

Bioactive chitosan/extract peppermint films to food packing in brisee dough: mechanic properties, antioxidant activity and shelf life

Filmes bioativos de quitosana / extrato de hortelã-pimenta para embalagem de alimentos em massa brisee: propriedades mecânicas, atividade antioxidante e validade comercial

Películas bioactivas de quitosano / extracto de menta para envasar alimentos en masa brisee: propiedades mecánicas, actividad antioxidante y vida útil

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Abstract

Besides practical and convenient products, the consumer has increasingly demanded safe and tasty foods, arousing interest in natural additives. This work aimed to develop chitosan films added with peppermint (*Mentha piperita L.*) hydroethanolic extract (EHH) for Briseé dough conservation. Films were prepared with chitosan (1% w/v) added with four EHH concentrations: 2.5% (EH2.5), 5% (EH5), 10% (EH10) and 25% (EH25). EH5 and EH10 films showed better antioxidant activity and bioactives retention, so were characterized by visual analysis, thickness, mechanical and optical properties, moisture, density, water vapour permeability, swelling, solubility. Moreover, by antimicrobial action against *S. aureus* and *Escherichia coli*, whose results showed they did not exhibit any activity against studied bacteria. For selected films, EHH addition improved mechanical, barrier and optical properties. They were used to pack pieces of dough, then results for DPPH and TBARS showed these films were effective in delaying lipid oxidation over ten days of storage.

Keywords: Bioactive compound; Plant extract; Films; Lipid oxidation; Pastry dough.

Resumo

Além de produtos práticos e convenientes, o consumidor tem demandado cada vez mais alimentos seguros e saborosos, despertando o interesse pelos aditivos naturais. Este trabalho teve como objetivo desenvolver filmes de quitosana com adição de extrato hidroetanólico (EHH) de hortelã-pimenta (*Mentha piperita L.*) para a conservação de massa Briseé. Os filmes foram preparados com quitosana (1% p/v) adicionada com quatro concentrações de EHH: 2,5% (EH2.5), 5% (EH5), 10% (EH10) e 25% (EH25). Os filmes EH5 e EH10 apresentaram melhor atividade antioxidante e retenção de bioativos, portanto foram caracterizados por análise visual, espessura, propriedades mecânicas e ópticas, umidade, densidade, permeabilidade ao vapor de água, intumescimento, solubilidade. Além disso, por ação antimicrobiana contra *S. aureus* e *Escherichia coli*, cujos resultados mostraram que não exibiram qualquer atividade contra as bactérias estudadas. Para os filmes selecionados, a adição de EHH melhorou as propriedades mecânicas, de barreira e ópticas. Eles foram usados para embalar pedaços de massa, então os resultados

para DPPH e TBARS mostraram que esses filmes foram eficazes em retardar a oxidação de lipídios em dez dias de armazenamento.

Palavras-chave: Composto bioativo; Extrato vegetal; Filmes; Oxidação de lipídios; Massa folhada.

Resumen

Además de productos prácticos y convenientes, los consumidores demandan cada vez más alimentos seguros y sabrosos, lo que despierta interés en los aditivos naturales. Este trabajo tuvo como objetivo desarrollar películas de quitosano con adición de extracto hidroetanólico (EHH) de menta piperita (*Mentha piperita* L.) para la conservación de la masa Briseé. Las películas se prepararon con quitosano (1% p/v) agregado con cuatro concentraciones de EHH: 2.5% (EH2.5), 5% (EH5), 10% (EH10) y 25% (EH25). Las películas EH5 y EH10 mostraron mejor actividad antioxidante y retención bioactiva, por lo que se caracterizaron por análisis visual, espesor, propiedades mecánicas y ópticas, humedad, densidad, permeabilidad al vapor de agua, hinchamiento, solubilidad. Además, por acción antimicrobiana frente a *S. aureus* y *Escherichia coli*, cuyos resultados mostraron que no presentaban actividad frente a las bacterias estudiadas. Para películas seleccionadas, la adición de EHH mejoró las propiedades mecánicas, de barrera y ópticas. Se utilizaron para envasar piezas de masa, por lo que los resultados de DPPH y TBARS mostraron que estas películas eran efectivas para ralentizar la oxidación de lípidos dentro de los diez días posteriores al almacenamiento.

Palabras clave: Compuesto bioactivo; Extracto vegetal; Películas; Oxidación de lípidos; Hojaldre.

1. Introduction

The food industries have increased and diversified the supply of food products, seeking to meet the needs of the growing market. In recent years, research on packaging has expanded, with emphasis on active packaging, a new technology line. (Barbosa-Pereira et al., 2014).

Active packaging is currently one of the most dynamic technologies used to preserve food quality through the release of active agents contained in the packaging. They alter the conditions of the product, increasing its shelf life, its safety and its quality, also improving sensorial characteristics (Morelli et al., 2015).

The goal of packaging, when considered as a food preservation technology, is to inhibit product contamination, delay its deterioration, extend shelf life, and maintain food quality and safety (López-Rubio et al., 2004). According to López et al. (2010), the incorporation of bioactive compounds with antimicrobial and antioxidant function in packaging can promote an increase in the shelf life of the food packaged in it. As examples of natural bioactive compounds, there are leaf and fruit extracts, which are substances of vegetable source and whose characteristics are volatile and organic (Gómez-Estaca et al., 2009). Some leaf extracts have significant antimicrobial activity (Norajit et al., 2010).

Because of the growing demand to reduce the use of chemical additives in the food industry, the interest in natural food additives with antimicrobial and antioxidant properties has increased (Crizel et al., 2018; Kalaycioglu et al., 2017; Kanatt et al., 2012;). Recently, researches and developments in active food packaging have focused on functional materials based on extracts that incorporate natural active compounds (Van Den Broek et al., 2015, Leceta, Guerrero & De La Caba, 2013).

Among the natural polymers, chitosan has aroused interest because it is biodegradable, renewable and can form flexible and resistant films, with efficient barriers against oxygen, in addition to having antimicrobial activity (Lisková et al., 2015). Chitosan is a functional biopolymer with intrinsic antimicrobial and antioxidant properties and therefore has high potential to be used as an active biodegradable alternative packaging (Guoa et al., 2015; Van Den Broek et al., 2015). Several researchers have carried out numerous studies on the use of incorporated chitosan extracts as an alternative to synthetic antimicrobial and antioxidants agents (Crizel et al., 2018; Genskowski et al., 2015, Siripatrawan & Vitchayakitti 2016). Herbal extracts and spices or agricultural byproducts also have shown antioxidant properties, which makes them interesting additives in foods.

Films containing plant extracts have been developed for use in packaging with potential antioxidant (Brewer et al., 2011) and antimicrobial activity (Moldovan, 2014, Mekinic, 2014). The use of Peppermint (*Mentha piperita* L.) extract is due to several compounds with antioxidant properties. These components, when incorporated into biopolymers, give a bioactive

character and can be applied in food packaging (Choudhury; Kumar; Garg, 2006). Given the above, this work aims to develop active films based on chitosan additived with peppermint extract applying them in brissé mass with the goal of extending its shelf life. Currently, the shelf-life of Brissé pasta is 24 hours. In addition to characterizing the films in terms of mechanical properties, barrier properties, optical properties, physicochemical properties, and antimicrobial and antioxidant action with the perspective of using them in refrigerated Briseé dough to delay lipid oxidation and extend the shelf life.

2. Methodology

This study was developed according to methodological support provided by Pereira et al. (2018), being a lab research, quantitative in nature.

2.1 Films preparation

The films were prepared using casting methodology. The filmogenic solution with chitosan (1,0%) and acetic acid (0.5%) was prepared homogeneously using a mechanical stirrer (Tecnal TE-102, Tecnal, Brazil) at 18,000 for 10 minutes. After six minutes of agitation, 0.2 mL of glycerol (0.2 mL) and hydroethanolic extract of peppermint at different conditions (0.0, 2.5, 5.0, 10.0, 25.0%) were added. Then the filmogenic solutions were dropped in the plates and dried at 35 ± 2 °C in an oven with circulation and air renewal (model MA035, Marconi Brazil) for 24 hours. After drying, the films were removed and stored in a desiccator covered with aluminum foil for further analysis.

The hydroethanolic extract of peppermint was obtained from the leaf of peppermint. The process consisted of maceration with alcohol until paste production. After that, it was shaken and added more 60 mL alcohol (60%). The mixture was kept under agitation for 20 minutes, centrifuged (Mdol CT 6000R, CIENTEC, Brazil) at 4000 rpm for 10 minutes. The supernatant was filtered and stored in amber glass for addition in chitosan film-forming solution.

2.2 Films characterization

2.2.1 Determination of total phenolics

The total phenolics of the films were measured by spectrophotometric method (Wettasinghe and Shahidi ,1999) and the standard curve for gallic acid (10 to 120 μ g / mL⁻¹, with $R^2 = 0.9999$). A spectrophotometer (Shimadzu UV-1650PC) recorded the absorption spectra at the wavelength of 725 nm, using the Folin-Ciocalteau reagent (Merck). The results were expressed in mg of total phenolics in gallic acid equivalent (EAG) / g⁻¹ of the film.

2.2.2 Determination of the antioxidant activity

The antioxidant capacity of the films to scavenge the 2,2-diphenyl-1-picrylhydrazyl radical was evaluated according to the method described by Brand-Williams et al. (1995), with some adaptations. A 0.5 mL aliquot of the solution extracted from the films, added with 3.5 mL of the DPPH (6 x 10⁻⁵ mol / L⁻¹) radical solution, had its absorbance recorded at 517 nm in a spectrophotometer (Shimadzu UV -1650PC) until reaching the plateau.

2.2.3 Thickness

The thickness of the film was measured using a manual micrometer (Mitutoyo / micrometer, Japan) with 0.001 mm precision. Five measurements were performed (quadrant and center point) and the mean values used for opacity and mechanical properties calculations.

2.2.4 Mechanical properties

For determination of the mechanical properties (tensile strength and percent elongation at break), ASTM D882-10 (2010) describes the methodology applied, using the equipment DL-500 MF-MARK: EMIC®.

2.2.5 Color and opacity parameters

Instrumental color analysis by colorimeter (CR-400 CHROMA METER -Konica Minolta®, Japan) was measured using the method of Siripatrawan and Harte (2010), with adaptations. The reflectance mode and illuminant C and standard observed of 2°, and the readings of L*; a* and b* were performed in five different points on the films, and the data submitted to the calculation of the arithmetic mean.

The determination of the opacity of the films followed the methodology described by Siripatrawan and Harte (2010).

2.2.6 Light transmission

The visible light barrier properties of the films were measured at wavelengths selected between 200 and 800 nm using a spectrophotometer (Shimadzu UV-1650 PC) according to the procedure reported by Fang et al. (2002).

2.2.7 Solubility and moisture

Water solubility tests performed by the method of Zamudio-Flores et al. (2010).

2.2.8 Water vapor permeability (WVP)

Measurement of water vapor permeability (WVP) was performed by the gravimetric method based on ASTM 969 (1995), with some modifications (Dick et al., 2015).

2.2.9 Swelling

The ability of the dry films to absorb water was analyzed according to the methodology described by Cao, Fu and He (2007).

2.3 Production of the pastry dough

The preparation of the dough was carried out according to the method described by Chef Profesional (2009) and some adaptations, with a mixture (for 10 minutes) of wheat flour (200g) and butter (132g) in 100 mL of water until obtaining a smooth and homogeneous dough. After 30 minutes of storage, the mass was opened with 25 × 6 cm articulated polypropylene roll and refrigerated (4°C ± 2) for analysis.

2.4 Application of the film to the *Briseé* dough

The application of the film carried out according to Figure 1. The dough was opened in a previously sanitized workbench (RDC N°216 / 2014 ANVISA) and cut in 3 x 5 cm with a thickness of 0.5 cm (Figure 1). The cut squares were packed and labeled with information regarding EHH concentrations, date of manufacture/packing and expiration date.

Figure 1 - Application of the chitosan film added with peppermint extract.



A: the opening of the dough, B: cut in 3 x 5 cm, C: the cut dough, D: packed dough, E: folding, F: placing in the hermetic closing bag. Source: Authors (2021).

2.4.1 Determination of lipid oxidation by determination of thiobarbituric acid reactive substances (TBARS)

The oxidative stability of the Briseé dough was determined by obtaining the content of reactive materials to thiobarbituric acid (TBARS), as described by Vyncke (1970), with adaptations, according to Sørensen and Jørgensen (1996).

2.4.2 Kinetics of antioxidant action, lipid oxidation of the Briseé dough

The Brissé dough was packed with chitosan films with 5 and 10% peppermint extract and stored under refrigeration (8°C) for ten days. Every 48 hours, according to CNNPA resolution 16 of June 28, 1978 / ANVISA, the films were collect to determine the antioxidant action and DPPH according to the method described by Brand-Williams et al. (1995).

2.5 Statistical analysis

All data were performed in triplicate and the data submitted to statistical analysis using Statistica v5.0. Values expressed as mean \pm standard deviation. The Student t-test and the Duncan test were applied to compare the means. Differences were considered significant when $p < 0.05$.

3. Results and Discussion

3.1 Antioxidant activity of chitosan films with peppermint extract

The results of the total phenolic contents are directly proportional to the amount of peppermint extract incorporated in the films with different concentrations of extract (Table 1). Vasco, Ruales & Kamal-Eldin (2008), for the concentration of phenolics, classified foods into three levels: low (0.21 up to 0.91mg EAG / g), intermediate (0.92 up to 10.1mg EAG / g) and high (above 10.1mg EAG/g). Considering this classification, it can be seen that the EH 2.5 film is classified as low TFT content (0.88 mg EAG / g), while the other films (EH5, EH10, and EH25) are considered intermediates with TFT content 1.44 mg EAG / g, 2.19 mg EAG / g and 4.14 mg EAG/g, respectively (Table 1).

Table 1 - Total phenolic content, antioxidant capacity (DDPH) and retention of chitosan films produced with four extract concentrations and their respective retention percentages.

Film	Phenolic content (mg EAG/g de film)	DPPH scavenging activity (%)	Retention (%)
Control (C)	--	0,83 \pm 0,06 ^e	--
EH2.5	0.88 \pm 0.006 ^d	23.04 \pm 6.03 ^d	13.63 \pm 1.87 ^a
EH5	1.44 \pm 0,022 ^c	54.36 \pm 2.06 ^c	11.06 \pm 0.27 ^b
EH10	2.19 \pm 0.002 ^b	72.26 \pm 0.68 ^b	8.83 \pm 0.03 ^c
EH25	4.14 \pm 0.043 ^a	82.69 \pm 1.69 ^a	6.73 \pm 0.33 ^d

EH2.5 = Chitosan with 2.5% extract; EH5 = Chitosan with 5% extract; EH10 = Chitosan with 10% extract; EH25 = Chitosan with 25% of extract. Values presented as mean and standard deviation. The averages followed by the same letters in the same column did not differ among the Duncan test ($p > 0.05$). Source: Authors (2021).

The antioxidant activity data of the films by the DPPH radical scavenging method revealed that the film with 25% of the peppermint extract presented higher antioxidant activity (Table 1), as well as in the other films (2.5%, 5%, 10%) the antioxidant action was proportional to the amount of extract inserted in the film.

The DPPH radical sequestration method was used to indicate the antioxidant activity of the film. This assay is based on the ability of DPPH, a stable free radical, to be degraded and thus discolored in the presence of antioxidants, resulting in a reduction in absorbance values (Enayat & Banerjee, 2009; Babbar et al., 2011). As the inhibition of the DPPH radical happens, the antioxidants reduce it, turning it from violet to a yellowish compound. The extent of the reaction depends on the hydrogen-donating capacity of antioxidants (Blois, 1958).

The film prepared without the addition of peppermint extract (C) presented an antioxidant activity of 0.83%. Kannatt et al. (2012) used chitosan / WVP blends as a biopolymer to obtain films with lyophilized extract of peppermint and lyophilized extract of pomegranate peel. In the control film, without the addition of the lyophilized extract, they found DPPH radical scavenging values of 0.78%, close to this study. Wang, Q. et al. (2015) also report antioxidant action in 1% chitosan films (0.92%). However, Kanatt et al. (2008) studied chitosan coating (1%) with mint extract applied on beef steaks, and they reported that no antioxidant activity was found in the control coat, without extract.

Bitencourt et al. (2014) observed that in fish gelatin films there was an improvement (78% compared to the control) of the antioxidant activity when adding ethanolic turmeric extract (200g of extract / 100g), which is proportional to the concentration of the added extract. This characteristic was also found in this study (Table 1) and corroborated by Hafsa et al. (2016), Bitencourt et al. (2014), and Li et al. (2014). The increase in antioxidant activity with the addition of natural compounds to the films was also observed by Moradi et al. (2012), in chitosan films with essential oil of *Zataria multiflora* and grape seed extract, and by Tongnuanchan, Benjakul and Podpan (2014) in a study with fish gelatin-based films plus extract of ginger and turmeric.

As the natural antioxidant was incorporated into the chitosan film, interactions with chitosan may also have affected its antioxidant property. Therefore, to interpret the antioxidant activity of chitosan films containing extracts, information is needed not only about the structure and antioxidant mechanism of the added compounds but also about the interactions between chitosan and antioxidants (Wang, Q. et al., 2015).

In relation to the retention of phenolics in the films, it was observed that with the lowest concentration of extract (2.5%) added, there was a higher retention percentage of the phenolic compounds (13.63%) while with the highest content (25%), this percentage reduced to 6.73% (Table 1). The results showed that to obtain significant retention of the phenolic compounds in the film, it is necessary to maintain a proportional relationship between the concentration of chitosan and the mint extract. The increase in the content of extract added to the film interferes with the retention of these compounds and must be a critical threshold. (Chiumarelli & Hubinger, 2014).

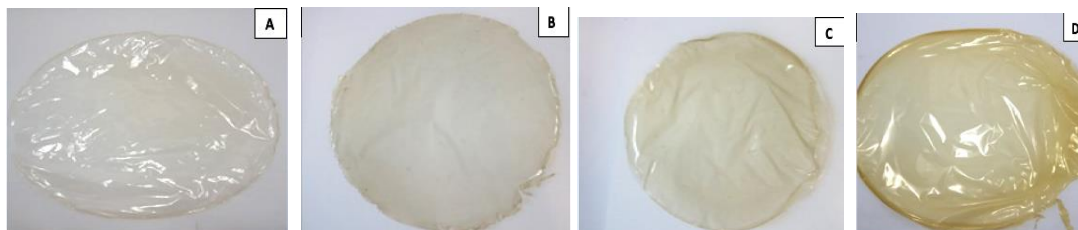
Another factor that interferes with the retention of the phenolic compounds is the amount of plasticizer used since its use in higher percentages in the film-forming solution provides higher retention of these compounds (Chiumarelli & Hubinger, 2014). However, despite the low retention of the phenolic compounds, the films showed good antioxidant action, especially in the films with higher concentrations of EHH. This suggested that the amount of TFTs and the chemical structure of the retained phenolics were sufficient to provide significant antioxidant action to the chitosan films.

3.2 Characterization of films

3.2.1 Visual aspects, color, opacity, and UV light transmission

All films presented (Figure 2) uniformity, transparency, flexibility, and handling, which indicates a good dispersion of the peppermint extract in the chitosan film, that is, the extract on the surface of the polymer.

Figure 2 - Chitosan films additive with peppermint hydroethanolic extract.



A) with 2.5% of extract, B) with 5% of extract, C) with 10% of extract, D) with 25% of extract. Source: Authors (2021).

In this sense, the properties of color, opacity and UV light transmission are significant for the appearance of the film, which, in turn, can influence the consumer acceptance of the packaged products (Barba De La Rosa et al., 2009; Pereda, Amica, & Marcovich, 2012). The color and brightness of a package are usually related to the product image. It is an important parameter for the characterization of the films, as it is related to the raw material used in their elaboration, for example, chitosan-based films show a tendency to yellowish color.

The addition of mint extract to the chitosan-based films influenced L *, a *, and b* parameters (Table 2). By correlating the different concentrations of the extract, it was noted that the addition of EHH caused a decrease in the L * parameter (light/dark).

Table 2 - Effect of addition of peppermint hydroethanolic extract (EHH) on luminosity (L *), chroma a *, chroma b * and color difference (ΔE^*) on chitosan-based films and Interference in the transmission of visible light (% T).

Films	L*	a*	b*	ΔE^*
Control (C)	90.95 ± 1.38 ^a	-0.37 ± 0.06 ^c	6.37 ± 0.19 ^c	7.24 ± 1.54 ^c
EH5	77.76 ± 3.77 ^b	-0.84 ± 0.17 ^b	24.01 ± 1.03 ^b	28.35 ± 0.52 ^b
EH10	73.21 ± 2.30 ^b	-1.34 ± 0.27 ^a	28.11 ± 1.14 ^a	36.54 ± 2.42 ^a

Films	Light Absorption (nm)								UV light transmission	Opacity (%T)
	200	280	350	400	500	600	700	800		
C	3.06	0.41	0.22	0.16	0.12	0.11	0.10	0.11	7.65 ^b	1.96 ^b
EH5	3.49	1.55	1.22	0.58	0.22	0.17	0.13	0.11	45.59 ^a	4.2 ^a
EH10	3.61	2.54	2.03	0.95	0.32	0.23	0.16	0.13	49.99 ^a	4.45 ^a

EH5 = Chitosan with 5% extract; EH10 = Chitosan with 10% extract. Values presented as mean and standard deviation. The averages followed by the same letters in the same column did not differ among the Duncan test (p < 0.05). Source: Authors (2021).

For parameter a*, the addition of extract resulted in lower values, which indicates greener tendency of the films. The EHH ratio interfered with the green color in the films; that is, the tonality accentuated by the increase in the concentration of mint extract (Table 2). When the parameter b*, which represents the color variations between blue and yellow, was evaluated at all concentrations of added extract, it was observed that its addition contributes to a more yellowish coloration of the films and that such tendency was also observed with addition of higher levels in the films.

The different extract concentrations in the films also influenced the color difference (ΔE^*) (Table 2), with higher values of ΔE^* for higher extract concentrations. The original color of the biodegradable films was directly influenced by the type and concentration of compounds added in the biomaterial. These compounds structurally bind to the film-forming solutions (Moradi et al., 2012). Similar changes in color parameters have been reported after incorporating other additives such as rosemary essential oil (Abdollahi et al., 2012), tea phenolic compounds (Wang et al., 2013) and green tea extract in films (Siripatrawan & Harte, 2010; Wang, L. et al., 2015).

Siripatrawan and Harte (2010), studying chitosan films with green tea extract, showed a decrease in the L^* value (from 87.50 ± 1.43 to 65.70 ± 0.97), similar to that observed in this study. The authors found a yellowish tendency (an increase of b^* value) for films added with green tea extract over the film without extract. However, the films with plant extracts showed more significant reddish tendencies (an increase in the value of a^*). Siripatrawan and Vitchayakitti (2016) obtained b^* values similar to those found in this study.

In the analysis of the opacity of the chitosan films with EHH (Table 2) presented higher values when compared to other studies. According to Santacruz and Castro (2015), the molecular weight of the matrix and the stirring time used to prepare the films may interfere with opacity. Another factor that may also interfere with opacity is the difference between the sources of chitosan and its production method (Aranaz et al., 2009).

An important parameter to be evaluated in food packaging films is opacity and UV light transmission. Because there are foods susceptible to photodegradation or photooxidation (Ojagh et al., 2010). A film is transparent when incident light passes through it with minimum of absorption or reflection. Likewise, an opaque film absorbs and/or reflects all incident light. In some products, protection against the incidence of light is necessary, as in the packaging of products sensitive to light-catalyzed deterioration reactions (Yuan et al., 2015). Films that contain ingredients that absorb light in the UV (200 to 400 nm) or visible (400 to 800 nm) spectrum as antioxidant compounds are alternatives to prevent such reactions (Silva-Weiss et al., 2013).

Concerning ultraviolet (UV) light transmission, the samples exhibited low light transmission at a wavelength of 280 nm (Table 2). The EHH film showed a higher protection index compared to the control film (without extract). Results suggest that the additive films would have a significant barrier to ultraviolet light and could be used as an antioxidant agent for high-fat foods (Kanatt et al. 2012; Souza et al., 2017).

Martins, Cerqueira and Vicent (2012) observed a similar behavior, with the incorporation of α -tocopherol in chitosan-based films, and in studies with other matrices as in the work of Norajit, Kim and Ryu (2010), which used alginate matrix incorporated with ginseng extract. Those by Wu et al. (2013), Bitencourt et al. (2014) and Li et al. (2014) who worked with gelatin films, incorporated with green tea extract, turmeric ethanolic extract and antioxidant compounds (grape seed extract, grape polyphenols, ginger extract, ginkgo leaf extract and green tea extract), respectively.

Chitosan films added with 5 and 10% EHH concentrations were darker in color than the control, pointing to an ability to help prevent oxidative deterioration from exposure to visible and ultraviolet light, which could lead to nutrient losses, discoloration and changes in the aroma and taste of packaged foods.

3.2.2 Thickness and mechanical properties

The mechanical properties of films define the ability to protect food integrity from physical damage (Martins, Cerqueira and Vicente, 2012; Rubilar et al., 2013). Chitosan, due to its positive charge, exhibits a versatility of adhesion to biological surfaces, being able to form stable materials (Moradi et al., 2012). However, they may offer fragility, requiring the use of plasticizer to reduce this (Ferreira et al., 2014; Leceta et al., 2015). The results of mechanical properties regarding thickness, tensile strength, elongation at break, and Young's modulus of EH5 and EH10 films are shown in Table 3.

Table 3 - Thickness of chitosan-based films with peppermint extract and Effect of the incorporation of peppermint hydroethanolic extract (EHH) on tensile strength (RT), elongation at break (AR) and Young's (E) modulus of chitosan-based films.

Films	Thickness (mm)	RT (MPa)	AR (%)	E (MPa)
Control (C)	0.04±0.004 ^a	14.05 ±0.65 ^c	5.64 ± 0.27 ^b	240.83 ± 25.53 ^c
EH5	0.04±0.001 ^a	17.27 ± 0.34 ^b	8.39± 0.45 ^a	615.00 ± 43.15 ^a
EH10	0.05±0.003 ^a	36.03 ± 0.57 ^a	8.47 ± 0.69 ^a	330.40 ± 8.41 ^b

EH5 = Chitosan with 5% extract; EH10 = Chitosan with 10% extract. Values presented as mean and standard deviation. The averages followed by same letters in the same column do not differ from each other by the Duncan test ($p > 0.05$). Source: Authors (2021).

Thickness is one of the parameters that influence the mechanical properties of films. Another important parameter to evaluate is homogeneity. This defines the uniformity of materials, repeatability of the measurements (Mahmoud & Savello, 1992; Gennadios et al., 1993).

In this study, the thickness of the films containing EHH did not exhibit any significant difference when compared to the chitosan film without the addition of extract (Table 3). Yuan et al. (2015) studied chitosan films supplemented with pomegranate peel extract and reported that there was no significant difference in film thickness. The results demonstrated that the phenolic compounds in the extract may have spreadability on the film surface without significantly changing the thickness. Similar results to this study were also reported by Bitencourt et al. (2014), in research with gelatin films with turmeric extract, and by Kanmani and Rhim (2014), on carrageenan films with grape seed extract.

Tensile strength increased when the EHH concentration increased from 0 to 10%, and the EH5 film had a significant difference when compared to the EH10 (Table 3). The improvement of this mechanical property of EHH films is attributed to interactions between the components of mint (for example, phenolic acids and their esters) having polar characteristics with the hydrophilic groups of the chitosan molecules. These interactions may result in increased interfacial adhesion between chitosan molecules and EHH in the film matrix, leading to a more effective resistance to mechanical stress (Pastor et al., 2010).

In films with 10% mint extract, RT values increased 2.6 times compared to pure chitosan films, results explained by the amount of EHH incorporated. Kalaycioğlu et al. (2017) obtained a 1.5-fold increase in RT when adding saffron extract to the chitosan film-forming solution. Liu et al. (2016) found similar results in chitosan films with curcumin. Kanatt et al. (2012) prepared chitosan-based films (1.0%, v / v) with different concentrations of mint extract and pomegranate extract and verified that RT increased when compared to the control, proving the influence of the concentration of bioactive compound on the RT value.

Tensile strength is the measure of the maximum force of a film to withstand the applied tensile stress (Park & Zhao, 2004). In the literature, trends in changing the RT values of chitosan films with the incorporation of different additives are frequent (Kalaycioğlu et al., 2017; Rubilar et al., 2013). With the addition of some additives, the RT can increase or decrease. Generally, an increase is attributed to a strong interaction between additives and chitosan chains, which causes an increase in film stiffness (Kalaycioğlu et al., 2017).

The elongation at breakage (AR) of the films had a significant increase ($p > 0.05$) when the added mint extract increased from 0 to 5% (Table 3). These results in film elongation can be attributed to the interactions between the peppermint components, which have polar characteristics with the hydrophilic chitosan groups. (Pastor et al., 2010; Siripatrawan and Vitchayakitti, 2016). These interactions may result in a greater adhesion between chitosan molecules and EHH, strengthening the biopolymer chain, increasing the AR ($p > 0.05$). However, there was no significant difference between EH5 and EH10, showing that HR does not change with an increase in EHH concentration above 5%. This factor is justified by the crystalline formation of excessive EHH components in the chitosan matrix, which leads to a stagnation of film flexibility (Pastor et al., 2010).

Similar findings were also observed by Shen and Kamdem (2015), who found elongation at an unchanged rupture with increasing concentration of two essential oils in chitosan films. Siripatrawan and Vitchayakitti. (2016) showed a decrease in AR in the chitosan film with 10% of propolis extract for the chitosan film with 25% of the extract. Bodini et al. (2013) suggest that interactions between bioactive components at high concentrations with chitosan can produce crosslinking, which can decrease free volume and mobility, causing a decrease in elongation.

Generally, different natural compounds showed different effects on the mechanical properties of the chitosan film. Hosseini et al. (2009) found that thyme and clove essential oil decreased tensile strength but improved elongation. Sanchez-Gonzalez et al. (2010) reported that bergamot essential oil decreased the tensile strength and elongation of the chitosan film. These differences can be attributed to the type of chitosan (solvent and molecular weight) used and to the particular interactions with natural components, which in turn are affected by relative humidity, presence of surfactants and temperature (Balti et al., 2017 Ma et al., 2016).

Young's modulus (commonly expressed in MPa) is the relationship between tensile strength and deformation in the elastic region, where the response of the specimen to elongation is increasing and linearly proportional to the imposed tension. The Young's modulus is an indicator of film stiffness, and so the higher the modulus, the more rigid the film is (Ferreira et al., 2014).

The control film favored the decrease of Young's modulus values (240.83 MPa), providing the formation of more elastic films (Table 3). The results also show that films incorporated with EHH (EH5 = 615.00 MPa and EH10 = 340.75 MPa) exhibit a significant increase ($p > 0.05$) in the Young modulus compared to pure chitosan film. The decrease in elasticity (increase in Young's value) is attributed to the interaction between chitosan molecules and mint extract (Kalaycioğlu, et al. 2017).

3.2.3 Moisture, solubility in water, water vapor permeability (WVP) and swelling

Chitosan films prepared with 5 and 10% of peppermint extract had a moisture content of $7.88 \pm 0.17\%$ and $8.43 \pm 0.40\%$, respectively (Table 4). These results indicated that the humidity of the EH5 films was not influenced by the addition of the extract ($P > 0.05$); however, EH10 films showed a significant difference comparing to the control. The moisture values of the films are in agreement with those obtained by several authors who used chitosan as a matrix (Rubilar et al., 2013; Pereda et al., 2011; Cerqueira et al., 2012).

Table 4 – Moisture, solubility, swelling and WVP of chitosan films with different concentrations of peppermint extract

Films	Moisture (%)	Solubility (%)	Swelling (%)	WVP (g.mm/h.m ² .kPa)
Control (C)	6.64±0.83 ^b	32.50±2.07 ^a	123.33 ± 11.29 ^c	0.059 ± 0.001 ^b
EH5	7.88±0.17 ^b	31.87±1.87 ^a	184.01 ± 8.53 ^b	0.074 ± 0.010 ^b
EH10	8.43±0.40 ^a	27.00±1.37 ^b	216.48 ± 6.58 ^a	0.113 ± 0.003 ^a

EH5 = Chitosan with 5% extract; EH10 = Chitosan with 10% extract. Values presented as mean and standard deviation. The averages followed by same letters in the same column do not differ from each other by the Duncan test ($p > 0.05$). Source: Authors (2021).

According to Cerqueira et al. (2012), the quantification of moisture in the films designates the fraction of water that has been inserted into them. It is also related to the vague gaps between the molecules of the biofilm microstructure that can be occupied by water molecules (Rubilar et al., 2013; Pereda et al., 2011). These authors denote that the portion of this element in the films is also dependent on the volume of the plasticizer (glycerol) present, which, due to its hydrophilicity, conserves water

in the film matrix. Thus, increasing the interrelationship between them, which may explain (Peng and Li, 2014; Wang et al., 2013), the amount of plasticizer used was around 50% lower, resulting in drier films.

Beigzadeh, Esmaili and Almasi (2017) achieved opposite results with chitosan films incorporated with milk thistle extract (*Silybum marianum*), reducing moisture in the films. This phenomenon is attributed to specific interactions between chitosan and milk thistle extract, which can stabilize the biofilm structure. Shojaee-Aliabadi et al., 2014 and Casariego et al., 2009 reported similar results in their studies.

The solubility values of chitosan films with peppermint extract are presented in Table 4. The solubility of the chitosan control film ($32.50 \pm 0.4\%$) was close to the value found by Pérez-Córdoba et al. (2017). Solubility is another important characteristic of films for food protection applications. Solubility is defined as the percentage of the resulting dry matter after 1 hour of immersion in distilled water. According to Wang, L. et al. (2015), the solubility of films is directly related to the properties of the incorporated bioactive components, such as the type of structure and their hydrophilicity/hydrophobicity.

All films added with mint extract had slightly lower solubility in distilled water. The values for EH5 (31.87 ± 1.8) and EH10 (27.35 ± 1.5) had a decrease in relation to the control, but only the EH10 film showed a significant difference ($p > 0.05$) compared to the control film. Pérez-Córdoba et al. (2017) reported a reduction in water solubility of chitosan/gelatin based films with canola oil nanoemulsion; α -tocopherol / cinnamaldehyde; α -tocopherol / garlic oil; α -tocopherol / cinnamaldehyde and garlic oil. The authors claimed that this was due to the non-polar compounds in the used oils, which resulted in substantial physical interference in the chitosan/gelatin polypeptide chains in the film matrix (Pérez-Córdoba et al., 2017; Ahmadi et al., 2012).

Wang, Q. et al. (2015), also observed a decrease in solubility of chitosan films with extract of the fruit *Lycium barbarum* (Goji berry). However, Khoshgozaran et al. (2012) obtained results contrary to those presented in this study, verifying an increase in the solubility of chitosan films added with Aloe vera oil. This difference in solubility can be explained by differences in additives, chitosan composition and film preparation (Khoshgozaran et al., 2012; Ojagh et al., 2010).

It is common to think that hydrophilic compounds should increase the solubility of a film, while hydrophobic compounds should decrease it. However, after incorporation of the mint extract into the chitosan film, the water solubility of the film was low, suggesting gel formation. These chitosan / extract interactions are responsible for the gel and may decrease water solubility. These data indicate that, as the amount of EHH increased, the hydrophilic groups available to bind to water became less available, indicating that more of these hydrophilic groups are involved in the interactions with the extract (Wang, Q. et al., 2015).

Swelling is related to the amount of water a dry film absorbs after a certain period of time. Generally, the marked occurrence of this property provides a reduction in the quality of mechanical properties (Muñoz et al., 2012). Films with a hydrophilic character tend to exhibit high water absorption rates under very humid conditions (Nagarajan et al., 2013). The swelling experiments (Table 4) carried out with chitosan films without extract (C) and with chitosan films EH5 and EH10. All films with extract showed a higher degree of swelling ($p < 0.05$) compared to the control film, indicating its greater hydrophilicity (Table 4). The swelling properties are influenced by the EHH content in the films.

The control swelling value, shown in Table 4, was equivalent to that found by Hajji et al. (2016). The authors studied films with different concentrations of chitosan and PVA (polyvinyl alcohol) and found that films containing 100% chitosan had similar swelling values to those presented in this study. Chitosan without extract contributes to reduce the hydrophilicity of the films since it has a lower content of hydrophilic groups per molecular unit when it does not have an additive (Costa-Júnior et al., 2009).

The EHH films showed higher swelling values in relation to the control. This fact can be attributed to the intermolecular interactions between the chitosan matrix and the phenolic compounds of the peppermint extract (Moradi et al., 2012). This property is desirable to absorb water from the external surface of foods that have high humidity (Hajji et al., 2016).

Kowalczyk and Biendl (2016) compared four types of biopolymers (carboxymethyl cellulose, oxidized potato starch, isolated soy protein and gelatin) with different concentrations of hop extract (0.1, 0.25, 0.50%). Gel extract films obtained higher swelling values than the control. As the extract concentration increased, there was also an increase in the swelling of the biopolymer film.

Another factor resulting from the increase in swelling of films with EHH is the release of the bioactive compound into the packaged product. According to Thakhiew et al. (2014), high swelling values may impair the release of the compound, being ineffective as an antioxidant or antimicrobial.

Water vapor permeability (WVP) is considered the most important property of edible films due to its close relationship with deterioration reactions (Ahmadi et al., 2012). The shelf life of food products is directly related to the transfer of water between the product and the external environment in which they are presented (Crizel et al., 2018). Control of moisture transfer can ensure product stability and safety during distribution and storage (Aloui et al., 2011). The results of WVP analysis of the films are shown in Table 4.

Chitosan films without EHH presented the lowest WVP among the tested films with no significant difference ($p > 0.05$) when compared to EH5 films. In the EH10 film, the WVP increased significantly ($P > 0.05$).

According to Silva et al. (2016), flexible films are classified according to the WVP values. Films with a WVP value between 0.4 and 4.2 g.mm/h.m².kPa are considered ineffective whereas those with moderate efficacy range from 0.004 to 0.4 g.mm/h.m² .kPa, and between 4×10^{-4} and 4×10^{-3} g.mm/h.m².kPa are considered effective. In this work, chitosan films with the two extract concentrations can be classified with moderate efficacy.

Similar to what was found in this study, Crizel et al. (2018) reported an increase in WVP when they incorporated olive-pomace flour and powder into the chitosan matrix. The increase in water vapor permeability is related to the increase in powder concentration. This fact is due to the irregular structure of the flour and its insolubility in the matrix, which can result in small cracks, which facilitate the transfer of water through the film. However, the same authors verified that the olive powder microparticle did not result in a significant difference ($p > 0.05$) in relation to the control (Crizel et al., 2018).

Martins et al. (2012) applied α -tocopherol to chitosan films and observed an increase in WVP values with an increase in the proportion of the bioactive compound, but with no significant difference between the films. The authors concluded that this increase in permeability can be attributed to the effect of α -tocopherol addition on the cohesive forces of the chitosan network (Bonilla et al., 2011).

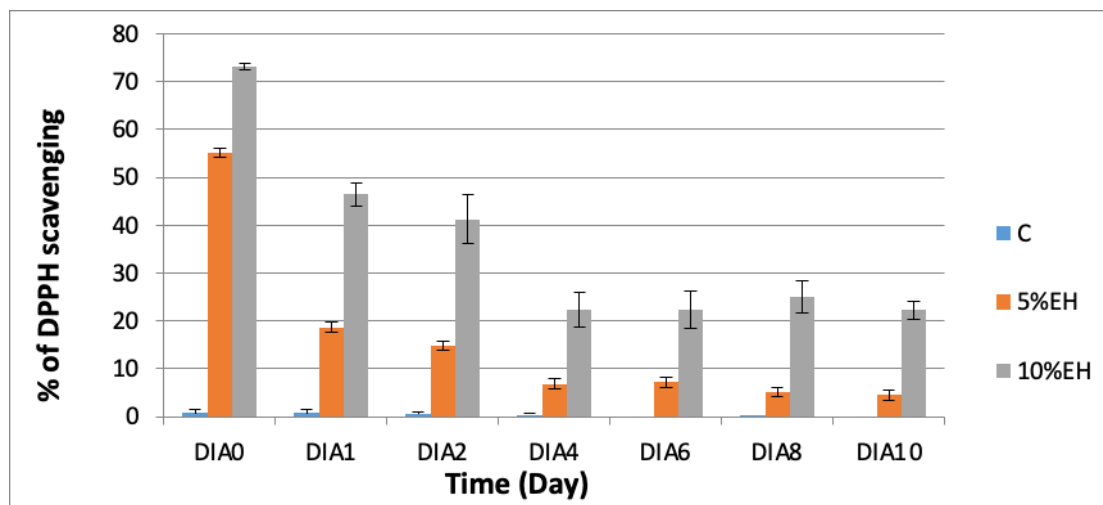
Unlike what was observed in this study, Bitencourt et al. (2014) showed that the addition of turmeric extract caused a significant decrease in water vapor permeability in gelatin-based films. However, there was no difference between films with different percentages of added extract.

3.3 Kinetics of antioxidant activity

One of the main causes of food spoilage is lipid oxidation (Siripatrawan & Vitchayakitti, 2016; Babbar et al., 2011; Brewer, 2011). Because of this, the interest in developing strategies that prevent food products from external interferences, aiding in food safety, increases. Aiming at this innocuousness, packaging with active properties is a good alternative for protection.

From the evaluation of antioxidant action of films added with EHH applied to Briseé mass for ten days, a good kinetic behavior of the films was observed (Figure 3).

Figure 3 - Kinetics of the free radical scavenging activity of the extract containing antioxidant bioactive compounds extracted from the chitosan-based films without EHH (C) and with 5% and 10% EHH.



C=Control film; EH5=Chitosan with 5% extract; EH10=Chitosan with 10% extract. Values are presented as mean and standard deviation. Source: Authors (2021).

The antioxidant action of EH10 films on day zero was higher than that found by Wang, Q. et al. (2015) (Table 1). In the present study, chitosan films were analyzed as follows: (1) the presence of polyphenols, whereas in this study it was at five minutes, demonstrating that EHH has a faster action, a desirable characteristic for an antioxidant. After 10 days of storage, the free radical scavenging activity of the chitosan / EHH films decreased by 51% and 50% in the EH5 and EH10 assays, respectively (Figure 3). This is probably a consequence of the loss of total phenolics in the films due to the migration of these compounds to mass (Crizel et al., 2018).

Crizel et al. 2018 applied three concentrations of olive flour (10%, 20% and 30%) to chitosan films. In the test with 10% olive flour, they obtained 14.08% DPPH radical scavenging on day zero. Chitosan films with EH10 were shown to have five times greater antioxidant action at the same time (Figure 3).

Peppermint has promising antioxidant properties and can be useful when incorporated into chitosan films. The phenolic compounds present in EHH provide antioxidant action, through polyphenols, which help to fight free radicals (Jayabalan et al., 2008). Thus, antioxidants play an important role in the preservation of foods, especially those rich in fat, because, besides protecting the product, they add value to its composition, improving and / or maintaining its functionality, making the product even more relevant for being a natural product (Kanatt et al., 2012).

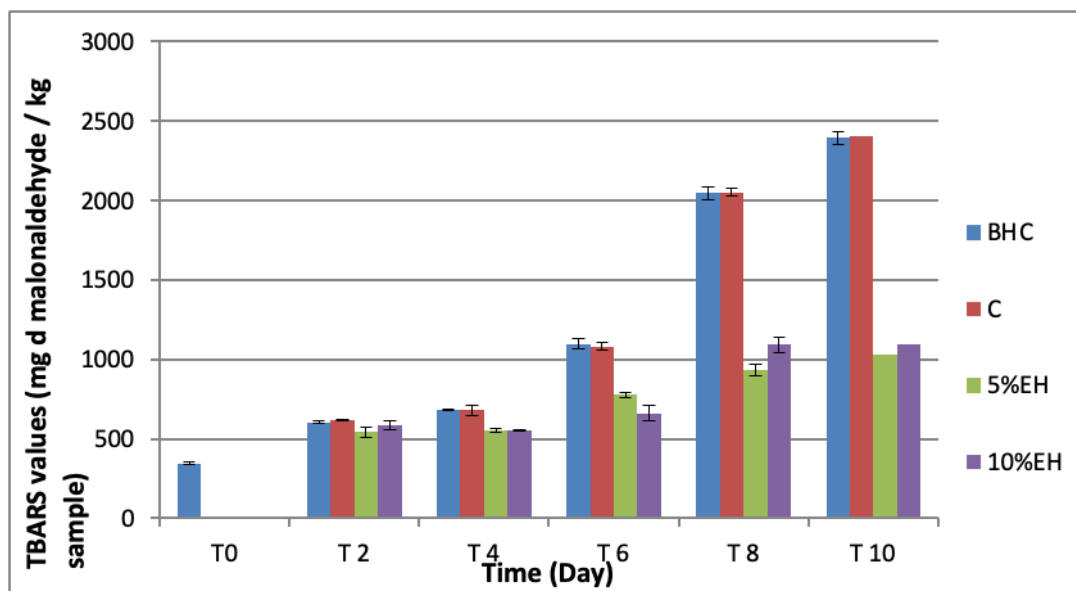
3.4 Kinetics of lipid oxidation by determination of thiobarbituric acid reactive substances (TBARS)

The decomposition of peroxides results in compounds of a very diverse nature, among them, aldehydes, ketones, hydroxy acids, hydrocarbons, polymers, which generally called by-products. Many of these by products have unpleasant odor and taste. 2-Thiobarbituric acid can react with fatty acid decomposition products, resulting in compounds that can be measured by spectrophotometry (Ashrafi et al., 2018).

The TBARS values found for Briseé dough packed with chitosan film with EH5 and EH10 stored at 4°C for ten days with protection from light are shown in Figure 4. The influence of four forms of packaging: polyethylene bags with hermetic closure, chitosan film without extract (C), chitosan film with 5% EHH and 10% EHH, on the TBARS values of the samples during storage. The masses with control packages (BHC and C) presented the highest values of TBARS, without, however, differing significantly from each other ($p < 0.05$). This fact confirms that these packages are not effective in protecting the

product against lipid oxidation. On the other hand, in masses packed with chitosan films with 5% and 10% of EHH, it is evident that there was a significant inhibition of lipid oxidation, demonstrating the effectiveness of these films in protecting the Briseé mass against the oxidation reaction.

Figure 4 - Results on kinetics with mean values of thiobarbituric acid reactive substances (TBARS) of Briseé mass packed with films added with EHH on different days of storage.



BHC = Bag with Hermetic Closure; C = Control; EH5 = Chitosan with 5% extract; EH10 = Chitosan with 10% extract. Values are presented as mean and standard deviation. Source: Authors (2021).

Lipid oxidation is one of the important factors related to food quality, which can result in anomalous flavors caused by rancidity. In addition, lipids are easily oxidized in the presence of light, heat and enzymes (Méndez-Cid et al., 2017; Ramirez et al., 2004). The determination of thiobarbituric acid reactive substances (TBARS) is a suitable method for the quantification of lipid oxidation by-products (Sathivel et al., 2007). The TBARS value measures the formation of by-products from oxidation when reacted with thiobarbituric acid (mainly malonaldehyde-MDA) (Darughe et al., 2012). During lipid oxidation, MDA, a minor component of fatty acids with three or more double bonds, is formed, resulting in the degradation of polyunsaturated fatty acids. MDA is probably formed from trienes of unsaturated peroxides (Wheatley, 2000).

Literature is scarce with regard to the application of additive chitosan films, with some bioactive compound that has antioxidant action, in cookies, pies, pizzas, breads or patties. However, there are reports of several applications of these films in meat products (Siripatrawan & Noipha, 2012; Sabaghi et al., 2015), fish (Gómez-Estaca et al., 2007) and beef (Vital et al., 2016, Camo et al., 2011, Oussalah et al., 2004). Özvural et al. (2016) reported application of chitosan coating added with microencapsulated green tea extract on hamburger patties. Empanadas, immersed in the added biopolymer solution and kept in a refrigerated environment for 8 days, presented a lower TBARS value compared to the control (chitosan coating without extract).

Some studies, however, report the inhibition of lipid oxidation in fat-rich masses that incorporated natural extract (antioxidant) to the product, as an ingredient. Bialek et al. (2016) studied the oxidative progression of cookie dough added with chocoberry extract (*Aronia melanocarpa*), over a period of 18 weeks, and verified an increase in mg MDA/kg⁻¹ dough, without, however, overcoming the cookie without the extract (control). Darughe et al. (2012) evaluated for a period of 60 days the antioxidant action of coriander essential oil applied in cakes, and found that cakes with concentrations of 0.05, 0.10 and 0.15% of essential oil had a delay in lipid oxidation when compared to control (with addition of BHT). Izzreen and Noriham

(2011) also demonstrated the efficacy of adding Malaysian leaf extract to cakes in controlling lipid oxidation, in view of a decrease in MDA/kg values over 15 days.

These three studies showed values of TBARS (up to 60 mg MDA / kg⁻¹), at time zero, much lower than those obtained in this study, whose TBARS value at the beginning of storage was 346.05 mg kg⁻¹ malonaldehyde, being a value much higher the established maximum level of acceptance of lipid oxidation (57.6 mg MDA/kg⁻¹) (Izzreen & Noriham, 2011). However, this value does not match the apparent sensory characteristics of the masses, which have been shown themselves to be unchanged. However, very high values may be due to interferences, since other substances can react with TBA, promoting an increase in absorbance and overestimation of values (Shahidi & Zhong, 2005). Thus, taking into account the above, a more in-depth study is essential to investigate with greater precision the existence of interferents in the tests.

4. Conclusion

Chitosan films added with peppermint hydroethanolic extract (EHH) showed to be malleable, easy to handle and detachable from the drying support. However, the addition of 5 and 10% of extract (EH5 and EH10) exhibited a better retention of bioactive compounds by the amount of extract added to the films that presented antioxidant action against the DPPH radical. In the films (EH5 and EH10), the addition of the extract improved the mechanical properties, especially those related to tensile strength and elongation, as well as the barrier and optical properties. The films did not exhibit antimicrobial action against the bacteria *S. aureus* and *Escherichia coli* at the concentrations studied. Applied to the Briséé mass, the chitosan films added with EHH, regardless of the extract concentration, demonstrated efficacy in inhibiting the lipid oxidation of the product for 10 days. Thus, developed biofilms have a strong potential for application in food packaging because they exert protection against lipid oxidation. It is recommended, in future research, to evaluate the properties of new films developed from the application of different active agents, such as silver or zinc oxide nanoparticles, in combination with peppermint extract. Detailed studies on different combinations can help to achieve an effective antimicrobial action, which in combination with the antioxidant activity already demonstrated by the studied films would bring new advances to food packaging technology.

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