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Physical, Mechanical, and Antimicrobial Properties of Carboxymethyl Cellulose Edible Films Activated with *Artemisia sieberi* Essential Oil

F. Salar Behrestaghi [1], S. Bahram [2*], P. Ariaii [1]

1. Department of Food Science and Technology, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran
2. Department of Fisheries, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran

HIGHLIGHTS

- Camphor, 1,8-cineole, β-Thujone, and camphanone were the main components of *Artemisia sieberi* Essential Oil (AEO).
- Carboxymethyl cellulose (CMC) film with 1.5% of AEO showed the highest "a" (greenness) and "b" (yellowness) values.
- The inhibition zones were 9.33, 11.5, and 17.30 mm for *Staphylococcus aureus*; and 8, 11.50, and 14.33 mm for *Escherichia coli* at AEO levels of 0.5, 1, and 1.5%, respectively.
- CMC films enriched with AEO could be beneficial in food packaging to retard food deterioration.

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Acronyms and abbreviations
AEO= *Artemisia sieberi* Essential Oil
CA=Contact Angle
CFU=Colony Forming Unit
CMC=carboxymethyl cellulose
E%=Elongation at break%
EO= Essential Oil
MC=Moisture Content
RH=Relative Humidity
TS=Tensile Strength
WVP =Water Vapor Permeability

ABSTRACT

Background: Edible films and coatings are biodegradable that can preserve the quality and extend the shelf life of foods. The aim of this study was to investigate the physical and mechanical properties, and antimicrobial activity of carboxymethyl cellulose (CMC) film containing *Artemisia sieberi* Essential Oil (AEO).

Methods: The studied parameters were the antibacterial activity and physical properties, including Water Vapor Permeability (WVP), Contact Angle (CA), solubility, Moisture Content (MC), and surface color; as well as mechanical properties including Elongation at break% (E%) and Tensile Strength (TS) of CMC incorporated with AEO at levels of 0 (control), 0.5, 1, and 1.5% v/v. Data were statistically analyzed by SPSS software.

Results: Camphor (36.38%), 1,8-cineole (15.89%), β-Thujone (6.7%), and camphanone (6.2%) were the main components of AEO. The edible CMC film showed increase in WVP, contact angle, E%, darker color, and yellowness, with decreases in film solubility, MC, and TS after the incorporation of AEO. CMC film with 1.5% of AEO showed the highest a* (greenness) and b* (yellowness) values. The inhibition zones were 9.33, 11.5, and 17.30 mm for *Staphylococcus aureus*; and 8, 11.50, and 14.33 mm for *Escherichia coli* at AEO levels of 0.5, 1, and 1.5%, respectively.

Conclusion: The overall results of this study showed that CMC films enriched with AEO could be beneficial in food packaging to retard food deterioration.

Introduction

An active packing film based on biodegradable materials is one of the innovative food packaging concepts, which has been introduced to drive toward renewable materials (Guillard et al., 2018). Edible films and coat-

* Corresponding author (S. Bahram)
✉ E-mail: bahramsomi@gmail.com
ORCID ID: https://orcid.org/0000-0003-0483-1138

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ings are great vehicles for combining a wide variety of additives such as antimicrobials, flavors, colorants, antioxidants, antifungal agents, and fortified nutrients (Rhim and Ng, 2007). These films or coatings can also preserve and improve food integrity and extend shelf life by minimizing microbial growth in the product (Abdollahi et al., 2012).

Cellulose and its derivative-based edible films are insoluble in water, very efficient oxygen, and hydrocarbon barriers, as well as aromatic compounds. Cellulose is esterified with aqueous caustic soda, and then with sodium monochloroacetate, methyl chloride, or propylene oxide to yield methylcellulose, hydroxypropyl methylcellulose, hydroxyl propylcellulose, and sodium carboxy methylcellulose in order to provide solubility (Jalali et al., 2016).

Carboxymethyl cellulose (CMC) films or coatings have some desirable characteristics such as perfect water-solubility, tastelessness, odorless, high viscosity, non-toxicity, moderate strength, transparency, flexibility, resistance to fats and oils, moderate moisture, and oxygen transmission (Ghanbarzadeh et al., 2010; Lan et al., 2018). However, they have poor moisture barrier properties due to their hydrophilic nature (Choi et al., 2016). Some studies have shown that using hydrophobic materials such as lipids and Essential Oils (EOs) can improve this property due to an increase in the hydrophobic compound fraction of the films (Bahram et al., 2014; Choi et al., 2016).

Incorporation of plant extracts and EOs into edible films provides a novel way to enhance food stability, functionality, and safety and to control the oxidation of fatty components and pigments, thereby contributing to food quality preservation (Martelli et al., 2017). EOs obtained from different parts of plant materials possesses antimicrobial and antioxidant activities, which minimize questions regarding their safe use in food products. The antimicrobial and antioxidant activities of plant EOs are related to a number of small terpenoids and phenolic compounds (e.g. thymol, carvacrol, eugenol), which also demonstrate high antibacterial and antioxidant activities in pure forms (Ahmad et al., 2012; Hosseini et al., 2009). The efficiency of EOs is limited because of interactions with food components, organoleptic deterioration, and severe odor, but incorporation of EOs into edible films instead of direct use may reduce required doses of EOs, while keeping their antimicrobial and antioxidant activities (Gómez-Estaca et al., 2010; Shojaee-Aliabadi et al., 2013).

A great number of aromatic and medicinal plants contain chemical compounds that exhibit antioxidant properties such as *Artemisia sieberi* belonging to Asteraceae family (Ghasemi-Pirbalouti et al., 2013; Mahboubi et al., 2015). Thirty-four species of *Artemisia* grow in Iran, from which some substances and have been reported to show antibacterial, antifungal (Mahboubi and Farzin, 2009), and antioxidant activities (Lopez-Lutz et al., 2008).

However, the effects of *A. sieberi* Essential Oil (AEO) on the physical, mechanical and antibacterial properties of CMC film have not been studied up to now. Thus, the principal purpose of the present research was to improve the properties of CMC-based on edible film by the incorporation of AEO, along with evaluating the physical, mechanical, and structural properties and antibacterial activities of the edible films.

Materials and methods

Materials

Commercial CMC was purchased from Sigma Aldrich, USA. Polyethylene glycol and tween 80 was acquired from Merck, Germany. Leaves of *A. sieberi* were provided from a local market (Kashan, Iran) in 2014 and verified at the Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran. AEO were extracted from the dried samples by hydro-distillation using a Clevenger apparatus, and the obtained AEO stored in a dark container at 4 °C until use.

Gas Chromatography-Mass Spectrometry (GC-MS)

GS-MS analyses of AEO were carried out on a Varian 3400 GC-MS (Agilent, USA) system equipped with a DB-5 fused silica column (30 m×0.25 mm, film thickness 0.25 μm; J and W Scientific Corporation). An initial oven temperature of 60 ºC was held for 4 min, then increased at a rate of 4 ºC/min to 220 ºC, and held for 15 min. Other operating conditions were as follows: carrier gas, helium at a linear velocity of 31.5 cm/s, split ratio 1:60, ionization energy 70 eV, scan time 1 s, and mass range 40-300 amu (Ariaii et al., 2015).

Film preparation

The method of Martelli et al. (2017) was applied to prepare CMC films. In brief, 33% (w/w) of the CMC was dissolved into distilled water and ethanol at a ratio of 2:1, with simultaneous rotary shaking for 30 min. As the edible CMC film was brittle, 33% of polyethylene glycol was added to the edible film solution. Then, Tween 80 (0.2% v/v of AEO), was added to aid EO dissolution in the film-forming solution. After 30 min of stirring, AEO at 0.5, 1, and 1.5% (v/v) concentrations was added to the CMC film-forming solution. The solution was homogenized using an Ultra-Turrax homogenizer (IKA T25-Digital Ultra-Turrax, Staufen, Germany) at 7000 rpm at room temperature for 2 min (Ojagh et al., 2010). The

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solution was kept overnight at 4 °C in order to remove all bubbles. Each film solution (14 g) was poured on glass plates, dried at room temperature, then collected from the plates, stored in a desiccator at 25-27 °C, and evaluated after receiving 50±2% Relative Humidity (RH).

Antibacterial activity

Antibacterial properties of the edible film solutions were studied using the agar diffusion method. Two different pathogenic as well as spoilage bacteria, viz. *Staphylococcus aureus* PTCC 1431 and *Escherichia coli* PTCC 3315 were used. Briefly, plates were seeded with 0.1 ml of an overnight broth culture, containing approximately 10^6-10^7 Colony Forming Unit (CFU)/ml of the bacteria. Film solutions (40 µl) were poured into 6 mm diameter Mueller-Hinton (Scharlau Chemie S.A., 08181 Sentmenat, Spain) agar wells. Next, the plates of the bacteria were incubated at 37 ˚C for 24 h. The inhibition zone was measured with a caliper and recorded in millimeter. Experiments were conducted in triplicate (Bahram et al., 2014).

Physical properties of films

- *Thickness*

Film thickness was determined using a handheld 0.001 mm digital micrometer (Mitutoyo, Mizonokuchi, Japan) at five random locations of the film sheets. Mean thickness values for each film sample were computed and used in Water Vapor Permeability (WVP) and Tensile Strength (TS) properties calculations (Bahram et al., 2014).

- *Contact Angle (CA)*

In a conditioned room, CA measurement was specified by a Goniometer (PG-X, Thwing-Albert Instrument Co., NJ). A small drop of distilled water was poured onto the surfaces of the edible films. Five measurements were done for each type of film (Ojagh et al., 2010).

- *Moisture Content (MC)*

MC of film samples was determined by measuring weight loss of the films (50 mg), upon drying in an oven at 110 °C during 24 h. After determining the weight loss of film samples, the MC was calculated as the percentage of water lost from the system (Bahram et al., 2014).

- *Solubility in water*

Each type of film was cut into pieces of 1-3 cm^2 and weighed to the nearest 0.0001 g. To measure water solubility (%) of the different films, they were immersed in 50 ml of distilled water at 25 °C under constant stirring for 6 h. After immersion, the remaining pieces of film samples were dried at 110 °C to constant weight (final dry weight). The initial dry weight was determined by thermal processing at 110 °C to constant weight (Ojagh et al., 2010). Water solubility (%) of films was calculated by the following formula:

$$\text{Solubility in water} = \left(\frac{\text{Initial dry weight} - \text{Final dry weight}}{\text{Initial dry weight}}\right)$$

- *WVP*

The film samples were sealed in glass cells containing anhydrous calcium chloride and 0% RH. A desiccator maintained RH gradient across the edible film at 100%. The air was stirred in the desiccator to retain uniform RH all over thereof. Transported water vapor was determined from the weight gain of the diffusion cell at a steady state of transfer. Weight changes of the cells were noted to the nearest 0.0001 g and drown as a function of time. To gain the Water Vapor Transmission Rate (WVTR), the effective film area was applied to divide the slope of the weight loss *vs* time (Martelli et al., 2017). The WVP was computed by the following formula:

$$WVP=(WVTR\times L)/\Delta P)$$

where L is the film thickness (mm) and ΔP is the difference in the water vapor pressure between the two sides of the film.

- *Surface color*

The color of the films was measured using a colorimeter (BYK Gardner, USA) with some modifications (Ojagh et al., 2010). Measurements are expressed as lightness (L*), chromaticity parameters a* (red/green), and b* (yellow/blue). Film samples were placed on a standard plate (L*=93.49, a*=-0.25 and b*=-0.09). Total color differences (ΔE) were computed with regard to standard plate parameters by the following formula:

$$\Delta E = \sqrt{(L* - L)2(a* - a)2(b* - b)2}$$

Mechanical properties

Elongation at break% (E%) and TS were tested using Instron Universal Testing Machine (Model 200; Hiwa Engineering Co., Tehran, Iran). The edible films were cut in rectangular samples (2.54×10 cm). Initial grip separation and crosshead speed were put at 50 mm and 50 mm/min, respectively. The measurements were repeated five times (Martelli et al., 2017).

Film microstructure

All the gold-coated film surfaces were scanned with Philips XL 30 Scanning Electron Microscope (SEM; Philips Research, Eindhoven, the Netherlands) under

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high vacuum condition at an electron voltage of 20.0 kV (Bahram et al., 2014).

Statistical analysis

The analysis was based on a completely randomized design using ANOVA by SPSS statistical software (version 16.0 for Windows, Chicago, IL, USA). Duncan's new multiple range test range test was used to compare the means of film characteristics at $p<0.05$. All data are presented as mean±standard deviation.

Results

Fourteen components were identified representing 95.32% of AEO (Table 1). Camphor (36.38%), 1,8-cineole (15.89%), β-Thujone (6.7%), and camphanone (6.2%) were the main components of AEO.

As a result of the CMC film solution, the control (film solution without AEO) did not show inhibitory effects against *E. coli* and *S. aureus*. The AEO-containing CMC film solution had antibacterial properties against both bacteria. The inhibition zones were 9.33, 11.5, and 17.30 mm for *S. aureus*; and 8, 11.50, and 14.33 mm for *E. coli* at AEO levels of 0.5, 1, and 1.5%, respectively. The antibacterial property of the film solution increased significantly ($p<0.05$) with increasing AEO concentration; however, they were more effective against the Gram-positive than the Gram-negative bacterial strains. *S. aureus* was the most sensitive strain at an AEO level of 1.5% with an inhibition zone of 17.30 mm.

Physical properties of CMC films incorporated AEO are shown in Table 2. The thickness of the different films did not change significantly ($p>0.05$), ranging from 0.028 to 0.033 mm. The control film had a high MC value (20.94%), and addition of AEO at levels of 0.5, 1, and 1.5% v/v of CMC film formulation decreased MC to 17.49, 15.23, and 12.33%, respectively ($p<0.05$). The results for WVP of different films were in agreement with those of the CA, with WVP values of 3.2 and 1.5% 4.72 (g.mm/kPa.day.m^2) in the CMC film and CMC+AEO, respectively. In fact, WVP in CMC+AEO 1.5% was significantly higher than the other films ($p<0.05$). Hunter lab color values (L*, a*, and b*) and total color difference (ΔE) were used to express optical properties. CMC film with 1.5% of AEO showed the highest a* (greenness) and b* (yellowness) values, and the lowest L value (Table 3).

Table 4 displays the mechanical properties of CMC films, showing that the addition of AEO to films resulted a significant increase in TS ($p<0.05$). However, different concentrations had no significant effects on the TS films ($p>0.05$). TS values of 23.8 MPa and 19.36-19.56 MPa were obtained for the CMC films and those containing AEO, respectively. Also, adding AEO to the film led to a significant increase in E ($p<0.05$). Furthermore, E values rose significantly ($p<0.05$) with increasing concentrations, with E values of 4.48 as well as 8.40 for the CMC films and the CMC+1.5% AEO films, respectively.

Table1: Percentage of chemical compounds identified in *Artemisia sieberi* essential oil studied by Gas Chromatography-Mass Spectrometry

Peak	Compound	GC area (%)
1	Camphor	36.38
2	1,8-cineole	15.89
3	β-thujone	6.7
4	camphanone	6.2
5	α-thujone	5.4
6	Cyclopropane carboxylic acid	5.2
7	4-methyl-1-1-methylethyl	4.5
8	3-thujanone	4.3
9	Camphenebicyclo heptane	3.2
10	Endopenyl acetate	2.4
11	propanal	2.2
12	α-pinene	1.4
13	ß-pinene	0.95
14	Myrtenol	0.6
	Total	**95.32**

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Table 2: Physical properties of carboxyl methylcellulose films incorporated with *Artemisia sieberi* essential oil

Physical properties*	AEO conc. (v/v) in carboxymethyl cellulose film (%)	
Thickness (mm)	Control	0.028±0.003 [a]
	0.5	0.031±0.002 [a]
	1	0.031±0.002 [a]
	1.5	0.033±0.005 [a]
Contact angle	Control	51.83±3.38 [c]
	0.5	53.61±2.34 [b]
	1	55.20±1.98 [b]
	1.5	64.88±3.78 [a]
Solubility (%)	Control	76.56±1.43 [a]
	0.5	74.63±0.89 [ab]
	1	72.56±1.12 [ab]
	1.5	70.34±0.88 [b]
Moisture content (%)	Control	20.94±1.11 [a]
	0.5	17.49±0.87 [b]
	1	15.23±1.11 [c]
	1.5	12.33±1.05 [d]
WVP (g/msPa) 10^{-10}	Control	3.2±0.15 [c]
	0.5	3.35±0.18 [c]
	1	4.1±0.08 [b]
	1.5	4.72±0.07 [a]

* Means in each column with different superscript letters are significantly different (p<0.05).

Table 3: Surface color of carboxyl methylcellulose films incorporated with *Artemisia sieberi* essential oil

Surface color*	AEO conc. (v/v) in carboxymethyl cellulose film (%)	
L	Control	77.98±0.52 [c]
	0.5	74.91±0.82 [bc]
	1	71.93±1.41 [ab]
	1.5	64.96±1.4 [a]
a	Control	-0.17±0.02 [c]
	0.5	-0.17±0.02 [cbc]
	1	-0.13±0.01 [ab]
	1.5	-0.1±0.01 [a]
b	Control	0.99±0.08 [b]
	0.5	1.07±0.05 [b]
	1	1.43±0.08 [a]
	1.5	1.46±0.06 [b]
ΔE	Control	0.34±0.01 [a]
	0.5	0.23±0.01 [b]
	1	0.21±0.02 [bc]
	1.5	0.19±0.01 [c]

* Means in each column with different superscript letters are significantly different (p<0.05).

Table 4: Mechanical properties of carboxyl methylcellulose films incorporated with *Artemisia sieberi* essential oil

Mechanical properties*	AEO conc. (v/v) in carboxymethyl cellulose film (%)	
Tensile strength (MPa)	Control	23.8±1.26 [b]
	0.5	19.36±1.17 [a]
	1	19.62±0.69 [a]
	1.5	19.56±0.48 [a]
Elongation at break (%)	Control	4.48±0.45 [c]
	0.5	5.1±0.92 [c]
	1	6.96±0.63 [b]
	1.5	8.4±0.85 [a]

* Means in each column with different superscript letters are significantly different (p<0.05).

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Microstructure of CMC-based films was studied with micrographs of the CMC and CMC incorporated with AEO (Figures 1-a, b, c, and d). By adding AEO at a concentration of 0.5% (Figure 1-b), the surface-profile of the film did not show much difference with the control film (Figure 1-a). The films had a smooth surface without cracks. Pores were observed on the surface of the films by adding AEO at a concentration of 1% (Figure 1-c); increasing AEO to a level of 1.5% resulted in cracks and heterogeneity in the film structure (Figure 1-d).

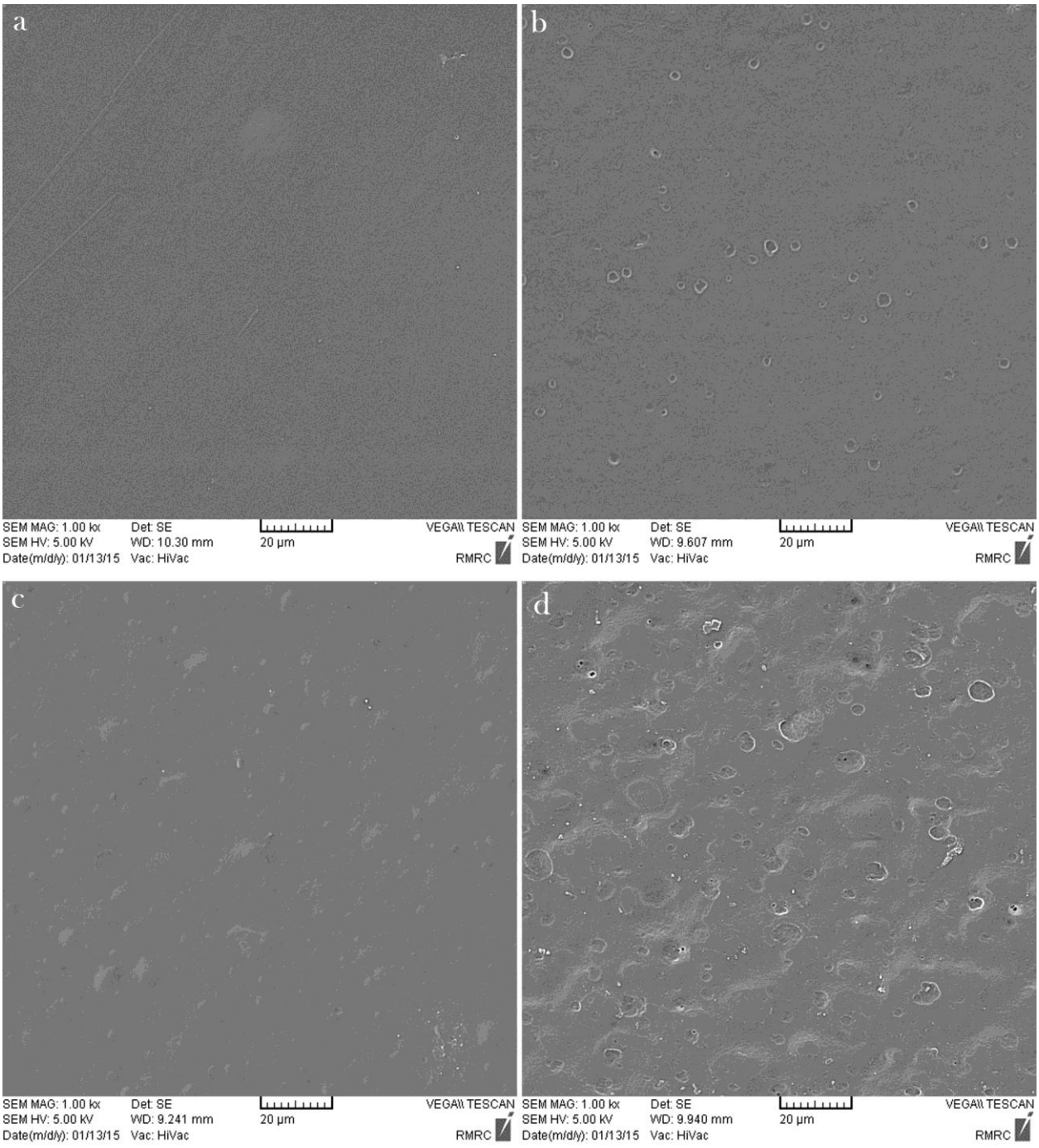

Figure 1: Microstructure of AEO incorporated CMC film by scanning electron microscope. (a) control, (b) 0.5%, 1%, and (c) 1.5% (d)

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Discussion

According to the results of this study, camphor (36.38%) and 1,8-cineole (15.89%) were the main components of AEO. Previous researches have shown that bornane derivatives (camphor, bornyle acetate, and borneol) and 1,8-cineole are the main components of *Artemisia* (Ghasemi-Pirbalouti et al., 2013; Lopez-Lutz et al., 2008; Rabiei et al., 2012). Our results are in accordance with those of Rabiei et al. (2012) and Assarzadeh et al. (2013) who analyzed *A. sieberi* grown in Iran. This agreement could be due to the similarity of seasonal or environmental factors at the time of harvest which may affect the components of plant EOs.

The film solution without AEO (control) did not show inhibitory effects against both *S. aureus* and *E. coli*, hence it needs to be combined with other substance such as EOs. This is in line with report of Dashipour et al. (2014) who revealed that CMC edible film could not inhibit the growth of bacteria, but incorporation of clove EO with CMC film was effective against *S. aureus* and *E. coli*. Our CMC film solution containing AEO showed antimicrobial activities against both tested bacteria. This can be explained by the fact that the addition of AEO to CMC resulted in diffusion through the agar gel and provided a clear zone surrounding the film solution.

The antimicrobial activity of each EO is related to its chemical components. Some components of *A. sieberi*, such as camphor and 1,8-cineole, have shown antibacterial properties (Lopez-Lutz et al., 2008; Mahboubi et al., 2015). The AEO-containing film solutions were considerably more effective against Gram-positive (*S. aureus*) than Gram-negative (*E. coli*) bacterial strains, and the inhibitory effect increased with increasing AEO concentrations. The results are in agreement with those of Mahboubi et al. (2015) who reported that AEO had excellent antimicrobial activities against *S. aureus* and *E. coli*. Also, Hedayati-Rad et al. (2013) showed that incorporation of AEO with pullulan film could inhibit the growth of these two bacteria. They reported that *S. aureus* was more sensitive than *E. coli*, which was attributed to the difference in the cell wall structure of the two bacteria. The cell wall of Gram-negative bacteria is complex and lipopolysaccharide is the essential constituent avoiding oil accumulation on the cell wall; instead, *S. aureus* has single cell layer making it be more sensitive (Dashipour et al., 2014; Hosseini et al., 2009).

The thickness of films did not change significantly by the incorporation of AEO, which is associated with the film casting technique. It is an important parameter for the calculation of mechanical and barrier properties. Siracusa et al. (2018) observed similar results for active edible films based on alginate and pectin with citral EO. These researchers reported a slight variation of thickness within different formulations and attributed it to the film casting technique, method of film preparation, the flatness of the dish surface, as well as film formation during drying. The value of CA with water indicates hydrophobicity of the film surface, which is generally used to estimate the resistance of the films to liquid moisture transfer. High values of the CA are typical of a hydrophobic surface, while low values are characteristic of a hydrophilic surface (Siracusa et al., 2018). According to Table 2, the control film had a low water CA (51.83°). Adding AEO increased water CA significantly to 64.88° at an AEO level of 1.5% v/v. AEO, therefore, increased the hydrophobicity of the CMC film, which may be due to hydrophobic nature of EO and hydrophilic functional groups of CMC film (amino and hydroxyl groups) covered with AEO (Ojagh et al., 2010).

In the current study, the MC and solubility values decreased as AEO was incorporated into the CMC based film, which may be related to the compactness of film network. The decrease in MC and solubility value of CMC film in the presence of AEO could be attributed to the ability of AEO in disrupting the structure of CMC film, interaction between the components of AEO, hydroxyl groups of CMC, and producing a new structure with hydrophobic properties. In other words, the incorporation of AEO increased the hydrophobicity of CMC films as confirmed by the CA test, showing an increase with rising AEO concentrations in the CMC film. Dashipour et al. (2014) revealed reduction in the MC and solubility of CMC film by incorporating clove EO. Hydrophobicity was mentioned to be the reason for this phenomenon. Another research showed that the addition of cinnamon EO into chitosan film decreased MC and solubility (Hosseini et al., 2009). By the formation of covalent bonds between the functional groups of chitosan chains, cinnamon EO decreased the availability of hydroxyl and amino groups resulting in decreases of MC and solubility of films.

Impeding the moisture transfer between food and the surrounding atmosphere or between two components of a heterogeneous food product is one of the main functions of edible films. Therefore, WVP should be as low as possible (Alves et al., 2007). We found that WVP increased from 3.2 to 4.72 (g.mm/kPa.day.m^2) at an AEO concentration of 1.5%. Many factors are important in WVP, including the ratio of hydrophilic and hydrophobic groups, tortuosity porosity, and cracks in the structure of the edible film. Although, substitution of hydrophobic ester groups by AEO instead of hydrophilic OH groups in CMC film could increase hydrophobicity, it does not always reduce the WVP; because the permeability is influenced by the steric hindrance channels through the matrix (Cheng et al., 2008) as confirmed by SEM micrographs. In some previous studies, meaningful decreases

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were found in WVP of edible films containing EO (Bahram et al., 2014; Sánchez-González et al., 2009), which were ascribed to the hydrophobicity of films. In contrast, some other researchers observed increase of WVP when an EO was added to edible films (Dashipour et al., 2014; Ghanbarzadeh and Almasi, 2011; Hosseini et al., 2009). These authors suggested that the existence of pores, cracks, and channels might be related to the volatility of the EO. These cracks were filled by EO that was evaporated from the surface of the film.

Color of the packaging materials is an important factor in terms of consumer acceptance of food products (Bourtoom and Chinnan, 2008). Based on the present investigation, the control film appeared clear, transparent, and colorless (the highest "L" value), but the addition of AEO affected the appearance and the color of CMC film with reduced transparency, which could probably be related to the phenolic compounds of AEO. Our result is consistent with two similar studies carried out regarding unicorn leatherjacket EO incorporation into gelatin films (Ahmad et al., 2012), and *Satureja hortensis* EO incorporation into k-carrageenan films (Shojaee-Aliabadi et al., 2013). Both studies suggested edible films incorporated with EO to be more opaque and darker than control films, and concluded that it might be because of phenolic compounds of EOs.

In order to protect the integrity of foods, mechanical properties represent the biodegradability or edible ability of films. The TS value (23.8 MPa) of CMC control film reduced by increasing AEO concentrations. The TS loss is related to the breakup of film network resulted from the addition of EO and partial replacement of weaker polymer-oil interactions instead of stronger polymer-polymer interactions in the film matrix (Hosseini et al., 2009; Shojaee-Aliabadi et al., 2013). The results are consistent with those of Hosseini et al. (2009) who reported that increasing concentration of thyme and clove EOs decreased TS of chitosan film. We found that the E% value (4.48%) in control film increased significantly by the incorporation of AEO (1.5% v/v) into CMC films, thereby making the films more flexible, elastic, and extensible. Our findings correspond to some previous studies (Cheng et al., 2008; Ghanbarzadeh and Almasi, 2011) that showed TS and E% values had reverse manners. These observations, however, are different from that of Dashipour et al. (2014) who reported that the addition of clove EO to CMC film decreased the elongation value due to a strong interaction between CMC film and EO. This discrepancy may be related to the type of EO, percentage of polymer, solvents, and other factors such as temperature and humidity affecting the preparation of such films (Bahram et al., 2014).

When control films were compacted, pores or cracks were not formed on the CMC film surface (Figure 1). A similar structure was reported by Ariaii et al. (2015) who found smooth and homogeneous surface on methylcellulose films. Dashipour et al. (2014) also observed a flat surface without pores and cracks on CMC films. However, when AEO was added into the CMC film, especially at higher concentrations, the microstructure was changed considerably and the films containing AEO showed a more pores on the surface, with a cracked and heterogeneous structure, which may be attributed to the evaporation of the AEO during the drying process of the film. In addition, it has been stated that the time of drying is important in determining the component arrangement during the formation of film and the final microstructure of the edible film (Siracusa et al., 2018).

Conclusion

AEO was successfully incorporated into CMC, which showed good physical properties as well as effective antimicrobial efficacy against *E. coli* and also *S. aureus*. This could have additional advantages such as antimicrobial effects without direct EO application on food products, requiring low doses of EO. In addition, the films containing AEO have a good potential for such applications as active packing materials. Nevertheless, it needs to be tested on selected food systems in the future researches.

Author contributions

S.B. designed the project of study and analyzed the data; F.S.B. conducted the experiments; S.B. and P.A. wrote the manuscript. All authors revised and approved the final manuscript.

Conflicts of interest

The authors declared no conflict of interest.

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