Chitin nanofibrils in antimicrobial coatings for cellulosic and bioplastic substrates

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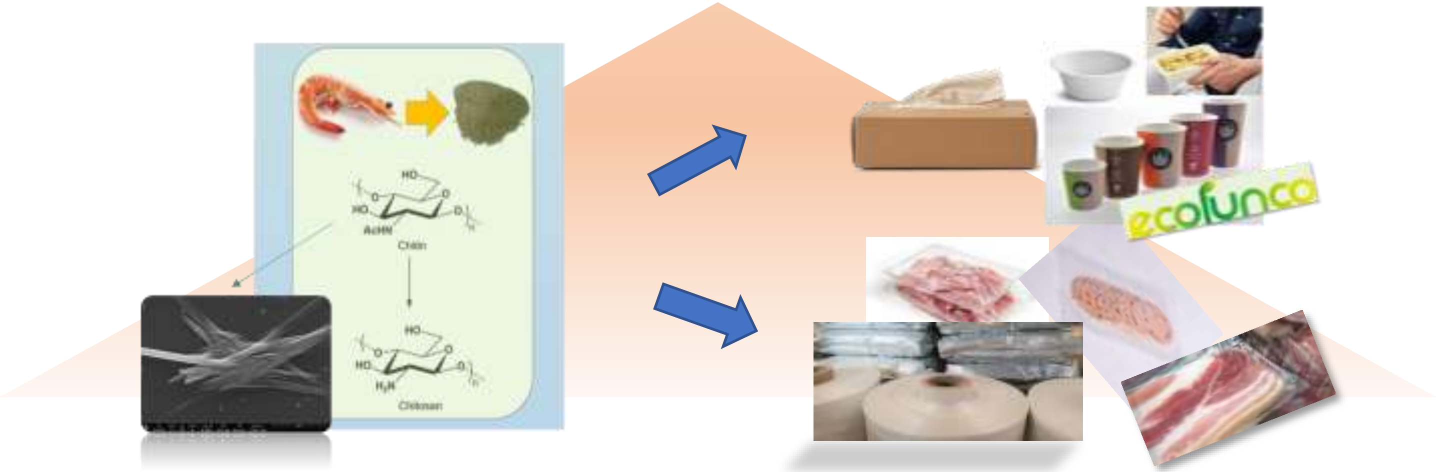

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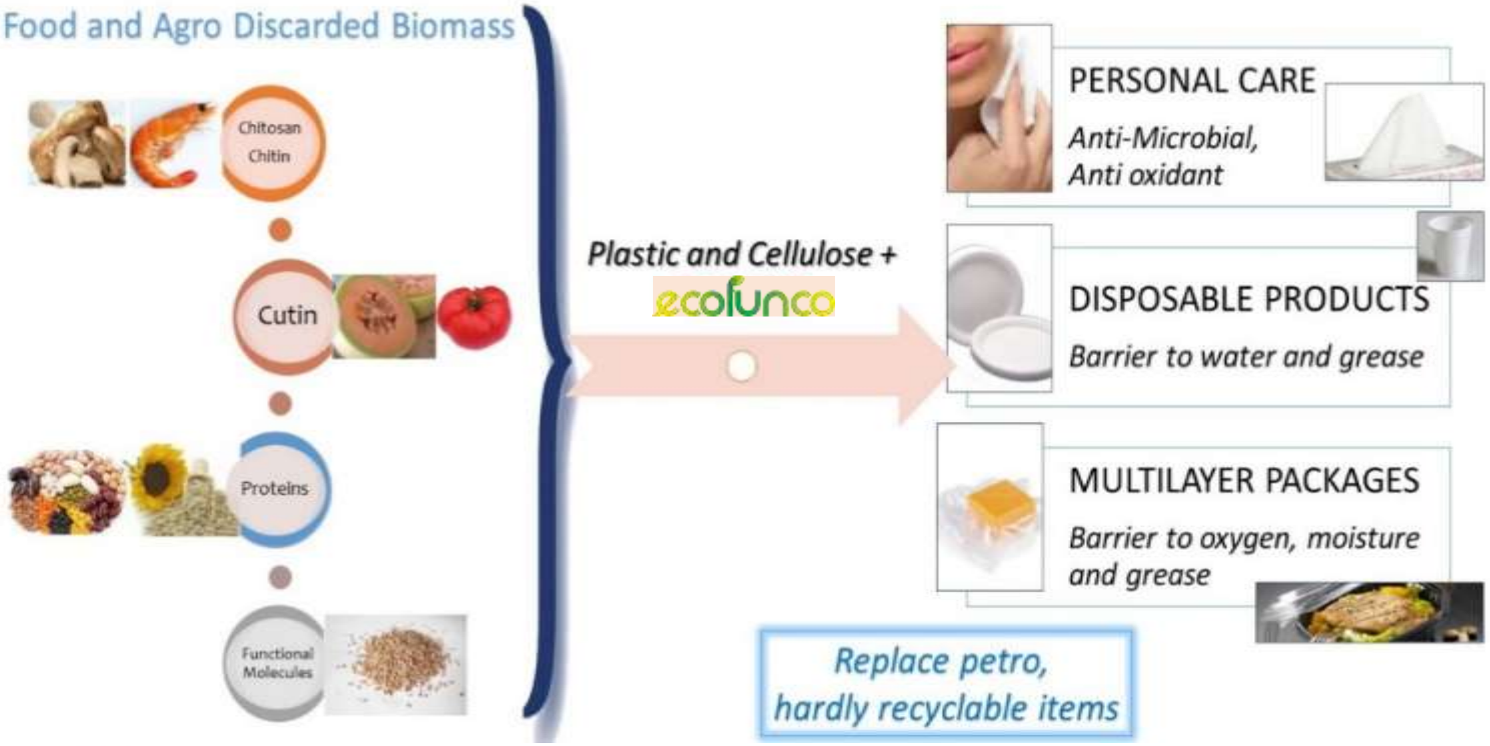
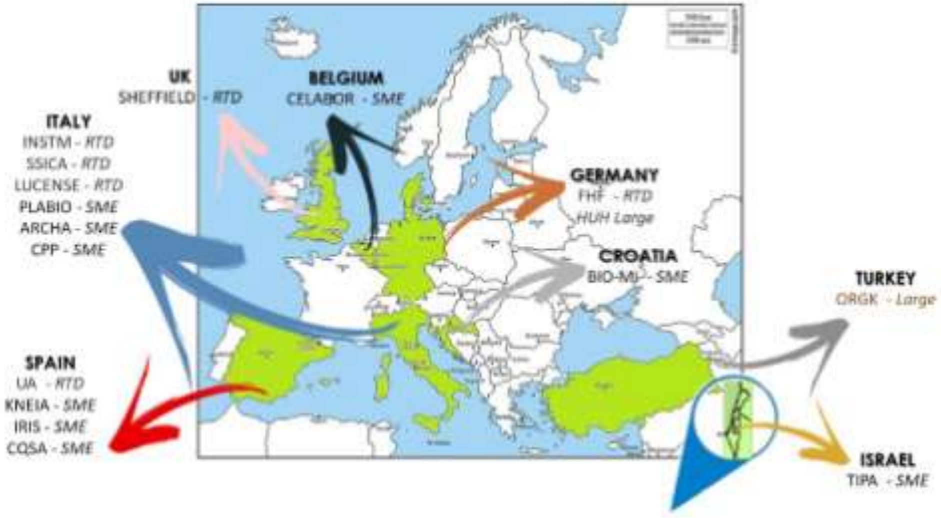
Horizon 2020
European Union Funding
for Research & Innovation

ECOFUNCO project: *Eco sustainable multifunctional biobased coatings with enhanced performance and end of life options*

- Highly performing, economically and environmentally sustainable bio-based coating for plastic and cellulosic substrates
- **17 Partners**, 39 months, started 1st May 2019
- **8 Countries** (Italy, Spain, UK, Belgium, Germany, Croatia, Turkey, Israel)
- **Partners** 6 RTO, 9 SME, 2 Large Enterprises
- **5.5 Million euro** (4.6 Million Euro BBIJU contribution)

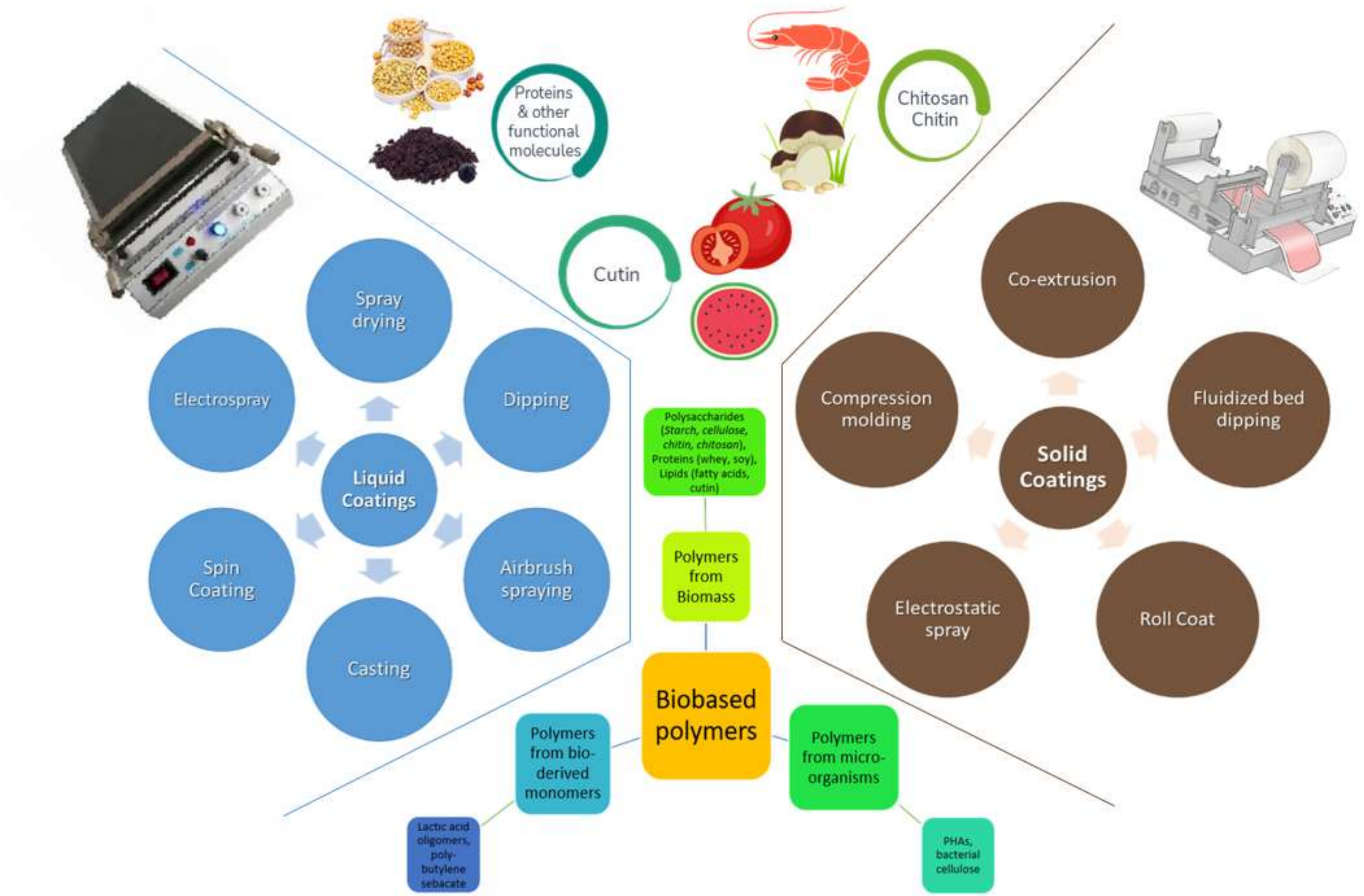




ORGANİK KİMYA · Kneia · celabor · Huhtamaki · PARMA · Universitat d'Alacant Universidad de Alicante · condensia · iris · ARCHA · LUCENSE · SSICA · Fraunhofer · bio-mi · The University Of Sheffield. · Università di Pisa

Methodologies and materials to be used for coating cellulose and bioplastics?

Gigante, V.; Panariello, L.; Coltelli, M.-B.; Danti, S.; Obisesan, K.A.; Hadrich, A.; Staebler, A.; Chierici, S.; Canesi, I.; Lazzeri, A.; Cinelli, P.
Liquid and Solid Functional Bio-Based Coatings.
Polymers **2021**, *13*, 3640.
https://doi.org/10.3390/polym13213640



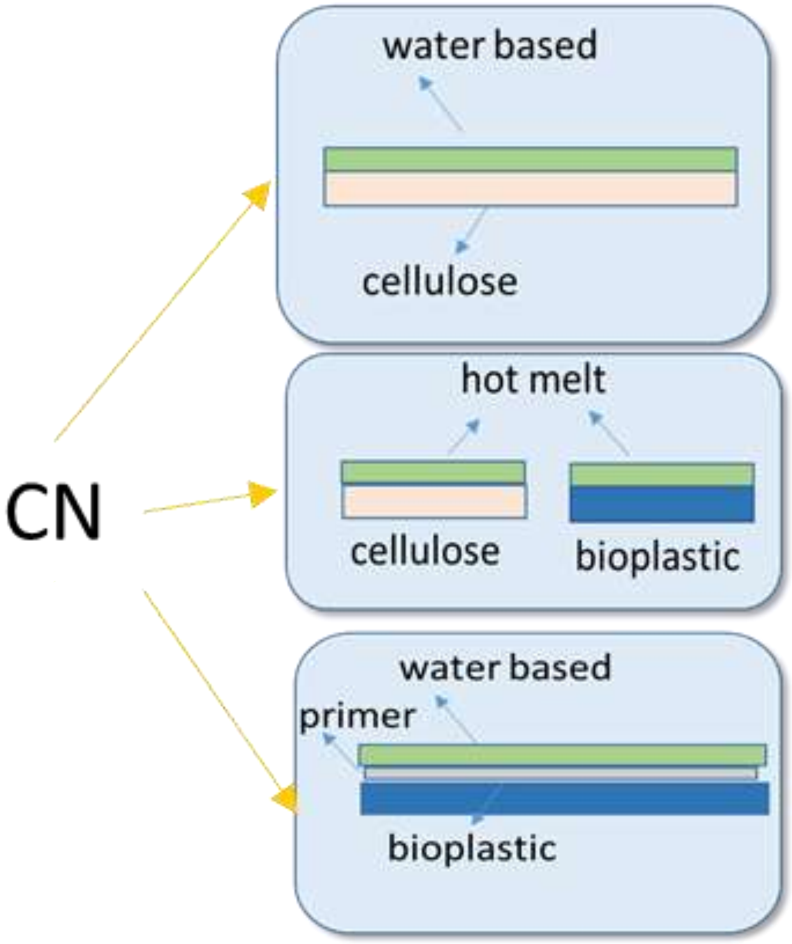
Methodologies and materials to be used for coating?

Gigante, V.; Panariello, L.; Coltelli, M.-B.; Danti, S.; Obisesan, K.A.; Hadrich, A.; Staebler, A.; Chierici, S.; Canesi, I.; Lazzeri, A.; Cinelli, P. **Liquid and Solid Functional Bio-Based Coatings.** *Polymers* **2021**, *13*, 3640. https://doi.org/10.3390/polym13213640

Table 1. Brief overview of biomolecules mostly used for coating formulations.

Bio-Based Polymer	Preparation	Application Method	Properties Improved and Main Results	REF
Chitin	0–2 wt.% chtin nanowhiskers dissolved in H_2SO_4 and glycerol.	Casting method on maize-starch films.	Evident antimicrobial resistance vs. Gram-positive *Listeria monocytogenes*.	[30]
	2 wt.% of water suspension of nanofibrils dispersed in PEG 8000.	Spray dryer on bioplastics films.	Antimicrobial and skin-regenerative improvements.	[31]
Chitosan	Chitosan (2 wt.%) and glycerol (2 wt.%) dissolved in a 1% (vol/vol) aqueous solution of acetic acid.	Chromatography plate coater application onto PP films. corona-treated	Evident antimicrobial resistance vs. *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli*.	[32]
	Chitosan concentration of 0.02 g/mL in acetic acid mixed in equal volumes with hydroxypropyl methylcellulose.	Thin-layer chromatography plate coater on plastic films.	Excellent long-term antilisterial effect.	[33]
Lignin	Dissolution in acetone of different amounts of softwood kraft lignin and evaporation of the solvent.	Erichsen coater on to a paperboard substrate.	Evident decrease in Oxygen Transmission Rate (OTR) value and a stable contact angle with respect to paperboard alone.	[34]
	Lignin estereified with palitic and lauric acid chloride in a mixture 3:1 ethanol/water.	Erichsen coater on a commercial paperboard substrate.	Good barrier properties against O_2 and H_2O	[35]
Cellulose derivates	Cellulose nitrate ester (CMCN) were dissolved in mixed solvents systems in different amounts.	Solvent casting method.	Gas and water barrier optimized.	[36]
	Hydroxypropyl methylcellulose acetate succinate plasticized with triethyl citrate and acetylated monoglyceride	Centrifugal granulator for feeding the coating powder and spraying simultaneously the plasticized.	Improved gastric resistance, coating efficiency, and processing stability	[37]
Proteins	Whey proteins with hydrolysed lactose at different contents	"Bird-type" applicator onto paperboard substrates	Good grease resistance and minimization of plasticizer migration	[38]
	12 g of whey proteins in 6 g of glycerol and 30 g of deionized water	Compression molding onto cellulosic substrates	Gas-barrier properties improvements	[39]

PLATFORM OF CN BASED COATINGS EXPLORED IN ECOFUNCO



Natural composites contain chitin

Excluding proteins, structural materials widely diffused in living beings are based on two polysaccharides: cellulose and chitin

POLYSACCHARIDES BIOMATERIAL FOR STRUCTURAL APPLICATION

wood: micro-composite of cellulose in a lignin resin

exoscheleton of arthropodes: micro-composite of chitin fibrils in a protein matrix (containing also calcium carbonate)

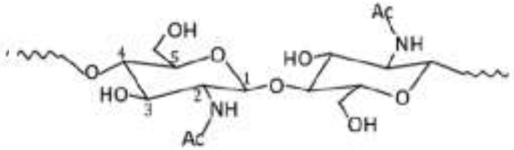
SOURCES OF CHITIN

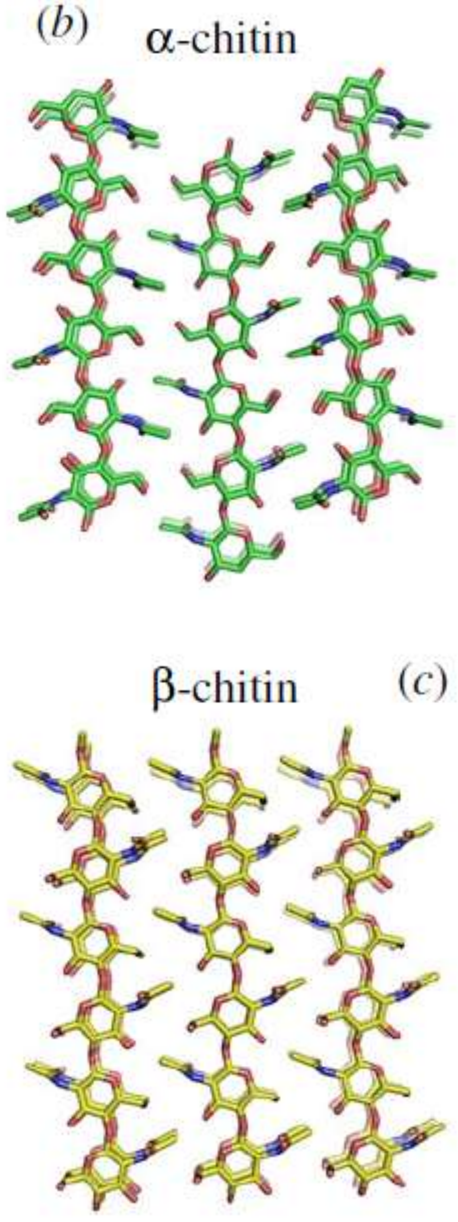
Worldwide chitin based waste material from the fishing industry, exceeds 25 billion tons/year

The processing of 1 kg of shrimp produces 0,75 kg of waste (e.g: chitin containing shells) and 0,25 Kg of final food [1]

High availability of chitin based waste from shrimp and crabs[2] as food industry produces them in huge amount

[1] *JG Fernandez et al., Adv. Funct. Mater. 2013, 4454-4466*
[2] *M Mincea et al., Rev. Adv. Mater. Sci. 30, 2012, 225-242*



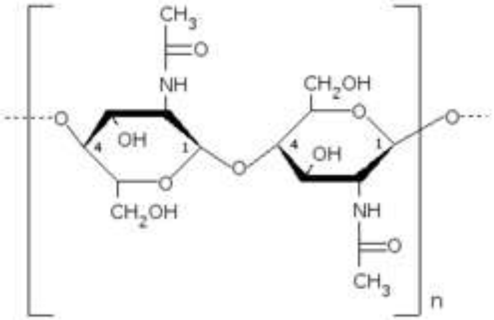


Chitin microfibrils are constituted of alternating crystalline and amorphous domains.
The most abundant kind of crystalline chitin is the α-chitin

CHITIN CRYSTALS	where	Structural features
α-CHITIN	     krill, insect cuticle, fungal and yeast cell walls	Molecules arranged in antiparallel fashion (strong H bonding)
β-CHITIN	 Squid pens  Tube worms	Molecules arranged in parallel fashion
γ-CHITIN	 Beetle cocoons	Molecules arranged in both parallel and anti-parallel fashion

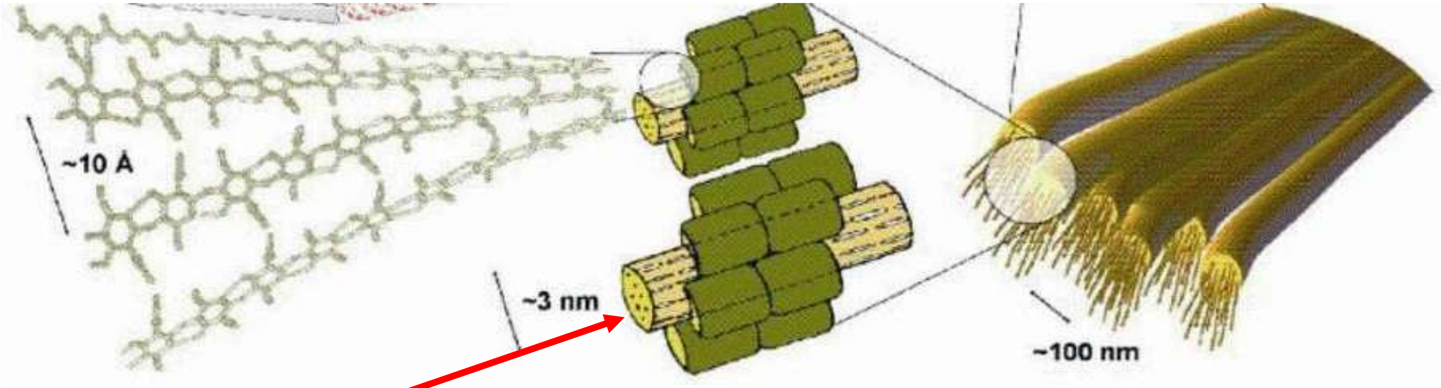
M Mincea et al., Rev. Adv. Mater. Sci. 30, 2012, 225-242

structure

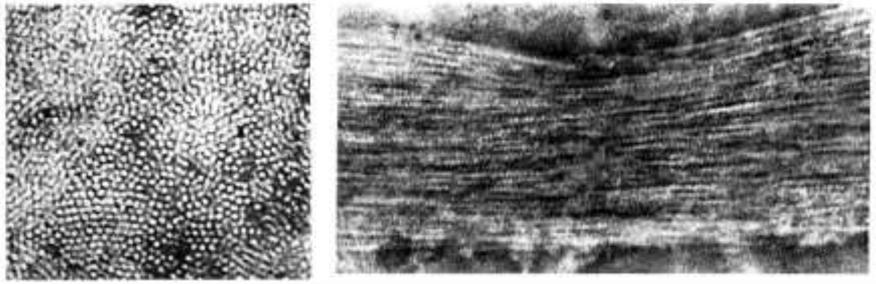


Inter-macromolecolar hydrogen bonding

nano-assembly

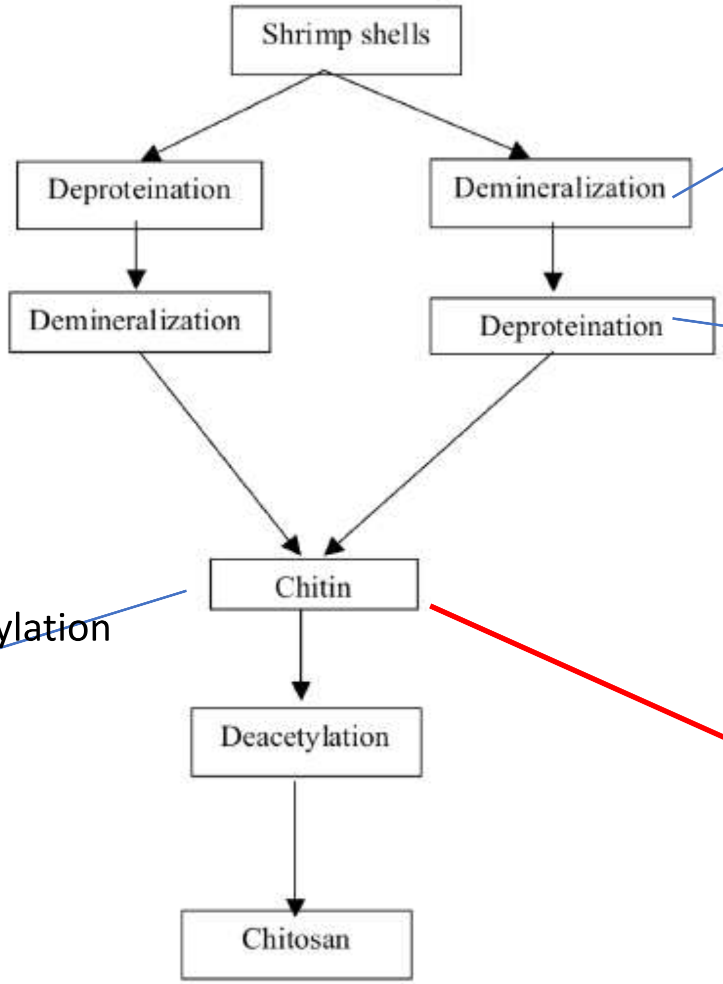


micro-assembly



the chitin resulting rigid substance is ***immersed in a matrix*** of proteins and calcium carbonate

CHITIN PURIFICATION



Acid treatment

Basic treatment

Mildly deacetylation

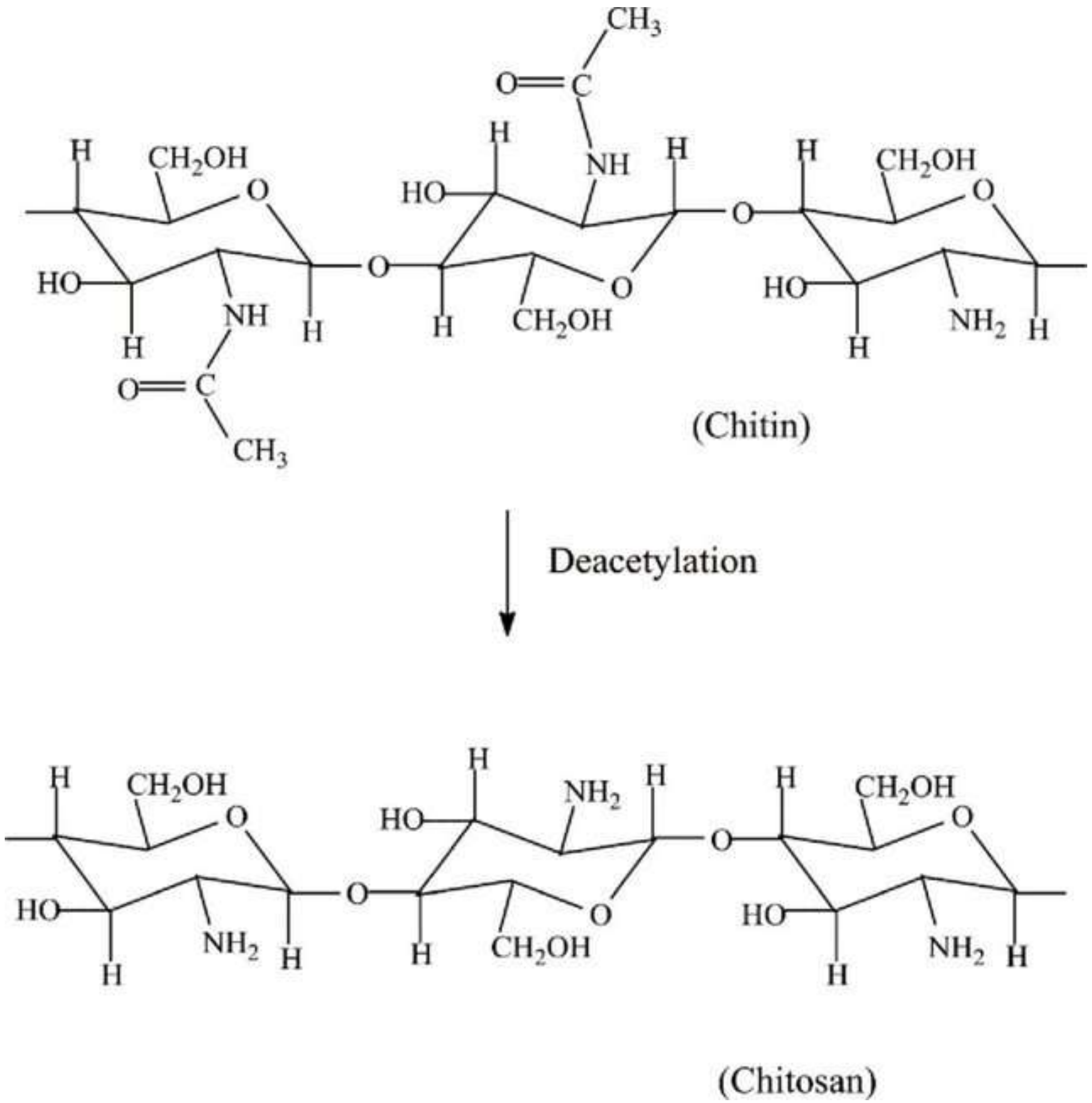
Chitin nanofibrils

Acetylation degree should be higher than 0,9 and molecular weight in the range 1-2,5·10 ^{6}

MNV Ravi Kumar, React. Funct. Polym, 46, 2000, 1-27

Microfibers based materials

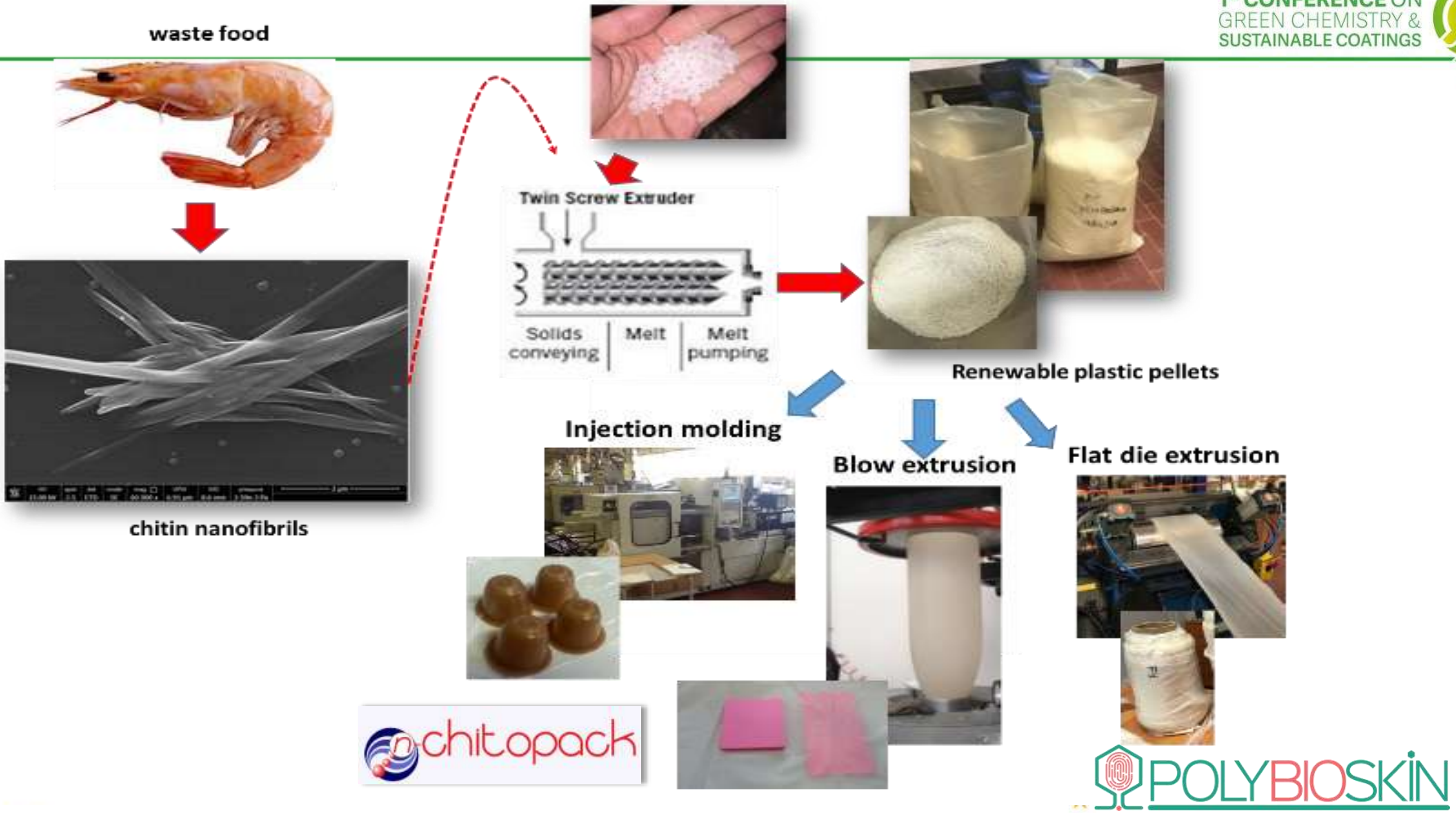
N. Van Toan, The Open Biomaterials Journal, 1, 2009

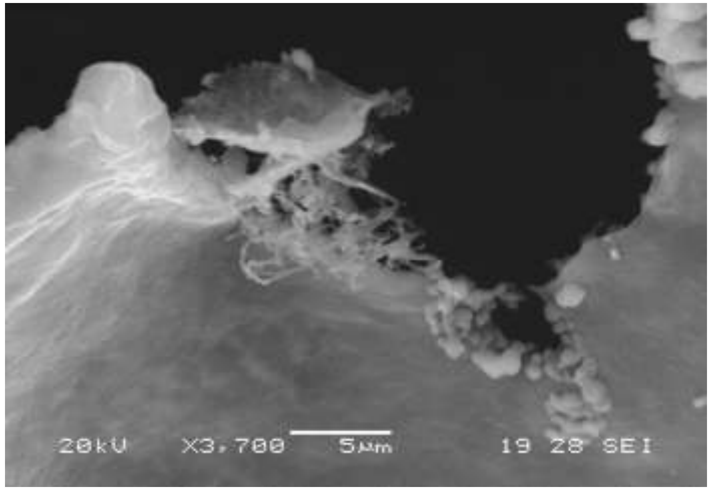


Removal by hydrolysis of acetyl groups that are converted in $-NH_2$ groups

Strong bases used at high T result in full deacetylation to chitosan.

Milder conditions can give chitin nanofibrils (P. Morganti patent, WO2006048829A3)

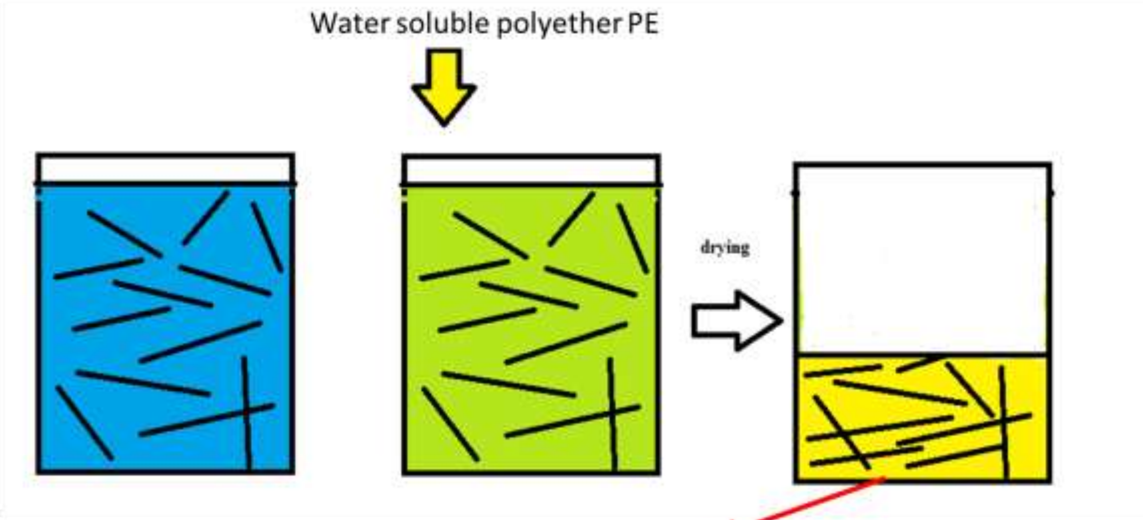

waste food
chitin nanofibrils
Twin Screw Extruder
Solids conveying
Melt
Melt pumping
Renewable plastic pellets
Injection molding
Blow extrusion
Flat die extrusion
n-chitopack
POLYBIOSKIN



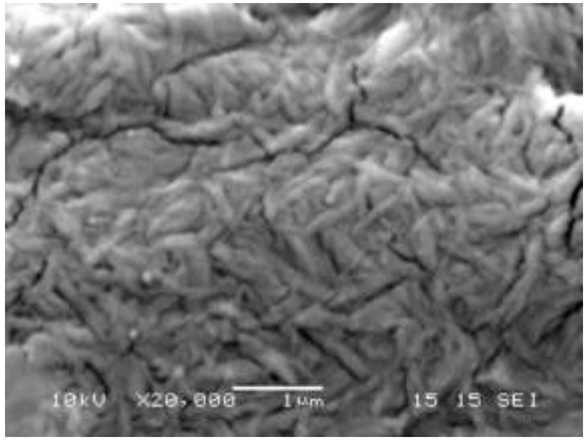
NANODISPERSION IN A BIOPOLYESTER MATRIX

MASTER-BATCH APPROACH

The preparation of a NC suitable for dispersing in PLA requires to keep separated nanofibrils during the drying.
Different polyethers were added to these water based suspensions.



Solid or semi-solid composites with CN maintaining nano-dispersion



Advanced Materials Letters

Research Article 2019, **10**(6), 425-430 Advanced Materials Letters

Chitin Nanofibrils in Renewable Materials for Packaging and Personal Care Applications

Maria-Beatrice Coltelli[1,2*], Vito Gigante[1,2], Luca Panariello[1,2], Laura Aliotta[1,2], Pierfrancesco Morganti[3], Serena Danti[1,2], Patrizia Cinelli[1,2], Andrea Lazzeri[1,2]

International Journal of *Molecular Sciences*

Article

Chitin Nanofibrils in Poly(Lactic Acid) (PLA) Nanocomposites: Dispersion and Thermo-Mechanical Properties

Maria-Beatrice Coltelli [1,2,*], Patrizia Cinelli [1,2], Vito Gigante [1,2], Laura Aliotta [1,2], Pierfrancesco Morganti [3,4], Luca Panariello [1,2] and Andrea Lazzeri [1,2,*]

https://www.mdpi.com/books/pdfview/book/3307

POLYBIOSKIN

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Guest Editors: Luca Di Palma, Elisabetta Petrucci, Marco Stoller

ISBN 978-88-95608- 50-1; ISSN 2283-9216

Degradability and Sustainability of Nanocomposites Based on Polylactic Acid and Chitin Nano Fibrils

Patrizia Cinelli[a,*], Maria Beatrice Coltelli[a], Norma Mallegni[a], Piefrancesco Morganti[b], Andrea Lazzeri[a]

Journal of *Functional Biomaterials*
MDPI
J. Funct. Biomater. **2020**, *11*, 21; doi:10.3390/jfb11020021

Article

Properties and Skin Compatibility of Films Based on Poly(Lactic Acid) (PLA) Bionanocomposites Incorporating Chitin Nanofibrils (CN)

Maria-Beatrice Coltelli [1,2,*], Laura Aliotta [1,2], Alessandro Vannozzi [1,2], Pierfrancesco Morganti [3], Luca Panariello [1,2], Serena Danti [1], Simona Neri [4], Cristina Fernandez-Avila [4], Alessandra Fusco [1,5], Giovanna Donnarumma [1,5] and Andrea Lazzeri [1,2]

International Journal of *Molecular Sciences*

MDPI

Article

Chitin Nanofibrils and Nanolignin as Functional Agents in Skin Regeneration

Serena Danti [1,2,*], Luisa Trombi [2], Alessandra Fusco [2,3], Bahareh Azimi [2], Andrea Lazzeri [1,2], Pierfrancesco Morganti [3], Maria-Beatrice Coltelli [1,2] and Giovanna Donnarumma [2,3]

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check for updates

nanomaterials

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Article

Chitin Nanofibril-Nanolignin Complexes as Carriers of Functional Molecules for Skin Contact Applications

Maria-Beatrice Coltelli [1,2,*], Pierfrancesco Morganti [3], Valter Castelvetro [1,4], Andrea Lazzeri [1,2], Serena Danti [1,2], Bouchra Benjelloun-Mlayah [5], Alessandro Gagliardini [6], Alessandra Fusco [1,7] and Giovanna Donnarumma [1,7]

Nanomaterials **2022**, *12*, 1295. https://doi.org/10.3390/nano12081295

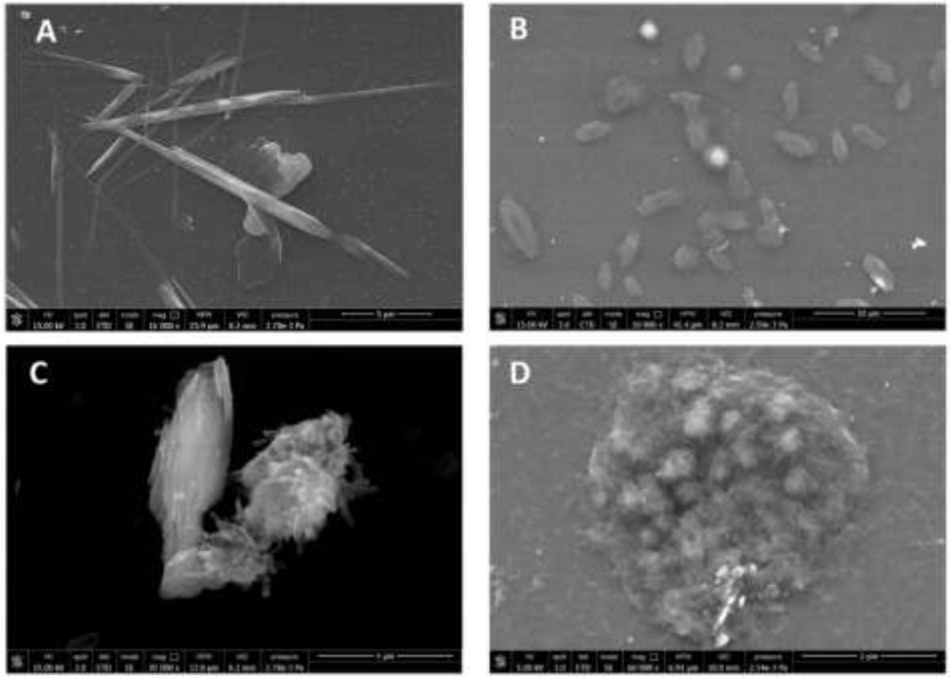


Figure 2. FE-SEM micrographs of (**A**) pure CN from deposition from diluted water suspension; (**B**) CN-NL complex; (**C**) and (**D**) CN-NL complex at higher magnification.

Tests about viability of cells and their immunomodulatory behavior showed that CN, NL and specifically CN-NL complexes are very promising for skin contact applications, such as for coating biomedical, personal care and cosmetic products

Application onto cellulosic board of chitin nanofibrils


European Polymer Journal 113 (2019) 328–339

Contents lists available at ScienceDirect

European Polymer Journal

journal homepage: www.elsevier.com/locate/europolj

Chitosan and nano-structured chitin for biobased anti-microbial treatments onto cellulose based materials

Luca Panariello[a], Maria-Beatrice Coltelli[a,b], Marco Buchignani[c], Andrea Lazzeri[a,b,*]

Components

Treatments preparation

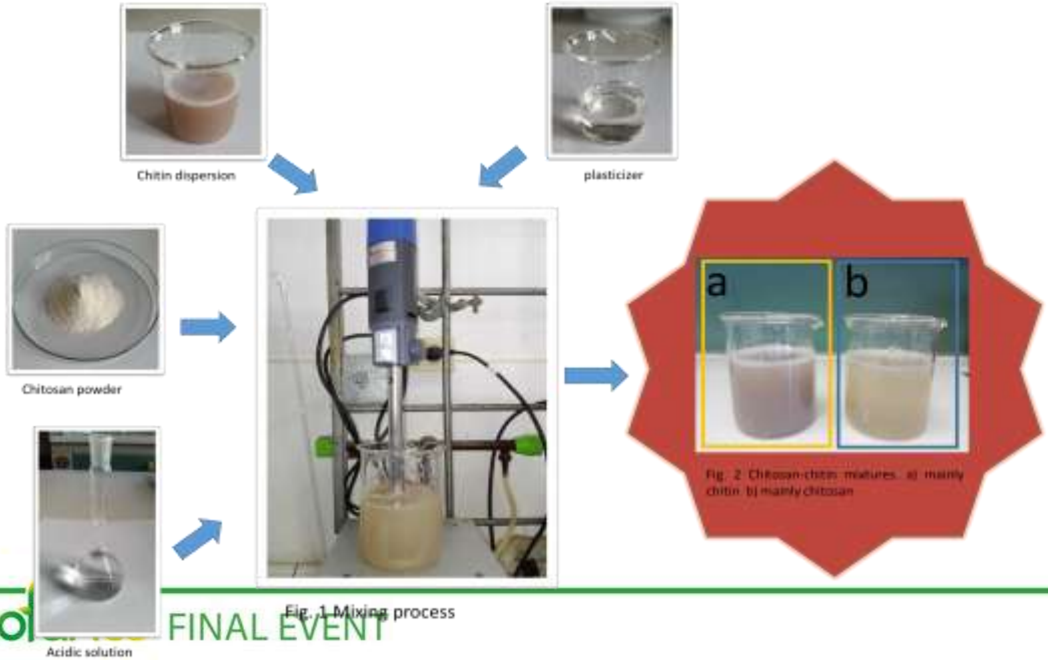
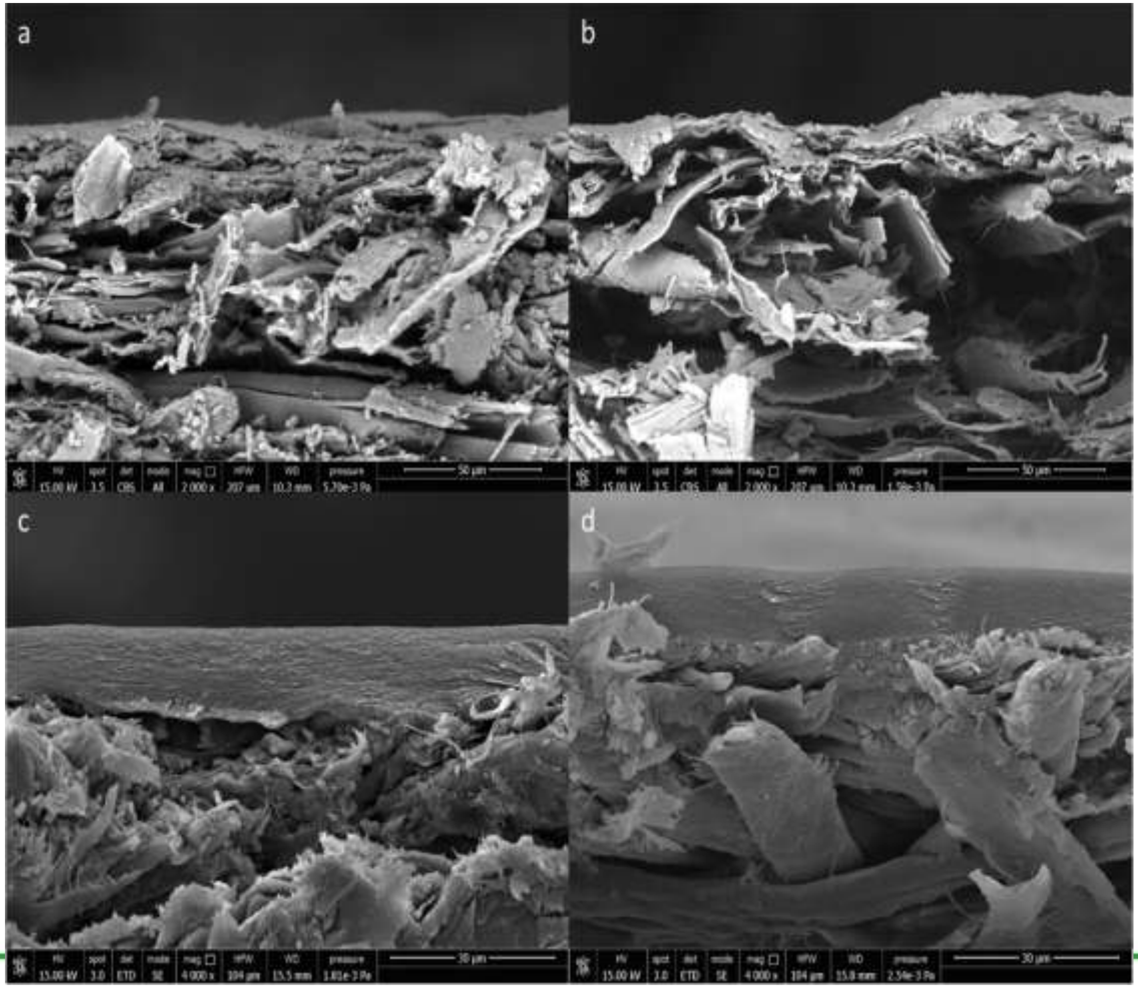


Fig. 1 Mixing process

Fig. 2 Chitosan-chitin mixtures a) mainly chitin b) mainly chitosan

INNOCARTOPACK project , funded by cassa di Risparmio di Lucca

recycled **virgin**

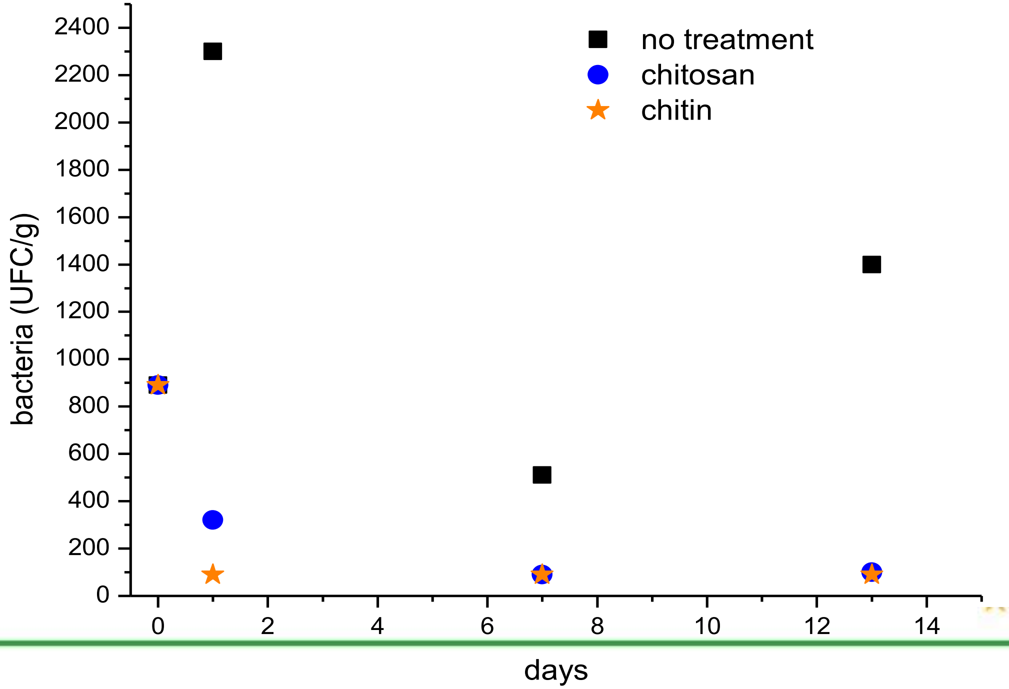


Treatments	Bacteria (UFC/m^2)	Molds/yeasts (UFC/m^2)
Without treatments	1875	1458
Chitin 2,5%	208	208
Chitin 2,5% + 30 % plast	208	208
Chitosan 2,5%	208	833
Chitosan 0,5%/chitin 2%	417	<200
Chitin 2,5%	<200	<200
Chitosan 0,5%/chitin 2% + 30% plast	833	10417

Data for virgin board

- Antimicrobial test by contact plate methodology on paperboard samples

- Antimicrobial tests onto fresh food stored in treated and untreated paperboards



Microbiological tests onto food

Improved packaging :
> Shelf life !!!!

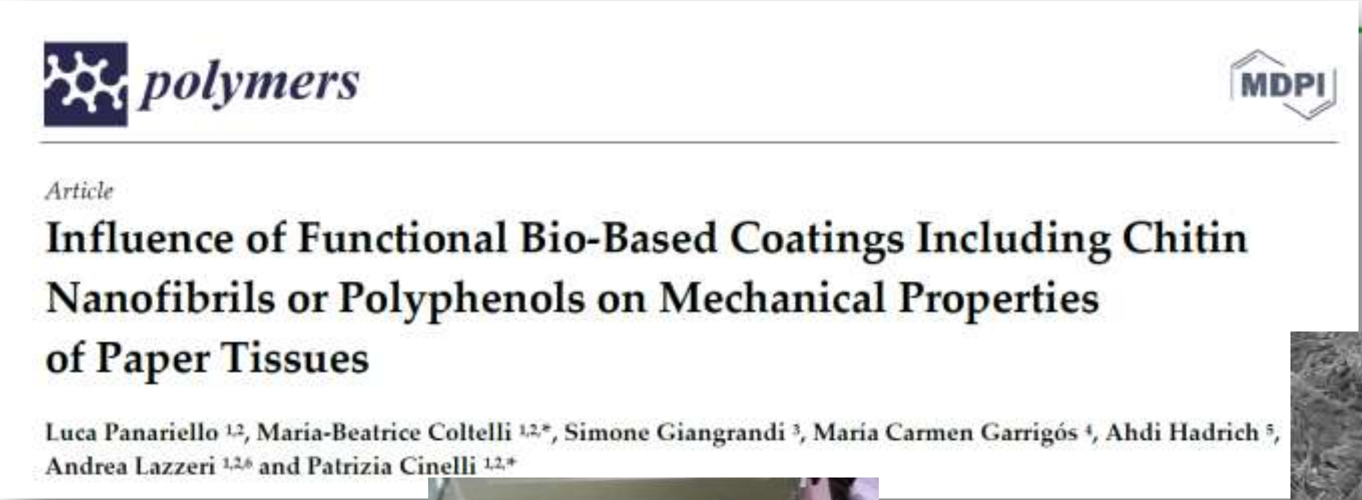
The suspension was obtained through a chemical pre-treatment followed by a mechanical defibrillation process using an ultra-fine friction grinder

From bacteria contact plate tests it can be noticed the antimicrobial effect of both chitin and chitosan on recycled board packaging.

Sample	TOTAL BACTERIA – CONTACT PLATE			MOLDS/YEASTS – CONTACT PLATE		
	Colonies	Area (cm^2)	**UFC/100 cm^2**	Colonies	Area (cm^2)	**UFC/100 cm^2**
Virgin pure	3	72	**4**	1	72	**1**
Recycled pure	835	72	**1.160**	4	72	**6**
Virgin + CELABOR	5	72	**7**	19	72	**26**
Recycled + CELABOR	13	72	**18**	17	72	**24**
Virgin + Chitosan	0	72	**0**	5	72	**7**
Recycled + Chitosan	28	72	**39**	30	72	**42**

Bending tests demonstrated that the treatments did not alter significantly the cardborad mechanical behaviour

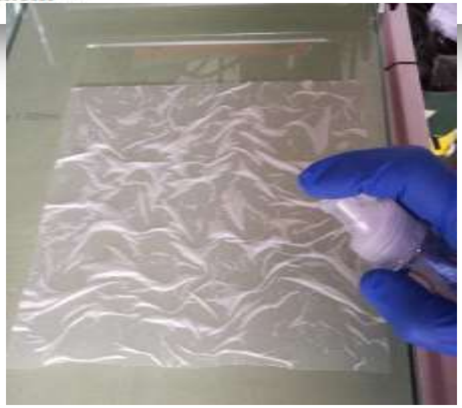
Application onto cellulose substrates of chitin nanofibrils: tissue


polymers
MDPI

Article

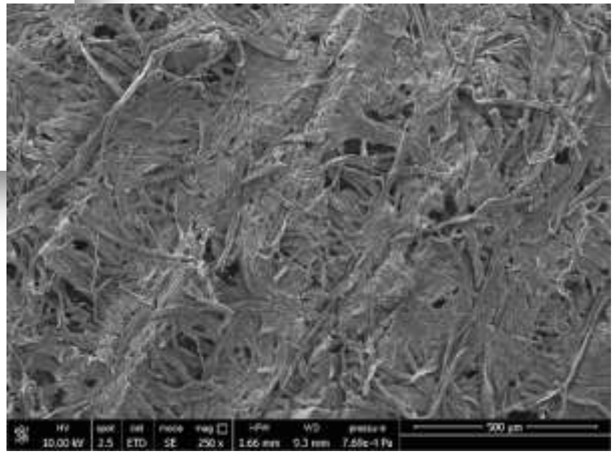
Influence of Functional Bio-Based Coatings Including Chitin Nanofibrils or Polyphenols on Mechanical Properties of Paper Tissues

Luca Panariello [1,2], Maria-Beatrice Coltelli [1,2,*], Simone Giangrandi [3], María Carmen Garrigós [4], Ahdi Hadrich [5], Andrea Lazzeri [1,2,6] and Patrizia Cinelli [1,2,*]

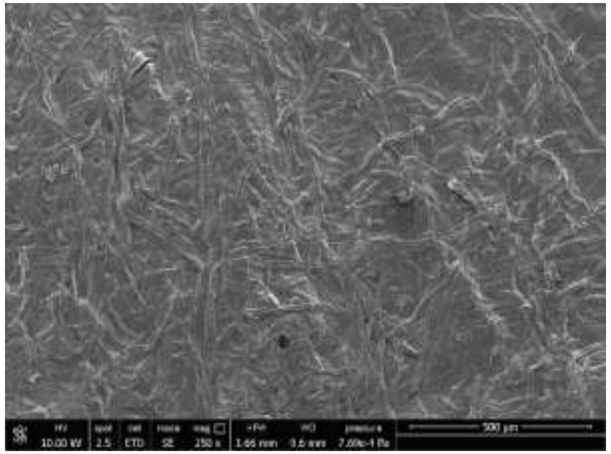


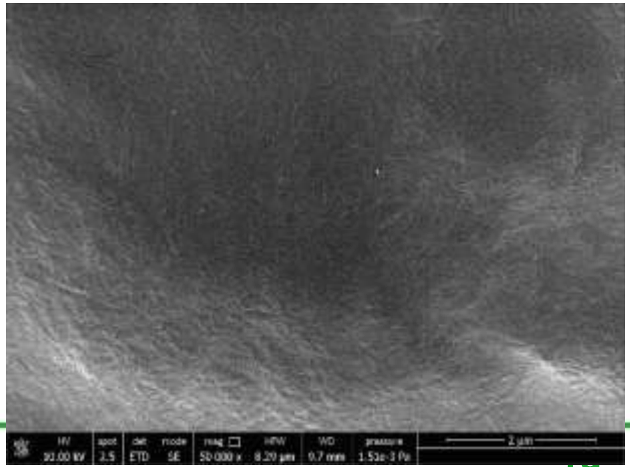
ATR-IR used to control homogenenity

paper tissue



paper tissue treated with CNs





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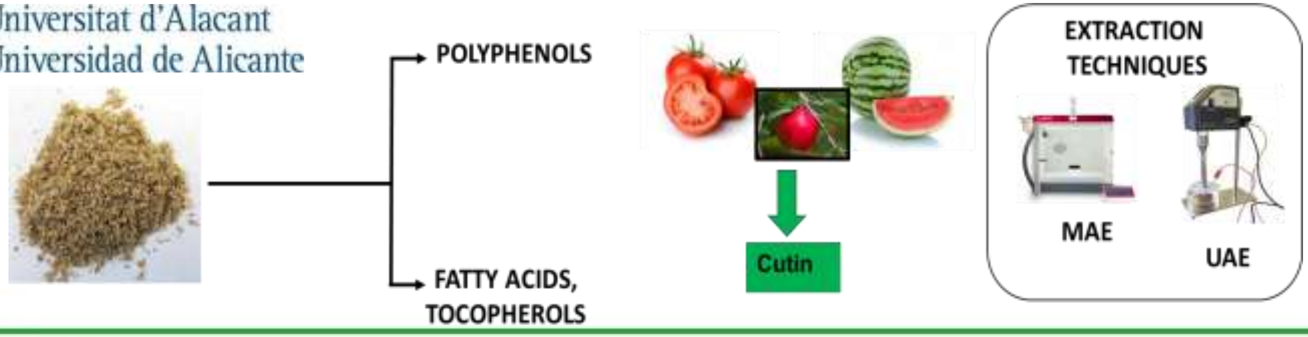
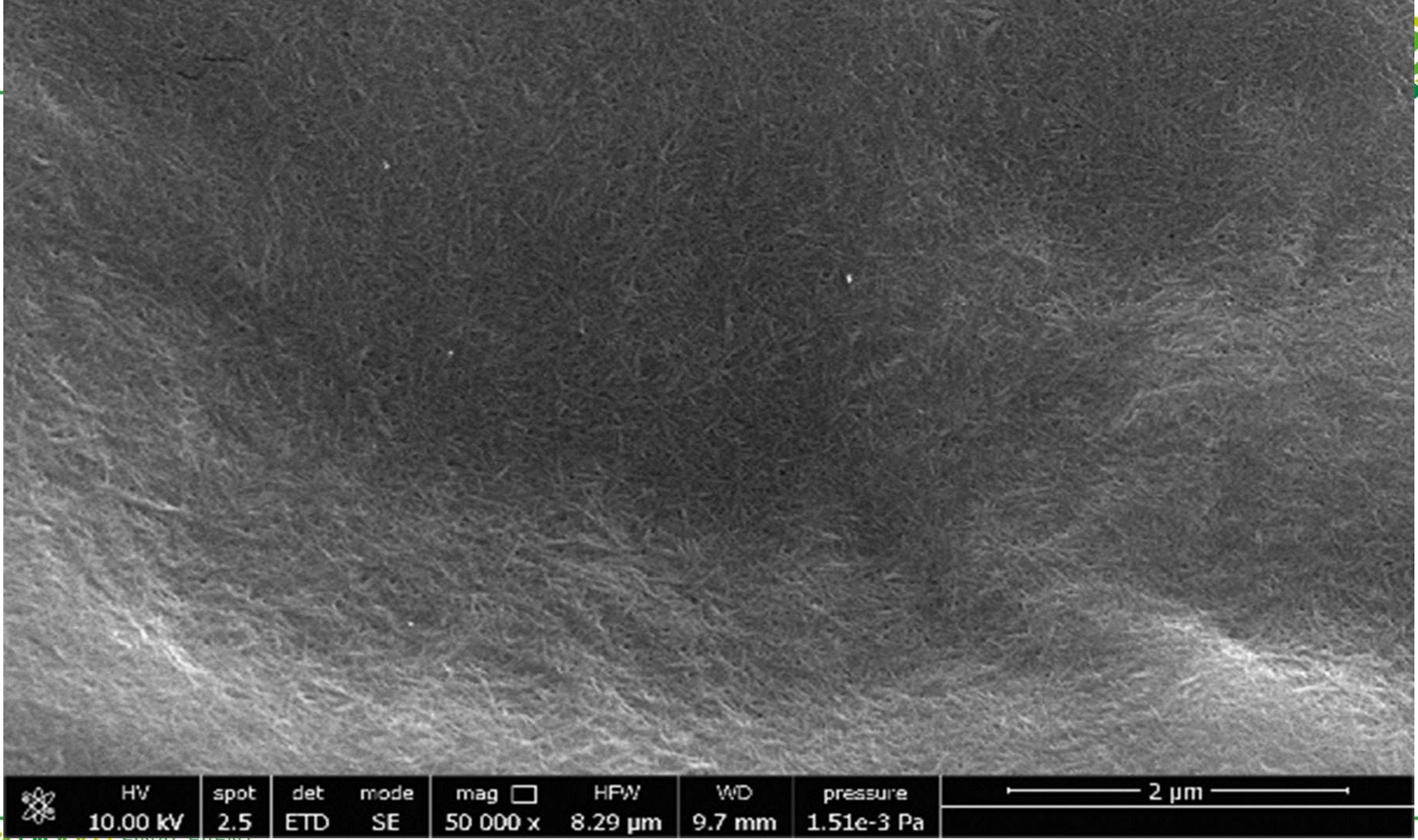
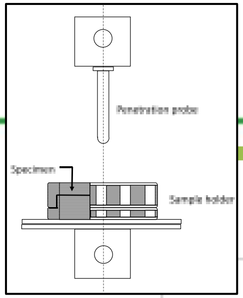
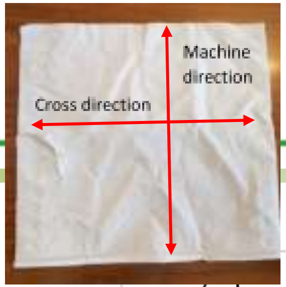
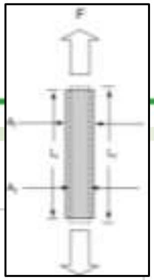
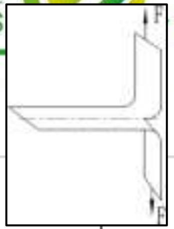



HV
10.00 kV
spot
2.5
det
ETD
mode
SE
mag
50 000 x
HFW
8.29 µm
WD
9.7 mm
pressure
1.51e-3 Pa
2 µm

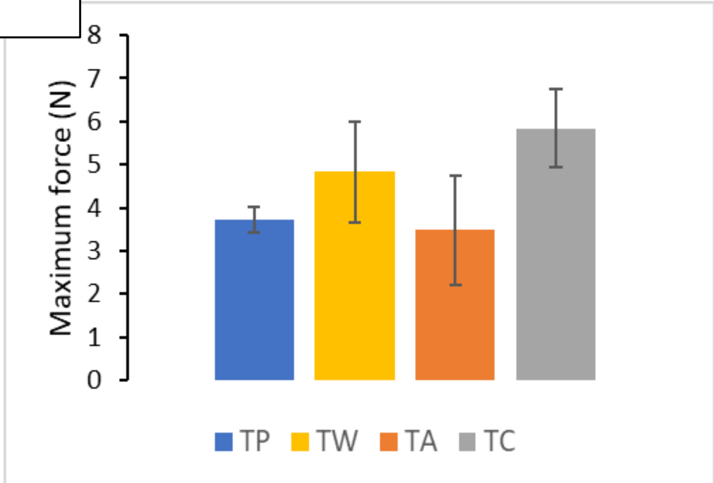


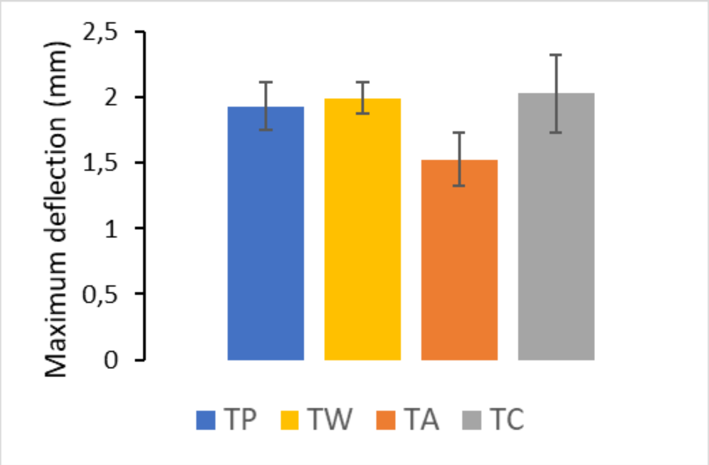




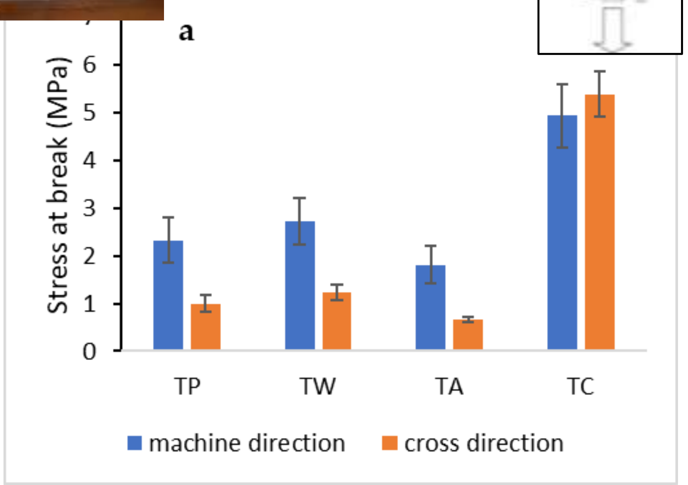


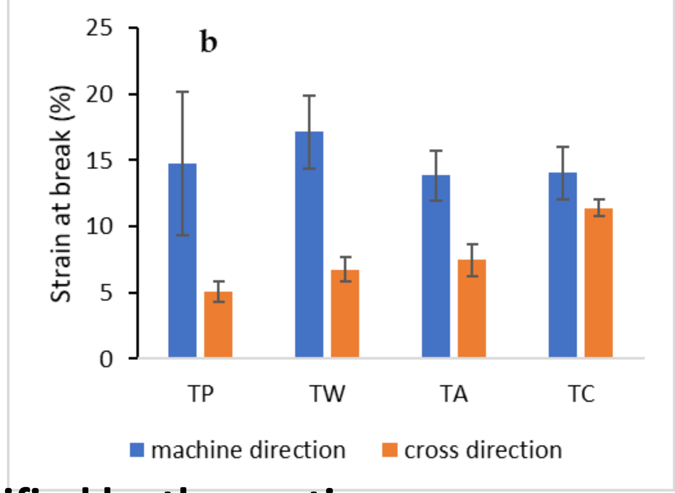
Puncture resistance test



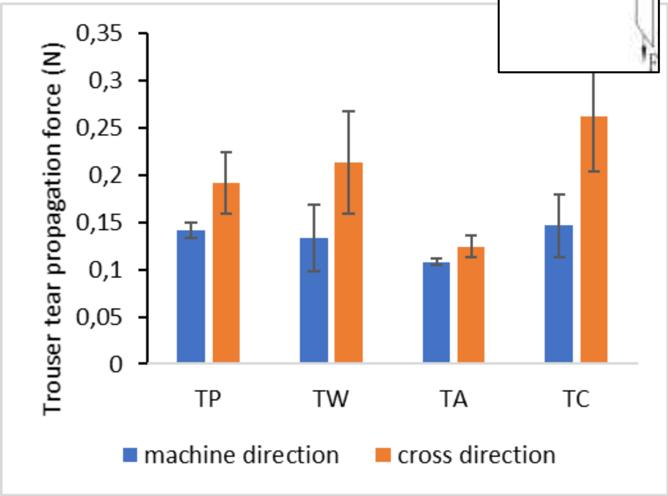


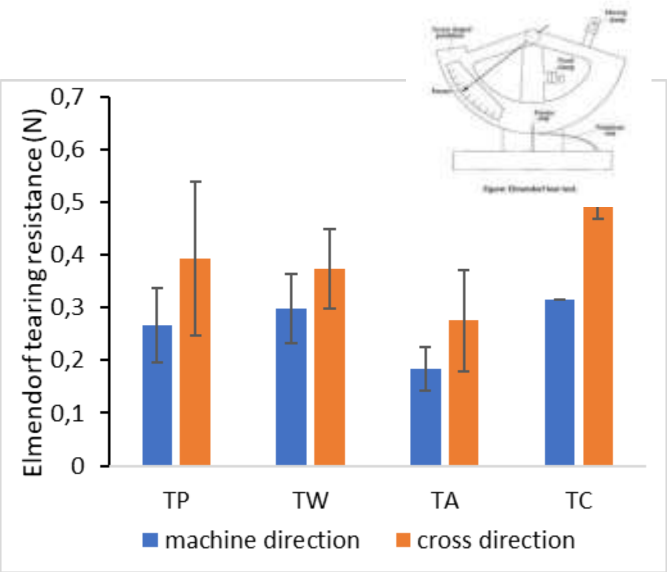
Tensile test





Tearing tests





- **Mechanical properties were only slightly modified by the coating**
- **Applications in personal care and packaging sector are possible**

Mushrooms as a source of chitin

Fungi are organisms with a chitin-containing cell wall.

Chitin is less than 10% of the total mass.

Chitin deacetylation results in chitosan. Chitin deacetylation in the cell wall of fungi is catalyzed by the chitin deacetylase enzyme.

Chitin and chitosan can be present in the cell wall as free macromolecules or **complexed to β-glucans**, forming chitin-glucan complexes (CGC) or chitosan-glucan complexes (ChGC), respectively.

Recently, attempts have also been made to extract chitin from **Aspergillus niger**, which is renowned for its massive biomass of mycelial waste materials after producing citric acid. Aspergillus niger has been engineered by regulating several genes involved in the biosynthesis of chitin producing around 250 g glucosamine/Kg cell wall.

BIOSYNTHESIS OF CHITIN

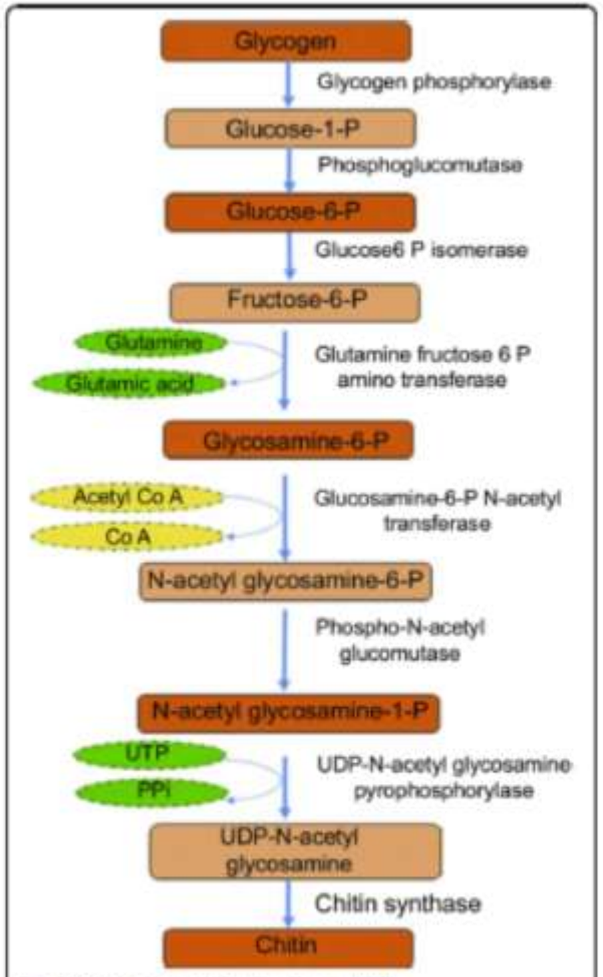
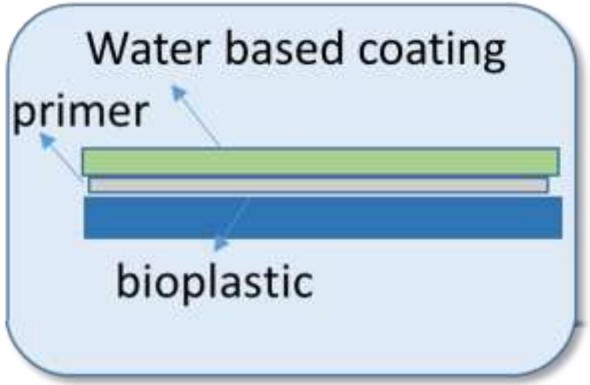


Fig. 3 Biosynthesis of chitin (Balaji et al. 2018)

Chitin nanofibrils in antimicrobial coatings for bioplastic substrates

Oligomers of lactic acid (OLA)

condensia
Passion for chemistry

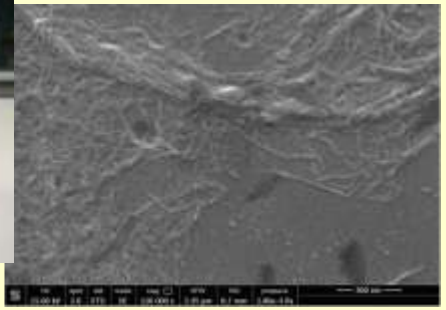


Polybutylene succinate/Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
(PBSA/PHBV)
-
Bioplastic sheet film

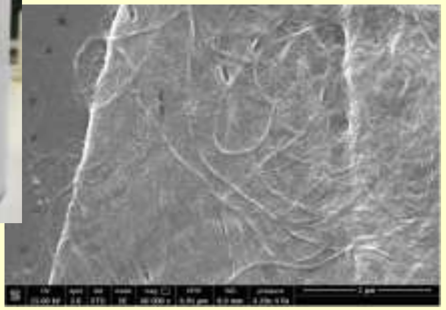
Chitin nanofibrils (ChNF) from different waste sources

Shrimps

celabor



Fungi



Chitin nanofibrils in antimicrobial coatings for bioplastic substrates

Is the primer really necessary to obtain an antimicrobial coating?

Oligomers of lactic acid (OLA)

Shrimps

Sample	Dry Coating	Coated surface	Adhesion
ChNF (Shrimps) (0.8 wt%)		78%	No
ChNF (Shrimps) (1.5 wt%)		86%	No
ChNF (Shrimps) (2.5 wt%)		96%	No

Application of ChNF from shrimps directly on bioplastic occur in a inhomogeneous coating with a shrinkage during the drying phase.

A more concentrated ChNF dispersion resulted better, but no adhesion can be achieved.

Chitin nanofibrils in antimicrobial coatings for bioplastic substrates

Is the primer really necessary to obtain an antimicrobial coating?

Oligomers of lactic acid (OLA)

Shrimps

Sample	Dry Coating	Coated surface	Adhesion
ChNF (Shrimp) (1,5 wt%) + OLA		100%	Yes

+OLA

The use of OLA can help to obtain an homogeneous coating.

Fungi

Sample	Dry Coating	Coated surface	Adhesion
ChNF (Fungi) (1,5 wt%)		100%	Yes

The use of ChNF from a different source can be a valid alternative to obtain an homogeneous coating.

Chitin nanofibrils in antimicrobial coatings for bioplastic substrates

ISO 22196:2011 - Measurement of antibacterial activity on plastics and other non-porous surfaces

R (Antibacterial activity) = Ut- At = log of bacteria number from the coated sample – log of bacteria number from the uncoated sample

Sample	Recovery after 24 h incubation (cells /cm^2)		Antibacterial activity (R)	
	S. aureus	E. coli	S. aureus	E. coli
Uncoated bioplastic film	6.87×10^2	15.6×10^3	0	0
Primer coating (OLA)	1.75×10^4	1.88×10^3	0	0
ChNF (Shrimps) (1.5 wt%)	3.56×10^2	1.25×10^5	0	0
ChNF (Shrimp) (1,5 wt%) + OLA	3.31×10^1	1.25×10^2	1.32	2.09
ChNF (Fungi) (1,5 wt%) + OLA	1.06×10^4	5.8×10^1	0.09	1.68

Primer results in no antimicrobial effect

Application of ChNF without primer cannot be classified as antimicrobial coating

Combination of OLA with the ChNF results in a good quality antimicrobial coating

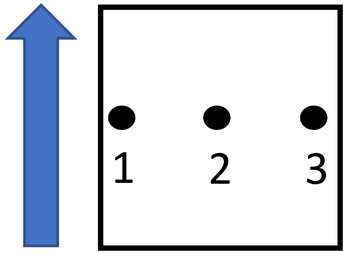
ARCHA

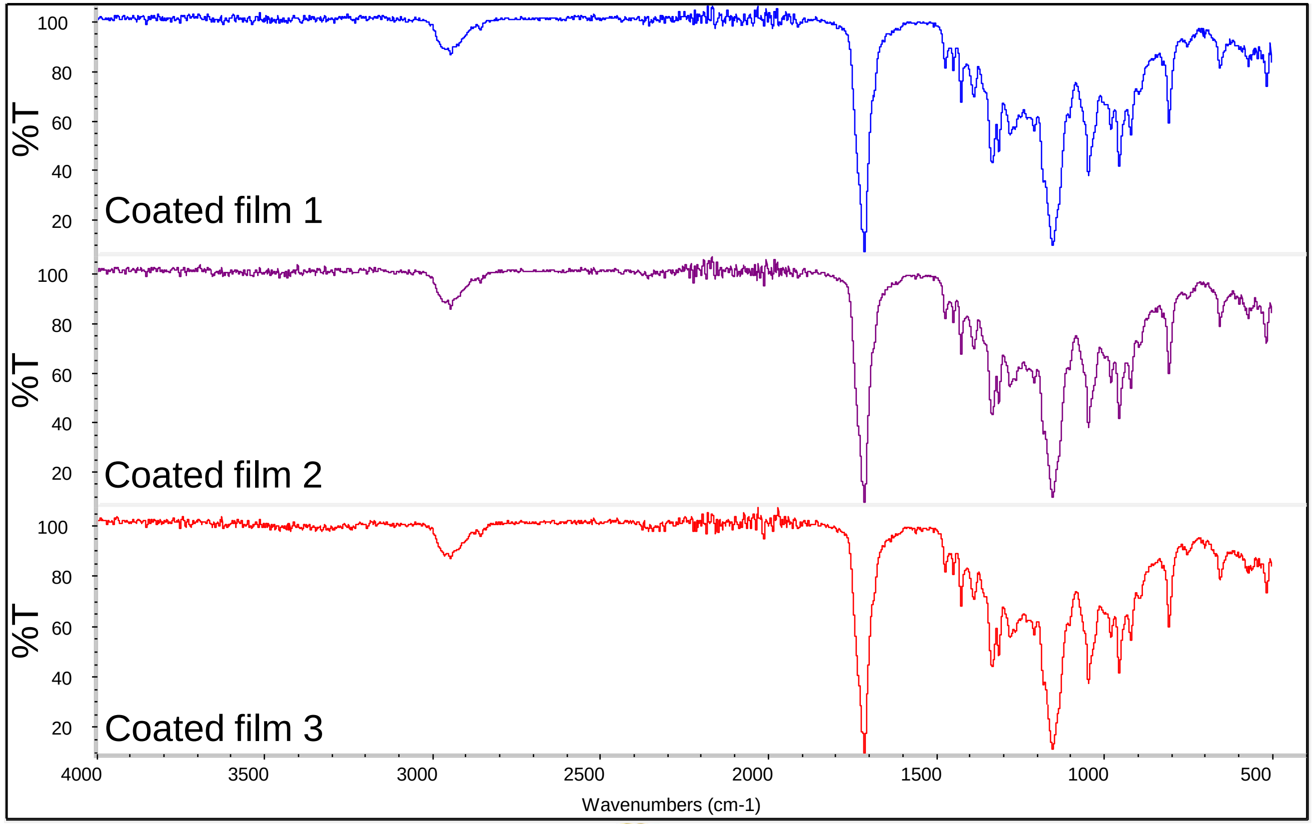
Chitin nanofibrils in antimicrobial coatings for bioplastic substrates

Industrial flexography of ChNF formulation

bio-mi SUSTAINABLE SOLUTIONS

Machine direction





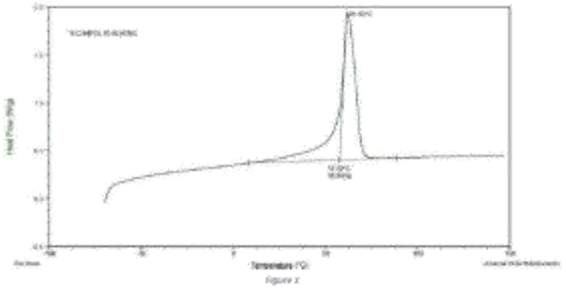
Hot melt coatings

- Hot-melt coatings (HMCs) are solvent-free thermoplastic materials.
- They are solid at low temperatures (generally below 80 °C), and they become low-viscosity fluids at high temperatures (generally above 80 °C), and rapidly set upon cooling[1].
- Hot melt coatings are compatible with most substrates, they can be easily spread or poured when heated, indeed when the hot melt is in a fluid state, it flows onto the substrate. Then, the hot melt is cooled, the coating solidifies and forms a bond to the substrate. They form a strong bond quickly, simply by cooling[2].
- Today, HMCs are used in a variety of manufacturing processes including *packaging*, bookbinding, product assembly, and box and carton heat-sealing as well as other pressure-sensitive applications such as disposable products, stamps, and envelopes like ashesives[3].

[1] Barreueso-Martinez, B.; Ferrandiz-Gomez, T.D.; Martin-Martinez, J.M.; Aran-Ais, F.; Torro-Palau, A.M.; Orgilés-Barceló, A.C. EVA hot-melt adhesives. *Adhes. Age* **2001**, *44*, 32–37
[2] Moody, V.; Needles, H.L. Hot Melt Coating. In Tufted carpet: Textile fibers, dyes, finishes and processes; William Andrew, 2004; p. 202 ISBN 0815519400
[3] Li, W.; Bouzidi, L.; Narine, S.S. Current research and development status and prospect of hot-melt adhesives: A review. Ind. Eng. Chem. Res. 2008, 47, 7524–7532.

HMCs Materials

HMC matrix
Polyester
T_m: *64°C*
Fully Biodegradable
Biobased content: *70%*



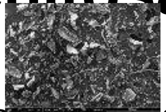
Plasticizers

GLYPLAST +

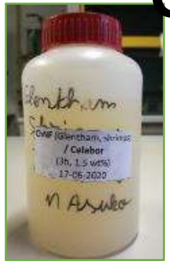
condensia

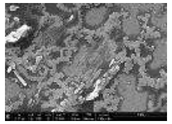
ACTIVE MOLECULES

Celabor Chitosan
from Shrimps



Glentham Chitin
from Shrimps



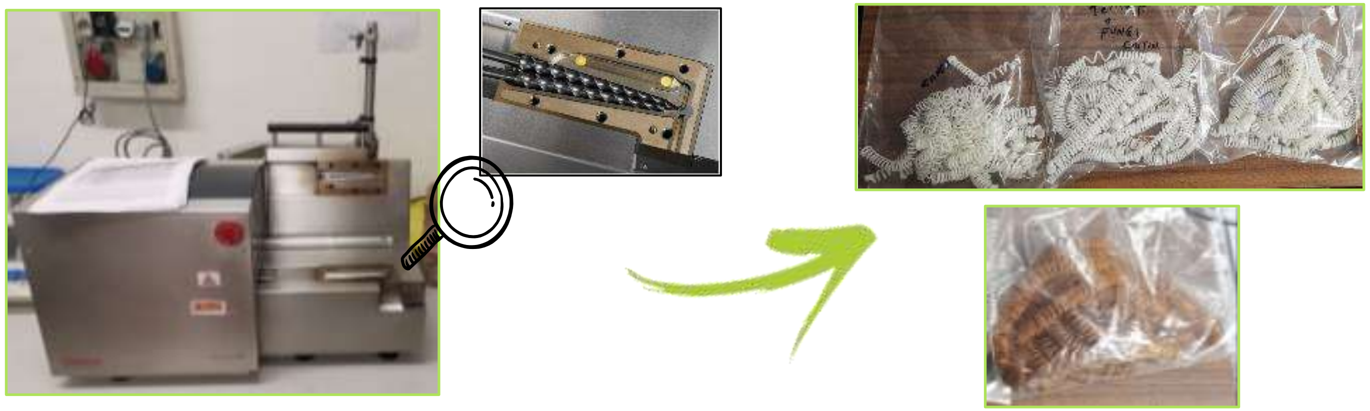


Lab-scale Processing operations

- In the images the experimental steps of the optimized procedure for the achievement of lab-scale extrudate chitin/chitosan based hot melt strands and the subsequent compression molding trial application onto cellulosic and PLA, PBAT and PBS based substrates.

- HMCs formulations were fed searching the optimal ratio into the micro-compounder at 110°C and screw speed of 80 rpm for one minute and the obtained strands were cut and applied (1 g) onto 150x150 mm cellulosic and plastic substrates (about 50 gsm)

- Compression molding trials results were *very homogenous* with *optimal adhesion*, covering the entire 15 cm x 15 cm substrate.



HMC were effective in making both bioplastic and cellulosic substrates, tested by ARCHA and LUCENSE respectively, slightly anti-microbial

Scale-up production

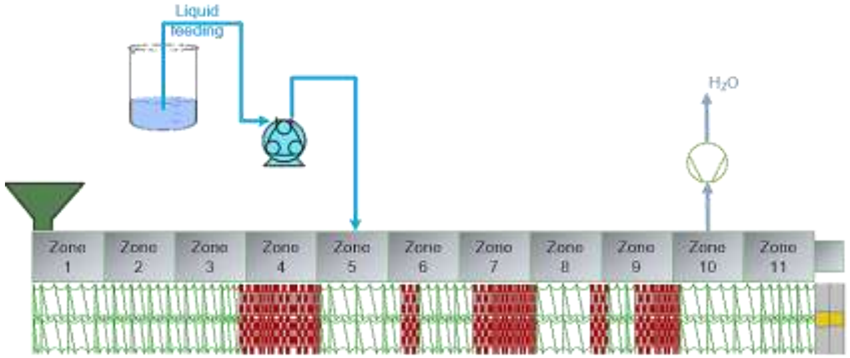
On the basis of lab-scale results, formulas of HMC based chitosan and chitin where chosen as the optimized formulation (and compared with the matrix alone):

HM Chitosan1 and HM Chitin1

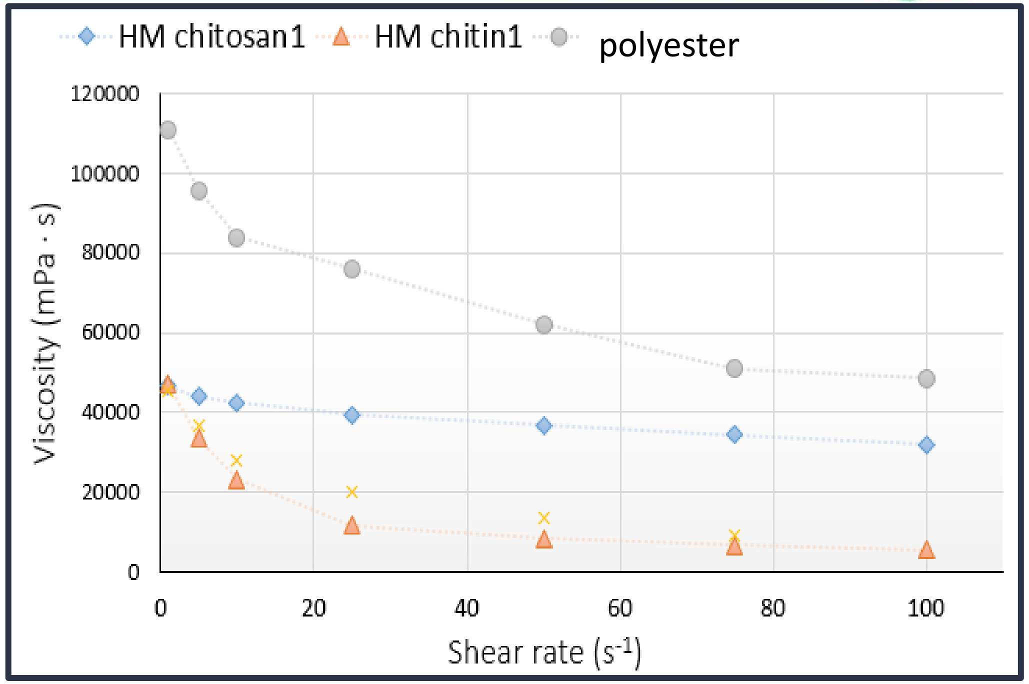
Extrusions were carried out with a **Comac semi-industrial co-rotating twin screw extruder EBC 25 HT, L/D 44.**

polyester matrix pellets have been fed by the principal feeder.

Pre-dispersion of chitin and chitosan were fed into one of the extruder vents.



Viscosimeter RM 100 equipped with spindle MK-SV427



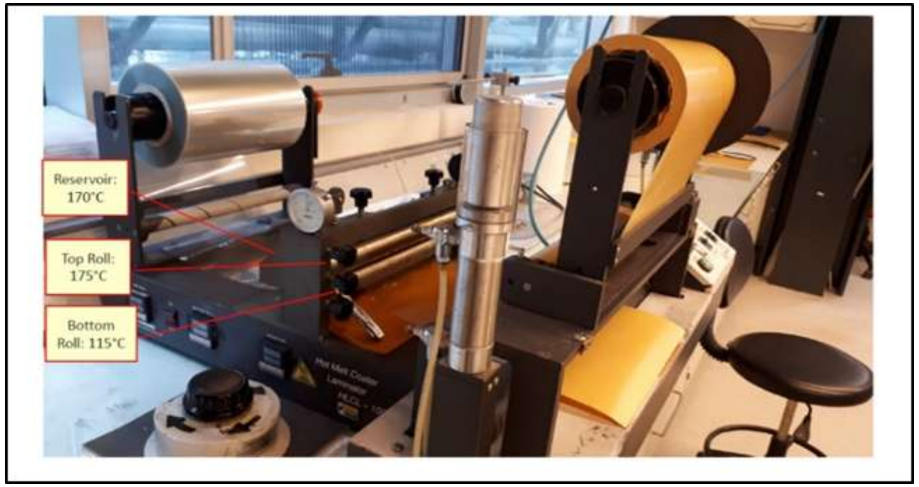
HM matrix

HM Chitosan1

HM Chitin1

Good processability with a tailored vacuum system to eliminate the water. “Humped” Temperature Profile with head temperature of 70°C and high shear rates of the screws to guarantee correct melting & dispersion. No degradation occurred. HMC granules have been sent to other partners for application on plastic and cellulosic substrates.

Finally ORGK produced coated cellulosic substrates with a hot melt laboratory scale coater and laminator, which is like a roller coating system.



ORGANİKKİMYA
the chemistry between us

✓ HM Chitosan 1 and HM Chitin 1 samples have been successfully applied on the HUTAMAKI paper substrate, obtaining reels in A4 width validated in the framework of ECOFUNCO activity.

Conclusions

- Chitin can be obtained from different sources and chitin nanofibrils represent its crystalline fraction. More research is required to compare chitin nanofibrils and chitosan coming from them

European Regulation 2017/893 allows the use of proteins derived only from seven insect species for fish feed (including the Hermetia illlucens)

scientific reports

OPEN **Characterization of chitin and chitosan derived from *Hermetia illucens*, a further step in a circular economy process**

Micaela Triunfo[1,6], Elena Tafi[1,6], Anna Guarnieri[1,6], Rosanna Salvia[1,2], Carmen Scieuzo[1,2], Thomas Hahn[3], Susanne Zibek[3], Alessandro Gagliardini[4], Luca Panariello[5], Maria Beatrice Coltelli[5], Angela De Bonis[1] & Patrizia Falabella[1,2]

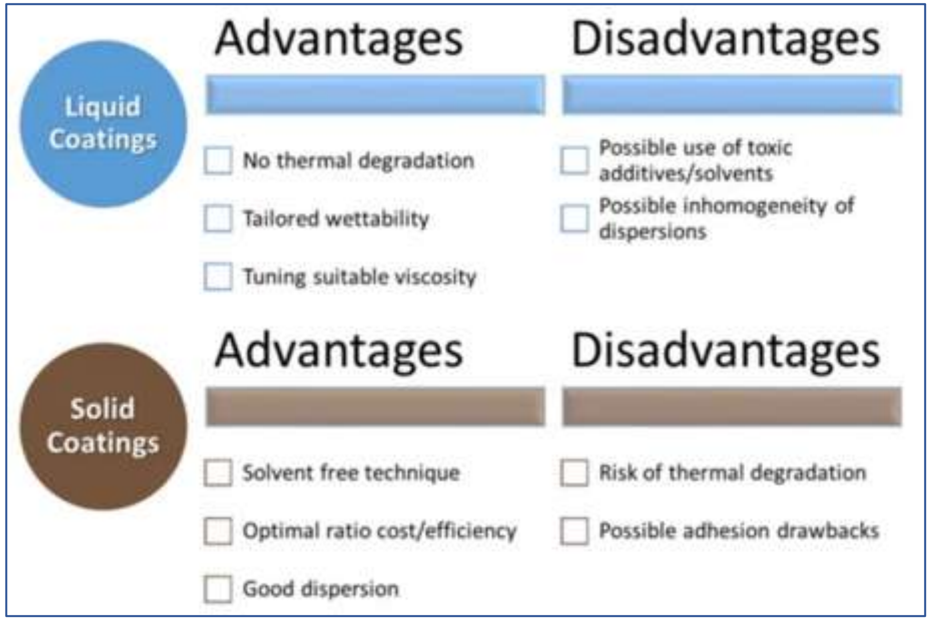
CHITIN

Conclusions

- Liquid coatings were successfully applied onto cellulosic board and tissue paper and anti-microbial properties were evidenced in both cases.
- The combination with other additives (such as anti-oxidants) seem possible and complementary.
- Mechanical properties were only slightly modified by the coating
- ***Applications in personal care and packaging sector are possible***

- Liquid coating can be applied on bioplastic surface to make it anti-microbial and barrier properties were also improved. In particular the treatment induced an increase in oxigen barrier.

- Solid versions of coatings incorporating chitin nanofibrils or chitosan were developed through an innovative methodology to be applied to bioplastic and cellulose for both gas barrier and anti-microbial properties



Thanks to a platform of CN based coatings ECOFUNCO developed and validated interesting cellulosic and bioplastic functional products

ECOFUNCO PARTNERS

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Fraunhofer

The University Of Sheffield.

IRIS TECHNOLOGY GROUP

Kneia

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TIPA

Universitat d'Alacant Universidad de Alicante

Università di Pisa

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Thank you for your attention

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