STSM Collaboration proposal

University of Milan – Grenoble INP Pagora

"Measurements of shelf life and anti-fog properties of functionalized cellulose nanocrystals materials for food packaging application"

Partners:

- Partner 1: Grenoble INP LGP2:

Laboratory of Pulp & Paper Science (LGP2) is a joined research unit (UMR 5518) with National Center for Scientific Research (CNRS). Its activity starts from wood science to converting & printing of packaging. Laboratory is then considered as expert in: Biorefinery (team n°1), Multiscale Biobased Materials (team n°2), Functionalization of surface & printing (team n°3). About 100 persons are working in this laboratory including 36 PhD Students & 5 post-doc.

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- Partner 2: University of Milan, *DeFENS* - Dept. of Food, Environmental and Nutritional Sciences, *PackLAB* - Food Packaging Labs of University of Milan:

The Food Packaging Laboratory (PackLAB) of University of Milan was established in 1985 at the Department of Food, Environmental and Nutritional Sciences – DeFENS (formerly DiSTAM) as the first Italian academic center exclusively devoted to packaging research. The very fast and worldwide growing importance of the food packaging sector pushed PackLAB to grow quickly as well. In these almost 25 years several collaborations have been established with other Universities and Research Centers in Italy and abroad, together fruitful industrial partnerships with in the food and polymeric materials sectors.

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ABSTRACT

Current investigations directed to the surface functionalization of cellulose-based substrates often have the purpose to widen the properties of the already available materials. In this sense, the use of cyclodextrins (CDs) seems to be a good method to functionalize cellulosic substrates. CDs can form complexes with an extensive diversity of molecules making them very attractive in pharmaceutics, biochemistry, food chemistry and textile areas. Hydroxypropyl-β-cyclodextrin (HPβ-CD) was grafted with TEMPO oxidized cellulose nanocrystals (TOCNC-COONa). Carvacrol, an antibacterial molecule, was included in HPβ-CD, previously graft onto TOCNC-COONa, by impregnation. The main objective of this structural modification was to obtain new surface-modified materials able to release antibacterial molecules over a prolonged period, considering its potential in food packaging.

1. **Introduction**

Current packaging materials are mainly composed of petroleum-based synthetic polymers due to their great thermal and mechanical properties. At the moment, there is an increasing pressure of society in developing eco-friendly and bio-based materials as alternatives to existing fossil resources, this development have motivated academic and industrial research.

Because of excellent mechanical properties, a physical resistance, low density, availability, biocompatibility, biological degradability and sustainability renewability, cellulose nanocrystals (CNC) have been widely used [1,2]. Although CNC have a great potential as mentioned, the high amount of OH on the surface of the crystals can promote the grafting with various molecules [3]. The above features give nanocellulose materials a great potential as a novel platform in the packaging field and a rapid development in recent years [4].

The growing concern for the decreasing of food waste worldwide has led to numerous attempts to develop active packaging, a material intended to extend the shelf-life, to maintain or improve the conditions of the packaged food [5,6]. Volatile substances with antimicrobial activities, such as natural essential oils are of great interest for the active packaging industry. Carvacrol (2-methyl-5-(1-methylethyl)-phenol) is a phenolic monoterpene presented in the essential oil of the family *Lamia- Ceae*, which includes the genera *Origanum* and *Thymus*[7], the effective encapsulation and release of this oil represent a main challenge, considering its high fugacity and the fact that it is very sensitive to heat, oxygen and light [8].

CDs are cyclic oligosaccharides containing 6 (α-CD), 7 (β-CD) or 8 (γ-CD) D-glucose units linked by α-1, 4 glycosidic bonds and having the molecular shape of a truncated cone [9]. They are produced by the enzymatic degradation of starch [10]. The hydroxyl groups located at the outside surface of the molecule make it hydrophilic, whereas the interior cavity of CDs is relatively hydrophobic and the most characteristic feature of CDs is the ability to form inclusion complexes through host–guest interactions, generally by hydrophobic or van der

Waals interactions [6]. Because of their non-toxicological and ecological properties, CDs has been widely used in many fields, such as pharmaceuticals, foods, cosmetics, chemical products and technologies [11]. In this study, Hydroxypropyl β-cyclodextrin (HPβ-CD) was used with carvacrol to form inclusion complexes (Figure 1). The formation of an inclusion complex between HPβ-CD and carvacrol has already been proved in few studies [12].

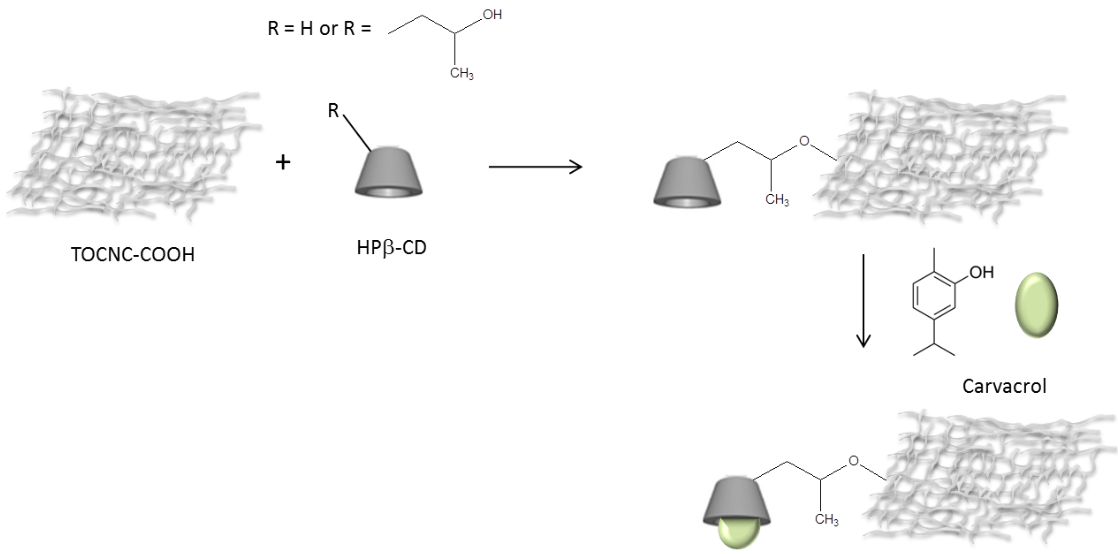


Figure 1. Schematic illustration of CNC surface-functionalized with HPβ-CD and impregnation with carvacrol.

As viable alternatives to existing fossil resources, sustainable, green, and environmentally friendly materials are in demand for several applications. In this context, a suspension of TEMPO oxidized cellulose nanocrystals (TOCNC-COONa) was functionalized with HPβ-CD and was coated in Poly(lactic acid) (PLA) and polyethylene terephthalate (PET). Polyesters have been extensively used for food-packaging applications. The aim of our study was to develop an active cellulose-based packaging in a pilot scale.

2. **Experimental**

2.1 Materials

TEMPO Cellulose nanocrystals (CNC) were acquired from the CelluloseLab (Canada). HPβ-CD, β-CD and carvacrol were commercial chemical grade products, supplied from Aldrich Chemicals (Saint Quentin Fallavier, France). They were used as received. Poly(ethylene terephthalate) (PET) film (thickness of 12 ± 0.5 μm) was provided by Sapici s.p.a (Cernusco sul Naviglio, Italy). Poly (lactic acid) (PLA) film (thickness of 25 ± 0.5 μm) was provided by EarthFirst (USA).

2.2 Coating Process

A 1 wt % TOCNC-COONa suspension was mixed by magnetic stirring for 1 h with HPβ-CD at TOCNC-COONa:HPβ-CD w:w 75:25. The suspensions were coated using an automatic film applicator (model 1137, Sheen Instruments, Kingston, UK) at a constant speed of 2.5 mm s^{-1} onto PET and PLA film, according to ASTM D823-07, after activation of the external side of the substrate by using a corona treater, (Arcotech GmbH, Monsheim, Germany). The coated films were placed at 105°C during 24 h in an oven for the grafting reaction between TOCNC-COONa and HPβ-CD.

2.3 Characterizations

2.3.1 Haze and Transparency

The transmittance of the sample was measured at a wavelength of 550 nm, according to the ASTM D 1746-70 using a Perkin-Elmer L650 UV–VIS spectro-photometer equipped with 150 mm integrating sphere.

Haze (%) was measured in accordance with ASTM D1003-61 with the same instrument equipped with a 150 mm integrating sphere. The haze values of uncoated and coated films were obtained as:

$$haze = \frac{T_d}{T_t} \times 100 \qquad (1)$$

Where T_d and T_t are the diffuse and total transmittance, respectively. At least triplicates were performed and average values calculated.

2.3.2 Coefficient of friction

The static (μ_S) and dynamic (μ_D) friction coefficients were measured by a dynamometer (model Z005, Zwick Roell, Ulm, Germany), according to the standard method ASTM D 1894-87. Firstly, the uncoated side of sample film was attached on a specific sled (6.2 x 6.2 cm^2, 197.99 g), while the uncoated film was covered on the sliding plane (exposing the un-treated side). Then the sled was connected to the force sensor of a dynamometer and horizontally pulled by the instrument on the covered sliding plane. The raw data (pulling force) was recorded and analyzed by TestXpert software V10.11 (Zwick Roell, Ulm, Germany).

2.3.3 Anti-fog properties

The boiling test was established and modified from the European Standard test (EN168). The coated and uncoated films were placed on top of a plastic pot with water at 95 °C and a black sponge. The black sponge was observed through the film by naked eye after the film was exposed to the steam from the white pot. The visibility of the black sponge was empirically evaluated for anti-fog performance and the images were captured using a Canon high-definition camera.

2.3.4 Optical properties

The uncoated and coated films were observed using an optical microscope (Micro Nikon Eclipse ME600 Laboratory Imaging; Nikon Instruments, Sesto Fiorentino, Italy), at 5X and 10X magnification. Pieces of film were mounted on a rectangular glass sample holder and observed after the anti-fog property was evaluated. Images were captured by NIS-Element software (Nikon Instruments, Sesto Fiorentino, Italy).

2.3.5 Permeability

The oxygen permeability (PO2) of coated and uncoated films was assessed (mL m^{-2} day^{-1} bar^{-1}) by an isostatic method (Multiperm, Extra_Solution S.r.l. Capannori, Italy) at 23 °C and 0% relative humidity, according to a standard method ASTM D-3985.

2.3.6 Antimicrobial activity assay

Carvacrol antimicrobial activity was tested for TOCNC-COONa and TOCNC-COONa/HPβ-CD in PLA and PET substrates employing two liquid cultures, in particular Tryptic Soy Broth (TSB) for bacteria and Malt Extract Broth (MEB) for yeasts and moulds. The media were aliquoted (5 mL) in tubes and sterilized at 118 °C for 20 min.

Microorganisms were inoculated (1% v/v) in form of a cell or spore suspensions, in the same culture medium, having an optical density at 600 nm of 0.300 ± 0.010. Cultures were incubated at 30 ± 1 °C, up to 72 h for bacteria and yeasts and up to 14 days for moulds.

Carvacrol was incorporated by impregnation of the samples, into a solution of carvacrol/ethanol in a ratio of 15/85 (v/v). The samples were impregnated for 10 min in the bath and dried at 50 °C for 60 min in a ventilated oven. Films of 36 cm^2 surface area and with 12 μm average thickness, after impregnation, were put horizontally into air-tight glass jar (0.5 L total capacity). 10 mL of molten agar medium was poured into sterile petri dishes of 5.5 cm diameter and inoculated, and then petri dishes were inserted inside an air-tight glass jar (Figure 2).

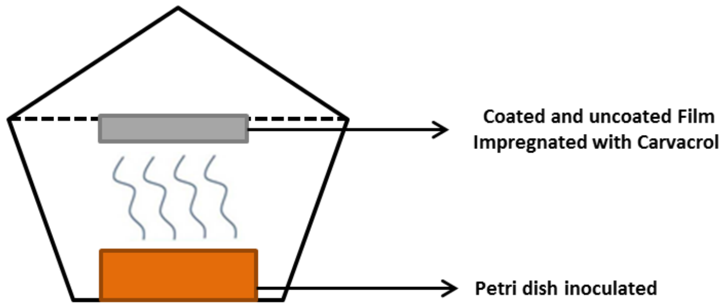


Figure 2. Schematic of air-tight glass jar used in the antimicrobial tests.

For each tested strain, a positive control sample was prepared by incubating the inoculated plate in a jar without carvacrol. Strains were incubated for 24-48 h at 30 °C. These tests were based on the presence or absence of growth, which was assessed visually.

3. Results and Discussions

Haze and Transparency

The transparency and haze values of PET films are 84–80 and 2.9–3.4 %, respectively. Table 1 shows that the TOCNC-COONa and TOCNC-COONa/HPβ-CD coated films still conserve great transparency and low haze and no significant influences on the optical properties of coated films, as requested to ensure easy evaluation of the product quality inside the package.

Table 1. Transparency at 550 nm and haze of coated and uncoated films.

Sample	Transparency	Haze
PET uncoated	84 ± 0.3	2.9 ± 0.1
PET TOCNC-COONa	82 ± 0.4	3.0 ± 0.5
PET TOCNC-COONa/HPβ–CD	80 ± 0.2	3.4 ± 0.7
PLA uncoated	82 ± 0.5	4.0 ± 0.3
PLA TOCNC-COONa	74 ± 0.7	4.2 ± 0.1
PLA TOCNC-COONa/HPβ–CD	77 ± 0.2	5.0 ± 0.3

The thickness of the PLA coating films (TOCNC-COONa and TOCNC-COONa/HPβ-CD) obtained was well below 1μ, didn't reduce the transparency and didn't increase the haze in comparison with the uncoated PLA film (T% 82, Haze 4.0).

Coefficient of Friction

The mechanical properties of polysaccharide-coated in plastic films can be influenced by biopolymers as a coating material with different molecular characteristics from the substrate[13,14].

The values for the coefficient of friction (COF) of TOCNC-COONa and HPβ-CD coated against films are presented in Table 2.

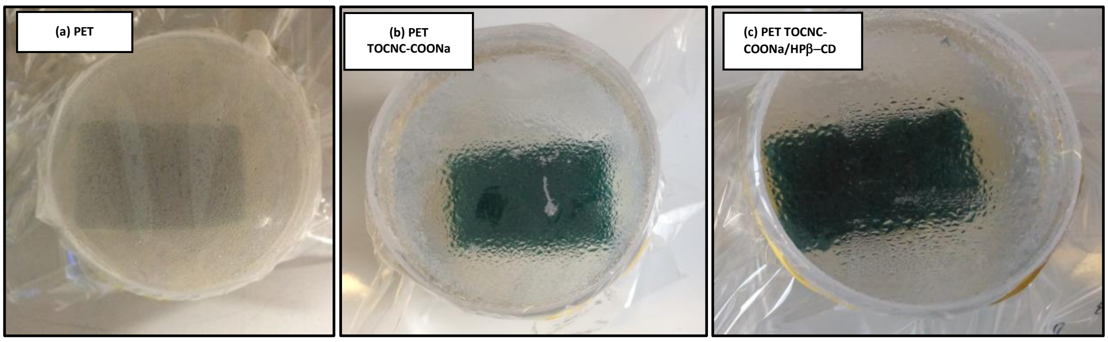
Table 2. The coefficient of friction (COF) of coated and uncoated films.

Sample	μ_S	μ_D
PET uncoated	0.38 ± 0.03	0.32± 0.02
PET TOCNC-COONa	0.28± 0.02	0.24± 0.01
PET TOCNC-COONa /HPβCD	0.29 ± 0.01	0.25 ± 0.01
PLA uncoated	0.33 ± 0.02	0.24 ± 0.02
PLA TOCNC-COONa	0.29 ± 0.02	0.24 ± 0.01
PLA TOCNC-COONa /HPβCD	0.20 ± 0.01	0.11 ± 0.01

It was possible to observe that the deposition of TOCNC-COONa/HPβ-CD created a new coating layer on PLA and PET substrates. Indeed, it can be clearly noted that TOCNC-COONa coated PLA was close to the PLA uncoated, probably due to hydrophobicity of PLA which leads to a weak adhesion with TOCNC-COONa coating and allowed the removal of this from substrate during dynamic measurements. These results can be interpreted as a possible improvement for practical applications on the automatic machineries.

Anti-fog properties

In Figure 3 a,b and c, it is possible to observe that the results for the coated and uncoated PET films were not different by eye inspection, possibly because of heterogeneous surface of the coatings and the presence of some holes. As shown in previous work [14], the fog is formed by small discontinuous water droplets that diffuse the incident light, thereby decrease the transparency substrates under investigation.



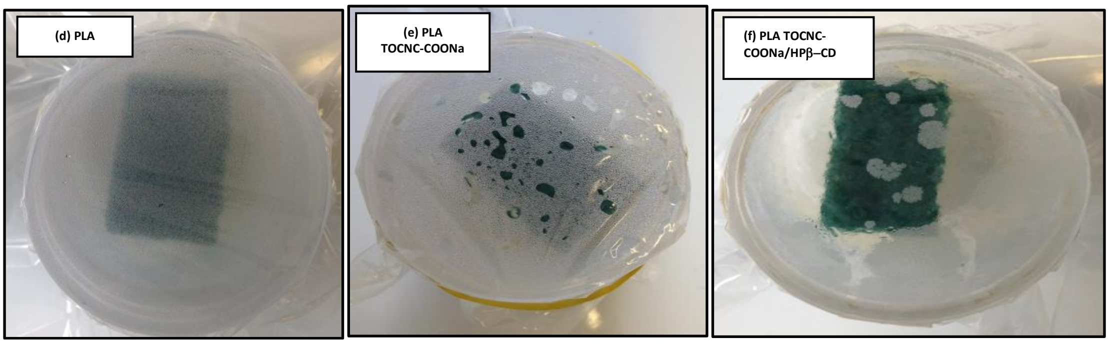


Figure 3. The foggy comparison between uncoated and coated films.

The TEMPO CNC/HPβ-CD coated in PLA films have good anti-fog properties, which are presented in Figure 3. Particularly, these properties are achieved by just using biopolymers instead of chemical additives included in the plastic film for which CNC coating indicates high potential of substitution[15]. This study opens an avenue for production of green, environmentally friendly, and economic materials. The optical microscopic (Figure 4) confirm the results of Figure 3.

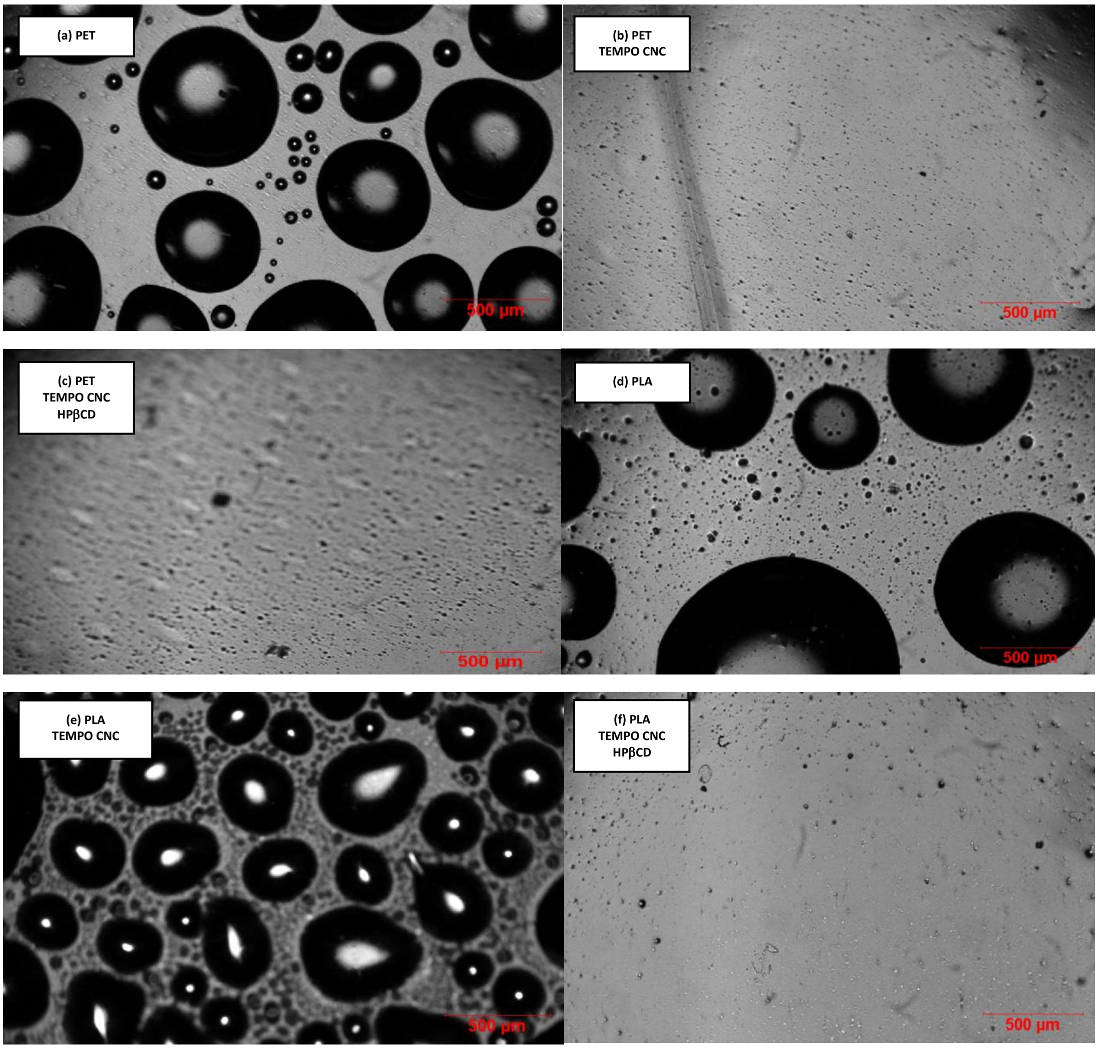


Figure 4. The foggy comparison between coated and uncoated films observed with an optical microscope.

Permeability

The uncoated and coated were subjected to oxygen permeability at 23 °C and 0% relative humidity (Figure 5).

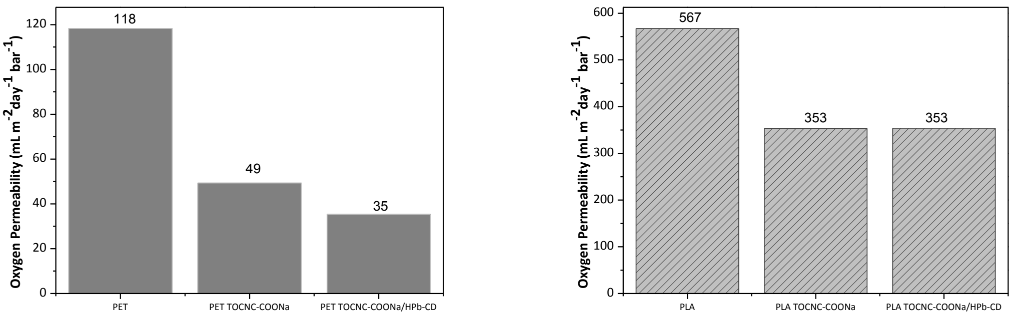


Figure 5. Oxygen permeability of coated and uncoated films.

The oxygen permeability of the PET films decreased from 118 to 35 mL m^{-2} day^{-1} bar^{-1} by coating with TOCN-COONa/HPβ-CD. Thus, the TOCN-COOH films had oxygen barrier properties clearly higher than those of PET and other petroleum-derived synthetic polymer films at 0% relative humidity. The PLA films coated with TOCN-COONa and TOCN-COONa/HPβ-CD had a low oxygen permeability value compared to bare PLA, but it was not possible to verify differences between coated films. However, it is conclusive that sodium carboxylate groups present on CNC surfaces in the presence of HPβ-CD can effectively improve the oxygen barrier properties of PLA and PET films.

Antimicrobial Test

Preliminary experiments were carried out to evaluate antimicrobial spectrum of activity of carvacrol. Trials were performed in liquid cultures, employing bacteria, yeasts and fungi selected among the most microorganisms that might be present in fresh food products. Obtained results are reported in Table 3.

Table 3. Antimicrobial activity of PLA and PET films after impregnation with carvacrol.

Microorganism	PLA TOCNC-COONa	PLA TOCNC-COONa/HPβ-CD	PET TOCNC-COONa	PET TOCNC-COONa/HPβ-CD
Pseudomonas putida	-	-	-	-
Listeria innocua	-	-	-	-
Escherichia coli	-	-	-	-
Staphylococcus aureus	-	-	-	-
Saccharomyces cerevisiae	-	-	-	-
Rhodotorula rubra	-	-	-	-
Pichia guilliermondii	-	-	-	-
Aspergillus niger	inhibition	inhibition	inhibition	inhibition
Penicillium chrysogenum	inhibition	inhibition	inhibition	inhibition

The antimicrobial activity of essential oils and their components has long been renowned, and interest has augmented in latest years. Carvacrol is a component of several essential oils and has been shown to exert antimicrobial activity [16]. Studies that have been performed on the antimicrobial activity of carvacrol have shown that it has a broad spectrum of antimicrobial activity against almost every Gram-positive and Gram-negative bacteria tested [17]. Besides this antibacterial activity, carvacrol has been described as antifungal [18], insecticidal [19], and antiparasitic [20].

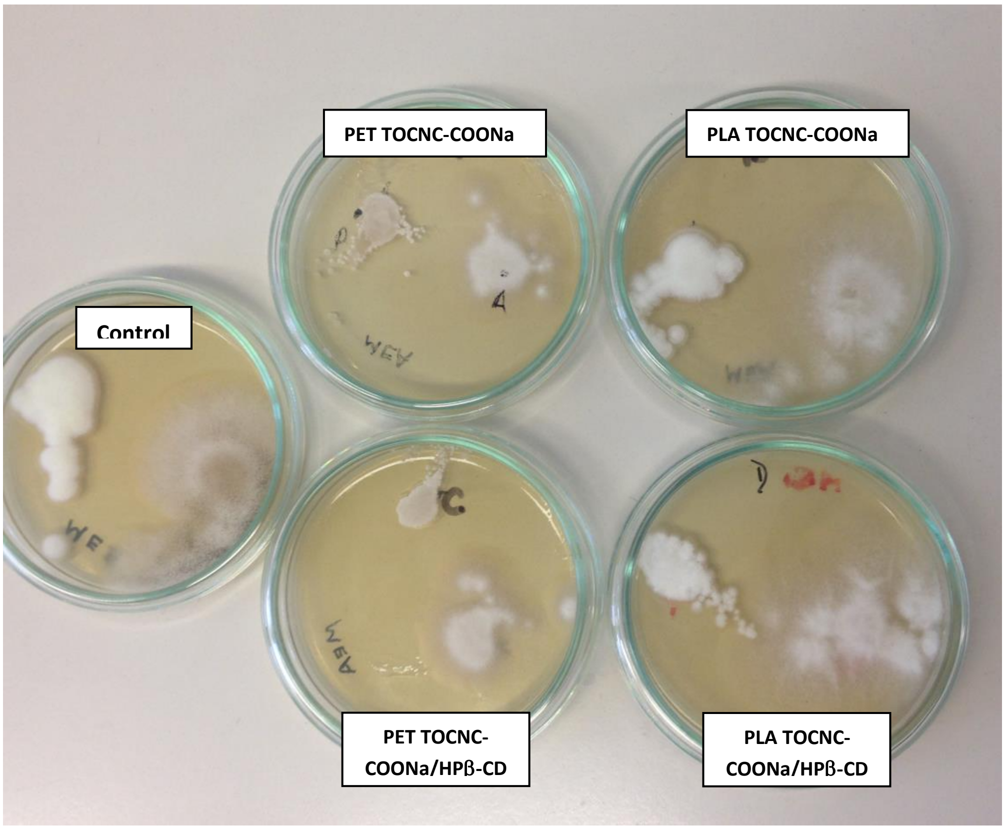


Figure 6. Antimicrobial activity of PLA and PET films immersed in liquid growth medium.

In this study carvacrol was found to be active against fungi for PLA and PET films (Figure 6), but not against bacteria and yeasts. The obtained films represent an example for the creations of an advanced antimicrobial delivery system to prevent the growth of fungi in food applications, for example on the internal surface of plastic salad packaging.

4. Conclusions

After the STSM in Italy, the cooperation between the Polytechnic Institute of Grenoble (INP) and the Food Packaging Laboratory (PackLAB) in the Department of Food, Environmental and Nutritional Sciences (DeFENS) at University of Milan was fortified. Indeed, with the results obtained project we are planning the publication of at least one scientific paper and a conference participation based on the data obtained during this project.

5. References

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