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Fabrication of Electrospun Nanofibres of Soy Protein Isolate/Polyvinyl Alcohol Embedded with *Cinnamon Zeylanicum* and *Zataria Multiflora* Essential Oils and their Antibacterial Effect

Mojtaba Raeisi [1], Masoud Aman Mohammadi [2], Vahid Bagheri [3], Soghra Ramezani [4], Marjan Ghorbani [5,6], Mahnaz Tabibiazar [7], Ozlem Emir Coban [8], Rahem khoshbakht [9], Sayed Mahdi Hossaeini Marashi [10,11], Seyyed Mohammad Ali Noori [12,13,*]

1 Food, Drug, and Natural Products Health Research Center, Golestan University of Medical Sciences, Gorgan, Iran
2 Student Research Committee, Department of Food Technology, Faculty of Nutrition Science and Food Technology, Nutritional and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3 Department of Food Science and Technology, Faculty of Agriculture, University of Tabriz, P.O. Box 51666-16471, Tabriz, Iran
4 Nanofiber Research Center, Asian Nanostructures Technology Co. (ANSTCO), Zanjan, Iran
5 Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
6 Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
7 Faculty of Nutrition and Food Science, Tabriz University of Medical Sciences, Tabriz, Iran
8 Faculty of Fisheries, Firat University, 23119 Elazig, Turkey
9 Department of Pathobiology, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran
10 College of Engineering, Design and Physical Sciences Michael Sterling Building (MCST 055), Brunel University London, Uxbridge, UB8 3PH, United Kingdom
11 School of Physics, Engineering and Computer Science, Centre for Engineering Research, University of Hertfordshire, Mosquito Way, Hatfield AL10 9EU, United Kingdom
12 Toxicology Research Center, Medical Basic Sciences Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
13 Department of Nutrition, School of Allied Medical Sciences, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
* Correspondence: noori-sma@ajums.ac.ir; sma.noori@gmail.com (S.M.A.N.);

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Abstract: The current study aimed to develop edible nanofibrous films embedded with essential oils (EOs) by the electrospinning method and assess their antimicrobial activity. At first chemical components of *Cinnamon zeylanicum* (Cz) and *Zataria multiflora* (Zm) EOs were evaluated, and their antimicrobial activities were investigated. Then, Cz and Zm EOs were added to Soy Protein Isolate (SPI)/Polyvinyl alcohol (PVA) by the electrospinning method. SEM, FTIR, and TGA tests were carried out to investigate the characteristics of fabricated nanofibers. The antimicrobial properties of nanofibers were investigated. SEM exposed that adding 50% PVA neutralized SPI charge, conducive to a significant decrease in nanofibers diameter by 130 nm. Moreover, TGA displayed SPI-PVA nanofibers' stability. Developed nanofibers showed excellent antimicrobial effects toward Gram-positive and -negative pathogens. For instance, SPI-PVA nanofibers containing 20% Cz reduced 72, 56, and 42% *S. aureus*, *B. cereus*, and *S. typhimurium*. However, in the case of *E. coli*, Zm showed the best result and reduced 42% of the bacterium. In gist, nanofibrous films containing Cz and Zm EOs can be utilized in active food packaging to increase the durability of food due to their excellent antimicrobial activity.

Keywords: essential oil; active food packaging; active food packaging; nanotechnology and nanofiber; bioactive film.

1. Introduction

Recently, researchers and industries have been attracted to active packaging due to its promising antibacterial activities, leading to better food preservation and shelf life. Despite all advances in food industries, there is still high demand for developing natural antimicrobial and antioxidant packaging materials [1,2]. Although traditional food packaging is assigned to perform simple tasks such as a barrier for dust or moisture, the purpose of applying active packaging is more advanced [3,4]. Active packaging can release antibacterial agents into foods and maintain the original quality of food products [5-8].

Nanotechnology, such as electrospinning, has provided a new approach to improve active packaging in food industries. In this method, nanofibers can be fabricated continuously with a very high surface area that makes a food barrier and acts as active packaging [9-11]. In addition, better mechanical resistance and porous structure of nanofibers fabricated by electrospinning make them perfect for application in food industries [12-14].

Essential oils are natural aromatic metabolites of plants that have been used in folk medicine for their therapeutic effects [15-17]. Some essential oils exhibited excellent antioxidant and antibacterial effects, such as cinnamon and thyme, which have been used in food models to prevent microbial spoilage and retard chemical reactions. Huang *et al*. [18] showed that cinnamon bark oil (containing 85.78% cinnamaldehyde and 7.30% diethyl malonate) has great antibacterial activity against the spoilage bacteria of fish. Pinto *et al*. [19] applied red thyme oil to the orange stored for 12 days in cold conditions to improve its shelf life and showed that the orange's shelf life improved significantly. Researchers have studied combining different essential oils to increase their beneficial effects. In another study, Lee *et al*. [20] showed that thyme thymol and oregano essential oil exhibited synergistic antibacterial effects, which was more effective compared to essential oil alone.

In addition, other approaches have been used to intensify the antibacterial and antioxidant activities of EOs [21,22]. Among all modern approaches [23,24], researchers have recently used the electrospinning method to obtain even more effective and durable natural preservatives with antibacterial activity. Zhou *et al*. [25] fabricated a nanofiber with encapsulated angelica essential oil and gelatin by electrospinning. Obtained nanofiber displayed great antibacterial effects toward Gram-negative and Gram-positive bacteria. Kamrudi *et al*. [26] fabricated polyamidoamine dendritic polymer incorporated with thyme essential oil using an electrospinning method to obtain nanofiber with antibacterial activity. The results showed that nanofiber is able to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*.

Moreover, some studies can be found in the literature which has used the electrospinning technique in food models. Göksen *et al*. [27] electrospun zein with *Rosmarinus officinalis* and *Laurus nobilis* essential oils and applied them in cheese slices to improve its shelf life. They observed that these nanofibers significantly reduced the aerobic mesophilic count and *Staphylococcus aureus* and *Listeria monocytogenes*. Vafania *et al*. [28] fabricated chitosan-gelatin nanofibrous films incorporated with thyme essential oil and applied them to

sausages to improve their shelf life. Fabricated nanofibers exhibited not only significant antibacterial activity but also long-lasting antioxidant effects.

Up to today, limited studies have evaluated the antibacterial effects of electrospun edible nanofibers embedded with EOs. Moreover, no study has investigated the characteristics and antimicrobial activity of electrospun SPI/PVA nanofibers incorporated with EOs. This study aimed to (1) fabricates SPI/PVA nanofibers combined with *Cinnamon zeylanicum* and *Zataria multiflora* and (2) evaluate the characteristics and antibacterial activity of electrospun nanofibers.

2. Materials and Methods

2.1. Materials.

Polyvinyl alcohol (PVA) and Soy Protein Isolate (SPI) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), and acetic acid was obtained from Merck Co., Germany. Throughout the study, deionized water was utilized.

2.2. Plant material.

Zm and Cz leaves were obtained from regional vendors in Gorgan, Iran, and the plant species were determined by Golestan University, Gorgan, Iran experts.

2.3. Extraction of essential oils

Provided leaves were dried, ground, and used for EO extraction by a Clevenger device. *Cinnamon zeylanicum* and *Zataria multiflora* leaves were immersed in distilled water. The extraction time was considered at the water boiling initiation and lasted 3 h. Sodium sulfate was added to accelerate dehydration. After obtaining EOs, filtration was carried out using a 0.22 μm filter and held in light-resistant vials at 4°C.

2.4. Gas chromatography-mass spectrometry (GC-MS) analysis to evaluate the components of Eos.

EOs of *C. zeylanicum* and *Z. multiflora* tested with an Agilent 6890N instrument (San Diego, United States) benefited HP-5MS capillary column (30 × 0.25 mm ID × 0.25 mm film thickness). The flow rate was 1 mL/min, and 50 °C was the initial temperature of the column, which was then increased gradually at a 2 °C per minute rate to reach 120 °C. The temperature remained at 120 °C for three minutes and reached a maximum temperature of 300 °C. The operation was performed at 70 eV. Identification of compounds was carried out by comparison of the retention indices with standard samples. The library of Wiley-VCH 2001, Weinheim, Germany, has been used to show mass spectral data.

2.5. Investigation of EOs antibacterial effects.

Four Gram-negative (*Salmonella typhimurium* ATCC 13311, *Escherichia coli* PTCC 1399, *Shigella dysenteriae* PTCC 1188, *Pseudomonas aeruginosa* PTCC 1616) and three Gram-positive (B. cereus PTCC 1154, *L. monocytogenes* PTCC 1298, and *S. aureus* PTCC 1112) bacteria were selected to investigate EOs antibacterial activity. Three different approaches were used for this purpose: agar well diffusion, agar disk diffusion, and microdilution methods. Mentioned bacteria were supplied by the IROST center in Tehran, Iran.

2.6. Agar disk diffusion test.

This method was accomplished as delineated by Evangelho *et al*. [29]. 0.1 mL of tested bacteria (1.5×106 cfu/mL) were poured on nutrient agar plates (Merck, Darmstadt, Germany) and spread adequately. Paper disks with six mm thickness containing EOs were set on described plates and incubated at 37 °C for 24 h. Gentamycin and vancomycin antibiotic disks were laid on plates and considered positive control. The negative control was a disk imbued with dimethyl sulfoxide (DMSO). Growth inhibition diameter was assessed with a caliper (Mitutoyo, Japan) and represented as mm.

2.7. Agar well-diffusion.

1.5 × 106 cfu/mL of bacteria were cultivated in broth media, and one mL was then poured into 100 mL of melted nutrient agar (Merck, Darmstadt, Germany). It was then mixed for 2 min, transferred into empty plates, and placed at room temperature to become solid. After that, by using a sterile cork borer, 4 wells were formed aseptically, and 10 µL of EOs were poured into each well. The negative control was DMSO, and plate incubation was done at 37 °C for 72h. The antibacterial activity was presented as millimeters of bacterial growth inhibition [30].

2.8. Broth microdilution assay.

The test was conducted in conformity with the technique described by Hosseini *et al*. with minor changes [30]. Firstly, the EOs were added to DMSO (10%) to the highest concentration (10,000 µg/mL), and within the range of concentration from 10 to 10,000 µg/mL, a two-fold serial dilution from each concentration was prepared. Subsequently, the EO solutions with 125 µL were poured into wells of a 96-well microplate (Sarstedt, Montreal, QC, Canada). At last, 15 µL of this culture media, which contains 106 CFU/mL of the bacteria, were transferred to each well, and the last volume which remained in the wells was 140 µL approximately. For each bacterium, three microplate rows were used. For negative controls, cultured bacteria were replaced with 15 µL normal saline. At the same time, the positive controls contained 15 µL of the cultured bacteria and 125 µL of the growth media. Incubation was performed at 37 °C for one day. The absorbance measurement was recorded at 595 nanometers by an absorbance microplate reader (Biotech ELX800O, Biotek Instruments Inc., Winooski, VT, USA).

The lowest concentration of an antibacterial substance to prevent bacterial growth is regarded as minimum inhibitory concentration (MIC). While the minimum concentration of antibacterial to remove a minimum of 99.9% of the original inoculum is defined as minimum bactericidal concentration (MBC). It was discovered by the subculturing from wells in nutrient agar plates with incubation conditions at 37 °C for 72 hours, and no sign of growth must be seen [30].

2.9. Preparation of solutions.

Because of the globular structure of SPI, it is non-spinnable. Therefore, PVA is mixed with fabricated SPI nanofiber. Acetic acid 65% was used to dissolve PVA and SPI (35% wt) by a magnetic stirrer, and stirring was continued for 120 min in a 60 °C water bath with three various ratios including 50:50, 60:40, 70:30, 80:20, 90:10, 100:0 respectively.

2.10. Electrospinning of solutions.

Electrospinning of solutions was done by 18 kV voltage power and a flow rate of 0.5 mL/h. They were then poured into a five mL plastic syringe with a stainless-steel needle and blunt-tipped 21-gauge. Fibers were collected using aluminum foil, and the distance between the needle tip and the aluminum foil collector was 12 cm. Relative humidity and temperature for electrospinning processes were 40 ± 4% and 25 ± 5 °C, respectively. The finest fiber, on the basis of spinnability and SEM analysis outcomes, was selected, and *C. zeylanicum* and *Z. multiflora* EOs were embedded into the polymer solution. The polymer solution was enriched with 20% w/v EOs concentration [31].

2.11. Scanning electron microscope (SEM).

In this research, the scanning electron microscope (SEM) (Amsterdam, Holland) has been applied to view the morphology and structure of electrospun fibers. All images have been captured at a voltage of 26 kV. To assess the fibers diameter distribution from obtained SEM images, approximately 100 fibers were measured randomly at a magnification of 5000× using the Image J software [32].

2.12. Fourier transform infrared spectrometry (FT-IR) analysis.

A Bruker-Tensor27 Spectrometer was applied to investigate the FT-IR electrospinning fibers spectra in KBr pellets. Interferograms have been accumulated over a spectral range of 500-4000/cm with a 2/cm nominal resolution and 100 scans [33].

2.13. Thermogravimetric analysis (TGA).

This test was completed by a thermogravimetric analyzer instrument (Linseis, STA PT1600, Germany). Devices were calibrated by utilization of calcium oxalate monohydrate. For this analysis, samples (15 mg) were heated under the controlled atmosphere of nitrogen gas at 10 °C/minute until the temperature of samples reaches approaches 600 OC.

2.14. Antibacterial evaluation by disc diffusion assay.

The antibacterial properties of SPI/PVA nanofiber embedded with *Cinnamon zeylanicum* and *Zataria multiflora* EOs against *L. monocytogenes*, *E. coli*, *S. aureus*, *B. cereus*, and *S. typhimurium* was evaluated by two methods as described by Wen *et al*. [34]. The first method was a disc diffusion assay. For negative control, SPI/PVA nanofibers were used. 50 mL of nutrient broth media was inoculated with the bacterium, and overnight incubation was performed at 37 °C. Subsequently, it was subcultured to another nutrient broth (50 mL) and incubated for 8 h at 37 °C. Afterward, a 10-fold serial dilution method was executed to reach the concentration of 107~108 CFU/mL, and then 100 µL of it was inoculated on the nutrient agar culture media. Mentioned fabricated films were slitted into disks with 6 mm diameter and radiated with UV for two hours for sterilization. They were then set on the nutritional agar previously inoculated with bacteria. Plates were then incubated at 37 ºC for 24 h, and the diameter of the inhibition zone was recorded. Considering that a less antibacterial agent was consumed for the equal inhibition zone diameter, it can be concluded that the stronger antibacterial property was exhibited. To avoid obtaining any random results, all tests were carried out with three repetitions for each film.

2.15. Investigation of antibacterial activity using AATCC 100 method.

AATCC 100 was applied as a standard analysis procedure to assess the potential antibacterial properties of electrospun nanofibers. For this purpose, two additional bacteria, including *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923) were used, along with other bacteria. To make standard samples, the electrospun nanofibers were cut into the shape of circular swatches (2.2 ± 0.2 cm diameter and 3 mg weight). In addition, UV light was used to sterilize all samples for 20 min.

Then, the cells were separated using a centrifuge at 10000 g for six min. According to the AATCC100 procedure, the cell densities of those bacteria were cultured using a spectrophotometric method in suitable media and should be modified to 0.5 McFarland at 630 nm wavelength (this provides 1-1.5 × 108 cells/mL of bacteria in stock suspension).

0.1 mL suspension bacteria for each concentration were poured on electrospun nanofibrous film, which in the previous step, were cut into circular shapes, sterilized with UV, and incubated for 24 h. Afterward, 10 mL phosphate-buffered saline was added to the inoculated swatches to make a pH of 7.4 then the samples were poured into the falcon tube to shake and mix for 1 min properly. In the next step, 10 µL of the above-mentioned solution was conveyed to plates containing nutrient agar to keep for 24 h at 37 °C inside an incubator. The following equation [35] was applied to calculate the reduction percentage of microbes:

$$\%R=(C-A)/C\times100$$

%R: reduction of bacteria

C: quantity of recovered bacteria from the inoculated untreated control

A: quantity of recovered bacteria from inoculated nanofibers after 24h.

2.16. Statistical analysis

To avoid any random results, all experiments were repeated three times, and the obtained results are indicated in the form of means ± standard deviation (SD). To determine the differences within a group, a one-way analysis of variance was conducted (Venglovska, Gresakova, Placha, Ryzner, & Cobanova), while for comparisons between the groups, Tukey's test was applied. All statistical analysis has been completed using GraphPad Prism (Windows version 6) (GraphPad Software, San Diego, California USA, www.graphpad.com).

3. Results and Discussion

3.1. GC-MS analysis of EOs

The components of essential oils have been revealed by GC-MS analysis. For *C. zeylanicum* EO, 97.13% of constituents were found, and the major component was cinnamaldehyde (75.47%), while other constituents were insignificant (below 4%). Tepe and Ozaslan [36] reported 81.39% Cinnamaldehyde for *C. zeylanicum* EO, which was in accordance with our study. However, other studies recorded different amounts of cinnamaldehyde. Ribeiro *et al.* [37] and Wang *et al.* [38] reported 31.0% and 57.97% Cinnamaldehyde, respectively. The GC-MS analysis showed 99.40% of *Zataria multiflora* EO components, and the major constituents were carvacrol (64.20%) followed by thymol (17.10%). Other studies reported that the highest amounts of *Z. multiflora* constituents belong to thymol and carvacrol. Pilevar *et al.* [39] reported 40.79% and 27.24% for thymol and carvacrol, respectively. However, Ardekani, Khorram, Zomorodian, Yazdanpanah, Veisi, and

Veisi [35] reported that major components of *Z. multiflora* are thymol (52.80%), cymene (13.89%), and carvacrol (5.97%). Variations in the levels of EOs component is due to plant ecotype, location, season, and part of the plant which was used to acquire EO.

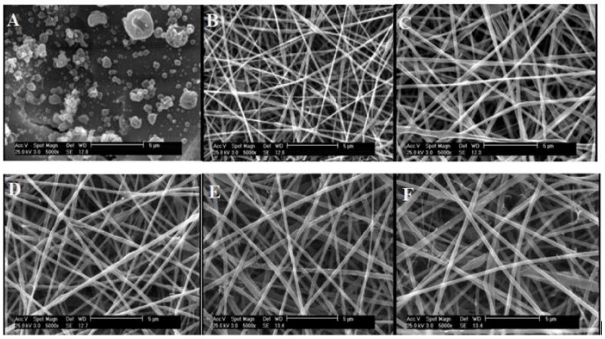


Figure 1. SEM image of electrospun fibers of a mixed solution of SPI- PVA at different concentrations **A** (100:0), **B**(50:50), **C**(60:40), **D**(70:30), **E**(80:20), **F**(90:10), respectively.

3.2. Evaluation of the antibacterial effect of EOs.

C. zeylanicum and *Z. multiflora* antibacterial potency have been investigated by agar well diffusion, micro-dilution, and agar disk diffusion method, as described in a previous study [40]. For this purpose, seven pathogenic microorganisms, including *E. coli*, *P. aeruginosa*, *S. thyphimurium*, *S. dysenteriae*, *B. cereus*, *L. monocytogenes*, and *S. aureus*, were chosen to evaluate the antibacterial effect of the EOs. The agar disk diffusion assay data revealed higher antibacterial activity of *Z. multiflora* compared to *C. zeylanicum* against all microorganisms. The highest activity of EOs was observed toward *S. dysenteriae* (16.32 ± 0.10 mm), while the highest resistant pathogen was *S. thyphimurium* (7.97 ± 0.19 mm). Almost similar results were observed by the agar well diffusion method. For example, *Z. multiflora* was more potent than *C. zeylanicum*. However, *B. cereus* was the most susceptible microorganism to *Z. multiflora* (12.02 ± 0.17 mm), and *P. aeroginosa* was the most resistant one (6.40 ± 0.04 mm) in the agar well diffusion method. In the case of microdilution assay, lower MBC and MIC were recorded for *Z. multiflora* toward *S. thyphimurium*, *E. coli*, *B. cereus*, and *S. aureus*, indicating more antibacterial activity of *Z. multiflora*. The same MIC and MBC for *Z. multiflora* and *C. zeylanicum* toward *P. aeroginosa* and *S. dysenteriae* were observed. Although two EOs showed equal MIC for *L. monocytogenes*, lower MBC was recorded for *C. zeylanicum* against this pathogen. Higher antibacterial activity of *Z. multiflora* compared to *C. zeylanicum* has been reported by Dohhi [41]. Although, some studies showed the most increased inhibition diameter zone for *C. zeylanicum* than other EOs against some microorganisms such as *E. coli* [42]. Moreover, different antibacterial activity has been demonstrated for EOs. The growth inhibition diameter for *Z. multiflora* measured 6.6 ± 0.1 and 6.4 ± 0.1 mm for *S. typhimurium* and *E. coli*, respectively [43]. In another study, *Z. multiflora* and *C. zeylanicum* growth inhibition in agar disk diffusion were recorded at 22.5 ± 1.3 mm and 32.3 ± 0.5 mm against *S. typhimurium*, which may ascribe to different constituents of EOs [44]. MIC of *C. zeylanicum* recorded 2500 against *S. typhimurium*, similar to our study [45]. The higher antibacterial potency observed for *C. zeylanicum* in this study may be attributed to high levels of carvacrol which act as a strong antibacterial agent. The presence of a hydroxyl group in the structure of carvacrol is critical for its antibacterial effects. The hydroxyl group provides a special ability to interact with bacterial membrane. Along with the hydrophobicity of carvacrol, it can damage the structure of the bacterial membrane, which cause an increase in its permeability, followed

by bacterial death [46]. Thus, a high amount of carvacrol in *Z. multiflora*, justifies its potent antibacterial effect in this study. Moreover, the synergistic effects of carvacrol and thymol may play a key role in the antibacterial properties of *Z. multiflora* EO.

3.3. SPI-PVA Nanofiber physical characteristics.

3.3.1. SEM results of SPI/PVA.

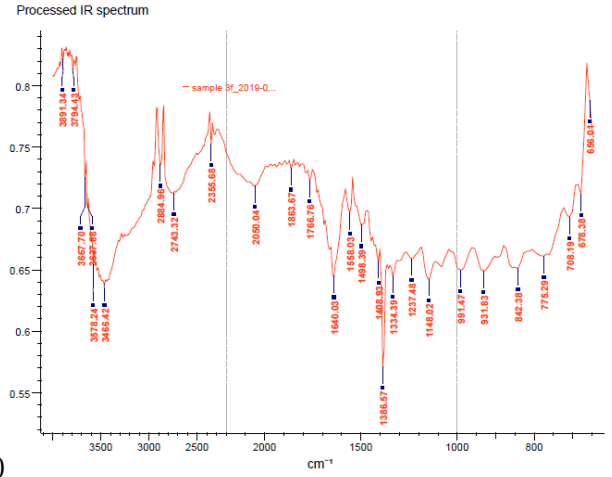
The structure of the nanofibers under different amount of SPI/PVA was presented by SEM images in Figure 1. Although the pure soy protein isolate solution has low surface tension and high electrical conductivity, this solution was not spinnable. On the other hand, it was not able to apply the higher SPI concentrations within the electrospinning process because the tip of the needles was drying immediately [47]. Hence, it can be stated that the complex network structure of pure soy protein has not allowed for fabricating the nanofibers because the pure protein has not had adequate entanglements to form the fibers. Nanofibers were formed by decreasing the SPI ratio and increasing the PVA ratio (90/10). By increasing the ratio of PVA (80/20, 70/30, 60/40, and 50/50) nanofibers' morphology changed from beaded to a uniform structure. It is important and necessary to evaluate the nanofiber diameters to determine the uniformity of the fibers because increasing the concentration of polymer influenced the nanofiber's diameters significantly. SPI/PVA nanofibers with a ratio of 60/40 and 50/50 produced good nanofibers with breakage at some points, low diameter, and a few aggregations. By increase of SPI content, the mean nanofibers diameter from 130 nm in 50/50 (SPI/PVA) to 181 nm in 60/ 40 (SPI/PVA), 230 nm in 70/30 (SPI/PVA), 308 nm in 80/20 (SPI/PVA) and 365 nm in 90/10 (SPI/PVA) were increased. Also, it can be seen that by an increase of SPI in the solution, the homogeneity was reduced, and the diameter of SPI/PVA nanofibers was increased. Therefore, the most uniform and homogenous SPI/PVA nanofibers at a 50/50 rate and low homogenous and high diameter at a 90/10 rate of SPI/PVA were obtained. Cho *et al.* [48] reported similar results and demonstrated that the mean diameters of nanofibers had been increased from 0.6 ± 0.2 to 4.5 ± 1.5 μm when the amount of soy protein content was increased from 9 to 13 wt%; this can be described by increasing the viscosity of the solution. Also, Maftoonazad *et al.* [49] obtained the same agreement results as our study about the properties of the SPI/PVA nanofibers. With the increasing amount of SPI, nanofibers' diameters were increased, and the homogeneity of nanofibers in high SPI was decreased. However, contradictory results with our study were observed in PVA/SPI hybrid nanofiber membranes produced by [50].

3.4. FTIR spectroscopy FTIR study of SPI-PVA.

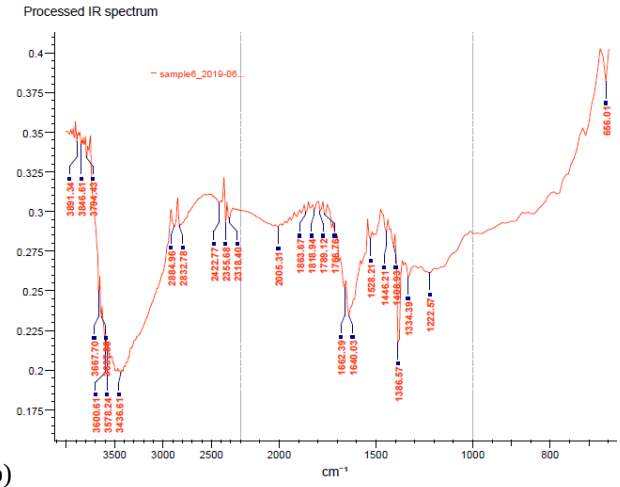
FTIR spectra analysis for SPI and PVA nanofibers are presented in Figure 2. In the case of the pure SPI sample, the wide absorption and sharp peak in the range of 3466 cm^{-1} were recorded because of the O-H bonds. This can be ascribed to the intermolecular hydrogen bonds and their stretching vibrations of the OH groups as well as the intramolecular free OH groups, which were adsorbed by water molecules. Also, there is a peak at 1654 cm^{-1}, assigned to C=O, a peak at 1537 cm−1 associated with N-H, and another peak at 1240 cm−1, ascribed to C-N bonds.

Furthermore, For the PVA sample, the FTIR spectra analysis recorded the peak for the stretching vibrations of O-H groups around 3300 cm^{-1}, and the spectra band from 2884 cm^{-1} to 2832 cm^{-1} was ascribed to C-H stretching vibrations from alkyl groups. The peak of C=O

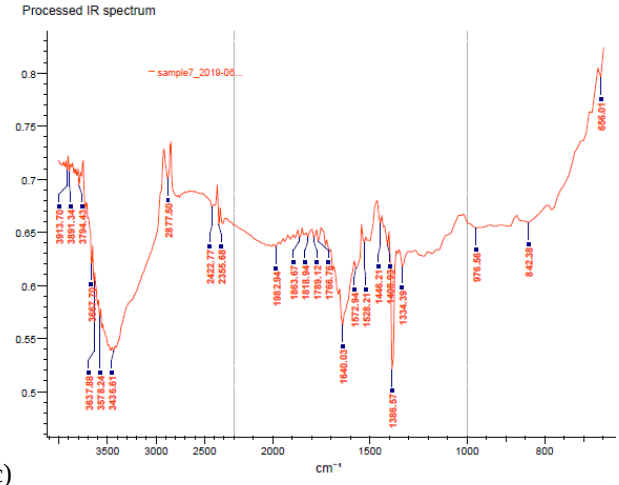
stretching of nonhydrolyzed vinyl acetate groups was recorded around 1766 cm−1. All absorbed peaks were detected for the samples of SPI/PVA, which showed that the electrospinning process effectively developed hybrid nanofibers. Because of the reaction between the PVA and SPI, the intensity of the O-H was considerably reduced from 3600 to 3000 cm−1. Also, the other recorded peaks indicated that intermolecular hydrogen bonds between SPI and PVA were possibly formed. Similar results were observed in PVA/SPI hybrid nanofiber membranes fabricated by Fang, Zhu, Yu, Sui, and Yang [50].



(a)



(b)



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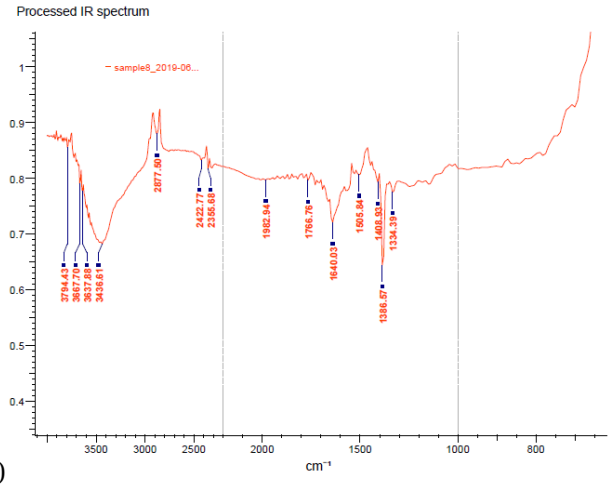

(d)

Figure 2. FTIR spectra of (**a**) Soy; (**b**) Soy/PVA; (**c**) Soy/Pva/ Zm 20%; (**d**) Soy/Pva/Cz 20%.

3.5. TGA.

Figure 3 shows thermal gravimetric curves for Soy/PVA, Soy/PVA/*Cinnamon zeylanicum*, and Soy/PVA/*Zataria multiflora* nanofibers. For all samples, thermal degradation was associated with three stages. In the beginning, the weight of samples was reduced because residual moisture was evaporated (below 200 °C). The second stage of thermal degradation was ascribed to the thermal degradation of polymers from 250 to 400 °C. Finally, the third phase starts at 400 and ends at 600 °C, which was attributed to the carbonization of polymeric materials.

Furthermore, by adding Cz and Zm EOs, the weight reduction percentage was increased, demonstrating that the additional content of Cz and Zm decreased the thermal stability of the nanofibers.

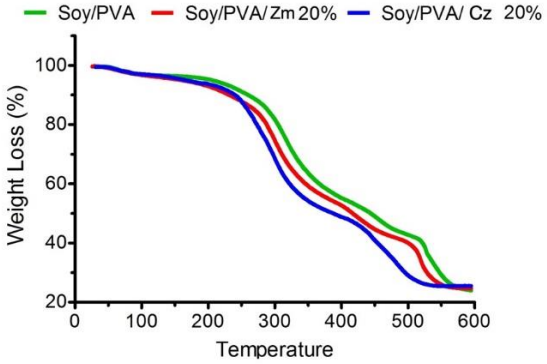

Figure 3. Thermal gravimetric (TG) curves of Soy/PVA, Soy/PVA/Zm and Soy/PVA/ Cz nanofibers.

Table 1. Inhibition zones (mm) of Soy/PVA nanofibers using ATCC 100 standard method.

Fibers	EOs	*E. coli*	*S. typhimurium*	*L. monocytogenes*	*B. cereus*	*S. aureus*
SPI/PVA	BLANK	0 [a]	0 [a]	0 [a]	0 [a]	0 [a]
SPI/PVA	%20 Zm	6 [b]	8 [b]	12 [b]	16 [b]	10 [b]
SPI/PVA	%20 Cz	7 [b]	5 [c]	10 [c]	13 [c]	8 [c]

Zm: *Zataria multiflora* essential oil; Cz: *Cinnamon zeylanicum* essential oil

Table 2. The antimicrobial activities of electrospun nanofibers in term of reduction (%) of colonies (CFU) against *S. aureus*, *B. cereus*, *E. coli* and *S. typhimurium*.

Fibers	EOs	*S. aureus*	*B. cereus*	*E. coli*	*S. typhimurium*
SPI/PVA	BLANK	12 [a]	8 [a]	----------	----------
SPI/PVA	20% Zm	68 [b]	50 [b]	42 [a]	35 [a]
SPI/PVA	20% Cz	72 [c]	56 [c]	38 [b]	42 [b]

Zm: *Zataria multiflora* essential oil; Cz: *Cinnamon zeylanicum* essential oil

3.6. Antibacterial activity of nanofibers.

Antibacterial properties of SPI/PVA nanofibers loaded with 20% *Cinnamon zeylanicum* and 20% *Zataria multiflora* and EOs were assessed using the ATCC 100 standard method and percent reduction of colonies. As presented in Table 1, SPI/PVA nanofibers without EOs showed no antibacterial effects on *L. monocytogenes*, *E. coli*, *S. aureus*, *S. typhimurium*, and *B. cereus*. While nanofibers incorporated with EOs exhibited well results. Overall, SPI/PVA nanofibers with *Z. multiflora* were more effective than the other EO, except for *E. coli*. The most susceptible pathogen to *Z. multiflora* was *B. cereus*, with an inhibition zone of 16 mm. The most resistant bacterium to *C. zeylanicum* was *S. typhimurium*, with a 5 mm diameter. The data obtained by the percent reduction of colonies are shown in Table 2. SPI/PVA nanofibrous films without EOs showed a slight reduction for *S. aureus* and *B. cereus* (12% and 8%, respectively). Still, it was ineffective against *E. coli* and *S. typhimurium*. 20% *C. zeylanicum* incorporated with SPI/PVA nanofibers exhibited more antibacterial activity compared to 20% *Z. multiflora*. The only exception was *E. coli* which *Z. multiflora* was more effective. The most reduction was demonstrated against *S. aureus* affected by 20% *C. zeylanicum* nanofibers (72% reduction).

Similar results were observed by other researchers. No antibacterial activity for crosslinked electrospun polyvinyl alcohol nanofiber was recorded toward *S. aureus* and *E. coli* [51]. Charernsriwilaiwat *et al.* [52] stated no impact for electrospun PVA nanofibrous films toward *S. aureus* and *E. coli*. However, pure electrospun PVA nanofibers showed a slight antibacterial impact on *S. aureus* and *E. coli* in the examination of Sekar *et al.* [53]. Some combination of electrospun nanofibers exhibits strong antibacterial properties, even without EOs. Zhang *et al.* [54] embedded silver nanoparticles into polyvinyl alcohol by using an electrospinning technique and reported its severe antibacterial activity.

The incorporation of electrospun nanofibers with EOs has recently drawn researchers' attention. Vafania, Fathi, and Soleimanian-Zad [28] fabricated chitosan/gelatin electrospun nanofibers embedded with thyme and asserted that mentioned nanofiber showed antibacterial activity against *C. perfringens*. In another study, electrospun sodium alginate/PVA was incorporated with three different EOs, including cinnamon, clove, and lavender, to investigate the antibacterial [55]. They asserted that cinnamon's best antibacterial activity was observed and exhibited the largest growth inhibition zone for microorganisms. Moreover, two studies used cinnamaldehyde with nanofibers. It incorporated chitosan/poly(ethylene oxide) to fabricate electrospun nanofibers, which were effective toward *P. aeruginosa* and *E. coli* [56]. Cinnamaldehyde was also embedded with zein to form electrospun nanofibers and showed antibacterial activity [57].

In addition, some studies applied nanofibers with EOs in food models. Lin *et al.* [58] electrospun thyme EO, gelatin nanofibers, and fabricated nanofibers. They were then applied in chicken to increase the durability as well as enhance the microbial feature. The data showed that electrospun thyme EO and gelatin nanofibers effectively reduced aerobic bacterial count and *Campylobacter jejuni*. PVA was used to fabricate nanofibers containing cinnamon to increase food product shelf life. It revealed that electrospun PVA/cinnamon nanofibers were effective, reduced the amounts of E. coli and S. aureus in mushrooms, and increased their shelf life period [51].

Moreover, PVA/cinnamon, fabricated by the electrospinning technique, was utilized to increase the storage duration of raw shrimp [59]. This nanofiber could decrease the total

bacterial count, *E. coli*, *P. aeruginosa*, and *S. aureus* count in shrimp during seven days of storage. Moreover, poly(ethylene oxide)/cinnamon nanofibers were utilized in meat, and antibacterial properties were demonstrated [60].

4. Conclusions

In this study, the electrospinning method has been used to fabricate SPI/PVA nanofibers incorporated with *Z. multiflora* and *C. zeylanicum* EOs. The morphology and structure of SPI/PVA nanofibers were analyzed by SEM and FT-IR. Produced SPI/PVA nanofibers with a ratio of 60/40 and 50/50 showed good properties of nanofibers with a low diameter and a few aggregations. The antibacterial activity of fabricated nanofibrous films was evaluated, and great outcomes were observed. SPI/PVA nanofibers incorporated with 20% *Zataria multiflora* EO exhibited a 16 mm inhibition zone diameter for *B. cereus*. Moreover, SPI/PVA nanofibers incorporated with 20% *Cinnamon zeylanicum* reduced 72% of *S. aureus* colonies. Overall, *Cinnamon zeylanicum* showed better antibacterial effects. The authors recommended further studies on the fabrication of nanofibers with other edible polymers and essential oils to obtain bioactive materials with antibacterial and antioxidant effects. Other characteristics of electrospun nanofibers, such as hydrophobicity, compatibility with the environment, and other criteria, should be noticed. It is worth mentioning that the adverse effects of these nanofibers on the human body must be considered prior to application as bioactive food packaging agents. We suggest further studies to evaluate the beneficial effects of electrospun nanofibers on foods such as meat, shrimp, fish, etc.

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Conflicts of Interest

The authors declare no conflict of interest.

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