

### Journal of Food Science and Technology (Iran)

Homepage: www.fsct.modares.ir

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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#### **ARTICLE INFO**

### **Article History:**

Received:2024/6/20

Accepted:2024/12/11

### **Keywords:**

Bunium persicum essential oil,

Antimicrobial activity,

Antioxidant activity,

Gelatin,

Chitosan,

Beef meat.

#### DOI: 10.22034/FSCT.22.163.90.

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### **ABSTRACT**

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 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 γ-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 gelatinchitosan 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 moisture content. abundance 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<sup>7</sup> 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 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 Chitosan-based coatings, derived from chitin from crustaceans extracted and 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

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,

coatings exhibit notable antioxidant properties

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 of antioxidant simultaneous use 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 γ -terpinene, cuminaldehyde, α-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 containing both emulsions and coating 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 high-pressure techniques, stirring, 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</i> persicum essential oil (%)	Nanoemulsion of <i>Bunium</i> persicum 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
<b>CH/G + BPNE 3%</b>	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 Coatings2.7.1. 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, γ -terpinene, βpinene, and p-cymene have been reported as the main components [21]. In a study on the active compounds of black cumin essential oil, Keykhosravy 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)
β-Phellandrene	0.50	10.02
β-Pinene	5.30	10.25
α-Phellandrene	1.31	11.47
p-Cymene	10.03	12.11
D-Limonene	6.63	12.62
γ-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

DOI: 10.22034/FSCT.22.163.90

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 size, although higher surfactant concentrations can reduce the antimicrobial activity of plant essential oils [23].

Table 3. Particle size. Z-average (nm) PDI  $125.10 \pm 1.95$  $0.27 \pm 0.03$ 

### 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 gelatinchitosan 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	
(a) Total viable count						
Con	$3.57 \pm 0.33~^{aE}$	$5.12\pm0.27~^{aD}$	$6.61\pm0.29~^{aC}$	$8.99 \pm 0.37~^{aB}$	$10.18\pm0.19~^{aA}$	
CH/G	$3.52 \pm 0.21~^{aE}$	$4.66\pm0.32~^{bD}$	$6.18\pm0.19~^{bC}$	$7.93\pm0.27~^{bB}$	$8.94\pm0.18~^{bA}$	
CH/G + BPE 1.5%	$3.56\pm0.37~^{\rm AE}$	$4.71\pm0.23~^{bD}$	$5.88\pm0.04~^{bC}$	$7.52\pm0.27~^{bcB}$	$8.43\pm0.11~^{cA}$	
CH/G +BPE 3%	$3.46\pm0.26~^{\rm AE}$	$4.52\pm0.11~^{bcD}$	$5.53\pm0.16~^{eC}$	$7.30\pm0.17~^{cB}$	$8.10\pm0.10~^{\rm dA}$	
CH/G + BPNE 1.5%	$3.63 \pm 0.27~^{aE}$	$4.34\pm0.16~^{bcD}$	$5.22\pm0.10~^{eC}$	$6.34 \pm 0.21~^{\mathrm{dB}}$	$7.89 \pm 0.17~^{dA}$	
CH/G + BPNE 3%	$3.55\pm0.32~^{\mathrm{AE}}$	$4.16\pm0.08~^{cD}$	$4.87\pm0.20~^{dC}$	$5.61\pm0.12~^{eB}$	$6.72\pm0.12~^{\text{eA}}$	
(b) Psychrotrophic bacteria						
Con	$2.55 \pm 0.18~^{aE}$	$4.52\pm0.32~^{aD}$	$6.35\pm0.23~^{aC}$	$7.57 \pm 0.17~^{aB}$	$8.95 \pm 0.24~^{aA}$	
CH/G	$2.49 \pm 0.22~^{aE}$	$4.34 \pm 0.17~^{abD}$	$5.71\pm0.14~^{bC}$	$6.88 \pm 0.20~^{bB}$	$8.59 \pm 0.26~^{\mathrm{bA}}$	
CH/G + BPE 1.5%	$2.47 \pm 0.23~^{aE}$	$4.09\pm0.13~^{bD}$	$5.26\pm0.21~^{eC}$	$6.42\pm0.20~^{cB}$	$7.70\pm0.12~^{cA}$	
CH/G +BPE 3%	$2.46 \pm 0.27~^{aE}$	$3.55\pm0.14~^{cD}$	$4.70\pm0.16~^{\text{dC}}$	$6.09\pm0.08~^{\mathrm{dB}}$	$7.29 \pm 0.11~^{dA}$	
CH/G + BPNE 1.5%	$2.51\pm0.32~^{aE}$	$3.24\pm0.09~^{cD}$	$4.47\pm0.18~^{dC}$	$5.70\pm0.15~^{\mathrm{dB}}$	$6.63\pm0.14~^{\text{eA}}$	
CH/G + BPNE 3%	$2.48 \pm 0.34~^{aE}$	$2.90\pm0.17~^{\mathrm{dD}}$	$3.40\pm0.21~^{eC}$	$5.07\pm0.19~^{\mathrm{fB}}$	$6.28\pm0.$ 13 $^{\mathrm{fA}}$	

*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).

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

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 meg/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 gelatinchitosan 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 gelatinchitosan 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, antioxidant evaluating the 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	)	8 1
	0	4	8	12	16
(a) PV					
Con	$1.35 \pm 0.24~^{aE}$	$3.95 \pm 0.21~^{aD}$	$5.94 \pm 0.59~^{aC}$	$8.31\pm0.19~^{aB}$	$9.43 \pm 0.34~^{\mathrm{aA}}$
CH/G	$1.27 \pm 0.15~^{aE}$	$3.29\pm0.33~^{bD}$	$5.14\pm0.16~^{bC}$	$7.09 \pm 0.31~^{bB}$	$8.27\pm0.24~^{bA}$
CH/G + BPE 1.5%	$1.34 \pm 0.33~^{aE}$	$2.57\pm0.34~^{cD}$	$4.16\pm0.16~^{cC}$	$6.36\pm0.35~^{cB}$	$7.27\pm0.18~^{cA}$
CH/G +BPE 3%	$1.44\pm0.41~^{aD}$	$2.07\pm0.39~^{\mathrm{dD}}$	$3.57\pm0.29~^{dC}$	$5.60\pm0.18~^{\mathrm{dB}}$	$6.33\pm0.41~^{dA}$
CH/G + BPNE 1.5%	$1.38 \pm 0.25~^{aD}$	$1.67 \pm 0.02~^{\mathrm{deD}}$	$2.66\pm0.15~^{eC}$	$4.19\pm0.32~^{eB}$	$5.40\pm0.17~^{eA}$
CH/G + BPNE 3%	$1.43\pm0.32~^{aD}$	$1.55\pm0.17~^{eD}$	$2.19\pm0.12~^{eC}$	$2.89\pm0.29~^{\mathrm{fB}}$	$4.61\pm0.30~^{\rm fA}$
(b) TBARS					
Con	$0.28 \pm 0.09~^{aE}$	$0.86 \pm 0.08~^{aD}$	$1.97 \pm 0.14~^{aC}$	$2.57 \pm 0.25~^{aB}$	$3.27 \pm 0.16~^{aA}$
CH/G	$0.26 \pm 0.07~^{aE}$	$0.60\pm0.06~^{bD}$	$1.48\pm0.25~^{bC}$	$2.00 \pm 0.21~^{bB}$	$2.86 \pm 0.17~^{\text{bA}}$
CH/G + BPE 1.5%	$0.22 \pm 0.03~^{aE}$	$0.49 \pm 0.01~^{cD}$	$1.10\pm0.10~^{cC}$	$1.58\pm0.09~^{cB}$	$2.30\pm0.14~^{cA}$
CH/G +BPE 3%	$0.24\pm0.05~^{aE}$	$0.40 \pm 0.04~^{cdD}$	$0.82\pm0.12~^{dC}$	$1.25\pm0.07~^{\mathrm{dB}}$	$1.98 \pm 0.08~^{dA}$
CH/G + BPNE 1.5%	$0.24\pm0.06~^{aD}$	$0.30 \pm 0.07~^{\mathrm{dD}}$	$0.56\pm0.10~^{deC}$	$1.01\pm0.11~^{dB}$	$1.59\pm0.13~^{eA}$
CH/G + BPNE 3%	$0.23\pm0.23~^{aCD}$	$0.29 \pm 0.05~^{dCD}$	$0.40\pm0.03~^{eC}$	$0.63\pm0.10~^{eB}$	$1.30\pm0.14~^{\rm fA}$

*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 γ-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 gelatinchitosan 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.

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### مجله علوم و صنایع غذایی ایران



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مقاله علمي\_پژوهشي

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

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### اطلاعات مقاله چكيده

تاریخ های مقاله:

تاریخ دریافت: ۱٤٠٣/٣/٣١

تاریخ پذیرش: ۱٤٠٣/٩/۲۱

### كلمات كليدى:

اسانس زیره سیاه، ژلاتین، فعالیت ضد میکروبی، فعالیت آنتی اکسیدانی، کیتوزان، گوشت گوساله.

DOI: 10.22034/FSCT.22.163.90.

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ترکیبات ضدمیکروبی گیاهی جایگزین بسیار مناسب و ایمنسی 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 ضدمیکروبی (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 ٤ درجه سانتی گردید. آنالیز اسانس توسط دستگاه GC-MS نشان داد که ترکیبات مـوثره اســانس کــومین اَلدئیــد و گامــا تــرپینن مــی باشــند. تــایج حاصل از بررسی اثر ضد میکروبی پوشش های تهیه شده نشان داد که بین نمونه شاهد و نمونه های پوشىش دادە شىدە اخىتلاف معنىي دارى وجىود دارد(p<0.05). افىزودن اسىانس زىىرە سىياە و افىزايش غلظت اسانس باعث افزايش خاصيت ضد ميكروبي گرديد. همچنين نتايج ارزيابي فعاليت ضدمیکروبی نشان داد نمونه حاوی پوشش ژلاتین-کیتوزان حاوی غلظت ۳٪ نانوامولسیون اسانس زیره سیاه دارای اثر ضدمیکروبی بالاتری در مقایسه با نمونه های دیگر بود. نتایج اندازه گیری شاخص پراکسید و اسید تیوباربیتوریک نشان داد که بین نمونه شاهد و نمونه های پوشش دهی شده اختلاف معنی داری وجود دارد (p<0.05). افزودن اسانس زیـره سیاه بـه دلیـل دارابــودن ترکیبــات آنتــی اكسيداني باعث بهبود و افرايش خاصيت آنتي اكسيداني پوشش ژلاتين-كيتوزان گرديـد و بـالاترين خاصيت آنتي اكسيداني مرتبط با پوشش ژلاتين- كيتوزان حاوي نانوامولسيون ١/٥٪ و ٣٪ اسانس زیره سیاه بود. در مجموع نتایج بدست آمده نشان داد که یوشش ژلاتین-کیتوزان حاوی اسانس زیره سیاه دارای قــدرت ضــدمیکروبی و آنتــی اکســیدانی بــالایی بــوده و مــی توانــد باعــث بهبــود مانــدگاری 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.