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RESEARCH

Elaboration and general evaluation of chitosan-based films containing terpene alcohols-rich essential oils

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Abstract

Recently, the scientific community is interested in the synthesis of biodegradable and bioactive packaging to replace oil-based ones. Therefore, the present study aims to elaborate an active and biodegradable material using chitosan (CS-film) combined with pelargonium, tea tree, marjoram, and thyme essential oils (EOs), and then evaluate their different properties and biological activities. The obtained data showed an augmentation in CS-film thickness and opacity following the addition of EOs ranging from 17 ± 3 to 42 ± 2 µm and from 1.53 ± 0.04 to 2.67 ± 0.09, respectively. Furthermore, a significant decrease in the water vapor transmission rate and moisture content parameters was recorded as regards the treated CS-films. On the other hand, the treatment with EOs engenders random modifications in the physicochemical and mechanical characteristics of the material. Concerning the biological activities, the treated CS-films scavenged around 60% of DPPH radical while the control CS-film exhibited a negligible antioxidant activity. Finally, the CS-films containing pelargonium and thyme EOs exhibited the strongest antibiofilm-forming activity against *Escherichia coli*, *Enterococcus hirae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* with values of inhibition greater than 70%. These encouraging results verify the effectiveness of CS-films containing EOs such as pelargonium and thyme EOs as biodegradable and bioactive packaging.

Keywords Bioactivities · Chitosan · Essential oils · Mechanical properties · Packaging · Physicochemical characteristics

Introduction

Recently, food loss represents a worldwide concern. The factors that mainly cause food spoilage are contact with oxygen (Cichello 2015) and water (Tapia et al. 2020), and the proliferation of microorganisms (Abd El-Hack et al. 2022). The latter is responsible for the deterioration of 1.3 billion tons of food, more than a half million sick, and 420.000 dead cases annually (Gustavsson et al. 2011; Fahey et al. 2022). As a result, various procedures such as refrigeration, freezing, and thermal processing were employed to prevent food spoilage and contamination (Huang et al. 2019). However, the freshness and minimally-processing of food are highly required criteria. Thereby, other alternatives are needed. The food industry relies principally on the utilization of physical barriers such as food packaging for food protection and

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conservation (Gupta et al. 2022). So far, fossil-based plastics e. g, polyethylene, polyvinyl chloride, polyethylene terephthalate, and polystyrene are the most utilized materials in the packaging sector due to their cost-effectiveness, abundance in nature, and great mechanical properties (Asgher et al. 2020). Nevertheless, fossil-based plastics remain ecologically harmful (Jem and Tan 2020). In this context, attention was given to the synthesis of biodegradable, natural, and bioactive packaging (Phothisarattana and Harnkarnsujarit 2022). Among the biopolymers used in packaging elaboration, various researchers have studied chitosan due to its abundance, biological activities, biodegradability, film-forming and mechanical properties, and visual transparency (Shahidi et al. 1999; Dutta et al. 2009; Wang et al. 2011). As regards food packaging, it is necessary to use films that are endowed with a good barrier property to water, UV light, and microorganisms in order to protect food and maintain its quality (Siracusa et al. 2008; Zhang et al. 2021). It was claimed that chitosan exhibits weaker bioactivities when it is used as film (Ouattara et al. 2000). Therefore, the introduction of bioactive agents into CS-film to improve the above-mentioned properties and activities is a research need. Previous investigations have studied the mixture of chitosan and other biopolymers e. g, gelatin and starch (Jovanović et al. 2021; Janik et al. 2022). Other researchers have discussed the impact of introducing silver and Fe_3O_4 nanoparticles on the CS-film activities and mechanical properties (Li et al. 2022; Yang et al. 2022; Zarandona et al. 2022). Meanwhile, Peng and Li (2014) and Azadbakht et al., (2018) confirmed the optimization of CS-film following EOs addition. EOs containing terpene alcohols as major constituents (pelargonium, tea tree, marjoram, and thyme EOs) are recognized for their great antibacterial activities (Tariq et al. 2019). Furthermore, several works have studied the combination of CS-film with some of these EOs (Sánchez-González et al. 2010; Cerempei et al. 2014; Sedlaříková et al. 2017; Vidács 2022; Mouhoub et al. 2023b). However, the studies were limited to a few properties and activities.

In this paper, we aimed to carry out a global characterization of CS-films incorporated with pelargonium, tea tree, marjoram, and thyme EOs and to highlight the potential application of these films as packaging for food.

Materials and methods

Materials

The tested strains were *S. aureus* ATCC29213, *E. hirae* CIP5855, *P. aeruginosa* ATCC53, and *E. coli* K-12 MG1655.

Pelargonium asperum (refractive index: 1.468, density: 0.895, optical rotation: − 11.16°), *Melaleuca alternifolia* (refractive index: 1.487, density: 0.898, optical rotation: + 9.9°), *Origanum majorana* (refractive index: 1.473, density: 0.895, optical rotation: + 22.5°), and *Thymus satureioides* EOs (refractive index: 1.483, density: 0.938, optical rotation: − 10.75°) were provided by Nectarome society. Chitosan C3646 (DDA > 75% and Mw = 400 kDa) was purchased from Sigma-Aldrich.

Growth conditions

Muller-Hinton Agar medium (MHA) and lysogeny broth (LB) supplemented with sucrose 1% were used in the agar diffusion and antiadhesion tests, respectively. The bacterial suspensions were adjusted to 10^8 cells/mL by a spectrophotometer (Jenway 6305, UK).

EOs analysis using GC-FID

The capillary column DB-Wax 127-7023 (20 m × 100 µm) was used for EOs analysis. The operating conditions details were highlighted in our previous study (Mouhoub, Guendouz, et al. 2022a, b). Briefly, carrier gas (Hydrogen) flow rate was 1 mL/min. The oven program was: 60 °C for 2 min, then 12 °C/min up to 248 °C, and finally held for 5 min.

Films synthesis

The film preparation was detailed in our previous work (Mouhoub et al. 2022a). Briefly, a combination of acetic acid 1%, chitosan 2%, glycerol, Tween 80, and EO (2% v/v) was continuously stirred for 6 h using magnetic stirrer, poured onto supports, and then placed in an oven and allowed to dry overnight at 30 ℃. The film without emulsifier and EO was considered as control. The films were collected and then stored for subsequent tests (Fig. 1).

Films' thickness and opacity

The thickness of the films was measured in 5 different areas by a digital caliper (INGCO HDCD01150, China). The films opacity was measured using an UV–Visible spectrophotometer (Jenway 6305, UK). The adopted blank was the empty cell (Siripatrawan and Harte 2010).

$$\text{Opacity} = \text{Abs}_{600}/\text{t}$$

where Abs_{600} and t refer to the absorbance at 600 nm and film thickness (mm), respectively.

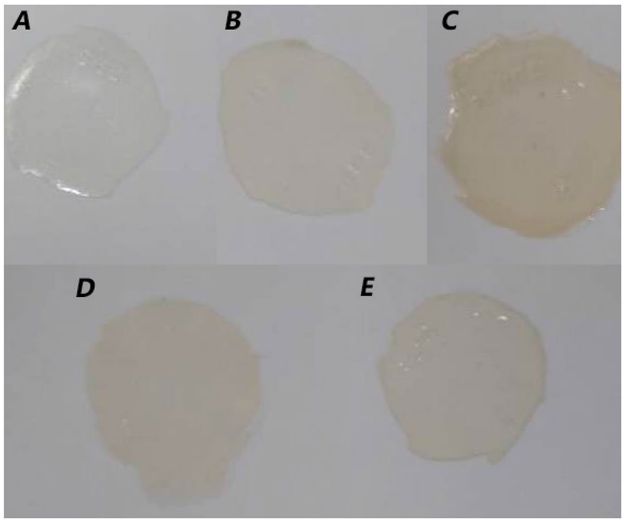

Fig. 1 Visual appearance of **A** control CS-film, **B** CS-film treated with pelargonium EO, **C** CS-film treated with tea tree EO, **D** CS-film treated with marjoram EO, and **E** CS-film treated with thyme EO

Determination of moisture content (MC), swelling level (SL), and hydrosolubility

The sample (1 × 1 cm) was weighed w_1 and then placed into the oven (24 h at 50 °C) to determine the dry weight w_2. The dried film was incubated in a beaker containing distilled water for 24 h. The swollen film was dried superficially and then weighed w_3. Finally, the sample was dried again for 24 h at 50 °C to define the final dry weight w_4. The experiment was performed in triplicate. The MC, SL, and hydrosolubility of the films were measured as follows:

$$\mathrm{MC}(\%) = 100 \times (w_1 - w_2)/w_1$$

$$\mathrm{SL}(\%) = 100 \times (w_3 - w_2)/w_2$$

$$\mathrm{Hydrosolubility}(\%) = 100 \times (w_2 - w_4)/w_2$$

Measurement of the water vapor transmission rate (WVTR)

The WVTR of the CS-films was measured based on He et al. (2021) procedure with minor modifications. Briefly, CS-film samples were firmly fixed over glass cups (3.14 cm^2 of transmission area) containing equal amounts of $CaCl_2$. The cups were placed inside a desiccator where the relative humidity and temperature were adjusted to 90% and 25 °C, respectively.

The cups were periodically weighed to the nearest 0.1 mg. The WVTR (g/h.m^2) was measured as follows:

$$WVTR = \frac{\left(\frac{\Delta w}{\Delta t}\right)}{A}$$

where $\frac{\Delta w}{\Delta t}$ and A represent the slope of the weight vs time and the effective film area, respectively.

Evaluation of surface free energy and hydrophobicity

The physicochemical characteristics of the CS-films surfaces were estimated based on the sessile drop method (Blanco et al. 1997). The contact angles of diiodomethane θ_d, distilled water θ_w, and formamide θ_f were monitored by a goniometer (GBX, France)- computer-camera system (Mouhoub et al. 2022a). The electron acceptor (γ^+), and donor (γ^-), the Lifshitz-Van der Waals component (γ^{LW}), and the free energy of interaction ($\Delta G iwi$) were determined following the Young-Van Oss equation (Oss 1995).

$$\gamma_L(1 + Cos\theta) = 2\left(\gamma_L^+\gamma_S^-\right)^{1/2} + 2\left(\gamma_S^+\gamma_L^-\right)^{1/2} + 2\left(\gamma_S^{LW}\gamma_L^{LW}\right)^{1/2}$$

where S refers to the solid surface and L to the liquid phases. The surface free energy ($\gamma_S{}^{Total}$) is calculated by the following equation:

$$\gamma_S^{Total} = \gamma_S^{LW} + \gamma_S^{AB} \mathrm{Where} \gamma_S^{AB} = 2\left(\gamma_S^-\gamma_S^+\right)^{1/2}$$

The hydrophobicity of films' surfaces can be predicted qualitatively based on the water contact angle (Lekbach et al. 2019). $\theta_w < 65°$ and $\theta_w > 65°$ refer to hydrophilic and hydrophobic surfaces, respectively (Vogler 1998). Otherwise, the procedure of Van Oss et al., (1988) was utilized to assess the hydrophobicity quantitatively. The surface is considered hydrophilic if $\Delta G iwi > 0$ and conversely for hydrophobic surfaces.

Where:

$$\Delta G iwi = -2[2((\gamma_i^+\gamma_i^-)^{1/2} + (\gamma_w^+\gamma_w^-)^{1/2} - (\gamma_w^-\gamma_i^+)^{1/2} - (\gamma_i^-\gamma_w^+)^{1/2}) + ((\gamma_i^{LW})^{1/2} - (\gamma_w^{LW})^{1/2})^2]$$

Films' mechanical properties

The tensile strength (TS) and elongation at break (EB) parameters of the films were evaluated using INSTRON 3369 electrohydraulic instrument. The films' strips (20 × 50 mm) were mounted between the grips and the selected cross-head speed was 2 mm/min. Data were treated using the Bluehill program. The experiment was realized in quintuplicate for each sample.

Films' antioxidant activity

The antioxidant activity of the CS-films was monitored as described by Siripatrawan and Harte, (2010). Two hundred milligrams of the film were submerged in 3 ml of methanol. The extract solution (0.9 mL) was sampled after 24, 48, and 72 h of the reaction and then mixed with 0.3 mL of the methanolic solution of DPPH 1 mM. The extract solution was replaced by methanol in the control. The vortexed mixture was incubated in dark for 30 min and then the absorbance was measured at 517 nm. For each sample, three repetitions were performed. The percentage of DPPH inhibition was calculated as follows:

$$DPPH\ \ scavenging\ (\%) = \left[1 - (Asample \div Acontrol)\right] \times 100$$

where A represents the absorbance at 517 nm.

Films' antibacterial activity

The antibacterial capacity of the CS-films was assessed using the agar diffusion method. The bacterial suspensions (1 mL) previously adjusted to 10^8 cells/mL were spread on the MHA surface and then the excess was withdrawn. The films' discs of 6 mm diameter were UV sterilized and then deposited on the medium surface. The diameter of inhibition zones (IZ) was measured after 24 h of incubation at 30 °C. The experiment was performed in triplicate.

Coating of microplate wells using CS-films

One hundred microliters of the solution used for the films' preparation were introduced in the bottom of 96-well microplate wells and dried at 30 °C overnight. The coated microplate was sterilized by UV light for the antiadhesion test.

Films' antiadhesion activity

Two hundred microliters of the bacterial suspensions (10^8 cells/mL) were introduced into each microplate well and then incubated at 30 °C. After 24 h, the wells content was transferred to another microplate and the turbidity was read at 630 nm by Bio-Tek ELx800 microplate reader. The experiment was conducted in triplicate. The coated microplate was rinsed multiple times with distilled water and then dried for 1 h to fix the sessile cells. The wells were dyed by adding 200 µL of crystal violet 0.1%. After 10 min, the microplate was rinsed to remove the dye, and then 200 µL of ethanol 95% were added to each well and allowed to react for 15 min. The wells content was transferred to a new microplate and the absorbance was read at 550 nm. The experiment was performed in triplicate.

Statistical analysis

Data were presented as mean ± standard deviation. The experimental results were subjected to an ANOVA test using SPSS software (version 25.0). The statistical significance was established at $P < 0.05$.

Results

EOs' chemical composition

As mentioned in Table 1. The analysis of *P. asperum*, *M. alternifolia*, *O.majorana*, and *T. satureioides* EOs by GC-FID revealed the presence of 40, 33, 30, and 33 compounds, respectively. Qualitative resemblances accompanied by quantitative disparities were noticed between the four EOs compositions. However, terpene alcohols were the main compounds for all EOs. Indeed, citronellol represented 33.98% of *P. asperum* EO. Whereas, terpinen-4-ol was the major component of *O.majorana* (23.37%) and *M. alternifolia* (40.33%). In contrast, *T. satureioides* contained mainly α-terpineol and borneol (43.90%).

Physical characteristics of the films

Table 2 presents the Hydrosolubility, SL, MC, opacity, WVTR, and thickness of the prepared films. Data showed high Hydrosolubility values (> 60%) for all tested films. The treatment by EOs increased significantly the opacity and thickness of the films while decreased the MC and WVTR parameters. Except for the film containing *M. alternifolia* EO, the recorded SL values were considerably high (> 600%).

Physicochemical characteristics of the films

Table 3 compares the physicochemical characteristics of the prepared films. Results revealed a drop in the water contact angle following the treatment by EOs. Whereas, only the film containing *M. alternifolia* EO was found to be quantitatively hydrophilic ($\Delta Giwi > 0$ mJ). The treatment by EOs reduces the γ^{AB} values of the control film and conversely for γ^{LW}.

Mechanical characteristics of the films

The mechanical characteristics of the different CS-films are presented in Table 4. Data showed that the treated films

Table 1 Chemical composition of the utilized EOs

Chemical constituents	EOs (concentration)	Chemical constituents	EOs (concentration)	Chemical constituents	EOs (concentration)
α-Pinene	Tea tree (1.13%) Marjoram (0.25%) Thyme (0.96%) Pelargonium (0.40%)	Germacrene D	Thyme (0.23%) Pelargonium (1.03%)	Caryophyllene oxide	Thyme (0.34%) Marjoram (0.06%)
Myrcene	Tea tree (0.86%) Marjoram (2.15%) Thyme (0.50%) Pelargonium (0.07%)	Geranyl acetate	Pelargonium (0.60%)	4-*Trans*-thujanol	Marjoram (6.17%)
α-Phellandrene	Tea tree (0.41%) Marjoram (0.36%) Thyme (0.11%) Pelargonium (< 0.05%)	Geranial	Pelargonium (0.66%)	γ-Cadinene	Thyme (0.43%)
Limonene	Tea tree (0.92%) Marjoram (2.15%) Thyme (1.11%) Pelargonium (0.21%)	Citronellol	Pelargonium (33.98%)	Camphor	Thyme (1.40%)
β-Phellandrene	Tea tree (0.80%) Marjoram (1.85%) Thyme (0.22%) Pelargonium (0.19%)	Nerol	Pelargonium (0.64%)	4-*Trans*-thujanol	Marjoram (4.14%)
6-Methyl-5-hepten-2-one	Pelargonium (0.05%)	Cadinene	Pelargonium (0.19%)	*Cis*-p-menth-2-en-1 ol	Marjoram (0.86%)
P-cymene	Tea tree (2.45%) Marjoram (1.20%) Thyme (3.51%) Pelargonium (0.09%)	Ceranyl isobutyrate	Pelargonium (0.69%)	Borneol	Thyme (9.84%) Marjoram (< 0.05%)
3-Octanone	Pelargonium (0.06%)	Citronellyl butyrate	Pelargonium (0.46%)	Tricyclene	Thyme (0.35%)
Cis-linalol-oxide	Pelargonium (0.16%)	Geraniol	Marjoram (0.08%) Thyme (0.22%) Pelargonium (11.04%)	Thymol methyl ether	Thyme (1.43%)
Trans-rose-oxide	Pelargonium (0.45%)	Geranyl tiglate	Pelargonium (1.23%)	Bornyl acetate	Thyme (2.07%) Marjoram (< 0.05%)
Cis-rose-oxide	Pelargonium (1.23%)	Geranyl isovalerate	Pelargonium (0.29%)	Bicyclogermacrene	Marjoram (1.13%)
Trans-linalol-oxide	Pelargonium (0.42%)	Geranyl butyrate	Pelargonium (1.34%)		Marjoram (< 0.05%)
Menthone	Pelargonium (1.20%)	Phenylethyl tiglate	Pelargonium (0.95%)	Spathulenol	Tea tree (0.14%) Marjoram (< 0.05%)
Citronellal	Pelargonium (0.11%)	10-Epi-γ-eudesmol	Pelargonium (3.62%)	Allo-aromadendrene	Tea tree (0.52%)
β-Bourbonene	Pelargonium (1.06%)	α-Thujene	Tea tree (2.44%) Marjoram (1.22%) Thyme (3.50%)	Viridiflorene	Tea tree (1.01%)
α-Copaene	Tea tree (0.13%) Thyme (0.22%) Pelargonium (0.45%)	β-Pinene	Tea tree (0.72%) Marjoram (0.46%) Thyme (0.81%)	*Trans*-α-farnesene	Tea tree (0.92%)
Isomenthone	Pelargonium (5.12%)	Sabinene	Tea tree (0.27%) Marjoram (8.01%) Thyme (0.07%)	δ-Cadinene	Tea tree (1.47%) Thyme (0.50%)
Linalool	Tea tree (0.07%) Marjoram (1.01%) Thyme (3.64%) Pelargonium (5.16%)	α-Terpinene	Tea tree (9.58%) Marjoram (8.47%) Thyme (0.62%)	Cadina-1,4-diene	Tea tree (0.23%)
β-Elemene	Pelargonium (0.14%)	1,8-Cineole	Tea tree (2.44%) Marjoram (0.16%) Thyme (0.88%)	Calamenene	Tea tree (0.17%)
β-Caryophyllene	Tea tree (0.46%) Marjoram (1.24%) Thyme (6.35%) Pelargonium (1.55%)	γ-Terpinene	Tea tree (20.51%) Marjoram (13.67%) Thyme (2.78%)	Cubenol	Tea tree (0.14%)

Table 1 (continued)

Chemical constituents	EOs (concentration)	Chemical constituents	EOs (concentration)	Chemical constituents	EOs (concentration)
Citronellyl acetate	Pelargonium (0.72%)	Terpinolene	Tea tree (3.42%) Marjoram (3.11%) Thyme (0.28%)	Camphene	Thyme (7.27%)
Citronellyl formiate	Pelargonium (6.91%)	α-Gurjunene	Tea tree (0.33%)	Globulol	Tea tree (0.34%)
Neral	Pelargonium (0.42%)	*Trans*-p-menth-2-en-1 ol	Tea tree (0.40%) Marjoram (1.19%)	δ-3-Carene	Thyme (< 0.05%)
α-Humulene	Tea tree (0.09%) Marjoram (< 0.05%) Thyme (0.33%) Pelargonium (0.43%)	Terpinen-4-ol	Tea tree (40.33%) Marjoram (23.37%) Thyme (1.77%)	Carvacrol	Thyme (7.07%)
α-Terpineol	Tea tree (3.00%) Marjoram (3.12%) Thyme (34.06%) Pelargonium (0.96%)	6,9-Guaiadiene	Tea tree (0.27%)	Thymol	Thyme (2.79%)
Geranyl formiate	Pelargonium (2.66%)	Aromadendrene	Tea tree (1.13%)	Viridiflorol	Tea tree (0.17%)
Linalyl acetate	Marjoram (12.38%)				

Table 2 Physical characteristics of the CS-films

CS-films	Thickness (µm)	Opacity	Moisture content (%)	Swelling level (%)	Hydrosolubility (%)	WVTR (g/h.m^2)
Control film	17 ± 3^{d}	1.53 ± 0.04^{d}	38.25 ± 0.52^{a}	758.64 ± 6.17^{b}	62.58 ± 0.88^{d}	69.38 ± 1.77^{a}
Film with *P. asperum* EO	33 ± 2^{b}	1.82 ± 0.06^{c}	18.20 ± 0.25^{d}	777.52 ± 8.70^{ab}	66.58 ± 0.38^{c}	55.92 ± 0.80^{c}
Film with *M. alternifolia* EO	22 ± 1^{c}	2.67 ± 0.09^{a}	21.87 ± 0.13^{b}	441.14 ± 21.37^{d}	67.89 ± 0.24^{bc}	60.81 ± 0.81^{b}
Film with *O. majorana* EO	23 ± 2^{c}	2.34 ± 0.04^{b}	18.07 ± 0.26^{d}	609.20 ± 23.20^{c}	69.05 ± 0.24^{b}	61.11 ± 1.01^{b}
Film with *T. satureioides* EO	42 ± 2^{a}	1.90 ± 0.05^{c}	20.97 ± 0.18^{c}	798.10 ± 22.66^{a}	72.94 ± 0.13^{a}	50.54 ± 1.72^{d}

Table 3 Physicochemical properties of the CS-films

CS-films	Contact angles (°)			Surface free energy parameters and components (mJ/m^2)				ΔG_{iwi} (mJ/m^2)	
	θ_F	θ_W	θ_D	γ^-	γ^+	γ^{LW}	γ^{AB}	γ^{Total}	
Control film	101.85 ± 2.19^{a}	96.55 ± 0.07^{a}	58.05 ± 0.35^{a}	16.68	7.27	29.64	22.02	51.66	− 10.32
Film with *P. asperum* EO	50.05 ± 0.35^{d}	64.30 ± 0.00^{c}	19.40 ± 0.00^{e}	18.31	0.09	47.86	2.57	50.43	− 24.84
Film with *M. alternifolia* EO	57.57 ± 0.64^{c}	60.20 ± 0.44^{c}	45.73 ± 0.31^{c}	29.60	0.04	36.54	2.18	38.72	3.75
Film with *O. majorana* EO	47.17 ± 0.42^{e}	59.73 ± 0.45^{c}	40.03 ± 0.06^{d}	21.73	0.17	39.51	3.84	43.35	− 12.48
Film with *T. satureioides* EO	67.70 ± 0.57^{b}	74.50 ± 0.28^{b}	47.50 ± 0.00^{b}	17.68	0.30	35.58	4.61	40.19	− 18.62

Table 4 Mechanical characteristics of the CS-films

CS-films	Tensile strength (MPa)	Elongation at break (%)
Control film	5.02 ± 0.13^{b}	153.97 ± 7.34^{a}
Film with *P. asperum* EO	3.71 ± 0.17^{c}	53.90 ± 7.91^{cd}
Film with *M. alternifolia* EO	1.53 ± 0.12^{d}	41.98 ± 3.28^{d}
Film with *O. majorana* EO	9.76 ± 0.61^{a}	97.20 ± 4.87^{b}
Film with *T. satureioides* EO	4.28 ± 1.63^{bc}	61.98 ± 13.86^{c}

had low stretching ability compared to the control film. As regards TS parameter, the treatment with tea tree and pelargonium EOs decreased the film resistance and conversely for marjoram EO treatment. On the hand, no significant modification in TS was recorded between the control film and the one treated with thyme EO ($P < 0.05$).

Films' antioxidant activity

The antioxidant activities of the different CS-films were statistically compared in Fig. 2. Results showed negligible activity in the case of the control. For all treatments, the antioxidant activity was stronger after 72 h of the material-methanolic solution contact (percentage of DPPH inhibition > 55%). The film treated with Thyme EO showed a slightly high activity compared to the other treated films.

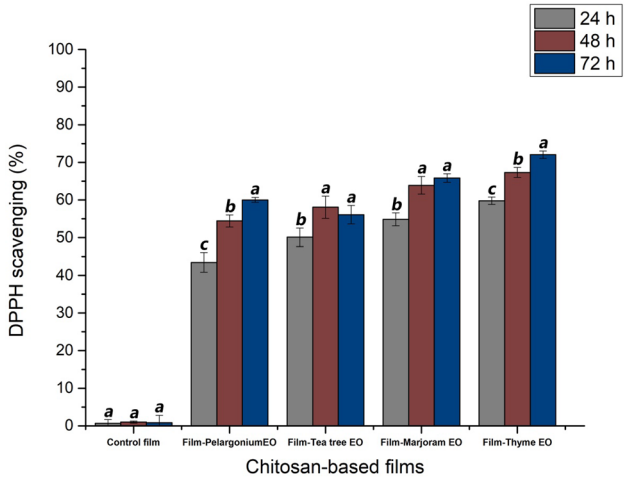

Fig. 2 DPPH inhibition (%) by the CS-films

Films' antibacterial activity

The methods of diffusion in liquid and solid mediums were utilized to assess the antibacterial effect of the prepared materials (Table 5). Among all tested films, only the ones treated with pelargonium and thyme EOs generated IZ against the four bacteria. The diameter of IZ variedbetween 8 and 12.67 mm depending on the bacterial strains. The diffusion in the liquid medium test revealed that the control film and the film treated with thyme EO exhibited the weakest and strongest activities, respectively.

Films' antiadhesion activity

Figure 3 compares statistically the antiadhesion activities of the prepared materials. From the data, three levels of activity can be defined, strong, moderate, and weak antiadhesion activity in which the inhibition percentages are around 20%, 50%, and 80%, respectively. The films containing thyme and pelargonium EOs showed strong activity while the ones containing marjoram and tea tree EOs exhibited moderate activity. On the other hand, the weakest antiadhesion activity was recorded in the case of the control film.

Discussion

The assessment of the physical, physicochemical, and mechanical characteristics of the materials is important to predict their usefulness as packaging. The physical characteristics of the material such as opacity and thickness are involved in product protection. Moreover, the film thickness is correlated to its mechanical parameters (Simsek et al. 2020). The results confirmed that the increase in

Table 5 Antibacterial activity of the CS-films against four foodborne bacteria expressed by the IZ diameter and turbidity calculation at 630 nm

CS-films	Antibacterial ativity							
	Pseudomonas aeruginosa ATCC53		*Escherichia coli* K-12 MG1655		*Staphylococcus aureus* ATCC29213		*Enterococcus hirae* CIP5855	
	IZ diameter (mm)	Absorbance (630 nm)	IZ diameter (mm)	Absorbance (630 nm)	IZ diameter (mm)	Absorbance (630 nm)	IZ diameter (mm)	Absorbance (630 nm)
Control film	–	0.28 ± 0.1^{b}	–	0.88 ± 0.01^{ab}	–	0.32 ± 0.01^{b}	–	0.38 ± 0.01^{b}
Film-Pelargonium EO	10.17 ± 0.29^{b}	0.24 ± 0.01^{c}	10.33 ± 0.58^{b}	0.57 ± 0.02^{c}	9.00 ± 0.50^{b}	0.24 ± 0.01^{d}	8.00 ± 0.00^{b}	0.26 ± 0.01^{d}
Film-Tea tree EO	–	0.24 ± 0.01^{c}	9.50 ± 0.50^{b}	0.56 ± 0.01^{c}	8.50 ± 0.50^{b}	0.28 ± 0.01^{c}	–	0.27 ± 0.01^{d}
Film-Marjoram EO	–	0.28 ± 0.02^{ab}	–	0.86 ± 0.02^{b}	–	0.29 ± 0.02^{bc}	–	0.32 ± 0.01^{c}
Film-Thyme EO	11.17 ± 0.29^{a}	0.08 ± 0.02^{d}	12.67 ± 1.04^{a}	0.42 ± 0.01^{d}	11.50 ± 0.50^{a}	0.22 ± 0.01^{d}	11.17 ± 0.29^{a}	0.26 ± 0.01^{d}
Positive control	–	0.31 ± 0.01^{a}	–	0.91 ± 0.01^{a}	–	0.38 ± 0.01^{a}	–	0.42 ± 0.01^{a}

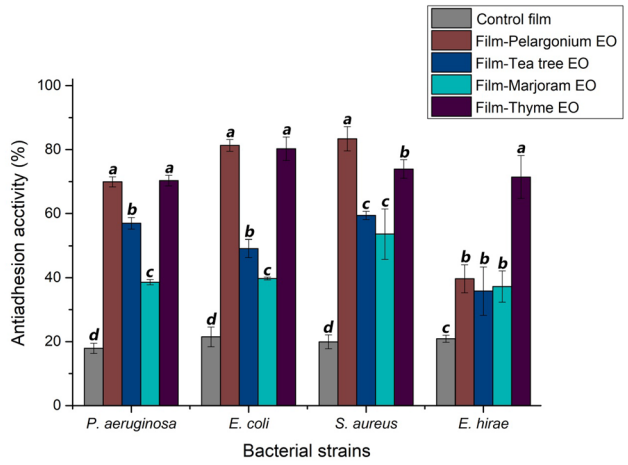

Fig. 3 Antiadhesion activity of the CS-films against four foodborne bacteria

film thickness following the treatment with EOs modified randomly the tensile strength property and caused a drop in the elongation at break parameter. It was reported that resistant polymers possess high stretching ability (Shaikh et al. 2021). Nevertheless, low film thickness led to a greater amount of dioxygen in the package's headspace and therefore the product oxidation (Del Nobile et al. 2007). As well as thickness, the reduction in film opacity negatively affects the light barrier parameter against harmful light (Zhang et al. 2021). Numerous studies have demonstrated an increase in the material opacity and thickness following the incorporation of pelargonium, tea tree, marjoram, and thyme EOs (Sedlaříková et al. 2017; Lian et al. 2019; Coyotl-Pérez et al. 2022; Jesser et al. 2022; Song et al. 2022; Wang et al. 2022). The modification of these parameters can be explained by the EOs impact on the material refractive index and microstructure, respectively. In addition to dioxygen and light barrier, a food packaging material should minimize the water-product contact. The evaluation of the WVTR parameter allows to define the capability of the packaging material to protect food in humid environment. The decrease in the WVTR values of the CS-film following the introduction of EOs could be related to the high thickness. Similar results were noticed in previous investigations (Priyadarshi et al. 2018; Akhter et al. 2019). Findings revealed that the treatment by the EOs decreased as well the moisture content of the CS-film 2-folds. Similar results were found in previous investigations (Ojagh et al. 2010; Hafsa et al. 2016). This drop in the moisture content could be associated to the interaction between the free OH groups of the chitosan and the EO components.

Basically, chitosan is known as an antimicrobial agent (Khan et al. 2020), The inhibitory effect against microorganisms is ensured by the presence of the positively charged NH_3^+ groups located on the carbon C2 of the chitosan molecule. These functional groups interfere with the negatively charged cell surface of the microorganism leading to its disorganization (Riaz Rajoka et al. 2020). It has been claimed that the antimicrobial behavior of the chitosan was higher against fungi than bacteria, with Gram-positive bacteria being less resistant in comparison with Gram-negative bacteria (Orzali et al. 2017). Xing et al., (2015) explained this variation in the antimicrobial activity by the difference in the cell wall constitution and cell structure. Moreover, chitosan can inactivate the replication of viruses and viroids (Kulikov et al. 2006). Nevertheless, numerous studies reported the limited activities of chitosan when applied as film (Wang et al. 2011; Mouhoub et al. 2023a).

In addition to the above-mentioned advantages of the EOs introduction into CS-film. These substances are well known to exhibit strong antioxidant and antimicrobial activities (Tariq et al. 2019). These biological activities essentially depend on the nature and concentration of the bioactive molecules present in the EO. The chemical assessment of the tested EOs indicates that citronellol, terpinen-4-ol, α-terpineol, and borneol which are all terpene alcohols, were the major constituents of the tested EOs. Previous results confirmed these findings (Fachini-Queiroz et al. 2012; Cerempei et al. 2014; Santana et al. 2014; Taoufik et al. 2017; Abbasi-Maleki et al. 2020; Song et al. 2022). In fact, terpene alcohols were defined as a good antioxidant and antimicrobial agents (Park et al. 2012; Ouedrhiri et al. 2018). Among all tested materials, the films containing pelargonium and thyme EOs exhibited the strongest bioactivities. These findings confirm the results of previous studies where the antioxidant and antimicrobial activities of pelargonium, tea tree, marjoram, and thyme EOs were compared (Teixeira et al. 2013; Alibi et al. 2020; Milenković et al. 2021; Mouhoub, Guendouz, et al. 2022a, b). This disparity in biological activities could be attributed to the qualitative and quantitative variation of the EOs' chemical components. The antimicrobial and antioxidant actions of citronellol which is the main constituent of pelargonium EO were previously highlighted (Santos et al. 2019; Sharma et al. 2020). Furthermore, An et al., (2019) demonstrated that terpinen-4-ol and α-terpineol exhibited the greatest antifungal activity against *Aspergillus niger* with slightly higher activity as regards α-terpineol. These findings could explain the effectiveness of thyme EO in comparison with tea tree and marjoram EOs and confirm that the stronger antimicrobial activity of tea tree EO, when compared to marjoram EO, was related to the high percentage of terpinen-4-ol (40.33%).

In natural conditions, It is well known that microorganisms attach to different surfaces and establish a complex structure called biofilm (Flemming and Wingender 2010). This arrangement of microorganisms enhances their resistance by 2000-folds against antimicrobial agents (Tabak et al.

2009; Wang et al. 2018). Moreover, biofilms engender different diseases including foodborne illnesses (Kulshrestha and Gupta 2022), and cause surfaces' damage (Lv et al. 2022) which leads to colossal economic losses. The contact angle analysis was realized to determine an eventual correlation between the bacterial adherence rate and the physicochemical characteristics of the CS-films. According to the obtained results, a drop in the qualitative hydrophobicity was recorded for all the treated CS-films. However, the quantitative hydrophobicity of the material was randomly modified following the treatment by EOs. From these findings, It is more accurate to attribute the antiadhesion activity of the treated CS-film to the direct action of the EOs. Numerous investigations highlighted the multiple mode of action of EOs and their components against biofilm formation (Guo et al. 2021). E. g, the anti-quorum sensing and bactericidal activities (Burt et al. 2014; Merghni et al. 2018; Tariq et al. 2019), the inhibition of adhesins and binding proteins production (Marinas et al. 2015; Kot et al. 2019), and the targeting of the extracellular polymeric substrates (Zhao et al. 2018; Kang et al. 2019).

Based on these promising findings, we conclude that the treatment of CS-film containing pelargonium, tea tree, marjoram, and thyme EOs improves the physical properties and enhances the antioxidant and antimicrobial activities.

Conclusion

In summary, pelargonium, tea tree, marjoram, and thyme EOs contain terpene alcohols as major components. The introduction of these EOs into CS-film improved its physical properties by increasing the thickness, opacity and water barrier property, and decreasing the moisture content. Moreover, the biological activities such as the antibacterial, antioxidant, and antibiofilm activities of the CS-film were considerably enhanced by the EOs introduction, especially in the case of pelargonium and thyme treatments. Overall, these promising results emphasize the eventual utilization of CS-film containing pelargonium and thyme EOs as biodegradable food packaging.

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Data availability The authors do not have permission to share data.

Declarations

Competing interests The authors declare no competing interests.

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