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ORIGINAL ARTICLE

Release of oregano essential oil from PHBV films in simulated food conditions[a]

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Abstract

Poly(hydroxybutyrate-*co*-hydroxyvalerate) – PHBV plays an important role in sustainability and food safety. In this work, active packaging with antimicrobial properties was analyzed for the controlled release of active compound in three environments (acidic aqueous foods, fresh foods, and fatty foods). The compositions were produced with the addition of sepiolite nanoparticles (Sep) and oregano essential oil (OEO). The GC-MS analysis detected the presence of 3-methyl-4-isopropyl phenol as the primary constituent of the OEO (71.7%). The characterization of the films by FTIR and SEM confirmed the presence of additives, and the quantification of OEO and thermal stability of the nanocomposites was verified by TGA. Four kinetic models were used to analyze the release profile. Our findings indicate that it is possible to adjust the kinetic release of the OEO by varying the composition of the films, which is a promising alternative for producing an antibacterial biomaterial for application in food packaging.

Keywords: *biodegradable polymer, controlled release, food packaging, polymer nanocomposites, oregano essential oil.*

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1. Introduction

The demand for plastic packaging has been increasing considerably in the last few years, aiming at the food security associated with epidemiological outbreaks and the control of the internal environment of packaging material, thus increasing the shelf life of food. In this scenario, active and biodegradable packaging development arises as a promising strategy for application in ready-to-eat and fresh foods[1]. These packages have as their main characteristics the use of an antimicrobial agent in the packaging material, as well as excellent degradation capacity under proper disposal[2-4].

Essential oils (EO) are highlighted in several active packaging researches due to their plasticizing effect[2] and excellent antimicrobial properties[5]. Regarding the degradation capacity, poly(hydroxybutyrate-*co*-hydroxyvalerate) – PHBV is a bacterial polyester from the polyhydroxyalkanoates family, known to be 100% biodegradable. This biopolymer has physical properties comparable to other thermoplastic materials, which allows its use in several areas, such as biomedical[6,7] and industrial applications[2,4,8]. Some drawbacks such as thermal stability, gas permeability, and brittleness might be overcome by incorporating additives[9,10]. One strategy might be the incorporation of clay nanoparticles, which results in the formation of a barrier to the permeation of gaseous molecules responsible for the loss of texture, color, and odor of food[2], besides being an alternative to control the release of antimicrobial agents.

The development of films for application in food packaging must strongly consider the type of food with which the polymeric material will come into contact since each food has specific polarity, acidity, and humidity conditions. Commission Regulation (EU) No. 10/2011[11] establishes normative conditions for simulation studies for application in food. For example, the food simulator based on 10% ethanol (v/v) should be used for hydrophilic foods. A 3% acetic acid (w/v) medium is used for food with a hydrophilic nature and a pH below 4.5, while a vegetable oil medium such as isooctane may be used for greasy foods[4]. Thus, the application of this normative allows the evaluation of the release of active compounds over time and the combined effect of the antimicrobial with nanoparticles, which might act as a barrier in PHBV films.

The diffusion kinetics of an active compound to a medium might be affected by the type and thickness of the polymer, as well as by the nature and initial concentration of additives in it, the type of surrounding medium, the time-temperature conditions of contact[7], and, particularly in the case of food packaging, by the method of food processing and preservation. As a low molecular weight

compound of lipophilic nature, essential oils tend to migrate into packaged foods, mainly those with high fat content[12]. Therefore, the release kinetics of active compounds into the food throughout its storage time is a decisive factor in guaranteeing antimicrobial effectiveness and food safety[13].

In this context, this work investigates the release of the active antimicrobial compound in different food simulating media of PHBV films modified with sepiolite nanoparticles and oregano essential oil. Developing these systems is a promising way to produce functional biodegradable materials for application in active packaging.

2. Materials and Methods

2.1 Materials

The PHBV was supplied by Ningbo Tianan Biologic Material Co., Ltd. (ENMAT Y 1000) with a viscosimetric molecular weight of 450,000 g mol^{-1} and a valerate content of 3.4 mol%[2]. The oregano essential oil (OEO) and sepiolite clay (Sep) were obtained from Sigma Aldrich. The acetic acid, ethanol, and isooctane (Vetec) were of analytical grade.

2.2 Melt processing and film production

The polymer and clay were previously dried in an air circulation oven at 80 ºC for 4 h. The PHBV, PHBV/Sep, PHBV/OEO, and PHBV/Sep/OEO compositions were prepared. For each formulation, 3 wt% of clay and 8 wt% of OEO were used. The PHBV nanocomposite films were prepared by melt processing using a Roller-Rotors R600, Rheomix 6002C mixer at 170 ºC and 165 ºC (for the compositions without and with OEO, respectively) at 100 rpm for 6 min[2]. The obtained compounds were then milled in a knife mill (SL-32 Solab Equipamentos) and compression molded at 190 ºC for 2 min and 1 ton in an electrically-heated hydraulic press (SL-11 Solab Equipamentos). All compression molded films showed a homogeneous surface, with an average diameter of 15 cm ± 1 cm and an average thickness of 0.23 mm ± 0.03 mm.

2.3 Characterization of the OEO and PHBV compositions

The OEO composition was evaluated by gas chromatography-mass spectrometry (GC-MS) in Agilent CG 7890A/Agilent 5975C equipment. An ionization voltage of 70 eV and mass ranging from 50 m/z to 300 m/z were used. Helium was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The injector temperature was adjusted from 40 ºC to 145 ºC at 3 °C min^{-1}, then heated again to 280 ºC at 10 ºC min^{-1}.

The morphology of the nanocomposites was examined by scanning electron microscopy (SEM) using JEOL JSM-6390LV equipment at 15 kV. The samples were fractured by immersion in liquid nitrogen and covered by a thin layer of gold. The thermal stability of the films was analyzed by thermogravimetric analysis (TGA) in PerkinElmer TGA 8000 equipment, in the range from 30 °C to 700 ºC at a heating rate of 20 ºC min^{-1} under an argon atmosphere (20 mL min^{-1}). The incorporation efficiency (*IE*) of OEO in the films after processing was determined by Equation 1, where V_O is the volatilized oil up to 270 °C and T_O is the theoretical oil content.

$$IE(\%) = \frac{V_O}{T_O} 100 \quad (1)$$

2.4 OEO release tests and kinetic studies

In order to study different food simulation environments, three immersion solutions were used: (a) 10% ethanol (v/v), (b) 3% acetic acid (w/v), and (c) isooctane. For the OEO release tests, 150 mg of each film composition were immersed in 30 mL of each medium. The system was maintained under continuous stirring in a MK1210 – TR orbital shaker at 24 ºC for 72 h, and aliquots of 2 mL were collected periodically. The amount of OEO released was analyzed by UV spectrophotometry in Shimadzu UV-1800 equipment at 272 nm. For the determination of the OEO release (%), the measured OEO concentration in each formulation from the results obtained in the TGA analysis was considered (Table 1). The experiments were performed in triplicate, and the average and standard deviation were calculated.

The OEO release data in the different food simulating media were computed using DDSolver, a MS-Excel extension plug-in written in Visual Basic for Applications, and the resulting data were fitted to different kinetic models[14]. The models available in DDSolver and applied to the experimental data were the first-order, Higuchi, Korsmeyer-Peppas, and Peppas-Sahlin models[15].

3. Results and Discussions

3.1 Chemical composition of the OEO

The OEO was analyzed according to GC-MS to verify the chemical structures of the major components of the oil. The chromatogram indicated that 71.7% of the composition refers to 3-methyl-4-isopropyl phenol. It was also possible to notice the presence of benzene (14.9%)

Table 1. Degradation temperatures and incorporation efficiency of additives after processing.

Sample	Td_{oil} (ºC)	$T_{5\%}$ (ºC)	Td_{max} (ºC)	OEO content (%)	IE (%)	Clay content (%)
PHBV	-	284	307	-	-	-
PHBV/Sep	-	297	319	-	-	2.8
PHBV/OEO	159	267	311	4.9	62	-
PHBV/Sep/OEO	170	273	317	4.9	61	2.7

Td_{oil}: volatilization temperature of the oil; $T_{5\%}$: initial decomposition temperature (5% of degradation); Td_{max}: maximum degradation rate temperature.

and terpinene (6.9%) in lower quantities. Although most studies indicate the prominent presence of carvacrol or thymol in the composition of oregano oil, it depends on where the plants were cultivated, the mechanism of its extraction, and the region of extraction of the oil[16]. It has been proven that 3-methyl-4-isopropyl phenol has antimicrobial characteristics, being marketed as Biosol®, an active compound with antiseptic properties used mainly in pharmaceutical and personal care products[17].

3.2 Morphology

The morphology of the clay nanoparticles was analyzed by SEM. This needle-shaped nanoparticle (as seen at 1000× magnification and made evident at higher magnifications – 5000× – Figure 1a-1b) has a high specific surface area due to the presence of longitudinal tunnels[18], allowing a small amount of Sep to alter specific properties of interest in PHBV films significantly. Although the reduced size of Sep might also result in some agglomeration in the nanocomposite formation, it was decided not to organically modify its surface to understand the behavior of the filler on the materials used and to produce a solvent-free film for application in food.

The morphology of the fractured surface of PHBV films (cross-section, 1000× magnification) and the distribution of Sep nanoparticles in the polymer matrix were also analyzed. The smooth surface of PHBV – typical of a brittle fracture – has been changed to a rougher surface due to the plasticizing effect of the essential oil. The good distribution of Sep nanoparticles in the polymer matrix is also worth noting. The presence of nanoparticles and their homogeneous distribution might influence the release characteristics of the OEO, as will be discussed. This good dispersion of Sep might be associated with the affinity between the components and the interactions between the hydroxyl groups on the Sep surface, the ester bonds of the PHBV chain, and the phenol group of 3-methyl-4-isopropyl phenol, the primary component of OEO.

3.3 Thermal properties and quantification of additives

The thermogravimetric results (Figure 2) revealed that the thermal decomposition of OEO starts at around 80 ºC, with a maximum mass loss at about 198 ºC. Above 260 ºC, all the oil present in the compositions had already been volatilized (DTG shown in the inset of Figure 2), and the degradation behavior follows the expected for PHBV films[19]. For PHBV, only one mass loss is observed in the range of 285 °C to 319 ºC associated with the degradation of the polymeric matrix.

In the TGA curve of compositions PHBV/OEO and PHBV/Sep/OEO, a mass loss was observed related to the volatilization of oregano essential oil in the initial stages of the analysis. This behavior does not compromise the application of interest, being fundamental for quantifying the OEO in the produced films.

The incorporation efficiency of the OEO was considered satisfactory, reaching 62% for the PHBV/OEO and 61% for the PHBV/Sep/OEO films (Table 1). The partial volatilization of OEO during the melt processing (165 °C) and compression molding (190 °C) of the films was expected due to the high temperature used and the time of exposure of the materials to this condition. The incorporation of Sep promoted an increase in the volatilization temperature of the oil (from 159 °C to 170 °C for PHBV/OEO and PHBV/Sep/OEO, respectively) and in the temperature of the maximum degradation rate of the polymer (from 311 °C for pure PHBV to 319 °C for PHBV/Sep). Even in the presence of OEO, this high degradation temperature was maintained (317 °C for PHBV/Sep/OEO), indicating that the incorporation of OEO did not affect the thermal stability of the polymer. Considering the theoretical clay content (3 wt%), one may also observe that the formulations maintained approximately the same percentage of inorganic filler, indicating minimal material loss during processing.

3.4 OEO release tests

Considering a real application in food packaging, the release of OEO must occur gradually so that the antimicrobial

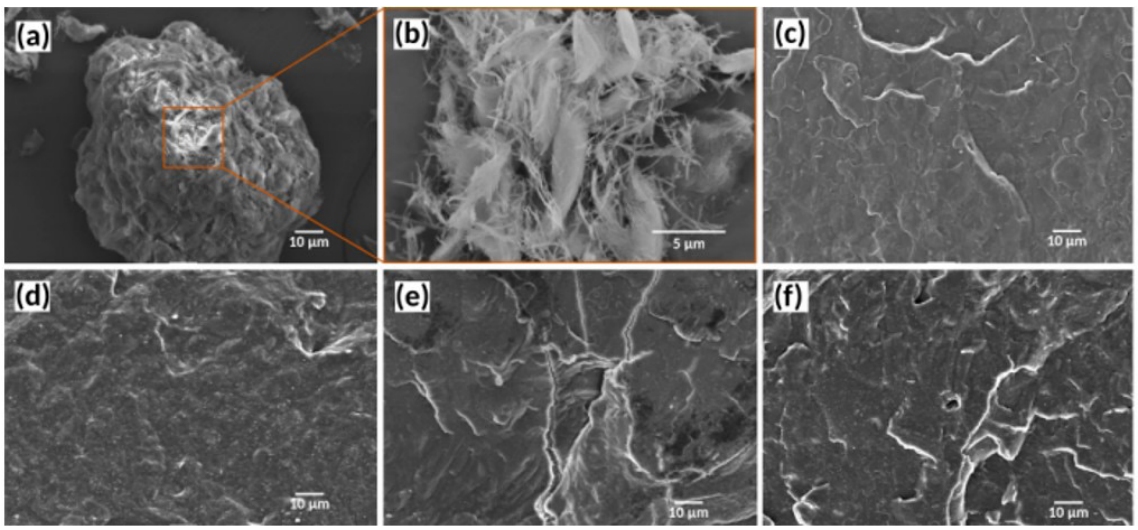

Figure 1. SEM of (a); and (b) sepiolite; and the films (c) PHBV; (d) PHBV/Sep; (e) PHBV/OEO; and (f) PHBV/Sep/OEO.

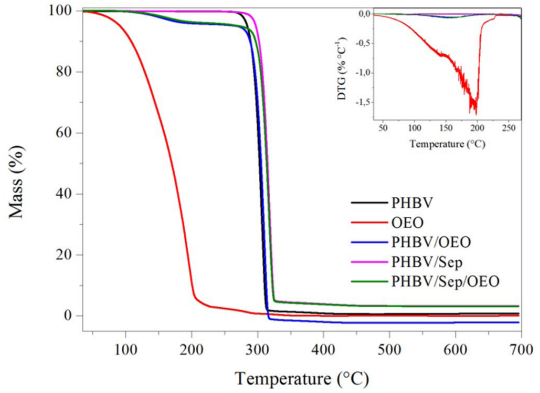

Figure 2. TGA curve of pure PHBV, OEO, and PHBV nanocomposites.

additive may remain active in the food for longer, preventing the growth of pathogenic microorganisms. If the release of the active compound occurs instantly, a minimal inhibitory concentration is not sustained for long periods. If the release is tardy, a minimum concentration in the early stages may not be achieved, and food spoilage is not controlled[20]. The OEO release was evaluated in different media in the absence or presence of sepiolite, and all compositions showed a gradual release of OEO (Figure 3).

Regarding the different food simulants, a trend was observed of a more considerable release of OEO with the decrease in the hydrophilicity of the simulant medium (isooctane > 10% ethanol > 3% acetic acid). This behavior was expected since the phenolic compounds present in the essential oil have more affinity to fatty foods.

The incorporation of Sep affected the diffusion of the OEO depending on the simulant. A similar trend was observed in the isooctane and 10% ethanol media: the percentage of OEO released was higher in the presence of fillers. Although studies have reported that the presence of nanoparticles may act as a physical barrier to the release of additives[8], in our study, a synergistic effect between the components allowed the essential oil to migrate easily toward the simulants. The presence of oil improved clay dispersion while the addition of clay at low levels improved the interaction between the components and distribution of OEO within the film. The combination of both resulted in a less crystalline composition[2] with a higher diffusion rate.

The migration of a substance from the packaging into the surrounding medium is a mass transfer phenomenon that depends on thermodynamics and kinetics aspects, such as the partition of the substance between the polymer and the food phase at equilibrium (depending on the polarity and solubility of the substances), and the diffusion of the substance into the polymer and the food phase, respectively[12]. The partition coefficient ($K_{P/L}$) is a parameter that may be used to evaluate the essential oil distribution in the polymer or the simulant. It takes into account the Hildebrand solubility parameter (δ) of the substances and may be determined by Equation 2, where $K_{P/L}$ is the partition coefficient, Δδ is the difference in the solubility parameter of two compounds, *a* is subscribed for the oregano essential oil, *P* for the PHBV, and *L* for the food simulant. From this equation, $K_{P/L}$ values

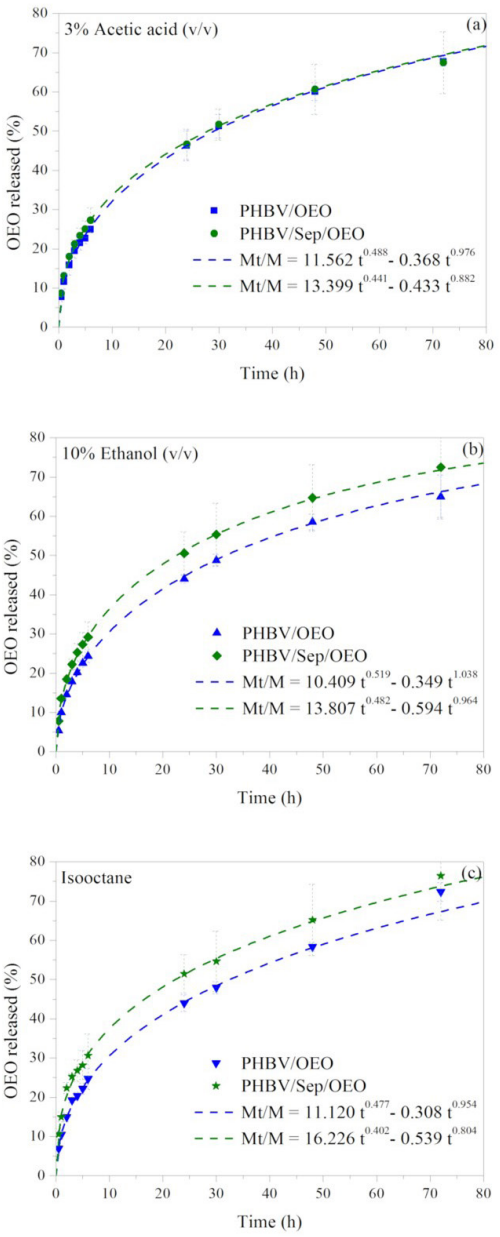

Figure 3. OEO release tests in three different food simulators (points) and approximation with the Peppas-Sahlin model (dashed lines): (a) 3% acetic acid (w/v); (b) 10% ethanol (v/v); and (c) isooctane.

≤ 1 correspond to migrants more soluble in the simulant medium than in the polymer[12].

$$K_{P/L(a)} = \frac{\Delta\delta_{L,a}}{\Delta\delta_{P,a}} \quad (2)$$

As a kinetic factor, the migration behavior of active compounds from polymer matrices depends on different factors such as i) the liquid diffusion into the polymer chains, ii) polymer solubility, and iii) the diffusion of the active

compound from the polymer matrix to the food (or simulating medium)[16]. The diffusion of low molecular weight molecules into the matrix may cause network weakening and structure changes. Therefore, the active compound diffuses through the polymer matrix towards the solvent by a concentration gradient.

In this study, two aspects might be influencing the release of the OEO to the isooctane medium: i) the swelling of the polymer matrix caused by the sorption of the organic solvent, thereby enlarging the intermolecular space between the molecules; ii) the OEO migration – confirmed by the similarity between δ values that indicates high thermodynamic compatibility between the essential oil and the solvent – associated with the lower $K_{P/L}$ value (0.15) in the PHBV/isooctane system, which indicates considerable movement to the simulant[12] (Table 2). In aqueous systems, the hydrophobic active compound presented a slower release, as observed when using acetic acid.

3.5 Kinetic studies

The study of the release mechanism of OEO from the PHBV matrix (with and without Sep) comprised four different mathematical models: first-order, Higuchi, Korsmeyer-Peppas, and Peppas-Sahlin. The coefficient of determination (Rsqr), Akaike Information Criterion (AIC), and Model Selection Criteria (MSC) parameters are presented in Table 3. The highest Rsqr and MSC represent the best model, and the model is considered appropriate when the MSC value is higher than 2. The model with the smallest AIC value is the most precise[14].

The first-order model (Equation 3, where M_O is the initial OEO concentration, M_T is the amount of released OEO at time t, k_1 is the first-order rate constant, and t is the time) is applied when the release of the active compound is proportional to the amount of OEO remaining in the interior of the matrix. In this case, the amount of OEO released reduces over time[23]. According to the results in Table 3, this model presented the worst fit to the experimental data.

$$M_t = M_o e^{-k_1 t} \quad (3)$$

In the Higuchi model, a linear relationship is proposed between the fraction of OEO released and the square root of time, as shown in Equation 4, where M_∞ is the absolute cumulative amount of drug released at an infinite time (which should be equal to the absolute amount of drug incorporated within the system at time t = 0) and k_H is a constant reflecting the design variables of the system. This model describes the OEO release as a diffusion process according to Fick's law[23] and assumes a one-dimensional diffusion, constant diffusivity of the compound, and that the swelling or dissolution of the polymer carrier is negligible, among other factors[24].

Table 2. Solubility parameters (δ) and partition coefficient ($K_{P/L}$) of OEO, PHBV, and food simulants.

Substance	Solubility parameter (δ) ($MPa^{1/2}$)	Partition coefficient ($K_{P/L}$)
PHBV	20.6[21]	-
OEO	15.1[22]	-
Isooctane	14.3[12]	0.15
10% Ethanol	45.5[12]	5.53
3% Acetic acid	47.1[12]	5.82

Table 3. Release kinetic models and associated parameters for the PHBV/OEO and PHBV/Sep/OEO films in different simulants.

Model	Formulation	Simulant	Rsqr	AIC	MSC
First-order	PHBV/OEO	3% Acid Acetic	0.7149	80.25	1.07
	PHBV/Sep/OEO		0.6262	82.50	0.80
	PHBV/OEO	10% Ethanol	0.7209	79.63	1.09
	PHBV/Sep/OEO		0.6911	82.24	0.99
	PHBV/OEO	Isooctane	0.7729	78.33	1.30
	PHBV/Sep/OEO		0.6203	84.19	0.79
Higuchi	PHBV/OEO	3% Acid Acetic	0.9668	56.60	3.22
	PHBV/Sep/OEO		0.9365	62.99	2.58
	PHBV/OEO	10% Ethanol	0.9721	54.28	3.40
	PHBV/Sep/OEO		0.9414	63.96	2.65
	PHBV/OEO	Isooctane	0.9863	47.43	4.11
	PHBV/Sep/OEO		0.9185	67.26	2.33
Korsmeyer-Peppas	PHBV/OEO	3% Acid Acetic	0.9981	20.97	5.87
	PHBV/Sep/OEO		0.9984	18.52	6.06
	PHBV/OEO	10% Ethanol	0.9967	26.41	5.31
	PHBV/Sep/OEO		0.9963	28.85	5.20
	PHBV/OEO	Isooctane	0.9981	20.11	5.88
	PHBV/Sep/OEO		0.9964	27.62	5.21
Peppas-Sahlin	PHBV/OEO	3% Acid Acetic	0.9990	16.54	6.31
	PHBV/Sep/OEO		0.9990	15.61	6.35
	PHBV/OEO	10% Ethanol	0.9984	20.93	5.85
	PHBV/Sep/OEO		0.9986	20.74	6.01
	PHBV/OEO	Isooctane	0.9987	18.14	6.07
	PHBV/Sep/OEO		0.9968	28.29	5.15

$$\frac{M_t}{M_\infty} = k_H \sqrt{t} \quad (4)$$

The Korsmeyer-Peppas model was used to investigate the Fickian and non-Fickian mechanisms, according to Equation 5, where M_T and M_∞ are the absolute cumulative amounts of drug released at time t and at an infinite time, respectively, k_{KP} is a constant incorporating structural and geometric characteristic of the matrix related to the diffusion process, and n is the diffusional exponent, indicative of the mechanism of the release process.

$$\frac{M_t}{M_\infty} = k_{KP} t^n \quad (5)$$

The power law may be viewed as a generalization of the superposition of two apparently independent transport mechanisms – a Fickian diffusion and a case-II transport. It is applied to investigate the mechanisms involved in the compound release process and the possible coupling of the relaxation/swelling of the polymer in contact with the solvent and the diffusion of the active compound through the polymer matrix. If the n value is lower than 0.45 (for cylinder specimens, for instance), a quasi-Fickian diffusion of the active release may be considered; for a n value of 0.45, the release occurs through Fickian diffusion; a n value between 0.45 and 0.89 indicates a non-Fickian model known as anomalous transport, where the diffusion and the polymer relaxation rates are coupled; and n = 0.89 represents an erosion mechanism (case-II transport with zero-order release)[13,23,25].

The Peppas-Sahlin equation (Equation 6) is an expanded version of the power law and may be applied to calculating two different contributions to the anomalous release process. The first term on the right-hand side represents the Fickian diffusional contribution (F), whereas the second term is the case-II relaxation contribution (R) (Equations 7-8)[26].

$$\frac{M_t}{M_\infty} = k_1 t^m + k_2 t^{2m} \quad (6)$$

$$F = \frac{1}{1 + \frac{k_2}{k_1} t^m} \quad (7)$$

$$R = F \frac{k_2}{k_1} t^m \quad (8)$$

In Equations 6-8, k_1, k_2, and m are the diffusion rate kinetic constant (Fickian), the erosion rate constant, and the Fickian diffusion exponent, respectively[25]. These equations, along with the Korsmeyer-Peppas model, may be used to analyze the first 60% of a release curve, regardless of the geometric shape of the specimen[26].

According to the results (Table 4), the Peppas-Sahlin model seems to be the best to describe the release kinetics of the OEO. This model decouples the Fickian diffusion and macromolecular relaxation contributions to an overall release mechanism over the process[27]. The greater value of k_1 compared to k_2 evinces that the Fickian behavior is the prevailing mechanism of OEO release in all media evaluated. The Korsmeyer-Peppas model also showed a very close approximation ($R^2 > 0.988$), and n values lower than 0.5 are also indicative of a Fickian diffusion process[7].

The diffusion coefficient (m) is applied to any geometrical shape that exhibits controlled release and may be determined from the aspect ratio of the sample ($2a/l$), where $2a$ is the diameter and l is the thickness. By the results obtained, m varies from 0.40 to 0.52, with a value between 0.45 and 0.89 indicating an anomalous transport[26].

By analyzing the values of F (Fickian mechanism) and R (relaxational mechanism) of the Peppas-Sahlin model (Figure 4), one may observe that the Fickian (case-I) contribution is prominent but decreases over time, while the contribution of

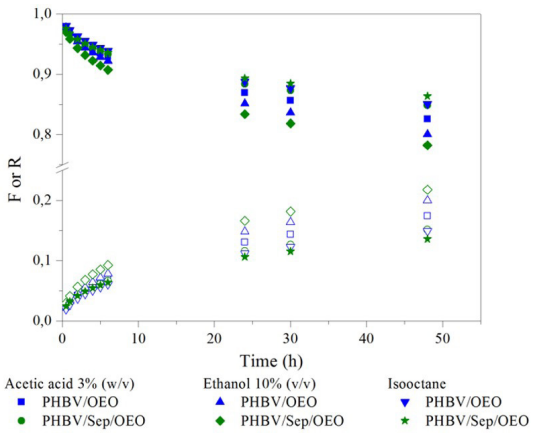

Figure 4. Diffusion (F – closed symbols) and Relaxation (R – open symbols) contributions to the OEO release mechanism in different simulants.

Table 4. OEO release parameters from PHBV/OEO and PHBV/Sep/OEO films in different simulants.

Formulation	Simulant	First-order	Higuchi	Korsmeyer-Peppas		Peppas-Sahlin		
		k_1	k_H	k_{KP}	n	k_1	k_2	m
PHBV/OEO	3% Acetic acid	0.03	8.83	11.75	0.43	11.56	-0.37	0.49
PHBV/Sep/OEO		0.03	8.98	13.39	0.39	13.40	-0.43	0.44
PHBV/OEO	10% Ethanol	0.03	8.46	10.72	0.44	10.41	-0.35	0.52
PHBV/Sep/OEO		0.03	9.62	14.07	0.40	13.81	-0.59	0.48
PHBV/OEO	Isooctane	0.02	8.78	11.22	0.43	11.12	-0.31	0.48
PHBV/Sep/OEO		0.03	9.93	16.06	0.36	16.23	-0.54	0.40

the polymer relaxation is increasing ($F + R = 1$). However, the overall phenomenon is diffusion-dominant in an anomalous process with a non-linear relationship. This may be related to the compatibility between PHBV and the solvents, especially isooctane and 10% ethanol, thus occurring the swelling of the polymer matrix along the experiment in contact with the medium, associated with the good affinity of the OEO with the medium, with both thermodynamic and kinetic factors facilitating the diffusion process.

Therefore, it is likely that the degradation of the polymer starts at later stages than when the analysis was performed. Therefore, this characteristic becomes promising for application in food since there is no interference from the erosion of the packaging material, and the OEO release to the different media occurs solely through the diffusion process through the PHBV matrix.

The set of results highlights that the dispersion of filler in the polymer matrix and the interaction with the active compound influenced by the presence of clay might interfere with the diffusion of the oil through the polymer. In this study, the PHBV/Sep/OEO exhibited a faster release profile in the first 8 h of the test compared to the PHBV/OEO composition and a gradual and slower release for higher periods when in contact with 10% ethanol and isooctane. In acetic acid, no differences were observed between the compositions. Since the PHBV/Sep/OEO presented a good dispersion of the filler and Sep nanoparticles did not significantly affect the crystallization characteristics of the polymer[2], these results suggest that Sep, when finely dispersed in the PHBV matrix, might improve the OEO dispersion, thus facilitating its release to the medium, especially in the early stages of the process. Furthermore, the formation of some preferential pathways due to the presence of the needle-shaped nanoparticles might have favored the diffusion of the solvent from the medium to the polymer and the diffusion of the OEO from the polymer to the medium.

4. Conclusions

The development of active packaging films using PHBV was successfully performed, and incorporating sepiolite and OEO rendered the material more functional, with associated antimicrobial properties. The oregano essential oil release in food simulants is a complex phenomenon that involves different factors such as film structure, simulant medium polarity, migrant solubility, and the presence of nanoparticles.

Therefore, the release of OEO is not governed only by kinetics aspects. The compatibility among all components plays an important role. While the presence of well-dispersed sepiolite influenced the diffusion of oil through the polymer matrix, the affinity of the OEO with the trapping network (PHBV) was more pronounced in hydrophilic media, thus causing a delay in the release process.

Tests in different media proved to be an important tool to fine-tune the release profile of the active compound according to the composition of the packaging material and predict its delivery to the food, thus allowing to establish the application of each film composition for a specific food. This strategy may affect the shelf life of the food, increasing the storage time and ensuring safety.

5. Author's Contribution

- **Conceptualization** – Larissa Nardini Carli.
- **Data curation** – NA.
- **Formal analysis** – Renata Cerruti da Costa; Ismael Casagrande Bellettini; Larissa Nardini Carli.
- **Funding acquisition** – Larissa Nardini Carli.
- **Investigation** – Renata Cerruti da Costa; Ana Paula Ineichen; Cristiano da Silva Teixeira.
- **Methodology** – Renata Cerruti da Costa.
- **Project administration** – Larissa Nardini Carli.
- **Resources** – Larissa Nardini Carli.
- **Software** – NA.
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