Food Research International 200 (2025) 115345

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Study of the release kinetics of Ethyl Lauroyl Arginate from poly (3-hydroxybutyrate-co-3-hydroxyvalerate) active films

Carola Nicosia [a,*], Fabio Licciardello [a,b]

[a] Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola 2, 42122 Reggio Emilia, Italy
[b] Interdepartmental Research Centre for the Improvement of Agro-Food Biological Resources (BIOGEST-SITEIA), University of Modena and Reggio Emilia, Via Amendola 2, 42122 Reggio Emilia, Italy

ARTICLE INFO

Keywords:
Antimicrobial packaging
Bioplastics
Food waste prevention
LAE
PHBV
Mathematical modeling

ABSTRACT

This study investigates the underexplored area of the release mechanism and kinetics of the antimicrobial Ethyl Lauroyl Arginate (LAE®) from an innovative active packaging system based on poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). We evaluated the impact of food simulants and temperatures on LAE® release, diffusion, and partition coefficients. Mathematical modeling was used to elucidate LAE® release kinetics, offering understanding of the release behaviour in food matrices. Results highlighted that temperature notably affected LAE® release into simulant A (10% EtOH) unlike the release into simulant D1 (50% EtOH). Although the release was faster in the less polar simulant, a greater partition coefficient demonstrated greater LAE® retention within the polymer matrix at equilibrium. Weibull models ensured robust fits, suggesting their usefulness for future studies on LAE® release kinetic. Finally, the active films were validated in food, showing significant reduction in microbial counts. These findings contribute to the design of effective antimicrobial food packaging and the selection of suitable food applications.

1. Introduction

Active packaging, at the forefront of innovative packaging solutions, involves materials that interact positively with protected food. Among active agents, antimicrobial compounds have been extensively studied for their role in reducing microbial growth, preserving food safety, and extending shelf life (Almasi et al., 2021; Dıblan & Kaya, 2018). The interest in antimicrobial active packaging aligns with Sustainable Development goals of the 2030 Agenda by the United Nations, particularly the reduction of food waste which constitute about one-third of the world's food production (FAO, 2011).

A growing interest has been directed toward Ethyl Lauroyl Arginate (LAE), a non-volatile antimicrobial compound synthesized from L-arginine and lauric acid. As a cationic surfactant, LAE can bind to anionic microbial proteins and cause their denaturation, exhibiting broad-spectrum activity against Gram-positive and Gram-negative bacteria, yeasts, and molds. LAE was authorized as a food ingredient by the Food and Drug Administration in 2005 (U.S. FDA, 2005) and endorsed by the European Food Safety Authority in 2007 as food additive E243 (EFSA, 2007; Regulation 1333/2008). Toxicological studies have shown that LAE is rapidly absorbed and metabolized into arginine, lauric acid, and ethanol, entering normal biochemical pathways (EFSA, 2007).

LAE has been incorporated into various polymers, including low-density polyethylene (LDPE) (Gaikwad et al., 2017), poly(γ-glutamic acid) (PGGA) (Gamarra-Montes et al., 2018), and ethylene vinyl alcohol (EVOH) (Muriel-Galet et al., 2014, 2015). Its incorporation has extended to biopolymers, such as polylactic acid (PLA) (Li et al., 2021; Patiño Vidal et al., 2022), zein (Kashiri et al., 2016), cellulose-based packaging (Bigi et al., 2023; Gracia-Vallés et al., 2022), chitosan-based systems (Bigi et al., 2023; Guo et al., 2014; Haghighi et al., 2019, 2020; Higueras et al., 2013; Hoa et al., 2022; Ma et al., 2016), gelatin (Haghighi et al., 2019; Moreno et al., 2017, 2018), poly(vinyl alcohol)/starch (Wu et al., 2021), pullulan-based coating systems (Hassan & Cutter, 2020; Pattanayaiying et al., 2015), and, very recently, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) (Bruni et al., 2024). These LAE-containing active packaging have shown promising applications in preserving a variety of food products, including raw meats like turkey breast, chicken breast, and beef as well as processed foods such as cured ham, deli meats, chicken stock, surimi sticks, and fresh spreadable cheese (Bruni et al., 2024; Gracia-Vallés et al., 2022; Guo et al., 2014; Higueras et al., 2013; Li et al., 2021; Moreno et al., 2018; Muriel-Galet et al., 2015; Pattanayaiying et al., 2015).

* Corresponding author.
E-mail address: carola.nicosia@unimore.it (C. Nicosia).

https://doi.org/10.1016/j.foodres.2024.115345
Received 5 July 2024; Received in revised form 8 November 2024; Accepted 13 November 2024
Available online 19 November 2024

PHBV is a biopolymer obtained through microbial processes from renewable carbon sources, including food by-products, and it is biodegradable across aerobic, anaerobic, and marine environments (Chea et al., 2016; Meereboer et al., 2020). PHBV is obtained by inserting units of 3-hydroxyvalerate (3-HV) into polyhydroxybutyrate (PHB), enabling the reduction of crystallinity, melting temperature, and brittleness, providing a wide temperature/time processing window and making PHBV more processable than PHB (Hernández-García et al., 2022; Soares da Silva et al., 2022). This thermoplastic polymer represents a viable candidate to replace traditional plastics used in packaging production, due to physical properties comparable to some petroleum-based polymers (da Costa et al., 2020). In this study we designed an active packaging system incorporating LAE into PHBV and investigated the kinetics and mechanism of release of the antimicrobial.

In active packaging where the active agent is directly incorporated into the polymer matrix, three release mechanisms may occur (Almasi et al., 2021): i) diffusion of the active compound through the polymer matrix until it reaches the film surface and diffuses into the food; ii) swelling of the polymer by the liquid medium, leading to an increase in the active compound's diffusion within the polymer matrix; iii) disintegration, cleavage, or deformation of the polymer due to the absorption of the liquid medium. Understanding the variables controlling the diffusion rate of the compound is necessary for designing effective active packaging (Rivera-Hernández et al., 2021).

The study of release kinetics involves calculating parameters such as the diffusion coefficient (through Fick's second law) and partition coefficient, which describe the diffusion rate of the active agent through the polymer matrix and the amount of active compound released at equilibrium, respectively (Marvdashti et al., 2019; Rivera-Hernández et al., 2021). Additionally, the release behavior of the antimicrobial agent from the polymer can be described through mathematical models (Kowalczyk, Pytka, et al., 2020). This comprehensive understanding enables the adaptation of the release rate of the active compound and the modulation of the antimicrobial activity of active films to satisfy the needs of various applications (Kowalczyk, Pytka, et al., 2020; Marvdashti et al., 2019).

Although the antimicrobial effectiveness of LAE has been demonstrated in several studies, its release kinetics from films has been little explored (Higueras et al., 2013; Muriel-Galet et al., 2014, 2015; Patiño Vidal et al., 2022). The elucidation of the release mechanism of LAE through mathematical modeling has not been performed in previous studies with other polymers: only recently, Bruni et al. (2024) estimated the diffusivity of LAE from melt-blended and coated PHBV by modelling with Fick's second law, however this paper was mainly focused on the design optimization in order to achieve effective concentrations in a specific application, while the present study offers an insight into the release mechanism of LAE from PHBV by comparing the effects of three food simulants and three temperatures on the release behavior of LAE from PHBV films obtained by solvent casting. The migration mechanism was elucidated studying kinetic parameters such as the diffusion and partition coefficients. Additionally, mathematical modeling was carried out to describe the release kinetics of LAE from PHBV and enable predicting its release in food matrices. These findings contribute to the design and development of biobased antimicrobial food packaging, potentially extending the shelf life of food products.

2. Materials and methods

2.1. Materials

Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV; NaturePlast PHI 003; 1 to 2 % HV; melt flow rate 15–30 g/10 min (190 °C, 2.16 Kg); density 1.24 g/cm^3) was kindly supplied by NaturePlast (Mondeville, France). Ethyl lauroyl arginate (LAE®) was kindly provided by Vedeqsa (Barcelona, Spain) as Mirenat®-GC formulation (20 % LAE®, 80 % glycerol). Formic acid (ACS reagent, ≥ 96 %), and the HPLC grade reagents acetic acid, ethanol, acetonitrile, and trifluoroacetic acid were supplied by Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).

2.2. Films preparation

PHBV film samples were produced using the solution casting method. The neat PHBV solution was obtained by stirring 5 % (w/v) PHBV in formic acid at 70 °C for 30 min (Nicosia et al., unpublished results). For active films compounding, LAE® was included at 5 % (w/w on a polymer dry weight basis). Since the LAE® formulation contains glycerol, a test control solution was prepared, adding 20 % (w/w) glycerol, maintaining the same polymer-to-glycerol ratio of the active solution. Solutions were cast onto glass Petri dishes under a fume hood at 80 °C until complete evaporation of the solvent. The resulting neat PHBV films, the active films (PHBV-LAE) and PHBV films with glycerol (PHBV-gly) were used for further testing.

2.3. Contact angle measurement

Surface wettability of control (PHBV, PHBV-gly) and active (PHBV-LAE) films was characterized through contact angle measurement using an OCA 15 EC contact angle meter and OCA 20 software (DataPhysics Instruments, Germany). Films (10 × 0.6 cm) were placed in the film holder, and 3 μL of each solvent (water, and food simulants A, B, and D1) were dispensed on the film surface at room temperature, using the sessile drop method. Contact angles were determined as the initial angle of contact between the liquid and the PHBV film surface. A minimum of five measurements were taken at different positions on each sample, with three samples analyzed for each film type.

2.4. Overall migration test

According to the European Commission Regulation (EU) No 10/2011, plastic materials and articles intended to come into contact with food shall not overcome the overall migration (OM) limit of 10 mg of total constituents released per dm^2 of food contact surface (mg/dm^2). To ensure compliance with the regulation, an overall migration test was conducted on the control (PHBV, PHBV-gly) and active (PHBV-LAE) films. Food simulants A (10 % ethanol) and B (3 % acetic acid) were used to simulate hydrophilic foods with pH above and below 4.5, respectively. Simulant D1 (50 % ethanol) is assigned for food with an alcohol content above 20 % and for oil-in-water emulsions (Regulation 10/2011). Rectangular strips of 60 cm^2 of each film were immersed in 100 mL of each food simulant, aligning with the specified surface to volume ratio of 6 dm^2 per kg of food.

The test was conducted under standardised testing condition OM1 (10 days of contact at 20 °C), designed for any food contact at frozen and refrigerated temperatures. After 10 days, film samples were removed, food simulants were separated using a rotary evaporator, and non-volatile residues were weighed using an analytical balance (± 0.1 mg). Overall migration was expressed as the residual content of migratable substances per kg of food simulant (mg/kg), applying a surface-to-volume ratio of 6 dm^2 per kg of food.

2.5. Release kinetics of LAE from PHBV

The mass transfer of LAE from the active films was assessed through a specific migration test. PHBV-LAE active films were cut into 3 cm^2 (1 × 1.5 cm, considering both sides) rectangular strips, and the thickness was measured using a digital micrometer with sensitivity of 0.001 mm (S.A. M.A. Italia S.r.l.®, Viareggio, Italia). Subsequently, the strips were entirely immersed in 5 mL of food simulants A, B, and D1 (surface-to-volume ratio of 6 dm^2/kg) at 6 °C, 20 °C and 30 °C. For each simulant, the samples were evaluated at intervals ranging from 0.33 to 48 h at 6 and 20 °C, while samples at 30 °C were analyzed from 0.08 to 40 h. At various contact times, the released LAE in the simulants was quantified

as previously reported in the literature (Kashiri et al., 2016; Patiño Vidal et al., 2022). The quantification was performed using an HPLC system (Jasco LC-Net II/ADC) equipped with a Jasco PU-2080 Plus pump and a Purospher® STAR RP-18 endcapped column (250 × 4.6 mm, 5 μm particle size, LiChroCART®). The mobile phase was acetonitrile/water (50:50, v/v) with 0.1 % trifluoroacetic acid, flowing at a rate of 1 mL/min, the temperature was 36 °C, and the injection volume 20 μL. LAE quantification was performed at 200 nm using a Jasco UV-2070 Plus detector. A calibration curve was obtained by injecting known LAE concentrations from 0.2 to 200 ppm, prepared by diluting LAE in the mobile phase. The analysis was performed in triplicate for each contact time, temperature, and food simulant.

The detection limit (LOD) and quantification limit (LOQ) of the HPLC method were determined based on the signal-to-noise ratio, where LOD and LOQ correspond to analyte amounts having a signal-to-noise ratio of 3 and 10, respectively (Vial & Jardy, 1999).

2.6. Mathematical modeling of LAE release

The DDSolver add-in program for Microsoft Excel (Zhang et al., 2010) was employed to model LAE release kinetics from PHBV films into food simulants A, B, and D1 at temperatures of 6, 20, and 30 °C. Experimental data from HPLC quantification were fitted using nine mathematical models (Table 1), that were selected based on their frequent application in release studies (Gouda et al., 2017; Kowalczyk, Kordowska-Wiater, et al., 2020; Marvdashti et al., 2019).

In all models, F represents the percentage of active compound released at time t (M_t) compared to the maximum fraction of the compound released at infinite time (M_∞) as illustrated in Eq. (1). The M_∞ fraction was calculated by a total extraction of LAE, after 5 days at 40 °C in simulant A.

$$F(\%) = \frac{M_t}{M_\infty} \times 100 \quad (1)$$

The selection of the model best fitting the experimental data was determined by comparing statistical criteria for model selection such as the adjusted coefficient of determination (R^2_{adj}), the Akaike Information Criterion (AIC), and the Model Selection Criterion (MSC), as recommended by DDSolver developers (Zhang et al., 2010).

Table 1
Mathematical models used for describing the release of active compounds (Zhang et al., 2010).

Model	Equation	Parameters
Zero order [a]	$F = k_0 \cdot t$	k_0
First order [b]	$F = 100 \cdot (1 - e^{-k_1 \cdot t})$	k_1
Higuchi (Hu) [c]	$F = k_H \cdot t^{0.5}$	k_H
Korsmeyer–Peppas (K-P) [d]	$F = k_{KP} \cdot t^n$	k_{KP}, n
Hixson–Crowell [e]	$F = \left[1 - (1 - k_{HC} \cdot t)^3\right]$	k_{HC}
Weibull (Wb) [f, g]	$F = 100 \cdot \left[1 - e^{-\frac{(t - Ti)^\beta}{\alpha}}\right]$	α, β, Ti
Two-parameter Weibull (2-Wb) [f]	$F = 100 \cdot \left(1 - e^{-\frac{t^\beta}{\alpha}}\right)$	α, β
Four-parameter Weibull (4-Wb) [f, g, h]	$F = F_{max} \cdot \left[1 - e^{-\frac{(t - Ti)^\beta}{\alpha}}\right]$	$\alpha, \beta, Ti, F_{max}$
Gompertz [i]	$F = 100 \cdot e^{-\alpha \cdot e^{-\beta \cdot \log(t)}}$	α, β

F is the fraction (%) of compound released in time t.
[a] k_0 is the zero-order release constant.
[b] k_1 is the first-order release constant.
[c] k_H is the Higuchi release constant.
[d] k_{KP} is the release constant incorporating structural and geometric characteristics of the matrix; n is the diffusional exponent indicating the release mechanism.
[e] k_{HC} is the release constant in Hixson–Crowell model.
[f] α is the scale parameter which defines the time scale of the process; β is the shape parameter which characterizes the curve as either exponential ($\beta = 1$; case 1), sigmoid, S-shaped, with upward curvature followed by a turning point ($\beta > 1$; case 2), or parabolic, with a higher initial slope and after that consistent with the exponential ($\beta < 1$; case 3).
[g] Ti is the location parameter which represents the lag time before the onset of the dissolution or release process and in most cases will be near zero.
[h] F_{max} is the maximum fraction of the compound released at infinite time.
[i] α is the scale factor in Gompertz model; β is the shape factor in Gompertz model.

2.7. Diffusion and partition coefficients

The release of an active compound from a polymeric matrix into a food simulant is strictly related to the diffusion coefficient (D) and the partition coefficient (K).

D determines the rate at which the migrant diffuses from the polymer to the solution until reaching the equilibrium, defined by Fick's second law (Crank, 1975) as illustrated in Eq. (2). Films can be considered as thin films with negligible edge effects, where one-dimensional release occurs in an axial direction, with the following assumptions: (1) D is dependent only on temperature (2) the initial concentration of the active compound throughout the film was homogeneous, (3) the initial LAE concentration in the food simulant was zero, (4) the LAE amount in the food simulant was the same as the amount released from the film (Rivera-Hernández et al., 2021).

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

where:
M_t represents the mass of the compound released at time t.
M_∞ represents the mass of the compound released at equilibrium.
n is the dummy variable.
D is the diffusion coefficient.
L is the thickness of film.

The D coefficient was calculated by fitting the experimental data to Equation (2) with 15 terms, using the Solver tool of Microsoft Excel to optimize the D values by minimizing the Sum of Squared Errors (SSE) (Kashiri et al., 2016; Requena et al., 2017). Thus, the calculated D represents the value providing the best fit of Eq. (2) to experimental data.

The partition coefficient (K) determines the extent of migrant release at the equilibrium stage and it is defined as the ratio of the migrant in the polymer matrix ($C_{P,\infty}$) to the migrant in the food simulant ($C_{F,\infty}$) after reaching the plateau as illustrated in Eq. (3). The K coefficients were obtained from concentration values after reaching equilibrium. The value of $C_{P,\infty}$ was calculated by difference between the total amount of LAE in the films (M_∞), obtained from a total extraction performed after 5 days in simulant A at 40 °C, and the $C_{F,\infty}$.

$$K = \frac{C_{p,\infty}}{C_{F,\infty}} \quad (3)$$

2.8. Demonstration of the effectiveness of active films

The developed active films were tested for their antimicrobial effectiveness against *Pseudomonas* sp. in broth medium and using a freshly opened UHT almond beverage as the food medium. The genus *Pseudomonas* was targeted as it is considered one of the main spoilage microorganisms found in dairy products and dairy alternative drinks (Lopez et al., 2018; Quintieri et al., 2021). The *Pseudomonas* sp. strain employed was isolated from naturally contaminated almond beverage and confirmed through DNA sequencing via amplification of the 16S rRNA gene. Genomic DNA extraction and sequencing were performed by

Bio-Fab Research (Rome, Italy).

A modified version of the liquid incubation method under stirring, also known as the dynamic shake flask test (ASTM E2149-01) was employed. Both the control and active PHBV-LAE films were cut into 2 × 4 cm pieces, UV-sterilized, and immersed in 20 mL of sterile Brain Heart Infusion (BHI) broth (Biolife, Milan, Italy) or UHT almond beverage (purchased locally). Afterwards, 10 μL of inoculum, standardized through OD_{600}, was added into each bottle containing either the control or active film. As a control, inoculated media without film were used. The initial microbial concentration quantified before incubation was 4.3 ± 0.1 $\log_{10}$ CFU/mL. To simulate a worst-case scenario and ensure consistency with the conditions used in the release kinetics test, samples were incubated at 30 °C with agitation at 200 rpm. After 16 h of incubation, samples were serially diluted, poured onto Plate Count Agar plates (Biolife, Milan, Italy), and incubated at 30 °C for 24–48 h.

2.9. Statistical analysis

Statistical analysis involved one-way and two-way analysis of variance (ANOVA), followed by Tukey's HSD post hoc test ($p < 0.05$) to assess significant differences among groups.

The relationships among variables were studied using the Pearson correlation test. RStudio (version 2022.12.0 + 353; RStudio, Boston, MA) and SPSS version 26 (IBM Corp., New York, NY, USA) were used for statistical analysis. Modeling of release data and calculation of the diffusion coefficient were performed using the DDSolver (Zhang et al., 2010) and Solver tool add-in programs for Microsoft Excel (Microsoft 365 MSO, Version 2304). Unless otherwise stated, all experiments were conducted in triplicate, and results are expressed as means ± standard deviations (SD).

3. Results and discussion

3.1. Films wettability with different simulants

Surface hydrophobicity of polymers is often assessed through water contact angle measurement, with values below 90° indicating hydrophilic polymers wetted by water (Muriel-Galet et al., 2014). Additionally, the physical–chemical stability of PHBV in contact with food simulants can be investigated by contact angle measurements (Chea et al., 2016).

The results (Table 2 and Fig. 1) indicate contact angle values below 75° with all the solvents tested. Neat PHBV had a water contact angle (WCA) of 63.6°, slightly lower than values reported in previous studies, ranging between 75.2° and 90.3° with decreasing HV content from 10 % to 3 % (Chea et al., 2016; Kim & Masuoka, 2009; Wang et al., 2006; Yoon et al., 2008; Zhu et al., 2009). The contact angle significantly decreased (54.8 and 20.5°, respectively) when drops of 10 % and 50 % ethanol were used. These findings align with values reported by Chea et al. (2016) and suggest that PHBV has an affinity for less polar solvents, such as 10 % and 50 % ethanol. This affinity is due to the hydrophobic groups on its surface: previous studies on PHBV films revealed the presence of C-C (or C-H), C-O, and C = O (COOR, COOH, or CONH) as main functional groups on the PHBV surface (Wang et al., 2006).

The addition of glycerol in the films resulted in a significant ($p < 0.05$) increase in the contact angles with all solvents tested. The percentage increase in contact angle ranged from 5 % (with 3 % acetic acid), to 7 % for water contact angle, up to 25 % increase in the case of 50 % ethanol contact angle. The PHBV-gly film exhibited a similar behavior to the neat PHBV film, where the contact angle of 50 % ethanol was considerably lower than that of water, 10 % ethanol, and 3 % acetic acid.

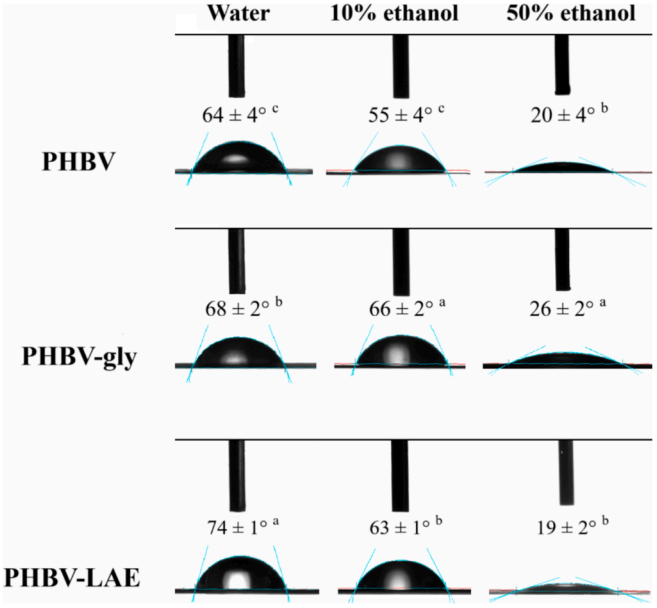

Fig. 1. Photographs of contact angle analysis of PHBV, PHBV-gly, and PHBV-LAE with drops of water, 10 % and 50 % ethanol (from left to right). Different letters in the same column indicate significant differences among formulations ($p < 0.05$).

The water contact angles of PHBV and PHBV-gly further significantly ($p < 0.05$) increased with LAE incorporation (16 % and 8 % increase compared to neat PHBV and PHBV-gly, respectively), suggesting an increase in the surface hydrophobicity of the films. A similar increase of WCA due to the addition of LAE has been reported in previous research on active packaging based on PLA, cellulose nanocrystals (CNC), and chitosan (Patiño Vidal et al., 2023). Similar to observations in neat PHBV, the contact angle of PHBV-LAE decreased proportionally with decreasing polarity of the solvent.

3.2. Overall migration test

Overall migration (OM) tests were performed to assess the compliance of PHBV films with the overall migration limit. The OM values of PHBV, PHBV-gly, and PHBV-LAE are presented in Fig. 2. The migration in neat PHBV was close to the OM limit of 60 mg/kg across all food simulants. These values were slightly higher than reported by Chea et al. (2016) in PHBV films prepared by extrusion and compression molding, possibly due to the different production method and different raw material. Neat PHBV demonstrated the highest OM in 3 % acetic acid, which may be attributed to polymer hydrolysis. Previous studies suggested that the hydrolysis of neat PHBV is favoured in acidic

Table 2
Contact angle analysis of PHBV, PHBV-gly, and PHBV-LAE with drops of water, 10% ethanol, 3% acetic acid, and 50% ethanol.

Sample	Water	Sim A (10 % EtOH)	Sim B (3 % acetic acid)	Sim D1 (50 % EtOH)
PHBV	63.57 ± 4.10° ^{cA}	54.82 ± 4.43° ^{cB}	61.53 ± 3.91° ^{cA}	20.48 ± 4.06° ^{bC}
PHBV-gly	68.13 ± 2.35° ^{bA}	65.70 ± 1.87° ^{aAB}	64.87 ± 3.87° ^{bB}	25.61 ± 2.08° ^{aC}
PHBV-LAE	73.70 ± 1.19° ^{aA}	62.63 ± 1.26° ^{bC}	68.82 ± 0.52° ^{aB}	19.48 ± 2.17° ^{bD}

Different lowercase letters in the same column denote significant differences among film formulations. Different uppercase letters in the same row indicate significant differences among solvents within the same film formulation.

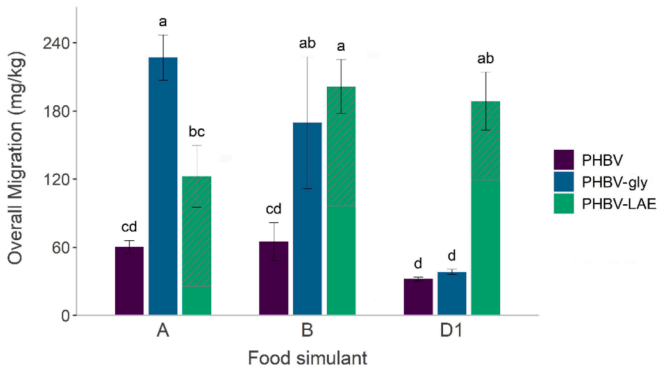

Fig. 2. Overall migration of PHBV, PHBV-gly, and PHBV-LAE in food simulants A (10% ethanol), B (3% acetic acid), and D1 (50% ethanol). The slanted lines within the PHBV-LAE bars represent the amount of LAE released, providing a visual representation of the LAE migration. Different lowercase letters indicate significant differences among samples and simulants, as determined by the two-way ANOVA.

environment, causing a significant decrease of PHBV molar mass and the release of water-soluble substances (Angellier-Coussy et al., 2020).

OM values significantly increased with the inclusion of both glycerol and LAE in neat PHBV, exceeding the limit, except for the migration observed from PHBV-gly in simulant D1. The overall migration from the active films includes the release of LAE, glycerol, and other residues or impurities from both the polymer and the active compounds (Aznar, Gómez-estaca, Vélez, Devesa, & Nerín, 2013). However, it should be underscored that the amount of a released active substance, such as LAE, should not be included in the measured OM value (Regulation 450/2009). Therefore, the amount of LAE released at equilibrium in each simulant was calculated from experimental data or through the best-fitting kinetic model (*Section 3.5*) and subtracted from the OM value of the PHBV-LAE films. The corrected OM values, which exclude the migration of LAE, can be used to evaluate the effect of LAE addition on the migration of unintended release substances. The corrected OM values showed that only in simulant A the migration of undesired substances was below the OML (accounting for 26 mg/kg), while the limit was exceeded in both simulant B (96 ppm) and D1 (119 ppm), as shown in Fig. 2.

3.3. Release kinetics profiles

3.3.1. Sensitivity of the analytical method by HPLC

The LAE calibration curve exhibited a linear trend and remarkable sensitivity, as indicated by R^2 of 0.999, LOQ of 2.5 ppm, and LOD of 0.5 ppm. The amount of LAE released into the food simulants was interpolated from the calibration curve.

3.3.2. Kinetics profiles

Kinetic profiles of LAE release from PHBV at 6, 20, and 30 °C are presented in Fig. 3. The maximum amount of LAE in the films (M_∞) was obtained from a total extraction performed after 5 days in simulant A at 40 °C and was used to calculate the percentage of active compound released at time t compared to the maximum fraction released at infinite time (Eq. 1). As expected, LAE release from the polymer matrix positively correlated with time ($p < 0.001$, Pearson's coefficient 0.64) and temperature ($p < 0.05$, Pearson's coefficient 0.23).

Elevated temperatures accelerate the release kinetics by enhancing molecular mobility (Marvdashti et al., 2019). Nevertheless, our study outscores that temperature impact varies with the food simulant. The migrated LAE correlated with temperature in simulant A (Pearson's correlation coefficient 0.36, $p < 0.001$), exhibiting a significantly different release at the three temperatures, and in simulant B (correlation coefficient 0.27, $p < 0.01$), where the migrated LAE at 20 °C and 30 °C was statistically similar and higher than that at 6 °C. No correlation was observed in simulant D1. This behavior is clear in the diffusion profiles, which overlap when no significant difference is observed.

Two-way ANOVA highlighted significant differences ($p < 0.001$) in release extent among simulants at all temperatures. Specifically, at 6 °C, the greatest LAE amount migrated towards simulant D1, while the lowest towards simulant B. At 20 °C and 30 °C, migration in simulants A and D1 was statistically similar, while significantly lower migration occurred in simulant B.

In simulant D1, after three hours, 56 %, 64 % and 68 % of LAE was released at 6 °C, 20 °C, and 30 °C, respectively, reaching a plateau at fifteen hours with 72 % of LAE released. The release profile in 50 % ethanol resembled a study by Patiño Vidal et al. (2022) from active PLA in 95 % ethanol, where LAE reached equilibrium after 2 h at 40 °C. The rapid release may be attributed to a greater polymer–solvent interaction, leading to greatest swelling and facilitating molecular diffusion.

In contrast, we noticed a slower release in simulants A and B, where the plateau was not reached at low temperatures within the experimental time, while at 30 °C the LAE migrated approaches equilibrium. The greatest LAE amount was released in simulant A at 30 °C, reaching a total release from the PHBV films, while in simulants D1 and B around 72 % and 67 %, respectively, was released after 40 h, even at the highest temperature.

3.4. Diffusion and partition coefficients

The calculation of the diffusion (D) and partition (K) coefficients requires the values of the migrant released at equilibrium. Despite not reaching equilibrium in simulants A and B at 6 °C and 20 °C within the

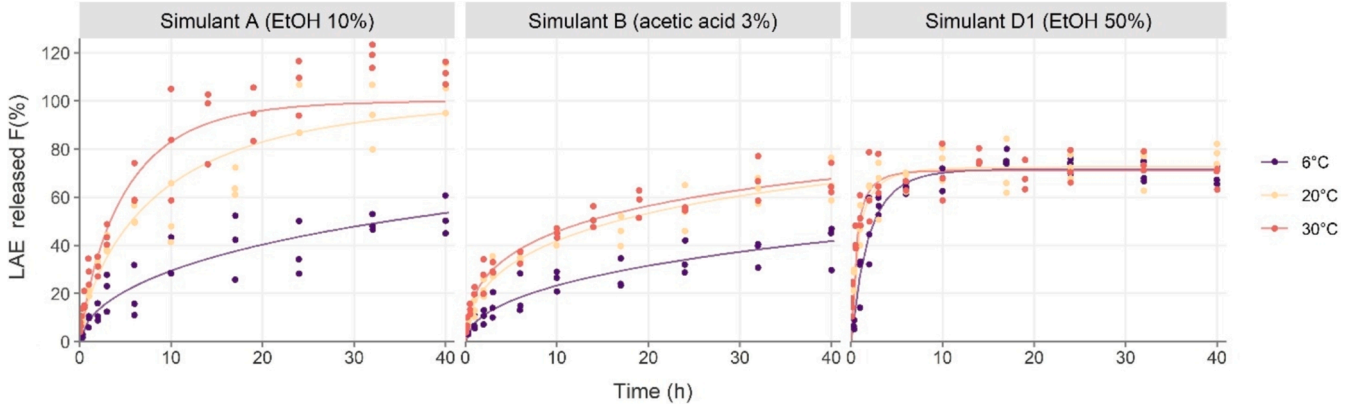

Fig. 3. LAE release kinetics from PHBV in food simulants A (10 % ethanol), B (3 % acetic acid), and D1 (50 % ethanol), at 6 °C, 20 °C, and 30 °C (Curve = predicted; Symbols = observed).

experimental time, the best-fitting kinetic models for each experimental condition (discussed in *Section 3.5*) were employed to predict the LAE migrated at equilibrium.

D and *K* coefficients of LAE released from PHBV in food simulants were calculated using either the experimental or predicted values of LAE migrated at equilibrium and are presented in Table 3. The films thickness used for *D* calculations was 34.6 ± 2.9 μm.

The release rate of a compound, described by the *D* coefficient, is related to the polymer swelling, a phenomenon observed in swellable delivery systems (Muriel-Galet et al., 2014). Upon immersion in a solvent and its diffusion into the matrix, polymer chains undergo increased mobility and polymer chain relaxation, resulting in plasticization and a glassy-to-rubbery-phase-transition, influencing the *D* coefficient. Driven by the concentration gradient, the active compound diffuses through the swollen polymeric matrix toward the solvent (Requena et al., 2017; Siepmann & Siepmann, 2008).

Our study reveals that, irrespective of temperature, *D* coefficients in simulants A and B were lower ($p < 0.001$) than in simulant D1, confirming the impact of the food simulant on release rate (Requena et al., 2017). Lower plasticization in polar solvents (simulants A and B) resulted in slower LAE diffusion within and from the PHBV matrix. In contrast, faster release in simulant D1 suggests higher plasticization and increased molecular mobility. The *D* coefficients of simulants A and B were in the same order of magnitude as those reported by Bruni et al. (2024) from LAE-coated PHBV. In line with our findings, Requena et al. (2017) observed the fastest release rate of carvacrol and eugenol from PHBV in simulant D1. Contact angle measurements (*Section 3.1*) support these findings, indicating much higher PHBV-LAE affinity to simulant D1 and a more intimate interaction, possibly causing greater swelling of the active films in contact with 50 % ethanol, with subsequent polymer structure modification, such as chain degradation and molecular rearrangement, facilitating rapid release regardless of storage temperature (Chea et al., 2016).

In simulant D1 and B, the diffusion coefficient at 6 °C was significantly lower ($p < 0.01$) than at 20 °C and 30 °C, while in simulant A, there was a significant difference among *D* values at the three temperatures ($p < 0.001$), confirming lower molecular mobility at lower temperatures (Marvdashti et al., 2019). However, no significant differences were observed in the amount of LAE released at equilibrium in simulants A and D1 at the various temperatures, as indicated by the *K* coefficient.

The solvent-polymer interaction determines solvent diffusion within the polymer matrix, influencing the diffusivity of the compound (Chea et al., 2016; Requena et al., 2017). In the present study, this assumption has been supported by the contact angle data, as reported above. Consequently, the diffusion of a specific compound varies with different polymers and solvents. LAE release has been previously studied from various polymers, such as PLA-CNC (Patiño Vidal et al., 2022), zein (Kashiri et al., 2016, 2019), chitosan (Higueras et al., 2013), EVOH (Muriel-Galet et al., 2014), and melt-blended or coated PHBV (Bruni et al., 2024), demonstrating that LAE release rate is affected by polymer hydrophobicity. A slower release has been reported in hydrophobic polymers as less polymer swelling occurs, in contrast to more hydrophilic polymers (Kashiri et al., 2016). Compared to the other polymers, PHBV exhibits the greatest presence of hydrophobic groups (–CH, $–CH_2$ and $–CH_3$) (Kunam et al., 2022; Muriel-Galet et al., 2014). This may explain the lower *D* observed both in 10 % ethanol and 3 % acetic acid compared to zein, as reported by Kashiri et al. (2016, 2019), reaching *D* in the order of 10^{-14} and 10^{-13} m^2/s at 30 °C and 37 °C, respectively. The release profile observed in 50 % ethanol resembled observations by Kashiri et al. (2019) from zein in 3 % acetic acid, where an even faster release than ours was reported, with LAE reaching equilibrium after 5 and 2 h at 4 and 37 °C, respectively. This may be due to higher zein affinity with polar simulants compared to PHBV, resulting in higher swelling. Furthermore, the diffusion rates of LAE from both PHBV (this study) and zein (Kashiri et al., 2016, 2019) into 10 % ethanol were substantially lower than those reported in PLA films (Patiño Vidal et al., 2022), exhibiting *D* of 4.7×10^{-13} m^2/s, confirming the higher hydrophilicity of PLA compared to zein and PHBV (Kim & Masuoka, 2009; Kunam et al., 2022).

Compared to other polymers exhibiting a fast and almost complete release of LAE in the first few hours, our study demonstrated lower release rates. These findings align with observations by Bruni et al. (2024), who reported low release rates of LAE from PHBV, occurring over days rather than hours, especially when the antimicrobial was incorporated within the bulk rather than applied as a PHBV coating. This slow release is advantageous in real conditions, ensuring a reservoir of antimicrobial compound and offering continued antimicrobial desorption into food.

The compound's diffusion through the plasticized polymer network progresses until equilibrium between the polymer and the food simulant is reached. The partition coefficient *K* depends on the active compound's affinity to the solvated polymer and its solubility in the simulant (Chea et al., 2016). Table 3 shows the calculated *K* values, demonstrating a strong dependence on the food simulant, consistent with previous research (Requena et al., 2017; Rojas et al., 2021). At 6 °C, the highest *K* was observed in simulant D1, while simulant A exhibited the lowest *K* value. At 20 and 30 °C, *K* values in simulants A and B were statistically similar, whereas simulant D1 exhibited significantly higher values, aligning with the release extent discussed in *Section 3.3*. Interestingly, *K* exhibited less variation with temperature, showing rather a stronger dependance on the simulant.

Overall, *K* values were very low in simulants A and B, indicating lower compound retention in the polymer matrix at equilibrium compared to simulant D1. This result may be attributed to the lower affinity of LAE with 50 % ethanol. The highest LAE amount released in simulant A suggests that this simulant can both swell PHBV, favoring LAE's diffusion into the matrix, and has the highest affinity to LAE compared to other simulants. As an amphiphilic compound, LAE exhibits affinity for both polar and non-polar solvents. We hypothesized that a possible explanation for the lower affinity of LAE with 3 % acetic acid could be the electrostatic repulsions between H^+ ions and LAE's positively charged groups. This hypothesis was based on the well-known nature of LAE as a cationic surfactant and its wide involvement in electrostatic interactions when it exerts its antimicrobial effect (Ma et al., 2020) and its emulsion-stabilizer effect (Su et al., 2024; Yu et al., 2022).

Interestingly, despite the faster release rate (higher *D*) in simulant D1, indicating a more pronounced interaction of the simulant with the

Table 3
Diffusion (*D*) and partition (*K*) coefficients of LAE released from active PHBV-LAE films towards food simulants A (10 % ethanol), B (3 % acetic acid), and D1 (50 % ethanol), at 6 °C, 20 °C, and 30 °C.

Temperature	*D* ($m^2/s \times 10^{-15}$)			*K*		
	A (10 % EtOH)	B (3 % Acetic Acid)	D1 (50 % EtOH)	A (10 % EtOH)	B (3 % Acetic Acid)	D1 (50 % EtOH)
6 °C	0.88 ± 0.67^{cB}	0.33 ± 0.05^{bB}	11.84 ± 2.50^{bA}	0.010 ± 0.001^{aC}	0.020 ± 0.004^{abB}	0.384 ± 0.003^{aA}
20 °C	2.68 ± 0.37^{bB}	0.89 ± 0.11^{aB}	28.88 ± 7.17^{aA}	0.001 ± 0.001^{aB}	0.006 ± 0.001^{bB}	0.384 ± 0.056^{aA}
30 °C	5.03 ± 0.86^{aB}	1.24 ± 0.25^{aB}	37.78 ± 8.47^{aA}	0.001 ± 0.001^{aB}	0.048 ± 0.027^{aB}	0.390 ± 0.056^{aA}

Different lowercase letters in the same column indicate significant differences between temperatures within the same simulant. Different uppercase letters in the same row denote significant differences between releases in the three simulants at the same temperature.

polymer matrix, the release extent at equilibrium was considerably lower (higher K coefficient) than in more polar solvents, indicating a lower affinity of LAE to simulant D1.

Similarly, Requena et al. (2017) reported a slower release rate of eugenol and carvacrol from PHBV in simulants A and B compared to simulant D1. However, in contrast to our findings, they reported a total extraction of the compounds in simulant D1, probably due to the lower polarity of essential oils compared to LAE and their greater affinity with ethanol. Additionally, Patiño Vidal et al. (2022) reported a lower extent of LAE release (higher K) in 10 % EtOH than 95 % EtOH, where practically total extraction of LAE from PLA was observed. This could be explained by a greater LAE affinity to ethanol than PLA matrix (Patiño Vidal et al., 2022). Although we did not investigate 95 % ethanol, our results indicate a higher extent of release towards 10 % ethanol than 50 %, possibly due to the greater hydrophobicity of PHBV compared to PLA (Kim & Masuoka, 2009), resulting in a higher LAE affinity to PHBV than to PLA. This implies that, despite LAE is soluble in ethanol, its affinity to PHBV may hinder its release.

3.5. Mathematical modeling

Table 4 presents the goodness of fit for the most suitable mathematical models applied to LAE migration data, specifically the Weibull models, which were selected based on statistical criteria such ad R^2_{adj}, AIC, and MSC. As mentioned in *Section 2.6*, nine models were preliminarily tested, and after statistical evaluation, six were discarded due to poor fitting. Table S1 (Supplementary Material) shows the statistical criteria for all the models tested, facilitating the selection of the best fitting model for each temperature and food simulant condition. The goodness of fit is determined by the highest value of R^2_{adj} and MSC, and lowest value of AIC (Zhang et al., 2010). However, the choice of the best-fitting model should consider both statistical accuracy and real limitations, ensuring that the amount of the active compound does not exceed 100 % of the compound loaded into the polymer. For instance, the Korsmeyer-Peppas model was statistically suitable for fitting data at 20 °C in simulant A, but its application led to unrealistic predictions of released LAE amount exceeding 100 % and never reaching an equilibrium. Same behavior was observed for Higuchi and four-parameter Weibull models at 30 °C and 6 °C in simulant A. In such cases, we selected the two-parameter Weibull model, showing good statistical criteria and respecting the actual limitation of a maximum released amount of the active compound of 100 %. The Weibull model has been applied to describe various non-linear phenomena, such as microbial destruction, chemical changes, and release kinetic of active compounds (de Jesús Martín-Camacho, Rodríguez-Barajas, Sánchez-Burgos, & Pérez-Larios, 2023; Marvdashti et al., 2019).

Based on both statistical accuracy and real limitations, we selected the Weibull models with two, three, and four parameters as the most suitable among the nine models tested for fitting release data. As shown in Table 4, a good fitting was achieved, with R^2_{adj} between 0.95 and 0.99, except for simulant A at 20 °C, with an R^2_{adj} of 0.88. The kinetic parameters obtained from the best-fitting models are presented in Table 5. These parameters, along with the equations of the kinetic models (Table 1), enable the prediction of LAE release from PHBV films in various foods, represented by the food simulants.

Fig. 3 illustrates diffusion profiles of LAE from PHBV films in various food simulants, showing both experimental and fitted data, calculated through the best-fitting model for each experimental condition, using kinetic parameters from Table 5.

The scale parameter (α) of the Weibull model defines the time scale of the process. We observed an increase in α with greater release extent in simulants A and B. The shape parameter (β), indicative of the release rate, was lower in simulant D1, having the fastest release rate. β values of the best-fitting models were below 1, indicating a parabolic curve (de Jesús Martín-Camacho, Rodríguez-Barajas, Sánchez-Burgos, & Pérez-Larios, 2023; Zhang et al., 2010). Additionally, $\beta < 0.75$ represents a Fickian diffusion, while $\beta = 1$ indicates that the Weibull model reduces to first-order kinetics, and for $\beta > 1$ the release process follows a sigmoidal curve (Liew et al., 2023; Marvdashti et al., 2019). As the Weibull models do not discriminate between Fickian and quasi-Fickian diffusion, considering the Korsmeyer-Peppas model, often used in drug release from swellable polymers, can enable the identification of the release mechanism through the diffusional exponent n (Siepmann & Siepmann, 2008). The n parameters of the Korsmeyer-Peppas model found in our study are shown in Table S2 (Supplementary File). For $n = 0.5$ the process represents Fickian diffusion, for $n < 0.5$ the diffusion is quasi-Fickian, while for $0.5 < n < 1$ the mechanism is non-Fickian or anomalous transport (Requena et al., 2017). For $n = 1$, solute diffusion directly correlates with time, and the mechanism is polymer swelling, while for $n > 1$, the solute is released in subsequent stages (Requena et al., 2017). In our study, the n value was below 0.5 across all temperatures and simulants, indicating a quasi-Fickian diffusion mechanism.

3.6. Effectiveness of active films

The antimicrobial effectiveness of the developed PHBV-LAE films against *Pseudomonas* sp. in BHI broth and almond beverage is illustrated in Fig. 4. The initial microbial concentration, quantified before incubation, was 4.3 ± 0.1 $\log_{10}$ CFU/mL.

After incubation for 16 h at 30 °C, the film-free inoculated medium and the control PHBV exhibited similar growth of *Pseudomonas* sp., with counts ranging from 7.4 to 8.7 $\log_{10}$ CFU/mL in both culture media. In BHI broth, the active PHBV-LAE films exhibited counts of 0.7 $\log_{10}$ CFU/

Table 4
Statistical criteria for the models selected for LAE migration, in simulant A (10 % ethanol), B (3 % acetic acid), and D1 (50 % ethanol) at 6 °C, 20 °C, and 30 °C. Values in bold represent the criteria of the best fitting model for each temperature and simulant combination.

Model	Statistical criterion	6 °C			20 °C			30 °C		
		D1	A	B	D1	A	B	D1	A	B
Weibull_1	R^2_{adj}	0.949	0.963	0.988	0.891	0.874	0.981	0.879	0.958	**0.990**
	AIC	64.354	57.523	38.974	63.728	85.058	52.509	86.532	91.269	**56.132**
	MSC	2.654	2.981	4.101	1.897	1.746	3.645	1.830	2.896	**4.340**
Weibull_2	R^2_{adj}	0.801	**0.965** *	**0.988**	0.724	**0.877** *	**0.982**	0.816	**0.956** *	0.990
	AIC	78.611	**56.334**	**37.874**	73.282	**84.061**	**51.310**	91.217	**91.253**	56.133
	MSC	1.358	**3.089**	**4.201**	1.029	**1.836**	**3.754**	1.470	**2.897**	4.340
Weibull_4	R^2_{adj}	**0.982**	0.961	0.988	**0.946**	0.903	0.980	**0.976**	0.984	0.989
	AIC	**53.373**	58.771	39.362	**56.482**	82.718	53.373	**66.063**	79.633	57.905
	MSC	**3.652**	2.868	4.066	**2.556**	1.959	3.566	**3.405**	3.791	4.204

Notes: R^2_{adj}: adjusted coefficient of determination; AIC: Akaike Information Criterion; MSC: Model Selection Criterion.
*Model selected due to the limitation of a maximum 100% of the compound released.

Table 5
Kinetic parameters of the Weibull models for LAE migration in simulants A (10 % ethanol), B (3 % acetic acid), and D1 (50 % ethanol) at 6 °C, 20 °C, and 30 °C. Values in bold represent the kinetic parameters of the best fitting model for each temperature-simulant combination.

Temperature	Simulant	Weibull_1			Weibull_2		Weibull_4			
		α	β	Ti	α	β	α	β	Ti	F_{max}
6 °C	D1	1.40	0.18	0.99	2.42	0.36	**1.62**	**0.80**	**0.23**	**71.67**
	A	9.40	0.53	0.27	**10.54**	**0.57**	2487.66	0.43	0.31	27759.51
	B	11.52	0.50	0.18	**12.32**	**0.51**	45715.90	0.42	0.25	410037.32
20 °C	D1	1.18	0.13	0.33	1.49	0.22	**0.81**	**0.57**	**0.16**	**72.49**
	A	40.34	1.33	−4.88	**5.16**	**0.74**	30365.56	0.40	0.22	732979.27
	B	5.30	0.47	0.17	**5.69**	**0.49**	11.91	0.38	0.24	230.99
30 °C	D1	1.43	0.21	0.08	1.61	0.26	**0.72**	**0.64**	**0.03**	**71.05**
	A	7.30	1.08	−0.72	**4.06**	**0.86**	5.14	0.75	−0.17	120.12
	B	**4.53**	**0.44**	**0.06**	4.76	0.46	5.20	0.42	0.07	114.79

Explanatory notes as in Table 1.

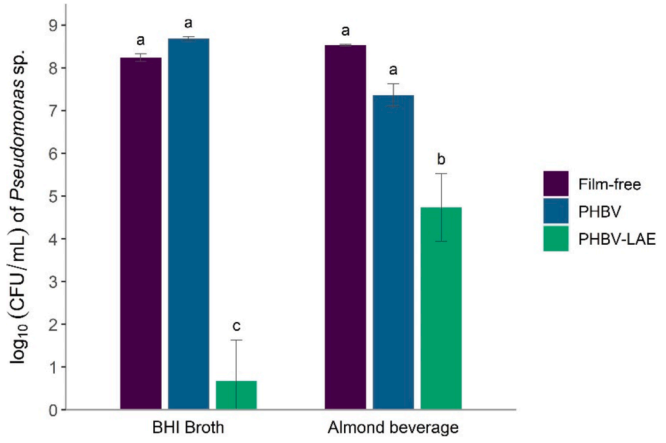

Fig. 4. Antimicrobial effectiveness of active PHBV-LAE films in BHI broth and almond beverage inoculated with *Pseudomonas* sp., compared to control PHBV films and film-free inoculated media after 16 h of incubation. The microbial count for all samples immediately after inoculation, quantified prior to incubation, was 4.3 ± 0.1 $\log_{10}$ CFU/mL. Different lowercase letters indicate significant differences among samples, as determined by the two-way ANOVA.

mL, which was 7.6 log lower than the control PHBV, indicating significant microbial inhibition ($p < 0.05$). When tests were performed in almond beverage as a real food system, the active films showed microbial counts of 4.7 $\log_{10}$ CFU/mL, indicating almost no growth of the inoculated microorganism. These results suggest that, even in almond beverage, *Pseudomonas* sp. counts were significantly reduced with PHBV-LAE films compared to neat PHBV ($p < 0.05$), specifically by 2.4 log. The lower effectiveness of active films in food compared to broth medium has been previously reported and is attributed to interactions between LAE and food components, such as fats, polysaccharides, and proteins, that may reduce the number of free LAE molecules available to act against bacteria in the aqueous phase (Loeffler et al., 2020; Ma et al., 2020).

Although the antimicrobial efficacy of the active films was reduced in the food matrix, the microbial inhibition attained was still satisfactory. In line with our findings, previous studies demonstrated that the growth of *Pseudomonas putida* decreased significantly with the application of active packaging incorporating LAE, specifically polystyrene pads (Manso et al., 2021) and chitosan films (Higueras et al., 2013), which resulted in reductions by 3 and 2.6 $\log_{10}$, respectively.

The LAE released into the media exerts an antimicrobial effect against *Pseudomonas* sp. due to its surfactant properties, which allow LAE to interact with lipopolysaccharides in the Gram-negative cell wall, as well as with anionic microbial proteins of cell membranes or enzymatic systems. This leads to denaturation, increased membrane permeability, and thus to cell death (Dong et al., 2023; Zhuang et al., 2020). Additionally, LAE can chelate iron, creating iron-limiting conditions that inhibit biofilm development in *Pseudomonas aeruginosa* (Kim et al., 2017).

4. Conclusions

This study introduces an active packaging system utilizing the bioplastic PHBV incorporating the antimicrobial compound LAE. The overall migration test revealed migration values for neat PHBV near to the limit, and overall migration values were exceeded for the active films due to glycerol included in the LAE formulation. Therefore, the utilization of different formulations without glycerol is recommended for future development of LAE-based active packaging systems.

Our results confirmed that the diffusion and release rate of the antimicrobial compound are affected by temperature, food simulant, by the physical interactions between polymer matrix and solvents and by the chemical affinity between the active agent and both the polymer and the simulant. Polymer and simulant polarity, as well as temperature, impact the polymer impregnation and molecular mobility, therefore the active compound diffusion and release rate. Additionally, the partition coefficient of the active compound is affected by its solubility in the solvent and its affinity to the polymer.

Contact angle measurements underscored a higher affinity of PHBV for ethanol, leading to enhanced polymer interaction with the solvent, higher swelling and increased release rates of the active compound, as testified by the higher D value observed for simulant D1. The partition coefficients pointed out a lower retention of the active compound in the polymer matrix at equilibrium and higher LAE released in more polar solvents (i.e. 10 % ethanol and 3 % acetic acid) compared to 50 % ethanol. Hence, PHBV-LAE active systems are expected to exhibit a faster release rate towards less polar foods (simulant D1), such as alcoholic beverages and oil-in-water emulsions, and a slower but higher extent of release towards more polar foods (simulants A and B) such as non-alcoholic beverages and juices.

Among the models tested, the Weibull models appeared as the most suitable for fitting release data, and the Korsmeyer-Peppas model enabled the identification of the LAE release mechanism from PHBV as quasi-Fickian diffusion.

Ultimately, the application of the developed PHBV-LAE active films under a worst-case scenario demonstrated significant antimicrobial efficacy in both broth medium and real food matrix, indicating promising applications for these films. Our findings underscored that, compared to more polar polymers studied previously, PHBV may enable a slower and sustained LAE release, ensuring an extended antimicrobial effect as microbial growth occurs in foodstuffs, enhancing food safety and shelf life extension. Our findings pave the way for the development and the

application of PHBV-LAE active films as a biodegradable and effective food packaging material.

CRediT authorship contribution statement

Carola Nicosia: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Fabio Licciardello:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Dr. Francesco Goto for his excellent assistance with the calculation of diffusion coefficients.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2024.115345.

Data availability

Data will be made available on request.

References

Almasi, H., Jahanbakhsh Oskouie, M., & Saleh, A. (2021). A review on techniques utilized for design of controlled release food active packaging. *Critical Reviews in Food Science and Nutrition, 61*(15), 2601–2621. https://doi.org/10.1080/10408398.2020.1783199

Angellier-Coussy, H., Kemmer, D., Gontard, N., & Peyron, S. (2020). Physical–chemical and structural stability of PHBV/wheat straw fibers based biocomposites under food contact conditions. *Journal of Applied Polymer Science, 137*(40), 1–15. https://doi.org/10.1002/app.49231

ASTM. (2013). ASTM E2149-01: Standard Test Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions. *ASTM International, 5–8*. https://doi.org/10.1520/E2149-13A

Aznar, M., Gómez-estaca, J., Vélez, D., Devesa, V., & Nerín, C. (2013). Migrants determination and bioaccessibility study of ethyl lauroyl arginate (LAE) from a LAE based antimicrobial food packaging material. *Food and Chemical Toxicology, 56*, 363–370. https://doi.org/10.1016/j.fct.2013.02.018

Bigi, F., Maurizzi, E., Haghighi, H., Siesler, H. W., Licciardello, F., & Pulvirenti, A. (2023). Waste Orange Peels as a Source of Cellulose Nanocrystals and Their Use for the Development of Nanocomposite Films. *Foods, 12*, 960. https://doi.org/10.3390/foods12050960

Bruni, J., Licciardello, F., Gaubiac, F., & Coffigniez, F. (2024). Tailored antimicrobial PHBV-based packaging for extended shelf life of processed cheese. *Food Packaging and Shelf Life, 44*, Article 101319. https://doi.org/10.1016/j.fpsl.2024.101319

Chea, V., Angellier-Coussy, H., Peyron, S., Kemmer, D., & Gontard, N. (2016). Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) films for food packaging: Physical–chemical and structural stability under food contact conditions. *Journal of Applied Polymer Science, 133*(2). https://doi.org/10.1002/app.41850

Crank, J. (1975). The mathematics of diffusion (2nd ed.). Clarendon Press, Oxford. doi: https://www-eng.lbl.gov/~shuman/NEXT/MATERIALS&COMPONENTS/Xe_damage/Crank-The-Mathematics-of-Diffusion.pdf.

da Costa, R. C., Daitx, T. S., Mauler, R. S., da Silva, N. M., Miotto, M., Crespo, J. S., & Carli, L. N. (2020). Poly(hydroxybutyrate-co-hydroxyvalerate)-based nanocomposites for antimicrobial active food packaging containing oregano essential oil. *Food Packaging and Shelf Life, 26*, Article 100602. https://doi.org/10.1016/j.fpsl.2020.100602

de Jesús Martín-Camacho, U. J., Rodríguez-Barajas, N., Sánchez-Burgos, J. A., & Pérez-Larios, A. (2023). Weibull β value for the discernment of drug release mechanism of PLGA particles. *International Journal of Pharmaceutics, 640*, Article 123017. https://doi.org/10.1016/j.ijpharm.2023.123017

Dıblan, S., & Kaya, S. (2018). Antimicrobials Used in Active Packaging Films. *Food and Health, 4*(1), 63–79. https://doi.org/10.3153/jfhs18007

Dong, Q., Yang, L., Xiang, L., Zhao, Y., & Li, L. (2023). Development of electrospun fish gelatin film containing lauroyl arginate ethyl and its application in large yellow croaker (Pseudosciaena crocea) preservation. *Food Control, 153*, Article 109959. https://doi.org/10.1016/j.foodcont.2023.109959

EFSA (European Food Safety Authority). (2007). Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to an application on the use of ethyl lauroyl arginate as a food additive. *EFSA Journal, 5*(7), 1–27. https://doi.org/10.2903/j.efsa.2007.511

FAO. (2011). *Global food losses and food waste – Extent, causes and prevention*. 1–204. http://www.fao.org/docrep/014/mb060e/mb060e00.pdf.

Gaikwad, K. K., Lee, S. M., Lee, J. S., & Lee, Y. S. (2017). Development of antimicrobial polyolefin films containing lauroyl arginate and their use in the packaging of strawberries. *Journal of Food Measurement and Characterization, 11*(4), 1706–1716. https://doi.org/10.1007/s11694-017-9551-0

Gamarra-Montes, A., Missagia, B., Morató, J., & Muñoz-Guerra, S. (2018). Antibacterial Films Made of Ionic Complexes of Poly(γ-glutamic acid) and Ethyl Lauroyl Arginate. *Polymers, 10*(1), 21. https://doi.org/10.3390/polym10010021

Gouda, R., Baishya, H., & Qing, Z. (2017). Application of Mathematical Models in Drug Release Kinetics of Carbidopa and Levodopa ER Tablets. *Journal of Developing. Drugs, 06*(02). https://doi.org/10.4172/2329-6631.1000171

Gracia-Vallés, N., Ruiz-Torrubia, F., Mitchell, S. G., Nerín, C., & Silva, F. (2022). Developing ethyl lauroyl arginate antimicrobial films to combat Listeria monocytogenes in cured ham. *Food Control, 141*, Article 109164. https://doi.org/10.1016/j.foodcont.2022.109164

Guo, M., Jin, T. Z., Wang, L., Scullen, O. J., & Sommers, C. H. (2014). Antimicrobial films and coatings for inactivation of Listeria innocua on ready-to-eat deli turkey meat. *Food Control, 40*(1), 64–70. https://doi.org/10.1016/j.foodcont.2013.11.018

Haghighi, H., De Leo, R., Bedin, E., Pfeifer, F., Siesler, H. W., & Pulvirenti, A. (2019). Comparative analysis of blend and bilayer films based on chitosan and gelatin enriched with LAE (lauroyl arginate ethyl) with antimicrobial activity for food packaging applications. *Food Packaging and Shelf Life, 19*, 31–39. https://doi.org/10.1016/j.fpsl.2018.11.015

Haghighi, H., Leugoue, S. K., Pfeifer, F., Siesler, H. W., Licciardello, F., Fava, P., & Pulvirenti, A. (2020). Development of antimicrobial films based on chitosan-polyvinyl alcohol blend enriched with ethyl lauroyl arginate (LAE) for food packaging applications. *Food Hydrocolloids, 100*, Article 105419. https://doi.org/10.1016/j.foodhyd.2019.105419

Hassan, A. H. A., & Cutter, C. N. (2020). Development and evaluation of pullulan-based composite antimicrobial films (CAF) incorporated with nisin, thymol and lauric arginate to reduce foodborne pathogens associated with muscle foods. *International Journal of Food Microbiology, 320*, Article 108519. https://doi.org/10.1016/j.ijfoodmicro.2020.108519

Hernández-García, E., Vargas, M., Chiralt, A., & González-Martínez, C. (2022). Biodegradation of PLA-PHBV Blend Films as Affected by the Incorporation of Different Phenolic Acids. *Foods, 11*(2), 243. https://doi.org/10.3390/foods11020243

Higueras, L., López-Carballo, G., Hernández-Muñoz, P., Gavara, R., & Rollini, M. (2013). Development of a novel antimicrobial film based on chitosan with LAE (ethyl-Nα-dodecanoyl-l-arginate) and its application to fresh chicken. *International Journal of Food Microbiology, 165*, 339–345. https://doi.org/10.1016/j.ijfoodmicro.2013.06.003

Hoa, V. B., Song, D. H., Seol, K. H., Kang, S. M., Kim, H. W., Kim, J. H., & Cho, S. H. (2022). Coating with chitosan containing lauric acid (C12:0) significantly extends the shelf-life of aerobically – Packaged beef steaks during refrigerated storage. *Meat Science, 184*, Article 108696. https://doi.org/10.1016/j.meatsci.2021.108696

Kashiri, M., Cerisuelo, J. P., Domínguez, I., López-Carballo, G., Hernández-Muñoz, P., & Gavara, R. (2016). Novel antimicrobial zein film for controlled release of lauroyl arginate (LAE). *Food Hydrocolloids, 61*, 547–554. https://doi.org/10.1016/j.foodhyd.2016.06.012

Kashiri, M., López-Carballo, G., Hernández-Muñoz, P., & Gavara, R. (2019). Antimicrobial packaging based on a LAE containing zein coating to control foodborne pathogens in chicken soup. *International Journal of Food Microbiology, 306*, Article 108272. https://doi.org/10.1016/j.ijfoodmicro.2019.108272

Kim, M. C., & Masuoka, T. (2009). Degradation properties of PLA and PHBV films treated with CO2-plasma. *Reactive and Functional Polymers, 69*(5), 287–292. https://doi.org/10.1016/j.reactfunctpolym.2009.01.013

Kim, T. S., Ham, S. Y., Park, B. B., Byun, Y., & Park, H. D. (2017). Lauroyl arginate ethyl blocks the iron signals necessary for Pseudomonas aeruginosa biofilm development. *Frontiers in Microbiology, 8*, 970. https://doi.org/10.3389/fmicb.2017.00970

Kowalczyk, D., Kordowska-Wiater, M., Karaś, M., Zięba, E., Mężyńska, M., & Wiącek, A. E. (2020). Release kinetics and antimicrobial properties of the potassium sorbate-loaded edible films made from pullulan, gelatin and their blends. *Food Hydrocolloids, 101*, Article 105539. https://doi.org/10.1016/j.foodhyd.2019.105539

Kowalczyk, D., Pytka, M., Szymanowska, U., Skrzypek, T., Łupina, K., & Biendl, M. (2020). Release kinetics and antibacterial activity of potassium salts of iso-α-acids loaded into the films based on gelatin, carboxymethyl cellulose and their blends. *Food Hydrocolloids, 109*, Article 106104. https://doi.org/10.1016/j.foodhyd.2020.106104

Kunam, P. K., Ramakanth, D., Akhila, K., & Gaikwad, K. K. (2022). Bio-based materials for barrier coatings on paper packaging. *Biomass Conversion and Biorefinery, 14*(12), 12637–12652. https://doi.org/10.1007/s13399-022-03241-2

Li, T., Liu, Y., Qin, Q., Zhao, L., Wang, Y., Wu, X., & Liao, X. (2021). Development of electrospun films enriched with ethyl lauroyl arginate as novel antimicrobial food packaging materials for fresh strawberry preservation. *Food Control, 130*, Article 108371. https://doi.org/10.1016/j.foodcont.2021.108371

Liew, W. C., Muhamad, I. I., Chew, J. W., & Karim, K. J. A. (2023). Synergistic effect of graphene oxide/zinc oxide nanocomposites on polylactic acid-based active packaging film: Properties, release kinetics and antimicrobial efficiency. *International*

Journal of Biological Macromolecules, 253(P6), Article 127288. https://doi.org/10.1016/j.ijbiomac.2023.127288

Loeffler, M., Schwab, V., Terjung, N., Weiss, J., & Julian McClements, D. (2020). Influence of protein type on the antimicrobial activity of LaE alone or in combination with methylparaben. *Foods, 9*, 270. https://doi.org/10.3390/foods9030270

Lopez, M. E. S., Gontijo, M. T. P., Boggione, D. M. G., Albino, L. A. A., Batalha, L. S., & Mendonça, R. C. S. (2018). Microbiological Contamination in Foods and Beverages: Consequences and Alternatives in the Era of Microbial Resistance. In *Microbial Contamination and Food Degradation* (pp. 49–84). Elsevier Inc.. https://doi.org/10.1016/b978-0-12-811515-2.00003-2

Ma, Q., Davidson, P. M., & Zhong, Q. (2020). Properties and potential food applications of lauric arginate as a cationic antimicrobial. *International Journal of Food Microbiology, 315*, Article 108417. https://doi.org/10.1016/j.ijfoodmicro.2019.108417

Ma, Q., Zhang, Y., & Zhong, Q. (2016). Physical and antimicrobial properties of chitosan films incorporated with lauric arginate, cinnamon oil, and ethylenediaminetetraacetate. *LWT - Food Science and Technology, 65*, 173–179. https://doi.org/10.1016/j.lwt.2015.08.012

Manso, S., Wrona, M., Salafranca, J., Nerín, C., Alfonso, M. J., & Caballero, M.Á. (2021). Evaluation of new antimicrobial materials incorporating ethyl lauroyl arginate or silver into different matrices, and their safety in use as potential packaging. *Polymers, 13*, 355. https://doi.org/10.3390/polym13030355

Marvdashti, L. M., Yavarmanesh, M., & Koocheki, A. (2019). In vitro release study of nisin from active polyvinyl alcohol-Alyssum homolocarpum seed gum films at different temperatures. *Polymer Testing, 79*, Article 106032. https://doi.org/10.1016/j.polymertesting.2019.106032

Meereboer, K. W., Misra, M., & Mohanty, A. K. (2020). Review of recent advances in the biodegradability of polyhydroxyalkanoate (PHA) bioplastics and their composites. *Green Chemistry, 22*(17), 5519–5558. https://doi.org/10.1039/d0gc01647k

Moreno, O., Atarés, L., Chiralt, A., Cruz-romero, M. C., & Kerry, J. (2018). Starch-gelatin antimicrobial packaging materials to extend the shelf life of chicken breast fillets. *LWT - Food Science and Technology, 97*, 483–490. https://doi.org/10.1016/j.lwt.2018.07.005

Moreno, O., Gil, À., Atarés, L., & Chiralt, A. (2017). Active starch-gelatin films for shelf-life extension of marinated salmon. *LWT - Food Science and Technology, 84*, 189–195. https://doi.org/10.1016/j.lwt.2017.05.005

Muriel-Galet, V., López-Carballo, G., Gavara, R., & Hernández-Muñoz, P. (2015). Antimicrobial Effectiveness of Lauroyl Arginate Incorporated into Ethylene Vinyl Alcohol Copolymers to Extend the Shelf-Life of Chicken Stock and Surimi Sticks. *Food and Bioprocess Technology, 8*(1), 208–217. https://doi.org/10.1007/s11947-014-1391-x

Muriel-Galet, V., López-Carballo, G., Hernández-Muñoz, P., & Gavara, R. (2014). Characterization of ethylene-vinyl alcohol copolymer containing lauril arginate (LAE) as material for active antimicrobial food packaging. *Food Packaging and Shelf Life, 1*, 10–18. https://doi.org/10.1016/j.fpsl.2013.09.002

Patiño Vidal, C., Luzi, F., Puglia, D., López-Carballo, G., Rojas, A., Galotto, M. J., & López De Dicastillo, C. (2023). Development of a sustainable and antibacterial food packaging material based in a biopolymeric multilayer system composed by polylactic acid, chitosan, cellulose nanocrystals and ethyl lauroyl arginate. *Food Packaging and Shelf Life, 36*, Article 101050. https://doi.org/10.1016/j.fpsl.2023.101050

Patiño Vidal, C., Velásquez, E., Galotto, M. J., & López de Dicastillo, C. (2022). Development of an antibacterial coaxial bionanocomposite based on electrospun core/shell fibers loaded with ethyl lauroyl arginate and cellulose nanocrystals for active food packaging. *Food Packaging and Shelf Life, 31*, Article 100802. https://doi.org/10.1016/j.fpsl.2021.100802

Pattanayaiying, R., H-Kittikun, A., & Cutter, C. N. (2015). Incorporation of nisin Z and lauric arginate into pullulan films to inhibit foodborne pathogens associated with fresh and ready-to-eat muscle foods. *International Journal of Food Microbiology, 207*, 77–82. https://doi.org/10.1016/j.ijfoodmicro.2015.04.045

Quintieri, L., Caputo, L., Brasca, M., & Fanelli, F. (2021). Recent Advances in the Mechanisms and Regulation of QS in Dairy Spoilage by Pseudomonas spp. *Foods, 10*, 3088. https://doi.org/10.3390/foods10123088

Regulation 1333/2008. *Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives*. https://eur-lex.europa.eu/search.html?scope=EURLEX&text=1333%2F2008&lang=it&type=quick&qid=1684425706771.

Regulation 450/2009. Commission Regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:135:0003:0011:EN:PDF.

Regulation 10/2011. *Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food*. https://eur-lex.europa.eu/search.html?scope=EURLEX&text=10%2F2011&lang=it&type=quick&qid=1684428637411.

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

Rivera-Hernández, L., Chavarría-Hernández, N., del López Cuellar, M. R., Martínez-Juárez, V. M., & Rodríguez-Hernández, A. I. (2021). Pectin-gellan films intended for active food packaging: Release kinetics of nisin and physico-mechanical characterization. *Journal of Food Science and Technology, 58*(8), 2973–2981. https://doi.org/10.1007/s13197-020-04800-z

Rojas, A., Velásquez, E., Piña, C., Galotto, M. J., & López de Dicastillo, C. (2021). Designing active mats based on cellulose acetate/polycaprolactone core/shell structures with different release kinetics. *Carbohydrate Polymers, 261*, Article 117849. https://doi.org/10.1016/j.carbpol.2021.117849

Siepmann, J., & Siepmann, F. (2008). Mathematical modeling of drug delivery. *International Journal of Pharmaceutics, 364*(2), 328–343. https://doi.org/10.1016/j.ijpharm.2008.09.004

Soares da Silva, F. A. G., Matos, M., Dourado, F., Reis, M. A. M., Branco, P. C., Poças, F., & Gama, M. (2022). Development of a layered bacterial nanocellulose-PHBV composite for food packaging. *Journal of the Science of Food and Agriculture, 103*(3), 1077–1087. https://doi.org/10.1002/jsfa.11839

Su, S., Yin, X., Du, W., Han, L., Wu, Q., & Zhang, H. (2024). Novel concentrated O/W emulsion co-stabilized by like-charged chitosan nanoparticle and ethyl lauroyl arginate (LAE) surfactant at very low dosages. *LWT - Food Science and Technology, 191*, Article 115692. https://doi.org/10.1016/j.lwt.2023.115692

U.S. Food and Drug Administration. (2005). *GRAS exemption claim for Lauramide Arginine Ethyl Ester (LAE) Added to Food to Inhibit Microbial Growth (GRN 000164)*. 3–240. doi: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=GRASNotices&id=164.

Vial, J., & Jardy, A. (1999). Experimental comparison of the different approaches to estimate LOD and LOQ of an HPLC method. *Analytical Chemistry, 71*(14), 2672–2677. https://doi.org/10.1021/ac981179n

Wang, Y., Lu, L., Zheng, Y., & Chen, X. (2006). Improvement in hydrophilicity of PHBV films by plasma treatment. *Journal of Biomedical Materials Research - Part A, 76*(3), 589–595. https://doi.org/10.1002/jbm.a.30575

Wu, F., Zhou, Z., Li, N., Chen, Y., Zhong, L., Law, W. C., & Tang, C. Y. (2021). Development of poly(vinyl alcohol)/starch/ethyl lauroyl arginate blend films with enhanced antimicrobial and physical properties for active packaging. *International Journal of Biological Macromolecules, 192*, 389–397. https://doi.org/10.1016/j.ijbiomac.2021.09.208

Yoon, Y. I., Moon, H. S., Lyoo, W. S., Lee, T. S., & Park, W. H. (2008). Superhydrophobicity of PHBV fibrous surface with bead-on-string structure. *Journal of Colloid and Interface Science, 320*(1), 91–95. https://doi.org/10.1016/j.jcis.2008.01.029

Yu, D., Luo, Q., Yang, C., Yang, C., Li, S., Wang, Z., Wang, Q., Liu, W., Wang, H., & Ji, D. (2022). Electrostatic complexes of ethyl lauroyl arginate/nano-montmorillonite as a food-grade pickering stabiliser: Emulsification performance and mechanism. *LWT - Food Science and Technology, 168*, Article 113934. https://doi.org/10.1016/j.lwt.2022.113934

Zhang, Y., Huo, M., Zhou, J., Zou, A., Li, W., Yao, C., & Xie, S. (2010). DDSolver: An add-in program for modeling and comparison of drug dissolution profiles. *The AAPS Journal, 12*(3), 263–271. https://doi.org/10.1208/s12248-010-9185-1

Zhu, X. H., Wang, C. H., & Tong, Y. W. (2009). In vitro characterization of hepatocyte growth factor release from PHBV/PLGA microsphere scaffold. *Journal of Biomedical Materials Research - Part A, 89*(2), 411–423. https://doi.org/10.1002/jbm.a.31978

Zhuang, S., Li, Y., Hong, H., Liu, Y., Shu, R., & Luo, Y. (2020). Effects of ethyl lauroyl arginate hydrochloride on microbiota, quality and biochemical changes of container-cultured largemouth bass (Micropterus salmonides) fillets during storage at 4 °C. *Food Chemistry, 324*, Article 126886. https://doi.org/10.1016/j.foodchem.2020.126886