

Nanoencapsulation of *Oliveria decumbens* Vent. and Basil Essential oils to Investigate Their Antibacterial Function in Vitro and Minced Beef Meat

Mahdis Gorzodin

Damghan Branch, Islamic Azad University, Damghan

Marjan Nouri (✉ Marjan.nouri@iau.ac.ir)

Roudehen Branch, Islamic Azad University, Roudehen, Iran

Sahar Javidi

Damghan Branch, Islamic Azad University, Damghan

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Abstract

Beef is a nutritious meat, which possesses biological factors such as protein and micronutrients. The purpose of present research is to investigate antioxidant and antibacterial activities for nanocapsules of *Oliveria decumbens* Vent. (OEO) and basil (BEO) essential oils in vitro and minced beef. OEO and BEO were extracted and their phytochemicals were identified by gas chromatography. These nanocapsules were produced using freeze drying and combination of maltodextrin and also gum arabic (1:1). Particle size, polydispersity index, encapsulation efficiency (EE), scanning electron microscopy, total phenolic (TPC) and flavonoid (TFC) contents, antioxidant and antibacterial activities, pH, thiobarbituric acid and also sensory were evaluated in vitro compared to free forms. The antibacterial functions were assessed against *S. aureus*, *E. coli*, *L. monocytogenes*, *S. typhimurium* and *P. aeruginosa* in minced meat during 10 days of cold storage. Thymol (28.12 %), carvacrol (23.97 %), γ -terpinene (19.56 %) and p-cymene (12.64 %) as well as estragole (48.93 %) and linalool (41.45 %) were the main in OEO and BEO, respectively. The TPC (30.43 to 32.41 mg GAE/g DW), TFC (27.72 to 30.10 mg GAE/g DW) and antioxidant capacity (25.97 to 26.42 %) were determined in free and encapsulated OEO and also antibacterial feature was observed, which were further than BEO. No significant effect was detected by nanoencapsulation on bioactive components and also antioxidant of OEO and BEO, however this process considerably improved antibacterial function ($p < 0.05$). Finally, OEO and BEO-loaded nanocapsules were applied to promote quality in beef, while potential of OEO nanocapsules was higher.

Introduction

Meat is a main food item in the human diet and extremely susceptible to microorganism growth due to various nutrient components and high moisture content [1]. One of the worrying issues about bacteria is their growth possibility in beef meat at refrigerator temperatures [2]. The consumption of foods containing synthetic preservatives brings several problems to consumers importantly carcinogenicity [3]. Nowadays, the possibility for replacing natural antimicrobial agents such as plant extracts and essential oils (EOs) instead of synthetic preservatives has been discussed [4].

Oliveria decumbens Vent. as an aromatic plant of the *Umbelliferae* family is usually found in southern regions of Iran and widely used to treat infectious diseases, diarrhea, fever and indigestion [5]. *Oliveria decumbens* Vent. essential oil (OEO) possesses several bioactive components especially thymol, carvacrol, p-cymene and γ -terpinene, which demonstrates remarkable antioxidant, radical scavenging and antimicrobial activities [6].

Basil or *Ocimum basilicum* is an annual and herbaceous plant from the *Lamiaceae* family that is applied in traditional medicine to treat convulsion, cancer, diarrhea, heart diseases, spleen enlargement, toothaches, sore throat and bronchitis [7]. Basil essential oil (BEO) is approximately 0.5 to 1.5% and consists of various phytochemicals such as estragole, eugenol, linalool, bergamotene and eucalyptol [8].

During encapsulation procedure, the bioactive and antimicrobial agents are covered by wall materials and protected against the deteriorating conditions of human body and also environment, which their solubility is also improved [9, 10]. Various biopolymers are applied individually or in combination as wall materials or coating agents [11]. Because these components can produce particles with high encapsulation efficiency (EE), the mixture of maltodextrin and gum arabic is the most prominent factor to cover EOs [12].

The aim of this research is to evaluate the antioxidant and antibacterial features of free and nanoencapsulated OEO and BEO prepared with freeze drying and also maltodextrin and gum arabic combinations (as wall materials) *in vitro*. Also, the antibacterial activities were assessed against *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Listeria monocytogenes* (*L. monocytogenes*), *Salmonella typhimurium* (*S. typhimurium*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) in minced beef meat during the cold storage.

Materials and Methods

Materials

Aerial parts of *Oliveria decumbens* Vent. were prepared from Fars province (Iran) and basil leaves were purchased from local market in Tehran (Iran). Microbial strains of *S. aureus* (PTCC1431), *E. coli* O157: H7 (ATCC35218), *L. monocytogenes* (ATCC19118), *S. typhimurium* (ATCC 14028) and *P. aeruginosa* (ATCC 27853) were obtained from the Food Science and Technology Department of Tehran University (Iran). Culture medium, maltodextrin, gum arabic and chemicals were gained from Merck Company of Germany in present study.

Extraction of OEO and BEO

The aerial parts for *Oliveria decumbens* Vent. and basil leaves were dried in shade; then, turned into powder and passed through a sieve (with a mesh of 250 μm) as well as water distillation was used to extract plant EOs. All plant powders were mixed with distilled water in a ratio of 1:3 and EO was extracted by the Clevenger apparatus at boiling temperature for 3 h. The collected EOs were dehydrated by anhydrous sodium sulfate and finally stored in dark glass at a refrigerator [13].

Identification of phytochemicals in OEO and BEO by gas chromatography–mass spectrometry (GC-MS)

The phytochemical constituents of OEO and BEO were analyzed using the Agilent GC-MS (America). This system was equipped by HP-5-MS column with dimensions of 60 m (length) $\times$ 0.25 mm (inner diameter) $\times$ 25 μm (thickness). The increase in temperature was considered to be 40°C/min and reached 290°C, carrier gas was helium (2 mL/min flow rate) and liquid with volume of 1 μL was the injection type. The temperatures were detected 280°C, 290°C and 70°C for injector, source and oven, respectively. The phytochemicals of OEO and BEO were identified and reported as percentage values [6].

Preparation of OEO and BEO-loaded nanocapsules

Initially, gum arabic (10 g) and maltodextrin (10 g) were mixed with 20 mL distilled water, then stirred for 1 h at 60°C (500 rpm) and also kept at refrigerator about 24 h to produce OEO and BEO nanocapsules. OEO or BEO at 6% (w/w) with 2% (w/w) tween 80 were added to coating solution and stirred at 500 rpm during 30 min; afterwards, the sonication of nanoemulsions was done at 24 kHz about 120 S and frozen for 1 day at -20°C. The capsules were kept at -50°C during 2 days by a freeze dryer and totally they were turned into final powders [10].

Characteristics of OEO and BEO-loaded nanocapsules

The particle size, polydispersity index (PDI) and zeta potential values of nanocapsules were evaluated through dynamic light scattering (Zetasizer Nano ZS®, Malvern Instruments Ltd., Malvern and Worcestershire, UK). The reproduced nanocapsules were achieved using stirring EOs (1 g) into deionized water (200 mL), which mixed by a magnetic blender at 500 rpm at ambient temperature about 30 min [11].

EE was determined using measurement of total phenolic content (TPC) for nanocapsules according to Folin-Ciocalteu method. For this purpose, capsules (200 mg) were stirred with 2 mL methanol:water:acetic acid mixture (50:42:8 v/v/v) and sonicated at 20 kHz for 20 min in 2 cycles. After that, mixture was centrifuged about 12 min at 5000 rpm and finally, TPC of supernatant was calculated through Eq. 1 [14]:

$$\text{Eq. 1 EE \%} = \frac{\text{TPCofEO} - \text{TPCofcapsule}}{\text{TPCofEO}} \times 100$$

Scanning electron microscopy (SEM, LEO 1450; America) was used to assay the microstructure or morphology of OEO and BEO-loaded nanocapsules [4].

Determination of TPC, total flavonoid content (TFC) and antioxidant activity

Folin-Ciocalteu method was applied to measure TPC of free or encapsulated OEO and BEO; briefly, sample (20 µL) and reagent (0.5 mL) were mixed, then distilled water (5 mL) was added and solution was kept for 10 min. Afterwards, 2% sodium carbonate (0.5 mL) was added to sample and incubation for 45 min, so the absorbance was read at 760 nm.

Sample (0.5 mL) was mixed with 80% methanol (1.5 mL) and then 10% AlCl₃ (0.1 mL), 1 M potassium sorbate (0.1 mL) and distilled water (2.8 mL) were added to measure TFC. The solution was kept for 30 min and absorbance against blank (without reactants) was recorded at 410 nm [15].

The antioxidant activity of free or encapsulated OEO and BEO was calculated by DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging assay. Briefly, the mixture EOs of each sample (1 mL) and 6×10^{-5} mol/L DPPH methanolic solution (3.5 mL) were stirred and also absorbance was recorded against blank

(methanol) at 517 nm after keeping for 30 min at 25°C in darkness. The DPPH radical scavenging capacity assay (%) for samples was obtained through Eq. 2 [16]:

$$\text{Eq. 2 DPPH (\%)} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

Analysis of antibacterial activity in vitro

Evaluation for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC of free or encapsulated OEO and also BEO against *S. aureus*, *E. coli* O157: H7, *L. monocytogenes*, *S. typhimurium* and *P. aeruginosa* were investigated by broth microdilution assay. Briefly, OEO and BEO (50 µL) at different concentrations were prepared and deposited in the wells and also bacteria strain (50 µL) at 6 Log CFU/mL was added. The incubation was done at 37°C for 18 h and after that, resazurin (10 µL) was inserted to the wells for accessing bacterial growth. The MIC was the lowest EO concentration that changes the color of resazurin from blue to pink. Negative subcultures (10 µL) was transferred to non-selective medium and after incubation, the MBC was obtained at 37°C for 24 h [13].

Assessment of antibacterial function using diffusion disc agar assay

The bacterial suspension (100 µL) was spread on surface of Muller Hinton agar (MHA) medium. Sterile blank discs were produced with 6 mm diameter and impregnated by 200 mg/mL dilution of EOs (40 µL). Furthermore, the discs were transferred on agar surface and impregnated in ethanol and also 10 µg gentamicin were used as negative and positive control, respectively. The prepared plates were finally incubated overnight at 37°C and the growth inhibition zone diameter (mm) around each disk was determined [17].

Antibacterial feature against some pathogenic bacteria in minced meat

The beef meat was purchased from the slaughterhouse and transported to the laboratory under hygienic conditions and at $5 \pm 1^\circ\text{C}$. After washing and chopping, the meats pieces were minced using a sterile grinder, divided to 25 samples (25 ± 5 g) and transferred into polyethylene bags. Then OEO, BEO, OEO/BEO-loaded nanocapsules and gentamicin in minced meat were treated by *L. monocytogenes*, *S. aureus*, *E. coli* O157:H7, *S. typhimurium* and *P. aeruginosa* (10^5 CFU/g final inoculation) and also homogenized at room temperature for 3 min. Afterwards, the free or encapsulated OEO and BEO were added to inoculated 2% (w/w) minced meats and homogenized for 5 min at normal speed (280 rpm) by a stomacher. The control sample indicated no EO concentrations and sterile water was added for replacement. The treatments were kept at $6 \pm 1^\circ\text{C}$ for 10 days and target bacteria were counted once every 2 days, so the results expressed as Log CFU/g meat. For this purpose, minced meats were removed from refrigerator and after preparing, desired dilution was cultured on medium of Listeria selective agar using

peptone water (0.1%) and incubation was done at 37°C for 24 h [18]. Baird-Parker medium supplemented with egg emulsion, cefixime-tellurite sorbitol MacConkey, xylose lysine deoxycholate and *Pseudomonas* agars were used for counting the populations of *S. aureus*, *E. coli* O157:H7, *S. typhimurium* and *P. aeruginosa*, respectively and plates were incubated at 37°C for 48 and 24 h [19].

pH, thiobarbituric acid (TBA) and sensory evaluation of minced meat

The minced meat was divided in 5 groups (25 ± 5 g) including OEO, BEO, their nanocapsules and control without EOs; afterwards, these samples were transferred into polyethylene bags to determine pH, TBA and sensory characteristics during 10 days of shelf life.

pH values were measured using a Metrohm pH meter (Switzerland) and 2 g minced meat were homogenized with 7.5% trichloroacetic acid solution (8 mL) for 3 min at 13,000 rpm and filtered. The solution of 0.02 mol/L TBA (2 mL) was then added to resulted mixture and heated about 40 min at 95°C in bath water. After cooling mixture until ambient temperature by a UV–Vis spectrophotometer (Jenway 7315, England), mixture absorbance was recorded at 532 nm [3].

Thirty experienced panelists in range of 25 to 40 ages were selected to evaluate sensory characteristics of minced meat samples including texture, color, odor, appearance and overall acceptability. The samples were randomly coded and sensory function were assessed based on a five-point scale from 5 = extremely good to 1 = extremely bad [20].

Results and discussion

Phytochemical compositions of OEO and BEO

The phytochemicals of OEO and BEO are presented in Table 1 and also the major chemicals of OEO were thymol (28.12 %), carvacrol (23.97 %), γ -terpinene (19.56 %) and p-cymene (12.64 %). While, the most important bioactive substances of BEO included estragole (48.93 %) and linalool (41.45 %) and other components in smaller amounts. Thymol, carvacrol, γ -terpinene and p-cymene were reported as major compounds of OEO [5, 15], which were in line with present research. The estragole (41.40 %) and 1,6-Octadien-3-ol, 3,7-dimethyl (29.49 %) were observed as the main components of BEO [6]. However, linalool (27.64 %), estragole (15.97 %), methyl cinnamate (10.49 %) and eucalyptol (5.49 %) were identified in other previous research [7]. In general, there are noticeable differences between the types and amounts of phytochemicals in different plant EOs and even in the same species [5]. These variations had been observed, which depend on whether conditions, cultivation programs, geographical region, plant parts, harvesting time and stage of plant growth [21].

#Approximate position of Table 1#

Characteristics of OEO and BEO-loaded nanocapsules

The characteristics of nanocapsules including particle size, PDI, zeta potential and EE are presented in Table 2 and Figure 1. The particle size is an important and effective factor in stability conditions and flow capability index [11]. EE is one of the prominent agents in determining the stability, which indicates EO presence on surface of powder particles and wall ability to prevent the internal oil from escaping [10]. The particle size and wall materials are major agents in assaying encapsulation, so particles with smaller sizes are dried without breaking that have higher efficiency and transfer to the surface is reduced [21]. No significant differences were distinguished between the particle size and EE of nanocapsulated OEO or BEO, which were 136.4 ± 3.7 nm and 135.8 ± 4.6 nm as well as 91.93 ± 3.14 % and 93.28 ± 2.75 %, respectively. Since, a similar method was used for drying nanocapsulation of EOs in another research and coating materials were the same, which was in line with present study [15].

The particle size of nanoencapsulated mandarin EO prepared by combination of maltodextrin and gum arabic was reported to be 578.58 nm [11], which was higher than values in the present study. The EE of nanocapsulated gurum seed oil and cinnamaldehyde content prepared with a combination of maltodextrin and gum arabic was reported to be higher than 90 % and 80 %, respectively [9, 12].

The researchers reported that maltodextrin is a hydrophilic and non-ionic component, but gum arabic had hydrophilic and hydrophobic functions [9, 15]. The mixture of these substances as coatings could be created a new wall with suitable and high efficiency for EO encapsulation [15].

#Approximate position of Table 2#

#Approximate position of Figure 1#

PDI demonstrates the degree for homogeneity and uniformity in distribution of particle size [22]. The PDI values for nanocapsulation of OEO and BEO were 0.376 ± 0.051 and 0.346 ± 0.028 as well as no significant difference was observed between these substances. In general, the PDI less than 0.5 illustrates the homogeneity in particles distribution [11]. The PDI for pequi oil emulsions prepared with a combination of maltodextrin and gum arabic was 0.890 [22], which was more than levels reported in the present study.

Zeta potential is an important index that expresses the electrical state of particle surface and shows the charge accumulation in immobile layer and adsorption intensity of opposite ions on surfaces [22]. As zeta potential increased, the electrostatic repulsion force and physical strength of the system improved as well as this factor was higher than 30 mV, which indicated adequate stability of particles in colloidal systems [11]. The results in present study demonstrated that zeta potential for nanocapsulation of OEO and BEO were -43.37 ± 0.86 mV and -45.10 ± 0.95 mV, which these particles had better stability. This index for mandarin EO nanocapsules prepared with combination of gum arabic and maltodextrin was -57.66 mV and the produced capsules had greater stability [11].

The microstructure of OEO and BEO-loaded nanocapsules is assessed using a SEM and its images are presented in Figure 2. The microstructure of particles depended on drying method and in this research,

the freeze drying technique was used to prepare OEO and BEO-loaded nanocapsules and produced capsules showed an asymmetric shape and had sharp edges. In the studies conducted by other researchers, the non-uniform shape of particles was prepared by freeze drying had been reported [23, 24]. Similarly, the capsules prepared by freeze drying with combination of maltodextrina and gum arabic had flaky and angular shapes [15].

#Approximate position of Figure 2#

TPC, TFC and antioxidant activity

The results of TPC, TFC and DPPH radical scavenging percentage for free or encapsulated OEO and BEO are given in Table 3. It showed that nanoencapsulation process led to decrease in TPC, TFC and antioxidant capacity of EOs; however, this reduction was not statistically significant. The lower bioactive compounds and antioxidant activity of plant extracts and EOs had been reported during encapsulation process in previous researches, but this decrease trend was minor and insignificant in some cases compared to others [4, 22]. The reduction in bioactive compounds was often due to the drying stage during the encapsulation process, which caused to loss of some active substances. Among OEO and BEO, the higher TPC and TFC were for OEO and therefore, this EO also indicated the more antioxidant features. TPC (31.28-33.05 mg GAE/g DW), TFC (6.53-6.81 mg QE/g DW) and DPPH radical scavenging (81.49-84.52 %) were detected; however, these values were 21.76-22.34 mg GAE/g DW, 4.72-4.76 mg QE/g DW and 60.33-62.19 % for free or encapsulated OEO and BEO, respectively. OEO was a mixture of various bioactive constituents such as phenols and terpenoids, which exhibited synergistic effects and created remarkable antioxidant activity [16]. The high antioxidant potential for OEO was attributed to presence of TPC and TFC [15]. Thymol and carvacrol have similar antioxidant activity, which were comparable to synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene [5]. Gamma-terpinene is another important bioactive constituents of OEO that antioxidant behavior and synergistic effect have been confirmed [14]. In general, the anti-radical and antioxidant traits of plant EOs were related to various bioactive materials and different types [3, 4]. The antioxidant function of BEO was attributed to the presence of eugenol and linalool; nevertheless, this factor has been reported to be lower than synthetic types [7].

#Approximate position of Table 3#

Antibacterial traits in vitro

The MIC and MBC values of free or encapsulated OEO and BEO are determined using micro-dilution test and results are presented in Table 4. OEO showed higher antibacterial activity and also MIC and MBC levels were also less than BEO. These values against different bacteria were 0.25 to 0.75 mg/mL and 0.50 to 1.25 mg/mL as well as 1.00 to 2.25 mg/mL and 1.25 to 3.00 mg/mL for free or encapsulated OEO and BEO, respectively. In general, the MBC of OEO and BEO was found to be further compared to MIC values. The antibacterial results were significant for free or encapsulated OEO and BEO prepared by diffusion disc agar against gram-positive and gram-negative bacteria; however, this activity in OEO was

higher than BEO (Table 5). The growth inhibition diameter of free and encapsulated OEO against *S. aureus*, *E. coli*, *L. monocytogenes*, *S. typhimurium* and *P. aeruginosa* were in the range of 28.94 to 31.34 mm, 19.09 to 20.52 mm, 26.17 to 28.44 mm, 27.48 to 29.84 mm and 17.33 to 19.06 mm; however, these values were 16.55 to 18.39 mm, 17.16 to 17.98 mm, 14.72 to 15.80 mm, 16.83 to 17.98 mm and 12.78 to 14.59 mm for free and encapsulated BEO, respectively. It has clearly indicated that EO and their active substances can destroy membrane due to hydrophobic nature and affect cell permeability and also lead to bacteria death with leakage of cellular compounds [21, 25]. The carvacrol could also prevent the flagellum synthesis and thus immobilization of bacteria [26]. Thymol and carvacrol are considered as major factors with strong antibacterial activity against pathogenic strains [4]. On the other hand, EOs rich in thymol and carvacrol have higher antimicrobial capacity compared to others consisting of linalool [26], which is consistent with present results. The p-cymene has also been identified as an important phytochemical of OEO with weak antibacterial ability, but synergistic effect with carvacrol could enhance this feature [27]. The estragole and eugenol of BEO also demonstrated a significant antibacterial function [21].

The present study outlined that nanocapsules of OEO and BEO had higher antibacterial activity than free types. The particle size was an effective factor on antimicrobial capacity of active agents and surface area, which were improved by smaller sizes [9]. Similarly, encapsulation process with reduction size led to an increase in antibacterial behavior of clove EO [25]. A decrease in MIC and MBC of oregano, rosemary and cinnamon EOs had also been reported owing to nanoencapsulation [24].

Overall, the antimicrobial function of EO against gram-negative bacteria is often lower than gram-positive bacteria on account of lipopolysaccharide outer layer around cell wall that limits penetration and reduces effectiveness with antimicrobial effects [19]. The higher sensitivity of gram-positive bacteria to EOs has been observed by other researchers [18, 21]. The antibacterial agent of free and encapsulated BEO was less than gentamicin; while, the opposite trend was observed OEO.

#Approximate position of Table 4#

#Approximate position of Table 5#

Antibacterial behavior in minced meat

The changes in *L. monocytogenes*, *S. aureus*, *E. coli*, *S. typhimurium* and *P. aeruginosa* counts of minced meat samples containing free or encapsulated OEO and BEO are depicted in Figure 3. During the storage in refrigerator, different variations of these bacteria numbers were observed in minced meat samples. In control, the count of *L. monocytogenes*, *S. aureus*, *E. coli*, *S. typhimurium* and *P. aeruginosa* gradually increased over time ($p < 0.05$) and reached their highest populations on the 10th day of cold storage (6.87, 7.38, 7.91, 7.86 and 8.39 Log CFU/g, respectively). Nevertheless, a decrease trend of these strains was observed in meat samples containing OEO and BEO due to antibacterial potential during the period. The populations of *L. monocytogenes* (1.39, 0.51, 3.43 and 2.87 Log CFU/g), *S. aureus* (1.09, 0.22, 2.17 and 1.37 Log CFU/g), *E. coli* (2.31, 2.00, 2.94 and 2.51 Log CFU/g), *S. typhimurium* (2.86, 2.00, 3.99, and 2.64

Log CFU/g) and also *P. aeruginosa* (2.87, 2.05, 4.89, and 3.59 Log CFU/g) in free or encapsulated OEO and BEO on the last day of cold storage, respectively. Among EOs studied in this research, antibacterial function of OEO against *L. monocytogenes*, *S. aureus*, *E. coli*, *S. typhimurium* and *P. aeruginosa* was significantly further than BEO. The antibacterial ability of OEO against *L. monocytogenes*, *S. aureus* and *E. coli* was considerably greater due to more phytochemicals especially thymol and carvacol compared BEO, which indicated lower MIC and MBC. The remarkable antibacterial behavior of thymol and carvacol against different bacteria has been confirmed in previous studies [13, 24]. The significant effect of carvacrol and thymol has also been detected in reducing gram-negative bacteria including *L. monocytogenes*, *E. coli* and *S. typhimurium* in marinated beef [28]. However, BEO was also able to decline gram-positive and gram-negative bacteria in meat samples during the cold storage, which was mostly due to more linalool [18].

As obtained results, nanoencapsules of OEO and BEO exhibited better antibacterial traits than free forms and led to a greater reduction of *L. monocytogenes*, *S. aureus*, *E. coli*, *S. typhimurium* and *P. aeruginosa* during the cold storage. The stability improvement of active substances had also been reported in food products during the period [8]. The antimicrobial agents in encapsulated forms are more effective to control microbial growth and reduce pathogens in food products compared to free types [11]. Because encapsulation process increases the stability of antimicrobial properties and causes less interference with food components by locking active agents in wall material, thus prevents inactivation [12]. The nanoencapsulation process can improve the effectiveness of EOs by lowering particle size and promoting surface area [24]. The significant effect of cinnamon and oregano EOs loaded with microcapsules was also reported in meat products (Italian salami) on decreasing *L. monocytogenes* [29].

#Approximate position of Figure 3#

pH, TBA and sensory characteristics of minced meat

The pH values of minced beef samples are compared in Table 6 during 10 days of cold shelf life. On production day, control had the highest pH (5.63) and this trend was declined by EOs addition in treated samples 5.57 to 5.59. The pH of all samples was increased by time ($p < 0.05$) and the lowest was observed on the 1st day of cold storage. This increase was due to meats, which were rich in proteins and as a results of microorganism growth and protein breakdown, alkaline products such as ammonia were manufactured that led to an enhancement in pH over time [16]. However, free and nanoencapsulated OEO and BEO were able to decrease the pH changes over time in meat samples by reducing growth rate and microorganism activity. The effect of plant EOs as natural preservatives in declining pH intensity for meat products was reported during storage [30].

#Approximate Position of Table 6#

The results of TBA in minced beef samples (Table 6) indicated that initially levels were 0.35 to 0.37 mg MDA/kg in samples and oxidative index increased significantly with time passage ($p < 0.05$). As expected, control had the highest TBA and free or encapsulated OEO and BEO led to a considerable

reduction in oxidation of secondary products for samples during cold storage, ($p < 0.05$). No statistically significant difference was detected between TBA for free or encapsulated OEO and BEO and also exhibited the lowest levels on 2 days of storage; however, the highest antioxidant effect was observed in encapsulated OEO on the other days. As reported in vitro study, OEO had further antioxidant activity than BEO and these results were also confirmed in meat assessment. Since, OEO was rich in thymol and carvacrol, which these active components illustrated significant antioxidant activity and synergistic effect [27]. The higher antioxidant of nanoencapsulated OEO and BEO in middle and also end of storage was owing to protective effect, which caused the controlled release of active constituents [11]. The encapsulation improvement on antioxidant activity of EOs in meat products had been confirmed by other researchers [5, 9, 23].

The mince beef samples are depicted in Figure 4 and the results of texture, color, appearance, odor and overall acceptability are given in Figure 5. There was no significant difference between texture scores and all samples got well; in terms of color, appearance, odor and overall acceptability, control had a higher score than others. As expected, encapsulation process masked specific organoleptic features of EOs, so minced beef samples containing nanoencapsulated OEO and BEO scored higher than free. Sensory scores of samples including OEO were higher than BEO, which received a medium score. One of the important advantage for encapsulation process was to mask distinctive sensory features in EOs and made them more desirable for use in food products [20].

#Approximate Position of Figure 4#

#Approximate Position of Figure 5#

Conclusion

The results of present research demonstrated that OEO and BEO nanocapsules prepared by freeze drying and combination of maltodextrin and gum arabic as wall materials created high efficiency and particles with remarkable antioxidant and antibacterial activities. EOs loaded with nanocapsules showed higher antibacterial traits than free forms in vitro and food model (minced meat). Therefore, prepared nanocapsules can be used to develop the quality and safety of meat and its products in current review.

Abbreviations

OEO	<i>Oliveria decumbens</i> Vent. essential oil
BEO	basil essential oil
EE	encapsulation efficiency
TPC	total phenolic content
TFC	flavonoid contents
EOs	essential oils
PDI	polydispersity index
MIC	minimum inhibitory concentration
MBC	minimum bactericidal concentration
MHA	Muller Hinton agar
TBA	thiobarbituric acid

Declarations

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Author Contributions Mahdis Gorzodin: Conceptualization, Writing, Software, Validation. Marjan Nouri: Methodology, Rewriting– original draft and review, Software editing. Sahar Javidi: Formal assessment, evaluation, Resources, Information curation.

Data Availability Available data will be expressed on request.

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Conflict of Interest No conflicts of interest was declared by authors.

Ethical Approval Not applicable.

Consent to Participate Not applicable.

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Tables

Table 1. Phytochemical composition of OEO and BEO

EO	RI	Composition	Values (%)
<i>Oliveira decumbens</i> Vent. essential oil (OEO)	938	α -Pinene	1.83
	988	β -Pinene	3.02
	1030	p-Cymene	12.64
	1031	Limonene	2.78
	1017	α -Phellandrene	3.65
	1068	γ -Terpinene	19.56
	1294	Thymol	28.12
	1299	Carvacrol	23.97
	1519	Myristicin	4.43
Basil essential oil (BEO)	923	α -Pinene	0.52
	982	Comphene	0.18
	1140	Linalool	41.45
	1182	Camphor	1.03
	1239	Estragole	48.93
	1255	Geraniol	1.34
	1358	Eugenol	3.13
	1487	γ -Caryophyllene	0.95
	1543	γ -Cadinene	2.47

OEO: *Oliveira decumbens* Vent. essential oil; BEO: Basil essential oil.

Table 2. Characteristics of encapsulated OEO and BEO

Samples	Particle size (nm)	PDI	Zeta potential (mV)	EE (%)
OEO-loaded nanocapsule	136.4 $\pm$ 3.7 ^a	0.376 $\pm$ 0.051 _a	-43.37 $\pm$ 0.86 ^a	91.93 $\pm$ 3.14 ^a
BEO-loaded nanocapsules	135.8 $\pm$ 4.6 ^a	0.346 $\pm$ 0.028 _a	-45.10 $\pm$ 0.95 ^a	93.28 $\pm$ 2.93 _a

Values represent mean (n=3) $\pm$ SD. Different letters represent significant difference at 5% level of probability among samples. OEO: *Oliveira decumbens* Vent. essential oil; BEO: Basil essential oil; PDI: Polydispersity index; EE: Encapsulation efficiency.

Table 3. TPC, TFC and antioxidant activity of free and encapsulated OEO and BEO

Samples	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	DPPH radical scavenging (%)
OEO	33.05 ± 0.96 ^a	6.81 ± 0.15 ^a	84.52 ± 1.37 ^a
OEO-loaded nanocapsule	31.28 ± 1.18 ^a	6.53 ± 0.32 ^a	81.49 ± 2.56 ^a
BEO	22.34 ± 0.51 ^b	4.76 ± 0.28 ^b	62.19 ± 1.82 ^b
BEO-loaded nanocapsules	21.76 ± 0.74 ^b	4.72 ± 0.19 ^b	60.33 ± 1.46 ^b

Values represent mean (n=3) ± SD. Different letters represent significant difference at 5% level of probability among samples. OEO: *Oliveira decumbens* Vent. essential oil; BEO: Basil essential oil; TPC: Total phenol content; TFC: Total flavonoid content.

Table 4. MIC and MBC values (mg/mL) of free and encapsulated OEO and BEO against some pathogenic food-borne bacteria

Samples	<i>S. aureus</i>		<i>E. coli</i> O157:H7		<i>L. monocytogenes</i>		<i>S. typhimurium</i>		<i>P. aeruginosa</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
OEO	0.50	0.75	0.75	1.25	0.50	0.75	0.75	1.00	0.50	1.00
OEO-loaded nanocapsule	0.25	0.50	0.75	1.00	0.50	0.50	0.50	0.75	0.50	0.75
BEO	2.00	2.50	2.00	2.50	2.25	3.00	2.00	2.25	2.50	3.25
BEO-loaded nanocapsules	1.00	1.25	1.50	1.50	1.50	1.75	1.00	1.50	1.50	2.00
Gentamicin	0.50	0.75	1.00	1.50	0.75	1.00	0.75	1.25	1.00	1.25

Values represent mean (n=3) ± SD. Different letters represent significant difference at 5% level of probability among samples. OEO: *Oliveira decumbens* Vent. essential oil; BEO: Basil essential oil; MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration

Table 5. Diameter of growth inhibition zone (mm) of free and encapsulated OEO and BEO against some pathogenic food-borne bacteria

Samples	<i>S. aureus</i>	<i>E. coli</i> O157:H7	<i>L. monocytogenes</i>	<i>S. typhimurium</i>	<i>P. aeruginosa</i>
OEO	28.94 ± 0.28 _b	19.09 ± 0.15 _b	26.17 ± 0.21 _b	27.48 ± 0.10 _b	17.33 ± 0.19 _c
OEO-loaded nanocapsule	31.34 ± 0.17 _a	20.52 ± 0.12 _a	28.44 ± 0.30 _a	29.89 ± 0.14 _a	19.06 ± 0.28 _a
BEO	16.55 ± 0.31 _e	17.16 ± 0.29 _e	14.72 ± 0.14 _e	16.83 ± 0.25 _e	12.78 ± 0.11 _e
BEO-loaded nanocapsules	18.39 ± 0.24 _d	17.98 ± 0.07 _d	15.80 ± 0.19 _d	17.98 ± 0.18 _d	14.59 ± 0.39 _d
Gentamicin	23.47 ± 0.36 _c	18.50 ± 0.22 _c	20.62 ± 0.25 _c	21.95 ± 0.45 _c	18.26 ± 0.17 _b

Values represent mean (n=3) ± SD. Different letters represent significant difference at 5% level of probability among samples. OEO: *Oliveira decumbens* Vent. essential oil; BEO: Basil essential oil.

Table 6. Changes in pH and TBA values of minced beef meat samples during cold storage period

Storage day	Control	OEO	NOEO	BEO	NBEO
	pH				
0	5.63 ± 0.02 ^{E,a}	5.58 ± 0.01 ^{F,b}	5.59 ± 0.01 ^{F,b}	5.57 ± 0.02 ^{F,b}	5.59 ± 0.00 ^{F,b}
2	5.98 ± 0.01 ^{D,a}	5.65 ± 0.02 ^{E,cd}	5.64 ± 0.02 ^{E,d}	5.68 ± 0.01 ^{E,b}	5.68 ± 0.02 ^{E,bc}
4	6.14 ± 0.02 ^{C,a}	5.78 ± 0.00 ^{D,d}	5.70 ± 0.02 ^{D,e}	5.84 ± 0.01 ^{D,b}	5.81 ± 0.01 ^{D,c}
6	6.28 ± 0.02 ^{B,a}	5.90 ± 0.01 ^{C,c}	5.82 ± 0.01 ^{C,d}	5.98 ± 0.00 ^{C,b}	5.88 ± 0.01 ^{C,c}
8	6.39 ± 0.01 ^{A,a}	5.96 ± 0.01 ^{B,c}	5.89 ± 0.02 ^{B,d}	6.07 ± 0.01 ^{B,b}	5.95 ± 0.03 ^{B,c}
10	6.42 ± 0.03 ^{A,a}	6.04 ± 0.01 ^{A,c}	5.94 ± 0.00 ^{A,d}	6.12 ± 0.02 ^{A,b}	6.03 ± 0.02 ^{A,c}
	TBA index (mg MDA/kg)				
0	0.37 ± 0.03 ^{F,a}	0.35 ± 0.02 ^{F,a}	0.37 ± 0.01 ^{F,a}	0.36 ± 0.01 ^{F,a}	0.36 ± 0.03 ^{F,a}
2	0.71 ± 0.02 ^{E,a}	0.42 ± 0.01 ^{E,c}	0.43 ± 0.03 ^{E,bc}	0.43 ± 0.01 ^{E,c}	0.48 ± 0.02 ^{E,b}
4	0.89 ± 0.03 ^{D,a}	0.65 ± 0.02 ^{D,c}	0.61 ± 0.01 ^{D,d}	0.70 ± 0.01 ^{D,b}	0.69 ± 0.01 ^{D,b}
6	1.14 ± 0.01 ^{C,a}	0.78 ± 0.02 ^{C,c}	0.73 ± 0.02 ^{C,d}	0.87 ± 0.03 ^{C,b}	0.79 ± 0.01 ^{C,c}
8	1.43 ± 0.01 ^{B,a}	0.90 ± 0.01 ^{B,d}	0.87 ± 0.01 ^{B,e}	1.02 ± 0.02 ^{B,b}	0.95 ± 0.03 ^{B,c}
10	1.95 ± 0.02 ^{A,a}	1.10 ± 0.03 ^{A,c}	1.01 ± 0.03 ^{A,d}	1.24 ± 0.02 ^{A,b}	1.07 ± 0.01 ^{A,c}

Values represent mean (n=3) ± SD. Small and big different letters indicate significant difference among samples and storage period at 5% level of probability, respectively. OEO: *Oliveira decumbens* Vent. essential oil; NOEO: *Oliveira decumbens* Vent. essential oil-loaded nanocapsules; BEO: Basil essential oil; NBEO: Basil essential oil-loaded nanocapsules

Figures

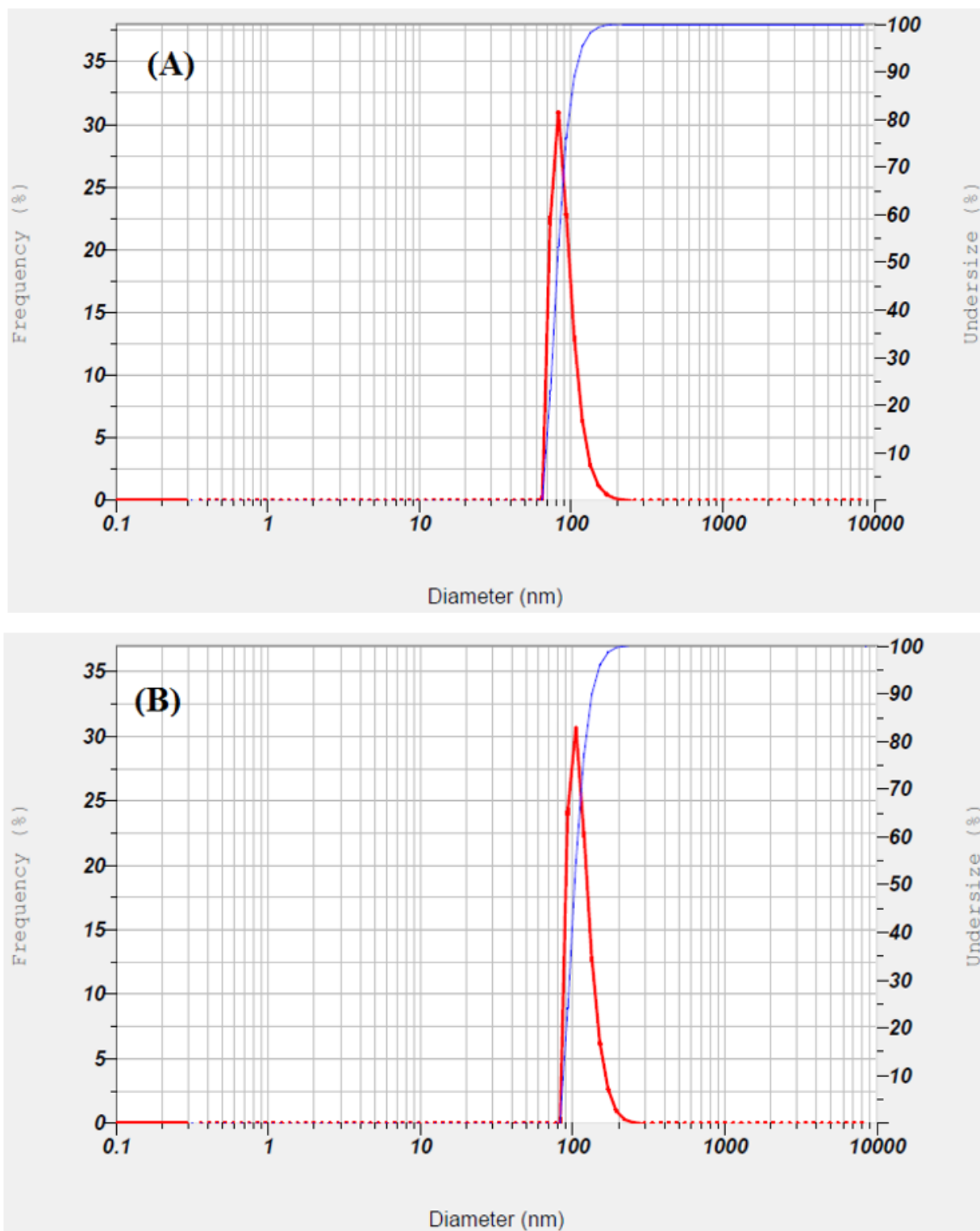


Figure 1

Particle size of (A) OEO-loaded nanocapsules, and (B) BEO-loaded nanocapsules

*OEO: *Oliveira decumbens* Vent. essential oil; BEO: basil essential oil

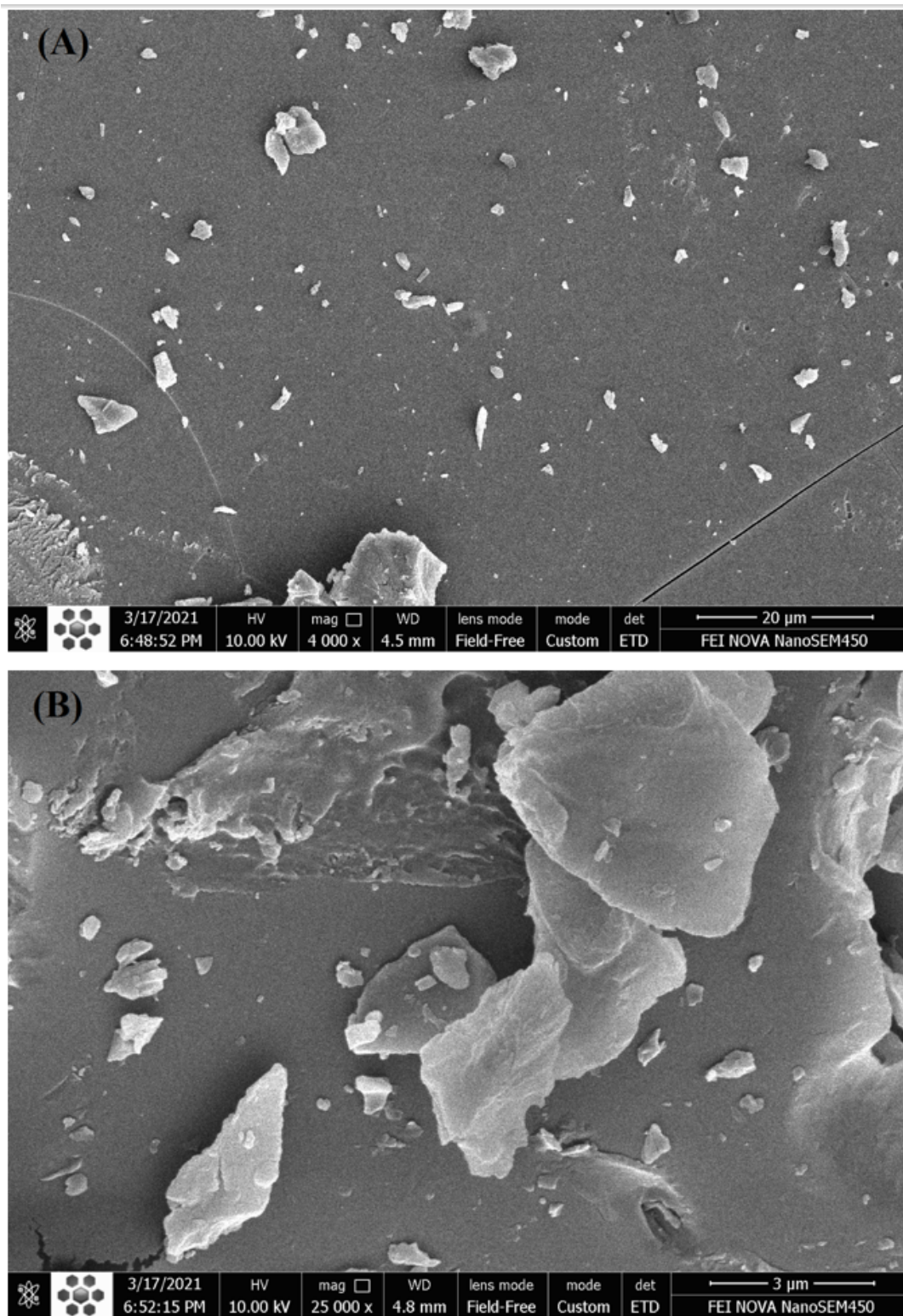


Figure 2

SEM image of (A) OEO-loaded nanocapsules, and (B) BEO-loaded nanocapsules

*OEO: *Oliveira decumbens* Vent. essential oil; NBEO: basil essential oil

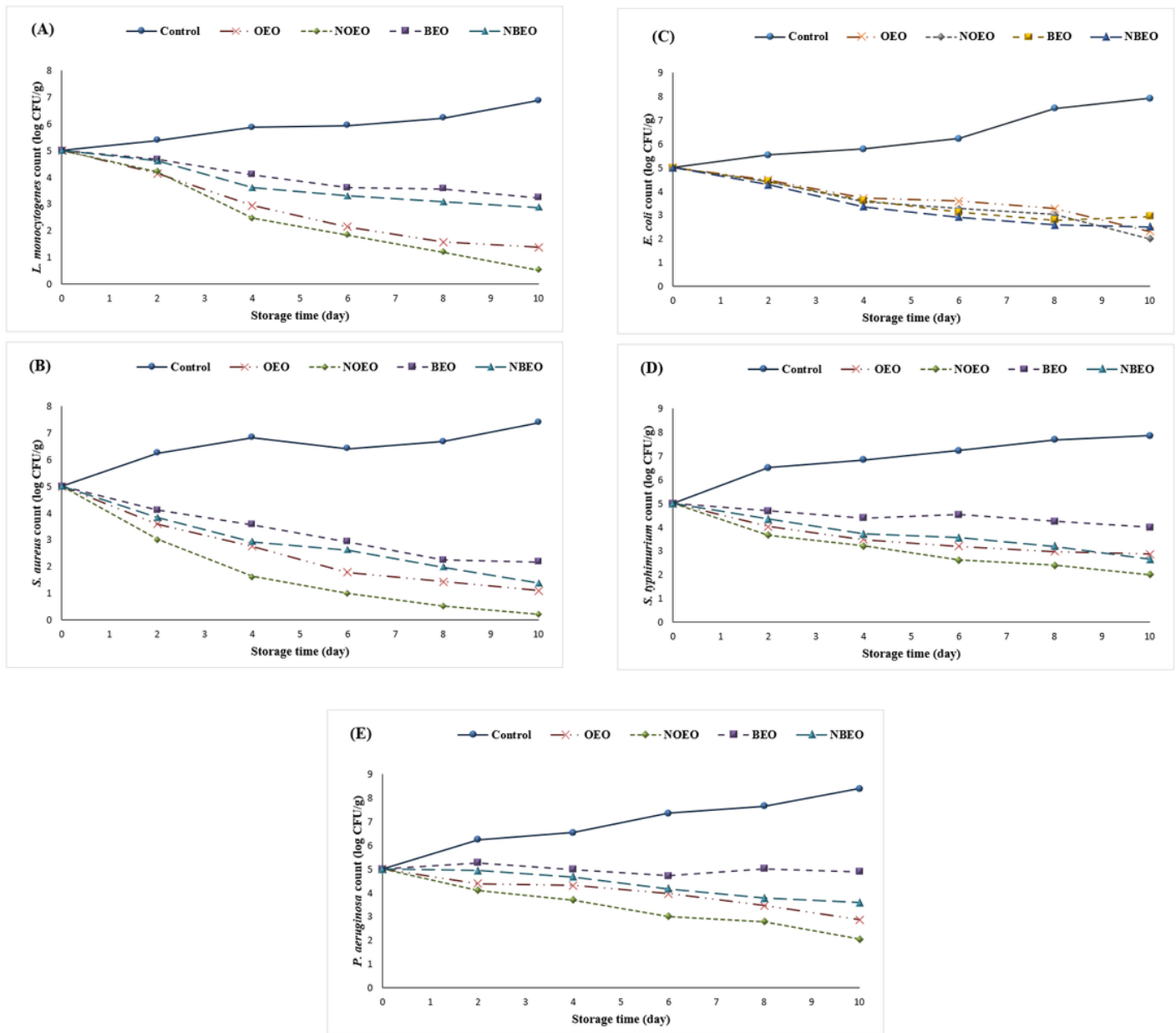


Figure 3

Changes in (A) *L. monocytogenes*, (B) *S. aureus*, (C) *E. coli* O157:H7, (D) *S. typhimurium*, and (E) *P. aeruginosa* counts (Log CFU/g) in minced beef meat during the 12-day cold storage period

* OEO: *Oliveira decumbens* Vent. essential oil; NOEO: *Oliveira decumbens* Vent. essential oil-loaded nanocapsules; BEO: Basil essential oil; NBEO: Basil essential oil-loaded nanocapsules

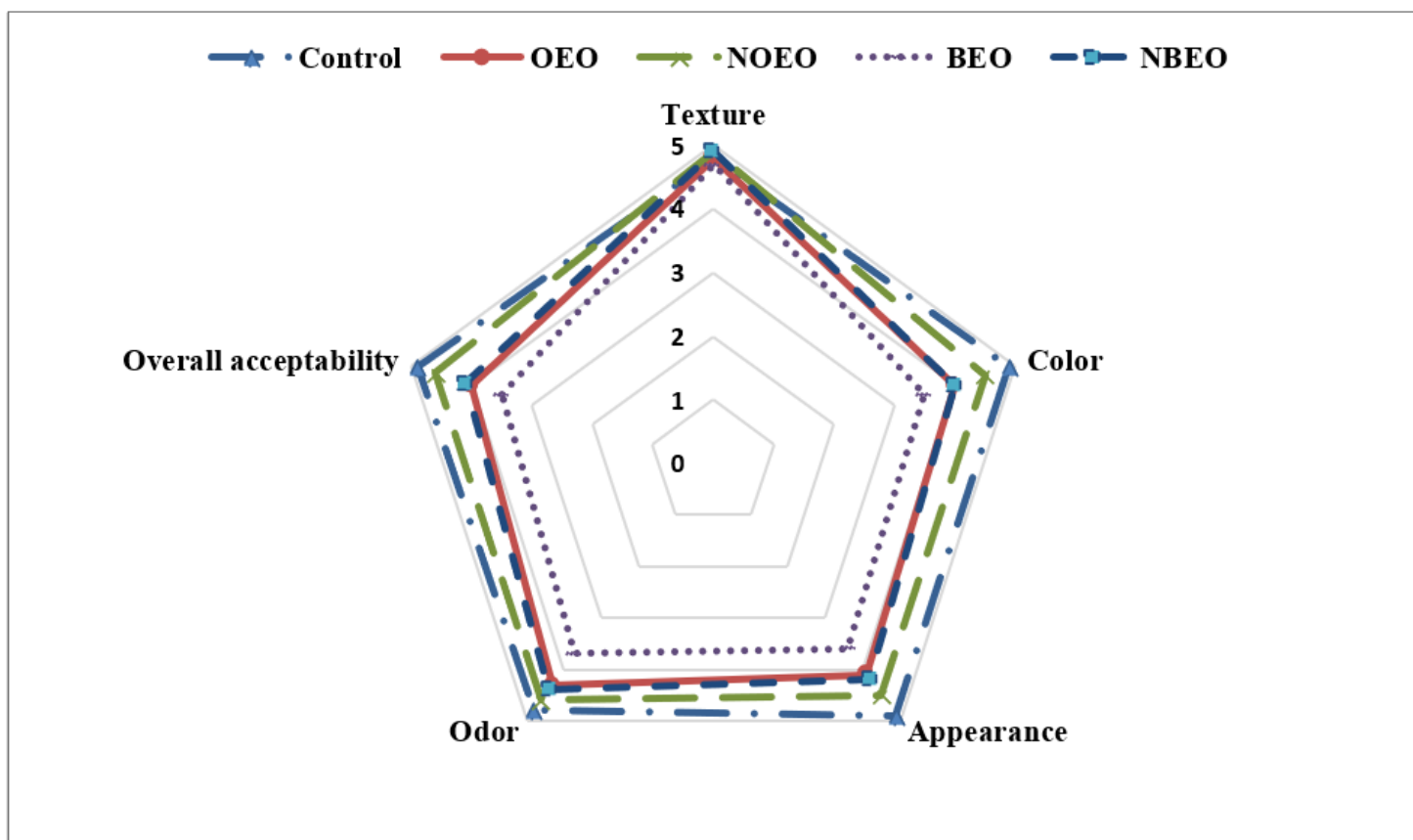


Figure 4

Sensory results of minced beef meat samples

* OEO: *Oliveira decumbens* Vent. essential oil; NOEO: *Oliveira decumbens* Vent. essential oil-loaded nanocapsules; BEO: Basil essential oil; NBEO: Basil essential oil-loaded nanocapsules

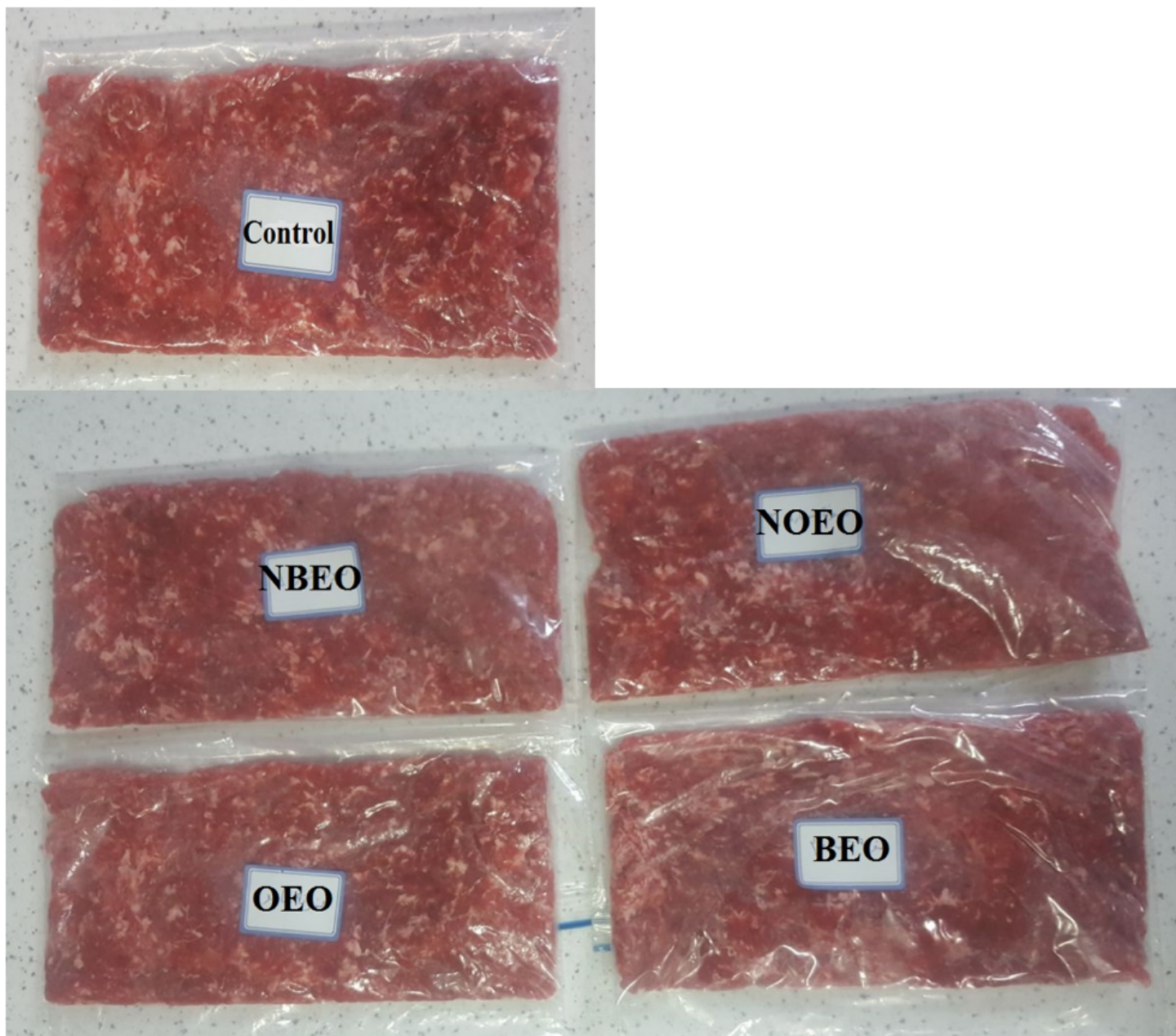


Figure 5

The minced beef meat samples

* OEO: *Oliveira decumbens* Vent. essential oil; NOEO: *Oliveira decumbens* Vent. essential oil-loaded nanocapsules; BEO: Basil essential oil; NBEO: Basil essential oil-loaded nanocapsules