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Evaluation of the mechanical and thermal properties of PHB/canola oil films

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Abstract

Packages are essential for the food processing industry. Among the innovative alternatives there is antimicrobial packaging, which aims to reduce or inhibit microbial growing on the food surface. One potential to produce this type of package is poly(3-hydroxybutyrate)-PHB additivated with canola oil. In this work, films of PHB additivated with canola oil were produced in different compositions. Mid-infrared records, tensile mechanical testing and thermal analyses were performed on the films. The results of the mechanical tests indicated that the addition of canola oil to the polymeric matrix of PHB increases the material flexibility. The thermal analyses results showed that the addition of canola oil changes the thermal properties of PHB, such as the melting and crystallization temperatures, maximum crystallization rate and relative crystallinity. The knowledge of these properties is fundamental for the manufacturing process of polymeric materials, due to the specifications required for these materials in the intended applications.

Keywords: *antimicrobial packaging, canola oil, mechanical properties, poly(3-hydroxybutyrate), thermal properties.*

1. Introduction

Packages are essential to the food processing industry since they are responsible for maintaining the quality of food products^[1,2]. Among the types of packaging used for foods, there are passive and active packaging^[3]. The latter is used due to its performance under changing environmental conditions to maintain the sensorial properties of the food contents, thus providing quality assurance, increase of shelf lifetime, besides considerations of hygiene and food safety^[4,5]. There are various types of active packaging^[3], among which antimicrobial stands out, developed in order to reduce the microbial growing on the food surface^[6].

One alternative for antimicrobial packaging is the use of biodegradable polymers with vegetable oils that present antimicrobial activity. Biodegradable packages have been studied as an alternative to those made with synthetic plastics, which are known to have a negative impact on the environment^[7]. Among the options of biodegradable polymers available for packaging^[8], there is the poly(3-hydroxybutyrate)-PHB, which is a potential thermoplastic used to replace polymers obtained from petrochemical industry^[9]. PHB is an aliphatic polyester that is biodegradable in water and carbon dioxide under environmental conditions, sustainable, durable, produced from several microorganisms, and with some characteristics similar to polypropylene, a synthetic polymer^[10-13].

Vegetable oils are derived from a larger group of chemical compounds known as fats or lipids^[14]. One of these is canola oil, which has a huge potential for use in the manufacturing of bioplastics^[15]. Canola oil is compounded mainly from oleic, linoleic and alpha-linolenic acids^[16]. These components are long chain unsaturated fatty acids that

have antibacterial activity^[17]. Thus, PHB films additivated with canola oil are an alternative for use in the production of antimicrobial packages. In the literature, there are studies that have evaluated the antimicrobial activity of eugenol in films of PHB/eugenol^[18] and the influence of d-limonene in the mechanical, thermal and barrier properties in the PHB/PLA/d-limonene system^[19].

In this work, we evaluated both the mechanical and thermal properties of PHB films additivated with canola oil in different compositions for purposes of antimicrobial packaging. Also in this work, we have evaluated the antibacterial activity of canola oil. The knowledge of these properties is fundamental in the manufacturing process of polymeric materials due to the specifications that these materials must meet in their intended applications.

2. Materials and Methods

2.1 Preparation of films

PHB in powder was donated by PHB Industrial S/A. The canola oil used was the Purilev trademark of the Cargill company. This oil is compounded mainly from oleic (50-70%), linoleic (15-30%) and alpha-linolenic acids (5-13%). The solvent used was chloroform from the Vetec brand. The films were produced by the solution casting technique using 1.3 g of PHB and 50 mL of chloroform. PHB films additivated with canola oil were prepared in different amounts (0, 2, 6 and 10% w/w). The films had an average thickness of 0.09 ± 0.02 mm. In total, 36 samples were prepared by casting.

2.2 Antimicrobial activity of canola oil

The activity of the canola oil was evaluated by disk diffusion assay^[20] with medium Plate Count Agar (PCA). Filter paper disks of 2 cm diameter were utilized. Aliquots of 0.5 ml of *E. coli* (ATCC 8739) in the order of 107 CFU/ml, were quantified by turbidity on the Mcfarland comparison scale. They were inoculated into the PCA by the pour plate method. After solidification of the PCA, these were placed on discs soaked with canola oil, in the center of a Petri dish. The plates were incubated at 35 °C for 48 h.

2.3 Mechanical tests

Mechanical tests of the films were made in the universal machine for tensile testing, model DL-500 MF from EMIC brand following the ASTM D882-12 standard^[21]. The tests took place at room temperature without humidity control. Assays were performed under the following conditions: load cell of 500 N; jaw speed of 5 mm/min; initial distance between the jaws of 40 mm; and specimen dimension of 2.5 x 7.5 cm. For the mechanical test, nine films for each composition were analyzed.

2.4 Spectral acquisition

Mid-infrared (MIR) spectra of the films and canola oil were recorded using a Spectrum 400 FT-IT/FT-NIR spectrometer from Perkin Elmer brand with Horizontal Attenuated Total Reflectance-HATR accessory under the following conditions: spectral region from 4000 to 650 cm⁻¹ with resolution of 4 cm⁻¹ and 16 scans. From the spectra recorded, a Principal Component Analysis-PCA was performed in the software The Unscrambler® version 9.7.

2.5 Thermal analyses

Thermal analyses of the films were performed using a calorimeter from Mettler Toledo brand, DSC STAR and SYSTEM model. Each sample used had mass of 4.0 ± 1.0 mg. The heating took place from -10 °C to 200 °C at a heating/cooling rate of 12 °C/min. The experiments were performed under a nitrogen cooling system. The data were processed using the software Integral® - Version B^[22]. It was assumed that the latent heat of the 100% crystalline PHB was 146 kJ/kg^[23].

The evolution of the relative crystallinity x observed at different temperatures was computed from the latent heat of the exothermic crystallization peaks as:

$$x = \frac{1}{E_0} \int_{t_1}^t |J(t') - J_0(t')| dt', E_0 = \int_{t_1}^{t_2} |J(t) - J_0(t)| dt \quad (1)$$

The variables in Equation 1 are: $J(t)$ is the heat flux recorded by DSC; $J_0(t)$ is a suitable virtual baseline; t_1 e t_2 are the onset and end times of the crystallization event; and t is an intermediate time related to a given value of x ($0 < x < 100\%$). E_0 represents the total heat for the observed crystallization process.

3. Results and Discussions

3.1 Mid-Infrared spectra of the films

Figure 1 illustrates representative spectra obtained from mid-infrared scans of canola oil, PHB film, and PHB film additivated with canola oil in the compositions of 2% w/w (PHB/2% CO), 6% w/w (PHB/6% CO) and 10% w/w (PHB/10% CO).

PHB has the following characteristic bands: 1720-1650 cm⁻¹ corresponding to the carbonyl stretching vibrations of ester groups of the polymer; 1460-1380 cm⁻¹ corresponding to asymmetric stretching and CH₂ groups; 1300-1100 cm⁻¹ corresponding to symmetric and asymmetric stretching of the COC group; 1274 and 1226 cm⁻¹ corresponding to crystalline phase of polymer; and 1261 and 1180 cm⁻¹ corresponding to amorphous phase of the polymer^[24]. Canola oil has the following characteristic bands: 3012 cm⁻¹ correspond to the C-H stretching vibration; 2920 and 2854 cm⁻¹ corresponding to the symmetric and asymmetric stretching vibration of the aliphatic CH₂ group, respectively; 1739 cm⁻¹ corresponding to the ester carbonyl functional group of triglycerides; 1457 cm⁻¹ corresponding to the bending vibrations of the CH₂ and CH₃ aliphatic groups; 1378 cm⁻¹ corresponding to the bending vibrations of CH₂ groups; 1244 and 1165 cm⁻¹ corresponding to the stretching vibration of the C-O ester groups; and 713 cm⁻¹ corresponding to the overlap of the CH₂ rocking vibration and the out-of-plane vibration of cis-disubstituted olefins^[25]. The incorporation of canola oil (CO) can be identified in the spectra of films of PHB/CO by increasing the maximum peak intensity corresponding to the ester carbonyl functional group which is detected between 1620 cm⁻¹ and 1810 cm⁻¹. In PHB films to which 10% canola oil was added, it is also possible to visualize bands with lower absorption intensities, such as those referring to the wavenumbers 2920 cm⁻¹ and 2854 cm⁻¹.

To confirm that it had the incorporation of canola oil into the matrix analysis, Principal Component Analysis (PCA) was performed. PCA^[26] is a chemometric technique used to evaluate the variations in the data. The PCA extracts the key information variations from the original variables and reduces the dimensional space, creating a reduced number of variables called principal components (PC's)^[27-29]. The PCA technique consists of decomposing the data matrix X (m samples and n absorbances) into a product of two matrices, the matrix of the scores (T) and loadings (P), together with the error matrix (E)^[30,31]. The graph of the scores represents the coordinates of the samples in the PC space and the loadings represent the relevance of the original variables in each PC. From the PCA it is possible to identify samples that have the same spectral characteristics. This can be verified by grouping the samples in a graph of the scores. The scores plot is illustrated in Figure 2.

Figure 2 shows that the PCA separated the samples into distinct groups. Groups identified by the colors dark blue, green, red and light blue refer to films of PHB, PHB/2% CO, PHB/6% CO and PHB/10% CO, respectively. The PCA results showed that there was an incorporation of canola oil in the polymer matrix since the samples were grouped according to the PHB/CO composition. Note that as the concentration of canola oil increases, a distancing of the

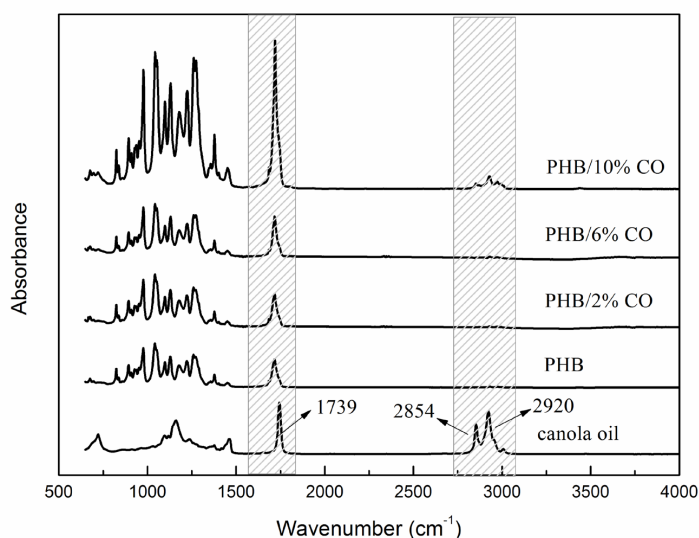


Figure 1. Mid-Infrared spectra of canola oil, PHB film and PHB/CO film in different compositions.

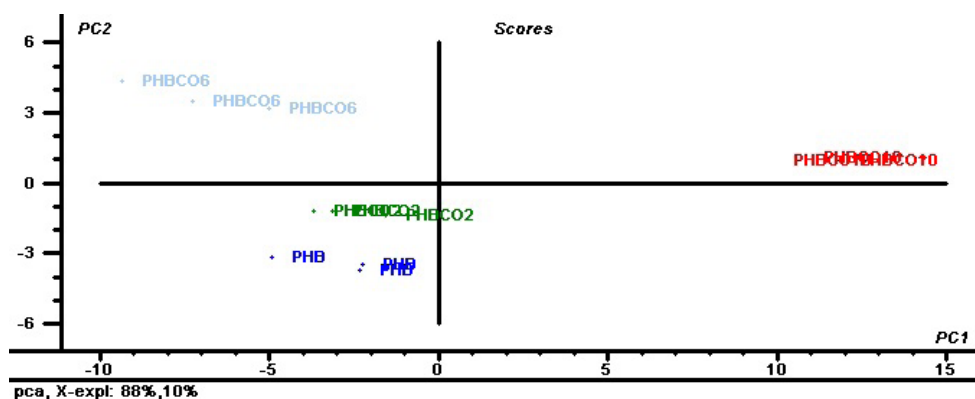


Figure 2. Score plots of PC1 x PC2 of PHB film and PHB/CO films.

samples in relation to the PHB/0% CO occurs, evidencing the incorporation of the oil into the polymeric matrix. The percentages of variance explained by PC1 and PC2 were 88% and 10%, respectively.

3.2 Mechanical properties

Table 1 shows the values obtained for the mechanical properties tensile strength (TS), percentage elongation at break (%E) and elastic modulus (EM). The mean values of the mechanical properties obtained through the mechanical tests were compared statistically by Duncan's test with a significance level of 5% ($p < 0.05$).

By the Duncan's test, the tensile strength the canola oil incorporation can be seen to cause a decrease in the value of this property. It can also be verified that the mean values obtained for the tensile strength with the addition of 6 and 10% showed no statistical differences considering a level of significance of 5%. The tensile strength expresses

the maximum strength of the material when under tension^[32]. The decrease in the value of this property, in PHB films, when the percentage of additive canola oil is increased, shows that the film has become mechanically less resistant when compared with the pure PHB film. The decrease in the tensile strength value was also observed in the work of Souza et al.^[33] with the incorporation of cinnamon essential oil in to polymer starch.

For the percentage elongation at break it can be seen that there was a decrease in the average value of this property only from the incorporation of 10% of canola oil. Elongation at break is a measure of the film's stretch ability prior to breakage^[34]. The other mean values were statistically equivalent when compared to the mean values of pure PHB films. The percentage elongation measures the ability of a film to stretch to its breakup. The incorporation of canola oil from 10% w/w led to a decrease in PHB's elongation ability. This decrease in the value of %EM

was also observed in the work of Hauser et al.^[35] with the incorporation of myrtle essential oil to methylcellulose.

There was a decrease in the value of the elastic modulus. An incorporation of 6 and 10% of canola oil showed no statistical differences for the 5% significance level. The elastic modulus is related to the stiffness of the material^[36]. The lower the value of the modulus of elasticity, the less rigid the film will be and vice versa. Thus, the decrease caused by incorporating canola oil results in less rigid films. As PHB is a rigid polymer, this change in stiffness is very positive when the films are intended for food purposes, whose packaging requires greater flexibility. The decrease in the value of EM was also observed in the work of Noronha et al.^[37] with the incorporation of nanocapsules of α -tocopherol in methylcellulose film.

3.3 Thermal properties

The output data from the Integral[®] program regarding the crystallization in the molten state and the cold crystallization are shown in Table 2 together with the melting temperature.

T_m shows the melting temperature of the material under study, $T_{c,i}$ is the temperature of crystallization, $\Delta H_{c,i}$ is the latent heat of crystallization and $X_{c,i}$ is the degree of crystallinity, considering $i=1$ for the crystallization from the molten state and $i=2$ for the cold crystallization. Regarding the melting temperature, a decrease is observed due to the increase in the percentage of canola oil in the polymeric matrix. The melting temperature of PHB is very close to its thermal degradation temperature ($\sim 180^\circ\text{C}$)^[38]. The decrease in the PHB melting temperature increases its possibilities for applications, because its applicability is limited due to their small window of processability^[39]. It is also observed that There was an increase in the crystallization temperature for the additivated films when compared to PHB films, both from the molten state as from the cold. On cooling, this implies an early crystallization and on heating a delayed crystallization. The values of the crystallized fraction from molten state of the PHB/6% CO and PHB/10% films are much smaller compared to PHB and PHB/2% CO films. This shows that the crystallization of PHB/CO and 6% PHB/10% films occurred, the most part, when cold.

3.3.1 Crystallization from the molten state

Figures 3a and 3b illustrate the curves of the rates of crystallization and the relative crystallinity versus temperature, respectively, for the crystallization from the molten state, with respect to the films of PHB and PHB additivated with canola oil in different concentrations.

Figure 3a shows that the maximum rate of crystallization occurred between 65°C and 75°C for the films additivated with canola oil, while for the PHB film the temperature was approximately 54°C . It is also possible to observe that the maximum crystallization rate values were lower for the films containing 2 and 6% of canola oil compared to PHB film. These results show that the percentage of canola oil has a direct influence on the polymer crystallization process. Figure 3b shows that the film additivated with 2% canola oil begins to crystallize at higher temperatures and finishes at higher temperatures than the PHB film, whereas the additivated films with 6 and 10% start to crystallize at lower temperatures, and finish at higher temperatures than PHB films.

3.3.2 Cold crystallization

For the analysis of cold crystallization behavior, Figures 4a and 4b illustrate the curves of the crystallization rate as a function of the temperature and the relative crystallinity as a function of the temperature, respectively, for films of PHB and PHB additivated with canola oil in different compositions.

Figure 4a shows that the maximum crystallization peaks of films were 2.2, 1.31, 1.31 and 1.27 for additions of 0, 2, 6 and 10% w/w CO. In this figure it is verified that there was a reduction in the value of the maximum crystallization rate for the additivated PHB film. Figure 4a also shows the temperatures that occur at the crystallization rate peaks. The temperatures are 41.9 , 48.5 , 48.5 and 50°C . This shows that each increase in temperature causes a faster crystallization rate, thus a higher peak, for the additivated films. Figure 4b shows that the increase in temperature causes a delay in the cold crystallization of the added PHB when compared to the non-additive film. This fact can be seen at a fixed temperature, where it is possible to see that the PHB has a greater relative crystallinity than the films with additives. Figures 4a and 4b show that the curves for

Table 1. Average values obtained for the mechanical properties tensile strength, percentage elongation at break and elastic modulus.

Sample	TS (MPa)	%E	EM (MPa)
PHB	20.87+0.85 ^a	4.12+0.41 ^a	801.30+24.46 ^a
PHB/2% CO	16.50+1.58 ^b	4.11+0.40 ^a	592.03+12.62 ^b
PHB/6% CO	10.45+0.15 ^c	4.01+0.12 ^a	375.20+22.06 ^c
PHB/10% CO	10.39+0.32 ^c	3.37+0.13 ^b	345.13+24.37 ^c

Means with the same letter in the same column do not differ with $p < 0.05$ for the Duncan's test.

Table 2. Data melting, crystallization from the molten state and cold crystallization of the samples.

Sample	T_m ($^\circ\text{C}$)	$T_{c,1}$ ($^\circ\text{C}$)	$\Delta H_{c,1}$ (kJ/kg)	$X_{c,1}$ (%)	$T_{c,2}$ ($^\circ\text{C}$)	$\Delta H_{c,2}$ (kJ/kg)	$X_{c,2}$ (%)
PHB	175.3	54.06	20.33	13.92	41.97	19.15	13.12
PHB/2% CO	174.8	72.17	26.56	18.19	48.72	9.83	6.74
PHB/6% CO	168.6	70.20	6.24	4.27	49.88	26.52	18.16
PHB/10% CO	167.6	66.77	3.61	2.47	48.63	29.33	20.09

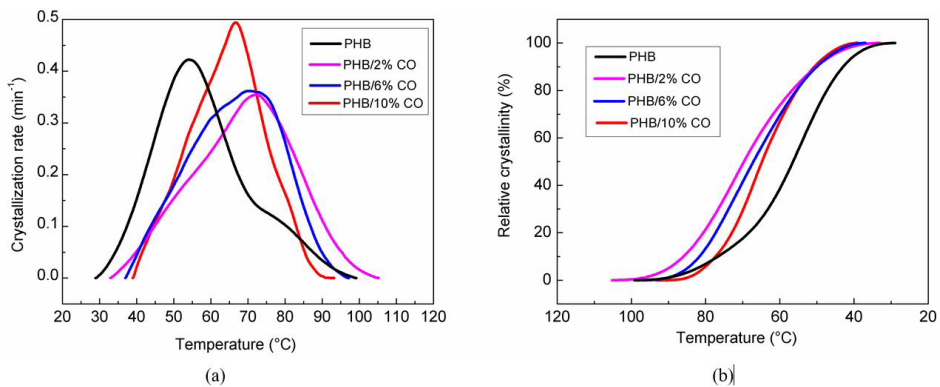


Figure 3. (a) Graph of the crystallization rate versus temperature of PHB film and PHB films additivated with canola oil in different compositions; (b) Graph of the relative crystallinity versus temperature of PHB film and PHB films additivated with canola oil in different compositions.

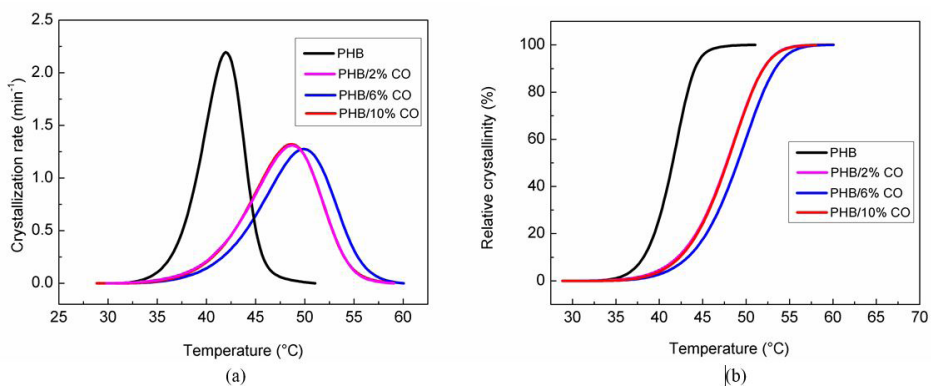


Figure 4. (a) Graph of the crystallization rate versus temperature of PHB film and PHB/CO films with different compositions; (b) Graph of the relative crystallinity versus temperature of PHB film and PHB/CO films with different compositions.

the PHB added with 2 and 6% w/w CO had a similar result, with overlaps occurring. This can be attributed to the additive values being very close and relatively low when compared to the mass of the polymer matrix.

3.4 Antimicrobial activity of canola oil

Figures 5a and 5b show the result of the antimicrobial activity of the sample without canola oil (control sample) and with canola oil, respectively, in the presence of *E. coli* bacteria. Figure 5a shows that there was no antimicrobial activity in the control sample. In Figure 5b the inhibition halo was observed, showing that there was antimicrobial activity in the presence of *E. coli* bacteria. The inhibition halo is observed around the sample, evidencing the absence of microorganisms. This result proves that canola oil is an antimicrobial agent and has the potential to be used in antimicrobial packages.

4. Conclusions

Infrared spectra in various compositions showed that canola oil was incorporated into a polymeric matrix of PHB. The results of an analysis of the mechanical properties showed

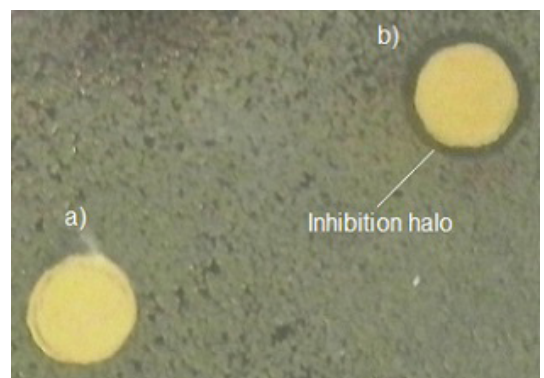


Figure 5. Antimicrobial activity in the presence in *E. coli* bacteria of the (a) sample without canola oil (control sample); and (b) sample with canola oil.

that the addition of canola oil increases the flexibility of the films. From the results of the thermal properties, it can be seen that the addition of canola oil caused changes in the melting and crystallization temperatures, in the maximum crystallization rate and in the relative crystallinity. Moreover,

the added oil accelerated crystallization from the molten state and delayed cold crystallization. The tested canola oil is an antimicrobial agent that can be used as a natural additive. These results indicate tendencies of PHB behavior when canola oil is added, suggesting a potential improvement for use in antimicrobial packaging.

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