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Physical Properties, Antioxidant and Antimicrobial Activity of Chitosan Edible Films Containing Essential oils

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ABSTRACT

Background: Herbs and spices, having antimicrobial effect, can be incorporated into edible films to extend product shelf life and to reduce the risk of microbial growth on food. Chitosan edible films have shown great promise for their application in food preservation and also are promising systems to be used as essential oils (EOs) carriers. This study aims to investigate the effect of EOs incorporation on the physical, antioxidant, and antimicrobial activities of chitosan films. **Methods:** Chitosan active films incorporated with cinnamon, clove, laurel, basil, and lemongrass EOs were investigated for physical parameters of color, thickness, opacity, moisture content, and water solubility in order to study the impact of the incorporation of EOs into the chitosan matrix. The antioxidant property of films was determined based on the method of DPPH to analyze and evaluate the quality of tested films. Furthermore, antibacterial activity was carried by agar diffusion method on food spoilage bacteria of Gram-positive *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus,* and Gram-negative *Escherichia coli (E.coli), Pseudomonas aeruginosa,* and *klebsiella pneumonia.* **Results:** In general, all chitosan EOs films presented yellowish coloration, slight thicknesses, good transparency in visible light, and high wettability. Compared to other tested films, chitosan film incorporated with clove EO had the highest total phenolic content of 0.303 mg/g Gallic acid equivalent with the highest antioxidant capacity of 89.93% value. All the films exhibited antibacterial activity against the studied food spoilage bacteria except of the chitosan films incorporated with Basil and Lemongrass EOs showed no inhibitory activity against *E.coli.* A significant antimicrobial bacterial inhibition zone of (20 mm) was assigned for clove chitosan film against *Bacillus cereus* and *Staphylococcus aureus*, Lemongrass chitosan film against *Pseudomonas aeruginosa*, and *klebsiella pneumonia*, and Basil chitosan films against *Pseudomonas aeruginosa*. **Conclusion:** Chitosan active films incorporated with the studied EOs assigned an effective antioxidant and antimicrobial activity to be used for active food packaging.

Keywords: *Antioxidant activity; Chitosan; Edible; Essential oils; Films*

Introduction

Consumers prefer good quality, fresh, and safe food. Therefore, the development of packaging technology is increasing rapidly (Irawan *et al.*, 2017). It relates to healthy foods and safe from all environmental influences that can damage the quality of the food. Food packaging aims to

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protect food against oxygen, moisture, UV light, and both chemical and microbial contamination (Miteluț *et al.*, 2015).

One of the packaging technologies that can be used to protect the food was active packaging. Active packaging is defined as an intelligent system that involves interaction between the components of the package and the food or the atmosphere and the internal gas in accordance with consumer demand for high quality, fresh-like, and safe products (Erkmen and Barazi, 2018). The main function of active packaging is to control moisture; slowing the loss of moisture that allows the microbes to grow. Controlled humidity prolongs shelf life or storage life a product (Dhandapani *et al.*, 2017). One of the active packaging is edible film that serves as a barrier to moisture, prevents the loss of aroma, and improves physical characteristics and carrier additives (D Antunes *et al.*, 2012). Hydrophobic substances such as resin, wax or protein are a good inhibitor for moisture, but the water-soluble hydrocolloid such as polysaccharides usually provides good mechanical properties such as tensile strength and elongation for edible films and coatings. Both are inhibitors of oxygen and CO_2 which are very good because of the hydrogen bonded network structure (Akram *et al.*, 2019). Edible films and coatings are prepared from biopolymers and are able to protect food products extending their shelf-life, the major constituents of which are polysaccharides, proteins, and lipids.

Chitosan, a cationic polysaccharide consisting of (1,4)-linked-2-aminodeoxy-β-D-glucan is the deacetylated form of chitin. It has attracted attention as a potential food preservative of natural origin and has been considered as an ideal biopolymer for the production of active edible films due to its non-toxicity, biocompatibility, biodegradability and film-forming ability (Ju *et al.*, 2019, Karimnezhad *et al.*, 2019). Many lipids like essential oils (EOs) have been incorporated to composite films, mainly with aim of optimizing characteristics (Ribeiro-Santos *et al.*, 2017). The antioxidant and antimicrobial or other biological activities of EOs are directly correlated to the presence of their bioactive volatile components. Chemically, EOs are composed of terpene compounds (mono-, sesqui- and diterpenes), alcohols, acids, esters, epoxides, aldehydes, ketones, amines, and sulfides. EOs such as cinnamon, clove, laurel, basil and lemongrass represented valuable antioxidant and antibacterial properties, due to their components including Cinnamaldehyde (87.30%), E ugenol (65.92%), 1, 8-Cineole (74.65%), Carene (23.93%), Octadienal (50.49%), respectively (Lee *et al.*, 2015). They are advised to be used in active food packaging in preventing the microbial spoilage to increase the food shelf life (Elsabee and Abdou, 2013).

The study aimed to develop composite films from chitosan incorporated with cinnamon, clove, laurel, basil, and lemongrass EOs as best bioactive common used spice and herbs. The study also investigated the effect of these EOs on the physical, antioxidant, and antimicrobial properties of the resulting films to be used in active food packaging as an interesting alternative to traditional chemical food preservatives (Bastarrachea *et al.*, 2015, Nwakaudu *et al.*, 2015, Raghav *et al.*, 2016).

Materials and Methods

Films preparation: Chitosan solution (1.5% w/v) was prepared and acetic acid (1% v/v) was added into the solution and stirred for 24 hours before filtration. Then, 1% (v/v) of each tested EOs (cinnamon, clove, laurel, basil, and lemongrass) was added into the chitosan filtrate with 0.5% (v/v) glycerol and 0.01% (v/v) Tween 80 before homogenization at 9,000 rpm for 4 min using homogenizer. The solutions (25 ml) were poured into the petri dish (90 mm × 15 mm) for film casting for 24 hours at 40 °C. Dried films were peeled manually (Zaman *et al.*, 2018) for the following characterization steps.

Thickness: The thickness of each film was measured manually using a digital micrometer which has a sensitivity of 0.001 mm. Measurements were taken at five different points with equal space on the film (Bonilla *et al.*, 2011).

Color analysis: Color of the tested films was

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determined using colorimeter. The film color was expressed as L* (lightness-darkness), a* (red-green), and b* (yellow-blue) values. Total color differences (ΔE) for the films were calculated according to the following equation:

$\Delta E = \sqrt{(L - L*)^2 + (a - a*)^2 + (b - b*)^2}$, *where L, a, and b were referring to the color perimeters of the film without EOs and L*, a*, and b* were the values for the chitosan incorporated with tested EOs, respectively* (Zaman *et al.*, 2018).

Opacity measurements: Opacity was determined by measuring the film absorbance at 600 nm using a UV spectrophotometer. The films were cut into rectangular shapes and directly placed on the internal side of the spectrophotometer cell. An empty test cell was used as the reference. The opacity of the films was calculated by the following equation:

O = Abs600/d, *where O is the opacity, Abs600 is the value of absorbance at 600 nm, and d is the film thickness (mm)* (Yuan *et al.*, 2015).

Moisture content and water solubility: To determine the moisture content of films, about 50 mg of film was dried at 105 °C during 24 h (until the equilibrium weight was attained). The weight loss of the sample was determined, from which the moisture content was calculated using the following equation:

Moisture content = Mi-Mf/Mi *100, *where Mi and Mf are the masses of initial and dried samples, respectively* (Hromis *et al.*, 2016).

The films solubility in water was determined according to the method reported by Cerqueira (Cerqueira *et al.*, 2012). Solubility is defined as the content of dry matter solubilized after 24 h immersion in water. The initial dry matter content of each film was determined by drying to constant weight in an oven at 105 °C. Disks of film (2 cm diameter) were cut, weighed (Mi), and immersed in 50 mL of water. After 24 h of immersion at 20 °C with agitation (60 rpm), the pieces of film were taken out and dried to constant weight (Mf) in an oven at 105 °C, to determine the weight of dry matter that was not solubilized in water. The solubility of the films was then determined as follows:

Water solubility = Mi-Mf/Mi *100, *where Mi is the initial mass and Mf is the final mass of the sample.*

Total phenolic content and antioxidant activity: Films (25 mg) were homogenized with ethanol during 5 min at 10,000 rpm and then centrifuged at 3000 rpm during 10 min. The extracts were used to determine the total phenolic content. Phenolic compounds were determined using Folin-Ciocalteu reagent method by reading the absorbance at 765 nm with a UV-Vis spectrophotometer (Yuan *et al.*, 2015). Gallic acid was used as a standard and the results were expressed as milligrams of gallic acid equivalent (GAE)/grams of film.

The antioxidant property of films was determined based on the method of free radical scavenging activity assay (DPPH method) (Karami *et al*., 2019). Briefly, 25 mg of each film was added into 3 ml of distilled water, and then a 2.8 ml of film extract solution was mixed with 0.2 ml of 1 mM methanolic solution of DPPH. The mixture was shaken and maintained at room temperature in the dark for 30 min. The absorbance was measured at 517 nm. Then, the percentage of DPPH radical scavenging activity was calculated as follows:

DPPH scavenging effect (%) = Abs DPPH – Abs film extract / Abs DPPH * 100.

Antimicrobial activity: The agar diffusion method was used to determine the antibacterial activity according to the method of Yum (Yuan *et al.*, 2015). Briefly, the nutrient agar medium in Petri dish was inoculated with 0.1 ml 10^5–10^6 cuf/ml bacteria Gram-positive *Bacillus subtilis, Bacillus cereus, and Staphylococcus aureus* and Gram-negative *Escherichia coli, Pseudomonas aeruginosa, and klebsiella pneumonia*. The prepared films were cut into 10 mm diameter disks using a hole-puncher, and then placed on microbial cultures. Bacterial strains were incubated at 37 °C for 24 h. The diameter of the zone of inhibition (mm) was measured using a caliper. The tests were performed in triplicate.

Data analysis: The results were reported as the mean ± SD for triplicate measurements. Statistical

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analysis of the data was carried out via one-way ANOVA followed by Tukey's test to compare the treatment means using MINITAB software (version 14). Statistical significance was expressed at P-value < 0.05.

Results

The color property of film for food packaging is an important criterion when selecting a suitable material. A lighter film packaging is generally preferred over a darker one, because it will not alter the original color of the contents. Moreover, the film should be clear and not heavily tinted, so that it will not affect the aesthetic of the food product during display. The parameters of L*, a*, and b* for each of the film samples are summarized in **Table 1**. The L* parameter which represents the lightness of the chitosan film samples, only exhibited a decrease when lemongrass EO was incorporated. For a* parameter, chitosan composite film with lemongrass EO was higher compared to the neat chitosan film. In addition, a significant increase in the b* parameter was observed with lemongrass EO in the films.

The effects of tested EOs incorporation on film thickness and opacity are shown in **Table 2**. The results showed that the thickness of the five films varied between 0.124 and 0.186 mm. The opacity of films incorporated with laurel EO was the highest. On the other hand, the opacity of film incorporated with cinnamon EO dramatically decreased compared to the control.

The summary of the moisture content of all tested chitosan films is shown in **Table 3**. In this study, it was observed that the incorporation of cinnamon EO caused a significant decrease in moisture content of the film with 24.65%. However, basil EO signed the highest moisture content 30.12%. As chitosan has high hydrophilic nature, the control chitosan film exhibited the highest moisture content. Water solubility of the material is one of the most important criteria when selecting an appropriate material to be used as food packaging. Chitosan on its own is highly water soluble due to its hydrophilic nature, which limits its use as a film packaging material. In present study, the addition of EO into chitosan decreased its water solubility from 28.89% in the chitosan basil film to 15.43% in chitosan cinnamon film.

All tested chitosan EOs films were investigated for their total phenolic content. The results clearly indicated high total phenolic content in clove chitosan film (0.303 mg/g Gallic acid) followed by 0.096, 0.069, 0.042, and 0.018 mg/g Gallic acid for basil, cinnamon laurel, and lemongrass, respectively, compared to chitosan control film with 0.009 mg/g Gallic acid as shown in **Figure 1**.

The DPPH test was performed on the chitosan films containing EOs. The results are shown in **Figure 2**, where clove EO film resulted in 89.93%, followed by 30.64%, 29.8%, 21.61%, and 16.32% for basil, cinnamon, laurel, and lemongrass, respectively, compared to 12.45% for chitosan control film.

The antibacterial activity of the films is shown in **Figure 3**. All tested films exhibited antibacterial activity against the studied food spoilage bacteria except for the chitosan films incorporated with basil and lemongrass Eos that showed no inhibitory activity against *E.coli*. The results showed that the chitosan films incorporated with clove EO had a significant inhibitory activity (20 mm) against Gram-positive *Bacillus cereus* and *Staphylococcus aureus*. However, those incorporated with lemongrass EOs had a significant inhibitory activity (20 mm) against Gram-negative *Pseudomonas aeruginosa*, and *klebsiella pneumonia*. Basil EO also showed a significant inhibitory activity (20 mm) against *Pseudomonas aeruginosa*.

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Table 1. Color values for chitosan films incorporated with tested essential oils.

Chitosan film with essential oils	Color			
	L*	a*	b*	ΔE
Chitosan	87.92±0.26 a	1.41±0.15 c	20.75±0.25 c	43.88
Cinnamon	87.06±0.29 a	1.50±0.47 c	34.16±0.53 b	44.30
Clove	87.38±0.15 a	1.61±0.09 c	20.17±0.59 c	34.29
Laurel	83.47±0.02 b	4.96±0.05 b	35.52±0.22 b	30.33
Basil	87.83±0.39 a	1.55±0.22 c	18.96±1.84 c	29.11
Lemongrass	68.00±1.25 c	17.71±0.54 a	55.50±1.47 a	61.40

L*: Lightness-darkness, a*: Red-green, b*: Yellow-blue, ΔE: Total color differences, Mean values in each column with different lower case letters are significantly different ($P < 0.05$).

Table 2. Thickness and opacity values for chitosan films incorporated with tested essential oils.

Chitosan film with essential oils	Thickness (mm)	Opacity (Abs600)
Chitosan	0.168±0.035 ab	0.527±0.103 d
Cinnamon	0.124±0.015 b	0.583±0.025 cd
Clove	0.182±0.025 a	0.692±0.005 bc
Laurel	0.162±0.025 ab	1.179±0.012 a
Basil	0.186±0.023 a	0.630±0.008 cd
Lemongrass	0.182±0.032 a	0.760±0.013 b

Mean values in each column with different lower case letters are significantly different ($P < 0.05$).

Table 3. Moisture content and solubility for chitosan films incorporated with tested essential oils.

Chitosan film with essential oils	Moisture content (%)	Solubility (%)
Chitosan	35.82	18.6
Cinnamon	24.65	15.43
Clove	28.65	20.51
Laurel	25.88	20.08
Basil	30.12	28.89
Lemongrass	26.36	20.23

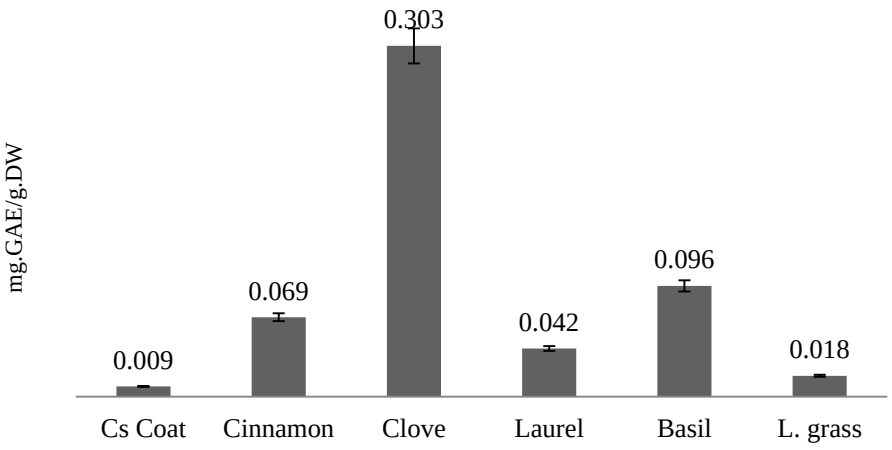

Figure 1. Total phenol contents for chitosan films incorporated with tested essential oils.

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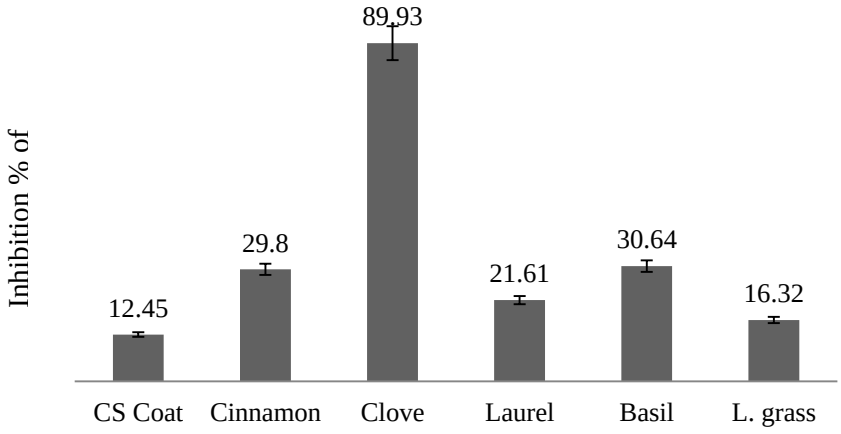
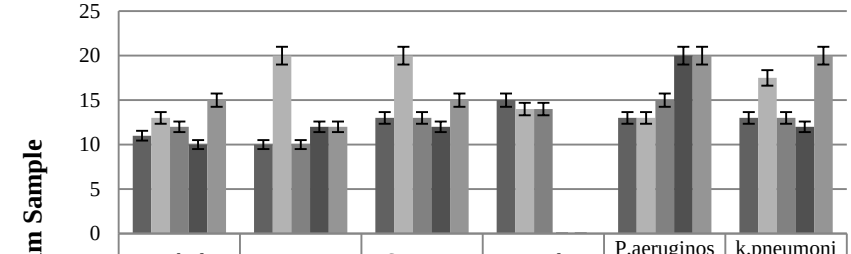


Figure 2. Antioxidant activity of chitosan films incorporated with tested essential oils.



Film Sample	B.subtilis	B.cereus	S.aureus	E.coli	P.aeruginosa	k.pneumoniae
Cin. Film	11	10	13	15	13	13
Clv. Film	13	20	20	14	13	17.5
Lau. Film	12	10	13	14	15	13
Bas. Film	10	12	12	0	20	12
Lg. Film	15	12	15	0	20	20

Figure 3. Antibacterial activity of chitosan films incorporated with tested essential oils.

Discussion

The changes in the color of the film were contributed by the color of lemongrass EO, which is bright yellow in nature as it is shown in ΔE. This finding is consistent with the study by Quesada (Quesada *et al.*, 2016), where the incorporation of plant EOs into polysaccharide-based films significantly increased the intensity of yellow color in the films. The effects of tested EOs incorporation on film thickness and opacity are in line with other study (Yuan *et al.*, 2015), which reported that the opacity of chitosan films incorporated with cinnamon EO showed a significant reduction. The change in opacity may be caused by the presence of polyphenols in films. The results of moisture content of all tested chitosan films can be explained by the higher molecular entanglement and viscosity in pure chitosan solutions, leading to higher retention of water molecules during drying of the films. The addition of EOs, which are hydrophobic, reduced the ability of the film to adsorb and retain water molecules, which is consistent with the findings of Yuan (Yuan *et al.*, 2015). The results of water solubility were due to the decrease in the number of OH bonds and the presence of aliphatic groups in the film when oil was added. Thus, the formation of hydrophobic portions of the film led to a less soluble material, causing the repulsion of water molecules, so that they were less able to penetrate and dissolve the films. The findings of the present study was supported by the study of Bonilla (Bonilla *et al.*, 2011).

The degree of antioxidant power of edible film

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is generally proportional to the amount of antioxidant additives added. Compared to the control, the antioxidant activity of the films significantly increased when incorporated with EOs. The highest antioxidant capacity for clove film shown in results reflects its highest total phenolic content and matches previous study (Yashaswini and Iyer, 2019). Nevertheless, lemongrass film suffered the lowest loss of antioxidant efficiency among the five types of active films (Sharafati-Chaleshtor and Sharafati-Chaleshtori, 2017, Sharafati Chaleshtori *et al.*, 2016). The loss of antioxidant activity may be attributed to the trapping of a portion of EOs inside the film matrix. It may be also affected by the evaporation of a portion of the incorporated EO during the drying stage of film preparation (Vazquez *et al.*, 2014).

The antimicrobial properties of EOs have been known for many centuries. In recent years, a large number of herbs and spices EOs and their constituents have been investigated for their antimicrobial properties against food spoilage bacteria (Yuan *et al.*, 2015). The antibacterial mechanism of EO could be able to disrupt and penetrate the lipid structure of the bacteria cell membrane, leading to its destruction. Chitosan naturally has antimicrobial property, enhancing by adding EOs. The significant antibacterial activity shown in results of chitosan films incorporated with clove, lemongrass EOs against Gram-positive bacteria matches previous studies (Vazquez *et al.*, 2014, Yashaswini and Iyer, 2019), respectively. In general, Gram-positive bacteria are considered more sensitive than Gram-negative bacteria to antimicrobial compounds. This is generally attributed to the differences in the structure of their cell walls as the cell walls of Gram-negative bacteria containing lipopolysaccharides, which may prevent active components from reaching the cytoplasmic membrane. The present results were in agreement with previous studies in which Gram negative *E.coli* bacteria seemed to present higher resistance against EOs chitosan films (Yuan *et al.*, 2015).

Conclusion

The present study indicates that all the studied chitosan EOs films are an effective antioxidant and antimicrobial activity can be used for active food packaging, with significance for the chitosan film incorporated with clove due to its highest total phenolic content of 0.303 mg/g Gallic acid equivalent with the highest antioxidant capacity of 89.93% value, in addition to a significant antimicrobial bacterial inhibition zone of (20 mm) against *Bacillus cereus* and *Staphylococcus aureus*. As a recommendation, chitosan-based films are the dynamic solution to the current need of biodegradable, active packaging material for food, where the EOs chitosan active films are an interesting alternative to traditional chemical food preservatives in preventing the microbial spoilage to increase the food shelf life.

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Authors' contributions

Each of the authors contributed equally in idea generation, study design, data collection and analysis, and manuscript writing. Also, all the authors of the article approved for publication.

Conflict of interest

The authors declared no conflict of interest.

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