



## Carboxymethyl Cellulose Film Incorporating *Satureja khuzistanica* and *Zataria multiflora* Essential Oils for Extending the Shelf Life of Chicken Legs

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Article History: Received: 23 January 2020/Accepted in revised form: 30 June 2020

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### Abstract

The aim of this study was to extend the shelf life of refrigerated chicken using carboxymethyl cellulose (CMC) film containing essential oils (EOs). The samples included a sample without wrapping film (control), a sample wrapped with pure film (CMC), one wrapped with CMC film containing *Zataria multiflora* Boiss EO (CMC-Z), and one wrapped with *Satureja khuzistanica* jamzad EO (CMC-S). The films containing 2.4% EOs were selected for this study. The shelf life of the CMC-Z and CMC-S treatments was extended from 6 to about 12 days when compared with the control sample. The film containing EOs reduced the TBA value compared to the CMC and control samples. Sensory evaluation of the cooked meat revealed that CMC-Z slightly decreased the overall acceptability compared to the control. The CMC-S samples did not show acceptable organoleptic properties ( $p < 0.05$ ). Packaging containing ZEO can extend the shelf life of refrigerated chicken with the least undesirable effect on sensory properties.

**Keywords:** Antimicrobial film, Chicken meat, Essential oil, Shelf life.

### Introduction

Chicken is considered an important and popular source of protein in many communities because of its high protein value, quick and easy cooking properties, and commercial availability. The growth of pathogenic microorganisms and lipid oxidation may occur in chicken meat during refrigerated storage [1]. Lipid oxidation can cause an unpleasant texture, color, and odor and also nutritional loss in meat products. Microbial growth as a serious hazard in the food industry is an alarm for public health [2].

Chicken can be preserved using different methods that include cooling, freezing, radiation, preservatives, and active packaging. Active packaging is a relatively new concept for extending the shelf life and maintaining the nutritional value of food products. Active agents are released onto the food surface to prolong shelf life and improve the nutritional and organoleptic properties [3]. Unlike traditional packaging in which there is minimum interaction between the products and packaging materials, active packaging is an interactive alternative. Antimicrobial packaging is a type of active packaging that can increase the shelf life and safety of food products [4]. This packaging

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is capable of decreasing the growth rate and increasing the lag phase of microorganisms, which can cause to reduce the risk of food poisoning [5]. Increased environmental contamination by the accumulation of plastic waste has increased the need for biodegradable packaging [6-7]. Many recent studies have investigated coatings and films based on carbohydrates (carboxymethyl cellulose (CMC), chitosan), lipids (waxes, acylglycerol) and proteins (zein, gelatine) or a combination of these polymers, because they are biodegradable and offer selective permeability and biocompatibility [8]. CMC as a water-soluble, non-toxic, odorless, tasteless, and non-allergenic derivative of cellulose has a good edible film forming properties [9]. Application and development of bio-based packaging can improve the quality and safety of fresh foods, and can be eaten as a part of the whole product by the consumer [10]. Edible film can carry active antimicrobial and antioxidant agents. One natural antimicrobial compound that can be used as an active ingredient in the edible films is essential oil (EO) [11]. They can help to keep the food quality and extend the consumption period [12]. Unlike chemical preservatives, EO with a high content of phenolic compounds can enhance the safety of food by preventing microbial growth and reducing lipid oxidation [13-14]. *Zataria multiflora* Boiss and *Satureja khuzistanica* Jamzad are members of the Lamiaceae family that grow primarily in Mediterranean regions. These plants are immensely popular because of their digestive, sedative, and analgesic properties in traditional Iranian medicine. They also are used as flavourings in various Iranian foods. It has been shown that these EOs, because of their phenolic content including thymol and carvacrol, can act as antimicrobial agents for meat and meat products to enhance shelf life [14-17]. Furthermore, regarding the fact that bacterial contamination mainly appear on the surface of meat, the slow release of antimicrobials from the film containing essential oils into the meat surface can be more effective than their direct addition [13]. Moreover, EOs can protect meat against reactive oxygen species and thus decreasing oxidative damages, to improve the shelf life of meat [13-18]. Although previous research has investigated the effect of EOs on meat and meat products by incorporating them into packaging materials, [18-19] to the best of our knowledge, there is no published study that evaluated the preservative

effect of *Z. multiflora* and *S. khuzistanica* essential oils in combination with CMC film to extend shelf life of chicken meat. Therefore, the aims of this study were to evaluate the effect of CMC film incorporated with *Z. multiflora* essential oil (ZEO) and *S. khuzistanica* essential oil (SEO) on the microbiological, physico-chemical and sensory properties of refrigerated chicken legs.

## Material and Methods

### Materials

The CMC was provided by Pharayandsazan Arian (Pharayandsazan Arian Company, Iran). *Z. multiflora* was obtained from local markets and *S. khuzistanica* was purchased from Khorraman Company (Khorraman Company, Iran). Fresh chicken legs were supplied from local retail shops. Glycerol, Tween 80 (analytical grade), Thiobarbituric acid (TBA), Brain Heart Infusion (BHI), Plate Count Agar (PCA), Baird-Parker Agar, de Man, Rogosa and Sharpe (MRS) Agar, Violet Red Bile Glucose Agar (VRBA), and *Pseudomonas* Agar were purchased from Merck Company (Merck, Germany).

### Extract of Essential Oil

The dried *S. khuzistanica* and *Z. multiflora* plants were powdered and the oils were extracted from the powder under water distillation for 3 h using a Clevenger apparatus. After separation, the oils were stored in dark vials until further use [20-21].

### Composition of Essential Oils

Gas chromatography–mass spectrometer (GC-MS) equipped with a 5975C mass selective detector network was used for identification of the EO components (Agilent Technologies (Palo Alto, CA, USA)). For the detection and quantification of the main compounds of the EO components, *S. khuzistanica* and *Z. multiflora* essential oils were diluted in 1 mL of solvent (cyclohexane), and then about 2  $\mu$ L of the dilute was injected into the GC-MS. Identification and quantification of the EO compounds were done by comparing their mass spectra with those of standard compounds and their retention times. The inlet temperature was set at 280 °C. The column temperature was as follows: initial temperature 50 °C for 1 min, increased (10 °C/min) to 140 °C and maintained for 2 min, followed by an increase (5 °C/min) to 160 °C for 1 min and then increased (30 °C/min) to 290 °C for 5

min. Helium was used as carrier gas at a flow rate of 1 mL/min. The compounds of the oil were identified by comparison of their retention indices (RI) and mass spectra fragmentation with those on the stored Wiley 7n,1 mass computer library, and NIST (National Institute of Standard and Technology).

#### Preparation of Films

The CMC film was prepared by dissolving 100 mL of distilled water in 1 g of CMC (1% w/v) at 70 °C for 45 min. Glycerol was added (50% w/w based on CMC) as a plasticizer and stirred for 10 min on a magnetic stirrer to achieve a clear solution and smooth. The film solution was cast onto the center of a glass plate that was 2 cm in height and 15 cm in diameter. The film solution was dried for 25 h at 30 °C and this film was applied as the control film. The EO-incorporated film also was prepared by adding SEO and ZEO separately to a clear solution of CMC at three concentrations (1.6%, 2.4%, 3.2% (v/v)). Tween 80 was added (based on EO) as an emulsifier and a homogenizer (IKA T25-Digital Ultra Turrax, Staufen, Germany) was applied for homogenization at 13,500 rpm for 4 min at 70 °C. The final emulsions were cooled to remove air bubbles that formed during homogenization. The dried film was peeled off of the plate and transferred to a desiccator containing saturated magnesium nitrate solution at 53% relative humidity at 25 °C [9].

#### Evaluation of the antimicrobial activity of films

##### Bacterial Strains

*Staphylococcus aureus* PTCC 25923, *Bacillus cereus* PTCC 1274, *Escherichia coli* PTCC 1399, *Pseudomonas aeruginosa* PTCC 1430, and *Salmonella enteritidis* PTCC 1231 were obtained from the Persian Type Culture Collection of the Iranian Research Organization for Science and Technology. They were revived in BHI broth at 37 °C for 24 h before further experimentation.

##### Disc Diffusion Assay

Film samples were aseptically cut into discs of 6 mm diameter (according to the Laboratory Standards Institute). To keep aseptic conditions before the experimentation, the all utensil applique laboratory were sterilized and working surface irradiated by ultraviolet light for 24 h. Then the discs were placed carefully on each plate (under laminar airflow condition) and seeded with

approximately  $10^7$  CFU/mL of pathogens. The plates were incubated for 24 h at 37 °C. The diameter of the clear zone as the area of inhibition around the disk film was measured using a caliper and reported as the 'zone of inhibition' [22]. All experiments were performed triplicate.

##### Sample Preparation

Fresh chicken fillets prepared from the local market were cut into 8×4×2 cm samples (weighing ca. 50 g). They were packed in polystyrene boxes and transferred to the laboratory on ice. All chicken meat pieces were wrapped with the test film, except the control sample, and all samples were packed into polyethylene pouches and kept at refrigerated for 12 days. Four treatment groups were prepared: control (without film, only polyethylene pouch), CMC, CMC film containing ZEO (CMC-Z), and CMC film containing SEO (CMC-S). The pH, TBA reactive substances, microbial counts and sensorial properties (taste, odor, color, texture, and overall acceptability) of the samples were assayed during storage [23]. All experiments were performed in triplicate.

##### Microbiological Analyses

The chicken meat samples (10 g) were aseptically removed from each package and transferred to 90 mL of sterile serum physiology solution (0.75%) before homogenization. They were 7-fold diluted and the bacteria were counted [24]. The total microbial count (TC) was done using Plate Count Agar (PCA) after incubation at 37 °C for 48 h. The psychrotrophic count (PTC) was done on PCA after incubation for 7 days at 10 °C [25]. The *Pseudomonas spp.* and *S. aureus* counts were measured on *Pseudomonas* agar base at 30 °C for 48 h and Baird-Parker agar at 37 °C for 48 h, respectively. The number of lactic acid bacteria (LAB) was determined using the MRS medium at 30 °C after 72 h of incubation. Finally, for coliforms, VRBA was used after incubation for 24 h at 37 °C [26-27]. The microbial counts were reported as colony forming units per gram (CFU/g) of chicken meat.

##### Physico-chemical Analyses

The moisture content and water activity ( $a_w$  Emode, Rotronic, Germany) of the chicken treatments were determined according to Shojaee-Aliabadi *et al.* [22] by direct contact of the probe into the homogenized meat. The chicken meat (10 g) was homogenized thoroughly in 90 mL of

distilled water and the homogenized samples were used for pH (pH meter, model Milwaukee) determination [28]. TBA was performed according to the method described by Natseba *et al.* [29] TBA quantities were expressed as mg of malondialdehyde (MDA) / kg of chicken meat.

#### Sensory Evaluation

The sensory quality of the chicken was evaluated by 30 untrained panellists aged 21-40 years (20 females and 10 males, all members of the Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Science, Tehran, Iran). Sensory evaluation was performed in a private room with eight cabins (adapted to perform a sensory analysis). Sensory evaluation room designing with ordinary lighting (normal fluorescents lighting) was applied throughout the test, odor free, air filtration, controlled temperature, and lighting. Panellists were instructed to fill the form and to evaluate the sample properly before the beginning test [30-31]. Meat samples were cut into pieces approximately 2.4 cm in diameter and 1.5 cm in thickness and cooked in a steam cooker for 60 min. The samples were labelled with 3-digit codes and included CMC-Z (2.4%) and CMC-S (2.4%), CMC and the control. The samples were individually presented to the panellists in random order and adequate privacy was provided to assure independent decisions.

The panellists evaluated the color, odor, texture, taste, and overall acceptability of the cooked samples. Between the samples, tap water was provided to neutralize the palate [32]. Sensory tests of cooked samples were done after 48 h of storage at 4 °C. The panel members also were asked to evaluate the odor, color, and overall acceptability of the raw samples just after opening the packaging (during storage). A 5-point hedonic scale was used for sensory evaluation. A score of 5.0 was excellent quality, a score of 1.0 indicated was bad quality and a score of 3.0 was considered to be acceptable level [23].

#### Statistical Analysis

The data were analysed statistically in SPSS 20 software. The mean and standard deviations were calculated at the 5% level ( $p < 0.05$ ) of significant and using Duncan's new multiple range test ( $p < 0.05$ ). The general linear model (GLM) was used to compare results on different days.

## Results and Discussion

#### Composition of Essential Oils

The compositions of the EOs are shown in Table 1. The highest amount of thymol (51.6%) was detected in ZEO followed by carvacrol (12.4%), p-cymene (9.5%), and  $\gamma$ -terpinene (7.1%). The main component of SEO was carvacrol with a concentration of 87.16%. Moradi *et al.* [33] evaluated the influence of various drying methods and distillation processes on the chemical composition of ZEO and indicated that thymol (64.87%), carvacrol (4.65%), p-cymene (5.63%), and  $\gamma$ -terpinene (9.11%) were the major compounds. Avaei *et al.* [34] reported that the chemical composition of ZEO was thymol (42.46%), p-cymene (10.62%), carvacrol (16.85%), and  $\gamma$ -terpinene (7.26%). Yousefzadi *et al.* [35] showed that the main components of the SEO were carvacrol (92.87%), and limonene (1.2%). However, Saei-Dehkordi *et al.* [36] reported that the main components of SEO were carvacrol (53.86%), and thymol (19.84%). This difference can be attributed to regional climate, harvest conditions, plant variety, and seasonal differences.

#### Antimicrobial Activity

The results of antimicrobial activity between various parameters are shown in Table 2. Antibacterial activity of essential oils was discerned by calculating their minimum bactericidal concentration (MBCs) and minimum inhibitory concentration (MICs). The MBCs was defined as the lowest concentration of test extract sample required to kill 99.9% of the bacterial cell by plating agar. The MICs was determined as the lowest concentration of sample that inhibition the visual growth of bacteria [37]. Our previous studies found that *Pseudomonas aeruginosa* was the most resistant to SEO and ZEO among tested bacteria (*P. aeruginosa*, *E. coli*, *S. aureus*, *Salmonella typhimurium*, *B. cereus*) [9-22] with minimum bactericidal concentration (MBCs) of 96000 and 92000 ppm, respectively (data not shown). Eftekhari *et al.* [38] reported the minimum inhibitory and bactericidal concentrations (MIC and MBC) values of ZEO against the isolate of *S. aureus* and gram-negative and gram-positive, which was in range of 0.66-2.64 mg/mL and *P. aeruginosa* was highly resistant to ZEO (42.2 mg/mL).

**Table 1** Different components identified in ZEO\* and SEO\*\*

Number	Type	Retention time (min)		Concentration (%)	
		ZEO	SEO	ZEO	SEO
1	$\alpha$ -pinene	10.313	10.319	1.6	0.63
2	camphene	10.814	10.803	-	0.05
3	$\beta$ -pinene	11.795	11.605	-	-
4	$\beta$ -myrcene	12.344	12.339	1.1	0.89
5	$\delta$ -3-carene	13.197	13.113	-	-
6	p-cymene	14.331	13.557	9.5	6.39
7	$\beta$ -phellandrene	12.802	12.915	-	-
8	$\gamma$ -terpinene	15.533	14.469	7.1	-
9	$\alpha$ -terpineol	19.119	19.113	-	0.06
10	thymol	23.512	22.581	51.6	0.25
11	carvacrol	23.712	22.953	12.4	87.16
12	caryophyllene	25.178	25.178	-	-
Total				83.3	95.43

\*ZEO: *Zataria multiflora* Boiss. essential oil.

\*\*SEO: *Satureja khuzistanica* Jamzad essential oil.

Hadian *et al.* [39] studied the MIC and MBC of four *Satureja* species EOs (*S. khuzistanica*, *S. rechingeri*, *S. mutica* and *S. bachtiarica*) against gram-negative and gram-positive bacteria (*P. aeruginosa*, *E. coli*, *B. cereus*, and *S. aureus*). *P. aeruginosa* showed good resistant, with MIC and MBC higher than 64 mg/mL. Moshayedi *et al.* [40] studied the antimicrobial properties of *Origanum vulgare* L (OVL), *Zataria multiflora* Boiss (ZMB), and *Mentha pulegium* (MP) essential oils. For Enterococcus the MICs of MP, OVL, and ZMB were 42000, 8000 and 2000 ppm, respectively.

In present study, the highest MBC was chosen for film preparation. In a preliminary evaluation, different concentrations of EOs were added to the film dispersions to obtain final concentrations of 1/2, 1/3, 1/4, 1/6, and 1/7 of MBC. The results showed that it was difficult to prepare a homogenous film containing EOs at concentrations above 1/3 MBC. Moreover, such film showed undesirable organoleptic properties (the scores were lower than 3 as an acceptable level). However, at concentrations of less than 1/6 MBC, the film showed no effective antibacterial activity. For these reasons, 1/3, 1/4, and 1/6 of MBC (equivalent to 1.6%, 2.4%, and 3.2% v/v, respectively) were selected for film preparation to study their properties.

The antimicrobial activity (Table 2) of CMC film at three concentrations of SEO and ZEO against five

selected bacteria indicating the CMC film (without EO) resulted no inhibitory activity, but CMC film containing SEO or ZEO significantly inhibited bacterial growth. As expected, antimicrobial activities were stronger at the higher EO concentrations. The films with 1.6% SEO or ZEO showed a weak antimicrobial activity. Although (based on t-test) there was a significant difference between the inhibitory action of the film with 2.4% and 3.2% EOs, the difference was not remarkable. *S. aureus* and *P. aeruginosa* were the most sensitive bacteria and resistant to EOs, respectively [41].

The antimicrobial activity of ZEO and SEO can be attributed to the phenolic compounds of carvacrol, thymol,  $\gamma$ -terpinene, and p-cymene. Phenolic compounds are hydrophobic in nature and can partition in the lipids of the bacterial cell membrane and disrupt the external membrane of the bacteria, increasing cytoplasmic permeability [9]. Ghasemlou *et al.* [8] showed that *Zataria multiflora* and *mentha pulegium* EOs were effective on *S. aureus* and *E. coli*.

Hashemi *et al.* [42] studied studied basil-seed gum edible film with oregano EO (1% to 6%) on bacteria growth and reported that an increase in EO content increased the inhibition zone around the film.

**Table 2** Antimicrobial activities of three concentration of CMC-Z\* and CMC-S\*\* in direct contact (mm) and seeded with approximately 10<sup>7</sup> CFU/mL of pathogens. *Staphylococcus aureus* PTCC 25923, *Bacillus cereus* PTCC 1274, *Escherichia coli* PTCC 1399, *Pseudomonas aeruginosa* PTCC 1430 and *Salmonella enteritidis* PTCC 1231 were obtained

Film	Inhibition zone (millimeter)				
	<i>P. aeruginosa</i>	<i>S. enteritidis</i>	<i>E. coli</i>	<i>B. cereus</i>	<i>S. aureus</i>
Control	0.00	0.00	0.00	0.00	0.00
CMC-Z 1.6%	6.00±0.00 a	17.50±1.44 a	16.50±2.08 a	17.67±0.21 a	18.50±0.86 a
CMC-Z 2.4%	22.27±1.7 b	26.50±0.86 b	24.23±0.39 b	27.80±0.15 b	32.73±1.03 b
CMC-Z 3.2%	24.43±1.72 b	27.60±0.3 b	27.33±2.68 b	28.30±0.18 b	36.50±1.44 b
CMC-S 1.6%	17.08±0.93 a	20.83±2.07 a	19.33±0.44 a	18.33±1.66 a	22.16±0.16 a
CMC-S 2.4%	27.50±0.50 b	33.66±2.52 b	32.66±0.72 b	28.33±0.73 b	36.76±0.13 b
CMC-S 3.2%	29.16±0.60 b	34.83±2.66 b	33.50±0.5 b	31.33±1.16 b	37.20±0.15 b

<sup>a</sup> means average values ± standard deviation

<sup>b</sup> Values within a column followed by a different small letter are significantly different from each other on a specific day ( $p < 0.05$ ) according to Duncan test.

\*CMC-Z: Carboxymethyl Cellulose film containing *Zataria multiflora* Boiss. essential oil

\*\*CMC-S: Carboxymethyl Cellulose film containing *Satureja khuzistanica* Jamzad essential.

*B. cereus* and *S. aureus* were the most sensitive bacteria and *P. aeruginosa* was the most resistant one. Shojaee-Aliabadi *et al.* [22] investigated K-carrageenan film containing different concentrations of *Satureja hortensis* EO, which showed an adverse effect on the growth of the tested pathogenic bacteria, *Pseudomonas aeruginosa* was the most resistant bacteria. Hu *et al.* [43] also reported that CMC/chitosan film showed a higher zone of inhibition for *S. aureus* compared to *E. coli* implying more sensitivity of *S. aureus*.

#### Effect of EO-incorporated Film on the Shelf Life of Chicken Meat

In the present study, to determine the effect of EO-incorporated film on the shelf life of chicken meat, a concentration of 2.4% was selected for films preparation. Because this concentration showed appropriate antibacterial inhibition in *in vitro* studies that were comparable to the 3.2% concentration and also it showed more desirable organoleptic properties than the 3.2% based on primary sensorial investigation (data are not shown) [41]. Raesi *et al.* [44] studied the shelf life of coated fish sample with CMC solution containing grape seed extract (GSE) 0.5% v/v, GSE 1%, ZEO 1%, ZEO 2%, ZEO 1% + GSE 0.5% and ZEO 1% + GSE 1%. They reported that an increase in concentration of GSE and ZEO resulted in stronger antimicrobial activities of coating. However, higher concentration led to undesirable sensorial properties of the samples. The fish fillets coated with ZEO + GSE 1% demonstrated the excellent organoleptic properties. Aliakbarlu and Sadaghia [45] studied the effect of *Zataria*

*multiflora* and clove (*Syzygium aromaticum*) (CEO) EOs, (0.25% v/w) on sensory properties of sheep meat at 4 °C during storage. CEO and ZEO had a noteworthy effect on color and odor attributed to meat, respectively. Unlike CEO, ZEO odor was pleasant and acceptable to panelists and achieved higher score throughout storage. In general, application of CEO gained higher overall acceptability. However, inhibitory effect of ZEO on lipid oxidation and microbial growth were higher than CEO.

#### Microbiological Analyses

The results of changes in TC (a), psychrotrophic bacteria (b), *Pseudomonas* (c), *Staphylococcus* (d), LAB (e), and coliform bacteria (f) in raw chicken meat during storage are shown in Fig. 1. The initial mesophilic bacteria population (TC on day 0) of chicken meat was 3.87 log CFU/g (Fig. 1a), indicating cross-contamination, probably during slaughter, transportation or retailing. The TC of all chicken meat samples increased during storage; for the control sample this parameter reached approximately to 7 log CFU/g (6.63 log CFU/g) on day 6. A value of 7 log CFU/g is the upper acceptability limit as defined by the ICMSF [46] (International Commission on Microbiological Specifications for Foods, 1986) for TC in fresh chicken meat [23].

For the CMC, CMC-Z, and CMC-S samples, this deadline was achieved after 6 and more than 9 days of storage, respectively. This result indicates a positive effect of the CMC film and both EO-incorporated films to provide good protection for meat against bacterial deterioration. The addition of either EO to CMC film resulted in a delay in the

microbial spoilage of chicken meat fillets. In accordance with our results, Mahdavi *et al.*[32] reported at chitosan film containing anise (*Pimpinella anisum* L.) EO (0%, 0.5%, 1%, 1.5%, and 2% v/v) extended the shelf life of ground chicken meat samples. The addition of EOs to chicken meat decreased the TC, which indicated antimicrobial effect of the EO. Anise EO at 2% had the lowest value TC and resulted in better shelf life among samples all over the storage period.

Raeisi *et al.* [47] reported that the application of sodium alginate incorporated with nisin *Cinnamomum Zeylanicm*, and rosemary EOs could retard the mesophilic bacteria population of chicken meat and improve its shelf life. Rocha *et al.* [48] demonstrated that agar film incorporated with clove EO (0.5 g EO/g agar) inhibited bacteria activity on flounder fillets over 15 days of refrigeration. The bacteria population (TC, *Pseudomonas spp.*, Lactic acid bacteria (LAB), and Enterobacteriaceae) increased in the control film compared to the film containing clove EO during storage.

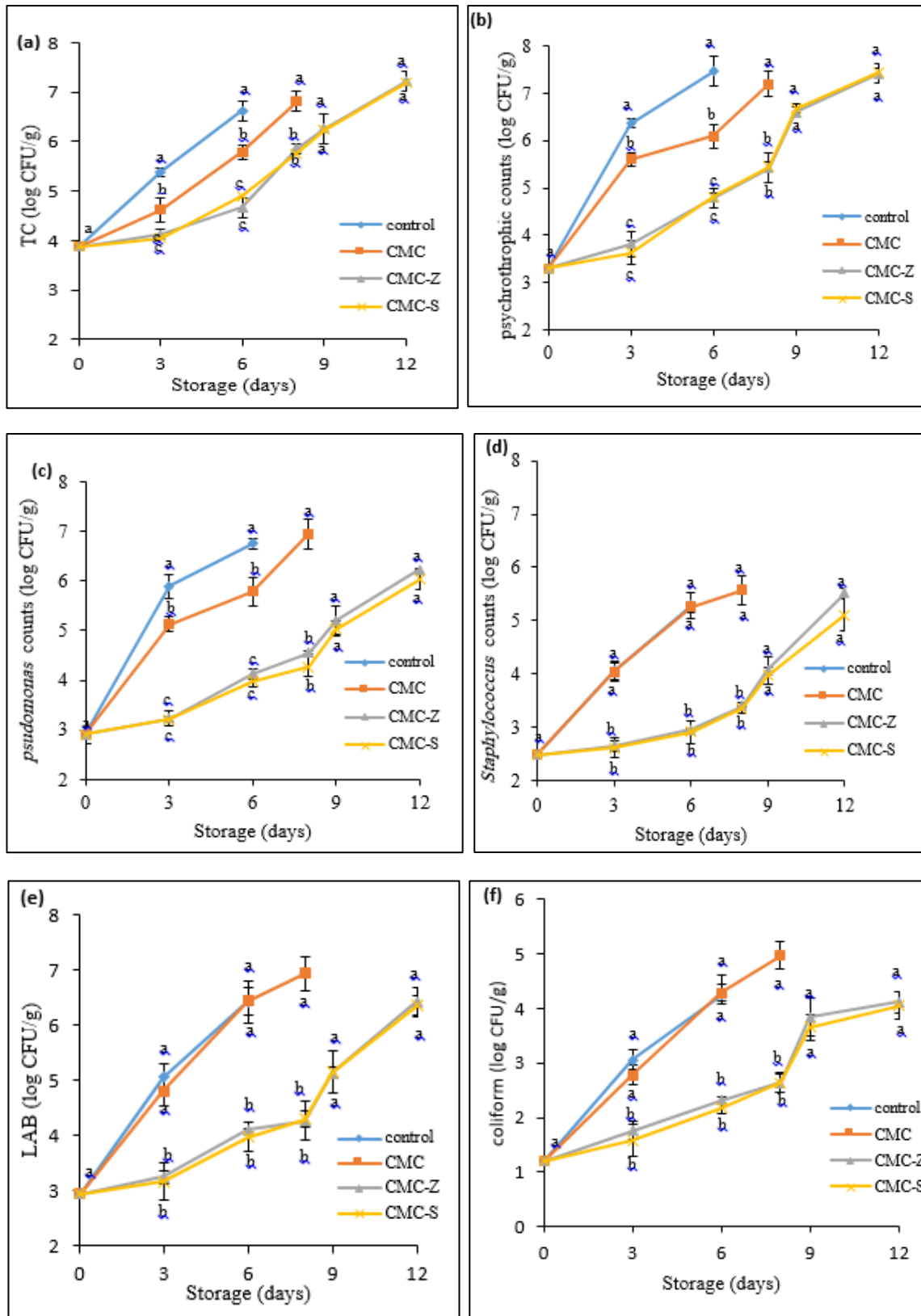
Psychrotrophic bacteria mostly grow on meat surfaces and are mainly gram-negative. The primary properties of psychrotrophic bacteria are lipolytic and proteolytic activities [32]. A similar increase was observed in the population of psychrotrophic bacteria in all samples during storage (Fig. 1b). Samples coated with CMC-Z and CMC-S film showed significant inhibition, resulting in about 7.41 and 7.45 log CFU/g, respectively, after 12 days of storage at 4 °C. Bazargani-Gilani *et al.* [49] observed that the psychrotrophic bacteria counts of chicken meat coated by chitosan film was 10.37 log CFU/g (day 20), whereas chitosan containing pomegranate juice and *Zataria multiflora* EO (2%) reduced the population to 7 log CFU/g.

*Pseudomonas spp.* are aerobic bacteria and a major cause of chicken meat spoilage during cold storage. The growth of this microorganism can cause putrid flavours in the meat. The initial population of *Pseudomonas spp.* was 2.90 log CFU/g (Fig. 1c), which significantly increased during storage for all samples ( $p < 0.05$ ). The count of *Pseudomonas spp.* showed a 2.63 and 2.78 log cycle reduction for CMC-Z and CMC-S samples compared to the control film (on day 6), respectively. During storage, samples wrapped in EO incorporated films had the lowest *Pseudomonas spp.* counts in

comparison with the control and CMC-based film ( $p < 0.05$ ). CMC-Z and CMC-S samples showed a 2.59 and 3.08 log cycle reduction, respectively, compared to the control ( $p < 0.05$ ) on day 6. Emiroğlu *et al.*[50] reported that initial *Pseudomonas spp.* populations was 6.36 log CFU/g in beef patties, which decreased to 5.62, 5.23, and 5.09 log CFU/g for wrapped samples with soy films containing 5% oregano, 5% thyme EOs, and mixture of oregano and thyme 5% EOs, respectively, after 12 days chilled storage. The results for psychrotrophic and *Pseudomonas spp.* populations were in accordance with the findings of Mahdavi *et al.* [32] on the effect of chitosan film incorporated with anise (*Pimpinella anisum* L.) EO on the increase in the shelf life of chicken meat over a period of 12 days at refrigerated temperature.

The initial level of contamination of *Staphylococcus spp.* was 2.48 log CFU/g (Fig. 1d). The *Staphylococcus* population increased steadily for all samples during the storage period. There was no significant difference between the control and CMC treated samples, indicating no suppressive effect of CMC film on the *Staphylococcus* count of chicken samples. However, the *Staphylococcus* population showed 39.06% and 42.65% reduction in the CMC-Z and CMC-S treatments on day 8, respectively, compared to the CMC film. A further decrease (7.60%) in the *Staphylococcus* count was seen in CMC-S compared to CMC-Z on day 12.

LAB are also natural bacteria on poultry meat. The initial LAB population was about 2.84 log CFU/g in meat samples. The LAB population in the CMC, CMC-Z, and CMC-S samples were 6.58, 4.27, and 4.3 log CFU/g on day 8, respectively (Fig. 1e). On day 12, it reached 6.43 and 6.35 log CFU/g in samples coated with CMC-Z and CMC-S, respectively. CMC treatment did not significantly inhibit LAB growth during storage at 4 °C. Raeisi *et al.*[44] observed that the LAB population in the control treatment of rainbow trout fillets coated with CMC film was 5.7 log CFU/g on day 20, while for samples coated with CMC containing a combination of ZEO and grape seed extract, the count was 3.1 log CFU/g. Bazargani-Gilani *et al.*[49] investigated the population of LAB in chicken fillets coated with into a chitosan (CH) solution alone or in combination with ZEO and/or pomegranate juice (PJ).



**Fig. 1** Changes in TC (a), psychrotrophic bacteria (b), *Pseudomonas* (c), *Staphylococcus* (d), LAB (e), and coliform bacteria (f) in raw chicken meat during storage (means average values  $\pm$  standard deviation; with different capital letters in the same sample and lowercase letters in the same day are significantly different ( $p < 0.05$ )).



Although all treatments significantly reduced the LAB population, the highest antibacterial effect was observed in samples coated with PJ-CH and PJ-CH-ZEO (1% and 2% ZEO).

The initial population of coliforms was 1.2 log CFU/g (Fig. 1f). There was a significant difference between the coliform counts of the control and CMC samples compared to the CMC-Z and CMC-S. In the control sample, the coliform count was Similarly, Sogut and Seydim [51] studied the effect of chitosan-based edible films containing grape seed extract (5%, 10%, and 15%) on chicken meat. The sample with grape seed extract (15%) had the lowest coliform population compared to the control sample (pure chitosan film) on the final storage period day (15). They also reported that all samples control sample, probably due to the barrier effect against oxygen permeability [52]. Balti *et al.* [53] reported that chitosan-based edible film blended with *spirulina* extract (2.5%, 5%, 10%, 15%, and 20% w/v) decreased the oxygen permeability rate as the concentration of *spirulina* extract increased. This probably was a function of the interaction between the phenolic compound and chitosan polymer. It should be noted that none of the tested bacteria could use pure CMC film as a substrate to sustain growth. This is in contrast to a milk protein-based film, which has been reported to increase *Pseudomonas spp.* and *E. coli* O157:H7 populations during storage [54]. However, no significant differences were observed in the LAB and *S. aureus* counts in the control and CMC treatments, which may be related to these bacteria are facultative anaerobes, which can grow under low pressure oxygen conditions.

The significant reduction in the population of all tested bacteria in the samples treated with CMC-Z and CMC-S during storage can be mainly attributed to the inhibitory effect of the EOs migrating from the film layer to the meat surface. There was no significant difference between the antimicrobial activity of treated samples (ZEO and SEO), which may be due to the similarity of the main chemical compounds such as thymol and carvacrol [6] EOs are hydrophobic by nature, which can penetrate the lipid layers of the mitochondria and cell membrane resulting in increasing cell permeability by

4.24 log CFU/g at 6 days of storage, while the value of CMC-Z and CMC-S showed a 45% and 48% decrease, respectively, on day 6. Zhang *et al.*[50] reported that the addition of rosemary, clove, and rosemary-clove extracts reduced the Enterobacteriaceae count in chicken meat (packed in polyethylene bags) to 4.46, 4.30, and 4.11 log CFU/g, respectively, compared to the control (4.80 CFU/g) after 15 days of refrigerated storage.

with more than 5% grape seed extract showed decreased bacterial growth in chicken fillets during chilled storage.

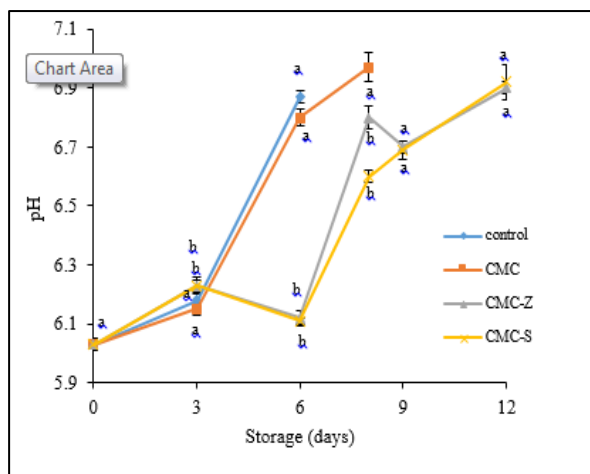
Therefore, coating chicken meat samples with CMC edible film could decrease the populations of TC, psychrotrophic bacteria and *Pseudomonas spp.* (aerobic bacteria) compared to the disturbing the cell membrane structure and causing cytoplasmic damage. The phenolic compounds can interact with enzymes in the bacterial cell wall causing rupture, which reduced the amount of cellular ATP leading to decreasing cell viability [48].

The EO-incorporated films probably acted as oxygen barriers and could release antimicrobial agents into the meat surface, which could increase the shelf life of the wrapped chicken meat.

#### Physico-chemical Changes

Fig. 2. shows the change in pH of the chicken meat samples during storage at 4 °C. The water activity and moisture content of the chicken legs were 0.97 and 79.24%, respectively.

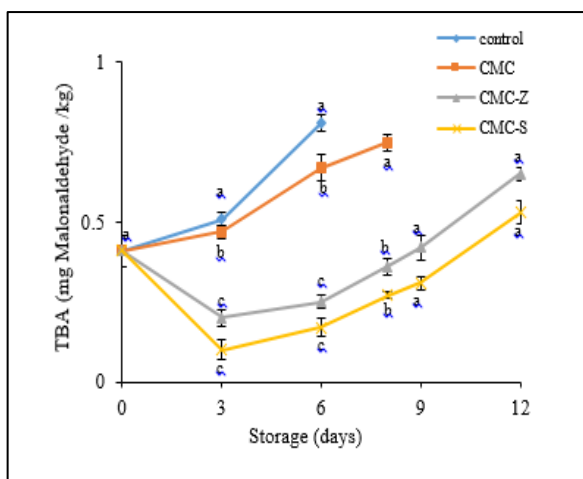
At first day, the pH of the samples was 6.03, which increased in the CMC and control samples during storage. For the CMC-Z and CMC-S treated samples the value significantly constant and lower compared to control ( $p < 0.05$ ). An increase in pH is considered a sign of microbial spoilage in meat products. This increase may be due to protein degradation, which occurs normally because of bacterial proteases and causes accumulation of break-down products such as amines and related compounds [50]. Giatrakou *et al.* [28] observed that the pH of ready-to-cook chicken meat treated with chitosan coating (1.5% v/w) containing thyme oil (0.2% v/w) was lower than that for the control samples at 4 °C.



**Fig. 2** Effect of essential oils on pH of chicken meat during storage (means average values  $\pm$  standard deviation; with different capital letters in the same sample and lowercase letters in the same day are significantly different ( $p < 0.05$ )).

The TBA value at day 0 was 0.41 mg MDA/kg (Fig. 3), indicating a low degree of oxidation in the fresh chicken meat. The TBA value of the control and CMC treatments increased significantly during storage ( $p < 0.05$ ). The CMC-Z and CMC-S coatings were able to reduce lipid oxidation compared to the CMC and control samples. The TBA value for the control sample was 0.81 mg MDA/kg on day 6 and reached 0.25 and 0.17 mg MDA/kg for the CMC-Z and CMC-S treatments, respectively. Zhang *et al.* [50] observed that the TBA value in chicken meat treated with spice extracts (rosemary and cloves) was lower than the control samples at 4 °C. This decrease can be probably attributed to lower oxygen permeability in all wrapped samples compared to the control. The effect was stronger for samples coated with films containing EOs probably due to the increased turbidity of the films, which reduced transparency and light transmission as main factors of photo-oxidation [22]. On the other hand, the phenolic compounds of the EO can also act as an antioxidant to reduce lipid oxidation during refrigerated storage [55].

Aliakbarlu and Sadaghiani [45] studied the TBA count of ground mutton containing ZEO and *Syzygium aromaticum* EO. The antioxidant properties of the ZEO caused complete inhibition of meat oxidation over the 9 days of storage and it was a more effective antioxidant than *Syzygium aromaticum* EO.



**Fig. 3** Effect of essential oils on TBA of chicken meat during storage (means average values  $\pm$  standard deviation; with different capital letters in the same sample and lowercase letters in the same day are significantly different ( $p < 0.05$ )).

Fig. 3 shows the effect of essential oils on TBA of chicken meat during storage. TBA test, which expresses the content of MDA, is widely used to estimate the amount of lipid oxidation in meat and meat products.

### Sensory

#### Evaluation

Table 3 shows the sensory properties of the raw chicken meat. The sensorial quality of the raw meat samples began to decrease on day 6 of storage in the control and CMC samples and caused an off odor, discoloration, and a slimy surface, indicating spoilage. It appears that the off odor was the main factor affecting the acceptability of the meat samples. The sensory results of the control sample were in agreement with those of microbiological analysis.

The CMC coating of the chicken meat could not maintain its sensorial quality during storage and a decrease in the organoleptic properties of samples was observed after 8 days. The CMC-Z and CMC-S treatments showed much higher acceptability than the CMC samples ( $p < 0.05$ ). Among the attributes, a higher rate of change was observed in the odor of the raw meat. Based on the odor scores, it can be stated that the shelf life of the fresh meat was extended by 2–3 days by using the active films.

**Table 3** Effect of CMC films containing essential oils on sensory evaluation of raw chicken meat during storage at 4 °C

Sample/day	0	3	6	9	12
<b>Odour</b>					
Control	0.52 a±4.50	0.39 a±4.0	0.10 a±2.9	0.10 a±1.2	0.11 a±1.1
CMC*	0.43 a±4.61	0.31 a± 4.1	0.33 b±3.7	0.14 a± 1.3	0.33 a± 1.2
CMC-Z**	0.55 a±4.45	0.30 b± 4.4	0.29 c±4.8	0.30 b± 4.3	0.27 b±3.1
CMC-S***	0.87 a±4.51	0.31 b± 4.1	0.22 c±4.6	0.33 b± 4.0	0.21 b±3.0
<b>Colour</b>					
Control	0.51 a±4.40	0.21 a±4.1	0.13 a± 3.0	0.20 a±1.4	0.16 a±1.1
CMC	0.78 a±4.39	0.24 a±4.2	0.21 b±3.8	0.23 a± 1.5	0.15 a± 1.2
CMC-Z	0.93 a±4.62	0.16 b± 4.6	0.16 c±4.6	0.33 b±4.0	0.21 b±2.3
CMC-S	0.71 a±4.56	0.13 b± 4.8	0.22 c±4.5	0.34 b± 3.9	0.16 b±2.6
<b>Overall acceptability</b>					
Control	0.48 a±4.30	0.34 a±4.1	0.13 a± 3.2	0.32 a±1.1	0.19 a±1.0
CMC	0.54 a±4.55	0.21 a±4.3	0.27 b±3.9	0.43 a± 1.0	0.17 a± 1.1
CMC-Z	0.79 a±4.21	0.16 b± 4.7	0.13 c±4.5	0.26 b± 4.1	0.33 b±2.4
CMC-S	0.68 a±4.40	0.16 b± 4.6	0.34 c± 4.4	0.32 b± 3.9	0.26 b±2.5

<sup>a</sup> means average values ± standard deviation, n = 30 panelists for sensory evaluation. A 5-point hedonic scale was used for sensory evaluation. A score of 5.0 was excellent quality, a score of 1.0 indicated bad quality and a score of 3.0 was considered to be acceptable level.

<sup>b</sup> Values within a column followed by a different small letter are significantly different from each other on a specific day ( $p < 0.05$ ) according to Duncan test.

\*CMC: Carboxymethyl Cellulose.

\*\*CMC-Z: CMC film containing *Zataria multiflora* Boiss. essential oil.

\*\*\*CMC-S: CMC film containing *Satureja khuzistanica* Jamzad essential oil.

Table 4 shows the sensory properties of cooked chicken meat. The meat samples were considered to be acceptable for human consumption until the sensory score reached 3 [23]. The sensory results of the cooked chicken meat samples (Table 4) showed that the addition of both EOs reduced all organoleptic scores of the coated samples. However, this decrease was slight in the film incorporating CMC-Z and this active film maintained all sensorial scores at an acceptable level. CMC-S treatment showed a greater antimicrobial effect, while resulted in an organoleptically undesirable product. Considering all other seasoning agents, the flavour of the EOs could be considered undesirable by some

consumers, which can cause to reduce the acceptability of the food products. However, for those who do not prefer the taste and odor of the meat itself, the presence of a suitable concentration of EOs could encourage them to consume the meat products. Gavahian *et al.* [56] also reported that the addition of Zenyan EO obtained by ohmic-assisted hydrodistillation (control, Z-0.015%, Z-0.03%, and Z-0.045%) to mayonnaise did not remarkably change its colour. Although all Zenyan EO-containing samples were different from the control based on sensory properties, there were no significant changes among the different concentrations of EO and some panellists preferred the new aromatic sauces.

**Table 4** Effect of CMC films containing essential oils on sensory evaluation of cooked chicken meat (steam cooker for 60 min) after 48 h storage at 4 °C

Sample	Texture	Colour	Odour	Taste	Overall acceptability
Control	0.26 a± 4.11	0.33 a± 4.80	0.22 a± 4.77	0.28 a± 4.90	0.20 a ±4.88
CMC*	0.32 a± 4.22	0.22 a± 4.82	0.20 a± 4.88	0.22 a± 4.84	0.23 a± 4.70
CMC-Z**	0.32 a± 4.22	0.26 b± 4.58	0.33 b± 4.10	0.33 b± 4.50	0.33 b± 4.20
CMC-S***	0.35 a± 4.28	0.36 b± 4.67	0.24 c± 3.55	0.10 c± 3.00	0.27 c± 3.17

<sup>a</sup> means average values ± standard

<sup>b</sup> Values within a column followed by a different small letter are significantly different from each other on a specific day ( $P < 0.05$ ) according to Duncan test.

\*CMC: Carboxymethyl Cellulose.

\*\*CMC-Z: CMC film containing *Zataria multiflora* Boiss. essential oil.

\*\*\*CMC-S: CMC film containing *Satureja khuzistanica* Jamzad essential oil.

Vital *et al.* [57] evaluated that alginate edible coating containing 0.1% oregano and 0.1% ginger EOs on fish. Treatments with oregano EO received better organoleptic scores for odor appraisal.

## Conclusion

Thymol and carvacrol are the main compounds in SEO and ZEO. The film containing these EOs had effective antimicrobial activities properties against *S. aureus*, *B. cereus*, *E. coli*, *P. aeruginosa*, and *S. enteritidis*. CMC-based films containing either of the EOs improved the shelf life of the chicken meat compared to the pure CMC film and the control by decreasing bacterial growth and oxidative deterioration. The organoleptic properties of the pure meat and meat treated with CMC did not differ significantly during storage; however, the raw chicken meat treated with CMC-based films containing EOs showed improved organoleptic properties during refrigerated storage. Cooked meat that had been coated with a film containing SEO received lower sensorial scores, but the scores for the ZEO film were acceptable in particular for those who did not like the taste and odor of pure meat. The film that was most applicable for industrial usage was the CMC film containing ZEO because it retarded meat spoilage (from 6 to 12 days) and preserved quality with fewest undesirable effects on the sensory properties after cooking and during cold storage. Final result of this study confirmed that potential utility of plant EOs for extending the shelf life of raw chicken meat. CMC film containing *Zataria multiflora* and *Satureja khuzistanica* can be studied to enhance the shelf life of other food products in further researches.

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