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ORIGINAL ARTICLE

Development of active PHB/PEG antimicrobial films incorporating clove essential oil

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Abstract

In this work were developed and evaluated new antimicrobial films of polyhydroxybutyrate (PHB) additivated with polyethyleneglycol (PEG) and clove essential oil (CEO). The PHB/PEG/CEO films were prepared using the solution casting technique. The CEO concentrations varied between 0.0 and 15% (w/w), related to the total mass (1.4 g), without considering the solvent used. The CG-MS analysis showed that the major component of the CEO was eugenol (72.96%). The antimicrobial activity from the CEO was evaluated against three bacteria (*E. coli, E. aerogenes and S. aureus*). The migration of CEO in the films occurred with all tested simulants. Thermal analysis has shown that the addition of 15% w/w of the CEO causes the biggest changes in the chemical structure of the material, resulting in less energy during film processing. The mechanical data demonstrated that the addition of 15% w/w of the CEO results in more flexible films.

Keywords: *active packaging antimicrobial films, clove essential oil, Polyhydroxybutyrate, Polyethyleneglycol.*

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1. Introduction

In the food industry, packaging are responsible for preserving food quality and safety[1-3]. They can be classified into passive, intelligent and active[4,5]. Recently, the attention to active packaging has been increased, due to its performance in changing the environmental conditions to maintain the sensory properties of the food, thus providing quality assurance, increasing its shelf life, in addition to hygiene and food safety[6,7,8]. Antimicrobial packaging is a kind of active packaging that is beneficial to the consumers and the food[9,10], which interfere in the lag period (growth period of the microorganisms) and inhibits microbial growth by the migration of the functional agents towards the food[11].

An ecologically friendly alternative for the production of antimicrobial packaging is the use of biodegradable polymers with natural antimicrobial agents. This combination can be an option to reduce the demand for degradable packaging

Polyhydroxybutyrate (PHB) is a well know biodegradable polymer used worldwide due to its crystalline structure, superior characteristics of aroma barrier, and water vapor permeability[9]. PHB is a thermoplastic polymer obtained from renewable natural sources that shown characteristics of biodegradability, biocompatibility, UV resistance and properties similar to the synthetic polypropylene[12-14]. However, the high crystallinity of PHB makes it very rigid and brittle, limiting its applications[15,16]. To overcome this limitation, plasticizers are added to the PHB. Poly (ethylene glycol) (PEG) is a polymer that can have a plasticizing effect when mixed with other polymers, in addition it is biodegradable and non-toxic[17]. According to the literature, PEG acts by decreasing intermolecular forces between PHB structures[18].

Regarding natural agents, there are essential oils (EO) that have been widely used in the food industry as natural antimicrobial agents in packaging material[19]. EOs are aromatic products of secondary plant metabolism, extracted

from leaves, flowers, stems, roots, seeds or fruit peels[20-22]. The antimicrobial activity of the EO is mainly related to its main components, and it can also have a synergistic effect with some trace compounds[23]. Among the essential oils with proven antimicrobial activity is the clove essential oil (CEO) of the *Eugenia caryophyllata* plant. This oil has eugenol as its main component[24]. It has antibacterial, antifungal, antioxidant, insecticidal and antiviral properties[25].

It has been cited in the literature some works that incorporated and evaluated the effect of CEO in different polymer matrices for antimicrobial packaging applications. Among them, Wang et al.[26], Mulla et al.[27], De Lima et al.[28] and Mupalla et al.[29], studied chitosan, low density linear polyethylene, polyvinyl chloride, and blends of carboxymethylcellulose/ polyvinyl alcohol, respectively. In all these works CEO showed great potential for applications in antimicrobial packaging.

The aim of this work was to develop a new antimicrobial films PHB/PEG-based incorporating CEO, and to determine its antimicrobial potential, as well as its migration towards food, in order to fulfill the requirements of an antimicrobial active packaging able to combat pathogenic microorganisms.

2. Materials and Methods

2.1 Materials

PHB was provided by PHB Industrial S/A (São Paulo - Brazil). Before the preparation of the polymeric films, the PHB powder was strained through a 150-mesh strainer. PEG with molecular mass of 6000g/mol was purchased from Sigma-Aldrich (San Luis - USA). CEO was provided by Solua Comercial Ltda (São Paulo - Brazil). Chloroform and ethanol were purchase from VETEC (Rio de Janeiro - Brazil), and acetic acid from Dinâmica (Indaiatuba - Brazil), all reagents were PA grade without further purification. The culture medium used Agar-agar was brand Kasvi (Paraná – Brazil).

2.2 Gas Chromatography Mass Spectrometry (CG-MS)

The identification and quantification of the constituents of CEO was performed using TRACE 1300 Series gas Thermo Xcalibur Instrument (Massachusetts, USA) equipped with a TGMS-5 (5% phenyl/ polydimethylsiloxane) capillary column. The temperature parameters were: oven ramp of CG: 60 °C for 3min and then a heating rate of 10 °C/min was used until the temperature of the oven reached 300 °C, and this temperature remained constant for 15min. Injector temperature: 270 °C. Temperature of the transfer line to the MS: 280 °C, and temperature of ions source of MS: 250 °C.

2.3 Antimicrobial activity of the clove essential oil

In order to evaluate the antimicrobial activity of CEO, three kinds of bacteria were used *Escherichia coli, Enterobacter aerogenes* and *Staphylococcus aureus* (Culture collection of the Department of Antibiotics, Federal University of Pernambuco-Brazil). Filter paper discs were used for the diffusion experiment in a solid environment for every specie. The discs had 52 mm diameter and were sterilized by autoclaving (121 °C for 15 minutes). The culture medium (agar-agar) was prepared in a Petri dish. Later, the discs were soaked with pure CEO and placed in a Petri dish. Finally, each Petri dish was inoculated with 0.1 mL of bacterial suspension (10^{-4} on MacFarland scale). The Petri dishes were incubated for 24 hours at 30 °C and then the inhibition halos were measured.

2.4 Film preparation

The polymeric films were produced using the solution casting technique[30]. The total weight of all formulated films was 1.4 g (polymer and CEO, without considering the weight of the solvent). Table 1 shows the weight of each component in the preparation of each film formulation.

For the film preparation, PHB was weighed and mixture with 50 mL of chloroform. The bottle containing the polymeric solution was closed, in order to avoid solvent evaporation. The mixture was homogenized under stirring for 5 hours at 60 °C.

The solution was left standing for 12 hours at room temperature. Afterwards, the mixture was again stirred for 4 hours at room temperature for complete solubilization. The film-forming solution was filtered and the PEG was added into the solution which was again stirred at room temperature for 1 hour. The mass of the CEO was weighed to prepared the following solutions: 5, 10 and 15% w/w, dissolved in 5 mL of chloroform, and added into the film-forming solution, mixing for 15 minutes at room temperature. PHB/PEG control films were prepared without CEO addition.

Finally, the solution was poured on a Petri dish with a 13 cm diameter until the solvent was completely evaporated.

2.5 Fourier-transform infrared spectroscopy (FTIR)

The study of the incorporation of CEO, and the evaluation of the migration on antimicrobial films were carried out through Fourier-transform infrared spectrometer (FTIR) of the company Perkin Elmer (Massachusetts, USA), using the Universal Attenuated Total Reflectance (UATR) accessory. All the spectra were acquired at spectral infrared region of 4000-650 cm^{-1}, at 4 cm^{-1} resolution and 16 scans[31].

Table 1. Weight of PHB, PEG and CEO for the preparation of each formulation of polymeric films.

Samples	CEO (g)	PHB (g)	PEG (g)	Total weight (g)
	% compared to 1.4g	9:1 aspect ratio		
PHB/PEG	-	1.260	0.140	1.4
PHB/PEG + CEO (5%)	0.070	1.197	0.133	1.4
PHB/PEG + CEO (10%)	0.140	1.134	0.126	1.4
PHB/PEG + CEO (10%)	0.210	1.071	0.119	1.4

2.6 Migration test

In order to investigate the migration of the essential oil (5, 10 and 15% (w/w) concentrations) in the antimicrobial films of the simulated food, the active films were cut into rectangles with sections of 1 cm x 3 cm width. For the migration test, the following simulant were prepared: a) acid: 3% (v/v) acetic acid solution, b) alcoholic: 10% (v/v) of ethanol, and c) neutral: distilled water. The assays were carried out in duplicate for each concentration of the CEO and kind of simulant, at a room temperature (25 °C) and cooled (5 °C). The antimicrobial films remained immersed in the 7 mL simulant solution for 90 hours. It is important to mention that every 18 hours the films were removed from the solution, washed with distilled water, dried in the oven, and two distinct points on the film were analyzed at the FTIR spectrometer. After the assay period of migration, the average of the spectra for each assay was analyzed. The baseline correction was applied. A fixed point was chosen in the region with the highest absorbance relative to the essential oil (1515 cm^{-1}) and its behavior was observed by applying the Equation 1 to convert the absorbance into concentration (%).

$$Concentration(\%) = \frac{AX_{hours}}{A0_{hours}} \times 100 \quad (1)$$

Where $A0_{hours}$ corresponds to the absorbance at the initial time of the experiment and AX_{hours} the absorbance at the end of the assay. Finally, the graphics for concentration x time for each assay were drawn.

2.7 Exploratory differential calorimetry (DSC)

The thermal parameters of PHB/PEG and samples containing 5, 10 and 15% (w/w) of essential oil were evaluated using differential scanning calorimetry (DSC) of the company Mettler Toledo (Ohio, USA) – Star System 1. The samples of 3 and 5 mg were introduced into an aluminum crucible under a nitrogen atmosphere at a flow rate of 50 mL/min. Thermal analysis was performed using a temperature range of 0 °C to 200 °C, which involved three stages regarding heating and cooling the samples, which were as follows: first stage: Heating from 0 – 200 °C with a heating rate of 30 °C/min; second stage: Cooling of 200 – 0 °C with a cooling rate of 10 °C/min; and third stage: heating from 0 – 200 °C with a heating rate of 10 °C/min.

2.8 Thermogravimetric analysis (TGA)

All the films were subjected to thermogravimetric tests in equipment of the company Shimadzu DTG 60H (Kyoto, Japan) in order to evaluate the rates of weight loss. Therefore, approximately 20 mg of the samples were introduced into a thermobalance. The thermogravimetric analyzes were performed in a temperature range of 35-600 °C, using a heating rate of 10 °C/min under a nitrogen atmosphere.

2.8 Tensile Test

Mechanical tensile tests were carried out according to the ASTM 882-12 standard[32]. For the analysis, EMIC 500 equipment was used (Paraná, Brazil). It was performed at room temperature without humidity control, and followed the procedure: claw speed of 5 mm/min; initial distance between 40 mm claws; size of the test piece of 2.5 x 7.5 cm. For each film manufactured, three samples were obtained. Since the tensile test was carried out in triplicate, it was obtained 9 samples for each concentration. Duncan's statistical test was used to evaluate statistically significant changes.

2.9 Statistical Analysis

Mechanical properties data were carried out by analysis of variance (ANOVA) using the Statistica software, version 10.0.228.8. Duncan's test was used to determine differences at a level of significance of 5% ($p \leq 0,05$).

3. Results and Discussion

3.1 Gas chromatography–mass spectrometry (GC-MS)

GC-MS was used in order to investigate the chemical composition of the CEO. Table 2 shows the percentage of the principal compounds present in the mixture of CEO. The major compound found in the oil was eugenol. This compound is well known for its great antimicrobial potential against various pathogenic microorganisms[33].

3.2 Evaluation of antimicrobial activity of the CEO

After the incubation period, the CEO exhibited inhibition of the microbial growth for the three studied bacteria, as shown in Figure 1. The bacterial colony *Escherichia coli* (Figure 1a) an average halo inhibition of approximately 9.0 mm, was observed; for the *Enterobacter aerogenes* (Figure 1b) the halo was 6.6 mm; and for the *Staphylococcus aureus* (Figure 1c) the halo inhibition was 8.1 mm. Using the classification of Ostrosky et al.[34], we observed that this indicates that the CEO is an excellent antimicrobial agent, because of its halo above 3.0 mm, emphasizing its potential as an active agent for food packaging. The CEO has been evaluated as antimicrobial additives in other polymers and the results show that this oil has a strong inhibitory potential in the metabolism of Gram negative as well as Gram positive bacterias[35-37].

3.3 Fourier-transform infrared spectroscopy (FTIR)

The antimicrobial films with 5, 10 and 15% (w/w) of CEO showed a characteristic peak between 1500-1535 cm^{-1} (Figure 2). This peak is associated with the C=C stretching due to the eugenol aromatic group[33]. It can be inferred through this analysis that the CEO was incorporated into the polymeric matrix PHB/PEG after total chloroform evaporation.

Table 2. Composition of the CEO obtained by CG-MS.

Compound	(%)
Eugenol	73.0
Caryophyllene	17.9
Humulene	4.9
Other compounds	4.2

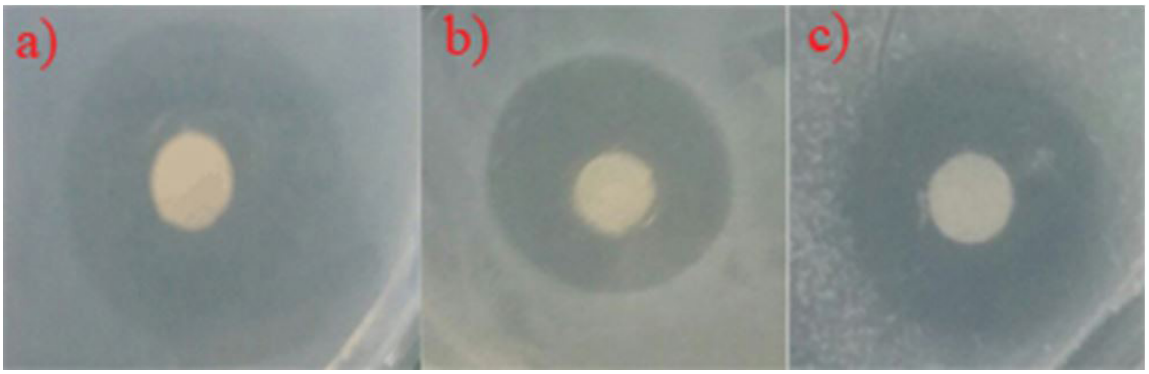

Figure 1. Microbial Inhibition test in solid state of clove essential oil: (a) *Escherichia coli* (b) *Enterobacter aerogenes* (c) *Staphylococcus aureus*.

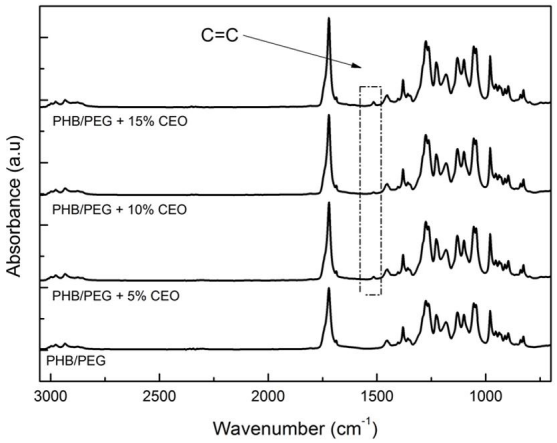

Figure 2. FT-IR spectra from PHB/PEG films: pure (bottom) and with 5, 10 e 15% (w/w) of CEO.

3.4 CEO migration kinetics

Figure 3 shows the graphs of the CEO migration kinetics in the PHB/PEG films. The films were subjected to two different temperature and three different simulants: neutral simulant at 25 °C (neutral simulant); acid simulant at 25°C (neutral simulant); alcoholic simulant at 25 °C (alcoholic simulant); neutral simulant at 5 °C (neutral simulant-cold); acid simulant at 5 °C (neutral simulant-cold) and alcoholic simulant at 5 °C (alcoholic simulant-cold).

Considering the effect of the simulant and the concentration of CEO in the films it was observed that the migration of the CEO occurs more quickly in the first 18 hours of exposure to the simulants, and more slowly after that period. Analyzing the effect of the temperature is verified that the films submitted to 25 °C resulted in a faster migration rate at the same period of time (18 hours) when compared to those submitted to 5 °C. Therefore, temperature is a variable that also contributes significantly to migration. According to Bart[38], several factors contribute to migration, among which we can highlight: starting concentration of migrant in the polymer; affinity and solubility of the migrant for the polymer or for the contact medium; molecular structure and molecular weight the migrant; and mobility of the migrant in the polymer.

At 5 °C, PHB/PEG films with 10% (w/w) CEO concentration showed the highest percentage of CEO migration in the 18 h period. In this composition, 46%, 44% and 39% of CEO occurred for the neutral, acid and alcoholic simulants, respectively, in the 18 h period. This behavior was similar for the other concentrations (5 and 15% w/w) in the PHB/PEG films at the same temperature.

At 25 °C, PHB/PEG films with a concentration of 15% w/w of CEO showed the highest percentage migration of CEO in the period of 18 hours for the acid and alcoholic simulants. In this composition, 81% and 72% of CEO migration occurred for the acid and alcoholic simulants, respectively. At 25°C, PHB/PEG films with 10% w/w CEO concentration showed the highest CEO migration (73%) in the 18 h period in the neutral simulant.

At the end of the 90 hs period, it can be observed that the PHB/PEG films with 15% w/w CEO concentration showed the highest percentage of CEO migration to the acid and alcoholic simulants at 25 °C. There was a percentage migration of CEOs of 80 and 90% for the alcoholic and acidic simulants, respectively. Also, for this composition, there was a 66% CEO match in the neutral simulant. According to Tawakkal et al.[39] the tendency towards migration is greater when submitted to the simulant with polarities closer to the antimicrobial agent. This explain the accentuated migration in acid and alcoholic simulants, when compared to the neutral simulator, which is polar in nature.

These results show either the CEO behavior in simulants to various types of food. The results shown that the CEO has the potential to migrate and therefore plays the role as an antimicrobial agent, an essential requirement to provide a protective action to the food and prevent its deterioration.

3.5 Exploratory differential calorimetry (DSC)

The crystallization temperature (T_c), cold crystallization temperature (T_{cc}), melting temperature (T_m), melting enthalpy change (ΔH_m) and degree of crystallinity (X_c) were obtained by the DSC technique, and the results are shown in Table 3.

According to the results shown in Table 3, can be observed that the melting temperature (T_m) of PHB/PEG polymer matrix decreases with increasing of the essential oil concentration. Silva et al.[40] used CEO as a plasticizer in bocaiuva (*Acromonia aculeata*) flour films. They observed

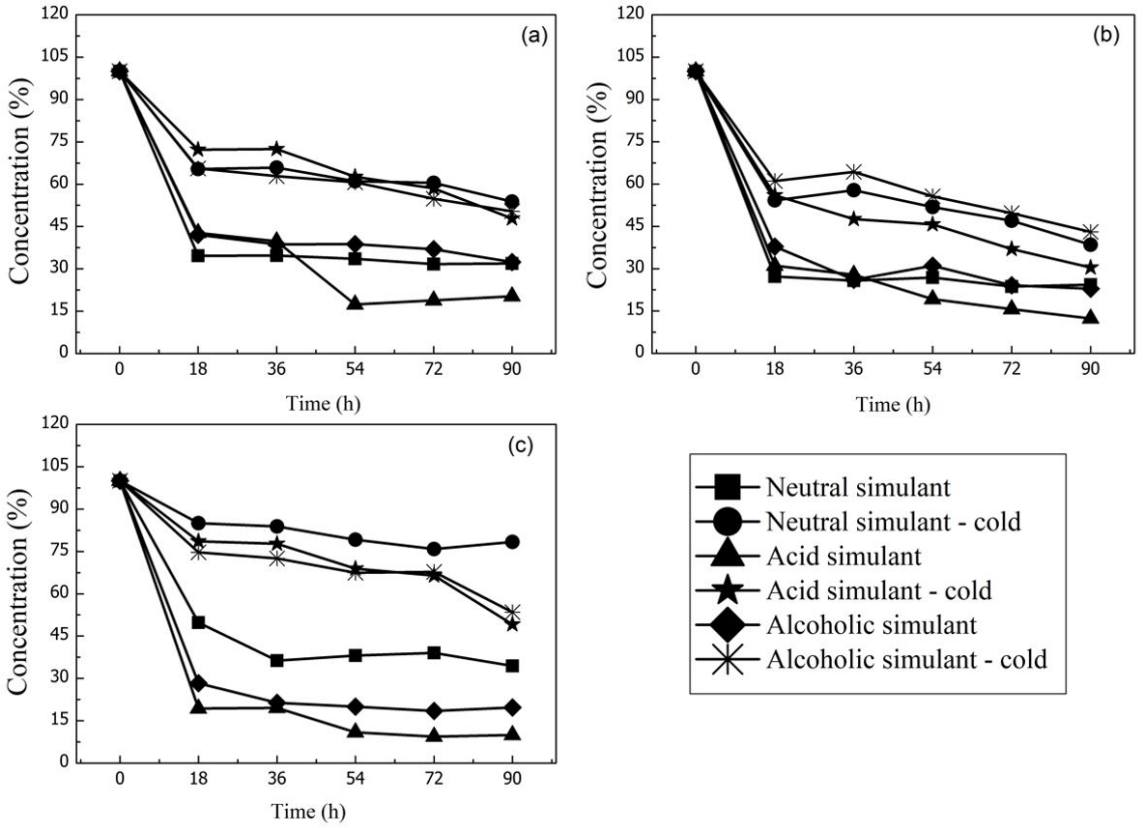

Figure 3. Migration test of active PHB / PEG films supplemented with (a) 5% w/w, (b) 10% w/w and (c) 15% w/w of the CEO.

Table 3. Thermal parameters of pure PHB / PEG samples and added with 5, 10 and 15% (w/w) of CEO.

Samples	T_c (°C)	T_{cc} (°C)	T_m (°C)	ΔH_m (J/g)	X_c (%)
PHB/PEG	56.64	-	171.38	70.21	48.09
PHB/PEG + CEO (5%)	46.77	21.59	172.47	72.26	49.49
PHB/PEG + CEO (10%)	-	39.52	168.64	72.18	49.44
PHB/PEG + CEO (15%)	-	31.41	160.80	31. 41	21.51

that the decrease of the melting temperature is related to the reduction of the secondary forces between the polymer structures caused by the incorporation of the additive. Therefore, the reduction of the melting temperature that occurred in the PHB/PEG blend with the addition of the CEO is likely related to a decrease in the secondary forces of the PHB polymer structures.

The degree of crystallinity (X_c) of the samples presented similar values, except for the polymer films with 15% (w/w) of CEO. For this sample the Xc value is smaller, showing that the polymer became less crystalline, an important feature for PHB processing. The decrease in degree of crystallinity is related to the mechanical properties of the polymers (the lower the degree of crystallinity, the greater the elasticity of the material) and the melting temperature (the higher the crystallinity, the higher the thermal energy required to melt the material).

It is possible to observe, from Table 3, a marked reduction in the melting enthalpy (ΔH_m) with the addition of 15% (w/w) of oil, and values close to the pure PHB / PEG in the other compositions. This behavior indicates that the addition of 15% (w/w) of CEO causes greater changes in the chemical structure of the material. In addition, with this change, less energy will be needed for this material to melt during processing[41].

As shown in Table 3, only films incorporated with essential oil presented the temperature of cold crystallization (T_{cc}). This effect indicates that the polymeric matrix has sufficient mobility to reorganize the amorphous phase, forming new crystallites. For crystallization temperature (T_c) it was only possible to observe this effect in samples of pure PHB/PEG and with 5% (w/w) of the CEO.

3.6 Thermogravimetric analysis (TGA)

In the graph of TGA (Figure 4a) and DTG (Figure 4b) was observed that the PHB/PEG blend showed two stages of degradation. The first stage occurs between the temperatures of 258-325 °C, and is related to the thermal degradation of PHB. The second stage is observed between 334-437 °C, caused by thermal decomposition of PEG.

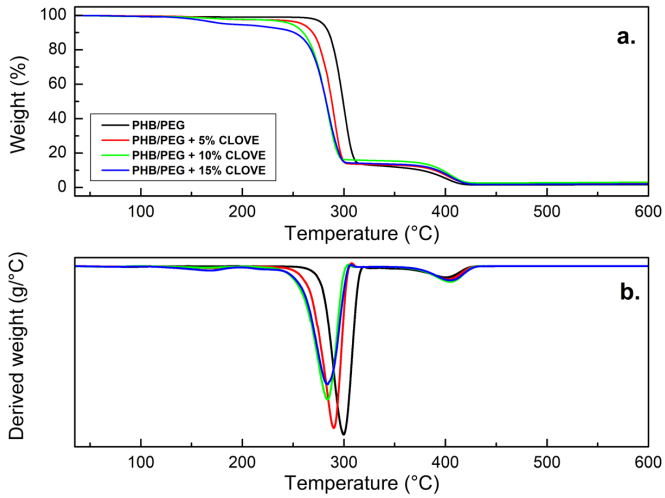

Figure 4. Thermogram of PHB / PEG samples and active films with 5, 10 and 15% (w/w) of CEO. (a) TGA curves in relation to mass percentage (b) Curves derived from TGA (DTG).

Table 4. Tensile stress, specific deformation and Young module values of PHB/PEG films at different CEO concentration.

Samples	Tensile stress (MPa)	Specific deformation (%)	Young module (MPa)
PHB/PEG	12.57[a]± 1.09	3.34[a]± 0.93	430.97[a]± 31.09
PHB/PEG + 5% CEO	13.76[a]± 0.35	3.70[a]± 0.49	405.27[a]± 25.46
PHB/PEG + 10% CEO	12.22[a]± 0.32	3.08[a]± 0.08	388.67[a]± 50.24
PHB/PEG + 15% CEO	8.10[b]± 1.84	9.77[b]± 1.01	301.90[b]± 20.29

Means followed by vertical letters do not differ significantly ($p > 0.05$) by the Duncan test.

In terms of the antimicrobial films, the presence of three degradation phases can be seen in the TGA and DTG, shown in Figure 4, and the first weight loss occurs in the temperature range of 140-225°C. According to Choi et al.[42], this stage refers to the thermo-oxidation and boiling temperature of eugenol present in CEO. The other stages of degradation are related to PHB / PEG blends.

Furthermore, by analyzing the results shown in Figure 4, may conclude that the CEO interferes with the degradation of the polymer because the increasing oil concentration decreases the initial PHB/PEG degradation temperature. The degradation of the PEG (third event) in the blend did not change regardless of the concentration of the CEO.

3.7 Tensile Test

Mechanical assays of active polymer films were performed to observe the influence of CEO on the mechanical properties of the PHB/PEG blend. Observing the results of the tensile test (Table 4) it can be seen that there was no significant change in tensile strength, specific deformation and modulus of elasticity of pure PHB / PEG samples and added with 5 and 10% (w/w) of the CEO.

However, the film with 15% (w/w) of the CEO showed a significant change in all the studied mechanical parameters, resulting in a more flexible film when compared with the lower percentage of essential oil in the film. The plasticizer effect of the essential oil was also observed in the other polymer films. According to Giménez et al.[43], CEO in the egg white gelatin film decreased the modulus of elasticity and increased its elongation to traction also contributing to the reduction of secondary forces between the polymer chains.

4. Conclusions

In this study, active packaging were prepared by incorporating CEO, in different concentrations, into polyhydroxybutyrate and polyethyleneglycol films, using the solution casting technique. Antimicrobial analysis by disc diffusion of CEO revealed the potential of CEO to inhibit pathogenic microorganisms, such as: *Escherichia coli, Enterobacter aerogenes* and *Staphylococcus aureus*. The migration test demonstrated the efficiency of the CEO as an active agent for active packaging, since these packaging must have a substance that migrates to the food, providing a protective barrier against the microorganisms. Therefore, as seen in the tests the migration occurred in all simulants, with a higher rate for the acid simulant. It has also been seen that the cooling of the system contributes to the reduction of the migration rate in all food simulants tested. From the mechanical and thermal analysis, it was observed that the increase in the CEO concentration (15% w/w) influences the decrease of the intermolecular interactions between the polymeric matrix structures, obtaining a less crystalline and consequently more elastic films. It can be concluded that PHB/PEG films, to which has been added with CEO, have potential for applications in antimicrobial protection packaging, contributing to the increase in the shelf life of perishable foods.

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6. References

1. Landim, A. P. M., Bernardo, C. O., Martins, I. B. A., Francisco, M. R., Santos, M. B., Melo, N. R. (2016). Sustainability concerning food packaging in Brazil. *Polímeros: Ciência e Tecnologia, 26*(no spe), 82-92. http://dx.doi.org/10.1590/0104-1428.1897
2. Wani, A. A., Singh, P., & Langowski, H. C. (2014). *Packaging.* In Y. Motarjemi & L. Gorris (Eds.), *Encyclopedia of food safety* (pp. 211-218). United Kingdom: Elsevier Science. http://dx.doi.org/10.1016/B978-0-12-378612-8.00273-0.
3. Muppalla, S. R., Kanatt, S. R., Chawla, S. P., & Sharma, A. (2014). Carboxymethyl cellulose–polyvinyl alcohol films with clove oil for active packaging of ground chicken meat. *Food Packaging and Shelf Life*, *2*(2), 51-58. http://dx.doi.org/10.1016/j.fpsl.2014.07.002.
4. Kuswandi, B., & Jumina. (2020). *Active and intelligent packaging, safety, and quality controls*. In M. W. Siddiqui (Eds.), *Fresh-Cut Fruits and Vegetables: Technologies and mechanisms for safety control* (pp. 243-294). United Kingdom: Academic Press. http://dx.doi.org/10.1016/B978-0-12-816184-5.00012-4
5. Lloyd, K., Mirosa, M., & Birch, J. (2018). *Active and Intelligent Packaging.* In L. Melton, F. Shahidi, & P. Varelis (Eds.), *Encyclopedia of Food Chemistry: Reference Module in Food Science* (pp. 177-182). United Kingdom: Academic Press. http://dx.doi.org/10.1016/B978-0-08-100596-5.22421-9
6. El-Wakil, N. A., Hassan, E. A., Abou-Zeid, R. E., & Dufresne, A. (2015). Development of wheat gluten/nanocellulose/titanium dioxidenanocomposites for active food packaging. *Carbohydrate Polymers*, *124*, 337-346. http://dx.doi.org/10.1016/j.carbpol.2015.01.076. PMid:25839828.
7. Gouvêa, D. M., Mendonça, R. C. S., Soto, M. L., & Cruz, R. S. (2015). Acetate cellulose film with bacteriophages for potential antimicrobial use in food packaging. *Lebensmittel-Wissenschaft + Technologie*, *63*(1), 85-91. http://dx.doi.org/10.1016/j.lwt.2015.03.014.
8. Wrona, M., Bentayeb, K., & Nerín, C. (2015). A novel active packaging for extending the shelf-life of fresh mushrooms (*Agaricus bisporus*). *Food Control*, *54*, 200-207. http://dx.doi.org/10.1016/j.foodcont.2015.02.008.
9. Zhong, Y., Godwin, P., Jin, Y., & Xiao, H. (2020). Biodegradable polymers and green-based antimicrobial packaging materials: A mini-review. *Advanced Industrial and Engineering Polymer Research*, *3*(1), 27-35. http://dx.doi.org/10.1016/j.aiepr.2019.11.002.
10. Takma, D. K., & Korel, F. (2019). Active packaging films as a carrier of black cumin essential oil: Development and effect on quality and shelf-life of chicken breast meat. *Food Packaging and Shelf Life*, *19*, 210-217. http://dx.doi.org/10.1016/j.fpsl.2018.11.002.
11. Espitia, J. P., Du, W.-X., Avena-Bustillos, R. J., Soares, N. F. F., & McHugh, T. H. (2014). Edible films from pectin: physical-mechanical and antimicrobial properties - A review. *Food Hydrocolloids*, *35*, 287-296. http://dx.doi.org/10.1016/j.foodhyd.2013.06.005.
12. Costa, A. R. M., Ito, E. N., Cavalho, L. H., & Canedo, E. L. (2019). Non-isothermal melt crystallization kinetics of poly(3-hydroxybutyrate), poly(butylene adipate-co-terephthalate) and its mixture. *Polímeros: Ciência e Tecnologia*, *29*(1), e2019006. http://dx.doi.org/10.1590/0104-1428.11217.
13. Li, Z., Yang, J., & Loh, X. Polyhydroxyalkanoates: opening doors for a sustainable future. *NPG Asia Materials, 8*, e265. http://dx.doi.org/10.1038/am.2016.48
14. Souza, G., Santos, A., & Vinhas, G. (2016). Avaliação das propriedades da blenda de poli(3-hidroxibutirato)/quitosana após esterilização térmica ou radiolítica. *Polímeros: Ciência Tecnologia, 26*(4), 352-359. http://dx.doi.org/10.1590/0104-1428.2215
15. Pachekoski, W. M., Dalmolin, C., & Agnelli, J. A. M. (2014). Blendas Poliméricas Biodegradáveis de PHB e PLA para Fabricação de Filmes. *Polímeros: Ciência Tecnologia*, *24*(4), 501-507. http://dx.doi.org/10.1590/0104-1428.1489.
16. Costa, A. R. M., Ito, E. N., Carvalho, L. H., & Canedo, E. L. (2019). Non-isothermal melt crystallization kinetics of poly(3-hydroxybutyrate), poly(butylene adipate-co-terephthalate) and its mixture. *Polímeros: Ciência Tecnologia*, *29*(1), e2019006. http://dx.doi.org/10.1590/0104-1428.11217.
17. Fiori, A. P. S. M., Camani, P. H., Rosa, D. S., & Carastan, D. J. (2019). Combined effects of clay minerals and polyethylene glycol in the mechanical and water barrier properties of carboxymethylcellulose films. *Industrial Crops and Products*, *140*, 11644. http://dx.doi.org/10.1016/j.indcrop.2019.111644.
18. Quental, A. C., Carvalho, F. P., Tada, E. S., & Felisberti, M. I. (2010). Blendas de PHB e seus copolímeros: miscibilidade e compatibilidade. *Quimica Nova*, *33*(2), 438-446. http://dx.doi.org/10.1590/S0100-40422010000200035.
19. Khaneghah, A. M., Hashemi, S. M. B., & Limbo, S. (2018). Antimicrobial agents and packaging systems in antimicrobial active food packaging: an overview of approaches and interactions. *Food and Bioproducts Processing*, *111*, 1-19. http://dx.doi.org/10.1016/j.fbp.2018.05.001.
20. Amorati, R., Foti, M. C., & Valgimigli, L. (2013). Antioxidant activity of essential oils. *Journal of Agricultural and Food Chemistry*, *61*(46), 10835-10847. http://dx.doi.org/10.1021/jf403496k. PMid:24156356.
21. Siddique, A. B., Rahman, S. M. M., & Hossain, M. A. (2012). Chemical composition of essential oil by different extraction methods and fatty acid analysis of the leaves of Stevia Rebaudiana Bertoni. *Arabian Journal of Chemistry*, *9*(2), 1185-1189. http://dx.doi.org/10.1016/j.arabjc.2012.01.004.
22. Scherer, R., Wagner, R., Duarte, M. C. T., & Godoy, H. T. (2009). Composição e atividades antioxidante e antimicrobiana dos óleos essenciais de cravo-da-índia, citronela e palmarosa. *Revista Brasileira de Plantas Medicinais*, *11*(4), 442-449. http://dx.doi.org/10.1590/S1516-05722009000400013.
23. Bagheri, L., Khodaei, N., Salmieri, S., Karboune, S., & Lacroix, M. (2020). Correlation between chemical composition and antimicrobial properties of essential oils against most common food pathogens and spoilers: in-vitro efficacy and predictive modelling. *Microbial Pathogenesis*, *147*, 104212. http://dx.doi.org/10.1016/j.micpath.2020.104212. PMid:32344178.
24. Hasheminejad, N., Khodaiyan, F., & Safari, M. (2019). Improving the antifungal activity of clove essential oil encapsulated by chitosan nanoparticles. *Food Chemistry*, *275*, 113-122. http://dx.doi.org/10.1016/j.foodchem.2018.09.085. PMid:30724177.

25. Chaieb, K., Hajlaoui, H., Zmantar, T., Kahla-Nakbi, A. B., Rouabhia, M., Mahdouani, K., & Bakhrouf, A. (2007). The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae): a short review. *Phytotherapy Research*, *21*(6), 501-506. http://dx.doi.org/10.1002/ptr.2124. PMid:17380552.
26. Wang, L., Liu, F., Jiang, Y., Chai, Z., Li, P., Cheng, Y., Jing, H., & Leng, X. (2011). Synergistic antimicrobial activities of natural essential oils with chitosan films. *Journal of Agricultural and Food Chemistry*, *59*(23), 12411-12419. http://dx.doi.org/10.1021/jf203165k. PMid:22034912.
27. Mulla, M., Ahmed, J., Al-Attar, H., Castro-Aguirre, E., Arfat, Y. A., & Auras, R. (2017). Antimicrobial efficacy of clove essential oil infused into chemically modified LLDPE film for chicken meat packaging. *Food Control*, *73*(Part B), 663-671. http://dx.doi.org/10.1016/j.foodcont.2016.09.018
28. Lima, M. S., Carvalho, D. S., Silva, S. H., Caetano, V. F., & Vinhas, G. M. (2017). Avaliação do efeito antimicrobiano do óleo essencial de cravo em filmes de poli (cloreto de vinila). *Revista Brasileira de Agrotecnologia, 7*(2), 294-298.
29. Mupalla, S. R., Kanatt, S. R., Chawla, S. P., & Sharma, A. (2014). Carboxymethyl cellulose–polyvinyl alcohol films with clove oil for active packaging of ground chicken meat. *Food Packaging and Shelf Life*, *2*(2), 51-58. http://dx.doi.org/10.1016/j.fpsl.2014.07.002.
30. Martelli, S. M., Sabirova, J., Fakhouri, F. M., Dyzma, A., de Meyer, B., & Soetaert, W. (2012). Obtention and characterization of poly(3-hydroxybutyricacid-co-hydroxyvaleric acid)/mcl-PHA based blends. *Lebensmittel-Wissenschaft + Technologie*, *47*(2), 386-392. http://dx.doi.org/10.1016/j.lwt.2012.01.036.
31. Giaquinto, C. D. M., Souza, G. K. M., Caetano, V. F., & Vinhas, G. M. (2017). Evaluation of the mechanical and thermal properties of PHB/canola oil films. *Polímeros: Ciência Tecnologia*, *27*(3), 201-207. http://dx.doi.org/10.1590/0104-1428.10716.
32. American Society for Testing and Materials – ASTM. (2012). *D882-12: Standard Test Method for Tensile Properties of Thin Plastic Sheeting*. Philadelphia: ASTM.
33. Narayanan, A., Neera, Mallesha, & Ramana, K. V. (2013). Synergized antimicrobial activity of eugenol incorporated polyhydroxybutyrate films against food spoilage microorganisms in conjunction with pediocin. *Applied Biochemistry and Biotechnology*, *170*(6), 1379-1388. http://dx.doi.org/10.1007/s12010-013-0267-2. PMid:23666640.
34. Ostrosky, E. A., Mizumoto, M. K., Lima, M. E. L., Kaneko, T. M., Nishikawa, S. O., & Freitas, B. R. (2008). Métodos para avaliação da atividade antimicrobiana e determinação da Concentração Mínima Inibitória (CMI) de plantas medicinais. *Revista Brasileira de Farmacognosia*, *18*(2), 301-307. http://dx.doi.org/10.1590/S0102-695X2008000200026.
35. Ahmed, J., Mulla, M., Jacob, H., Luciano, G., Bini, T. B., & Almusallam, A. (2019). Polylactide/poly(ε-caprolactone)/zinc oxide/clove essential oil composite antimicrobial films for scrambled egg packagings. *Food Packaging and Shelf Life*, *21*, 100355. http://dx.doi.org/10.1016/j.fpsl.2019.100355.
36. Song, N., Lee, J., Mijan, M., & Song, K. B. (2014). Development of a chicken feather protein film containing clove oil and its application in smoked salmon packaging. *Lebensmittel-Wissenschaft + Technologie*, *57*(2), 453-460. http://dx.doi.org/10.1016/j.lwt.2014.02.009.
37. Cui, H., Zhao, C., & Lin, L. (2015). The specific antibacterial activity of liposome-encapsulated Clove oil and its application in tofu. *Food Control*, *56*(2), 128-134. http://dx.doi.org/10.1016/j.foodcont.2015.03.026.
38. Bart, J. C. J. (2006). *Polymer Additive Analytics: Industrial Practice and Case Studies*. Italy: Firenze University Press. http://dx.doi.org/10.36253/8884533783
39. Tawakkal, I. S. M. A., Cran, M. J., & Bigger, S. W. (2016). Release of thymol from poly(lactic acid)-based antimicrobial films containing kenaffibres as natural filler. *Lebensmittel-Wissenschaft + Technologie*, *66*, 629-637. http://dx.doi.org/10.1016/j.lwt.2015.11.011.
40. Silva, A. O., Cortez-Veja, W. R., Prentice, C., & Fonseca, G. G. (2019). Development and characterization of biopolymer films based on bocaiuva (*Acromonia aculeata*) flour. *International Journal of Biological Macromolecules*, *155*, 1157-1168. http://dx.doi.org/10.1016/j.ijbiomac.2019.11.083. PMid:31726125.
41. Andrade, M. F., Silva, I. D. L., Silva, G. A., Cavalcante, P. V. D., Silva, F. T., Almeida, Y. M. B., Vinhas, G. M., & Carvalho, L. H. (2020). A study of poly (butylene adipate-co-terephthalate)/orange essential oil films for application in active antimicrobial packaging. *Lebensmittel-Wissenschaft + Technologie*, *125*, 1-22. http://dx.doi.org/10.1016/j.lwt.2020.109148.
42. Choi, M., Soottitantawat, A., Nuchuchua, O., Min, S., & Ruktanonchai, U. (2009). Physical and light oxidative properties of eugenol encapsulated by molecular inclusion and emulsion-diffusion method. *Food Research International*, *42*(1), 148-156. http://dx.doi.org/10.1016/j.foodres.2008.09.011.
43. Giménez, B., Gomez-Guillén, M. C., López-Caballero, M. E., Gomezestaca, J., & Montero, P. (2012). Role of sepiolite in the release of active compounds from gelatin-egg white films. *Food Hydrocolloids*, *27*(2), 475-486. http://dx.doi.org/10.1016/j.foodhyd.2011.09.003.

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