Research, Society and Development, v. 9, n. 10, e3849108722. 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.8722

Characterization of films of low density polyethylene incorporated with oregano essential oil

Caracterização de filmes de polietileno de baixa densidade incorporados com óleo essencial de orégano

Caracterización de películas de polietileno de baja densidad incorporadas con aceite esencial de oregano

Received: 09/23/2020 | Reviewed: 09/27/2020 | Accept: 09/29/2020 | Published: 09/30/2020

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Abstract

The purpose of this study was to develop active films of low-density polyethylene incorporated with different concentrations of oregano essential oil (1, 2, 3, 4, 5 and 6% w / w). The production of the films was carried out in a modular mono screw extruder and the antimicrobial activity was evaluated by the agar diffusion test. The films were analyzed for mechanical, optical and gaseous barrier properties to evaluate changes in the films due to the addition of an antimicrobial agent. As a result, it was found that the control film had a higher luminosity, compared to the others. The opacity was not affected. Saturation, however, had a tendency to transform more vivid colors, while the tone had a decrease tendency towards yellow. In terms of mechanical properties, elongation was not affected, Young's modulus decreased and tension increased. The CO_2 permeability decreased. There were no inhibition halos around the films.

Keywords: Antimicrobial; Active film; Polymeric matrix.

Resumo

O objetivo deste estudo foi desenvolver filmes ativos de polietileno de baixa densidade incorporados com diferentes concentrações de óleo essencial de orégano (1, 2, 3, 4, 5 e 6% p/p). A produção dos filmes foi realizada em extrusora modular mono rosca e a atividade antimicrobiana avaliada pelo teste de difusão em ágar. Os filmes foram analisados quanto às propriedades mecânicas, ópticas e de barreira gasosa, para avaliar suas alterações devido à adição de um agente antimicrobiano. Como resultado, constatou-se que o filme controle apresentou maior luminosidade, em relação aos demais. A opacidade não foi afetada. A saturação, no entanto, teve uma tendência a transformação em cores mais vívidas, enquanto a tonalidade tendeu a decrescer, em sentido a cor amarela. Para as propriedades mecânicas, a elongação não foi afetada, o módulo de Young diminuiu e a tensão aumentou. A permeabilidade ao CO_2 decresceu. Não houve formação de halo de inibição ao redor dos filmes.

Palavras-chave: Antimicrobiano; Filme ativo; Matriz polimérica.

Resumen

El objetivo de este estudio fue desarrollar películas de polietileno activo de baja densidad incorporadas con diferentes concentraciones de aceite esencial de orégano (1, 2, 3, 4, 5 y 6% p / p). La producción de las películas se llevó a cabo en una extrusora monohusillo modular y la actividad antimicrobiana se evaluó mediante la prueba de difusión en agar. Las películas se analizaron en busca de propiedades mecánicas, ópticas y de barrera a los gases, para evaluar sus cambios debido a la adición de un agente antimicrobiano. Como resultado, se encontró que la película de control mostraba mayor brillo en comparación con las demás. La opacidad no se vio afectada. La saturación, sin embargo, tendió a cambiar a colores más vivos, mientras que el tono tendió a disminuir, hacia el amarillo. Para las propiedades mecánicas, el alargamiento no se vio afectado, el módulo de Young disminuyó y la tensión aumentó. La permeabilidad al CO_2 ha disminuido. No hubo formación de un halo de inhibición alrededor de las películas.

Palabras clave: Antimicrobiano; Película activa; Matriz polimérica.

1 Introduction

Studies on the antimicrobial activity of essential oils have reported reduction in the damage caused by the growth of undesired microorganisms (Lee et al., 2020; Galindo et al., 2019; Bouzidi et al., 2013; Muriel-Galet et al., 2013). Antimicrobial agents are able to extend the microorganisms lag phase. The effect is directly proportional, higher the concentration of the antimicrobial agent, greater the latency time (Nobile et al., 2009). Antimicrobial agents are able to extend the microorganisms lag phase (Menezes et al., 2018).

One of the many essential oils is oregano oil, in which carvacrol is the most important component. It has antimicrobial activity and can act as a food preservative (Du et al., 2015; Martucci et al., 2015; Veldhuizen et al., 2007).The antimicrobial action of the essential oil of oregano is usually attributed to damaging the structure and function of the cytoplasmic membrane properties of microorganisms. The carvacrol acts directly degradating the phospholipids layer. This causes a loss of structure and consequently lysis, leakage of cellular material, and cell death (Ultee; Bennik & Moezelaar, 2002).

The use of antimicrobial agents employed directly in packaging reduces the amount of chemical additives added to the products. Therefore, it meets the requirements of consumers who prefer healthier products, free of added preservatives and other products used for this purpose (Braga & Silva, 2017). Active films of low-density polyethylene incorporated with essential oils are usually produced by two different processing methods, neither impairing the antimicrobial activity. The ionizing method is characterized by the oil incorporation after electrical ionization in the film. In the extrusion method, the mixture of the resin and oil is done directly in the extruder. Comparing these two treatments, the extrusion method has a better antimicrobial activity, due to better incorporation of the active compounds in the polymer matrix (Solano & Gante, 2012).

When incorporating compounds in plastic, often their characteristics are changed. That is why it is necessary to study the new developed material (Oliveira & Oliveira, 2004). In the case of essential oils, they normally act as plasticizers, increasing elongation (Hosseini; Razavi & Mousavi, 2009), and gas permeability. However, they decrease the strength and elastic modulus (Ramos et al., 2012; Persico et al., 2009).

Our objective was to develop films of low-density polyethylene, incorporated with oregano essential oil. As well as, evaluating their antimicrobial potential and the optical, mechanical, and barrier properties.

2. Methodology

2.1 Materials selection and film production

Low-density polyethylene (LDPE, Braskem, Brazil) and oregano essential oil (EO, Ferquima Ind. e Com. Ltda, Brazil) were used to produce the films. The thickness was standardized between 95 and 105 micrometers.The production was performed with a modular single screw extruder (Model AX 16:26 AX Plastics, Brazil) with temperature profiles in the heating zones at 170, 180 e 190ºC (Coelho et al., 2020). Seven treatments were produced: LDPE control (without oil) and six films with essential oil contents (1, 2, 3, 4, 5 and 6%) (w/w).

2.2 Colorimetric evaluation

Color was measured using the Collor QUEST colorimeter (Hunter Lab) and CIELab system with a D65 light source and observation angle of 10° (Ravi; Prakash & Bhat, 2005). The following parameters were used: Opacity (O), O=(Ob/Ow)x100%, opacity of the film superposed on the black standard (Ob) and opacity of the film superposed on the white standard (Ow); L* (level of light or dark); a*(redness or greenness); b*(yellowness or blueness); Chrome (C), $C=(a^2+b^2)^{1/2}$; Hue (H), $H=\tan^{-1}(a/b)$.

2.3 Mechanical properties

The mechanical properties were determined using a Universal Testing Machine (Instron, model 3367, USA). The dimensions for each film were 50x20mm and the parameters were load cell of 1 kN and a speed of 500 mm/min. The tensile (MPa), elongation (%) and Young's modulus (MPa) values were calculated (ASTM, 2010).

2.4 Gas Barrier

The gas barrier test of CO_2 was done using a permeability tester (Labthink, model PERME Vac 1, China). The dimensions for each film disk were 70mm of diameter. (ASTM, 2009).

2.5 Antimicrobial activity

The antimicrobial activity against *Salmonella choleraesuis* (ATCC 10708) was tested based on the method disk-agar diffusion method (NCCLS, 2003). The films (1cm in diameter) sterilized (ultra violet light for 15 minutes each side) were placed on plates containing Mueller-Hinton agar (Merck) previously inoculated with 0.1 ml of standardized bacterial cultures (106 CFU/ml) with McFarland scale. Then the plates were incubated at 35°C for 48 hours. To evaluate the activity on *Aspergillus niger* (ATCC 16404), the microorganism was inoculated on potato dextrose agar plates (Acumedia) acidified and incubated for 5 days at 25°C (Botre et al., 2010). The growth of microorganisms in the contact area with the surface of the film was visually examined.

2.6 Statistical Analysis

The experiment was conducted in a completely randomized design with ten replicates. Data regarding treatments were subjected to regression analysis using the software Statistica7.0 (Statsoft, 2004). For color analysis, we performed analysis of variance (ANOVA) and the differences between treatments were tested using Tukey test at 5% probability.

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3 Results and Discussion

3.1 Colorimetric Evaluation

The results of the color parameters (L*, a* and b*) and opacity of the films produced are shown in Table 1.

The control presented greater value of luminosity than the other treatments. The incorporation of EO caused reduction of luminosity in the films up until the concentration of 2%. The higher concentrations of EO did not affect the variable L*, in comparison with the 2% concentration.

Table 1. Color parameters (L*, a* and b*) and opacity in films of low-density polyethylene incorporated with oregano essential oil (mean ± standard error).

Film (% EO)	L*	a*	b*	Opacity
Control (0)	91.18 ± 0.10[a]	-0.87 ± 0.01[a]	0.46 ± 0.02[d]	12.33 ± 0.45[a]
Film (1)	90.89 ± 0.13[b]	-0.87 ± 0.01[a]	0.59 ± 0.04[c]	12.08 ± 0.47[a]
Film (2)	90.65 ± 0.31[bc]	-0.88 ± 0.01[a]	0.69 ± 0.03[b]	12.17 ± 0.40[a]
Film (3)	90.13 ± 0.26[c]	-0.88 ± 0.08[a]	0.79 ± 0.10[b]	12.48 ± 0.30[a]
Film (4)	90.28 ± 0.22[c]	-0.88 ± 0.03[a]	0.92 ± 0.10[a]	12.36 ± 0.45[a]
Film (5)	89.72 ±0.49[c]	-0.89 ± 0.05[a]	1.08 ± 0.14[a]	11.18 ± 1.01[a]
Film (6)	90.13 ± 0.35[c]	-0.87 ± 0.04[a]	1.07 ± 0.10[a]	12.46 ± 0.60[a]

The same lowercase (column) letters indicate differences that are not significant (P>0.05), according to Tukey's test.
Source: Authors

The values of a* were not significantly changed by the addition of essential oil. The negative values found suggest a tendency to the color green. However, the values of b* distinguished themselves significantly among most treatments, tending to yellow with the increase of essential oil concentration in the film.

The increased concentrations of essential oil did not alter the film's transparency. The opacity of the films is associated with several factors, including the concentration of lipids (Liporacci; Mali & Grossmann, 2005; Taqi et al., 2011). Although, sometimes, the opacity is not significantly affected (Pires et al., 2013).

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The tone decreased with the incorporation of essential oil of oregano in the films. The values ranged from 150.5 to 126.6 for control and 6% EO film, respectively. This total variation represents a reduction of approximately 16%. Thus, the film of low-density polyethylene has a hue between yellow and green, tending to yellow with higher concentrations of added essential oil.

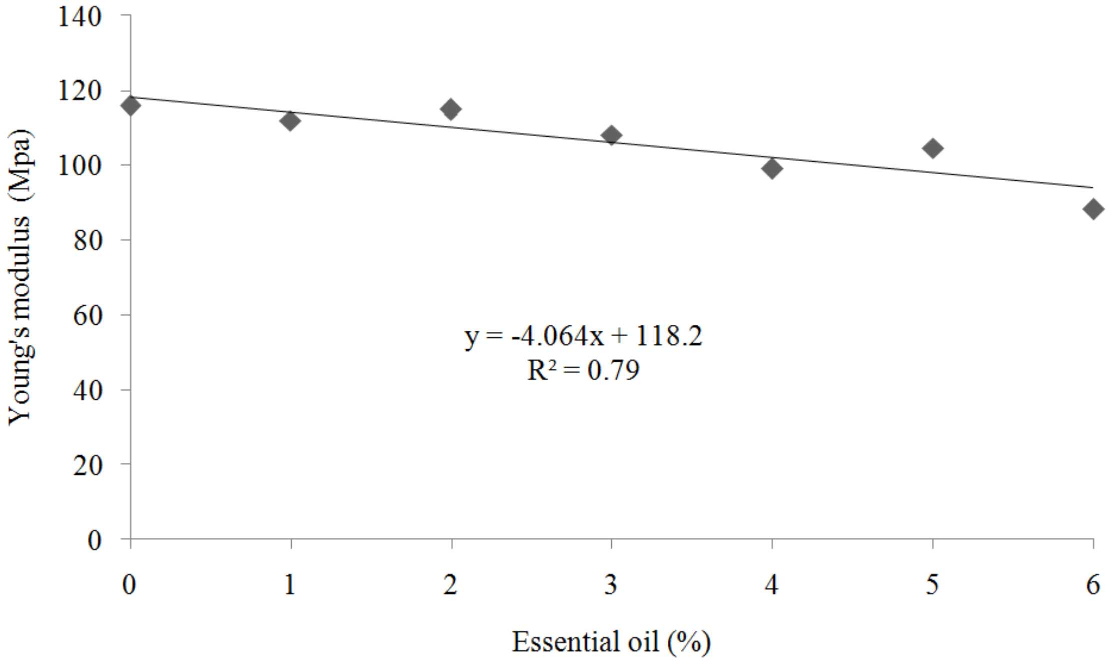
For the parameter saturation, the incorporation of EO in the films increases the values. There is a tendency to the emergence of sharper and more vivid colors. Values ranged from 0.980 to 1.412 for the control film and with 6% EO, respectively, with a considerable variation of 44.08%. However, the values of C* remained next to zero, which indicates a low, or barely noticeable, saturation (Mendonça et al., 2003).

3.2 Mechanical Properties

For property elongation we observed no significant difference between treatments ($p < 0.05$), with mean values ranging from 311.4 to 348.2%. However, some studies indicate that the addition of EO causes a plasticizing effect in the polymeric matrix and increase elongation and ductility (Pelissari et al., 2009; Persico et al., 2009; Ramos et al., 2012). This is because it allows the material to stretch more, which may lead to a large deformation before breaking. However, it is understood that the incorporation of essential oil does not always act with plasticizing effect, whereas can cause an increase of intermolecular forces and reduce the mobility of the chains decreasing the elongation property (Dias et al., 2013).

For Young's modulus there was a linear decreasing tendency ranging from 118.2 to 93.816, for the control film and 6% EO film, respectively (Figure 1). This difference corresponds to approximately 20% of reduction in elastic deformation. That is, as the essential oil and their constituents are incorporated, the films became less rigid (Ramos et al, 2012; Pelissari et al., 2009; Zinoviadou; Koutsoumanis & Biliaderis, 2009).

Figure 1. Values of Young's modulus (MPa) of the films of low-density polyethylene incorporated with 0, 1, 2, 3, 4, 5 and 6% of essential oil of oregano.



Source: Authors.

The values of tension had an increasing variation of 16.28% with the increment of EO in films (Figure 2). Some studies show that this incorporation tends to increase the values of tension when there is an interaction between the polymer chains and the components present in the oil. It turns the film into a structure with greater capacity to accommodate the voltage and reduce the matrix's mobility (Ojagh et al., 2010; Kechichian et al., 2010).

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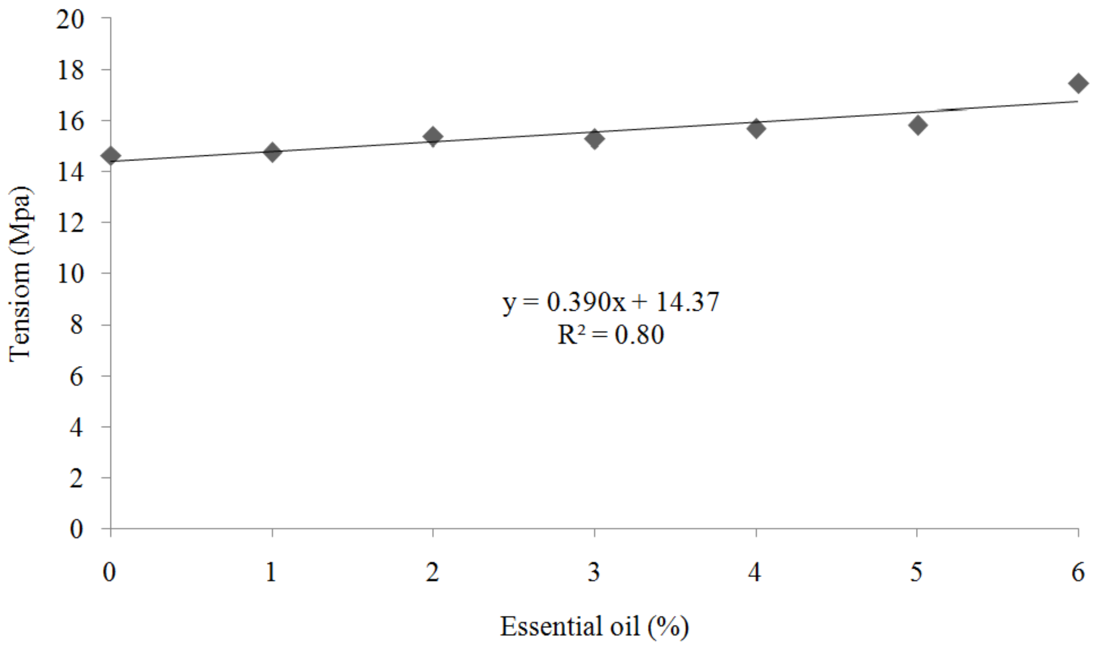
Figure 2. Maximum Values Tensile (MPa) of the films of low-density polyethylene incorporated with 0, 1, 2, 3, 4, 5 and 6% of essential oil of oregano.



Source: Authors.

Despite the known plasticizing effect generated by the presence of oils, sometimes the films do not achieve these changes in mechanical properties. The polymer properties are a reflection of the interactions between the polymer matrix and antimicrobial agents in the oils (Hosseini; Razavi & Mousavi, 2009).

3.3 Gas Barrier

The addition of EO in polyethylene films resulted in a decrease tendency in CO_2 permeability (Figure 3). The values ranged from 7172.9 to 5904.9 cm^3/m^2.24h.0.1MPa respectively for the control film and film with 6%of essential oil. This corresponds to a 17.68% decrease. The permeability of a film is usually associated with many factors. Among them, we can cite the type of polymer base employed for the film production, as well as other elements incorporated in the mixture (Pinheiro et al., 2010). The morphological structure and porosity of the films are also factors determining the permeation through the membrane (Mousavi et al., 2010; Sothornvit & Krochta, 2000).

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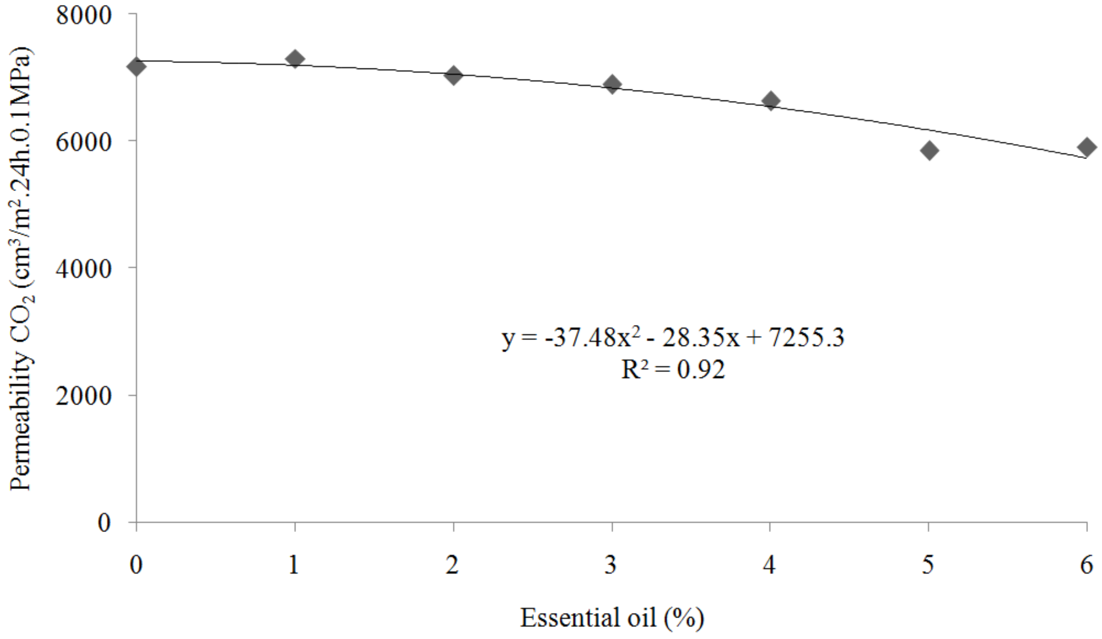
Figure 3. Permeability values of CO_2 ($cm^3/m^2.24h.0.1MPa$) of the films of low-density polyethylene incorporated with 0, 1, 2, 3, 4, 5 and 6% of essential oil of oregano.



Source: Authors.

3.4 Antimicrobial Activity

Independently of microorganisms and oil concentration used, there was no formation of inhibition zones. It was expected that the EO could disseminate radially from the polymeric matrix creating halos. However, there was inhibition of microorganism growth in direct contact with the surface of the film. It was possible to observe visually a lower concentration of colonies on the films themselves.

In some cases, even after prolonged storage, active films may continue inhibiting the growth of microorganisms depending on storage temperature. The incorporation of highly volatile compounds, like essential oils, in polymeric films is advantageous and should be studied further (Suppakul et al., 2011).

4 Conclusion

We concluded that the incorporation of essential oil of oregano in films of low-density polyethylene alters their properties by reducing the values of hue and increase the values of luminosity and saturation. The opacity and elongation property do not change with the addition of essential oil. However, Young's modulus, and the permeability decrease and the tension increases. The insertion of the essential oil did not form a microorganism inhibition halo for *A.niger* and *S.choleraesuis* in the concentrations evaluated.

New studies may be carried out with the incorporation of higher concentrations of oregano essential oil in the films and even tests directly in real food systems, to evaluate their antimicrobial, antioxidant and sensory efficiency.

Acknowledgements

We acknowledge Federal University of Goiás and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the financial support.

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