

Development of antimicrobial bioaerogel based on Zataria multiflora essential oil to extending ground meat shelf life

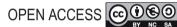
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Academic Editor: Mohammad Hashem Yousefi, PhD., Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

Received: 9 September 2024; Accepted: 28 December 2024; Published: 16 January 2025 © 2025 Codon Publications



ORIGINAL ARTICLE

Abstract

Active packaging has become increasingly popular in recent years due to its ability to prolong the shelf life of food. This study used a novel bioaerogel composite consisting of Salep and cellulose derived from grape stalk waste as a carrier for loading and then gradually releasing essential oil during storage in active meat packaging. Then the characteristics of two types of aerogels including morphology, density, porosity, water vapor absorption capacity, and viscosity of their precursor hydrogel were investigated. A total of 99.5% of these compounds were identified in Zataria multiflora essential oil (ZMEO). X-ray diffraction and thermogravimetric analysis of cellulose and cellulose-Salep aerogels showed the crystalline structure of cellulose aerogel, with a thermal decomposition temperature of 350 °C. X-ray diffraction analysis of cellulose revealed three prominent peaks at 16°, 22°, and 34°, corresponding to the (110), (200), and (004) crystal planes, respectively. Then, different amounts of ZMEO were loaded on aerogels, and the minimum inhibitory dose (MID) of ZMEO in vapor phase on meat microbiota and Pseudomonas aeruginosa was reported as 256 ($\mu L/L_{headspace}$). Application of cellulose and cellulose-Salep aerogels with $10 \times MID$ ZMEO per liter headspace of meat package reduced 1.8 and 1.4 \log_{10} CFU/g of total mesophilic bacteria, 2 and 1.6 log₁₀ CFU/g of total psychrotrophic bacteria, and 1.4 and 0.8 log₁₀ CFU/g of total coliform during the 9-day storage in the refrigerator compared to the control samples. Low-value byproducts from renewable resources can be used to produce bioaerogels for packaging applications. These bioaerogels offer cost-effective solution for antimicrobial and antioxidant food packaging.

Keywords: antimicrobial emitting; cellulose-aerogel; minced beef; vapor phase essential oil; Zataria multiflora

Introduction

Active packaging is packaging modified to include additional features designed to improve food safety and quality. (Ghosh *et al.*, 2024). The most common types of active packaging include oxygen absorbers, carbon dioxide absorbers or emitters, taste and odor absorbers

or emitters, humidity modifiers, and packaging containing antioxidants, and antimicrobial compounds (Roopa et al., 2023). Antimicrobials, often volatiles, are added to carriers, such as sachets, pouches, or pads, within the package without touching the food. This mechanism relies on the evaporation of antimicrobials into the packaging headspace (Otoni et al., 2016). The carrier is

typically affixed inside the package or positioned in a distinct area, such as a compartmentalized package, to prevent direct contact with food. This concept has been effectively researched for different food products (Colín-Chávez *et al.*, 2024; Matan *et al.*, 2023; Shafiei *et al.*, 2024; Sohrabi *et al.*, 2024).

Agro-waste materials such as cellulose, hemicellulose, chitin, lignin, starch, pectin, alginate, and protein can possibly be transformed into food packaging. By offering benefits such as being nontoxic, easily degradable, widely accessible, and compatible with other materials, it can enhance the quality of packaged items and prolong their shelf life (Varghese et al., 2023). It is crucial in the food industry to develop eco-friendly and effective food packaging systems and new options that are sustainable and offer acceptable performance. Recently, there has been a strong focus on developing innovative methods for fabricating food packaging materials that can effectively absorb both oil and water, exhibit thermal stability, and release active ingredients into food products in a controlled manner (Almasi et al., 2021). Aerogels are promising candidates for this purpose (Selvasekaran and Chidambaram, 2022). The primary standout characteristic of aerogels for food packaging is their porous structure, which results in lightweight and large specific surface area. These properties offer significant opportunities for mechanical protection, thermal insulation, and active packaging materials that can absorb or emit certain compounds (Sen et al., 2022). These cutting-edge materials feature a threedimensional framework filled with air, offering several notable benefits such as an extremely lightweight, high porosity, and an exceptionally large surface area (Sohrabi et al., 2024). Aerogels are primarily produced using synthetic materials; however, recent research has increasingly shifted toward bioaerogels derived from renewable resources such as whey proteins (De Berardinis et al., 2024), gelatin/gum Arabic (Li et al., 2024), gelatin (Wang et al., 2024), wheat starch (da Silva et al., 2020), and chitosan (Sohrabi et al., 2024). Different sources of cellulose have been used to produce bioaerogels for food-active and intelligent applications (Ciuffarin et al., 2023; Duong et al., 2023; Liebner et al., 2012; Mirmoeini et al., 2023a).

Essential oils (EOs) play a crucial role in the controlled release of food packaging materials and offer numerous benefits for food preservation and safety (Zubair *et al.*, 2022). These natural compounds possess potent antimicrobial, antioxidant, and antifungal properties, making them excellent alternatives to synthetic additives in food packaging materials (Sharafi *et al.*, 2023; Wani *et al.*, 2022). Although EOs are highly effective antimicrobial agents, their volatility, low water solubility, and

susceptibility to oxidation can limit their direct use in packaging (Rout *et al.*, 2022). To overcome these challenges, controlled-release materials can be developed to improve the stability and controlled-release of EOs in food packaging applications. Bioaerogels located in the packaging headspace can encapsulate EOs and control their release. Using volatile antimicrobials eliminates the chances of food directly touching the packaging material, resulting in minimal changes to its sensory properties. Furthermore, the antimicrobials in volatile form can permeate nearly all types of food matrices found within the packaging system.

In our recently published work (Mirmoeini et al., 2023a), we developed a porous intelligent bioaerogel using cellulose and Salep to monitor meat freshness. The results demonstrated that starch concentration is a critical factor in the porosity of bioaerogels. Because a dense matrix is appropriate for the controlled release of EOs, in the current study, a new dense bioaerogel using cellulose and Salep with a different formulation was fabricated for the controlled release of Zataria multiflora essential oil (ZMEO). The developed material was thoroughly characterized, and its antimicrobial activity against Pseudomonas aeruginosa and meat microbiota containing native meat microbiota, as well as its impact on the shelf life of meat, was investigated. ZMEO was selected because of its significant antimicrobial activity against a wide range of microbial strains (Sajed et al., 2013), which makes it a valuable natural antimicrobial for food applications.

Materials and Methods

Extraction and analysis of EO

Zataria multiflora plants were purchased from a local market in Urmia, Iran. ZMEO was extracted from dried leaves using a Clevenger. The collected TDEO was stored in a dark glass bottle at 4 °C until use. The chemical composition of ZMEO was analyzed using Agilent GC-MS, GC-7890B/ mass detector MS-5975C (Agilent Technologies, Santa Clara, CA, USA). The HP-5MS column, with a diameter of 0.25 mm and a thickness of 0.25 μ m, was used, with high pure helium (99.999%) serving as the carrier gas at a linear flow rate of 1 mL/min. The split injection was done at a ratio of 500:1 and fragmentation was conducted with a 70 eV electron energy. The initial temperature of the oven was set to 80 °C and maintained for 3 min before gradually increasing to 180 °C at a rate of 8 °C/min, where it was kept for 15 min. HP Chemstation was in charge of controlling the instrument, while the processing of data and labeling of compounds were determined using the in-house library Wiley 2007 and the NIST 2005 database.

Bioaerogels fabrication

Cellulose was extracted from grape stalks using the method described by Mirmoeini et al. (2023a). To prepare the cellulose aerogel, cellulose powder was dissolved in water (1.5% w/v) and homogenized at 17,500 rpm for 15 min to prepare a consistent colloidal solution. Separately, Salep powder was dissolved in water to prepare a (3% w/v) solution. A composite aerogel was prepared by mixing equal parts of cellulose and Salep solution (1:1). The cellulose and cellulose-Salep aerogels were then freeze-dried at -45 °C) and lyophilized under vacuum pressure for 48 h. Aerogels were prepared with different concentrations of cellulose and Salep and different mixing ratios, and the porosity, density, and pore diameter distribution were calculated for each (Mirmoeini et al., 2023a). To address the need for a denser aerogel in active packaging, it was hypothesized that such a structure would allow the essential oil to be released gradually throughout food storage. Based on the findings from experiments reported in two previous studies on this topic, the specified concentrations were selected for aerogel preparation.

Antimicrobial bioaerogels fabrication and minimum inhibitory dose (MID) determination

To determine the optimal amount of ZMEO for antimicrobial bioaerogels, a series of experiments were conducted. Different concentrations of ZMEOs (4, 8, 16, 32, and 64 $\mu L/L_{headspace}$) were added to the aerogels (2 cm in diameter) and the minimum inhibitory dose (MID) of the ZMEO vapor was determined. P. aeruginosa (ATCC27853) suspension (108 CFU/mL) was prepared using a spectrophotometer. In this way, a 0.5 McFarland concentration was made from the bacteria and its OD was set between 0.8-1.0 at a wavelength of 600 nm and cultured on Mueller-Hinton agar (Ibresco, Karaj, Iran) plates. To assess the effect of bioaerogels on the meat microbiota, minced beef (3 g) was mixed with peptone water (1% w/v), centrifuged (3 min at $3000 \times g$), and aliquots (100 µL of the meat suspension) were cultured on agar plates. Aerogels were placed inside petri dish lids, and bacterial lawns were prepared on agar. The plates were sealed, and EO-free aerogels were used as controls. The plates were incubated at 36 °C for 48 h in ambient humidity (Mirmoeini et al., 2023a). The MID represents the threshold concentration of TDEO necessary to achieve 99.9% bacterial inhibition. The ability of ZMEO to restrict bacterial growth was quantified as a percentage of inhibition. This was accomplished by dividing the diameter of the inhibition zone by the diameter of the petri dish. To calculate MID in meat extract, colonies were counted, and the concentration that caused 99.9% reduction was considered as MID.

Bioaerogels characterization

Viscosity

The viscosity of a combination of cellulose (1.5%), Salep (3%) + cellulose (1.5%) in 250 mL of solution was measured at 25 °C using a viscometer (Brookfield DV-II, Middleborough, USA) with a 62 cylindrical spindle at a shear rate of 30 rpm (Kuntzler *et al.*, 2020).

Water vapor sorption capacity (WVSC), density, and porosity WVSC was determined according to the method of Mirmoeini *et al.* (2023b), while density and porosity were assessed based on Fontes-Candia *et al.* (2019) methodology

Scanning electron microscopy (SEM)

The structures of the aerogels (with and without ZMEO) were examined using a scanning electron microscope (S360-Cambridge-1990) at 20 kV acceleration and 500x magnification. To capture images, the samples were coated with gold using the Fison sputtering technique.

Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD)

An FTIR spectrometer (Thermo Nicolet, Nexus* 670, USA) within the range of 400–4000 cm⁻¹ and a Shimadzu-6000 diffractometer (Tokyo, Japan) were used to investigate functional groups and X-ray diffraction patterns of developed materials (Mirmoeini *et al.*, 2023a).

Thermogravimetric analysis (TGA)

TGA was used to assess the thermal characteristics of the aerogels fabricated with and without ZMEOs. Each sample (6 mg) was weighed and placed into a standard cup. The samples were heated from 22 to 600 °C at a rate of 10 °C/min. Weight loss occurred in an argon gas atmosphere (Zia *et al.*, 2021).

Meat application of antimicrobial bioaerogel

Forty grams of minced meat was placed in a polyethylene container (6.5 \times 5.5 \times 3.5 cm), and a bioaerogel was attached to the lid. Different types of aerogels, including ZMEO-free and ZMEO-loaded aerogels, were used at 1 \times MID and 10 \times MID concentrations. To prevent EO leakage, the containers were sealed and stored at 7 °C for 18 days. The samples were analyzed every 3 days.

Microbial analysis

The populations of mesophilic, psychrotrophic, and coliform microorganisms in meat samples were determined by diluting 10 g of sample in 90 mL of 0.1% peptone water (Quelab, Montreal, Canada), homogenizing using a stomacher (Stomacher, Seward, London, UK),

and preparing decimal dilutions. Cultured plate count agar (Ibresco) plates were incubated at 7 °C for 7 days for psychrotrophic and at 36 °C for 48 h for mesophilic microbial enumeration. Violet red bile agar (Ibresco) was used to count coliforms (at 36 °C after 48 h of incubation) (Ghorbani *et al.*, 2024).

Chemical analysis

Determination of pH, total volatile nitrogen (TVN), and thiobarbituric acid (TBA)

To determine pH, 5 g of the meat sample was mixed thoroughly with 45 mg of 0.1% peptone water, and the pH was assessed using a digital pH meter (Metrohm Herisau, Switzerland). The volatile nitrogenous bases were measured according to the method described by Ezati et al. (2020). To assess lipid oxidation in meat samples, 10 g of meat sample was blended with 4% perchloric acid (Merck, Darmstadt, Germany) using Ultra-Turrax (IKA, Ultra-Turrax, T25 basic, Deutschland, Germany). The homogenate was passed through a Whatman No. 54 filter paper. The filtrate was made up to a volume of 50 mL using 4% perchloric acid. This method relies on the reaction between one malondialdehyde (MA) molecule and two TBA molecules to produce a red MA-TBA complex, which can be analyzed using spectrophotometry at 532 nm. The results were reported in mg MA/kg of the sample (Ghorbani et al., 2024).

Sensory evaluation

Sensory evaluation was conducted by a panel of 10 semitrained food hygiene students and researchers (chosen among food science researchers and students; age range 25–35 years) at Urmia University with a foundational understanding of meat quality, who participated in a training program before the evaluation. Every sample was assigned a random code, and rectangular sections were sliced from the middle portion of the samples for sensory assessment. Meats were evaluated based on the color, odor, and overall acceptability using a 9-point hedonic scale, ranging from 1 (extremely disliked) to 9 (extremely liked).

Statistical analysis

Each test was conducted in triplicate. The data were analyzed using GraphPad software (version 5.00 for Windows, GraphPad Software, San Diego, California, USA) with one-way ANOVA and Tukey's multiple comparison test at a significance level of P < 0.05. For shelf life estimation, storage durations were treated as fixed variables, while replication was included as a random factor within the model. In the sensory evaluation, panelists were assigned as the random factor, whereas sampling intervals were considered fixed effects.

Results and Discussions

ZMEO chemical composition

Different studies have revealed that the amount and chemical composition of EO can differ significantly based on factors such as geographical location, plant age, harvest time, season, processing techniques, drying method, storage conditions, and extraction method (Kashiri *et al.*, 2017). A total of 99.5% of these compounds were identified in ZMEO (Table 1). The main compounds detected were carvacrol (55.29%), thymol (19.63%), and γ -terpinene (3.61%). Carvacrol, the primary active compound found in ZMEO, restricts the growth of foodborne bacteria when released in vapor form. This suggests

Table 1. Chemical composition of Zataria multiflora essential oil.

No.	Compound	Percentage	Retention time (min)
1	alpha-Thujene	0.15%	11.20
2	alpha-Pinene	2.07%	11.59
3	Camphene	0.11%	12.48
4	beta-Pinene	0.18%	13.93
5	1-Octen-3-ol	0.12%	14.23
6	beta-Myrcene	1.50%	14.50
7	alpha-Phellandrene	0.17%	15.45
8	alpha-Terpinene	1.09%	15.98
9	Cymene	7.96%	16.48
10	Limonene	0.29%	16.64
11	Eucalyptol	0.40%	16.82
12	gamma-Terpinene	3.61%	18.18
13	3-Crene	0.16%	18.92
14	Terpinolene	0.08%	19.57
15	Linalool	0.67%	20.39
16	Borneol	0.11%	24.25
17	Terpinen-4-ol	0.46%	24.59
18	alpha-Terpineol	0.35%	25.39
19	Carvone, dihydro-	0.09%	25.60
20	Thymol, methyl ether	0.18%	26.85
21	Carvacrol, methyl ether	1.69%	27.26
22	Bornyl acetate	0.11%	29.45
23	Thymol	19.63%	30.02
24	Carvacrol	55.29%	30.47
25	Thymol acetate	0.29%	32.22
26	Carvacrol acetate	0.91%	33.08
27	Caryophyllene<(E)>	1.22%	35.33
28	Aromadendrene	0.20%	36.14
29	Viridiflorene	0.12%	38.34
30	Caryophyllene oxide	0.29%	42.18
31	Total	99.5%	_

its possible use as an antimicrobial agent emitted from packaging films into the headspace of food packages (Tao et al., 2022). Previous studies have shown that thymol possesses various bioactive properties, including antioxidant, antimicrobial, immunomodulatory, anticancer or antitumor, and antihypertensive effects. In addition to its bioactive properties, thymol is commonly used in various food products via vapor deposition, direct addition, and edible coatings (Zhou and Kong, 2023). The combination of thymol and carvacrol has been reported to have synergistic antimicrobial effects (Cid-Pérez et al., 2024).

Aerogel characterization

Viscosity, density, porosity, WVSC

Table 2 displays the viscosity of the precursor composition of the aerogels along with the density, porosity, and water vapor absorption capacity of the developed bioaerogels. In the realm of food applications, the structural, mechanical, and functional characteristics of aerogels are significantly influenced by their specific surface area, density, and porosity. Mirmoeini *et al.* (2023b) demonstrated that altering the starch concentration within hybrid aerogels effectively modifies their porosity. Their findings revealed a direct correlation between increased starch concentration and decreased porosity, resulting in denser aerogels.

To achieve a denser aerogel with reduced porosity and ensure controlled release of ZMEO, this study employed a 1:1 blend of cellulose and Salep at concentrations of 1.5s and 3% to fabricate the aerogel. Table 2 indicates that the 1.5% cellulose solution has a viscosity of 3899 mPas, compared to 17.2 mPas for the cellulose-salep aerogel. Surprisingly, the aerogel produced from the high-viscosity solution was less dense and more porous. However, the dried cellulose aerogel demonstrated a higher density and lower porosity, likely owing to the stabilization of the precursor solution, which inhibited shrinkage during freezing. Pantić et al. (2020) observed that incorporating chitosan into pectin aerogels led to an increase in porosity, which is consistent with the results of the current study. In a separate study, Xing et al. (2022) used supercritical drying to develop a starch-cellulose hybrid aerogel and found that by adjusting the cellulose concentration from 0.1 to 1%, the aerogel density could be modulated within a range of 2–12 mg/cm³. Given the hydrophilic properties of Salep, it was anticipated that introducing Salep into the cellulose aerogel would substantially enhance its water vapor absorption capacity. Owing to the hydrophilic nature of Salep, a significant increase in WVSC was observed upon the addition of salep to the cellulose aerogel (Table 2). This is consistent with the amorphous structure of the cellulose–Salep aerogel, as revealed by X-ray diffraction patterns, compared to the cellulose aerogel.

Morphology

SEM was used to investigate the morphological characteristics of the aerogels before and after the incorporation of ZMEO (Figure 1). Both categories of aerogels exhibited porous structures that were well-suited for ZMEO encapsulation. Introducing Salep to the cellulose aerogel resulted in a more uniform and consistent structure, whereas the addition of ZMEO to the aerogels had a negligible impact on the porous network or structural integrity. A previous study revealed minor structural alterations when liposomes were incorporated into alginate aerogels (Trucillo *et al.*, 2020). In contrast, Mirmoeini, *et al.* (2023b) observed no significant changes in the structure of a starch–cellulose aerogel upon the introduction of EO.

XRD

X-ray diffraction analysis of cellulose revealed three prominent peaks at 16°, 22°, and 34°, corresponding to the (110), (200), and (004) crystal planes, respectively (Figure 2). The XRD pattern of the cellulose aerogel exhibited three primary peaks. These findings suggest that the crystalline structure of cellulose remains intact throughout homogenization and lyophilization processes. In contrast, the cellulose-Salep aerogel displayed a broad peak at 2θ angles ranging from approximately 10-30°, which indicated an amorphous phase. The absence of distinct cellulose peaks, in this case, can be attributed to the masking effect of Salep or the formation of novel chemical bonds within the cellulose aerogel. Zhang et al. (2022) previously reported a similar phenomenon in cellulose nanofibers and carboxymethyl cellulose aerogels, in which specific cellulose peaks were not observed. Subtle modifications occurred upon

Table 2. Viscosity of polymeric paste with density, porosity, and water vapor sorption capacity (WVSC) of fabricated aerogels.

Sample	Density (mg/ cm³)	Porosity (%)	Viscosity (mPa.s)	WVSC (g/g aerogel)
Cellulose	48.7 ± 0.9 ^a	34.7 ± 1.1 ^a	17.2 ± 1.4 ^a	0.08 ± 0.01 ^a
Cellulose–Salep	28.1 ± 1.4 ^b	62.7 ± 1.5 ^b	3899.0 ± 0.1 ^b	5.8 ± 0.1 ^b

Data are reported as mean of triplicate ± standard deviation (P < 0.05). Different letters in each column indicate the existence of a significant difference at the 5% level (P < 0.05).

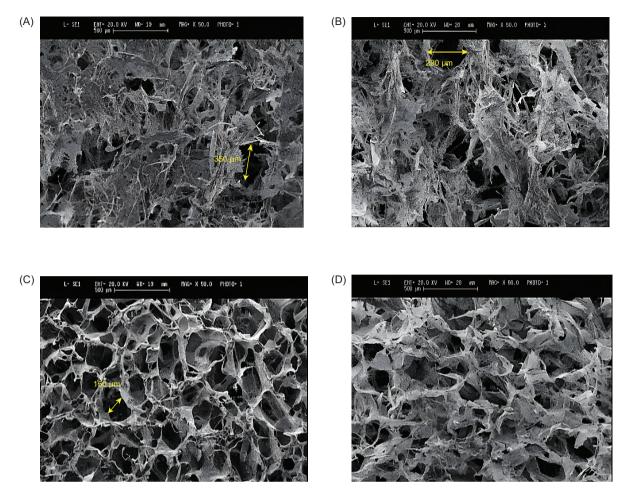


Figure 1. Scanning electron microscope (SEM) image of cellulose aerogel (A); cellulose aerogel loaded with *Zataria multiflora* essential oil (EO; B); cellulose-Salep aerogel (C); and cellulose-Salep aerogel loaded with EO (D).

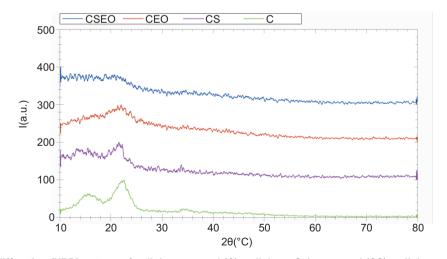


Figure 2. X-ray diffraction (XRD) pattern of cellulose aerogel (C), cellulose–Salep aerogel (CS), cellulose aerogel loaded with essential oil (CEO) and cellulose–Salep aerogel laded with EOs (CSEO).

incorporation of ZMEO into the aerogels, leading to a decrease in the intensity of the cellulose peaks. These changes are likely associated with variations in the polymer microstructure (Ullah *et al.*, 2021). Similarly, studies have shown that the addition of EO to starch foam results in a reduction in peak intensity. The direct interaction of EO with the starch polymer structure enhances the rigidity of the amorphous region relative to the crystalline region, consequently weakening intermolecular interactions. This reduction in starch chain mobility subsequently diminishes the mechanical strength of the foams (Cruz-Tirado *et al.*, 2020).

TGA

TGA of the cellulose aerogel and cellulose–Salep aerogel revealed two distinct stages of weight loss (Figure 3). A slight weight loss occurred between ambient temperature (25 °C) and 100 °C, primarily attributed to the evaporation of moisture within the samples. Thermal degradation or decomposition below 100 °C was deemed insignificant in the TGA study (Tuan Mohamood *et al.*, 2021). In the cellulose aerogel, thermal degradation occurred within the temperature range of 250–350 °C, whereas in the cellulose–Salep aerogel, it occurred between 250 and 300 °C. Approximately 25% of the residual ash in the cellulose–Salep aerogel was associated with the presence of mineral components in the Salep (Kurt and Kahyaoglu, 2017).

The weight loss profiles of the ZMEO-loaded aerogels differed markedly from those of cellulose aerogels. Three distinct stages of weight loss were observed for the ZMEO-containing aerogels. The initial stage, encompassing the evaporation and decomposition of EO, occurred between 100 and 200 °C. In the cellulose aerogels, the second stage of weight loss, which is related to the depolymerization and degradation of the cellulose backbone, occurred between 200 and 340 °C. In the cellulose-Salep aerogel containing ZMEO, the second stage of weight loss commenced at 200 °C, primarily owing to the extraction and subsequent decomposition of water from the Salep polysaccharide rings. The third stage of weight loss in the ZMEO-containing aerogels is attributed to the decomposition and destruction of organic matter and the remaining carbonaceous components of the EO, such as terpenoids (Volić et al., 2022). This final stage of weight loss was not observed in the thermograms of aerogels without ZMEO.

FTIR

Figure 4 presents the FTIR absorption spectra of ZMEO, cellulose aerogel, ZMEO-loaded cellulose aerogel, cellulose-Salep aerogel, and ZMEO-loaded cellulose-Salep aerogel. The primary objective of this analysis was to confirm the presence of ZMEO in the aerogels. The FTIR spectrum of ZMEO exhibited a prominent peak at 3424 cm⁻¹, indicative of the stretching vibrations of the

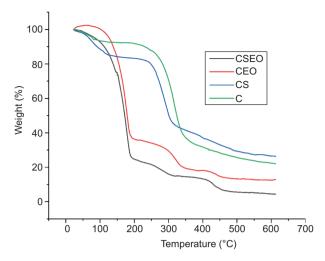


Figure 3. Thermogravimetric analysis (TGA) thermogram of cellulose aerogel (C), cellulose–Salep aerogel (CS), cellulose aerogel loaded with essential oil (CEO) and cellulose–Salep aerogel loaded with EOs (CSEO).

O-H bond in the hydroxyl group. This functional group is characteristic of alkyl groups or carboxylic acids commonly found in ZMEO. Additionally, asymmetric and symmetric stretching vibrations of methyl C-H bonds were observed at wavenumbers of 2961 cm⁻¹ and 2865 cm⁻¹, respectively. These wavenumbers are typically associated with alcoholic compounds present in ZMEO. Furthermore, the absorption peak at approximately 1700 cm⁻¹corresponds to the stretching vibrations of the carbonyl group (C=O), suggesting the presence of aldehydes (Moradinezhad et al., 2023). The absorption peak at 812 cm⁻¹ is attributed to the angular deformation of the CH2 group (Alipanah et al., 2022). In the FTIR spectrum of the cellulose aerogel, the broad peak at 3420 cm⁻¹ was associated with the stretching vibrations of the hydroxyl group. This functional group, derived from the polysaccharide structure of cellulose, plays a crucial role in stabilizing antimicrobial agents within the cellulose aerogel structure through mechanisms such as surface adsorption (electrostatic bonding) or chemical bond formation (Ghorbani et al., 2022).

Antimicrobial properties of ZMEO vapor phase loaded on aerogels

The antimicrobial efficacy of bioaerogels, both with and without ZMEO, against *P. aeruginosa* (Figure 5) and meat extract (Figure 6) was evaluated. This study aimed to identify the appropriate ZMEO loading on bioaerogels for food applications. The MID required to achieve a 99.9% reduction in meat extract microorganisms. Mukurumbira *et al.* (2023) investigated the antimicrobial activity of Tasmanian mountain pepper (*Tasmannia lanceolata*), lemon myrtle (*Backhousia citriodora*), and common herb thyme (*Thymus vulgaris*) EOs in the vapor

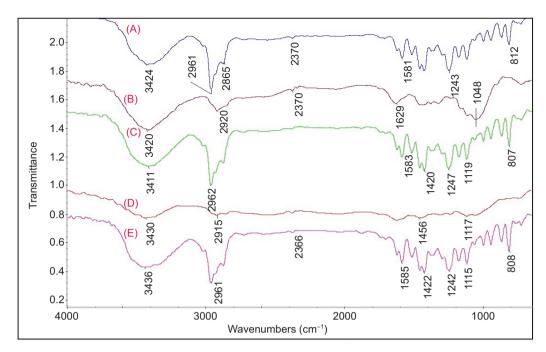


Figure 4. Fourier transform infrared spectroscopy (FTIR) spectra of *Zataria multiflora* essential oil (ZMEO) (A); cellulose aerogel (B); cellulose aerogel loaded with ZMEO (C), cellulose—Salep aerogel (D) cellulose—Salep aerogel loaded with ZMEO (E).

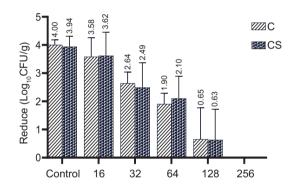


Figure 5. Effects of different concentrations of *Zataria multiflora* essential oil in the vapor phase loaded onto cellulose aerogel (C) and cellulose–Salep aerogel (CS) on the microbial load of meat extracts.

phase against *E. coli* and *P. aeruginosa*. While thyme EO exhibited a 25 mm zone of inhibition against *E. coli*, none of the three EOs demonstrated any inhibitory effect on *P. aeruginosa*. López *et al.* (2007) reported varying inhibition rates for *E. coli* when exposed to thyme, cinnamon, and mint EOs, ranging from 40 to 68% at a concentration of 175 μ L/L headspace. Furthermore, Wu *et al.* (2019) determined the MIDs for various EOs against *E. coli*, including 200 μ L/L for tea tree oil, 500 μ L/L for cassia, marjoram, rosemary, eucalyptus, and star anise, 1000 μ L/L for clove EO, 1500 μ L/L for *Mentha arvensis* and *Litsea cubeba* EO, and 2500 μ L/L for clary sage EO. It is important to note that there is currently no standardized method for assessing the antimicrobial effects

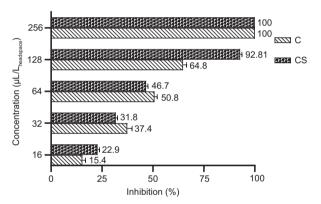


Figure 6. Percentage inhibition of *Pseudomonas aeruginosa* by cellulose (C) and cellulose–saline aerogel (CS) with different concentrations of *Zataria multiflora* essential oil.

of EOs in the vapor phase. Laboratory studies have suggested that the antimicrobial activity of EO vapors may be attributed to either direct absorption of volatile compounds onto microorganisms or indirect diffusion of the vapors into the microorganisms after adsorption in the surrounding environment (Mukurumbira *et al.*, 2023).

Food application

EOs are commonly used as antimicrobials in food packaging and preservation owing to their antimicrobial and antioxidant properties. However, their use has several disadvantages. One of the primary drawbacks is the potential

impact on sensory properties of food. The strong aroma of EOs can alter the taste and smell of packaged foods, potentially affecting their consumer acceptance (Ju et al., 2019). An effective method, as followed in this study, involves the incorporation of EOs into packaging systems rather than directly into food products. This approach allows for the gradual release of EOs while maintaining their preservative effects without significantly altering the taste and aroma of food. In this study, starch and starch-Salep bioaerogels were fabricated and treated with various concentrations of ZMEO. The results demonstrated that a MID of 256 µg/L headspace was effective against the natural microbiota of the meat. Subsequently, bioaerogels incorporating this concentration (1 × MID and 10 × MID) were developed and applied to the headspace of meat packaging to investigate their effects on various microbial, chemical, and sensory properties of the meat.

Microbial analysis

The growth patterns of mesophilic, psychrophilic, and coliform bacteria in the control and treated samples were monitored over a 9-day storage period at 7°C (Figure 7). As observed, the total mesophile count (TMC) increased from 1.4 log₁₀ CFU/g on Day 0 to 9.8 log₁₀ CFU/g on Day 9 of storage. Although the growth trend was also upward in the treated samples, it was slower than that in the control sample, and this difference was statistically significant (P > 0.05). Specifically, on the ninth day of storage, the counts in packages containing cellulose aerogel and cellulose-Salep aerogel loaded with 1 × MID concentrations reached 9.7 and 8.04 log₁₀ CFU/g, respectively, although the difference between these two treatments was not statistically significant (P < 0.05). In packages containing cellulose aerogel and cellulose-Salep aerogel loaded with 10 × MID concentrations, the counts reached 7.03 and 7.5 log₁₀ CFU/g, respectively. Because the permissible limit for mesophilic microorganisms in meat is 7 log₁₀ CFU/g (Ghorbani et al., 2024), the control sample was considered spoiled with a count of 7 log₁₀ CFU/g on the sixth day of storage. In contrast, none of the treated samples exceeded the permissible limit for TMC on the sixth day of storage, and the maximum reduction in the number of TMC compared to the control samples was observed in the cellulose aerogel loaded with a 10 × MID concentration of ZMEO. In a similar study (Zhang et al., 2020), authors used two types of cellulose nanofiber foams (enzymatically hydrolyzed and TEMPO-pretreated) hybridized with thyme nanoemulsion to investigate the release of EO and shelf life of beef steak. In this study, when TMC exceeded 7 log₁₀ CFU/g on the 15th day of meat storage in the control sample, the TMC in the samples packaged with cellulose nanofiber foams containing 0.3 and 0.6% EO did not exceed the permissible limit.

Studies have shown that total psychrophilic counts (TPC) in ground beef stored under refrigeration reach

the maximum permissible limit (7 \log_{10} CFU/g) after 5–7 days (Nurul Syahida *et al.*, 2021). As shown in Figure 7, on Day 9 of storage, the TPC in the control sample and packages containing cellulose aerogel and cellulose–Salep aerogel loaded with 1 × MID concentrations was 7.63, 6.93, and 7 \log_{10} CFU/g, respectively, rendering them unsuitable for consumption. However, on the ninth day of storage, there was a reduction of 2 and 1.6 log cycles in TPC in treatments containing cellulose aerogel and cellulose–Salep aerogel loaded with 10 × MID concentrations, respectively, compared with the control. Oral *et al.* (2009) used a pad impregnated with 1.5% thyme EO to package chicken meat. After 7 days of refrigerated storage, a reduction of over two log cycles in TPC compared to the control group was reported.

The initial total coliform count (TCC) on Day 0 was 2.79 log₁₀ CFU/g, which increased to 5.6 log₁₀ CFU/g in the control sample on Day 9 (Figure 7). In samples treated with cellulose aerogel and cellulose-Salep aerogel loaded with 10 × MID concentrations, there was a reduction of 1.55 and 0.96 log₁₀ CFU/g, respectively, compared to the control sample. As shown in the figures, on the third day of storage, a slight decrease in the number of mesophilic, psychrophilic, and coliform bacteria was observed in treatments containing cellulose aerogel and cellulose-Salep aerogel loaded with 10 × MID, likely due to the rapid release of ZMEO from the aerogels in the early days of storage. From the third day of storage, gradual growth of microorganisms occurred, but the rate was slower than that of the control owing to the presence of released ZMEO in the headspace of the packages. A comparative analysis of the release rates of ZMEO from the cellulose aerogel and cellulose-Salep aerogel showed a more effective release from the cellulose aerogel. Several factors, such as the aerogel embedded in food packaging, can influence the release rate of EOs from the pads. For example, it has been shown that EO release occurs at a much faster rate at higher temperatures than at lower temperatures. Additionally, in a study, increasing the amount of konjac glucomannan in the konjac glucomannanpolyacrylic acid-polyvinyl alcohol absorbent pad loaded with lemon EOs increased the water absorption capacity (Li et al., 2021). In agreement with the present study, the addition of Salep (rich in glucomannan) to the cellulose aerogel significantly increased its water vapor absorption capacity.

pH, TVN, and TBA

TVB-N and pH are standard indicators of meat freshness. These parameters are associated with the growth of spoilage microorganisms and subsequent breakdown of proteins. The pH values increased during refrigerated storage of the meat (Table 3), a trend consistent with the increase in microbial growth and production of volatile bases in raw meat. The initial pH of the ground beef

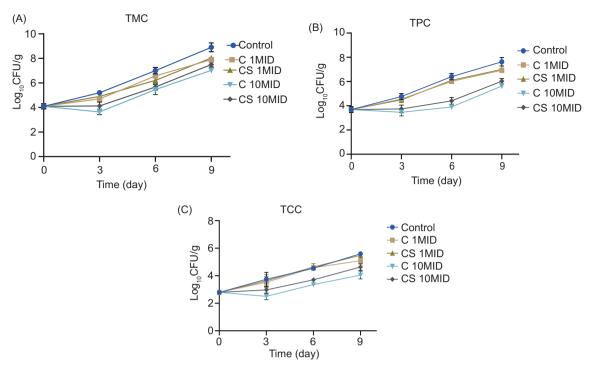


Figure 7. Impact of using cellulose and cellulose-Salep aerogels with different essential oil concentrations (1MID, 10 MID; MID stands for minimum inhibitory dose) on the total mesophilic count (TMC), total psychrotrophic count (TPC), and total coliform count (TCC) in minced meat during 9 days of refrigerated storage.

sample was 5.76 ± 0.05 . At the end of the storage period, the pH of the control sample had increased to 5.57 ± 0.07 . Statistically, the difference in pH between treatments containing cellulose aerogel and cellulose-Salep aerogel loaded with 10 × MID concentrations compared to the control sample was significant (P < 0.05). When TVB-N exceeds 15 mg/100 g and the pH rises above 6.3, meat enters a secondary stage of freshness (Zhang et al., 2020). The trend in TVN values (Table 3) was in accordance with the increase in pH. The TVN limit was reached on Day 6 for the control group and aerogels treated with 1 × MID concentration of ZMEO, while it was reached on Day 9 for samples treated with 10 × MID concentration of ZMEO. Table shows that Lipid oxidation (TBA values) was significantly lower in samples treated with cellulose aerogel and cellulose-Salep aerogel loaded with higher concentrations of essential oil (10 × MID) than in the control and those with lower concentrations (1 \times MID). This aligns with a previous study (Chen et al., 2018), which found that the antioxidant effect of the clove EO vapor phase on lipid oxidation in fish meat is concentration-dependent.

Sensory analysis

During storage, the overall acceptance of all sensory parameters (color, odor, and overall acceptability) decreased (Figure 8). The results of this study clearly showed an increase in odor acceptability in the treated samples but had less effect on the appearance of the samples. The slight effects on color are likely related to delayed spoilage of the treated samples and slower color changes in the meat during storage. In samples treated with aerogels containing ZMEO on the third day, the odor scores were almost similar; however, over time and with the progression of spoilage, the higher concentration of ZMEO received higher scores in terms of the sensory parameter of odor. This was likely related to a slower spoilage rate or the greater ability of higher ZMEO concentrations to mask the odor of spoilage in the samples. Overall, in treatments containing aerogels loaded with a higher concentration of ZMEO (10 × MID), a higher overall acceptability was found, and the differences between these two treatments were not significant. Because the sensory acceptance threshold was three (Liu et al., 2020), on the last day of storage, only the control sample had scores less than three for the odor and overall acceptability parameters. It is important to note that sensory evaluation is subjective and can be influenced by individual preferences and biases. Therefore, future studies could benefit from incorporating a larger panel of trained sensory evaluators to minimize variability and increase the reliability of the sensory data.

Despite the positive results obtained in this work, the large-scale production of cellulose–Salep aerogels faces several challenges. The cost of raw materials, including

Table 3. The effects of using cellulose and cellulose–Salep aerogels loaded with *Zataria multiflora* essential oil in different concentrations (μL/Lheadspace) on the chemical properties of minced meat during 9 days of storage at 7 °C.

	Day	Control	C 1MID	CS 1MID	CS 10MID	C 10MID
pН	0	5.76 ± 0.05 ^a	5.76 ± 0.05°	5.76 ± 0.05°	5.76 ± 0.05°	5.76 ± 0.05 ^a
	3	6.25 ± 0.07 ^a	6.2 ± 0.1 ^a	6.3 ± 0.0^{a}	6.25 ± 0.07 ^a	6.00 ± 0.07^{b}
	6	6.95 ± 0.07^a	6.85 ± 0.07^{a}	6.9 ± 0.1 ^a	6.55 ± 0.07 ^b	6.35 ± 0.07 ^b
	9	7.55 ± 0.07 ^a	7.5 ± 0.1 ^a	7.6 ± 0.0^{a}	6.95 ± 0.07 ^b	6.75 ± 0.07°
TVN (mg/100g)	0	8.6 ± 0.4^{a}	8.6 ± 0.4^{a}	8.6 ± 0.4a	8.6 ± 0.4a	8.6 ± 0.4^{a}
	3	12.6 ± 0.7 ^a	11.4 ± 0.4 ^{ac}	12.4 ± 0.4 ^a	10.3 ± 0.4bc	8.9 ± 0.4^{b}
	6	17.2 ± 0.4 ^a	16.3 ± 0.4 ^a	17.0 ± 0.4 ^a	12.1 ± 0.4 ^b	10.3 ± 0.4°
	9	27.3 ± 2.5^{a}	25.6 ± 1.1	25.8 ± 0.8 ^a	21.7 ± 0.7 ^b	18.9 ± 0.7 ^b
TBA (mg/100g)	0	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}
	3	0.59 ± 0.06 ^a	0.47 ± 0.01 ^a	0.56 ± 0.01 ^b	0.35 ± 0.02^{bc}	0.30 ± 0.01°
	6	0.70 ± 0.04^{a}	0.53 ± 0.02^{a}	0.63 ± 0.01 ^a	0.47 ± 0.03^{a}	0.41 ± 0.03 ^b
	9	1.32 ± 0.04^{a}	1.15 ± 0.04 ^a	1.28 ± 0.03 ^{ac}	1.00 ± 0.09bc	0.8 ± 0.1°

^{*}Different letters in each column indicate the existence of a significant difference at the 5% level (P < 0.05). MID, TVN, TBA, and MDA stand for minimum inhibitory dose, total volatile nitrogen, thiobarbituric acid, and malondialdehyde, respectively.

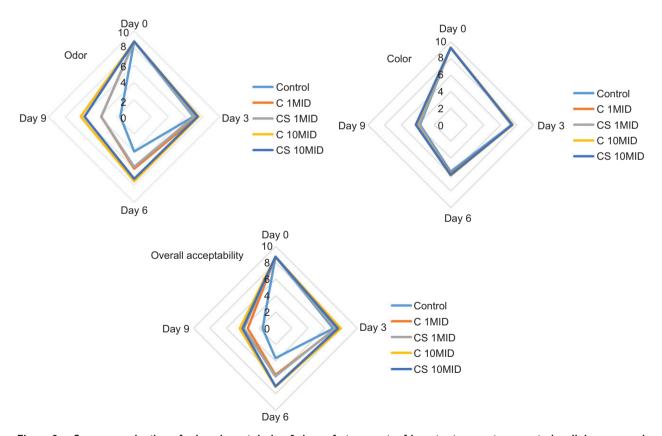


Figure 8. Sensory evaluation of minced meat during 9 days of storage at refrigerator temperature, control, cellulose aerogel containing 1 × MID concentration of essential oil (C 1MID), cellulose-Salep aerogel containing 1MID concentration of EOs (CS 1MID) and cellulose aerogel containing 10 × MID EOs (C 10MID), cellulose-Salep aerogel containing 10 × MID EOs (CS 10MID). MID stands for minimum inhibitory dose.

high-purity cellulose and Salep, could limit the economic feasibility of industrial-scale production. Furthermore, the manufacturing process, including the drying methods required to produce dense aerogels, might necessitate specialized equipment, leading to increased operational costs. From a storage stability point of view, over extended periods, the volatilization of ZMEO or its interaction with environmental factors such as humidity and temperature may reduce its antimicrobial activity. Additionally, the porous structure of the aerogels might be affected by prolonged exposure to varying storage conditions, potentially altering their ability to release ZMEO in a controlled manner. Future studies should address drawbacks for commercial use.

Conclusions

In this study, the grape stalk was used as a cellulose source, Salep as a copolymer to modify density and porosity, and ZMEO as an antimicrobial agent in antimicrobial bioaerogels. The resulting dense bioaerogels were used in the active packaging of meat as headspace packaging material to release ZMEO in a controlled manner. Cellulose and cellulose-Salep aerogels were effective on meat microbiota and *P. aeruginosa* with MID of 256 μL/L headspace. Aerogels loaded with 10 times the MID concentration of ZMEO in active packaging of ground beef effectively reduced the number of aerobic mesophilic, psychrophilic, and coliform bacteria. Results indicated a decrease in malondialdehyde levels from 0.27 to 1 and 0.8 mg/kg in meat packages containing cellulose and cellulose-Salep aerogel, respectively, after nine days of storage. Furthermore, the sensory evaluation revealed that treated samples received higher scores for odor and overall acceptability. The newly developed dense bioaerogels could serve as excellent carriers for volatile antimicrobials, making them ideal for packaging and preservation. The present study highlights the importance of conducting future research on the potential of bioaerogels for bio-based active packaging. Future studies should explore the role of active bioaerogels in extending the shelf life of frozen foods, the incorporation of antimicrobial agents into their structure, and their application as antimicrobial packaging for direct food contact. These findings cover the way for the industrial-scale production and implementation of these eco-friendly and effective bioaerogelbased active packaging systems in the food industry.

Authors' Contributions

Seyedeh Sahar Mirmoeini contributed to the investigation, formal analysis, and writing of the original draft. Hossein Tajik and Hadi Almasi were involved in supervision and visualization, while Mehran Moradi contributed to the conceptualization, methodology, formal analysis, and writing and review and editing of the manuscript.

Data Availability Statement

The data supporting the findings are available upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Funding

This work was funded by the Faculty of Veterinary Medicine, Urmia University, and the Iran National Science Foundation (INSF) under Project No. 4003314.

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