An Acad Bras Cienc (2022) 94(4): e20200142 DOI 10.1590/0001-3765202220200142
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

CROP SCIENCE

Development and characterization of antioxidant and antimicrobial poly (butylene adipate-co-terephtalate) (PBAT) film incorporated with oregano essential oil and applied in sliced mozzarella cheese

LUCAS G. CARDOSO, JÂNIA B.A. DA SILVA, JULIANA A. DA SILVA, GEANY P. CAMILLOTO, CAROLINA O. DE SOUZA, JANICE I. DRUZIAN & ALAÍSE G. GUIMARÃES

Abstract: The objective was to develop and characterize biodegradable films with antimicrobial and antioxidant action, using poly(butylene adipate-co-terephthalate) (PBAT) incorporated with OEO - essential oil (*Origanum vulgare*). The degradation temperature of the OEO increased after incorporation into the PBAT matrix, however, the degradation of the matrix did not undergo considerable changes. The films showed increase in elongation and modulus of elasticity with presence of OEO, however, it reduced the maximum tension. The permeability of the films was reduced with OEO presence. The spectra (FTIR) showed the presence of the functional groups attributed to the bioactive compounds (Carvacrol) of OEO. The films presented high antioxidant activity and effective antimicrobial action, reducing *Staphylococcus aureus* in 53 days and psychrotrophic microorganisms in up to 28 days of storage. The films showed to be efficient with antioxidant activity and antimicrobial action with indication to be used as packaging of sliced mozzarella cheese.

Key words: Active packaging, *Origanum vulgare L.*, antioxidant, antimicrobial, mozzarella cheese.

INTRODUCTION

The widespread use of plastic films as food packaging is due to the fact that they are chemically and mechanically resistant, lightweight, heat-solderable, can be printed on and are available in large quantities at low cost. However, the main disadvantage of plastic materials is that they are not biodegradable, causing environmental pollution due to their inappropriate disposal (Sousa 2013). Recently, environment problems and food safety have caused many concerns to the public, and green chemistry appeals to many researchers (Wang 2018).

In view of the health concerns expressed by consumers and current environmental problems, research is now focusing on the development of sustainable packaging materials based on annually renewable natural biopolymers such as polysaccharides and proteins or synthetics such as poly(butylene adipate co-terephthalate) or PBAT (Gómez-Estaca et al. 2009).

Among the biopolymers, the PBAT is biodegradable synthetic aliphatic-aromatic copolyester, alternative to replace synthetic polymers, petroleum derivatives. It is polymerized from adipic acid, butanediol, and terephthalic acid, in which the aromatic monomers contribute good thermal stability

and mechanical properties and the aliphatic monomers provide flexibility and good biodegradability (Ki & Park 2007).

Bioactive food packaging is one of the innovative concept that have been emerged as a response to the continuous variations in current consumer demands for natural, minimally processed and preservative-free food products (Nisar et al. 2018, Kasai et al. 2019). As a form to introduce active agents to increase food preservation, the area of antimicrobial packaging has become one of the major areas of research in food packaging (Pelissari et al. 2009).

The incorporation of plant essential oils, with bioactive action into these films represents an interesting alternative. Because, 85% of the components of essential oils have biological properties such as antioxidant and antimicrobial. These properties can be attributed to the high content of terpenic compounds (-pinene, -pinene, 1,8-cineol, menthol, linalool) or phenolic compounds such as carvacrol, eugenol and thymol (Burt 2004, Rojas-Grau et al. 2006). Thus, they can be substitutes for synthetic antioxidant-antimicrobial agents that cause damage to health, since agencies like FDA have stimulated the use of natural compounds beneficial to health due to the migration of the packaging - food compounds. Therefore, the use of essential oils contributes to food security (Bakkali 2008).

Conventional packaging materials cannot actively control reactions within the food product. Barriers to oxygen, moisture, and light offer good protection for the most sensitive foods. Developments in materials science and engineering have resulted in a novel packaging technique which include the use of natural and safe anitmicrobials. By using polymers which have characteristics suitable for application in foods such cheese that has a rapid deterioration, resulting in damages to the industry foods and risk to consumer health (Khaneghah 2018). In addition, according to Kokoszka et al. (2010) essential oils can play the role of the plasticizers that 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. 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 in mozzarella cheese by controlling microbial development and oxidation.

MATERIALS AND METHODS

Materials

The active packaging was produced using poly(adipate co-terephthalate) - PBAT, under the commercial name Ecoflex (film grade), was supplied by BASF (Ludwigshafen, Germany) and oregano essential oil (Ferquima, Brazil), main Components: Carvacrol = 71wt%; Thymol = 3 wt%; Gamma-terpinene = 4.5 wt%; Para-cymene: 3.5 wt% and Beta-caryophyllene: 4 wt%. Appearance: Clear Liquid; Color: Brown; Yellow Greenish to Dark Brown; Free from impurities; Characteristic Odor and Density (20 °C): 0.938 g/cm^3.

Development of active films

The films were obtained according to the methodology described by Rhim et al. (2006) with adaptations, using the casting method. For the development of the films, different concentrations (w/w) of OEO (0.0, 2.5, 5.0 and 7.5) incorporated into 5.0 g of PBAT and dissolved in 25 g of Chloroform were used, the filmogen solution was vigorously homogenized, then placed in an ultrasonic (Unique, Brazil) bath for 30 min and subsequently sealed and maintained at room temperature (± 25°C) within

24 hours. The solutions formed were poured onto a glass plate (24 × 30 cm), evenly spread with a glass rod, allowed to dry for about 24 h at room temperature, thereby conferring the film format allowing the evaporation of the solvent.

Mechanical properties

The thickness of the films were measured using a digital micrometer (Mitutoyo Corp.) and then subjected to mechanical tests to evaluate the maximum tensile strength, rupture deformation and modulus of elasticity, using the Universal Mechanical Testing Machine (EMIC DL-200MF, BRA) In accordance with ASTM D882-09 (ASTM 2009a). It was carried out with ten proof body of each formulation, following the dimensions 9.0 cm (size of the proof body); 5.0 cm (distance between the claws) and 1.5 cm (width). The machine was operated with 1 kN load cell at a traction speed of 500 mm·min^{-1}.

Microstructural characterization

Cross-section and surface images of the films were obtained by Scanning Electron Microscopy (SEM) using a JEOL (6390LV, Tokio, Japão). A 0.5 cm^2 sample of each formulation was fixed in stubs and covered with a gold coating of 20 to 30 nm thickness, performed on the Sputter Coater (Balzers - Model SCD 010). Soon after, the samples were placed in order to focus and capture the respective photomicrographsin tension 50 kV.

Fourier-transform infrared (FTIR) spectroscopy

The FTIR spectra of the films were recorded at wavenumber range of 600 – 4000 cm^{-1} at resolution of 2 cm^{-1} using a Spectrum 100 da Perkin Elmer, by attenuated total reflectance (ATR).

Thermogravimetric analysis (TGA)

The thermal behavior of the conditioned film samples at 54% RH was analyzed using a thermogravimetric analyzer (TGA, Pyris 1, Massachusetts, EUA). Approximately 5mg of film samples were gradually heated at 10 °C·min^{-1} from room temperature, starting at 25 to 700 °C, under nitrogen flow (50 ml·min^{-1}). The initial degradation temperature (T_{onset}), i.e. the temperature at which 5% mass loss is registered, was recorded. The temperature at which the maximum degradation rate was observed (T_{max}), i.e. the temperature of the peak in the first derivative graphs, as well as the percentage of mass loss at the end of the test (700 °C), were also registered.

Water vapour permeability (WVP)

The desiccant method, according to the methodology ASTM E96/E96M - 09 (ASTM 2009b), was applied with some modifications. Pots containing 15 g of anhydrous calcium chloride were hermetically sealed and packed in a desiccator chamber at a mean temperature of 22.4 °C and a mean relative humidity of 71.3% containing 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. (1), wherein e is film thickness (m) and WVTR is its water vapor transmission rate (Eq. (2)):

P (g/ hm mm Hg) = WVTR x e *Equation 1*

TTVA (g/h m^2) = G/t x A *Equation 2*

e = film thickness (m);

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).

Antimicrobial efficiency in sliced mozzarella cheese storage

The films were tested for microbiological efficiency by direct contact with sliced mozzarella cheese. The films were cut into sizes sufficient to cover the surfaces of the sliced cheese (20 x 20 cm) and all sides were sterilized in UV light for about 2 minutes, 25g the mozzarella cheese were packed in the films, sealed and later stored in a refrigerator at 7°C. The study of microorganisms from the total coliforms group, psychrotrophic and *Staphylococcus aureus* was performed according to the methodology described by APHA (2001) at times 0, 7, 14, 21, 28 and 35 days.

The packaged 25 g of mozzarella cheese were aseptically collected from the respective packaging and transferred to 225 ml of 0.1% sterile peptone water and homogenized in Stomacker, corresponding to a 10^{-1} dilution, from which subsequent dilutions were made for microbiological analyzes. For the total coliform group count, 0.1 ml of sample dilutions were surface inoculated in the chromogenic medium Chromocult Coliform Agar® (CCA) and incubated at 37 °C for 24 hours. As for the psychrotrophic microorganisms, 0.1 mL of each dilution was inoculated in surface in PCA and incubated for 7 days with inverted plates at refrigeration temperature (7 °C). For counting *Staphylococcus aureus*, 0.1 ml of sample dilutions were inoculated into Baird Parker Agar (BP) with surface inoculation, incubated for 48 hours at 37 °C with inverted plates. All results were expressed in $LogUFCg^{-1}$ (APHA 2001).

DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to the method of Jongjareonrak et al. (2008) with a slight modification. Film (0.1 g) was 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. Then, 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 methanol extract (500 µL) was mixed with 2 mL of 0.06 mM DPPH in methanol. The mixture was vigorously vortexed for 1 min and allowed to stand at room temperature in the dark for 30 min. The absorbance was measured at 517 nm using a UV spectrometer (Model UV-2101PC, Shimadzu, MD, USA). The methanol was used as control and mixed with 0.12 mM DPPH. DPPH radical scavenging activity was calculated as follows (Singh & Ragini 2004):

$$Radical\ scavenging\ activity\ (\%) = (1 - (A_{sample} - A_{control})) \times 100$$

Where, A_{sample} = absorbance of sample; $A_{control}$ = absorbance of the control.

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).

RESULTS AND DISCUSSION

Microstructural characterization

Figure 1(a) shows the surface of OEO0.0 film, where it is possible to observe the presence of pores. OEO films presented surfaces with lower pores (Figure 1a,b and c) the fracture surface of OEO-containing films (Figure 1d, e and f) shows uniform films.

Mechanical properties

The tensile strength at break, maximum deformation and modulus of elasticity of the PBAT: OEO films are shown in Figure 2. The statistical treatment showed that the elastic modulus was the only mechanical parameter

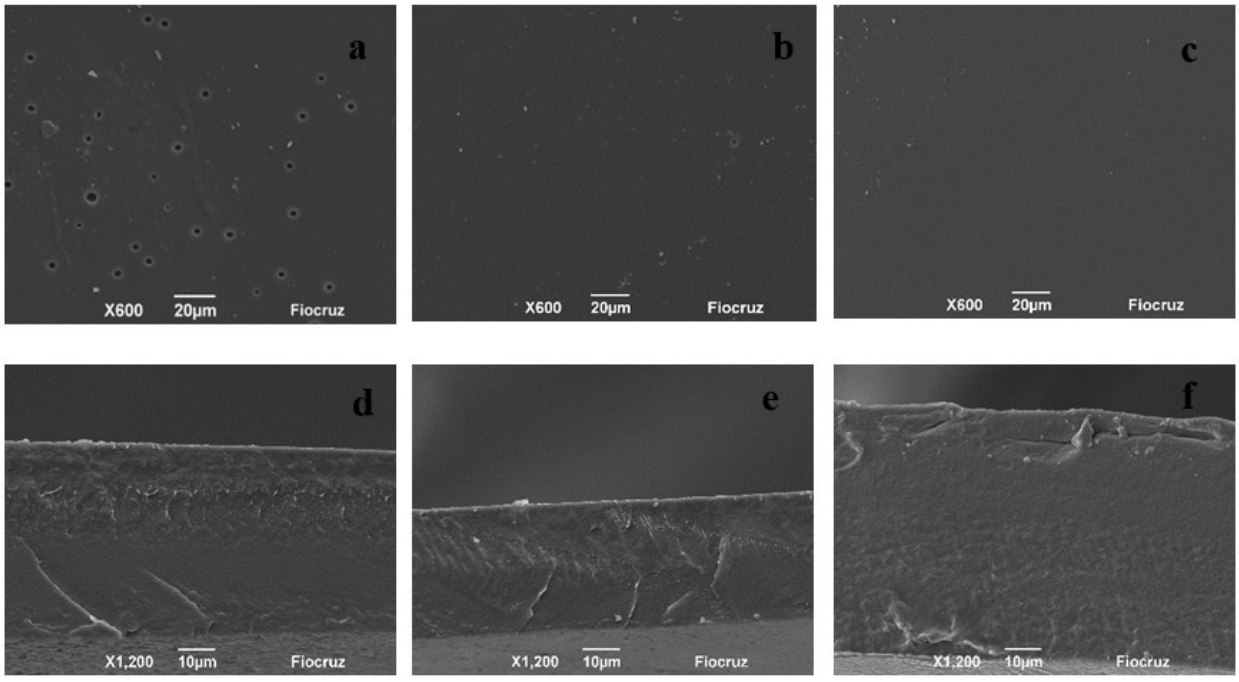

Figure 1. Surface (top) and cross-sectional (bottom) scanning electron microscopy (SEM) images of PBAT films produced by casting: OEO0.0 (left; a and d), OEO5.0 (middle; b and e), and OEO7.5 (right; c and f).

missing from a mathematical model. Thus, the discussion considered a tendency to increase the observed modulus in the acquired curve. The control film presented a tensile strength of 17.83, the films containing OEO showed a considerable reduction in this parameter, showing a reduction of approximately 44% for film (5.0OEO) and 32% (7.5OEO) (Figure 2a).

The incorporation of the OEO in the PBAT film increased the elongation at the break considerably (Figure 2b), presenting an increase of up to 1300% with the 7.5OEO incorporation in the film. The modulus of elasticity showed an increasing tendency in relation to the control film (OEO0.0). The OEO5.0 film presented a higher increase in this parameter with approximately 52%, with the incorporation of OEO7.5 the increase was approximately 18% in relation to the control film (Figure 2c).

Fourier-transform infrared spectroscopy (FTIR)

In Figure 3(a,b) it is possible to observe that all the films presented similar spectra. In the Control film (OEO0.0) and in the other films containing OEO, the vibration band 1458 cm^{-1}. It is possible to observe the band at 810 cm^{-1} in the OEO spectrum and in the prepared films, associated with the presence of bands (1600, 1581 and 1400 cm^{-1}).

Thermogravimetry (TGA)

The thermal stability of the prepared films was determined by TGA curves (Figure 4a,c) and (DTG) (Figure 4b). It is possible to clearly observe in the TGA and DTG curves of the OEO a single event of mass loss, between 29 – 179 °C, referring to the degradation of the oil. The TGA and DTG of the PBAT film (OE0.0) showed a single mass loss event, with event start at 327 °C (T_{onset}) and maximum degradation rate at 426 °C.

In the TGA of the films containing OEO, data evidences two mass loss stages, the first

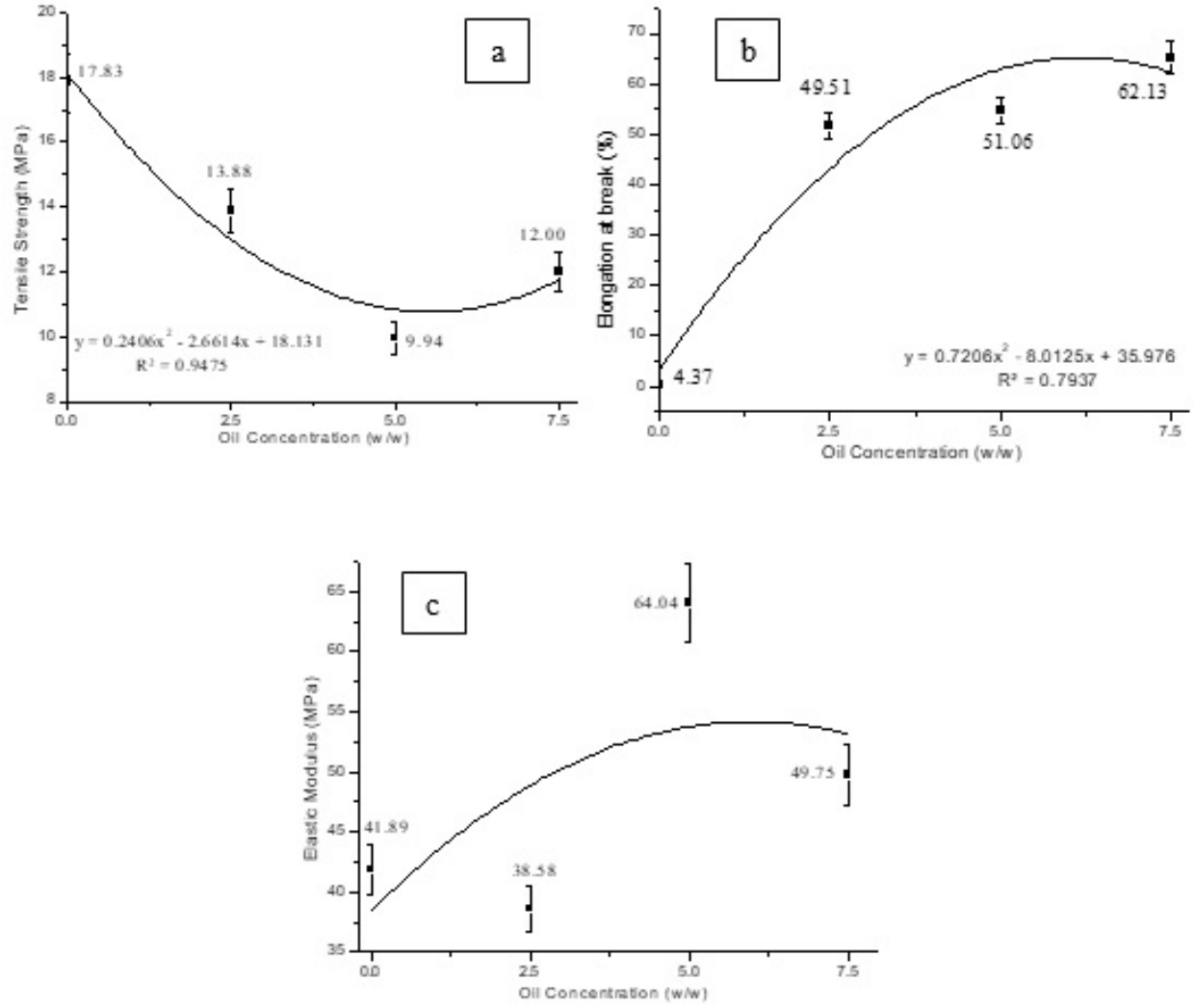

Figure 2. Mechanical properties of PBAT films produced by casting, where axis x = oil concentration (g) and axis y = (a) maximum strain; (b) elongation at break, and (c) elastic modulus.

one being attributed to OEO degradation, since it is the volatile compound with lower onset degradation (98 °C). The second, in turn, is attributed to the degradation of organic compounds present in PBAT because of its molecular arrangement which is composed of several carbon atoms, with a degradation peak at 425 °C, indicating that the addition of OEO to PBAT did not change considerably this parameter. The thermal stability of the OEO was changed to higher temperatures after incorporation into the PBAT matrix, indicating possible interaction between the OEO and PBAT structures. The T_{onset} temperature of OEO increased from approximately 29 to 98 °C. This effect was observed in all films.

Water vapor permeability

The water vapor permeability (WVP) of films incorporated with different OEO concentrations ranged from 62.63 g/(h.m.mmHg) in the control film (OE0.0) to 37.24 g/(h.m.mmHg) (OEO5.0), Figure 5. The formulations OEO2.5 and OEO5.0 presented lower WVP values than the control (OEO0.0). However, the formulation OEO7.5 presented a WVP value close to the control.

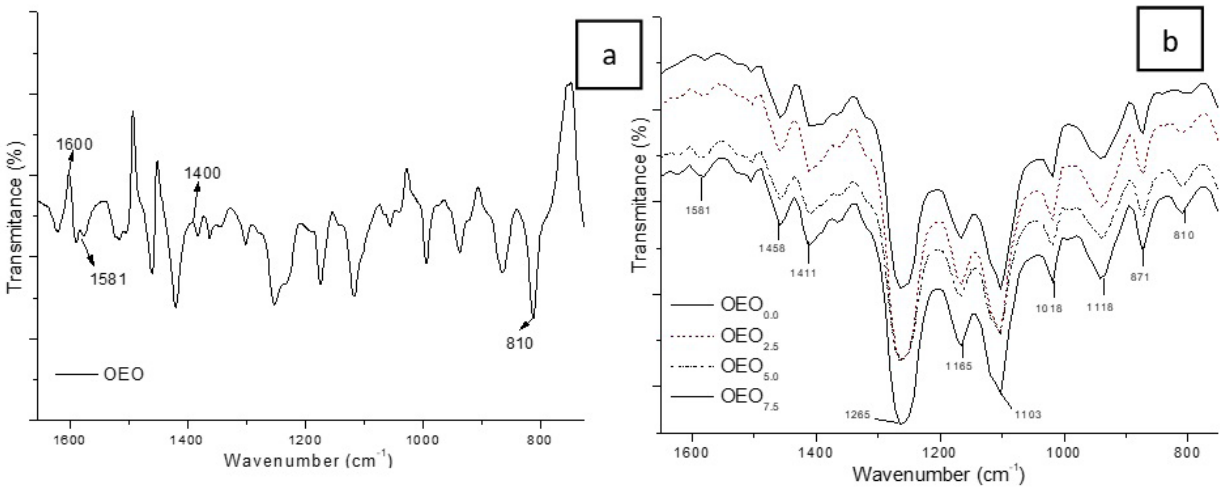

Figure 3. FTIR spectra of PBAT films incorporated with different OEO concentrations: (a) OEO and (b) OEO0.0 to OEO7.5."

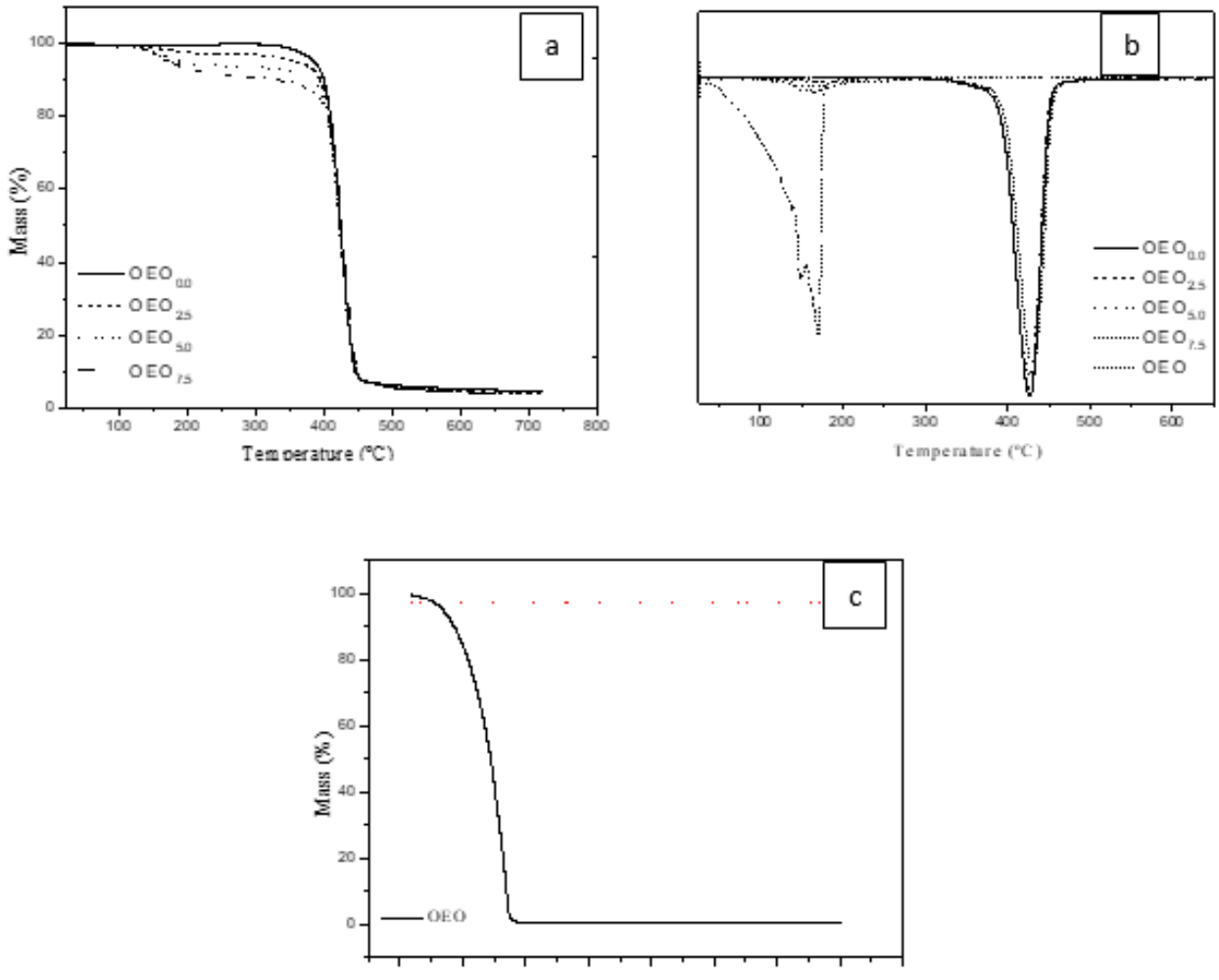

Figure 4. Curves of TGA (a and c) and DTG (b) OEO and formulated films."

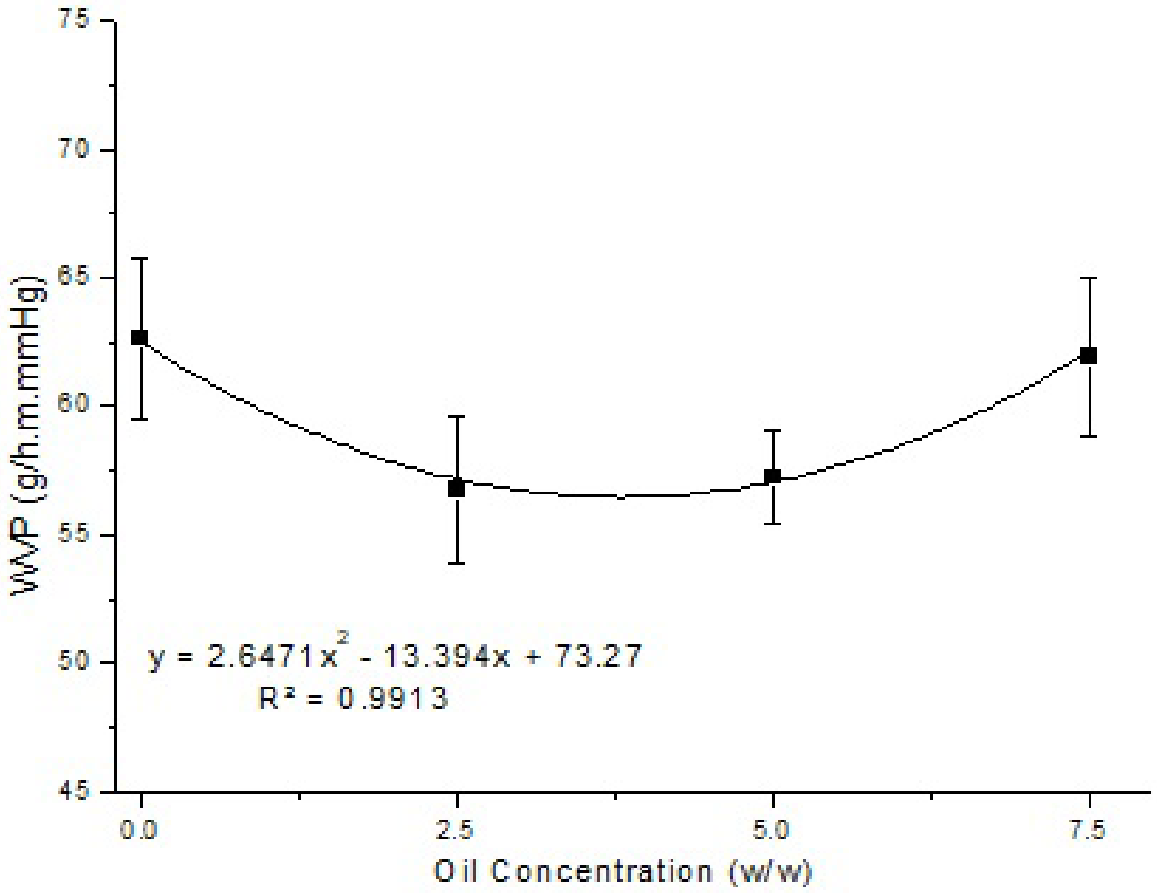

Figure 5. Water vapor permeability (WVP) of PBAT films produced by casting.

DPPH radical scavenging activity

According to the obtained data, it was observed that all formulations incorporated with OEO showed ability to capture stable DPPH radicals, Figure 6 and that the antioxidant capacity was proportional to OEO concentration. The inhibition rate of OEO2.5, OEO5.0, and OEO7.5 formulations were 51.49, 54.66, and 65.09, respectively.

Antimicrobial efficiency in sliced mozzarella cheese storage

The results of the microbiological analyses demonstrate that the films were efficient in reducing *Staphylococcus aureus* (Figure 7) and psychrotrophic (Figure 7a) microorganisms. Brazilian legislation RDC No. 12 establishes the following microbiological standards for mozzarella cheese: *Staphylococcus aureus* (< 5.10^3CFU/g), coliforms at 45 ºC (< 5.10^3CFU/g), *Listeria monocytogenes* and *Salmonella*: absent (Romani, 2017). *Staphylococcus aureus* (4.06 logCFU/g), total and thermotolerant coliforms (absent), psychrotrophic bacteria (4.93 logCFU/g), *Salmonella* (absent), and *Listeria monocytogenes* (absent) was considered as time zero. The investigated cheese samples already had a count above that allowed by current legislation (*Staphylococcus aureus*: 4.06 logCFU/g), therefore being unsuitable for consumption.

In Figure 7(b), it is possible to observe that the graphs present a tendency of reducing *Staphylococcus aureus* cells towards inhibition. The OEO2.5 and OEO5.0 formulations were able to extend cheese shelf-life up to 35 days. Regarding the counts of psychrotrophic bacteria, the formulations OEO2.5 and OEO5.0 presented reduced initial loads up to 28 days of storage, from which there was an increase of 0.13 and 0.09 log cycles until the end of the storage period (35 days), Figure 7(b).

DISCUSSION

The presence of pores, possibly attributed to the rapid evaporation of the solvent used in the process of preparing the films. Lower pores indicating that the presence of the oil made it difficult to evaporate the solvent, reducing the presence of micropores. Uniform films, corroborating with the micrographies of the film surfaces. A similar outcome was reported by Branderelo et al. (2015) when studying starch/poly(vinyl alcohol)/alginate blends incorporated with copaiba and lemongrass essential oils. Control films (i.e., absent of oil) presented higher amounts of micropores than their OEO-containing analogues. The authors attributed this behavior to film-forming technique used in such study, i.e., casting, which promotes continuous water evaporation through film and creates the observed microstructures. The presence of OEO in the film decreases the evaporation rate of the solvent, be it water or chloroform (solvent used in this study), which reduces the incidence of micropores in the film.

As OEO incorporation increased, films became more homogeneous. Similar results were found by Lee et al. (2016) in films made up of protein from red pepper seeds (RMP) incorporated with OEO. In this study, the films showed a smooth, homogeneous texture because pores and cracks that were noticed in the control film disappeared in the presence of the additive.

According to Martucci et al. (2015) the mechanical properties can be affected by the type and concentration of the essential oil. In the present study, tensile strength was reduced as the OEO concentration increased. On the other hand, the elongation and the elasticity modulus showed an increasing trend as the OEO concentration increased.

According to Pelissari et al. (2009), the addition of OEO into films reduce its tensile strength, probably due to the plasticizing effect of the additive. The similar behavior observed here is also attributed to the plasticizing effect of OEO, which separates polymer chains apart, therefore reducing their tensile strength. Films containing OEO showed a considerable reduction the tensile strength possibly indicating that concentrations higher than 5wt% OEO favors the anti-plasticizing effect, providing an increase in tension, an effect reported by some authors especially for the use of glycerol as plasticizer in polymer matrices (Santana et al. 2018, Turhan & Sahbaz 2004, Mali et al. 2005). Martucci et al. (2015) reported that the addition of oils into polymers can reduce the tensile strength of the resulting films due to the reduction of the intermolecular forces. Similar results were found by Shojaee-Aliabadi et al. (2013), who reported that films containing low oil concentrations had increased extensibility, and reduced the tension due to the plasticizing effect of the oil plays on the polymer chains. Increased the elongation was also found by Pelissari et al. (2009) with an increase in this parameter of up to 48% with the incorporation of 1% OEO. Zivanovic (2005) observed an increase in elongation of chitosan films combined with essential oils.

According to Mali et al. (2010), the weakening effect of intermolecular interactions on films

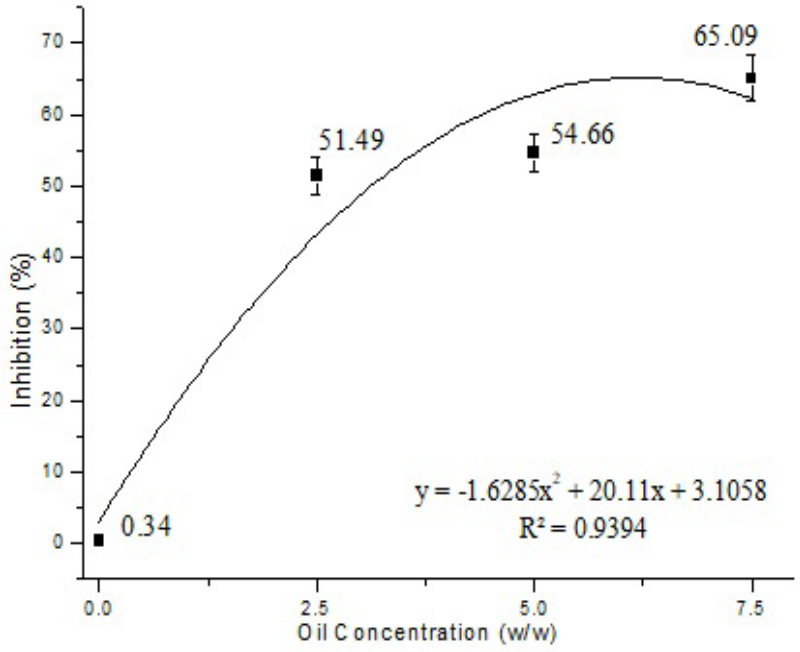

Figure 6. Antioxidant activity of PBAT films produced by casting.

with high OEO concentrations may increase the free space between the chains, triggering an anti-plasticizer effect. However, the use of the plasticizer in small concentrations interacts with the polymer matrix, but not sufficient to increase molecular motility, only increases the degree of interactions and stiffness of this matrix. Similar behavior was reported by Cardoso et al. (2017) for films with the same components, however obtained by extrusion. Films containing lipid compounds (oils) have limitations to form a cohesive layer. This behavior implies a reduction in the levels of intermolecular cohesion, which, in turn, are responsible for the force as for the rigidity of the material (Espitia et al. 2014). Therefore, depending on the concentration of OEO in PBAT films, the effect on the parameters of mechanical strength (maximum tension, tear strength and modulus of elasticity) changes considerably.

Bands present at the wavenumber between 1600 and 1400 cm^{-1} can be attributed to the existence of the aromatic ring in the carvacrol molecule (Arrieta et al. 2013). However, as the molecular structures of PBAT and OEO have aromatic ring, OEO incorporation led to an increase in the intensity of this band in 1581 cm^{-1} (Cardoso et al. 2017). The vibration band 1458 cm^{-1} can be attributed to the vibration of C-H in CH_3 groups; 1265, 11685 and 1103 cm^{-1} represent the vibration stretch of C = O at the ester bond (Silva et al. 2019) all attributed to the molecular structure of the PBAT.

This band (810 cm^{-1} is attributed to OEO's main bioactive agent carvacrol (Hosseini et al. (2015), presence of bands (1600, 1581 and 1400 cm^{-1}) attributed the OEO functional compounds, demonstrate that the casting method using chloroform as the solvent did not affect the availability of these compounds in the film. Guo et al. (2015) thermal stability of the PBAT up to 350 °C, with maximum degradation speed at 400 °C. Ouajai & Shanks (2009) reported that organic compounds are degraded between 350 and 500 °C. In thermograms of starch-chitosan-OEO films (1wt%) three mass loss events were observed, first between 72 – 99 °C attributed to low molecular weight compounds, and the aromatic structures present in the OEO because they were highly stable due to benzene ring

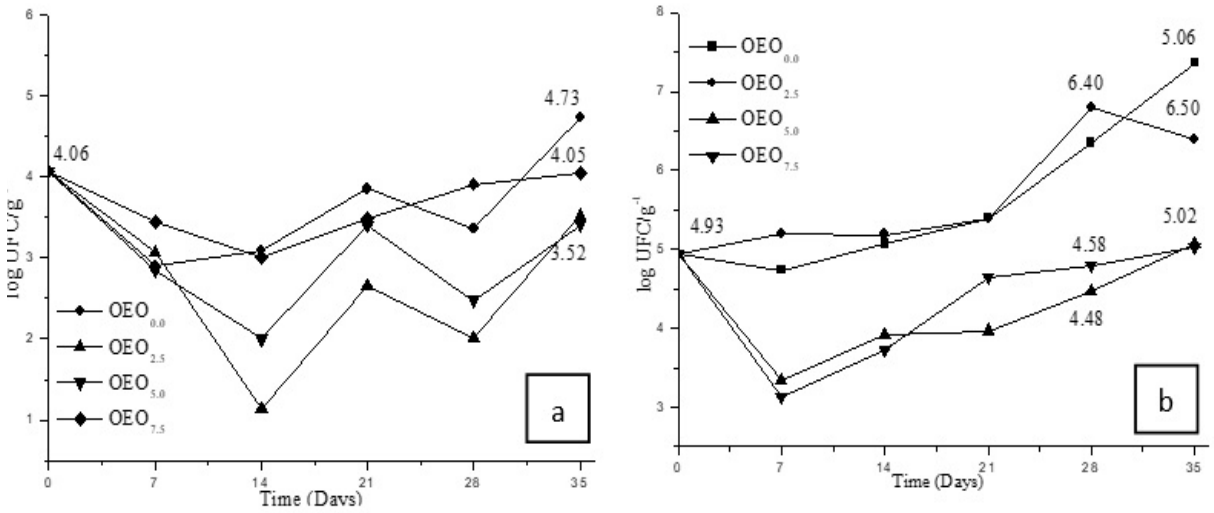

Figure 7. Antimicrobial activity of PBAT/OEO films produced by casting against *Staphylococcus aureus* (a) and psychrotrophic (b).

resonance resulted in decomposition at higher temperatures (380 °C) (Pelissari et al. 2009).

The PBAT:OEO films produced by casting presented a thermal profile similar to that produced by extrusion (Cardoso et al. 2017). DTG curves exhibited an event of maximum degradation rate at approximately 170 °C, attributed to the degradation of OEO compounds. The peak at 405 °C, in turn, is attributable to the thermal degradation of PBAT. This indicates that the technique used to prepare the film does not adversely affect the thermal stability thereof, and that evaporation of the solvent used does not impair or influence the thermal stability of the film.

The formulation OEO7.5 presented a WVP value close to the control this behavior can be attributed to the casting process, which may have led to poor homogenization of OEO in the PBAT matrix, since the opposite behavior was reported by Cardoso et al. (2017) for PBAT films with the same percentage of OEO, but produced by extrusion. The reduction of WVP in films can be attributed to the presence of oregano oil (OEO), since OEO can enter the structure of the PBAT matrix and limit the passage of liquid molecules through the films, increasing their hydrophobicity. Similar result Nisar et al. (2018) investigated the permeability of pectin films incorporated to clove essential oil and observed that the WVP films decreased with the addition of oil. Similar results were found by Sánchez-González et al. (2011) when studying the effect of essential oils on hydroxypropyl methylcellulose and chitosan films. The authors observed that WVP values decreased as OEO concentration increased. Regardless of the polymer, such behavior is expected since blends having a hydrophobic fraction usually improve the water barrier properties of films.

The reduction in WVP values observed here may be related to the role that OEO incorporation plays on the aggregation forces among the macromolecules as well as their location within the polymer chains. The nature of OEO combined with its compatibilizing action reduces water vapor permeation through the chains, thus altering the hydrophilic/hydrophobic balance of the film (Teixeira et al. 2014).

The concentration of phenolic compounds is an important approach for packaging development, especially for its active functions (Knapp et al. 2019). Aromatic terpenes containing hydroxyl groups are those responsible for free radicals inhibition (Alvarez et al. 2019). Gómez-Estaca et al. (2009) reported a similar outcome for tuna and bovine skin gelatin films incorporated with oregano and rosemary essential oils. The authors observed that the antioxidant potential of such biodegradable films was proportional to the concentration of the antioxidant additives (Teixeira et al. 2014).

Essential oils are complex mixtures of various compounds with different structures, polarities, and functional groups. Due to this structural complexity, Wang et al. (2008) stated that the antioxidant effect of an essential oil cannot be attributed to one or some of its constituents since, in addition to the main compounds, the constituents found at lower concentrations may contribute significantly to the oil activity. The main active compounds present in OEO are carvacrol, thymol, C-therpinene, and p-cymene, all of these featuring a broad spectrum of antioxidant capacity (Bakkali 2008).

Shojaee-Aliabadi et al. (2013) analyzed the antioxidant power of biodegradable kappa-carrageenan films incorporated with *Satureja hortensis* oil and observed that the polymer itself showed low antioxidant activity, probably as a function of the polyphenols present in the polymer matrix. Here, antioxidant action was not observed in the control film (OEO0.0), allowing one to infer that PBAT does not comprise any compound featuring antioxidant capacity.

Romani (2017) analyzed the antioxidant activity of rice starch and fish protein films and found that, after OEO incorporation, the films presented antioxidant power by capturing DPPH radicals. Serrano-Leóna et al. (2018) developed films incorporating natural antioxidants from peanut skin and pink pepper residue extracts. Peanut skin (776.46 µmol trolox/g) had higher antioxidant activity compared to pink pepper (535.74 µmol trolox/g). In this way, the produced materials present potential to be applied as active antioxidant packaging. In the present study, PBAT was also presented as a suitable polymer matrix for OEO incorporation, since DPPH tests confirmed its active function. In this sense, one may suggest its use as active antioxidant biodegradable packaging.

Carvacrol is the major constituent in oregano OEO and most effective antimicrobial components, considering the large number of different groups of chemical compounds present in OEOs, it is most likely that their antibacterial activity is not attributable to one specific mechanism (Alvarez et al. 2019). The extend cheese shelf-life up to 35 days, keeping it within the microbiological standards recommended by the current legislation and significantly reducing the initial contamination, both formulations having a similar effect (Romani 2017).

In a study carried out by Emiroğlu et al. (2010), increasing OEO and thyme essential oil concentrations in edible films had similar inhibitory effects. The lowest tested essential oil content (i.e., 3%) was then selected as the most antimicrobially effective because it represents a better cost-to-benefic ratio. According to Burt (2004) intrinsic food properties (e.g., fat and protein contents, pH, etc.) as well as environmental conditions in which food is stored (e.g., temperature, packaging, etc.) can influence the effect of essential oils on microbiota. Thus, factors such as low pH, storage temperature, decreased O_2 concentrations, and high salt contents increase the antimicrobial efficiency of the oils.

According to Yuan (2016), the release and availability of the active compounds of the EOs could also be affected by the food matrices. Films

with high permeability had a lower antimicrobial effect, which can be observed in the formulation (OEO7.5), where the higher OEO content did not represent a higher antimicrobial activity, justified by films high O_2 permeation. Studies related to the incorporation of antimicrobial agents into PBAT films are recent. The shelf-life of fresh-cut lasagne intercalated with extruded films from blends of rice flour, PBAT, and glycerol, when 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*, and psychrotrophic microorganisms (Sousa et al. 2013).

The films demonstrated a better antimicrobial action against Gram-positive bacteria (*S. aureus*) the greater efficiency of carvacrol against Gram-negative bacteria occurs due to high hydrophobicity, being capable of disintegrating the outer membrane and allowing lipopolysaccharide leakage by means of increasing the permeability of the cytoplasmic membrane (Burt 2004). Moreover, Cox et al. (2000) has shown that OE hinders the growth of Gram-negative bacteria, by changing the permeability of the cell, increasing the intracellular escape of K^+ ions and disturbing cellular respiration. Likewise, the antibacterial action of thymol, menthol and linalyl acetate causes a disturbance of the lipid fractions of the bacterial plasma membranes. In response, membrane permeability is affected by causing escape of intracellular materials. Hosseini et al. (2015) stated that the nature and structural characteristics of the matrix in which the essential oil is dispersed, together with the film-forming method, play a crucial role in the antimicrobial activity of the resulting material. In this sense, when extrusion is compared with casting for the production of PBAT/OEO blends, the antimicrobial action is affected, as demonstrated by Cardoso et al. (2017). In this study, except for OEO2.5, all formulations were able to extend the shelf-life of the slices for up to 10 days. Thyme essential oil incorporated chitosan coating applied to sliced fresh Channa argus retarded the decay of the fish fillets and significantly extended the shelf life by approximately 4–5 days (Yuan 2016, Yang et al. 2015). The formulation containing 2.5 g of OEO did not present antimicrobial activity. Here, this activity was observed in OEO-containing films.

Future trends of essential oils in food packaging

According to Souza et al. (2016) studies related to the incorporation of antimicrobial agents into PBAT films are recent. For this reason, other essential oils must be incorporated into the PBAT. The present study has the future perspective to analyze the percentage of migration between the packaging and the cheese. In addition, test the efficiency of the packaging against other food matrices. According to Santos et al. (2017) numerous food products require protection against microbial spoilage and lipid oxidation during their shelf-life.

An important perspective is the use of emerging technologies such as nano and micro encapsulation of essential oils. When protected through encapsulation, they can be used in techniques such as extrusion, based on the use of high temperatures to melt polymers such as PBAT. Thus, perform the fusion between the polymer and the oil, without degrading the bioactive compounds present. According to Shojaee-Aliabadi et al. (2013) films incorporated with OEO are generally prepared by casting method, based on the evaporation of the solvent, without using high temperatures.

CONCLUSION

The casting technique proved to be efficient to produce PBAT:OEO films, obtaining homogeneous films with low porosity. Depending on the OEO concentration in the PBAT matrix, the same causes a change in the mechanical properties, being able to reduce the tension, increase the elongation and the modulus of elasticity. The thermal behavior of the films underwent minor changes with the OEO incorporation. The permeability to water vapor decreased with the presence of OEO in the films until a certain concentration. Due to the high antimicrobial and antioxidant action, the produced PBAT:OEO films demonstrated efficiency as sliced mozzarella cheese packaging, since this material controlled both microbial growth and oxidation.

Therefore, the results of the mechanical, antimicrobial and antioxidant properties point out the formulations OEO2.5 and OEO5.0 for use as active packaging for food. Thus, the use of such films in Mozzarella cheese storage led to counts lower than those established by the current Brazilian legislation and extended the shelf life of cheese reducing *Staphylococcus aureus* in 35 days and psychrotrophic microorganisms in up to 28 days of storage in refrigeration.

Acknowledgments

This work was supported by Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) project (process 311392/2016-4 and 400710/2014-5).

REFERENCES

ALVAREZ MV, ORTEGA-RAMIREZ L, SILVA-ESPINOZA GA, GONZALEZ-AGUILAR GA & AYALA-ZAVALA JF. 2019. Antimicrobial, antioxidant, and sensorial impacts of oregano and rosemary essential oils over broccoli florets. J Food Process Preserv 43: 13889.

APHA - AMERICAN PUBLIC HEALTH ASSOCIATION. 2001. Compendium of Methods for the Microbiological Examination of Foods. Washington, D.C.

ARRIETA MP, PELTZER MA, GARRIGOS MC & JIMÉNEZ A. 2013. Structure and mechanical properties of sodium and calcium caseinate edible active films with carvacrol. J Food Eng 114: 486-494.

ASTM - AMERICAN SOCIETY FOR TESTING AND MATERIALS. 2009a. Designation D882-09: Standard test method for tensile properties of thin plastic sheeting. Annual Book of ASTM Standards. ASTM, Philadelphia. Available at http://www.anvisa.org.br. Accessed on Jully 25th, 2019.

ASTM - AMERICAN SOCIETY FOR TESTING AND MATERIALS. 2009b. Designation 96/E 96M—05: Test Method for Water Vapor Transmission of Materials. Annual Book of ASTM Standards. ASTM, Philadelphia. Available at http://www.anvisa.org.br. Accessed on Jully 25th, 2019.

BAKKALI FS, AVERBECK D & IDAOMAR M. 2008. Biological effects of essential oils - A review. Food Chem Toxicol 46: 446-475.

BRANDELERO RPH, ALMEIDA FM & ALFARO A. 2015. Microestrutura e Propriedades de Filmes de Amido-Álcool Polivinílico-Alginato adicionados de Óleos Essenciais de Copaíba e Capim Limão. Quim Nova 38: 910-916.

BURT S. 2004. Essential oils: their antibacterial properties and potential application in foods: a review. Int J Food Microbiol 94: 223-253.

CARDOSO LG, SANTOS JCP, CAMILLOTO GP, MIRANDA AL, DRUZIAN JI & GUIMARÃES AG. 2017. Development of active films poly (butylene adipate coterephthalate) - PBAT incorporated with oregano essential oil and application in fish fillet preservation. Ind Crops Prod 108: 388-397.

COX SD, MANN CM, MARKHAM JL, BELL HC, GUSTAFSON JE, WARMINGTON JR & WYLLIE SG. 2000. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). J Appl Microbiol 88: 170-175.

EMIROĞLU ZK, YEMIŞ GP, COŞKUN BK & CANDOĞAN K. 2010. Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. Meat Sci 86: 283-288.

ESPITIA PJP, AVENA-BUSTILLOS RJ, REINALDO WD, TEÓFILO F, SOARES NFF & HUGH TH. 2014. Optimal antimicrobial formulation and physical-mechanical properties of edible films based on açaí and pectin for food preservation. Food Packag 2: 38-49.

GÓMEZ-ESTACA J, BRAVO L, GOMEZ-GUILLEN MC, ALEMAN A & MONTERO P. 2009. Antioxidant properties of tuna-skin and bovine-hide gelatin films induced by the addition of oregano and rosemary extracts. Food Chem 112: 18-25.

GUO G, ZHANG C, DU Z, ZOU W, TIAN Z, XIANG A & LI A. 2015. Structure and property of biodegradable soy protein isolate/PBAT blends. Ind Crops Prod 74: 731-736.

HOSSEINI SF, REZAEIA M, ZANDIB M & FARAHMANDGHAVI F. 2015. Bio-based composite edible films containing *Origanum vulgare* L. essential oil. Ind Crops Prod 67: 403-413.

JONGJAREONRAK A, BENJAKUL S, VISESSANGUAN W & TANAKA M. 2008. Effects of plasticizers on the properties of edible films from skin gelatin of big eye snapper and brown stripe red snapper. Eur Food Res Technol 222: 229-235.

KASAI D, CHOUGALE R, MASTI S, CHALANNAVAR R, MALABADI RB, GANI R & GOURIPUR G. 2019. An Investigation into the Influence of Filler *Piper nigrum* Leaves Extract on Physicochemical and Antimicrobial Properties of Chitosan/Poly (Vinyl Alcohol) Blend Films. Environ. Polym Degrad 27: 472-488.

KHANEGHAH M, BAGHER SM & LIMBO HS. 2018. Antimicrobial agents and packaging systems in antimicrobial active food packaging: An overview of approaches and interactions. Food Bioprod Process 111: 1-19.

KI HM & PARK B. 2007. Preparation and rheological characteristics of ethylene-vinyl acetate copolymer/oregano clay nanocomposites. Int J Biol Macromol 46: 261-273.

KNAPP MA, SANTOS DF, PILATTI-RICCIO D, DEON VG, FIDELIS GH & PINTO VZ. 2019. Yerba mate extract in active starch films: Mechanical and antioxidant properties. J Food Process Preserv 43: 1-12.

KOKOSZKA S, DEBEAUFORT F, HAMBLETON A, LENART A & VOILLEY A. 2010. Protein and glycerol contents affect physico-chemical properties of soy protein isolate-based edible films. Innov Food Sci Emerg Technol 11: 503-510.

LEE S, JEONG E, LEE MY, LEE M & LEE Y. 2016. Improvement of the mechanical and thermal properties of polyethersulfone-modified epoxy composites. J Ind Eng Chem 33: 73-79.

MALI S, DEBI F, GROSSMANN MVE & YAMASHITA F. 2010. Starch, sugarcane bagasse fibre, and polyvinyl alcohol effects on extruded foam properties: A mixture design approach. Ind Crops Prod 32: 353-359.

MALI S, SAKANAKA SLS, YAMASHITA F & GROSSMANN MVE. 2005. Water sorption and mechanical properties of cassava starch films and their relation to plasticizing effect. Carbohyd Polym 60: 283-289.

MARTUCCI JF, GENDE LM & NEIRA RA. 2015. Oregano and lavender essential oils as antioxidant and antimicrobial additives of biogenic gelatin films. Ind Crops Prod 71: 205-213.

NISAR T, WANGA Z, YANGA X, TIAN Y, IQBAL M & GUO Y. 2018. Characterization of citrus pectin films integrated with clove bud essential oil: Physical, thermal, barrier, antioxidant and antibacterial properties. Int J Biol Macromol 106: 670-680.

OUAJAI S & SHANKS RA. 2009. Biocomposites of Cellulose Acetate Butyrate with Modified Hemp Cellulose Fibres. Macromol Mater Eng 294: 213-221.

PELISSARI FM, GROSSMANN MV, YAMASHITA F & PINEDA EAG. 2009. Antimicrobial, mechanical, and barrier properties of cassava starch-chitosan films incorporated with oregano essential oil. Food Chem Toxicol 57: 7499-7750.

RHIM JW, HONG S, PARK PHK & NG W. 2006. Preparation and Characterization of Chitosan-Based Nanocomposite Films with Antimicrobial Activity. J Agric Food Chemistry 54: 5814-5822.

ROJAS-GRAU MA, AVENA-BUSTILLOS RJ, FRIEDMAN M, HENIKA PR & MCHUGH TH. 2006. Mechanical, barrier, and antimicrobial properties of apple puree edible films containing plant essential oils. J Agric Food Chemistry 54: 9262-9267.

ROMANI VP, PRENTICE-HERNÁNDEZ C & MARTINS VG. 2017. Active and sustainable materials from rice starch, fish protein and oregano essential oil for food packaging. Ind Crops Prod 97: 268-274.

SÁNCHEZ-GONZÁLEZ LCA, GONZÁLEZ-MARTÍNEZ C & CHÁFER M. 2011. Effect of essential oils on properties of film forming emulsions and films based on hydroxyl propyl methylcellulose and chitosan. J Food Eng 105: 246-253.

SANTANA JS, COSTA ÉKC, RODRIGUES PR, CORREIA PRC, CRUZ RS & DRUZIAN JI. 2018. Morphological, barrier, and mechanical properties of cassava starch films reinforced with cellulose and starch nanoparticles. J Appl Polym 47: 1-10.

SANTOS RR, ANDRADE M, MELO NR & SANCHES-SILVA A. 2017. Use of essential oils in active food packaging: Recent advances and future trends. Trends Food Sci Technol 61: 132-140.

SERRANO-LEÓNA JS, BERGAMASCHIA KB, YOSHIDA CMP, SALDAÑA E, SELANIC MM, RIOS-MERAA JD, ALENCARA SM & CONTRERAS-CASTILLO CJ. 2018. Chitosan active films containing agro-industrial residue extracts for shelf-life extension of chicken restructured product. Food Res Int 108: 93-100.

SHOJAEE-ALIABADI S, HOSSEINIA H, MOHAMMADIFARA MA, MOHAMMADIA A, GHASEMLOU M, OJAGHC SM, HOSSEINIA SM & KHAKSAR R. 2013. Characterization of antioxidant-antimicrobial-carrageenan films containing *Satureja hortensis* essential oil. Int J Biol Macromol 52: 116-124.

SILVA JBA, SANTANA JS, LUCAS AA, PASSADOR FR, COSTA LAS, PEREIRA FV & DRUZIAN JI. 2019. PBAT/TPS-nanowhiskers

blends preparation and application as food packaging. J Appl Polym 47699: 1-10.

SINGH N & RAGINI PS. 2004. Free radical scavenging activity of an aqueous extract of potato peel. Food Chem 85: 611-616.

SOUSA GM, SOARES MS & YAMASHITA F. 2013. Active biodegradable films produced with blends of rice flour and poly(butylene adipate co-terephthalate): Effect of potassium sorbate on film characteristics. Mater Sci Eng 33: 3153-3159.

TEIXEIRA B, MARQUES A, PIRES C, RAMOS C, BATISTA I, SARAIVA JA & NUNES ML. 2014. Characterization of fish protein films incorporated with essential oils of clove, garlic and origanum: physical, antioxidant and antibacterial properties. LWT - Food Sci Technol 59: 533-539.

TURHAN KN & SAHBAZ F. 2004. Water vapor permeability, tensile properties and solubility of methylcellulose-based edible films. J Food Eng 61: 459-466.

WANG H, QIAN J & DING F. 2018. Emerging Chitosan-Based Films for Food Packaging Applications. J Agr Food Chem 66: 395-413.

WANG N, MA X, CHANG PR & YU J. 2008. Preparation and properties of biodegradable poly(propylene carbonate)/ thermoplastic dried starch composites. Carbohydr Polym 74: 229-234.

YUAN G, CHENA X & LI D. 2016. Chitosan films and coatings containing essential oils: The antioxidant and antimicrobial activity and application in food systems. Food Res Int 89: 117-112.

ZIVANOVIC S, CHI S & DRAUGHON AF. 2005. Antimicrobial activity of chitosan films enriched with essential oils. J Food Sci 70: 45-51.

How to cite

CARDOSO LG, DA SILVA JBA, DA SILVA JA, CAMILLOTO GP, DE SOUZA CO, DRUZIAN JI & GUIMARÃES AG. 2022. Development and characterization of antioxidant and antimicrobial poly (butylene adipate-co-terephtalate) (PBAT) film incorporated with oregano essential oil and applied in sliced mozzarella cheese. An Acad Bras Cienc 94: e20200142. DOI 10.1590/0001-3765202220200142.

Manuscript received on February 06, 2020;
accepted for publication on September 09, 2020

LUCAS G. CARDOSO[1]
https://orcid.org/0000-0002-6674-338X

JÂNIA B.A. DA SILVA[2]
https://orcid.org/0000-0003-2442-2833

JULIANA A. DA SILVA[5]
https://orcid.org/0000-0002-9397-1580

GEANY P. CAMILLOTO[3]
https://orcid.org/0000-0002-3924-7823

CAROLINA O. DE SOUZA[4]
https://orcid.org/0000-0002-8028-5418

JANICE I. DRUZIAN[4]
https://orcid.org/0000-0001-8940-6098

ALAÍSE G. GUIMARÃES[4]
https://orcid.org/0000-0001-6020-1283

[1]Programa de Graduação em Biotecnologia, Universidade Federal da Bahia (UFBA), Instituto de Ciências da Saúde, Av. Reitor Miguel Calmon, s/n, 40110-100 Canela, BA, Brazil

[2]Universidade Federal do Recôncavo da Bahia (UFRB), Centro de Ciência e Tecnologia, Colegiado de Engenharia Mecânica, Rua Rui Barbosa, 710, 44380-000 Cruz das Almas, BA, Brazil

[3]Universidade Estadual de Feira de Santana, Departamento de Tecnologia, Colegiado de Engenharia de Alimentos, Av. Transnordestina, s/n, Novo Horizonte, 44036-900 Feira de Santana, BA, Brazil

[4]Programa de Pós-Graduação em Ciência dos Alimentos, Universidade Federal da Bahia (UFBA), Faculdade de Farmácia, Rua Barão de Geremoabo, s/n, Campus de Ondina, 40170-210 Salvador, BA, Brazil

[5]Universidade Federal da Bahia, Departamento de Engenharia Química, Rua Aristides Novis, 2, Federação, 40210-360 Salvador, BA, Brazil

Correspondence to: **Lucas G. Cardoso**
E-mail: lucaseng.pesca@yahoo.com.br

Author contributions

Lucas Guimarães Cardoso: Methodology, Validation, Investigation, Writing-Original draft preparation; Jânia Betânia Alves da Silva Investigation, Writing-Original draft preparation; Juliana Albuquerque da Silva and Geany Peruch Camilloto: Investigation, Writing-Original draft preparation Carolina Oliveira de Souza and Janice Izabel: Conceptualization, Supervision, Funding acquisition; Alaíse Gil Guimarães: Conceptualization, Supervision, Methodology, Writing.