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Additional Information

Antimicrobial properties and release of cinnamaldehyde in bilayer films based on polylactic acid (PLA) and starch

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ABSTRACT

Cinnamaldehyde (CIN) loaded amorphous PLA films were obtained by casting, using ethyl acetate as solvent. Likewise, bilayer films were obtained by thermocompression of the PLA active layer and compression moulded cassava starch (S) films or semi crystalline PLA films. Starch-PLA laminated materials were considered to improve the barrier capacity (high oxygen barrier through the starch layer and high water vapour capacity through the polyester layer), while CIN incorporation confers antimicrobial activity on the films. The PLA bilayers were obtained for comparison purposes. The antimicrobial activity of the CIN loaded PLA films and S bilayer films was proved against *Escherichia coli* and *Listeria Innocua* through *in vitro* tests, which indicates that the active amount released into the growth medium exceeded the minimum inhibitory concentration (MIC) of both bacteria. The release kinetics of the active compound in different food simulants demonstrated that a part of CIN was tightly bonded to the PLA matrix, whereas the free compound diffused more easily through the starch layer, making S bilayers more active against the bacteria when the starch layer was in direct contact with the culture medium. CIN entrapped in PLA bilayers did not exhibited any antibacterial effect due to its release inhibition, associated to its bonding within the PLA matrix and the lower degree of relaxation in the semi crystalline PLA layer in contact with the food simulants.

Key words: PLA, cassava starch, cinnamaldehyde, compression moulding, antimicrobial activity, release kinetics.

INTRODUCTION

Antimicrobial biodegradable packaging materials are of great interest for the food industry and consumers not only as a means of enhancing food preservation and shelf life extension but also for the purposes of reducing the environmental problems caused by synthetic plastic packaging. Natural, non-toxic antimicrobials were also required in order to enhance the product health properties, avoiding the toxicity of other antimicrobials. Therefore, the development of biodegradable antimicrobial

materials for food packaging applications, using natural antimicrobial substances, such as essential oils or their active compounds, is a current alternative for food preservation and shelf life extension [1]; [2].

Cinnamaldehyde, one of the major constituents of cinnamon bark oil (~ 60-90 %) [3]; [4] is a phenolic terpenoid classified as GRAS (Generally Recognized As Safe) by the FDA (Food and Drug Administration), with high antibacterial, antifungal, antiinflammatory and antioxidant activity. It is widely used in cosmetic, food and pharmaceutical industries since it is a natural antimicrobial substance [5]; [6]; [7]. Different studies analysed the effects of CIN on the bacterial membranes, showing that CIN permeabilizes the internal membranes of different bacteria, such as *E. coli* and *Staphylococcus aureus*, [8] or *Staphylococcus epidermidis* and *Enterococcus* [9], altering their structure. It has also been reported that CIN is an inhibitor of β -(1,3) glucan synthase and quitin synthase 1, which are important during the synthesis of enzymes in the cell wall of yeasts and moulds [10].

Several studies have demonstrated that CIN could be potentially used, in different concentrations, as an effective antimicrobial agent. This was observed for *E. coli* O157: H7 in apple juice and cider [11], for *Enterobacter sakazakii* in reconstituted milks for breastfed babies [12], for *E. coli* in bovine meat pasties [13], for *Salmonella spp* in tomato washing (alternative to sodium hypochlorite) [14] or for a mix of strains of bacteriophage and *E. coli* O157:H7 in [green-leafy greened](#) vegetables [15]. Cinnamon essential oil or CIN also caused the inhibition of the growth of psychotropic strains of *Bacillus cereus* (EPSO-35As y el INRA TZ415) in minimally processed products, such as carrot [16]. CIN has also been incorporated into different polymer matrices to obtain antimicrobial packaging materials or food coatings. Polymers, such as cellulose [17], pectin [18], PLA [19]; [20]; [21], starch [22], proteins [23] or alginates [24], have been used as CIN carriers. Studies about the antimicrobial effect of the active films, *in vitro* as well as in food matrices, showed antimicrobial activity against different microorganisms. Nevertheless, the antimicrobial action depended not only on the active compound and microorganism, but also on the effective release of antimicrobial compounds onto the contaminated product where microbial growth occurs.

Both thermoplastic starch and PLA are biodegradable polymers with easy availability and relatively low cost whose bilayer combination could enhance the film functional properties, taking advantage of their complementary barrier capacity. Starch exhibits very good barrier capacity to oxygen [25] and PLA to water vapour [26]. Likewise, mechanical resistance of PLA is higher than that of starch, while being more brittle, and their combination could improve the film mechanical behaviour [24]. On the other hand, due to its highly hydrophilic nature, starch film properties greatly change in contact with high moisture foods and its application onto a more hydrophobic layer, such as a PLA layer, could improve its performance as a food coating. Sanyang et al. [27] characterized sugar palm starch/PLA bilayers obtained by casting, and found that the starch/PLA bilayers exhibit reduced water vapour permeability and enhanced mechanical properties in comparison with starch films. The incorporation of an active compound into starch/PLA bilayers could, moreover allow an active, biodegradable food packaging material with optimised functional properties to be obtained.

The objective of this study was to obtain bilayer films with PLA and starch, incorporating cinnamaldehyde as antimicrobial compound into the PLA layer prepared by casting, analysing both the release kinetics of CIN in different food simulants and the *in vitro* antimicrobial properties against Gram+ (*E. coli*) and Gram- (*L. innocua*) bacteria. For comparison purposes, the study of these properties in CIN loaded monolayers and PLA-CIN loaded PLA bilayers was also carried out.

MATERIALS AND METHODS

1. Materials

PLA resins: Crystalline PLA LL700, density of 1.25 g/cm³, purchased from Ercros (Spain) and amorphous PLA 4060D, density of 1.24 g/cm³, for heat seal layer in coextruded oriented films, purchased from Natureworks (U.S.A) were used to obtain films by compression moulding and casting, respectively. Cassava starch was produced by Asia CO., LDT (Kalasin, Thailand) and purchased from Quimidroga S.A. (Barcelona, Spain) and glycerol, used as a plasticizer for starch, was provided by Panreac Química SLU (Castellar del Vallés, Barcelona, España). Ethyl acetate, used as solvent for amorphous PLA, was obtained from Indukern S.A. (Barcelona, Spain) and trans-cinnamaldehyde and Poly(ethylene glycol) (PEG 4000 g/mol) were provided by Sigma-Aldrich (Madrid, Spain). Magnesium nitrate (Mg(NO₃)₂), used to reach a relative humidity (RH) of 53% for starch films, was supplied by Vidra Foc S.A. (Barcelona, Spain) and phosphorus pentoxide (P₂O₅), used to generate a 0% RH, was purchased from Panreac (Barcelona). As regards the quantification and kinetics release of cinnamaldehyde, pure methanol, ethanol, acetic acid and isooctane, UV-IR-HPLC quality, were obtained from Panreac (Barcelona, Spain). For the microbiological analysis, stock cultures of *Listeria innocua* (CECT 910) and *Escherichia coli* (CECT 101) were purchased from the *Colección Española de cultivos tipo* (CECT; Valencia, España) and culture medias: Tryptic Soy Broth (TSB), Trypcasein Soy Agar (TSA), buffered peptone Water ISO were supplied by Scharlau (Barcelona, España).

2. Film preparation

Monolayer films of starch and PLA, with or without cinnamaldehyde, were obtained and combined to obtain S-PLA and PLA-PLA bilayer films.

2.1. PLA monolayers obtained by casting method (M and M_{CIN})

To obtain active PLA monolayers with CIN, amorphous PLA pellets were used. Films were prepared by means of the solvent casting procedure after the preparation of film forming dispersions. PLA pellets were first dried at 60°C overnight to remove residual water and then dissolved in ethyl acetate at 10% (w/w), for 4 hours while being stirred. CIN-free layers (M) were obtained as control films and, in the case of active layers (M_{CIN}), CIN was dispersed at 2.5%, which means a PLA:CIN ratio of 10:2.5. The resulting dispersions were poured into Teflon plates of 15 cm diameter at a surface density of solids of 1.5 g/plate and the solvent was evaporated overnight. Consequently, dried films were peeled off the casting surface and stored in a

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desiccator with silica gel (SiO_2) at room temperature until further analysis or use to obtain bilayer films. Then, a nominal concentration of CIN of 20 g/100 g of dried film can be assumed.

2.2. PLA monolayers obtained by compression moulding (PLA)

Crystalline PLA pellets were first dried at 60°C overnight and then hot-mixed in a two-roll mill (Model LRM-M-100, Labtech Engineering, Thailand) at 200°C and 10 rpm for 10 min. The resulting paste was then compression moulded in a hydraulic press (Model LP20, Labtech Engineering, Thailand). 3 g of sample were placed onto steel sheets and pre-heated at 200°C for 4 min. Then, compression was performed at 200°C and 100 bars for 4 min, followed by a cooling cycle of 3 min until the temperature reached about 70°C. The obtained films were maintained in a desiccator with SiO_2 at room temperature until their use to obtain bilayer films.

2.3. Cassava starch monolayers obtained by compression moulding (S)

Starch was first hand mixed with glycerol (30 g/100 g of starch), water (55 g/100 g of starch) and 0,5 g of PEG. The dispersion was then hot-mixed in a two-roll mill at 160°C at 10 rpm for 25 min. The resulting thermoplastic starch was stored in a desiccator with an oversaturated solution of $\text{Mg}(\text{NO}_3)_2$ at room temperature for 10 days, in order to reach a 53 % HRH. S films were then obtained the same way as compression moulded PLA films. 4 g of sample were placed onto steel sheets and pre-heated in a hydraulic press at 160°C for 1 min. Then, compression was performed at the same temperature and 50 bars for 2 min, then 100 bars for 6 min, followed by a cooling cycle of 3 min until the temperature reached about 70°C. The obtained films were maintained in a desiccator with an oversaturated solution of $\text{Mg}(\text{NO}_3)_2$ at room temperature until their use to obtain bilayer films.

2.4. Cassava starch (S-M, S-M_{cin}) and PLA bilayer films (PLA-M, PLA-M_{cin})

Bilayer films were obtained through thermocompression of the different monolayers. Either compression moulded PLA or S films were hot pressed with PLA films obtained by casting method with and without CIN. Films were hot pressed at 160°C and 100 bars for 3 min and cooled down until reaching a temperature of 70°C. Films were maintained in a desiccator with SiO_2 at room temperature until analyses were carried out.

3. Film characterization

3.1. Concentration of cinnamaldehyde in the films

Samples containing CIN (M_{cin} , S-M_{cin} and $\text{PLA-M}_{\text{cin}}$) were analysed in-as to their content by methanol extraction and spectrophotometric determination. Square samples of each film (about 1 cm side) were weighed and extracted in 100 mL of methanol under constant stirring for 24 h (previously determined maximum time of extraction previously determined) at 20°C. Then, absorbance was measured using a UV-visible spectrophotometer (Thermo Scientific Evolution 201, EEUU) at a wavelength of 286 nm (maximum of absorption of the cinnamaldehyde in methanol). The extract of the corresponding control film without cinnamaldehyde was used as a blank in each case. The calibration curve was obtained

from the absorbance measurements of standard solutions of cinnamaldehyde and was used to determine the concentration of cinnamaldehyde in the films.

3.2. *In vitro* antimicrobial activity of the films

L. innocua and *E. coli*, provided with 30 % glycerol, were regenerated in Tryptic Soy Broth (TSB) and were incubated at 37°C for 24 h. A 10-μL aliquot from this culture was then transferred to 10 mL of TSB and grown again at 37°C for 24 h. 10 g of TSA were poured into petri dishes of 5.5 cm diameter and used as a model solid food system (TSA-NaCl). After the culture medium solidified, 100 μL of the appropriate dilution of the TSB culture was inoculated on the surface of the agar plate in order to obtain an inoculum of 10⁷CFU/g. Then, circles of the different active films (5.5 cm diameter) sterilized with UV light for 15 min, were placed in contact with the agar. The corresponding films without CIN were used as control samples. The plates were incubated at 10°C for 13 days. Inoculum controls (C) with no film were also analysed. The bacterial counts of each sample were carried out in duplicate at 0, 2, 6, 9 and 13 days. To this end, 10 g of sample were diluted in 90 mL of peptone water and were homogenized in a stomacher (IUL, Spain) for 3 min. Serial dilutions were then made and 1 mL of each adequate dilution was inoculated in petri dishes with 15 mL of TSA. These plates were incubated at 37°C for 24 h. In the case of the bilayer films, the antimicrobial test was carried out by placing both sides of the film in contact with the bacteria (for example S-M_{cin} or M_{cin}-S for the starch or active layer in contact with the bacteria, respectively). All the manipulations were made in a sterile environment underneath a laminar flow cabinet (Telstar, Bio II Advance, Barcelona, España).

3.3. Release kinetics of cinnamaldehyde in food simulants

The release kinetics of the active compound from the films into four food simulants was analysed according to the Commission regulation (EU) 2015/174 amending and correcting Regulation (EU) No 10/2011 [28] on plastic materials and articles intended to come into contact with food. The four simulants were 10 % ethanol (v/v) (A), 3 % acetic acid (w/v) (B), 50 % ethanol (v/v) (D1) and isooctane (D2). Film samples (from 50 to 500 mg depending on both film and food simulant) were cut, weighed and placed in contact with 100 mL of each simulant at 20°C, under constant stirring. The absorbance of the solutions was measured throughout time by means of spectrophotometry at the wavelength corresponding to the maximum of absorption of cinnamaldehyde in each simulant (between 280 and 286 nm, depending on the solvent). The calibration curves were obtained in each simulant. Each film sample was analysed in triplicate and the corresponding films without cinnamaldehyde, submitted to the same release process, were used to obtain the blank solution for the absorbance measurements. The amount of CIN delivered at each time (M_t) was fitted to Peleg's model (1980) (equation 1), and the parameters k₁ (inverse of the initial release rate) and k₂ (inverse of the asymptotic release) were obtained. The delivered amount at the equilibrium (M_∞) was deduced from k₂ (equation 2).

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

$$M_{\infty} = \frac{1}{k_2} \quad (2)$$

Fick's second law was considered to model the diffusion process of CIN from the films towards the food simulants. Film samples can be considered as infinite plane sheets where the active compound diffuses only in an axial direction, with the characteristic half thickness characteristic-dimension. The diffusional long-time equation for an infinite plane sheet [29] with ten terms was used to determine the values of diffusion coefficient (D) of CIN into the different solvents (equation 3), by using the Solver tool (Microsoft Excel 2016®) to optimize the D values, by minimizing the Sum of Squared Errors (SSE), and considering the following boundary conditions:

$$\begin{array}{lll} t = 0 & 0 < x < L & c = c_0 \\ t > 0 & x = 0 & x = L & c = 0 \end{array}$$

$$M_t = M_{\infty} \left(\frac{8}{\pi^2} \sum_{n=0}^{\infty} \left[\frac{1}{(2n+1)^2} \exp \left(\frac{-n^2 D (2n+1)^2 t}{L^2} \right) \right] \right) \quad (3)$$

where:

M_t is the mass of compound released at time t

M_{∞} is the mass of compound released at equilibrium

L : half thickness of film

The thickness of films was measured at six random positions using a hand-held digital micrometer (Electronic Digital Micrometer, Comecta SA, Barcelona, Spain).

3.4. Statistical analysis

Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, MD, U.S.A.) was used to carry out a statistical analysis of data through an analysis of variance (ANOVA). Fisher's least significant difference (LSD) was used at the 95 % level.

RESULTS AND DISCUSSION

Table 1 gives the values of the concentration of CIN in the active films, both the nominal value and that quantified through extraction with methanol. The surface concentration was also shown, this being equivalent from to the nominal value, but greatly reduced when the extracted amount was considered. The extraction method gave values below those corresponding to the quantity incorporated into the PLA monolayer (11.7 g against 20 g per 100 g of film). Nevertheless, the final weight of the monolayers did not reflect notable losses of CIN by evaporation, taking into account the solid mass of the casted film forming solution for each film. Then, Nevertheless, the 58.5 % extraction with methanol should only correspond to the extractable compound from PLA, whereas a part of CIN could remained bonded to the polymer matrix, despite that fact that the compound solubility in methanol was not overcome exceeded. The extractable amount in the bilayer films was also lower than the theoretical one, which was estimated

from the mass fraction of each layer in the laminated film (33.7 and 53.4 % for S bilayer and PLA bilayer films, respectively). ~~In that case, a significant difference between the weight of the films before and after the thermocompression process was observed, especially for the starch bilayers.~~ The ~~W~~weight losses ~~of in~~ the starch bilayer and PLA bilayer films during thermocompression were 28.5 and 4.4 %, respectively, which could be associated ~~to with~~ the water loss ~~of in~~ the S layer and ~~the~~ CIN losses in both cases. During ~~the~~ heating and compression of the bilayers, diffusion of the compound could occur to the CIN-free layer. Likewise, the polymer radial flow could imply ~~a~~ lower concentration of the compound per surface unit. The radial flow was especially relevant in ~~the~~ starch layer, which in turn dehydrates, promoting a steam drag effect of the CIN. On the other hand, the compound diffusion to the CIN-free PLA layer could imply ~~aided~~ ~~tigh~~higher bonding of this active to the PLA chains, reducing the ~~amount of available~~ compound ~~available~~ to be effectively released ~~into~~ the solvent. All of these factors could greatly reduce the CIN methanol extraction. Nevertheless, ~~it could be assumed that the bilayer films have a~~ higher contents of CIN than ~~that~~ obtained from the methanol extraction ~~could be assumed in the bilayer films~~, although the CIN-polymer interactions could affect its deliver capacity ~~into~~ the medium and, thus, its antimicrobial potential.

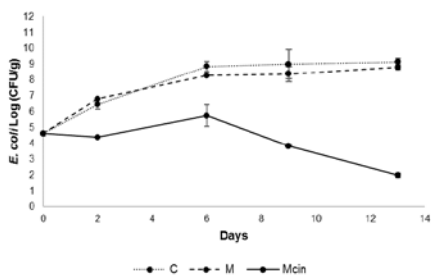
Table 1. Film thickness, concentrations (C) of theoretical and extractable CIN in mono (M_{cin}) and bilayer films with starch (S- M_{cin}) and PLA (PLA- M_{cin}) and percentage of CIN extraction in methanol.

Film	Thickness (μm)	Theoretical C		Extractable C		Extraction %
		(g/100 g of film)	(mg/cm ²)	(g/100 g of film)	(mg/cm ²)	
M_{cin}	77 \pm 13	20	1.7	11.7 \pm 0.8	1.0	58.5
S- M_{cin}	183 \pm 12	8.1	1.7	2.7 \pm 0.2	0.6	33.7
PLA- M_{cin}	188 \pm 13	7.6	1.7	4.0 \pm 0.1	0.9	53.4

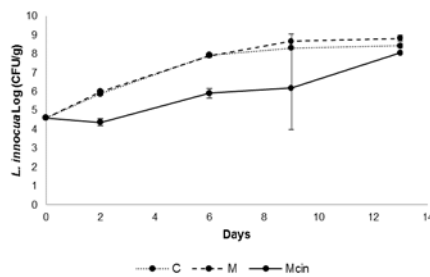
Antimicrobial properties of the films

The ~~A~~antimicrobial properties of the monolayer and bilayer films were analysed against Gram+ (*L. innocua*) and Gram- bacteria (*E. coli*). In the case of bilayer films, the film contact with the agar plate containing the bacteria was through both ~~the~~ PLA active layer with CIN and ~~the~~ PLA or S layer, ~~into~~ which CIN was not directly incorporated, but where the compound diffusion could have occurred. Figure 1 shows microbial counts for *E. coli* ~~and~~ *L. innocua* for the different samples and controls (the corresponding films without CIN (M, M-S and M-PLA) and the uncoated plate (C)) as a function of incubation time. For monolayer films with CIN (M_{cin}) applied on plates with *E. coli* (a), a bacteriostatic effect was observed during the first 6 days, in comparison with the controls; ~~however, whereas,~~ from 6 days ~~onwards,~~ a bactericidal effect occurred, with a reduction of nearly 4 Log (CFU/g) with respect to the initial counts at 13 days of incubation. In the case of *L. innocua*, an inhibition of the growth of the bacteria was observed during the first 9 days but afterwards, very similar counts to those of the control were obtained (b). That could be explained by the progressive evaporation of the active compound during the incubation time, reaching levels lower than the minimum inhibitory concentration (MIC) for ~~this bacterium,~~ this bacteria, or also to the adaptation of the bacteria, becoming less sensitive to the active agent.

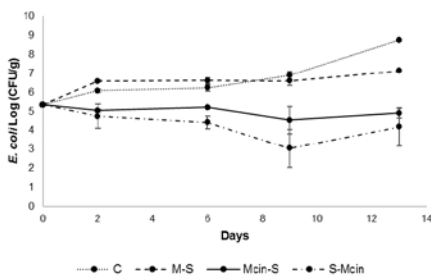
In the case of the S bilayer films, a growth inhibition of both bacteria (c-d) was observed throughout the incubation time, even when films were in contact with from the starch layer (S-M_{cin}) where no active was incorporated. This indicates that CIN effectively diffuses from the PLA to the starch layer and then through the starch layer to the agar medium. The latter was probably enhanced by the swelling of the S layer swelling in contact with the wet medium, which favours the CIN migration to the plate surface and resulted in greater antimicrobial effectiveness. In fact, for *E. coli*, a bactericidal effect was observed throughout the incubation time when the starch layer was in contact with the medium (S-M_{cin}), whereas the active compound had a bacteriostatic effect when the contact occurred through the M_{cin} layer (c). Coherent behaviour was observed for *L. innocua*, where the S layer contact also conferred greater antibacterial effect than that with the M_{cin} layer. In the last case, cells growth was observed at the end of the incubation time, as observed for the M_{cin} monolayer. This could be associated to with an insufficient release of CIN from the PLA layer, which allows the bacterial adaptation, recovering the growth capacity at long contact times. This was also confirmed by with the counts obtained for PLA bilayers, where no growth inhibition of either bacteria was observed for either contact types, the PLA or M_{cin} layer (e-f). The lack of antibacterial activity when the M_{cin} layer was in contact with the agar medium, despite the initial load of CIN, could be explained by the CIN diffusion and partition between the two PLA layers and its binding to the polymer matrix with the subsequent loss of availability for the antibacterial action.



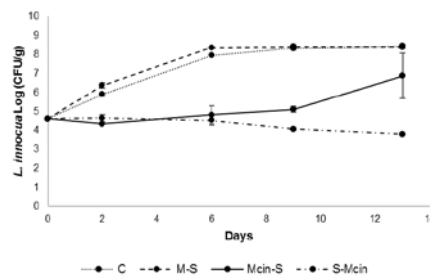
a



b



c



d

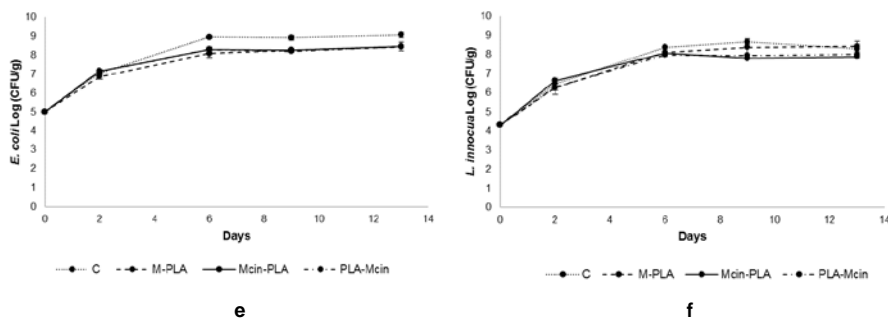


Figure 1. Microbial growth at the different incubation times for *E. coli* and *L. innocua* in samples with monolayer PLA films (a-b) and bilayer films with S (c-d) and PLA (e-f), in comparison with their respective control films without CIN and with the inoculum controls (C). For the bilayer films, count values are given for the culture medium in contact with the starch layer (S-M_{cin} data), compression moulded PLA layer (PLA-M_{cin} data) and CIN-loaded PLA layer (M_{cin}-S or M_{cin}-PLA data).

The results obtained results suggest that CIN is strongly tightly bonded to the PLA matrix, which limits its release rate into the medium and, so its antibacterial capacity. In the PLA monolayer, the incorporated amount of CIN incorporated seems to be enough to deliver a CIN concentration above the MIC of both bacteria, but when this amount was distributed in a thicker PLA bilayer, not enough active compound is released occurs to affect bacterial growth. In contrast, the S layer offers much less resistance to the CIN migration, thus enhancing the antimicrobial capacity of the bilayer films. In that sense, since starch is a hydrophilic material, its hydration in contact with the medium would favour the molecular mobility and thus the diffusion of CIN into the medium. To corroborate this hypothesis, the next section analyses the release kinetics of CIN from mono and bilayer films in different food simulants.

Release kinetics of cinnamaldehyde

The release kinetics of the active compound into food simulants with of differing polarities was analysed. Aqueous (A: 10 % ethanol (v/v) and B: 3 % acetic acid (w/v)) and less polar simulants (D1: 50 % ethanol (v/v) and D2: isooctane) were considered. The Released mass of CIN delivered at different contact times with simulants has ve been determined and figure 2 shows the mean values, referred to the maximum value (at equilibrium) for each case. This ratio represents the fraction of active released at each time with respect to the final amount delivered at equilibrium in each simulant. Table 2 shows the maximum values (M_∞), referred per mass unit of the initial film, which were estimated by applying Peleg's model to the experimental data for the CIN release. The values of 1/k₁ parameter, related to the release rate, were also included in table 2. A good fitting of the model was achieved in all every cases (R² > 0.97). Both the deliver rate and asymptotic value were greatly affected by the polarity of the food simulant and the kind of film, yielding different values depending on both factors. For every kind of film, the fastest release of CIN was observed in 50 % ethanol

(simulant D1) (c), whereas the slowest delivery occurred in the non-polar solvent (D2: isoctane) (e) for all kinds of films, as previously observed by several authors for other essential oil compounds in polymer matrices [30]; [31]; [32]; [33]; [34]. However, although the different films were observed to exhibit great differences in the CIN release rate were observed among the different films for a determined simulant, while in all cases the PLA bilayer (PLA-M_{CIN}) exhibited the slowest release rate in every case.

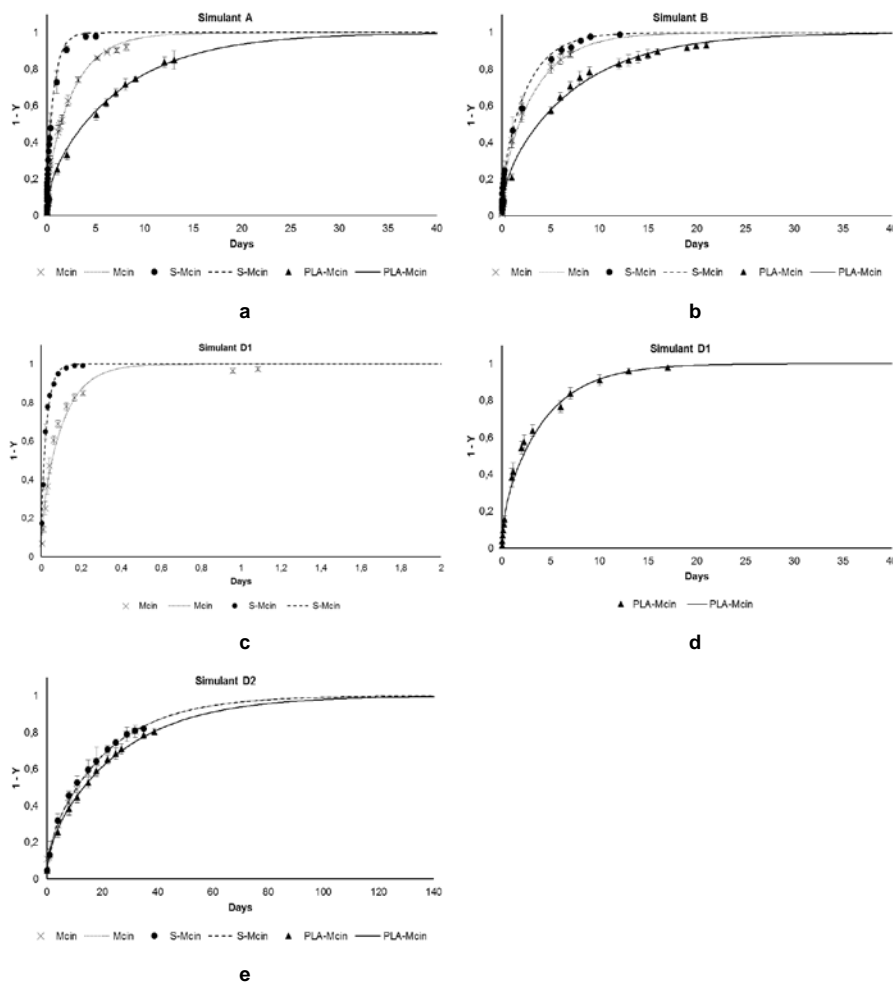


Figure 2. Ratio of the active compound released in each food simulant, with respect to the equilibrium value, as a function of contact time (points) and Fick's fitted Fick's model (lines). A: 10 % ethanol (v/v), B: 3 % acetic acid (w/v), D1: 50 % ethanol (v/v), and D2: isoctane.

As concerns the maximum active compound delivered, table 2 also shows the maximum release ratio (M_{∞}/M_0) for each compound, related with its partition coefficient (defined as the mass of active released at equilibrium in the simulant (M_{∞}) with respect to the corresponding residual mass of the active in the film ($M_0 - M_{\infty}$)). This ratio was referred to the M_0 value of the theoretical amount incorporated into the films and also to the amount determined by methanol extraction. The highest M_{∞}/M_0 values occurred in the starch bilayer (S- M_{cin}) and PLA monolayer samples (M_{cin}) in almost all every simulants, except in D1, where no significant differences were observed for the different films; if when considering the extractable CIN in methanol is considered. In this case, a total release can be assumed, considering the methanol-extractable CIN in methanol of the films SE PUEDE QUITAR?? although only about 50 % of the incorporated compound was delivered from the PLA mono and bilayers. The S starch bilayers exhibited the greatest variability in this parameter, thus indicating more stochastic losses of the active compound during the bilayer processing, which could be due to the coupled effects of water loss from the S layer and the potential steam drag of CIN. In the non-polar simulant (D2), a very low-small release of CIN occurred in all every cases, with being the PLA bilayer being the film that more than most strongly retains the active compound.

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Table 2. Parameters of Peleg's model: amount of active compound released at equilibrium in the simulant (M_{∞}) and its release rate ($1/k_1$), and maximum release ratio (M_{∞}/M_0): mass of active delivered at equilibrium in the simulant related to the initial mass of the active (theoretical and extractable in methanol). Values of the Diffusion coefficient (D) were also included. Mean values \pm standard deviation.

Simulant	Film	$1/k_2 = M_{\infty}$ (g CIN/100 g film)	$1/k_1$ ($\mu\text{g}/\text{min}$)	M_{∞}/M_0 theoretical (%)	M_{∞}/M_0 extractable in methanol (%)	$D \times 10^{11}$ (cm^2/s)
A	M_{cin}	9.7 ± 0.3^e	4.3 ± 0.6^{ab}	48 ± 2^{cd}	83 ± 3^c	2.4 ± 0.2^a
	S- M_{cin}	3.1 ± 0.7^c	4.5 ± 0.9^{ab}	35 ± 8^b	113 ± 25^e	53 ± 20^{bc}
	PLA- M_{cin}	0.57 ± 0.12^{ab}	0.9 ± 0.4^a	7.5 ± 0.4^a	14.2 ± 0.7^{ab}	5 ± 1^a
B	M_{cin}	9.0 ± 0.2^e	4.0 ± 0.8^{ab}	45 ± 1^c	77 ± 2^c	1.9 ± 0.3^a
	S- M_{cin}	2.6 ± 0.6^c	1.6 ± 0.3^a	29 ± 7^b	95 ± 22^{cde}	15 ± 4^{ab}
	PLA- M_{cin}	0.278 ± 0.012^a	0.47 ± 0.07^a	3.7 ± 0.2^a	6.9 ± 0.3^{ab}	5.4 ± 0.5^a
D1	M_{cin}	10.9 ± 0.2^f	97 ± 8^d	55 ± 1^d	93 ± 2^{cde}	63 ± 5^c
	S- M_{cin}	2.3 ± 0.9^c	49 ± 12^c	27 ± 10^b	86 ± 33^{cd}	1378 ± 106^d
	PLA- M_{cin}	4.3 ± 0.7^d	8.9 ± 0.6^b	56 ± 9^d	107 ± 17^{de}	9 ± 2^a
D2	M_{cin}	1.11 ± 0.03^b	0.18 ± 0.05^a	5.5 ± 0.2^a	9.5 ± 0.3^{ab}	0.31 ± 0.08^a
	S- M_{cin}	0.7 ± 0.2^{ab}	0.11 ± 0.04^a	8 ± 3^a	26 ± 9^b	1.78 ± 0.12^a
	PLA- M_{cin}	0.046 ± 0.006^a	0.014 ± 0.002^a	0.61 ± 0.08^a	1.2 ± 0.2^a	1.7 ± 0.2^a

Different superscript letters within the same column indicate significant differences among samples (films or simulants ($p < 0.05$)).

In relation with As regards the active agent release process, three steps can be assumed: a) the solvent diffusion into the polymer matrix, b) the network relaxation in line with solvation and plasticization and c) the diffusion of the compound through the relaxed polymer network until the

thermodynamic equilibrium between phases (polymer/food system) is reached. The compound affinity with the solvated polymer and its solubility in the liquid food system will determine the partition coefficient at equilibrium for the delivered compound. Thus, the compound diffusion through the matrix will be affected by the solvent impregnation into the polymer network and the interactions established among the components. Considering bilayer films, two polymer matrices were involved in the film (amorphous PLA and starch or crystalline PLA, in S-M_{cin} or PLA-M_{cin} samples respectively). Therefore, ~~different behaviours of~~ the two layers could be expected ~~to behave differently~~. Nevertheless, ~~there was~~ an overall ~~behaviour tendency was~~ observed ~~CHECK~~ when bilayer films were submitted to the release studies. Previous studies ~~for into~~ similar compounds in different polymer matrices revealed no coupling of the polymer relaxation and compound diffusion steps. ~~Then So~~, the diffusional mechanism controlled the compound release in different polymer films. This was the case of thymol in poly(butylene succinate) [32] and PLA [33] or lemongrass essential oil in sodium alginate films [35]. Taking these considerations into account, the apparent diffusion coefficient of the different mono and bilayer films were obtained.

Figure 2 shows ~~the fitted~~ Fick's model ~~fitted~~ to the experimental points in each simulant. A good fitting of the model was observed in ~~all every cases~~ (SSE < 0,076) as well as the different pattern of the curves depending on the film and the simulant. ~~In each simulant, The the~~ time required to reach the equilibrium value greatly varied ~~among from film to the films in each simulant;~~ from ~~a few hours in the case of the starch bilayer (S-M_{cin})~~ to several days; ~~in that of being shorter for the starch bilayer (S-M_{cin}) and much longer for the PLA bilayer (PLA-M_{cin})~~ ~~CHECK~~. This agrees with that ~~previously commented a forementioned~~ CIN bonding to the PLA chains, which greatly retards the CIN deliver ~~into~~ the food simulants, regardless ~~of~~ their polarity. ~~The D~~ differences were more marked for ~~the~~ simulant D1 (50 % ethanol), where a much faster release was observed for ~~both~~ monolayer and S bilayer films (c), in comparison with PLA bilayers (d). The decrease ~~of in~~ the simulant polarity, related to the rise ~~of~~ ~~in the ethanol ratio, promoted boosted~~ the CIN release rate of both ~~the~~ PLA monolayer and starch bilayer, probably due to the greater affinity of CIN with the solvent ~~(-----)~~ and the greater relaxation of the PLA layer in contact with this ethanol-~~rich~~ solvent. Finally, the three kinds of films exhibited ~~a very similar behaviour in isooctane (D2), with a very slow release until reaching the equilibrium is reached at~~ longer contact times (nearly 120 days).

The values of the apparent diffusion coefficient (D) in each film and simulant are shown in table 2, where both the polymer matrix and solvent influence can be observed. As the solvent penetrates ~~the polymer matrix~~ to a different extent ~~in the polymer matrix~~, providing it ~~with~~ different relaxation effects, the compound diffusion was affected not only by the kind of polymer but also by the relaxation induced by the different solvents. In ~~all every cases~~, D values were higher for the S bilayer, thus reflecting the ~~low poor~~ binding capacity of starch chains to bond CIN, which can be released more easily. No significant differences in D values ~~were observed for each in either film were observed~~ for the two most ~~fre~~ aqueous simulants (A and B). Nevertheless, the increase ~~of in the ethanol ratio in the~~ simulant D1 enhanced ~~the~~ CIN diffusion in ~~all every~~ films, which can be attributed to a greater relaxation of the PLA matrix in both mono and bilayers. Finally, the lowest values of D were obtained in ~~the~~ simulant D2 due to both the low solvent chemical affinity with CIN and the polymers, which will imply low relaxation

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effects in the film matrices. It is remarkable that the D value in the PLA bilayer was significantly lower than in the PLA monolayer when the simulant D1 was used, which can be attributed to the lower relaxation of the crystalline PLA layer in contact with the ethanol-rich simulant.

The obtained results confirm the great retention of CIN in the PLA matrix, which can be explained by the enhanced compound-chain interactions with great chemical affinity. This will limit both its release ratio and rate in food systems, which could compromise for the CIN antimicrobial action. This retention seems to be enhanced after the thermocompression in bilayer films, as also revealed by the antimicrobial test. In the S bilayer, the thermocompression step seems to involve the CIN diffusion into the starch layer, where no CIN binding occurred due to the lesser-poorer polymer-compound chemical affinity, and the migrated CIN could effectively be delivered into the simulants. This migration also occurred for the crystalline PLA layer in the PLA bilayer films, which contributes to the compound binding. The different relaxation of crystalline and amorphous PLA films in contact with the medium affected the CIN diffusion, limiting its release when it is entrapped in the more crystalline layer.

Active release prediction over during/throughout the plate incubation time.

Based on the kinetics analysis, the active agent release concentration vs. time was predicted in the plates where in which the antimicrobial test was performed, assuming bulk diffusion into the culture medium and assuming the simulant A (10 % ethanol) behaviour for the medium. Figure 3 shows the predicted CIN concentration (mg/mL TSA medium) when each one of the films was placed/put in contact with the plate as a function of time. The CIN MIC range reported by several authors for *E. coli* and *L. innocua* [36]; [37]; [38]; [39]; [40]; [41] was reflected in the plot (shadow areas). As observed in the figure 3, different CIN concentrations will be present in the plate at the different incubation times. The S starch bilayer released the active compound faster, whose concentration reached the maximum level after about two days of contact, whereas the CIN release was slower from the PLA monolayer active films. For both films, the MIC value was exceeded/overcome, which explains the effective antimicrobial action observed and commented on previously. Nevertheless, the CIN delivered from the PLA bilayer film did not exceed/overcome the MIC value throughout the incubation time (13 days), which explains the lack of antibacterial action found in the antimicrobial test. Despite the fact that release kinetics provided information about the overall release from both sides of the films, the comparative analysis of both bilayers allows for obtaining information to be obtained about the different release kinetics of starch and semi crystalline PLA layers. This information is completely coherent with the obtained antimicrobial response obtained of from the three types of films. When the S bilayer is considered, CIN migration to the culture media is promoted by the diffusion of the compound into the starch layer where the compound is faster-delivered faster. When this diffusion occurred into the semi crystalline PLA layer, CIN was highly-closely retained into the polymer network, which becomes less relaxed in contact with the medium, both factors inhibiting the compound release. The initially free CIN in the amorphous PLA layer will be greatly reduced by its distribution and partial bonding to the new PLA layer, when the bilayer was formed by thermocompression, and its availability to be delivered into the agar medium becomes more limited,

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thus not reaching the MIC value for the tested bacteria when the films were ~~are~~ in contact with the plates.

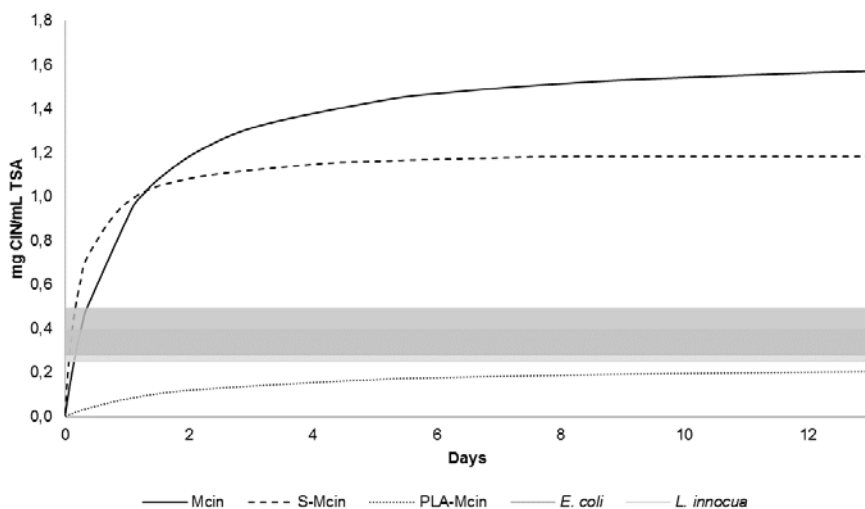


Figure 3. Predicted concentration of CIN released throughout the incubation time into the TSA culture medium for the three different types of film, considering the simulant A. Shaded areas correspond to the range of the MIC of CIN against *E. coli* (dark grey) and *L. innocua* (faded grey).

So, the kinetics analysis of the CIN release from the different films contribute helps to explain the different antimicrobial effects of the compound, according to its different retention ratios in the polymer matrix. The obtained results show great differences in the active compound release properties, affected by the polymer retention-entrapping capacity of the active compounds and the polymer-medium interactions, which greatly affect its relaxation capacity and compound diffusion. Crystalline PLA offers high great resistance to CIN release, ase CIN and soe will limitings its antimicrobial action in active packaging materials. However, amorphous PLA and starch delivered CIN faster and at a higher ratio, which favoured the antibacterial activity. Of both polymers, starch seems both to retain CIN to a lesser extent and provide a higher CIN content to the culture medium, thus being more effective at to controllling bacterial growth.

CONCLUSION

Antimicrobial films based on starch and PLA could be obtained by the thermocompression of a CIN loaded amorphous PLA layer and compression moulded thermoplastic starch. Films exhibited antibacterial effect in *in vitro* tests against *E. coli* and *L. innocua*, when the culture medium was in contact with both starch and PLA layers, being more effective for in the case of S contact, despite the fact that CIN was loaded into the PLA layer. Both the antimicrobial test and CIN release kinetics

suggest that a part of the incorporated CIN was ~~strongly-tightly~~ bonded to the PLA ~~m~~Matrix and the free CIN diffuses ~~in~~to the initially free layer in the bilayer films. In the starch bilayers, CIN migrates ~~in~~to the food system faster through the starch side due to its ~~lower-poorer/fewer~~ interactions with the starch chains and the ~~fact that the~~ polymer swells~~ing~~ in contact with the wet medium, which favours ~~the~~ compound diffusion. When bilayers~~s~~ were formed with CIN loaded amorphous PLA and ~~a~~ semi crystalline PLA layer, CIN migration was greatly inhibited, in line with the increase in the bonded/free CIN ratio and the ~~lower-milder~~ solvent relaxation effects in the crystalline regions of the film. This implied ~~the-a~~ lack of antimicrobial action of CIN ~~of-in~~ these bilayers, where the released active compound did not ~~overcome-exceed~~ the MIC of both tested bacteria. Therefore, the lamination of CIN loaded amorphous PLA with thermoplastic starch represents a good strategy to obtain antimicrobial films for food applications, which can be applied through starch or PLA food contact, depending on the interest ~~for-in~~ a determined kind of food. In both cases, ~~an~~ antimicrobial activity can be expected, while films show complementary barrier properties associated ~~to-with~~ the combination of both materials: ~~-~~ ~~The~~ films facing the oxygen through the starch layer, whereas the PLA layer would ~~bring-provide~~ good ~~water vapour~~ barrier ~~properties-capacity~~. Nevertheless, *in vivo* tests are required to assess the antimicrobial and functional effectiveness of these bilayer films in real foods.

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REFERENCES

- [1] M. Corrales, A. Fernández, J.H. Han, Chapter 7 - Antimicrobial packaging systems. *Innovations in Food Packaging* (2nd ed., pp. 133-170) (2014). Plano, TX, U.S.A.: Academic Press.
- [2] Lanciotti, R., Gianotti, A., Patrignani, F., Belletti, N., Guerzoni, M. E., & Gardini, F. (2004). Use of natural aroma compounds to improve shelf-life and safety of minimally processed fruits. *Trends in food science & technology*, 15(3), 201-208.
- [3] Y-G. Kim, J-H. Lee, S-I. Kim, K-H. Baek, J. Lee, Cinnamon bark oil and its components inhibit biofilm formation and toxin production, *Int. J. Food Microbiol.* 195 (2015) 30-39.
- [4] V.A. Parthasarathy, B. Chempakam, T.J. Zachariah, *Chemistry of Spices* p.128, CABI, Biddles Ltd, King's Lynn, UK, 2008.
- [5] Q. Sun, L. Wang, Z. Lu, Y. Liu, *In vitro* anti-aflatoxigenic effect and mode of action of cinnamaldehyde against aflatoxin B1. *Int. Biodeterior. Biodegrad.* 104 (2015) 419-425.
- [6] H. Wang, H. Yuan, S. Li, Z. Li, M. Jiang, Synthesis, antimicrobial activity of Schiff base compounds of cinnamaldehyde and amino acids, *Bioorg. Med. Chem. Lett.* 26 (2016) 809-813.
- [7] F. Xing, H. Hua, J. Nimal Selvaraj, Y. Zhao, L. Zhou, X. Liu, Y. Liu, Growth inhibition and morphological alterations of *Fusarium verticillioides* by cinnamon oil and cinnamaldehyde, *Food Control* 46 (2014) 343-350.
- [8] S. Shen, T. Zhang, Y. Yuan, S. Lin, J. Xu, H. Ye, Effects of cinnamaldehyde on *Escherichia coli* and *Staphylococcus aureus* membrane, *Food Control* 47 (2015) 196-202.

- [9] K.A. Hammera, K.A. Heel, Use of multiparameter flow cytometry to determine the effects of monoterpenoids and phenylpropanoids on membrane polarity and permeability in staphylococci and enterococci, *Int. J. Antimicrob. Agents* 40 (2012) 239-245.
- [10] K.H. Bang, D.W. Lee, H.M. Park, Y.H. Rhee, Inhibition of fungal cell wall synthesizing enzymes by *trans*-cinnamaldehyde, *Biosci. Biotechnol. Biochem.* 64 (2000) 1061-1063.
- [11] S.A. Baskaran, M.A. Roshni Amalaradjou, T. Hoagland, K. Venkitanarayanan, Inactivation of *Escherichia coli* O157:H7 in apple juice and apple cider by *trans*-cinnamaldehyde, *Int. J. Food Microbiol.* 141 (2010) 126-129.
- [12] M.A. Roshni Amalaradjou, T.A. Hoagland, K. Venkitanarayanan, Inactivation of *Enterobacter sakazakii* in reconstituted infant formula by *trans*-cinnamaldehyde, *Int. J. Food Microbiol.* 129 (2009) 146-149.
- [13] M.A. Roshni Amalaradjou, S.A. Baskaran, R. Ramanathan, A. Kollanoor Johny, A.S. Charles, S. Rao Valipe, T. Mattson, D. Schreiber, V.K. Juneja, R. Mancini, K. Venkitanarayanan, Enhancing the thermal destruction of *Escherichia coli* O157:H7 in ground beef patties by *trans*-cinnamaldehyde, *Food Microbiol.* 27 (2010) 841-844.
- [14] T.E. Mattson, Anup Kollanoor Johny, Mary Anne Roshni Amalaradjou, Karen More, David T. Schreiber, Jitu Patel, Kumar Venkitanarayanan. Inactivation of *Salmonella spp.* on tomatoes by plant molecules, *Int. J. Food Microbiol.* 144 (2011) 464-468.
- [15] S. Viazis, M. Akhtar, J. Feirtag, F. Diez-Gonzalez, Reduction of *Escherichia coli* O157:H7 viability on leafy green vegetables by treatment with a bacteriophage mixture and *trans*-cinnamaldehyde, *Food Microbiol.* 28 (2011) 149-157.
- [16] L.A. Hernández-Herrero, M.J. Giner, M. Valero, Effective chemical control of psychrotrophic *Bacillus cereus* EPSO-35AS and INRA TZ415 spore outgrowth in carrot broth, *Food Microbiol.*, 25 (2008) 714-721.
- [17] N. Sanla-Ead, A. Jangchud, V. Chonhenchob, P. Suppakul, Antimicrobial Activity of Cinnamaldehyde and Eugenol and Their Activity after Incorporation into Cellulose-based Packaging Films, *Packag. Technol. Sci.* 25 (2012) 7-17.
- [18] C.G. Otoni, M.R. de Moura, F.A. Aouada, G.P. Camilloto, R.S. Cruz, M.V. Lorevice, N. de F.F. Soares, L.H.C. Mattoso, Antimicrobial and physical-mechanical properties of pectin/papaya puree/cinnamaldehyde nanoemulsion edible composite films, *Food Hydrocolloids* 41 (2014) 188-194.
- [19] J. Ahmed, N. Hiremath, H. Jacob, Antimicrobial, Rheological, and Thermal Properties of Plasticized Polylactide Films Incorporated with Essential Oils to Inhibit *Staphylococcus aureus* and *Campylobacter jejuni*, *J. Food Sci.* Vol. 81 (2016) Nr. 2.
- [20] S. Makwana, R. Choudhary, N. Dogra, P. Kohli, J. Haddock, Nanoencapsulation and immobilization of cinnamaldehyde for developing antimicrobial food packaging material, *LWT Food Sci. Technol.* 57 (2014) 470-476.
- [21] Y. Qin, J. Yang, J. Xue, Characterization of antimicrobial poly(lactic acid)/poly(trimethylene carbonate) films with cinnamaldehyde, *J. Mater. Sci.* 50 (2015) 1150-1158.
- [22] A.C. Souza, G.E.O. Goto, J.A. Mainardi, A.C.V. Coelho, C.C. Tadini, Cassava starch composite films incorporated with cinnamon essential oil: Antimicrobial activity, microstructure, mechanical and barrier properties, *LWT Food Sci. Technol.* 54 (2013) 346-352.
- [23] M.P. Balaguer, G. Lopez-Carballo, R. Catala, R. Gavara, P. Hernandez-Munoz, Antifungal properties of gliadin films incorporating cinnamaldehyde and application in active food packaging of bread and cheese spread foodstuffs, *Int. J. Food Microbiol.* 166 (2013) 369-377.
- [24] M.A. Rojas-Graü, R.J. Avena-Bustillos, C. Olsen, M. Friedman, P.R. Henika, O. Martín-Belloso, Z. Pan, T.H. McHugh, Effects of plant essential oils and oil compounds on mechanical, barrier and antimicrobial properties of alginate-apple puree edible films, *J. Food Eng.* 81 (2007) 634-641.
- [25] R. Ortega-Toro, I. Morey, P. Talens, A. Chiralt, Active bilayer films of thermoplastic starch and polycaprolactone obtained by compression molding, *Carbohydr. Polym.* 127 (2015) 282-290.

- [26] J. Muller, A. Jiménez, C. González-Martínez, A. Chiralt, Influence of plasticizers on thermal properties and crystallization behaviour of poly(lactic acid) films obtained by compression moulding, *Polym. Int.* 65 (2016) 970-978.
- [27] M.L. Sanyang, S.M. Sapuan, M. Jawaid, M.R. Ishak, J. Sahari, Development and characterization of sugar palm starch and poly(lactic acid) bilayer films, *Carbohydr. Polym.* 146 (2016) 36-45.
- [28] Commission Regulation (EU) 2015/174 of 5 February 2015 amending and correcting Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food. Official Journal of the European Union.
- [29] J. Crank, *The Mathematics of Diffusion*, second ed., Clarendon Press, Oxford, UK, 1975.
- [30] A. Narayanan, K.V. Ramana, Synergized antimicrobial activity of eugenol incorporated polyhydroxybutyrate films against food spoilage microorganisms in conjunction with pediocin, *Appl. Biochem. Biotechnol.* 170 (6) (2013) 1379-1388.
- [31] L. Sánchez-González, M. Cháfer, C. González-Martínez, A. Chiralt, S. Desobry, Study of the release of limonene present in chitosan films enriched with bergamot oil in food simulants, *J. Food Eng.* 105 (1) (2011) 138-143.
- [32] N. Petchwattana, P. Naknaen, Utilization of thymol as an antimicrobial agent for biodegradable poly (butylene succinate), *Mater. Chem. Phys.* 163 (2015) 369-375.
- [33] I.S. Tawakkal, M.J. Cran, S.W. Bigger, Release of thymol from poly (lactic acid)-based antimicrobial films containing kenaf fibres as natural filler, *LWT Food Sci. Technol.* 66 (2016) 629-637.
- [34] R. Requena, M. Vargas, A. Chiralt, [Release kinetics of carvacrol and eugenol from poly\(hydroxybutyrate-co-hydroxyvalerate\) \(PHBV\) films for food packaging applications](#), *Eur. Polym. J.* 92 (2017) 185-193.
- [35] S.B. Matiacevich, Edible antimicrobial films based on microencapsulated lemongrass oil, *J. Food Sci. Technol.* 53 (1) (2016) 832-839.
- [36] R-S Pei, F. Zhou, B-P Ji, J. Xu, Evaluation of Combined Antibacterial Effects of Eugenol, Cinnamaldehyde, Thymol, and Carvacrol against *E. coli* with an Improved Method, *J. Food Sci.* Vol. 74 (2009) Nr. 7.
- [37] R. Becerril, R. Gómez-Lus, P. Goñi, P. López, C. Nerín, Combination of analytical and microbiological techniques to study the antimicrobial activity of a new active food packaging containing cinnamon or oregano against *E. coli* and *S. aureus*, *Anal Bioanal. Chem.* 388 (2007) 1003-1011.
- [38] S-T Chang, P-F Chen, S-C Chang, Antibacterial activity of leaf essential oils and their constituents from *Cinnamomum osmophloeum*, *J. Ethnopharmacol.* 77 (2001) 123-127.
- [39] J. Han, M.E. Castell-Perez, R.G. Moreira, The Influence of Electron Beam Irradiation on the Effectiveness of Trans-cinnamaldehyde-coated LDPE/Polyamide Films, *J. Food Sci.* Vol. 71 (2006) Nr. 5.
- [40] N. Trinh Thi Thahn, E. Dumas, M. Le Thanh, P. Degraeve, C. Ben Amara, A. Gharsallaoui, N. Oulahal, Effect of a Vietnamese *Cinnamomum cassia* essential oil and its major component *trans*-cinnamaldehyde on the cell viability, membrane integrity, membrane fluidity, and proton motive force of *Listeria innocua*, *Can. J. Microbiol.* 61 (2015) 263-271.
- [41] N. Trinh Thi Thanh, K. Bensadi, E. Dumas, A. Gharsallaoui, S. Gouin, M.H. Ly-Chatain, P. Dagraeve, M. Le Thanh, N. Oulahal, Comparison of the antibacterial activity of Vietnamese *Cinnamon* essential oil and its chemotype (*trans*-cinnamaldehyde) in tryptone soya broth (TSB) and in an oil in water emulsion containing TSB: consequences for its use in food preservation, *Scientific Works Volume LX "Food Science, Engineering and Technology"*, 2013.

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