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**Original Article**

# Production and Characterization of Antimicrobial Carboxymethyl Cellulose (CMC) Films Containing Essentiol Oils of *Satureja khuzistanica*, *Zataria multiflora*, *Allium sativum* and *Bunium persicum*

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## Abstract

The aim of this study was to evaluate antimicrobial effects of *Zataria multiflora* Boiss. *Satureja khuzistanica* Jamzad, *Bunium persicum* (Boiss.) B. Fedtsch. and *Allium sativum* L. essential oils and the use of the most effective one in packaging and evaluation of the film properties. The antimicrobial activity against *P. aeruginosa*, *S. aureus*, *E.coli*, *B. cereus*, *S. enteritidis* was studied using the disk diffusion method and MIC and MBC was determined. Then CMC films produced with the addition of *Zataria multiflora* Boiss. And *Satureja khuzistanica* essential oils (1.6%, 2.4%, and 3.2%). The addition of essential oils caused to reduce the moisture content to 16.25% and 17.91%, solubility to 69.01% and 64.78%, the color difference to 21.50 and 12.86 and tensile strength to 54.12 and 29.26 Mpa, respectively. An increase in concentration of *Satureja khuzistanica* essential oil made the film more resistant to water diffusion by $1.05\times10^{-13}$(g/s m Pa) compared to *Zataria multiflora*. Regarding the possibility of producing the degradable film with antimicrobial essential oils, it is desirable to use these materials to increase food shelf life.

**Keywords:** Carboxymethyl Cellulose, Essential Oil, *Zataria multiflora*, *Satureja khuzistanica*, Antimicrobial Packaging

## Introduction

It is reported that the world production of food packaging materials which are often polyethylene or other plastics is more than 180 million tons per year. Considering that these materials are not recyclable and decomposable naturally, they remain in the environment and cause a lot of health risks to consumers [1]. In recent years, a new approach to the use of polymer-based materials has become widespread, which is not only harmless for consumers, but also improves the shelf life of food due to improved conditions. Various compositions can be used to produce biodegradable polymers that are generally considered as eatable and safe compound by United States Food and Drug Administration (FDA) [1]. Nowadays, the use of edible films has become prevalent to create a barrier against the entry and exit of moisture, gases, odor, fat, and prevention of microorganism's growth, and also, the preservation of qualitative and mechanical characteristics of the food. Antimicrobial packaging is a special type of active packaging that can increase the shelf life of food products and provide more microbial safety to the consumer [2].

Today, the antimicrobial properties of essential oils are widely used. Essential oils and extracts from medicinal herbs which contain antimicrobial,

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anticancer, antioxidant and free radical compounds are powerful natural preservatives in processed foods. Essential oils are volatile aromatic substances, including complex mixture of organic chemicals such as terpenoids, aldehydes, alcohols, esters and ketones [3,4]. *Zataria multiflora* is called 'Wild Marjoram' and its scientific name is *Zataria multiflora,* belongs to the Labiatae mint family [5,6]. This plant with the vernacular name of *Avishan Shirazi* in Iran has several traditional uses such as antiseptic, anesthetic and antispasmodic. It has a limited distribution in the world and it exclusively grows in Iran, Afghanistan and Pakistan [5]. Its most important compounds are thymol and carvacrol [7]. Cumin is a perennial plant of the umbrella family that is known by the scientific name *Bunium persicum* (Boiss.) B. Fedtsch and in English called Black cumin. Two important components of *Bunium persicum* essential oil are limonene and carvone [8]. Phytochemical profile of *Bunium persicum* has shown flavonoids, phenolic acids, and aldehydes as well as a high content of mono-terpenes and sesquiterpenes contained in the essential oil and extracts of this plant. In recent years, application of natural compounds particularly medicinal plants has increased in food due to their potential to increase the food safety and shelf life [9].

*Satureja khuzistanica Jamzad* is an herbaceous plant belonging to the family of Lamiaceae and growing in southern Iran. This plant is a subshrub, branched stem about 30 cm high, densely leafy, and broadly ovaiate-orbicular covered with white hairs [10]. The essential oil and extract of *Satureja khuzistanica* have biological properties including antibacterial, antifungal, antioxidant, anti-inflammatory, anti-diabetic, anti-leishmanial, anti-hyperlipidemic, anti-spasmodic [11]. Garlic is a perennial plant from the Liliaceae *Allium Sativam* L. family. Its therapeutic and anti-bacterial effects are due to the presence of oregano-sulfonic compounds, the most important of which is allicin. Other compounds such as allian, polysulfides, ajoene, mercaptans, polysulfides, thiosulfatites and adenosine are also found in *Allium sativam* [12]. The aim of this study was to evaluate the antimicrobial effect of essential oils of *Zataria multiflora, Satureja khuzistanica, Bunium persicum, Allium sativum* and effective use of essential oils in the production of packaging and evaluation of antimicrobial carboxymethyl cellulose is produced feature-packed.

## Material and Methods

### Materials

Carboxymethyl cellulose (CMC) with a molecular weight of 41,000 g /mol was bought from Aria Company and *Satureja khuzistanica* by the pharmaceutical company Khorman (Lorestan) and *Bunium persicum* by Agriculture Organization Qazvin (Qazvin, Iran). *Allium sativum and Zataria multiflora* were bought from the local market (Tehran, Iran). Glycerol, Tween 80 (analytical grade), medium (BHI broth) and Agar were purchased from Merck (Darmstadt, Germany).

### Extraction of Essential Oils from Plant

Water Distillation (WD): 100 g of dried leaves *Zataria multiflora*, *Satureja khuzistanica*, *Allium sativam* and *Bunium persicum* were submitted to water distillation with a Clevenger-type apparatus, according to the European Pharmacopoeia, and extracted with 1 liter of water (until no more essential oil was obtained). The essential oil was collected; the oils were stored in dark vials and stored at 4 °C until used [13].

### Antimicrobial Assay

Antimicrobial activity was performed using paper disk diffusion method with microbial population $10^8$ CFU/mL. BHI Agar was used as microbial inoculum medium and the film disks were placed on medium and incubated at 37 °C for 24 h. Antimicrobial activity was measured by the diameters of inhibition growth zones [14].

### Minimum Inhibitory Concentration (MIC)

Dilution method using BHI Broth as a culture medium was used to determine MIC of essential oils. Then tubes containing microbial population $10^8$ CFU/ mL were incubated at 37 °C for 24 hours. The first tube that showed no visible growth was selected as MIC [15].

### Minimum Bactericidal Concentrations (MBC)

MBC of essential oils was determined according to the results of MIC. The MBC was determined by sampling the tube without any signs of growth in the MIC. The suspension was inoculated onto plate containing culture media. Then it was placed in an incubator for 24 hours at 37 °C. After incubation, the MBC was considered as the lowest concentration of essential oils that killed 99.9 percent of bacteria. Therefore, the essential oil was

used in the film packaging based on the results of MIC and MBC [15].

Films Preparation

To prepare the films, 2 g carboxymethyl cellulose (CMC) in 200 mL of distilled water was continuously stirred by a magnetic stirrer at 70 °C for 45 minutes. It was done to dissolve CMC and make a transparent solution. Then 1 mL of glycerol as a lubricant was added. Stirring was continued for 10 minutes at 70 °C, until bubbles created in the dispersion film by the vacuum pump. Then, 50 mL of this solution was slowly poured into the center of the glass plates to dry samples and the thin film create. It takes 30 hours to dry the samples at 35 °C. However this time is variable between 24 to 40 hours based on the film. Dried films were separated from plates slowly and preserved in a desiccator at 25 °C and relative humidity of 53% to reach equilibrium moisture (constant weight) and be ready for tests. To reach humidity 53%, desiccators containing a saturated solution of magnesium nitrate were used.

Determination of Physical Properties of Films

Thickness

Film thickness was determined by a micrometer with a sensitivity 0.01 mm. Film thickness measurement is essential for mechanical tests. Seven points on sheets of the film was randomly tested to get results.

Moisture Content

To determine the moisture content, films were cut out in square shapes with the dimensions 2cm x 2cmand weighed before and after drying. A laboratory oven (Shimi Co., Iran) was employed at 110 °C to reach constant weight (dried sample weight). Every film treatment was done three times to calculate the moisture content [16, 17].

Water Solubility (WS)

After determining the amount of moisture in all the films, the amount of solids into them can be measured. Pieces of the film with dimensions 2cm×2cm were cut and weighed to a precision scale of 0.0001 g. Then, they were placed into 50 mL of distilled water and mixed slowly to reach a constant weight at 25 °C. Then the film and water mixture was percolated down through a filter paper that had already reached a constant weight and weighed precisely. Then the filter paper with a sample was heated again at 110 °C to reach constant weight. The water solubility of the film was calculated according to the following equation [16, 17].

$$WS\ (\%) = ((WO - WF) / WO) \times 100 \quad (1)$$

Water Vapor Permeability (WVP)

WVP tests were carried out at 25 °C and 75% RH (Relative Humidity) using the standard ASTM E96-95(Standard Test Methods for Water Vapor Transmission of Materials.) with some modifications [18]. For this purpose calcium chloride powder without water was placed in a laboratory oven at 130 °C for 24 hours. Circular cups with an average diameter of 6 cm and a depth of 10 cm containing anhydrous calcium chloride (0% RH, assay cup) or nothing (control cup) were sealed by the test films. Each cup was placed inside a desiccator with sodium chloride saturated solution (Merck, Darmstadt, Germany) that was maintained at 75% RH. This difference in RH corresponds to a driving force of 1753.55 Pa, expressed as water vapor partial pressure. The desiccator was kept at 25 °C. Cups were weighed every 24 hours and water vapor transport was determined by the weight gain of the cup. Slopes were calculated by linear regression (weight change vs. time). The water vapor transmission rate (WVTR) was defined as the slope (g/h) divided by the transfer area (m2). WVP (g/m h Pa) was calculated as follows in eq. 2:

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

Where P is the saturation vapor pressure of water (Pa) at the test temperature (25 °C), R1 is the RH in the desiccator, R2 is the RH in the cup and X is the film thickness (m). Under these conditions, the driving force [P (R1 − R2)] is 1753.55 Pa. All measurements were performed three times [19].

Mechanical Properties

Mechanical testing, including tensile strength in terms of MPa, percent elongation at break was determined by Testometric (M350-10CT model made in England). These tests were conducted using ASTM method no. D882-91 [20]. Before carrying out the tensile test; all films (10×1cm) were placed inside the desiccator which includes a magnesium nitrate saturation solution at 25 °C and relative humidity of 53%, for 48 hours until they reached the equilibrium moisture. The films were cut rectangular. The distance between the two jaws was 5cm, the speed of movement of the jaws 30

mm per minute, and the force was 500 pounds. Tensile strength (TS) was calculated by dividing the peak load by the cross-sectional area of the initial film specimen. Elongation at break (ELB) was obtained by the percentage change in length of the specimen from the original distance between the grips (30 mm) [17, 18].

$$\mathbf{TS} = \frac{F}{w \times d} \quad (3)$$

TS is tensile strength in MPa, F is a force in terms of N, and D and W are the thickness and width of the film respectively in meters.

Optical Properties

To measure surface colors, three factors of the film (L, a and b) were read on a white plate (reference) by the Minolta Chroma Meter CR 360 (Minolta Co., Ltd, Japan). Values indicated the lightness with range from 0 (black) to 100 (white), redness–greenness (a) from −80 (greenness) to 100 (redness), and blueness-yellowness (b) from −80 (blueness) to 70 (yellowness) [21]. To calculate the difference between the sample and the color of white plate obtained factors (L*, a* and b*, standard parameters) and L, a and b (parameter of the sample) were used [16,17].

Measurements were taken on white standard backgrounds (L* = 93.49, a* = −0. 25 and b* = −0. 09). Films were conditioned in desiccators at 25 °C and 53% RH before optical measurements. Total color difference (ΔE) and whiteness index (WI) were calculated using the following

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

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

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

The color of samples in terms of lightness (L), redness (a), and yellowness (b) was measured by the Minolta Chroma Meter CR400 (Minolta Co., Ltd, Japan).

Scanning Electron Microscopy (SEM)

The microstructure of film surface and cross-sections were observed by SEM (model: KYKY–EM3200, China). The electron beam was used instead of light. Because of the short wavelength, it can magnify the images highly [22, 23]. The Film samples were cut to a 2×2 cm and attached to double side adhesive tape and mounted on the specimen holder. The films were coated of 100 Å thickness of gold using sputter coater (Model: KYKY–SBC12, China) under vacuum. The images of samples were captured with an accelerating beam voltage of 30 Kv [16, 17].

Statistical Analysis

Analysis Tables Data Analysis of variance (ANOVA) was performed by SPSS software version 20 (SPSS Inc., Chicago, IL). Duncan's mean comparison was used to examine the significant difference between the data at the significance level of 0.05.

## Results and Discussion

Antimicrobial Activity of Essential Oils

Antibacterial activities of the essential oils including *Zataria multiflora*, *Satureja khuzistanica*, *Bunium persicum* and *Allium sativum* against five selected bacteria have been shown in Table 1. In the nature, herbal essential oils play a significant role in the protection of the plant against microorganisms. These essences are composed of two or three main components with high concentrations (20-70%) and a low percentage of other components [22].

Obtained results showed that *Zataria multiflora* and *Satureja khuzistanica* had highest inhibitory activity which can be due to their phenolic compounds. *Zataria multiflora* essential oil contain phenolic compound such as thymol (51.6%) and carvacrol (12.4%) and trace amounts of non-phenolic components. The main compound of *Satureja khuzistanica* was carvacrol (87.16%) as detected in previous study (data not shown). Rahimi *et al.* studied on chemical composition of *Zataria multiflora*. The obtained results indicated that the most substantial compounds were thymol (34.44%) and carvacrol (33.45%) [23]. Similarly, Raeisi *et al.* revealed the inhibitory effect of *Zataria multiflora* essential oil on *Listeria monocytogenes* and the most substantial compounds were phenolic monoterpene carvacrol (63.20%), followed by thymol (15.10%) and γ-terpinene (2.70%). Fatemi *et al.* studied on chemical composition and antioxidant properties of Iranian *Zataria multiflora* extracts, the major compounds were thymol (61.8%), carvacrol (10.5%), p-cymene (7.5%), and γ-terpinene (4.4%) [24, 25]. The essence of wild Marjoram from

Lamiaceae, family contains two main compounds of thymol, and carvacrol. The most vital component is carvacrol (71.12%) [26]. The bactericidal and antioxidant properties of the wild Marjoram have attracted the attention of many researchers. Thymol as an active ingredient is a phenolic compound, and carvacrol is also compound which is well soluble in alcohol and organic solvents, and these materials are mainly stored in young leaves during plant growth [7]. Thymol by the rate of 10.64% and carvacrol by the rate of 2-11% are regarded as the most active components of wild Marjoram. Both of these ingredients possess an extensive bactericidal spectrum. Gamma tripnin and P. Simen are regarded as the two main components of the essence extracted from wild Marjoram [27]. It has been proved that phenolic compounds destruct the external membranes of bacteria which cause the gradual leakage of the Lipopolysaccharides leading to enhanced permeability of cytoplasmic membrane to ATP. The propulsion of ATP, in turn, results in the depletion of the potential cell energy and final death of the cell [22].

Hadian *et al.* studied on bactericidal effects of four different species of savory, *Satureja hortensis* L. of Bakhtiari and Khuzistanica origins, *Satureija* rishengary and Motica origins, as the native shrubs of Iran [28]. The obtained results indicate that the most substantial compounds of these herbs are thymol and carvacrol. They had the highest bactericidal activities against the gram-negative microorganisms of P. *aeruginosa* and E.*coli* versus the Gram-positive microorganisms of S. *aureus*, and B. *cereus* using paper disk diffusion method [28].

Essential oils of *Zataria multiflora* and *Satureija khuzistanica* had the most efficacious influences on the Gram-positive bacteria compared to the Gram-negative bacteria. The Gram-positive bacteria possess thick cell walls, containing a high grade of peptidoglycan and tiquic acid. Essential oils disintegrate the outer membrane of Gram-positive bacteria, and thus increase the cytoplasmic membrane permeability. The Gram-negative bacteria possess an outer membrane containing lipopolysaccharides with hydrophilic-hydrophobic nature which is sensitive to penetration and thus disintegration activities of essential oils [29]. Similar results were obtained by Ghasemi *et al.* on the MIC of marjoram against S. *aeruginosa*, as the S. *aureus*, E. *coli*, and L. *monocitogenes* [29].

MIC and MBC of Essential Oils

Among essential oils, *Zataria multiflora*, *Satureja khuzistanica* had the highest antimicrobial activity and they were selected to determine MIC and MBC. *Salmonella enteritidis* and P. *aeruginosa* had been determined as the most resistant bacteria against *Satureja khuzistanica* and *Zataria multiflora* microorganisms, respectively. MIC and MBC of *Satureja khuzistanica* essential oil against *Salmonella enteritidis* and *Zataria multiflora* essential oil against P. *aeruginosa* are shown in Table 2. The antimicrobial impact of essential oils and its various components extracted from medicinal plants has been well documented. Essential oils have been extracted from complex mixture of volatile molecules produced by the secondary metabolism of medicinal plants [30].

The antimicrobial activities showed that *Zataria multiflora* and *Satureja khuzistanica* essential oils are more effective on Gram-positive bacteria than Gram-negative bacteria related to the cell wall.

In 2011, Eftekhar *et al*. studied antimicrobial activity of essential oil of *Zataria multiflora* some microorganisms such as B. *subtilis*, E. *facalis*, E. *coli*, S. *aureus*, K. *penumonia*, P. *aeruginosa* [31]. The result showed that all tested bacteria were susceptible to the essential oil except *Pseuedomonas aeruginosa.* (Eftekhar, Zamani, Yusefzadi, Hadian, and Nejad Ebrahimi, 2011). Owlia *et al*. evaluated antimicrobial characteristics of some essential oils of *Matricaria chamomilla*, *Artemisia persica*, *Zataria multiflora*, *Myrtus communi*, *Ruta graveolens*, *Eucalyptus camaldulensis* and *Ferula gummosa* against P. *aeruginosa* (ATCC 27853) [32]. In this regard, three essential oils of Z. *multiflora*, M. *communis* and E. *camaldulensis* had the highest diameter of inhibition zone 0.57-10.66, 0.57-12.33, 1.7-12 mm, respectively. So these 3 essential oils have the most antimicrobial activity against P. *aeruginosa.*

Hadian *et al.* studied the composition and antibacterial activity of essential oils of four *Satureja* species (S. *bachtiarica*, S. *khuzistanica*, S. *mutica* and S. *rechingeri*) growing in Iran. Inhibition zone diameter of essential oils against S. *aureus*, B. *cereus*, E.*coli*, P. *aeruginosa* microorganisms was determined. Inhibition zone diameter of *Satureja* essential oil of *khuzistanica* were 12, 45, 41, 32 mm, respectively, in Bakhtiari were 11, 32, 45, 39 mm, in mutica are 12, 40, 50, 37 mm and in Rechingeri were 11, 40, 46, 40 mm,

respectively. *Pseudomonas aeruginosa* had the least amount of inhibition zone diameter and the highest amount of MIC and MBC [28].

Physical Properties of Films

All films were easily removed from the plate surfaces. Thickness incorporated with essential oils, varies from 0.14 to 0.20 mm, and from 0.14 to 0.17 mm for films incorporated with *Zataria multiflora* and *Satureja khuzistanica* essential oils, respectively. The thickness of the control film was 0.13 mm. The thickness of the films significantly ($p$ <0.05) increased with the addition of essential oils and proportional to different concentrations. Essential oils are natural, volatile liquid, complex compounds characterized by a strong odor, rarely colored, soluble in lipid and organic solvents [30]. The increase in the thickness of the films containing essential oils is due to the increase in the amount of its solids. Also, how to position the essential molecule and the amount of tween 80 in the films can be effective in increasing the thickness due to the trammeling of the droplets of the essences, within the film lattices. Films containing essential oil, in particular, have a texture of 3.2% concentration similar to that of a compact sponge that can increase the thickness of the film, thereby producing films with a concentration of 3.2 in both the most essential oil and the non-essential oil control film showed the smallest thickness ($p$ <0.05). Similar results were reported by Dashipour *et al.* and Shojaee Aliabadi *et al.* [16, 17].The Zivanovic study in 2005 demonstrated that the thickness of chitosan films significantly increases by addition of different essences [33].

Moisture Content and Solubility of the Film

By adding pertinent essences, the moisture content of the film was significantly decreased ($p$ <0.05). Therefore, the film with a concentration of 3.2% essential oil and the control film without essential oil had the lowest and highest moisture content, respectively ($p$ <0.05). The water solubility rate of the control film was 81.50%. However, the solubility rate in the film containing the essential oils was significantly reduced by the presence of higher amounts of the essences, in proportion with pertinent concentration rates [34]. Essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from the plant. The advantages of essential oils are their flavor concentrations and their similarity to their corresponding sources. The majority of them is fairly stable and contains natural antioxidants and natural antimicrobial agent [35]. The reason for this decrease in moisture and solubility in the film can be attributed to the hydrophobic nature of the essential oils, which reduces the ratio of the hydrophilic section to the film hydrophobic regions. Ghasemlou *et al.* implemented research on the film which was produced from lotus with addition of oleic acid [36]. The result of this research is in line with the findings of the present study, indicating that by the addition of oleic acid to the film, the humidity rate is reduced by the significance level of ($p$ <0.05). Atares *et al*. studied on the Film cinnamon and ginger-flavored soy protein by addition of the essence, the water solubility rate and moisture content in the film significantly decreased ($p$ <0.05) [37]. The results were according to the Dashipour findings, on the bactericidal and antioxidant properties of the carboxylmethyl cellulose film, containing the essence of *Zataria multiflora* [16]. These findings are also in line with Shojaee Aliabadi results, on the bactericidal and antioxidant properties of carrageenan film, containing the essence of *Satureja hortensis* [17].

Water Vapor Permeability (WVP)

WVP of the Essential Oil of *Zataria multiflora* Boiss

Depending on the type of food, packaging must prevent moisture transfer or minimize transfer of moisture between food and the environment. Comparison of mean permeability data to steam showed that in the film containing essential oil of *Zataria multiflora*, the presence of higher concentrations of the essential oil of *Zataria multiflora* increases the permeability to water vapor. As a result, the film with a concentration of 3.2% of the essential oil of *Zataria multiflora* showed the highest amount and the control film lacking essential oil showed the lowest rate of water vapor permeability (WVP) ($p$ <0.05).

In 2012, Ahmad *et al.* identified the concentration and type of essential oil as an important factor in reducing and increasing WVP to film [38]. They showed while lime grass essential oil reduces the permeability to water vapor in the gelatin film, bergamot essential oil ingresses WVP in the gelatin film at high concentrations of 0.5% increases WVP because non-hydrophilic molecules of bergamot essential oil locate in the chain of gelatin and disrupt continuous network of the film. While the

presence of higher levels of lime grass oil because of hygroscopic property, (adsorption and desorption of moisture) reduces WVP different from bergamot essential oil. The obtained results are according with Dashipor results in 2015 as well as the results were also confirmed by the results of Atares *et al.* in 2011 [16, 39].

WVP of the Essential Oil of *Satureja khuzistanica*

The results show that the amount of WVP of films decreased significantly by adding various concentrations of essential oils of *Satureja khuzistanica* to the film-forming solution. So the control film (without essential oil) showed the most amount of WVP and the film with the greatest amount, concentration of 3.2 of *Satureja khuzistanica* essential oil showed the least result ($p$ <0.05).

Adding essential oil droplets as a dispersed hydrophobic phase in continuous phase film network causes to increase the Tortuosity factor in continuous film matrix and this process leads to a reduction in the water vapor transfer rate. The transfer of water vapor from hydrophilic films depends on the solubility and diffusion of water molecules in the film matrix.

The effect of essential oils on reducing the rate of vapor permeability in the film can be attributed to the creation of zigzag paths against the penetration of water vapor molecules, thereby reducing the emission factor and decreasing the solubility of water molecules in the film matrix [16, 17]. In 2005, Pranto expressed the proportion of hydrophilic to hydrophobic compounds of the film material is an important factor in the moisture permeability into edible films [40].

Possibly, the presence of 0.4% concentration of essential oil in the film network significantly reduced permeability to moisture in the carboxymethyl cellulose edible film, but at lower concentrations of essential oil, these compounds did not have a significant effect on the structure carboxymethyl cellulose film. In 2010, Atares *et al.* did not suppose that adding hydrophobic compounds such as essential oils is not enough reason for reducing moisture permeability to edible films [37]. They stated that the interactions of the protein and essential oil network cause the essential oil to be ineffective on the water vapor permeability and thus reduces the essential oil's hydrophobicity. The results of this study corresponded with the result of Kechichanin 2010 and Sanchez-Gonzalez *et al.* in 2010 in which they examined the physical and antimicrobial properties of chitosan and tea tree essential oil, and concluded that adding 2% tea tree essential oil into chitosan film causes to distribution and release of essential oil on the film surface, and significantly, decrease the permeability (g/s mPa× $10^{-11}$) 124 to 74.8 [41, 42].

Mechanical Properties of Films

The mechanical properties are one of the most important factors in choosing the type of food packaging. Knowing information about the mechanical parameters (tensile strength and elongation percentage) of biodegradable films is very important for designing packaging process and predicting their ability to maintain their integrity during use as a package [36]. The results showed that the mechanical properties of both essential oils are almost equal and with increasing concentration of *Zataria multiflora* and *Satureja khuzistanica* essential oils from 1.6 to 3.2%, tensile strength of the films compared to the control film decreased significantly($p$ <0.05). Addition of essential oil of *Zataria multiflora* to the carboxymethyl cellulose film reduces tensile strength and elongation percent at rupture while addition of *Satureja khuzistanica* essential oil reduces tensile strength and increase elongation percent at rupture.

With the addition of essential oils of *Zataria multiflora* to the film at a concentration of about 3.2%, tensile strength and elongation percent at break decreased about 54.12 MPa and 3.95%, respectively compared to control films. In fact, addition of the essential oil reduces strength (density) of carboxymethyl cellulose polymer filaments due to the accumulation of oil droplets in the inter-polymeric spaces (the presence of large amounts of oil within the films spreads incomplete oil into film) and finally it creates weak bonds between the polar polymer and non-polar essential oil, and this reduces the creation of points with intolerance to stretching on the film, which reduces the tensile strength and the percentage stretch at break of polymer filaments. In the case, this mode occurs at low concentrations of essential oils [16].

**Table 1** Antimicrobial activities of different essential oils[1]

| Essential oil | *Salmonella enteritidis* | *Bacillus cereus* | *E.coli* | *Pseudomonas aeruginosa* |
|---|---|---|---|---|
| *Bunium Persicum* | 9.16±0.44 b | 8.66±0.72 c | 1.83±0.16 b | 17 ±4.80 b |
| *Zataria multiflora* | 25.27±4.05 a | 40.83±4.49 a | 29.50 ± 6.52 a | 29±9.96 a |
| *Satureja khuzestanica* | 9.62± 0.23 b | 21.66± 0.44 b | 10.75 ±1.12 b | 15.37±6.06 b |
| *Allium Sativam* | 11±0.28 b | 10.66± 0.33 c | 10.66 ±0.88 b | 12± 1.00 b |

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

**Table 2** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of different essential oils[1]

| Essential oils | Microorganism | MIC(mg/L) | MBC(mg/L) |
|---|---|---|---|
| *Zataria multiflora* | *Pseudomonas aeruginosa* | 763.66±69.33 b | 90666.67±3335.33 b |
| *Satureja khuzestanica* | *Salmonella enteritidis* | 260.00±52.00 a | 56000.00±4000.00 a |

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

**Table 3** Physical properties of control and EO containing films [1]

| Film | Thickness (mm) | Moisture content (%) | Solubility in water (%) | WVP ($gs^{-1}m^{-1}Pa^{-1}$) | Tensile strength(MPa) | Elongation at break (%) | ΔE | WI |
|---|---|---|---|---|---|---|---|---|
| ZEO1.6 | 0.14± 0.002 d | 19.80± 0.14 de | 80.27± 0.31 f | $1.40\times10^{-13}$± 0.003 c | 93.08± 8.24 a | 13.12± 2.64 a | 11.39±0.39cd | 82.10±0.39 c |
| ZEO2.4 | 0.17± 0.002 b | 17.35± 0.22 ab | 76.76± 0.32 e | $2.03\times10^{-13}$ ± 0.002 d | 69.88±8.60 b | 11.07± 1.92 b | 15.28± 0.36 b | 78.42±0.34f |
| ZEO3.2 | 0.020± 0.002 a | 16.25± 0.14 a | 69.01± 0.01 b | $2.69\times10^{-13}$± 0.018 e | 54.12± 3.70 e | 3.95± 0.68 f | 21.50± 0.23 a | 72.38± 0.23 h |
| SEO1.6 | 0.14± 0.003 d | 20.64± 0.17 e | 72.85± 0.02 d | $1.22\times10^{-13}$± 0.005 b | 55.34± 5.95 d | 4.14± 0.31 e | 11.30± 0.30 cd | 82.36±0.01 b |
| SEO2.4 | 0.15± 0.003 c | 18.96± 0.49 cd | 70.36± 0.09 c | $1.17\times10^{-13}$± 0.003 b | 40.50± 4.78 f | 4.16± 1.15 d | 11.86± 0.20 c | 81.77± 0.02 d |
| SEO3.2 | 0.17± 0.003 b | 17.91± 0.75 bc | 64.78± 0.17 a | $1.05\times10^{-13}$± 0.002 a | 29.26± 3.44 h | 6.92± 1.35 c | 12.86± 0.50 b | 80.78± 0.02 e |
| Control | 0.13± 0.003 e | 28.60± 0.07 f | 81.50± 0.20 h | $1.28\times10^{-13}$±0.23 b | 55.81± 3.77 c | 2.38± 0.42 h | 10.75± 0.35 d | 82.70± 0.34 a |

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

Dashipor *et al*. studied the properties of the carboxymethyl cellulose film containing essential oil and in 2009, Sanchez-Gonzalez *et al*. investigated the effects of essential oil of tea in the film HPMC, which confirmed our results in the tensile properties of the film containing essential oils of *Zataria multiflora* [16, 43]. Changes in length increase percent in the film containing the essential oil of *Satureja khuzistanica* showed that with increasing concentration of essential oil compared to the control film, tension capacity increased significantly and it seems that the presence of oil causes the effects of softening or flexibility in the film, and it changes the balance of interaction forces and reduces cohesion forces in the films matrix. It also reduces the cohesion and adhesion of the film network forces and facilitates the movement of the chains while dragging as well as our study is as some other studies like Shojaee Aliabadi *et al*. and Sánchez González *et al*. concluded that increased essential oil concentration in the film decreases the tensile strength and increases the capacity of traction ($p$<0.05) [17,42].

Color

The color specification of packaging is one of the important factors in the attractiveness and choice of product by the customer and depends on the type of composition and process used in making the film [44]. The total color difference ΔE was obtained from Equation 4which L*, a*, b* represents black to white, green to red, blue and yellow respectively [37]. In this study, white plate used as the color reference. The optical properties, ΔE, L, a, b showed whiteness index in the carboxymethyl cellulose film with and without essential oils of *Zataria multiflora* and *Satureja khuzistanica*. The control films lack transparent, colorless while adding various concentrations the essential oil of *Zataria multiflora* and *Satureja khuzistanica* increased ΔE and reduced white index in the film.

So, the transparency of the film containing the essential oil was significantly decreased by adding different concentrations of essential oil ($p$ <0.05) and the appearance color became darker. Essential oils are usually colorless, particularly when fresh. Nevertheless, with age essential oil may oxidize which resulting the color becomes darker. Therefore, essential oil needs to be stored in a cool, dry place tightly stoppered and preferably full in amber glass containers [35]. The reason for this color change can be due to the presence of phenolic compounds in the oil. The oil has almost yellowish color. Adding the essence to the film reduces the transparency of the glass film, which is due to creation of a rough surface on the film surface during drying, because the essence is usually accumulated on the film surface, causing heterogeneity in the film's surface [44]. Increased light scattering in the matrix film is distributed by drops of essential oil. The distribution property depends on the amount and size of the dispersed particles (essential oil droplets). Due to the greater dispersion and diffusion intensity, transparency is reduced [44]. These results are consistent with previous study by Dashipor *et al.* on the video CMC contains essential oil and Shojaee Aliabadi *et al.* on the film carrageenan-containing oil of *Satureja khuzistanica* [16,17].

Films' Microstructure

Figures 1 and 2 show microstructures from the cross-section of the control films (without oil) and films containing *Zataria multiflora* and *Satureja khuzistanica* essential oils at concentrations of 1.6%, 2.4%, and 3.2%.

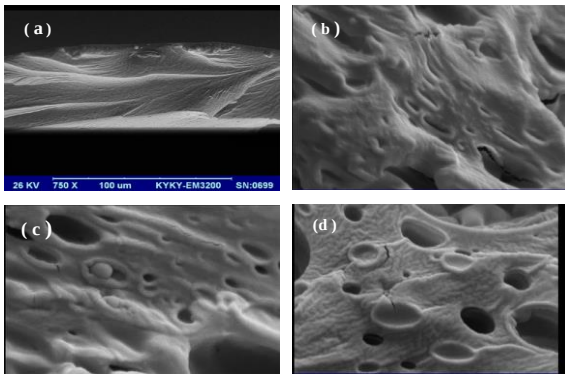


**Fig. 1** Cross section of the film microstructure control (a) films containing of essential oil *Zataria multiflora* at a concentration of 1.6 (b), the concentration of 2.4 (c), the concentration of 3.2 (d)

Microstructure development in the film emulsion distribution during drying determines the composition of the compound in the dried film. Changes in film microstructures were studied to visualize the surface topography and the cross-section of all films prepared from carboxymethyl cellulose. The control film (without essential oil) had a compact, smooth structure with continuous microstructures and without any irregularities. The films containing both essential oils showed that the addition of essential oils creates a heterogeneous structure in the film, especially when the concentration of essential oil was increased. The addition of *Satureja khuzistanica* essential oil creates a heterogeneous structure in the film because the essential oil droplets are continuously trapped in the film [17].

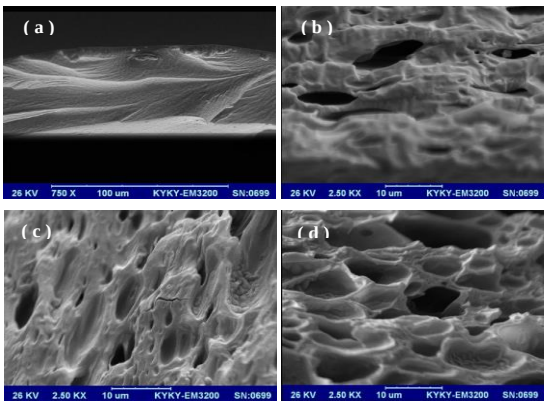


**Fig. 2** cross section of the film microstructure control (a) films containing of essential oil *Satureja khuzestanica* at a concentration of 1.6 (b), the concentration of 2.4 (c), the concentration of 3.2 (d)

Based on microscopic images, it seems that the homogenization process has been able to produce almost equal particles even at high concentrations of essential oils. Even in high concentrations, no cream state is observed in the emulsion film. Mobility of the oil droplets decreases due to the high viscosity of the film-forming dispersion and increasing this viscosity during drying. At low concentrations, the particle size of the emulsion films is small which has also increased with increasing concentrations of essential oils in the film. So in a concentration of 3.2%, the large droplets of essential oil are visible on the surface with magnification. Similar results have been reported by Shojaee Aliabadi *et al.* on carrageenan film containing *Satureja khuzistanica* essential oil [17]. Microscopic images show more discontinuity in films with essential oils of *Zataria multiflora*

than *Satureja khuzistanica* which may be due to the different behavior of essential oils during homogenization and drying processes. Similar results have been reported by Dashipor *et al.* on the CMC film containing the essential oil and Atares *et al.* on the film cinnamon and ginger-flavored soy protein [16,37].

## Conclusion

*Zataria multiflora* and *Satureja khuzistanica* essential oils had the most antimicrobial activity among *Zataria multiflora*, *Satureja khuzistanica*, *Bunium persicum* and *Allium sativum* against the microorganism *Pseudomonas aeruginosa*, *E.coli* and *Salmonella Enteritidis* (Gram-negative), *Staphylococcus aureus* and *Bacillus cereus* (Gram-positive) due to the presence of two inhibitors thymol and carvacrol. In general, the film containing essential oils of *Satureja khuzistanica* due to favorable reduction moisture content (%) and solubility in water (%) and good resistance to water vapor permeability and a favorable increase in elongation at break (%) is more efficient in comparison with essential oil of *Zataria multiflora*. Due to the possibility of producing biodegradable films with essential oils as natural antimicrobial compounds, it is recommended to reduce the microbial load and increase shelf life of food products.

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