Journal of Agricultural Science and Technology (JAST), 28(5)
In Press, Pre-Proof Version

Physical, Mechanical, and Antifungal Properties of Ethyl cellulose/Polycaprolactone Electrospun Nanofibers Incorporated with Gallic Acid and Natamycin for Cheese Packaging

Samira Beikzadeh[1], Ali Ehsani[2*], Amir Mohammad Mortazavian[3*], Zhian Sheikhi[2], Marjan Ghorbani[4], and Soghra Ramezani[5]

Abstract

Electrospinning is a process used to produce nanofibers that can be applied in active food packaging. In this study, polymers of ethyl cellulose (ECL) and polycaprolactone (PCL) were blended in ratios of 70:30, 80:20, and 90:10 (% w/w), and combined with natamycin (NAT) at concentrations of 1% and 2% (w/w) and gallic acid (GAL) at 10% and 30% (w/w). Nanofibers were produced via electrospinning under the following conditions: voltage of 16 kV, flow rate of 1 ml/hour, and a tip-to-collector distance of 15 cm. The nanofibers were characterized for their morphology, FTIR, thermal, mechanical, antioxidant, water contact angle**,** and antifungal properties. The results indicated that ECL90/PCL10, ECL80/PCL20, and ECL70/PCL30 nanofibers had average diameters of 1250.86 ± 18 nm, 1174 ± 21.4 nm, and 754.73 ± 23 nm, respectively. The ECL70/PCL30 ratio was selected and combined with NAT and GAL. The average diameter of these nanofibers ranged from 779.32 to 924.63 nm. The addition of NAT and GAL enhanced the thermal stability, mechanical properties, and antifungal efficacy of the ECL/PCL nanofibers. Notably, nanofibers containing 2% NAT combined with either 10% or 30% GAL completely inhibited the growth of molds. Cheese samples packaged with nanofibers containing NAT and GAL exhibited a significantly lower increase in mold and yeast counts during storage compared to control samples. Furthermore, sensory evaluation revealed no significant differences in scores among the packaged samples. Therefore, ECL/PCL nanofibers incorporated with 2% NAT and 10% GAL present an effective alternative to the direct incorporation of NAT in cheese formulations.

Keywords: Cheese, Ethyl cellulose, Gallic acid, Natamycin, Polycaprolactone.

[1] Department of Nutrition and Food Sciences, Maragheh University of Medical Sciences, Maragheh, Islamic Republic of Iran.
[2] Department of Food Science and Technology, Faculty of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Tabriz, Islamic Republic of Iran.
[3] Department of Food Science and Technology, Faculty of Nutrition Sciences and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Islamic Republic of Iran.
[4] Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Islamic Republic of Iran.
[5] Faculty of Textile Engineering, Urmia University of Technology, Urmia, Islamic Republic of Iran.
*Corresponding authors; e-mails: ehsaniali@yahoo.com and mortazvn@sbmu.ac.ir

Introduction

Fungi are among the primary agents of food spoilage, rendering food unfit for human consumption by diminishing its nutritional value and producing harmful mycotoxins (Kumar, Mishra et al. 2007). Molds belonging to the *Aspergillus* and *Penicillium* genera are among the most frequently encountered contaminants during the production, ripening, curing, and refrigerated storage of food products prior to consumption (Gandomi, Misaghi et al. 2009, Beikzadeh, Hosseini et al. 2021). Natamycin (also known as pimaricin) is a polyene macrolide antifungal compound produced by species of *Streptomyces*, and is commonly classified within the polyene macrolide fungicide group (Delves-Broughton, Thomas et al. 2005). It is widely used as an antimicrobial agent for surface preservation in various sausages and cheeses (Regulation 1333/2008/EC). Compared to other fungicides, natamycin demonstrates superior efficacy, with most fungi being inhibited or destroyed at concentrations ranging from 0.5 to 6 µg/mL (Koontz and Marcy 2003, Delves-Broughton, Thomas et al. 2005). However, the efficacy of natamycin may be diminished when directly incorporated into food matrices, due to interactions with other food components (Fajardo, Martins et al. 2010). Therefore, incorporating NAT into polymer matrices can enhance its stability and enable controlled release, thereby maintaining effective concentrations of the compound on the food surface.

GAL (3,4,5-trihydroxybenzoic acid) and its derivatives, including catechin and tannin, are naturally present in a variety of plants, as well as in wine, tea, cereals, citrus fruits, and berries (Laura, Alvarez-Parrilla and González-Aguilar 2009). Owing to its potent antioxidant and antimicrobial properties, gallic acid is widely utilized as an additive in the food and pharmaceutical industries. However, its application in food products is often limited by its inherent bitterness and astringency. Additionally, GAL is sensitive to environmental factors such as light, oxygen, pH, and temperature, which can affect its stability and efficacy (Fang and Bhandari 2010, Li, Kim et al. 2016). Encapsulation techniques are employed to prevent oxidation, enhance stability, and mask the undesirable taste of gallic acid. Common encapsulation methods include lyophilization, cocrystallization, liposome entrapment, coacervation, extrusion, spray cooling or chilling, and spray drying (Fang and Bhandari 2010).

Novel encapsulation technologies, such as electrospinning, offer significant advantages by eliminating the need for toxic solvents and high processing temperatures. Electrospinning is particularly suitable for encapsulating food and pharmaceutical compounds for several reasons.

Notably, the process occurs at room temperature, which helps preserve the integrity of sensitive active compounds. This technique enables the formation of nano-sized fibers, providing a high surface-area-to-volume ratio that can enhance the release of encapsulated agents. Furthermore, the polymer matrix within the nanofibers facilitates sustained or controlled delivery of the active compounds (Mohammadi, Rostami et al. 2018, Beikzadeh, Hosseini et al. 2021, Beikzadeh, Ehsani and Ramezani 2025). Moreover, a limited surface area can slow the release of active substances. By reducing fiber size from the microscale to the nanoscale, electrospun fibers provide a significantly increased surface area, which facilitates more efficient and controlled release of encapsulated compounds (Neo, Swift et al. 2013, Aytac, Ipek et al. 2017, Beikzadeh, Hosseini et al. 2021). In the electrospinning process, both synthetic and natural polymers can be utilized to fabricate nanofibers (Beikzadeh, Hosseini et al. 2021). The accumulation of packaging waste, especially from synthetic polymers that exhibit long environmental persistence, poses a major worldwide environmental challenge. To address this issue, the use of biodegradable biopolymers in packaging has emerged as a viable approach to reducing plastic waste (Pilevar, Bahrami et al. 2019, Beikzadeh, Ghorbani et al. 2020, Beikzadeh, Khezerlou et al. 2020, Javdani, Abedi-Firoozjah et al. 2025). Among biopolymers, ethyl cellulose stands out as one of the most important and widely utilized cellulose ether derivatives. It is characterized by its hydrophobic nature and resistance to salts and alkaline conditions (Beikzadeh, Hosseini et al. 2021). Blending different polymers is one of the most economical and practical approaches to producing materials with enhanced mechanical and thermal properties (Gregorova, Machovsky and Wimmer 2012, Beikzadeh, Hosseini et al. 2021). PCL is recognized for its compatibility with various polymers. It is a six-carbon polymer featuring hydroxyl end groups. However, its relatively low melting point (~60 °C) limits its thermal performance. To overcome this constraint, PCL is often blended with other polymers to enhance its thermal and mechanical properties (Chrissafis, Antoniadis et al. 2007).

In terms of degradability, Electrospinning enables rapid drying because the fibers produced are extremely fine, which limits the time available for crystal growth. Additionally, since the annealing process is performed below the melting point, it further contributes to reduced crystallinity. Consequently, the brief annealing of the electrospun mats appears to be a promising strategy for creating materials that degrade quickly (Salević-Jelić, Lević et al. 2024). Also, PCL demonstrates favorable biodegradability under appropriate environmental conditions. Different

microorganisms, including bacteria and fungi, produce extracellular depolymerases that enable them to break down PCL. The breakdown of PCL-based films happens through the attachment of compost microorganisms to the surface, followed by enzymatic action, fragmentation of the film, and the release of degradation byproducts (Salević-Jelić, Lević et al. 2024). The incorporation of ethyl cellulose into polymer matrices has been reported to enhance biodegradability. For instance, Girija et al. (2010) demonstrated that blending ethyl cellulose with poly(ethylene-co-vinyl acetate) (EVA) led to improved biodegradation characteristics (Girija, Sailaja et al. 2010).

In this study, ECL/PCL nanofibers containing varying concentrations of NAT and GAL were fabricated. The thermal, mechanical, morphological, and antifungal properties of the nanofibers were evaluated. Subsequently, cheese samples were packaged with the optimized nanofibers, and their mold growth and overall sensory acceptance were assessed.

Experimental Methods

Required Materials

Fungal strains were obtained from the Iranian Research Organization for Science and Technology. Polymers, including PCL and ECL, along with NAT and GAL, were procured from Sigma-Aldrich.

Production Steps of Electrospinning Solutions

Approximately 12.5% (w/v) solutions of ECL and PCL were prepared in a 50:50 (v/v) ethanol/chloroform solvent mixture at 25 °C. Polymer blends with ECL/PCL ratios of 90:10, 80:20, and 70:30 were subsequently formulated. Ultimately, the ECL/PCL ratio of 70:30 was selected (Beikzadeh, Hosseini et al. 2021). NAT at 1% and 2% (wt%) and GAL at 10% and 30% (wt%) were then incorporated into the nanofiber solutions (Amjadi, Almasi et al. 2020).

Electrospinning Operation

The electrospinning process was conducted at an applied voltage of 16 kV, a flow rate of 1 ml/hour, and a tip-to-collector distance of 15 cm. The operation was performed at a temperature of 25 ± 2 °C and relative humidity of 30 ± 1%. The resulting nanofibers were collected on the grounded collector (Amjadi, Almasi et al. 2020, Beikzadeh, Ehsani and Ramezani 2025).

Characteristics of Produced Nanofibers

Scanning Electron Microscopy

Scanning electron microscopy (SEM) operated at 26 kV was employed to examine the microstructure of the nanofibers. Fiber diameters were quantified using NIS Elements 0.3 image analysis software.

Fourier Transforms Infrared

Fourier-transform infrared (FTIR) spectra were recorded using a Tensor 27 spectrometer (Ettlingen, Germany). Interferograms were collected over the spectral range of 1000–4500 cm^{-1}(Sheikhi, Mirmoghtadaie et al. 2020).

Antioxidant Activity

DPPH radical scavenging method evaluates antioxidant activities (Altan, Aytac and Uyar 2018). The antioxidant activity of the nanofibers was evaluated using the DPPH radical scavenging assay. Briefly, 100 mg of nanofibers were suspended in 2 mL of water and stirred. Subsequently, 1 mL of the resulting solution was mixed with 0.2 mL of DPPH solution. After incubating the mixture at 25 °C for 30 minutes, the absorbance was measured at 517 nm using a spectrophotometer. The scavenging activity was then calculated using the appropriate equation to determine the antioxidant capacity.

Water Contact Angle (WCA)

A drop of water (5 µL in volume) was deposited onto the nanofiber surface, after which the contact angle was photographed and analyzed (Heidari, Bahrami et al. 2019, sheikhi, Farhoodi et al. 2020).

Mechanical Properties

To assess the mechanical properties, the nanofibers were cut into dumbbell-shaped specimens (0.6 cm × 9 cm) and subjected to tensile testing at a crosshead speed of 1.0 mm/min (Amjadi, Emaminia et al. 2019).

Thermogravimetric Analysis (TGA)

For the TGA, approximately 15 mg of nanofibers were heated under a nitrogen atmosphere at a rate of 10 °C/min up to 600 °C using a thermogravimetric analyzer.

Release Kinetics of NAT and GAL

The release profiles of gallic acid (GAL) and natamycin (NAT) were evaluated by immersing nanofibers (15.1 cm² area) in 10 mL of distilled water at 4 °C. At predetermined time intervals (0, 250, 500, 750, 1000, and 1250 hours), the absorbance of GAL and NAT was measured at 260 nm and 320 nm, respectively, using a UV-Visible spectrophotometer. The concentration of each compound was determined based on calibration curves constructed from standard solutions with concentrations ranging from 1 to 50 mg/L (Titone, Ceraulo et al. 2025).

Antifungal Properties

To evaluate antifungal activity, *Aspergillus niger* (PTCC 5012) and *Penicillium notatum* (PTCC 5014) strains were cultured on potato dextrose agar (PDA) media. A spore suspension (10^6 spores/mL) was prepared, and 1 mL of this suspension was homogenized with 99 mL of PDA. Nanofiber samples (1 × 1 cm) were sterilized under ultraviolet light for 1 hour, then placed onto the inoculated plates. The plates were incubated for 5 days, after which the diameter of the inhibition zone around the nanofibers was measured to assess antifungal efficacy (Veras, Roggia et al. 2016).

UF cheese packaging

Based on the evaluation of the morphological, thermal, mechanical, and antifungal properties of the produced nanofibers, those exhibiting optimal characteristics were selected for packaging ultrafiltrated (UF) cheese. For this purpose, aluminum foils coated with nanofibers (both with and without preservatives) were sterilized using ultraviolet (UV) light for 40 minutes. Subsequently, UF cheese was produced and cut into pieces measuring 5 × 25 × 30 mm. The cheese samples were sterilized under UV light for 20 minutes before being packaged using the nanofiber-coated aluminum foils (Beikzadeh, Ehsani and Ramezani 2025).

Desired tests on packaged cheeses

Microbial analysis of produced cheeses

Mold and yeast counts were performed using Sabouraud dextrose agar culture medium via the pour plate method, with a 0.01 dilution, under refrigerated storage conditions throughout the storage period.

Sensory analysis of manufactured cheeses

The sensory evaluation was conducted by a panel of 30 trained assessors. Cheese samples were removed from refrigeration one hour prior to testing to equilibrate to room temperature and were coded using a random number table. Each panelist received the samples along with a standardized evaluation questionnaire and assessed them based on texture, taste, aroma, and overall acceptance. Evaluations were performed using a 5-point hedonic scale.

Statistical Evaluation

Data obtained from three independent replicates were expressed as mean ± standard deviation. Statistical differences among groups were analyzed using one-way analysis of variance (ANOVA) performed in SPSS software. Duncan's multiple range test was applied to determine significant differences at a confidence level of $p < 0.05$.

Results and Discussion

Scanning Electron Microscopy

The SEM images and corresponding mean diameter distributions of the electrospun ECL/PCL nanofibers are presented in Figure 1. The average fiber diameters for ECL90/PCL10, ECL80/PCL20, and ECL70/PCL30 were 1250.86 ± 18 nm, 1174 ± 21.4 nm, and 754.73 ± 23 nm, respectively. For comparison, Wang et al. (2020) reported an average diameter of 649 nm for pure ECL nanofibers (Wang, Li et al. 2020). Liu et al. (2018) reported nanofibers with a spindle-like morphology and an average diameter of 413 nm (Liu, Deng et al. 2018). In contrast, PCL nanofibers have been reported to exhibit an average diameter of approximately 419 nm. As shown in Figure 2, the average diameters of nanofibers containing ECL70/PCL30 with varying concentrations of NAT and GAL (ECL70/PCL30/NAT1/GAL10, ECL70/PCL30/NAT2/GAL10, ECL70/PCL30/NAT1/GAL30, and ECL70/PCL30/NAT2/GAL30) were 779.32 ± 35 nm, 886.27 ± 13.5 nm, 805.45 ± 12 nm, and 924.63 ± 19 nm, respectively. The highest average diameter was observed for the ECL70/PCL30/NAT2/GAL30 sample. Overall, the incorporation of NAT and GAL led to an increase in fiber diameter. Neo et al. (2013) reported that the addition of GAL to zein solutions resulted in increased fiber diameters. This effect was attributed to the elevated solution viscosity caused by GAL, which enhances molecular entanglement and consequently leads to larger fiber diameters (Neo, Swift et al. 2013). Similarly, Veras et al. (2020) reported that

the incorporation of NAT into the polycaprolactone electrospinning solution led to an increase in fiber diameter. Notably, tripling the NAT concentration compared to the initial sample resulted in a 64% increase in the average fiber diameter (Veras, Ritter et al. 2020). These findings are consistent with the results obtained in the present study.

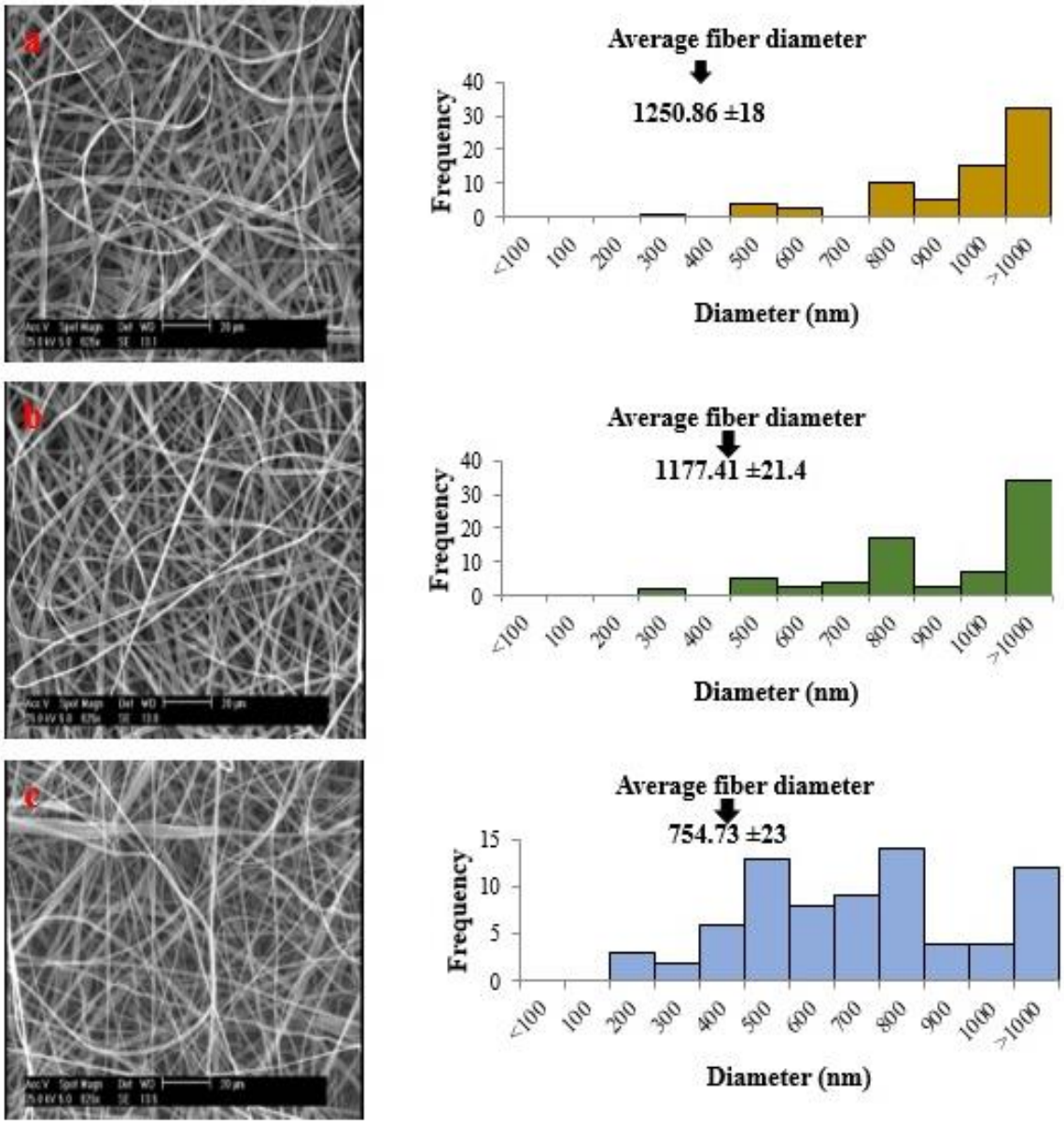


Figure 1. Fiber diameter distributions of electrospun nanofibers: (a) ECL90/PCL10, (b) ECL80/PCL20, and (c) ECL70/PCL30. Abbreviations: NAT, Natamycin; GAL, Gallic acid; ECL, Ethyl cellulose; PCL, Polycaprolactone.

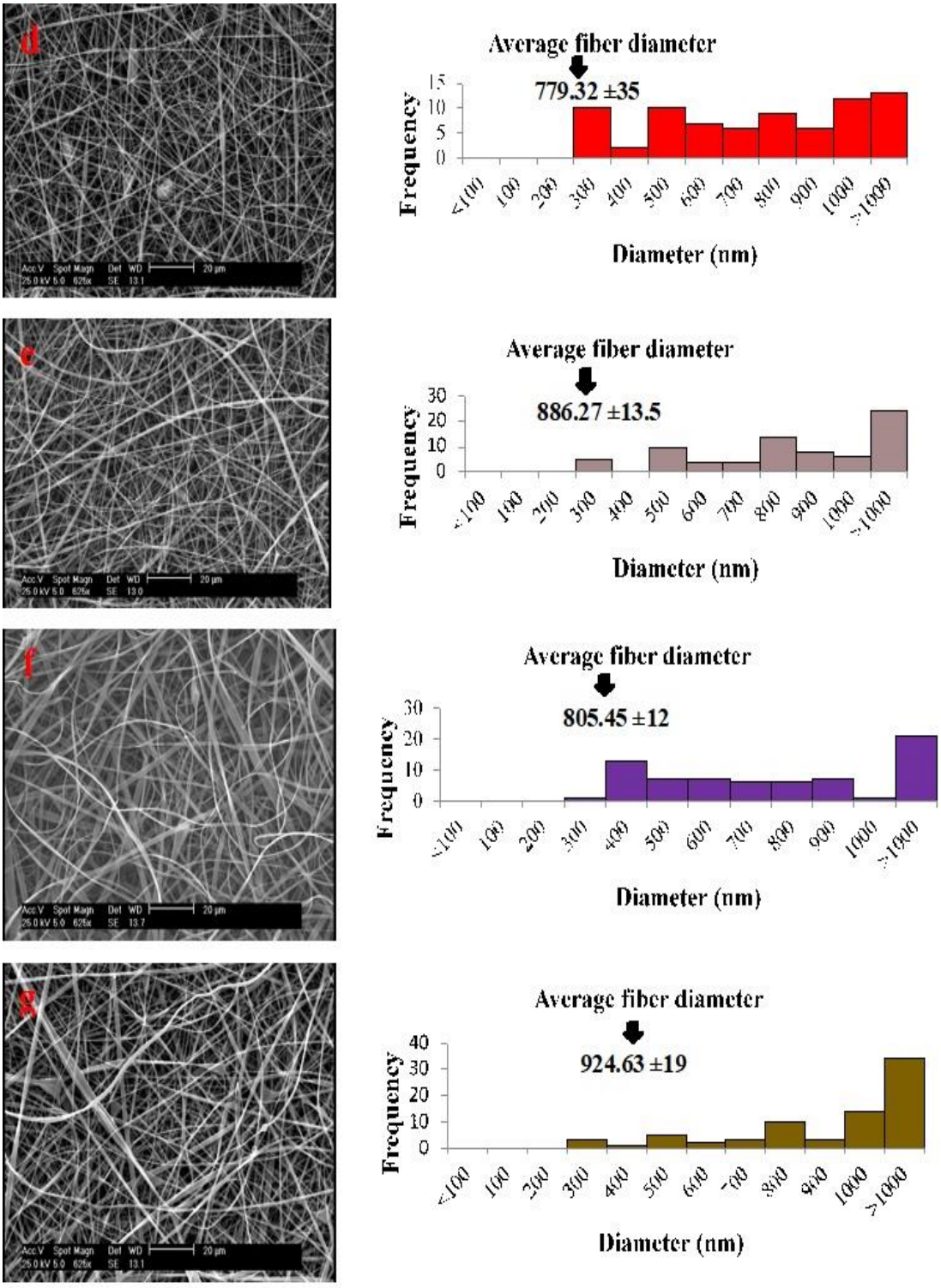


Figure 2. SEM images and average nanofiber diameters of ECL70/PCL30/NAT1/GAL10 (d), ECL70/PCL30/NAT2/GAL10 (e), ECL70/PCL30/NAT1/GAL30 (f), and ECL70/PCL30/NAT2/GAL30 (g). Abbreviations: NAT, Natamycin; GAL, Gallic acid; ECL, Ethyl cellulose; and PCL, Polycaprolactone.

Fourier Transforms Infrared (FTIR)

Table 1 and Figure 3 present the wavenumbers of characteristic peaks observed in the nanofibers. For the ECL nanofiber, the peaks at 2879, 3450–3741, 2621–2742, and 1249 cm^{-1} correspond to $–CH_3$ stretching vibration, O–H vibration, CH_2 and CH stretching, and C–O–C linkage, respectively (Beikzadeh, Hosseini et al. 2021). For PCL, the peaks observed at 2889–3030 cm^{-1}, 1743 cm^{-1}, 1375 cm^{-1}, and 1047–1257 cm^{-1} can be attributed to CH_2 stretching vibrations, C=O stretching, C–C stretching, and C–O–C stretching vibrations, respectively (Beikzadeh, Hosseini et al. 2021, Beikzadeh, Ehsani and Ramezani 2025). For NAT, the presence of peaks in the range of 1100–1750 cm^{-1} was attributed to the C=O bond. In the case of GAL, the peaks at 3490–3748 cm^{-1} corresponded to O–H stretching vibrations. The peaks observed at 1520–1710 cm^{-1} were related to the stretching vibrations of C–C and C–H aromatic groups. Additionally, the peaks at 1193–1411 cm^{-1} were assigned to C–O stretching vibrations. Changes in the ECL spectrum were observed following the addition of PCL, with the peaks at 2870 and 3450 cm^{-1} shifting to higher wavelengths. Furthermore, a new peak appeared at 2504 cm^{-1}. Upon the incorporation of NAT and GAL, further modifications were detected in the ECL70/PCL30 spectrum, including the emergence of novel peaks at 3380, 3220, and 3114 cm^{-1}, while the peak at 2804 cm^{-1} shifted to lower wavelengths. Veras et al. (2020) reported that mixing NAT with PCL did not cause significant changes in the nanofiber spectra, likely due to the absence of chemical bonding between the polymers and antifungal compounds (Veras, Ritter et al. 2020).

Table 1. Types of nanofibers, connections, and wavenumber of specified peaks of nanofibers.

Nanofiber types	Connection	Wavenumber (1/cm)
ECL	$–CH_3$ connection vibration	2879
	O–H vibrations	3450-3741
	CH_2 and CH stretches	2621-2742
	C–O–C connection	1249
PCL	CH_2 stretching vibration	2889-3030
	CO connection vibration	1743
	CC connection vibration	1375
	C–O–C connection vibration	1047-1257
NAT	C=O connection	1100-1750
GAL	O–H stretching vibration	3490-3748
	C–C/C–H stretching vibration	1520-1710
	C–O stretching vibration	1193-1411

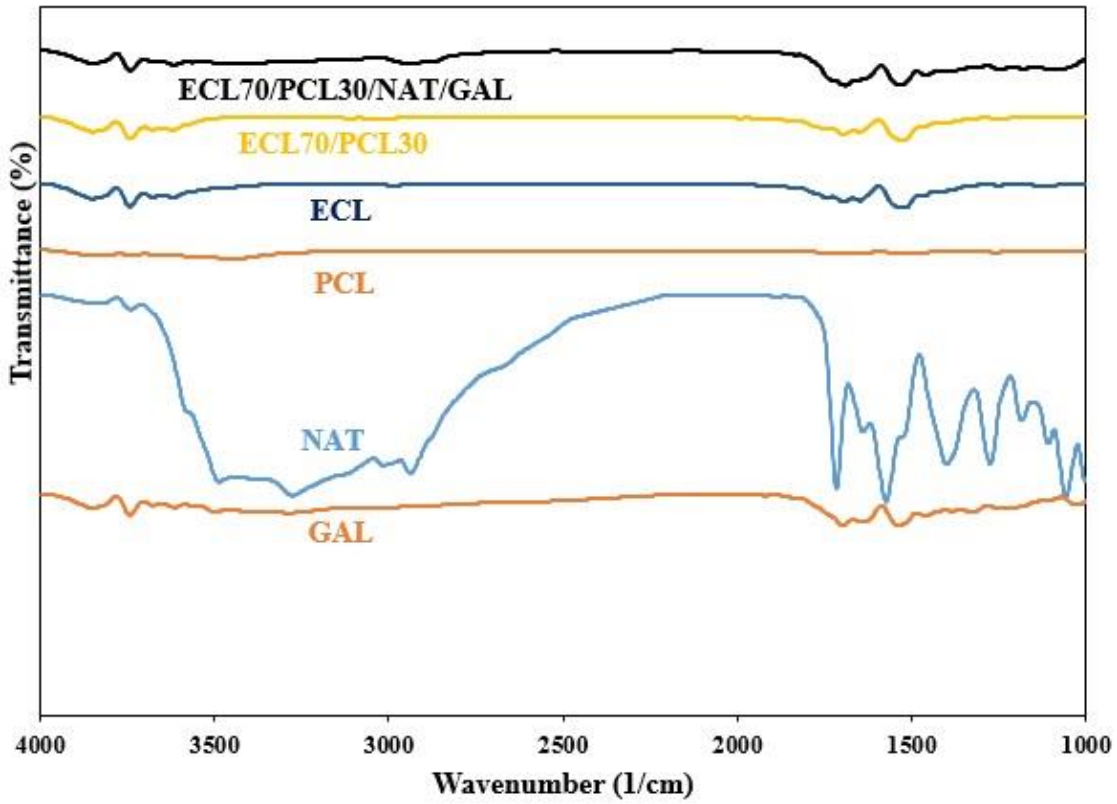


Figure 3. FTIR results of various nanofibers. PCL, Polycaprolactone; ECL, Ethyl cellulose; NAT, Natamycin; GAL, Gallic acid.

Mechanical Properties

The mechanical properties of fibers containing NAT and GAL are presented in Table 2. For ECL70/PCL30 nanofibers, thickness, tensile strength, and elongation at break were measured as 0.112 ± 0.007 mm, 8.22 ± 0.4 MPa, and 4.49 ± 0.3%, respectively. ECL70/PCL30 exhibited superior mechanical properties compared to ECL90/PCL10 and ECL80/PCL20. The increase in tensile strength is attributed to the formation of new interactions between the polymers (Liu, Deng et al. 2018), , a phenomenon also supported by the FTIR results. Additionally, the incorporation of NAT and GAL further enhanced the mechanical properties of the nanofibers.

Table 2. Mechanical properties of nanofibers.

Sample	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)
ECL90/PCL10	0.108 ± 0.005 ^{b}	5.93 ± 0.6 ^{d}	2.66 ± 0.2 ^{c}
ECL80/PCL20	0.109 ± 0.001 ^{b}	6.78 ± 0.4 ^{c}	3.12 ± 0.4 ^{c}
ECL70/PCL30	0.112 ± 0.007 ^{b}	8.22 ± 0.4 ^{b}	4.49 ± 0.3 ^{b}
ECL70/PCL30/NAT/GAL	0.124 ± 0.003 ^{a}	9.12 ± 0.5 ^{a}	6.32 ± 0.4 ^{a}

Mean ± Standard deviation.

Water Contact Angle (WCA)

WCA analysis was conducted to assess the surface wettability of the fabricated nanofibers, with the results presented in Figure 4. The WCA values for ECL90/PCL10, ECL80/PCL20, and ECL70/PCL30 were measured as 49.03°, 54.84°, and 59.77°, respectively. An increase in PCL content corresponded with a higher WCA, indicating enhanced hydrophobicity. This behavior may be attributed to hydrogen bonding interactions between ECL and PCL polymers (Beikzadeh, Hosseini et al. 2021). Additionally, the incorporation of NAT and GAL into the ECL70/PCL30 nanofibers further increased the WCA values. Shen et al. (2021) reported that increasing the weight of encapsulated natamycin led to a rise in the WCA of gelatin/zein/polyurethane nanofibers, attributed to the hydrophobic nature of NAT (Shen, Cao et al. 2021).

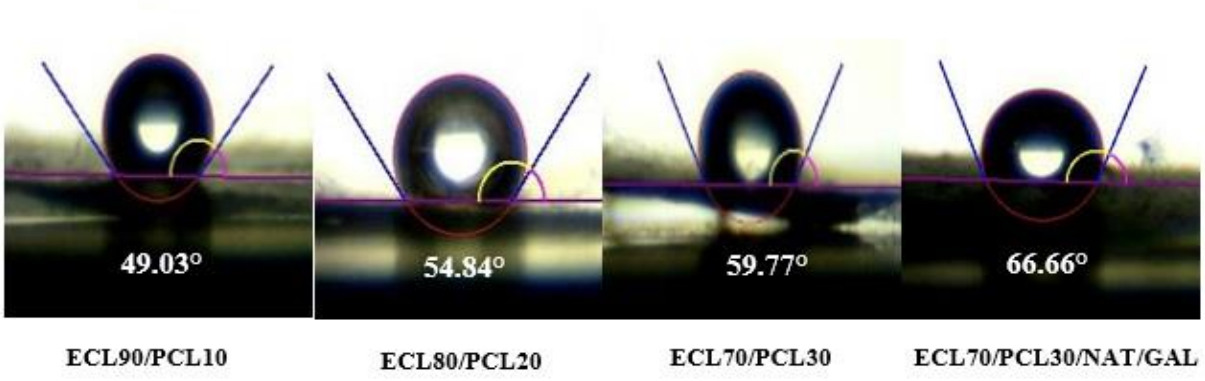


Figure 4. Water contact angles of the formed nanofibers: ECL, ethyl cellulose; PCL, polycaprolactone; NAT, natamycin; GAL, gallic acid.

Thermogravimetric Analysis (TGA)

The thermogravimetric analysis (TGA) results of the nanofibers containing natamycin and gallic acid are presented in Figure 5. The decomposition temperature of pure ECL was approximately 310.56 °C, whereas the ECL/PCL and ECL/PCL/NAT/GAL nanofibers exhibited decomposition temperatures around 315 °C and 325.3 °C, respectively. These results indicate that the thermal stability of the nanofibers improved with the incorporation of NAT and GAL into the ECL matrix. Additionally, the observed shift in thermal decomposition profiles between the neat ECL/PCL blend and the NAT/GAL-loaded nanofibers confirms the successful incorporation of the active compounds. Similarly, Neo et al. (2013) reported that blending GAL with zein enhanced the decomposition temperature of the resulting nanofibers (Neo, Swift et al. 2013).

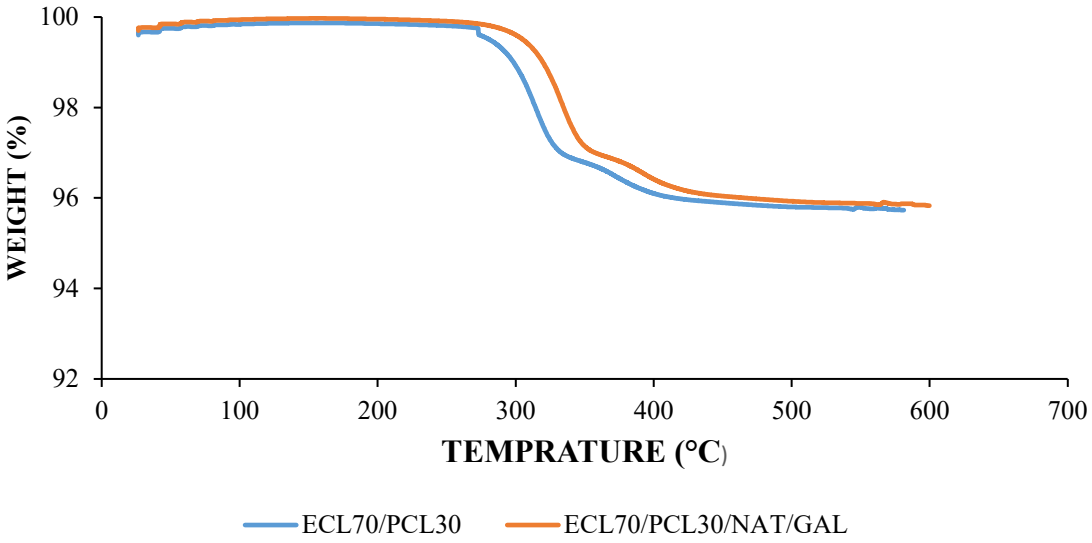


Figure 5. TGA curves of ECL70/PCL30 and ECL70/PCL30/NAT/GAL nanofibers. ECL: Ethyl cellulose, PCL: Polycaprolactone, NAT: Natamycin, GAL: Gallic acid.

Antioxidant Activity Measurements

The antioxidant activity of the nanofibers in 80% ethanol aqueous solutions is presented in Figure 6. GAL reduces free radicals by acting as a hydrogen or electron donor (Chuysinuan, Chimnoi et al. 2009). ECL70/PCL30 electrospun nanofibers incorporated with 10% and 30% GAL exhibited antioxidant activities of approximately 62.38 ± 3.5% and 78.23 ± 2.9%, respectively. In contrast, the neat ECL70/PCL30 nanofiber showed only 4.12 ± 1.8% antioxidant activity. Neo et al. (2013) reported antioxidant properties ranging from 58% to 89% for zein nanofibers containing GAL.

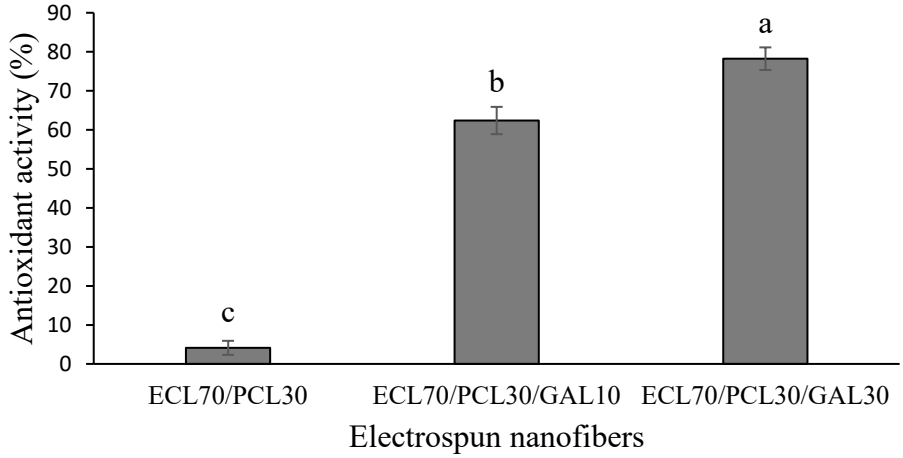
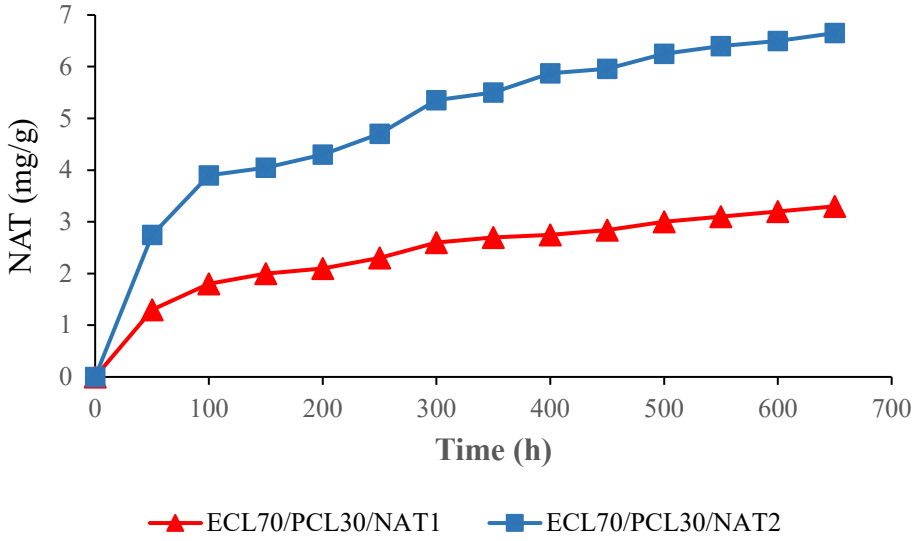


Figure 6. Antioxidant properties of electrospun nanofibers. ECL: Ethyl cellulose, PCL: Polycaprolactone, NAT: Natamycin, GAL: Gallic acid.

Release Kinetics of NAT and GAL

Figure 7(a) illustrates the release profiles of natamycin (NAT) from the ECL70/PCL30/NAT1 and ECL70/PCL30/NAT2 nanofibers. The NAT2 formulation demonstrated a higher cumulative release of approximately 6.7 mg/g, compared to around 3.3 mg/g for NAT1, over a period of nearly 700 hours. Both samples exhibited an initial burst release within the first 100 hours, which is characteristic of electrospun nanofibers and is likely caused by the rapid desorption of NAT molecules located near the fiber surface (Sill and Von Recum 2008). Following this phase, the release continued at a slower, sustained rate, consistent with diffusion-controlled mechanisms commonly observed in polymeric nanofiber systems (Li and Xia 2004). The differences in release profiles may be attributed to variations in fiber morphology, polymer crystallinity, and drug-polymer interactions influencing the diffusion rate of NAT. The U.S. Food and Drug Administration (FDA) regulation No. 155-172 (200) authorizes the application of NAT on cheese surfaces, with the stipulation that its concentration in the final product dose not exceed 20 ppm (Türe, Eroğlu et al. 2008).

Also, the release profiles of GAL from ECL 70/PCL 30/GAL10 and ECL 70/PCL 30/GAL30 are shown in Figure 7 (b). Both samples exhibited an initial burst release within the first 100 hours, followed by a sustained and gradual release over the subsequent period (up to 700 hours). The GAL30 formulation released a significantly higher amount of GAL (~5.2 mg/g) compared to GAL10 (~3.6 mg/g), indicating that increasing the GAL loading enhances cumulative release. The burst release phase is commonly attributed to the presence of GAL near the fiber surface, allowing for rapid diffusion into the release medium (Sill and Von Recum 2008). After this stage, the release rate decreased, suggesting that the migration of GAL molecules through the polymer matrix was primarily governed by diffusion (Kenawy, Bowlin et al. 2002). Variations between the two formulations might result from modifications in the nanofiber structure due to elevated GAL content. A higher concentration of GAL can impact the fiber's porosity, hydrophilic properties, and degree of crystallinity, which collectively affect the release behavior (Kenawy, Bowlin et al. 2002). Additionally, the small molecular size and hydrophilic characteristics of GAL facilitate its prolonged release from the hydrophobic PCL/ECL mixture, as this blend restricts water penetration and slows down drug diffusion (Bhardwaj and Kundu 2010). These controlled and sustained release characteristics are beneficial for antifungal drug delivery, as they help maintain the prolonged bioactivity of both NAT and GAL (Agarwal, Wendorff and Greiner 2008).



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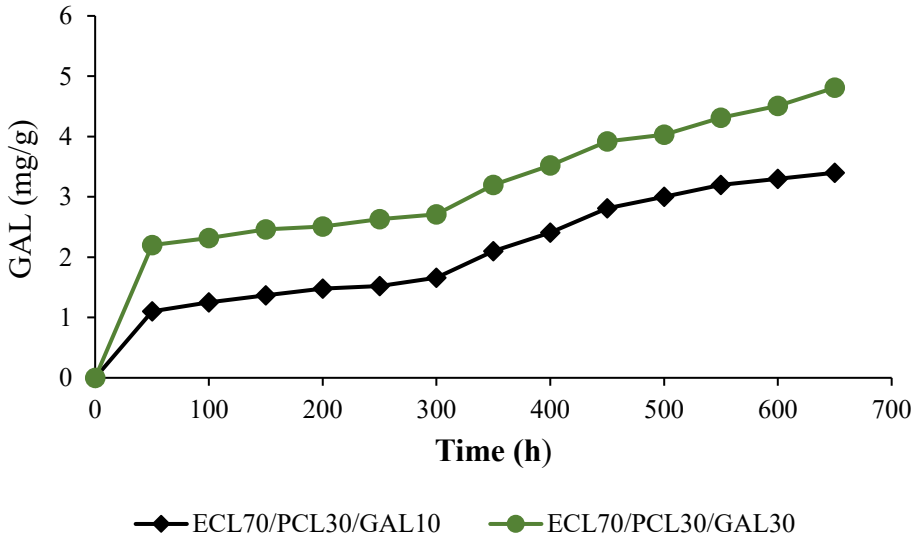

b

Figure 7. (a) Cumulative NAT release from ECL/PCL/NAT, (b) Cumulative GAL release from ECL/PCL/GAL. ECL: Ethyl cellulose, PCL: Polycaprolactone, NAT: Natamycin, GAL: Gallic acid.

Antifungal Properties

The results of the antifungal activity of nanofibers containing NAT and GAL are shown in Table 3 and Figure 7. Control samples (ECL70/PCL 30) and samples containing 1% NAT and 10% or 30% GAL did not exhibit antifungal activity against the growth of *Aspergillus niger* and *Penicillium notatum*. However, nanofibers containing 2% NAT inhibited fungal growth. ECL/PCL /NAT/GAL nanofibers also showed lower antifungal activity against *Aspergillus niger* compared

to *Penicillium notatum*. Overall, the results indicate that the antifungal properties of the nanofibers improve with increasing amounts of NAT. Additionally, adding 2% NAT combined with 10% or 30% GAL completely prevented the growth of both molds. Veras et al. (2020) reported that the inhibition zones of PCL nanofibers containing 1% to 4% NAT ranged from approximately 6.6 to 24 mm against *Aspergillus flavus* and *Penicillium citrinum*. With increasing amounts of NAT, the antifungal properties of nanofibers increased (Veras, Roggia et al. 2016).

Table 3. The colony diameter of *Aspergillus niger* and *Penicillium notatum.*

Nanofiber samples	Colony Diameter (mm)	
	Aspergillus niger	*Penicillium notatum*
ECL70/PCL30	38 ± 1.4	36 ± 1.6
ECL70/PCL30/NAT1/GAL10	32 ± 0.9	25 ± 1.1
ECL70/PCL30/NAT2/GAL10	0	0
ECL70/PCL30/NAT1/GAL30	18 ± 0.7	12 ± 1.3
ECL70/PCL30/NAT2/GAL30	0	0

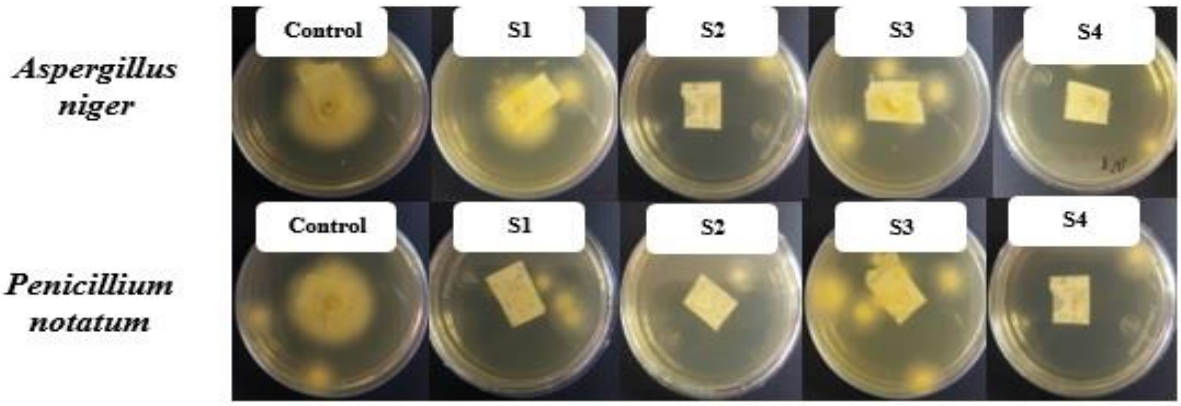

Figure 7. Antifungal properties of nanofibers: (ECL70/PCL30 (control), ECL70/PCL30/NAT1/GAL10 (S1), ECL70/PCL30/NAT2/GAL10 (S2), ECL70/PCL30/NAT1/GAL30 (S3), and ECL70/PCL30/NAT2/GAL30 (S4) nanofibers. ECL: Ethyl cellulose, PCL: Polycaprolactone, NAT: Natamycin, GAL: Gallic acid.

Mold and Yeast Analysis of Cheeses Packaged by Nanofibers

Changes in the amount of mold and yeast in cheeses packaged with nanofibers are shown in Table 4. In all treatments, the levels of mold and yeast increased over time, with the control sample (cheese packed with nanofibers without NAT and GAL) showing a higher increase compared to the other samples.

Fajardo et al. (2010) packaged semi-hard cheese (Saloio cheese) with chitosan and chitosan coatings containing natamycin for 37 days at 4°C. Microbial analysis of the cheese samples

showed that the amount of mold and yeast ranged from 4.53 to 6.06 Log (CFU g^{-1}). The shelf life of Saloio cheese is about 50 days, but after 37 days, fungal growth on the surface of the cheese ended the microbial analysis (Fajardo, Martins et al. 2010). These findings are consistent with our results. Also, Jalilzadeh et al. (2020) used whey protein containing NAT to package ultra-refined cheese, and the results showed that the amount of mold and yeast in the control samples was higher than in the coated samples after 28 days (Jalilzadeh, Hesari et al. 2020). The lower levels of mold in cheeses packaged with nanofibers containing NAT and GAL, compared to the control sample, demonstrate the stability of these active compounds during storage and their antimicrobial effects.

Table 4. Mold and yeast levels Log (CFU g^{-1}) of cheeses packaged with nanofibers.

Packaging type	Storage period (days)			
	1	14	28	56
Without packaging	5.16 ± 0.11^{aC}	6.29 ± 0.43^{aB}	7.55 ± 0.54^{aA}	-
ECL70/PCL30	5.09 ± 0.26^{aC}	5.33 ± 0.47^{bBC}	5.98 ± 0.29^{aAB}	6.28 ± 0.61^{aA}
ECL70/PCL30/NAT2/GAL10	5.12 ± 0.09^{aC}	5.28 ± 0.16^{bBC}	5.78 ± 0.24^{aAB}	6.03 ± 0.27^{aA}
ECL70/PCL30/NAT2/GAL30	5.04 ± 0.17^{aB}	5.15 ± 0.46^{bB}	5.69 ± 0.15^{aA}	5.97 ± 0.35^{aA}

Overall Acceptance of Cheeses Packaged by Nanofibers

Overall acceptance of cheese samples packed with nanofibers during refrigerated storage is shown in Table 5. The results of the sensory evaluation scores for the packaged cheese samples did not show a significant difference between samples packaged with NAT-free nanofibers, nanofibers containing 2% NAT and 10% GAL, and nanofibers containing 2% NAT and 30% GAL. The control sample (without packaging) had a lower score in terms of overall acceptance. Nottagh et al. (2019) used chitosan containing natamycin to package ultra-refined cheese for 6 weeks. The highest scores for taste, smell, and overall acceptance were observed in the packaged samples at the third week compared to the control sample (without packaging) (Nottagh, Hesari et al. 2020).

Table 5. Sensory evaluation score of cheeses packaged with nanofibers.

Overall acceptance	Storage time (days)		
	1	14	28
Without packaging	4.0 ± 0.18^{aA}	3.8 ± 0.35^{aA}	3.6 ± 0.16^{bA}
ECL70/PCL30	3.8 ± 0.14^{aA}	4.0 ± 0.15^{aA}	4.1 ± 0.37^{abA}
ECL70/PCL30/NAT2/GAL10	4.2 ± 0.17^{aA}	3.9 ± 0.39^{aA}	4.2 ± 0.32^{aA}
ECL70/PCL30/NAT2/GAL30	3.9 ± 0.36^{aA}	4.0 ± 0.10^{aA}	4.0 ± 0.29^{abA}

Conclusions

This study investigated the antioxidant, antifungal, morphological, and thermal properties of ECL/PCL nanofibers containing NAT and GAL. The results revealed that ECL70/PCL30

exhibited the desired morphological and mechanical properties compared to ECL90/PCL10 and ECL80/PCL20. Additionally, mixing NAT with GAL increased the average fiber diameter, mechanical characteristics, water contact angle (WCA), and thermal stability. Electrospun ECL70/PCL30 nanofibers containing 30% GAL had the highest antioxidant content. Furthermore, ECL70/PCL30/NAT2/GAL10 and ECL70/PCL30/NAT2/GAL30 nanofibers completely prevented the growth of *Aspergillus niger* and *Penicillium notatum*.

Acknowledgements

This research can be introduced with ethics code IR.SBMU.NNFTRI.REC.1398.067 from the Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

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Journal of Agricultural Science and Technology (JAST), 28(5)
In Press, Pre-Proof Version

بررسی ویژگیهای فیزیکی، مکانیکی و ضد قارچی نانوالیاف الکتروریسی شده اتیل سلولز/پلی کاپرولاکتون حاوی گالیک اسید و ناتامایسین برای بسته‌بندی پنیر

سمیرا بیک زاده، علی احسانی، امیر محمد مرتضویان، ژیان شیخی، مرجان قربانی، و صغری رمضانی

چکیده

الکتروریسی فرآیندی برای تولید نانوالیاف است که می‌تواند برای بسته‌بندی فعال مواد غذایی به کار رود. در این مطالعه، از پلیمرهای اتیل سلولز (ECL) و پلی‌کاپرولاکتون (PCL) با نسبت‌های وزنی 70:30، 80:20 و 90:10 درصد و ناتامایسین (NAT)(1 و 2 درصد وزنی/وزنی) و گالیک اسید (GAL) (10 و 30 درصد وزنی/وزنی) استفاده شد. نانوالیاف پس از فرآیند الکتروریسی (ولتاژ 16 کیلوولت، سرعت 1 میلی‌لیتر در ساعت، فاصله 15 سانتی‌متر) تولید شدند. سپس، نانوالیاف از نظر ویژگیهای مورفولوژیکی، FTIR، حرارتی، مکانیکی، آنتی‌اکسیدانی، زاویه تماس آب و خواص ضد قارچی بررسی شدند. نتایج نشان داد که ECL90/PCL10، ECL80/PCL20 و ECL70/PCL30 دارای قطر متوسط 18 ± 1250/86 نانومتر، 21/4 ± 1174 نانومتر، 23 ± 754/73 نانومتر بودند. ECL و PCL با نسبت 70 به 30 انتخاب و با NAT و GAL ترکیب شدند. قطر متوسط نانوالیاف تولید شده بین 779/32 تا 924/63 نانومتر بود. همچنین با افزودن NAT و GAL، پایداری حرارتی، خواص مکانیکی و ضد قارچی ECL/PCL افزایش یافت. افزودن 2 درصد NAT به همراه 10 یا 30 درصد GAL به طور کامل از رشد هر دو کپک جلوگیری کرد. نمونه‌های پنیر بسته‌بندی شده با نانوالیاف حاوی NAT و GAL در تمام تیمارها، افزایش کمتری در میزان کپک و مخمر در طول دوره نسبت به نمونه کنترل داشتند. همچنین، نتایج ارزیابی حسی تفاوت معنی‌داری بین نمونه‌های بسته‌بندی شده نشان نداد. بنابراین، نانوالیاف ECL/PCL ترکیب شده با 2 درصد NAT و 10 درصد GAL می‌توانند به عنوان جایگزینی مؤثر برای افزودن مستقیم NAT به فرمولاسیون پنیر مورد استفاده قرار گیرند.