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Physical, Morphological, Structural, Thermal and Antimicrobial Characterization of Films based on Poly(Lactic Acid), Organophilic Montmorillonite and Oregano Essential Oil

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This work developed ternary films based on Poly(Lactic Acid) (PLA), Cloisite 30B (C30B) and Oregano Essential Oil (OEO) as proposal to be applied in food packaging. The films thus manufactured were unscathed, with reduced surface defects. Their thickness and moisture content varied according to the composition and content of each component in the formulation. Optical micrographs indicated a homogeneous morphology, with good distribution of clay in the matrix. FTIR data confirms the incorporation of OEO into the films. TGA analysis indicated that the thermal stability of the films was not significantly affected by the incorporation of C30B and OEO. OEO incorporation did not promote an efficient antimicrobial action which is probably due to its retention by the clay. Further studies are needed to confirm this hypothesis. The combination between C30B and OEO in the PLA polymeric matrix is a promising proposal.

Keywords: *Food Packaging, Oregano Essential Oil, Poly(Lactic Acid).*

1. Introduction

Food shortages led society to recognize the need for strategies on food transport and storage, giving rise to the packaging industry. Initially, simple containers developed from wood, clay, animal skin, among others, were used for this purpose, However, over the years, new materials have been discovered, tested and applied for packaging manufacture, especially glass, paper, metals and plastics[1]. It is important to emphasize that despite the variety of current materials available for packaging, several materials are combined to explore their functional and/or aesthetic properties[2]. Food packaging aims is indispensable for the modern food industry to preserve the quality of the product from production to consumption[3] as it allows the transport of products over long distances and provides the necessary information on its label to consumers[4]. Packaging has a transitory nature as in most applications its shelf life is short since it is usually discarded after use[5].

Extending the shelf life of perishable foods by choosing a suitable packaging material, taking into account its susceptibility to oxidation and microbiological deterioration, are important considerations to preserve the quality of packaged foods throughout their life cycle[6]. Food packaging materials, traditionally known on the market, only offer barrier and protection functions (passive function), and other preservation techniques (refrigeration) do not guarantee the quality and safety of all foods. Numerous preservation techniques are being researched and developed, and among them a promising option is Active Packaging (AP)[7]. The development of an AP is based on the release of an active compound from the packaging that will inhibit the growth of microorganisms in the food. The use of natural antimicrobials instead of synthetic ones, such as Essential Oils (EOs) from plants, is stimulated.

According to Cirqueira[8], in recent years, the rise in the use of spices and their Eos considerably improved preservation techniques against microbiological deterioration. Thus, the

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use of antimicrobial films has become an attractive option for many food industry applications. Different types of chemical compounds were incorporated into polymeric matrices to act as antimicrobial agents, such as metal ions (copper and silver) and organic acids and their salts (malic, tartaric and lactic acids). However, pathogenic bacteria acquired antibiotic resistance and this led to investigations on the potential of plant-derived antimicrobials as a viable and promising option[9-11]. Among a variety of EOs, the Oregano Essential Oil (OEO) showed the highest antimicrobial activity against several foodborne pathogenic bacteria, and this behavior was attributed to the various active components present in it[12]. Thus, OEO is considered to be a good alternative to be used in packaging materials for food preservation.

The packaging industry is the largest industry of single-use (disposable) products made from plastics, which strengthens research on the use of biodegradable (sustainable) polymers as a substitute for conventional polymers for these applications, thus reducing the environmental impact caused by their inadequate disposal[13]. The most important biodegradable polymers are aliphatic polyesters, such as Poly(Lactic Acid) (PLA), Poly(ε-Caprolactone) (PCL) and Poly(3-Hydroxybutyrate) (PHB), with PLA standing out due to its properties[14,15]. PLA is a linear aliphatic thermoplastic polyester derived from lactic acid and produced from 100% renewable vegetable sources, being the most attractive among the biodegradable polymers available on the market for large-scale application as a packaging material. It is an easy to process biopolymer produced in an industrial scale with an annual growth rate of 17.2% from 2018 to 2023[16-18]. Films produced based only the neat polymer have several limitations, which can significantly and effectively be overcome by the incorporation of clay into the polymer matrix, with Montmorillonite clay being the most researched and used for that purpose[19].

Although montmorillonite is an efficient reinforcement for polymer matrices, its hydrophilic nature renders it difficult to disperse in most polymer matrices rendering it essential to chemically modify montmorillonite clays via organophilization reactions[20,21]. Pristine montmorillonite clay is polar while the organophilic clay is non-polar, which increases its affinity with polymers, since the vast majority of organic polymers are non-polar[22,23]. The most used commercial organophilic clays are Cloisite 10A, Cloisite 15A, Cloisite 20A, Cloisite 93A and Cloisite 30B[24], with Cloisite 30B (C30B) being the most used one to improve the properties of biodegradable polymers[25]. Just as PLA[26] and OEO[27], C30B was approved by the Food and Drug Administration (FDA) of the United States to be used in food packaging formulations[28].

Few studies have reported the development of ternary systems consisting of natural additives and organophilic clays in biodegradable polymer matrices, which justifies conducting further research on the potential applications of these systems in packaging. Thus, based on the information available and this being a promising system for food packaging, this work aims to develop and characterize PLA/C30B/OEO films. All films were characterized by visual evaluation, thickness, moisture content, optical microscopy, infrared spectroscopy, thermogravimetric analysis and *in vitro* antimicrobial assay.

2. Experimental

2.1. Materials

PLA 2003D produced by the NatureWorks Company (Minnetonka, Minnesota, EUA) was used as a matrix. The filler employed was a commercial organophilic clay, C30B, provided by Southern Clay Products (Gonzales, Texas, USA) with cation-exchange capacity (CEC) of 90 meq/100 g clay, organophilized with the surfactant MT2EtOH (methyl, tallow, bis-2-hydroxyethyl, quaternary ammonium chloride). According to FERQUIMA Technical Report (Vargem Grande Paulista, São Paulo, Brazil), the OEO employed has carvacrol (72%), gamma-terpinene (4.5%), linalool (4%), para-cymene (4%) and thymol (2%) as its main components.

2.2. Preparation of PLA/C30B systems and film manufacture

Prior to processing, PLA and C30B were dried in an air convection oven operating at 60 °C for 24 h as proposed by Zaidi et al.[29]. A PLA/C30B (85%/15% w/w) masterbatch was prepared in an internal mixer (Rheomix 3000, HAAKE™) operating with a fill factor of 70% and roller type rotors at 180 °C, 50 rpm for 7 min. These operational conditions were chosen based on the work of Zembouai et al.[30]. The material obtained was ground in knife mill from PROJEMAQ Engenharia. The ground material was dried at 60 °C for 24 h in an oven and then diluted in the polymer matrix in quantities necessary for the production of systems with 2, 4 and 6% w/w C30B in a bench-top single screw extruder (26 L/D), model AX-16 from AX Plásticos, operating with a temperature profile of 170, 175 and 180 °C and screw speed of 50 rpm.

Table 1 shows materials employed, their amounts and coding. The amounts of C30B and OEO used were determined based on the work of Ketkaew et al.[31].

Prior to film manufacture by compression molding, the concentrates were dried in an air circulation oven at 60 °C for 24 h. The material needed for the production of each film was placed between two teflon sheets positioned over an aluminum plate and then compression molded at 170 °C in a hydraulic press (Solab, Model SL-11/15) operating for 2 min at atmospheric pressure and, subsequently, at 4 tons for 2 min before demolding. The film produced was allowed to cool for 1 min at room temperature. For the films incorporated with the OEO, an additional step was carried out, which was the previous contact of the pellets contained in a beaker with the OEO.

2.3. Characterization of films

Tactile and visual analysis of the macroscopic films were performed in order to select those with good surface finish, that is, without apparent impurities, holes, scratches and bubbles around the clay particles. Sample thickness was determined according to the methodology adapted from the work of Souza et al.[32].

A thickness gauge (Model 130.125, DIGIMESS, São Paulo, Brazil) with a 0.01 mm graduation and an accuracy of ±0.02 mm was used to determine film thickness. The analysis was performed on 5 film specimens (3 x 3 cm) for each system

Table 1. Systems produced and respective coding.

Systems	Content			Coding
	PLA (%)	C30B (%)	OEO (%)	
Neat PLA	100	0	0	P
PLA/2%C30B	98	2	0	P/2C
PLA/4%C30B	96	4	0	P/4C
PLA/6%C30B	94	6	0	P/6C
PLA/2%C30B/2%OEO	96	2	2	P/2C/2O
PLA/4%C30B/4%OEO	92	4	4	P/4C/4O
PLA/6%C30B/6%OEO	88	6	6	P/6C/6O

and the thickness was measured in 10 different locations of each sample: two in the central part and eight along its perimeter. Average results are reported.

The moisture content of the films was determined by sample mass loss after drying in an oven as described (adapted) in works of Medina-Jaramillo et al.[33] and Song et al.[34]. Film samples (1.7 x 1.7 cm) were weighed before and after drying at 105 °C for 24 h and their moisture content was determined using Equation 1. The results reported are an average of 5 measurement per system.

$$Moisture\,content = \frac{\left(w_i - w_f\right)}{w_i} \times 100\% \quad (1)$$

Where: w_i and w_f are, respectively, the sample mass (g) before and after drying.

Optical microscopy (OM) was performed in a binocular optical microscope (Model ICC50 E, Leica Microsystems) operating in the transmission mode, 40X magnification and 500 μm scale. The samples analyzed sample were taken from the region between the perimeter and the center of the film.

Fourier-transform infrared spectroscopy (FTIR) spectra were obtained using a SHIMADZU Prestige-21 Spectrophotometer operating at wavelengths in the range of 3100 to 600 cm^{-1}.

The OEO was analyzed under Argon (Ar) atmosphere with a flow of 10 ml/min, from room temperature to 200 °C and heating rate of 10 °C/min, using a TG 209 F1 equipment from NETZSCH. The polymer films were analyzed in a TA Instruments equipment, Model SDT Q600 V20.9 Build 20, under an Ar atmosphere with a flow of 100 ml/min, from room temperature to 600 °C and heating rate of 10 °C/min. The thermogravimetric analysis (TGA) curve of the PLA/C30B/OEO based films was used to determine the actual content of OEO in the films after the compression molding step.

For the preparation of the inoculum for the *in vitro* antimicrobial assay, a culture of *Staphylococcus aureus* (*S. aureus*) (ATCC 25923) was obtained by transferring a loop of bacteria to a test tube with 3 ml of BHI Broth (Brain Heart Infusion) and, subsequently, this solution was incubated at 37 °C for 24 h. After this period, 0.1 ml of the previously prepared solution was removed and transferred to a test tube containing saline solution, and then the turbidity of the obtained solution was compared to that of a 0.5 standard on the McFarland scale, which represents 10^8 UFC/ml. The inner surface of seven petri dishes containing Müeller-Hinton Agar (growth medium) was inoculated with 0.1 ml of *S. aureus* with the aid of a sterile swab, and a 1 cm in diameter sample for each of the systems was deposited on the surface of the dishes. The plates were incubated in an Incubator (Model LUCA-161/01, LUCADEMA) for 37 °C/24 h after which they were inspected to determine for the formation of an inhibition halo.

3. Results and Discussion

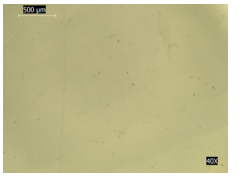
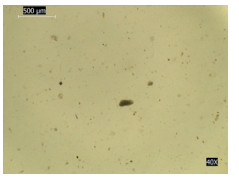
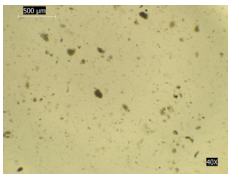
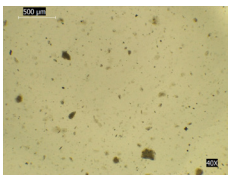
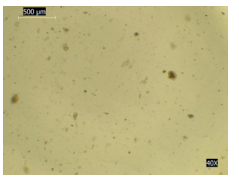
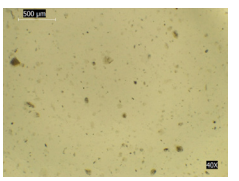
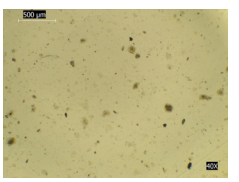
3.1. Visual evaluation, Thickness, Moisture content and Optical microscopy (OM)

The physical properties of the films produced were studied through visual evaluation, as well as measurements of thickness and moisture content. These characterizations, combined with other techniques, such as optical microscopy, allowed a relevant evaluation of fundamental characteristics of the films presented in Table 2.

In general, the films produced had a good surface finish and were free of irregularities that could compromise their performance. This is taken as an indication that the compression molding processing parameters were adequate, as they enabled the production of intact films with very few surface defects. The films produced are fairly flexible and strong and do not break during handling and bending. Our data shows that PLA film opacity to increase with C30B incorporation. The incorporation of OEO into the PLA/C30B films resulted in the production of less uniform films with a larger diameter, supposedly, due to the nature of the OEO that acts as a plasticizer. After processing, the films containing OEO had a characteristic odor. The films obtained in this work did not ooze OEO as they were not wet or moist to the touch.

As shown in Table 2, films with 0.15–0.19 mm thickness were obtained. The average thickness value for the neat PLA films was 0.1908±0.003 mm. Incorporation of 2% of C30B in the polymer matrix did not lead to changes in film thickness. On the other hand, reductions in film thickness of 9.64 and 13.73%, respectively, were observed for the systems with 4 and 6% of C30B. It was also observed that the diameters of compression molded films with 4 and 6% of C30B was greater than those of neat PLA or of those with 2% C30B, which accounts for the observed reduction in film thickness, suggesting that the flow of polymer chains was influenced by C30B particles. Clay particles influenced the diameter of the films produced, however, this influence was more evident with the increase in clay content from 4%. This change in diameter occurs because the chains of the quaternary ammonium salt used in the organophilization process of the natural montmorillonite clay can play the role of plasticizer

Table 2. Visual evaluation, thickness, moisture content and OM of the films produced.

Systems	Visual evaluation	Thickness (mm)	Moisture content (%)	OM (500 µm/40X)
P		0.1908 ± 0.003	0.5058 ± 0.111	
P/2C		0.1916 ± 0.001	0.6313 ± 0.104	
P/4C		0.1724 ± 0.001	0.6773 ± 0.066	
P/6C		0.1646 ± 0.003	0.9859 ± 0.077	
P/2C/2O		0.1742 ± 0.001	1.8719 ± 0.123	
P/4C/4O		0.1662 ± 0.004	2.5065 ± 0.225	
P/6C/6O		0.1514 ± 0.001	3.6293 ± 0.220	

and can be responsible for the mobility of polymer chains in higher particle content[35]. OEO addition in PLA/C30B films led to further decreases in film thickness, which was taken as an indication that OEO incorporation leads to increased flow of the molten material during processing.

For a material to be used as food packaging, it is essential to assess its water retention capacity, as when this is high it can be a relevant factor for the degradation of the packaging that comes into contact with food that has a high moisture content. The moisture content of the films manufactured was determined and shown in Table 2. Our data is in agreement with that reported by Van de Velde & Kiekens[36] which the average moisture content of neat PLA films being approximately 0.5%. Film moisture content increased with clay incorporation and content increasing from 0.5% for neat PLA to about 1% with 6% of C30B. Although organophilic montmorillonite

is less polar than natural (pristine) montmorillonite, there are still polar groups on its surface after treatment which allow for interactions of the inorganic clay layers with the water molecules through hydrogen bonds. The incorporation of OEO in the PLA/C30B films led to a more significant increase in the moisture content of the films, especially for higher oil contents. Moisture contents ranged from 1.8–3.6% as OEO concentration increased from 2 to 6%. Possible explanations for the observed results are: 1) despite being hydrophobic, these oils do have some hydroxyl groups in their structure which could interact with water molecules or 2) the observed irregularities in film surfaces with OEO addition could assist in trapping water molecules.

Still regarding the moisture content of the films, the ideal result will depend on the type of food to be packaged, as each food interacts with the package in a specific way. According to Cozmuta et al.[37], the film with a high moisture content is more effective for packing aqueous foods and with a low content it is more effective for packing fatty foods. It is noteworthy that moisture influences several food deteriorations processes, such as lipid oxidation, microbial growth, non-enzymatic browning reactions and pigment destruction[38]. Souza[39] highlighted that increased humidity can cause undesirable effects on products, namely: cookies lose their crunchiness, powdered foods become hard and minimally processed vegetables have their structure altered due to mass loss.

Regarding the results of OM, the neat PLA sample showed a smooth surface, without voids or bubbles. The PLA/C30B films displayed a similar morphology with the absence of bubbles, but a few clusters of C30B were detected. Although the size and quantity of these clay agglomerates increased with increasing C30B content in the PLA matrix, particle distribution was good throughout the film area in all systems. The results obtained by OM did not allow for a detailed analysis on the effects of OEO incorporation on the morphology of PLA/C30B films. Despite the fact that the analysis of the surface of the films by OM could not provide in-depth conclusions, their contribution was relevant to this work, as they allowed us to observe that C30B particle distribution in the PLA matrix was a good.

3.2. Fourier-transform infrared spectroscopy (FTIR)

The interaction between PLA, C30B and OEO was observed by changes in FTIR characteristic band. According to Vlachos et al.[40] the FTIR technique allows the qualitative determination of different organic compounds, since the specific vibrational mode of each molecular group causes the appearance of a band at a characteristic wavelength. Figure 1 shows the spectra of the films manufactured.

According to the spectra, vibrations assigned to PLA chemical bonds were verified at the following

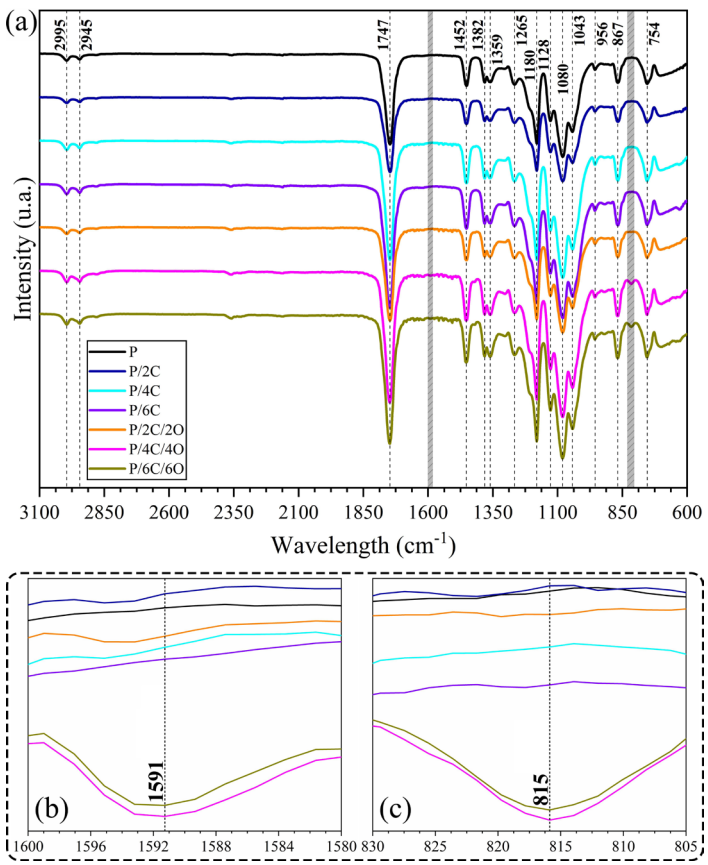


Figure 1. FTIR spectra of the films produced: (a) bands in the 3100–600 cm^{-1} region; (b) emphasis on the bands in the 1600–1580 cm^{-1} region and (c) 830–805 cm^{-1}.

wavelengths: 2995 cm^{-1} (─CH─ asymmetric stretch)[41]; 2945 cm^{-1} (─CH─ symmetric stretch)[42]; 1747 cm^{-1} (─C=O carbonyl asymmetric stretch)[43]; 1452 cm^{-1} (─CH_3 asymmetric deformation)[44]; 1382 cm^{-1} (─CH─ symmetric deformation)[45]; 1359 cm^{-1} (─CH_3 symmetric deformation)[46]; 1265 cm^{-1} (─C─O─ stretch)[47]; 1180 cm^{-1} (─C─O─ lactide stretch/─C─O─C─ asymmetric stretch)[48]; 1128 cm^{-1} (─C─O─ stretch)[49]; 1080 cm^{-1} (─C─O─ stretch)[50]; 1043 cm^{-1} (─OH bending)[51]; 956, 867 and 754 cm^{-1} (─C─C─ stretch)[52,53].

In comparison with the spectra of the P/2C, P/4C and P/6C films, it was found that there was no change in the positioning of the PLA bands in the presence of C30B, however, the intensity of the bands increased with clay content, especially for systems with 4 and 6% C30B. The spectra of the PLA/C30B films showed that no new band appeared in the region of 3100–600 cm^{-1} due to the incorporation of C30B into the polymer matrix, which in principle may indicate that there was only a physical interaction between PLA and C30B. Alves et al.[54] stated that C30B clay exhibits bands at 515 and 460 cm^{-1} that are attributed to the O─Si─O bending vibration of the montmorillonite structure, but the verification of possible interactions below the 600 cm^{-1} wavelength was not feasible. However, an increase in band intensity of 1747 cm^{-1} relative to carbonyl was found with incorporation from 4% C30B, which can be attributed to the interactions between the hydroxyl of the clay organic modifier and the PLA carbonyl[55].

With the incorporation of OEO, two new bands were observed, mainly for the films with 4 and 6% OEO, which allowed the identification of functional groups of OEO. Cardoso[56] stated that the identification of bands referring to the essential oil is a satisfactory result, as the high temperatures used in film processing can degrade the oil components. It was found that the intensity of all bands of the PLA matrix increased in the presence of OEO, and this result is corroborated by Pola et al.[57]. The new bands that appeared at 1591 and 815 cm^{-1} (Figure 1b and 1c, respectively) confirmed the efficient incorporation of OEO into the films, being attributed to the vibrations of the bonds in aromatic compounds. The band located around 1591 cm^{-1} refers to the aromatic unsaturation of the carvacrol molecule, specifically, to the C─C stretch and its presence provided a strong indication of its incorporation into the PLA matrix, as carvacrol it is the main active component of the OEO[58,59]. The band at 815 cm^{-1} refers to C─H ring bonds in carvacrol[60,61]. Therefore, FTIR spectra indicated that OEO was incorporated in the PLA/C30B films.

3.3. Thermogravimetric analysis (TGA)

The thermal property of the film reflects its ability to withstand degradation at elevated temperatures and is commonly analyzed by TGA. T_{onset} refers to the initial thermal degradation temperature and T_{endset} represents the final degradation temperature. The maximum degradation temperature (T_{dmax}) was determined from the maximum peak temperature in the 1st derivative of the TGA curve. Figure 2 illustrates the OEO TGA and DTGA curve.

TGA of the OEO, showed only one thermal event in the range of 136 and 180 °C with a T_{dmax} of 168 °C. The determination of the temperature range for the OEO mass loss was an important result for the definition of the film processing temperature, as one of the main problems in the incorporation of essential oils in polymeric matrices is the high temperatures used in polymer processing, which associated with other processing conditions, can affect the chemical stability of the compounds present in the OEO, thus reducing their antimicrobial effect. This statement is based on the high processing temperature discussed above, since at low temperatures the primary quality of the essential oil is conserved with minimal change. It is also important to mention that the processing time for film production directly influences the thermal stability of the OEO, as the longer the processing time, the greater the mass loss of this component. A single thermal degradation event is a common feature of numerous essential oils.

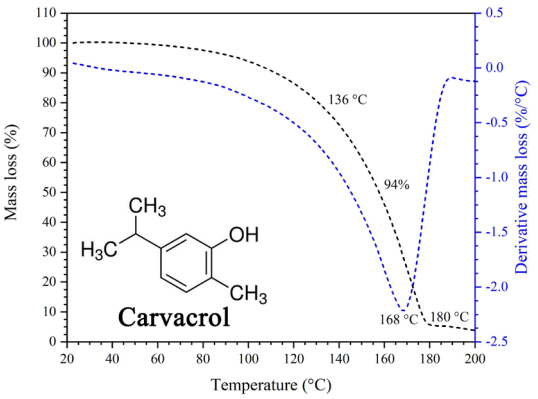


Figure 2. TGA and DTGA curve of OEO.

The carvacrol molecule was highlighted in Figure 2 due to its predominance in the chemical composition of OEO and according to Yahyaoui et al.[62], the thermal degradation of essential oils is associated with their chemical composition. Several authors reported different values for the thermal stability of OEO and carvacrol. Keawchaoon and Yoksan[63] analyzed carvacrol via TGA and observed mass loss starting from 183.8 °C with a T_{dmax} of 213.9 °C. Hosseini et al.[64] state that OEO starts losing mass at 171 °C and obtained T_{dmax} at 195 °C. The OEO characterized by Kaya et al.[65] showed a mass loss at room temperature and at 100 °C the oil lost around 80% of mass. In the work carried out by Guimarães et al.[66] carvacrol showed a mass loss of 100% up to 168 °C. According to Ferrándiz et al.[67], OEO displays a mass loss attributed to evaporation starting at 60 °C and T_{endset} at 190 °C. The OEO employed in the work by Llana-Ruiz-Cabello et al.[68] exhibited mass loss from room temperature to 180 °C.

In Figures 3 and 4, the TGA and DTGA curves of the films without and with the incorporation of OEO are presented, respectively, and in Table 3 the parameters of each thermal event extracted from the curves are shown.

In general, the TGA curves shown in Figure 3 exhibited a common trend for mass loss for neat PLA and PLA/C30B films (one thermal event), however, for PLA/C30B/OEO films two thermal events were observed, this result being more noticeable in the curve of the system with 6% OEO. For the films incorporated with OEO, the 1st thermal event refers to the degradation of the oil itself and the 2nd refers to the

Table 3. Thermal parameters obtained by TGA and DTGA.

Systems	1st thermal event					2nd thermal event				
	T_{onset} (°C)	T_{endset} (°C)	T_{dmax} (°C)	PM[a] (%)	R[b] (%)	T_{onset} (°C)	T_{endset} (°C)	T_{dmax} (°C)	PM[a] (%)	R[c] (%)
P	-	-	-	-	-	338,48	370,02	357,05	99,33	0,539
P/2C	-	-	-	-	-	335,60	368,94	357,83	97,32	2,550
P/4C	-	-	-	-	-	332,53	363,64	352,79	97,66	2,180
P/6C	-	-	-	-	-	336,40	370,09	358,86	94,40	5,414
P/2C/2O	133,27	153,99	144,47	1,692	97,03	332,62	360,79	348,80	97,67	2,125
P/4C/4O	123,51	147,97	139,96	3,119	96,76	332,47	367,60	355,33	97,28	2,574
P/6C/6O	109,97	149,29	133,30	4,822	95,13	331,54	369,12	357,46	98,03	1,910

Caption: [a]mass loss; [b]residue at 190 °C; [c]residue at 600 °C.

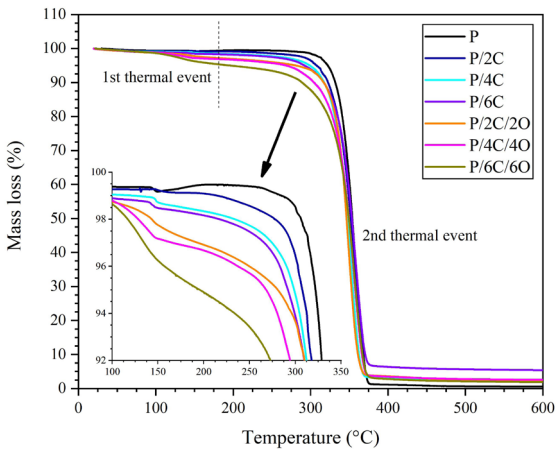


Figure 3. TGA curves of the produced films showing the two observed thermal events. The 1st event is evident only in films incorporated with OEO, being more pronounced with the 6% OEO content.

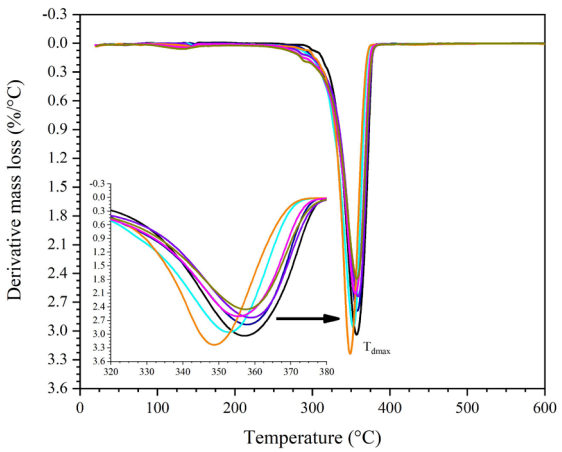


Figure 4. DTGA curves of the films produced, with emphasis on the T_{dmax}, which refers to the maximum degradation temperature of the films.

degradation of PLA with C30B. The 1st curve event of the PLA/C30B/OEO films confirms the incorporation of OEO into the PLA/C30B films after processing. It is believed that interactions of PLA with OEO and C30B leads to a different arrangement of the matrix molecular chains, influencing the thermal performance of the films.

It was expected that the incorporation of clay in the polymer matrix would lead to an increase in its thermal stability compared to that of the neat matrix, and this increase should be reflected in the T_{onset} of the polymer that would take longer to start its thermal degradation in the presence clay[69]. It is commonly accepted that this increase in thermal stability occurs because clay layers hinder the release of gaseous products generated during matrix degradation[70], as the clay particles are impermeable to the products formed in the degradation process. For a broader understanding of the thermal degradation of PLA and its pyrolysis mechanism, references Arrieta et al.[71] and Pires et al.[72] should be consulted. Consequently, the incorporation of clay into the matrix should reduce the rate of release of these products and the longer they remain in the matrix, the more likely they are to bind to the residual non-degraded matrix. This understanding is acceptable since reactive radicals are often produced during degradation[73]. The results presented in Table 3 and in the curves indicated a slight opposite effect, in which a small reduction in T_{onset} was observed with the incorporation of C30B clay in the matrix. Values for T_{endset} and T_{dmax} parameters of the films were similar that of the neat PLA matrix.

The data in Table 3 indicates that different C30B clay contents did not result in an expressive change in the thermal stability of the PLA/C30B films. According to the literature, this slight reduction in the stability of polymer/clay materials may be associated with dispersion of clay particles in the matrix, thermal instability of cations and catalytic action of clay (organophilic). Bitinis et al.[74] produced PLA/Cloisite Na^+ based materials and observed that clay decreased T_{dmax}. The authors attributed this result to the poor dispersion of clay in the matrix, causing the formation of tactoids, thus, the barrier against gases was inefficient, which accelerated the degradation of the material. The optical micrographs shown in Table 2 for the PLA/C30B systems showed clay agglomerates, and it is believed that these agglomerates also directly influenced the reduction of the thermal stability of the films produced. Hsieh et al.[75] observed that the increase in clay content caused a reduction in T_{onset}, which was attributed to the thermal instability of the quaternary ammonium cations that accelerated matrix degradation. In other works, the reduction in stability occurred because

the organophilic clay acted as catalyst for matrix degradation at high temperature[76,77].

The incorporation of OEO to the films did not result in significant changes in the thermal parameters although the values for PLA/C30B/OEO films were slightly lower than those of PLA/C30B films. The result obtained is in accordance with the work of Rodriguez et al.[78] who stated that the thermal stability of the matrix is generally greater with the incorporation of clay than with the incorporation of essential oil, since that the volatilization of the oil favors matrix degradation. The plasticizing effect of OEO may also have contributed to the reduction in thermal stability of PLA/C30B/OEO films, as the incorporation of a plasticizer in a polymer matrix leads to a reduction in interactions between polymer chains and, as a result, thermal stability also decreases[79]. Despite the slightly reduced thermal stability, the combined incorporation of C30B clay and OEO in the PLA matrix is a promising feature, since C30B can function as an OEO carrier and ensure its preservation in the matrix at high processing temperatures. Other works also found that no significant changes in the thermal stability of the film produced with the incorporation of essential oil[80,81].

Figure 5 illustrates the difference between the nominal OEO content (content incorporated in the formulation of the films) and real (content quantified by means of TG curves). These results show that the real content of OEO differed from the nominal content, which indicated that although a small quantity of the additive was lost during processing, most of it remained in the films as approximately 85% (P/2C/2O), 78% (P/4C/4O) and 80% (P/6C/6O) of the nominal OEO content was measured by TGA. This is considered a satisfactory result if one takes into account the volatility of OEO.

The quantification of the essential oil content through the TG curve was also performed by other authors. In the work by Ramos et al.[82], the films developed from PLA/Thymol/Organophilic Montmorillonite showed that 30% of the initial thymol was lost during processing by evaporation or degradation due to the high temperature used during polymer melting. However, the thymol content after processing was slightly higher in the clay-incorporated films, because the clay retarded the evaporation of thymol during processing. For the films produced by Krepker et al.[83] based on Polypropylene/Carvacrol and Polypropylene/(Haloysite Nanotubes-Carvacrol) a real carvacrol content of 3.1% (nominal content of 4%) was obtained, suggesting that around ~80% of the nominal content remained after processing. Finally, irrespective of composition, all films exhibited considerably low residual mass at 600 °C, indicating almost complete degradation of the films.

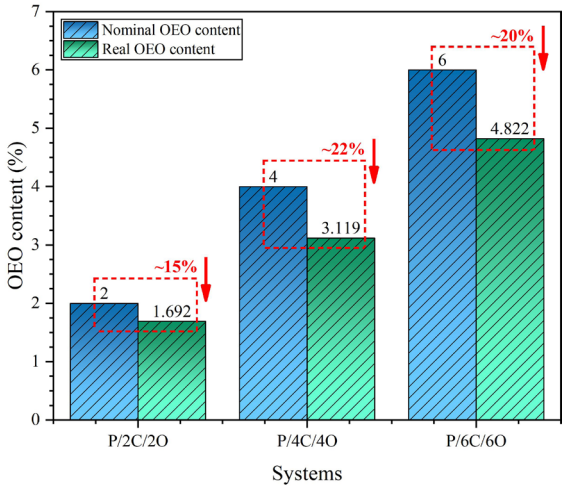


Figure 5. Nominal and real OEO content*. *The values are an estimate based on the mass loss, therefore, it is not an absolute result.

3.4. In vitro antimicrobial assay

In this work, the *in vitro* antimicrobial activity of the films was considered a preliminary analysis as this analysis reports the result directly compared to the *in vivo* analysis (in a food). The result of the efficiency of the films against *S. aureus* inhibition is shown in Figure 6.

The agar diffusion method has as its basic principle the diffusion of an antimicrobial agent through the culture medium. According to Martínez et al.[84], the efficiency of this method is based on the measurement of a clear zone formed around the film disk, representing the region where there was inhibition of bacterial growth called "inhibition halo". Halo formation is related to the spread of the antimicrobial and the rate of growth of the bacteria. As to the bacterium used in this work, it is characterized as a Gram-positive pathogen capable of contaminating several foods, such as minimally processed vegetables and meat products, and it is a concern for the health of consumers if they consume the contaminated food[85,86].

The neat PLA film showed no trace of antimicrobial activity against *S. aureus*, allowing its growth around and on the film surface and, as a result, there was no formation of an inhibition halo. A similar result was observed with the incorporation of C30B clay, although some traces of antimicrobial activity were noticeable in the films containing 4 and 6% of C30B. A low density of bacterial growth was observed in the contact area of the film with 2% of C30B. The antimicrobial activity of PLA/C30B films is mainly attributed to the quaternary ammonium salt present in the chemical structure of C30B[87]. According to Sadeghianmaryan et al.[88] interactions that take place between the salt, that exhibits a positive charge and the anionic molecules (negative charge) on the cell surface, affect the permeability of the bacterial cell membrane leading to cell content leakage and death occur of the cell.

It is possible that the lack of continuous and uniform inhibition halo observed in PLA/C30B films can be attributed to the loss of the antimicrobial function of C30B due to thermal degradation of the clay quaternary ammonium organic modifier during processing at high temperatures, as the clay was subjected to high temperatures and/or shear in the production of the masterbatch (internal mixer) and in its dilution (single screw extruder), as well as in the development of the films (compression molding). Zehetmeyer[89] observed that films produced based on Polypropylene/Cloisite 15A did not exhibit a halo against the bacteria: *S. aureus*, *Listeria monocytogenes* (*L. monocytogenes*), *Bacillus cereus* (*B. cereus*), *Escherichia coli* (*E. coli*), *Salmonella enterica* (*S. enterica*) and *Pseudomonas aeruginosa* (*P. aeruginosa*). Despite the limited inhibition of the films against *S. aureus*, the results showed that C30B clay particles can be incorporated

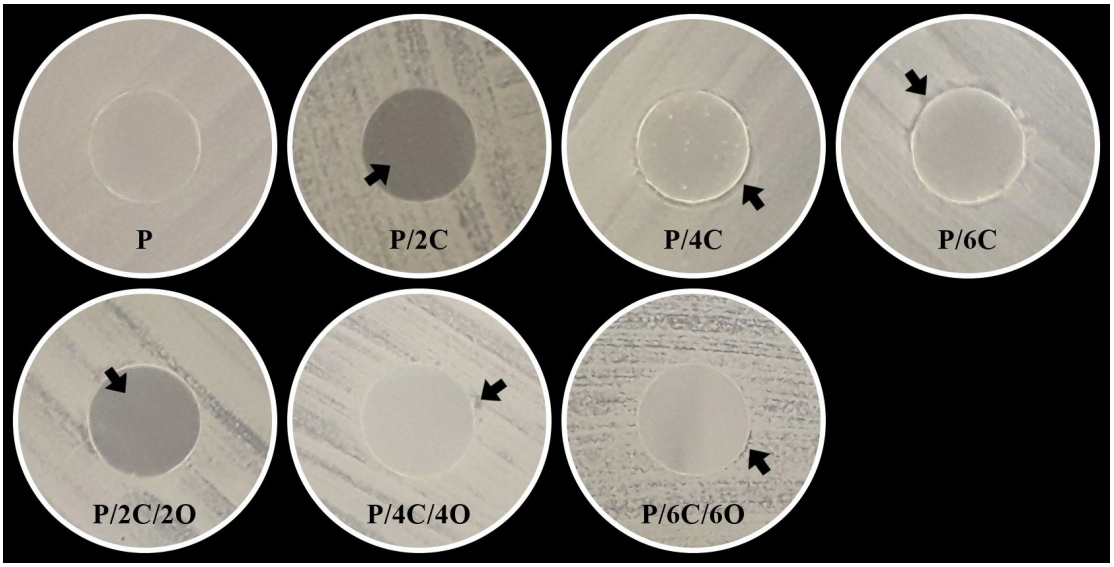


Figure 6. Antimicrobial activity of the films evaluated by the agar diffusion method against *S. aureus* bacteria.

into the PLA matrix not only as a reinforcement, but also as an antimicrobial aid to provide functional properties.

Although OEO is recognized as a powerful antimicrobial agent for several types of microorganisms, the PLA/C30B/OEO films did not show an antimicrobial activity against *S. aureus*, since an inhibition halo was not observed. It was explained in the literature that clay can act to control the diffusion and increase the retention of antimicrobials in polymers. Mascheroni et al.[90] observed that the increase in the montmorillonite content enabled a greater retention of carvacrol in wheat gluten matrix. The lack of a inhibition halo with the incorporation of an antimicrobial agent was also reported in the literature. Ramos et al.[91] produced films based on Polypropylene/Thymol-Carvacrol and found only antimicrobial activity on the film surface against *E. coli*. Scudeler et al.[92] stated that there was no halo formation against the bacteria *S. aureus*, *Staphylococcus epidermidis* (*S. epidermidis*), *E. coli*, *P. aeruginosa* and *S. enterica* in film produced from Nile tilapia protein isolate/Glycerol/OEO/Clove Essential Oil/Montmorillonite. Thus, the data obtained highlights the need for further studies to analyze the antimicrobial activity of the PLA/C30B/OEO films as a function of OEO and C30B content as well as on the king of pathogen incorporated.

4. Conclusions

In this work, compression molded flexible films based on neat PLA, PLA/C30B and PLA/C30B/OEO were obtained. Macroscopically, the films were unscathed and had reduced surface defects, showing that the processing parameters were adequate for film production. The thickness of the films varied according to the composition and content of each component of the formulation, with the OEO containing films being the thinnest. The moisture contents obtained were also influenced by these variables, with the highest values observed in films containing OEO. Optical micrographs showed a good distribution of C30B particles in the PLA matrix. FTIR results confirmed the effective incorporation of OEO into the PLA/C30B films. TGA analysis showed that the incorporation of C30B and OEO did not significantly affect the thermal stability of the films. Despite the low inhibition of PLA/C30B films against *S. aureus*, the results showed that this clay can act as an antimicrobial agent to provide functional properties. Regarding the incorporation of OEO, it was observed that there was no effective antimicrobial activity against *S. aureus*, making further studies essential. Although the combination of C30B and OEO in PLA to be used in food packaging is a promising proposal, other analyzes must be carried out to better understand the performance of these films.

5. Acknowledgments

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