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Evaluation of antimicrobial activity and properties of gelatin nanofibers containing lavender essential oil

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ABSTRACT

Lavender essential oil has herbal uses in cosmetic, food and pharmaceutical industries. In this research, for the first time, the encapsulation of lavender essential oil by gelatin biopolymer was done by electrospinning method. At first, the chemical compounds of lavender essential oil were identified with the help of gas chromatography associated with a mass spectrometer (GC-MS) and then 0, 2.5, 0.5 and 0.10% v/v were added to the electrospinning solution. The prepared nanofibers have been evaluated using scanning electron imaging (SEM), thickness determination (Image J), X-ray diffraction, mechanical properties, and essential oil loading efficiency. Also, the antibacterial activity of nanofibers against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Salmonella typhimurium* were investigated by disk diffusion method. The most important components of lavender essential oil were linalool (35.21%) and linalyl acetate (26.47%). All nanofibers had a uniform and continuous shape. By increasing the concentration of essential oil in the electrospinning solution, the values of thickness (431.5 - 705.3 nm) and essential oil content (4.7-14.6%) increased. Lavender essential oil had no effect on crystallinity of nanofibers, but caused a significant decrease in Young's modulus and stiffness. The nanofiber sample containing 10% lavender essential oil recorded the highest hardness, Young's modulus and elasticity. Evaluation of antibacterial activities showed that nanofiber samples containing essential oils had appropriate antibacterial activities against all target bacteria. The effectiveness of gelatin nanofiber + lavender essential oil (10%) against *S. aureus* and *B. cereus*. According to the results of the research, it is possible to use gelatin nanofibers containing lavender essential oil for active food packaging. Expressing the definitive result requires conducting clinical and a real environment tests.

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1. Introduction

Pathogenic microbes with food origin play an important role in causing people to suffer from various infectious diseases and food poisoning. According to the statistics of the World Health Organization, about 420,000 people die from food-borne pathogenic microbes, which causes huge economic losses [1]. One of the most important food-borne pathogenic bacteria is*Staphylococcus aureus*، *Listeria monocytogenes*، *Bacillus cereus*، *Escherichia coli*، *Pseudomonas aerogenes* And*Salmonella typhimurium* pointed out [2]. Adding chemical preservatives is one of the most common ways to control food spoilage, which has caused increasing concerns due to its adverse effects on human health and the emergence of resistant strains. For this reason, nowadays natural preservatives are considered by food producers and consumers due to their durability, antimicrobial properties and few side effects [3]. Essential oils are compounds obtained from the secondary metabolites of plants and are usually used as flavoring in the preparation of various foods. Numerous studies have confirmed the antimicrobial, anti-parasitic, antioxidant and insecticidal effects of essential oils [4, 5]. Lavender is one of the medicinal plants belonging to the Laminaceae family, which is cultivated as an ornamental plant in many countries of European regions, North Africa, Southwest Asia, as well as India, Iran, and China [4]. Lavender essential oil is known for its pleasant aroma and is widely used in the perfume industry, cosmetics and as a flavoring. It has been reported that lavender essential oil has calming, anti-depressant, anti-inflammatory, antioxidant and antimicrobial effects against many fungal species and bacteria resistant to antibiotics [4, 6].

However, due to the effect of essential oils on the taste of food, their direct addition is limited. On the other hand, essential oils are hydrophobic in nature, which reduces their effectiveness in hydrophilic products. The electrospinning method is of interest due to its low production costs, ease, flexibility and ability to produce fibers with a thickness of 1000-1000 nm. This technique is based on the stretching of the polymer solution (synthetic or natural) under electric force, which leads to the production of very thin fibers at the nanoscale [7, 8].

Gelatin is a biodegradable, biocompatible and edible polymer obtained from the hydrolysis of collagen. One of the most important applications of gelatin can be mentioned in the food industry, tissue engineering, and as a carrier of active compounds [5, 9]. In recent years, several studies have been conducted on the production of electrospun gelatin fibers containing essential oils or their derivatives. Production of microcapsules of angelica essential oil [5], cinnamaldehyde, limonene (LEO), eugenol [9] and sesamol in gelatin fibers [10] are among them. The results showed that the mechanical and antimicrobial properties of nanofibers depend on the type and concentration of essential oil, the type of polymer and the preparation method.

The aim of the current study is to produce new edible nanofibers with the properties of controlling the growth of food spoilage bacteria. Based on this, gelatin bio-reinforcement and lavender essential oil were used. In this research, after identifying the chemical compounds of lavender essential oil using gas chromatography equipped with a mass spectrometer (GC-MS), gelatin nanofibers containing lavender essential oil were produced for the first time, and then its antibacterial properties and its most important features were investigated.

2- Ingredients for soups

2-1- Raw materials

Edible gelatin (Type A, 200 Bloom) was obtained from Gel-Tec (made in South Korea), glacial acetic acid (purity 99%), dimethyl sulfoxide (99%) and n-hexane were obtained from Merck (made in Germany). Bristol A9596 bacteria (*S. aureus*) *Staphylococcus aureus*،*Bacillus cereus* ATCC 9634 (*B. cereus*) ، (*E. coli*) ATCC 25922 *Escherichia coli*And*Salmonella typhimurium* ATCC 14028 (*S. typhimurium*) from the Department of Food Science and Industry, Faculty of Agriculture, Zabol University, Zabol, Iran, all culture media were obtained from Sigma Aldrich brand (made in USA) and lavender essential oil from Raiha Salamat Company (Iran).

2-2- Identification of essential oil compounds using GC-MS[1]

Compositions of lavender essential oil using an Agilent 7890A gas chromatograph (USA) connected to a mass detector (Agilent 5975C), equipped with a column(mm 250 × m 30) HP-5MS was determined Helium gas (99.99% purity) was injected into the column with a variable temperature of 40 to 280 °C as a carrier at a rate of 0.9-1.1 ml/min. temperature ofInjector and detector respectively 280 and°C250 were set. The ionization energy of the mass spectrometer was ev70, and essential oil compounds were identified using the NIST'08 spectrum library (National Institute of Standards and Technology, Gaithersburg, MD, USA) [11].

2-3- Preparation of nanofibers by electrospinning method

In order to produce electrospun gelatin nanofibers (GNF), gelatin solution at a w/v ratio of 15% in glacial acetic acid (40%) under temperature°C40 was prepared for 30 min. Based on the initial tests, lavender essential oil was replaced with acetic acid

1-Gas chromatography–mass spectrometry

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solution in concentrations of zero, 2.5, 0.5 and 0.10 v/v%. The electrical conductivity of the nanofiber solution was measured using a digital conductivity meter (model 103, Taiwan) and it was electrospun under a voltage of Kv15, a flow rate of 0.4 mL/h, a distance of 170 mm from the tip of the needle to the collector. In order to remove the remaining solvent, the produced fibers were kept in a desiccator for 24 hours [5].

2-4- Structure of nanofibers

Appearance and thickness of nanofibers produced using scanning electron imaging (SEM[2]) Done. First, the nanofibers were covered by a mixture of gold and palladium with a thickness of 20 nm, and then they were imaged under a voltage of ev20 and a working distance of 10 mm with the help of SEM (model LEO 1450 VP, made in Germany). The diameter of nanofibers was determined using Image J software and by averaging the thickness of 50 fibers [8].

2-5- X-ray diffraction (XRD[3]

Nanofiber samples prepared using an X-ray machine (Panalytical, Poland) with a wavelength of 1.54 A and under an angle of 45°– 5 were reviewed [7].

6-2- Mechanical characteristics

The mechanical behavior of nanofibers was studied using a tissue analysis device (model RS-232 LLOYD, Amtec, England). The thickness of the samples (10 x 60 mm) was measured at 10 points and in order to equalize the humidity, the pieces of nano fibers were placed inside a desiccator with a relative humidity of 75% (containing saturated sodium chloride solution) for 24 hours. Then the samples were placed inside the jaws of the machine with a distance of 50 mm, head speed of 6 mm/min and load cell N 50. The test was performed in 3 repetitions [8].

2-7- The efficiency of micro-coating

Microcoating efficiency and lavender essential oil content in nanofibers were evaluated according to the method of Tavasli et al. (2018) with some modifications [12]. First, the free essential oil on the nanofiber surface was removed with the help of ethanol solvent. Then, the amount of 50 mg of nanofibers was dissolved in 5 mL of ethanol and absorbed using a spectrophotometer (Gold Spectrumlab 54, USA) at a wavelength of 275 nm. Essential oil concentration was measured using a calibration curve (R2 = 0.99) and encapsulation efficiency and oil content were calculated using the following formulas:

Weight of primary essential oil / weight of essential oil in nanofibers = encapsulation efficiency (%)) x 100

Weight of essential oil of nanofibers before washing with ethanol / weight of essential oil in nanofibers) = oil content (%)) x 100

8-2- Antibacterial activity of nanofibers by disc diffusion method

Antibacterial activity of nanofibers prepared by disk diffusion method according to Ghasemi et al. (2022) against two gram positive strains (*S. aureus* And*B. cereus*(and two gram-negative strains)*E. coli* And*S. typhimurium*) was done [8]. First, in order to remove possible microbial contamination, nano fiber discs (diameter 8 mm) were exposed to UV light (λ=254 nm) for 1 hour. Then on the surface of nutrient agar culture medium inoculated with 100 µL of target bacteria containing 10 CFU/mL^{6}-10^{5} (0.5 McFarland) were transferred. Plates in temperature°C 37. For 24 h, incubation and antibacterial activity were evaluated based on the diameter of the inhibitory zone (in mm).

9-2- Statistical analysis

Statistical analysis of the data was done using one-way analysis of variance (ANOVA) and comparison of means was done with the help of Tukey's test. For this purpose, SAS software version 9.1 (SAS Institute INC., America) was used at the confidence level of 95%. All experiments were performed in three repetitions and the results were averaged± Standard deviations were expressed.

3. Results and Discussion

3-1- GC-MS test

The results of GC-MS analysis of lavender essential oil are given in Table 1. In total, 27 different chemical compounds were identified with a share of 98.83% of the total essential oil components.

Table 1 Chemical composition of LEO using GC–MS chromatography

No	Compounds	RT (min)	Area (%)
1	α-Thujene	9.84	0.29
2	α-Pinene	9.98	0.50
3	β -Pinene	11.66	0.22
4	3-Octanone	11.93	0.51
5	β -Myrcene	11.93	0.68
6	n-Hexyl acetate	12.53	0.73
7	D-Limonene	13.09	0.62

2- Scanning electron microscope

3- X-ray diffraction

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8	Eucalyptol	13.17	0.85
9	α-trans-Ocimene	13.28	4.73
10	β -trans-Ocimene	13.41	0.72
11	1,6-Octadien-3-ol, 3,7-dimethyl	13.49	1.62
12	cis-Ocimene	1357	2.02
13	Linalool	15.15	35.21
14	Norbonan	15.68	0.96
15	Camphor	15.82	0.72
16	β -Myrcene	16.63	0.85
17	Borneol	16.94	1.23
18	lavandulol	17.43	1.05
19	α-Terpineol	18.01	1.83
20	Linalyl acetate	19.30	26.47
21	Nerol	20.11	3.94
22	Geranyl acetate	22.64	0.86
23	Santalol	23.75	0.59
24	Trans-β - Caryophyllene	23.92	5.39
25	β - The Farnese	24.52	4.83
26	Germacrene-D	25.45	0.64
27	Caryophyllene oxide	28.04	0.77
	Total	98.83	

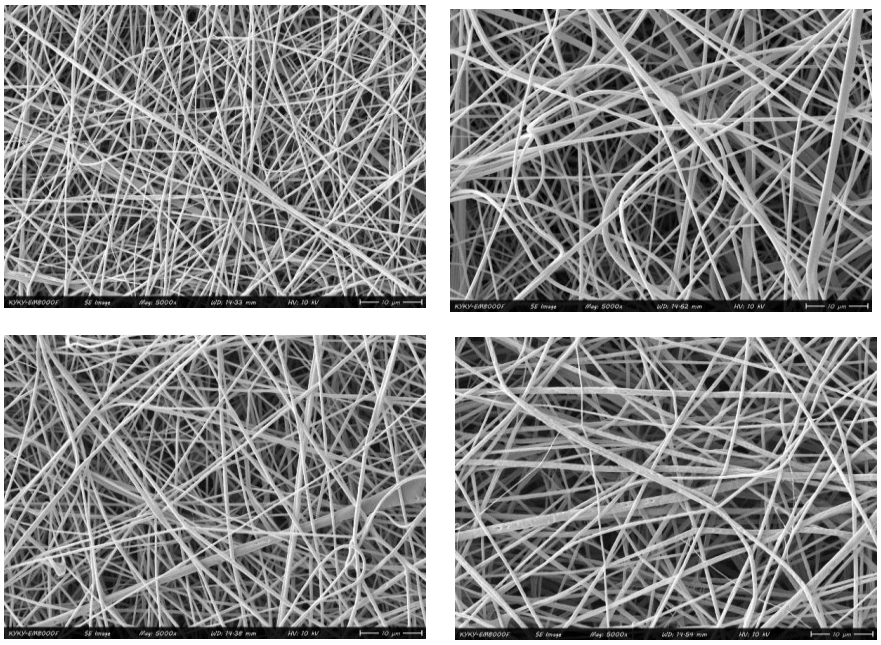

Fig 1 SEM images of the nanofiber with different LEO concentrations (A: 0%; B: 2.5% ; C: 5% ; and D: 10%).

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Two chemical compounds linalool (35.21%) and linalyl acetate (26.47%) accounted for more than 60% of the compounds. Similar studies have shown that linalool and linalyl acetate are the main components of lavender essential oil, although they showed differences in the type and amount of some compounds [13, 14]. The difference in the chemical composition of the essential oils of a plant can be attributed to various factors such as the breed, the part of the plant used, weather conditions, geographical region, cultivation and harvesting conditions, as well as the method of extracting and preserving the essential oil [9, 11, 15].

2-3- Structure, diameter and gelatin nanofibers

Table 2 shows the results of evaluating nanofiber thickness and electrical conductivity of electrospinning solution. By increasing the concentration of essential oil from zero to 10%, the diameter of nanofibers increased from 431.5 to 705.3 nm, which can be caused by the decrease in the electrical conductivity of the electrospinning solution from 43.4 to 30.7 µS.cm. In general, reducing the electrical conductivity of the solution causes a reduction in the electric charge distribution in the jet area of the device, which, as a result, reduces the tensile forces on the nanofibers and leads to an increase in thickness [5, 8]. In similar reports, the thickness of electrospun gelatin nanofibers increased by increasing the concentration of angelica essential oil [5] and Portugal [12].

Electron microscope images of nanofibers containing different concentrations of lavender essential oil are shown in Figure (A-C) 1.

The images show that all the nanofibers are without fibers and have a uniform and continuous structure, which shows that the essential oil does not affect the shape of the nanofibers.

Table 2. Electrical conductivity (EC) of LEO loaded gelatin solutions and average diameter of nanofibers.

Nanofiber	EC (µS/cm)	Average diameter (nm)
Gelatin+ LEO (0.0 %)	43.4 ± 0.3^{A}	431.5 ± 15.2^{A}
Gelatin+ LEO (2.5 %)	36.6 ± 0.1^{B}	477.4 ± 14.3^{A}
Gelatin+ LEO (5 %)	34.9 ± 0.6^{C}	528.6 ± 12.7^{A}
Gelatin+ LEO (10 %)	30.7 ± 0.2^{D}	705.3 ± 18.6^{A}

The values are the means ± SD, (n=3). Means followed by different uppercase indicate significant differences in column ($P < 0.05$).

3-3- XRD results

The crystal structure of gelatin nanofiber components (Figure 2) was investigated by XRD test. Comparison of the obtained graphs shows the same behavioral similarity. All graphs in the region of $\theta 2 = 13\text{-}28°$ show an important prominence, which is related to the triple helix crystal structure of the gelatin macromolecule [7]. The addition of essential oil caused a slight increase in the area of the triple helix, which is probably due to the change in the structure of the triple helix under the influence of lavender essential oil, which can also affect the mechanical properties of nanofibers. A similar report of the addition of peppermint essential oil to gelatin nanofibers is available [16]. In another report, it has been stated that the addition of curcumin to gelatin/chitosan nanofibers had no effect on the structure of nanofibers [7]. This difference in the results is probably due to the difference in the chemical properties of the biospasar and the active agent [8, 17]..

Fig 2: XRD diffractograms of different GNFs, **a:** Gelatin+ LEO (0 %), **b:** Gelatin+ LEO (2.5 %), **c:** Gelatin+ LEO (5 %), **d:** Gelatin+ LEO (10 %).

Fig 2: XRD diffractograms of different GNFs, **a:** Gelatin+ LEO (0 %), **b:** Gelatin+ LEO (2.5 %), **c:** Gelatin+ LEO (5 %), **d:** Gelatin+ LEO (10 %).

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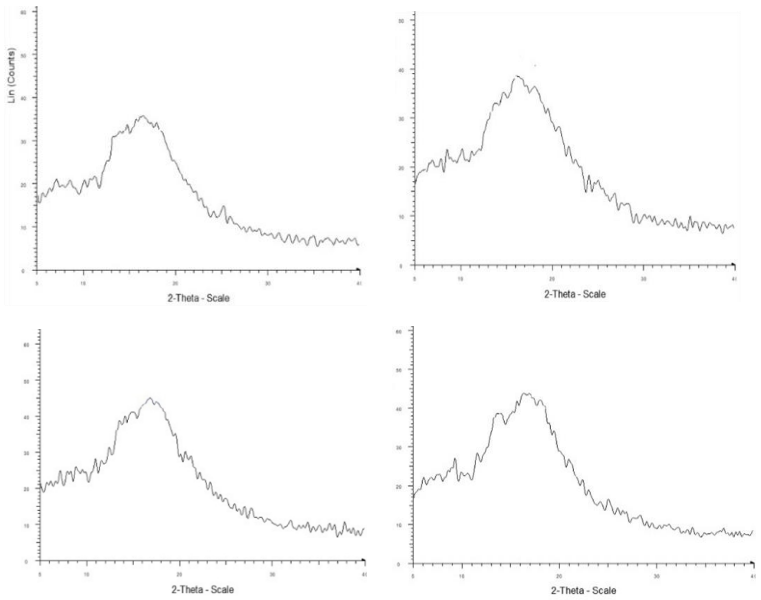

Fig 2: XRD diffractograms of different GNFs, **a:** Gelatin+ LEO (0 %), **b:** Gelatin+ LEO (2.5 %), **c:** Gelatin+ LEO (5 %), **d:** Gelatin+ LEO (10 %)**.**

3-4- Mechanical properties of gelatin nanofibers

One of the important factors in choosing a food packaging wrapper is its mechanical properties. The evaluation of the mechanical indices of prepared nanofibers is given in Table 3. The highest values of hardness (N/m 4.31230) and Young's modulus (Mpa 0.460) were recorded by nanofibers without essential oil (Gelatin + LEO (0%) ($p<0.05$). Addition of essential oil causes a significant decrease in tensile strength values and Compared to the sample without essential oil, the Young's modulus increased ($p<0.05$). Essential oils have softening properties of biopolymers, which exert this role by being placed between the macromolecules of the polymer structure [16]. However, it has been reported that the intensity of the effect It depends on the type, concentration of the essential oil and the degree of interaction of the components added to the biopolymer [16, 8, 18].

Table 3. Mechanical characteristics of nanofibers containing different concentration of LEO

Nanofiber	Stiffness (N/m)	Tensile Strength (MPa)	Modulus (Mpa)
Gelatin+ LEO (0 %)	31230.4 ± 12.7^{A}	0.11 ± 0.04^{C}	462.0 ± 8.1^{A}
Gelatin+ LEO (2.5 %)	954.6 ± 18.3^{D}	0.17 ± 0.03^{BC}	14.8 ± 6.8^{C}
Gelatin+ LEO (5 %)	2694.3 ± 23.5^{C}	0.20 ± 0.05^{AB}	35.3 ± 9.5^{B}
Gelatin+ LEO (10 %)	3979.2 ± 29.6^{B}	0.25 ± 0.05^{A}	37.2 ± 5.3^{B}

The values are the means ± SD, (n=3). Means followed by different uppercase indicate significant differences in column ($P < 0.05$).

With the increase in the concentration of the essential oil of the electric solution from 2.5 to 10%, the increasing trend in the hardness values (3979.2 N/m– 954/6) and Young's modulus (37/2 MPa– 14.8) nanofibers were observed ($p<0.05$). The results of the tensile strength values (Table 2) show that increasing the concentration of essential oil from 0 to 10% has a slow increasing trend (0.25 MPa– 0.11), so that the highest values were obtained in Gelatin+LEO (5%) and Gelatin+LEO (10%) samples ($p<0.05$). This phenomenon is probably due to the limited interaction of essential oil components with the protein structure, which has changed the arrangement of biopolymer chains and improved the tensile strength [16, 19]. Similar reports of changes in the mechanical properties of the gelatin wrapper are available [16, 18, 20].

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3-5- Microcoating efficiency and nanofiber essential oil content

Microcoating efficiency and essential oil content of nanofibers are important parameters for evaluating antimicrobial activity [8]. Table 4 shows the results of measuring the efficiency and essential oil content of prepared nanofibers. The highest (99.1%) and lowest (87.4%) microcoating efficiency was observed in Gelatin + LEO (2.5%) and Gelatin + LEO (10%) samples. With the increase in the percentage of essential oil in the electrospinning solution, the essential oil content of nanofibers also showed a significant increase ($p<0.05$), so that the highest amount (14.6%) was recorded in Gelatin + LEO sample (10%) ($p<0.05$). Pilicheva et al [17] reported the microencapsulation efficiency of lavender essential oil in maltodextrin about 91%. In another report, the efficiency of microcoating and the content of orange essential oil in gelatin biorepository were reported as 69% and 26%, respectively [12]. These results show that the electrospinning of gelatin protein has a suitable efficiency for microencapsulation of lavender essential oil.

Table 4. The encapsulation efficiencies (EE) and LEO contents (LC) of gelatin nanofibers.

Nanofiber	EE (%)	LC (%)
Gelatin+ LEO (2.5 %)	99.1±0.2 ^{A}	4.7 ± 0.1 ^{C}
Gelatin+ LEO (5 %)	96.9±0.2 ^{B}	8.9 ± 0.2 ^{B}
Gelatin+ LEO (10 %)	87.4±0.3 ^{C}	14.6 ± 0.2 ^{A}

The values are the means ± SD, (n=3). Means followed by different uppercase indicate significant differences in column ($P < 0.05$).

6-3- Antibacterial activity

Controlling the growth of spoilage microbes plays a key role in the food application of nanofibers. The results of evaluating the antibacterial activity of gelatin nanofibers containing lavender essential oil against some bacteria causing food spoilage.*S. aureus, B. cereus, E. coli and S. typhimurium*) is given in Table 5. Gelatin + LEO sample (0%) did not show antibacterial effect, while increasing the concentration of essential oil in gelatin nanofibers from 2.5 to 10%, the antibacterial effect was enhanced. In total, nanofibers containing lavender essential oil have a better inhibitory effect on Gram-positive bacteria (*S. aureus* And*B. cereus*(compared to gram negative)*E. coli* And*S. typhimurium*) Showed. The greatest antibacterial effect (21.4-22.2 mm) by Gelatin + LEO sample (10%) against bacteria*S. aureus* And*B. cereus* Was recorded. The reported antibacterial activity of lavender essential oil is attributed to the high content of linalool and linalyl acetate [8, 21].

Table 5- Encapsulation efficiency and Inhibition zone values (mm) of gelatin nanofibers against target bacteria

Nanofiber	Inhibition zone (mm)			
	S. aureus	*B. cereus*	*E. coli*	*S. typhimurium*
Gelatin+ LEO (0 %)	-	-	-	-
Gelatin+ LEO (2.5 %)	1 2.9 ± 0.7 ^{C,c}	1 2.6 ± 0.4 ^{C,c}	1 1.8 ± 0.6 ^{C,c}	12. 2 ± 0.3 ^{C,c}
Gelatin+ LEO (5 %)	1 7.6 ± 0.6 ^{B,b}	1 7.2 ± 0.8 ^{B,b}	1 6.7 ± 0.4 ^{B,b}	16. 9 ± 0.5 ^{B,b}
Gelatin+ LEO (10 %)	2 2.2 ±0.4 ^{A,a}	2 1.4 ± 0.6 ^{A,ab}	2 0.6 ± 0.5 ^{A,b}	20. 9 ± 0.4 ^{A,ab}

The values are the means ± SD, (n=3). Means followed by different uppercase and lowercase letters indicate significant differences in column and row, respectively ($P < 0.05$).

Terpenic and phenolic compounds of essential oils can pass through the cell wall of bacteria and enter the cell due to their hydrophobic properties. The presence of lipopolysaccharide compounds in the outer membrane of Gram-negative bacteria partially prevents the entry of these compounds, which results in increased cell resistance against antibacterial agents. However, small molecule and hydrophilic compounds pass through purine channels and cause cell death by disrupting intracellular activities [15]. Also, the compounds in essential oils increase the permeability by destroying the molecular order of the cytoplasmic membrane, which results in the depletion of ions and ATP, disturbance in the activity of the cytoplasmic membrane and DNA synthesis. However, attention should also be paid to the type, concentration and interaction of essential oil compounds with environmental components [22]. So far, the antibacterial effect of gelatin nanofibers containing lavender essential oil has not been reported. Hajiali et al. (2016) investigated the antibacterial effect of alginate nanofibers containing lavender essential oil against*S. aureus* reported [23]. In another study, the antibacterial effects of lavender essential oil nanoemulsion against some Gram-negative and Gram-positive bacteria have been published [24]. These reports are in line with the results obtained in this research.

4 - Conclusion

In this research, for the first time, lavender essential oil (at concentrations of 0 to 10%) was loaded into gelatin nanofibers by electrospinning method. The most important chemical components of lavender

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essential oil were linalool and linalyl acetate. From the point of view of structure, all the nanofibers made had a uniform texture and did not have cells. Also, the addition of essential oil did not cause any significant change in the structure of amorphous nanofibers, which was confirmed by X-ray diffraction. By increasing the concentration of essential oil in the electrospinning solution, the values of thickness and essential oil content of nanofibers increased. The samples containing lavender essential oil recorded less hardness compared to the sample without essential oil. The results of the antibacterial test showed that nanofibers containing lavender essential oil have antibacterial activity against gram-positive and gram-negative bacteria. According to the results of the present research, it can be said that electrospun nanofibers containing lavender essential oil can be considered as an active packaging for food such as cheese, meat and some other food products. Obviously, the final conclusion requires detailed clinical investigations in real environments.

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6- Resources

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ارزیابی فعالیت ضدمیکروبی و ویژگی‌های نانوالیاف ژلاتین حاوی اسانس اسطوخودوس

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چکیده

چکیده: اسانس گیاه اسطوخودوس کاربردهای گسترده‌ای در صنایع آرایشی، غذایی و دارویی دارد. در این پژوهش برای نخستین بار، ریزپوشانی اسانس اسطوخودوس توسط زیست بسپار ژلاتین به روش الکتروریسی انجام شد. در ابتدا ترکیبات شیمیایی اسانس اسطوخودوس به کمک گازکروماتوگرافی متصل به طیف سنج جرمی (GC-MS) شناسایی و سپس در غلظت‌های صفر، ۲/۵، ۵/۰ و ۱۰٪/۰ v/v به محلول الکتروریسی اضافه گردید. نانوالیاف تهیه شده با استفاده از آزمون‌های تصویربرداری الکترونی روبشی (SEM)، تعیین ضخامت (Image J)، پراش اشعه ایکس، خواص مکانیکی و راندمان بارگذاری اسانس مورد بررسی قرار گرفتند. همچنین خواص ضدباکتریایی نانوالیاف در برابر باکتری‌های *Staphylococcus aureus*، *Bacillus cereus*، *Escherichia coli* و *Salmonella typhimurium* به روش انتشار دیسک بررسی گردیدند. مهمترین ترکیبات اسانس اسطوخودوس اجزاء لینالول (۳۵/۲۱ درصد) و لینالیل استات (۲٦/٤۷ درصد) بودند. تمامی نانوالیاف دارای شکل یکنواخت و پیوسته بودند. با افزایش غلظت اسانس در محلول الکتروریسی ضخامت (۷۰۵/۳ nm – ٤۳۱/۵) و محتویات اسانس (۱٤/٦–٤/۷٪) نانوالیاف افزایش یافت. اسانس اسطوخودوس تاثیری بر ایجاد ساختار کریستالی در نانوالیاف نداشت، اما باعث کاهش شدید مقادیر مدول یانگ و سختی گردید. نمونه نانوالیاف حاوی ۱۰٪ اسانس اسطوخودوس بیشترین سختی، مدول یانگ و کشش‌پذیری ثبت نمود. ارزیابی فعالیت ضدباکتریایی نشان داد نمونه‌های نانوالیاف حاوی اسانس دارای فعالیت ضدباکتریایی در برابر تمامی باکتری‌های هدف بودند. بیشترین تاثیرگذاری توسط نانوالیاف ژلاتین + اسانس (۱۰٪) در برابر *S.aureus* و *B. cereus* بدست آمد. با توجه به نتایج پژوهش حاضر احتمالا بتوان نانوالیاف ژلاتین حاوی اسانس اسطوخودوس را جهت بسته‌بندی فعال مواد غذایی بکاربرد. بیان نتیجه قطعی مستلزم انجام آزمایشات بالینی و در محیط واقعی است.

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