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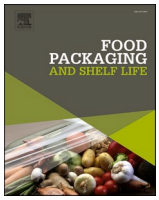

Release and antibacterial action of phenolic acids incorporated into PHBV films

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ABSTRACT

Ferulic or p-coumaric acids were incorporated (3, 6 or 9%) into PHBV films by melt processing or surface anchoring (3–4% in the film). The release kinetics of phenolics and the films' antibacterial effect against *Escherichia coli* and *Listeria innocua* were analysed. At equilibrium, a near 100% release of phenolics was obtained from melt processed films in a low polar simulant (D1: 50% v/v ethanol in water), whereas it ranged between 10% and 38% in a more polar simulant (A: 10% v/v ethanol in water). The diffusion coefficient of FA or PCA in the matrix also rose by 4–30 times in contact with simulant D1. The limited release of the phenolics in contact with aqueous systems hindered the film's antibacterial effect in contact with the inoculated culture media. In contrast, surface-loaded films exhibited a complete release of phenolics in aqueous media and a significant (near 2 log CFU) bacterial growth inhibition.

1. Introduction

Food packaging is key for the ensuring of food quality and safety, preserving products from physical, chemical and microbial spoilage during the food chain (Wikström et al., 2018). This makes the food industry one of the main consumers of single use plastic packages (Hale et al., 2020), contributing heavily to the environmental problem caused by the prolonged bad management of non-degradable plastics. To solve this problem, to reduce the environmental impact of plastics, different approaches are needed, such as responsible use, recycling management or the use of biodegradable materials in packaging development (Muller et al., 2017).

Although biodegradable polymers are plastic materials that have great potential to be used as replacements for conventional plastics, their production is still low scale while their cost is high and their properties must be fitted to the packaging requirements of different food products (Vert et al., 2012). These polymers disintegrate and biodegrade in compost media or even in natural environments (soil or water systems), thus integrating them into the organic matter cycle (Peelman et al., 2013; Siracusa et al., 2008). Polyhydroxy-alkanoates (PHAs) have gained importance in recent decades due to their particular advantages. These are semi-crystalline aliphatic polyesters produced by a variety of bacteria as intracellular carbon or energy stores. They are fully biosynthetic and biodegradable (Briassoulis et al., 2021; Corre et al., 2012; Li et al., 2016) and can be synthesized from carbon-rich waste, such as waste and by-products from the food industry itself, which includes the production process within the concept of the circular economy (Koller et al., 2017).

Polyhydroxy-butyrate (PHB) is the most common polymer of the PHAs family. However, its crystalline structure makes it a rigid and brittle material. Moreover, its difficult processability, due to thermal instability, has prevented its widespread use (Fabra et al., 2014; Modi et al., 2011). To overcome these drawbacks, PHB has been copolymerised with 3-hydroxyvalerate (HV) to form poly (3-hydroxybutyrate-co-3-hydroxyvalerate) or PHBV. This new copolymer has a lower melting point, which widens the window for thermoprocessing (Anderson & Dawes, 1990; Laycock et al., 2014; Tebaldi et al., 2019). In addition, it exhibits good water vapour and oxygen barrier properties, making it a very promising biopolymer for application as a packaging material (Jost & Langowski, 2015).

Active packaging materials containing antimicrobial/antioxidant compounds represent an efficient alternative for food preservation purposes, avoiding the direct incorporation of preservatives into the food, which is aligned with the actual consumer demands (Carocho et al., 2014). Antioxidant and/or antimicrobial compounds of natural origin, such as phenolic acids, could be incorporated into the polymeric packaging materials to obtain active packages that may extend the shelf life of food products (Ordoñez et al., 2022a). Phenolic acids exhibit

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antioxidant and antimicrobial capacity depending on their molecular structure (Lima et al., 2019; Ordoñez et al., 2022a; Pernin et al., 2019). Specifically, ferulic and p-coumaric acids (the most abundant isomer from coumaric acid) are phenolic compounds isolated from various types of plant products, which exhibit antioxidant, antibacterial, anti-inflammatory and other health-promoting properties (Dędek et al., 2019; Lou et al., 2012; Mitani et al., 2018; Miyague et al., 2015; Shi et al., 2016; Takahashi et al., 2013; Useyin Boz, 2015).

Previous studies (Andrade et al., 2022; Aragón-Gutiérrez et al., 2020; Hernández-García et al., 2022a; Ordoñez et al., 2021a, 2022b) reported antibacterial activity for ferulic, cinnamic, p-coumaric or protocatechuic acids when incorporated in different biodegradable or non-biodegradable polymeric matrices, such as starch, PLA, PVA, PLA-PHBV or EVOH films. To exert their antimicrobial action, the adequate release (rate and proportion) of the active compounds into the food system is required in order to reach the minimum inhibitory concentration (MIC) of the bacteria present. The release of an active compound from a polymer matrix to the target medium is determined by its diffusion coefficient (*D*) through the matrix and the partition coefficient (ratio between the mass of active compound released at equilibrium (M_∞) and that retained in the polymer matrix (M_0-M_∞)). The partition coefficient, in turn, is affected by the balance of interactions of the active compound with the polymer matrix and the product in contact with the film, depending on the chemical affinity between the compound and polymer matrix and the compound solubility in the target product (Requena et al., 2017). Likewise, D is also affected by the compound's interactions with the polymer matrix and its molecular mobility. The latter may be affected by the changes in the matrix structure (swelling) promoted by the penetration of the compounds of the in-contact medium (mainly solvent) into the polymer network. Therefore, all these factors will affect the antimicrobial action of the active compounds included in the polymeric package, which must be released in the correct ratio to inhibit the bacterial growth. No previous studies reported the release kinetics of phenolic acids, as active compounds, from PHBV films in order to evaluate their potential antibacterial action as food packaging materials.

The aim of the present study was to produce PHBV films with ferulic acid (FA) and p-coumaric acid (PCA) with antibacterial activity. Thus, melt processed films with different phenolic concentrations and films with surface-anchored phenolics were obtained. The release kinetics of the compounds incorporated into PHBV films, using food simulants of different polarities, and the films' antibacterial action against *Listeria innocua* and *Eschericia Coli* were analysed by *in vitro* tests with inoculated culture media.

2. Materials and methods

2.1. Materials

Poly(3-hidroxybutyrate−3-co-(hydroxyvalerate) (PHBV), with 2% HV fraction, was supplied in pellet form (ENMAT Y1000P) by TianAn Biologic Materials (Ningbo, China). Ferulic acid (FA) and P-coumaric acid (PCA), used as active compounds in the films, were supplied by Sigma-Aldrich, Saint Louis, USA. Phosphorus pentoxide (P_2O_5), from Panreac Química, Barcelona, Spain, was used for conditioning the samples at 0% relative humidity. UV-grade methanol and ethanol (99.9% purity), used for the extraction of active compounds and simulant formulations, were obtained from Sigma-Aldrich Chemie (Steinheim, Germany). Ethanol 96%, from Panreac Química (Barcelona, Spain) was used to obtain the spraying solutions of active compounds.

Listeria innocua (CECT 910) and *Escherichia coli* (CECT 101) strains were supplied by the Spanish Type Collection (CECT, University of Valencia, Spain). Soy tryptone broth (TSB), peptone buffered water (BPW) and bacteriological agar were purchased from Scharlab (Barcelona, Spain) for microbial tests. The selective media for plate counts (red-violet bile agar (VRBA) for *E. coli* and palcam base agar (PAB) enriched with palcam selective supplement for *Listeria innocua*) were also obtained from Scharlab (Barcelona, Spain).

2.2. Film preparation

PHBV films were obtained by melt blending and compression moulding by incorporating different ratios of ferulic or p-coumaric acid into the blend (0, 3, 6 or 9 g phenolic acid/100 g blend). Melt blending (50 g per batch) was prepared using an internal mixer (Haake PolyLab QC, Thermo Fisher Scientific, Germany) at 180 °C and 50 rpm for 5 min. The obtained blends were stored in a desiccator with P_2O_5 until use. Films were obtained by compression moulding of 3.5 g of blend powder, using a hot-plate press (Model LP20, Labtech Engineering, Thailand), applying preheating at 180 °C for 5 min, and thermocompression for 4 min at 180 °C and 100 bar, and final cooling for 3 min to 70 °C. PHBV films with the different ratios of ferulic acid are named 3FA, 6FA and 9FA, for concentrations of 3%, 6% and 9% respectively of ferulic acid in the blend, while those with p-coumaric acid were named 3PCA, 6PCA and 9PCA for the same concentrations of this acid.

PHBV films with FA or PCA were also obtained by spraying an ethanol solution of the respective phenolic acid onto the already thermoformed PHBV films, as previously described by other authors (Hernández-García et al., 2022b; Ordoñez et al., 2022b) in order to obtain active films with more available active compounds. For this purpose, 5% (w/w) solutions of ferulic or p-coumaric acid in 96% (v/v) ethanol were prepared and loaded into an airbrush (E4182, Elite pro). PHBV films, positioned 4 cm away from the airbrush nozzle, were sprayed for 6 s at an average flow rate of 65 mg/s. The sprayed films were dried at room temperature for 24 h. These films were named SFA and SPCA, respectively.

All of the films were stored at 0% relative humidity until characterization.

2.3. Scanning electron microscopy (FESEM)

The cross-section of the melt blended films was observed by field emission scanning electron microscopy (FESEM, Ultra 55, Zeiss, Oxford Instruments, UK). For this analysis, the films were cryofractured by immersion in liquid nitrogen. The surface of the pulverized films was also observed. Samples were coated with platinum before the microscopic observations, which were carried out at an accelerating voltage of 1.5–2 kV.

2.4. Quantification of the active compound retention in melt blended films

The total content of both FA and PCA in the films was quantified by extraction with methanol and the subsequent spectrophotometric determination (Ordoñez et al., 2021b). For this purpose, 100 mg of film (at 0% RH) was cut into thin strips and diluted in 10 ml of methanol, under stirring for 48 h at 20 °C. Absorbance measurements of the methanolic extracts were obtained at 321 and 309 nm, respectively for FA and PCA, using a UV–visible spectrophotometer (Thermo Scientific Evolution 201, USA). Calibration curves were previously obtained for the methanolic solutions of each phenolic acid. In all cases, the methanolic extract of the active-free PHBV films was used as blank. The measurements were taken in quadruplicate for each film sample.

In the films obtained by spraying, the mass difference between the initial and dried films after spraying was used to quantify the mass ratio of the surface incorporated compound.

2.5. Release kinetics of FA and PCA in different media

To analyse the release kinetics of the active compounds from the melt blend films, two food simulants of different polarities were used, as previously described by Requena et al., (2017). Food simulant A (10% ethanol in water; v/v) was used to emulate neutral aqueous foods, while

simulant D1 (50% ethanol in water; v/v) was selected to simulate foods with a high alcohol content (>20%) or oil in aqueous emulsions (Commission Regulation (EU) 10/2011). For this purpose, 500 mg film samples were immersed in 100 ml of each simulant at 20 °C, with continuous stirring of samples located on a magnetic stirrer plate (IKA-RT 15, IKA Werke, Germany). The amount of active released as a function of contact time was quantified spectrophotometrically through the solution absorbance as a function of time, until a constant value. Measurements were taken at the absorbance maximum of each compound in the corresponding simulant (321 and 291 nm in simulant D1, and 311 and 288 nm in simulant A, respectively for FA and PCA). Concentration of each compound were obtained from the corresponding calibration curves obtained in each simulant. Each assay was carried out in triplicate.

Two mathematical models were considered to describe the release of active compounds into the food simulants. First, the Peleg model (Peleg, 1988) was fitted to the experimental data by linear regression (Eq. 1), obtaining the concentration released at equilibrium.

$$\frac{t}{M_t/M_o} = k_1 + k_2 \cdot t \quad (1)$$

where:

M_t (g) is the mass of the active compound released in the simulant after a contact time t; M_o (g) is the initial mass of the compound in the film; k_1 (h) and k_2 are the constants of the model, where $1/k_1$ is the initial release rate of the process and $1/k_2$ is the ratio of active compound released at equilibrium (M_∞/M_o).

From the k_2 Peleg parameter, the partition coefficient of each compound in each simulant can be deduced as the mass of active released at equilibrium in the simulant (M_∞) relative to the residual mass of active in the film (M_0-M_∞).

Likewise, Fick's second law was fitted to the experimental data to obtain the effective diffusion coefficient (D) of each compound in the different films, for each simulant. To this end, film samples were considered as infinite plane sheets where the active compound diffuses perpendicularly from the centre of the film towards the surface, considering the film half-thickness (e) as a characteristic dimension. The diffusional long-time equation for an infinite plane sheet (Crank, 1979) with ten terms (Eq. 2, with n = 10) was used to determine the values of diffusion coefficient (D) of FA and PCA in the different simulants, by using the Solver tool (Microsoft Excel 2013®) to optimize the D values, by minimizing the Sum of Squared Errors (SSE). The boundary conditions for applying Eq. (2) were: i) a t = 0, the initial distribution of active compound into the films was homogenous (M_0, being the initial mass of active compound in the film) while no active compounds are present in the simulant, ii) at t > 0, the mass of active compound released from the films into the simulant is M_t, assuming no degradation of active compounds during the migration process. The mass of active compound released from the film when equilibrium was reached is M_∞, related with the partition coefficient: M_∞/ (M_0-M_∞).

$$\frac{M_t}{M_\infty} = \frac{8}{\pi^2}\sum_{n=0}^{\infty}\left[\frac{1}{(2n+1)^2}\exp\left\{\frac{-D(2n+1)^2\pi^2 t}{4e^2}\right\}\right] \quad (2)$$

The release of the active compounds from the different films in pure water (solvent to film ratio of 8:5) was also analysed at 10 °C, without agitation, to simulate the compound release in the culture medium (with a_w near 1) during the *in vitro* antibacterial test. The experimental data were only fitted to the Peleg's model since no internal diffusion occurs through the films.

2.6. Assessment of antimicrobial activity

To test the antibacterial efficacy of the films, the method previously described by other authors for *in vitro* tests was used (Ordoñez et al., 2022b; Requena et al., 2019; Tampau et al., 2018). Briefly, 55 mm diameter Petri dishes, containing 10 ml of TSA, were inoculated with 100 μL of bacterial suspensions (*E. coli* or *L. innocua*) (10^6 CFU/ml) on the plate surface, evenly spreading the bacteria with an L-shaped rod. The plates were completely covered with film samples (about 300 mg films) and incubated for 6 days at 10 °C. After this time, the contents of each plate were homogenized with 100 ml of buffered peptone water using a Masticator paddle blender (IUL Instruments, Barcelona, Spain). Serial dilutions of each sample were plated with selective media: violet red bile agar (VRBA) for *E. coli* and palcam agar base (PAB) with palcam selective supplement for *L. innocua*. Plates were incubated at 37 °C for 48 h and the number of colonies was counted.

All of the films with active compounds (melt blend and surface loaded) were submitted to the antibacterial test, using the active-free films and non-covered plates as controls. Each film sample was tested in duplicate.

2.7. Statistical analysis

The statistical analysis of the data was carried out by analysis of variance (ANOVA) using Statgraphics Centurion XVIII software (Statgraphics Technologies, Inc. The Plains, Virginia). Fisher's Least Significant Difference at 95% confidence level was used. Significant differences are indicated in the tables with different letters (abc.).

3. Results and discussion

3.1. Final concentration and distribution of active compounds in the films

Although the final concentration of FA in the melt processed films differed significantly from the amount incorporated, it was nearer the ratio added for PCA. Specifically, 2.68 ± 0.02; 4.54 ± 0.07 and 7.62 ± 0.04 g FA/100 g film were obtained, respectively, for 3FA, 6FA and 9FA formulations, which supposed an average compound retention value of about 85 ± 6% (with respect to the amount incorporated). In contrast, the concentrations of PCA determined in melt processed films were 2.90 ± 0.02, 6.10 ± 0.02 and 8.73 ± 0.04 g PCA/100 g film, respectively for the 3PCA, 6PCA and 9PCA formulations, which practically implied a total retention of the ratio incorporated (98 ± 6% retention). These results suggest that FA was more thermosensitive than PCA and degraded to a greater extent during the formation of the films. Other authors (Hernández-García et al., 2022a; Ordoñez et al., 2021b) also observed small losses of phenolic acids in films obtained by melt processing, which were attributed to the partial degradation of these compounds when submitted to the thermal and shear stress of processing.

Differences in the thermal stability of FA and PCA in the melt processed films could be attributed to their different thermal behaviour and degree of integration in the film matrix. The melting point of pure FA was between 173 and 176 °C, with the peak at 173 °C, whereas thermal degradation occurs between 200 and 240 °C, with the peak at 240 °C; these values shift when blended with compatible solids (Bezerra et al., 2017). These data suggest that FA could be melted at the process temperature (180 °C) and homogeneously blended with PHBV while partially degraded by local overheating in the internal mixer. In contrast, the melting point of PCA is higher (210–216 °C), simultaneously degrading during melting (Vilas-Boas et al., 2020). This suggests that PCA could remain in a solid state during the thermal process at 180 °C in the internal mixer, thus being more resistant to degradation during this step. This was reflected in the film's microstructure shown in Fig. 1. Films with FA exhibited a homogeneous cryofracture surface where no FA separation was observed. In contrast, the small particles that may be appreciated in the film's cross section micrographs can be attributed to PCA particles that are non-homogeneously integrated in the PHBV matrix. This was more evident when the PCA concentration rose in the films (Fig. 1g). The different structural integration of the phenolic acids in the polymer matrix could also affect their release

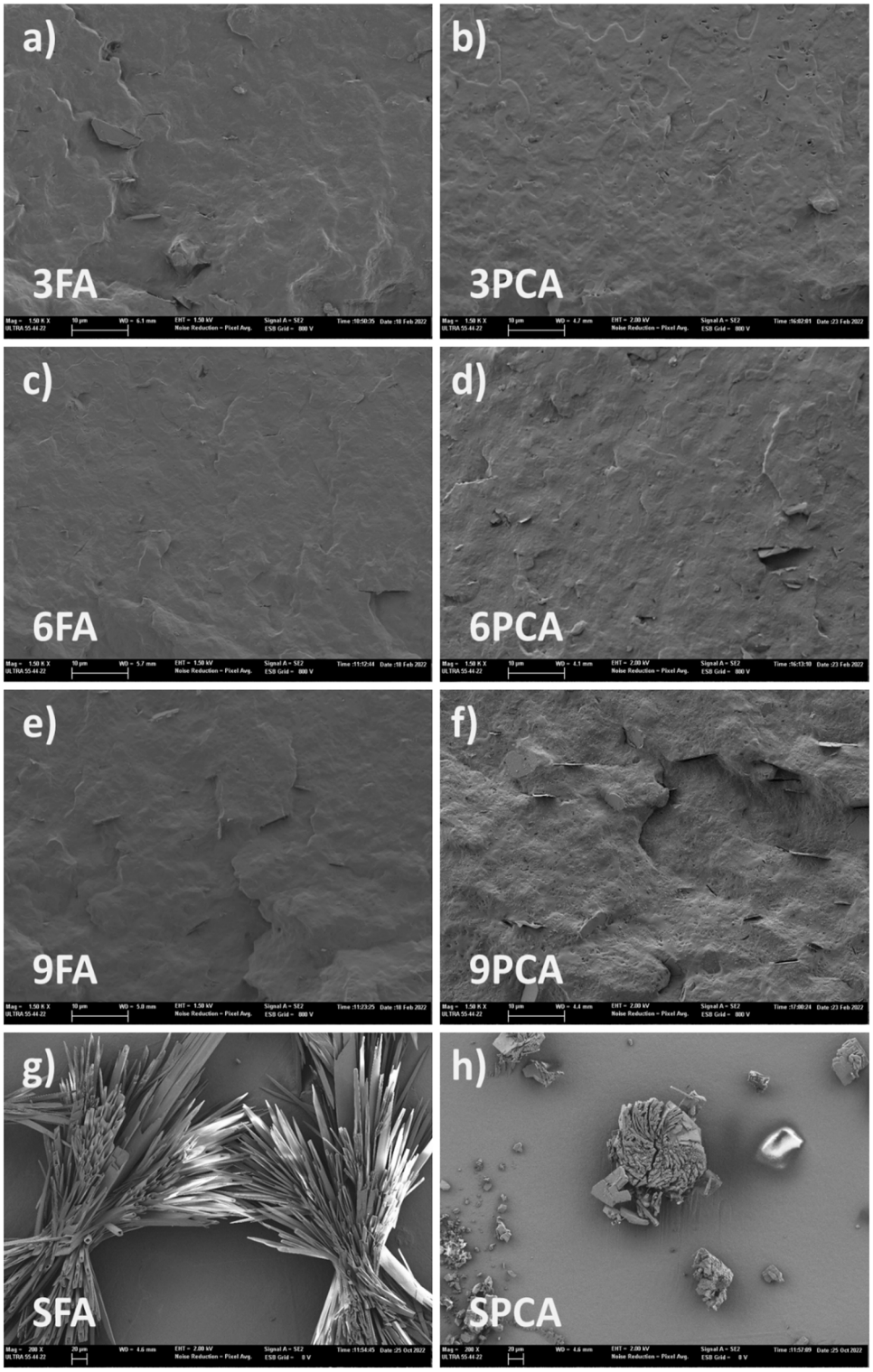

Fig. 1. Field Emission Scanning Electron Microscope (FESEM) micrographs of the cross sections of melt blended PHBV films with different ratios (3, 6 or 9% w/w) of FA (a, c and e) and PCA (b, d and f) (Magnification: 1.500X; bar: 10 μm) and surface micrographs of PHBV films surface loaded by spraying with the compound ethanolic solutions (g and h) (Magnification 200X; bar: 20 μm).

kinetics when in contact with food systems or simulants.

The quantities of FA and PCA surface anchored in the sprayed PHBV films were 4.6 ± 0.8 and 3.3 ± 0.8 g/100 g film, respectively, despite the identical spraying conditions applied. This can be attributed to the partial crystallization of p-coumaric acid at the nozzle edge, which partially obstructed the solution flow, in spite of the greater solubility of PCA in ethanol (188 g/L against 116 g/L for FA; Vilas-Boas et al., 2020). Differences in crystallisation rate of both compounds when over-saturation occurs in the nozzle edge during spraying could explain the different obstruction level produced by FA and PCA. In every case, the total compound load occurred on the film surface, where the phenolics crystallized in line with the solvent evaporation and solution

oversaturation. The FESEM images of the film's cross-section did not reveal significant internal diffusion of FA or PCA, but their crystallisation on the film surface. This can be explained by the fact that the diffusion time was longer than the solvent evaporation time. Therefore, most of the FA and PCA molecules were anchored on the film surface as crystalline formations, as shown in Fig. 1g and h. The needle-like morphology of the FA crystals, reported by other authors (Chen et al., 2020; Ordoñez et al., 2022b), could be observed, whereas the crystals of p-coumaric acid are monoclinic, composed of parallel molecular layers, joined together mainly by weak van der Waals forces (Utsumi et al., 1967). This structural arrangement suggests that both FA and PCA could be easily and totally released when the films are put in contact with any food or culture media, where crystals can be solved, thus promoting the antimicrobial activity.

3.2. Release kinetics of phenolic acids

The release kinetics of FA and PCA incorporated by melt processing into the PHBV matrices at different concentrations was analysed in two food simulants of differing polarities (simulants A and D1), attempting to emulate different kinds of food. Fig. 2a and b show the ratio of active released with respect to the initial amount in the film (M_t/M_0) as a function of the contact time with each simulant. Likewise, the Peleg model ($R^2 > 0.98$) fitted to each series of experimental points is shown. Different release behaviour was observed in simulants A and D1 for both phenolic compounds. In simulant D1 (50% v/v ethanol), with lower polarity, a higher release ratio and rate of both active compounds could be observed, whereas both the release ratio and rate were more limited in the more polar simulant, A (10% v/v ethanol in water). Table 1 shows the Peleg parameters (initial release rate ($1/k_1$) and the delivery ratio at equilibrium (M_∞/M_0) of the two phenolic acids in each simulant. In simulant D1, practically the total amount of both compounds (96–100%) was delivered faster than in simulant A, in which a smaller amount (10–40%) of both was released more slowly. Therefore, the partition coefficient (mass of the active released at equilibrium relative to that remaining in the polymer matrix) was much higher in the less polar simulant, D1, than in the more polar simulant, A.

The nature and concentration of the compound also affected the release kinetics, depending on the simulant. The release rate always increased when the compound concentration rose in the polymer matrix, due to the higher driving force (concentration gradient) for the mass transfer process and to the different structural changes promoted in the matrix by the different compound concentration. However, for a combination of simulant and active film of a given compound concentration, FA exhibited lower initial release rates than PCA. Previous studies also showed similar release behaviour for different active compounds incorporated into polyester matrices to food simulants A and D1. Thus, Requena et al. (2017) reported a higher release rate and ratio of carvacrol and eugenol from PHBV matrices to simulant D1 than to simulant A. Likewise, Tawakkal et al., (2016) reported a greater amount of thymol released from PLA/Kenaf fibre composite matrices to food simulants with a high ethanol ratio. A greater amount of FA, PCA and protocatechuic acid was released faster from PLA-PHBV (75:25) blend films when the ethanol concentration rose in the food simulants in contact with the films (Hernández-García et al., 2022a). The release promotion from polyester films in contact with aqueous systems with a higher concentration of ethanol has been attributed to the swelling and relaxation of the polymer matrix when ethanol diffuses into the film, promoting the polymer degradation (Jamshidian et al., 2012). The increase in polymer free volume due to the ethanol penetration also promotes diffusion of water molecules into the polyester matrix, which accelerates the polymer hydrolysis (Iñiguez-Franco et al., 2016), which facilitates mass transfer in the film.

The diffusion coefficient (D) of FA and PCA through the PHBV matrix was also determined by fitting the Fick model to the experimental data (M_t/M_∞ *vs.* t), and the corresponding D values are shown in Table 1. The

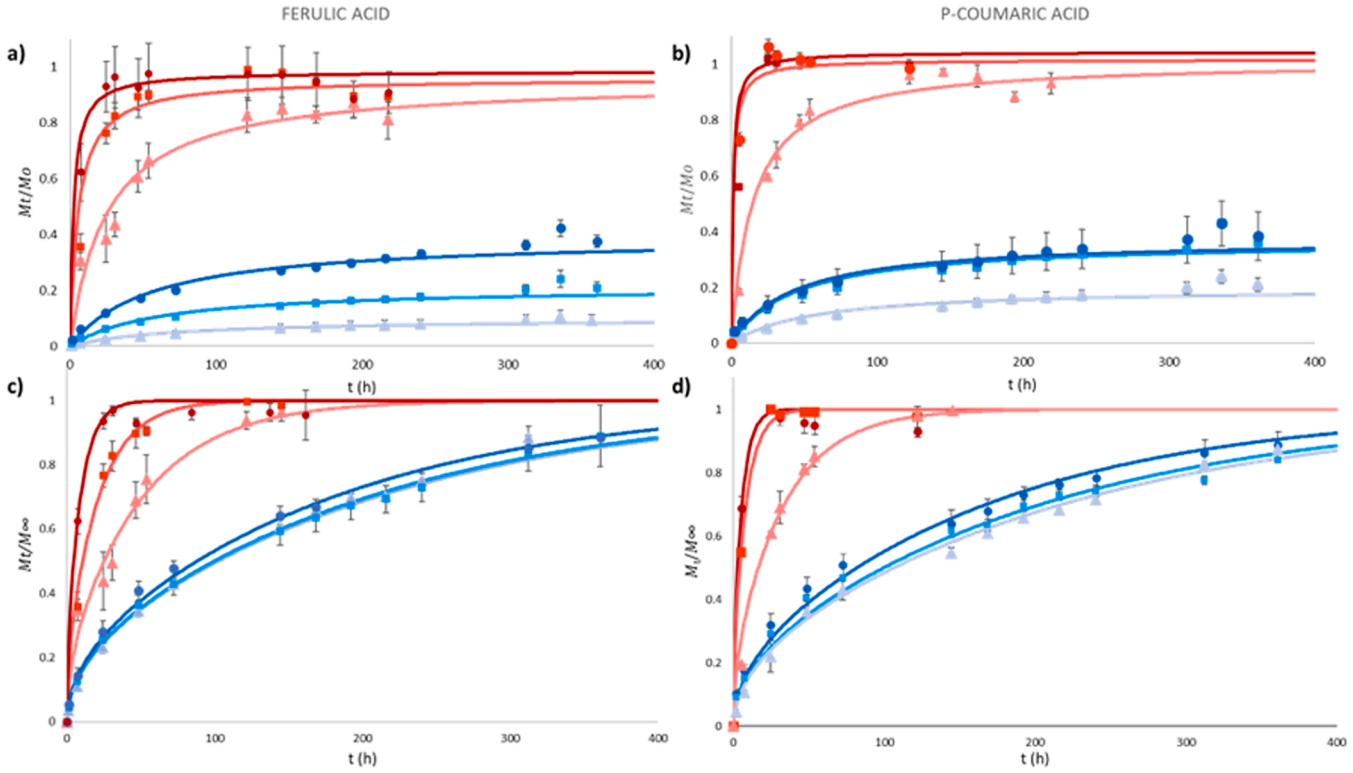

Fig. 2. Release of ferulic acid (a,c) and p-coumaric acid (b,d) as a function of contact time, expressed with respect to the initial film content (M_t/M_o) (a,b) or to the released equilibrium value (M_t/M_∞) (c,d), in simulants A (blue) and D1 (red), for the three concentrations of incorporated active compounds. Experimental values (triangle: 3%; square: 6%; circle: 9%) and fitted models (a,b: Peleg; c,d: Fick).

Table 1
Diffusion coefficient (D) and parameters of the Peleg model: initial release rate ($1/k_1$) of the active compound in the simulant and maximum release ratio (with respect to the amount incorporated in the film) of active compound in the simulant (M_∞/M_0).

	Simulant A				Simulant D1			
Formulation	$Dx10^{15}$ (m^2/s)	$(1/k_1)x10^3$ (h^{-1})	(M_∞/M_0) (%)	R^2	$Dx10^{15}$ (m^2/s)	$(1/k_1)x10^3$ (h^{-1})	(M_∞/M_0) (%)	R^2
3AF	2.9 ± 0.2^{ab}	1.4 ± 0.2^{cd}	10 ± 2^{c}	0.984	11.0 ± 2.0^{f}	43 ± 18^{c}	96 ± 9^{a}	0.983
6AF	2.8 ± 0.4^{b}	3.5 ± 0.1^{cd}	21 ± 2^{b}	0.985	27.0 ± 4.0^{d}	180 ± 40^{bc}	96 ± 2^{a}	0.993
9AF	2.7 ± 0.2^{b}	7.0 ± 1.0^{b}	39 ± 2^{a}	0.988	50.0 ± 4.0^{c}	430 ± 90^{b}	99 ± 11^{a}	0.996
3APC	2.4 ± 0.3^{b}	3.6 ± 0.3^{c}	20 ± 2^{b}	0.993	16.0 ± 1.0^{e}	69 ± 8^{c}	101 ± 3^{a}	0.990
6APC	3.4 ± 0.2^{a}	8.4 ± 0.3^{ab}	37 ± 1^{a}	0.986	82.2 ± 0.8^{b}	490 ± 170^{b}	105 ± 6^{a}	0.998
9APC	3.4 ± 0.3^{a}	9.0 ± 2.0^{a}	38 ± 7^{a}	0.980	102.0 ± 14.0^{a}	1000 ± 300^{a}	104.1^{a}	0.999

obtained *D* values for both phenolic acids were in the range of those previously reported (Requena et al. 2017) for other phenolic compounds (carvacrol and eugenol) incorporated into PHBV films, with the expected differences associated to the specific molecular structure and molecular interactions within the polymer matrix. The obtained *D* values were also affected by the and concentration of phenolic compound in the polymer matrix and the food simulant in contact with it. In general, the *D* values were higher in simulant D1 than those in simulant A and increased when the compound concentration rose in the matrix. Likewise, FA had lower values of *D* than PCA for a determined simulant and compound concentration level in the matrix. Although no effect of compound concentration would be expected on *D* values, since this was considered in the Fick's law, the different structural changes promoted by the incorporated amount of active compound into the polymer matrix could modify its mass transfer properties, affecting the molecular diffusion of the compounds.

The differences in the mass transfer behaviour of FA and PCA could be explained by different factors, such as the compound molecular mass and structure, film's microstructure and the binding forces of the compound in the matrix, the compound solubility in the simulant and the simulant-polymer interactions, which modify the polymer chain cohesion forces (*e.g.*, matrix swelling and relaxation). Both phenolic acids are more soluble in ethanol than in water, PCA being slightly more soluble than FA. Vilas-Boas et al., (2020) reported the solubility values of PCA in water at 25 °C (0.56 g/L), which were much lower than those in ethanol (188 g/L), and reported similar behaviour for FA, which had an ethanol solubility of 116 g/L and a water solubility of 0.60 g/L. Considering the maximum amount of each compound delivered to the simulants, in no case was the solubility limit of phenolic acids in water exceeded in simulant A (richer in water). Therefore, the solubility of compounds did not limit their release in the simulants but their slow diffusion through the polymer matrix. Other studies also reported very low diffusion of the other compounds encapsulated in small PHBV particles into aqueous media during the first contact period, whereas much longer times and polymer degradation are involved in the total release of the encapsulated compound (Levett et al., 2019). The slow and sustained release into aqueous media of organic molecules encapsulated in PHBV microspheres is governed by the diffusion and relaxation of the polymer chains (Grillo et al., 2011).

The effect of the simulant on the mass transfer behaviour of FA and PCA was clearly reflected in the obtained *D* values. The *D* values were between 4 and 30 times higher for simulant D1 than for simulant A, depending on the kind and concentration of phenolic acid incorporated into the polymer matrix. The increase of *D* values in simulant D1 was higher for PCA than for FA and *D* values were higher with the increased compound concentration in the film as well. This suggests that the structural changes promoted by each phenolic acid and concentration in the polymer network could affect its sensitivity to the ethanol penetration and the subsequent changes in the free volume and molecular mobility of the polymer. FA that was better integrated in the polymer by melt blending, as shown in Fig. 1, could better protect the polymer chains from swelling probably due to the formation of interchain hydrogen bonds between phenolic and carboxylic -OH groups and the oxygens of ester groups in PHBV chains. This could affect the polymer-ethanol interactions that determine the changes in the molecular mobility of the matrix in contact with the ethanol-richer simulant. PCA is more poorly integrated in the polymer matrix (as shown by the particle separation in Fig. 1) and so, more limited polymer-PCA interactions are expected, which favour its release in contact with simulants.

The differences in the release behaviour of active compounds may affect the antibacterial activity of the films. Greater activity would be expected from films with a higher concentration of phenolic acid, whereas PCA was more likely to release, always depending on the polarity of the system in contact with the films. It should be considered that what is sought in an active packaging is the release capacity of the active compound according to the needs of the system, because if it is released too quickly or too slowly, the antioxidant and antibacterial effect of the active compound may not have positive consequences for the food (Mascheroni et al., 2010). The released amount of active compound must reach the minimum inhibitory concentration of the target bacteria in order to ensure their growth inhibition (Ordoñez et al., 2022a, 2022b).

3.3. Antibacterial action of films obtained by melt blending

The antimicrobial capacity of ferulic acid and p-coumaric acid has been demonstrated for both Gram-positive and Gram-negative bacteria (Boz, 2015; Lou et al., 2012; Mitani et al., 2018; Miyague et al., 2015; Shi et al., 2016; Takahashi et al., 2013). However, the minimum inhibitory concentration (MIC) of a target bacterial strain must be reached in the medium to ensure that films can exert antimicrobial activity by inhibiting their growth. The antimicrobial capacity of the films obtained were tested with *Listeria innocua* and *Escherichia coli* (Gram--positive and Gram-negative bacteria, respectively). Fig. 3 shows the bacterial counts (log CFU) after 6 days of incubation, at 10 °C, of the plates covered with the different films containing PCA or FA, in comparison with that covered with active-free PHBV films and uncovered plates. In no case was the growth inhibition of inoculated bacteria significant with respect to the film without active compounds (PHBV), which suggests the lack of an effective release of phenolic acids into the culture medium. This could be attributed to the highly polar nature of the aqueous culture medium and the limited release of these compounds from the films in contact with polar systems, as deduced from the release kinetics analyses, carried out at higher temperature than microbial test where the release would be still more slowed.

Hernández-García et al. (2022a) reported the MIC values of the tested strains (*L. Innocua* and *E. Coli*) for FA (700 mg/L and 800 mg/L respectively) and PCA (900 mg/L and 800 mg/L, respectively). These values could not be reached in the culture media due to the limited release of FA and PCA in the aqueous media. This was corroborated by the additional release study, with 9FA and 9PCA films, in distilled water, at 10 °C for 6 days, without stirring, simulating the antibacterial *in vitro* test. Only 3.6% of the phenolics present in the films were released into water. The amount released did not exceed the water solubility of the active compounds, so the limiting factor was the diffusion of the phenolic acids from the films into the aqueous medium. Assuming this

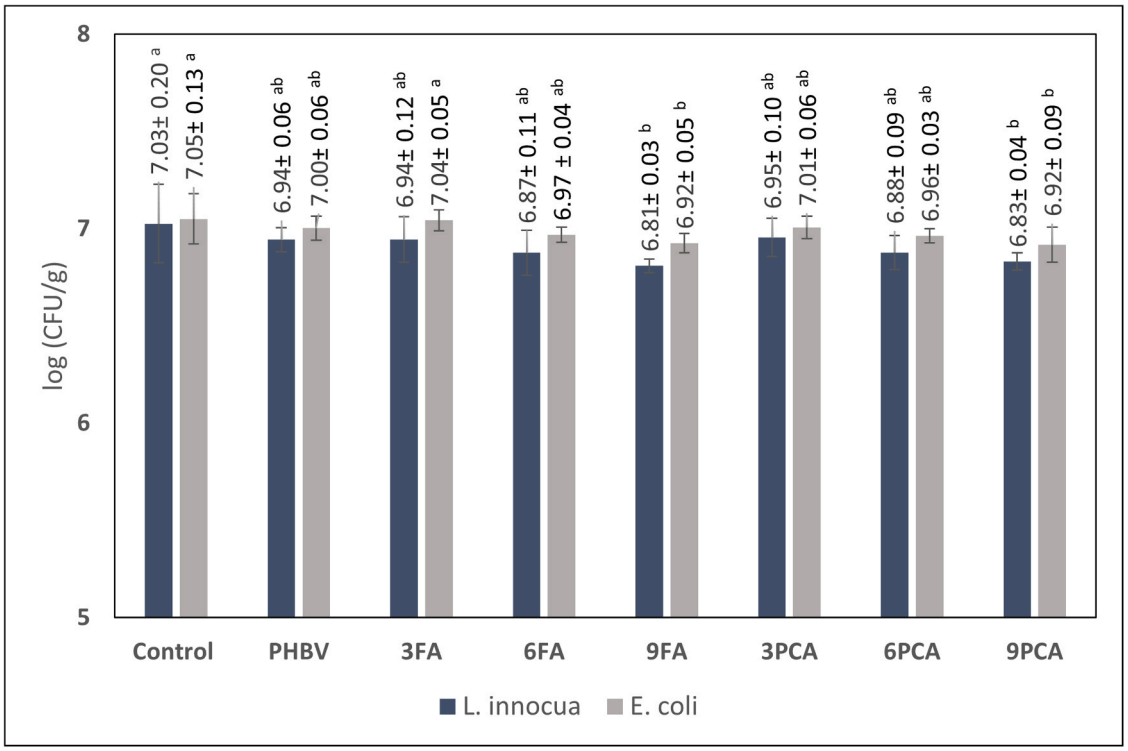

Fig. 3. Microbial counts of L. innocua and E. coli, obtained after 6 days incubation at 10 °C of the culture medium (*in vitro* test) with an initial inoculum of 10^4 CFU/ml, covered with PHBV films with 3% (3FA), 6% (6FA) or 9% (9FA) of ferulic acid, or 3% (3PCA), 6% (6PCA) and 9% (9PCA) of p-coumaric acid. Controls: PHBV films without active and uncovered plates. Mean values ± standard deviation. Different superscript letters (a-b) indicate significant differences between formulations ($p < 0.05$).

release ratio in the inoculated culture media, and taking the phenolic content of the plate-covering film into account, the respective concentrations of phenolics reached in the plate would be 82 ± 6 mg/L for FA and 105 ± 28 mg/L for PCA. Both these two values were much lower than the MIC values of both inoculated bacteria. Therefore, PHBV films with FA or PCA obtained by melt processing, tightly encapsulate the compounds in the polymer matrix, hindering their effective release in polar systems, such as the culture medium or aqueous food, thus inhibiting the antibacterial action.

3.4. Release kinetics in water and antibacterial action of surface loaded films

The surface incorporated active compounds in PHBV films were analysed both as to their ability to be released into aqueous systems and as to their antibacterial action. Surface loaded compounds are readily available, their delivery only depending on the solubilization kinetics of the crystallised compound in the medium. Fig. 4a shows the mass ratio of the released compound, with respect to the amount incorporated into the film, as a function of contact time with water, at 10 °C (simulating the culture medium). The experimental points were well fitted to Peleg's model (R^2 =0.99) to obtain the initial release rate ($1/k_1$=3.3 ± 0.5 h^{-1}

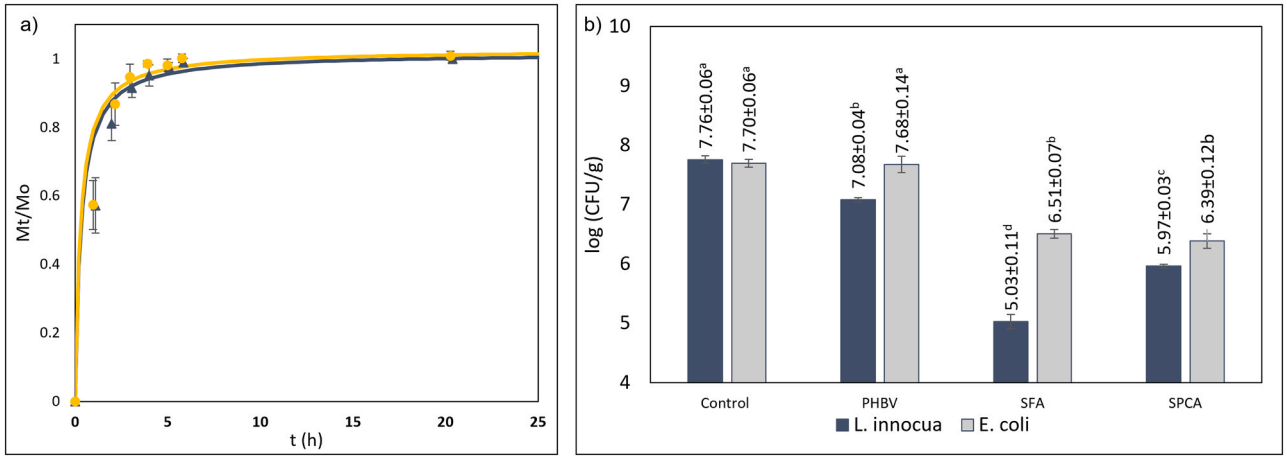

Fig. 4. a) Release kinetics of PCA and FA in aqueous media from surface loaded PHBV films, by spraying. Experimental points (triangle: FA; circle: PCA) and Peleg's fitted model b) Microbial counts of L. innocua and E. coli after 6 days of incubation at 10 °C (initial inoculum 10^4 CFU/ml). Control (without film), PHBV without active (PHBV), Mean values ± standard deviation. Different superscript letters (a-b) indicate significant differences between formulations ($p < 0.05$).

and 4.0 ± 1.2 h^{-1}, respectively for SFA and for SPCA films) and the asymptotic release ratio (M_∞/M_0=104 ± 4 (%) and 102 ± 1 (%) for SFA and SPCA films, respectively). Both compounds were almost completely released in approximately 6 h of contact time, with a similar release rate. The concentration reached for both compounds in the aqueous media was about 64.8 mg/L, which was lower than the compound's solubility in water. Similar results were obtained by Hernández-García et al. (2022b) for ferulic acid surface loaded onto PLA-PHBV (75:25) blend films by film spraying and solvent evaporation.

Fig. 4b shows the bacterial counts reached for each bacterium after 6 incubation days, at 10 °C, for plates covered with surface loaded films with FA and PCA, in comparison with plates covered with active-free PHBV films and uncovered plates. Significant bacterial growth inhibition (nearly 2 log CFU) was observed for surface loaded films with both FA and PCA, whereas PHBV films without active compounds did not provoke bacterial growth inhibition. The differences between active films were coherent with the MIC values of both compounds for the bacteria; a similar growth inhibition of *E. coli*, whose MIC values were equal for both compounds (800 mg/L), was observed; however, the antilisterial action of FA was greater than that of PCA, according to the lower MIC value of FA (700 mg/L, compared to 900 mg/L of PCA). No clear effect of the small difference in the surface compound concentration (4.6 ± 0.8 and 3.3 ± 0.8 g/100 g film, respectively for FA and PCA) was observed on the antibacterial action, which suggests that the compound solubility in the culture medium could attenuate the differences in the theoretical compound concentration that could be reached in the culture medium as a result of the different surface load. Table 2 shows the estimated theoretical active compound concentration (TAC) that would be reached in the culture medium if a complete compound delivery occurred from the corresponding covering film. For surface loaded films, the TAC values exceed the compound solubility in water (500–600 mg/L), which, in turn, are lower than the MIC values. Therefore, local overconcentration of the active compounds could explain their effective antibacterial action. All of the films obtained by melt processing could deliver a sufficient amount of active compound to exceed the bacteria MIC values, regardless of the concentration incorporated (3, 6 or 9%) into the film. However, no effective release occurred from the melt processed films and only the surface loaded films containing less than 5% of active compound led to significant antibacterial action, as shown by the respective bacterial growth inhibition obtained in the *in vitro* tests (Table 2). The surface incorporation by spraying and solvent evaporation is an easily scalable process at industrial level, but it would be necessary to protect the surface formed crystals so as to ensure the stability of the film's antibacterial action.

Therefore, the easily available phenolic acids, crystallised onto the film surface, were effective at controlling bacterial growth, whereas their incorporation into the film matrix did not produce active films for application in aqueous foods, similar to the used culture medium. The diffusion-controlled release from the melt processed polymer network was overly hindered and slow and was only enhanced when the film was in contact with less polar media that can penetrate into the matrix, relaxing the polymer chains and increasing the molecular mobility. To predict the antibacterial action of the melt blended films in food system simulated by solvents A and D1 at 20 °C, the theoretical delivered amounts from the films for 6 days of food contact were also included in the Table 2. In simulant A, only films with 9% of FA or PCA reached released concentration values near the MIC values of both bacteria. However, in simulant D1, every film would deliver enough active compound to overcome the MIC values and, then the antibacterial action of the films would be expected.

Previous studies (Hernández-García et al., 2022a; Ordoñez et al., 2022b) also observed a very limited release of the phenolic acids incorporated into melt processed polyester films in polar systems, which made them non-effective for antibacterial action. In contrast, more polar and water sensitive polymers, such as EVOH (Aragón-Gutiérrez et al., 2020) or starch (Ordoñez et al., 2021b) effectively delivered ferulic acid

Table 2
Theoretical active compound concentration (TAC, mg/L) that would be reached in the culture media if the release occurred from the covering films was total or partial according to that predicted in simulants A and D1 for 6 d contact time at 20 °C. The real growth inhibition (difference in log CFU with respect to the control sample) reached in each case is also shown.

Formulation	TAC (mg/L)			*Growth inhibition* (log *CFU/ml*)	
	Total	Simulant A	Simulant D1	*L. innocua*	*E. coli*
3FA	950	64	790	0.00 ± 0.12	0.08 ± 0.05
6FA	1600	237	1481	0.07 ± 0.12	0.03 ± 0.04
9FA	2700	759	2631	0.13 ± 0.04	-0.04 ± 0.05
SFA	1600	-	-	2.05 ± 0.12	1.17 ± 0.07
3PCA	1000	144	909	-0.01 ± 0.10	0.00 ± 0.06
6PCA	2400	680	2366	0.07 ± 0.09	0.04 ± 0.04
9PCA	3000	882	2979	0.11 ± 0.04	0.09 ± 0.09
SPCA	1200	-	-	1.12 ± 0.03	1.29 ± 0.12

in aqueous culture media, inhibiting the bacterial growth, due to the polymer swelling and relaxation in contact with the aqueous media. The surface loaded PHBV films exhibited great antibacterial activity due to the availability of the surface crystallised active compounds placed in contact with the culture media. A similar antilisterial effect was also observed for FA when surface loaded onto blend films of PLA-PHBV (75:25) (Hernández-García et al., 2022a). This activity was maintained in stored films at 25 °C and 53% relative humidity for at least 2 months, which indicates that superficially crystallised FA was stable under these storage conditions.

4. Conclusions

The release kinetics of FA or PCA incorporated into PHBV films by melt processing showed a higher release rate and ratio in less polar food simulants (D1: 50% ethanol in water) than in polar simulants (A: 10% ethanol in water). The diffusion of the phenolic compounds through the polymer matrix was hindered when in contact with the most aqueous systems when the polyester network did not swell and relax to promote mass transfer. This implied a lack of any antibacterial effect of the melt processed films, since phenolic acids were not effectively delivered in the polar culture media.

The surface loading of phenolic acids onto the PHBV films was necessary to promote both the effective compound release in the culture media and antibacterial activity. Spraying the film surface with ethanolic solutions of FA or PCA led to the compound crystallizing onto the film surface while the formed crystals solubilised in the culture media, promoting a significant antibacterial effect (near 2 log CFU growth inhibition) against *E. coli* and *L. innocua.* The compound diffusion in the culture media from the surface crystals occurred within the adequate time range so as to ensure bacterial growth inhibition for both FA and PCA. The surface protection of the formed crystals would be necessary to avoid losses caused by friction or degradation.

Therefore, FA or PCA, with antimicrobial, antioxidant and health promoting properties, could be used to produce PHBV active films, using different incorporation technologies that allow for an adequate availability of the compounds and their release into the biological media. Further studies are necessary to prove the compound's bioactivity when films are applied in the packaging of real foods both to extend their shelf-life as well as to ensure the stability of the compound in the films throughout time.

CRediT authorship contribution statement

Eva Moll: Investigation, Conceptualization, Methodology, Formal analysis, Writing – original draft. **Chelo González-Martínez:** Conceptualization, Data curation, Supervision, Project administration, Writing - review & editing. **Amparo Chiralt:** Conceptualization, Methodology, Data curation, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Eva Moll Montaner reports financial support was provided by Ministerio de Ciencia e Inovación.

Data Availability

The authors do not have permission to share data.

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References

Anderson, A. J., & Dawes, E. A. (1990). Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates. *Microbiological Reviews, 54*(4), 450–472. https://doi.org/10.1128/mr.54.4.450-472.1990

Andrade, J., González-Martínez, C., & Chiralt, A. (2022). Antimicrobial PLA-PVA multilayer films containing phenolic compounds. *Food Chemistry, 375*, Article 131861. https://doi.org/10.1016/J.FOODCHEM.2021.131861

Aragón-Gutiérrez, A., Rosa, E., Gallur, M., López, D., Hernández-Muñoz, P., & Gavara, R. (2020). Melt-Processed bioactive EVOH films incorporated with ferulic acid. *Polymers, 13*(1), 68. https://doi.org/10.3390/polym13010068

Bezerra, G. S. N., Pereira, M. A. V., Ostrosky, E. A., Barbosa, E. G., de Moura, M., de, F. V., Ferrari, M., Aragão, C. F. S., & Gomes, A. P. B. (2017). Compatibility study between ferulic acid and excipients used in cosmetic formulations by TG/DTG, DSC and FTIR. *Journal of Thermal Analysis and Calorimetry, 127*(2), 1683–1691. https://doi.org/10.1007/s10973-016-5654-9

Boz, H. (2015). Coumaric acid in cereals: presence, antioxidant and antimicrobial effects. *International Journal of Food Science & Technology, 50*(11), 2323–2328. https://doi.org/10.1111/ijfs.12898

Briassoulis, D., Tserotas, P., & Athanasoulia, I. G. (2021). Alternative optimization routes for improving the performance of poly(3-hydroxybutyrate) (PHB) based plastics. *Journal of Cleaner Production, 318*, Article 128555. https://doi.org/10.1016/J.JCLEPRO.2021.128555

Carocho, M., Barreiro, M. F., Morales, P., & Ferreira, I. C. F. R. (2014). Adding Molecules to Food, Pros and Cons: A review on synthetic and natural food additives. *Comprehensive Reviews in Food Science and Food Safety, 13*(4), 377–399. https://doi.org/10.1111/1541-4337.12065

Chen, H., Wang, C., Kang, H., Zhi, B., Haynes, C. L., Aburub, A., & Sun, C. C. (2020). Microstructures and pharmaceutical properties of ferulic acid agglomerates prepared by different spherical crystallization methods. *International Journal of Pharmaceutics, 574*, Article 118914. https://doi.org/10.1016/j.ijpharm.2019.118914

Commission Regulation (EU) 10/2011. (n.d.). 10/2011 of 14 January 2011 on Plastic Materials and Articles Intended to Come into Contact with Food.

Corre, Y. M., Bruzaud, S., Audic, J. L., & Grohens, Y. (2012). Morphology and functional properties of commercial polyhydroxyalkanoates: a comprehensive and comparative study. *Polymer Testing, 31*(2), 226–235. https://doi.org/10.1016/J.POLYMERTESTING.2011.11.002

Crank, J. (1979). *The mathematics of diffusion* (Second ed.). Oxford University Press (1979).

Dędek, K., Rosicka-Kaczmarek, J., Nebesny, E., & Kowalska, G. (2019). Characteristics and biological properties of ferulic acid. *Biotechnology and Food Science, 83*(1), 71–85.

Fabra, M. J., Lopez-Rubio, A., & Lagaron, J. M. (2014). Nanostructured interlayers of zein to improve the barrier properties of high barrier polyhydroxyalkanoates and other polyesters. *Journal of Food Engineering, 127*, 1–9. https://doi.org/10.1016/J.JFOODENG.2013.11.022

Grillo, R., Pereira, A., do, E. S., de Melo, N. F. S., Porto, R. M., Feitosa, L. O., Tonello, P. S., Filho, N. L. D., Rosa, A. H., Lima, R., & Fraceto, L. F. (2011). Controlled release system for ametryn using polymer microspheres: Preparation, characterization and release kinetics in water. *Journal of Hazardous Materials, 186*(2–3), 1645–1651. https://doi.org/10.1016/J.JHAZMAT.2010.12.044

Hale, R. C., Seeley, M. E., Guardia, M. J., la, Mai, L., & Zeng, E. Y. (2020). A global perspective on microplastics. *JGR: Oceans, 125*(1). https://doi.org/10.1029/2018JC014719

Hernández-García, E., Vargas, M., & Chiralt, A. (2022a). Effect of active phenolic acids on properties of PLA-PHBV blend films. *Food Packaging and Shelf Life, 33*, Article 100894. https://doi.org/10.1016/j.fpsl.2022.100894

Hernández-García, E., Vargas, M., & Chiralt, A. (2022b). Active starch-polyester bilayer films with surface-incorporated ferulic acid. *Membranes, 12*(10), 976. https://doi.org/10.3390/membranes12100976

Iñiguez-Franco, F., Auras, R., Burgess, G., Holmes, D., Fang, X., Rubino, M., & Soto-Valdez, H. (2016). Concurrent solvent induced crystallization and hydrolytic degradation of PLA by water-ethanol solutions. *Polymer, 99*, 315–323. https://doi.org/10.1016/J.POLYMER.2016.07.018

Jamshidian, M., Tehrany, E. A., & Desobry, S. (2012). Release of synthetic phenolic antioxidants from extruded poly lactic acid (PLA) film. *Food Control, 28*(2), 445–455. https://doi.org/10.1016/J.FOODCONT.2012.05.005

Jost, V., & Langowski, H. C. (2015). Effect of different plasticisers on the mechanical and barrier properties of extruded cast PHBV films. *European Polymer Journal, 68*, 302–312. https://doi.org/10.1016/J.EURPOLYMJ.2015.04.012

Koller, M., Maršálek, L., de Sousa Dias, M. M., & Braunegg, G. (2017). Producing microbial polyhydroxyalkanoate (PHA) biopolyesters in a sustainable manner. *New Biotechnology, 37*, 24–38. https://doi.org/10.1016/j.nbt.2016.05.001

Laycock, B., Arcos-Hernandez, M. v, Langford, A., Pratt, S., Werker, A., Halley, P. J., & Lant, P. A. (2014). Crystallisation and fractionation of selected polyhydroxyalkanoates produced from mixed cultures. *New Biotechnology, 31*(4), 345–356. https://doi.org/10.1016/j.nbt.2013.05.005

Levett, I., Pratt, S., Donose, B. C., Brackin, R., Pratt, C., Redding, M., & Laycock, B. (2019). Understanding the mobilization of a nitrification inhibitor from novel slow release pellets, fabricated through extrusion processing with PHBV biopolymer. *Journal of Agricultural and Food Chemistry, 67*(9), 2449–2458. https://doi.org/10.1021/acs.jafc.8b05709

Li, Z., Yang, J., & Loh, X. J. (2016). Polyhydroxyalkanoates: opening doors for a sustainable future. *NPG Asia Materials, 8*, 4.

Lima, M. C., Paiva de Sousa, C., Fernandez-Prada, C., Harel, J., Dubreuil, J. D., & de Souza, E. L. (2019). A review of the current evidence of fruit phenolic compounds as potential antimicrobials against pathogenic bacteria. *Microbial Pathogenesis, 130*, 259–270. https://doi.org/10.1016/j.micpath.2019.03.025

Lou, Z., Wang, H., Rao, S., Sun, J., Ma, C., & Li, J. (2012). p-Coumaric acid kills bacteria through dual damage mechanisms. *Food Control, 25*(2), 550–554. https://doi.org/10.1016/j.foodcont.2011.11.022

Mascheroni, E., Guillard, V., Nalin, F., Mora, L., & Piergiovanni, L. (2010). Diffusivity of propolis compounds in Polylactic acid polymer for the development of anti-microbial packaging films. *Journal of Food Engineering, 98*(3), 294–301. https://doi.org/10.1016/j.jfoodeng.2009.12.028

Mitani, T., Ota, K., Inaba, N., Kishida, K., & Koyama, H. A. (2018). Antimicrobial activity of the phenolic compounds of Prunus mume against enterobacteria. *Biological and Pharmaceutical Bulletin, 41*(2), 208–212. https://doi.org/10.1248/bpb.b17-00711

Miyague, L., Macedo, R. E. F., Meca, G., Holley, R. A., & Luciano, F. B. (2015). Combination of phenolic acids and essential oils against Listeria monocytogenes. *LWT - Food Science and Technology, 64*(1), 333–336. https://doi.org/10.1016/j.lwt.2015.05.055

Modi, S., Koelling, K., & Vodovotz, Y. (2011). Assessment of PHB with varying hydroxyvalerate content for potential packaging applications. *European Polymer Journal, 47*(2), 179–186. https://doi.org/10.1016/j.eurpolymj.2010.11.010

Muller, J., González-Martínez, C., & Chiralt, A. (2017). materials combination of Poly (lactic) acid and starch for biodegradable food packaging. *Materials*. https://doi.org/10.3390/ma10080952

Ordoñez, R., Atarés, L., & Chiralt, A. (2021a). Physicochemical and antimicrobial properties of cassava starch films with ferulic or cinnamic acid. *LWT, 144*, Article 111242. https://doi.org/10.1016/J.LWT.2021.111242

Ordoñez, R., Atarés, L., & Chiralt, A. (2021b). Physicochemical and antimicrobial properties of cassava starch films with ferulic or cinnamic acid. *LWT, 144*, Article 111242. https://doi.org/10.1016/J.LWT.2021.111242

Ordoñez, R., Atarés, L., & Chiralt, A. (2022a). Biodegradable active materials containing phenolic acids for food packaging applications. *Comprehensive Reviews in Food Science and Food Safety, 21*(5), 3910–3930. https://doi.org/10.1111/1541-4337.13011

Ordoñez, R., Atarés, L., & Chiralt, A. (2022b). Antilisterial action of PLA films with ferulic acid as affected by the method of incorporation. *Food Bioscience, 49*, Article 101865. https://doi.org/10.1016/j.fbio.2022.101865

Peelman, N., Ragaert, P., de Meulenaer, B., Adons, D., Peeters, R., Cardon, L., van Impe, F., & Devlieghere, F. (2013). Application of bioplastics for food packaging. *Trends in Food Science & Technology, 32*(2), 128–141.

Peleg, M. (1988). An empirical model for the description of moisture sorption curves. *Journal of Food Science, 53*(4). https://doi.org/10.1111/j.1365-2621.1988.tb13565.x

Pernin, A., Bosc, V., Maillard, M.-N., & Dubois-Brissonnet, F. (2019). Ferulic acid and eugenol have different abilities to maintain their inhibitory activity against listeria monocytogenes in emulsified systems. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.00137

Requena, R., Vargas, M., & Chiralt, A. (2017). Release kinetics of carvacrol and eugenol from poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) films for food packaging applications. *European Polymer Journal, 92*, 185–193. https://doi.org/10.1016/J.EURPOLYMJ.2017.05.008

Requena, R., Vargas, M., & Chiralt, A. (2019). Eugenol and carvacrol migration from PHBV films and antibacterial action in different food matrices. *Food Chemistry, 277*, 38–45. https://doi.org/10.1016/J.FOODCHEM.2018.10.093

Shi, C., Zhang, X., Sun, Y., Yang, M., Song, K., Zheng, Z., Chen, Y., Liu, X., Jia, Z., Dong, R., Cui, L., & Xia, X. (2016). Antimicrobial activity of ferulic acid against cronobacter sakazakii and possible mechanism of action. *Foodborne Pathogens and Disease, 13*(4), 196–204. https://doi.org/10.1089/fpd.2015.1992

Siracusa, V., Rocculi, P., Romani, S., & Rosa, M. D. (2008). Biodegradable polymers for food packaging: a review. *Trends in Food Science & Technology, 19*(12), 634–643.

Takahashi, H., Kashimura, M., Koiso, H., Kuda, T., & Kimura, B. (2013). Use of ferulic acid as a novel candidate of growth inhibiting agent against Listeria monocytogenes in ready-to-eat food. *Food Control, 33*(1), 244–248. https://doi.org/10.1016/j.foodcont.2013.03.013

Tampau, A., González-Martínez, C., & Chiralt, A. (2018). Release kinetics and antimicrobial properties of carvacrol encapsulated in electrospun poly-(ε-caprolactone) nanofibres. Application in starch multilayer films. *Food Hydrocolloids, 79*, 158–169. https://doi.org/10.1016/J.FOODHYD.2017.12.021

Tawakkal, I. S. M. A., Cran, M. J., & Bigger, S. W. (2016). Release of thymol from poly (lactic acid)-based antimicrobial films containing kenaf fibres as natural filler. *LWT - Food Science and Technology, 66*, 629–637. https://doi.org/10.1016/J.LWT.2015.11.011

Tebaldi, M. L., Maia, A. L. C., Poletto, F., de Andrade, F. V., & Soares, D. C. F. (2019). Poly(-3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV): Current advances in synthesis methodologies, antitumor applications and biocompatibility. *Journal of Drug Delivery Science and Technology, 51*, 115–126. https://doi.org/10.1016/j.jddst.2019.02.007

Useyin Boz, H. (2015). *Review p-Coumaric acid in cereals: presence, antioxidant and antimicrobial effects*. https://doi.org/10.1111/ijfs.12898.

Utsumi, H., Fujii, K., Irie, H., Furusaki, A., & Nitta, I. (1967). The crystal structure of p-coumaric acid. *Bulletin of the Chemical Society of Japan, 40*(2). https://doi.org/10.1246/bcsj.40.426, 426–426.

Vert, M., Doi, Y., Hellwich, K.-H., Hess, M., Hodge, P., Kubisa, P., Rinaudo, M., & Schué, F. (2012). Terminology for biorelated polymers and applications (IUPAC recommendations 2012). *Pure and Applied Chemistry, 84*(2), 377–410.

Vilas-Boas, S. M., Alves, R. S., Brandão, P., Campos, L. M. A., Coutinho, J. A. P., Pinho, S. P., & Ferreira, O. (2020). Solid-liquid phase equilibrium of trans-cinnamic acid, p-coumaric acid and ferulic acid in water and organic solvents: experimental and modelling studies. *Fluid Phase Equilibria, 521*, Article 112747. https://doi.org/10.1016/J.FLUID.2020.112747

Wikström, F., Verghese, K., Auras, R., Olsson, A., Williams, H., Wever, R., Grönman, K., Pettersen, M.K., Møller, H., Soukka, R. (2018). *Packaging Strategies That Save Food A Research Agenda for 2030*. https://doi.org/10.1111/jiec.12769.