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Physical, Antioxidant and Antimicrobial Characteristics of Carboxymethyl Cellulose Edible Film Cooperated with Clove Essential Oil

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Abstract

***Background*:** Carboxymethyl cellulose (CMC) is one of the most common cellulose derivatives. It has many applications such as edible films and coating. Antioxidant and antimicrobial of essential oils and their direct or indirect usage in foods have been investigated. This study focuses on the physical, chemical, antioxidant and antimicrobial characteristics of CMC edible film incorporated with clove essential oils (CEO).

***Materials and Methods*:** In this experimental study, CMC edible films with or without clove essential oil were prepared by casting method and many characteristics such as thickness, water vapor permeability (WVP), tensile strength, elongation at break, optical characteristics, microstructure, antimicrobial and antioxidant properties of the films were assessed.

***Results*:** Tensile strength values were higher when compared with those of control film (pure CMC film), especially in 1% EO concentration. Elongation at break value in 1% EO was higher than control film, but by increasing of EO portion, it decreased. Antioxidant properties and total phenolic compounds as expected increased in higher concentration of EO. Antimicrobial properties of the films showed that films incorporated with EO are effective against selected pathogen bacteria, especially in the higher concentration of EO. Some variations in the structure of various films were shown by scanning electron microscopy (SEM). Additions of EO into CMC film disrupted condense structure of film and produced a heterogeneous structure.

***Conclusion*:** As antimicrobial and antioxidant properties of CEO retain when it used in CMC edible film, it could be beneficial in food packaging to retard of deterioration.

Introduction

In the past years using of petroleum polymers on the packaging has been widely increased [1]. In the recent decades, extensive investigation into biodegradable films or coatings prepared from polymers such as polysaccharides, lipids and proteins or their combinations were investigate [2]. Edible films and coating are biodegradable and protect food products and prevent of quality deterioration and increase their shelf-life [3]. One of the most common cellulose derivatives that used in edible films and coating is CMC. It has some desirable characteristics such as water-soluble, odorless, tasteless, high viscosity, non-toxic, non-allergenic, flexible, moderate strength, transparent, resistance to oil and fats, moderate to moisture and oxygen transmission [4, 5].

Synthetic and natural antimicrobial or antioxidant substances including organic acids, enzymes, bacteriocins and essential oils [6] are used to prevent and decrease the food deterioration. Some side effects that related to the using of synthetic additives such as carcinogenic effects in the living organisms are the reasons that consumers prefer natural additives into food products [7, 8]. On the other hand, a trend towards 'green' consumerism, using of synthetic food additives decreased and products that have less environmental waste-disposal problems were increased [9]. Antioxidant and antimicrobial activity of essential oils (EOs) have been demonstrated. The majority of EOs that are used as flavor additives in the food are classified as generally recognized as safe (GRAS) [10] and obtained from different parts of plant materials. They are aromatic oily liquids with mixtures of several components deleted. Today the possibilities of adding them into the foods in order to prevent or retard the spoilage have been investigated [11-13]. Direct applications of EOs effective doses are often limited because led to organoleptic deterioration or rapid diffusion and partial inactivation. An alternative way to prevent of this problem is the incorporation of essential oil within edible films [14-17]. Therefore, decreased the amounts of EOs to achieve the target shelf life and fallow it unlike flavor decreased [18]. Cloves are the aromatic dried flower buds of a tree in the family of Myrtaceae, *Syzygium aromaticum*. They are native to the Maluku Islands in Indonesia and used as a spice in culinary over the world. Moreover, clove oil has many uses as dental analgesic, numbing, carminative, stimulant and antiseptic [19]. The major components of (clove essential oils) CEO

which have antioxidant and antimicrobial characteristics are phenolic compounds, especially eugenol and eugenyl acetate [20]. The aim of this study was to test the antimicrobial and antioxidant activities of CEO incorporated into CMC film, and determine the physical and chemical properties of these films in various CEO concentrations.

Materials and Methods

In this experimental study, carboxymethyl cellulose (CMC) with average molecular weight 41,000 (practical grade) was purchased from Caragum Parsian Co. (Tehran, Iran). CEO supplied by Barij Essence Pharmaceutical Co, (Kashan, Iran), Glycerol, Tween 80 (analytical grade), Mueller–Hinton agar (MHA) and Mueller–Hinton Broth (MHB) were purchased from Merck Co. (Darmstadt, Germany). All other reagents used were of analytical grade.

Preparation of films: CMC based films incorporated with CEO were prepared by the method of Shojaee-Aliabadi [20] with some modifications. Preliminary experiments showed that how much ratio of CMC and glycerol are needed to achieve a film that easily peeled off from the plate. One gram of CMC slowly dissolved in 100 mL (1% w/v) of distilled water meanwhile continues magnetic stirring at 70ºC. The clear film solution was achieved after about 45 min. Then, under continues magnetic stirring 0.5 mL (50% v/w) glycerol (based on CMC weight) as plasticizer was added and stirring continued for the further 10 min at 70ºC. Emulsions of CMC incorporated with essential oil were obtained by adding concentrations of 1, 2 and 3% (v/v) CEO and Tween 80 as emulsifier in 0.1%, 0.2% and 0.3% (v/v) based on essential oil. Rotor-stator Homogenizer (IKA T25-Digital Ultra Turrax, Staufen, Germany) was used in emulsion homogenization at 13,500 rpm for 3 min at 70ºC. The emulsions were cooled to 55ºC and remained for a while to exhaust air bubbles constituted during homogenization, as possible. Glass plates with 15 cm diameter (about 177 cm^2), leveled and rimmed circular were used for preparation films. The 50 mL of the film-forming dispersions (FFDs) was cast in the center of glass plates and dried at 35ºC for about 30 h (it was varied 24-40 h based on the kind of FFDs). Dried films peeled off from the glass plates and stored inside desiccators at 25ºC and 53% relative humidity (RH) until evaluation. Saturated magnesium nitrate was used to achieve this RH. FFDs without any essential oils were also prepared as control film. Homogenization and Tween 80 were not used in control film preparation. Thickness: Film thicknesses were measured with a handheld micrometer (Mituto, Tokyo, Japan) having a sensitivity of 0.001 mm. Measurements were made at different points (at least seven random locations) and results reported as mean and standard deviation for each film sheet.

Moisture content: One gram of each sample film was dried in an oven at 110ºC until constant weight was reached. The water content was calculated as: % water content $= \frac{Wo-Wf}{Wo} \times 100$ (1)

Where Wo was the sample weight before drying, Wf was its weight after drying. Three replications of each film measurement were performed

Water vapor permeability (WVP): WVP tests were carried out at 25ºC and 75% relative humidity (RH) gradient according to the standard ASTM method E96 with some modifications [21]. About one third of the volume of the circular cups with 5 cm diameter and 8 cm depth were placed of a hydrous calcium chloride (0% RH). They were covered by the circle films with a diameter slightly larger than the diameter of the cup. Each cup was placed in desiccators with sodium-chloride-saturated solution (Merck, Darmstadt, Germany) that maintained at 75% RH. The difference of RH induced driving force of 1753.55 Pa and water transports into the cups. The desiccators were kept at 25ºC. Cups were weighed in initially and every 24 h (with an accuracy of 0.0001 g). The slope weight gains of the cup vs. time were calculated by linear regression. The water vapor transmission rate (WVTR) was defined as the slope (g/h) divided by the transfer area (m^2). WVP (g m-1 h-1 Pa-1) was calculated as:

$$WVP = \frac{WVTR}{P(R1-R2)}X \qquad (2)$$

Where WVTR is water vapor transmission rate while P is the saturation vapor pressure of water (Pa) at the test temperature (25ºC), R1 and R2 are RH inside and outside the cup, respectively and X is film thickness (m). In these conditions, driving force [P (R1-R2)] is 1753.55 Pa. All measurements were performed in triplicates [22].

Solubility in water: The percentage of the dry matter of film which solubilized after a time immersion in the water considered as solubility in water [23]. In this study the water solubility was assessed based on method of Ojagh et al. [24] with some modifications. Pieces of film (1×3 cm) prepared of each film and dried at 110ºC to reach constant weigh, after then dried film weighed to the nearest 0.0001 g. Pieces immersed in 50 mL of distilled water for 6 h at 25ºC under constant agitation. Whatman filter paper NO 3 (that reached a dry constant weight before) was used to separate unsolved pieces film from the water. Filter papers and remained pieces on filter papers were placed back at 110ºC to reach constant weight. The equation 3 was used to calculate film water solubility (WS) percentage. WS (%)=[(Wo-Wf)/Wo]×100 (3) Where Wo is the initial dry weight of the film sample and Wf is dry weight of unsolved film.

Mechanical properties: The mechanical properties including tensile strength (TS) and elongation at break (ELB) of the film samples were evaluated according to ASTM standard method D882 [25] by Testometric Machine M350-10CT (Testometric Co., Ltd., Rochdale, Lancs., England). Film strips (1.5×10 cm) were cut from each of the samples that prior to testing were equilibrated at 25ºC and 53% RH in desiccators containing Mg $(NO3)_2$ saturated solutions for 48 h. Film strips were mounted

between the grips. The initial separation of 50 mm was used and the crosshead speed was set at 10 mm/min with 500 N load cells.

Optical properties: The color of samples includes "L" value, that indicate the lightness [black (L=0) and white (L=100)], "a" value that indicates redness–greenness [total red (a=100) and total green (a=-100)] , and "b" value that indicates yellowness–blueness [total yellow (b=100) and total blue (b=-100)] were measured by the Minolta Chroma Meter CR-400 (Minolta Co., Ltd, Japan). Yellowness index (YI) achieved by YI=(145.86*b)/L equation [26]. White standard background (L*=93.49, a*=-0.25 and b*=-0.09) was used as a standard. Before evaluation, films conditioned at 25ºC and 53% RH for 48 hours in a desiccator containing Mg $(NO3)_2$ saturated solutions. Total color difference (ΔE) and whiteness index (WI) were calculated using the equations 4 and 5, respectively.

$$\Delta E = \sqrt{\left(L^* - L\right)^2 + \left(a^* - a\right)^2 + \left(b^* - b\right)^2} \quad (4)$$

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \quad (5)$$

Where L, a, and b are the color parameter values of the film and L*, a*, and b* are the color parameter values of the standard.

Scanning electron microscopy (SEM): Surface and cross-sections microstructure of the films were evaluated by scanning electron microscopy (SEM) (Model: KYKY-EM3200, China). Film samples were attached to double-sided adhesive tape and assemble on the specimen holder and sputtered with gold coating under vacuum with 100 A thicknesses by sputter coater (Model: KYKY-SBC12, China). The images of coated film samples were captured with an accelerating beam voltage of 25 kV.

DPPH radical-scavenging activity: The radical scavenging activity of the films incorporated essential oils were measured by the method of Brand-Williams et al. [27]. This test was based on the hydrogen atom or electron donation abilities and evaluated by measuring the colorimetric changes (from deep-violet to light-yellow) on methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent [28].

Briefly, 25 mg of each film sample was dissolved in 5 mL of distilled water by continuous stirring, and 0.1 mL of film extract solution was added to 3.9 mL of the DPPH solution (0.1 mM methanol solution) and incubated in a dark place at ambient temperature for 60 min. The absorbance was measured against Methanol (blank) at 517 nm and the percentage of DPPH radical scavenging activity was achieved by following equation:

DPPH scavenging activity (%) = (Ablank-Asample/ Ablank)×100(6) Where A is the absorbance.

Total phenolic (TP) compounds content: TP content of the films incorporated with or without essential oils was measured by Siripatrawan and Harte method [29]. Briefly, 25 mg of each film sample was added into the 5 mL of distilled water, then 0.1 mL of extract solution, 7 mL distilled water, and 0.5 mL Folin-Ciocalteu reagent were mixed together and stored at room temperature for 8 min then 1.5 mL sodium carbonate (2%, w/v) and distilled water were added to obtain a final volume of 10 mL. The mixture was stirred thoroughly and kept at room temperature for 2 h. UV Spectrophotometer (Shimadzu UV-VIS 1601, Japan) was used to absorbance reading at 765 nm distilled water that considered as blank. The equation (7) was used to calculate mg gallic acid equivalents (GAE mg/g) per gram of dried film.

T=CV/M (7) Where T is the total content of phenolic compound (mL/g dried film, in GAE), C is the concentration of gallic acid obtained from the standard calibration curve (mg/mg), V is the volume of film extract (mL) and M is the weight of dried film (g).

Bacterial strains: Strain bacteria of *Staphylococcus aureus* ATCC 25923; *Bacillus cereus* PTCC 1154, *Escherichia coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhimurium* ATCC 14028 were purchased by Iranian Research Organization for Science and Technology (Tehran, Iran). Mueller Hinton agar (MHA) was used to preparation of stock cultures bacteria at 37ºC for 24 h before the tests.

Disc diffusion method: Antimicrobial activity of the film samples was determined by using the agar diffusion method. Film samples with 10 mm diameter were aseptically cut into disc shape and then placed on MHA plates, which had been previously smeared with 100 µL of broth culture containing approximately 10^8 CFU/mL of some food pathogens.

The plates were incubated at 37ºC for 24 h. Diameter of inhibition zone was measured by using a caliper to the nearest 0.02 mm. As diameter of inhibition zone was not same in the entire circle, a mean of at least three diameters for each circle considered as diameter of inhibition zone. Antimicrobial index [30] of the film samples was measured based on the total area by using the equation below:

$$\text{Antimicrobial index} = \frac{\text{Area of inhibition zone} - \text{Area of film}}{\text{Area of film}} \quad (8)$$

Statistical analysis: Each experiment repeated at least three times. Descriptive analysis was used to express mean and standard deviation, and Analysis of variance (ANOVA) followed by the Duncan's multiple range test was used to determine any significant differences among the treatments at a 95% confidence level. SPSS-16 statistical software (SPSS Inc., Chicago, IL) was used.

Results

Film thickness: Film thickness varied from 0.034 to 0.09 mm (Table 1). The lowest thickness was achieved in film prepared without EO, and by increasing of EO concentration in the film structure, thickness was

increased and the highest thickness was found in film with 3% EO. These variations were significant (p<0.0001).

Moisture content: Incorporation of EO into the CMC matrix was able to decrease (p<0.0001) the moisture content percent of the films as shown in table1.

Water solubility: Determination of water solubility in control film was impossible based on a method that mentioned before, because after immersion in water produced a gel that not be filtered with Whatman filter paper No.3. This phenomenon was found slightly in film incorporated 1% of EO. In other films by increasing of concentration, water solubility decreased.

Water vapor permeability (WVP): WVP is an important property for films which is used as edible food coatings, because most natural biopolymers are very sustain to water absorption [31]. As shown in table 1, the WVP of the CMC film was $3.02\pm0.28\times10^{-7}$ (g Pa^{-1} $h^{-1}m^{-1)}$ and by increasing of EO portion in the films, WVP increased. The films containing 3% (V/W) EO showed the highest WVP value and differences were significant (p<0.0001).

Mechanical properties: Table.1 shows the effect of various percent of essential oil incorporation in CMC-based film on the mechanical properties. Variation in TS and ELB were found from 18.21 MPa to 51.34 MPa and 10.35% to 31.04% respectively. TS and ELB drastically increased when 1% essential oil was used in CMC-based film. Although in higher concentration of EO TS decreased, they were higher than pure CMC film. The differences were transported to discussion.

Color: Hunter Lab color values ("L", "a" and "b"), total color difference (ΔE), whiteness (WI) and yellowness (YI) values were used to express optical properties. The results have shown in table 2. CMC film without essential oil was colorless, clear and has a transparent appearance (highest "L" value), but the incorporation of EO in the CMC film led to the decrease of clarity and transparency. "L" value showed significant difference between the control (without EO) and 3% CEO film (p=0.017). CMC film with 3% CEO showed the high negative "a" (greenness) and positive "b" (yellowness) values (-0.07 and 2.17, respectively). "a" value among the control and incorporated of EO films were significant (p<0.0001).

Films Microstructure: The scanning of electron micrographs of CMC and CMC incorporated with 1 to 3% (v/v) EO films, in surface (S) and cross section (Cr), are shown in the right and left in figure 1 respectively. As shown in figure 1, CMC film is compact with flat surface without pores or cracks. However, when EO was added into the CMC film, especially at higher concentrations, the microstructure was changed considerably and in surface and cross section, the films containing EO showed a more heterogeneous structure.

Total phenolic (TP) compounds: The Folin–Ciocalteu reagent assay is the base of methods that used in determination of total phenolic compounds and the results of total phenolic compounds were described as milligrams of gallic acid equivalent (mg GAE) per mL of extract. As shown in figure. 2a, the lowest TP content with 0.23 mg GA/g, was found in control film (pure CMC film) and the highest TP content was 16.16 mg GA/g that achieved in film with 3% CE. Results showed that although the interaction between matrix (CMC) and EO could be occur, phenolic compounds are available and the amount of EO concentration, phenolic compound content increased significantly.

DPPH radical scavenging activity: Figure 2B shows that the CMC film has the lowest antioxidant activity (0.32%) and the increase in CEO portion in the CMC film led to the increase of antioxidant activity and 3% CEO incorporated film has the highest antioxidant activity (71.76%).

Antimicrobial activity: As table 3 shows all films containing CEO have bacterial inhibitory effect when compared with CMC film and in higher concentration, inhibitory effect increased, although they were more effective against positive bacteria, especially *Staphylococcus aureus*.

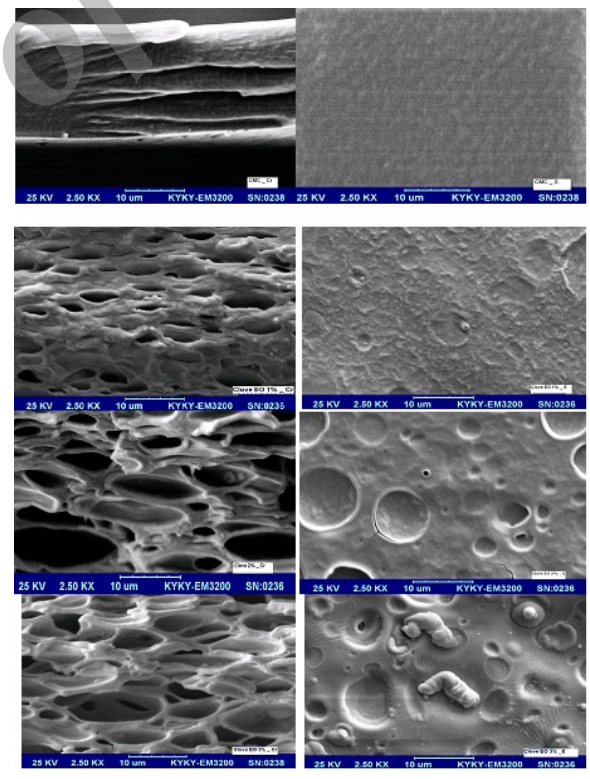

Figure 1. SEM micrographs of the surfaces (right, -S) and cross-sections (left, -Cr) of the CMC films with various percent of clove essential oil and control (without EO)

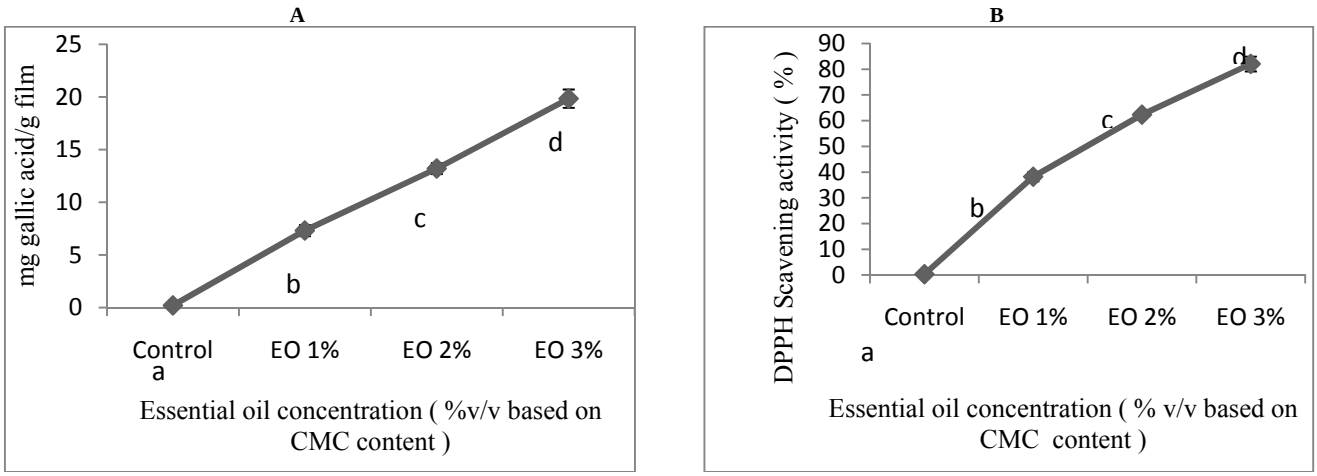

Figure 2. DPPH-scavenging activities (A) and phenolic content (B) of CMC_based films incorporated with CEO and control (without EO)

Table 1. Physical, WVP and mechanical properties of CMC films formulated with different concentrations of CEO [a]

Film	Thickness (mm)	Moisture content (%)	Solubility in water (%)	WVP ($g\ s^{-1}\ m^{-1}\ Pa^{-1}\times10^{-10}$)	Tensile strength (MPa)	Elongation at break (%)
Control	0.034±0.011[a]	23.01±0.68[a]	-	3.02±0.28[a]	18.21±2.3[a]	25.14±0.56[a]
CEO1	0.058±0.009[b]	20.07±0.27[b]	34.39±3.09 [a]	4.36±0.23[b]	51.34±5.5 [b]	31.04±0.13 [b]
CEO2	0.069±0.012[b]	18.35±0.29[c]	31.03±1.22[a]	5.48±0.25[c]	38.67±4.42[c]	12.32±0.87 [c]
CEO3	0.090± 0.005[c]	17.47±0.71[cd]	30.78±3.51[a]	6.75±0.14[d]	26.07±2.42[d]	10.35±0.82 [c]

[a] Data reported are average values ± standard deviations. Values within each column with different letters are significantly different ($p< 0.05$).

Table 2. Effect of different concentrations of CEO on optical properties of CMC films

Film	L	a	b	Δ E	YI	WI
Control	76.76±3.02[a]	1.01±0.02[a]	-3.25±0.07[a]	17.04±2.96[a]	-6.05± 0.39[a]	76.55±3.02[a]
CEO1	74.25±0.72[a]	0.52±0.01[b]	-0.95±0.1[b]	16.73±7.13[a]	-1.85±0.36[b]	74.22±7.18[ab]
CEO2	70.38±3.8[ab]	0.23±0.02[c]	1.02±0.32[c]	16.74±3.81[a]	2.05±0.54[c]	70.36±3.79[ac]
CEO3	60.67±6.14[b]	-0.07±0.01[d]	2.17±0.1[d]	16.85±2.54[a]	5.13±0.46[d]	60.61±2.49[c]

[a] Data reported are average values ± standard deviations. Values within each column with different letters are significantly different ($p< 0.05$).

Table 3. Antimicrobial activities of different concentrations of CEO incorporated in CMC based films in direct contact [a, b]

	Film	P. aeruginosa	S. typhimurium	E. coli	B. cereus	S. aureus
Inhibition Zone (mm)	Control	0[a]	0[a]	0[a]	0[a]	0[a]
	CEO1	14±1[b]	11.33±1.57[b]	14.66±2.57[b]	13.06±1.57[b]	31.66±3.07[b]
	CEO2	20.67±2.57[c]	19.66±2.56[c]	24±3.1 [c]	24.05±2.03 [c]	38.97±3.08[c]
	CEO3	26.56±3.47[d]	23.33±2.62[d]	24.33±2.32[d]	29.33±4.08[d]	37.66±2.15[d]
Inhibition Factor	Control	0[a]	0[a]	0[a]	0[a]	0[a]
	CEO1	6.86±1.12[b]	4.14±0.53[b]	7.61±0.66[b]	5.78±1.04[b]	39.12±1.45[b]
	CEO2	16.09±0.94[b]	14.48±0.9[c]	22.06±1.92[c]	22.06±1.92[b]	56.78±3.04[c]
	CEO3	27.1±1.49[c]	19.26±1.27[d]	23.02±1.38[d]	33.82±1.66[c]	58.3±2.17[d]

[a] Data reported are average values±standard deviations. [b] Values within each column with different letters are significantly different ($p< 0.05$). Diameter (mean and SD) of inhibition zone (mm) including film (10 mm)

Discussion

EO incorporated CMC film maintained their antioxidant and antimicrobial activity. The physical structure in combined films, showed some different properties when compared with CMC film.

Using of EO in edible film structure increase the thickness of combined film. This result was found by others [20, 22]. When EO added in CMC film, solubility in water was decreased. It is related to incorporation of EO in CMC structure and interaction between the components of EO and the hydroxyl groups of CMC that lead to the increase of the hydrophobic nature of the films. Consequently the availability of hydroxyl groups for interaction with water molecules reduced that led to a more water-resistant film. These results had same trend with moisture content and showed that EO can disrupt the structure of CMC film and produce a new structure with hydrophobic properties [20, 32]. Many factors such as the ratio of hydrophilic and hydrophobic groups, tortuosity,

porosity, and cracks in the structure of the film are important in WVP. Although substitution of hydrophobic ester groups by EO instead of hydrophilic OH groups in CMC-based film could be increased hydrophobicity but, it does not assure the reduction of WVP, because film permeability is influenced by the existence of pores, voids, cracks and channels [31, 33]. Garcia et al. [34] showed that different portion of lipid incorporated starch-based films, and coatings have various effects in WVP. They showed although the concentration 2.2 (g/L) of sunflower oil improved WVP, lower or higher concentration increased it. Bonilla et al. [3] and Ghanbarzadeh et al. [35] reported an increase in the WVP of Chitosan film incorporated thyme and basil essential oils and CMC film with oleic acid when concentrations of EOs or oleic acid increased. Tortuosity was mentioned the reason of this phenomenon. In contrast other authors [20, 36, 37] reported a significant increase in the moisture-barrier properties of films containing essential oil. Hydrophobicity of film induced by EO was expressed as its reason. Tensile strength (TS) and elongation at break (ELB) are important factors in food packaging. They indicate the maximum tensile stress that the film can maintain and the maximum change in length of a test sample before breaking [31].

Our findings were same as Ghanbarzadeh et al. [35] when oleic acid used in CMC edible film. It seems that, EO can improve the film strength. A strong interaction between the carbohydrate in the low concentration of EO produced a cross linking effects. This phenomenon was previously reported by other researchers in Chitosan incorporated with oleic acid films [24, 31, 38]. These finding are different with studies that showed TS is weaker when EO used in the film structure than compared with control film [20]. This effect can primarily be explained by increasing of weaker polymer-oil interactions, development of a heterogeneous film structure and discontinuities in the polymer network in higher concentration of EO. Against of previous studies, [35, 39 40] that showed TS and ELB have reverse manner, in this study variation in both of them had the same trend approximately, although the maximum ELB was revealed in 1% EO. The findings of Pereda et al. [31] and Jimenez et al. [41] are in agreement with our findings. Optical properties of edible films and coating are important factors that attract consumers and affect on food packaging applications. Optical properties depend on the internal structure developed during the film drying, the kind of dispersion, miscible phase, the initial structure of the emulsions, the size of aggregates dispersed, the color of portion added and etc. [42, 43].

Blue appearance in control film and yellowness in CEOs were higher and showed that CMC-based films incorporated of CEO are darker, more opaque and yellowish. The differences were significant. Visual observations approved these findings too. This phenomenon is probably due to the phenolic compounds [20] of CEO. ΔE value showed CMC-based films incorporated with CEO and pure CMC films are the same when compared with standard color plate. Microstructure of the films depends on methods that used in preparation, ingredients, the time of drying and etc. Evaporation of water is faster in the surface of the film, hence the top layer sets and dries faster than the inner center layer [44, 45]. Thus, this phenomenon increased duration drying time and heterogeneous, bumpy and craters surface in films incorporated with EO. In usual oil/water emulsions, we expect that the oil droplets to be spherical but in these films they were not seen. It could be due to the traction forces induced by the CMC network, emulsifier, interaction between the CMC structure and EO globules, preparation method, water and solvent rate evaporation. Nevertheless, weak interaction between the CMC film and EO in higher concentration gave rise coalescence between EO globules [40, 46, 47] as shown in the figure 1. Different methods were used to determine the antioxidant activity [48]. One of them is DPPH radical scavenging assay. Depending on EO antioxidant activity and the reduction of DPPH, the absorbance at 515 nm decreases and antioxidant activity measures as the percent of inhibition absorbance [49]. Antioxidant activity of EOs has been extremely studied [49-51].

The antioxidant potential of the film could be modified following interactions between the matrix and phenolic compounds additives. Although interaction between EO and matrix as vehicles such as edible film could block the active site [52], antioxidant activity exists in edible films incorporated with EO. The antioxidant activities of these biodegradable films are related to the kind and concentration of EO. Phenolic compounds are responsible of antioxidant activity in EOs [20, 53-54]. Eugene (72.2%), ß-caryophyllen (11.4%) and acettaugenol (9%) were the most phenolic compounds that revealed by our GC-MS study (results were not shown). Although our findings showed variation with some studies, variability in compounds content of EOs was largely due to seasonal and environmental factors, stage of harvest, methods of extraction and etc. [15, 55]. Spices and plant essential oils or extracts have been used as food additives, pharmaceutical, medicine substitute, antioxidant, and antimicrobial agents for many years. Recently antimicrobial essential oil properties especially, against pathogen bacteria, have investigated and the antimicrobial activity of them classified as strong, medium or weak [19]. CEO inhibitory effects have been shown in several studies [52, 56].

In this study, eugenol followed ß-caryophyllen and acettaugenol were major components that probably have more antimicrobial activity. Results showed that CMC edible films incorporated with different CEO percent have inhibitory effects against both positive and negative bacteria and inhibitory effect raised when EO percent increased. Essential oils have hydrophobicity characteristics, which enable them to interact with lipid structure such as Gram-negative bacterial cell membrane, mitochondria and other intracellular component, and subsequently, disruption of cell structure, leakage, ions exchange, inhibition of respiration, alteration of permeability and death could be occur [56, 57]. Although it seems that Gram negative bacteria are more sensitive to

EOs, it is not general rule and different results have been found [19, 58]. Prabuseenivasan et al. [59] and Oussalah et al. [19] reported *E. Coli* and *B. subtilis* are more resistant than *S. auruse* against EO respectively. Lipopolysaccharide (LPS**)** is the essential compound in the cell wall structure of Gram negative bacteria. Hence the accumulation of the oils on the cell membrane avoided [60]. *Staphylococcus aureus* has single layer wall which makes it to be more sensitive than others bacteria [19, 59]. When essential oils incorporated in edible films, their properties may be converted. This is may be with advantage or disadvantage. Results showed that addition of EO into CMC-based film increased tortuosity, pores and cracks in the structure of the film that increased WVP. EO in lowest concentration (1%) in CMC-based film showed highest TS and ELB but by increasing of EO concentration in CMC film, TS decreased although TS was higher than pure CMC film in all concentration and ELB was higher in pure CMC after 1% EO concentration film. EO produced opaque appearance and reduced transparency and increased total color difference (ΔE), Yellowness (YI), and whiteness (WI) values in CMC-based film. Incorporation of EO in CMC film disrupted the compact and smooth structure it and produced pores and crack with more heterogeneous structure. Incorporation of EO in CMC film showed antioxidant and antimicrobial properties and these characteristics increased in higher concentration of EO.

Our findings showed that antimicrobial and antioxidant properties of EO are useful characteristics that give antimicrobial and antioxidant potential to CMC film when used in it and produce an active biodegradable substance to postpartum deterioration, oxidation, and microbial growth. Autorse propose that the effects of these combined films survey in the various food packaging.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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