Industrial Crops & Products 108 (2017) 388–397

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Development of active films poly (butylene adipate co-terephthalate) – PBAT incorporated with oregano essential oil and application in fish fillet preservation

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ARTICLE INFO

Keywords:
Active packaging
Origanum vulgare
Antimicrobial activity
Biopolymer

ABSTRACT

The food industry has been using antimicrobial active packaging as a tool for maintaining the quality of highly perishable products such as fish products. The use of synthetic materials, though, causes serious environmental impacts due to inappropriate disposal. Therefore, eco-friendly polymer materials from renewable sources stand out as alternatives to reduce such impacts. This study set out to develop biodegradable antimicrobial films comprising poly(butylene adipate-co-terephthalate) (PBAT) and oregano (*Origanum vulgare*) essential oil (OEO) for application in fish fillet storage. Films were produced by hot-melt extrusion and characterized as to morphological, mechanical, physical, water barrier, and structural properties. Their bioactive, antioxidant, and antimicrobial activities were also analyzed. OEO incorporation did not affect films' thermal properties, but the mechanical attributes tensile strength, elongation at break, and elastic modulus were impaired. Scanning electron microscopy images indicated heterogeneous, rough surfaces and cross-sections upon OEO addition. The higher the OEO content, the greater the water vapor permeability. Fourier-transform infrared spectroscopy corroborated the presence of OEO bioactive compounds in the extruded films. Microbiological assays demonstrated the effectiveness of the films in lessening total coliforms, *Staphylococcus aureus* and psychrotrophic microorganisms. Concerning antioxidant properties, the higher the OEO concentration, the stronger the antioxidant activity. The films produced here were found to be efficient as an active packaging system to control microbial growth in fish fillets. Additionally, their mechanical, thermal, and water barrier properties were demonstrated to be suitable for food packaging applications.

1. Introduction

The environmental impact of non-biodegradable plastic materials is an ever-growing global concern. In order to partially replace such materials, research on eco-friendly polymer materials from renewable sources that can improve food has been carried out (Khwaldia et al., 2010).

An alternative for minimizing the environmental impacts of the disposal of non-biodegradable plastics is poly(butylene adipate-co-terephthalate) (PBAT), which is a biodegradable aromatic-aliphatic polyester commercialized as Ecoflex®. It degrades within a few weeks and is processable through hot-melt extrusion into films featuring good mechanical properties (Gu et al., 2008).

These characteristics allow the use of PBAT in novel technological trends related to packaging, given that packaging has been developed for many years only for protecting food without any interaction with it. It should be mentioned that an extensive research is underway on in-package antimicrobial, including the incorporation of active agents onto the polymer, these agents can be, e.g., antimicrobials, antioxidants or enzymes that can act either on the surface of a solid food or in the bulk of liquid foods (Dainelli et al., 2008). This concept – so called active packaging – is a promising technology for the control of spoilage and pathogenic microorganisms in food products (Kapetanakou et al., 2013).

Active packaging can be comprised by active agents and environmentally friendly polymers, thus reducing the negative environmental impacts of synthetic food packaging. Meanwhile, these materials are able to enhance the safety of food consumption by lessening

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http://dx.doi.org/10.1016/j.indcrop.2017.06.058
Received 20 January 2017; Received in revised form 18 May 2017; Accepted 26 June 2017

the risk of the development of spoilage and/or pathogenic microorganisms, therefore prolonging food shelf life (Woranuch et al., 2015).

Active packaging may increase the shelf life and maintain the quality of fish fillets, which typically exhibit limited shelf life due to intrinsic characteristics, including neutral pH, high water activity, high contents of nutrients that may be metabolized by microorganisms, and high metabolic activity of natural microbiota (Silva et al., 2008).

Small contents of the antimicrobial agent migrate in a controlled fashion from active packaging to food instead of its direct addition into the product (Andrade-Molina et al., 2013). From the food safety standpoint, the antimicrobial substances used in the development of active films must be approved for human consumption because of their migration towards food (Sousa et al., 2016). In this context, the trend of reducing the use of chemical additives in foods has encouraged the search for natural antioxidant and antimicrobial additives that do not have negative effects in human health (Alves-Silva et al., 2013). Examples of these compounds include essential oils, which are volatile organic plant metabolites obtained via physical processes (Botre et al., 2010).

Among plant essential oils, that extracted from oregano (*Origanum vulgare*) has widely known antimicrobial and antioxidant activities, which are attributed to phenolic compounds, such as thymol, carvacrol, and eugenol (Burt, 2004). Several studies have demonstrated the efficacy of oregano essential oil (OEO)-incorporated films in inhibiting microbial development and synthesis of microbial metabolites, including pathogenic microorganisms such as *Staphylococcus aureus, Listeria monocytogenes, Salmonella* Enteritidis, and *Escherichia coli* (Hosseini et al., 2015), *S. aureus* (Oliveira et al., 2010), *S. aureus* and *E. coli* (Javidi et al., 2016), as well as yeasts and molds.

The objective of the present study was to develop active biodegradable films of PBAT incorporated with different concentrations of OEO to function as an active packaging system and promote the preservation of fish fillets by controlling microbial development and oxidation.

2. Materials and methods

2.1. Materials

The active film was based on (PBAT) (BASF, Germany), commercially known as Ecoflex®, and OEO (Ferquima, Brazil).

2.2. Film production

The films were produced using a formulation of 100 g of PBAT at different concentrations of oregano essential oil (0.0, 2.5, 5.0, 7.5 and 10.0 g). All components were manually mixed and then processed by hot melt extrusion in a Ø 16 mm L/D 40 mm twin screw extruder and a DR 1640 extruder, AX Plastics, Brazil, a flat matrix (45,5 × 10,4) operating with eight heating zones. The temperature profile was 80, 120, 130, 130, 140, 140, 145, 145, and 130 °C and the screw speed was 70 rpm, conditions that had been determined in preliminary tests (data not shown).

2.3. Apparent opacity

This test was performed in the spectrophotometer (FEMTO model 700 PLUS, Brazil). The films were cut into squares and adhered to the inner wall of the pail to be positioned perpendicular to the light beam. The visibly clear band was scanned at 600 nm for each film and the opacity was calculated according to Eq. (1):

$$Apparent\ \ opacity = \frac{A\,600}{T} \quad (1)$$

2.4. Mechanical properties

Film thickness was measured using a digital micrometer (Mitutoyo Corp, Brazil) prior to tensile assay in order to determine the mechanical attributes tensile strength, elongation at break, elastic modulus. The tests were carried out in the Universal Mechanical Testing Machine (EMIC DL-200MF, Brazil) equipped with a 1-kN load cell, in accordance with ASTM D882-09 (ASTM, 2009). Ten 9.0-cm-long, 1.5-cm-wide film strips of each formulation were mounted onto grips originally separated by 5.0 cm and then stretched at 500 mm·min^{-1} until rupture.

2.5. Microstructural characterization

Cross-section and surface images of the films were obtained by scanning electron microscopy (SEM) using a JEOL series 6390LV (Tokyo, Japan). A 0.5-cm^2 sample of each formulation was fixed in a stub, coated with a 20- to 30-nm-thick gold layer in a Sputter Coater (Balzers, model SCD 010), and imaged through SEM.

2.6. Fourier-transform infrared (FTIR) spectroscopy

FTIR spectra of the films were recorded in attenuated total reflectance (ATR) mode at wavenumbers ranging from 600 to 4000 cm^{-1} at a resolution of 2 cm^{-1} in a Spectrum 100 (Perkin Elmer, Brazil).

2.7. Thermogravimetric analysis (TGA)

The thermal behavior of films previously conditioned at 54% RH for 24 h was obtained using a thermogravimetric analyzer (Pyris 1, Massachusetts, USA). Samples of approximately 5 mg heated at 10 °C min^{-1} from 25 to 700 °C within an atmosphere of nitrogen flowing at 50 mL min^{-1}. The onset degradation temperature (T_{onset}) – *i.e.*, the temperature at which 5% of the mass was lost – was recorded. The temperature at which the maximum degradation rate occurred (T_{max}) – *i.e.*, temperature of the peak in the first derivative curves – as well as the percentage of mass lost at 700 °C were also registered.

2.8. Differential scanning calorimetry (DSC)

Approximately 10 mg of film was sealed in aluminum pans. An empty pan was used as reference. A heating scan was carried out between 25 and 700 °C at 10 °C min^{-1}. The tests were performed in a DSC equipment (model DSC 7, Perkin Elmer, Brazil) within an atmosphere of nitrogen flowing at 20 mL min^{-1}. The glass transition (T_g) and melting (T_m) temperatures were calculated from the thermogram generated in the scan.

2.9. Water vapor permeability (WVP)

The desiccant method proposed in ASTM E96/E96M-09 (ASTM, 2009) was applied with some modifications. Vessels containing 15 g of anhydrous calcium chloride were hermetically sealed with the film coupled between the vessel and the cap containing a circular aperture, rendering the film the only barrier between the vessel interior and the external medium. After sealed the films were stored in a desiccator chamber at 22.4 °C and 71.3% RH, the latter being achieved through the use of a saturated sodium chloride solution. The weight of the capsules was recorded twice a day for 15 days. Permeability (P) was then calculated according to Eq. (2), wherein e is film thickness (m) and $WVTR$ is its water vapor transmission rate (Eq. (3)).

$$P\,(\mathrm{g/h\ m\ mm\ Hg}) = WVTR \times e \quad (2)$$

$$TTVA\,(\mathrm{g/h\ m^2}) = \frac{G}{t} \times A \quad (3)$$

Where:

G/t = slope coefficient of the linear stretch of the weight gain versus time (g/h) gradient of a line

A = film area where water vapor can permeate (m^2).

2.10. Antimicrobial efficiency in fish fillet storage

Films large enough to cover the surfaces of fish fillets were sterilized by ultraviolet (UV) radiation for about 2 min in each side. The fillets were wrapped with the films, heat sealed, and stored at 7 °C. Total coliforms, Staphylococcus aureus, and psychrotrophic microorganisms were investigated in accordance with the methodology described by APHA (2001) after 0, 2, 4, 6, 8, 10, and 12 days of storage.

2.11. DPPH-radical scavenging activity

DPPH-radical scavenging activity was determined according with the method proposed by Jongjareonrak et al. (2008), with a slight modification. Film samples (0.1 g) were cut into small pieces and mixed with 2 mL of methanol. The mixture was vigorously vortexed for 3 min and allowed to stand at room temperature for 3 h. Subsequently, it was vigorously vortexed for another 3 min and centrifuged at 2300 rpm for 10 min. The supernatant obtained was analyzed for DPPH-radical scavenging activity. An aliquot of the centrifuged methanol extract (500 μL) was mixed with 2 mL of a 0.06 mM DPPH solution in methanol. The mixture was vortexed for 1 min and allowed to rest at room temperature and in the dark for 30 min. Absorbance was measured at 517 nm using a UV spectrophotometer (model UV-2101PC, Shimadzu, MD, USA). Methanol was mixed with 0.12 mM DPPH and used as blank. DPPH-radical scavenging activity was calculated by Eq. (4) (Singh and Ragini, 2004), wherein A_{sample} is sample absorbance and A_{blank} is the absorbance of the blank sample.

$$\text{Radical scavenging activity}(\%) = \left(1 - \frac{A_{sample\,(517\,nm)}}{A_{control\,(517\,nm)}}\right) \times 100 \quad (4)$$

2.12. Statistical analysis

The influence of OEO content on film properties was evaluated through Regression Analysis using the Statistical Analysis System software (SAS, USA, version 9.1).

3. Results and discussion

Hot-melt extrusion was suitable for producing OEO-containing PBAT films (Fig. 1). The films presented a homogeneous aspect, were continuous and did not exhibit surface defects. The observed yellowish hue is attributable to OEO addition.

3.1. Apparent opacity

The consumer acceptability of biodegradable films as food coatings may be affected by their optical properties (Shojaee-Aliabadi et al., 2013). The optical properties of the film are relevant because they play an important role in the coated product aspect, modifications in the optical characteristics of the films, suggest that the incorporation of essential oil influenced the color of the film, although the changes depend on the type of essential oil. (Martucci et al., 2015).

The films obtained by means of the casting technique presented a yellowish coloration. It was observed that as the concentration of OE was incorporated, the films had a more yellowish coloration, resulting from the hue of OE (Fig. 2).

This reduction of the opacity of the films is justified by the dispersion of the OEO in the PBAT, because it is a lipid phase, the increase of the reflectance occurs and the low rugorisity of the films after the incorporation of OEO, causes an increase of the brightness. According to Fabra et al., 2009 films with lipid fractions cause a light scattering due to the distribution of fat droplets. The brightness of the films is related to the roughness of the surface: in general, the rougher the surface, the lower the brightness (Atarés et al., 2012). In the present study, the following relation can be observed: the higher the OEO incorporation the greater the reflectance and the brightness of the films with the polymer matrix of PBAT.

3.2. Mechanical properties

Considering the mathematical models to which the acquired data on mechanical properties were fitted (Fig. 3), the tensile strength presented a maximum value at an OEO concentration of 4.01 g. For any higher OEO content, lower tensile strength is achieved. A similar behavior was observed for elastic modulus, but in this case the maximum value was led by an OEO content of 3.46 g.

According to Kokoszka et al. (2010), the plasticizers weaken the intermolecular interactions between polymer chains, increasing the mobility of the molecules and leading to films with less rigidity, as well as greater extensibility and flexibility. However, this weakening effect of intermolecular interactions on films with high concentrations of OEO can increase the free space between the chains, triggering an anti-plasticizer effect, an inverse behavior than expected, since the plasticizer in small concentrations interacts with the polymer matrix, But not enough to increase molecular mobility, only increases the degree of interactions and stiffness of this matrix (Mali et al., 2010).

The opposite was observed for elongation at break. The minimum value of this attribute was obtained for an OEO concentration of 6.66 g. Thus, small OEO contents were found to decrease film deformability, whereas the formulation comprising the highest OEO content ($OEO_{10.0}$) presented a higher elongation than $OEO_{5.0}$ and $OEO_{7.5}$. The opposite was observed for elongation at break. The minimum value of this attribute was obtained for an OEO concentration of 6.66 g. Thus, small OEO contents were found to decrease film deformability, whereas the formulation comprising the highest OEO content (OEO10.0) presented a higher elongation than OEO5.0 and OEO7.5. Was shown reduced at all non-zero OEO concentrations, when compared to the control film.

This effect may be related to the plasticizing action of OEO, which increases the free volume and consequently the free spaces, however, in films with high OEO concentrations, there is a great distance between the polymer chains due to the high concentration of plasticizer, reducing The elongation. Several studies reported similar behaviors, including those carried out by Guo et al. (2015) – who analyzed soy protein isolate (SPI) and PBAT films – and Martucci et al. (2015) – on biogenic gelatin films incorporated with OEO.

According to Bonilla et al. (2012), higher molecular contact between C—H compounds and oil may weaken aggregation forces between polymer chains, resulting in a less compact matrix, reducing the kinetic cohesion between them and, as a result, impairing the mechanical strength of the material. In this context, the increased elastic moduli were observed for low OEO concentrations, whereas higher concentration resulted in materials less rigid. The arrangement of PBAT molecular show three molecular CH bonds. Those points are undermined due to OEO action, which reduces the elastic modulus and tensile strenght after its maximum point (4.01).

According to Espitia et al. (2014), films containing lipids present limited ability to form a cohesive layer. This fact implies in reduced levels of intermolecular cohesion, which in turn are responsible for both strength and stiffness of the material. Overall, intermediate OEO concentrations ($OEO_{2.5}$ and $OEO_{5.0}$) showed no effect on tensile strength and elastic modulus of PBAT films.

3.3. Microstructural characterization

Fig. 4 presents SEM images of the surface (A) and cross-sections (B) of the produced films. No oil droplets were observed in the films.

Fig. 1. Poly(butylene adipate-co-terephthalate) (PBAT) films produced through hot-melt extrusion technique: (A) $OEO_{0.0}$, (B) $OEO_{2.5}$, (C) $OEO_{5.0}$, (D) $OEO_{7.5}$, and (E) $OEO_{10.0}$.

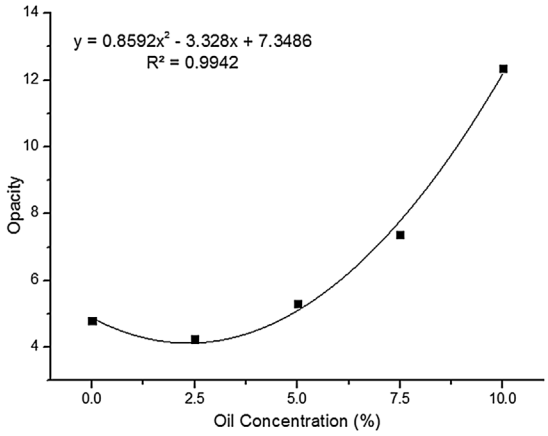

Fig. 2. Aparent opacity of poly(butylene adipate-co-terephthalate) (PBAT) films incorporated with 0 (a), 2.5 (b), 5.0 (c), 7.5 (d), and 10.0 g (e) of oregano essential oil (OEO).

According to Bonilla et al. (2012), this is due to homogenization during hot-melt extrusion. The thread design allows a more efficient, homogeneous effect, making it difficult for OEO drops to appear in the films. No oil droplets were observed in the films, according to Bonilla et al., 2012 the reduction of EO droplets is related to the technique of homogenization, in the case of films obtained by extrusion, the drawing of the thread allows a homogeneous effect more efficient, thus, making it difficult for OEO drops to appear in the films.

Session (A) presents the control film (OEO0.0) that, in turn, showed a smooth, compact surface that is characteristic of pure PBAT films. When incorporated with OEO, though, a rough, heterogeneous surface was observed. Javidi et al. (2016), when analyzing OEO-containing polylactide (PLA) films produced by casting, noticed that the interaction between both components was not strong enough to avoid phase separation during solvent (chloroform) evaporation, leading to surface discontinuities. Thus, because there is no thorough interaction among PBAT and OEO, regions at which OEO was unable to interact with the polymer matrix were observed.

Similar results to the present study were reported by Atarés et al. (2011) in hydroxypropylmethylcellulose (HPMC) films, Javidi et al. (2016) reported discontinuities were reported upon the addition of 0.5–1.5% (m/m) of OEO. This behavior is related to the OEO availability within the matrix, which in turn is due to the molecular arrangement after PBAT melting alongside OEO throughout the hot-melt extrusion process.

Like film surfaces, cross-sections of the film presented harsher cuts after the OEO addition. The same behavior was observed by Martucci et al. (2015) in biogenic gelatin films, in which the essential oil addition led to slightly coarser materials. The discontinuities observed in the cross-sectional and horizontal cuts are related to the mechanical properties of the films. These discontinuities may damage the mechanical properties of materials added by OEO due to the plasticizing action of the latter (Li et al., 2014).

3.4. Fourier-transform infrared (FTIR) spectroscopy

FTIR analyses (Fig. 5) were necessary to understand the interactions between OEO and PBAT. The spectra allowed the identification of some functional groups constituent of OEO. This is a positive outcome because the high temperatures employed in hot-melt extrusion could degrade such compounds.

Several bands were observed at wave-lengths ranging between 1265

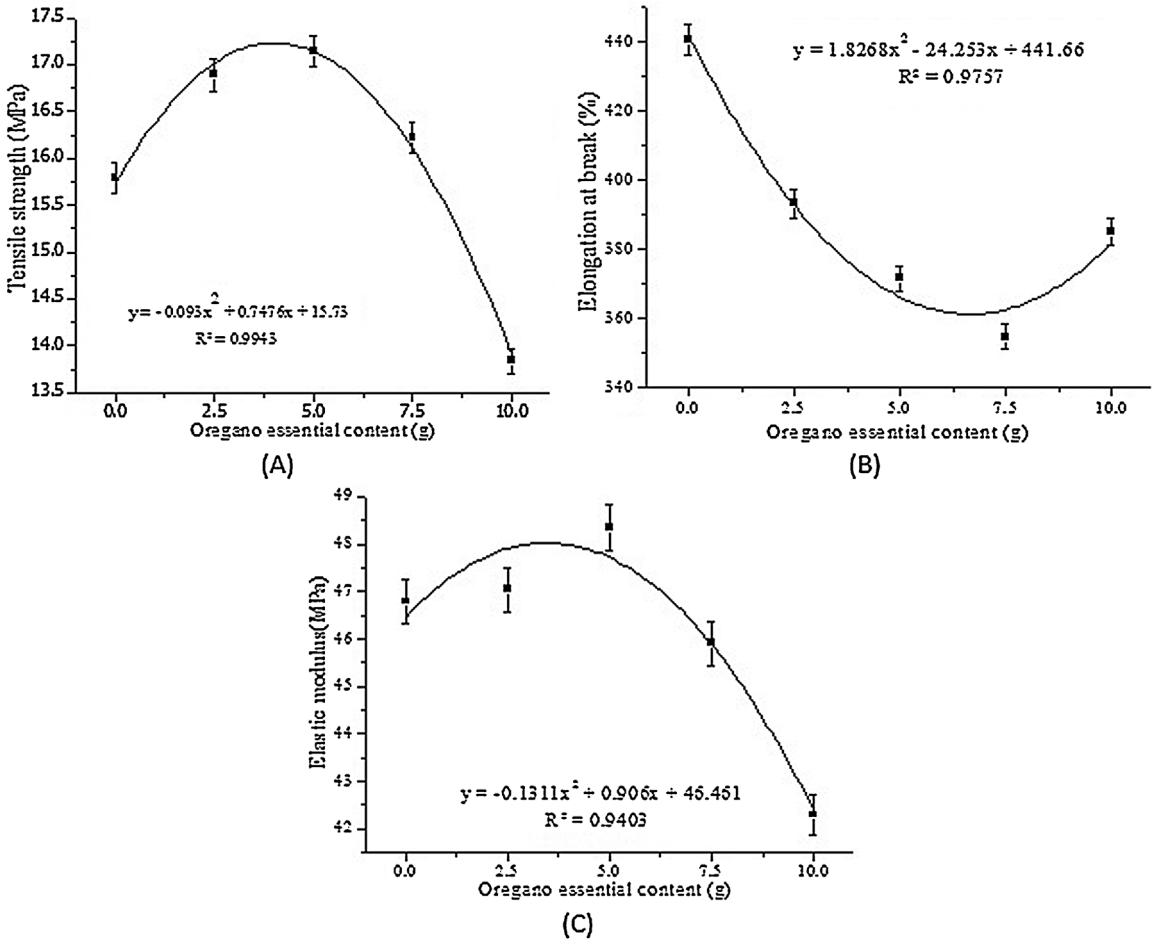

Fig. 3. Mechanical properties – tensile strength (A), elongation at break (B), and elastic modulus (C) – of poly(butylene adipate-co-terephthalate) (PBAT) films produced by hot-melt extrusion.

and 1118 cm^{-1} that according to Al-Itry et al. (2012) are attributed to the C—O bond, as from the carboxyl groups (C—O—C), causing increases and decreases in the spectra due to the elongation vibrations. However, no spectral changes were observed in the present study.

Increased OEO concentration in the films led to the formation of a peak at 810 cm^{-1}, which has been attributed by Hosseini et al. (2015) to carvacrol – the major antioxidant and antimicrobial agent in OEO – spectrum. The presence and formation of peaks in bands related to OEO functional compounds demonstrates their resistance to high temperatures. Once they are present in the final materials, the latter exhibit active function.

According to Arrieta et al. (2013), bands at wavenumbers ranging from 1600 to 1400 cm^{-1} can be attributed to the existence of an aromatic ring in carvacrol molecule. However, PBAT also has an aromatic ring in its structure, intensifying the peak at 1581 cm^{-1} as a result of increasing OEO concentrations. This suggests a presence of the aromatic rings in the polymer matrix.

3.5. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC)

Thermal stability was determined by TG, DTG, and DSC curves (Fig. 6). The TG profiles of all films were similar, regardless of OEO content (Fig. 6a). OEO-free PBAT samples ($OEO_{0.0}$) were thermally stable up to 310 °C (T_{onset}). In the TGA performed by Guo et al. (2015), pure PBAT samples presented stability up to 350 °C, temperature from which there was a remarkable mass loss that was corroborated by the DTG peak at 400 °C. This event is attributable to the thermal degradation of PBAT macromolecules.

This observation indicates that the addition of OEO reduced the thermal stability of films because this first mass loss occurs because OEO is degraded first The PBAT, modifying the composition of the films. This is supported by DTG curves (Fig. 6b), which exhibit a peak at *ca.* 170 °C. Again, this first event is related to OEO degradation. Similar results were reported by Guo et al. (2015) for PBAT/soybean protein isolate (SPI) blends. The first event (310 °C) in the DTG curves is assigned to PIS degradation.

Thermal analyses allowed the establishment of a relationship between decomposition temperatures and OEO's major antimicrobial compounds – *i.e.*, carvacrol – ensuring that hot-melt extrusion did not degrade this compound, since both OEO and films presented degradation temperatures higher than 170 and 200 °C, respectively. Keawchaoon and Yoksan (2011) reported carvacrol to have a degradation temperature of 186.4 °C, supporting one to infer that hot-melt extrusion at the temperature profile used here does not affect the availability of the bioactive compound in the resulting films.

The thermal behavior of the films was also examined by DSC (Fig. 6c), which showed two endothermic peaks in all formulations. According to Gan et al. (2004), the peak at approximately 60.4 °C is attributed to the melting of the crystalline fraction AB-butyl adipate of PBAT. In the present study, OEO-free PBAT presented a melting temperature of approximately 52.56 °C.

Fig. 5 elucidates OEO thermal behavior. The TG curve shows a decomposition temperature around 171.08 °C. The DSC curves present two endothermic events, the first at 56.33 °C – responsible for the increased the melting temperature in the films – and the second at 186.06 °C – referring to the complete decomposition of the oil. Portella et al. (2014) characterized the thermal behavior of the essential oil of

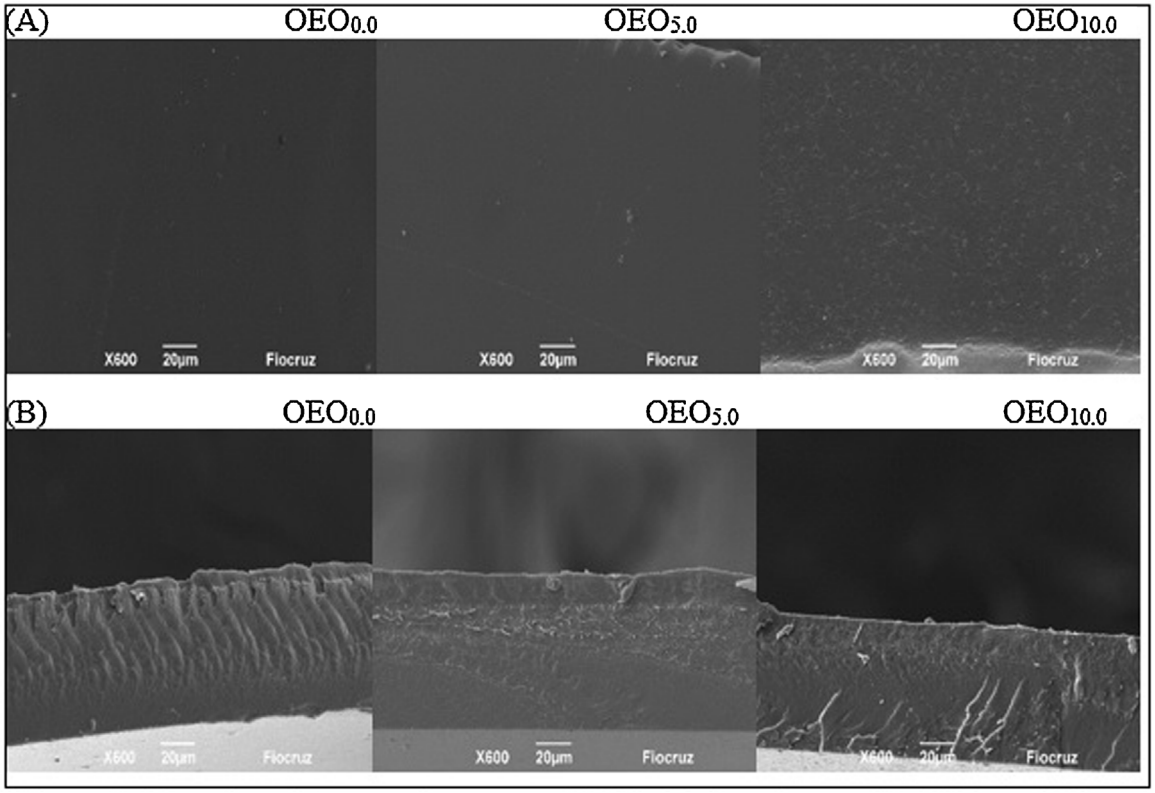

Fig. 4. Scanning electron microscopy (SEM) images of the surfaces (A) and cross-sections of (B) of samples $OEO_{0.0}$, $OEO_{5.0}$, and $OEO_{10.0}$.

Siparuna guianensis. The authors reported a marked decomposition between 100 and 225 °C – indicated by TG. Also, DSC revealed an exothermic event at 148.3 °C – possibly because of the partial oxidation of the sample, – as well as an endothermic peak at 194.7 °C – attributed to sample volatilization or decomposition.

The other endothermic event found in the films (403.91 °C) is related to PBAT decomposition. According to BASF (2009), PBAT presents an initial decomposition temperature of 280 °C. Here, the TG analysis revealed a final degradation temperature of *ca.* 450 °C. OEO addition did not affect PBAT thermal degradation profile because OEO degrades at temperatures much lower than PBAT.

The thermal profile of the films was similar to that found by Jouki et al. (2014), who observed a partial phase separation (fusion and decomposition) by analyzing the thermograms of mucilage films incorporated with different OEO concentrations. In that study, two endothermic peaks – at 167 and 230 °C – have been found.

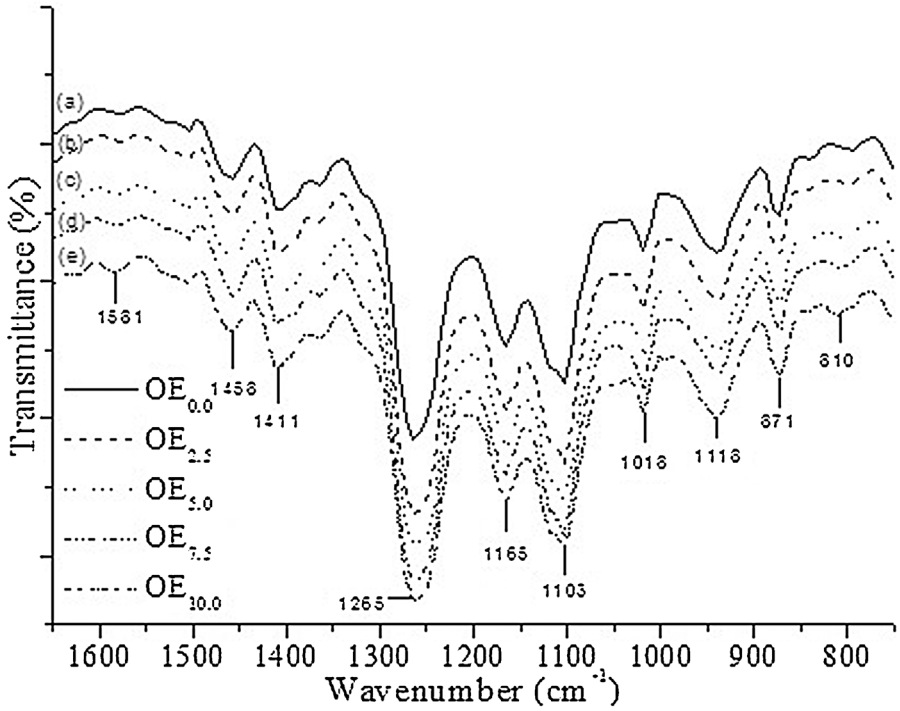

Fig. 5. Fourier-transform infrared spectroscopy (FTIR) spectra of poly(butylene adipate-co-terephthalate) (PBAT) films incorporated with 0 (a), 2.5 (b), 5.0 (c), 7.5 (d), and 10.0 g (e) of oregano essential oil (OEO).

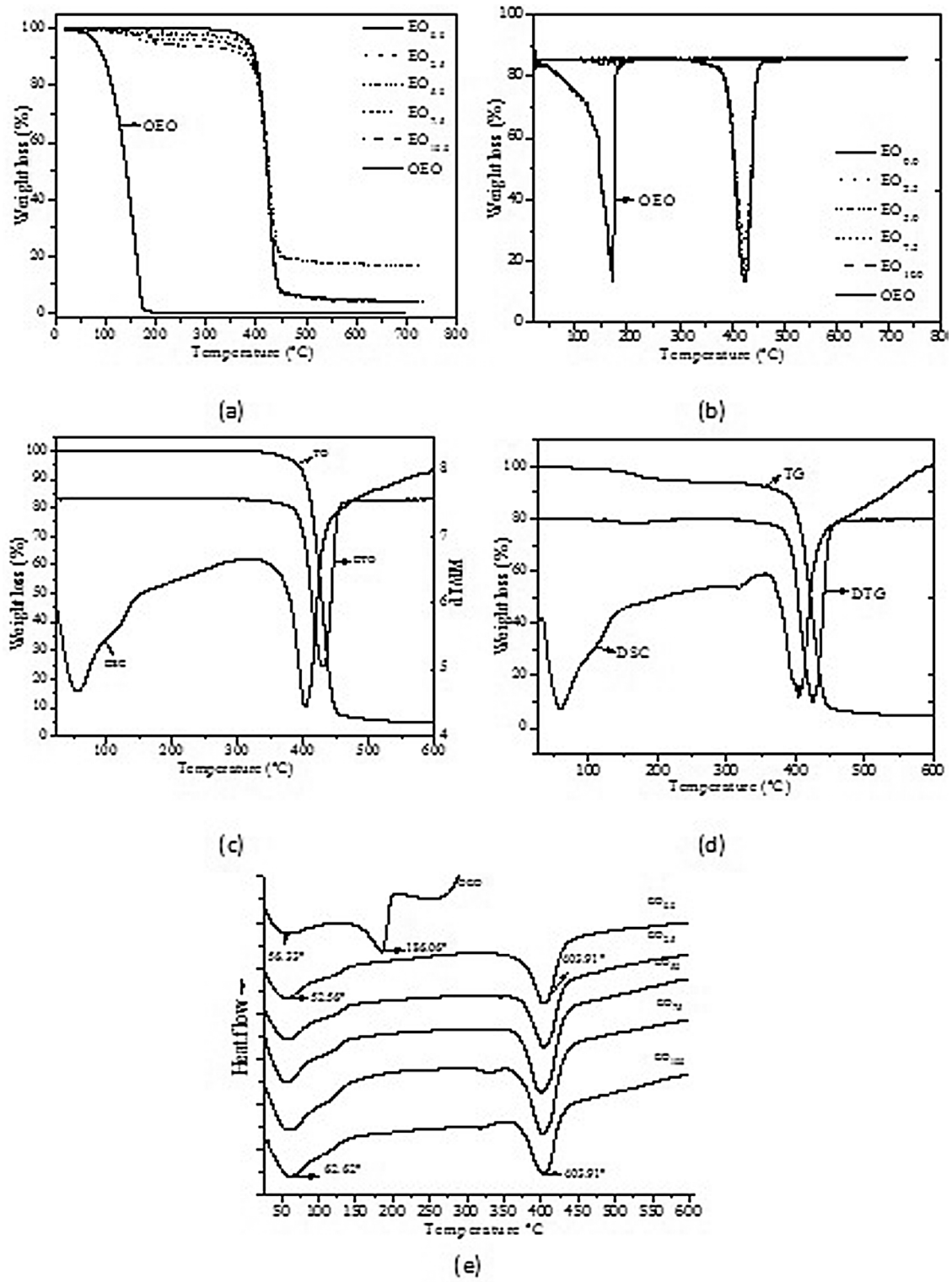

Fig. 6. Thermal behavior of poly(butylene adipate-co-terephthalate) (PBAT) films incorporated with different oregano essential oil (OEO) contents: (a) thermogravimetric (TG) curves of all formulations; (B) derivative TG (DTG) curves of all formulations; (C) TG, differential scanning calorimetry (DSC), and DTG curves of formulation $OEO_{0.0}$; (D) TG, DSC, and DTG curves of formulation $OEO_{10.0}$; and (e) DSC curves of all formulations.

3.6. Water vapor permeability (WVP)

WVP analysis revealed a linear profile as well as a tendency towards increased values after the addition of increasing OEO concentrations (Fig. 7). The WVP of the $OEO_{10.0}$ significantly increased (*ca.* 1.2 g/h·m·mmHg) in relation to control. Andrade-Molina et al. (2013) found a contrasting behavior when investigating PBAT and starch films incorporated with potassium sorbate. These authors reported reduced permeability upon film additivation, which is probably a result of starch aging and subsequent recrystallization. Similarly, Javidi et al. (2016) observed reduced WVP of OEO-incorporated PLA films and attributed such decrease to the hydrophobic nature of the essential oil.

Generally, polymer hydrophilicity and oil hydrophobicity are used to justify the reduction in WVP in films. However, Atarés et al. (2010)

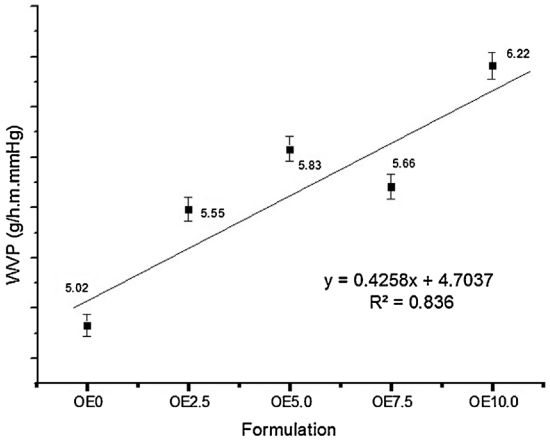

Fig. 7. Water vapor permeability (WVP) of poly(butylene adipate-co-terephthalate) (PBAT) films incorporated with 0 ($OEO_{0.0}$), 2.5 ($OEO_{2.5}$), 5.0 ($OEO_{5.0}$), 7.5 ($OEO_{7.5}$), and 10 g ($OEO_{10.0}$) of oregano essential oil.

analyzed SPI films incorporated with ginger and cinnamon essential oils and attributed the reduction in films' WVP to the interactions between oil components and proteins, which could promote the reduction of the hydrophobic character of the protein matrix. One of the reasons for the increased WVP found here may be related to the interaction between PBAT and OEO, where the oil due to its hydrophobic character increases the diffusion of water molecules through the films and impairs the barrier properties.

Pranoto et al. (2005), when studying edible alginate films incorporated with garlic oil, observed that the oil contributed to extend the intermolecular interactions of the structural matrix in the films due to its hydrophobic character, increasing the amount of water that diffused through the film. Another possible explanation is the increase in film permeability, which is related to additives that do not bind to the polymer matrix, but probably entrap within the intermolecular space – *i.e.*, between adjacent polymer chains. In this way, because no interaction among PBAT and the bioactive compound is expected, these spaces may not be completely filled by OEO and therefore serve as a channel for water permeation (Cooksey, 2005).

The amount of OEO used in the preparation of the films is relatively lower than the amount of PBAT (100 g), since in researches with whey protein films with beeswax emulsion, it was found that the low amount of emulsion caused Discontinuities of the lipids in the protein matrix and are not relevant to increase tortuosity, thus facilitating the permeability of water molecules, responsible for the increase in water vapor permeability (Perez-Gago and Krochta, 2001). In the present study, it is believed that the added OEO contents reduced the intermolecular tortuosity and, therefore, facilitated water permeation through films.

3.7. Evaluation of the efficiency of antimicrobial films in contact with food

The microbiological outcome (Fig. 8) shows that the produced films were efficient in lessening the counts of total coliforms, staphylococcus aureus, and psychrotrophic microorganisms. The current Brazilian legislation (RDC No. 12) establishes the following microbiological sanitary standards for refrigerated fish: staphylococcus aureus (counts lower than 5×10^3 CFU/g) and *Salmonella* (absent) (Brasil, 2001). When fillets were packaged with the produced films, there was performed a counting of staphylococcus aureus (3.85 log CFU/g), total coliforms (3.34 log CFU/g), and psychrotrophic microorganisms (4.94 log CFU/g), considered as time zero.

Fig. 7c evidences a tendency of reducing and inactivating staphylococcus aureus cells. Except for $OEO_{2.5}$, all formulations were able to extend the shelf life of the fillets for up to 10 days, taking into account the microbiological standards recommended by the current legislation in Brazil (Brasil, 2001).

Studies related to the incorporation of antimicrobial agents into PBAT films are recent. The shelf life of fresh lasagne pasta intercalated with extruded films from blends of rice flour, PBAT, and glycerol, added by 3 g of potassium sorbate has been evaluated. The film presented antimicrobial action against molds and yeasts, coliforms at 45 °C, *S. aureus*, *Bacillus cereus* psychrotrophic microorganisms (Sousa et al., 2016).

Javidi et al. (2016) tested the antimicrobial efficiency of PLA films incorporated with 1.5% (wt.) of OEO in direct contact with rainbow trout. The films demonstrated a better antimicrobial action against Gram-positive bacteria (*S. aureus*), result which is similar to that found here. However, the greater efficiency of carvacrol against Gram-negative bacteria has been widely reported and is due to its high hydrophobicity, being capable of disintegrating the outer membrane of these bacteria and allowing lipopolysaccharide leakage by means of increasing the permeability of the cytoplasmic membrane (Burt et al., 2007).

Similar results were reported by Sousa et al. (2016) for extruded PBAT films, considering the employed technique, demonstrating that it may affect the form of action of the bioactive compound. Hosseini et al. (2015) stated that the nature and structural features of the matrix in which the essential oil is dispersed, together with the method of preparation of the film, play a crucial role on the antimicrobial activity of the resulting material. In this regards, the behavior OEO in the present study can be justified by these characteristics.

Among the Gram-negative bacteria, the total coliforms presented greater sensitivity to $OEO_{5.0}$, $OEO_{7.5}$, and $OEO_{10.0}$, but no sensitivity to control and $OEO_{2.5}$ formulations. In a study by Shiv Shankar and Rhim (2016) in composite films of PBAT and silver nanoparticles (AgNPs), a distinct antimicrobial activity was observed against E. coli and *L. monocytogenes*, however, showed a stronger antimicrobial activity against E. coli (± 2 log CFU/g) than *L. monocytogenes* (± 1 log CFU/g) within 15 h. In the present study, the inactivation of bacterial cells after a 10-day storage reached values close to 3 log CFU/g.

Generally, increasing storage temperature accelerates the migration of active agents from film to food. Conversely, refrigeration reduces migration rates (Quintavalla and Vicini, 2002). This effect was not observed here because fish fillets were stored at 7 °C and the interactions between the antimicrobial agent, the coating material, the target bacteria, and the food matrix itself could be interrelated, thus influencing active compound release rate.

Concerning the inhibition of Staphylococcus aureus, the antimicrobial films $OEO_{5.0}$, $OEO_{7.5}$, and $OEO_{10.0}$ showed a direct proportionality between bacterial inhibition and OEO content. It was verified that the antimicrobial agents did not bond to the polymer structure. In accordance to Cooksey (2005), the antimicrobial agents are probably entrapped within the spaces between adjacent polymer chains, which allow a greater diffusion of increased OEO concentrations. Regarding total a psychrotrophic coliforms, the patterns were slightly divergent for the same concentrations.

3.8. DPPH-radical scavenging activity

The major bioactive active compounds of OEO are carvacrol, thymol, C-therpinene, and *p*-cymene, which provide it with a broad spectrum of antioxidant capacity (Burt, 2004). Fig. 9 shows a linear relationship between antioxidant capacity and OEO concentration. Shojaee-Aliabadi et al. (2013) analyzed the antioxidant power of biodegradable κ-carrageenan films incorporated with *Satureja hortensis* oil and observed that the polymer itself had low antioxidant activity, probably because of the polyphenols present in the polymer matrix. Here, the control film ($OEO_{0.0}$) had no antioxidant action, allowing one to conclude that PBAT does not comprise any compound featuring antioxidant activity in its structure.

From the linearity presented here, it can be stated that the higher

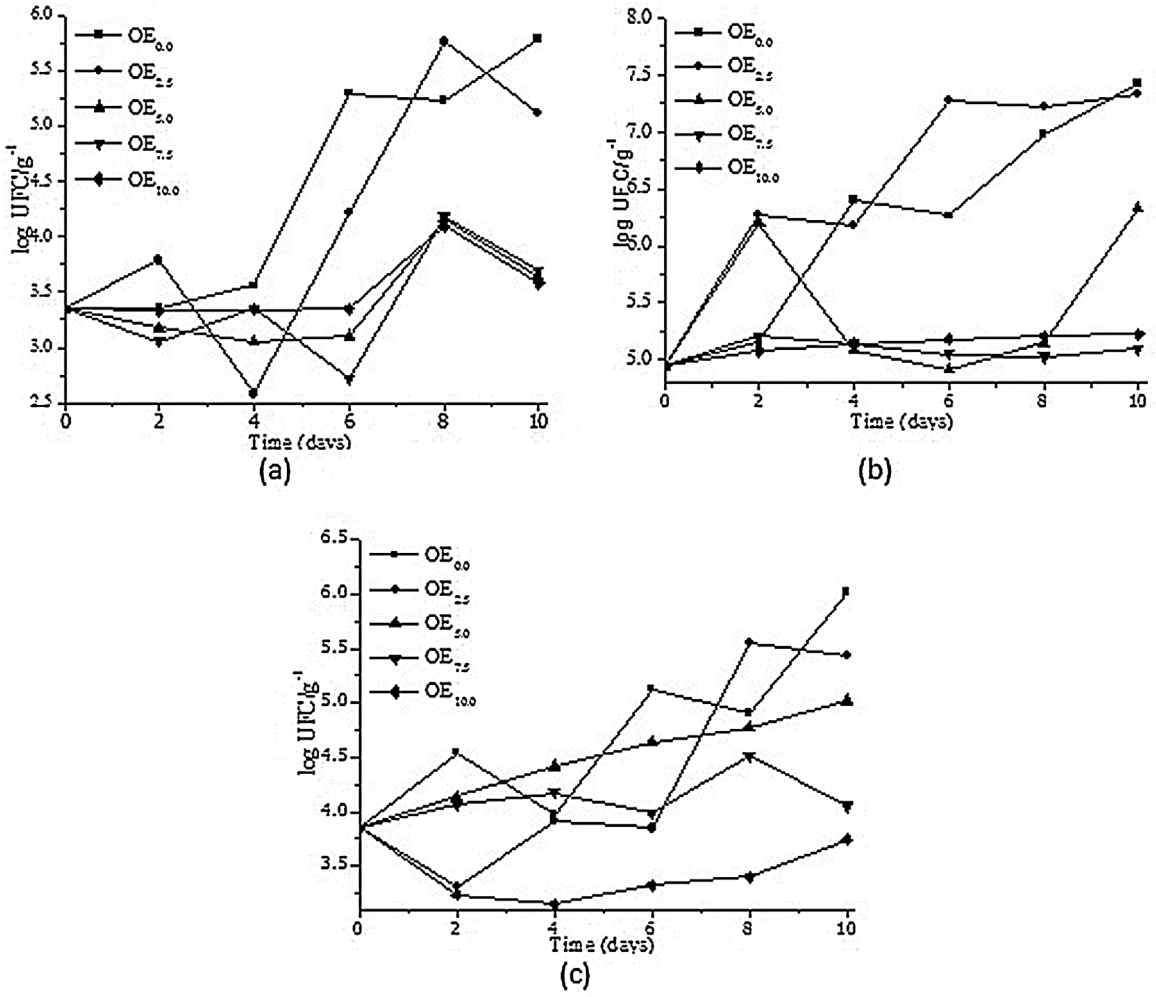

Fig. 8. Antimicrobial action against total coliforms (a), psychrotrophs (b), and staphylococcus aureus (c) of poly(butylene adipate-co-terephthalate) (PBAT) films incorporated with different concentrations of oregano essential oil (OEO) in fish fillet storage for 10 days.

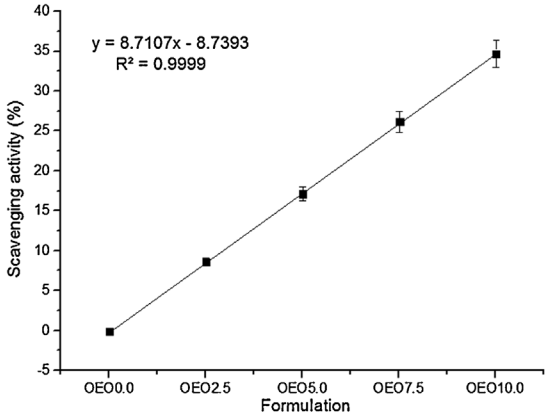

Fig. 9. Antioxidant activity of poly(butylene adipate-co-terephthalate) (PBAT) films incorporated with 0 ($OEO_{0.0}$), 2.5 ($OEO_{2.5}$), 5.0 ($OEO_{5.0}$), 7.5 ($OEO_{7.5}$), and 10 g ($OEO_{10.0}$) of oregano essential oil.

the OEO concentration in the films, the greater its antioxidant activity. Jouki et al. (2014) drew a similar conclusion when quantifying the antioxidant activity in mucilage films, which was found to be significantly increased upon the addition of greater OEO concentrations. Gómez-Estaca et al. (2009) also reported that the antioxidant potential of biodegradable films is generally proportional to the amount of antioxidant additives.

4. Conclusions

Biodegradable films produced from OEO and PBAT composite were demonstrated to be an efficient active packaging system for fish fillet preservation purposes as they controlled the microbial development in such food matrix. As for the mechanical, thermal, and water barrier properties, the films presented suitable characteristics for application as food packaging, provided that OEO incorporation did not affect the essential PBAT properties.

The antioxidant action of the films produced here was high. Additionally, it was antimicrobially efficient by lessening the counts of total coliforms, Staphylococcus aureus, and psychrotrophic microorganisms. The use of such films in fish fillet storage led to counts lower than those established by the current Brazilian legislation and extended the shelf life of fish fillets by up to 10 days under refrigeration.

Acknowledgements

This work was supported by FAPESB – Foundation for Research Support of Bahia, project REDE 0017/2013 and grant of master's degree to the first author of the manuscript.

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