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Antimicrobial and antioxidant effects of chitosan edible film containing nanoemulsion of *Melissa officinalis L.* extract and *Buniumpersicum* essential oil on *Listeriamonocytogenes* inoculated into camel meat

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ABSTRACT

The aim of this study was to investigate the antimicrobial and antioxidant effects of chitosan edible film containing nanoemulsion of *Melissa officinalis* L. extract and *Buniumpersicum* essential oil on *Listeriamonocytogenes* inoculated into camel meat. The studied films were prepared using 2% chitosan and 2.5% and 5% of nanoemulsion of *Buniumpersicum* essential oil and 4% of *Melissa officinalis* L.extract. The antimicrobial and antioxidant effects of coated camel meat during 16 days of storage at 4 °C with a 4-day interval (0, 4, 8, 12, 16) were evaluated. The coated portions were chemically evaluated. Most of the essential oil compounds include: cuminaldehyde (24.37%), γ-Terpinene(19.99%), and P-cymene (9.71%). The MIC of *Melissa officinalis* L. extract and *Buniumpersicum* essential oil against *L. monocytogenes* were 1% and 0.25%, respectively. The antioxidant effects of films by DPPH method showed that the addition of essential oils and extracts increases the antioxidant properties of films. The antimicrobial effect of films by disk diffusion method, the largest diameter of growth inhibition zone (17.15 ± 0.16) was related to chitosan film containing 5% *Buniumpersicum* essential oil and 4% *Melissa officinalis* L. extract. The average count of *L.monocytogenes* in the control treatment was higher than the other treatments. The results of TBARS showed that the antioxidant properties of films containing *Buniumpersicum* essential oil and *Melissa officinalis* L. extract was higher than the control sample. The pH level in the samples coated with chitosan film containing 5% *Buniumpersicum* essential oil and 4% *Melissa officinalis* L. extract was lower than the other treatments. In general, the prepared films have good antimicrobial and antioxidant properties against *L. monocytogenes*, which increase with the addition of plant compounds.

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1. Introduction

Meat and meat products are considered as one of the most important human food sources [1]. Camel meat is a type of red meat that has low fat and cholesterol [2]. The composition of camel meat depends on the breed, age, gender and geographical location. Camel meat has a higher moisture content than the meat of other domesticated animals [3]. Among food ingredients, meat is a very suitable environment for the growth of spoilage and disease-causing microorganisms due to its high protein, moisture, and nutrient compounds [4, 5]. All the measures that have been taken from the past until today are aimed at preserving the meat and its products from any corruption and contamination.

One of the ways to preserve food is to use chemical preservatives. But due to the harmful effects of these compounds on human health, the attention of food producers has been directed to the use of natural antimicrobial compounds, including essential oils and plant extracts [6].

Another way to increase the shelf life of food is to use suitable covers and packaging. Plastic materials made from petroleum compounds have been used in food packaging for many years [7]. But nowadays, due to various health problems, the use of biodegradable and useful edible coatings and films has attracted the attention of producers [8].

Edible films and coatings can not only be eaten by the consumer, but also protect the food from moisture, dust, oxygen and any other harmful compounds. Edible films and coatings can be protein, polysaccharide, lipid and derivatives of these compounds. Edible films and coatings can be biodegradable or edible depending on the formulation and materials used in their formation [9, 10].

Polysaccharide chitosan has a positive charge, which is produced by alkaline deacetylation of chitin. This compound is non-allergenic and biodegradable, and in addition to having antimicrobial and antioxidant activity, it also delays the loss of food moisture [11, 12]. Chitosan has antimicrobial properties due to the presence of positively charged amino groups, so that it reacts with the cell membrane of microorganisms and causes disruption in the cell membrane [13].

The use of natural antimicrobial compounds, including plant essential oils, in the composition of edible films and coatings has increased their preservation properties and today has attracted the attention of many researchers. Many researches have been done in relation to chitosan film and the use of natural antimicrobial substances of animal and plant origin with the aim of increasing antioxidant, antimicrobial and antifungal activity[14].

Caraway (*Bunium persicum*), belongs to the Apiaceae family. It is a medicinal plant and is natively found in the southeast of Iran. This plant is used in traditional medicine to relieve muscle cramps, increase appetite, increase expectoration, and increase milk production in the food industry as a flavoring [15].

Melis (lemon) plant, Banamalemi*Melissa officinalis*It belongs to the Lamiales order and the Labiatae family. It is a fragrant, herbaceous and perennial plant whose height reaches 100 cm and its habitat is southern Europe, the Mediterranean and some parts of Azerbaijan and northern Iran. This plant has a lemon smell because of that*Lemon balm* It is also said In traditional medicine, this plant is used to treat insomnia, nervous system diseases, toothache, high blood pressure and headache [16]. Melissa essential oil and extract are used as an antibacterial agent, antifungal agent, pain reliever and Alzheimer's disease treatment drug[17].

Recent studies have shown better physical properties of nanoemulsions containing essential oils compared to their normal emulsions[18]. In addition, more antibacterial activity has been observed in nanoemulsions containing plant essential oils [19]. Hyper nanoemulsion based on polysaccharides such as alginate and essential oils is used as an antimicrobial agent to form edible films, which can be considered a new generation of edible packaging [20].

bacteria*Listeria monocytogenes*, gram positive and catalase positive, which causes listeriosis, which is one of the important diseases that can be transmitted between humans and animals, and enters the human body through eating contaminated food products. Due to being cold-oriented, it can grow in refrigerator temperature and can easily grow in food stored in refrigerator [21].

This research aims to produce an edible chitosan film containing nanoemulsion of black seed essence and lemon balm extract and to investigate the antioxidant and antimicrobial properties of the films.*Listeria monocytogenes* It was done as a foodborne pathogen.

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2- Materials and methods

2-1-Preparation of the studied bacteria

bacteria*Listeria monocytogenes*(ATCC 7644) was obtained from the microbial collection of the Department of Food Hygiene, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad and was prepared for inoculation according to Marshii et al. (2017) [22]. containing BHI agar medium were cultured and kept warm for 24 hours at 37°C. Then, a suspension equal to 0.5 McFarland (light absorption rate equal to 0.08-0.1 at 600 nm wavelength) was prepared from the desired bacteria. To be sure, the prepared suspension was diluted and counted on BHI agar medium.

2-2-Preparation and analysis of black cumin essential oil

200 grams of black cumin seeds (*Bunium persicum*) after collecting from Kerman province, essential oil was extracted with water distillation method by Cloninger machine and dried by anhydrous sodium sulfate [23]. Essential oil analysis was done by GC-MS machine. Gas chromatograph (Agilent HP-6890 Palo Agilenttechnologies, Alto, CA, USA) with HP-5MS capillary column, column length 30 m, inner diameter 0.25 mm and thickness 0.25 µm connected to a mass spectrometer (Agilent AHP-5973) was done. Helium flow rate was 1 ml/min. The temperature of the oven was initially 50°C, then it increased by 2°C every minute until it reached 120°C, it was kept at this temperature for 3 minutes, and at the end the temperature increased to 300°C. The mass spectrometer was also performed with an ionization angle of 70 electron volts [24].

2-3- Preparation of lemon balm extract and drying of aqueous extract by freeze dryer method

The aerial parts of the Melissa plant (*Melissa officinalis*) after being procured from medicinal plant suppliers in Mashhad city, they were cleaned and dried in the shade and powdered by a mill and kept in suitable conditions until use. To prepare the aqueous extract of Melissa, 10 cc of boiled distilled water was added to each gram of powder and boiled for 15 minutes. The obtained extract was filtered using a Buchner funnel and filter paper, then dried with a freeze dryer (Christ, Osterode, Germany). In this way, the extract was poured on steel plates and freeze-dried at -70 degrees Celsius for at least 6 hours. When freezing takes place at a temperature lower than -70 degrees Celsius, very small ice crystals are formed in the product, which will prevent tissue damage during freezing and sublimation. After complete freezing, the samples were weighed with a digital scale with an accuracy of 0.001 grams. During drying, it was possible to observe weight loss. The sublimation of the samples was done by gradually heating the environment inside the vacuum chamber. The high vacuum conditions caused the sublimation of the crystals inside the sample. Most of the water in the sample was removed in 33 hours, which made it easy to store the obtained product in the environment.

2-4-Preparation of nanoemulsion of black cumin essential oil and lemon balm extract

To prepare nanoemulsion of black cumin essential oil (BPNE) and nanoemulsion of lemon balm extract (MONE), the method of Moghimi et al. (2016) was used with some changes [25]. In this way, to prepare nanoemulsion of black cumin (10%), 10% by weight of essential oil of black cumin and 5% by weight of tween 80 and 85% by weight of deionized distilled water and to prepare nanoemulsion of lemon balm extract (4% by weight of extract powder, 2% by weight of Tween 80 and 94% by weight of deionized distilled water were mixed. The solutions of black cumin essential oil and lemon balm extract were separately subjected to ultrathorax (3 min at 3000 rpm) and ultrasonic probe (50 °C, pulse; 45s and rest; 15s) for 15 minutes and the particle size was determined by Light scattering (DLS) was measured.

5-2- Determination of minimum inhibitory concentration (MIC) and minimum lethal concentration (MBC) of nanoemulsions of black cumin essential oil and Melissa extract

Broth microdilution method was used to determine the minimum inhibitory concentration of nanoemulsions of black cumin essential oil and lemon balm extract. Neem McFarland suspension (CFU/ml $10^8$1.5 ×) from bacteria*Listeria monocytogenes* was prepared, then until the bacteria reached 10 CFU/ml^6 diluted Different

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concentrations of black cumin essential oil nanoemulsion, from 0.031% to 1% concentration, and different concentrations of lemon balm extract nanoemulsion, from 0.125% to 4% concentration were prepared. In order to determine MIC, 160 microliters of BHI broth, 20 microliters of nanoemulsion of black cumin essence and lemon balm extract and 20 microliters of bacteria were poured into each well. All experiments were performed in 3 replicates. For each replication, one well was considered as a negative control (without adding bacteria) and another as a positive control (without adding essential oil and extract). The plates were kept in a greenhouse at 37°C for 24 hours. After this period, the wells were examined macroscopically for turbidity and the minimum growth inhibitory concentration (MIC) was determined by visual method and observation of turbidity.

To determine the minimum lethal concentration (MBC), bacteria from the wells where turbidity was not observed were cultured on BHI agar medium. After incubation for 24 hours at 37°C, the lowest concentration in which the bacteria did not grow was considered as the minimum lethal concentration (MBC).

6-2-Preparation of studied films

To prepare 2% chitosan film, 2 grams of chitosan powder (Sigma Aldrich) was dissolved in 100 ml of 1% acetic acid solution (Merck, Germany). Glycerol (Merck, Germany) was added to the solution at a ratio of 30% by weight/weight of chitosan [26]. Antimicrobial films were prepared by combining different amounts of black cumin essential oil nanoemulsion (concentration 2.5% and 5%) and lemon balm extract nanoemulsion (4%) in the optimum solution of 2% chitosan film. A homogenizer (12000 rpm and 5 minutes) was used to mix essential oils in solutions. The drying of the prepared solution was done in a period of 48 hours at an ambient temperature of 25±2 degrees Celsius and a relative humidity of 50% by casting method on Teflon molds.

7-2-Evaluation of antimicrobial properties of edible films prepared by the disk extraction method

To evaluate the antimicrobial properties of prepared edible films on bacteria*Listeria monocytogenes*Agar diffusion method was used. For this purpose, a microbial suspension equivalent to half McFarland was prepared using the turbidity method and 0.1 ml of the suspension was cultured on a plate containing Mueller Hinton agar. Then the prepared films were cut into 6 mm discs and placed in the center of the plates [27]. After 24 hours of incubation and creation of no growth halo, the diameter of the no growth halo was measured.

8-2-Evaluation of the antioxidant activity of prepared edible films

Free radical inhibitory activity in films using 2 and 2 diphenyl, 1 picrylhydrazyl[1](DPPH), based on the red or purple color of methanolic DPPH solution was determined as the reactant. In this method, 25 mg of the film sample was dissolved in 5 ml of distilled water and then 0.1 ml of the film was mixed with 3.9 ml of methanolic DPPH solution (0.1 mmol) and the obtained solution was kept for He stayed in the dark house for an hour. After this time, the absorbance of the solutions was measured against the absorbance of pure methanol at a wavelength of 517 nm using a spectrophotometer based on the following equation.

= free radical inhibitory activity (%)
100x (absorption of blank/absorption of sample-absorption of blank)

9-2- Preparation of camel meat samples

Meat samples were purchased from the market and transported to the laboratory on ice. After separating the fats into equal pieces for bacterial inoculation*Listeria monocytogenes* were divided First, they were washed completely with water to remove its waste.

2-10-Inoculation of bacteria into camel meat

Pieces of 25 grams of camel meat were sterilized in 70 degree alcohol for 15 minutes. In order to clean the alcohol from the surface of the meat, the meat pieces were washed with sterile distilled water and passed over the flame. After drying the surface water, the parts were placed in small sterile zip packs. per piece CFU/g10^5It was inoculated with the desired bacteria. In the next step, the parts were dried for 30 minutes under the microbiology hood. 3 pieces of meat were considered for Hertimar.

[1]. 2,2 Diphenyl 1—picrylhydrazyl

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2-11-Coating camel meat pieces for microbial evaluation

Meat pieces were divided into 7 equal groups for coating (Table 1). The first group was the control group without any coverage. The second group covered with 2% chitosan film, the third group covered with 2% chitosan film containing 2.5% black cumin essential oil nanoemulsion, the fourth group covered with 2% chitosan film containing 5% black cumin essential oil nanoemulsion, group The fifth group covered with 2% chitosan film containing 4% essential oil nanoemulsion of lemon balm extract, the sixth group covered with 2% chitosan film containing 2.5% black cumin essential oil nanoemulsion and 4% nanoemulsion lemon balm extract and the seventh group covered with chitosan film 2 % containing 5% black cumin essential oil nanoemulsion and 4% lemon balm extract nanoemulsion were coated. Then the treatments were kept at 4 degrees Celsius for microbial evaluation on days 0, 4, 8, 12 and 16 during the storage period.

Table 1 List of treatments in the present study.

NO	Treatment	Description
1	With	Samples without any coating solution
2	CH	Samples coated with Chitosan solution
3	CH+BPNE1	Samples coated with Chitosan solution containing 2.5% BPEO
4	CH+BPNE2	Samples coated with Chitosan solution containing 5% BPEO
5	CH+MONE	Samples coated with Chitosan solution containing 4% MOE
6	CH+BPNE1+MONE	Samples coated with Chitosan solution containing 2.5% BPEO and 4% MOE
7	CH+BPNE2+MONE	Samples coated with Chitosan solution containing 5% BPEO and 4% MOE

2-12-Microbial analysis of samples

to count*Listeria monocytogenes* 25 grams of camel meat sample was mixed and homogenized with 225 ml of 0.1% peptone water under sterile conditions, and then the desired dilutions were prepared. 0.1 ml of each dilution was cultured on the surface of the specific agar culture medium. To count bacteria*Listeria monocytogenes* Palcom Agar (Merck, Germany) specific culture medium was used. After cultivation, it was placed in a warm house at 37 degrees Celsius for 24 hours.

2-13- Measurement of thiobarbituric acid (TBARS)

TBARS measurement was expressed as milligrams of malondialdehyde per kilogram of camel meat. 10 grams of the sample was mixed with 90 ml of distilled water. The resulting mixture was transferred to Arlene distillation. 2.5 ml of 4 normal hydrochloric acid along with anti-foam and anti-boiling materials were added to the mixture and Erlenmeyer was added to the distillation apparatus. The mixture was heated and 50 mL of the distillate was collected from the mixture after boiling time. 5 ml of the distilled substance and 5 ml of TBARS reagent (10 ml of 90% glacial acetic acid and 0.2883 g of TBARS powder) were transferred to closed tubes and placed in boiling water after complete shaking for 35 minutes. were given At the same time, all the steps were repeated for the witness. After 35 minutes at boiling temperature, the samples were cooled for 10 minutes and the optical density was read in 1 cm cells in front of the control at a wavelength of 538 nm.

TBA (mg of malonaldehyde per kg) = optical density x 7.8

2-14-pH measurement

10 grams of the sample was homogenized with 90 ml of distilled water and the pH of the sample was measured by a digital pH meter.

2-15-Statistical analysis

In this research, SPSS16 software was used to analyze the results. All experiments were performed in 3 repetitions. Comparison of means was done by ANOVA test. In all evaluations>0.05*p*It was considered as a significant limit.

3. Results and Discussion

3-1-Chemical compounds of black cumin essential oil

The constituent compounds of the essential oil were checked by a gas chromatography device connected to a mass spectrometer, and 25 compounds were identified in black cumin essential oil. The constituent compounds of the essential oil are listed in Table 2. In this table, the abundance of the most important components of the essential oil and the separation time of these compounds are specified.

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Table 1 Composition of *Bunium persicum*essential oil.

RT	Concentration (%)	Constituents	NO
8.24	0.28	β-Thujene	1
8.38	0.26	Cyclohexane, (1-methylethylidene)-	2
9.16	0.18	Camphene	3
10.07	0.49	β-Phellandrene	4
10.28	5.28	β-Pinene	5
10.73	0.52	β-Myrcene	6
11.46	1.32	α-Phellandrene	7
11.91	0.14	α-Terpinene	8
12.33	**9.71**	**p-Cymene**	**9**
12.49	6.29	D-Limonene	10
12.62	0.48	Eucalyptol	11
12.75	0.10	trans-β-Ocimene	12
13.84	**19.99**	**γ-Terpinenes**	**13**
14.98	0.50	Terpinolene	14
19.49	0.31	4-Terpineol	15
19.76	0.13	Dill ether	16
20.20	0.14	α-Terpineol	17
20.37	0.24	Spoil	18
22.62	**24.37**	**Cuminaldehyde**	**19**
24.07	0.18	Phellandral	20
24.56	8.75	Carbamodithioic acid, formyl-, methyl ester	21
24.82	**18.96**	**1,4-p-Menthadien-7-al**	**22**
30.07	0.16	Caryophyllene	23
34.47	0.12	Myristicine	24
59.57	0.98	Heptasiloxane, hexadecamethyl-	25
	99.88	Total	

The main constituents of Bartandaz essential oil: cumin aldehyde (24.37%), alpha-terpinene (19.99%), 1-4-paramethane (DN-7-L) (18.96%) and paracymene (9.71%). The results of the present study are consistent with the studies conducted by other researchers. This difference in chemical compounds and the amount of their effective substance can change under conditions such as geographical region, place of growth, age, the part of the plant used and the way of extracting essential oils.

Kihosravi et al. (2020) stated cumene aldehyde (38.39%), paracymene (5.23%) and gamma terpinene (4.4%) as the most compounds of black cumin essential oil [28]. Talebi et al. (2017) reported that the most constituent compounds of black cumin essential oil are propanal 2-methyl-3-phenyl (34.08%), simene (18.23%) and myrtnal (12.37%) [29]. Arouj Alian et al. (2010) introduced gammaterpinene (44.2%), cumin aldehyde (19.6%) and gamma-terpinen-7-ol (10.5%) as the most important components of black cumin essential oil[30]. Hakir al-Sadat et al. (2013), the most compounds of black cumin essential oil were gammaterpinene (21.86%), cumene aldehyde (17.28%) and paracymene (6.21%)[31].

3-2- MIC and MBC nanoemulsion of black cumin essential oil and lemon balm extract

The results of minimum inhibitory concentration and minimum lethality concentration of nanoemulsion of black cumin essential oil and lemon balm extract are shown in Table 3. Evaluation results of MIC and MBC of black

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cumin essential oil nanoemulsion against bacteria*Listeria monocytogenes* 0.25% and 0.5% were reported respectively. Kikhsravi et al. (2020) the minimum inhibitory concentration and the minimum lethal concentration of black cumin essential oil by microbroth dilution method against a number of strains*Salmonella enteritidis* And*Listeria monocytogenes* investigated, the minimum inhibitory concentration of black cumin essential oil nanoemulsion against*Salmonella enteritidis* And*Listeria monocytogenes* 2 mg/ml and 1 mg/ml, respectively, the minimum lethality concentration of black cumin essential oil nanoemulsion against*Salmonella enteritidis* And*Listeria monocytogenes* 4 mg/ml and 2 mg/ml were reported respectively [28]. In the study of Oruj Alian et al. (2010), the minimum inhibitory concentration of black cumin essential oil against several food pathogens including*Staphylococcus aureus*، *Bacillus cereus*، *Escherichia coli*O157:H7،*Salmonella enteritidis* And*Listeria monocytogenes* It was reported in the range of 0.03-0.05 mg/ml and the results showed the antimicrobial properties of black cumin essential oil against Gram-positive and Gram-negative bacteria, which is consistent with the results of the present study [30].

Evaluation results of MIC and MBC of lemon balm extract nanoemulsion against bacteria*Listeria monocytogenes* 1% and 2% were reported respectively. A study related to the antimicrobial property of lemon balm extract nanoemulsion and the extract of this plant in general has not been done so far. But a number of articles indicate the antimicrobial and antifungal properties of this plant. Kamali et al. (2015) Antimicrobial effect of alcoholic extract of Melissa against bacteria*Bacillus cereus* checked by disk diffusion method. The results showed that the extract of this plant has a good antimicrobial effect against bacteria*Bacillus cereus*is[32].

Table 3 MIC and MBC of *Bunium persicum*nanoemulsions(BPN) and*Melissa officinalis*L. extract (MOE) against *Listeriamonocytogenes* by microdilution broth method.

MBC(%)	MIC (%)	Nanoemulsion
0.50	0.25	*Bunium persicum*
2	1	*Melissa officinalis*L.

3-3- Antimicrobial activity of the films prepared by the disc diffusion method

The antimicrobial effect of chitosan film containing black cumin essential oil and lemon balm plant extract against two food-borne bacterial species was tested using disk diffusion method. As the results show, chitosan film containing 5% black cumin essential oil nanoemulsion and 4% lemon balm extract has the most antimicrobial effect on bacteria.*Listeria monocytogenes* And chitosan film containing 2.5% black cumin essential oil nanoemulsion had the least antibacterial effect, which is probably due to the high amounts of gamma terpene and cumin aldehyde (Table 2). Also, empty chitosan was used as a control, which showed the lowest antibacterial effect compared to when it contained essential oil and extract. Essential oil and extract were more effective against gram positive bacteria than gram negative bacteria. The reason for this is the difference in the cell wall structure of these two types of bacteria. The main composition of the cell wall of Gram-positive bacteria is peptidoglycan along with a small amount of protein. However, the cell wall of Gram-negative bacteria is more complex, despite being less thick, and besides peptides and glycans, it contains different polysaccharides, proteins and lipids. Also, the cell wall of gram-negative bacteria has an outer membrane that covers the outer surface of the wall. The combination of these factors increases the resistance of Gram-negative bacteria compared to Gram-positive bacteria. By increasing the concentration of essential oil, the inhibitory effect increased. In different articles, different concentrations of essential oils have been used, which is due to the type of plant, the method of preparing the essential oil, the concentration of the effective compounds in the essential oil, and also the type of material that forms the film [33].

Table 4 Antibacterial activity of chitosan films containing BPNEand MONE against *Listeriamonocytogenes* by the agar well diffiusion assay

Listeria monocytogenes	Treatment
11.30±0.17^{g}	CH

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13.46±0.18[f]	CH+BPNE1
15.66±0.18[lt is]	CH+BPNE2
14.30±0.15[d]	CH+MONE
16.47±0.14[c]	CH+BPNE1+MONE
17.15±0.16[b]	CH+BPNE+MONE
30.19±0.14[a]	GM

Different letters indicate a statistically significant difference ($p < .05$).

As mentioned, chitosan has antimicrobial properties [34]. Although no growth was observed under the surface of the films, but the control films (without essential oil) showed a slight aura. The reason for this phenomenon is that the antimicrobial nature of chitosan is an intrinsic property due to the presence of positively charged amino groups, so the antimicrobial effect is without Migration of the active substance occurred and only the growth of bacteria that were in contact with the film surface was prevented [13].

In a study conducted by Soleimani et al. (2009), the results showed that the highest amount of aura of non-growth of bacteria was related to*Bacillus cereus*with a diameter of 45 mm,*Bacillus subtilis* 21 mm,*Staphylococcus aureus*20 mm,*Shiglaflexion* 18 mm,*Escherichia coli*16 mm and*Salmonella latifimorium* It was 8 mm. They stated that the main reason for the antibacterial properties of cumin essential oil is the presence of cumin aldehyde [35].

In a study, Kamali et al. (2015) showed that Alki Melis extract has an antibacterial effect on*Bacillus cereus*and the maximum diameter of the halo of growth inhibition in this study was 12 mm [32].

3-4- Evaluation of antioxidant activity of films

Addition of essential oil and extract to DPPH solution caused a rapid decrease in absorbance at 517 nm. The degree of color change indicates the radical scavenging capacity of essential oil and extract. Free radicals cause the oxidation of unsaturated fats in food. Based on the results of the antioxidant test shown in Figure 1, with increasing the amount of black cumin essential oil, the antioxidant activity of the edible film increased, so that the highest antioxidant activity was related to the chitosan film containing 5% black cumin essential oil and 4% plant extract. It was Melissa. The lowest amount of antioxidant property was related to the chitosan film containing 2.5% black cumin essential oil. Also, blank chitosan was used as a control, which had the lowest amount of antioxidant property compared to chitosan containing essential oil and extract. Many studies have been conducted in the field of antioxidant properties of black cumin essential oil, all of which indicate high antioxidant effects and the ability to eliminate free radicals for these plants [36 and 37].

In a study by Pereira et al. (2009) in the investigation of DPPH free radical inhibition in aqueous and alkyd extracts of Melissa, they found that the ethanolic extract has more free radical inhibition power. They found that among the pure compounds, quercetin has the highest antioxidant activity, the results of these researchers are consistent with the results of the present study [38].

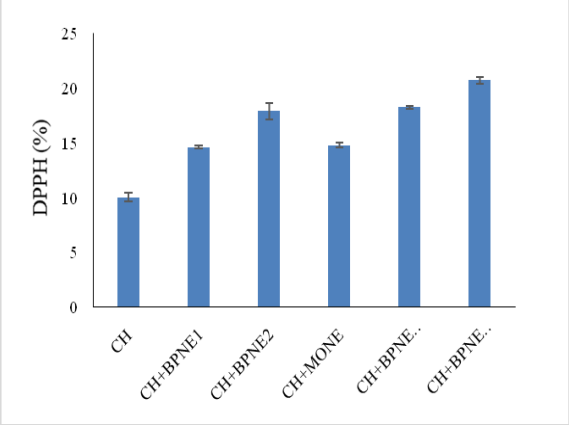

Fig 1 Antioxidant activity of chitosan films containing BPNE and MONE.

5-3-Evaluation of the antimicrobial properties of the samples covered with the studied films against Listeria monocytogenes bacteria

Antimicrobial effects of chitosan film containing nanoemulsion of black cumin essential oil and Melissa extract against bacteria*Listeria monocytogenes* It is shown in figure 2 in camel meat stored at 4°C for 16 days. Pairwise comparison of logarithmic reduction of bacterial counts*Listeria monocytogenes* It is stated in the studied samples in Table 5. The results showed that the number of bacteria*Listeria monocytogenes* In the control treatment, there was an increasing trend during the 16-day period,

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and the average bacterial count in the control treatment was higher than the rest of the samples covered with the studied films. In all treatments, except the control treatment, during the storage period, the average bacterial count decreased until the 12th day of storage and increased on the 16th day of storage. Chitosan treatment containing 5% black cumin essential oil nanoemulsion and 4% lemon balm extract had more antimicrobial properties than other studied treatments. All treatments had significant differences with the control group.$p<0.05$). There was no significant difference between chitosan treatment containing 5% black cumin nanoemulsion and chitosan treatment containing 4% lemon balm extract.$p>0.05$).Results of microbial evaluation against bacteria*Listeria monocytogenes* In the present study, it was shown that edible chitosan films containing nanoemulsion of black cumin essential oil and lemon balm extract have good antimicrobial properties against*Listeria monocytogenes* inoculated into camel meat, and as the concentration of the herbal antimicrobial compound to the chitosan films increases, this property should increase. The results of the present study are consistent with the results of other researchers' studies. Wang et al. (2020), the antimicrobial effect of chitosan film containing apricot kernel essential oil against bacteria*Listeria monocytogenes* In beef stored in the refrigerator in a 15-day period of storage with three-day time intervals, they were examined. In this study, the number of bacteria increased over a period of fifteen days. The count of samples containing chitosan films was significantly lower than the control group. The results showed that counting in samples coated with a higher concentration of essential oil has a stronger antimicrobial effect on bacteria*Listeria monocytogenes* They had [39]. In the study of Mehdizadeh et al. (2012), the antimicrobial and antioxidant effect of kakuti essential oil with concentrations of 0 to 2% included in the edible starch-chitosan composite film was analyzed. Antimicrobial and antioxidant effects increased significantly with the addition of essential oil concentration. So that the most antimicrobial effect on bacteria*Listeria monocytogenes*And the least related to bacteria*Salmonella enteritidis* Was. The results of this study showed that the addition of kakuti essential oil as a natural antimicrobial in the form of starch-chitosan composite film can have a high potential in the development of edible films for use in active packaging [40]. Khazarian et al. (2017), application of chitosan and carboxymethyl cellulose nanocomposite film containing thyme essential oil and fig extract in minced camel meat against*Listeria monocytogenes* And*Escherichia coli*O157:H7 were investigated. Bacterial count rate*Listeria monocytogenes* There was an increase in the use of both films containing plant compounds in the control group and the chitosan and carboxymethyl cellulose film alone, but in the case of the use of avian essential oil and fig extract, this trend was decreasing and they had a lower count rate than the control group. [41].

Table 5 Average reduction rate of *Listeriamonocytogenes* count (log CFU/g) among treatments when compared together during 16 days of storage.

Group I	Mean Difference I-J					
	Group J					
	CH	CH+BPNE1	CH+BPNE2	CH+MONE	CH+BPNE1+ MONE	CH+BPNE2+ MONE
With	1.06*	1.33*	1.69*	1.59*	1.91*	2.12*
CH		0.26*	0.63*	0.52*	0.84*	1.05*
CH+BPNE1			0.36*	0.26*	0.58*	0.79*
CH+BPNE2				-0.10	0.21*	0.42*
CH+MONE					0.32*	0.53*
CH+BPNE1+MONE						0.21*

∗Indicates a statistically significant difference (p <0.05).

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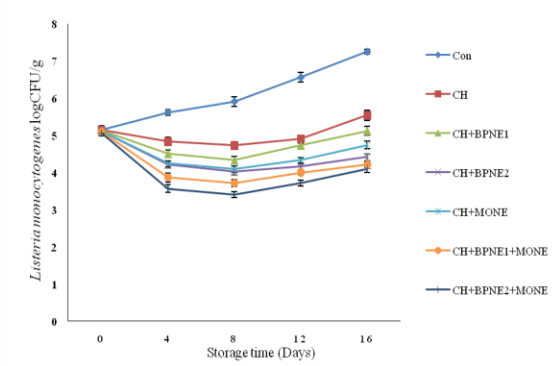

Fig 2 Changes of *Listeria monocytogenes*count of camel meat samples in different treatments during 16 days of storage at 4 °C.

3-6-TBARS measurement

The trend of changes in the amount of TBARS in the studied samples is shown in Table 6. The results show that the amount of thiobarbituric acid index at the beginning of the period in all samples is in the range of 0.20-0.22 mg MDA/kg, but during the period this index had an increasing trend, so that in the amount

Thiobarbituric acid in the control sample reached 1.77 mgMDAlkg at the end of the period. However, in treatments containing black cumin essential oil and lemon balm extract, the rate of increase of thiobarbituric acid was low. In the best treatment, i.e. chitosan film treatment containing 5% black cumin essential oil and 4% lemon balm extract, this index reached 0.88 mgMDA/kg at the end of the period. All samples had significant differences with the control group (P<0.05). Mehdizadeh et al. (2017) investigated the antioxidant effect of starch-chitosan edible composite film containing the combination of pomegranate peel extract and kakuti oil essence on the shelf life of red meat during 21-day storage. The evaluation of the oxidation rate showed that using the film alone and together With plant extract and essential oil, it has a significant effect on the amount of meat oxidation, and the treatment containing a higher concentration of extract and essential oil had a greater effect in preventing the formation of malonaldehyde[42].

Table 6 Average reduction rate of TBARS among treatments when compared together during 16 days of storage.

Group I	Mean Difference I-J					
	Group J					
	CH	CH+BPNE1	CH+BPNE2	CH+MONE	CH+BPNE1+ MONE	CH+BPNE2+ MONE
With	0.27 *	0.36*	0.43*	0.34*	0.43*	0.47*
CH		0.09	0.15*	0.06	0.15*	0.20*
CH+BPNE1			0.06	-0.02	0.06	0.10
CH+BPNE2				-0.09	0.001	0.45
CH+MONE					0.09	0.13
CH+BPNE1+MONE						0.04

∗Indicates a statistically significant difference (p <0.05).

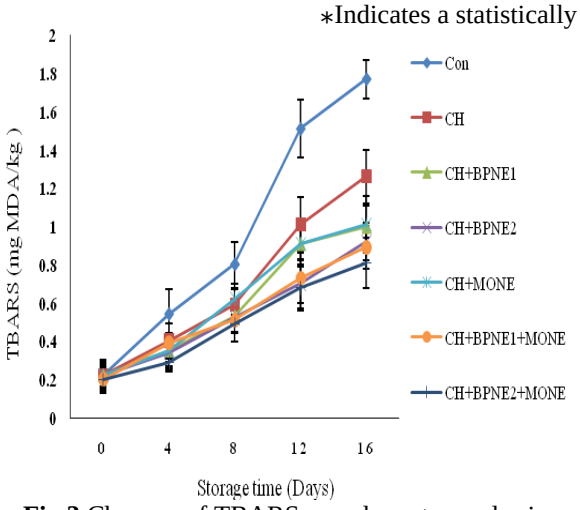

Fig 3 Changes of TBARS camel meat samples in different treatments during 16 days of storage at 4 °C.

Kane et al. (2013) investigated the effect of chitosan film with tea polyphenol on the quality and shelf life of pork meat, the results showed that the amount of TBARS increased in all samples during the 12-day storage period at 4 degrees Celsius, but The amount of TBARS in the samples covered with chitosan film containing tea polyphenol was significantly lower than the control group [43]. Georgentlis et al. fresh pork meat at 4 degrees Celsius, showed that films containing rosemary extract have more antioxidant properties compared to chitosan film alone due to the presence of phenolic compounds.

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The amount of malondialdehyde showed an increasing trend in all samples. Level

TBARS in the control sample was significantly higher than the samples containing rosemary extract and α-tocopherol in pork sausage [44].

3-7-pH measurement

The average pH changes in the studied samples during the storage period are shown in Table 7. The average pH in the control treatment and the samples covered with the studied films had an increasing trend during the storage period. The increase in pH in the control treatment increased faster than other samples so that the average pH at the beginning of the period in the control treatment reached 5.14 and at the end of the period it reached 6.64. The results showed that the samples coated with chitosan alone have a higher pH than the samples treated with chitosan film containing black cumin essential oil and lemon balm extract. There was no significant difference between the samples covered with chitosan film containing 5% black cumin and 4% lemon balm and the samples covered with chitosan film containing 2.5% black cumin and 4% lemon balm extract. Also, there was no significant difference between the treatments of chitosan film containing 2.5% black cumin and 4% lemon balm extract, chitosan film containing 4% lemon balm extract and chitosan film containing 5% black cumin.P>0.05). The main reason for the increase in pH in meat is due to the production of alkaline compounds such as ammonia and trimethylamines caused by the breakdown of meat proteins by the proteolytic activity of microorganisms and microbial enzymes [45].

Table7 Average reduction rate of pH among treatments when compared together during 16 days of storage.

Group I	Mean Difference I-J					
	Group J					
	CH	CH+BPNE1	CH+BPNE2	CH+MONE	CH+BPNE1+ MONE	CH+BPNE2+ MONE
With	0.14*	0.23*	0.33*	0.32*	0.41*	0.48*
CH		0.08*	0.18*	0.18*	0.26*	0.34*
CH+BPNE1			0.10*	0.09*	0.17*	0.25*
CH+BPNE2				0.006	0.07	0.15*
CH+MONE					0.08	0.15
CH+BPNE1+MONE						0.07

∗Indicates a statistically significant difference (p <0.05).

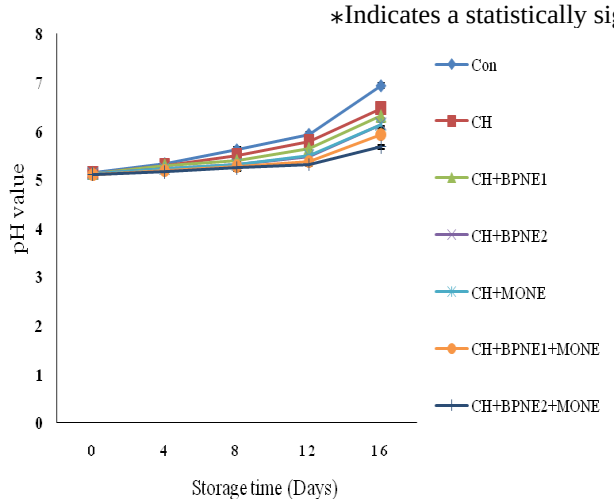

Fig 4 Changes of pH camel meat samples in different treatments during 16 days of storage at 4 °C.

Karimnejad et al. (2017), stated that the chitosan film containing 1% and 2% of zenian essential oil in chicken meat had a lower pH than the control sample and the film without essential oil, which could be due to the antimicrobial effect of zenian essential oil on spoilage-producing proteolytic bacteria. It can be [46]. In the research of Kin et al. (2013), the presence of chitosan film containing tea polyphenols in pork reduced the pH during storage compared to the control group[43]. Khazarian et al. (2017) investigated the application of chitosan and carboxymethyl cellulose nanocomposite film containing natural preservatives in minced camel meat, the pH level in all meat samples increased from about 5.9 to 6.6, but the pH in the edible film containing essential oil and extract was significantly lower than the control group during the 14-day storage period [41].

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4 - Conclusion

The results of this study showed that chitosan edible film containing nanoemulsion of black cumin essence and lemon balm extract has good antimicrobial effects against food-borne pathogens including*Listeria monocytogenes*is. It also has very good antioxidant properties. Therefore, the edible film obtained from these compounds can be used to increase the storage time of camel meat and maintain its quality during storage in the refrigerator. The use of edible chitosan film containing nanoemulsion of black cumin essential oil and lemon balm extract obtained in this study or in combination with other plant essential oils is suggested to increase the shelf life of other food products such as fish and chicken.

5- Resources

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بررسی اثرآنتی اکسیدانی و ضد میکروبی و فیلم خوراکی کیتوزان حاوی نانوامولسیون اسانس زیره سیاه (*Buniumpersicum*) و عصاره گیاه ملیس (*MelissaofficinalisL.*) علیه باکتری *لیستریامونوسیتوژنز* تلقیح شده به گوشت شتر

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چکیده

این مطالعه با هدف بررسی اثرضد میکروبیو آنتی اکسیدانی فیلم کیتوزان حاوی نانوامولیسیون عصاره گیاه ملیس و اسانس زیره سیاه علیه باکتری *لیستریا مونوسیتوژنز* تلقیح شده به گوشت شتر انجام گردید. فیلم‌های مورد مطالعه با استفاده از کیتوزان ۲٪ و غلظت های ۲/۵٪ و ۵٪ نانوامولسیون اسانس زیره سیاه و ٤٪ عصاره ملیس تهیه شدند. اثرات آنتی اکسیدانی و ضدمیکروبی قطعات گوشت شتر پوشش داده شده در طول ١٦ روز نگهداری در دمای ٤ درجه سانتیگراد با فاصله زمانی ٤ روزه (٠،٤،٨،١٢،١٦) مورد ارزیابی قرار گرفت. قطعات پوشش داده شده مورد ارزیابی شیمیایی قرار گرفتند. بیشترین ترکیبات تشکیل دهنده زیره سیاه شامل کومین آلدهید (٢٤/٣٧ ٪)، گاما ترپینن (١٩/٩٩ ٪) و پارا سایمن (٩/٧١ ٪) بود. حداقل غلظت مهارکنندگی عصاره ملیس و زیره سیاه علیه باکتری *لیستریامونوسیتوژنز* به ترتیب ١٪ و ٠/٢٥ ٪ تعیین گردید. نتایج ارزیابی خاصیت آنتی اکسیدانی فیلم ها به روش DPPH نشان داد که افزودن اسانس و عصاره باعث افزایش خاصیت آنتی اکسیدانی فیلم ها می گردد. در بررسی اثر ضدمیکروبی فیلم ها به روش انتشار از دیسک بیشترین قطر هاله عدم رشد (٠/١٦ ± ١٧/١٥) مربوط به فیلم کیتوزان حاوی ٥٪ نانوامولسیون اسانس زیره سیاه و ٤٪ عصاره ملیس بود. میانگین شمارش تعداد باکتری در تیمار کنترل در طول دوره ١٦ روزه از سایر تیمار های مورد مطالعه بالاتر بود. نتایج TBARS بیانگر خواص آنتی اکسیدانی بیشتر در فیلم های حاوی اسانس زیره سیاه و عصاره ملیس نسبت به نمونه کنترل بود. میزان pH در نمونه های پوشش داده شده با فیلم کیتوزان حاوی٥٪ نانوامولسیون اسانس زیره سیاه و ٤٪ عصاره ملیس نسبت به بقیه نمونه ها پایین تر بود. در مجموع فیلم‌های تهیه شده دارای خاصیت ضدمیکروبی و آنتی اکسیدانی خوبی علیه باکتری *لیستریا مونوسیتوژنز* بوده که با افزودن ترکیبات گیاهی افزایش پیدامی کند.

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