Celal Bayar University Journal of Science

Preparation and Characterization of Polylactic Acid Based Nanofiber Loaded with Tangerine Peel (*Citrus Unshiu*) Essential Oil

Tuğba Güngör Ertuğral*[1], Sevim Akçura[2]

[1]Department of Food Technology, Faculty of Canakkale Applied Sciences, Canakkale Onsekiz Mart University, Türkiye
[2]Department of Field Crops, Agriculture Faculty, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye
*tugbagungor@comu.edu.tr
*Orcid No: 0000-0002-1306-3399

Received: 27 July 2023
Accepted: 22 September 2023
DOI:10.18466/cbayarfbe.1333674

Abstract

Electrospinning method used in nanofiber production is an economical method applied with high voltage electricity. Essential oils obtained from natural sources have antioxidant, antimicrobial and antifungal properties and are preferred for production of nanomaterials by electro-spinning method. In this study, tangerine (*Citrus unshiu*) peel essential oil (TPEO) was obtained by hydro-distillation method and D-Limonene (77.45%) was one of 10 main components in Gas Chromatography Mass Spectrometry (GC-MS) analysis. The composite nanofiber was prepared with 25% TPEO and polylactic acid (PLA). Nanofibers were characterized by Scanning Electron Microscopy (SEM) and Thermogravimetric Analysis (TGA). Essential oil is composited with prominent spheroidal structures in PLA nanofiber according to SEM results, and nanofiber diameter obtained is approximately 76.8 nm. Its decomposition temperature is about 324.8°C. Nanofibers obtained from tangerine peels and their antibacterial properties, can be recommended use for food preservation and medicine fields.

Keywords: Tangerine peel, Hydro-distillation, Essential oil, PLA, Electrospin, Food preservation, Nanofiber.

1. Introduction

Due to slow degradation rate of waste plastic materials, environmental problems have increased interest in biodegradable polymers [1]. Prevention of increasing environmental pollution and necessity of foodstuffs to have a long shelf life for many reasons have made biopolymer and edible films attractive in food and packaging industry [2]. Polylactic acid (PLA), obtained from corn, beet and cane products, is a lactic acid condensation polymer with catalytic ring opening method, and it is preferred in food packaging applications for than hydrophobic and biodegradable [3,4]. Natural bioactive agents can be an alternative to synthetic products in food industry [5]. Important advantage of electrospinning technique is large surface area, nanometer fine fiber production, good mechanical properties and ease of processing [6]. Adjusting (reducing) voltage in applications can affect the targeted structures and density, and it is possible to determine some structure of nanofibers in a short time by observing the electrical current data [7]. As a protective materials and sensors nanofiber reinforced composites, where electrospinning technique is used, are important application areas [8].

There is evidence in literature that antimicrobial additives can effective as food additives when used for food packaging materials [9].

Stable shapes are formed by electrically charging the suspended polymer solution droplets between flat plate and electric potential difference. These stable shapes are due only to electric forces balance and surface tension in non-viscous, Newtonian and viscoelastic fluids. The fluid takes a conical and known as Taylor cone [10].

Colorless, volatile, strong-smelling, natural products essential oils, called essential oils or essential oils that are obtained from leave, fruit, bark, root please of plants [11]. Antimicrobial and antioxidant properties of essential oils are used in food packaging. Antimicrobial effect of hydrosols obtained from orange grown in three different regions was investigated against both Gram (+) and Gram (–)

Celal Bayar University Journal of Science
Volume 19, Issue 3, 2023, p 283-287
Doi: 10.18466/cbayarfbe.1333674

bacteria. Hydrosols and essential oils obtained from Dalaman, Köyceğiz and Finike oranges showed inhibitory properties against *Staphylococcus aureus* and *Escherichia coli* bacteria. [12]. Essential oils obtained from tangerine peel extract feature as a glazing material in cold preservation of fish. Essential oils obtained from *Citrus reticulata*, *Citrus bigarradia* and *Citrus sinensis* can be effective in aquaculture preservation [13]. Antioxidant extracts obtained from herbal material are important research areas [14]. In different uses of essential oils, for example, peppermint and peppermint essential oils against weeds, bio-herbicides can replace chemical herbicides [15]. Distillation methods are divided into 3 as hydro-distillation distillation (Hydrodistillation - HD), steam distillation and vacuum distillation [16]. Microwave assisted hydro-distillation method can also be used to obtain essential oil by adjusting microwave parameters [17]. Hydro-distillation method was successful in examining to components of Anatolian Sage (*Salvia fruticosa Mill*.) essential oil.[18]. hydro-distillation is a process performed with a Clevenger type apparatus and is carried out in retorts in industrial applications [16].

The basis of method; balloon Neo-Clevenger assembly is placed in heated jacket and vertical graduated glass tube is connected to glass balloon at bottom and upper part to cooler system, and pure water is filled into graduated and inclined tube and hydro-distillation is performed for approximately 2 hours after cooling system and heater are turned on. After essential oil is separated from aqueous phase, it is determined in ml and amount of essential oil in a 100 g sample is calculated as a percentage (%) according to sample amount (g) weighed [18, 19].
In this study, essential oil composition obtained from tangerine (*C. unshiu*) peels by hydro-distillation was investigated by GC-MS analysis and composite nanofibers were formed with this obtained essential oil and PLA using electrospinning method. Nanofibers formed were characterized.

2 Materials and Methods

2.1. Samples and Reagents

Poly(lactic acid) (PLA) (Mn=160000 g/mol) was purchased from Natureworks LLC (4043 D Nebraska, USA) and NN-Dimethylformamide - anhydrous, 99.8% (DMF) and n- hexane 99% were purcased from Sigma-Aldrich (Taufkirchen Germany) and deionized water. October 2022 harvest tangerines were obtained from Antalya / Turkey. Tangerine peel essential oil was obtained by hydro-distillation method. Essential oil of tangerine was achieved with Modified-Clevenger device according to water vapor distillation principle.

2.2. Methods

2.2.1. Essential Oil Extraction

Fresh tangerine peels were washed and peeled to cut into pieces of 1.5x1x1 cm in size and added directly to a transparent flask that was containing distilled water at room temperature. Obtaining essential oil was done with SH-Clevenger device according to water vapor distillation principle. 242 g of tangerine peels were taken, crushed and placed in a swollen balloon, 900 mL of deionized water was added, and distillation was carried out for 3 hours . The extracted distillate was stored in a closed glass tube and wrapped in aluminum foil [20].

2.2.2. GC-MS Analysis Of Tangerine Peel Essential Oil

TPEO components were determined by Shimadzu GC/MS QP-2010. Esesential oil sample was analyzed with HP-88 100m(length)-(0.20µm thickness, 0,25mm diameter) column. Column oven temperature is 100 °C and injection temperature was 220 °C and 30 split ratio. Carrier gas was He Prim Press. (500-900) and 232.8 kPa. Total flow was 37.2 mL/min and column flow was 1.10 mL/min. Mass Spectrometer ion source and interface temperatures were 230 °C and 250 °C. Solvent cutoff time was 10 min. [21].

2.2.3. Preparation of the PLA/TPEO Electrospun Nanofiber

Nanofibers were prepared according to method specified by Zhang et al. Before electrospinning, 0.4 g of PLA was added to 5 ml of DMF (v/v) and dissolved [22]. After obtaining a homogeneous mixture, 1 g of essential oil was added and mixed again for 1 hour. Solution placed in a 5 ml syringe was attached horizontally to electrospinning device and PLA/EO nanofibers were prepared at 17.2 kV with NE 100 electrospinning system (Inovenso LLC. Istanbul, Turkey). Solution progressed at 0.62 mL/h (New Era Pump Systems, Farmingdale, NY) and between of needles -collector distance was 15.5 cm and drum collecting nanofibers was covered with an aluminum foil. [23].

3. Characterization of PLA/TPEO Nanofiber

3.1. Thermogravimetric (TGA) Analysis

Thermal degradation graphs of PLA/TPEO nanofibers were determined with a thermogravimetric analyzer (SDT Q600 V20.9 Build 20) under nitrogen atmosphere at a heating rate of 10 °C min^{-1} and between 0-700 °C [24].

3.2. Scanning Electron Microscopy (SEM) Analysis

Surface properties and morphology of PLA/TPEO nanofiber was observed by scanning electron microscope (SEM; Carl Zeiss 300VP). In SEM analysis, gold plating was applied and investigated with an acceleration voltage of 5 kV [25, 26].

4. Results and Discussion

4.1. GC-MS Analysis

Fatty acid composition of essential oil obtained by steam distillation in Clevenger system is examined that is high terms of oleic acid (Table 1). Among essential fatty acid components defined in Table 2, D-Limonene and γ-Terpinene are important compounds with antibacterial effect [27] and D-Limonene was determined to be 77.45% and γ-Terpinene 12.2% (Table 2).

Table 1. Fatty acid components identified in *C. unshiu* type tangerine peel essential oil.

Fatty acid components	(%)
Palmitic Acid (C16:0)	16.60
Stearic Acid (C18:0)	6.32
Oleic Acid (C18:1) (n-9)	67.60
Linoleic Acid (C18:2) (n-9, 12)	9.48

Table 2. Essential fatty acid components identified in *C. unshiu* type tangerine peel essential oil

Essential fatty acid components	(%)
β-Myrcene	3.56
D-Limonene	77.45
γ-Terpinene	12.2
α-Terpinolene	0.75
p-Cymene	0.44
Δ-Elemene	0.35
β-Linalool	1.7
β-Elemene	0.56
α-Farnesene	1.79
α-Terpineol	1.18

4.2. TGA analysis

According to TGA results of PLA/TPEO nanofiber is seen decomposes between approximately 145.8°C-358.4°C and degradation peak is at an average of 324.8°C (Figure 1,a) and degradation temperature of pure PLA nanofiber is about 320°C (Figure 1,b).and higher temperature degradation of PLA/TPEO nanofiber can be explained with thermal stability of PLA/TPEO nanofiber is better than PLA-containing nanofiber after addition of essential oil [28, 29].

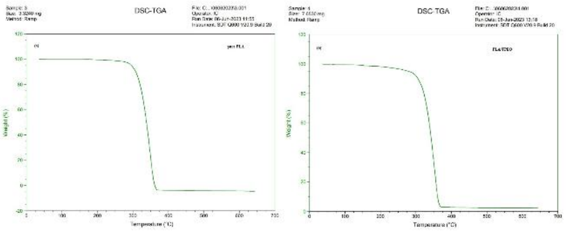

Figure 1. TGA curve of pure PLA nanofiber (a) and PLA/TPEO nanofiber (b).

4.3. SEM Analysis

According to SEM images of 25% TPEO loaded into PLA nanolife, 47nm and 76.8 nm diameter fibers were observed (Figure 2;c,d). Spherical formations (Figure 2; e, f) and their homogeneous distribution on fiber are evident in essential oil added PLA nanofiber compared to pure PLA (Figure 2; a, b). Pure PLA nanofiber diameter can average up to 512 nm [30], but PLA/TPEO composite nanofiber diameter has decreased, which can be due to hydrogen bonding [28].

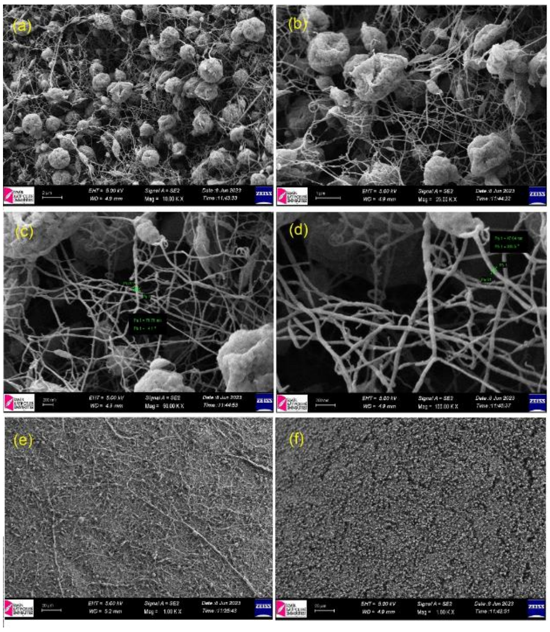

Figure 2. PLA/TPEO nanofiber SEM images and scales; 2 µm (a), 1 µm (b), 200nm 50.00 KX magnification (c) and 200nm 100 KX magnification (d), pure PLA nanofiber 20 µm (e) and PLA/TPEO nanofiber 20µm (f).

5. Conclusion

Biodegradable polymers are often preferred over synthetic polymers in terms of environment and health. PLA/TPEO nanofibers were obtained by using telectrospinning method with success. TPEO essential oil composition was investigated by GC-MS and obtained nanofiber was characterized by TGA and

SEM. It was determined that TPEO contains 77.45% high level of D-Limonene fatty acid and 12.2% γ-Terpinene. Degradation temperature of PLA/EO nanofiber is 324.8°C and nanofiber diameters were measured as 47nm and 76.8nm. PLA/TPEO composite nanofiber can be nominated as a candidate for food packaging industry and medical application.

Acknowledgement

This study was supported by Çanakkale Onsekiz Mart University Scientific Research (BAP) project code FHD-2022-3969.

Author contributions

Tuğba Güngör Ertuğral: Wrote draft of article, including preparation of composition and production of nanofibers by electrospinning method, and carried out characterization of nanofiber and analyzed results.
Sevim Akçura: Obtained essential oil with SH-Clevenger from tangerine peel.

Ethic

There are no ethical problems after publication of this publication.

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