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DEVELOPMENT OF WALNUT AND PUMPKIN SEED OIL-LOADED PHBV NANOFIBROUS MATS AND NANOFIBROUS SPONGES

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ABSTRACT: Nanofibrous polymeric biomaterials that incorporate bioactive agents have emerged as a focal point in various applications due to their distinctive properties. Walnut and pumpkin seed oils, which are rich in bioactive compounds, significantly enhance antioxidant capacity, exhibit anti-inflammatory effects, and support skin hydration. This study aimed to produce and characterize (poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) nanofibrous mats and sponges loaded with varying concentrations of walnut and pumpkin seed oils, utilizing electrospinning and wet-electrospinning techniques. The morphologies of the developed biomaterials were investigated through scanning electron microscopy (SEM), revealing that the mats presented smooth, continuous fibers free of beads, while the sponges showcased a three-dimensional, porous structure that was equally bead-free. Fiber diameter analysis using ImageJ software indicated an average range of 500 to 800 nm. Additionally, the percent porosity of the mats was approximately 60%, whereas the sponges exhibited about 75% porosity. To further analyze the chemical structure and confirm the presence of the oils, Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was employed. The findings from this research demonstrated the successful production of PHBV nanofibrous mats and sponges loaded with walnut and pumpkin seed oils for the first time. These innovative materials show considerable promise for biomedical applications, including bioactive packaging, facial masks, and wound dressing.

Keywords: PHBV, electrospinning, oil-loaded biomaterials, nanofiber

CEVİZ VE KABAK ÇEKİRDEĞİ YAĞI ENTEGRE EDİLMİŞ PHBV NANOLİFLİ YÜZEY VE NANOLİFLİ SÜNGERLERİN GELİŞTİRİLMESİ

ÖZ: Biyoaktif maddeler içeren nanolifli polimerik biyomalzemeler benzersiz özellikleri nedeniyle çeşitli alanlarda büyük ilgi çekmektedir. Zengin biyoaktif bileşikleriyle bilinen ceviz ve kabak çekirdeği yağları, antioksidan kapasiteyi artırır, anti-inflamatuar etki gösterir ve cilt nemlenmesini destekler. Bu çalışma, farklı konsantrasyonlarda ceviz yağı ve kabak çekirdeği yağı entegre edilmiş poli (3-hidroksibutirat-ko-3-hidroksivalerat) (PHBV) nanolifli yüzey ve nanolifli süngerlerin elektroeğirme ve ıslak elektroeğirme teknikleri kullanılarak üretilmesini ve karakterize edilmesini amaçlamaktadır. Üretilen biyomalzemelerin morfolojileri taramalı elektron mikroskobu (SEM) ile karakterize edilmiştir. Sonuçlar, matların pürüzsüz, boncuksuz ve sürekli lifler sergilediğini, süngerlerin ise üç boyutlu, gözenekli ve boncuksuz lifli bir yapıya sahip olduğunu göstermiştir. Liflerin ortalama çapı SEM görüntülerinden Image J programı ile 500-800 nm olarak bulunmuştur. Ek olarak, nanolifli yüzeylerin gözeneklilik yüzdesi yaklaşık %60 iken, süngerler yaklaşık %75 gözeneklilik sergilemiştir. Kimyasal yapıyı analiz etmek ve yağların malzeme içerisine dahil edildiğini doğrulamak amacı ile zayıflatılmış toplam yansıma Fourier dönüşümlü kızılötesi spektroskopisi (ATR-FTIR) kullanılmıştır. Bulgular, ceviz ve kabak çekirdeği yağları içeren PHBV nanolifli yüzeylerin ve süngerlerin ilk kez başarıyla üretildiğini göstermiştir. Bu malzemeler biyoaktif ambalaj, yüz maskeleri ve yara örtüsü gibi biyomedikal uygulamalar için umut verici bir potansiyele sahiptir.

Anahtar Kelimeler: PHBV, elektroeğirme, yağ entegre edilmiş biyomalzemeler, nanolif

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1. INTRODUCTION

Nanofibrous materials exhibit distinct characteristics, such as a high surface area-to-volume ratio, adjustable porosity, exceptional mechanical strength, and the capacity to integrate different substances [1]. These properties are well-suited for miscellaneous applications, including textile engineering, tissue engineering, wound dressing, filtration, and sensors [2-8]. Electrospinning is a highly efficient technique used to produce nanofibers due to its availability, convenience, cost-effectiveness, and wide range of applications. This method produces two-dimensional electrospun mats with large specific surface area, high porosity, and facile functionalization properties [9].

Various techniques have been developed to produce three-dimensional fibrous materials, including multilayer electrospinning, post-processing of 2D membranes, using a 3D template, or employing a liquid collector [10,11]. The technique utilizing a liquid collector is called a wet-electrospinning technique. This technique entails electrospinning the polymer solution into a coagulation bath that contains a low-surface tension solvent, such as tertiary-butyl alcohol, methanol, or ethanol. The resulting homogenously suspended fibers in the bath are then collected and freeze-dried to preserve their three-dimensional structure [12,13].

Different polymers, including natural and synthetic ones, can be used in the spinning process depending on the specific requirements [14]. In addition, one of the key advantages of electrospinning technology is its ability to incorporate a wide range of active agents into the fibers. By directly adding these bioactive materials to the polymeric solution prior to electrospinning, a uniform distribution within the resulting fibers can be achieved. This method opens new possibilities for advanced biomedical solutions [15,16].

Poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) (PHBV) is a copolymer that combines polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV). It is as a member of the polyhydroxyalkanoates (PHAs), composed of naturally occurring biodegradable polyester synthesized by microorganisms. PHBV has many advantages over other types of PHA polymers such as toughness and elasticity. In addition, it can be easily formed into a nanofibrous structure [17-19].

Pumpkin seed and walnut oils are types of fixed oils which are naturally occurring bioactive materials extracted from plant seeds or fruits primarily composed of fatty acids. Culinary, cosmetic, and medicinal applications widely utilize fixed oils due to their nutritional and therapeutic properties [20]. Pumpkin seed oil is recognized for its rich nutritional profile and diverse bioactive compounds. It is characterized by a high lipid content, typically around 60%, with a significant proportion of polyunsaturated fatty acids, predominantly linoleic acid (C18:2) and oleic acid (C18:1), which are essential for human health. In addition to fatty acids, pumpkin seed oil is rich in bioactive compounds such as tocopherols (vitamin E), phytosterols, and carotenoids. Tocopherols are known for their antioxidant properties, which help in protecting the oil from oxidative degradation and contribute to its health-promoting effects. The carotenoid content enhances the oil's nutritional value, providing additional antioxidant benefits. The mineral content of pumpkin seeds, including potassium, magnesium, and phosphorus, also adds to the overall health benefits of the oil, supporting various physiological functions [21-25]. Walnut oil is rich in polyunsaturated fatty acids, particularly linoleic acid and alpha-linolenic acid, which are essential for human health and play a significant role in cardiovascular health. The fatty acid profile of walnut oil typically shows a favorable ratio of omega-6 to omega-3 fatty acids, approximately 4:1, which may be beneficial in reducing the risk of cardiovascular diseases. From a chemical perspective, walnut oil is characterized by its high content of bioactive compounds, including tocopherols, phytosterols, squalene, and polyphenols. These compounds enhance its antioxidant ability, essential for protecting cells against oxidative stress [26-29].

In the literature, few studies concern nanofibers produced with fixed oils. The study of Ribes [30] aimed to produce cellulose nanofiber/chitosan membranes using essential vegetable oils (Thymus vulgaris, Anethum graveling, Origanum vulgare, Laurus nobilis, and Cominum cyminum) and fixed vegetable oil based on Myrocarpus fronds, for use as wound dressings. In the study of Rezk [31], incorporating pumpkin seed oil into chitosan/polyvinyl alcohol electrospun nanofibers demonstrated structural integrity while enhancing the material's bioactivity, as evidenced by color changes and morphological observations.

This study focused on the production, development, and characterization of walnut oil and pumpkin seed oil-loaded PHBV nanofibrous mats obtained through electrospinning and nanofibrous sponges produced via the wet-electrospinning technique. While a study examined a polymeric mat with pumpkin seed oil, there was no corresponding sponge, nor was there one with walnut oil. Nevertheless, research on nanofibrous mats and nanofibrous sponges that incorporate walnut oil and/or pumpkin seed oil is absent. Walnut and pumpkin seed oil are Türkiye's two most recognized and utilized oils. With this study, these materials can enter into new application areas, such as nanoscale cosmetics and biomedical materials.

2. EXPERIMENTAL STUDIES

2.1. Materials

PHBV (PHV content 3 wt%, M_n=80 kDa, $T_{melting}$ =78 °C, $T_{decomposition}$=297 °C) was purchased from Helian Polymers, Netherlands. Organosoluble salt, benzyl triethylammonium chloride (BTEAC), and chloroform were obtained from Sigma-Aldrich. Walnut and pumpkin seed oils were purchased from Arifoğlu, Türkiye.

2.2. Production of Natural Oil-Loaded Nanofibrous Mats and Nanofibrous Sponges

2.2.1. Preparation of the Solutions

The optimum PHBV concentration was determined to be 3% (w/v) in our preliminary study [32]. PHBV solution was prepared by dissolving PHBV and BTEAC (0.2% (w/v)) in chloroform at 50 °C for 2 h and at room temperature overnight through stirring. BTEAC, organosoluble salt, was added to increase the electrical conductivity of the PHVB solution. The increased electrical conductivity of the solution subjects the fiber jet to a higher tensile force with the applied electric field, resulting in smaller fiber diameters [33]. Natural oil/PHBV solution was prepared as follows: walnut and pumpkin seed oils were dissolved in chloroform at two ratios (1% and 1.5% w/v). The resulting mixture was then added to a PHBV solution and agitated at 50 °C for 2 hours, followed by overnight stirring at room temperature.

2.2.2. Electrospinning Process

Electrospinning was used to prepare nanofibrous mats made of natural oil and PHBV. Individual solutions of walnut/PHBV or pumpkin seed oil/PHBV were injected into a 10 mL plastic syringe equipped with a 21-gauge stainless steel needle. The solutions were introduced into the needle tip using a syringe pump (NE-1000, New ERA Pump System, Inc., USA) at a flow rate of 1.0 mL/h. A sheet of aluminum foil, utilized as the collector, was positioned at a distance of 15 cm from the tip of the needle. Nanofibers were collected by providing a positive high voltage of 20 kV to the needle using a Gamma High Voltage ES30 power source. Moreover, the manufacturing conditions were the same as those of the PHBV mat. Figure 1 illustrates all of the steps involved in producing nanofibrous mats.

2.2.3. Wet-electrospinning Process

The combination of wet-electrospinning and freeze-drying processes was employed to fabricate 3D nanofibrous sponges. At first, individual uniform natural oil/PHBV or PHBV solutions were subjected to wet electrospinning using particular parameters: a distance of 10 cm between the collector and the spinneret, a flow rate of 2 ml/h, and a voltage of 20 kV, in contrast to the traditional electrospinning arrangement, a collector glass container was employed, which contained a coagulation bath consisting of a mixture of ethanol and distilled water in a ratio of 9:1 (v/v). An electrically conductive copper plate was positioned beneath the container to sustain the electric field. Subsequently, nanofibers evenly distributed in a suspension were gathered and meticulously rinsed with distilled water. Ultimately, they underwent freeze-drying using FreeZone Freeze Dry Systems to achieve a 3D structure at a temperature of -78°C for 24 hours. Figure 2 depicts the sequential stages involved in the production of nanofibrous sponges.

2.3. Characterization Techniques

The morphology of the produced nanofibrous mats and nanofibrous sponges was observed by scanning electron microscopy (SEM, JEOL JSM-6060), and all the scaffolds were sputter-coated (Quorum Technologies, SC7620) with a thin layer of gold/palladium before imaging. The average diameter of the fibers was estimated from the SEM images (500x) via the Image J program by taking measurements from 100 different points for each of the samples.

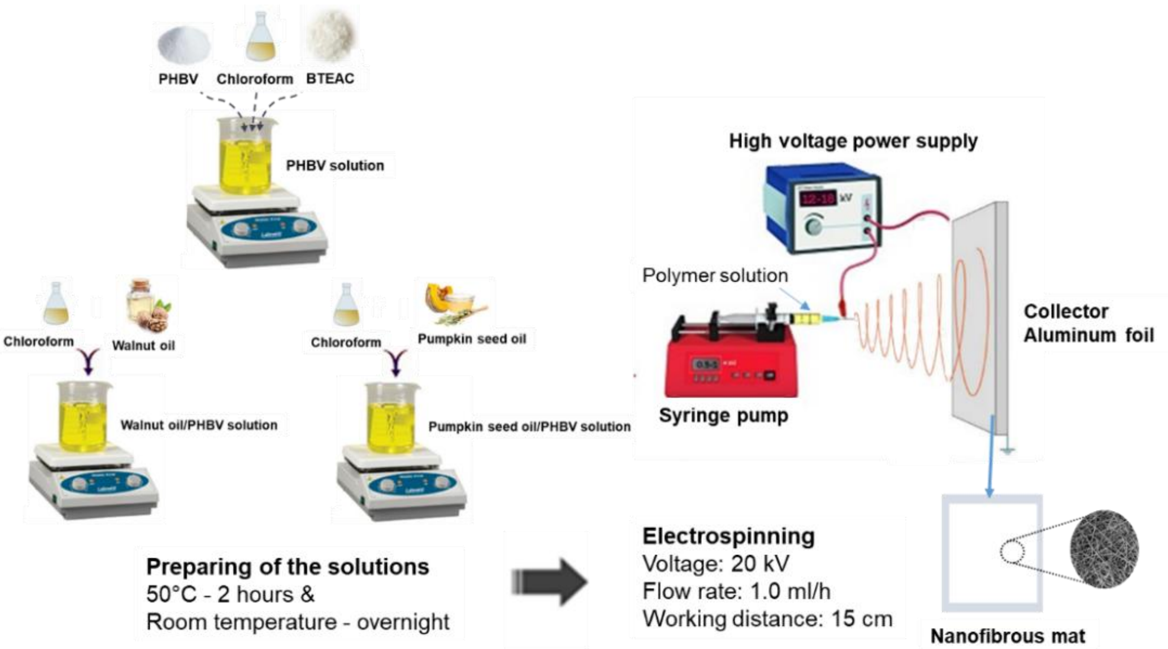

Figure 1. Schematic illustration of the production steps of nanofibrous mats

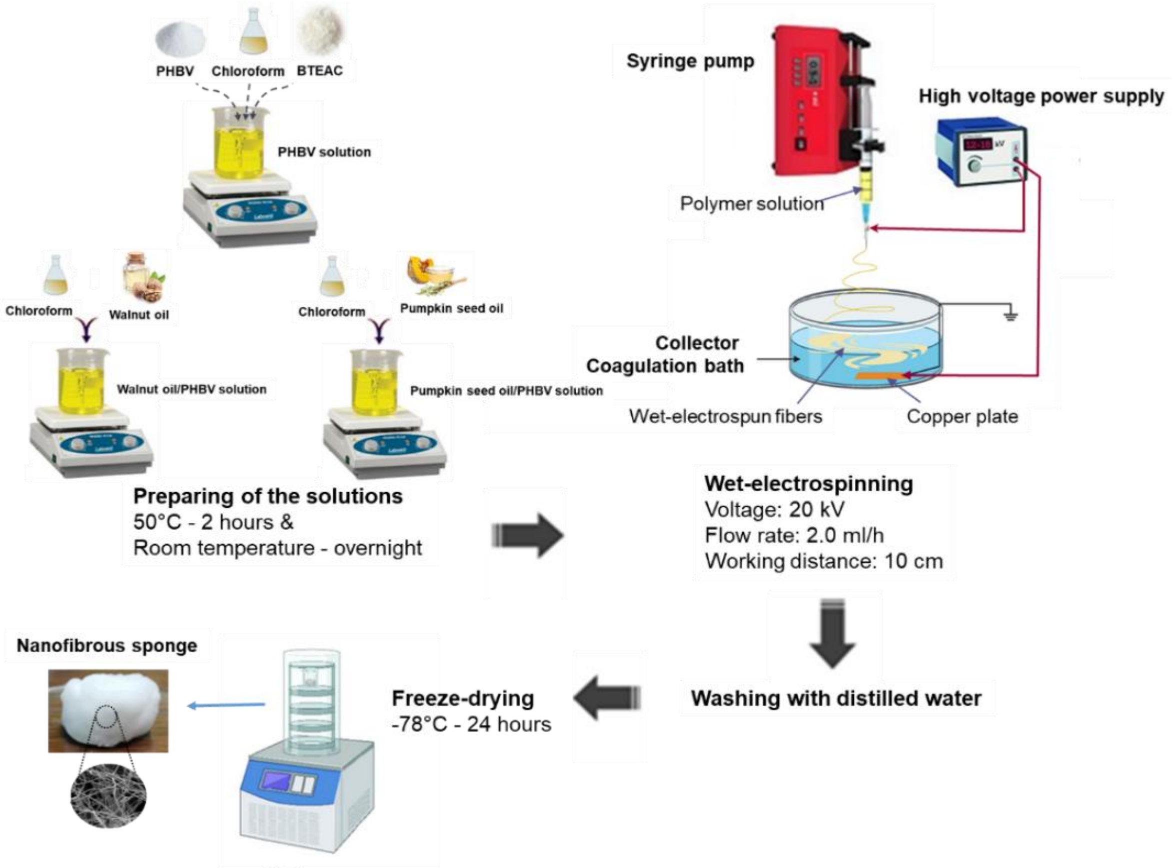

Figure 2. Schematic illustration of the production steps of nanofibrous sponges

Percent porosity values were obtained from the SEM images (500x) using the Image J program in triplicate (n=3). The process involved using brightness and contrast (B&C) adjustment to remove the noise in the background of the images and automatic thresholding to convert images to binary, resulted in a clear distinction, with the black areas representing the pores and the white areas representing the material surface, keeping the audience informed and engaged.

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) (Perkin Elmer Spectrum BX) was used to determine the chemical structures of the nanofibrous mats and nanofibrous sponges in the wavenumber range of 4000-650 cm^{-1} with a resolution of 4 cm^{-1} and 25 scans per sample.

3. RESULTS AND DISCUSSIONS

3.1. Morphology Characterization

Natural oil/PHBV mats and natural oil/PHBV sponges were produced by electrospinning and wet electrospinning in conjunction with freeze-drying. Table 1 names them based on the natural oils in the spinning polymer solution and their w/v% ratios in the solution.

Table 1. Sample codes and average fiber diameters of the nanofibrous mats and sponges

Sample code	Oil name – concentration (w/v %)	Fiber Diameter (nm)	Porosity (%)
PHBV mat	-	636 ± 95	70.1 ± 0.7
W1/PHBV mat	Walnut oil - 1	690 ± 85	69.3 ± 0.3
W1.5/PHBV mat	Walnut oil - 1.5	710 ± 102	68.4 ± 0.4
PS1/PHBV mat	Pumpkin seed oil – 1	680 ± 76	69.6 ± 0.3
PS1.5 /PHBV mat	Pumpkin seed oil – 1.5	702 ± 86	67.7 ± 0.7
PHBV sponge	-	549 ± 35	80.1 ± 0.5
W1/PHBV sponge	Walnut oil - 1	580 ± 53	78.2 ± 0.9
PS1/PHBV sponge	Pumpkin seed oil – 1	583 ± 67	77.6 ± 0.2

The porosity data (Table 1) indicate that the sponges had a greater porosity level of approximately 80%, whilst the mats displayed porosity levels of around 70%. The higher porosity of nanofibrous sponges compared to nanofibrous mats are attributed to differences in fabrication techniques. The sublimation process during freeze-drying creates pore spaces [34]. The produced biomaterials exhibit significant promise for application as wound dressings, with porosity values of nanofibrous materials identified within the optimal range (60-90%) for such uses, as supported by existing literature [35]. Studies have shown that incorporating oils can increase the diameter of nanofibers, which may lead to a reduction in the overall porosity of the fibrous mats [36, 37]. The oils tend to create a more intricate porous structure by filling voids and modifying the fibers' interconnectivity [38]. However, in this particular study, the addition of natural oil does not significantly alter the porosity values of the resulting biomaterials. A suitable level of porosity is essential for the successful support of cell adhesion and growth, as it allows for the distribution of cells throughout the biomaterial [39].

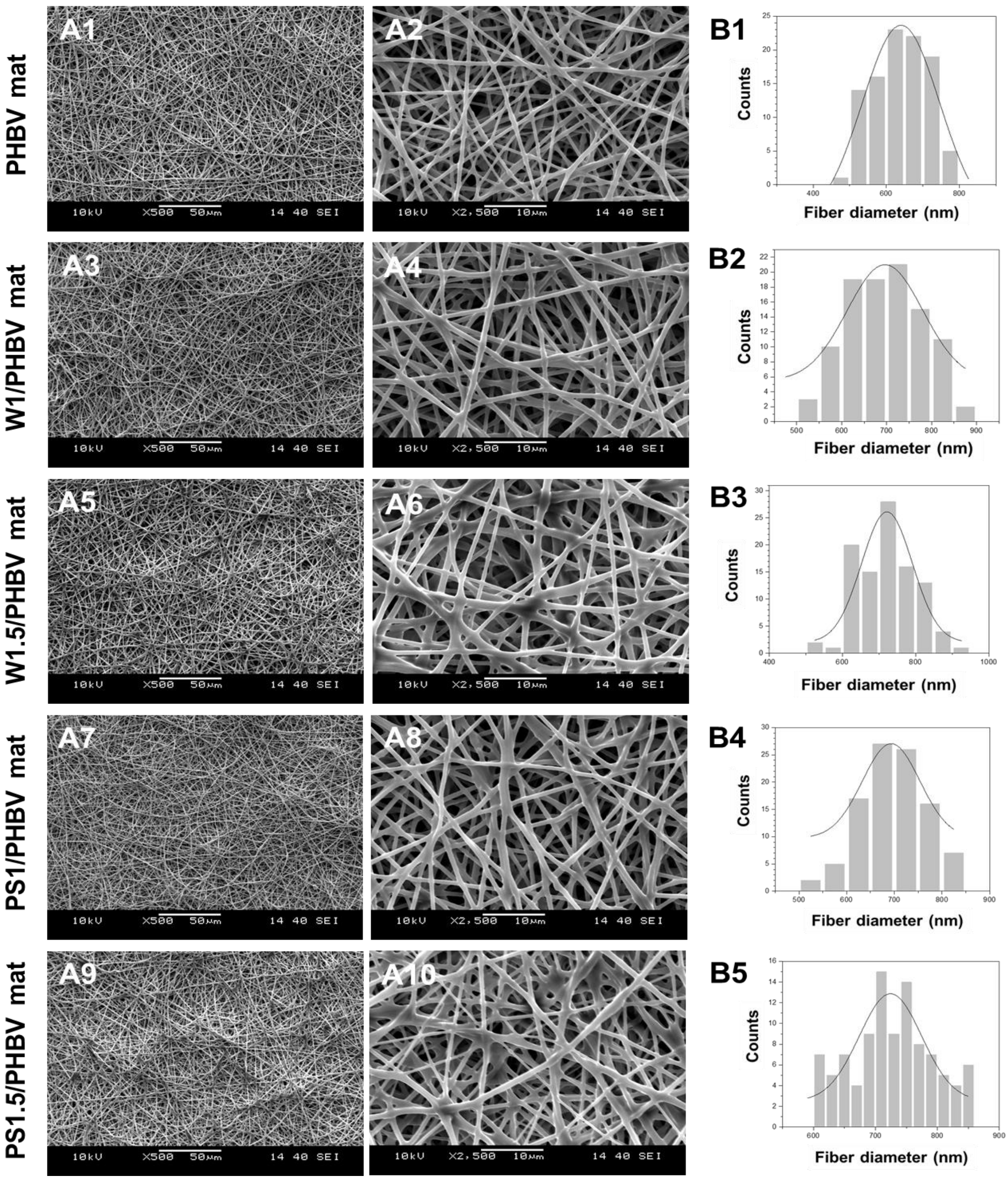

Figure 3. SEM images (A1-A10) and fiber diameter distribution (B1-B5) of the PHBV, W1/PHBV, W1.5/PHBV, PS1/PHBV, PS1.5/PHBV nanofibrous mats

Table 1 presents the average fiber diameters of the mats and sponges, which range between 500 and 800 nm. The PHBV mats have an average diameter of 636 ± 71 nm. Introducing walnut and pumpkin seed oil increased fiber diameter, adding 1 w/v% of these oils, leading to an average diameter of 693 nm. Moreover, a higher concentration of natural oils correlated with a more significant increase in fiber diameter. The average fiber diameters for the W1.5/PHBV mat and the PS1.5/PHBV mat were measured at 721 ± 80 nm and 726 ± 65 nm, respectively. Thus, the optimal oil concentration for creating nanofibrous sponges was 1 w/v%. Existing literature supports the observation that more additives in polymer solutions tend to increase fiber diameters [40, 41]. In addition, studies have indicated that the incorporation of oils can alter the morphology of nanofibers, leading to increase in fiber diameter [42, 43].

Figure 3 (A1-A10) presents the SEM images of the PHBV and natural oil/PHBV mats at magnifications of 500x and 2500x. Maintaining a bead-free morphology is crucial during the electrospinning process [44]. All the mats produced display smooth, bead-free, and continuous fibers. The fibers composed of natural oil/PHBV (Figures 3 A4, A6, A8, and A10) show a rougher surface texture compared to the PHBV nanofibers (Figure 3 A2), suggesting effective integration of the oil into the fibers. Additionally, Figure 3 B1-B5 depicts the distribution of fiber diameters across various mats. The PHBV mat (Figure 3 B1) demonstrates a narrower diameter distribution, while the integration of oil results in a broader distribution, particularly noticeable at a 1.5 ratio (Figures 3 B3, B5).

The SEM images of the produced sponges are shown in Figure 4. They demonstrate a three-dimensional, porous, continuous, bead-free nanofibrous structure. The integration of oil did not affect this porous structure. It's important to maintain such a structure because higher porosity is essential for cell behavior, influencing adhesion, proliferation, and differentiation. Furthermore, the porosity of biomaterials significantly affects the diffusion of nutrients and oxygen [45]. Figure 4 A3-E3 also presents the fiber diameter distribution of the produced sponges. It is evident that sponges loaded with natural oil have a wider fiber diameter distribution when compared to PHBV mats. In contrast, the natural oil/PHBV sponges have comparable fiber diameters both above that of PHBV sponges (see Table 1).

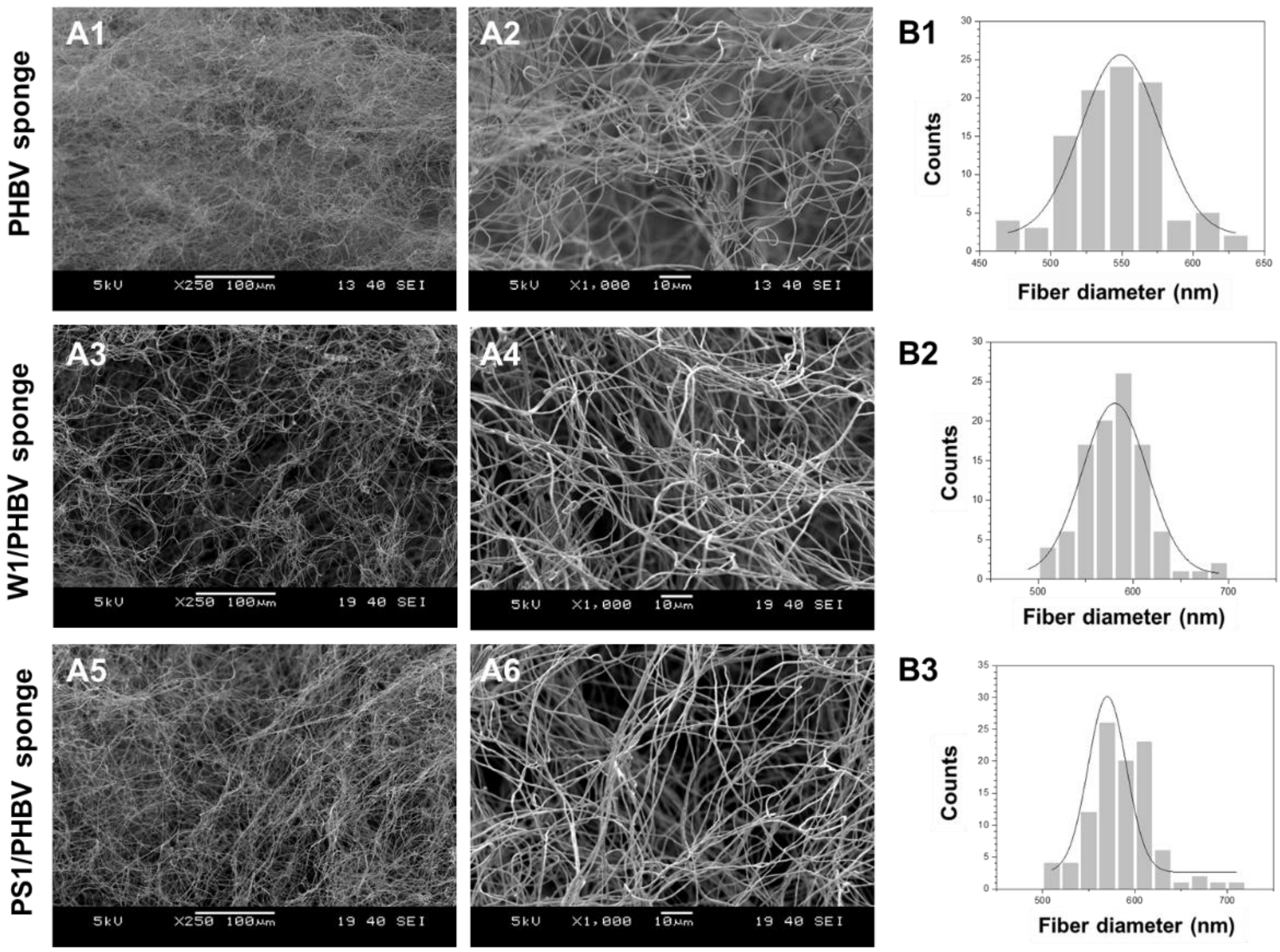

Figure 4. SEM images (A1-6) and fiber diameter distribution (B1-B3) of the PHBV, W1/PHBV, and PS1/PHBnanofibrous sponges

3.2. Chemical Structure Characterization

ATR-FTIR analysis was performed to examine the presence of natural oil on the surface of the produced mats and sponges. Figure 5A displays the characteristic band of the PHBV mats and sponges, along with walnut oil and pumpkin seed oil. As expected, PHBV mat and PHBV sponges were observed to have the same FTIR spectrum. The C=O stretching vibration of PHBV was at 1721 cm^{-1}, and the C-O stretching bands were at 1278 and 1055 cm^{-1}, respectively. C-H stretching bands were around 2978 and 2934 cm^{-1}, besides C-H bending vibrations at 1453 and 1380 cm^{-1} [46]. Both walnut and pumpkin seed oils exhibit peaks similar to those of PHBV, with a C=O stretching band at 1743 cm^{-1}, a C-O stretching band at 1163 cm^{-1}, and a C-H bending band at 1457 cm^{-1} identifiable in the natural oils. Distinctive triple C-H stretching bands appear at 3007, 2923, and 2856 cm^{-1} for walnut oil and 3010, 2926, and 2854 cm^{-1} for pumpkin seed oil [47, 48].

Figure 5B shows the FTIR spectrum of PHBV mats and sponges containing walnut and pumpkin seed oil. The peaks that appeared at 1721, 1453, 1380, 1278, and 1055 cm^{-1} were assigned to the characteristic vibrations of PHBV. The triple C-H stretching bands, the unique components of the oils, provide evidence of the presence of natural oil in the mats and sponges. The graph magnifies and repeats these parts across all spectra. Since PHBV also had absorption peaks at 2978 and 2934 cm^{-1} in this range, it was observed that the peaks overlapped. However, the characteristic band of oil at 2856 cm^{-1} in the W1/PHBV mat, PS1/PHBV mat, and PS1/PHBV sponge proved that walnut oil and pumpkin seed oil were successfully loaded into the materials. This peak could not be observed for the W1/PHBV sponge, but the peak of PHBV at 2978 cm^{-1} shifted to a higher wave number (2987 cm^{-1}), as well as the peak of oil at 2856 cm^{-1}, and the peak of PHBV at 2934 cm^{-1} merged and appeared at 2940 cm^{-1}, which was attributed to the presence of walnut oil.

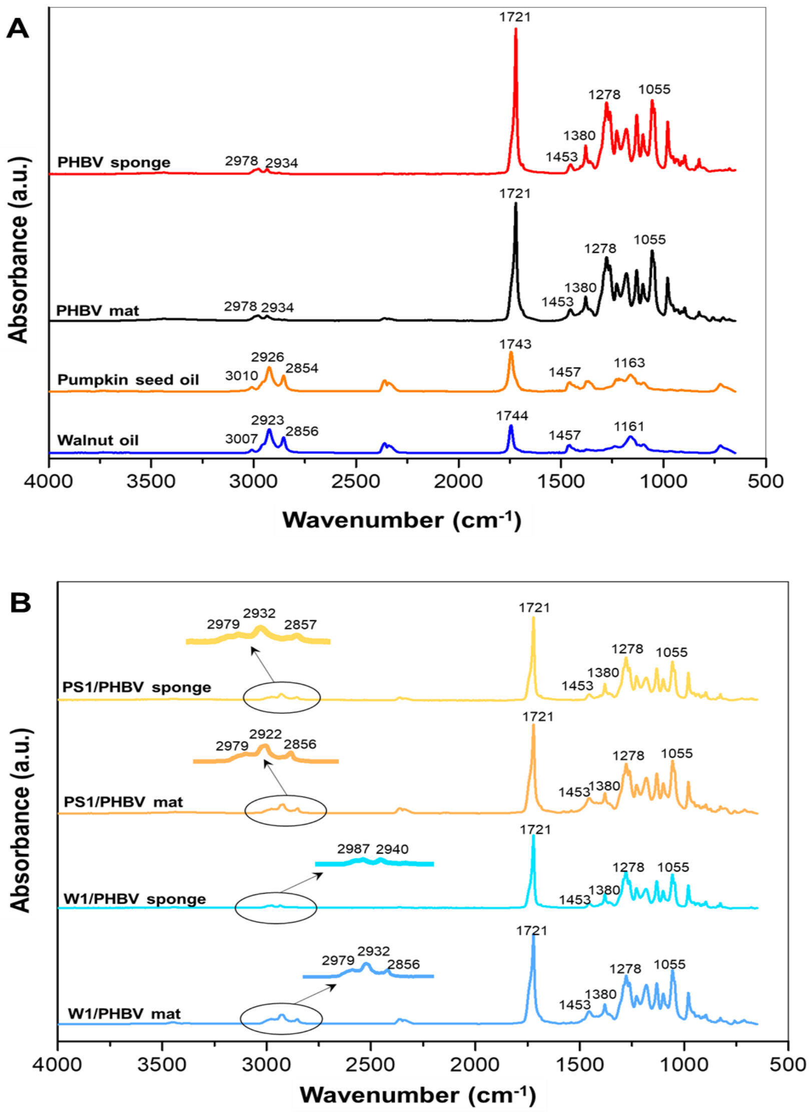

Figure 5. ATR-FTIR spectra of the walnut oil, pımpkin seed oil, PHBV mat and PHBV sponge (A), produced natural oil loaded mats and sponges (B)

4. CONCLUSION

This research focused on incorporating walnut and pumpkin seed oils into 2D fibrous mats and 3D fibrous sponges using electrospinning and wet-electrospinning techniques. Two different concentrations of oil-loaded PHBV nanofibrous mats were produced and subjected to morphological analysis. Both concentrations yielded continuous and bead-free nanofibers, but for sponge production, a concentration of 1 wt% (w/v) was chosen due to its smaller fiber diameters. SEM images revealed a rougher surface on the mats and sponges compared to the PHBV nanofibers, indicating successful oil incorporation. ATR-FTIR spectroscopy was employed to investigate the chemical structure of the materials, confirming that no undesirable reactions occurred during the formation of the polymer solution. Additionally, ATR-FTIR analysis confirmed the presence of walnut and pumpkin seed oils within the structures of the mats and sponges. Given the beneficial properties of walnut and pumpkin seed oils, along with PHBV, these nanofibrous mats and sponges have promising applications in food packaging, wound dressings, and biomedical and pharmaceutical fields.

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REFERENCES

1. Wei, L., Yu, H., Sun, R., Liu, C., Chen, M., Liu, H., Xiong, J., Quin, X., (2021), *Experimental investigation of process parameters for the filtration property of nanofiber membrane fabricated by needleless electrospinning apparatus*, Journal of Industrial Textiles, 50, 9, 1528-1541.
2. Dirlik-Uysal, D., Mínguez-García, D., Bou-Belda, E., Gisbert-Payá, J., Bonet-Aracil, M. (2024), *Thermo-Regulated Cotton: Enhanced Insulation through PVA Nanofiber-Coated PCM Microcapsules*. Applied Sciences, 14, 4725.
3. Sanchaniya, J. V., Lasenko, I., Kanukuntla, S. P., Mannodi, A., Viluma-Gudmona, A., & Gobins, V. (2023), *Preparation and characterization of non-crimping laminated textile composites reinforced with electrospun nanofibers*. Nanomaterials, 13, 13, 1949.
4. Kara, A., Colpankan Gunes, O., Ziylan Albayrak, A., Bilici, G., Erbil, G., Havitcioglu, H., (2020), *Fish scale/poly(3-hydroxybutyrate-co-3-hydroxyvalerate) nanofibrous composite scaffolds for bone regeneration*, Journal of Biomaterials Applications, 34, 9, 1201-1215.
5. Colpankan Gunes, O., Kara, A., Baysan, G., Husemoglu, R. B., Akokay, P., Ziylan Albayrak, A., Ergur, B. U., Havitcioglu, H. (2022), *Fabrication of 3D Printed poly(lactic acid) strut and wet-electrospun cellulose nanofiber reinforced chitosan-collagen hydrogel composite scaffolds for meniscus tissue engineering*, Journal of Biomaterials Applications, 37, 4, 683-697.
6. Fahimirad, S., Khaki, M., Ghaznavi-Rad, E., Abtahi, H., (2024), *Investigation of a novel bilayered PCL/PVA electrospun nanofiber incorporated Chitosan-LL37 and Chitosan-VEGF nanoparticles as an advanced antibacterial cell growth-promoting wound dressing*, International Journal of Pharmaceutics, 661, 124341.
7. Desai, K., Kit, K., Li, J., Davidson, P. M., Zivanovic, S., Meyer, H., (2009), *Nanofibrous chitosan non-wovens for filtration applications*, Polymer, 50, 15, 3661-3669,
8. Chowdhury, S. A., Saha, M. C., Patterson, S., Robison, T., Liu, Y., (2019), *Highly conductive polydimethylsiloxane/carbon nanofiber composites for flexible sensor applications*, Advanced Materials Technologies, 4, 1800398.
9. Kalbali, N., Hashemi-Najafabadi, S., Bagheri, F., (2023), *Improving pore size of electrospun gelatin scaffolds containing graphene oxide using PEG as a sacrificial agent for bone tissue engineering*, International Journal of Polymeric Materials and Polymeric Biomaterials, 73, 12, 1068-1077.
10. Sun, B., Long, Y., Zhang, H., Li, M., Duvail, J., Jiang, X., Yin, H., (2014), *Advances in three-dimensional nanofibrous macrostructures via electrospinning*, Progress in Polymer Science, 39, 862-890.
11. Kim, M. S., Kim, G., (2014), *Three-dimensional electrospun polycaprolactone (PCL)/alginate hybrid composite scaffolds*, Carbohydrate Polymers, 114, 213-221.
12. Kostakova, E., Seps, M., Pokorny, P., Lukas, D., (2014), *Study of polycaprolactone wet electrospinning process*, Express Polymer Letters, 8, 8, 554-564.
13. Taskin, M. B., Xu, R., Gregersen, H. V., Nygaard, J. V., Besenbacher, F., Chen, M., (2016), *Three-dimensional polydopamine functionalized coiled microfibrous scaffolds enhance human mesenchymal stem cells colonization and mild myofibroblastic differentiation*, ACS Applied Materials and Interfaces, 8, 25, 15864-15873.
14. Ahmadian, A., Shafiee, A., Aliahmad, N., Agarwal, M., (2021), *Overview of nano-fiber bundles fabrication via electrospinning and morphology analysis*, Textiles, 1, 2, 206-226.
15. Dong, B., Smith, M., Wnek, G. E., (2009), *Encapsulation of multiple biological compounds within a single electrospun fiber*. Small, 5, 13, 1508-1512.
16. Amorim, L. F. A., Mouro, C., Gouveia, I. C., (2024), *Electrospun fiber materials based on polysaccharides and natural colorants for food packaging applications*. Cellulose, 31, 10, 6043-6069.
17. Sultana, N., Wang, M. J., (2008), *Fabrication of HA/PHBV composite scaffolds through the emulsion freezing/freeze-drying process and characterization of the scaffolds*. Journal of Materials Science: Materials in Medicine, 19, 7, 2555-2561.
18. Li, Z., Yang, J., Loh, X., (2016), *Polyhydroxyalkanoates: opening doors for a sustainable future*, NPG Asia Materials, 8, e265.
19. Kaniuk Ł., Stachewicz U., (2021), *Development and Advantages of Biodegradable PHA Polymers Based on Electrospun PHBV Fibers for Tissue Engineering and Other Biomedical Applications*. ACS Biomaterials Science and Engineering, 7, 12), 5339-5362.
20. Piras, A., Rosa, A., Marongiu, B., Porcedda, S., Falconieri, D., Dessì, M. A., Ozcelik, B., Koca, U., (2013), *Chemical composition and in vitro bioactivity of the volatile and fixed oils of Nigella sativa L. extracted by supercritical carbon dioxide*, Industrial Crops and Products, 46, 317-323.
21. Oliveira, R. L., Gomes, R. S., Almeida, C. F., Júnior, R. M., Rocha, J. R., Silva, D. J. H., Carneiro, P. C. S. (2021), *Multitrait selection of pumpkin genotypes aimed at reducing the growth habit and improving seed production*, Crop Science, 61, 3, 1620-1629.

22. Bardaa, S., Halima, N., Aloui, F., Mansour, R., Jabeur, H., Bouaziz, M., Sahnoun, Z., (2016), *Oil from pumpkin (cucurbita pepo l.) seeds: evaluation of its functional properties on wound healing in rats*, Lipids in Health and Disease, 15, 73.

23. Setsiripakdee, A., Lourith, N., Kanlayavattanakul, M., (2019), *In vitro and in vivo removal efficacies of a formulated pumpkin seed oil makeup remover*. Journal of Surfactants and Detergents, 22, 6, 1461-1467.

24. Seymen, M., Uslu, N., Türkmen, Ö., Juhaimi, F. A., Özcan, M. M., (2016), *Chemical compositions and mineral contents of some hull-less pumpkin seed and oils*. Journal of the American Oil Chemists' Society, 93, 8, 1095-1099.

25. Lisa, S. A., Kabir, M. A., Khan, S. (2022), *Utilization of seed from cucurbita maxima, a pumpkin variety of bangladesh, converting into refined oil and oilcake*. Discover Food, 2, 19.

26. Liao, J., Nai, Y., Li, F., Chen, Y., Mei, L., Xu, H., (2020), *Walnut oil prevents scopolamine-induced memory dysfunction in a mouse model*, Molecules, 25, 7, 1630.

27. Gao, P., Cao, Y., Liu, R., Jin, Q., Wang, X., (2018), *Phytochemical content, minor-constituent compositions, and antioxidant capacity of screw-pressed walnut oil obtained from roasted kernels*, European Journal of Lipid Science and Technology, 121, 1, 1800292.

28. Özkan, G., Koyuncu, M., (2005), *Physical and chemical composition of some walnut (Juglans regia L) genotypes grown in Turkey*, Grasas Y Aceites, 56, 2.

29. Tian, L., Zhang, S., Yi, J., Zhu, Z., Decker, E., McClements, D., (2022), *The impact of konjac glucomannan on the physical and chemical stability of walnut oil-in-water emulsions coated by whey proteins*, Journal of the Science of Food and Agriculture, 102, 10, 4003-4011.

30. Ribes, D.D., de Avila Delucis, R., Acosta, A.P., Barbosa, K. T., Piva, E., Alberto Gatto, E. D., Lund, R. G., Beltrame, R., (2024), *Exploring diverse vegetable oils in crafting next-gen bio-curatives containing cellulose nanofibrils and chitosan*. Biomass Conversion and Biorefinery, DOI: 10.1007/s13399-024-05391-x

31. Rezk, M. Y., Ibrahim, S., Khalil, E. A., Saba, D. A., Abdellatif, M., Abdellatif, A., Allam, N. K., (2023), *Pumpkin seed oil -loaded chitosan/polyvinyl alcohol electrospun nanofiber scaffold for dermal and oral wound dressing*. ChemistrySelect, 8, 26.

32. Colpankan Gunes, O., Unalan, I., Cecen, B., Ziylan Albayrak, A., Havitcioglu, H., Ustun O., Ergur, B. U., (2019), *Three-dimensional silk impregnated HAp/PHBV nanofibrous scaffolds for bone regeneration*, International Journal of Polymeric Materials and Polymeric Biomaterials, 68, 5, 217-228.

33. Choi, J. S., Lee, S. W., Jeong, L., Bae, S. H., Min, B. C., Youk, J. H., Park, W. H., (2004), *Effect of organosoluble salts on the nanofibrous structure of electrospun poly(3-hydroxybutyrate-co-3-hydroxyvalerate)*, International Journal of Biological Macromolecules, 34, 249-256.

34. Yin, M., Liu, M., Cao, Q. Y., Wu, J., Liu, T., Liu, J., (2012), *Effect of pre-freezing rate on porosity ratio and mechanical property of pig aorta*, Frontiers in Bioscience, 17, 1, 575.

35. Salehi, M., Niyakan, M., Ehterami, A., Haghi-Daredeh, S., Nazarnezhad, S., Abbaszadeh-Goudarzi, G., Mousavi, S., (2019), *Porous electrospun poly(ε-caprolactone)/gelatin nanofibrous mat containing cinnamon for wound healing application: in vitro and in vivo study*. Biomedical Engineering Letters, 10, 1, 149-161.

36. Berechet, M. D., Gaidău, C., Miletić, A., Pilić, B., Râpă, M., Stanca, M., Lazea-Stoyanova, A., (2020), *Bioactive properties of nanofibres based on concentrated collagen hydrolysate loaded with thyme and oregano essential oils*, Materials, 13, 7, 1618.

37. Chomachayi, M. D., Solouk, A., Akbari, S., Sadeghi, D., Mirahmadi, F., Mirzadeh, H., (2018), *Electrospun nanofibers comprising of silk fibroin/gelatin for drug delivery applications: thyme essential oil and doxycycline monohydrate release study*. Journal of Biomedical Materials Research Part A, 106, 4, 1092-1103.

38. Cloete, W. J., Hayward, S., Klumperman, B., (2019), *Degradation of proteins and starch by combined immobilization of protease, α-amylase and β-galactosidase on a single electrospun nanofibrous membrane*, Molecules, 24, 3, 508.

39. Tomić, S. L., Nikodinović-Runić, J., Vukomanović, M., Babić, M. M., Vuković, J. S., (2021), *Novel hydrogel scaffolds based on alginate, gelatin, 2-hydroxyethyl methacrylate, and hydroxyapatite*, Polymers, 13, 6, 932.

40. Hosseini, F., Miri, M. A., Najafi, M., Soleimanifard, S., Aran, M. (2021), *Encapsulation of rosemary essential oil in zein by electrospinning technique*, Journal of Food Science, 86, 9, 4070-4086.

41. Ansarifar, E., Moradinezhad, F., (2022), *Encapsulation of thyme essential oil using electrospun zein fiber for strawberry preservation*, Chemical and Biological Technologies in Agriculture, 9, 1, 1–11.

42. Arik, N., Horzum, N., Truong, Y. B., (2022), *Development and Characterizations of Engineered Electrospun Bio-Based Polyurethane Containing Essential Oils*, Membranes, 12, 2, 209.

43. Unalan, I., Endlein, S. J., Slavik, B., Buettner, A., Goldmann, W. H. Detsch, R., Boccaccini, A. R., (2019), *Evaluation of electrospun poly (ε-caprolactone)/gelatin nanofiber mats containing clove essential oil for antibacterial wound dressing*, Pharmaceutics 2019, 11, 11, 570

44. Unalan I, Colpankan O, Albayrak, A. Z, Gorgun, C., Sendemir Urkmez, A., (2016), *Biocompatibility of plasma-treated poly(3-hydroxybutyrate-co-3-hydroxyvalerate) nanofiber mats modified by silk fibroin for bone tissue regeneration*, Materials Science and Engineering: C, 68, 1, 842-850.

45. Lee, B. L. P., Jeon, H., Wang, A., Yan, Z., Yu, J., Grigoropoulos, C. P., Li, S., (2012), *Femtosecond laser ablation enhances cell infiltration into three-dimensional electrospun scaffolds*, Acta Biomaterialia, 8, 7, 2648-2658.

46. Colpankan Gunes, O., Ziylan Albayrak, A., Tasdemir, S., Sendemir, A., (2020), *Wet-electrospun PHBV nanofiber reinforced carboxymethyl chitosan-silk hydrogel composite scaffolds for articular cartilage repair*, Journal of Biomaterials Applications, 35, 4–5, 515–531.

47. Liang, P., Chen, C., Zhao, S., Ge, F., Liu, D., Liu, B., Fan, Q., Han, B., Xiong, X., (2013), *Application of Fourier Transform Infrared Spectroscopy for the Oxidation and Peroxide Value Evaluation in Virgin Walnut Oil*, Journal of Spectroscopy, 138728.

48. Irnawati Riyanto, S., Martono, S., Rohman, A., (2020), *The employment of FTIR spectroscopy and chemometrics for authentication of pumpkin seed oil from sesame oil*, Food Research, 4, 1, 42-48.