

**Biodegradable nanocomposite poly(lactic acid) foams containing
carvacrol-based cocrystal prepared by supercritical CO₂
processing for controlled release in active food packaging**

Simón Faba ^{a,*}, Marina P. Arrieta ^{b,c}, Julio Romero ^d, Ángel Agüero ^{b,c}, Alejandra Torres ^a,
Sara Martínez ^a, Emilio Rayón ^f, María José Galotto ^{a,*}

^a Packaging Innovation Center (LABEN), Department of Food Science and Technology,
Faculty of Technology, Center for the Development of Nanoscience and Nanotechnology
(CEDENNA), University of Santiago de Chile (USACH), Santiago 9170201, Chile.

^b Departamento de Ingeniería Química Industrial y del Medio Ambiente, Escuela Técnica
Superior de Ingenieros Industriales, Universidad Politécnica de Madrid (ETSII-UPM), Calle
José Gutiérrez Abascal 2, 28006 Madrid, Spain.

^c Grupo de Investigación: Polímeros, Caracterización y Aplicaciones (POLCA), 28006
Madrid, Spain.

^d Laboratory of Membrane Separation Processes (LabProSeM), Department of Chemical
Engineering and Bioprocesses, Engineering Faculty, University of Santiago de Chile
(USACH), Santiago 9170201, Chile (USACH) 9170201 Santiago, Chile.

^e Institut de Tecnologia de Materials (ITM), Universitat Politècnica de València (UPV),
Plaza Ferrándiz y Carbonell 1, 03801 Alcoy, Spain.

^f Institut de Tecnologia de Materials, Universitat Politècnica de València (UPV), Camino de
Vera, s/n, Código Postal 46022, Valencia, Spain.

* Corresponding Authors: María José Galotto and Simón Faba.

E-mail addresses: maria.galotto@usach.cl (MJ. Galotto), simon.faba@usach.cl (S. Faba).

Abstract

Compounds derived from essential oils have been used in active packaging, but their volatility and degradability negatively affect stability and leads to high release rates. The present study aimed to develop PLA bionanocomposite foams loaded with carvacrol cocrystal by supercritical CO₂ and its release into a food simulant for control release in food packaging. For this purpose, 4,4'-bipyridine was used as coformer and carvacrol as active agent. Cocrystallized closed cell foams were obtained using supercritical CO₂ and were characterized in terms of their physicochemical and mechanical properties, and release kinetics to a D1 simulant were evaluated as well as the antioxidant ability. A better overall mechanical behavior due to the nanoclay promoting a higher interfacial adhesion with the polymeric matrix was revealed. A higher incorporation of carvacrol was observed in samples with higher C30B content. The incorporated cocrystals showed a decrease of one order of magnitude in the estimated effective diffusion coefficient of carvacrol and showed antioxidant activity. These results suggest that the nanocomposite foam containing carvacrol-based cocrystals could be used in active packaging systems with controlled release characteristics, especially with highly volatile compounds, and can be proposed for other fields such as biomedical applications.

Keywords: PLA, biocomposite, foams, cocrystal, supercritical CO₂, controlled release.

1. Introduction

The incorporation of active compounds in natural and synthetic polymers is a strategy to develop active packaging to increase the shelf life of packaged food products. [1]. Among this category, the incorporation of active agents with antimicrobial and/or antioxidant capacity in the structure of the packaging material stands out. The renowned antimicrobial and antioxidant agents widely used in active packaging are mainly essential oil derivatives (EOs). Carvacrol is one of the most studied EOs compounds, which presents antifungal, phytotoxic, insecticidal, antioxidant, antitumor, antimutagenic, antiparasitic and antimicrobial activity [2–5]. However, most of the active substances present in EOs, including carvacrol, are highly volatile (high vapor pressure) and thermosensitive, negatively affecting their chemical stability and release rates and thus decreasing their biological activity [6–8].

A new alternative to address the problem of high volatilization of active agents derived from EOs is the use of Cocrystallization Technology. According to the US Food and Drug Administration (FDA), cocrystals are defined as crystalline materials composed of two or more molecules within the same crystal lattice. In the pharmaceutical industry, cocrystals are widely used to address different issues of some drugs or active pharmaceutical ingredients (APIs) and are formed from a coformer that is in solid state under ambient conditions [9,10]. Cocrystals exhibit different physicochemical properties to the API, due to differences in molecular structure and the nature of the interaction between the API and the coformer [11]. The melting point of a cocrystal is an example of how the selection of a coformer can modify a drug with desirable properties [12,13]. [14]. Cocrystals have been used in applications to develop new solid-state compounds with improved or tunable properties, such as reducing

hygroscopicity, modifying the solubility of compounds, and improving the stability of energetic materials, agrochemicals, among others [15–17].

There are different techniques for the formation of cocrystals, such as solvent-based cocrystallization, extrusion, microwave irradiation, and supercritical fluid technology, among others [13]. One of the new technologies for the development of cocrystals is the one based on the use of supercritical fluids (SCF), especially the use of supercritical carbon dioxide (scCO₂), since it is non-toxic, non-flammable, inexpensive and considered a green solvent [18,19]. Traditional cocrystal production techniques present some disadvantages when developing cocrystals associated with an active agent derived from EOs, since these techniques use large amounts of solvents or high temperatures that could degrade the active compounds.

On the other hand, bio-based and biodegradable plastics such as polylactic acid (PLA) have been introduced in the packaging sector, thus reducing the consumption of non-renewable materials and avoiding their accumulation in landfills after their useful life [20–22].

In this sense, in the development of foams, it has been shown that the incorporation of nanoclay to the polymer matrix delivers more heterogeneous cell nucleation sites, in turn improving the thermo-mechanical and barrier performance of PLA [23–25]. Many researchers have shown that the use of both organic (e.g., cellulose nanofibers) and inorganic (e.g., nanoclay) nanoparticles as nucleation agents can effectively improve the thermo-mechanical and barrier performance of PLA [26–28]. Hence, it was shown that incorporating only 1 wt.% of nanoclay (Cloisite 30B) as a nucleation agent in the PLA matrix can reduce the density of the foam, obtaining small and uniform cells and a growth in the Young modulus [29–31]. Therefore, the operating conditions as well as the characteristics of the fillers (i.e.:

their size, surface chemistry and shape) play an essential role in the final foam morphology [32], leading to the manufacturing of foams with specific desired properties. Therefore, this research aims to study the supercritical cocrystallization of carvacrol in PLA loaded with C30B biocomposite foams as a technological alternative that allows modifying the physicochemical properties of this compound. The main novelty in the present research is the supercritical cocrystallization process used to encapsulate an active compound inside the polymeric matrix, contributing to the development of eco-friendly packaging solutions that promote the preservation of the environment and sustainable practices, as well as further their potential use in control release in active food packaging and other fields such as biomedical applications.

2. Materials and Methods

2.1. Materials

Polylactic acid (PLA), Ingeo™ Biopolymer 2003D (specific gravity 1/4 1.24; MFR g/10 min (210 °C, 2.16 kg)), was acquired from Natureworks® Co. (Plymouth, MN, USA). The organoclay-modified (montmorillonite), Cloisite® 30B (C30B) (100 meq/100 g) was purchased by Southern Clay Products (Texas, TE, USA). Carvacrol ($\geq 99.5\%$) and 4,4'-bipyridine were provided from Sigma Aldrich® Chemistry (St. Louis, MO, USA). HPLC grade solvents, such as chloroform (CHCl₃), Acetonitrile (CH₃CN), Methanol (CH₃OH), ultrapure water and ethanol (EtOH), were purchased from Sigma Aldrich (Chile). Carbon dioxide gas (CAS No: 124-38-9) was supplied by Linde (Santiago, Chile).

2.2. Preparation of PLA and bionanocomposite foams

2.2.1. Extrusion process

Neat PLA films and biocomposite PLA/C30B films (1 wt.%, 2 wt.% and 3 wt.%) were prepared via hotmelt extrusion process. A Scientific Labtech® LTE20 twin-screw extruder (Samut-prakarn, Thailand) was used at temperature profiles varying from 175 to 200 °C with a screw speed of 30 rpm. PLA films were collected using a Scientific Labtech® LBCR-150 chilled roller attachment (Samut-prakarn, Thailand) at a rotation speed of 1.8 rpm. The PLA powder and C30B clay were pre-dried at 60 °C for 24 h. The films obtained had an average thickness of 600 µm and were stored in a desiccator until they were used for the supercritical foaming process.

2.2.2. Supercritical foaming

Supercritical foaming of PLA and bionanocomposites films were performed using the system schematically described in **Fig. 1**. Samples of the PLA films (1,5 x 4 cm) were placed into a 100 mL high-pressure cell, separated between them by a metal support which avoids the direct contact. Subsequently, supercritical CO₂ was loaded in the system through a ISCO 500D syringe pump (Lincoln, NE, USA) operating at a constant set pressure. The experiments were carried out at 25 MPa and 130 °C for 25 min, after this time the system was rapidly depressurized reaching atmospheric pressure in 1 s [33].

2.2.3. Supercritical cocrystallization

The supercritical cocrystallization of carvacrol (CAR) was carried out using the same experimental setup implemented for the preparation of the PLA foams (**Fig. 1**). This process was carried out in the high-pressure cell where a glass vessel containing 1 mL of CAR and another one containing 500 mg of 4,4' bipyridine (BI) as conformer were placed. The cocrystallization assays were carried out pressurizing the high-pressure cell with CO₂ until

to reach 10 MPa. This pressure and a temperature equal to 40°C were maintained constant during 3 h. After this time the system was depressurized to a rate of 0.1 MPa min⁻¹.

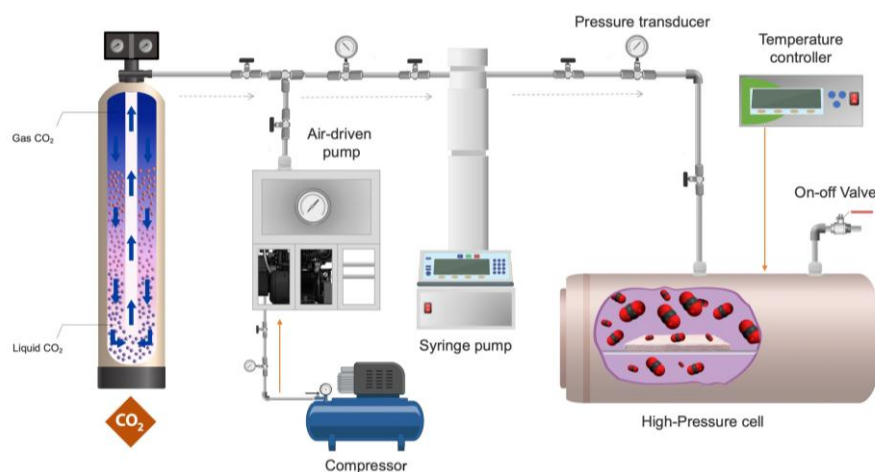


Fig. 1. Experimental setup for supercritical foaming and cocrystallization process.

2.3. Characterization of cocrystallized bionanocomposite foam

2.3.1. Scanning electron microscopy (SEM)

The morphology of the foam samples prepared by supercritical processing was analyzed by scanning electron microscopy (SEM) before and after the exposition to the food simulant D1 for 10 during 10 days at 40 °C using a Jeol JSM-5410 scanning microscope with an accelerating voltage of 10 [kV]. These samples were frozen, fractured in liquid nitrogen and sprayed with gold/palladium using a Hummer 6.2 sputtering system, some samples were coated with 10 nm gold RVBG. SEM micrographs were obtained at 1.0 kx, 5.0 kx and 20.0 kx magnifications.

2.3.2. Viscosity molecular weight

Intrinsic viscosity (η) of PLA biocomposite foams was measured using an Ubbelohde capillary viscometer (type C) according to ISO 1628. Samples previously dissolved in chloroform in different concentrations were introduced into the viscometer in a thermoregulated bath at 25 [°C] and at least five concentrations for each formulation were measured. Finally, the viscosity molecular weight (M_v) was calculated using the Mark-Houwink relationship (**Eq.1**):

$$[\eta] = K \times M_v^a \quad (1)$$

Where K and a are constant, $1,53 \cdot 10^{-2}$ and 0,759 respectively, for the standard PLA polymer [34].

2.3.3. Water uptake

Since the behavior against water is a key factor for materials intended to be in contact with foodstuffs, water absorption analysis of the PLA/C30B cocrystallized foams was carried out as recommended by the standard ISO 62:2008. Briefly, three square samples of 15 x 15 mm² of each material were immersed in distilled water for 84 days at a controlled temperature of 23 ± 1 °C and once a week its mass gain was measured. For this, samples were removed from the bath, dried with absorbent paper to remove water excess, weighed on a Sartorius QUINTIX-125D-1S analytical balance (Göttigen, Germany), and then samples were immersed again. The amount of absorbed water is expressed as a mass percentage calculated according to **Eq. 2**.

$$\Delta m_t(\%) = \left[\frac{W_t - W_0}{W_0} \right] \cdot 100 \quad (2)$$

where W_t is the sample weight after an immersion time t and W_0 is initial weight of the dry sample.

2.3.4. *Attenuated total reflectance Fourier Transforms Infrared (ATR-FTIR) spectroscopy*

Fourier Transform Infrared (FTIR) spectra of cocrystallized foam samples were recorded using a Bruker Alpha IFS 66V spectrometer (Wismar, Germany) equipped with an attenuated total reflexion diamond crystal accessory (Bruker, Platinum). FTIR spectra were obtained with a resolution of 4 cm^{-1} in a wavenumber range from 4000 to 400 cm^{-1} with 100 scans. The spectra analysis was performed using OPUS Software Version 7.0.

2.3.5. *X-ray diffraction*

X-ray diffraction (XRD) analysis of neat foams and cocrystal-loaded foams was performed using a Bruker D8 Advance X-ray diffractometer at 40 kV using CuK α radiation (wavelength $\lambda=1.54[\text{\AA}]$) at room temperature. Scanning was performed over the surface of foams which were mounted in an appropriate sample holder. Samples were scanned from 5° to 50° $[2\theta]$ angle at a rate of $0.02^\circ [\text{sec}^{-1}]$. Experimental data were refined using Pawley method from optimized single-crystal X-ray diffraction (SCXRD) scans.

2.3.6. *Tensile test*

Mechanical properties before and after the exposition of foams to the food simulat D1 for 10 during 10 days at 40°C were analyzed by tensile testing by means of a Shimadzu AGS-X 100 N universal testing machine (Kyoto, Japan) equipped with a 100 N load cell, using 30 mm jaw spacing as initial length, and operating at a crosshead speed of 10 mm min^{-1} . Rectangular specimens of foams were cut in $5\text{ mm} \times 50\text{ mm}$ and at least five specimens were assayed for each foam formulation. the mean percentage elongation at break ($\epsilon\%$), Young's modulus (E) and tensile strength (TS) were calculated from the stress-strain curves obtained.

2.4. *Quantification of carvacrol in bionanocomposite foams*

To study the supercritical cocrystallization process in the polymer matrix, the effective concentration of CAR in PLA bionanocomposite foams was determined. The analysis was carried out by a gravimetric method before and after the cocrystallization process, where the amount of carvacrol was quantified according to the amount of BICAR cocrystal. CAR-based reference cocrystals were quantified by e2695 Waters Alliance high performance liquid chromatography (HPLC) (Ohio, USA) equipped with photodiode array detector (2998 PDA detector - Waters) and an Inertsil® ODS-3 C18, 5 [μm], 4.6 x 250 mm column. The mobile phase was acetonitrile: water (60:40) at a constant flow rate of 1 [mL/min], with an injection of 10 [μL]. The oven was held constant at 30 [$^{\circ}\text{C}$].

The calibration curve of peak area versus CAR concentration of standard solutions from 100 to 1000 mg kg^{-1} (ppm) was performed with three samples for each CAR concentration. All results were expressed as CAR wt.% with respect to polymer weight.

2.5. *Kinetic release of carvacrol from bionanocomposite foams*

2.5.1. *Release test procedure*

The release tests of carvacrol from the bionanocomposite foams to a food simulant were performed by specific migration experimental analysis according to the current European standard of the European Committee for Standardization for migration testing [36], following the methodology already applied in previous studies on release kinetics of active compounds [37,38]. Samples of the PLA and nanocomposite foams containing BICAR cocrystals were sized with a surface area of 25 [cm^2]. Subsequently, these samples were fully immersed in glass tubes with 130 [mL] of a fatty food simulant (50 [% v/v] ethanol aq.

solution). The samples of foams were immobilized into the tubes using a metal holder to avoid floating as well as the direct contact with the tube wall.

The tubes were kept in an oven at a constant temperature of 40 [°C], for 7 days. 1 mL aliquots of the simulant solution were collected at different times. These samples were analyzed by high performance liquid chromatography (HPLC) to determine the concentration of active compound released as a function of time, according to procedure of **section 2.5**.

2.5.2. Mathematical modeling of effective carvacrol release kinetic from the bionanocomposite foam containing BICAR cocrystals

The release kinetics of CAR from neat PLA and nanocomposite foams can be explained by a mass transfer based on the one-dimensional simplification diffusion process (Fick's Law) through the material coupled to convective transport in the receiving phase (food simulant) if this phase is a fluid. This approach based on classical phenomenological equations and thermodynamic considerations has been previously developed and validated in several published studies [3,38,39].

This mathematical description involves the definition of an effective diffusion coefficient of carvacrol through the PLA-based foam, which is immersed in food simulant. Thus, the instantaneous transfer of carvacrol through the polymeric matrix can be explained by molecular diffusion. The overall transfer of this compound through the foam can be estimated by the following expression (**Eq. 3**):

$$J_{CAR} = \frac{D_p}{L/2} \left[C_{CAR}^p(x=0, t) - C_{CAR}^p\left(x = \frac{L}{2}, t\right) \right] \quad (3)$$

where J_{CAR} is the mass transfer flux ($\text{kg m}^{-2} \text{s}^{-1}$) of carvacrol through the PLA-based foam, D_p represents the effective diffusion coefficient of carvacrol in the foam ($\text{m}^2 \text{s}^{-1}$), C_{CAR}^p (kg m^{-3}) is the concentration of carvacrol in the polymeric foam core, and $L/2$ is the foam sample

233 thickness half (m). It is worth noting that the effective diffusion coefficient could represent
 234 a more complex transfer phenomenon considering the biphasic nature of the foam where its
 235 porosity and tortuosity as well as other morphological characteristics can affect the mass
 236 transfer and it will be quantified by this parameter. Nevertheless, in this work this coefficient
 237 has been quantified as a single parameter describing the overall process in this phase.
 238 On the other hand, the thermodynamic equilibrium of carvacrol between the polymeric and
 239 food simulant phases can be described by a distribution coefficient of carvacrol between the
 240 polymeric foam and the food simulant. Thus, this distribution coefficient can be estimated
 241 by the following equation (Eq. 4):

$$K_{P/FS} = \frac{C_{CAR}^P \left(x = \frac{L}{2}, t \right)}{C_{CAR}^{FS} \left(x = \frac{L}{2}, t \right)} \quad (4)$$

242 where $C_{CAR}^P (x=L/2, t)$ and $C_{CAR}^{FS} (x=L/2, t)$ are the concentrations of carvacrol at the
 243 interface in a specific time on the side of the polymeric foam (P) and the food simulant (FS),
 244 respectively. In this study, this distribution coefficient will be considered constant.
 245 Finally, the second mass transfer step in the release of carvacrol is its transport through the
 246 boundary layer of the food simulant phase at the proximities of the interface. This process
 247 can be described by a natural convection mechanism where the instantaneous mass transfer
 248 flux of carvacrol can be estimated by the Eq. 5.

$$J_{CAR} = k_{FS} \left[C_{CAR}^{FS} \left(x = \frac{L}{2}, t \right) - C_{CAR}^{FS} (x = \infty, t) \right] \quad (5)$$

249 where k_{FS} is the mass transfer coefficient of carvacrol through the food simulant boundary
 250 layer and $C_{CAR}^{FS} \left(x = \frac{L}{2}, t \right)$ and $C_{CAR}^{FS} (x = \infty, t)$ are the concentrations of carvacrol in the food
 251 simulant at the interface with the polymeric foam and the concentration of carvacrol at the
 252 bulk of the food simulant, respectively.

This mass transfer model has been solved in MatLab® combining equations 1, 2 and 3 with the mass balance of carvacrol for each step time in order to simulate the release kinetics estimating the variation of the concentration of carvacrol in the food simulant as a function of time.

The model was solved under the following assumptions: i) the initial concentration of carvacrol in the polymeric foam is known and homogeneously distributed ($C^P_{CAR}(x,t=0)=C^{P0}_{CAR}$, $0 \leq x \leq L/2$); ii) the food simulant is initially carvacrol free ($C^{FS}_{CAR}(x,t=0)=0$); and iii) there is no transfer limitation in the food simulant, then carvacrol is homogeneously distributed in this phase.

The value of the partition coefficient ($K_{P/FS}$) has been obtained from the experimental assays of release kinetic, quantifying the concentration of carvacrol at equilibrium for both phases. Meanwhile, the value of the effective diffusion coefficient of carvacrol through the polymeric foam (D_P) is estimated by the curve fitting finding the lowest values of RMSE (root of the mean-square error) between experimental release kinetic data and the predicted ones by the mathematical model.

2.6. Antioxidant activity of the foams

For the determination of the antioxidant activity, foams were subjected to a double-sided total immersion specific migration test, by total immersion of the foams in a glass vial containing a fatty food simulant (Simulant D1 = ethanol 50% v/v) at 40 °C for 10 days (area-to-volume ratio = 6 dm²/L) [40]. After 10 days, the foams were removed and the food simulant D1 was used to determine the antioxidant activity of the foams. For this purpose, the antioxidant effectiveness of the released CAR into the food simulant was measured by

determination of the radical scavenging activity (RSA) through the DPPH method using Eq. 6.

$$RSA (\%) = \frac{A_{control} - A_{foam}}{A_{control}} \times 100\% \quad (6)$$

Where $A_{control}$ is the absorbance of 0.9 mL of food simulant D1 after 10 days at 40 °C mixed with 0.1 mL of 2mM 2,2-difenil-1-picrylhydrazyl (DPPH) in ethanolic solution after 15 min at darkness and A_{sample} the absorbance of 0.9 mL of the food simulant D1 containing the foam samples after 10 days at 40 °C mixed with 0.1 mL of 2mM DPPH after 15 min in darkness [41] The reduction in the maximum absorbance of radical DPPH was measured by means of a UV-Vis Varian Cary spectrophotometer.

2.7. Disintegration under standard composting conditions

All foams were disintegrated under composting conditions according to the international standard ISO 20200. In a plastic container, solid synthetic waste was prepared and demineralized water was then added at a ratio of 45:55 and mixed. Water was periodically added to the reaction container to maintain relative humidity in the compost [43]. Samples of cocrystallized PLA foams were cut in squares of 1.5 x 1.5 cm² and buried at 6 cm in deep in the composting reactors containing the wet solid synthetic waste. Individually foam sample was contained in a textile mesh to permit easy recovery of disintegrated samples after the exposition to the composting medium but allowing access of moisture as well as microorganisms. The reactors were placed in a Memmert GmbH air circulation oven (Schwabach, Germany) at 58 °C for 30 days. An aerobic environment was ensured by periodic mixing of the solid synthetic wet waste [33,42,44]. Foam samples were then recovered at different days (days 1, 4, 7, 11, 18, 22, 25, 28 and 30), washed using distilled water, dried in a vacuum oven at 40 °C for 24 h and reweighed on a Sartorius

297 QUINTIX125D-1S analytical balance (Göttingen, Germany). The foam samples were
298 photographed after removal from the composting test to qualitatively check the visual
299 appearance of the samples as a consequence of the disintegration phenomenon. Finally, the
300 degree of disintegration was quantified by normalizing the weight of the foam at each day of
301 exposition to the composting medium to the initial weight of the foam, while 90% of
302 disintegration was used as the target of the foams disintegrability [45].

303 2.8. *Statistical analysis.*

304 Experimental data have been statistically analyzed by Statgraphics Plus 5.1 (StatPoint
305 Inc., Herndon, VA, USA). This software was used to implement variance analysis ANOVA
306 test. The experimental design was random-type in order to detect differences between the
307 samples with a 95% confidence level ($p < 0.05$).

3. Results and discussions

3.1. Processing of cocrystallized PLA bionanocomposite foams

PLA bionanocomposite foams loaded with 1.0, 2.0 and 3.0 wt.% C30B were prepared by hot melt extrusion simulating the industrial conditions and subsequent foaming with scCO₂ as described in section 2.2.2. Fig. 2 shows the result of foams cocrystallized with BICAR cocrystal by the supercritical cocrystallization process. Preliminary tests yielded processing conditions of 40 °C temperature and 10 MPa pressure. The depressurization rate was kept constant 0.1 MPa/min, allowing slow cocrystallization between the system components. As previously reported, asymmetric shapes are due to biaxial growth during foam expansion [33]. As can be seen in Fig. 2, the resulting foams were white coated with macrocrystals of BICAR cocrystals due to the rearrangement of the BI coformer and its supramolecular interactions, mainly hydrogen bridging, next to the active compound (CAR).

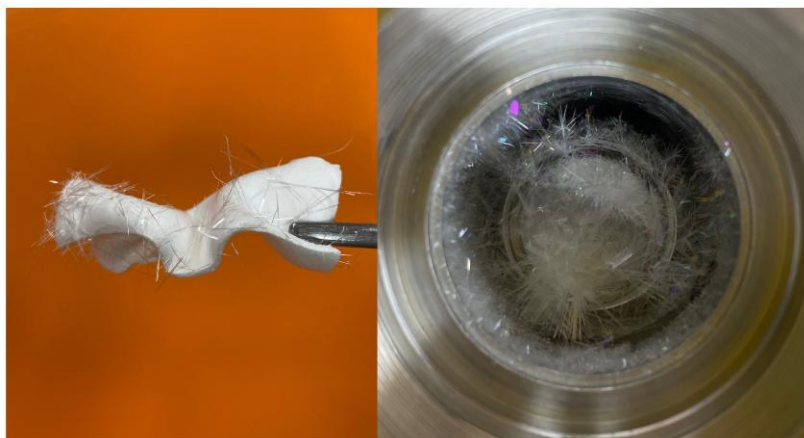


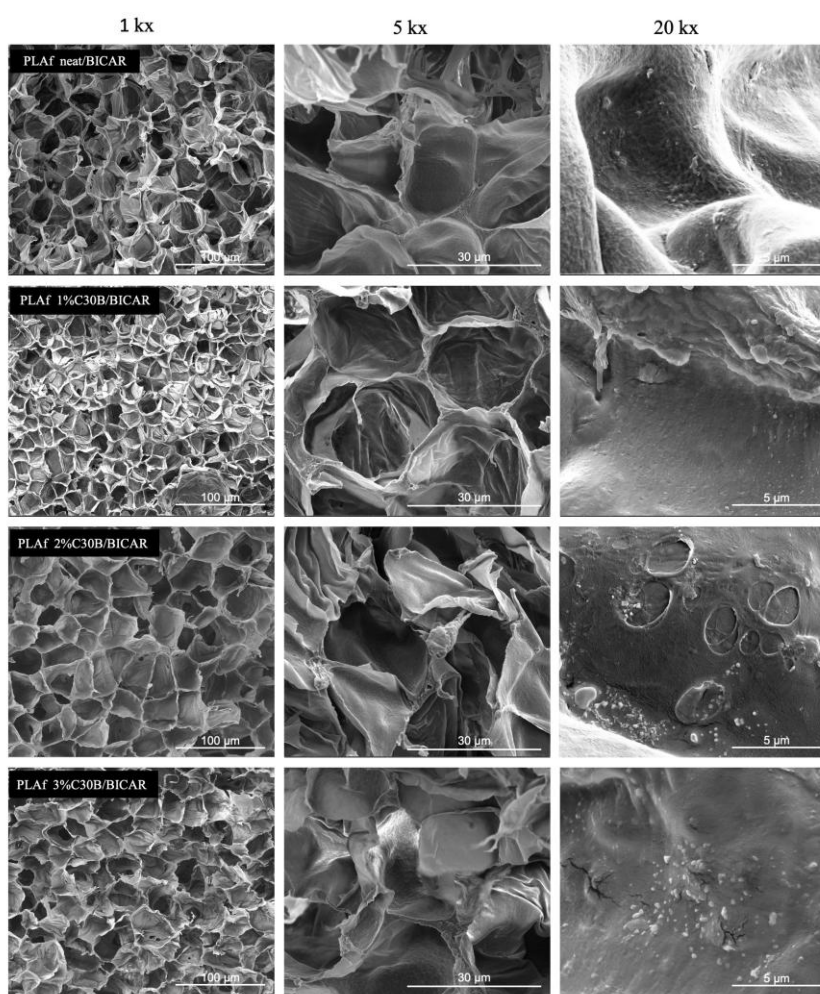
Fig. 2. Supercritical cocrystallization of bionanocomposite PLA foams with BICAR cocrystal.

3.2. *Microstructure bionanocomposite foams*

The incorporation of nanoclay and its proper distribution in the polymeric matrix structure of the different formulations as well as the effect on the cocrystallization structure of PLA foams was analyzed by SEM analysis. In **Fig. 3**, SEM micrographs of the cross-section of freeze-fractured samples of all formulations with BICAR cocrystals are shown. Enlargements made at 1.0 kx, 5.0 kx and 20.0 kx cross sections of each of the foams are shown.

It could be observed that the cocrystallized PLA foams showed a mostly rough surface due to the compression exerted by the high pressure in the system (10 MPa). However, no cell rupture was observed in the cells of these, indeed the samples maintained a closed cell structure. This was due to the addition of C30B and its alignment along the cell walls, increasing their resistance to the stretching force, thus inhibiting cell rupture [29,46]. The cocrystallized foams formed from nanocomposites, like the uncrytallized foams, presented a texture, due to the presence of C30B, more prominently in the 3 wt.% reinforced samples. In general, the samples presented a smooth and soft surface, with no evidence of material agglomerations. This result is in agreement with that reported by Najafi et al. (2015) who, through morphological observations by SEM, concluded that a more uniform, fine and dense cell structure was achieved in PLA foams reinforced with commercial clay. From the micrographs, it can be concluded that the cell structure was influenced by the addition of nanoclay, because the PLA cells with C30B were relatively smaller and more uniform in size and distribution, which in turn is due to the induction of crystals caused by the presence of nanoclay, acting as an effective nucleating agent and increasing the low melting strength of virgin PLA [32,48]. The results indicated that foaming conditions, including foaming pressure, temperature and scCO₂ dissolution time, influence the foam morphology [49,50].

347 On the other hand, at the surface level of the foams, small crystals of a size between 150-300
 348 nm were observed, similar to those observed on the surface of the BICAR-SC reference
 349 cocrystals. With this evidence and the results obtained by XRD, it could be inferred a
 350 formation of cocrystals inside the cells, but at a nanometer or molecular scale that is able to
 351 modify the crystallinity of the foam and form small crystals on the surface of the material.



352
 353 **Fig. 3.** SEM micrographs of cross sections of cryofractured samples at 1.0 kx, 5.0 kx and 20.0 kx.

3.3. Structural characterization (FTIR-XRD)

FTIR analysis was performed to identify the main interactions between the functional groups of each compound of the different formulations, as well as the formation of new functional groups as a consequence of the resulting interactions. **Fig. 4(a)** shows the FTIR spectra of the formulations obtained for each process. The most characteristic peaks of PLA are at 2944 cm^{-1} and 2995 cm^{-1} , attributed to symmetrical and asymmetrical vibrations of axial -CH groups in saturated hydrocarbons (CH_3) at 1082 cm^{-1} and 1128 cm^{-1} , where peaks of the C-O stretching bond of the polymer can be seen [51]. The characteristic carbonyl group (-C=O) of PLA can be seen at approximately 1748 cm^{-1} . Lastly, peaks at 749 cm^{-1} and 870 cm^{-1} were assigned to the stretching of the C-C bond attributed to the crystalline and amorphous PLA phases [52]. A slight shift of the peak of the carbonyl group is observed, which is possibly due to the stretching of the crystalline carbonyl group, as a consequence of the addition of C30B and its nucleating effect [33]. Weak peaks are also observed between 2924 cm^{-1} and 2842 cm^{-1} , associated to the symmetric and asymmetric vibration of the C-H bond of the methylene group present in the nanoclay structure [53]. From the above analysis, it is possible to deduce that the addition of the C30B nanoclay in the polymeric matrix does not affect the chemical structure of the PLA. On the other side, the spectrum between the crystal structure of PLA foams loaded with different amounts of C30B and their supercritical cocrystallization with BICAR presented significant changes. Carvacrol shows out of plane stretching peaks due to aromatic C-H bonds in the 900–650 cm^{-1} region [54], and as shown in **Fig. 4(b)** the three cocrystal impregnated materials (PLAf 1%C30B/BICAR, PLAf 2%C30B/BICAR and PLAf 3%C30B/BICAR) shows well-defined peaks between 812 and 866 cm^{-1} and can be attributed to the C=C stretching of the aromatic ring of carvacrol [55,56]. On the other hand, as shown in the **Fig. 4(c)** for the cocrystal impregnated materials (PLAf

2%C30B/BICAR and PLA/3%C30B/BICAR) peaks are observed between 2900 and 3100 cm^{-1} are attributed to the asymmetric stretching of C-H bond for pyridine rings and methyl groups. The peaks between 3053-3078 cm^{-1} and 3076-3101 cm^{-1} correspond to the pyridine ring frequencies, while the peaks at 2882 cm^{-1} and 3081 cm^{-1} correspond to the asymmetric bending of CH_3 vibrations [57]. In addition, there is a direct relationship between the amount of nanoclay incorporated into the polymer and the intensity of the peaks, the latter being higher when there is a higher percentage of C30B in the material as shown in the **Fig. S1** (Supplementary) between peaks 1720 cm^{-1} and 1800 cm^{-1} . This could be explained due to the establishment of hydrogen bonds between C=O groups of PLA and -OH groups of C30B, which indicates that the oxygen of carbonyl group is involved in hydrogen bonding interactions with the hydroxyl groups of C30B [27,33]. The supercritical impregnation process effectively incorporated both compounds, as the formation of cocrystals results in changes in positions, intensities, and shapes in the 400–4000 cm^{-1} range of the spectra [58]. Therefore, the proper incorporation of BICAR in PLA/C30B foams was demonstrated due to the generation of characteristic peaks of both compound in the FTIR spectra of the developed materials.

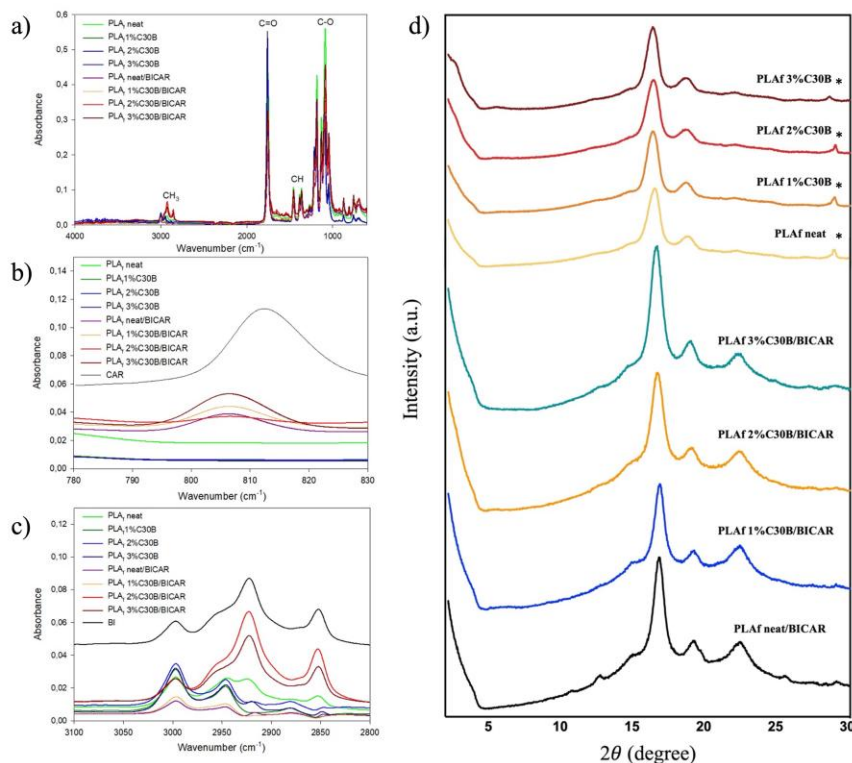


Fig. 4. (a-c) FTIR-ATR spectra and (d) X-ray diffraction patterns of PLA composite foams with and without BICAR cocrystals. *The X-ray diffraction patterns of PLA neat, PLA 1% C30B, PLA 2% C30B and PLA 3% C30B have been previously reported [33] and have been included here for comparison.

X-ray diffraction analysis was performed to study the crystal structure of PLA foams loaded with C30B and their supercritical cocrystallization with BICAR. **Fig. 4 (d)** shows the X-ray diffraction patterns of the samples with and without the BICAR cocrystal. It can be seen that both conditions showed peaks centered at $2\theta = 16.5^\circ$ and $2\theta = 18.3^\circ$, characteristic of PLA crystalline forms [59]. However, a new broad peak centered at $2\theta = 22.4^\circ$ was observed, attributable to the deposition of BICAR cocrystals in the polymer matrix, indicating its amorphous nature [60].

In addition, PLA composite foams showed a larger peak shift centered at $2\theta = 4.82^\circ$ of pure C30B at even lower 2θ angles than in the case of composite films, furthermore with a higher intensity, showing a greater effect of nanoclay on the composite structures when processed by supercritical foaming. The large displacements of these peaks were already observed by Lee et al. (2009), who developed foams of C30B-loaded starch/PLA composites and attributed this behavior as indicative of the intercalation of a small amount of polymer chains in the gallery space between silicate platelets [62]. In the present work, the peaks that appeared around $2\theta = 5.3^\circ$ in the PLA composite films disappeared in the XRD pattern of the foams, suggesting that exfoliation of the clay in the matrix was achieved, which can be attributed to the plasticizing effect of $scCO_2$ in the composite matrices by increasing the mobility of the polymer chains, enhancing the dispersion of C30B in the polymer matrix and allowing greater interaction between PLA and C30B.

3.4. *Viscosity molecular weight*

The estimated viscosity molecular weight (M_v) of the PLA pellet previously calculated was around $\sim 186,000$ g/mol [33]. The intrinsic viscosity (η) and the calculated molecular weight of the PLA and nanocomposite foams containing BICAR cocrystals are gathered in **Table 1**. Values related to neat PLA foam and PLA/C30B foams without BICAR cocrystals are from our previous work [33] and they were included here for comparison. where the diminution of the estimated M_v of the PLA produced by melt-extrusion and supercritical foaming process was discussed. In addition, it was concluded that in the PLA/C30B foams the presence of the nanoclay as a nucleating agent allows the formation of a porous structure more homogeneous during the foaming process, which turns into a less noticeable molecular weight reduction with respect to the counterpart nanocomposite film.

428 As was described in **section 2.2.3** of this work, to obtain the BICAR cocrystals into the
429 PLA/C30B foams, a second scCO₂ process was performed with established conditions of 10
430 MPa pressure at 40 °C for 3 h and a depressurization rate of 0.1 MPa/min. In the foaming
431 process the gas pressure, time and temperatures are factors that act directly over foams
432 parameters such as cell morphology, density or crystallinity. Besides, it has been studied how
433 PLA trend to foam as well during the gas release stage [63]. This is totally in concordance
434 with the M_v obtained here for all the foams after cocrystallization. After the second
435 interaction with supercritical CO₂ and a slow gas release, it was obtained a M_v value of
436 109,598 g/mol for the cocrystallized neat PLA foam which represents a reduction of 24%
437 respecting to the neat PLA foam without BICAR cocrystals. A similar molecular weight
438 decrease was observed for the PLA/C30B foams, especially for PLAf 3%C30B/BICAR
439 whose M_v value of 89,966 g/mol represents a noticeable reduction compared to the 123,040
440 g/mol of the PLAf 3%C30B sample. Nevertheless, with smaller C30B contents the
441 modification of the molecular weight is less remarkable which is related to the slightly higher
442 crystallinity obtained for the starting PLAf 1%C30B and PLAf 2%C30B foams. Corre et al.
443 (2011) have studied the influence of rheological properties on the cellular structure of chain-
444 extended PLA foams and observed a reduced CO₂ sorption and diffusion due to the presence
445 of highly ordered chain packaging. Besides, DRX results indicated an increase in the
446 crystallinity of the foams by the cocrystallization process and this is more evident for the
447 PLAf 3%C30B/BICAR. Hence, the crystallinity increase, and a significant modification of
448 the molecular weight could be related to the greater interaction between the pressurized gas
449 and a high porosity structure, and over a considerable time which lead in shorter polymer
450 chains.

451 **Table 1.** Intrinsic viscosity (η), Viscosity Molecular Weight (Mv) and mechanical properties of PLA foams before and after 10 contact days with the food
 452 simulant D1.

Sample	η (mL g ⁻¹)	M_v (g mol ⁻¹)	E (MPa)	E_{D1} (MPa)	TS (MPa)	TS_{D1} (MPa)	ε (%)	ε_{D1} (%)
PLA f neat	121.7*	144,753*	22.8 ± 2.7 ^{a*}	17.2 ± 2.3 ^a	1.7 ± 0.2 ^{a*}	1,6±0,1 ^a	18.3 ± 5.2 ^{a*}	32,5±4,3 ^a
PLAf 1%C30B	113.9*	129,964*	51.8 ± 6.6 ^{b*}	18.3±2.3 ^a	3.6 ± 0.2 ^{c*}	2,0±0,2 ^{ab}	49.6 ± 4.3 ^{c*}	24,6±2,2 ^b
PLAf 2%C30B	105.8*	117,736*	44.7 ± 2.7 ^{b*}	24.5±0.7 ^b	2.3 ± 0.2 ^{b*}	2,2±0,4 ^c	31.8 ± 3.7 ^{b*}	13,7±0,9 ^c
PLAf 3%C30B	110.6*	123,040*	47.0 ± 3.2 ^{b*}	81.9±6.4 ^c	4.0 ± 0.1 ^{d*}	4,1±0,3 ^d	32.9 ± 2.9 ^{b*}	8,6±1,6 ^d
PLAf neat/BICAR	99.29	109,598	16.7 ± 2.1	8.82±1,1 ^d	3.6 ± 0.3	0,7±0,1 ^c	76.5 ± 2.6	13,1±0,6 ^c
PLAf 1%C30B/BICAR	102.45	114,386	17.2 ± 1.8	16.5±0,8 ^a	2.5 ± 0.2	1,7±0,2 ^{ab}	64.9 ± 3.2	14,2±1,4 ^c
PLAf 2%C30B/BICAR	101.16	111,894	27.9 ± 2.4	16.9±1.2 ^a	3.0 ± 0.1	2,2±0,2 ^c	68.1 ± 2.3	14,5±0,9 ^c
PLAf 3%C30B/BICAR	86.19	89,966	45.5 ± 3.8	33.±2.4 ^c	3.8 ± 0.2	3,1±0,2 ^c	68.8 ± 2.4	14,1±0,5 ^c

453 *The Intrinsic viscosity (η), Viscosity Molecular Weight (Mv) and tensile test results before 10 contact days with the food simulant D1 of previously reported PLA

454 and PLA/C30 foam have been included for comparison [33]

455 ^{a-d} Different superscripts within the same column indicate significant differences (p < 0.05).

456 D1 Indicates the mechanical properties after 10 contact days in simulant D1 at 40°C.

3.5. *Water uptake*

As the cocrystallized nanocomposite foams developed here are proposed as materials with potential applicability in the food packaging sector, the study of its behavior against humidity and/or in direct contact with water is pertinent. With this purpose, water absorption of the PLA/C30B foams with BICAR cocrystals during an immersion time of 84 days at a controlled temperature of 23 ± 1 °C was measured. The water absorbed represented as a percentage of mass gain curve versus the immersion time of each sample is plotted in **Fig. 5**. During the first week under water immersion, it can be observed in the graph how the mass gain of all the foam samples is relatively small compared with the values achieved at the end of the assay. Although is worth noting that this weight increment produced by water absorption represents 25% for the cocrystallized foams with nanoclay, while for PLAf neat/BICAR is approximately 15%. This high hydrophilicity of the cocrystallized PLA/C30B foams is correlated with the presence of well-dispersed nanoclay particles that as it was previously mentioned promote a nucleating effect during the foaming process and a more porous inner structure is formed. As the void content of the foam is increased more space is available for water molecules to contact the material surface and the water diffusion through the material is facilitated [65].

Then, in the second week, the high capacity to absorb water of the nanocomposite foams compared to neat PLA foam becomes more evident and this trend remains until the end of the immersion time. After this initial period, PLAf neat/BICAR remains with a moderately growing amount of water absorbed, reaching a 50% of weight increment after the studied immersion time. Though, this value does not seem as the saturation mass of the PLAf neat/BICAR, curves apparently is near to being completely asymptotic after these 84 days.

480 In regard to the presence of nanoclay particles, small contents affected significantly the
481 hydrophilic behavior of the foams, the mass of water absorbed during the first half of the test
482 grow considerably up to 150% after 35 days for the foams containing 1 and 2 wt% of C30B.
483 While with the upper C30B content after the same period, the saturation mass is almost
484 reached, exceeding 300% of the weight gained by water uptake. Finally, when the studied
485 immersion time was over, the asymptotic aspect of the curve of all the PLA/C30B
486 cocrystallized foams indicates that the mass saturation was reached.

487 Despite the saturation mass of all the cocrystallized nanocomposites foams resulted
488 extremely high, the sample with 2 and 3 wt.% of C30B absorbed almost 350% in weight of
489 water while PLA/C30B /BICAR remains around 175%. One of the aims of this work is
490 to study the effectiveness of incorporating carvacrol into cocrystallized foams with variable
491 content of nanoclays. Water uptake results indicate that the incorporation of BICAR
492 cocrystals affects the hydrophilicity of the nanocomposite foams and the higher incorporation
493 of carvacrol induces greater water absorption capacity. Probably due to the higher mobility
494 acquired by the polymer chains in these formulations, due to the plasticizer effect that
495 occurred which will be further discussed during the tensile test discussion, which facilitates
496 the water diffusion throughout the polymeric foam.

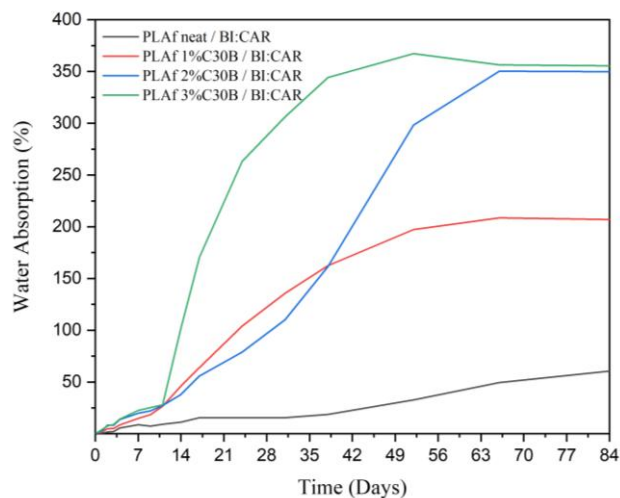


Fig. 5. Water uptake of the neat and bionanocomposite cocrystallized foams.

Comentado [MA1]: Esta figura creo que quedaría mejor con el formato de las Figuras de cinética de liberación y de desintegración en compost (con un punto/triángulo en cada día que lo han pesado)

3.7. Supercritical cocrystallization and release kinetics of CAR from cocrystallized bionanocomposite foams

Supercritical cocrystallization tests were performed following the procedure described in section 2.2.3. Table 2 shows the effective cocrystal incorporation and quantified by HPLC at the time of obtaining the BICAR reference cocrystals (section 2.5). Furthermore, impregnated foams with carvacrol were tailored under the same conditions mentioned for the foam cocrystallization process (section 2.2.3), in order to compare the kinetics between foams impregnated with the active compound (CAR) and foams cocrystallized with BICAR. Table 2 shows the CAR amount and incorporation percentages of the impregnated foams (control). Those results show that there is a higher incorporation of cocrystals in the foams of PLA composites with 2 (wt.%) and 3 (wt.%) of C30B, 22.55 (wt.%) and 24.30 (wt.%) respectively, which is also in agreement with the effective incorporation of carvacrol, 12.28 (wt.%) and 13.23 (wt.%) respectively. In addition, a higher incorporation of carvacrol could

be observed, reaching almost 47.07 (wt.%) for the 3% C30B foams. It was observed that the impregnated foams increased the amount of CAR incorporated by 2.9 to 4.2 times with respect to the foams with BICAR cocrystals. The difference in the effective incorporation of BICAR in foamed PLA composites and the higher incorporation of carvacrol in impregnated foams is mainly due to the chemical affinity of the components, since PLA presents in its chemical structure hydroxyl groups (-OH) in each terminal branch and in the C30B layered silicate, in addition to carboxyl groups characteristic (-C=O) of the polymer [27]. These functional groups improve the supramolecular-type chemical interactions, especially hydrogen-bridging ones, with both compounds of the BICAR cocrystal (coformer and active compound).

On the other hand, the morphology of the material could favor the contact area between the components by increasing the cell density of the foams with the addition of C30B. As shown in previous results, the foams increased their cell density with increasing clay content, which increased the surface area available for the molecular interaction between PLA-C30B and the active compound. Finally, these results are in agreement with those reported in the literature, since the presence of clay allows the incorporation of higher amounts of carvacrol into the polymer matrix [66].

3.8. Release kinetics of carvacrol to a food simulant

In this work, the study of release kinetics of carvacrol from PLA and PLA/C30B foams to a food simulant was carried out according to the procedure described in **section 2.6.1**. The results of carvacrol concentrations in the food simulant obtained as a function of time are shown in **Fig. 6**, for each condition according to standardized protocols for specific migration tests. In this figure, the dots represent the experimental results; meanwhile, the black lines

close to experimental data represent the adjusted mass transfer model, which was obtained through an iterative estimation of the effective diffusion coefficient (D_P in **Eq.1**) according to the procedure described in **section 2.6.2**. The carvacrol release can be characterized by two steps: (i) a sustained release stage where the diffusion of the carvacrol through the polymeric foam is acting and (ii) the stage when the thermodynamic equilibrium between the polymeric phase and the simulant. This last stage is reached at average times of 7.5 h (**Fig. 6a**) and 24 h (**Fig. 6b**) for impregnated foams and BICAR cocrystallized foams, respectively. Thus, there is a significant change in the release rate of carvacrol when the carvacrol is cocrystallized slowing down the transfer rate because of the molecular interactions between the active agent (CAR), the conformer (BI) and the polymeric matrix. These interactions have been explained in literature by supramolecular effects such as hydrogen bonds [67–69].

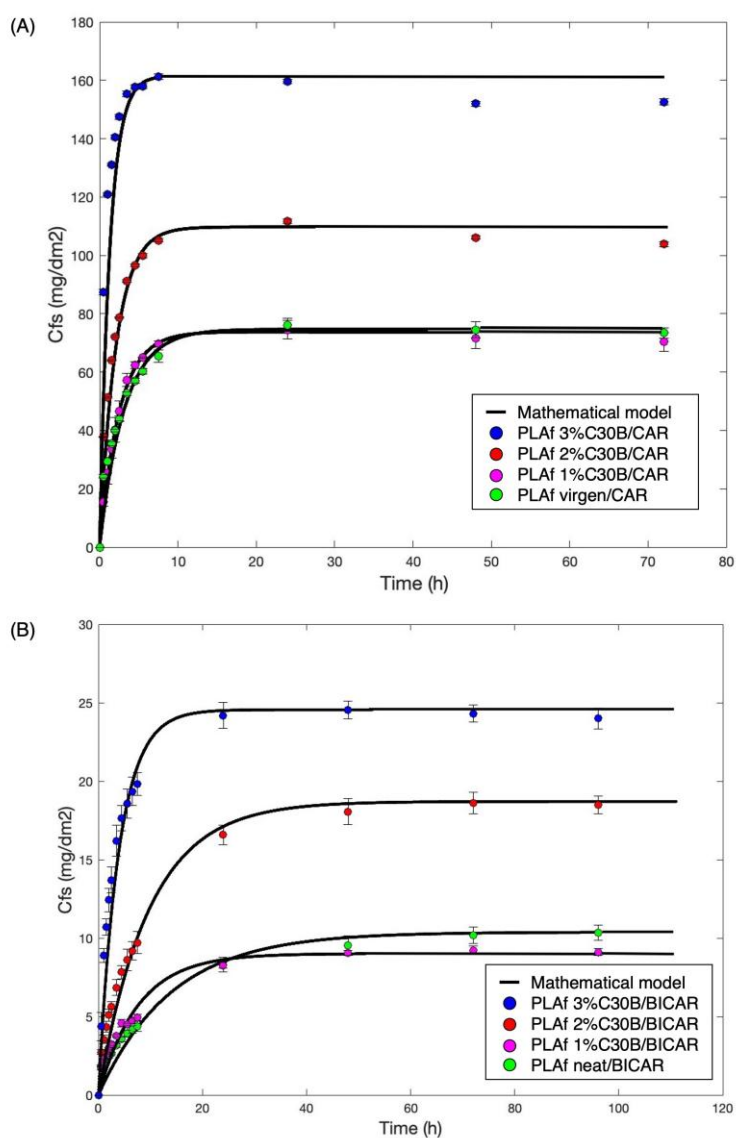
The highest concentration of released carvacrol was estimated in impregnated foams (**Fig. 6a**) reaching equilibrium values varying from 76 [mg dm⁻²] to 150 [mg dm⁻²]; meanwhile, the BICAR cocrystallized foam samples (**Fig. 6b**) released carvacrol reaching equilibrium concentrations ranged from 10 [mg dm⁻²] to 24 [mg dm⁻²] in the food simulant. In this way, **Table 2** shows the values of the distribution coefficient ($K_{P/FS}$) estimated from the experiments as well as the values of the effective diffusion coefficient of carvacrol through the polymeric matrix (D_P) estimated from the transfer model adjustment.

From the comparison of the experimental data with the transfer model it is possible to observe that the value of $K_{P/FS}$ increased as the concentration of C30B increased in the composite material foams, from 718 to 1590 for the direct incorporation of carvacrol and from 1300 to 3050 for the incorporation of carvacrol as BICAR cocrystals. These results show a higher affinity of carvacrol with C30B which retains the active agent in the composite material

558 limiting its release to the food simulant in terms of the final concentration. This higher
559 retention of carvacrol due to the higher content of C30B could be explained by the interaction
560 of the -OH site of the nanoclay with the phenol group of carvacrol. This has been observed
561 in other nanocomposite systems containing carvacrol [66].

562 On the other hand, **Table 2** shows the estimated values of D_P obtained from the adjustment
563 of the transfer model. These values varied between $3.5 \cdot 10^{-13} \text{ [m}^2 \cdot \text{s}^{-1}\text{]}$ and $9.0 \cdot 10^{-13}$
564 $\text{[m}^2 \cdot \text{s}^{-1}\text{]}$ for foams containing BICAR cocrystals. These values were at least one order of
565 magnitude lower than the values observed for foams with direct impregnation of carvacrol,
566 where the value of D_P varied between $5.2 \cdot 10^{-12} \text{ [m}^2 \cdot \text{s}^{-1}\text{]}$ and $9.0 \cdot 10^{-12} \text{ [m}^2 \cdot \text{s}^{-1}\text{]}$. It is
567 worth noting that the value of D_P reported for the foam with 3wt% of C30B containing
568 carvacrol (CAR) was reported as $1.0 \cdot 10^{-3} \text{ [m}^2 \cdot \text{s}^{-1}\text{]}$ because this value is the highest estimation
569 of effective diffusion coefficient that can be calculated with convergence in the adjustment
570 of the mass transfer model using the MatLab script developed in this study then it represents
571 an underestimation of its real value. This extremely high value of D_P for this specific system
572 is not coherent with the transport phenomena observed in all the other cases studied herein
573 and it could be explained by a possible saturation of carvacrol on the surface of the composite
574 foam with the highest concentration of C30B. Thus, the effective diffusion coefficient value
575 estimated by the model adjustment is not representing a gradual release from the composite
576 phase as the proposed theoretical approach supposes and it cannot be directly compared with
577 the other values that represent a predominant diffusive phenomenon through the foam. This
578 release behavior has also been documented in the presence of active compounds with
579 hydrophobic characteristics, such as thymol and carvacrol, where not only release is favored

580 in fatty simulants [37], but also in polymeric media that present a higher charge and lower
581 foam cell density, significantly favoring the release rate of this type of compounds [70].



582

583 **Fig. 6.** Effect of BICAR cocrystallized bionanocomposite foams on CAR release process in simulant D1.

584

585 **Table 2.** Incorporation of BICAR cocrystal and carvacrol in PLA foams, partition coefficients ($K_{P/FS}$) and
 586 effective diffusion coefficients (D_p) of carvacrol in PLA bionanocomposite foams in contact with fatty
 587 simulant at 40°C.

Sample	C30B [wt. %]	BICAR incorporated [wt. %]	CAR incorporated [wt. %]	$K_{P/FS}$	$D_p [m^2 \cdot s^{-1}]$
<i>Impregnated foams</i>	0	-	18.39 ± 0.03^a	718	$5.2 \cdot 10^{-12}$
	1.0	-	25.99 ± 0.40^a	825	$6.8 \cdot 10^{-12}$
	2.0	-	37.74 ± 0.32^b	925	$9.0 \cdot 10^{-12}$
	3.0	-	47.07 ± 1.25^c	1590	$1.0 \cdot 10^{-3*}$
<i>Cocrystallized foams</i>	0	8.83 ± 0.04^a	4.81 ± 0.02^a	1600	$3.5 \cdot 10^{-13}$
	1.0	11.11 ± 0.41^a	6.05 ± 0.22^a	1745	$6.5 \cdot 10^{-13}$
	2.0	22.55 ± 0.89^b	12.28 ± 0.48^b	2015	$4.2 \cdot 10^{-13}$
	3.0	24.30 ± 0.62^b	13.23 ± 0.64^b	3050	$9.0 \cdot 10^{-13}$

588 *: This value is the highest estimation of D_p that can be developed with convergence in the
 589 adjustment of the mass transfer model using the MatLab script developed in this work, but this
 590 value underestimate the value observed in the experimental test.

591 3.9. Antioxidant activity of cocrystallized foams

592 With the aim to confirm the effectiveness of the cocrystallized foams incorporated with
 593 BICAR as active materials the reduction of stable free radical DPPH as a consequence of
 594 carvacrol presence was determined after the release from the foamed PLA-based composites
 595 in the fatty food simulant D1 after 10 contact days at 40°C.

596 The obtained results of the percentage of the radical scavenging activity (RSA%) were: PLAf
 597 neat/BICAR = 41.6 ± 0.1 %, PLAf 1%C30B/BICAR = 34.3 ± 1.6 %, PLAf 2%C30B/BICAR
 598 = 60.8 ± 0.1 % and PLAf 3%C30B/BICAR = 86.2 ± 2.1 %. The PLAf neat/BICAR foam
 599 showed higher antioxidant activity than that of the PLAf 1%C30B/BICAR, even when it
 600 contains less amount of BICAR incorporated in the polymeric matrix (**Table 2**). As it was

already commented C30B is able to better retain the active agent in the PLA matrix limiting its release from the composite PLA/C30B/BICAR to the fatty food simulant ascribed to the interaction of the -OH of C30 with the phenol group of carvacrol. The results of the antioxidant performance of the cocrystallized foams are in good agreement with the carvacrol concentrations released in the fatty food simulant as a function of time already discussed (see **Fig. 6b**) as they showed a comparative tendency. The scavenging effect obtained here for composite loaded with 2 wt.% of C30B, and thus containing 12.28 ± 0.48 t.% of carvacrol, are in the range of PLA-based polymers incorporated with carvacrol [71]. In this sense, Burgos et al. (2017) prepared PLA-PHB blends plasticized with oligomeric lactic acid (OLA) and incorporated with 10 wt.% of carvacrol as active compound by melt blending, studied the antioxidant activity in an ethanolic solution (100% ethanol) and found RSA values between 56 and 64 %. Similarly, Altan et al. (2018) developed electrospun PLA fibers loaded with 10 wt.% of carvacrol and put them in direct contact with a DPPH solution for 30 minutes, obtaining RSA values around 60%. Therefore, our results show that carvacrol is able to interact with fatty food protecting food products from oxidative processes and extending their shelf-life and, thus, confirming once again the effective incorporation of BICAR in foamed PLA nanocomposites.

3.6. *Mechanical properties*

The PLA/C30B nanocomposite foams loaded with BICAR cocrystals developed in the present work are proposed as promising food packaging material some features such as good flexibility or enough strength play a key role in successful applicability in the food packaging field. Therefore, the mechanical performance of all foam samples before and after 10 days at

623 40°C in contact with the fatty food simulant D1 was studied by means of tensile test
624 measurements.

625 With the aim of evaluating the mechanical behavior of the cocrystallized PLA/C30B foams,
626 the mean values of the tensile test results are gathered in **Table 1**. For a proper comparison,
627 tensile test values of the PLA/C30B foams before the cocrystallization process were
628 recovered from previous work [33] and are also summarized in the same table.

629 It is well known the plasticization effect that supercritical CO₂ processes exert in PLA-based
630 materials [27,33,37]. The good solubility of the CO₂ in PLA [73] together with high pressures
631 and temperatures allow the gas molecules to induces polymer chain swelling which
632 increments the interchain distance and facilitates their movement [74]. This was evidenced
633 in the elongation at break values obtained for the foams which are around 20-50 %. On the
634 other hand, when a polymer matrix is plasticized Young's modulus and the tensile strength
635 tend to decrease due to the ductility enhanced of the material. But in the case of the
636 PLA/C30B nanocomposite foams, this effect is countered by the good dispersion of the
637 nanoclay achieved during the foaming process [33]. Thus, in the nanocomposite foams the
638 Young's modulus values resulted in higher than neat PLA foam.

639 Regarding the foams with BICAR cocrystals, the second scCO₂ process to produce the
640 cocrystals subjected PLA matrix to a further plasticization effect. After cocrystallization,
641 PLAf neat/BICAR showed a high increase in elongation at break, up to 76.54 %, and the
642 Young's modulus decreased from 22.8 MPa in PLA neat foam to 16.75 MPa in PLAf
643 neat/BICAR. The values obtained for the elongation at break of the cocrystallized
644 PLA/C30B/BICAR foams are also significantly higher than their counterpart nanocomposite
645 foams and keep closer to neat PLA cocrystallized foam (PLAf neat/BICAR). Although a

646 reduction of Young's modulus is also observed for the nanocomposite cocrystallized foams,
647 this is practically insignificant for the upper C30B contents. While the modulus value of the
648 PLAf 1%C30BBICAR dropped to 17.20 MPa from 51.79 MPa of the same foams before
649 cocrystallization, the Young's modulus of the PLAf 3%C30B remained around 46 MPa after
650 and before the cocrystallization. Tensile strength values of all the foams after and before of
651 the second scCO₂ process remain in the same range despite the plasticization effect exerted
652 by the CO₂. As was mentioned during the discussion of SEM results, the presence of the
653 C30B particles promoted the formation of a more uniform, fine and dense cell structure [33].
654 When foam is under mechanical requirements, the cell structure affects significantly,
655 improving the tensile strength and Young's modulus if a homogenous distribution of small
656 closed-cells is formed [75].

657 The tensile test results after the exposition to the fatty food simulant showed that in general
658 the Young's modulus mainly decreased, being this effect particularly marked in PLA/C30B
659 foams, while it was less pronounced in cocrystallized PLA/C30B/BICAR foams. In fact, the
660 Young's modulus of PLAf 1%C30B/BICAR was mainly maintained after the foam
661 exposition to the food simulant. Similarly, the tensile strength values decreased in
662 cocrystallized foams after 10 days in contact with the fatty food simulant. It should be
663 highlighted that while PLAf neat/BICAR showed a drastic diminution of the tensile strength
664 value (a reduction of around 80%), this reduction was less pronounced in cocrystallized
665 PLA/C30B/BICAR nanocomposite foams (a reduction of around 18-30%). These results
666 highlight the importance of adding C30B to produce foams with better overall mechanical
667 performance. In fact, the microstructural analysis by SEM observations after the
668 cocrystallized foams after 10 days exposed to simulant D1 (Supplementary **Fig. S2**) also

shows that higher microstructural changes are produced in PLA neat/BICAR, while PLA/C30B/BICAR nanocomposites although showed somewhat deformation on the closed-cell foams, those changes were less marked due to the already mentioned positive effect of the nanoclay as a cell nucleation agent. Regarding the elongation at break, in general, all foams showed a diminution of the flexibility of the foams after ten days in contact with fatty food simulant. However, this behavior was considerably marked in cocrystallized foams containing BICAR suggesting that not only the CO₂ is producing a plasticizer effect, but also carvacrol is plasticizing the system. The plasticizing effect of carvacrol has been already observed by other authors [54,76].

3.10. Disintegration under composting

PLA foams and their C30B-loaded nanocomposites were disintegrated under standard laboratory-scale composting conditions. **Fig. 7** shows the disintegration results, samples' visual appearances for 30 days exposed to the composting medium are shown in **Fig. 7a**, while the mass loss prompted by the composting conditions in the disintegrability test as a function of time is displayed in **Fig. 7b**.

It is well known that PLA disintegration under composting conditions occurs in two main consecutive stages: (i) hydrolytic and (ii) enzymatic degradation processes [27,45,51]. From the results obtained here, it is clear that the disintegration phenomenon starts first in the cocrystallized foams. During the beginning of the PLA disintegration phenomenon, it is firstly attacked by water, the cocrystallized foams allowed the penetration of water faster since the polymer chains acquired higher mobility due to the plasticizing effect this is why the cocrystallized foams started the disintegration phenomenon earlier than the foams

692 without cocrystallization treatment showing mass loss at the first day of the disintegration
693 test. Although the mass loss of the foams not subjected to the cocrystallization process started
694 around 18 days, they previously showed some signs of disintegration. Moreover, from the
695 water absorption test it can be seen that the foams started water absorption between 10 and
696 14 days (**Fig. 5**); and it can be concluded that after water enters the structure of the foams, it
697 hydrolyzes the polymer chains into shorter ones which are susceptible to the microorganism
698 attack [45]. Generally, biodegradation is slower with increasing the molecular weight of the
699 polymer [77], this is in agreement with the results shown in **Table 1**, where cocrystallized
700 foams presented lower molecular weights than untreated foams. Therefore, once the
701 disintegration phenomenon started, it quickly resulted in a greater disintegration rate than in
702 the foams that were not subjected to the cocrystallization process. This behavior can be
703 ascribed to the structure of the foam which has a larger exposed surface area in contact with
704 water [33]. Foams rapidly and homogeneously allow the penetration of water and then the
705 hydrolyzes of the polymeric chains into smaller ones in the whole material to be further
706 enzymatically degraded by the microorganisms existing in the compost medium [45,78,79].
707 All the samples analyzed reached disintegration percentages above 90% before 30 days,
708 reaching the frequently used value as a disintegrability target [27,45,80] and demonstrating
709 their sustainable character.

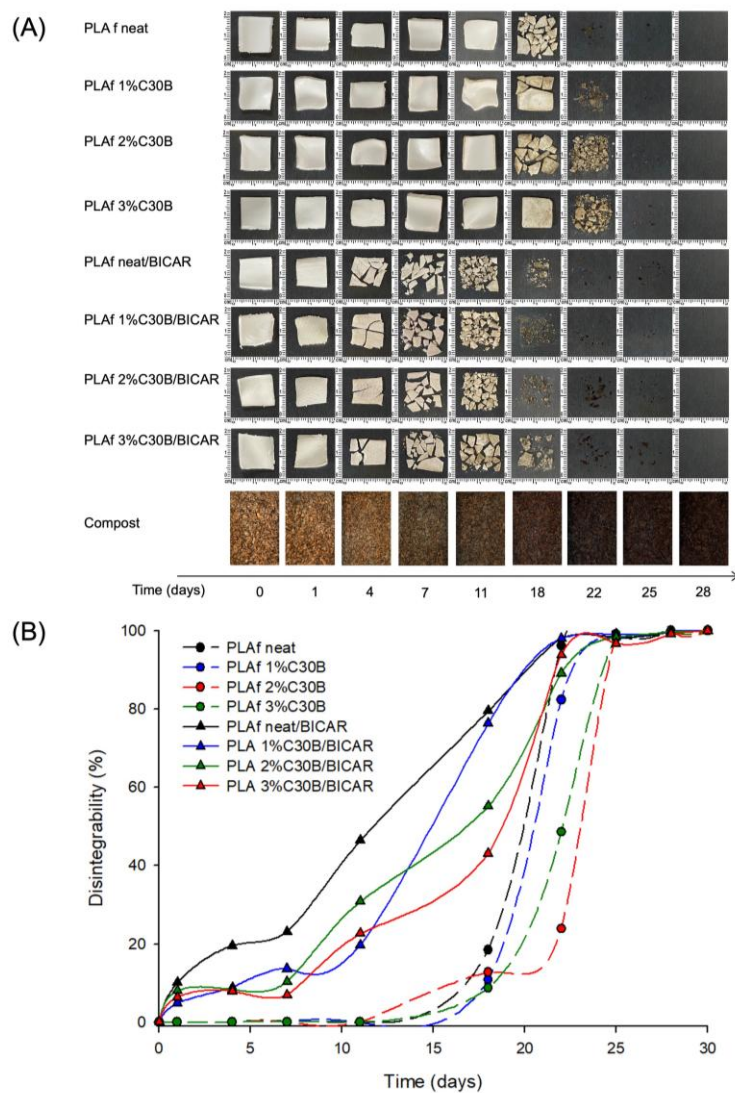


Fig. 7. A) Visual appearance of foam samples before and after different incubation days under composting conditions. B) Foam disintegration degree under composting conditions as a function of time.

4. Conclusion

In this work, the effect of the BICAR cocrystals and their incorporation in a nanocomposite foam by supercritical processing over the structural, mechanical and antioxidant properties of the obtained material has been studied. Through the experimental work developed herein, microcellular foams were obtained from PLA-based bionanocomposite films. SEM analysis showed that closed-cell foams were obtained, in which the PLA/C30B foam stood out for having small cells, thick walls and the highest cell density, due to the role of the nanoclay as a cell nucleation agent. SEM micrographs of the cocrystallized foams showed the effect of cocrystallization processing on the structure. The samples showed roughness in the cell walls, but no breaks in the material were evident, resulting in closed-cell foams. No BICAR microcrystals were observed inside the foams, however, at surface level small crystals of a size between 150-300 [nm] were observed.

The effect of C30B clay incorporation on the content of carvacrol incorporated in PLA foams was evidenced, as PLA/C30B/BICAR foams presented the highest incorporation percentage of the crystallized foams with 13.23 %[w/w] and a cocrystal incorporation percentage of 24.3 %[w/w].

XRD patterns of the cocrystallized foams revealed the incorporation of BICAR cocrystals into the polymer matrix, the peaks of the PLA foam samples with cocrystals were more elongated, demonstrating an increase in the crystallinity of the samples by the supercritical cocrystallization process.

When comparing the release kinetics of PLA foams impregnated with CAR and foams with BICAR cocrystals, differences are observed in terms of interfacial equilibrium concentrations in the foam-food simulant distribution. Bionanocomposite foams impregnated with carvacrol show a higher incorporation of the active compound (2.9 to 4.2 times higher)

737 compared to the system containing BICAR cocrystals, as well as a faster release of the active
738 agent than the observed one in foams containing BICAR cocrystals. The presence of
739 cocrystals implies a decrease of at least one order of magnitude in the value of the effective
740 diffusion coefficient of carvacrol through the PLA bionanocomposite foams, which means a
741 significant slowing down of the release under the experimental conditions considered in this
742 research. In addition, a higher affinity of carvacrol could be observed for those foams with
743 higher C30B content, which could be explained by the chemical affinity between the active
744 compound and the nanoclay. The antioxidant effectiveness of the carvacrol released from
745 cocrystallized foams was demonstrated. The disintegrability assessment under composting
746 medium confirmed the biodegradable character of all foamed formulations and also showed
747 that the cocrystallized foams disintegrated faster as the CO₂ plasticizes the system and speeds
748 up the hydrolysis process. Consequently, this study contributes to relating the interaction
749 between the active compound with a conformer, the polymer and the nanoclay to develop
750 biodegradable composite foams with tuned release characteristics to be used in antioxidant
751 active food packaging or other potential systems.

752 This study considered standard tests to characterize the mass transfer in the carvacrol release
753 process. However, further research will be conducted to test these materials with specific
754 foods.

756 CRediT authorship contribution statement

757 **Simón Faba:** Writing original draft, Formal analysis, Data curation & editing. **Marina**
758 **Arrieta:** Formal analysis, Data curation, Writing review & editing, Funding acquisition.
759 **Ángel Agüero:** Formal analysis, Data curation, Writing review & editing. **Sara Martínez:**

760 Data curation, Writing review & editing. **Alejandra Torres:** Supervision, Formal analysis.
761 **Emilio Rayón:** SEM Resources, Formal analysis, Writing review & editing. **Julio Romero:**
762 Supervision of supercritical processing and experimental and theoretical aspects of release
763 kinetics, Resources. **María José Galotto:** Conceptualization, Project administration,
764 Supervision, Funding acquisition.

765 **Declaration of Competing Interest**

766 The authors declare that there is no conflict of interest.

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784 **Supplementary materials**

785 Supplementary material associated with this article can be found in the online version.

786 **References**

- 787 [1] M.J. Galotto, A. Guarda, C.L. de Dicastillo, Antimicrobial Active Polymers in Food
788 Packaging, *Functional Polymers in Food Science: From Technology to Biology*. 1
789 (2015) 323–353. <https://doi.org/10.1002/9781119109785.ch10>.
- 790 [2] K. Cooksey, Antimicrobial food packaging materials, 2001.
791 [https://doi.org/10.1016/S0306-3747\(01\)80187-1](https://doi.org/10.1016/S0306-3747(01)80187-1).
- 792 [3] A. Guarda, J.F. Rubilar, J. Miltz, M.J. Galotto, The antimicrobial activity of
793 microencapsulated thymol and carvacrol, *Int J Food Microbiol*. 146 (2011) 144–
794 150. <https://doi.org/10.1016/j.ijfoodmicro.2011.02.011>.
- 795 [4] M.P. Arrieta, M.A. Peltzer, J. López, M. del C. Garrigós, A.J.M. Valente, A.
796 Jiménez, Functional properties of sodium and calcium caseinate antimicrobial
797 active films containing carvacrol, *J Food Eng*. 121 (2014) 94–101.
798 <https://doi.org/https://doi.org/10.1016/j.jfoodeng.2013.08.015>.
- 799 [5] A. Satpayeva, A. Rojas, M. Tyrka, E. Ksepko, M.J. Galotto, I. Zizovic, Supercritical
800 Foaming and Impregnation of Polycaprolactone and Polycaprolactone-
801 Hydroxyapatite Composites with Carvacrol, *Processes*. 10 (2022).
802 <https://doi.org/10.3390/pr10030482>.
- 803 [6] F. Reyes-Jurado, T. Cervantes-Rincón, H. Bach, A. López-Malo, E. Palou,
804 Antimicrobial activity of Mexican oregano (*Lippia berlandieri*), thyme (*Thymus*
805 *vulgaris*), and mustard (*Brassica nigra*) essential oils in gaseous phase, *Ind Crops*
806 *Prod*. 131 (2019) 90–95.
807 <https://doi.org/https://doi.org/10.1016/j.indcrop.2019.01.036>.
- 808 [7] A. Rojas, D. Cerro, A. Torres, M.J. Galotto, A. Guarda, J. Romero, Supercritical
809 impregnation and kinetic release of 2-nonanone in LLDPE films used for active
810 food packaging, *Journal of Supercritical Fluids*. 104 (2015) 1–9.
811 <https://doi.org/10.1016/j.supflu.2015.04.031>.
- 812 [8] C. Turek, F.C. Stintzing, Stability of Essential Oils: A Review, *Compr Rev Food Sci*
813 *Food Saf*. 12 (2013) 40–53. <https://doi.org/10.1111/1541-4337.12006>.
- 814 [9] M. Guo, X. Sun, J. Chen, T. Cai, Pharmaceutical cocrystals: A review of
815 preparations, physicochemical properties and applications, *Acta Pharm Sin B*. 11
816 (2021) 2537–2564.
817 <https://doi.org/https://doi.org/10.1016/j.apsb.2021.03.030>.
- 818 [10] O. Shemchuk, S. d'Agostino, C. Fiore, V. Sambri, S. Zannoli, F. Grepioni, D. Braga,
819 Natural Antimicrobials Meet a Synthetic Antibiotic: Carvacrol/Thymol and
820 Ciprofloxacin Cocrystals as a Promising Solid-State Route to Activity
821 Enhancement, *Cryst Growth Des*. 20 (2020) 6796–6803.
822 <https://doi.org/10.1021/acs.cgd.0c00900>.

- [11] A. Karagianni, M. Malamatar, K. Kachrimanis, Pharmaceutical cocrystals: New solid phase modification approaches for the formulation of APIs, *Pharmaceutics*. 10 (2018) 1–30. <https://doi.org/10.3390/pharmaceutics10010018>.
- [12] M. Karimi-Jafari, L. Padrela, G.M. Walker, D.M. Croker, Creating cocrystals: A review of pharmaceutical cocrystal preparation routes and applications, *Cryst Growth Des.* 18 (2018) 6370–6387. <https://doi.org/10.1021/acs.cgd.8b00933>.
- [13] S.A. Ross, D.A. Lamprou, D. Douroumis, Engineering and manufacturing of pharmaceutical co-crystals: a review of solvent-free manufacturing technologies, *CHEMICAL COMMUNICATIONS*. 52 (2016) 8772–8786. <https://doi.org/10.1039/c6cc01289b>.
- [14] M. Rodrigues, B. Baptista, J.A. Lopes, M.C. Sarraguça, Pharmaceutical cocrystallization techniques. Advances and challenges, *Int J Pharm.* 547 (2018) 404–420. <https://doi.org/10.1016/j.ijpharm.2018.06.024>.
- [15] N. Schultheiss, A. Newman, Pharmaceutical Cocrystals and Their Physicochemical Properties, *Cryst Growth Des.* 9 (2009) 2950–2967. <https://doi.org/10.1021/cg900129f>.
- [16] A.S. Aakeöy, C.B. Sihnh, Co-crystals, *The Royal Society of Chemistry*, 2018.
- [17] I. Sathisaran, S.V. Dalvi, Engineering cocrystals of poorlywater-soluble drugs to enhance dissolution in aqueous medium, *Pharmaceutics*. 10 (2018). <https://doi.org/10.3390/pharmaceutics10030108>.
- [18] M. Chauvet, M. Sauceau, J. Fages, Extrusion assisted by supercritical CO₂: A review on its application to biopolymers, *Journal of Supercritical Fluids*. 120 (2017) 408–420. <https://doi.org/10.1016/j.supflu.2016.05.043>.
- [19] A. Rojas, A. Torres, M.J. Galotto, A. Guarda, R. Julio, Supercritical impregnation for food applications: a review of the effect of the operational variables on the active compound loading, *Crit Rev Food Sci Nutr.* 0 (2019) 1–12. <https://doi.org/10.1080/10408398.2019.1567459>.
- [20] G. Ghoshal, Chapter 10 - Recent Trends in Active, Smart, and Intelligent Packaging for Food Products, in: A.M. Grumezescu, A.M.B.T.-F.P. and P. Holban (Eds.), *Handbook of Food Bioengineering*, Academic Press, Cambridge, MA, USA, 2018: pp. 343–374. <https://doi.org/https://doi.org/10.1016/B978-0-12-811516-9.00010-5>.
- [21] N. Singh, O.A. Ogunseitan, M.H. Wong, Y. Tang, Sustainable materials alternative to petrochemical plastics pollution: A review analysis, *Sustainable Horizons*. 2 (2022) 100016. <https://doi.org/https://doi.org/10.1016/j.horiz.2022.100016>.
- [22] A.L. Patrício Silva, J.C. Prata, T.R. Walker, A.C. Duarte, W. Ouyang, D. Barcelò, T. Rocha-Santos, Increased plastic pollution due to COVID-19 pandemic: Challenges and recommendations, *Chemical Engineering Journal*. 405 (2021) 126683. <https://doi.org/https://doi.org/10.1016/j.cej.2020.126683>.
- [23] S.T. Lee, *Polymeric foams: Innovations in processes, technologies, and products*, 1st Ed., Taylor & Francis, 2017. <https://doi.org/10.1201/9781315369365>.

- [24] S. Iannace, C.B. Park, *Biofoams- Science and Applications of bio-based cellular and porous materials*, 1st ed., Taylor & Francis, 2016. <https://doi.org/10.1201/b19333>.
- [25] M. Nofar, Effects of nano-/micro-sized additives and the corresponding induced crystallinity on the extrusion foaming behavior of PLA using supercritical CO₂, *Mater Des.* 101 (2016) 24–34. <https://doi.org/10.1016/j.matdes.2016.03.147>.
- [26] S. Molinaro, M. Cruz Romero, M. Boaro, A. Sensidoni, C. Lagazio, M. Morris, J. Kerry, Effect of nanoclay-type and PLA optical purity on the characteristics of PLA-based nanocomposite films, *J Food Eng.* 117 (2013) 113–123. <https://doi.org/10.1016/j.jfoodeng.2013.01.021>.
- [27] C. Villegas, M.P. Arrieta, A. Rojas, A. Torres, S. Faba, M.J. Toledo, M.A. Gutierrez, E. Zavalla, J. Romero, M.J. Galotto, X. Valenzuela, PLA/organoclay bionanocomposites impregnated with thymol and cinnamaldehyde by supercritical impregnation for active and sustainable food packaging, *Compos B Eng.* 176 (2019) 107336. <https://doi.org/10.1016/j.compositesb.2019.107336>.
- [28] N. Najafi, M.C. Heuzey, P.J. Carreau, Polylactide (PLA)-clay nanocomposites prepared by melt compounding in the presence of a chain extender, *Compos Sci Technol.* 72 (2012) 608–615. <https://doi.org/https://doi.org/10.1016/j.compscitech.2012.01.005>.
- [29] Y. Fujimoto, S. Sinha Ray, M. Okamoto, A. Ogami, K. Yamada, K. Ueda, Well-controlled biodegradable nanocomposite foams: From microcellular to nanocellular, *Macromol Rapid Commun.* 24 (2003) 457–461. <https://doi.org/10.1002/marc.200390068>.
- [30] L. Chen, D. Rende, L.S. Schadler, R. Ozisik, Polymer nanocomposite foams, *J Mater Chem A Mater.* 1 (2013) 3837–3850. <https://doi.org/10.1039/c2ta00086e>.
- [31] S.-K. Yeh, Y.-C. Liu, C.-C. Chu, K.-C. Chang, S.-F. Wang, Mechanical Properties of Microcellular and Nanocellular Thermoplastic Polyurethane Nanocomposite Foams Created Using Supercritical Carbon Dioxide, *Ind Eng Chem Res.* 56 (2017) 8499–8507. <https://doi.org/10.1021/acs.iecr.7b00942>.
- [32] J.A. Villamil Jiménez, N. Le Moigne, J.C. Bénézet, M. Sauceau, R. Sescousse, J. Fages, Foaming of PLA composites by supercritical fluid-assisted processes: A review, *Molecules.* 25 (2020) 3408. <https://doi.org/10.3390/molecules25153408>.
- [33] S. Faba, M.P. Arrieta, Á. Agüero, A. Torres, J. Romero, A. Rojas, M.J. Galotto, Processing Compostable PLA/Organoclay Bionanocomposite Foams by Supercritical CO₂ Foaming for Sustainable Food Packaging, *Polymers (Basel)*. 14 (2022). <https://doi.org/10.3390/polym14204394>.
- [34] M.P. Arrieta, J. López, E. Rayón, A. Jiménez, Disintegrability under composting conditions of plasticized PLA–PHB blends, *Polym Degrad Stab.* 108 (2014) 307–318. <https://doi.org/https://doi.org/10.1016/j.polymdegradstab.2014.01.034>.
- [35] UNE-EN-ISO, *Plastics-Determination of water absorption (ISO 62:2008)*, 2008. <https://asrecomposite.comWebwww.iso.orghttps://asrecomposite.com>.

- [36] UNE-EN 13130-1, Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Part 1: Guide to test methods for the specific migration of substances from plastics to foods and food simulants and the determination of substances in plastics and the selection of conditions of exposure to food simulants., 2005.
- [37] A. Torres, E. Ilabaca, A. Rojas, F. Rodríguez, M.J. Galotto, A. Guarda, C. Villegas, J. Romero, Effect of processing conditions on the physical, chemical and transport properties of polylactic acid films containing thymol incorporated by supercritical impregnation, *Eur Polym J.* 89 (2017) 195–210. <https://doi.org/10.1016/j.eurpolymj.2017.01.019>.
- [38] J. Sepulveda, C. Villegas, A. Torres, E. Vargas, F. Rodríguez, S. Baltazar, A. Prada, A. Rojas, J. Romero, S. Faba, M.J. Galotto, Effect of functionalized silica nanoparticles on the mass transfer process in active PLA nanocomposite films obtained by supercritical impregnation for sustainable food packaging, *Journal of Supercritical Fluids*. 161 (2020). <https://doi.org/10.1016/j.supflu.2020.104844>.
- [39] A. Torres, J. Romero, A. Macan, A. Guarda, M.J. Galotto, Near critical and supercritical impregnation and kinetic release of thymol in LLDPE films used for food packaging, *Journal of Supercritical Fluids*. 85 (2014) 41–48. <https://doi.org/10.1016/j.supflu.2013.10.011>.
- [40] Commission Regulation (EU), No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food, *Off. J. Eur. Union*. L12 (2011) 1–89.
- [41] M.P. Arrieta, M. del M. Castro-López, E. Rayón, L.F. Barral-Losada, J.M. López-Vilaríño, J. López, M.V. González-Rodríguez, Plasticized Poly(lactic acid)–Poly(hydroxybutyrate) (PLA–PHB) Blends Incorporated with Catechin Intended for Active Food-Packaging Applications, *J Agric Food Chem*. 62 (2014) 10170–10180. <https://doi.org/10.1021/jf5029812>.
- [42] UNE-EN-ISO, Plastics - Determination of the degree of disintegration of plastic materials under simulated composting conditions in a laboratory-scale test (ISO 20200:2015), (2016).
- [43] O. Gil-Castell, J.D. Badia, S. Ingles-Mascaros, R. Teruel-Juanes, A. Serra, A. Ribes-Greus, Polylactide-based self-reinforced composites biodegradation: Individual and combined influence of temperature, water and compost, *Polym Degrad Stab.* 158 (2018) 40–51. <https://doi.org/10.1016/j.polymdegradstab.2018.10.017>.
- [44] M.P. Arrieta, M. Perdigüero, S. Fiori, J.M. Kenny, L. Peponi, Biodegradable electrospun PLA-PHB fibers plasticized with oligomeric lactic acid, *Polym Degrad Stab.* 179 (2020). <https://doi.org/10.1016/j.polymdegradstab.2020.109226>.
- [45] M.P. Arrieta, E. Fortunati, F. Dominici, E. Rayón, J. López, J.M. Kenny, PLA-PHB/cellulose based films: Mechanical, barrier and disintegration properties, *Polym Degrad Stab.* 107 (2014) 139–149. <https://doi.org/10.1016/j.polymdegradstab.2014.05.010>.

- [46] I. Tsivintzelis, G. Sanxaridou, E. Pavlidou, C. Panayiotou, Foaming of polymers with supercritical fluids: A thermodynamic investigation, *J Supercrit Fluids*. 110 (2016) 240–250. <https://doi.org/https://doi.org/10.1016/j.supflu.2015.11.025>.
- [47] N. Najafi, M.-C. Heuzey, P.J. Carreau, D. Therriault, C.B. Park, Mechanical and morphological properties of injection molded linear and branched-poly(lactide) (PLA) nanocomposite foams, *Eur Polym J*. 73 (2015) 455–465. <https://doi.org/https://doi.org/10.1016/j.eurpolymj.2015.11.003>.
- [48] E.J. Jeong, C.K. Park, S.H. Kim, Fabrication of microcellular polylactide/modified silica nanocomposite foams, *J Appl Polym Sci*. 137 (2019) 48616. <https://doi.org/https://doi.org/10.1002/app.48616>.
- [49] X. Liao, A. V. Nawaby, H.E. Naguib, Porous poly(lactic acid) and PLA-nanocomposite structures, *J Appl Polym Sci*. 124 (2012) 585–594. <https://doi.org/10.1002/app.34994>.
- [50] X. Wang, J. Mi, H. Zhou, X. Wang, Transition from microcellular to nanocellular chain extended poly(lactic acid)/hydroxyl-functionalized graphene foams by supercritical CO₂, *J Mater Sci*. 54 (2019) 3863–3877. <https://doi.org/10.1007/s10853-018-3120-8>.
- [51] C. Villegas, S. Martínez, A. Torres, A. Rojas, R. Araya, A. Guarda, M.J. Galotto, Processing, Characterization and Disintegration Properties of Biopolymers Based on Mater-Bi® and Ellagic Acid/Chitosan Coating, *Polymers (Basel)*. 15 (2023). <https://doi.org/10.3390/polym15061548>.
- [52] S.-M. Lai, Y.-H. Liu, C.-T. Huang, T.-M. Don, Miscibility and toughness improvement of poly(lactic acid)/poly(3-Hydroxybutyrate) blends using a melt-induced degradation approach, *Journal of Polymer Research*. 24 (2017) 102. <https://doi.org/10.1007/s10965-017-1253-0>.
- [53] J.M. Cervantes-Uc, J. V Cauich-Rodríguez, H. Vázquez-Torres, L.F. Garfias-Mesías, D.R. Paul, Thermal degradation of commercially available organoclays studied by TGA–FTIR, *Thermochim Acta*. 457 (2007) 92–102. <https://doi.org/https://doi.org/10.1016/j.tca.2007.03.008>.
- [54] M.P. Arrieta, M.A. Peltzer, M. del C. Garrigós, A. Jiménez, Structure and mechanical properties of sodium and calcium caseinate edible active films with carvacrol, *J Food Eng*. 114 (2013) 486–494. <https://doi.org/https://doi.org/10.1016/j.jfoodeng.2012.09.002>.
- [55] M. Altan, Thermoplastic Foams: Processing, Manufacturing, and Characterization, Recent Research in Polymerization. (2018). <https://doi.org/10.5772/intechopen.71083>.
- [56] H. Wu, L. Ma, S. Li, J. Wang, T. Li, L. Peng, S. Li, Q. Li, X. Yuan, M. Zhou, Z. Zhang, Y. Liu, Sustained-release antibacterial gelatin films: Effects of diatomite/carvacrol complex on their structure, physicochemical and antibacterial properties, *Food Packag Shelf Life*. 35 (2023) 101019. <https://doi.org/https://doi.org/10.1016/j.fpsl.2022.101019>.
- [57] M. Alp, S. Yurdakul, Experimental and theoretical vibrational spectroscopic, quantum chemical analysis, and electronic properties investigations of novel ruthenium complexes (RuLCl₂·2H₂O; L: 4,4'-Dimethoxy-2,2'-Bipyridine, 4,4'-

- Dimethyl-2,2'-Bipyridine), Polyhedron. 234 (2023) 116322. <https://doi.org/https://doi.org/10.1016/j.poly.2023.116322>.
- [58] Y.-N. Zhang, H.-M. Yin, Y. Zhang, D.-J. Zhang, X. Su, H.-X. Kuang, Preparation of a 1:1 cocrystal of genistein with 4,4'-bipyridine, *J Cryst Growth*. 458 (2017) 103–109. <https://doi.org/https://doi.org/10.1016/j.jcrysgro.2016.10.084>.
- [59] N.M. Barkoula, B. Alcock, N.O. Cabrera, T. Peijs, Flame-Retardancy Properties of Intumescent Ammonium Poly(Phosphate) and Mineral Filler Magnesium Hydroxide in Combination with Graphene, *Polymers and Polymer Composites*. 16 (2008) 101–113. <https://doi.org/10.1002/pc>.
- [60] M. Sun, J. Lv, H. Xu, L. Zhang, Y. Zhong, Z. Chen, X. Sui, B. Wang, X. Feng, Z. Mao, Smart cotton fabric screen-printed with viologen polymer: photochromic, thermochromic and ammonia sensing, *Cellulose*. 27 (2020) 2939–2952. <https://doi.org/10.1007/s10570-020-02992-3>.
- [61] L.Y. Lee, S.H. Ranganath, Y. Fu, J.L. Zheng, H.S. Lee, C.H. Wang, K.A. Smith, Paclitaxel release from micro-porous PLGA disks, *Chem Eng Sci*. 64 (2009) 4341–4349. <https://doi.org/10.1016/j.ces.2009.07.016>.
- [62] H.W. Xiao, P. Li, X. Ren, T. Jiang, J.-T. Yeh, Isothermal crystallization kinetics and crystal structure of poly(lactic acid): Effect of triphenyl phosphate and talc, *J Appl Polym Sci*. 118 (2010) 3558–3569. <https://doi.org/https://doi.org/10.1002/app.32728>.
- [63] W. Zhai, Y. Ko, W. Zhu, A. Wong, C.B. Park, A study of the crystallization, melting, and foaming behaviors of polylactic acid in compressed CO₂, *Int J Mol Sci*. 10 (2009) 5381–5397. <https://doi.org/10.3390/ijms10125381>.
- [64] Y.-M. Corre, A. Maazouz, J. Duchet, J. Reigner, Batch foaming of chain extended PLA with supercritical CO₂: Influence of the rheological properties and the process parameters on the cellular structure, *J Supercrit Fluids*. 58 (2011) 177–188. <https://doi.org/https://doi.org/10.1016/j.supflu.2011.03.006>.
- [65] B. Kord, A. Varshoei, V. Chamany, Influence of chemical foaming agent on the physical, mechanical, and morphological properties of HDPE/wood flour/nanoclay composites, *Journal of Reinforced Plastics and Composites*. 30 (2011) 1115–1124. <https://doi.org/10.1177/0731684411417200>.
- [66] R. Scaffaro, A. Maio, E.F. Gulino, M. Morreale, F.P. La Mantia, The effects of nanoclay on the mechanical properties, carvacrol release and degradation of a pla/pbat blend, *Materials*. 13 (2020). <https://doi.org/10.3390/ma13040983>.
- [67] D. Capucci, D. Balestri, P.P. Mazzeo, P. Pelagatti, K. Rubini, A. Bacchi, Liquid Nicotine Tamed in Solid Forms by Cocrystallization, *Cryst Growth Des*. 17 (2017) 4958–4964. <https://doi.org/10.1021/acs.cgd.7b00887>.
- [68] K.Z. Barikah, Traditional and Novel Methods for Cocrystal Formation: A Mini Review, *Systematic Reviews in Pharmacy*. 9 (2018) 79–82. <https://doi.org/10.5530/srp.2018.1.15>.
- [69] A. Bacchi, P.P. Mazzeo, Cocrystallization as a tool to stabilize liquid active ingredients, *Crystallogr Rev*. 27 (2021) 102–123. <https://doi.org/10.1080/0889311X.2021.1978079>.

- [70] S. Milovanovic, D. Markovic, A. Mrakovic, R. Kuska, I. Zizovic, S. Frerich, J. Ivanovic, Supercritical CO₂ - assisted production of PLA and PLGA foams for controlled thymol release, *Materials Science and Engineering: C*. 99 (2019) 394–404. <https://doi.org/https://doi.org/10.1016/j.msec.2019.01.106>.
- [71] N. Burgos, I. Armentano, E. Fortunati, F. Dominici, F. Luzi, S. Fiori, F. Cristofaro, L. Visai, A. Jiménez, J.M. Kenny, Functional Properties of Plasticized Bio-Based Poly(Lactic Acid)_Poly(Hydroxybutyrate) (PLA_PHB) Films for Active Food Packaging, *Food Bioproc Tech*. 10 (2017) 770–780. <https://doi.org/10.1007/s11947-016-1846-3>.
- [72] A. Altan, Z. Aytac, T. Uyar, Carvacrol loaded electrospun fibrous films from zein and poly(lactic acid) for active food packaging, *Food Hydrocoll*. 81 (2018) 48–59. <https://doi.org/https://doi.org/10.1016/j.foodhyd.2018.02.028>.
- [73] M.P. Fernández-Ronco, R. Hufenus, M. Heuberger, Effect of pressurized CO₂ and N₂ on the rheology of PLA, *Eur Polym J*. 112 (2019) 601–609. <https://doi.org/https://doi.org/10.1016/j.eurpolymj.2018.10.023>.
- [74] L. Yu, H. Liu, K. Dean, L. Chen, Cold crystallization and postmelting crystallization of PLA plasticized by compressed carbon dioxide, *J Polym Sci B Polym Phys*. 46 (2008) 2630–2636. <https://doi.org/https://doi.org/10.1002/polb.21599>.
- [75] J. Chen, L. Yang, D. Chen, Q. Mai, M. Wang, L. Wu, P. Kong, Cell structure and mechanical properties of microcellular PLA foams prepared via autoclave constrained foaming, *Cellular Polymers*. 40 (2021) 101–118. <https://doi.org/10.1177/0262489320930328>.
- [76] I. Armentano, E. Fortunati, N. Burgos, F. Dominici, F. Luzi, S. Fiori, A. Jiménez, K. Yoon, J. Ahn, S. Kang, J.M. Kenny, Bio-based PLA_PHB plasticized blend films: Processing and structural characterization, *LWT - Food Science and Technology*. 64 (2015) 980–988. <https://doi.org/https://doi.org/10.1016/j.lwt.2015.06.032>.
- [77] T. Standau, C. Zhao, S. Murillo Castellón, C. Bonten, V. Altstädt, Chemical Modification and Foam Processing of Polylactide (PLA), *Polymers (Basel)*. 11 (2019) 306. <https://doi.org/10.3390/polym11020306>.
- [78] G. Kale, R. Auras, S.P. Singh, Comparison of the degradability of poly(lactide) packages in composting and ambient exposure conditions, *Packaging Technology and Science*. 20 (2007) 49–70. <https://doi.org/https://doi.org/10.1002/pts.742>.
- [79] Zeenat, A. Elahi, D.A. Bukhari, S. Shamim, A. Rehman, Plastics degradation by microbes: A sustainable approach, *J King Saud Univ Sci*. 33 (2021) 101538. <https://doi.org/https://doi.org/10.1016/j.jksus.2021.101538>.
- [80] F. Luzi, E. Fortunati, A. Jiménez, D. Puglia, D. Pezzolla, G. Gigliotti, J.M. Kenny, A. Chiralt, L. Torre, Production and characterization of PLA_PBS biodegradable blends reinforced with cellulose nanocrystals extracted from hemp fibres, *Ind Crops Prod*. 93 (2016) 276–289. <https://doi.org/https://doi.org/10.1016/j.indcrop.2016.01.045>.