Seminar

ADVANCES IN CELLULOSE-BASED MATERIALS IN FOOD PACKAGING

In collaboration with:

Aarhus University

VTT Technical Research Centre of Finland

Seminar under the scope of COST Action CIRCUL-A-BILITY CA19124, supported by COST (European Cooperation in Science and Technology).

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FROM FOSSIL TO FOREST

CIRCUL-A-BILITY

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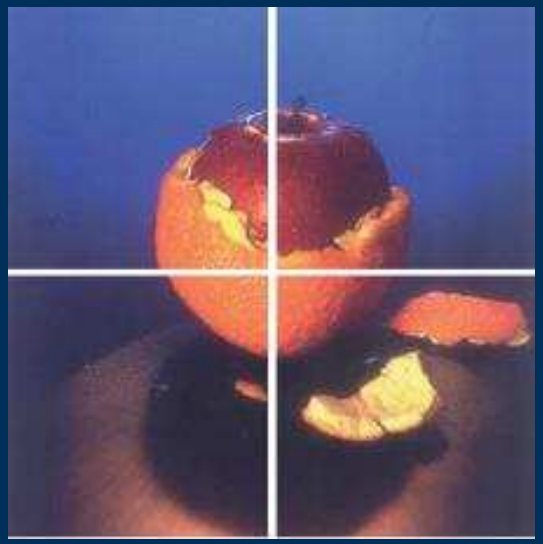

Applications of bacterial nanocellulose in active packaging

Francisco A.G. Soares da Silva, Miguel Gama, Paula Teixeira, Fátima Poças

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Agenda

1. Food Packaging
    1. Requirements
    2. Synthetic vs Bio-based
2. Active & Intelligent Food Packaging
    1. Active agents
    2. Nanocellulose application
3. Plant vs Bacterial nanocellulose
    1. Production & characterization
    2. BNC production and properties
4. Nanocellulose composite processing
    1. Solvent vs Coating vs Impregnation
5. Safety of NCs based composites
    1. Cellulose vs NCs
    2. Migration onto food
6. Case study
    1. BNC_{ZnO} as active packaging

1. Food packaging

- Aiming to protect the food from the environment
  - Food packaging requirements
    - Food intrinsic properties (Water activity)
    - Extrinsic factors (temperature, humidity, light)
    - Shelf-life
  - Properties to be monitored
    - Permeability water vapour, oxygen and other gases
    - Mechanical performance

Packaging requirements

Product	WVTR ($g/m^2.day$) 23 °C	Oxygen permeance ($cm^3_{STP}/m^2.day.Pa$) 23 °C	Shelf-life (months)	Materials typically used
	Low moisture foods, a_w < 0.6			
Nuts, snacks, chips	0.093 - 3.0	$1.6x10^{-6}$ – $9.6x10^{-5}$	3 – 12	Metallised films, Laminates with Ethylene vinyl alcohol (EVOH), Polypropylene (PP)
	High moisture foods, a_w > 0.9			
Fruit juices, soft drinks	0.47 - 12.2	$6.1x10^{-6}$ – $6.14x10^{-4}$	1 - 18	Glass, PET, Metal cans, bag in box, Aseptic multilayer
Fats	5.2 – 9.2	$6.8x10^{-5}$ – $8.0x10^{-4}$	3	Fat resistant paper, PP
	Fresh foods			
Fruits, vegetables, fresh salads	10 – 4 000	$1x10^{-1}$ - 2	0.25	Low-density polyethylene (LDPE), PP
Meat and meat based product	2 - 100	$2x10^{-4}$ – $1x10^{-1}$	0.25 – 0.5	Polystyrene (PS) and PET trays

Food packaging

Plastics dominating the industry!!

- ✓ Lightweight
- ✓ Low production cost
- ✓ Good mechanical properties
- ✓ Good barrier properties

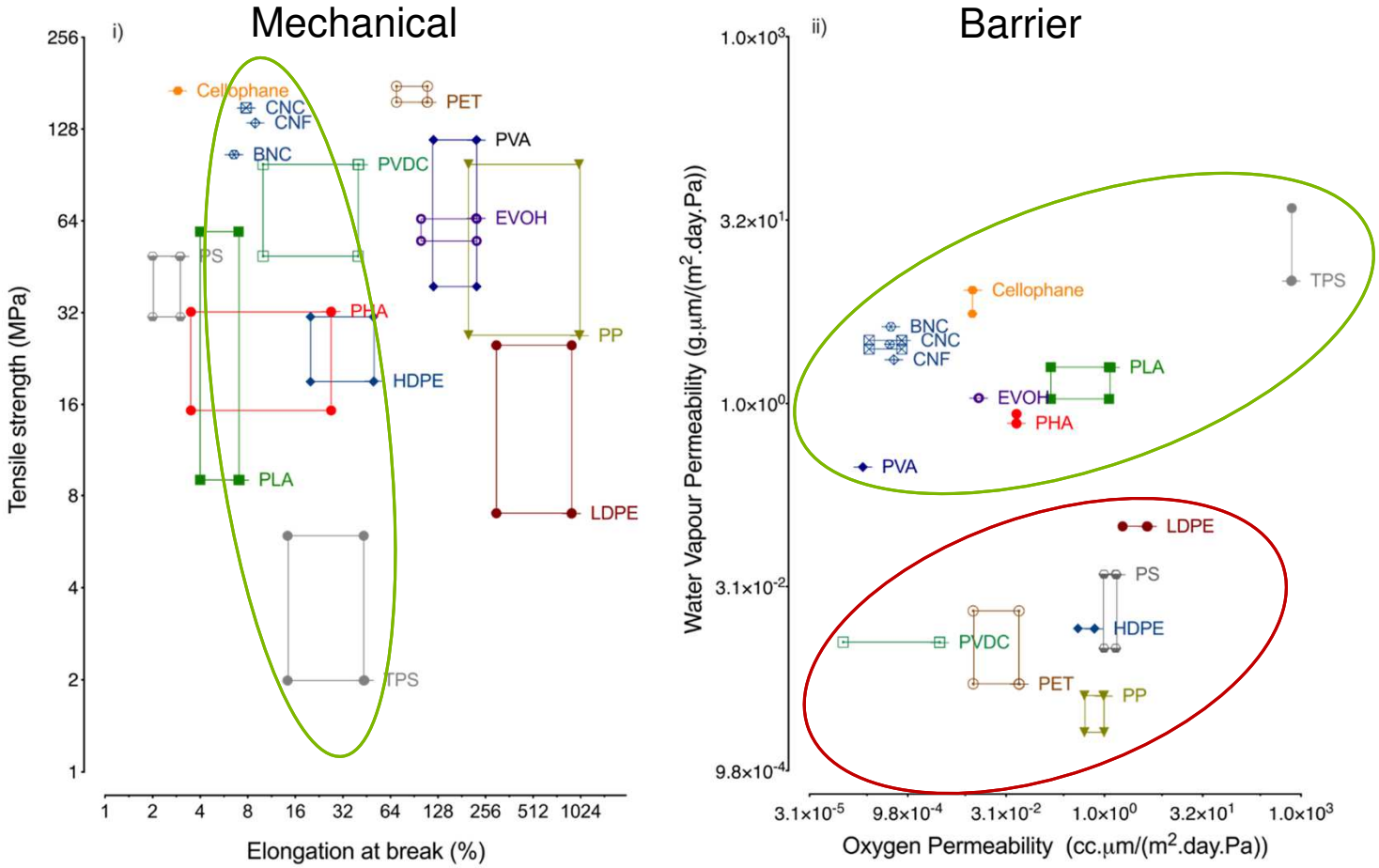
- ✗ Petroleum based material
- ✗ Low recycling rate
  - ✗ 79% of the plastic in landfills/waterways
- ✗ Microplastics in the air, soil and water
- ✗ Non degradable

Alternatives needed!!

Bio based materials?

Cellulose, PLA, PHAs, TPS…

Synthetic vs Bio-based



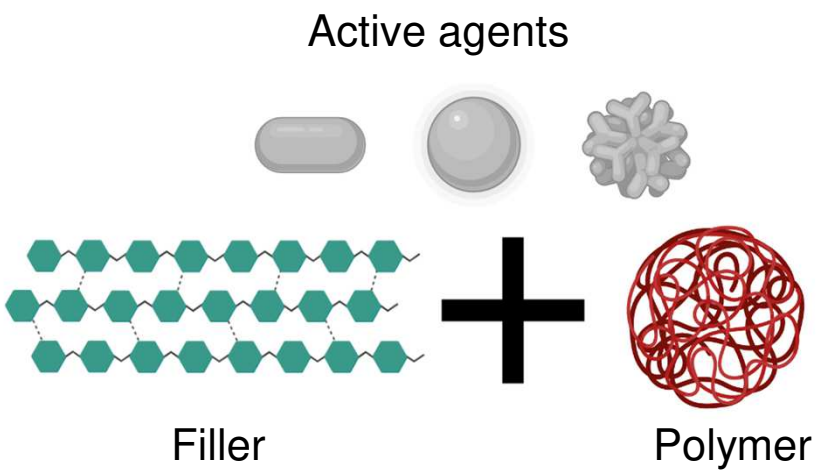
Nanocomposite technology

- ✓ Mechanical properties
- ✓ Barrier properties
- x High production cost
- x Low production capacity

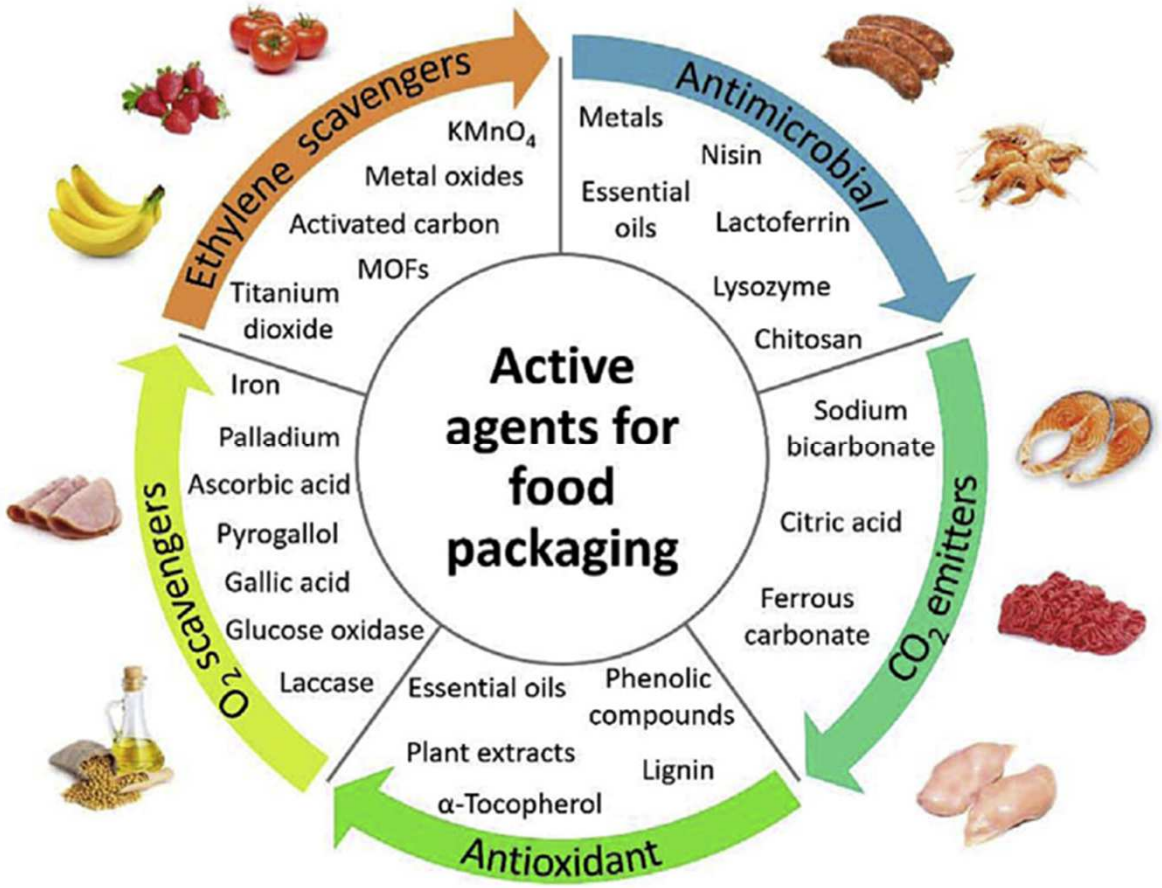
2. Active & Intelligent Food packaging

- System that interacts with food to **extend shelf life**, **preserve freshness** and **enhance safety**
- Active - system that **absorbs or releases substances**
  - Antimicrobial & Antioxidant
  - Oxygen and ethylene scavengers
  - CO_2 emitters
- Intelligent - monitors the condition of packaged food
  - Information on **freshness of the food**

Nanocomposite technology



Active Packaging



Vilela *et al.* 2018

Incorporated by blending, coating…

Commercial active packaging
- *Biomaster*
- *Foodtouch*
- …

Based on silver, glucose oxidase, natamycin,…

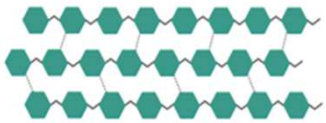
Active Packaging

Nanocellulose as a support in active packaging

Nanocellulose	Active agent	Activity	Reference
Cellulose nano fibres	Feruloylated arabinoxylo-oligosaccharides	UV-barrier Antioxidant Antimicrobial	Moreirinha et al., 2020
	Tannins	Antioxidant	Missio et al., 2018
	Bromothymol Blue/methyl red	Freshness indicator (pH sensor)	Lu et al., 2020
Cellulose nanocrystals	Titanium dioxide NPs	Antimicrobial	Wakil et al., 2015
Bacterial nanocellulose	Lactoferrin	Antimicrobial	Padrão et al., 2016
	Sorbic acid	Antimicrobial	Jipa et al., 2012
	Methyl red	Freshness indicator (pH sensor)	Kuswandi et al., 2013
	Silver NPs	Antimicrobial	Wang et al., 2020
	Silymarin-zein NPs	Antioxidant; Antimicrobial	Tsai et al., 2018
	Zinc oxide NPs	Antimicrobial	Dinca et al., 2020 Pirsa et al., 2018 Mocanu et al., 2019 Wahid et al., 2019

3. Plant vs Bacterial nanocellulose

Cellulose

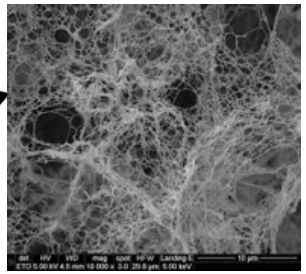


Plant origin

Extraction methods

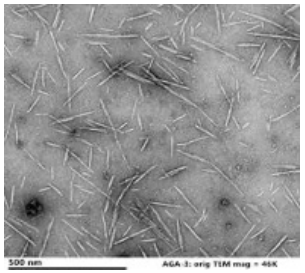
- Steam explosion
- Acid hydrolysis
- High pressure homogenization
- Microfluidization

Cellulose nanofibres (CNF)

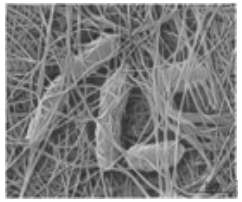


Diameter 5-20 nm
Length 2-10 um

Cellulose nanocrystals (CNC)



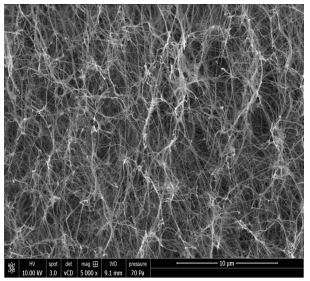
Diameter 4-70 nm
Length 100-6000 nm



Komagataeibacter bacteria

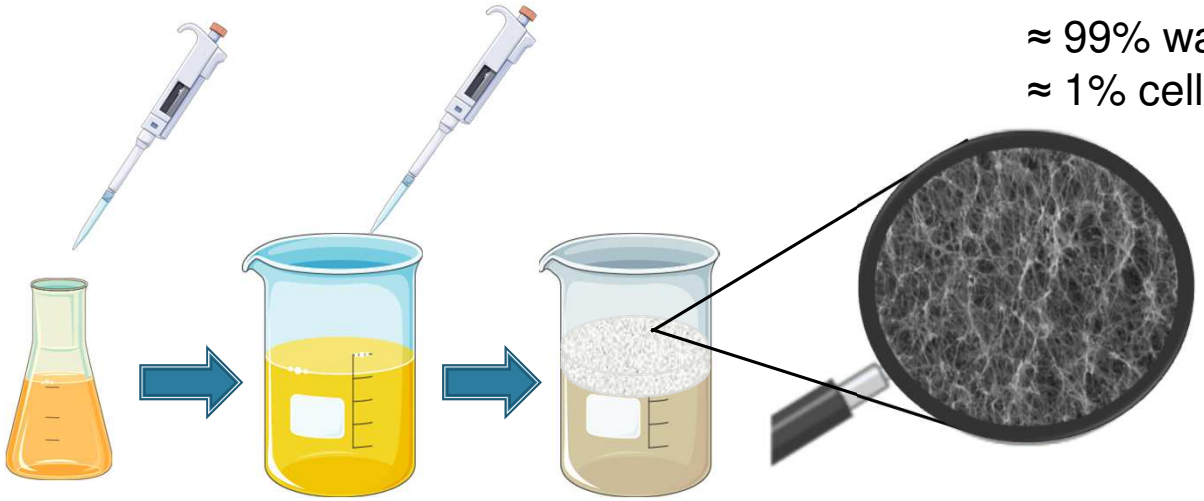
Bacterial origin (by fermentation)

Bacterial nanocellulose (BNC)



Diameter <100 nm
Length 100 um

BNC Production & Characterization



Cellulose membrane forming from surface to bottom

≈ 99% water
≈ 1% cellulose

Main properties of BNC

- High degree of polymerization
- High crystallinity
- High purity
- Biodegradability
- Biocompatibility
- **High water holding capacity**

Feature for absorbing and release of **active substances**

Strains for BNC synthesis: *Komagataeibacter xylinum, K. hansenii; K. rhaeticus*

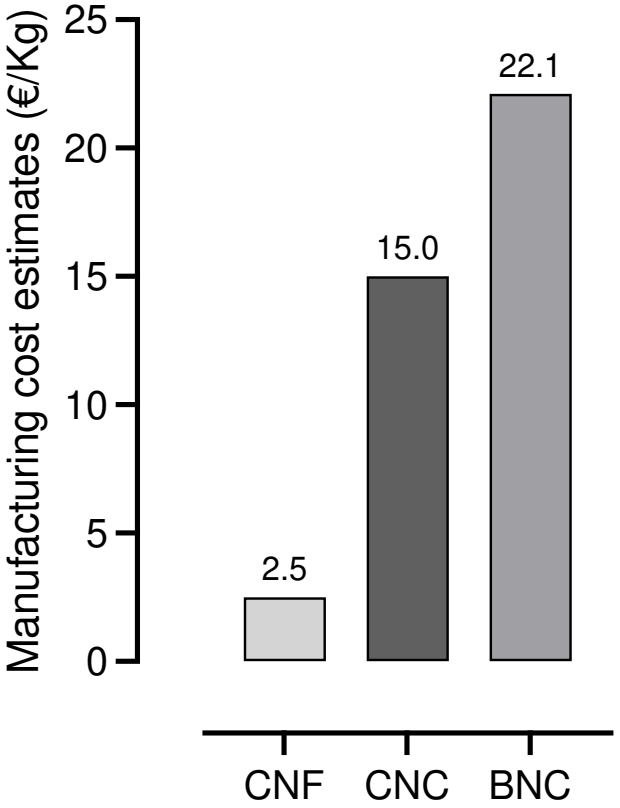
Culture media: Hestrin-Schram; modified HS… (depend on the strain)

Conditions: ≈ 30ºC, 7-30 days of incubation (depend on the surface area to volume ratio)

BNC Production & Characterization

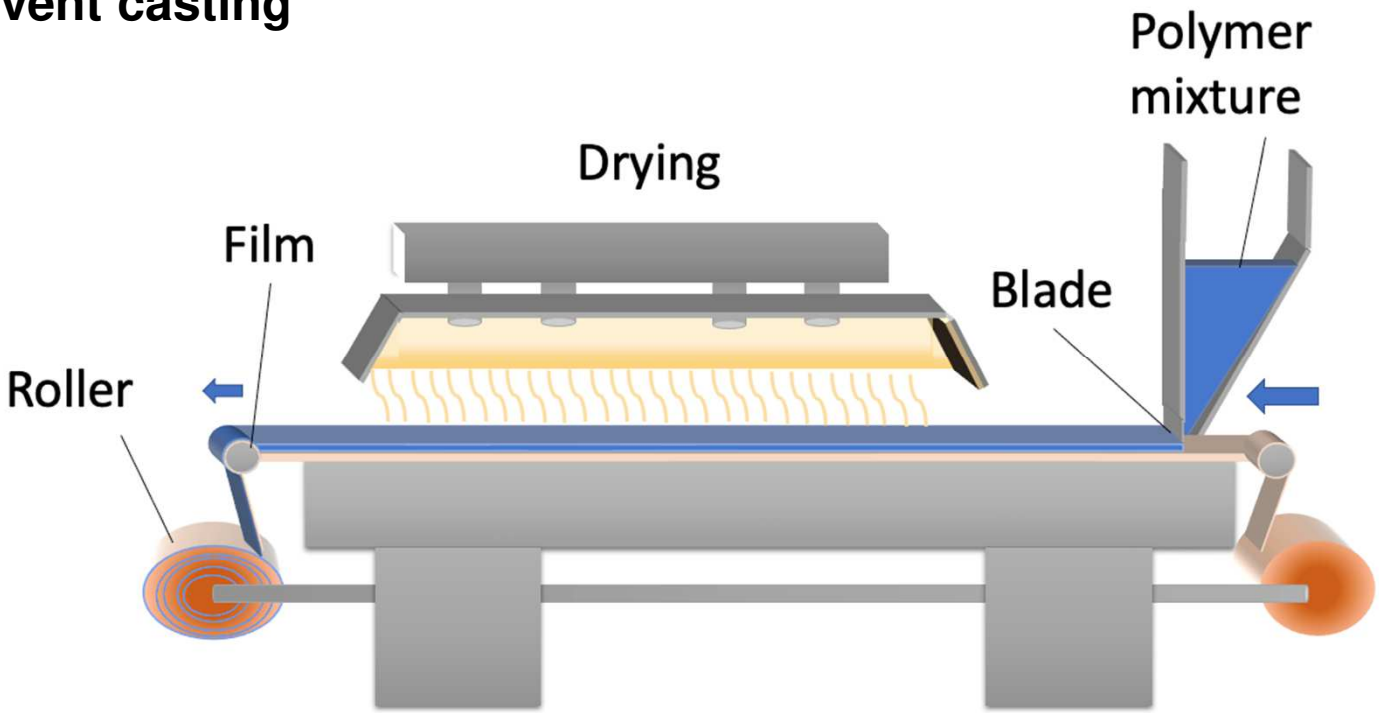
Setbacks

- Low yields of BNC (max 10 $g.L^{-1}$)
- High manufacturing cost
  - Strategies to lower the manufacturing



4. Nanocellulose composite processing

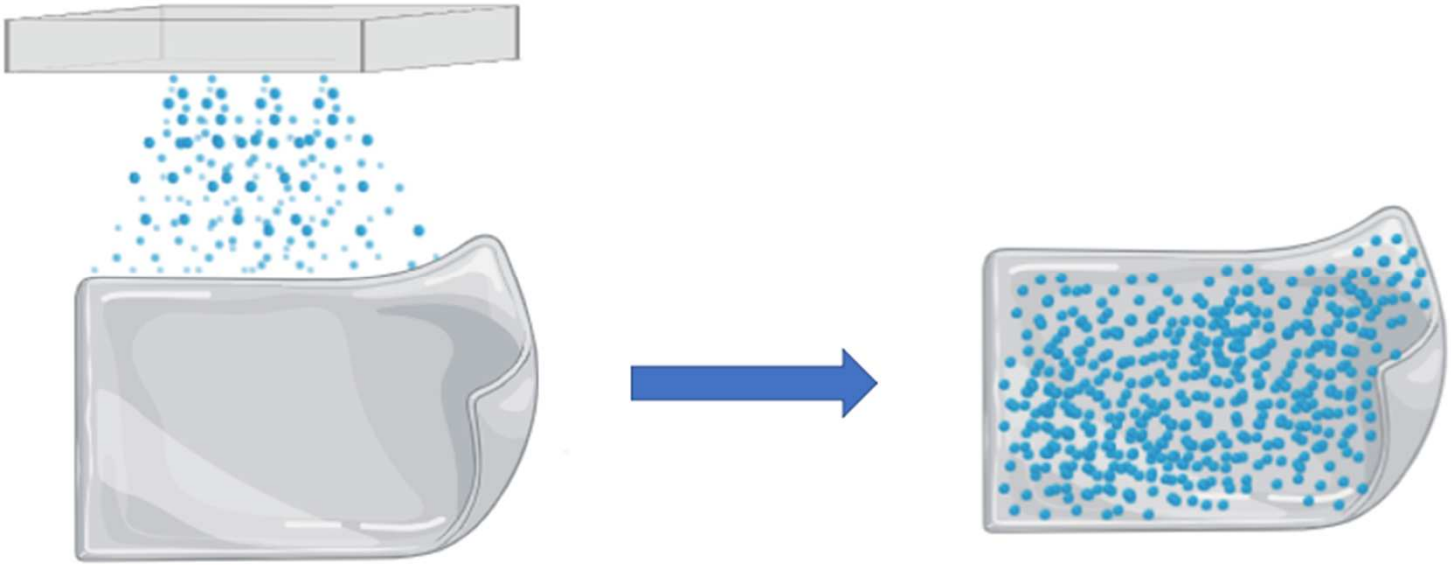
Solvent casting



- ✓ Simple method
- ✓ Easy upscaling
- ✗ **Dispersibility**
  - ✗ Mechanical
  - ✗ Lower activity

4. Nanocellulose composite processing

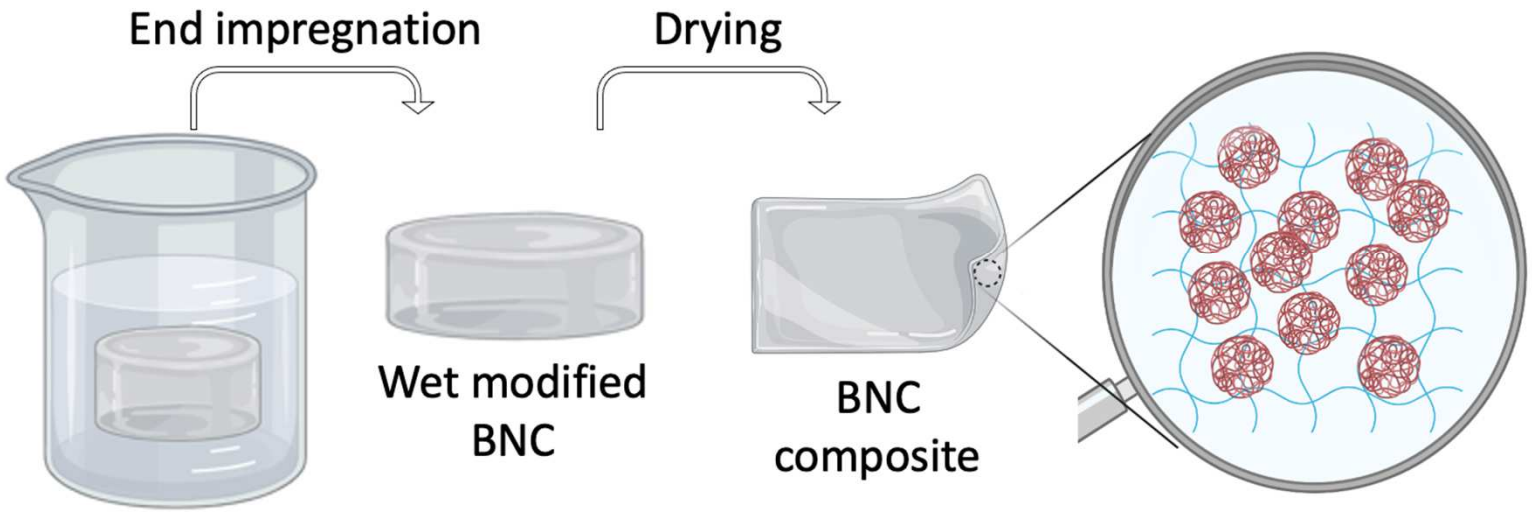
Coating



- ✓ Easy upscaling
- ✓ Barrier performance
- ✓ Good dispersibility (on the surface)
- ✗ Mechanical
- ✗ **Rapid release**

Nanocellulose composite processing

Impregnation



- ✓ **Direct** absorption
- ✓ Mechanical
- ✓ Homogenous distribution
- ✓ **Delayed release**
- ✗ Nano-sized compounds
- ✗ **Limited scalability**

5. Safety of NCs based composites

Plastic materials on active food packaging

Authorised by EFSA

- Cellulose
- Cellulose acetate butyrate
- Hydroxyalkyl celluloses
- Nitrocellulose
- Lignocellulose

Nanocellulose (including BNC) **not yet** authorised for food contact applications

COMMISSION REGULATION (EU) No 10/2011

of 14 January 2011

on plastic materials and articles intended to come into contact with food

(Text with EEA relevance)

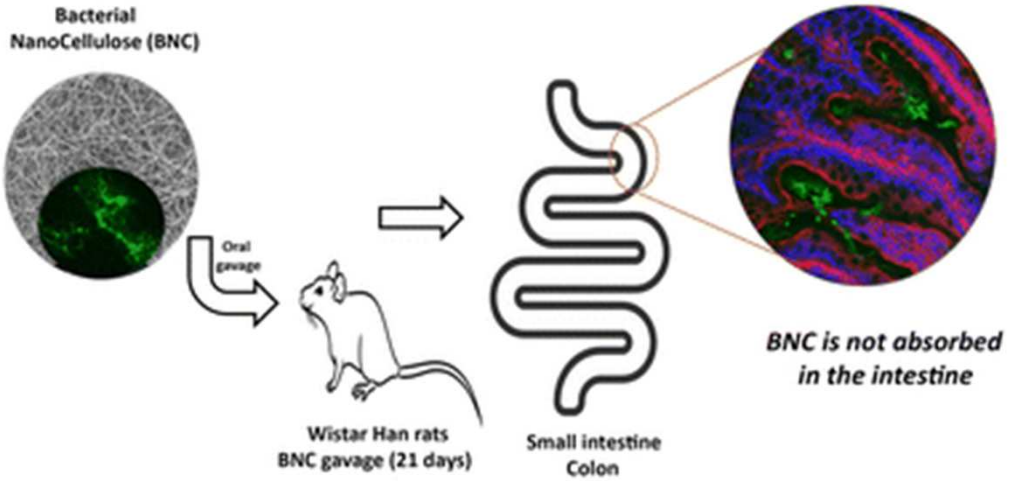
*"**Nanoparticles** interaction (…) may be different from **conventional particle size scale**, leading to **different exposure** and different **toxicological** properties".*

- NC toxicity through inhalation (Inconsistent results)
  - **Variability** of biological systems/conditions
  - NCs characteristics (dimension, shape..)
- NC toxicity in the gastrointestinal tract - no evidence (?)
  - CNCs (not CNF or BNC) **may generate ROS**
  - NC long term effects (absorbance of micronutrients)

Safety of NCs based composites

Assessment of the gastrointestinal fate of bacterial nanocellulose and its toxicological effects after repeated-dose oral administration†

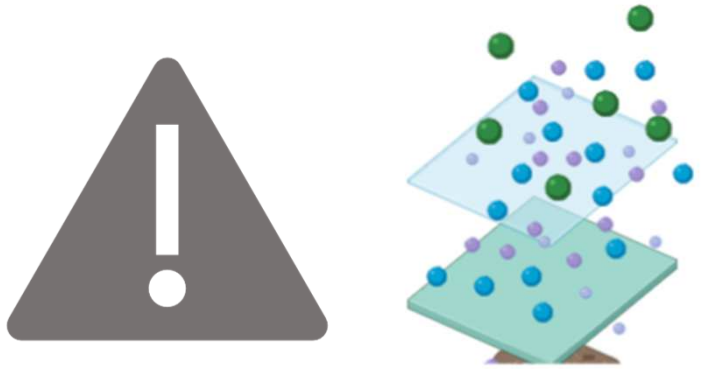
Ana Cristina Rodrigues,‡[ab] Lígia Costa,‡[ab] Ricardo Silva-Carvalho,[ab] Renato Mota,[ab] Sara Duarte-Silva,[cd] Andreia Teixeira-Castro,[cd] Nuno Lamas,[cde] Gonçalo N. P. Oliveira,[f] Yizao Wan,[gh] Fernando Dourado[ab] and Miguel Gama *[ab]



- BNC administred dayly for 21 days
  - **Not absorbed** at the intestine
    - No **hazardous effects**

Promising for BNC!

NCs composite migration



Migration rate depending on:

- Physico-chemical interactions between NCs and actives substance
- Amount, properties and mobility of migrating substance
- Temperature and time of contact
- Type of food (acidic, alcholic or fatty);

NCs composite migration

List of food simulants

Food simulant	
Ethanol 10 % (v/v)	Food simulant A
Acetic acid 3 % (w/v)	Food simulant B
Ethanol 20 % (v/v)	Food simulant C
Ethanol 50 % (v/v)	Food simulant D1
Vegetable oil (*)	Food simulant D2
poly(2,6-diphenyl-p-phenylene oxide), particle size 60-80 mesh, pore size 200 nm	Food simulant E

- Foods with hydrophilic character;
- Acidic foods (pH<4.5);
- Alcoholic foods; lipophilic character;
- Alcoholic foods; oil in water emulsions;
- Foods with free fats at the surface;
- Dry foods

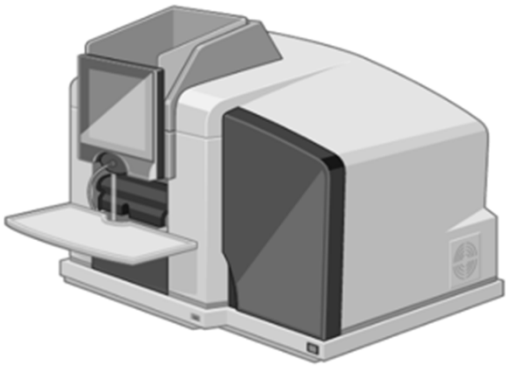
AND

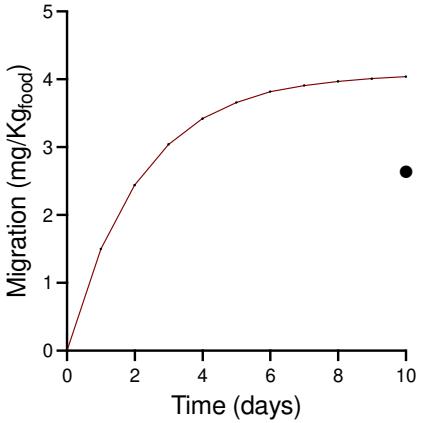
Real food model

- e.g. Pork, Chicken

NCs composite migration

Specific migration



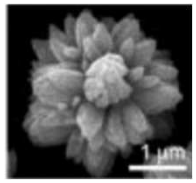


- Most active substances authorised by EFSA
  - Specific migration limit (SML) ($mg_{substance}.Kg^{-1}{}_{food}$)
  - Substances without SML – 60.0 $mg.Kg^{-1}$

BNC as support may decrease migration rate!

Substances incorporation using impregnation method!

6. Case study



ZnO

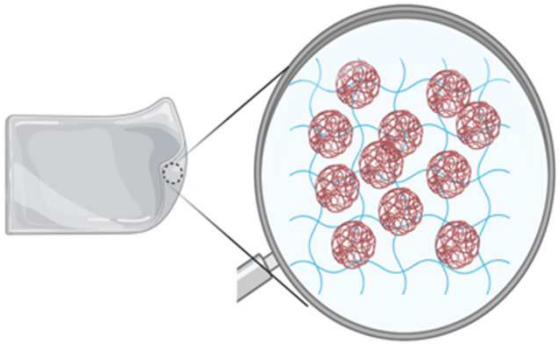
- Low production cost
- Easily scalable
- EFSA approved
- Bio-compatible & non-toxic
- ***Anti-microbial properties***

Efficiency of ZnO NPs highly depends on:
- NPs size
- Morphology
- ZnO dosage

Aims & Scope

Active pad for chicken meat

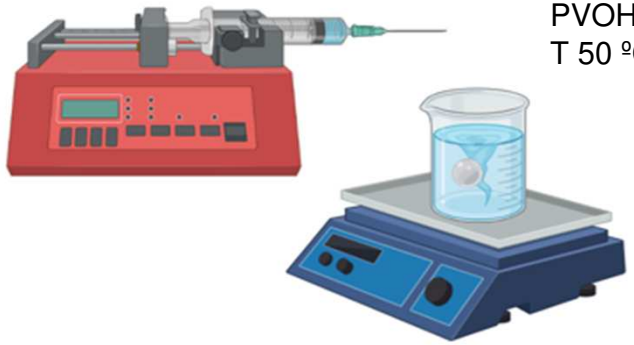
ZnO *In situ* synthesis on BNC



- ZnO optimization
  - NPs size on BNC
  - Dosage on BNC
- Anti-microbial (AM) properties
  - Viable cell count (on chicken skin)
- Migration assay (on chicken skin)

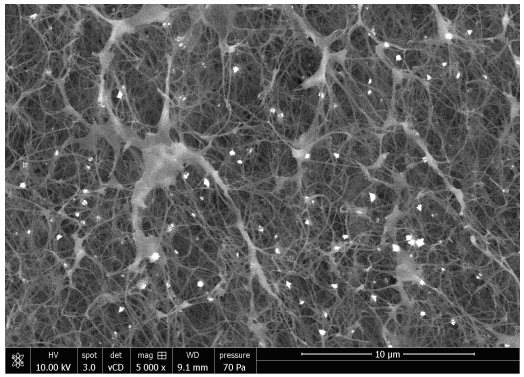
Results – BNC_{ZnO} production & characterization

20 mL NaOH (1M)
0.67 mL/min



BNC in:
$Zn(CH_3COO)_2$
PVOH 0.5% m/v
T 50 ºC; 200 rpm

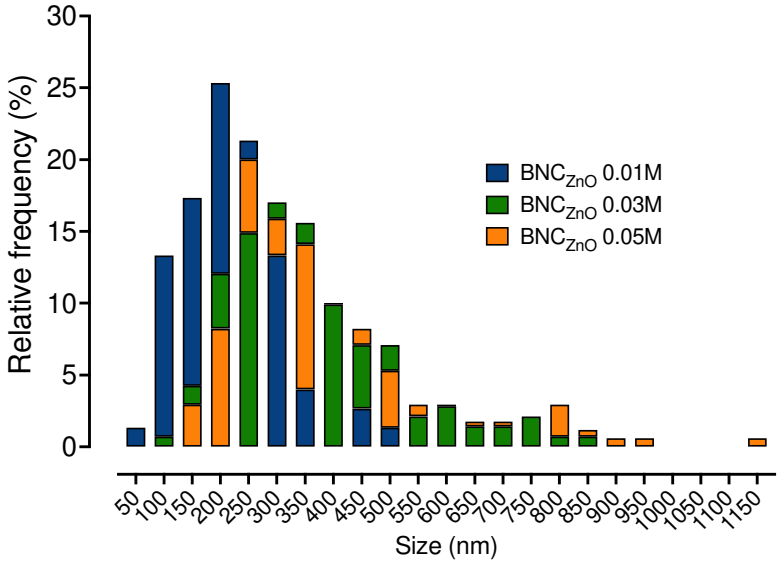
Morphology of BNC_{ZnO}



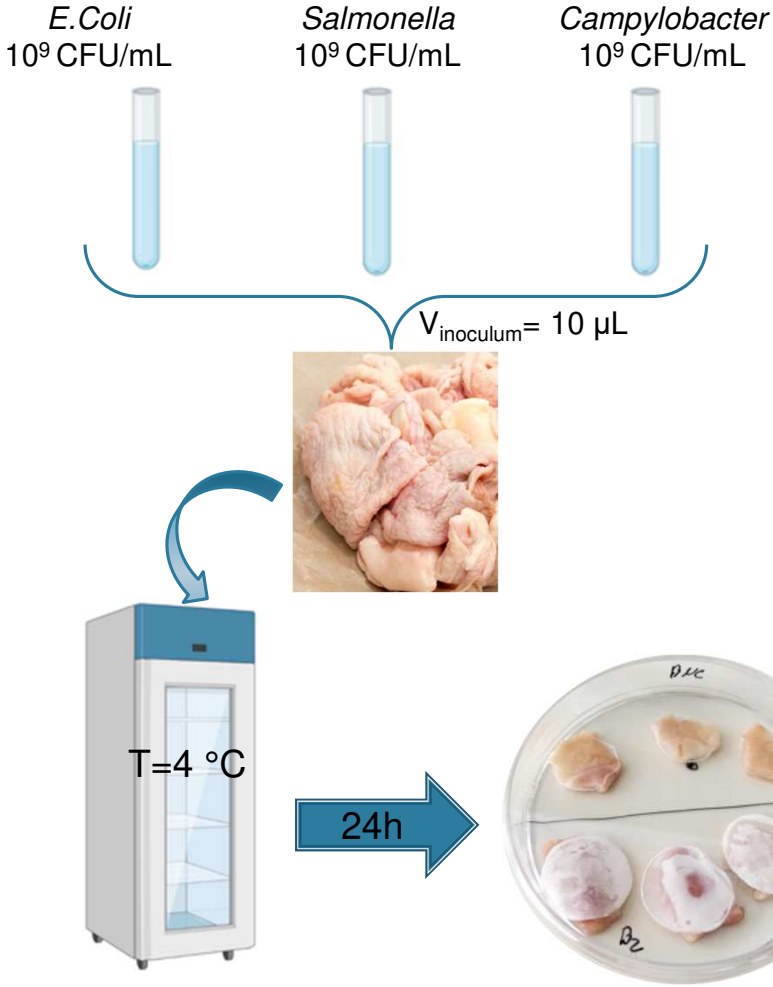
Quantification of Zn by Atomic absorption spectrometer

$Zn(CH_3COO)_2$ (M)	Theorical ZnO (mg)	Zn % (m/m_{BNCZnO}) (AAS)
0.01	0.68	**11.05**
0.03	0.96	**21.12**
0.05	1.48	**27.10**

Particle size measurement from SEM-EDS

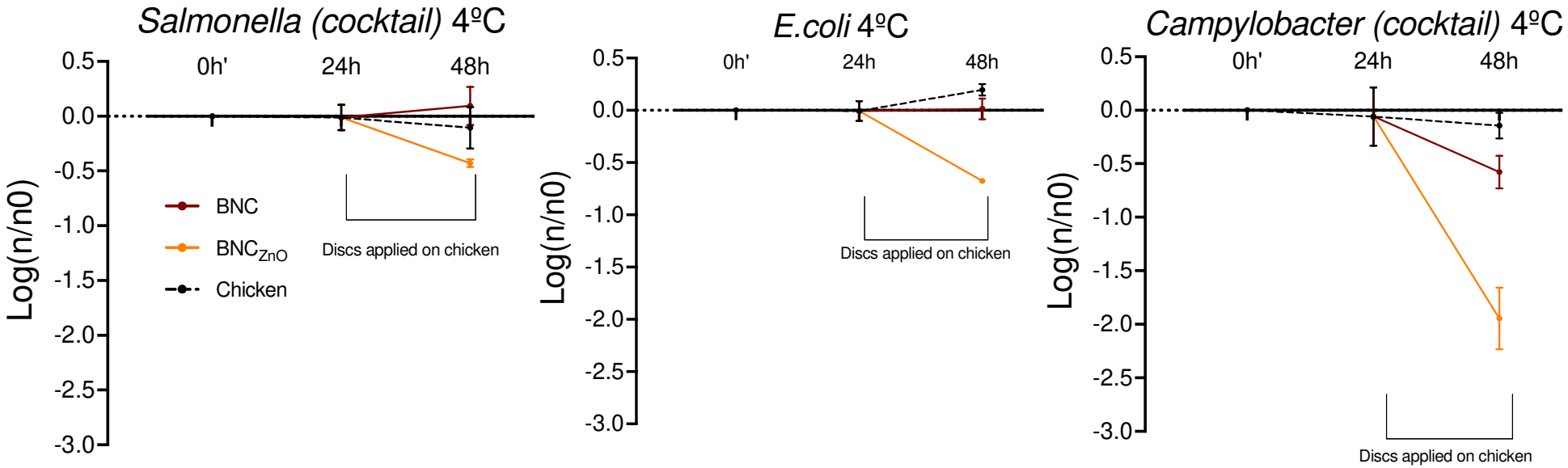


Chicken skin as food model



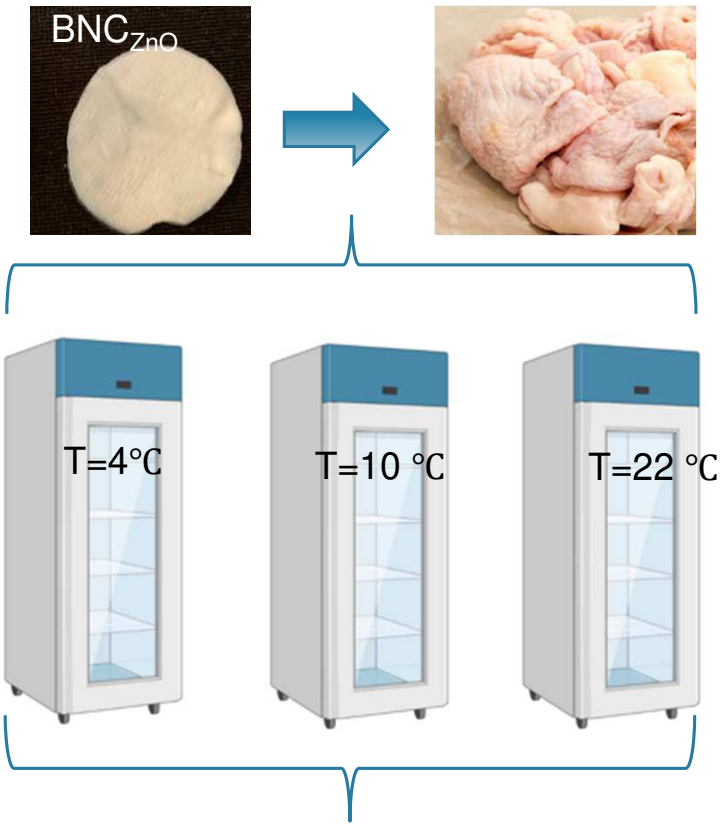
- *Salmonella, Escherichia coli* and *Campylobacter* were inoculated (10µL; 10^9 CFU/mL) into chicken skin and incubated for 24h at 4 °C.
- BNC and BNC_{ZnO} were placed on top of chicken skin samples and incubated for 24h
- CFUs/mL were measured at 0 h' (after 15 min), 24 h and 48h

Chicken skin as food model

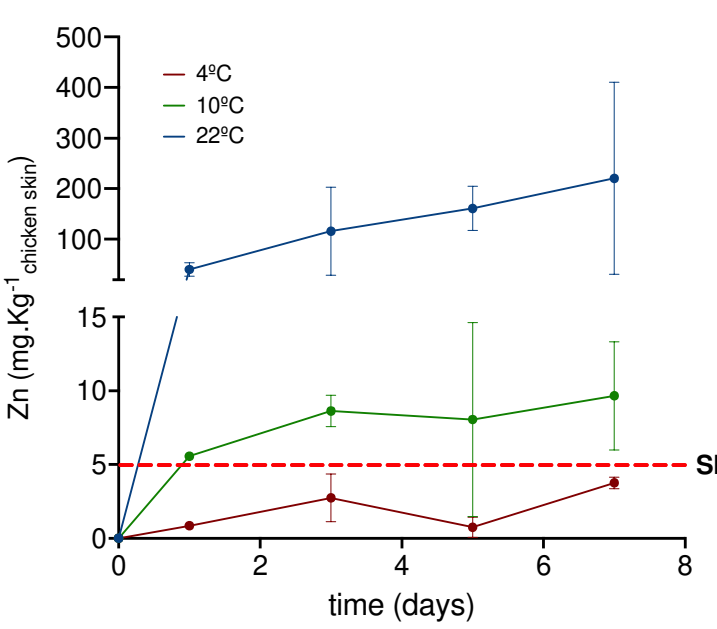


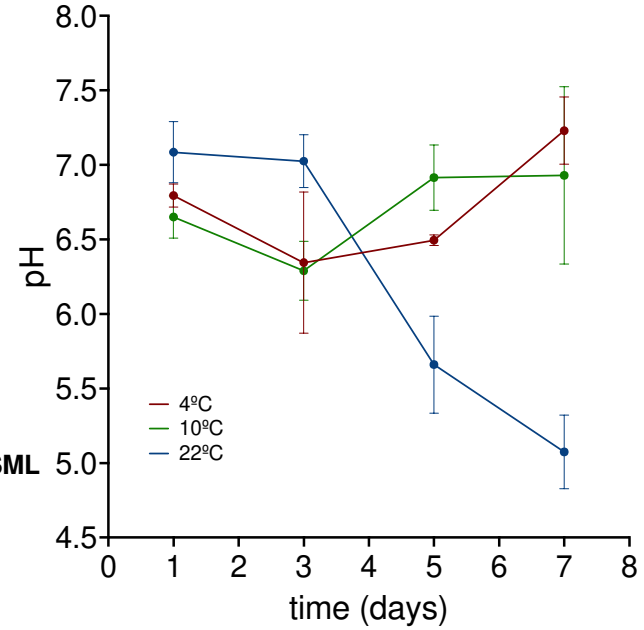
- Activity against *Salmonella and E.Coli*
  - **0.5-1.0 log reduction**
- Highly effective against *Campylobacter*
  - **2.0-2.5 log reduction**

Zn migration on chicken skin



1d, 3d, 5d, 7d, Zn migration





- Zn migration exponentially increased with T
  - Zn concentration equilibrium at lower T;
- Zn migration levels surpassed **SML**
  - > **5.0 mg/Kg.day** at T>10 C
  - Acidification promoted higher migration

Conclusions of the case study

Antimicrobial activity of BNC_{ZnO}

Chicken skin as food model

- BNC_{ZnO} effective on ***Escherichia coli*** and ***Salmonella*** (0.5-1.0 log reduction) and even more effective on ***Campylobacter species*** (2.0 log reduction);

Zn Migration into chicken skin

- Zn migration was temperature and pH dependent;
  - Increased with temperature increase
- Zn migration surpassed SML, at T higher than 10°C;
  - Acidic pH increased Zn solubility

Acknowledgements

- BIOPROTECT - Development of Biodegradable Packaging Material with Active Properties for Food Preservation POCI-01-0247-FEDER-069858;
- Portuguese Foundation for Science and Technology (FCT) through the project UID/BIO/04469/2013;
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COST (European Cooperation in Science and Technology) is a funding agency for research and innovation networks. Our Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research, career and innovation.

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3. Plant vs Bacterial nanocellulose

Yearly cellulose yield (per ha) of different sources

Biotech unit 100m^3/day

COTTON	TREES	BAMBOO	BACTERIAL CELLULOSE
3 tons	2,5 tons	15 tons	300 tons

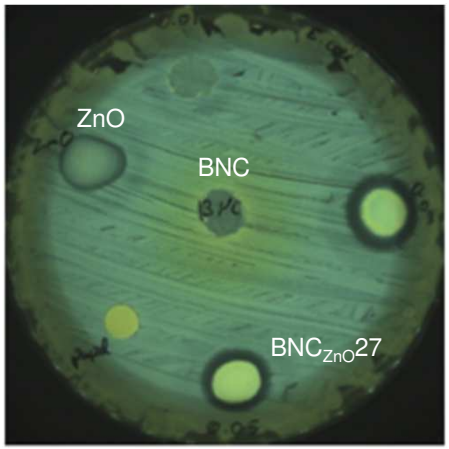
6. – Agar diffusion assay

Inibition of bacterial growth of ZnO on BNC

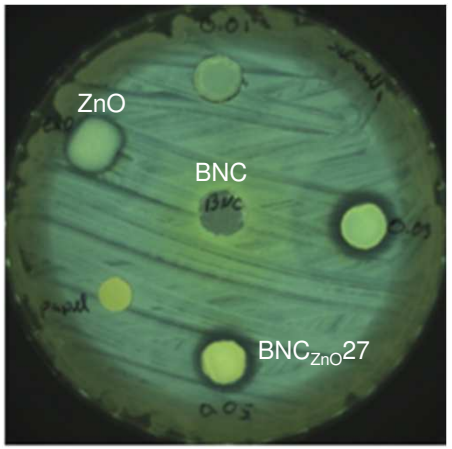
	Bacterial strains	BNC	BNC_{ZnO} **27%m/m**
Gram-	*Salmonella* Enteritidis	✗	✓
Gram-	*Escherichia coli*	✗	✓
Gram-	*Yersinia enterocolitica*	✗	✓
Gram-	*Pseudomonas aeruginosa*	✗	✓
Gram-	*Campylobacter coli*	✗	✓✓
Gram+	*Staphylococcus aureus*	✗	✓
Gram+	*Bacillus cereus**	✗	✓✓

- ZnO on BNC had antibacterial activity
- BNC_{ZnO} effective
  - All Gram- bacteria
  - Gram+
    - *Staphylococcus aureus*
    - *Bacillus cereus*

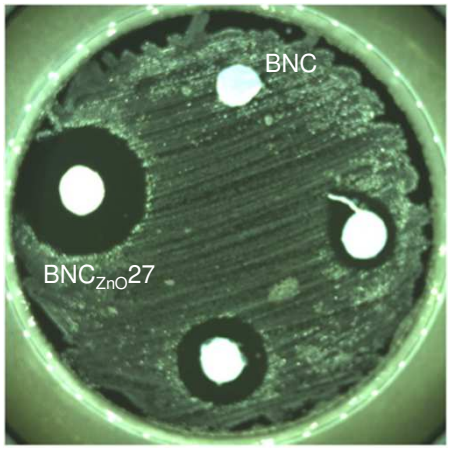
Escherichia coli



***Salmonella* Enteritidis**



Campylobacter coli



6. Zn migration – using food simulants