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Fabrication of Mandarin (*Citrus reticulate L.*) peel essential oil and nano-calcium carbonate incorporated polylactic acid/polyvinylpyrrolidone electrospun webs

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ABSTRACT: Fibrous materials from polylactic acid (PLA) and polyvinylpyrrolidone (PVP) containing mandarin peel essential oil (MPEO) and/or nano-calcium carbonate were prepared by electrospinning process. The electrospun webs were characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and microbiological tests. IR spectra results indicated the existence of interaction among additives and polymer matrix. Obtained SEM results showed that incorporation of essential oil to the polymer matrix have a remarkable effect on the fiber morphology. Essential oil incorporation increased the fiber diameters. The electrospun composite webs were ineffective against *Acinetobacter baumannii, Bacillus subtilis, Escherichia coli, Enterococcus fecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Keywords: Citrus essential oil, electrospinning, nanofibers, bioactive polymer composite

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INTRODUCTION

The advances in electrospinning in recent years have led to various studies dedicated to the improvement of nanofibers which present a bright future for several industrial applications (Kohsari et. al. 2016). One of the widely used areas of electrospun nanofibers is biomedical applications. In most biomedical application antibacterial materials are needed to combat bacterial infections.

As a promising bio-renewable polymer, polylactic acid (PLA, $[CH(CH_3)COO]_n$) is a hydrophobic, biocompatible and biodegradable polyester (Maroufi et al. 2021). It is preferred to be used in multipurpose biomedical applications because the degraded monomers of PLA can be removed from the body via metabolic pathways (Ahmadian et al. 2020). Additionally, PLA has good electrospinning processability resulting in nanofibrous webs with good mechanical features. The hydrophobic nature of PLA could provide good interaction with essential oils or lipophilic drugs (Sinsup et al. 2021). However, PLA should be modified to adjust its wettability to enhance biomedical performance based on the application (Bulbul et al. 2019). Therefore, the combination of PLA with hydrophilic polymers seems to be an effective strategy (Hou et al. 2021). Among hydrophilic polymers, polyvinyl pyrrolidone (PVP) is also a good candidate for diverse bio-related applications due to its unique properties. It is a biocompatible, non-toxic as well as water-soluble polymer (Güler et al. 2019). Therefore, much attention has been drawn to PLA/PVP hybrid fibers that exhibits the combination of properties of each individual polymer (Bulbul et al. 2019). Furthermore, poloxamers, known as kolliphors or pluronics, can enhance the hydrophilicity feature in polymer systems (Oliveira et al. 2019).

Electrospun nanofibers can be activated with the addition of bioactive compounds (Mohammadi et al. 2021). Essential oils are aromatic oils derived from different parts of plants that have been properly investigated over the past decade due to their biological activities (Güler et al. 2019). Mandarin (*Citrus reticulate*) peel, an agro-industrial by-product, is evaluated as a good essential oil source (Abdal-Aziz et al. 2019). *Citrus reticulate* essential oil is considered as generally recognized as safe (GRAS) by the US Food and Drug Administration (FDA) with showing antioxidant and antimicrobial activities (Mahdi et al. 2021).

In addition, there is an increasing interest to combine nanofillers and essential oils to develop composite electrospun fibers with improved performances as well as antibacterial activities. Zinc oxide (Amjadi et al. 2020), magnesium oxide (Eghbalian et al. 2021), and silver (Sofi et al. 2019) nanoparticles are among the investigated ones.

The aim of the present contribution was to prepare novel electrospun webs based on PLA/PVP matrix incorporated with organic (Mandarin essential oil) and inorganic (nano-calcium carbonate) additives. Kolliphor was used as a non-ionic biocompatible surfactant. The influence of the composition of the webs on the structural, morphological and biological behavior upon contact with pathogenic microorganisms (bacteria: *Acinetobacter baumannii, Bacillus subtilis, Escherichia coli, Enterococcus fecalis, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Staphylococcus aureus,* yeast: *Candidatropicalis, and Candida parapsilosis*) was examined.

MATERIALS AND METHODS

Materials

PLA filaments were purchased from ABG plastics (İstanbul, TÜRKİYE), and PVP powders (Mw ~ 1 300 000 g mol^{-1}) and Kolliphor RH40 were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Dichloromethane (DCM) and N,N-Dimethylformamide (DMF) were

also purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Nano-$CaCO_3$ (50 nm average particle size, 24 $m^2 g^{-1}$ BET surface area, 99 % purity) was a kind gift from Adaçal Industrial Minerals Company (Afyon, TÜRKİYE).

Experimental procedure

Extraction of mandarin essential oil

Mandarins were collected from Köyceğiz region of Muğla, Türkiye in 2021. Hydrodistillation was used for the extraction of mandarin essential oil from the fresh peels. After subjecting the peels (200 g) to hydrodistillation for 2 hours in distilled water, the essential oil was collected and dried with anhydrous $MgSO_4$. The essential oil was put into sealed vials and stored in a refrigerator (4 °C) until experiments.

Preparation of the fibrous webs

An electrospinning machine (INOVENSO NE300 Multinozzle Electrospinning Machine, Turkey) was used to fabricate PLA/PVP fibrous blend webs. PLA filaments were dissolved in a binary-solvent system of DCM and DMF (1:1 v/v) to obtain a 10% (w/v) PLA solution by stirring at room temperature overnight. Meanwhile, PVP powders were dissolved in DMF to obtain a 15% (w/v) PVP solution by stirring at room temperature overnight. Then, PLA and PVP polymer solutions were mixed to prepare a blend solution of 4/1 (v/v). $CaCO_3$ (3 wt%) was added to the blend polymer solution in proportion to the total mass of polymer and mixed until the solution was homogeneous. Thereafter, the quantity of mandarin peel essential oil (MPEO) in the polymer was calculated to be 10% of the total polymer weight. Once the homogenous polymer solution was prepared, it was placed in a plastic syringe (10 mL). The webs were electrospun at 26 kV voltage and 2 mL h^{-1} flow rate with a rotating collector spinning at 200 rpm. The distance between the tip and the collector was 122 mm, and the collection was covered with aluminuim foil (Fig.1).The residual solvent was evaporated overnight by placing the nanofibers in a fume hood. The process parameters were the same as for the neat blend polymers. The compositions of fibers were given in Table 1. The samples were coded as PLA, PLA-PVP, PLA-PVP/$CaCO_3$, PLA-PVP/MPEO, and PLA-PVP/$CaCO_3$/MPEO, respectively.

Table 1. The compositions of PLA / PVP based electrospun web

Sample code	PLA (%v/v)	PVP (%v/v)	Kolliphor (%w/v)	Mandarin essential oil (%v/v)	Nano-$CaCO_3$ (%w/v)
PLA	80	-	3	-	-
PLA-PVP	80	20	3	-	-
PLA-PVP/$CaCO_3$	80	20	3	10	3
PLA-PVP/MPEO	80	20	3	-	-
PLA-PVP/$CaCO_3$/MPEO	80	20	3	10	3

Figure 1. Schematic representation of the fabrication of bioactive composite electrospun webs

Characterization of the fibrous webs

Scanning electron microscopy

The nanofiber surface morphologies were examined using a Carl Zeiss/Gemini 300 Scanning Electron Microscope (SEM) (ZEISS Ltd., Germany) with a 10 kV voltage. Before analysis, the samples were coated with gold for 20 minutes. The diameters of the fibers were measured using Image J (version 1.520 software) by selecting 100 individual fibers at random for each sample.

FT-IR spectral analysis

To investigate the formation of interactions between polymers, mandarin peel essential oil (MPEO), nano-$CaCO_3$, and also nanofibers were evaluated using a Thermo Nicolet iS50 FT-IR (USA) spectrometer with an ATR (Attenuated Total Reflectance) adaptor (Smart Orbit Diamond, USA). The frequency of all fibers in the range of 4000–500 cm^{-1} was recorded with 16 scans at 4 cm^{-1} resolution. The spectra were performed with the Omnic 9 program.

Antimicrobial assay

The agar well diffusion method and spectrophotometric microbroth dilution were performed to determine the inhibition zone (IZ) and minimum inhibitory concentration (MIC), respectively.

The 0.1 mg of electrospun fibers were dissolved in 1 mL of chloroform. The inoculums of *Escherichiacoli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus fecalis*, *Klebsiellapneumoniae*, *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus*, *Acinetobacterbaumannii* bacteria strains in Tryptic soy Broth and *C. tropicalis*, *C. parapsilosis* in Sabouraud dextrose broth were prepared and incubated at 37ºC overnight. The microbial suspensions were adjusted to 0.5 McFarland Standard and stored at +4ºC until tests. The ampicillin (AMP) for bacteria and fluconazole (FLC) for yeast were used as a positive control. Chloroform was used as negative control. To determine IZ, microorganisms were spread onto Mueller Hinton Agar plates using sterile swab sticks. Then, 6 mm-wells were drilled in the middle of the plate, and 40 µL of fiber solutions were poured into it. After the microorganisms were incubated at 37ºC for 24 h, the clear zone was measured using a digital caliper. For spectrophotometric microbroth test, 50 µL of Mueller Hinton Broth medium was added to 96-Well Microtiter. Then, a two-fold serial dilution of 50 µL of fibers was carried out over all the eight lines of the plate. Negative (sterile distilled water) and positive controls was added in columns 11 and 12 of each line, respectively. Finally, 10 µL of each microorganism was added to the wells and incubated at 37ºC for 24 hours. Then microbial turbidity was measured at 615 nm and the experiments were triplicated. MICs were evaluated by inhibition of visible growth (Erdoğan Eliuz, 2021).

Chloroform, which was used as a negative control in the study, had no antimicrobial effect against pathogens.

RESULTS AND DISCUSSION

Morphology and Mean Fiber Diameter of the Fibrous Webs

The evaluation of the microstructures of the electrospun webs is considered as one of the most significant characterizations (Bulbul et al. 2019). Variations in the initial polymer composition can remarkably influence the fiber diameter and morphology (Parin et al. 2021a; Parin et al., 2021b; Parın and Yıldırım, 2021). The morphology and size distribution of fibers in the PLA/PVP hybrid webs are represented in Figure 2. PLA and PLA-PVP fibers had a smooth and beadless fiber morphology without any cracks and pores (Fig. 2. a1 and b1). The average diameter of PLA fibers was 452.12 ± 114 nm. The average diameter was increased to 951.9 ± 253 nm with PVP addition compared to neat PLA fibers. This result is consistent with the study of Bonan et al. (2015) about poly(lactic acid) (PLA) and polyvinylpyrrolidone (PVP) micro- and nanofiber webs developed by solution blow spinning.

It is clearly observed that $CaCO_3$ nanoparticles were present as agglomerates on some of the fiber surfaces (Fig.2. c1 and e1). The diameter of PLA-PVP fibers with $CaCO_3$ addition was determined as 796.9 ± 231 nm. The addition of $CaCO_3$ to PLA-PVP matrix slightly decreased the fiber diameter.

SEM micrographs revealed that there were significant differences in the morphology of the PLA-PVP/MPEO and PLA-PVP/$CaCO_3$/MPEO fibers. The addition of essential oil caused the fibers to stick together (Fig.2. d1 and e1). Similarly, a sticky PVP/Gelatine electrospun mat was obtained at 5 wt % thyme essential oil incorporation (Çallıoğlu et al. 2019). The average fiber diameter of PLA-PVP/MPEO and PLA-PVP/$CaCO_3$/MPEO samples were 1358 ± 453 nm and 1002.5 ± 319 nm, respectively. The addition of essential oil into spinning solutions resulted in larger diameters of the fibers, as previously reported in zein fibers by incorporation of rosemary essential oil (Hosseini et al. 2020). Also, the result is consistent with the study of Zhou et al. (2020) on Angelica essential oil loaded electrospun gelatin nanofibers.

IR-spectra of the fibrous webs

FT-IR spectra of the webs were obtained to evaluate all the possible interactions between the polymers and the additives (Figure 3).The characteristic peaks of PLA are observed as 1746 cm^{-1} (-C = O strength vibration), 1380 (-CH bonds asymmetric strength vibration), 1358 (-CH bonds symmetric strength vibration), 1266 cm^{-1} (-C = O bending vibration), 955 cm^{-1} (C-C groups) and 754 cm^{-1} (-C = O torsion vibration) (Sepahi et al. 2021). Additionally, the absorption peaks located at 1127, 1042 and 867 $cm^{-1}$are attributed to the strength vibration of C-O groups. FT-IR spectra comparison of PLA-PVP and PLA fibers clearly show the presence of PVP structure in blend fibers. The PLA-PVP fiber spectrum exhibits the dominant peak of PVP at 1663 cm^{-1} related to the C=O stretching vibration (Wang et al. 2022). Small peaks at 2872, 647, and 576 cm^{-1}which were absent in PLA; however, available in PVP pointed out that there was an association between PLA and PVP. These peaks are also in accordance with the studies of Ranimol et al. (2021) who reported similar results for neat PVP. A slight shift was noted in the peak from 1269 cm^{-1} to 1291 cm^{-1} upon essential oil incorporation to the PLA-PVP fiber. Moreover, the small peak between 696-591 cm^{-1}showed the interaction of essential oil with the polymer matrix in the FT-IR spectra of PLA-PVP/MPEO.

FT-IR analysis results verify the existence of $CaCO_3$ in the structure of the nanofibers. The increase in peak intensities at 1452 and 867 cm^{-1} is the evidence which ascribed to -CH_2 bending stretching and β-1, 4 glycosidic bonds, respectively (Terzioğlu, 2021).

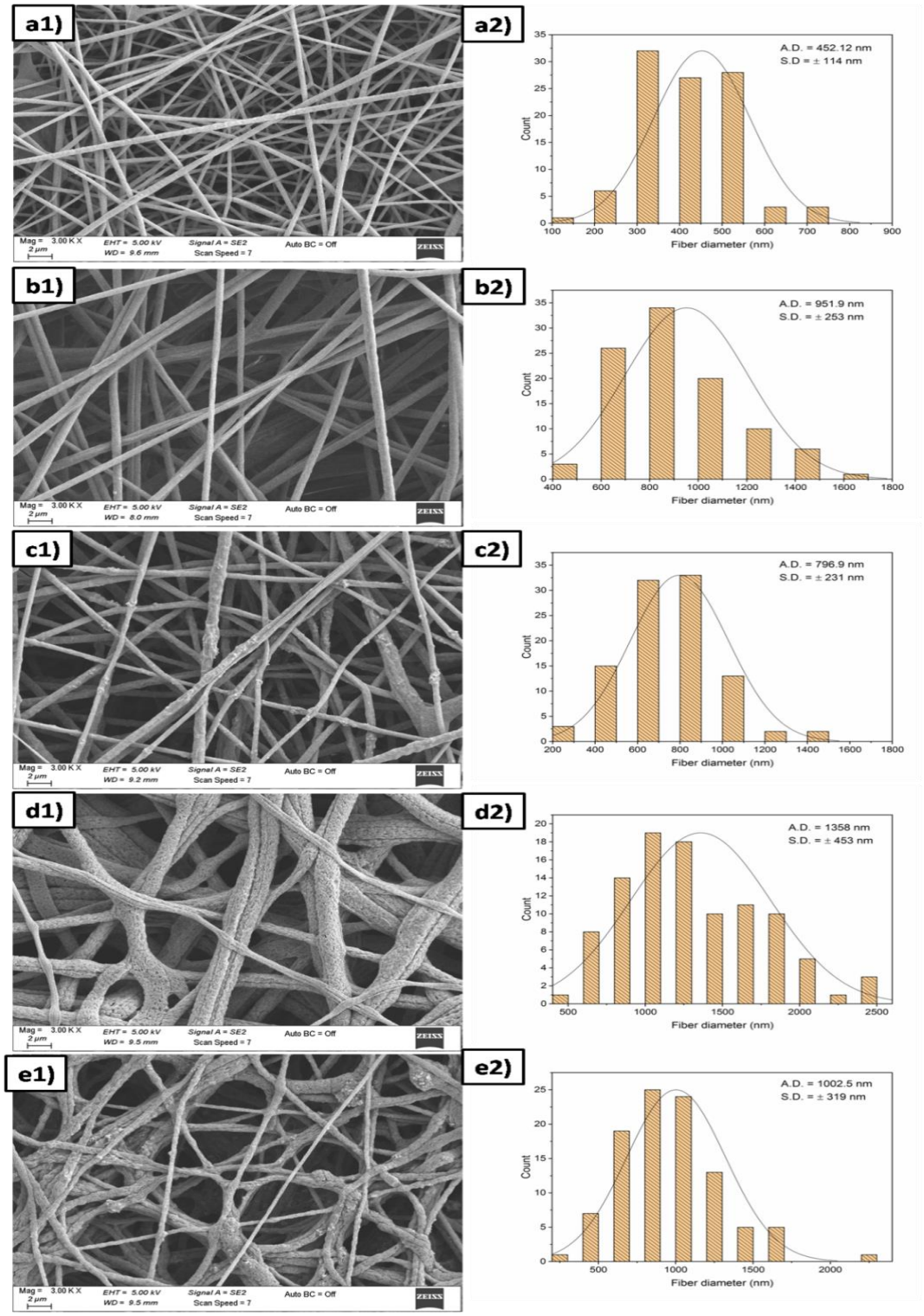

Figure 2. SEM micrographs of electrospun webs a1) PLA, b1) PLA-PVP, c1) PLA-PVP/$CaCO_3$, d1) PLA-PVP/MPEO, e1) PLA-PVP/$CaCO_3$/MPEO (Magnification: 3 kX, scale; 2 µm) and fiber diameters (a2-e2)

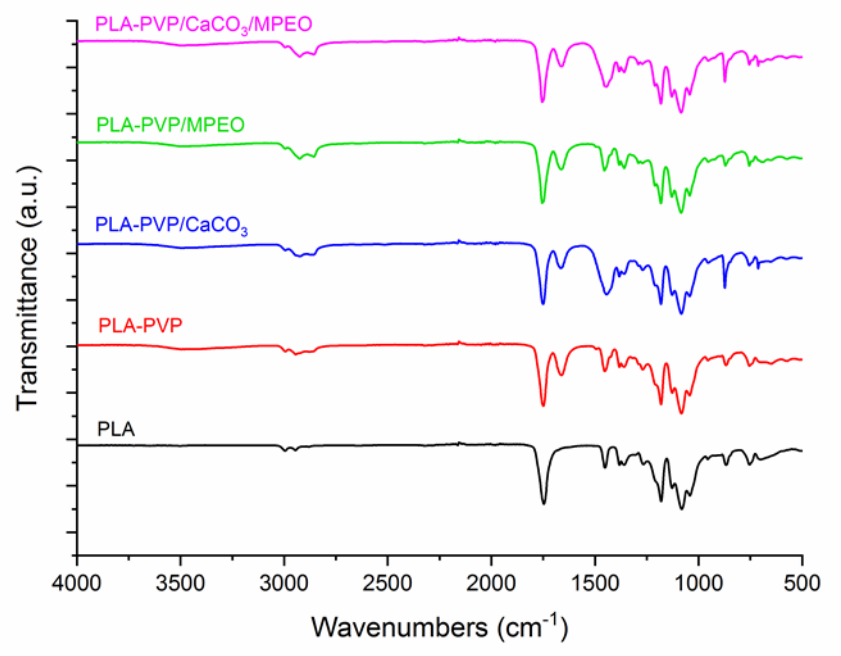

Figure 3. FT-IR spectra of electrospun webs

Antimicrobial activity of the fibrous webs

Fruit peels are considered as valuable raw materials due to including various bioactive compounds and being low-cost materials. Essential oils are among the components present in agro wastes which may be a natural and safe source of antimicrobials (Saleem and Saeed, 2020). Various studies reported the antimicrobial potential of essential oil of *Citrus reticulate* L.peels against different microorganisms (Viuda-Martos et al. 2008, Song et al. 2020). In recent years, research studies focused on the development of mandarin essential oil loaded polymer-based antimicrobial materials (Song et al. 2020, Yabalak et al. 2021). Unfortunately, the fibers obtained in this study did not show any antimicrobial effect. This may be due to insufficient concentration of *C. reticulata* studied or minimal effects of solvents.

CONCLUSION

Electrospinning provides a convenient platform to incorporate bioactive agents to fabricate active non-woven webs. In this research, bioactive composite fibers were successfully produced based on PLA/PVP, mandarin peel essential oil and nano-$CaCO_3$ by electrospinning. The fabricated composite fibers were characterized with SEM, FT-IR and antibacterial analyses. The incorporation of mandarin peel essential oil and nano-$CaCO_3$ remarkably affected the morphology of fibers. Slight changes were observed in the FT-IR spectra of essential oil and nano-$CaCO_3$ loaded fibers compared to neat PLA-PVP fibers indicating the interaction of additives and polymer matrix.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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