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Preparation and characterization of active chitosan/bacterial cellulose nano-whisker composite film enriched with lauroyl arginate ethyl for food packaging applications

Hossein Haghighi[1*], Salvatore La China[1], Maria Gullo [1*], and Andrea Pulvirenti[1]

[1] *Dept. of Life Sciences, University of Modena and Reggio Emilia, 42122, Reggio Emilia, Italy*

* Shared correspondence: Hossein.Haghighi@unimore.it; Maria.Gullo@unimore.it

UNIMORE UNIVERSITÀ DEGLI STUDI DI MODENA E REGGIO EMILIA

Introduction

Acetic acid bacteria are recognized for their wide range of industrial applications. Recently, applying bacterial cellulose (BC) from acetic acid bacteria as a packaging material has received considerable attention from food packaging industries. This is mainly due to its unique properties such as biocompatibility, biodegradability, water holding capacity and remarkable mechanical properties (1). Active packaging is intended to extend the shelf-life of food products and assure their safety and quality inside the packaging systems. In this context, lauroyl arginate ethyl (LAE) is considered as one of the most effective antimicrobial substances among novel food additives which is also classified as GRAS (generally recognized as safe) by the Food and Drug Administration and the European Food Safety Agency (2). Thus, the main aim of this study was to characterize the microstructural, physical, mechanical and optical properties of chitosan/bacterial cellulose nano-whisker (BCNW) composite film enriched with LAE for potential application as active food packaging. Moreover, antimicrobial activity of this film against four common food bacterial pathogens including *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella typhimurium*, was investigated.

Material and Methods

BC was produced from *Komagataeibacter xylinus* (strain K2G30 = UMCC 2756) due to its high yield and purity (3). Then, BC was subjected to acid sulfuric hydrolysis with the aim of breaking the hierarchical structure into rod-like crystalline structures called bacterial cellulose nano-whiskers (BCNW). Finally, the BCNW (5- 10% w/w with respect to chitosan) was incorporated into the chitosan film-forming solution containing (5% w/v) lauroyl arginate ethyl (LAE) as an antimicrobial compound to form an active nano-composite film. Following parameters were characterized:

- Microstructure: using scanning electron microscopy (SEM)
- Physical: FT-IR using ATR/FT-IR
- UV-Vis light barrier: spectrophotometer
- Mechanical including tensile strength (TS), elongation at break percentage (E%) and elastic modulus (EM): using dinamometer
- Antimicrobial: disk diffusion assay.

Statistical Analysis

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 3 times. The data was showed as the mean ± SD (standard deviation).

Results and Discussion

Microstructure

- The surface morphology of chitosan film was completely compact, smooth and homogenous and did not show any pores or cracks (Fig. 2a)
- The surface of chitosan film containing 5% BCNW showed similar microstructure to pure chitosan film indicating a good compatibility between chitosan and BCNW and uniform distribution of BCNW in chitosan film forming solution(Fig. 2b).
- The surface of chitosan film containing 10% BCNW was rough with small particles and aggregations. This was mainly due to the agglomeration of BCNW in the polymer matrix at high concentration (Fig. 2c).

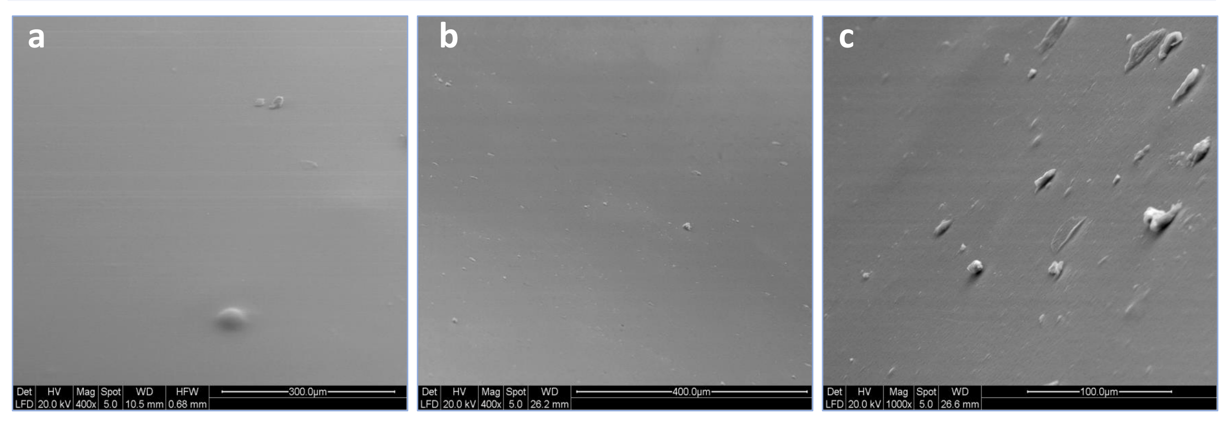

Fig. 1. SEM images on cross-section of films based on a: Chitosan, b: Chitosan-BCNW5%, c: Chitosan-BCNW10%

FT-IR Spectroscopy

- ATR/FT-IR spectroscopy was performed to characterize the structural and spectroscopic changes due to the incorporation of the BCNW into the chitosan film matrix (Fig. 2)
- Characteristic chitosan bands are mainly assigned to amide I, amide II, saccharide structures and intra- and intermolecular OH vibrations (Fig. 2a).
- FT-IR spectrum of Chitosan-BCNW film showed a sharp peak at 3342 cm^{-1} suggesting hydrogen bond formation between chitosan and BCNW. In addition changes in intensity and position of bands at 1300-1550 cm^{-1} and an increase in the intensity of the absorption bands at 1054 and 1032 cm^{-1} due to BCNW incorporation confirmed intermolecular interactions between chitosan and BCNW (Fig. 2b and 2c).

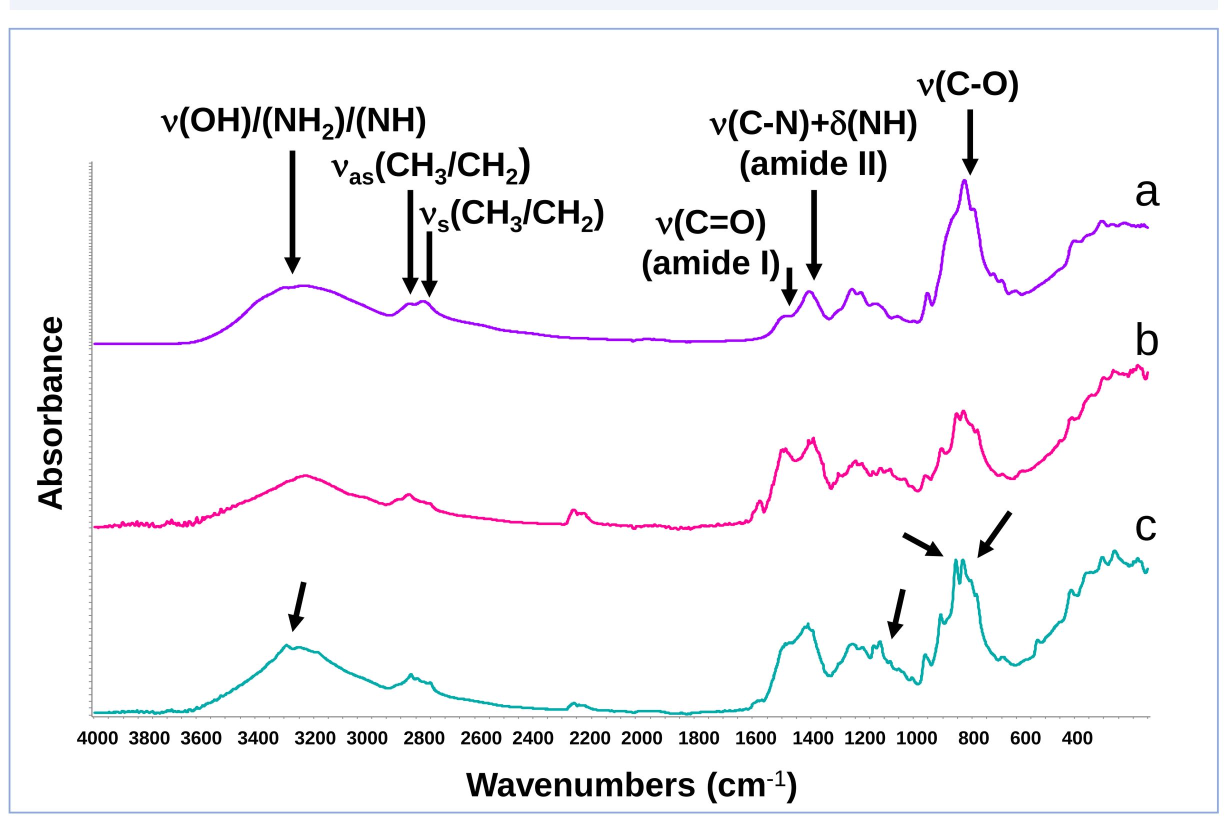

Fig. 2. ATR/FT-IR spectra of films based on a: Chitosan, b: Chitosan/BCNW5% and c: Chitosan/BCNW10%.

Results and Discussion

UV-Vis Light Barrier Properties

- Incorporation of BCNW to chitosan films effectively inhibited penetration of UV light (200-350 nm) to the packaging system.
- Despite the reduction of transparency in BCNW incorporated films, they were still transparent enough to be used for food packaging applications (Fig. 3)

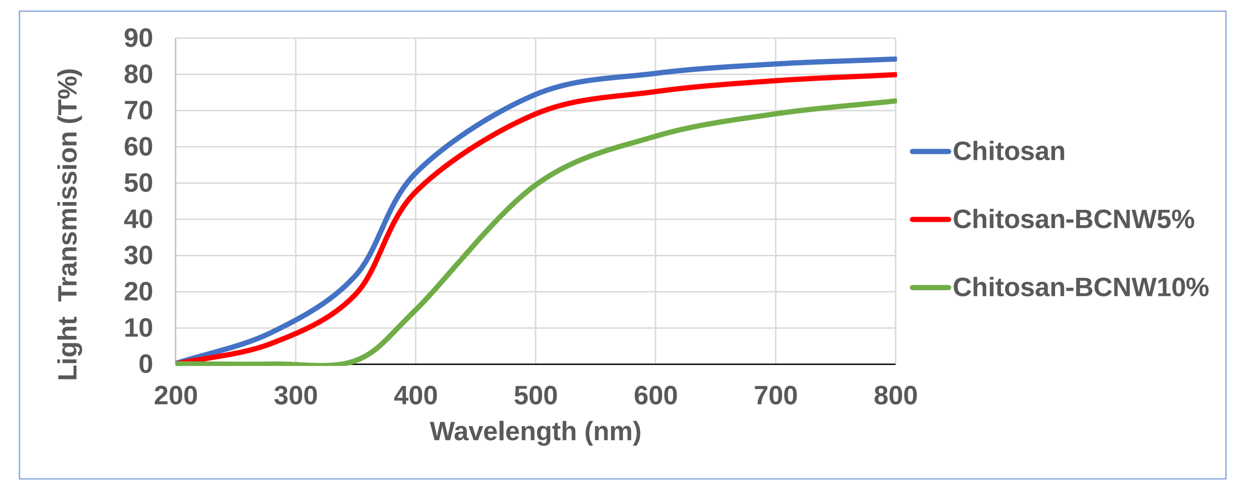

Fig. 3. UV-Vis light transmittance spectra

Mechanical Properties

- Chitosan film incorporated with BCNW showed higher TS and EM than pure chitosan film.
- Elasticity of chitosan film reduced upon addition of BCNW indicated strong interactions between BCNW and film matrix due to the hydrogen bond formation, which restricted the motion of the matrix and hence decreased elasticity (Tab. 1).

Tab. 1. Mechanical properties of films including tensile stength (TS), elongation at break percentage (EAB%) and elastic modulus (EM).

Film	TS (MPa)	E (%)	EM (MPa)
Chitosan	25.0 ± 1.9^{a}	4.8 ± 0.7^{a}	1827 ± 145^{a}
Chitosan-BCNW 5%	29.2 ± 1.9^{b}	2.0 ± 0.3^{b}	2060 ± 190^{b}
Chitosan-BCNW 10%	27.0 ± 2.1^{ab}	2.0 ± 0.4^{b}	1926 ± 133^{ab}

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

Antimicrobial property

- LAE incorporation into Chitosan-BCNW films revealed an antimicrobial effect and inhibited the growth of four major food bacterial pathogens including *C. jejuni*, *E. coli*, *L. monocytogenes* and *S. typhimurium* *(Data not shown)*.
- 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 causing a disturbance in membrane potential and resultingin cell growth inhibition and loss of viability (2).

Conclusions

- SEM and ATR/FT-IR analysis showed a good compatibility between chitosan and BCNW to form blend film.
- Chitosan film incorporated with BCNW demonstrated higher TS and EM values and effectively inhibited the UV light.
- Active films containing LAE showed antimicrobial activity against four major food bacterial pathogens.

References

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