



## Scientific Research

## Evaluation of antibacterial and antioxidant activity of gelatin-chitosan coating containing emulsion and nanoemulsion of *Bunium persicum* essential oil on beef meat

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## ABSTRACT

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Plant antimicrobial compounds are a very suitable and safe alternative for synthetic preservatives. On the other hand, nanotechnology increases and improves the effect of the effective compounds of plants on the target cells. In this study, the antimicrobial effect (TVC and Psychrotrophic bacteria) and antioxidant effect (TBARS and PV) of edible gelatin-chitosan coating containing emulsion and nanoemulsion of *Bunium persicum* essential oil (1.5% and 3%) on beef meat over a 16-day period (0, 4, 8, 12 and 16) at 4 ° C was investigated. Analysis of the essential oil by GC-MS showed that the effective compounds of the essential oil are Cuminaldehyde and  $\gamma$ -Terpinene. The results of the antimicrobial effect of the prepared coatings showed that there is a significant difference between the control sample and the coated samples ( $p < 0.05$ ). Adding *Bunium persicum* essential oil and increasing the concentration of essential oil increased the antimicrobial activity. Also, the results of the evaluation of antimicrobial activity showed that the sample containing gelatin-chitosan coating containing 3% nanoemulsion of *Bunium persicum* essential oil had a higher antimicrobial effect compared to other samples. The results of peroxide index and thiobarbituric acid showed that there is a significant difference between the control sample and the coated samples ( $p < 0.05$ ). The addition of *Bunium persicum* essential oil due to its antioxidant compounds improved and increased the antioxidant activity of gelatin-chitosan coating, and the highest antioxidant activity associated with gelatin-chitosan coating containing 1.5% and 3% nanoemulsion of *Bunium persicum* essential oil. The results of this study showed that the gelatin-chitosan coating containing emulsion and nanoemulsion of *Bunium persicum* essential oil has high antimicrobial and antioxidant properties and can improve the shelf life of beef meat stored in the refrigerator. Also, this prepared coating can be used as a biodegradable packaging in the food industry.

## 1. Introduction

In modern societies, the increasing population, greater diversity in dietary habits, and the complex processes involved in food production and distribution have led to a heightened risk of contamination by pathogenic and spoilage microorganisms. As a result, researchers in the food industry are increasingly turning to innovative and efficient methods to preserve food products and ensure their quality and safety [1].

Depending on the composition, nutritional value, storage methods, and the surrounding environment in contact with food, the microbial diversity in food products varies. Accordingly, various methods and compounds are employed for food preservation based on these characteristics. Most food products particularly protein-rich ones such as red meat provide a favorable environment for microbial growth and are highly susceptible to spoilage [2].

Red meat, which is rich in proteins, minerals, trace elements, and vitamins, is derived from a variety of animals including calves, cattle, buffalo, camels, sheep, goats, and deer [3]. The high moisture content, abundance of micronutrients, and elevated protein levels in beef make them highly perishable under improper storage conditions, resulting in rapid microbial spoilage and contamination by pathogenic microorganisms.

Spoilage indicators such as discoloration, gas formation, off-odors, and slime production typically appear when the microbial load exceeds  $10^7$  CFU/g. In this context, the poorer the storage conditions and the higher the moisture and micronutrient levels, the faster the spoilage occurs and the more rapidly and intensely the signs of spoilage emerge [4]. Therefore, researchers and food industry managers are adopting various strategies to extend shelf life and protect food products from pathogenic and spoilage microorganisms. One of the traditional yet still widely used methods for extending the shelf life of food products particularly meat is the application of low temperatures, including refrigeration (0 to 7°C) and freezing (below 0°C). Most pathogenic and spoilage microorganisms are unable to grow at such low temperatures, and their activity is effectively halted under cold conditions.

Moreover, at lower temperatures, enzymatic and chemical reactions within the food are either significantly slowed down or completely

inhibited. As a result, the spoilage and contamination of meat occur at a much slower rate [5]. Among the modern methods increasingly adopted in the food industry is the use of edible, biodegradable coatings and compounds. Edible coatings are considered thin layers of bioactive and food-grade materials that are applied to food surfaces via spraying or dipping. These coatings serve to control microbial populations and inhibit the growth of pathogenic and spoilage microorganisms in food products [6].

Chitosan-based coatings, derived from chitin extracted from crustaceans and other invertebrates, are among the most common polysaccharides used in the production of biodegradable edible packaging. The films formed from chitosan act as effective barriers to oxygen transmission, provide a protective shield against microbial invasion, and exhibit moderate antimicrobial properties. As a result, chitosan coatings contribute to extending shelf life and maintaining the quality of food products [7, 8].

Gelatin-based coatings are proteinaceous in nature and are derived from collagen. These coatings exhibit notable antioxidant properties and, depending on the concentration used, are capable of forming effective gels [9]. Unlike chitosan, gelatin does not possess inherent antimicrobial activity and is therefore often used in combination with other antimicrobial agents to enhance its preservative effects [10].

The combined use of gelatin and chitosan coatings improves the physicochemical and mechanical properties of these coatings, making them particularly effective for the preservation of food products, especially meat (such as fish, poultry, and beef). Since this combined coating lacks strong antimicrobial and antioxidant properties, and considering the benefit of enhancing these coatings through the simultaneous use of antioxidant and antimicrobial agents like emulsions and Nano emulsions of essential oils and plant extracts, these compounds are typically employed together to coat food products and improve their shelf life [4].

Essential oils and plant extracts are compounds derived from various parts of plants (such as roots, stems, leaves, fruits, and seeds) and are extracted using different methods. These plant-derived essential oils are rich in antimicrobial and antioxidant compounds, such as terpenes and phenols. In various studies, these compounds have been utilized for their antibacterial,

antifungal, antiviral, and antiparasitic properties [11].

One of the valuable and native plants of Iran is the perennial black cumin (*Bunium persicum*), which belongs to the Apiaceae family and is found in Asia and Southeastern Europe [12]. This plant is rich in therapeutic properties and is used for the prevention, management, and treatment of various ailments, such as lowering bad cholesterol levels, improving bone fractures, reducing fever, and treating gastrointestinal and respiratory diseases. It is also a rich source of antioxidant and antibacterial compounds, including  $\gamma$ -terpinene, cuminaldehyde,  $\alpha$ -pinene, and limonene [13], and is utilized in the food industry as a flavoring agent and preservative [14].

In this study, a gelatin-chitosan coating containing nanoemulsion and emulsion of black cumin essential oil was used to extend the shelf life of beef stored in refrigeration. After identifying the active compounds of the essential oil, the antimicrobial and antioxidant properties of the edible coating were assessed over a 16-day storage period of beef in the refrigerator. Since no previous studies have been conducted on a gelatin-chitosan edible coating containing emulsions and nanoemulsions of black cumin essential oil, the aim of this study was to investigate and compare the antimicrobial and antioxidant effects of the gelatin-chitosan coating containing both emulsions and nanoemulsions of black cumin essential oil on beef meat.

Emulsions are mixtures of two or more immiscible liquids, which tend to have relatively low stability. To enhance their stability, emulsifiers are used [15]. On the other hand, due to recent advancements in nanotechnology, scientists are eager to apply this technology in various fields, particularly in the development of drug delivery systems and food products, owing to its ease of preparation and favorable functional properties [16]. Nanoemulsions are stable liquids with particle sizes ranging from 10 to 100 nanometers. Various methods are employed to produce nanoemulsions, such as high-speed stirring, high-pressure techniques, and ultrasonication. Among these, ultrasonication is the most common and widely used method for producing nanoemulsions [17].

In this study, a gelatin-chitosan coating containing nanoemulsion and emulsion of black cumin essential oil was used to extend the shelf life of beef stored in refrigeration. After

identifying the active compounds of the essential oil, the antimicrobial and antioxidant properties of the edible coating were assessed over a 16-day storage period of beef in the refrigerator. Since no previous studies have been conducted on a gelatin-chitosan edible coating containing emulsions and nanoemulsions of black cumin essential oil, the aim of this study was to investigate and compare the antimicrobial and antioxidant effects of the gelatin-chitosan coating containing both emulsions and nanoemulsions of black cumin essential oil on beef meat.

## 2. Materials and Methods

### 2.1. Preparation and Analysis of Black Cumin Essential Oil

After obtaining black cumin seeds from the Kerman province, essential oil extraction was carried out using a Clevenger apparatus. The chemical analysis of the extracted essential oil was performed using a Gas Chromatography-Mass Spectrometry (GC-MS) system (Agilent HP-6890, Palo Alto, Agilent Technologies, CA, USA).

### 2.2. Preparation of Black Cumin Essential Oil Nanoemulsion and Particle Size Determination

To prepare the black cumin essential oil nanoemulsion, concentrations of 1.5% and 3% essential oil were mixed with deionized water and Tween 80. The nanoemulsion was then prepared using a Sonicator (Sonopuls, Bendelin, Berlin, Germany) at a frequency of 30 kHz for 15 minutes at 15°C. The particle size of the nanoemulsion was measured using a Dynamic Light Scattering (DLS) particle size analyzer.

### 2.3. Preparation of Gelatin-Chitosan Coating Containing Emulsion and Nanoemulsion of Black Cumin Essential Oil

The edible coating solution was prepared by mixing 3% fish gelatin (Sigma, Aldrich) in distilled water and allowing it to dissolve for 30 minutes. After heating the solution to 70°C for 10 minutes under magnetic stirring, 3% chitosan (Sigma, Aldrich) in 1% acetic acid solution

(Merck, Germany), glycerol (Merck, Germany), and 1.5% and 3% emulsion and nanoemulsion of black cumin essential oil were added, and the mixture was homogenized thoroughly. The coating was then immediately applied to the veal samples [18].

## 2.4. Preparation of Beef Meat

Beef meat was purchased from reputable centers within the city and transported to the laboratory on ice. The meat was then washed with tap water to remove any residual fat, and after separating the fatty pieces, it was cut into 10-gram portions.

## 2.5. Preparation of the Experimental Treatments

For coating the beef meat samples, the samples were classified into different groups according to Table 1. The samples were then immersed in the desired solutions for 3 minutes and allowed to drain for 2 minutes. Afterward, the samples were stored in polyethylene bags at 4°C for 16 days. The samples were evaluated every 4 days (at 0, 4, 8, 12, and 16 days).

Table 1. The treatment studied

Treatment	Chitosan (%)	Gelatin (%)	Emulsion of <i>Bunium persicum</i> essential oil (%)	Nanoemulsion of <i>Bunium persicum</i> essential oil (%)
Con	0	0	0	0
CH/G	3	3	0	0
CH/G + BPE 1.5%	3	3	1.5	0
CH/G + BPE 3%	3	3	3	0
CH/G + BPNE 1.5%	3	3	0	1.5
CH/G + BPNE 3%	3	3	0	3

## 2.6. Evaluation of Antimicrobial Activity of the Prepared Coatings

For microbiological analysis, 10 grams of the samples were homogenized in 90 mL of 0.1% peptone water. Serial dilutions were then prepared, and bacterial counts were conducted. To count the mesophilic aerobic bacteria, surface plating was performed on PCA (Plate Count Agar) media using the prepared dilutions. The plated samples were incubated at 30°C for 48 hours. For the psychrotrophic bacteria, surface plating was also performed on PCA media with the prepared dilutions. The plates were then incubated at 7°C for 10 days.

## 2.7. Evaluation of Antioxidant Activity of the Prepared Coatings

### 2.7.1. Peroxide Value (PV) Measurement

The primary oxidation products were determined using the iodometric method. The amount of free iodine released was titrated with sodium thiosulfate, and the peroxide value was expressed as milliequivalents of peroxide per kilogram of fat [19].

### 2.7.2. Thiobarbituric Acid Reactive Substances (TBARS) Measurement

The secondary oxidation products were measured using the TBARS assay, according to the method of Jafari Nea et al. (2022), and expressed as milligrams of malondialdehyde per kilogram of beef meat [19].

## 2.8. Statistical Analysis

All experiments were conducted in triplicate, and the results are expressed as means  $\pm$  standard deviations. Data analysis was performed using SPSS statistical software version 26 (SPSS Inc., Chicago, IL, USA). One-way ANOVA was used for comparing the data, and Duncan's post hoc test was used for pairwise comparisons between groups. A p-value of  $\leq 0.05$  was considered statistically significant for all evaluations.

### 3. Results and Discussion

#### 3.1. Analysis of Black Cumin Essential Oil

The results of the identification of the components of black cumin essential oil using GC-MS are presented in Table 2. The major

active compounds in the black cumin essential oil were cuminaldehyde (11.25%) and  $\gamma$ -terpinene (15.20%). Factors affecting the type and concentration of active compounds in plants include geographical conditions, climate, season, and the method of essential oil and extract preparation [20]. In studies on black cumin essential oil, cuminaldehyde,  $\gamma$ -terpinene,  $\beta$ -pinene, and p-cymene have been reported as the main components [21]. In a study on the active compounds of black cumin essential oil, Keykhosravi et al. (2020) reported that cuminaldehyde (39.38%), p-cymene (23.5%), and  $\gamma$ -terpinene (44.4%) accounted for the highest proportion of the essential oil, which is consistent with the results of the present study [22].

**Table2. Chemical composition of *Bunium persicum* essential oil.**

Compounds	Concentration (Peak area %)	Retention time (min)
$\beta$ -Phellandrene	0.50	10.02
$\beta$ -Pinene	5.30	10.25
$\alpha$ -Phellandrene	1.31	11.47
p-Cymene	10.03	12.11
D-Limonene	6.63	12.62
$\gamma$ -Terpinene	20.15	14.11
Terpinolene	0.56	15.21
4-Terpineol	0.43	19.82
Cuminaldehyde	25.11	21.41
Carbamodithioic acid, formyl-, methyl ester	9.23	23.82
1,4-p-Menthadien-7-al	18.36	24.56
Carvacrol	0.91	28.31
Myristicine	0.39	43.47
Total	98.91	

#### 3.2. Particle Size and Polydispersity Index (PDI) of the Black Cumin Essential Oil Nanomulsion

The average particle size and PDI index of the

black cumin essential oil nanomulsion are presented in Table 3. In this study, the nanomulsion of black cumin essential oil was prepared using the ultrasonic method, resulting in a particle size of 125.10 nm and a PDI value

of 0.27. In a study by Keykhosravy et al. (2020), the black cumin essential oil nanomulsion was obtained by combining black cumin essential oil, water, Tween 80, and lecithin, using an ultrasonic device. The results showed that the average particle size and PDI index of the black cumin essential oil were 154.26 nm and 0.24, respectively, which was consistent with the

results of the present study [22]. According to research, the concentration and type of surfactant can influence the droplet size. An increase in the surfactant concentration results in a decrease in particle size, although higher surfactant concentrations can reduce the antimicrobial activity of plant essential oils [23].

**Table 3. Particle size.**

Z-average (nm)	PDI
125.10 ± 1.95	0.27 ± 0.03

### 3.3. Evaluation of Antimicrobial Activity of the Coatings Studied

The results of evaluating the antimicrobial activity of the gelatin-chitosan coating containing emulsion and nanoemulsion of black cumin essential oil are presented in Table 4. According to the obtained results, the average count of aerobic mesophilic bacteria (TVC) and psychrotrophic bacteria in beef samples in the control group were log CFU/g 3.57 and log CFU/g 2.55, respectively, indicating good quality and freshness of the meat. The counts of TVC and psychrotrophic bacteria increased in all coated samples over the study period, reaching log CFU/g 10.18 and log CFU/g 8.95, respectively, on day 16 in the control group. The average counts of TVC and psychrotrophic bacteria in the samples coated with gelatin-chitosan without essential oil were significantly lower than those in the control group throughout the storage period ( $p < 0.05$ ), which could be attributed to the antimicrobial properties of chitosan. According to previous studies, the antimicrobial activity of chitosan has been proven, and many researchers have investigated the effect of chitosan coatings in combination with plant-based antimicrobial compounds to improve the shelf life of food products [24]. On day 12 of the study, the counts of TVC and psychrotrophic bacteria in the control group

reached above the permissible limit of log CFU/g 7 [25], while in the samples containing nanoemulsion of black cumin essential oil, the bacterial count was below the standard permissible limit. Based on the results, adding essential oil in both emulsion and nanoemulsion forms enhanced the antimicrobial properties of the gelatin-chitosan coating. However, when nanoemulsion of the essential oil was used, particularly at a higher concentration (3%), the antimicrobial activity increased significantly ( $p < 0.05$ ). Overall, the results showed that the most effective treatment was the gelatin-chitosan coating containing 3% nanoemulsion of black cumin essential oil. In line with the current study, Keykhosravy et al. (2020) found similar results when investigating the antimicrobial properties of a chitosan film containing nanoemulsion of black cumin and Shirazi thyme essential oils. The initial counts of TVC and psychrotrophic bacteria in their study were CFU/g 3.35 and log CFU/g 2.55, respectively, and increased over the 18-day study period. They stated that using nanoemulsion of thyme and black cumin essential oils limited microbial growth, including aerobic mesophilic and psychrotrophic bacteria, and the lowest bacterial counts were associated with the samples coated with chitosan solution containing the plant essential oil nanoemulsions [22]. In another study on chitosan and gelatin coatings containing brassica essential oil, the

researchers indicated that gelatin alone did not have antimicrobial properties, but due to the antimicrobial properties of chitosan, adding it to the gelatin coating controlled microbial growth throughout the study period in fish meat [26]. Other studies have examined the effect of chitosan-gelatin edible coatings containing natural antimicrobial compounds on microbial growth during storage of food products,

including turkey and fish meat. All of these studies indicated a positive and significant impact of packaging food products with chitosan-gelatin coatings containing plant essential oils, confirming their antimicrobial activity and improving the shelf life of food products through coating with these compounds [27, 28].

**Table 4. Antimicrobial activity of the studied treatments on beef meat during the storage period**

Treatment	Storage time (days)				
	0	4	8	12	16
<b>(a) Total viable count</b>					
Con	3.57 ± 0.33 <sup>aE</sup>	5.12 ± 0.27 <sup>aD</sup>	6.61 ± 0.29 <sup>aC</sup>	8.99 ± 0.37 <sup>aB</sup>	10.18 ± 0.19 <sup>aA</sup>
CH/G	3.52 ± 0.21 <sup>aE</sup>	4.66 ± 0.32 <sup>bD</sup>	6.18 ± 0.19 <sup>bC</sup>	7.93 ± 0.27 <sup>bB</sup>	8.94 ± 0.18 <sup>bA</sup>
CH/G + BPE 1.5%	3.56 ± 0.37 <sup>AE</sup>	4.71 ± 0.23 <sup>bD</sup>	5.88 ± 0.04 <sup>bC</sup>	7.52 ± 0.27 <sup>bcB</sup>	8.43 ± 0.11 <sup>cA</sup>
CH/G + BPE 3%	3.46 ± 0.26 <sup>AE</sup>	4.52 ± 0.11 <sup>bcD</sup>	5.53 ± 0.16 <sup>cC</sup>	7.30 ± 0.17 <sup>cB</sup>	8.10 ± 0.10 <sup>dA</sup>
CH/G + BPNE 1.5%	3.63 ± 0.27 <sup>aE</sup>	4.34 ± 0.16 <sup>bcD</sup>	5.22 ± 0.10 <sup>cC</sup>	6.34 ± 0.21 <sup>dB</sup>	7.89 ± 0.17 <sup>dA</sup>
CH/G + BPNE 3%	3.55 ± 0.32 <sup>AE</sup>	4.16 ± 0.08 <sup>cD</sup>	4.87 ± 0.20 <sup>dC</sup>	5.61 ± 0.12 <sup>eB</sup>	6.72 ± 0.12 <sup>eA</sup>
<b>(b) Psychrotrophic bacteria</b>					
Con	2.55 ± 0.18 <sup>aE</sup>	4.52 ± 0.32 <sup>aD</sup>	6.35 ± 0.23 <sup>aC</sup>	7.57 ± 0.17 <sup>aB</sup>	8.95 ± 0.24 <sup>aA</sup>
CH/G	2.49 ± 0.22 <sup>aE</sup>	4.34 ± 0.17 <sup>abD</sup>	5.71 ± 0.14 <sup>bC</sup>	6.88 ± 0.20 <sup>bB</sup>	8.59 ± 0.26 <sup>bA</sup>
CH/G + BPE 1.5%	2.47 ± 0.23 <sup>aE</sup>	4.09 ± 0.13 <sup>bD</sup>	5.26 ± 0.21 <sup>cC</sup>	6.42 ± 0.20 <sup>cB</sup>	7.70 ± 0.12 <sup>cA</sup>
CH/G + BPE 3%	2.46 ± 0.27 <sup>aE</sup>	3.55 ± 0.14 <sup>cD</sup>	4.70 ± 0.16 <sup>dC</sup>	6.09 ± 0.08 <sup>dB</sup>	7.29 ± 0.11 <sup>dA</sup>
CH/G + BPNE 1.5%	2.51 ± 0.32 <sup>aE</sup>	3.24 ± 0.09 <sup>cD</sup>	4.47 ± 0.18 <sup>dC</sup>	5.70 ± 0.15 <sup>dB</sup>	6.63 ± 0.14 <sup>eA</sup>
CH/G + BPNE 3%	2.48 ± 0.34 <sup>aE</sup>	2.90 ± 0.17 <sup>dD</sup>	3.40 ± 0.21 <sup>eC</sup>	5.07 ± 0.19 <sup>IB</sup>	6.28 ± 0.13 <sup>fA</sup>

Values represent mean ± standard deviation (n = 3).

Means in the same row with different capital letters are significantly different ( $p < 0.05$ ).

Means in the same column with different small letters are significantly different ( $p < 0.05$ ).

### 3.4. Evaluation of Antioxidant Activity of the Studied Coatings

The PV (Peroxide Value) indicates the increase in peroxide and hydroperoxide levels produced in the early stages of lipid oxidation, and the TBARS (Thiobarbituric Acid Reactive Substances) is related to the production of secondary metabolites resulting from secondary lipid oxidation [13]. The values of PV and TBARS are reported in Table 5. The peroxide index value between meq/kg 10 and mg MDA/kg

20, and TBARS value lower than mg MDA/kg 5 have been reported as the acceptable limit [22]. In this study, the initial PV and TBARS in the beef control sample were meq/kg 1.32 and mg MDA/kg 0.28, respectively, and by the end of day 16, they increased to meq/kg 9.43 and mg MDA/kg 3.27. The study results showed that during the storage period, both lipid oxidation indexes increased, with the rate of increase being higher in the control sample, which lacked any antioxidant compounds or coatings. The results



indicated that the rate of increase in PV and TBARS in the samples coated with gelatin-chitosan was significantly lower than in the control group ( $p < 0.05$ ), which reflects the antioxidant properties of the gelatin-chitosan coating. These results are consistent with those of other researchers and demonstrate the antioxidant properties of these compounds [25]. Adding black cumin essential oil to the coating solution increased its antioxidant activity. With higher concentrations of the essential oil and the reduction of essential oil emulsion particles using ultrasound, the antioxidant activity of the gelatin-chitosan coating increased, which is consistent with the results of Amiri et al. (2019). They stated that using nanoemulsion and reducing the particle size of Shirazi thyme essential oil nanoemulsion stabilized oxidative stability in ground beef [29]. As reported in Table 5, the lowest levels of PV and TBARS were observed in the gelatin-chitosan coating containing 3% nanoemulsion of black cumin essential oil, where the PV and TBARS values at the end of the study were meq/kg 4.61 and mg MDA/kg 1.30,

respectively. Several studies have been conducted on the antioxidant effect of black cumin essential oil, all of which indicate the high antioxidant activity of this essential oil. The study on the antioxidant activity of the chitosan coating containing nanoemulsion of black cumin essential oil in turkey meat showed that the rate of increase in PV and TBARS in the control group was significantly higher than in the coated samples ( $p < 0.05$ ), and the presence of the black cumin nanoemulsion effectively controlled the oxidation process [22]. Additionally, in evaluating the antioxidant property of biodegradable starch film containing nanoemulsion of black cumin essential oil, Taami et al. (2022) stated that due to the presence of antioxidant compounds in black cumin essential oil, the starch coating containing black cumin essential oil exhibited excellent antioxidant properties and could be used as an active packaging material for food preservation [30].

**Table 5. Antioxidant activity of the studied treatments on beef meat during the storage period**

Treatment	Storage time (days)				
	0	4	8	12	16
<b>(a) PV</b>					
Con	1.35 ± 0.24 <sup>aE</sup>	3.95 ± 0.21 <sup>aD</sup>	5.94 ± 0.59 <sup>aC</sup>	8.31 ± 0.19 <sup>aB</sup>	9.43 ± 0.34 <sup>aA</sup>
CH/G	1.27 ± 0.15 <sup>aE</sup>	3.29 ± 0.33 <sup>bD</sup>	5.14 ± 0.16 <sup>bC</sup>	7.09 ± 0.31 <sup>bB</sup>	8.27 ± 0.24 <sup>bA</sup>
CH/G + BPE 1.5%	1.34 ± 0.33 <sup>aE</sup>	2.57 ± 0.34 <sup>cD</sup>	4.16 ± 0.16 <sup>cC</sup>	6.36 ± 0.35 <sup>cB</sup>	7.27 ± 0.18 <sup>cA</sup>
CH/G + BPE 3%	1.44 ± 0.41 <sup>aD</sup>	2.07 ± 0.39 <sup>dD</sup>	3.57 ± 0.29 <sup>dC</sup>	5.60 ± 0.18 <sup>dB</sup>	6.33 ± 0.41 <sup>dA</sup>
CH/G + BPNE 1.5%	1.38 ± 0.25 <sup>aD</sup>	1.67 ± 0.02 <sup>deD</sup>	2.66 ± 0.15 <sup>eC</sup>	4.19 ± 0.32 <sup>eB</sup>	5.40 ± 0.17 <sup>eA</sup>
CH/G + BPNE 3%	1.43 ± 0.32 <sup>aD</sup>	1.55 ± 0.17 <sup>eD</sup>	2.19 ± 0.12 <sup>eC</sup>	2.89 ± 0.29 <sup>fB</sup>	4.61 ± 0.30 <sup>fA</sup>
<b>(b) TBARS</b>					
Con	0.28 ± 0.09 <sup>aE</sup>	0.86 ± 0.08 <sup>aD</sup>	1.97 ± 0.14 <sup>aC</sup>	2.57 ± 0.25 <sup>aB</sup>	3.27 ± 0.16 <sup>aA</sup>
CH/G	0.26 ± 0.07 <sup>aE</sup>	0.60 ± 0.06 <sup>bD</sup>	1.48 ± 0.25 <sup>bC</sup>	2.00 ± 0.21 <sup>bB</sup>	2.86 ± 0.17 <sup>bA</sup>
CH/G + BPE 1.5%	0.22 ± 0.03 <sup>aE</sup>	0.49 ± 0.01 <sup>cD</sup>	1.10 ± 0.10 <sup>cC</sup>	1.58 ± 0.09 <sup>cB</sup>	2.30 ± 0.14 <sup>cA</sup>
CH/G + BPE 3%	0.24 ± 0.05 <sup>aE</sup>	0.40 ± 0.04 <sup>cdD</sup>	0.82 ± 0.12 <sup>dC</sup>	1.25 ± 0.07 <sup>dB</sup>	1.98 ± 0.08 <sup>dA</sup>
CH/G + BPNE 1.5%	0.24 ± 0.06 <sup>aD</sup>	0.30 ± 0.07 <sup>dD</sup>	0.56 ± 0.10 <sup>deC</sup>	1.01 ± 0.11 <sup>dB</sup>	1.59 ± 0.13 <sup>eA</sup>
CH/G + BPNE 3%	0.23 ± 0.23 <sup>aCD</sup>	0.29 ± 0.05 <sup>dCD</sup>	0.40 ± 0.03 <sup>eC</sup>	0.63 ± 0.10 <sup>eB</sup>	1.30 ± 0.14 <sup>fA</sup>



Values represent mean  $\pm$  standard deviation ( $n = 3$ ).

Means in the same row with different capital letters are significantly different ( $p < 0.05$ ).

Means in the same column with different small letters are significantly different ( $p < 0.05$ ).

#### 4. Conclusion

Black cumin essential oil, due to its components such as cuminaldehyde and  $\gamma$ -terpinene, exhibits good antimicrobial and antioxidant properties. On the other hand, the use of nanotechnology reduced the particle size of the essential oil to approximately 125 nanometers, which enhanced its antimicrobial and antioxidant properties. The use of a gelatin-chitosan coating containing black cumin essential oil emulsion and nanoemulsion significantly inhibited the growth of mesophilic aerobic microorganisms and psychrotrophic bacteria compared to the control sample. Additionally, by delaying lipid oxidation, the peroxide and thiobarbituric acid indices in the coated samples were significantly lower than those in the control sample. According to the results, increasing the concentration of black cumin essential oil and its nanoemulsion from 1.5% to 3% enhanced its antimicrobial and antioxidant activities. Overall, the findings of this study showed that the use of a gelatin-chitosan coating containing black cumin essential oil, especially when it includes nanoemulsion, significantly limited microbial spoilage and lipid oxidation, leading to improved shelf life of refrigerated veal. Based on the results, the use of gelatin-chitosan coating containing nanoemulsion of black cumin essential oil is recommended to enhance the shelf life of food products.

#### 5. Reference

- [1] Thakur M, Olafsson S, Lee JS, Hurburgh CR. 2010. Data mining for recognizing patterns in foodborne disease outbreaks. *Journal of Food Engineering*, **97**(2):213-27.
- [2] Takma DK, Korel F. 2019. Active packaging films as a carrier of black cumin essential oil: Development and effect on quality and shelf-life of chicken breast meat. *Food Packaging and Shelf Life*, **19**:210-7.
- [3] Ahmad RS, Imran A, Hussain MB. 2018. Nutritional composition of meat. *Meat Science and Nutrition*, **61**(10.5772):61-75.
- [4] Panahi Z, Mohsenzadeh M. 2022. Sodium alginate edible coating containing *Ferulago angulata* (Schlecht.) Boiss essential oil, nisin, and NaCl: Its impact on microbial, chemical, and sensorial properties of refrigerated chicken breast. *International Journal of Food Microbiology*, **2**;380:109883.
- [5] Rawat S. 2015. Food Spoilage: Microorganisms and their prevention. *Asian Journal of Plant Science and Research*. **5**(4):47-56.
- [6] Suhag R, Kumar N, Petkoska AT, Upadhyay A. 2020. Film formation and deposition methods of edible coating on food products: A review. *Food Research International*, **136**:109582.
- [7] Baldwin EA, Hagenmaier R, Bai J, editors. 2011. Edible coatings and films to improve food quality. CRC pres.
- [8] Raghav PK, Agarwal N, Saini M. 2016. Edible coating of fruits and vegetables: A review. *Education*. **1**(2):188-204.
- [9] López-Caballero ME, Gómez-Guillén MC, Pérez-Mateos M, Montero P. 2005. A chitosan-gelatin blend as a coating for fish patties. *Food Hydrocolloids*, **19**(2):303-11.
- [10] Pereda M, Ponce AG, Marcovich NE, Ruseckaite RA, Martucci JF. 2011. Chitosan-gelatin composites and bi-layer films with potential antimicrobial activity. *Food Hydrocolloids*, **25**(5):1372-81.
- [11] São Pedro A, Santo I, Silva C, Detoni C, Albuquerque E. 2013. The use of nanotechnology as an approach for essential oil-based formulations with antimicrobial activity. *Microbial Pathogens and Strategies for Combating Them*. **2**:1364-74.
- [12] Moravvej G, Of-Shahraki Z, Azizi-Arani M, Yaghmai F. 2009. Fumigant Toxicity of *Bunium persicum* Boiss.(Umbelliferae) and *Elletaria cardamomum* Maton.(Zingiberaceae)

oils against *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae). *Journal of Plant Protection*, **19**(23):2.

[13] Taheri Mirghaied A, Abiavi T, Hassani F, Shafiei S. 2018. Antibacterial effects of essential oil of Black Cumin (*Bunium persicum*) against some pathogenic bacteria of fish. *Journal of Aquatic Ecology*, **7**(4):159-65.

[14] Shahsavari N, Barzegar M, Sahari MA, Naghdibadi H. 2008. Antioxidant activity and chemical characterization of essential oil of *Bunium persicum*. *Plant Foods for Human Nutrition*, **63**:183-8.

[15] Hosseinzadeh H, Ramezani M, Salmani GA. 2000. Antinociceptive, anti-inflammatory and acute toxicity effects of *Zataria multiflora* Boiss extracts in mice and rats. *Journal of Ethnopharmacology*, **73**(3):379-85.

[16] Moghimi R, Ghaderi L, Rafati H, Aliahmadi A, McClements DJ. 2016. Superior antibacterial activity of nanoemulsion of *Thymus daenensis* essential oil against *E. coli*. *Food Chemistry*, **194**:410-5.

[17] Jerobin J, Makwana P, Suresh Kumar RS, Sundaramoorthy R, Mukherjee A, Chandrasekaran N. 2015. Antibacterial activity of neem nanoemulsion and its toxicity assessment on human lymphocytes in vitro. *International Journal of Nanomedicine*, **10**(sup2):77-86.

[18] Cardoso GP, Dutra MP, Fontes PR, Ramos AD, de Miranda Gomide LA, Ramos EM. 2016. Selection of a chitosan gelatin-based edible coating for color preservation of beef in retail display. *Meat Science*, **114**:85-94.

[19] Jafarinia S, Fallah AA, Dehkordi SH. 2022. Effect of virgin olive oil nanoemulsion combined with ajowan (*Carum copticum*) essential oil on the quality of lamb loins stored under chilled condition. *Food Science and Human Wellness*, **11**(4):904-13.

[20] Raut JS, Karuppayil SM. 2014. A status review on the medicinal properties of essential oils. *Industrial Crops and Products*, **62**:250-64.

[21] Bansal S, Sharma K, Gautam V, Lone AA, Malhotra EV, Kumar S, Singh R. 2023. A comprehensive review of *Bunium persicum*: a

valuable medicinal spice. *Food Reviews International*, **39**(2):1184-202.

[22] Keykhosravy K, Khanzadi S, Hashemi M, Azizzadeh M. 2020. Chitosan-loaded nanoemulsion containing *Zataria Multiflora* Boiss and *Bunium persicum* Boiss essential oils as edible coatings: Its impact on microbial quality of turkey meat and fate of inoculated pathogens. *International Journal of Biological Macromolecules*, **150**:904-13.

[23] Li J, Hwang IC, Chen X, Park HJ. 2016. Effects of chitosan coating on curcumin loaded nano-emulsion: Study on stability and in vitro digestibility. *Food Hydrocolloids*, **60**:138-47.

[24] Sarfraz MH, Hayat S, Siddique MH, Aslam B, Ashraf A, Saqalein M, Khurshid M, Sarfraz MF, Afzal M, Muzammil S. 2024. Chitosan based coatings and films: A perspective on antimicrobial, antioxidant, and intelligent food packaging. *Progress in Organic Coatings*, **188**:108235.

[25] Raeisi M, Tabaraei A, Hashemi M, Behnampour N. 2016. Effect of sodium alginate coating incorporated with nisin, *Cinnamomum zeylanicum*, and rosemary essential oils on microbial quality of chicken meat and fate of *Listeria monocytogenes* during refrigeration. *International Journal of Food Microbiology*, **238**:139-45.

[26] Ahmadi K, Mohsenzadeh M. 2023. Evaluation of antibacterial and antioxidant effect of gelatin-chitosan bilayer edible coating containing nanoemulsion of *Perovskia abrotanoides* Kar. essential oil on growth control of *Aeromonas hydrophila* inoculated into rainbow trout fillet. *Journal of Food Science and Technology (Iran)*, **19**(133):29-41.

[27] Beigmohammadi F, Naseri HR, Mohammadi R, Sadeghi E. 2021. Production of edible film based on chitosan-gelatin, containing *Ferulago angulate* essential oil and evaluation of optical, sensory features and shelf life of packaged Turkey meat in it. *Journal of Food Research*, **30**(4):169-79.

[28] Saki J, Khodanazary A, Hosseini SM. 2017. The effect of chitosan-gelatin composition and bi-layer coating and film on physicochemical, microbial and sensory

properties of *Johnius belangerii* stored at refrigerator. Journal of Research and Innovation in Food Science and Technology, **6(1)**:71-86.

[29] Amiri E, Aminzare M, Azar HH, Mehrasbi MR 2019. Combined antioxidant and sensory effects of corn starch films with nanoemulsion of *Zataria multiflora* essential oil fortified with cinnamaldehyde on fresh ground beef patties. Meat Science. **153**:66-74.

[30] Taami B, Rostami Zadeh K, Aminzare M, Hassanzad Azar H. 2022. Antioxidant Efficacy of Biodegradable Starch Film Containing of *bunium persicum* Essential Oil Nanoemulsion Fortified with Cinnamaldehyde. Journal of Medicinal Plants and By-products, **11(Special)**:67-75.



## ارزیابی فعالیت ضد میکروبی و آنتی اکسیدانی پوشش ژلاتین-کیتوزان حاوی امولسیون و نانوامولسیون اسانس زیره سیاه بر روی گوشت گوساله

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اطلاعات مقاله	چکیده
<p><b>تاریخ های مقاله :</b></p> <p>تاریخ دریافت: ۱۴۰۳/۳/۳۱</p> <p>تاریخ پذیرش: ۱۴۰۳/۹/۲۱</p> <p><b>کلمات کلیدی:</b></p> <p>اسانس زیره سیاه، ژلاتین، فعالیت ضد میکروبی، فعالیت آنتی اکسیدانی، کیتوزان، گوشت گوساله.</p> <p><b>DOI: 10.22034/FSCT.22.163.90.</b></p> <p>* مسئول مکاتبات: b.hajirostamloo@iauneyshabur.ac.ir</p>	<p>ترکیبات ضد میکروبی گیاهی جایگزین بسیار مناسب و ایمنی نسبت به نگهدارنده های سنتزی هستند. از طرفی تکنولوژی نانو اثرگذاری ترکیبات مؤثره گیاهان بر روی سلول های هدف را افزایش داده و بهبود می بخشد. در این مطالعه اثر ضد میکروبی (باکتری های مزوفیل هوازی و باکتری های سرماگرا) و آنتی اکسیدانی (TBARS و PV) پوشش خوراکی ژلاتین-کیتوزان حاوی امولسیون و نانوامولسیون اسانس زیره سیاه (۱/۵٪ و ۳٪) در نمونه های گوشت گوساله تحت شرایط دمایی ۴ درجه سانتی گراد در طول دوره ۱۶ روزه (۰، ۴، ۸، ۱۲ و ۱۶) بررسی گردید. آنالیز اسانس توسط دستگاه GC-MS نشان داد که ترکیبات مؤثره اسانس کومین آلدئید و گاما ترپین می باشند. نتایج حاصل از بررسی اثر ضد میکروبی پوشش های تهیه شده نشان داد که بین نمونه شاهد و نمونه های پوشش داده شده اختلاف معنی داری وجود دارد (<math>p&lt;0.05</math>). افزودن اسانس زیره سیاه و افزایش غلظت اسانس باعث افزایش خاصیت ضد میکروبی گردید. همچنین نتایج ارزیابی فعالیت ضد میکروبی نشان داد نمونه حاوی پوشش ژلاتین-کیتوزان حاوی غلظت ۳٪ نانوامولسیون اسانس زیره سیاه دارای اثر ضد میکروبی بالاتری در مقایسه با نمونه های دیگر بود. نتایج اندازه گیری شاخص پراکسید و اسید تیوباریتوریک نشان داد که بین نمونه شاهد و نمونه های پوشش دهی شده اختلاف معنی داری وجود دارد (<math>p&lt;0.05</math>). افزودن اسانس زیره سیاه به دلیل دارا بودن ترکیبات آنتی اکسیدانی باعث بهبود و افزایش خاصیت آنتی اکسیدانی پوشش ژلاتین-کیتوزان گردید و بالاترین خاصیت آنتی اکسیدانی مرتبط با پوشش ژلاتین-کیتوزان حاوی نانوامولسیون ۱/۵٪ و ۳٪ اسانس زیره سیاه بود. در مجموع نتایج بدست آمده نشان داد که پوشش ژلاتین-کیتوزان حاوی اسانس زیره سیاه دارای قدرت ضد میکروبی و آنتی اکسیدانی بالایی بوده و می تواند باعث بهبود ماندگاری گوشت گوساله نگهداری شده در یخچال گردد. همچنین می توان از این پوشش تهیه شده به عنوان یک بسته بندی زیست تخریب پذیر در صنعت غذایی بهره برد.</p>