

In-vitro antibacterial and antioxidant properties of starch/chitosan edible composite film incorporated with thyme essential oil

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Background/aim

Nowadays, packaging research is receiving considerable attention because of the development of ecofriendly materials made from natural polymers such as starch and chitosan. This study aimed to prepare composite films from starch–chitosan, incorporated with *Thymus vulgaris* essential oil (S-CH-Th), and to evaluate their antibacterial, antioxidant and optical properties.

Materials and methods

The molecular weight of chitosan was (~400 kDa, 75–85% deacetylated). 27% amylose corn starch, Tween 80, DPPH (2, 2-diphenyl-1-picrylhydrazyl) and Folin–Ciocalteu reagent were used to carry out research. Antibacterial activity testing was performed using the disk method. Antioxidant activity test was performed using a spectrophotometric method with DPPH as the radical source. Essential oil concentrations ranging from 0 to 2%, incorporated into a starch–chitosan composite (S-CH) film, were used.

Results

Antibacterial and antioxidant properties increased significantly with the incorporation of Essential oils (EO) ($P < 0.05$). On incorporating EO, there was an increase in the total colour differences (ΔE), yellowness index and whiteness index, which were significantly higher than those of the control, and the transparency was reduced. Also, the results showed that chitosan edible films incorporated with *T. vulgaris* EO could be used as active films because of their excellent antibacterial and antioxidant activities.

Conclusion

S-CH edible films incorporated with thyme EO as a natural antibacterial and antioxidant agent may potentially be used as an active packaging to enhance the safety of foods and food products.

Keywords:

antimicrobial, antioxidant, composite film, starch–chitosan, thyme essential oil

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Introduction

New biobased packaging materials such as edible and biodegradable films have been used to address environmental issues and concurrently extend the shelf-life of food [1]. This edible/biodegradable packaging can be consumed along with the food, providing additional nutrients, and enhancing sensory characteristics and quality [2,3]. The use of an edible film with antimicrobial agents is one of the approaches to prevent contamination of pathogens on the surface of food products.

Biodegradable polymers based on natural polysaccharides, particularly starch, can be produced at a low cost and on a large scale. Starch-based materials can reduce the use of nonrenewable resources. Furthermore, starch is one of the most abundant, natural biopolymers. The starch granule is essentially composed of two polysaccharides, amylose and amylopectin, and some minor components such as lipids and proteins. This polymer has attracted considerable attention as

a biodegradable thermoplastic polymer [4]. The films prepared from starch represent promising applications in food packaging because of their biodegradability, low cost, flexibility and transparency. However, several authors have observed that, despite their ease of preparation, starch films have some drawbacks, such as their poor mechanical properties. For this reason, starch films require the addition of plasticizing compounds [5].

Starch is a water-soluble polysaccharide with well-known biodegradable and edible film-forming properties. Starch-based packaging materials are available widely in a variety of botanical sources such as corn, wheat, potatoes, yam and tapioca, and can be produced at a low cost and on a large scale from different surplus of harvesting and raw material industrialization [6].

Chitosan is a cationic polysaccharide derived from the deacetylation of chitin, a component of the shells of crustaceans. Several studies have indicated the

antimicrobial and antioxidant characteristics and nontoxicity of chitosan. In addition, chitosan has immense advantages as an edible packaging material owing to its good film-forming properties [7]. However, the wide application of starch films is limited by their water solubility and brittleness. Therefore, chitosan films have relatively poor water vapour barrier characteristics [8]. One of the effective strategies to overcome the poor mechanical properties of these films, while preserving the biodegradability of the materials, is the use of composite films that can be formulated to combine the advantages of each component [9,10]. Films with varying proportions of starch and chitosan have been manufactured, and it was observed that films with a chitosan proportion of 20% (w/w) showed good antimicrobial properties and improved mechanical properties compared with pure starch films [11].

There have been consumer demands for more natural preservatives, mainly because of safety concerns, in that the residual chemicals might be hazardous. In this respect, incorporation of natural preservatives such as plant extracts and essential oils with antimicrobial and antioxidant properties into biobased packaging materials provides an innovative means of improving the safety and shelf-life of food [12,13].

The aromatic and medicinal properties of the genus *Thymus* have made it one of the most popular plants worldwide. Thymus essential oils and extracts with antimicrobial and antioxidant properties are used widely in pharmaceutical, cosmetic, herbal tea, flavouring agents and perfume industries, and also for flavouring and preservation of several foods [14]. Because of the effect of the direct addition of essential oils to food on the sensory characteristics of added food, incorporation of essential oils into edible films may have supplementary applications in food packaging [15,16]. Some studies have examined the antimicrobial properties of films based on starch or chitosan incorporated with various essential oils, with good results [17–23].

The addition of essential oils (thyme and basil) and other components with antioxidant activity, such as α -tocopherol (liposoluble antioxidant) or citric acid, can improve the functional properties of edible films and increase their potential use in the preservation of foods with a high fat content. Despite the huge potential of essential oils, their use in food preservation remains limited mainly because of their intense aroma, toxicity and possible changes in the organoleptic properties of the food [24]. The use of edible coatings to carry essential oils could minimize the required doses by the encapsulation effect in the polymer matrix, which limits their volatilization and controls the release of the

compound, thus reducing the negative impact of these ingredients. Although the incorporation of bioactive compounds can modify the barrier properties (water vapour and oxygen) of the films, doing so provides additional advantages, such as protection against microbial growth and lipid oxidation [25,26].

The aim of this study was to prepare composite films from starch–chitosan, incorporated with *Thymus vulgaris* essential oil (S-CH-Th), and to evaluate their antibacterial, antioxidant and optical properties.

Materials and methods

Plant material and gas chromatography-mass spectrometry analysis

The dried leaves and aerial parts of *T. vulgaris* were purchased from the same local market (Cairo, Egypt). Essential oil was obtained by hydrodistillation for 3 h using a Clevenger-type collector. The oils were dried over anhydrous sodium sulphate and, after filtration, stored at 4°C until tested and analysed. The constituents of EO were identified by gas chromatography-mass spectrometry (GC-MS) [Hewlett-Packard (Agilent Technologies, Wilmington, DE, USA) model (5890)]/mass spectrometry [Hewlett-Packard MS (5970)]. The ionization voltage was 70 eV and mass range was m/z 39–400 amu. The GC condition was as mentioned above. The isolated peaks were identified by matching with data from the library of mass spectra (National Institute of Standard and Technology) and compared with those of authentic compounds and published data [27]. Quantitative determination was carried out on the basis of peak area integration.

Preparation of films

A chitosan-based film was prepared by dissolving medium-molecular-weight chitosan (~400 kDa, 75–85% deacetylated) (Fluka; Sigma-Aldrich (Fluka, Sigma-Aldrich, St. Louis MO, USA)) in an aqueous solution (1% v/v) of glacial acetic acid (Merck, Darmstadt, Germany) to a concentration of 2% (w/v) while stirring on a magnetic stirrer-hot plate. The solution was stirred with low heat (at 50°C), which typically required 3 h of stirring. The resultant chitosan solution was filtered through a Whatman No. 3 filter paper, followed by vacuum filtration to eliminate insolubles and remove any undissolved particles. Starch solutions with concentrations of 3.5% (w/v) were prepared by dispersing 27% amylose corn starch (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in distilled water and heating the mixtures on hotplates 95°C for 30 min with stirring until it gelatinized, and then cooling to 40°C [28].

Starch–chitosan (S-CH) composite films were prepared by mixing 100 ml of a 2% chitosan solution with 100 ml of 3.5% starch solutions. Glycerol (Sigma Chemical Co., St. Louis, MO, USA) was added as 30% (w/w) of the total solid weight in solution. Tween 80 at the level of 0.2% (v/v) of EO was added in film-forming solutions to aid essential oil dissolution, and then EO was added to the S-CH solution to reach a final concentration of 0, 1, 1.5 and 2% (w/w). The solution was homogenized at 8000 rpm for 3 min to obtain an emulsion. The mixtures were cast on to flat, level polytetrafluoroethylene casting plate. After drying the films at room temperature for at least 72 h, they were peeled from the plates. Dried films were conditioned at 50% RH and 25°C for 48 h before testing.

Determination of the antibacterial effects of films

For the antibacterial activity test, *Staphylococcus aureus* (ATCC 43300), *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 27325) and *Listeria monocytogenes* (ATCC 35152) from the culture collections of the Microbiological Department, National Research Center (Dokki, Giza, Egypt) were used. The bacterial cultures were grown on the nutrient agar slant and kept at 4°C. In the preparation of seeding culture, a loopful of bacteria from agar slant was taken and inoculated into 50 ml of tryptone soy broth in a 125 ml flask. The flask was then incubated at 125 rpm in an incubator at 37°C for 24 h. A dilution series was used to obtain the required bacterial population for seeding using sterile-distilled water. The agar diffusion method was used to determine the antibacterial effects of films on bacterial strains. Disks (1 cm diameter) cut from the films were placed on tryptone soy agar plates, previously surface spread with 0.1 ml of inoculums containing approximately 10^5 – 10^6 CFU/ml of tested bacteria. The plates were then incubated at 37°C for 24 h. The diameter of the zone of inhibition was measured using a caliper to the nearest 0.01 mm. The entire zone area was calculated and then subtracted from the film disc area and this difference in area was reported as the 'zone of inhibition'. The contact area was also examined visually to evaluate growth inhibition underneath the film disk contact [29].

Determination of antioxidant activity

The antioxidant activity of the film samples was evaluated using a DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. Briefly, 3 ml of film extract solution was mixed with 1 ml of methanolic solution of DPPH (Merck). The mixture was vortexed and incubated in the dark at ambient temperature for 30 min. When the DPPH solution was mixed with the sample mixture acting as a hydrogen atom donor, a

stable nonradical form of DPPH was obtained, with a simultaneous change in the violet colour to pale yellow. The absorbance was then measured at 517 nm using a spectrophotometer (UV-160-IPC; Shimadzu, Japan). The percentage of DPPH free radical quenching activity was determined using the following equation:

$$\text{DPPH scavenging effect \%} = \frac{A_{\text{DPPH}} - A_{\text{extract}}}{A_{\text{DPPH}}} \times 100,$$

where A_{DPPH} is the absorbance value at 517 nm of the methanolic solution of DPPH and A_{extract} is the absorbance value at 517 nm for the sample extracts [30].

Total phenolic assay

For this purpose, 25 mg of each film sample was dissolved in 3 ml of distilled water. Phenolic compound content in each film extract was determined according to the Folin–Ciocalteu procedure as described by Singleton *et al.* [31], with slight modifications by Siripatrawan and Harte [30]. Briefly, 0.1 ml of film extract solution was mixed with 7 ml distilled water and 0.5 ml of Folin–Ciocalteu reagent (Merck). The mixture was incubated for 8 min at room temperature before the addition of 1.5 ml of sodium carbonate solution and 0.9 ml of distilled water. The mixture was stored in a dark chamber at room temperature for 2 h. The absorbance of the mixture was then measured at 760 nm using a spectrophotometer. Gallic acid solutions (Sigma-Aldrich) in the specific concentration range were used to construct a calibration curve. The concentration of total phenolic compounds in the samples is expressed as gallic acid equivalents, which reflect the phenolic content as the amount of gallic acid in mg/g dry weight of the sample, calculated using an equation that was obtained from the standard graph ($R^2 = 0.991$):

$$A_{760} = 0.912 \text{ mg gallic acid} + 0.041.$$

Film solubility in water

A modified method from Jutaporn *et al.* [32] and Rhim *et al.* [33] was used to measure film solubility. Film portions measuring 1.3 cm² were cut and dried at 110°C in a vacuum oven for 24 h and then weighed to the nearest 0.0001 g for the initial dry weight. Then, films were placed in a glass beaker with 50 ml of distilled water and shaken gently at 25°C for 24 h. The solution was then filtered through a Whatman No. 1 filter paper to recover the remaining undissolved film. The remaining pieces of film after immersion were dried at 110°C to a constant weight (final dry weight). Tests for each type of film were carried out in three replicates [32,33]. Solubility in water (%) was calculated using the following equation:

$$\text{Solubility in water (\%)} = \frac{\text{Initial dry weight} - \text{final dry weight}}{\text{Initial dry weight}} \times 100$$

Surface colour and opacity measurements

Film colour was determined using a colorimeter. The Hunter colour scale was used; lightness (L) and chromaticity parameters a (red–green) and b (yellow–blue) were measured. Measurements were performed by placing the film sample over the standard white plate ($L = 91.35$, $a = 0.31$ and $b = -1.21$). Total colour difference (ΔE), yellowness index (YI) and whiteness index (WI) were calculated according to the following equation [31]:

$$\Delta E = [(L_{\text{standard}} - L_{\text{sample}})^2 + (a_{\text{standard}} - a_{\text{sample}})^2 + (b_{\text{standard}} - b_{\text{sample}})^2]^{0.5}$$

$$\text{YI} = 142.86b \times L^{-1}$$

$$\text{WI} = 100 - [(100 - L)^2 + a^2 + b^2]^{0.5}$$

Transparency was determined according to the method of Siripatrawan and Harte [30] by measuring the film absorbance at 600 nm using a UV spectrophotometer. The films were cut into a rectangle piece and directly placed in a spectrophotometer test cell. An empty test cell was used as the reference. The transparency of the films was calculated as follows:

$$\text{Transparency} = \log(T_{600}) \times x^{-1},$$

where T_{600} is the transmittance at 600 nm and x is the film thickness (mm). According to this equation, the high values of T indicate lower transparency and higher degree of opacity.

Statistical analysis

The statistical analysis of the data was carried out using SPSS version 18.0 (Statistical Package for the Social Sciences Inc., Chicago, IL, USA). Quantitative data were represented in form of mean $\pm$ SD. Analysis of variance was used in the analysis of the results. Duncan's multiple range test was used to detect differences among the mean values of films. The P -value of the test was 0.05 or less.

Results

Identification of volatile components from essential oil

The results of GC-MS analytical data of compounds in *T. vulgaris* EO are shown in Table 1. The major constituents were thymol (GC peak area%, 33.14%), carvacrol (19.59%), linalool (10.55%), α -caryophyllene (2.03%), α -terpineol (0.42%), α -terpinene (0.72%),

eucalyptol (1,8-cineole and limonene) (2.95%), p-cymene (10.30%) and g-terpinene (2.43%).

Antimicrobial activity of edible starch–chitosan composite films

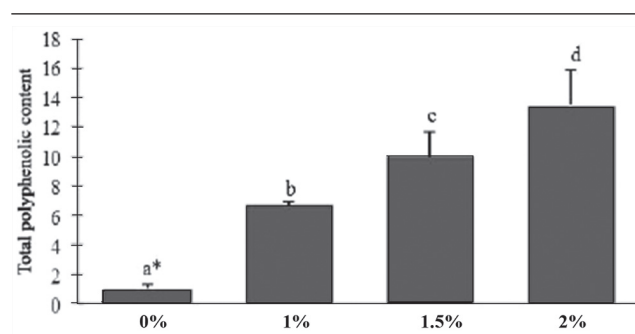
The growth-inhibition zones were measured using an agar disc diffusion assay. The effects of *T. vulgaris* EO addition on the antimicrobial properties of S-CH composite-based films are shown in Table 2. When antimicrobial agents are incorporated into films, these materials diffuse through agar gel and result in a clear zone around the film cuts. *T. vulgaris* EO showed different inhibition levels against *S. aureus*, *L. monocytogenes*, *E. coli* and *S. typhimurium* as shown in Table 2. In this study, the inhibition zone was increased with increasing concentration of EO, but this was not significant for all concentrations in four tested microorganisms ($P < 0.05$). A S-CH composite film without EO was not effective against *S. typhimurium* and a clear zone of inhibition was not observed.

Total phenolic content and antioxidant activity

Foline–Ciocalteu phenol reagent is used for a crude estimate of the amount of phenolic groups present in an S-CH composite film. The results showed that the total phenolic content in the S-CH films significantly was increased ($P \leq 0.05$) with increasing EO concentration (Fig. 1).

The DPPH scavenging assay was used to determine the antioxidant activity of the film. This assay was based on the ability of DPPH, a stable free radical, to be quenched and thereby decolourize in the presence of antioxidants, resulting in a reduction in absorbance values [30]. The results showed that the DPPH scavenging activity of the S-CH films increased significantly ($P \leq 0.05$) with increasing EO concentration as shown in Fig. 2.

Figure 1



Total polyphenolic content (mg/g of gallic acid) in 1 g of an starch–chitosan composite film incorporated with *Thymus vulgaris* EO. Values are given as mean $\pm$ SD. Different letters indicate significant differences ($P < 0.05$) when analysed by Duncan's new multiple range test.

Table 1 Chemical composition of *Thymus vulgaris* essential oil

Compounds	Concentrations (%)	RT	RI	Method of identification
α -Thujene	2.84	8.36	931	a,b
α -Pinene	2.97	8.58	939	a,b,c
β -Pinene	0.71	10.15	980	a,b,c
Octan-1-en-0-o1	0.48	10.37	988	a,b
Myrcene	2.45	10.82	991	a,b
α -Phellandrene	0.42	11.23	1005	a,b
α -Terpinene	0.72	11.71	1018	a,b
π -Cymene	10.30	12.04	1026	a,b,c
Limonene	1.23	12.17	1031	a,b
1,8-cineole	1.72	12.23	1033	a,b
γ -Terpinene	2.43	13.36	1062	a,b
Cis-sabinene hydrate	0.19	13.54	1068	a,b
α -Terpineol	0.42	14.29	1088	a,b
Linalool	10.55	14.78	1096	a,b,c
Terpinen-4-ol	0.25	17.29	1177	a,b
Thymol methyl ether	1.78	19.14	1235	a,b
Thymol	33.14	21.33	1290	a,b,c
Carvacrol	19.59	21.33	1298	a,b,c
Terpinyl acetate	0.68	22.60	1352	a,b
Eugenol	0.10	22.77	1356	a,b
β -Bourbonene	0.09	23.66	1386	a,b
β -Elemene	0.14	23.83	1391	a,b
methyl eugenol	0.21	24.05	1401	a,b
β -Caryophyllene	1.64	24.64	1418	a,b,c
β -Copaene	0.16	24.88	1430	a,b
α -Humulene	0.17	25.53	1454	a,b
Germacrene D	0.40	26.27	1480	a,b
β -Bisabolene	0.33	26.93	1509	a,b
δ -Cadinene	0.14	27.33	1520	a,b
α -Caryophyllene	2.03	28.89	1581	a,b

^aRetention index in comparison with the literature value; ^bMass spectra; ^cCoinjection with an authentic sample.

Table 2 Antibacterial activity of edible starch–chitosan composite films disks with various concentrations (0, 1, 1.5 and 2%) of thyme EO against different bacteria

Test organisms	Inhibition zone diameters (mm ²)			
	0	1	1.5	2
<i>Staphylococcus aureus</i> (ATCC 43300)	14.22 $\pm$ 6.12 ^d	74.56 $\pm$ 5.45 ^c	150.65 $\pm$ 11.65 ^b	265.12 $\pm$ 21.43 ^a
<i>Listeria monocytogenes</i> (ATCC 35152)	34.02 $\pm$ 12.24 ^d	77.70 $\pm$ 6.94 ^c	165.89 $\pm$ 14.91 ^b	290.60 $\pm$ 16.06 ^a
<i>Escherichia coli</i> (ATCC 27325)	12.43 $\pm$ 3.36 ^d	63.69 $\pm$ 9.13 ^c	143.08 $\pm$ 15.05 ^b	259.55 $\pm$ 20.17 ^a
<i>Salmonella typhimurium</i> (ATCC 13311)	0.00 $\pm$ 0.00 ^d	53.24 $\pm$ 9.23 ^c	110.60 $\pm$ 10.82 ^b	188.33 $\pm$ 18.54 ^a

Reported values correspond to the mean $\pm$ SD; Different letters in the same column indicate significant differences [$P < 0.05$].

Table 3 Hunter colour values (*L*, *a* and *b*), opacity (*T*), yellowness index, whiteness index, total colour difference (ΔE) and solubility in water of starch–chitosan films as a function of *Thymus vulgaris* EO concentration

EO%	<i>L</i>	<i>a</i>	<i>b</i>	<i>T</i>	WI	YI	ΔE	Solubility in water (%)
0	88.72 ^d	−1.27 ^a	12.36 ^a	2.01 ^a	85.11 ^d	20.55 ^a	14.13 ^a	13.55 ^a
1	85.56 ^c	−1.43 ^b	13.11 ^b	3.29 ^b	82.01 ^c	23.35 ^b	15.54 ^b	17.54 ^b
1.5	84.55 ^c	−1.85 ^c	14.75 ^c	4.66 ^c	79.05 ^b	28.15 ^c	19.04 ^c	21.25 ^c
2	81.35 ^a	−1.99 ^d	18.39 ^d	5.13 ^d	75.23 ^a	35.12 ^d	22.74 ^d	24.28 ^c

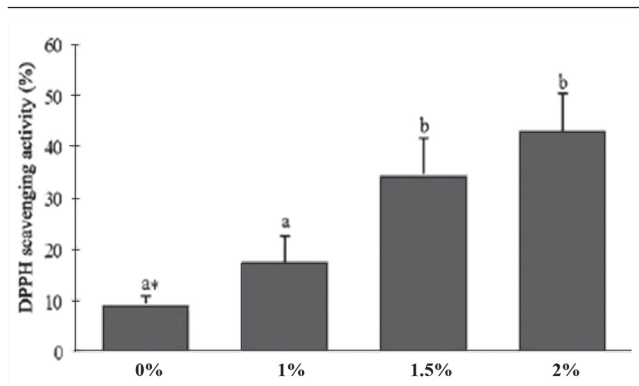
Each column with different superscript letters are significantly different [$P < 0.05$].

Film solubility in water

The water solubility of the S-CH composite films as a function of EO content is shown in Table 3. The addition of EO, at all concentrations, increased the water solubility of films. The percentage

of water solubility was 13.55% for the samples without EO, which was increased to 24.28 for the films containing 2% EO. However, a significant ($P \leq 0.05$) increase in solubility was observed at high levels of EO.

Figure 2



DPPH scavenging of an starch–chitosan composite film incorporated with *Thymus vulgaris* EO. Values are given as mean $\pm$ SD. Different letters indicate significant differences ($P < 0.05$) when analysed by Duncan's new multiple range test.

Surface colour and opacity

The effects of EO concentration on L , a and b Hunter Lab colour values, total colour difference (ΔE), YI, WI and opacity of films are shown in Table 3. The addition of EO to chitosan films significantly affected ($P \leq 0.05$) L (lightness/darkness), a (redness/greenness) and b (yellowness/blueness) values of the film surface. Films without EO were lighter (higher L value). L values of the films decreased from 88.72 to 81.35, but a decreased from -1.27 to -1.99 (Negative numbers) because $-1.99 < -1.27$ (an indicator of the tendency towards redness), and b values increased from 12.36 to 18.39 (an indicator of the tendency towards yellowness) as the EO concentrations increased from 0 to 2%.

Discussion

The results of GC-MS analytical data of compounds in *T. vulgaris* EO showed that EO is rich in monoterpene phenols, especially thymol and carvacrol, which have antibacterial and antioxidant properties. The results showed that *L. monocytogenes* was the most sensitive bacteria against *T. vulgaris* EO-incorporated films, followed by *S. aureus*, *E. coli* and *S. typhimurium*. As the concentration of EO increased, the inhibition zone increased significantly ($P < 0.05$).

The inhibitory effects of essential oils on the types of bacteria such as Gram-positive or Gram-negative bacteria are still controversial. Emiroğlu *et al.* [34] determined the antibacterial activity of soy protein edible films incorporated with oregano and thyme essential oils and showed that although *E. coli* and *S. aureus* were significantly inhibited by antimicrobial films, *Lactobacillus plantarum* and *Pseudomonas aeruginosa* appeared to be the more resistant bacteria. Solomakos *et al.* [35] reported that 0.60% thyme

essential oil had an inhibitory effect against *E. coli* when applied directly to minced meat during refrigerated storage at 10°C. Seydim and Sarikus [29] evaluated the antimicrobial activity of whey protein isolate-based edible films incorporated with essential oils and reported greater inhibitory effects of whey protein isolate-based edible films containing 2% oregano oil against *L. monocytogenes* than *E. coli*. Oussalah *et al.* [36] showed that the addition of 1% oregano essential oil to milk protein-based edible films inhibited *E. coli*. In another study, carvacrol containing tomato-based edible films inactivated the *E. coli*, with the inactivation related to carvacrol levels in the films [37]. Thymol and carvacrol are considered to be the major active compounds present in thyme and oregano EO, and have been reported to have inhibitory effects against microorganisms through breakdown of the outer membrane of microorganisms and lead to an excessive leakage of essential elements, and to cause bacterial death [38]. The results showed that the *T. vulgaris* EO incorporated into S-CH films exerted significant inhibitory effects against common foodborne pathogenic bacteria such as *L. monocytogenes*, *E. coli*, *S. aureus* and *S. typhimurium*.

Folin–Ciocalteu colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. Phenolic compounds undergo a complex redox reaction with phosphotungstic and phosphomolybdic acids present in the Folin–Ciocalteu reactant [39]. On the basis of Folin–Ciocalteu results, the total phenolic content in the S-CH films increased significantly ($P \leq 0.05$) with increasing EO concentration (Fig. 1). The DPPH scavenging assay was used to determine the antioxidant activity of the film. As the concentration of EO increased, the DPPH scavenging activity of the films increased significantly ($P \leq 0.05$), but this was not significant between the concentrations of 1–1.5 and 1.5–2%. In the films containing 2% EO, the antioxidant activity increased 4.5-folds more than the control samples. In a study by Amiri [40], *T. vulgaris* EO showed 117 mg GEs/mg of extract Phenolic content and 278 mg/ml DPPH IC50 antioxidant activity. In another study, a chitosan film incorporated with 1 and 2% *Zataria multiflora* Boiss EO showed 33.98 and 37.77% DPPH scavenging activity, respectively, and 5.6 and 11.2 mg gallic acid/g film phenolic content [41].

The chitosan films with no EO showed some scavenging activity on DPPH (9.10%). This is because free radicals can react with the residual free amino (NH_2) groups of chitosan to form stable macromolecule radicals, and the NH_2 groups can form ammonium (NH_3^+) groups by absorbing a hydrogen ion from the solution [42]. However, the results of this study

showed that incorporation of GTE into chitosan films improved the polyphenolic content and antioxidant activity of the films.

In both edible and inedible films, colour is an important factor in terms of consumer acceptance. The addition of *T. vulgaris* EO affected the colour and transparency of S-CH edible films. Edible S-CH films without EO appeared clear and transparent and S-CH composite films incorporated with EO showed significantly higher ΔE , b value (yellowish) and lower L value (darker) than control films ($P \leq 0.05$). In one study, chitosan-based films containing cinnamon essential oil were investigated by Ojagh *et al.* [20], and similar results were reported. Pranoto *et al.* [15] showed that the addition of garlic EO affected the appearance of edible film in both colour and transparency. When garlic oil at 0.30% or a higher concentration was incorporated, the colour changed to yellowish as indicated by the increase in the b value. L values were decreased and the colour of the edible film tended to darken [15].

The addition of EO, in all concentrations, increased the water solubility of S-CH composite films. Although a higher solubility of edible film is required during cooking of food products coated with edible film, a low solubility is required during storage [38]. Laohakunjit and Noomhorn [43] showed that the inclusion of 0.40% lemongrass EO in a starch film increased the water solubility of films. This was attributed to the interference of EO with the arrangement of polymer chains and hydrogen binding and this led to less interaction between the starch molecules. Furthermore, leaching of amylase from the starch component in the film can increase percent of water solubility [43]. These findings are in contrast to the Ojagh *et al.* [20] study that showed that incorporation of CEO into the a chitosan film formulation at a level of 1.5 and 2% (v/v) led to 41.00 and 55.00% reductions in solubility in water, respectively.

S-CH, as natural polymers, have great potential for use in biobased packaging materials. The results showed that incorporation of *T. vulgaris* EO improved the antibacterial and antioxidant properties of an S-CH composite film. *T. vulgaris* EO exerted significant inhibitory effects against four common foodborne pathogenic bacteria used in this study. The colour of edible films was darker and more yellowish as the *T. vulgaris* EO increased.

Conclusion

An antibacterial and antioxidant S-CH composite film incorporated with *T. vulgaris* EO is promising

and has good potential to enhance the safety of foods and food products. Future research should be carried out to evaluate the sensory aspects of using these natural essential oil compounds in edible films and coatings, as well as to characterize their stability and other physicochemical properties. Moreover, the antimicrobial effect of CEO-enriched films should be determined on an entire model food.

Acknowledgements

Conflicts of interest

None declared.

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