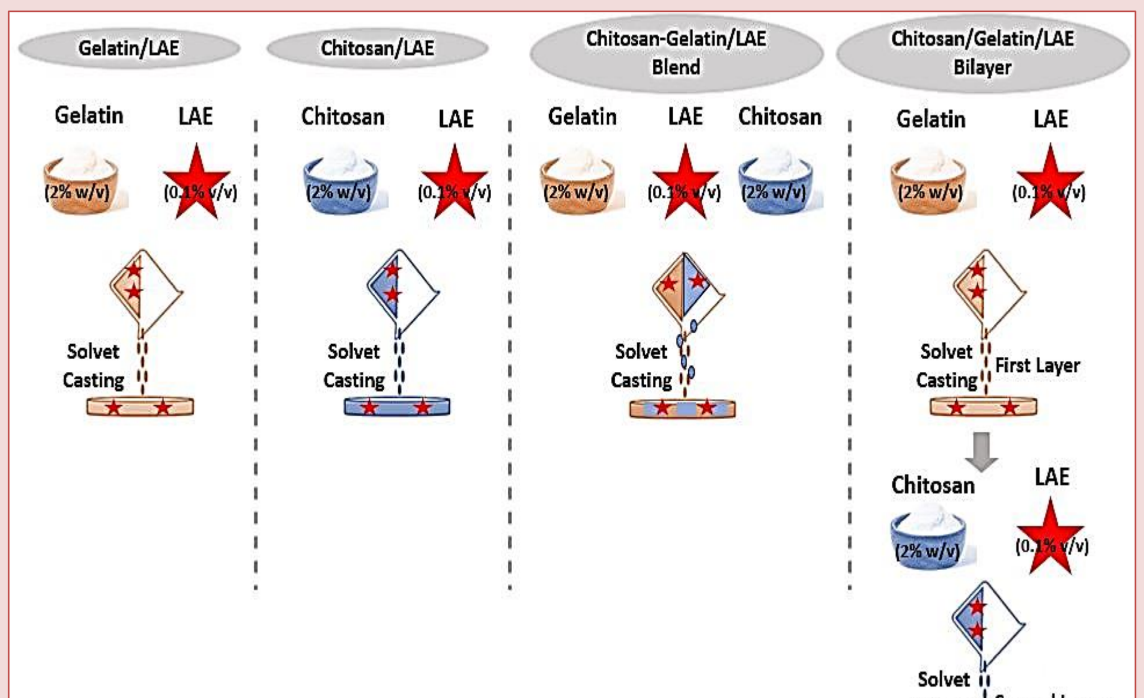

Fig 1. Schematic workflow of the study.

Different properties were determined:

- **Optical:** UV- and visibile- light barrier.
- **Physical**: thickness and FT-IR spectroscopy.
- **Mechanical**: tensile strength (TS), elongation at break percentage(EAB%) and elastic modulus (EM).
- **Microstructure**: scanning electron microscopy (SEM).
- **Antimicrobial:** disk diffusion assay.

Statistical analysis

The statistical analysis was performed through analysis of variance (ANOVA) using SPSS statistical program (SPSS 20 for windows, SPSS INC., IBM, New York). The differences between means were evaluated by Tuckey's multiple range test (p<0.05). All tests were repeated three times. The data was expressed as the mean ± SD (standard deviation).

Results and Discussion

Optical property

- Blend and bilayer films (Tab. 1) showed significantly lower transparency values than CS/LAE film (p<0.05).
- Blend films showed UV barrier values such as single component films, while bilayer films showed lowest values. This might be due to the higher thickness of bilayer film compare to the blend and single component films.

Tab 1. Optical (Transmission%) and transparency values.

Film	200	280	350	400	500	600	700	800	Transparency
CS/LAE	0.20	37.94	52.43	62.82	70.54	74.76	77.46	88.48	3.59±0.41[b]
GL/LAE	0.23	60.66	89.45	91.97	91.78	91.15	90.26	90.20	1.34±0.16[a]
Blend/LAE	0.23	61.17	82.83	89.28	90.25	89.86	89.24	89.06	1.43±0.22[a]
Bilayer/LAE	0.16	36.72	62.27	79.23	83.86	85.86	86.14	86.33	1.30±0.32[a]

Mechanical properties

- Blend film (Tab. 2) showed significantly higher TS and EM (p<0.05). This could be attributed to the formation of intermolecular hydrogen bonds between ammonium of the CS backbone and hydroxyl of the GL in the blend formulations.

Tab 2. Mechanical properties of films including tensile stength (TS), elongation at break percentage (EAB%) and elastic modulus (EM).

Film	Thickness	TS	EAB%	EM
CS/LAE	35 ± 1.56[b]	22.83 ± 0.60[b]	28.88 ± 0.55[a]	565.23 ± 23.68[c]
GL/LAE	29 ± 1.97[ab]	09.29 ± 0.04[a]	29.37 ± 1.81[a]	361.30 ± 08.95[a]
Blend/LAE	32 ± 1.30[ab]	31.86 ± 1.66[c]	28.69 ± 0.54[a]	534.61 ± 06.37[c]
Bilayer/LAE	58 ± 3.06[c]	24.34 ± 3.25[b]	31.61 ± 0.63[a]	462.61 ± 10.18[b]

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

Microstructure

- Both CS (Fig. 2a) and GL (Fig. 2b) films were homogenous.
- Blend films (Fig. 2c) showed dense, homogeneous and compact structures. Phase separation could not be detected in blend indicating a high compatibility and homogeneity between CS and GL due to associative interactions.
- Bilayer film (Fig. 2d) revealed sheets stacked in layers. Interactions could take place at the interface of the bilayer film, increasing the bonding strength between layers which prevent manual peeling of single film components**.**

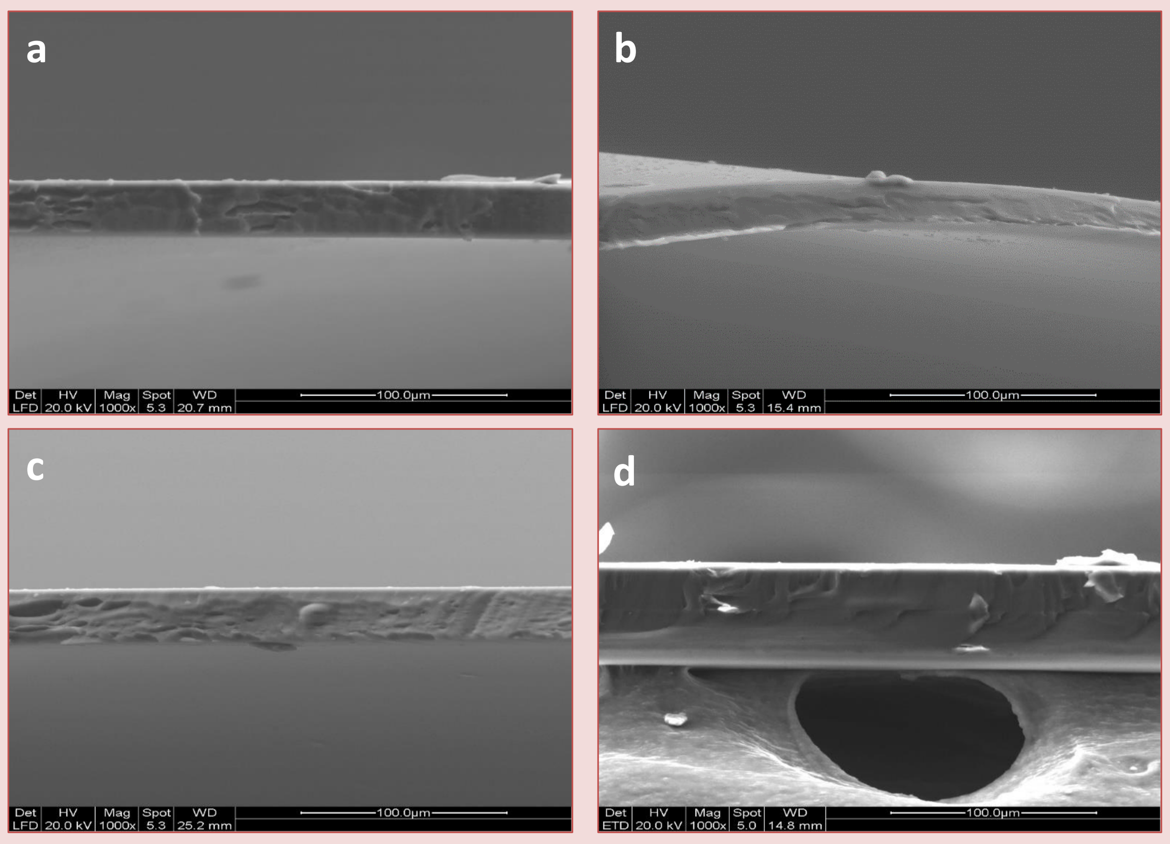

Fig. 2. SEM images on cross-sections of films based on a: CS/LAE, b: GL/LAE, c: blend/LAE, d: bilayer/LAE.

FT-IR spectroscopy

Analysis of FT-IR spectrum of blend/LAE film (Fig. 3c) revealed a slight shift of the ν(N-H) band to 3289 cm^{-1} which indicates the formation of additional intermolecular hydrogen bonds between CS and GL. Moreover, the amide-I band shifted from 1631 cm^{-1} to 1638 cm^{-1} in the blend. This change can be interpreted as a conformational change of the secondary structure of GL. The amide-II peak slightly shifts from 1545 to 1550 cm^{-1} in the blend and indicates the formation of further hydrogen bonds. Furthermore, the shift of the amide-II peak of CS from 1586 to 1550 cm^{-1} confirms the presence of the electrostatic interactions between the carboxyl groups of GL and amino groups of CS.

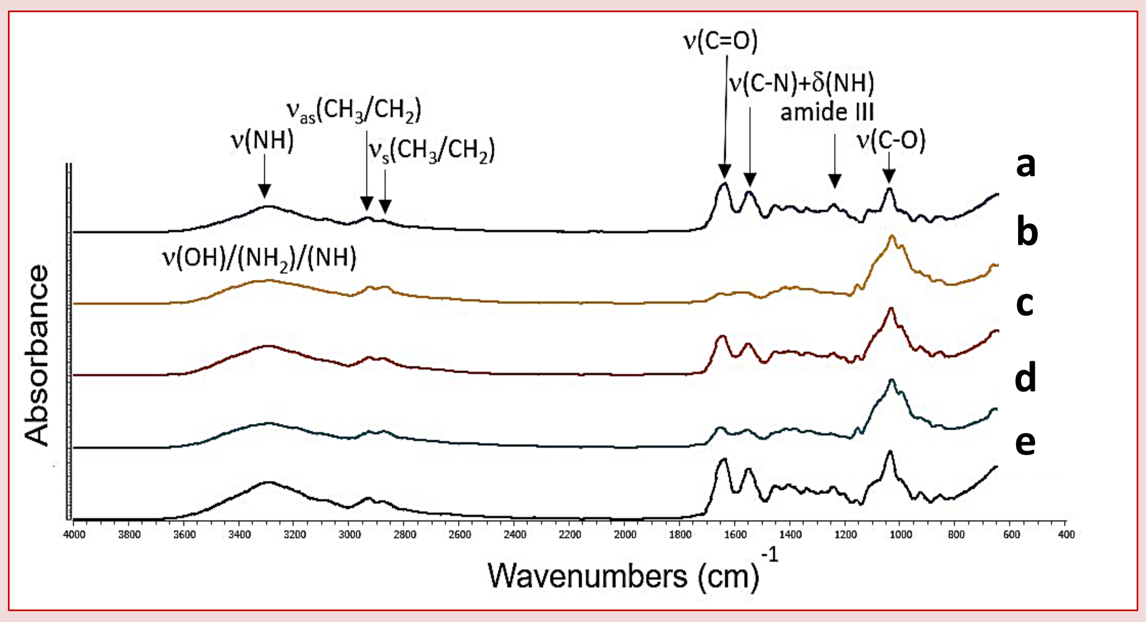

Fig 3. ATR-FT-IR spectra of films based on a: gelatin/LAE, b: chitosan/LAE, c: blend/LAE, d: bilayer/LAE (top layer) and e: bilayer/LAE (bottom layer).

Antimicrobial property

Incorporating LAE into films even at low concentrations (0.1%) revealed an antimicrobial effect and inhibited the growth of four major food bacterial pathogens including *C. jejuni, E. coli, L. monocytogenes* and *S. typhimurium* (Tab. 3).
The high antimicrobial activity of LAE has been attributed to its action as a cationic surfactant by increasing the permeability of the cytoplasm and membrane of microorganisms and inhibition of cellular ATP generation.

Tab 3. Inhibition zone diameters of the film disks (15 mm diameter) based on CS/LAE, GL/LAE, blend/LAE and bilayer/LAE.

Film	*L. monocytogensis*	*E. coli*	*S. typhimurium*	*C. jejuni*
CS/LAE	16.36 ± 0.55[aA]	17.66 ± 1.08[aA]	16.05 ± 0.08[aA]	23.33 ± 2.30[aB]
GL/LAE	16.66 ± 0.57[aA]	16.33 ± 0.57[aA]	16.00 ± 1.00[aA]	23.33 ± 1.52[aB]
Blend/LAE	16.70 ± 0.51[aA]	18.00 ± 1.73[aA]	17.03 ± 0.05[aA]	23.66 ± 0.57[aB]
Bilayer/LAE	16.70 ± 0.60[aA]	17.66 ± 2.08[aA]	16.03 ± 1.00[aA]	24.33 ± 0.57[aB]

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

Conclusions

The results of this study demonstrated that blend and bilayer films based on CS and GL enriched with low amount of LAE (0.1%) presented as an alternative in the development of biodegradable and edible packaging with an additional bioactive function to ensure food safety and to extend the shelf-life of foods.

References

1) Haghighi, H., De Leo, R., Bedin, E., Pfiefer, F., Siesler, HW., & Pulvirenti, A. (2019) Comparative analysis of blend and bilayer films based on chitosan and gelatin enriched with LAE (lauroyl arginate ethyl) with antimicrobial activity for food packaging applications. *Food Packaging and Shelf Life*, (19), 31-39.
2) Rubilar, J. F., Candia, D., Cobos, A., Díaz, O., & Pedreschi, F. (2016). 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.