

Experimental and theoretical characterization of the release kinetic of carvacrol as inclusion complexes with β -cyclodextrin in poly(lactic acid) and Mater-Bi processed by supercritical impregnation

Patricia Rivera^{1,2}, Alejandra Torres², Julio Romero^{1*}, Francisco Rodríguez², Marina Arrieta^{3,4}, Felipe Olea⁵, Tannia Silva², Paola Maldonado², Esteban Quijada-Maldonado⁵, Andrea Tapia²

¹ *Laboratory of Membrane Separation Processes (LabProSeM). Department of Chemical Engineering and Bioprocess. Engineering Faculty. University of Santiago de Chile. Santiago, Chile.*

² *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.*

³ *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.*

⁴ *Grupo de Investigación: Polímeros, Caracterización y Aplicaciones (POLCA), 28006, Madrid, Spain.*

⁵ *Laboratory of Separation Process Intensification (SPI), Department of Chemical Engineering and Bioprocess, University of Santiago de Chile, Santiago, Chile.*

*Corresponding author: julio.romero@usach.cl (J. Romero)

ABSTRACT

The incorporation of active compounds into polymeric matrices using traditional methods has several drawbacks mainly due to the high volatility and thermal sensitivity of these substances. A solution to this problem could be the incorporation of bioactive compounds forming inclusion complexes (ICs) as a strategy to improve the chemical stability, bioactivity and achieve controlled release.

In this work, ICs of carvacrol with β -cyclodextrin were prepared by spray drying to be incorporated into poly(lactic acid) (PLA) and Mater-Bi® films by supercritical CO₂ impregnation. The impregnation process was carried out at pressures of 10, 15 and 20 MPa and at 40°C. Both polymers showed the highest amount of incorporated IC at 15 MPa, where the percentage of impregnation varied from 0.6% to 7.1% in Mater-Bi® and PLA, respectively.

Release tests for PLA films impregnated with IC showed a slow release of the active compound, which did not reach equilibrium after 350 h under the experimental conditions. This prolonged release was not observed in Mater-Bi® due to the lower incorporation of the IC. The release rate was described herein by a comprehensive phenomenological model considering the decomplexation kinetics combined with the equilibrium and mass transfer expressions.

Keywords: *Inclusion complex; supercritical impregnation; β -cyclodextrin; kinetic release; mass transfer.*

1. INTRODUCTION

Industry and consumers are constantly demanding products with new features and environmentally friendly. The food packaging industry is no stranger to these trends. As a result, the development of biodegradable materials containing active ingredients that can be released gradually to protect the packaged products has motivated a significant number of studies [1–6].

Among the effective and well-established strategies for incorporating active compounds that can be released or delivered from polymeric matrices are the use of cyclodextrins as agents to form inclusion complexes. Cyclodextrins are a family of cyclic oligosaccharides, formed by a macrocyclic ring of glucose subunits joined by α -1,4 glycosidic bonds. Cyclodextrins can be produced from starch by enzymatic conversion. They are used in food, pharmaceutical, drug delivery, and chemical industries, as well as agriculture and environmental engineering [7]. The correct combination of active compounds forming inclusion complexes (ICs) with cyclodextrins has proven to be an effective technique to improve chemical stability, controlled release and also enhanced bioactivity of the active agents[8–10].

On the other hand, the development of materials for active food packaging requires comprehensive information on the morphology, thermal, mechanical and barrier properties of different polymer matrices. A wide range of properties and advantages of natural-derived polymers has been extensively studied [11], including polycaprolactone [12,13], poly(lactic acid) [14–16] and Mater-Bi® [6,17]. These polymers have compostable properties, making them excellent candidates for use in the food industry.

In this framework, supercritical fluid technology has emerged as a promising alternative to conventional polymer processing methods. A pure component is in a supercritical state when its temperature and pressure exceed critical values. Carbon dioxide (CO₂) is the most

commonly used compound as a supercritical fluid due to its cost effectiveness, biocompatibility with the human body, and chemical inertness. The Food and Drug Administration (FDA) has classified it as a generally recognized as safe (GRAS) biomaterial [11], and it is chemically inert. In addition, supercritical carbon dioxide (scCO₂) can dissolve a wide range of organic molecules under supercritical conditions ($P > P_c = 73.8$ bar and $T > T_c = 304.15$ K) [12]. This ability allows processes to occur at near ambient temperatures that avoid thermally induced degradation of organic compounds (e.g., drugs, antimicrobials, antioxidants, etc.) [13]. Among the applications that highlight the use of scCO₂ is impregnation, which allows the incorporation of active compounds into polymeric matrices for various applications [14], where the influence of operating variables such as temperature, pressure, contact time, and depressurization conditions on the incorporation of the active agent has been intensively studied [15-17].

The main objective of controlled release packaging is to allow continuous and prolonged diffusion of active compounds, thus reducing the need for certain preservatives [14, 18]. Among the biopreservatives is carvacrol, a phenolic monoterpene with high antioxidant [19–21] and antimicrobial activity [22–25], commonly found in the essential oils of oregano and thyme [27]. Its hydrophobic nature, volatility and susceptibility to light or heat mediated oxidation limit its use as a food preservative.

Within this framework, this study aims to establish the experimental and phenomenological characterization in the controlled release of carvacrol, which was incorporated as an IC with β -cyclodextrin in PLA and Mater-Bi® films using the supercritical impregnation technique. This research combines the experimental characterization of the release kinetics of carvacrol with a comprehensive theoretical model, which combines a decomplexation rate expression with the mass transfer equations, the thermodynamic equilibrium

expression between the polymeric film phase and a food simulant, and the mass balance to describe the transient process of active compound release.

To the best of our knowledge, this is the first study on the supercritical impregnation of inclusion complexes with β -cyclodextrin in polymeric films to explain the combined thermodynamic and transfer phenomena related to the release of an active compound.

2. MATERIALS AND METHODS

2.1. Materials

β -Cyclodextrin pharma grade ($MW = 1,134.98 \text{ g mol}^{-1}$, 98.5% purity) was obtained from Cyclolab, Ltd., carvacrol (> 98 %) was supplied by Sigma Aldrich, poly (lactic acid) (PLA) 2003D (specific gravity 1.24; MFR g/10min) was purchased from Natureworks® Co (Minnetonka, USA) and Mater-Bi® EF51L from Novamont (Novara, Italy). Meanwhile, carbon dioxide (99.9% purity) was supplied by Linde (Santiago, Chile). Chromatographic grade acetonitrile and absolute ethanol analytical grade were purchased from Merck S.A.

2.2. Formation of inclusion complex β -cyclodextrin/carvacrol

β -cyclodextrin/carvacrol inclusion complexes with a 1:1 molar ratio were prepared by spray drying according to the general methodology proposed by Muñoz-Shugulí and coworkers [35], with some modifications. For this purpose, a solution of β -cyclodextrin (20 g) was prepared in 200 mL of an ethanolic aqueous solution (1:3 ethanol/water ratio) at 55 °C with constant stirring for 3.5 h. Then, carvacrol, previously dissolved in 5 mL of 10% ethanol, was added to the β -cyclodextrin solution and the mixture was stirred for 30 min. After this time, the mixture was cooled to room temperature for 1 hour with stirring. Finally, the mixture was dried in a Büchi B-290 spray dryer at a flow rate of approximately 6 mL/min with an inlet temperature of 150°C and an outlet temperature of 75°C.

2.3. Extrusion of PLA and Mater-Bi films

Dry PLA powder (dried at 60 °C for 24 h) and Mater-Bi® were extruded using a Scientific Labtech LTE20 (Samutprakarn, Thailand) 20 mm laboratory twin-screw extruder. For the PLA films obtention, the extruder temperature profiles from zone 1 to zone 5 extruder temperature profiles were maintained between 185 and 195 °C with a die temperature of 195 °C [36]. In the case of Mater-Bi® films, the temperature profile was set between 120 and 180 °C. Screw speed and feed were set at 30 rpm, and the films were collected on a Labtech LBCR-150 chill roll attachment (Samutprakarn, Thailand) at 3.5 rpm [6].

2.4. Supercritical impregnation of carvacrol and IC in PLA and Mater-Bi films

The supercritical fluid impregnation process of carvacrol pure and ICs was carried out using the system, as shown in **Fig. 1**. The impregnation process was performed in a high-

pressure cell of 100 mL. Previous tests were performed to determine the initial mass of the IC and carvacrol pure to be loaded into the cell to achieve a saturation state in the dense CO₂ phase. A 25 cm² film was placed in the cell on a metal support in order to avoid direct contact with the cell and to ensure homogeneous impregnation on both sides. The temperature inside the high-pressure cell was controlled using a thermostatic electrical resistance around the cell. CO₂ was loaded into the system using an ISCO 500D syringe pump (Nebraska, United States), which was operated at a constant pressure regime during the impregnation runs.

The impregnation experiment was repeated for each kinetic experiment in order to verify the reproducibility and reliability of this procedure. Supercritical impregnation runs were performed at 10, 15 and 20 MPa. at a constant temperature of 40° C for 3 h to reach the equilibrium conditions. After this time, the cell was depressurized at 1 MPa/min. and the active material was recovered for its characterization and evaluation of release kinetics.

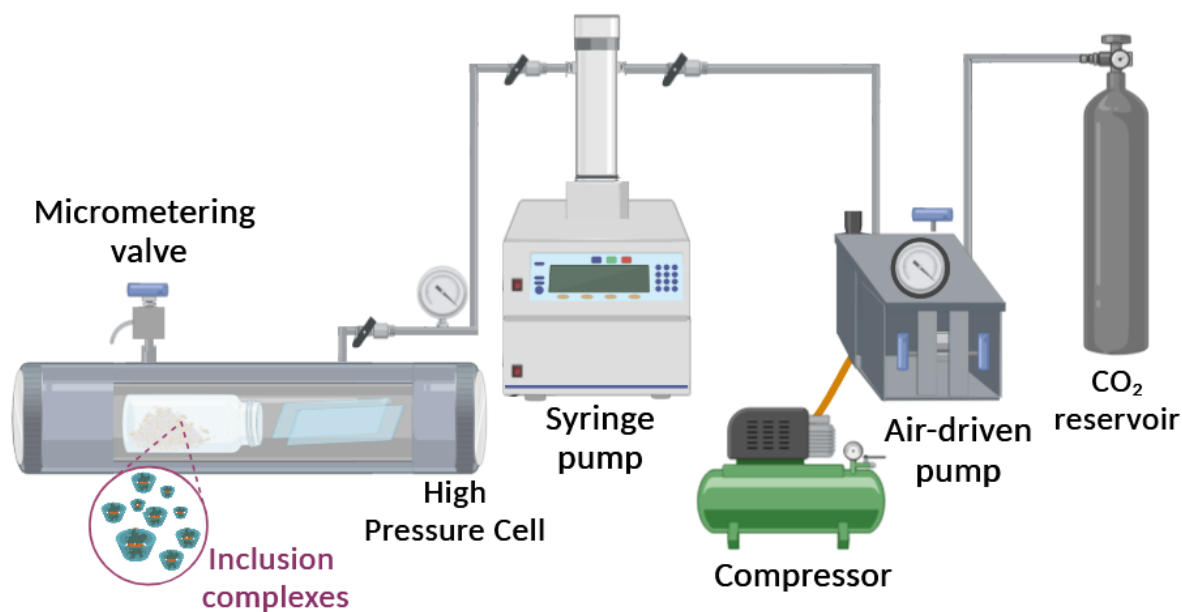


Figure 1. Scheme of experimental setup for supercritical impregnation.

2.5. Characterization of inclusion complexes and impregnated films

2.5.1. Entrapment efficiency and carvacrol content in the inclusion complex (IC)

The quantification of the complexed carvacrol content was performed according to the method proposed by Muñoz and coworkers [35], with some modifications. The active compound carvacrol was extracted from 5 mg of IC with acetonitrile (5 mL) in hermetically sealed vials, using an incubator shaker IST-4075 (Jeio Tech, Korea) at 150 rpm and 25°C for 24 h. The insoluble phase (β -cyclodextrin) was separated by centrifugation, and the supernatant was analyzed with a high-performance liquid chromatography in a HPLC (Water Alliance e2695 Milford, MA, USA) equipped with model 2998 diode detector (PDA) and model e2995 auto-sampler. The chromatographic column used was a Waters Symmetry C18 analytical column, 5 μ m, 3.9 x150 mm.

The compound was quantified using a calibration curve previously established with different solutions of carvacrol in acetonitrile with concentrations ranging from 0 to 200 ppm ($R^2 = 0.99$). From this analysis, the entrapment efficiency (EE) was determined according to **Eq. 1**:

$$EE (\%) = \frac{\text{released CRV content}}{\text{theoretical CRV content}} \cdot 100 \quad (\text{Eq. 1})$$

where real complexed carvacrol content was the carvacrol content in the IC, and the theoretical carvacrol content correspond to the initial amount of carvacrol used to prepare the IC. The experiments were performed in duplicate.

2.5.2. Nuclear magnetic resonance (NMR)

¹H-NMR spectra were recorded for β-cyclodextrin and inclusion complex dissolved in D₂O using Varian Mercury 400 MHz spectrometer. Chemical shifts were measured relative to the peak at 4.80 ppm, due to the solvent (D₂O). (dos Passos 2016 <https://doi.org/10.1016/j.molstruc.2016.06.062>)

The ¹H NMR chemical shifts for the inclusion complex and β-cyclodextrin were determined. The difference in chemical shift value was considered by **Eq. 2**, where δ_{free} is the chemical shift of the pure component and δ_{complex} is the chemical shift of the complex (Simsek 2019 <https://doi.org/10.1007/s11224-019-01347-4>).

$$\Delta\delta = \delta_{complex} - \delta_{free} \quad \textbf{(Eq. 2)}$$

2.5.2. Quantification of active agent in impregnated films

The pure carvacrol or IC loaded mass was determined gravimetrically as proposed by Milovanovic and coworkers [37], considering the mass difference of the impregnated polymer and the polymer at the beginning of the process. Active loading was calculated as the mass percentage of active loaded in the impregnated sample. Experiments were performed in duplicate.

2.5.3. Fourier-Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of β-cyclodextrin, carvacrol and IC, were performed using the KBr disk technique. In the case of the impregnated films, the spectra were obtained by direct contact

of a piece of material, using an attenuated total reflection (ATR) diamond crystal accessory (Bruker® Platinum). All analyses were performed on an Alpha IFS 66 V spectrometer (Bruker, Germany), using OPUS v7 software, programmed to perform 64 scans per sample over a wavelength range between 4000 and 400 cm⁻¹.

2.5.4. Scanning Electronic Microscopy (SEM) analysis

To observe the cross-section of the film samples, they were frozen in liquid N₂, cryo-fractured and further coated with a gold alloy under vacuum in a Q150 metallizer (Quorum Technologies, Sussex, UK). The microstructure of the cryo-fractured cross-sections of the films were then observed with a JEOL 6400 scanning electron microscope (SEM) (Tokyo, Japan). Image acquisitions were conducted at an accelerating voltage of 25 kV.

2.5.5. Thermogravimetric Analysis (TGA)

TGA analyses of β -cyclodextrin, carvacrol, IC and impregnated films were carried out on a TGA/DSC 1 analyzer (Mettler Toledo, Switzerland). Samples (≈ 7 mg) were heated in an alumina crucible (70 μ L) from 20 to 600°C at a rate of 10°C min⁻¹ in nitrogen atmosphere. Samples were measured in duplicate.

2.5.6. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry tests of the obtained materials were performed on a Mettler Toledo DSC model 822e (Schwarzenbach, Switzerland) in a temperature range from 20 °C to 200 °C. Thermal scanning of the samples was performed at 10 °C min⁻¹ and samples weighing between 5-7 mg were placed in hermetically sealed capsules. All experiments were carried out in N₂ atmosphere. Analyses were performed in duplicate. The glass transition (T_g), cold crystallisation (T_{cc}) and melting (T_m) temperatures, as well as the cold crystallization (ΔH_{cc}) and melting (ΔH_m) enthalpies, were determined from the second heating process. On the other hand, the degree of crystallinity (X_c) of PLA was derived from the DSC data using the following equation (3):

$$X_{C\text{ PLA}} = \% \text{ Cristalinity PLA} = 100 * \left[\frac{(\Delta H_m - \Delta H_{cc})}{\Delta H_m^0} \right] \quad (\text{Eq. 3})$$

where ΔH_m is the specific melting enthalpy of the sample (J g⁻¹); ΔH_{cc} is the specific cold crystallization enthalpy of the sample (J g⁻¹) and ΔH_m^0 is the specific melting enthalpy of a wholly crystalline PLA (93.6 J g⁻¹) [38].

2.5.7. Statistical analysis

Impregnation experiments were randomized using an experimental design. Meanwhile, analysis of variance (ANOVA) was applied on the results using the statistical program Statgraphics Plus 5.1 (StatPoint®, Inc., USA) and the Fisher test was applied to assess average differences (at a 95% of confidence interval).

2.6. Molecule modeling in COSMO-RS

The molecules of carvacrol, β -cyclodextrin, and IC (β -cyclodextrin/carcacrol) were optimized before being used in COSMO-RS. Molecular optimizations were carried out in Turbomole® (version 4.4.1) using a basis set with triple-zeta valence polarization (def-TZVP) [32], a density functional theory (DFT) with the DGA functionality Becke-Perdew (BP86-D4) [33,34]. After geometry optimization, a vibrational frequencies analysis was performed in Turbomole® for every molecule to ensure that all were stable molecules with zero negative frequencies. Subsequently, the COSMO-RS calculations were conducted using COSMOTermX® (version C30_1705) software.

To know which conformer of the inclusion complex was the most stable (Eq. 4), a Born-Haber cycle was calculated using the chemical energy in the vacuum plus the free energy of solvation in the CO₂ phase media according to Eq. 5. For this purpose, the chemical potential for each molecule was calculated based on a vibrational frequency analysis in Turbomole® and the solvation energy in the CO₂ media with COSMOTermX®.

$$\Delta G_{conf} = \Delta G_{IC} - \Delta G_{carvacrol} - \Delta G_{\beta-CD} \quad (\text{Eq. 4})$$

$$\Delta G_i = \Delta G_{vacuum} + \Delta G_{CO_2} \quad (\text{Eq. 5})$$

The partition coefficient ($K_{p/scCO_2,i}$) of the molecule (i) between the polymer (P) and the supercritical carbon dioxide (scCO₂) phase is defined according with **Eq. 6**:

$$K_{p/scCO_2,i} = \frac{x_{P,i}}{y_{scCO_2,i}} = \frac{\gamma_{scCO_2,i}}{\gamma_{P,i}} \quad (\text{Eq. 6})$$

Where $x_{P,i}$ and $y_{scCO_2,i}$ represent the mole fraction in the polymer and CO₂ respectively, and γ is the activity coefficient at infinite dilution in the polymer or the scCO₂, and these values were conducted using COSMOThermX®. For the polymer a value of 10.000 [g/mol] as the molecular weight was used.

2.7. Quantification of transport properties and thermodynamic equilibrium in the release of carvacrol from impregnated films

2.7.1. Experimental procedure for release rate quantification of carvacrol from impregnated films

The release rate of carvacrol from the impregnated films to the food simulant was determined according to the standards of the European Committee for Standardization [43]. For this purpose, the method proposed by Torres and coworkers [36] was used with some modifications. PLA or Mater-BI® films with a contact area of 50 cm², which had the highest amount of carvacrol or IC impregnation, were contacted with 42 mL of a 50% v/v ethanol solution, which is considered to be a fatty food simulant (D1 simulant). The films were placed on metal supports inside the glass tubes filled with the simulant solution to avoid direct contact between the film and the tube walls. The scheme of these experiments is shown in **Fig. 2a**. The glass tubes were stored in an oven at a constant temperature of 40 °C for at least 2 weeks, and 1 mL samples of the solution were taken

periodically at different times to determine the concentration of carvacrol released as a function of time. Quantification of carvacrol was performed by HPLC. An acetonitrile:water (60:40) mobile phase was used at a flow rate of 1 mL/min with an injection volume of 10 μ L. These experiments were performed in duplicate until a constant concentration of carvacrol was observed as a function of time.

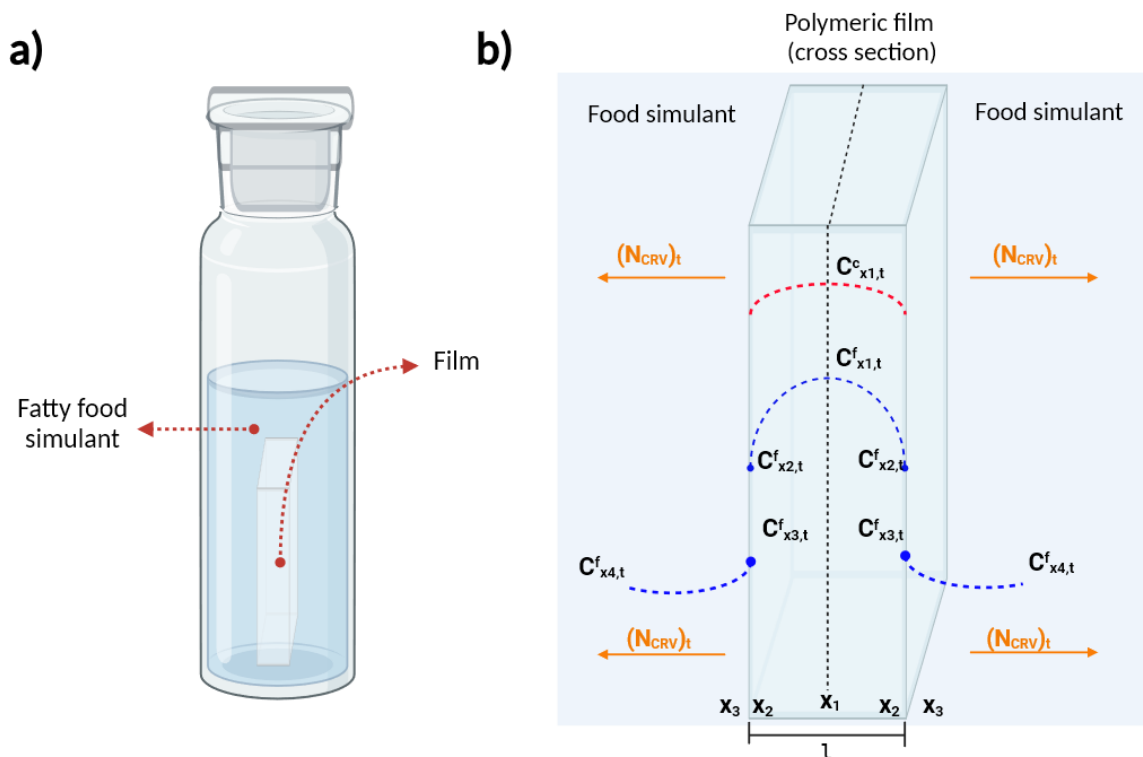


Figure 2. Outline of the experiments for the quantification of the transport properties and equilibrium conditions during the release of carvacrol from the impregnated films. These experiments were implemented for samples containing carvacrol as IC and neat carvacrol. This last one as a reference system.

2.7.2. Theoretical description of the mass transfer of carvacrol release

In this work, a novel approach is proposed to describe the carvacrol release that is contained in the impregnated polymeric films since the active compound is complexed with β -cyclodextrin an effective transfer model is proposed considering the following assumptions:

i) A quantifiable amount of free and complexed carvacrol is present in the polymeric films. The concentration of free and complexed carvacrol in the film depends on the physicochemical properties of the polymeric medium, including the concentration of complexed and free active compound.

ii) Mass transfer from the polymeric material to the food simulant is considered only for free carvacrol, which is gradually released from the complexed species as well as the free compound present in the polymeric phase at the onset of release.

iii) Free carvacrol is present in the polymeric material as the release progresses, the amount of carvacrol remaining in the polymeric phase depends on the concentration of free carvacrol that is released from the complexed species and subsequently transferred through the polymeric phase to the food simulant.

iv) Complexed carvacrol is gradually released as a free compound. This decomplexation kinetics is given by the following relationship:

$$\frac{dC^c}{dt} = -(k_1 C^c - k_2 C^f) \quad (\text{Eq. 7})$$

where C^c is the concentration of complexed carvacrol and C^f is the concentration of free carvacrol present in the polymer phase. Thus, the rate of decrease of the concentration of complexed carvacrol is directly proportional to the concentration of the complexed species according to a kinetic constant k_1 , while this rate of decrease is limited in a relationship which is directly proportional to the concentration of free

(non-complexed) carvacrol present in the polymeric phase according to a kinetic constant k_2 .

v) In this model, the kinetic release of carvacrol depends on the concentration of free carvacrol present in the polymeric phase and transferred to the food simulant as well as the availability of carvacrol released from the complex, according to the following expressions:

$$\left(\frac{dC^f}{dt}\right)_{diffusion} = -2 \frac{N_{CRV}A}{V_p} \quad (\text{Eq. 8})$$

$$\left(\frac{dC^f}{dt}\right)_{decomplexation} = -\frac{dC^c}{dt} \quad (\text{Eq.9})$$

where N_{CRV} is the mass transfer flux of carvacrol through the polymeric film to the food simulant, A is the surface area of a film face available for mass transfer (one side), and V_p is the volume of the polymeric film. In equation 9, the increasing rate of the concentration of free carvacrol due to the decomplexation of the complexed one is equal to the decreasing rate of the concentration of the complexed specie.

From these assumptions, the change in concentration of free carvacrol in the polymer as a function of the time depends on the transfer rate to the food simulant as well as the availability of free compound from the decomplexation process. Thus, this concentration change in the polymer could be represented by the following equation:

$$\frac{dC^f}{dt} = \left(\frac{dC^f}{dt}\right)_{diffusion} + \left(\frac{dC^f}{dt}\right)_{decomplexation} \quad (\text{Eq. 10})$$

315 Replacing equations 6, 7 and 8 in equation 9 it is possible to obtain:

$$\frac{dC^f}{dt} = -2 \frac{N_{CRV} A}{V_p} + (k_1 C^c - k_2 C^f) \quad (\text{Eq. 11})$$

316

317 The outline shown in **Fig. 2b** describes the mass transfer phenomenon of carvacrol from the
318 polymer to the food simulant during the experimental release tests.

319 On the other hand, the carvacrol transport rate estimated by the mass transfer flux through
320 the polymer phase could be described by equation 12:

$$\left[(N_{CRV})_t = D_{CRV-P} \left(\frac{dC^f}{dx} \right) \right]_P \quad (\text{Eq. 12})$$

321

322 which can be rewritten in a discrete form using the outline shown in **Fig. 2b**:

$$\left[(N_{CRV})_t = \frac{D_{CRV-P}^{eff}}{l/2} (C_{x1,t}^f - C_{x2,t}^f) \right]_P \quad (\text{Eq. 13})$$

323

324 where $(N_{CRV})_t$ is the mass transfer flux of carvacrol at a given time t ; D_{CRV-P}^{eff} is the
325 effective diffusion coefficient of carvacrol in the polymer; l is the thickness of the polymer
326 film; $C_{x1,t}^f$ and $C_{x2,t}^f$ are the concentrations of free carvacrol in the bulk of the polymer and
327 at the interface with the food simulant on the polymer side, respectively.

328 The phase equilibrium of the carvacrol between the polymer and food simulant phases can
329 be estimated by the following expression:

$$K_{P/FS} = \frac{C_{x2,t}^f}{C_{x3,t}^f} \quad (\text{Eq. 14})$$

330

where $C_{x2,t}^f$ and $C_{x3,t}^f$ are the concentrations of free carvacrol at the interface with the food simulant on the polymer side and on the food simulant side at a given time t , respectively.

In these calculations, the value of $K_{P/FS}$ is assumed to be constant.

On the other hand, the mass transfer flux of carvacrol through the food simulant boundary layer in the vicinity of the polymer/food simulant interface can be quantified by equation 14:

$$[(N_{CRV})_t = k_{CRV-FS}(C_{x3,t}^f - C_{x4,t}^f)]_{FS} \quad (\text{Eq. 15})$$

where k_{CRV-FS} is the mass transfer coefficient of carvacrol in the food simulant estimated under natural convection conditions. Meanwhile, $C_{x3,t}^f$ and $C_{x4,t}^f$ are the concentrations of carvacrol in the food simulant at the interface with the polymer film and in the bulk at a given time t , respectively.

The calculation of the concentration of free carvacrol available for the release in the bulk of the polymer phase at the next time step can be obtained from the discrete form of equations 10 and 11 as follows:

$$C_{x1,t+\Delta t}^f = \frac{(C_{x1,t}^f V_p - 2(N_{CRV})_t A \Delta t)}{V_p} + (k_1 C_{x1,t}^c - k_2 C_{x1,t}^f) \Delta t \quad (\text{Eq. 16})$$

Thus, at each time step, the concentration at the next step time $C_{x1,t+\Delta t}^f$ is recalculated to simulate the carvacrol transfer during release in a pseudo-steady state. The solution of this system of equations was developed in Matlab® by writing a script based on a Regula Falsi algorithm. The solution of these equations allows to obtain the instantaneous value of the carvacrol flux ($\text{kg m}^{-2} \text{s}^{-1}$) at each time step.

The main challenge in solving this system of equations is the quantification of some key parameters to characterize the mass transfer condition at the beginning and during the release process. These parameters are the initial concentrations of free and complexed carvacrol in the polymer film, $C_{xI,t=0}^f$, and $C_{xI,t=0}^c$, the value of the effective diffusion coefficient of free carvacrol through the polymer film, D_{CAR-P}^{eff} (Eq. 13), the partition coefficient at the polymer/food simulant interface, $K_{P/FS}$ (Eq. 14), and the constants k_1 and k_2 described in the decomplexation kinetic equation (Eq. 7). These six parameters are determined by a screening approach that estimates the release kinetic curve for a combination of values of these six parameters. Thus, the screening was implemented in the Matlab script to track the solution of the system that obtains the best fit with the release kinetic curves obtaining the lowest value of RMSE (root of the mean square error) between the experimental release kinetic data and the predicted ones (concentration of carvacrol in the food simulant as a function of time) by the mathematical model described above. This procedure was implemented for five levels per parameter obtaining a calculation set of $5^6 = 15625$ combinations. The script developed in Matlab® R2023b was executed in a computer Apple® Macbook Pro M1 16 Gb RAM estimating an average value for the calculation of a kinetic curve. In this way, the calculations for the whole set of parameter combinations could be achieved in 28.94 days. After these calculations a second screening was performed at five levels of the six parameters in the intervals with the lowest RMSE values during an additional time of 30 days. After selecting narrower intervals of the six parameters, a third and fourth screening was performed at three levels for a total of two weeks to estimate the set of parameters with the lowest RMSE. **Table 1** summarizes the values of the screening intervals considered for each parameter:

Table 1. Intervals of parameters used in the calculation of the release kinetic curve of carvacrol from the polymer film to the food simulant.

Parameter	$C_{xl,t=0}^f$ (kg m ⁻³)	$C_{xl,t=0}^c$ (kg m ⁻³)	D_{CRV-P}^{eff} (m ² s ⁻¹)	$K_{P/FS}$	k_1 (s ⁻¹)	k_2 (s ⁻¹)
Value interval	0 - 15	0 - 15	1.0*10 ⁻¹⁵ – 1.0*10 ⁻¹³	1 - 100	0 – 5*10 ⁻⁶	0 – 5*10 ⁻⁶

3. RESULTS AND DISCUSSIONS

3.1. Characterization of the inclusion complex (β-cyclodextrin/carvacrol)

The carvacrol content in cyclodextrin is limited because it is formed from a β-cyclodextrin/carvacrol complex in a 1:1 molar ratio, where the complexes used in this study were characterized by having an entrapment efficiency equal to 77%, corresponding to a carvacrol content of 0.124 g/g β-cyclodextrin.

To molecular structure of the inclusion complex was corroborated by one-dimensional proton nuclear magnetic resonance (¹H-NMR), in which the bands associated with the hydrogens belonging to the host and guest were analyzed. Figure 3 shows the partial ¹H-NMR of the inclusion complex along with that of the β-cyclodextrin. Table 2 presents a summary of the chemical shifts (δ) of the internal (H-3 and H-5) and external (H-1, H-2, H-4 and H-6) protons of the β-cyclodextrin and the inclusion complex (Herrera 2019 <https://doi.org/10.1016/j.foodres.2019.03.026>).

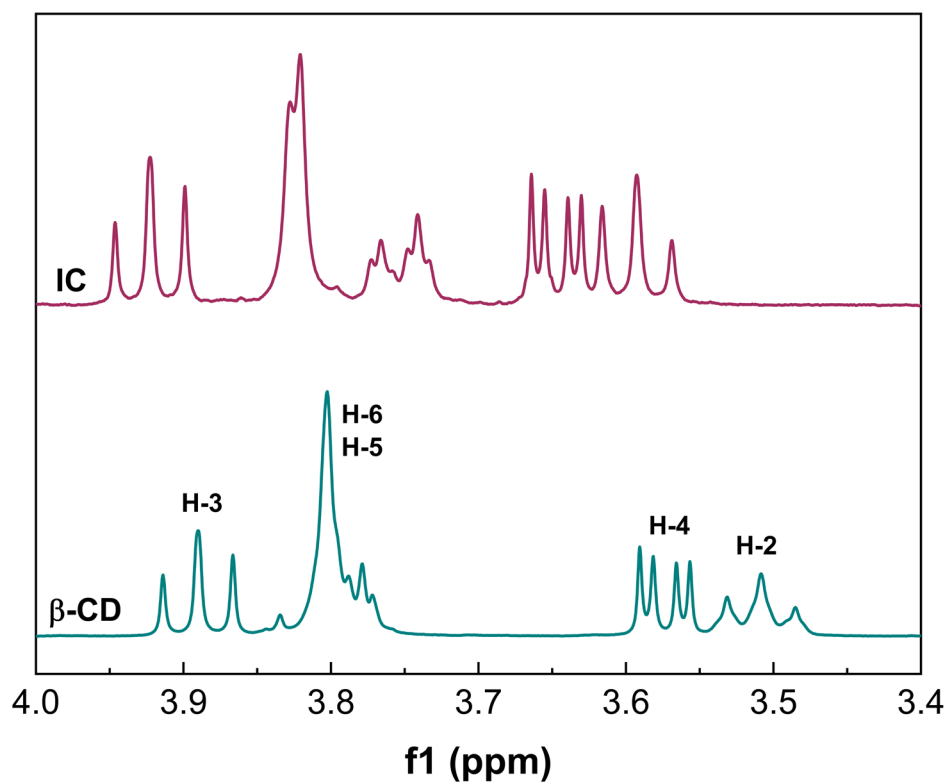


Figure 3. Partial ¹H NMR inclusion complex (IC) and β-cyclodextrin (β-CD).

Table 2. Proton chemical shift data (ppm) of β-CD in free state and in the complex in D₂O solution.

	δ_{free}	δ_{complex}	$\Delta\delta_{\text{complex}}$
H-1	5.00	4.98	-0.02
H-2	3.59	3.58	-0.01
H-3	3.89	3.83	-0.06
H-4	3.51	3.5	-0.01
H-5	3.78	3.65	-0.13
H-6	3.80	3.73	-0.07

While, no chemical shifts were observed in the external protons (H-1, H-2, H-4 and H-6) of the β-cyclodextrin, there was somewhat chemical shifts (δ) in the case of the internal

protos (H-3 and H-5) of the β -cyclodextrin when carvacrol is in its cavity. The downward displacement of the H-3 and H-5 protons from the cavity of the β -cyclodextrin may be attributed to the interaction of carvacrol with the internal cavity of the β -cyclodextrin. As previously reported by Liu and co-workers, the variations in the chemical shifts of protons along the internal cavity are due to their proximity to the aromatic ring, which is rich in π electrons (Liu 2021 <https://doi.org/10.1016/j.foodcont.2021.108296>). This suggests that the aromatic ring of carvacrol is located within the cyclodextrin cavity, which is in agreement with the findings of other researchers (Lavoine 2014 <https://doi.org/10.1016/j.ifset.2014.06.006> ; Dos Passos 2016 <https://doi.org/10.1016/j.molstruc.2016.06.062>).

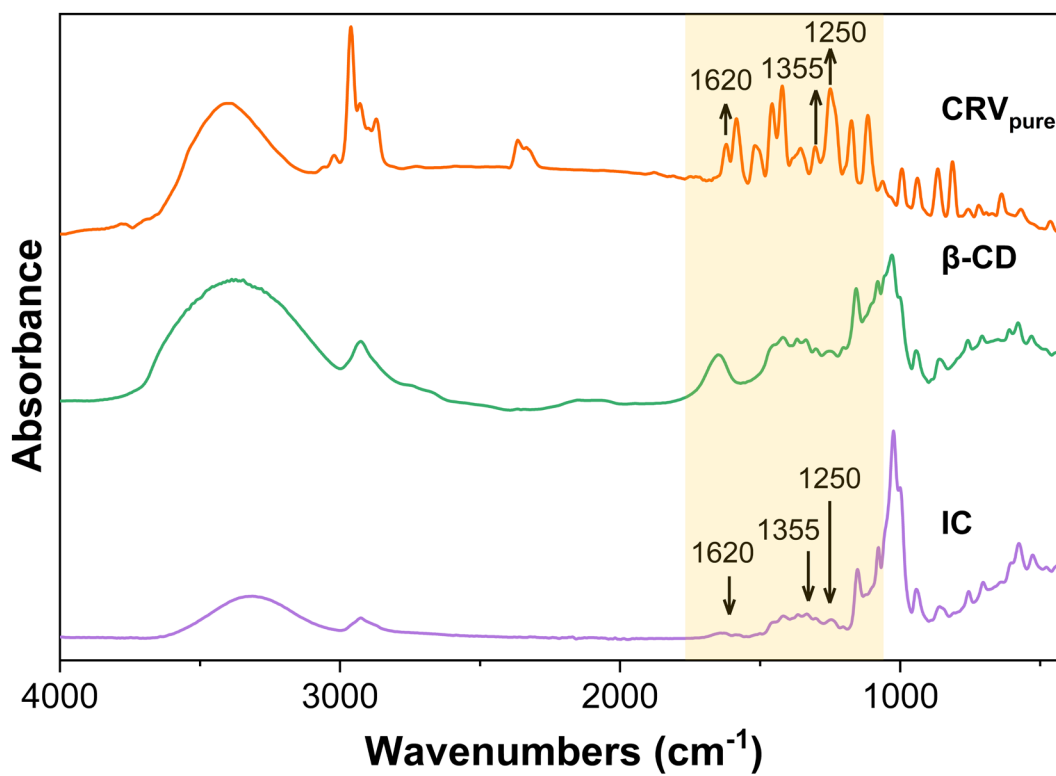


Figure 4. FTIR spectra of carvacrol (CRV), β -cyclodextrin (β -CD) and β -cyclodextrin/carvacrol inclusion complex (IC) samples.

On the other hand, FTIR analysis enabled the detection and identification of the specific interactions between the guest (carvacrol) and host (β -cyclodextrin) molecules during the inclusion process. The significant changes in the shape and position of the characteristic absorbance bands associated with the active agent, cyclodextrin, and the inclusion complex were observed.

Fig. 4 shows the FTIR spectra of the carvacrol, β -cyclodextrin and IC. The carvacrol showed the characteristic absorption bands around $3500\text{--}3300\text{ cm}^{-1}$ corresponding to the stretching vibrations of the phenolic OH- group. It was also observed that the intense peaks at 1620 cm^{-1} correspond to the presence of aromatic C=C stretching vibrations. In addition, the presence of a peak at 1355 cm^{-1} corresponds to the isopropyl group and an intense band at 1250 cm^{-1} is due to the CO- stretching vibration. In the 800 cm^{-1} region, a peak corresponding to aromatic CH-bending was observed, which can be attributed to the substitution of the aromatic ring [40]. The FTIR spectrum of the β -cyclodextrin showed a broad band at 3386 cm^{-1} corresponding to OH- stretching vibrations, a band at 2926 cm^{-1} related to the C-C bond tension of the polysaccharide, at 1649 cm^{-1} coming from the H-O-H angular deformation, at 1251 cm^{-1} attributed to the asymmetric stretching vibration assigned to the glycosidic bond, and an intense absorption at 1029 cm^{-1} due to the C-O-C stretching of the alcohol [8]. It is worth mentioning that the results obtained are in agreement with those reported [9]. The FTIR spectra of the inclusion complex showed that the bands of the active compound were obscured by very intense and broad β -cyclodextrin

bands. However, the absorption bands at 1620 cm^{-1} , 1355 cm^{-1} , and 1250 cm^{-1} of carvacrol were slightly shifted and decreased in intensity. Therefore, it can be concluded that the encapsulation of the active compound in the β -cyclodextrin by the formation of inclusion complexes was confirmed, in good accordance with the ^1H NMR results

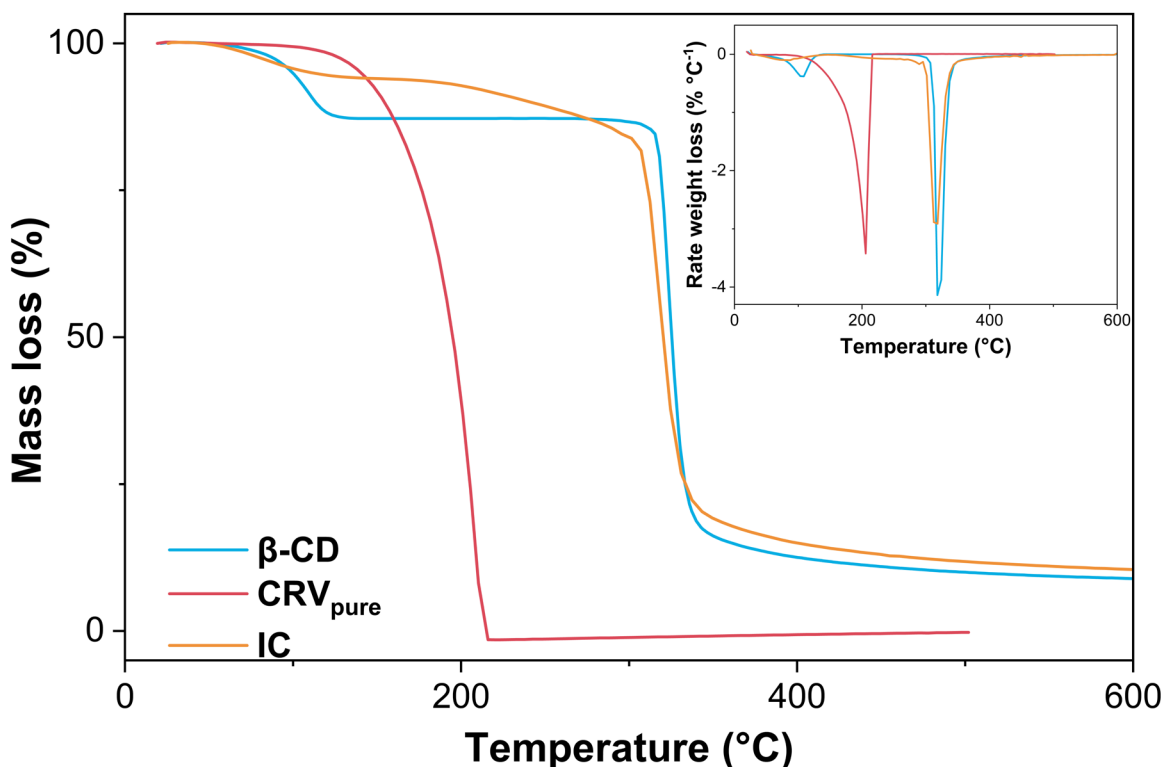


Figure 5. TG (a) and DTG (b) curves obtained for carvacrol (CRV), β -cyclodextrin (β -CD) and β -cyclodextrin/carcvacrol inclusion complex (IC) samples.

The thermal stability of carvacrol, β -cyclodextrin and the inclusion complex was determined by TGA analysis. This involved monitoring mass changes as a function of temperature, which is key for the confirmation of the formation of the inclusion complex. **Fig. 5** shows the mass loss of the samples as a function of temperature and that derived from thermogravimetric analysis. The β -cyclodextrin showed a mass loss at 2 different

temperatures i) below 100 °C, attributed to the release of adsorbed water molecules, and ii) near 330 °C, corresponding to the thermal decomposition of the β -cyclodextrin. [41]. In contrast, carvacrol showed a single-step mass loss between 100 °C and 200 °C, corresponding to the evaporation of the compound [42]. The inclusion complex showed a slight mass loss below 100 °C, which is attributed to the release of water molecules that are absorbed by the material due to the system used to form the inclusion complex. In addition, degradation of the β -cyclodextrin was observed, demonstrating that although the material can absorb water from the environment, it still confers thermal protection to the carvacrol active compound. The TGA results once again confirm the correct incorporation of active compound into the β -cyclodextrin structure.

3.2.Characterization of impregnated films

3.2.1. Quantification of active agent in impregnated films

As is depicted in **Fig. 6a** higher impregnation of pure carvacrol than inclusion complex (IC) was observed for both polymeric matrices, which could be related to the nature of the active compound, since it has a high solubility in scCO₂ [43] and therefore a higher permeability capacity in the polymeric matrix because its higher concentration gradient as driving force during the impregnation process.

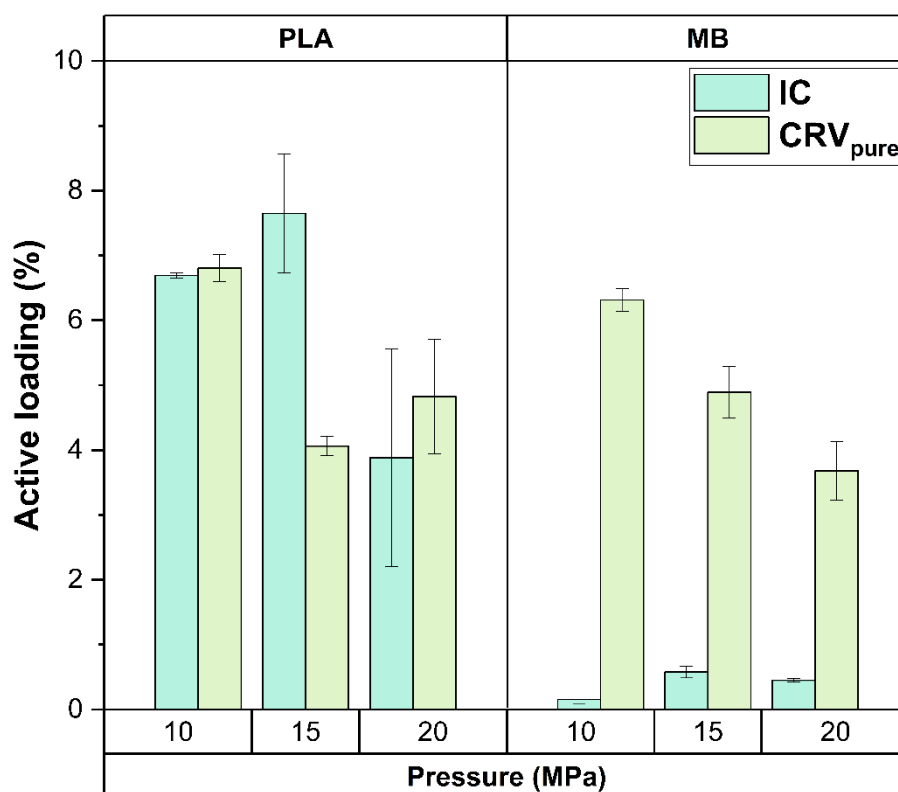


Figure 6 Quantification of inclusion complex (IC) and carvacrol (CRV) in impregnated films of PLA and Mater-Bi® (MB) at different scCO₂ pressures.

On the other hand, the lower incorporation of the inclusion complex in the films could be due to the molar mass of the IC and the polarity of the molecule as such, which hinders its solubility in the supercritical phase and, at the same time, its diffusion in the polymeric matrix. In addition, it should be noted that both for the samples with pure carvacrol and those with IC, PLA showed a higher incorporation than Mater-Bi®, which can be explained by the fact that the supercritical impregnation process is highly dependent on the interaction and equilibrium in the ternary system (polymer matrix/active agent/scCO₂), including the

effect of pressure, temperature and/or polymer structural change on the solubility, diffusivity and partitioning in the scCO₂/polymer/active agent system [44].

3.2.2. Morphological properties

The films with the highest amount of pure carvacrol or IC incorporated were observed by scanning electron microscopy (**Fig. 7**), while neat PLA and neat Mater-Bi® were also observed for comparison. Neat PLA (**Fig. 7a**) show a rigid fracture with a regular surface, whereas neat Mater-Bi® (**Fig. 7d**) shows a smoother surface typical of a more plastic behavior, which could be attributed to the presence of very flexible polymers such as poly(butylene adipate-co-terephthalate) (PBAT), since the Mater-Bi® used is composed of three polymer matrices (namely PLA, thermoplastic starch (TPS) and PBAT) [5]. Meanwhile, pure PLA exhibits the typical stiff aspect of an amorphous polymer with low plastic behavior [34]. All film formulations with scCO₂-incorporated pure carvacrol (**Fig. 7b and 7e**) and IC (**Fig. 7c and 7f**) showed mostly homogeneous cryo-fractured surfaces with no apparent phase separation, suggesting good compatibility between carvacrol and IC with both polymer matrices. The incorporation of carvacrol into PLA (PLA/carvacrol/10MPa, **Fig. 7b**) and to Mater-Bi® (Mater-Bi/carvacrol/10MPa, **Fig. 7e**) at an impregnation pressure of 10 MPa practically did not change the cryo-fractured surface aspect of the pristine polymer matrices. Meanwhile, the incorporation of IC (at an impregnation pressure of 15 MPa) resulted in a slightly more roughened surface in both Mater-Bi/IC/15MPa (**Fig. 7f**) and slightly more pronounced in PLA/IC/15MPa (**Fig. 7c**), but without showing IC agglomeration. These results are in good agreement with the results reported by Byun et al. [35], who developed materials based on PLA-β-cyclodextrin

inclusion complex by solvent casting method using 1, 3, 5 and 7% loading levels of IC with respect to the polymeric matrix and observed that the PLA surface became rougher as the amount of IC increased.

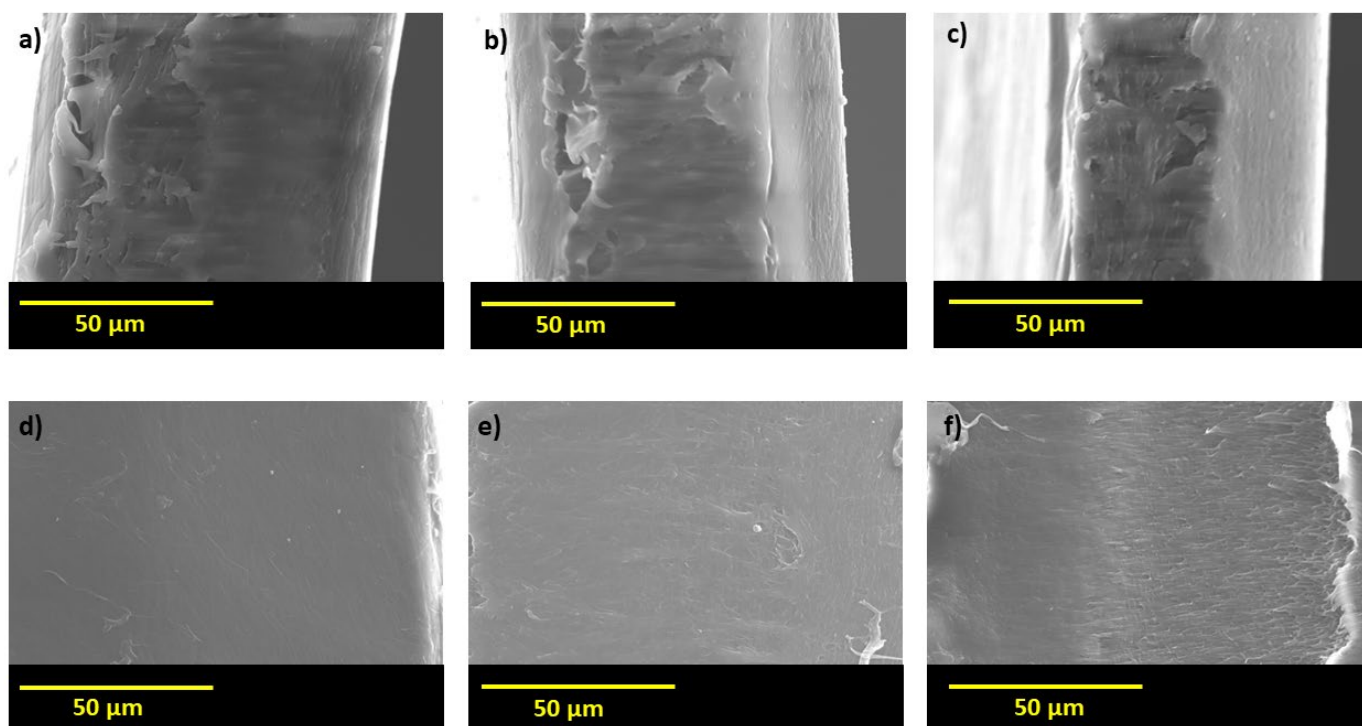


Figure 7. SEM images of materials with and without active compound at 1.00kX: a) PLA; b) PLA/pure carvacrol/10MPa; c) PLA/IC/15MPa; d) Mater-Bi; e) Mater-Bi/pure carvacrol /10MPa; f) Mater-Bi/IC/15MPa.

3.2.3. Structural properties

Fourier transform infrared spectroscopy analysis (**Fig. 8 and 9**) of the films with and without the presence of active (pure carvacrol, IC) was performed to identify chemical functionalities and to confirm the incorporation of carvacrol pure and IC into the impregnated polymer matrices. Characteristic peaks were observed in the PLA spectrum

(Fig. 8), at 1747 cm^{-1} associated with the carbonyl group C=O, while the weak bands at 1453 and 1380 cm^{-1} are assigned to the stretching of the methyl groups (CH₃) [45,46]. The peaks at 1180 and 1079 cm^{-1} correspond to the asymmetric C-O-C and C-O vibrations. [47].

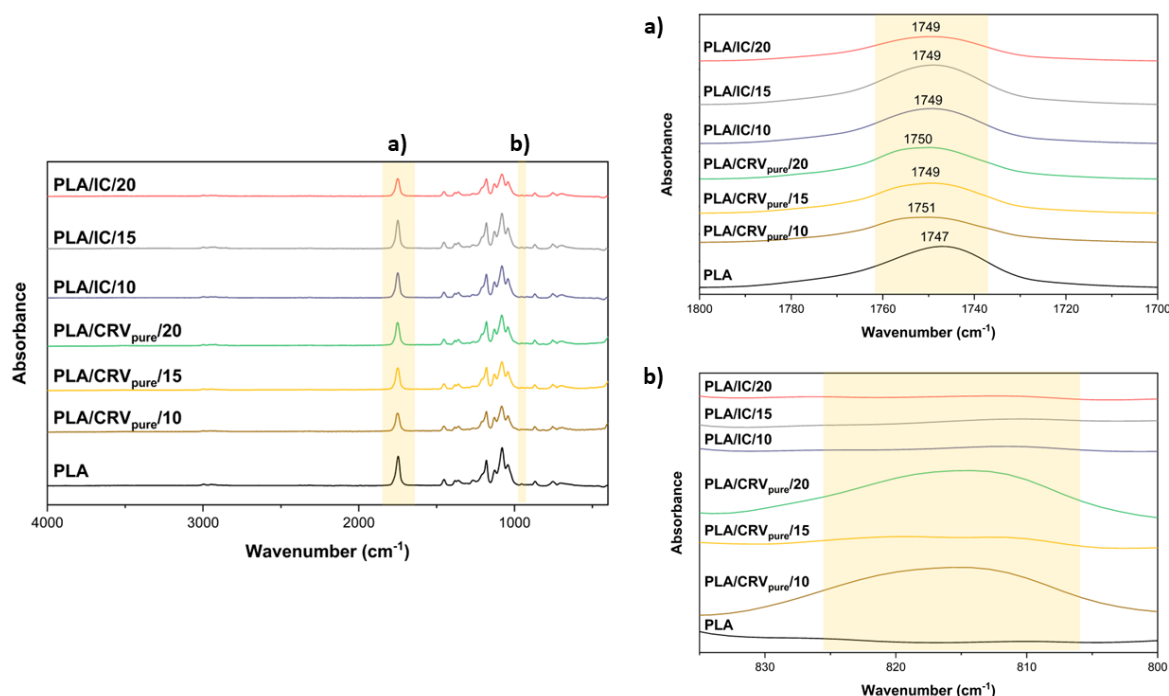


Figure8. FTIR spectra of PLA with pure carvacrol (CRV) or inclusion complex (IC), (a) range between $1800 - 1700\text{ cm}^{-1}$ (b) range between $830 - 800\text{ cm}^{-1}$. The number next to each sample indicates the impregnation pressure (10, 15 or 20 MPa).

Fig. 8a shows that the vibration of the PLA carbonyl group (pure PLA film) presented a maximum at 1747 cm^{-1} , while the PLA/carvacrol pure samples at 10 and 20 MPa shifted to 1751 and 1750 cm^{-1} , respectively. This case is similar to that reported by Villegas et al. [48] where they impregnated cinnamaldehyde on PLA films and the samples that were in contact with scCO_2 as well as those impregnated with the active compound showed the same band shift. This is related to changes in the crystallization behavior of PLA-based

materials, where crystalline and amorphous stretching of the carbonyl group appear around 1735 cm⁻¹ and 1750 cm⁻¹, respectively [49].

On the other hand, the Mater-Bi® spectrum (**Fig. 9**) showed the presence of bands attributable to three polymers, PLA, poly(butylene adipate-co-terephthalate) (PBAT) and starch. The most characteristic peaks of PLA were already mentioned above. The functional groups of PBAT, such as the presence of phenyl rings at 1015 cm⁻¹ [4], in addition to the 1720 cm⁻¹ band due to the presence of C=O carbonyl groups corresponding to the stretching of the ester bonds, together with the 1267 cm⁻¹ band assigned to the stretching of the C-O ester groups. An intense signal at 727 cm⁻¹ corresponds to the stretching of CH₂ [51]. Finally, the absorption bands related to the starch-based fraction are present at 1445-1325 and 1250-900 cm⁻¹[6].

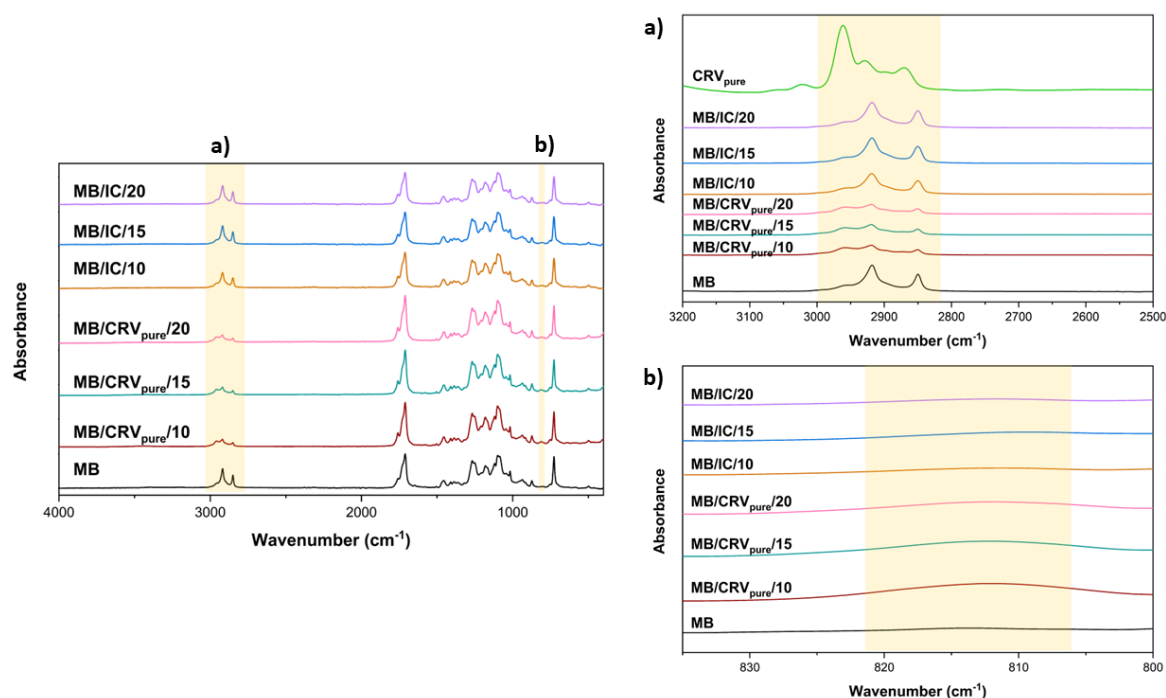


Figure 9. FTIR spectra of Mater-Bi (MB) with pure carvacrol (CRV_{pure}) or inclusion complex (IC). a) range between 3200 – 2500 cm⁻¹ (b) range between 830 – 800 cm⁻¹. The number next to each sample indicates the impregnation pressure (10, 15 or 20 MPa).

With respect to the samples impregnated with carvacrol, an overlapping of the bands present between 3000 - 2800 cm⁻¹ was observed in the Mater-Bi® films (**Fig. 8a**), corresponding to the symmetric and asymmetric vCH vibrations present in the polymer matrix and in the active, which could be related to the impregnation of carvacrol in the polymer, which is similar to the results reported by other authors [6,19,52].

In addition, the presence of pure carvacrol in PLA and Mater-Bi® films could be detected by monitoring the signal centered at ~812 cm⁻¹ (**Fig. 8b and 9b**), which corresponds to the characteristic aromatic ring of a polyphenol [13, 50, 53], that in PLA films this could be

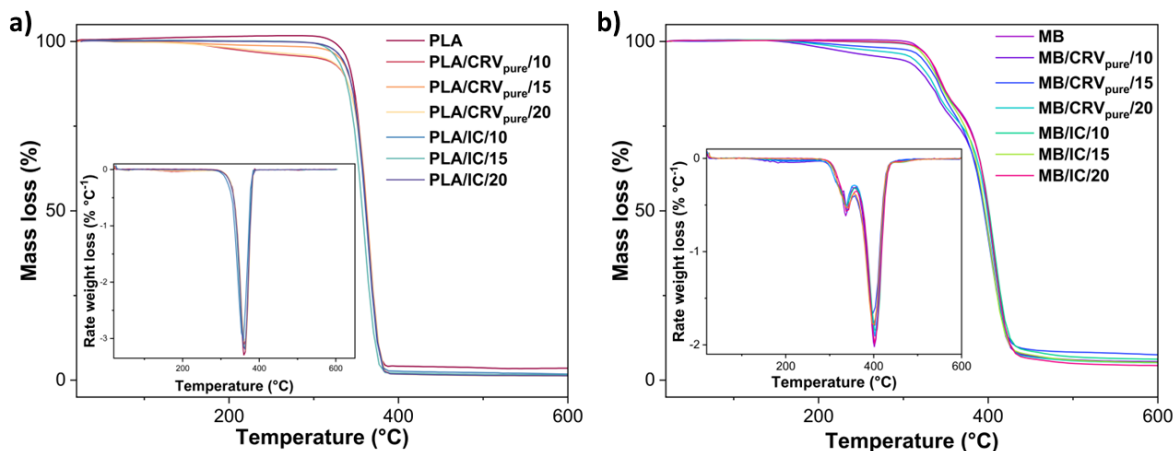
clearly seen in the samples impregnated at 10 and 20 MPa, where a higher concentration of pure carvacrol was impregnated, while for Mater-Bi® samples the intensity increased with higher concentrations of the active agent in the film. This would confirm the presence of the active agent in the polymer matrix.

Finally, the PLA samples impregnated with inclusion complex did not present the band associated with the aromatic ring of the polyphenol (**Fig. 8b**), which could be due to the fact that it is included within the β -cyclodextrin molecule, causing this band not to appear in the spectrum. While the Mater-Bi® films presented this signal (**Fig. 9b**), it was a lower intensity compared to the samples that were impregnated with carvacrol without complexing, which could be due to the fact that a large part of the impregnated IC is free carvacrol, i.e., that it is not trapped in the β -cyclodextrin.

3.2.4. Thermal properties

3.2.4.1. Thermogravimetric Analysis (TGA)

Fig. 10 shows the initial degradation temperatures (initial T^0) and the temperature of the maximum decomposition rate (T^0 max) for each degradation process of the different samples obtained after the pure carvacrol and IC impregnation process in PLA and Mater-Bi®.



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Figure 10. TG and DTG curves obtained for pure and impregnated materials (a) PLA; (b) Mater-Bi (MB). Results are reported for both materials (PLA or Mater-Bi) impregnated with pure carvacrol (CRV_{pure}) or inclusion complex (IC). The number next to each sample indicates the impregnation pressure (10, 15 or 20 MPa).

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Regarding the PLA samples with the presence of pure carvacrol (**Fig. 10a**), it can be observed that the degradation occurred in two stages, the first weight loss being related to the evaporation of the carvacrol (153 - 188 °C) and the second to the degradation of the PLA with temperatures ranging between 329 and 331 °C, considering that pure PLA presented a decomposition onset temperature of 335°C, the incorporation of pure carvacrol in the films generated a decrease in thermal stability [54], which was directly related to the amount of carvacrol impregnated in the polymer matrix. On the other hand, the increase in the thermal stability of the carvacrol impregnated in the samples compared to its pure state (148°C) could be explained by the interaction between PLA chains and carvacrol molecules, mainly through hydrogen bonds, results similar to those reported by Alvarado

and co-workers [55] in the supercritical impregnation of PLA films containing natural nanofibers with thymol. On the other hand, the samples impregnated with inclusion complex, unlike PLA films with carvacrol, presented a one-step degradation in a range of 313-319°C, this would confirm that carvacrol is trapped inside the β -cyclodextrin, in addition to the fact that the incorporation of IC in the polymeric matrix generates a greater decrease in thermal stability compared to when pure carvacrol is impregnated. On the other hand, the Mater-Bi® samples (**Fig. 10b**), in the case of the unimpregnated material, showed a 3-stage degradation where the first degradation inflection (312 °C) is attributed to the degradation of thermoplastic starch (TPS), while the second (336 °C) corresponds to the decomposition of PLA and the third inflection (400 °C) corresponds to PBAT, results similar to those reported by Villegas and collaborators [6]. With respect to the samples impregnated with pure carvacrol, an additional stage in the degradation of the polymer was observed between 170 -180 °C, associated with the presence of carvacrol, contrary to what happened with the samples impregnated with IC, which did not present the degradation of carvacrol.

3.2.4.2. Differential scanning calorimetry (DSC)

By means of DSC analysis, the effect on the transition temperatures of films impregnated with pure carvacrol and IC has been determined. Tables 2 and 3 show the results obtained for PLA and Mater-Bi® samples, respectively, in addition to the films without CO₂ exposure, in order to identify the possible structural modifications induced by the impregnation of the active agent.

Table 2. Thermal properties of PLA and PLA films impregnated with pure carvacrol (CRV pure) or inclusion complex (IC). The number next to each sample indicates the impregnation pressure (10, 15 or 20 MPa).

Sample	T _g (°C)	T _{cc} (°C)	T _m (°C)	ΔH _{cc} (J/g)	ΔH _m (J/g)	%X _c
PLA	57.6 ± 0.6 ^a	117.0 ± 0.7 ^a	148.2 ± 0.4 ^a	32.2 ± 0.7 ^a	36.3 ± 0.4 ^a	4.4 ± 0.3 ^a
PLA/CRV _{pure} /10	54.1 ± 0.4 ^b	113.0 ± 0.2 ^b	146.8 ± 0.1 ^b	37.4 ± 4.4 ^b	37.9 ± 4.2 ^a	0.5 ± 0.2 ^c
PLA/CRV _{pure} /15	56.5 ± 0.2 ^a	114.8 ± 0.5 ^{a,b}	148.4 ± 0.2 ^{a,b}	30.1 ± 0.5 ^b	32.1 ± 0.6 ^{b,c}	2.1 ± 0.1 ^b
PLA/CRV _{pure} /20	54.9 ± 0.3 ^b	112.3 ± 2.0 ^b	146.5 ± 1.0 ^b	35.7 ± 0.3 ^b	37.1 ± 0.4 ^{a,b}	1.5 ± 0.1 ^d
PLA/IC/10	57.0 ± 1.1 ^a	116.6 ± 1.7 ^a	149.7 ± 1.3 ^a	31.5 ± 1.2 ^a	33.1 ± 0.7 ^a	2.4 ± 0.1 ^b
PLA/IC/15	57.1 ± 0.9 ^a	116.3 ± 1.1 ^a	148.6 ± 1.0 ^{a,b}	32.7 ± 1.6 ^a	34.7 ± 1.7 ^a	2.1 ± 0.1 ^b
PLA/IC/20	57.2 ± 0.3 ^a	117.2 ± 0.8 ^a	149.3 ± 0.5 ^a	27.3 ± 1.2 ^c	29.5 ± 0.8 ^c	2.4 ± 0.5 ^b

*Superscript letters a, b, and c indicate significant differences between the values of each thermal parameter.

The thermograms obtained for the pure PLA films showed a glass transition (T_g) around 57 °C, which is associated with changes in the amorphous region of the polymers, which depends on the mobility of the polymer chains. A cold crystallization temperature of 117°C (T_{cc}), which corresponds to the transition from the amorphous to the crystalline state, where the molecules in a given phase can reach sufficient freedom of movement to spontaneously arrange themselves in a crystalline form. In addition to the melting temperature (T_m) of 148°C, which is determined by the transition of the crystalline regions and is specific to each layer, the melting temperature (T_m) of 148°C is determined by the transition of the crystalline regions [56,57]. In the case of the samples impregnated with carvacrol, a significant decrease in T_g was observed for the samples treated at 10 and 20 MPa, which presented a greater amount of incorporated active ingredient, due to the plasticizing effect

exerted by the active ingredient in the polymer matrix, which causes a greater movement between the polymer chains, similar to the results reported by other authors [27,48,58]. This effect was also observed in the T_{cc} , which also showed a decrease from 117°C to 112°C, due to the reorganization of the crystalline structure of the polymer by increasing the free volume of the polymeric matrix [36], which favors the crystallization of PLA into less stable α' crystals. This is also confirmed by the appearance of a double endothermic melting peak (Fig. 11a) in all the impregnated samples, generated by the crystalline polymorphism that PLA presents when impregnated with pure carvacrol in the matrix [59].

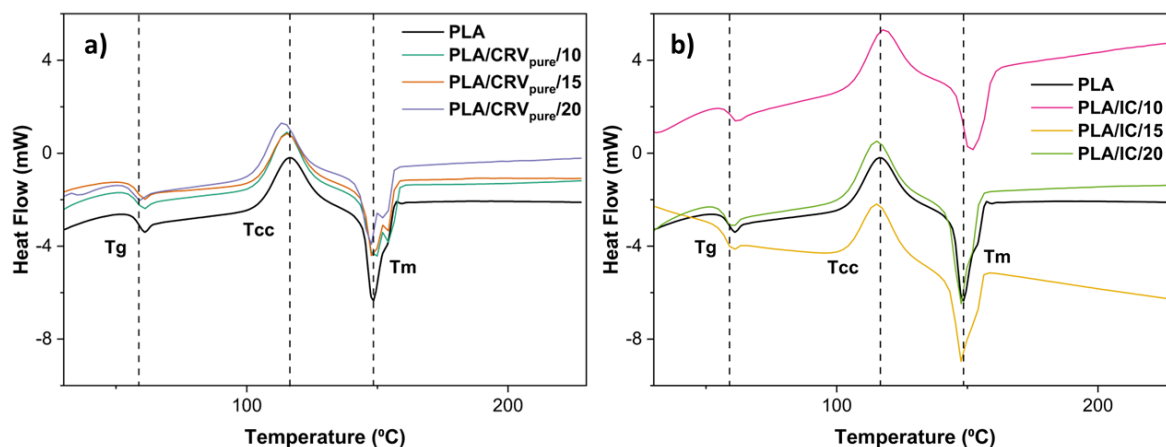


Figure 11. DSC thermogram of PLA films with and without active agent. a) pure carvacrol (CRV_{pure}) and b) inclusion complex (IC). The number next to each sample indicates the impregnation pressure (10, 15 or 20 MPa).

The PLA samples impregnated with IC showed only a decrease in the degree of crystallinity, which could be related to the presence of a small amount of free carvacrol that could be incorporated during the IC impregnation, as evidenced by the appearance of a double endothermic melting peak in the thermograms (Fig. 11b), as in the samples

impregnated with carvacrol without complexing. In addition, they did not show significant changes in the glass transition temperature, cold crystallization and melting, which could be due to the increase in the mobility of the polymeric matrix chain, these results are similar to those reported by Friné et al. [60], who developed PLA films with β -cyclodextrin/carvacrol complexes by the extrusion process. These results would confirm the incorporation of the complexes into the polymeric matrix.

However, pure Mater-Bi® films (**Table 3**) showed a T_g of 58°C associated with PLA[6] and two endothermic peaks at 120°C and 165°C associated with the melting temperatures of poly(butylene adipate-co-terephthalate) (PBAT) (T_{m1}) and thermoplastic starch (T_{m2}), respectively. This behavior is consistent with that reported in other studies, including that of Hejna and coworkers, who investigated the biodegradability of Mater-Bi® [17,61].

Table 3. Thermal properties of Mater-Bi (MB) and Mater-Bi films impregnated with pure carvacrol (CRV_{pure}) or β -cyclodextrin/carvacrol inclusion complex (IC). The number next to each sample indicates the impregnation pressure (10, 15 or 20 MPa).

Sample	T_g (°C)	T_{m1} (°C)	T_{m2} (°C)	ΔH_{m1} (J/g)	ΔH_{m2} (J/g)
MB	58.4 ± 0.5^a	120.4 ± 0.5^a	165.5 ± 0.7^a	4.11 ± 0.5^a	1.9 ± 0.1^a
MB/CRV _{pure} /10	51.4 ± 1.3^c	$118.5 \pm 0.2^{b,c}$	$163.8 \pm 0.5^{b,c}$	$7.1 \pm 1.2^{a,b,c}$	2.2 ± 0.2^a
MB/CRV _{pure} /15	56.8 ± 0.1^b	117.6 ± 0.2^c	$163.6 \pm 0.1^{b,c}$	9.4 ± 0.8^c	2.0 ± 0.1^a
MB/CRV _{pure} /20	56.3 ± 0.4^b	119.0 ± 0.8^b	$164.7 \pm 0.4^{a,b}$	8.8 ± 0.9^c	2.0 ± 0.2^a
MB/IC/10	58.3 ± 0.5^a	$118.6 \pm 0.4^{b,c}$	$164.4 \pm 0.5^{b,c}$	$8.1 \pm 0.6^{b,c}$	1.6 ± 0.2^b
MB/IC/15	57.8 ± 0.6^b	117.7 ± 0.1^c	163.5 ± 0.3^c	$5.3 \pm 2.7^{a,b}$	$1.1 \pm 0.4^{b,c}$
MB/IC/20	58.9 ± 1.3^a	$118.4 \pm 0.6^{b,c}$	$164.2 \pm 0.7^{b,c}$	$7.5 \pm 1.6^{b,c}$	1.4 ± 0.1^b

*Superscript letters a,b, and c indicate significant differences between the values of each thermal parameter.

On the other hand, the samples impregnated with pure carvacrol showed a decrease in Tg and melting temperature associated with the fraction of PLA present in the blend (T_{m1}). In particular, the samples MB/CRV_{pure}/10 and MB/CRV_{pure}/15, which presented a higher amount of incorporated active. This variation could be due to the plasticizing effect exerted by the incorporation of carvacrol in the matrix, which generates a greater mobility of the macromolecules of Mater-Bi® [62].

3.3.Molecule modeling in COSMO-RS

3.3.1. Computational study of the conformational position of the carvacrol in the β -cyclodextrin

 **Fig. 12** shows the two possible conformations for the inclusion of the carvacrol into the β -cyclodextrin, designated IC-1 and IC-2. In the conformer (1) the CH(CH₃)₂ in the carvacrol's ring is in the same plane as the terminal CH-OH groups in the β -cyclodextrin, whereas in the case of the conformer (2) the CH(CH₃)₂ group in the carvacrol's ring is opposite to the CH₂OH alkyl group in the β -cyclodextrin. According to Table 4 the conformer β -cyclodextrin-carvacrol(1) is more favorable in CO₂. The bulkiest terminal functional group CH(CH₃)₂ in the carvacrol produces less electron repulsion in the open part of the β -cyclodextrin where the CH-OH functional group is located in its ring. The same orientation preference is found by other authors [39], but in this case in the CO₂ phase media, where this conformation is also the most stable.

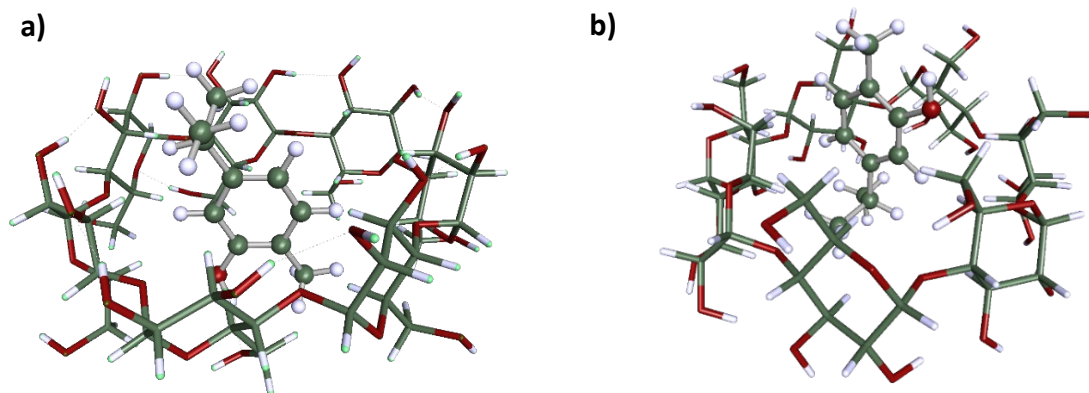


Figure 12. Possible conformational structure of the carvacrol in the β -cyclodextrin: a) IC-1, b) IC-2 (Carbon=green, Hydrogen= white, Oxygen= red).

Table 4. Stability of the inclusion complex for the different conformers. Calculations were performed at T=298 [K] and 1 [bar].

Molecule	G in vacuum (kJ/mol)	G in scCO ₂ (kJ/mol)	ΔG inclusion in scCO ₂ (kJ/mol) (Eq. 1)
Carvacrol	453.58	-45.31	-
β -cyclodextrin	2803.18	-260.55	-
IC-1	3324.66	-232.18	141.6
IC-2	3331.68	-232.78	148.0

3.3.2. Prediction solubility test

Due to the impossibility of knowing the exact composition and the complex polymer network of the commercial polymer blend Mater-Bi®, each molecule of the inclusion complex (IC) and free carvacrol is confronted with the main components of this polymer,

such as starch, poly(lactic acid) (PLA) and poly(butylene adipate-co-terephthalate) (PBAT), in order to know the distribution between this and scCO₂ (**Table 5**).

Table 5. Partition coefficient ($K_{P/scCO_2}$) between the polymer (P) and the CO₂ obtained at 298 [K].

Molecule	Log ($K_{P/scCO_2}$)		
	Starch	PLA	PBAT
IC	1.96	2.03	-2.20
Carvacrol	-3.35	-2.52	-2.61

According to these results, low values of partition coefficients are obtained for the carvacrol in all polymers, due to the high affinity of this molecule for CO₂. When the carvacrol is introduced in β -cyclodextrin to form the IC, the host molecule provides a more hydrophobic environment that increases the solubility of the carvacrol for all studied polymers and for starch and PLA the equilibrium is shifted towards solubilization of the inclusion complex, showing positive values of log $K_{P/scCO_2}$, the estimation of the log $K_{P/scCO_2}$ being higher for PLA when compared to the other components of Mater-Bi®. This result predicts a better incorporation of the inclusion complex from the supercritical phase to the polymeric phase for PLA, although the individual solubility values of carvacrol and IC in scCO₂ must be considered, PLA seems to be the polymer with the highest availability to incorporate the inclusion complex according to these simulations.

3.4. Mass transfer model to describe the release kinetic of carvacrol

The mass transfer during the release of carvacrol from the impregnated films (PLA and Mater-Bi®) was studied experimentally through specific migration tests described in section 2.7.1. From the migration tests of PLA and Mater-Bi® films impregnated with carvacrol reported in **Fig. 12**, it could be observed that the release kinetics of the active compound can be explained by a diffusion process through the polymers.

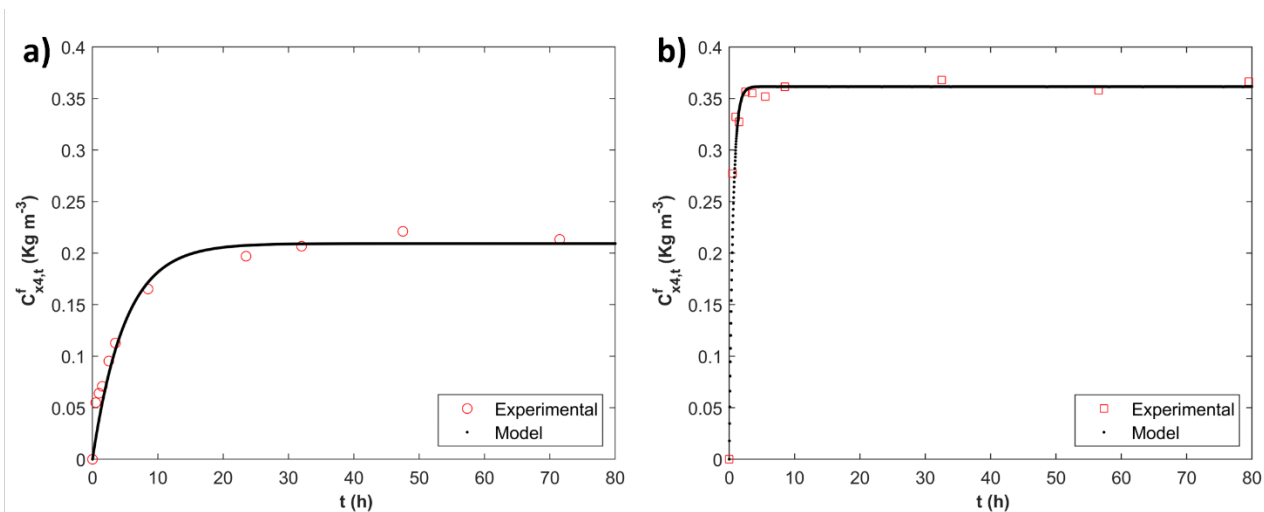


Figure 13. Adjustment of the mass transfer model to describe the carvacrol release from the polymer to the food simulant. a) PLA; b) Mater-Bi.

Thus, the transfer of carvacrol through the polymer during the release process can be described by Fick's law. Considering the above, the selection of parameters to fit the obtained experimental data was carried out as reported by Torres et al [36]. The obtained values (initial concentration of the active compound in the polymer, effective diffusion coefficient in the polymer phase and polymer/food simulant partition coefficient) are presented in **Table 6**.

Table 6. Parameter values estimated from the experimental kinetic curve fitting of carvacrol (CRV) release from PLA and Mater-Bi to the food simulant.

Samples	$C_{xI,t=0}$ (kg m^{-3})	D_{CRV-P}^{eff} ($\text{m}^2 \text{s}^{-1}$)	$K_{P/FS}$
PLA/carvacrol	90.3	$5.0 \cdot 10^{-14}$	240
Mater-Bi/carvacrol	79.6	$9.9 \cdot 10^{-13}$	38

730 The kinetic curves in **Fig. 13** show that carvacrol is released more slowly from PLA (**Fig.**
731 **13a**) than from Mater-Bi® (**Fig. 13b**). Both reach equilibrium at approximately 32 hours.
732 In addition, the polymer/food simulant partition coefficient, $K_{P/FS}$ (Table 6), shows a lower
733 value for Mater-Bi® films, indicating a higher affinity of carvacrol for PLA and food
734 simulant. The effective diffusion coefficient of carvacrol from PLA and Mater-Bi® films
735 was $5 \cdot 10^{-14}$ and $9.9 \cdot 10^{-13}$, respectively.

736 The kinetic results of carvacrol from PLA would be related to the chemical interactions
737 between the polymer, the active compound and the food simulant. In the polymer/simulant
738 case, this is due to the competitive equilibrium between the swelling effect of ethanol,
739 which expands the PLA network and allows maximum water sorption into the PLA matrix
740 and the scission of the polymer main chain due to hydrolysis [63]. While carvacrol and
741 ethanol exhibit high affinity as both are molecules with non-polar groups [64].

742 Moreover, the mass transfer model described in this work (equations 6-15) was fitted to
743 estimate the release of carvacrol from the polymer film to the food simulant when carvacrol
744 is incorporated into the material as an inclusion complex with β -cyclodextrin. The selection
745 of parameters to fit the model to the experimental release kinetic data was performed
746 according to the procedure described in section 2.7.2. Thus, the initial concentration of
747 complexed carvacrol in the bulk of the polymer was estimated to be 7.85 kg m^{-3} and
748 $3.9 \cdot 10^{-13} \text{ kg m}^{-3}$ for PLA and Mater-Bi®, respectively, while the initial concentration of
749 free carvacrol in the same phase was estimated to be 4.15 kg m^{-3} and 6.5 kg m^{-3} ,
750 respectively. The value of the effective diffusion coefficient of carvacrol through PLA and

Mater-Bi® is $3.0 \cdot 10^{-14} \text{ m}^2 \text{ s}^{-1}$ and $9.9 \cdot 10^{-13} \text{ m}^2 \text{ s}^{-1}$, respectively. The latter value is in agreement with the data reported in the literature [20, 65].

The value of the carvacrol partition coefficient between polymer and food simulant ($K_{P/FS}$) was estimated to be 15 for PLA and 3 for Mater-Bi®, the values of the decomplexation kinetic constants k_1 and k_2 were estimated to be $1.98 \cdot 10^{-6} \text{ s}^{-1}$ and $9.48 \cdot 10^{-7} \text{ s}^{-1}$, respectively for PLA films, while for Mater-Bi® they were $1 \cdot 10^{-8} \text{ s}^{-1}$ and $3.5 \cdot 10^{-8} \text{ s}^{-1}$, respectively.

These values of the parameters allowing the estimation of the lowest RMSE are summarized in **Table 7**:

Table 7. Parameter values estimated from the adjustment with the experimental release kinetic curve of carvacrol from PLA and Mater-Bi film to the food simulant. Carvacrol incorporated into the material as an inclusion complex (IC) with β -cyclodextrin.

Samples	$C'_{xI,t=0}$ (kg m^{-3})	$C^c_{xI,t=0}$ (kg m^{-3})	D^{eff}_{CRV-P} ($\text{m}^2 \text{ s}^{-1}$)	$K_{P/FS}$	$k_1 \text{ (s}^{-1}\text{)}$	$k_2 \text{ (s}^{-1}\text{)}$
PLA/IC	4.15	7.85	$3.0 \cdot 10^{-14}$	15	$1.98 \cdot 10^{-6}$	$9.48 \cdot 10^{-7}$
Mater-Bi/IC	6.50	$3.90 \cdot 10^{-13}$	$9.9 \cdot 10^{-13}$	3	$1.01 \cdot 10^{-8}$	$3.51 \cdot 10^{-8}$

Fig. 14a and 15a show the kinetic release curves of carvacrol from PLA and Mater-Bi®, respectively, as the concentration of carvacrol in the food simulant as a function of time. The black line represents the model fitted to the experimental data (red dots) using the parameters listed in **Table 7**. In addition, **Fig. 14b and 15b** show the change in concentration of complexed (red line) and free (blue line) carvacrol in PLA and Mater-Bi®, respectively, as a function of time estimated by the model using the values of the parameters reported in **Table 1** for the fitted model.

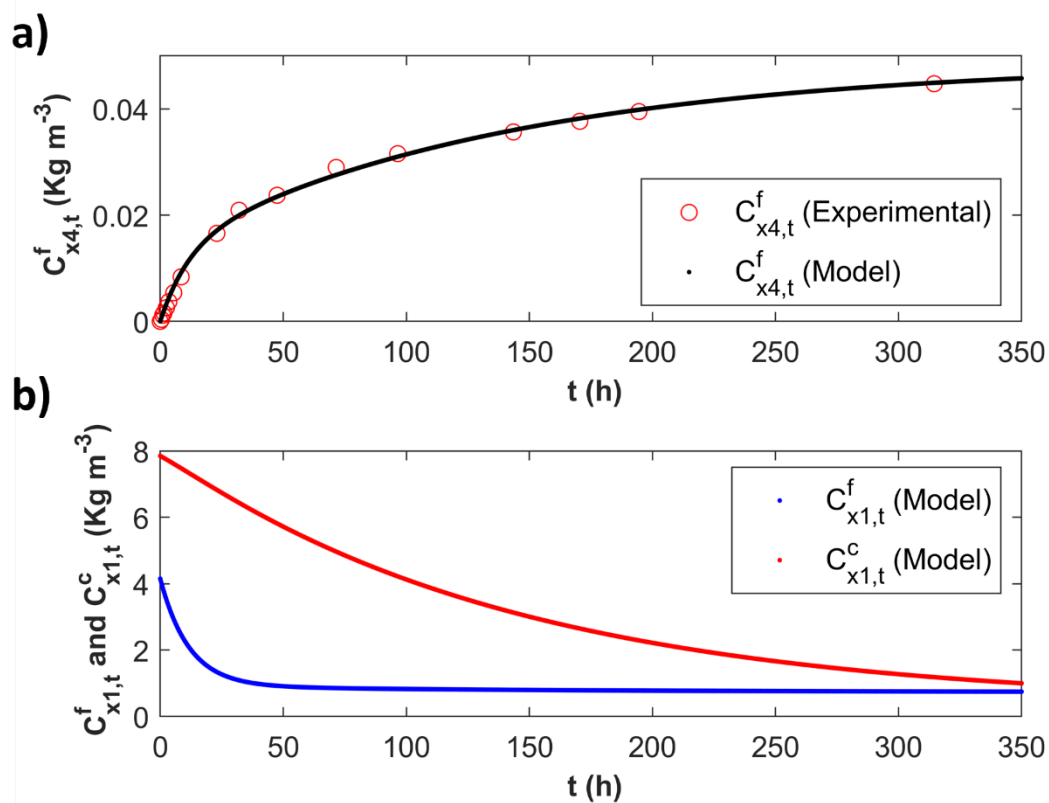


Figure 14. Fitting of the mass transfer model to describe the release of carvacrol from PLA containing the β -cyclodextrin/carvacrol inclusion complex to the food simulant: a) carvacrol release kinetics represented as the concentration of carvacrol in the food simulant as a function of time (experimental and estimated from the fitted model); b) concentration of complexed and free carvacrol in the polymer as a function of time (estimated from the model).

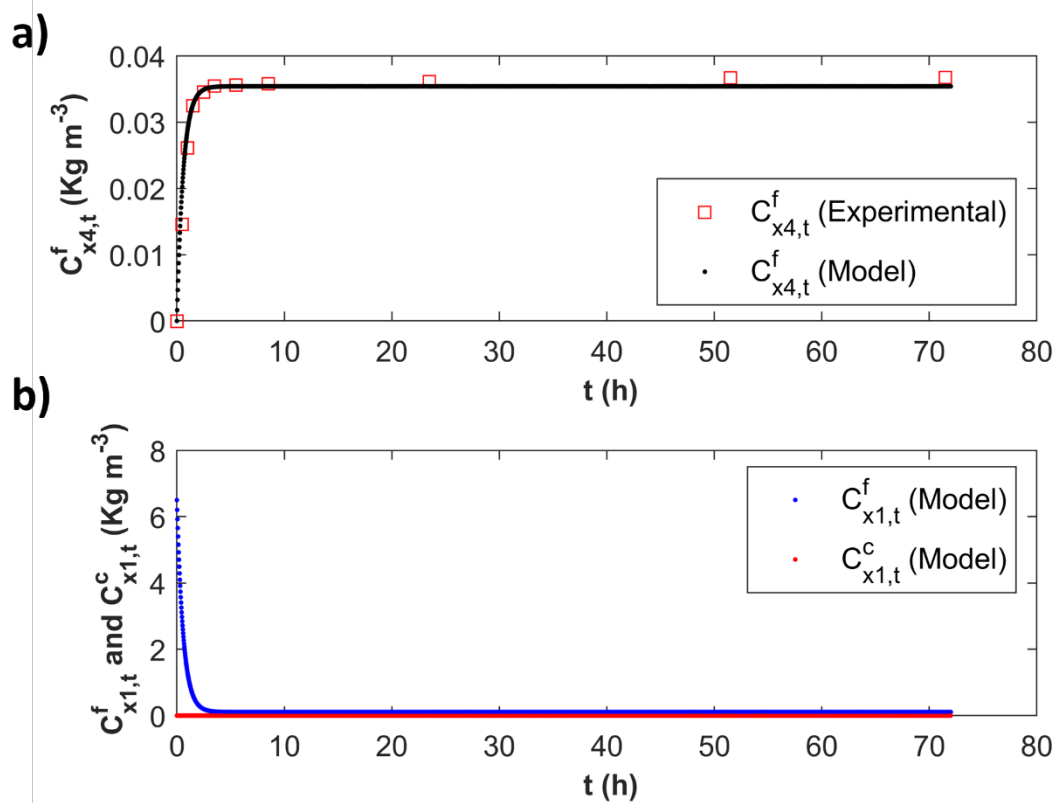


Figure 15. Fitting of the mass transfer model to describe the release of carvacrol from Mater-Bi containing the β -cyclodextrin/carvacrol inclusion complex to the food simulant: a) carvacrol release kinetics represented as the concentration of carvacrol in the food simulant as a function of time (experimental and estimated from the fitted model); b) concentration of complexed and free carvacrol in the polymer as a function of time (estimated from the model).

Figures 14 and 15 show the different behavior of the two materials, where PLA shows a slow and sustained release of carvacrol up to 350 hours, and it is possible to observe in Fig. 14a a change in the slope at about 25 hours, taking into account the stability in the concentration of free carvacrol at the same time (blue line in Fig. 14b); meanwhile, the

concentration of a considerable amount of complexed carvacrol gradually decreases (red line in **Fig. 14b**). Thus, it can be observed that the incorporation of the β -cyclodextrin/carvacrol inclusion complex involves a slow release of the active compound, which, under the experimental conditions chosen in this work, does not reach the equilibrium after 350 hours.

The values of the initial concentration of complexed carvacrol ($C_{cx1,t=0}$) and the initial concentration of free carvacrol ($C_{fx1,t=0}$) in the bulk of the PLA film, estimated from the model fit, for PLA show that a significant amount of free carvacrol is available in the polymeric phase from the beginning of the release process. This amount of free carvacrol, representing almost 35% of the total concentration of the complex and free species, could be explained by a decomplexation during the supercritical impregnation of the β -cyclodextrin/carvacrol inclusion complex and/or a decomplexation of the active compound after the impregnation of the inclusion complex in the polymeric matrix.

On the other hand, regarding **Fig. 15a**, it can be seen that the incorporation of IC has no effect on the release compared to the release of carvacrol from Mater-Bi® shown in **Fig. 13b**, since the concentration of free carvacrol was significantly higher than that of complexed carvacrol. In addition, the presence of TPS in Mater-Bi® could cause a more accelerated release, both for the samples impregnated with carvacrol and with inclusion complex, since this polymer seems to have a higher affinity with the simulant, which contributes to the swelling of the polymeric matrix and to a faster diffusion of the active compound. This interaction could also explain the rapid decomplexation of carvacrol and the negligible effect of its incorporation on the release rate.

Finally, the model proposed in this work could be applied to other systems containing inclusion complexes, since the structure of the equation describing the decomplexation rate (Eq. 7) is a general relationship involving the presence of the complexed and free active compound. Further experimental and theoretical characterization using this model, coupled with analysis of the chemical composition in the polymer, could represent a step forward in the design of a wide range of applications of materials that release active compounds from the inclusion complexes.

4. CONCLUSIONS

This work reports the characterization of two polymeric materials (PLA and Mater-Bi®) impregnated with an inclusion complex of carvacrol and β -cyclodextrin to analyze the controlled release of the active compound from the polymeric matrix to a food simulant, understanding the decomplexation and transport processes that explain the observed release rates.

The results obtained from the FTIR spectra and thermal properties of the PLA and Mater-Bi® samples show that the incorporation of the β -cyclodextrin/carcacrol inclusion complex by impregnation with scCO₂ can be verified at different levels. The DCS analysis of these samples shows the presence of a quantifiable amount of free and complexed carvacrol in the polymer samples. Meanwhile, the morphological properties observed by SEM show results in agreement with the characteristics of these polymeric materials reported in the literature using similar impregnation procedures.

Furthermore, these results are complemented by a predictive analysis of the conformational position of the carvacrol in the β -cyclodextrin obtained by molecular modeling made by

COSMO RS, verifying the orientation and preference reported in the literature, estimating that this conformation is the most stable in a pressurized CO₂ medium. Meanwhile, the prediction of the partition coefficients of the inclusion complex between the polymers or their components and scCO₂ shows that PLA seems to be the material with the highest relative availability to host the β -cyclodextrin/carvacrol inclusion complex in its structure. This fact was confirmed in the impregnation and release tests.

On the other hand, a novel model to describe the release kinetics of carvacrol impregnated with scCO₂ in polymeric films of PLA and Mater-Bi® is proposed here to take into account the coupled phenomena that explain the decomplexation rate of the active compound in the polymeric material with the diffusion rate. This model includes the presence of complexed and non-complexed active compound. In this case, the model allows the coherent description of a decomplexation phenomenon coupled with a diffusional transport through the PLA to a food simulant up to 350 hours of release. Meanwhile, the low concentration of the complexed carvacrol in Mater-Bi® is associated with a negligible effect of the complex on the gradual release.

The structure of this model can be applied to any other similar material system containing active compounds incorporated as inclusion complexes. These calculations, coupled with an appropriate experimental characterization, could even allow the design of controlled release systems by selecting the best conditions for the appropriate combination of active compound, complexing agent, and host polymer.

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