CATÓLICA FACULTY OF BIOTECHNOLOGY PORTO

UNIVERSITÀ DEGLI STUDI DI MILANO

MICROBIOTEC 21 Webconference — CONGRESS OF MICROBIOLOGY AND BIOTECHNOLOGY 2021

Preparation and Characterization of Bioactive Chitosan-Based Films Incorporated with Olive Leaves Extract for Food Packaging Applications

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1

Motivation and objectives

- Microbiological contamination accounts for up to 25% of the waste of fresh, highly perishable food prior to consumption
- *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, and *Escherichia coli* are relevant for food safety

- Active packaging is receiving increased interest to protect the food
- Polymers of renewable origin instead of oil-based plastics makes these systems more appealing for a sector increasingly looking for environmentally sustainable solutions

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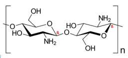
2

Motivation and objectives

- Biopolymer: large availability, biodegradability
- Extracted from the shell of crustaceans

Chitosan



- High value by-product
- Active compounds (Oleuropein as main compound)

Olive leaf extract (OLE)

Development of active coatings with both antimicrobial and antioxidant properties

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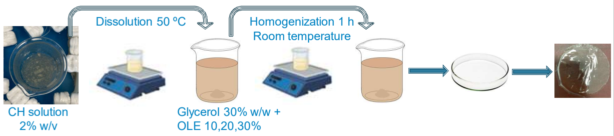
3

Methods

- Preparation of **Chitosan-Olive Leaf Extract Films** at different concentrations (10, 20, 30% OLE relative to the chitosan)



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4

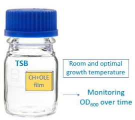
Methods

- **Characterized films properties:**
  - Solubility - % dry matter of film solubilized at 23 °C/ 24 h immersion in simulants
  - Water vapour transmission rate (WVTR) - gravimetric method (ISO 2528)
  - Tensile properties (ASTM D 882)

- **Evaluation of active properties**

Antimicrobial against *L. monocytogenes*, *E. coli*, *C. jejuni*

In vitro
- measuring the optical density at 600 nm, ASTM E2149



In situ
- sterile agar with OLE 2% in contact with pre-inoculated chicken pieces
- *C. jejuni* counts on pieces

Antioxidant activity (ABTS assay)

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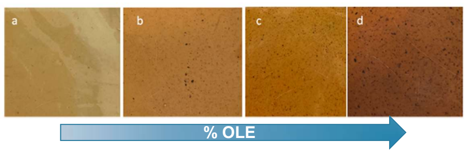
5

Results

Films characterization

Visual appearance of the films



✓ Films are transparent with a yellowish taint increasing with the concentration of the OLE and showing some insoluble particles

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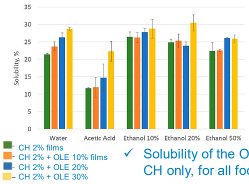
6

Results

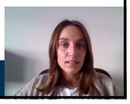
Solubility of films in food simulants



Moisture permeability of films and tensile strength and elongation at break

Sample	WVP ($g \cdot Pa^{-1} \cdot s^{-1} \cdot m^{-1}$)	TS (MPa)	E (%)
CH 2% film	$(4.92 \pm 0.12) \times 10^{-11}$	17.02 ± 2.27	4.34 ± 1.25
OLE film (10%)	$(3.97 \pm 0.32) \times 10^{-11}$	25.13 ± 1.62	18.7 ± 1.33
OLE film (20%)	$(2.90 \pm 0.20) \times 10^{-11}$	18.12 ± 3.45	12.4 ± 3.04
OLE film (30%)	$(2.72 \pm 0.30) \times 10^{-11}$	21.97 ± 3.14	11.6 ± 0.63

- ✓ Solubility of the OLE-CH films was higher than that of the CH only, for all food simulants
- ✓ Permeability of CH film decreased with OLE incorporation
- ✓ Tensile strength was not significantly affected by the OLE incorporation while elongation at break increased with OLE incorporation



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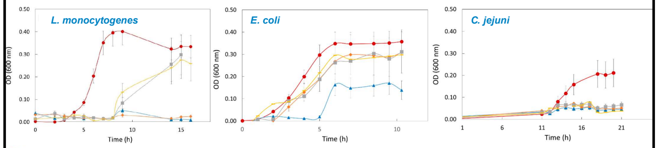
7

Results

Evaluation of antimicrobial properties

Growth curves at optimal growth temperature



- ✓ ***L. monocytogenes* and *C. jejuni***: substantial antimicrobial activity
- ✓ ***E. coli***: lower level of inhibition
- ✓ OLE seems not to improve the intrinsic antimicrobial properties of the chitosan itself, except for ***C. jejuni***

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8

Results

Evaluation of antimicrobial properties

In situ

- ✓ 6-log reduction was achieved for *C. jejuni*
- ✓ Removing the meat from OLE contact, some growth was observed after incubation

- *C. jejuni* growth is reduced but some cells remain viable
- OLE affects either the number of viable bacteria (by killing some) or affects the cells metabolism (cells viable but not culturable)

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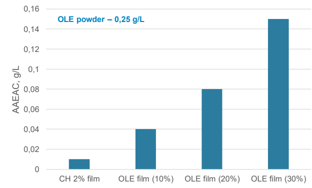
9

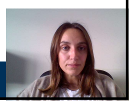
Results

Evaluation of antioxidant properties

Antioxidant activity expressed in ascorbic acid eq.



- ✓ The chitosan films with OLE exhibited antioxidant activity, increasing with the OLE concentration



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10

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Overall, the combination of OLE and chitosan allows to obtain a promising active bio-based packaging solution for addressing safety and quality issues

Thank you!

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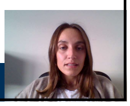

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11