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Original research

Polymethacrylate coated electrospun chitosan/PEO nanofibers loaded with thyme essential oil: a newfound potential for antimicrobial food packaging

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ABSTRACT

Development of antimicrobial nanofibers by the electrospinning process is one of the most emerging trends in food bio-packaging systems. In this study, the blend of chitosan (CS) and polyethylene oxide (PEO) was employed to fabricate electrospun nanofibrous mats. Thyme essential oil (TEO) was embedded into the electrospun CS/PEO mats at concentrations of 0.5%, 1%, and 1.5% to create nanofibers with antibacterial properties. Adding functional groups to the surface of the CS/PEO/TEO electrospun mat was achieved by dip-coating the mat into a poly (MMA-co-MAA) solution with two different compositions to enhance bacteria immobilization. The morphology and diameter of CS/PEO/TEO nanofibers before and after coating were investigated by field emission scanning electron microscopy (FE-SEM). Atomic force microscopy (AFM) and water contact angle (WCA) measurements demonstrated the physical properties of coated and uncoated electrospun mats. Fourier transform infrared spectroscopy (FTIR) was further utilized to reveal the chemical structure of nanofibrous mats. The inhibition zone diameter was employed as an indicator of antibacterial activity through the disk diffusion test. The study results showed that the TEO-loaded nanofibrous mats, fabricated by an electrospinning system and coated by poly (MMA-co-MAA) at a 7:3 ratio of MMA: MAA can effectively inhibit the growth of bacteria. This novel biopolymer-based electrospun mat proved to be a promising candidate for antimicrobial packaging material due to the remarkable properties of biocompatibility, biodegradability, and excellent antibacterial performance.

Keywords: Electrospinning; Chitosan; Polyethylene oxide (PEO); Antimicrobial activity; Food packaging

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1. Introduction

Recently, there has been significant progress in the application of polymer fibers in the field of biotechnology (Zhang et al., 2020). Electrospinning is the most popular and effective method for producing polymeric fibers. It has been known as a simple, versatile, and superior strategy in nanotechnology (Rostamabadi et al., 2020; Zhao et al., 2020). Polymeric fibers can be fabricated in the range of nanometers to micrometers by using this approach (Frenot & Chronakis, 2003). A great advantage of electrospinning can be ascribed to the mild experimental conditions, which make it suitable for sensitive materials. Electrospinning setup can also be made at quite a low cost (Hajikhani & Lin, 2022). Moreover, the electrospun fibers have revealed advantages of high porosity, large specific surface area, and 3D structures (Hosseini et al., 2015; Zhao et al., 2020). Electrospun fibers have been used in a variety of applications such as drug delivery (Luraghi et al., 2021; Zelkó et al., 2019), tissue engineering (Peranidze et al., 2021; Rahmati et al., 2021), biosensors, and biomaterials (Ahmed, 2021; Majumder et al., 2022), food packaging (Sameen et al., 2022; Wu et al., 2022), etc. It has been proven that electrospun nanofibers can be a great expectant for application in food and packaging systems (Zhao et al., 2020).

Application of natural polymers in electrospinning has been broaden in the food industry as their beneficial properties such as nontoxicity or low-toxicity, biocompatibility and biodegradability (Khalid & Arif, 2022). These properties are the general requirement of electrospun nanofibers for utilizing in food packaging. Furthermore, the specified functionalities of the fibers such as antimicrobial property, being edible and etc. have been required for

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the food quality maintenance and shelf-life extension (Amna et al., 2015; Zhao et al., 2020; Zhu et al., 2019).

Chitosan is a polysaccharide-based natural polymer, which is derived from deacetylation of chitin. Chitosan films have the good properties of transparency, elasticity and oxygen resistant (Guan et al., 2020). It also exhibits the advantages of nontoxicity, biocompatibility, and biodegradation in a short time, anti-microbial and antifungal activities. Thus, the nanofibers based on chitosan have been significantly applied in food packaging technology (Amna et al., 2015; Surendhiran et al., 2020).

In a study by Lin et.al (2018), ε-polylysine/chitosan nanofibers were used to inhibit *Salmonella typhimurium* and *Salmonella enteritidis* on chicken. The nanofibers showed brilliant antibacterial activity and increased the shelf life, whilst maintained the color and flavor of the chicken (Lin et al., 2018).

Gel formation can be one of the main challenges of using natural polymers in electrospinning. Gel can be formed by hydrogen binding. Furthermore, a minimum concentration of biopolymer is required. Very low polymer concentrations may not produce fibers due to inadequate entanglement, resulting in beads and droplets (Shenoy et al., 2005). It also should be noted that an optimum polymer concentration is required to form uniforms fibers without increasing solution viscosity. A popular approach to overcome these obstacles is adding synthetic polymers like polycaprolactone (PCL), propylene carbonate (PPC), polylactic acid (PLA) and polyvinyl alcohol (PVA) and polyethylene oxide (PEO) (Göksen et al., 2021). The synthetic polymers can enhance molecular entanglement by gelation disruption (Koushki et al., 2018; Lamarra et al., 2020).

PEO is of great interest in food packaging applications due to the low toxicity, biocompatibility and biodegradation (Kayaci & Uyar, 2012; Koushki et al., 2018; Zhao et al., 2020). The combination of PEO and chitosan can achieve the marked functionalized electrospun fibers in food packaging systems. Arkoun et.al (2017), studied the antimicrobial function of nanofibers made of chitosan and PEO in food packaging system. Electrospun chitosan/PEO nanofibers had the potential of trapping bacteria (*Escherichiacoli (E.coli), Salmonella typhimurium, Staphylococcus aureus (S. aureus) and Listeria innocua*) by electrostatic interactions attribute to high porosity and surface to mass ration (Arkoun et al., 2017). Duan et al. (2004) fabricated the ultrafine fibers through electrospinning of chitosan and PEO solution (Duan et al., 2004).

Active packaging in the food industry aims to maintain food quality and extend the shelf-life of food (Karabagias et al., 2011). It can be achieved by controlling oxygen, moisture, flavor release, and antimicrobial activities. Antimicrobial packaging changes the packaging condition to extend shelf-life by prevention of food pathogens' growth. Antimicrobial materials can prolong the period of bacterial stagnation, leading to a reduction in the growth rate. Direct application of antimicrobial substances on the food surface can cause for neutralizing or inactivation of active constituent and fast diffusion from the surface into the food mass. Hence, applying antimicrobial substances into the packaging fiber mats can overcome the pitfalls of direct surface application (Kumar et al., 2019). The antimicrobial function of chitosan/PEO/lauric arginate (LAE) nanofibrous films were studied by Deng et al. (2018). Their result showed the ultrafine 3D porous structures of electrospun fibers and good antimicrobial activities against *S. aureus* and *E. coli* (Deng et al., 2018).

Essential oils are increasingly evolving into the public eyes, which have the antimicrobial/antioxidant function for active packaging (Sharma et al., 2020; Sharma et al., 2021., Varghese et al., 2020).

Thyme essential oil (TEO) has the outstanding antibacterial potential against a broad range of bacteria such as *Salmonella* and *E. coli* O157:H7 (Wen et al., 2021). It is usually prepared by steam distillation of *thymus vulgaris* plant. However, Special flavor, volatility and hydrophobicity have become an important issue which restricts application of TEO in food industry. Encapsulation is an effective way to overcome these limitations (López-Rubio et al., 2012). Essential oils can be encapsulated in electrospun fibers to preserve their bioactivity. Encapsulation via electrospinning has the advantages of direct encapsulation of hydrophobic and hydrophilic compounds into the polymeric electrospun fibers (Yao et al., 2016). Also, taking place of elecrospining in the ambient temperature, make it a suitable approach to encapsulate thermal sensitive bioactive compounds in comparison to the conventional encapsulation methods (Drosou et al., 2017; Yao et al., 2016). Encapsulation efficiency (EE) would significantly improve in electrospun nanofibers and burst release could be reduced. Also, the diffusion of encapsulated compounds into the surrounding medium would be facilitated by electrospun fibrous mats comparing to the packaging films produced by the conventional techniques, leading to more effective release (Zamani et al., 2013).

Performance of electrospun fibers can be developed by adding desirable functionalities to the surface of nanofibers via post treatment methods including surface modification, dip-coating, and etc (Zhang et al., 2020). Dip-coating method exhibits the advantages of simple operation and high production efficiency. Therefore, dip-coating electrospun fibers with functionalized polymer can increase bimolecular interaction. Presence of effective functional groups such as carboxyl (–COOH), amine ($–NH_2$), hydroxyl (–OH) and sulfhydryl (–SH) is effective for physical and covalent immobilization (Farahmand et al., 2015).

Polymethyl methacrylate-co-methacrylic acid, poly(MMA-co-MAA), can be used as the functionalized polymer with presence of surface carboxyl (–COOH) groups from MAA segments, which has the potential for covalent and physical immobilization of pathogens (Fig. 1) (Farahmand et al., 2015).

In this study, we are going to introduce a novel active food packaging material that can be effectively used for food quality maintenance and shelf-life extension. Chitosan/PEO hybrid is desirable for application in an electrospinning system, which can offer nanofibers with good properties, including transparency, biodegradability, biocompatibility, low toxicity, elasticity, and antimicrobial properties (Zhao et al., 2020). TEO, as the natural antimicrobial compound, was encapsulated in Chitosan/PEO nanofibers via the electrospinning method. Adding functional groups to the surface of Chitosan/PEO/TEO electrospun mats was achieved by dip-coating into the poly (MMA-co-MAA) solution to enhance bacteria immobilization. The electrospun fibrous mats were characterized through scanning electron microscopy (SEM), atomic force microscopy (AFM), Fourier transform infrared spectroscopy (FTIR) and water contact angle (WCA). The antimicrobial function of electrospun mats was also studied.

2. Material and Methods

2.1. Materials

Chitosan (CS, medium molecular weight, degree of deacetylation 75-80%), polyethylene oxide (PEO, the molecular weight of 100,000 g/mol), acetic acid (glacial, ≥99.85%), methyl methacrylate (MMA), methacrylic acid (MAA) and Tween 80 were

obtained from Sigma-Aldrich, US. Tetrahydrofuran (THF), solvent for coating procedures was brought from Thermo Fisher Scientific Inc, US. Thyme essential oil (TEO) was purchased from Barij Essential Pharmaceutical Company, Iran. *Staphylococcus aurous (ATCC 29213)* and *Escherichia coli (ATCC 25922)* as food-born pathogenic bacteria were used in this study.

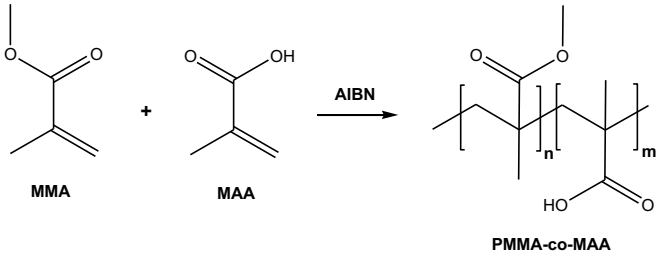


Fig. 1. Chemical structure of poly(MMA-co-MAA).

2.2. Preparation of polymer solutions for electrospinning

First, chitosan solution (CS) (4% w/v) was prepared by dissolving the powdered chitosan in acid acetic (50% v/v). It was stirred for 1 h at room temperature to dissolute completely. PEO powder (20% w/v) was also dissolved in acid acetic (50% v/v) and magnetically stirred for 2 h. Then, CS and PEO solutions were mixed together continuously for 1 h in room temperature in three different ratios of CS: PEO (20/80, 40/60 and 50/50). To prepare the active electrospun nanofibers, TEO at concentrations of 0.5, 1 and 1.5% was further added to CS/PEO mixture under stirring for 1 h at room temperature. TEO was mixed with Tween 80 prior adding to the CS/PEO solution in order to distribute completely in the mixture solution.

2.3. Electrospinning of Chitosan/PEO/TEO solutions

Nanofibrous mats were fabricated through electrospinning process using electrospinning device. Briefly, the polymer solutions were placed into a 1 ml syringe with a stainless-steel blunt needle 19 G. The polymer solution was ejected out of the needle at a flow rate of 0.1 ml/h and 20 KV voltage. The distance between the needle tip and the wrapped collector with an aluminum foil was considered 15 cm. After spinning, all the electruspun mats were peeled off from the collector for further studies. The prepared electrospun mats were designated as CS/PEO, CS/PEO/TEO (0.5%), CS/PEO/TEO (1%) and CS/PEO/TEO (1.5%) based on the TEO concentration incorporated into the mats.

2.4. Poly (MMA-co-MAA) synthesis and coating procedure

Two different compositions of poly (MMA-co-MAA) were polymerized by free-radical polymerization reaction based on the previously reported method (Drosou et al., 2017; Hosseini et al., 2015). For ease of discussion, copolymer compositions were presented further in text as follows: comp.(5:5), indicating 50% of MMA and 50% of MAA in reaction mixture and comp.(7:3), indicating 70% of MMA and 30% of MAA in polymerization reaction. Synthesis reaction was carried out for 6 hours in THF by using azobisisobutyronitrile (AIBN) as an initiator in 90 °C. Washing procedure of the coated electrospun mats was conducted by using distilled water to eliminate the excess of unreacted monomers. Polymer composition dried in vacuum oven and stored in refrigerator.

Polymer coatings were prepared on electrospun nanofibrous mats by dip-coating procedure. Cleaned electrospun mats were coated by immersing them into the poly (MMA-co-MAA) solutions (5% in THF). Coated nanofibrous mats were taken out after three seconds and dried at ambient temperature (Drosou et al., 2017; Farahmand et al., 2015). Section 2.2- 2.4 have been depicted in scheme 1.

2.5. Morphology analysis by field emission scanning electron microscopy (FE-SEM)

Surface morphology of coated and uncoated electrospun nanofibrous mats was analyzed by FE-SEM (ZEISS Sigma 300). The samples were mounted on a double-sided conductive tape and coated with a thin layer of gold before imaging. Frontal view images of the samples were recorded in the secondary electron mode. ImageJ software (Image J, NIH, Maryland, USA) was used for fiber diameter measurement. Fifty randomly chosen nanofibers from SEM images were considered for each sample.

2.6. Atomic force microscopy (AFM)

Surface topology and roughness of the electrospun mats was recorded by AFM (Q-Scope 250; Ambios Company, Santa Cruz, CA, USA) in non-contact mode. The calculated roughness is presented as a root mean-square (RMS) value.

2.7. Fourier-transform infrared spectroscopy (FTIR) analysis

Surface topology and roughness of the electrospun mats was recorded by AFM (Q-Scope 250; Ambios Company, Santa Cruz, CA, USA) in non-contact mode. The calculated roughness is presented as a root mean-square (RMS) value.

2.8. Water contact angle assay

Wettability of the fibrous mats was measured by an optical contact angle goniometer (DataPhysics OCA20, Germany). WCA evaluation was carried out by depositing water drops (1 µL) on the surfaces of electrospun mats in room temperature. The average result from five different position of droplet on the surface was calculated. WCA measurements was possessed in triplicate for each sample (n=15).

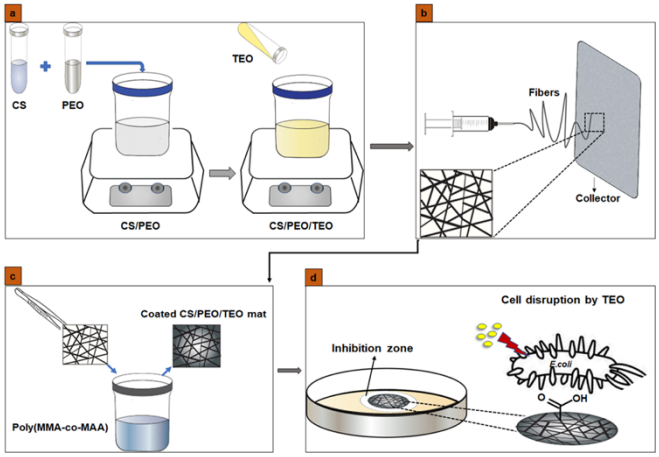
2.9. Antibacterial activity assay

The antimicrobial activity of the Chitosan/PEO/TEO electrospun mats was examined via inhibition zone method (Humphries et al., 2021; Mehdizadeh et al., 2012; Pecarski et al., 2014) on *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) bacteria. Electrospun mats were cut into 6 mm diameter disks and placed on Mueller Hinton agar plates (scheme 1). The agar plates' surface had been earlier inoculated with 0.1 mL of a broth culture containing approximately 10^5–10^6 CFU mL^{-1} of tested bacteria. Plates were incubated at 37 °C for 24 h. CS/PEO mats without TEO were considered as a control. A caliper was used for measuring the

diameter of the inhibition zone. The tests were carried out in triplicate.

2.10. Statistical analysis

In order to compare between the differences, statistical analysis was performed by using analysis of variance (ANOVA) and Tukey's test to establish if a significant difference exists ($p< 0.05$).



Scheme. 1. Schematic illustration of the procedure including a) preparation of CS/PEO and CS/PEO/TEO solutions, b) electrospinning of the polymer solutions to fabricate nanofibers, c) dip-coating by immersing CS/PEO/TEO electrospun mat into the poly(MMA-co-MAA) solution, d) assessment of antimicrobial activity by determination of inhibition zone for coated electrospun mats.

3. Results and Discussion

3.1. SEM results

The fiber distribution and size were measured using FE-SEM. Figures 2 to 4 show SEM images of electrospun mats with different CS/PEO ratios: 20/80, 40/60, and 50/50. CS/PEO (20/80) fibers showed the largest average fiber diameter at about 163 ± 31.02 nm (Fig. 2). CS/PEO (20/80) fibers showed the largest average fiber diameter at about 163 ± 31.02 nm (Fig. 2). CS/PEO (40/60 ratio) fibers were smooth and randomly oriented with a narrow distribution of diameters (Fig. 3), measuring 100 ± 19.16 nm. CS/PEO hybrid mat in a 50/50 ratio, however, showed the smallest fiber diameter, but it suffered from beads and droplets (Fig. 4). CS/PEO-prepared mat a ratio of 40/60 was selected for further assessments due to its slender, smooth, uniform, and bead-free fibers.

Parameters of the electrospinning process were optimized at a flow rate of 0.1 mL/h, voltage of 20 kV, and a tip-to-collector distance of 15 cm.

SEM images were further used to scan the surface of CS/PEO mats at the ratio of 40/60 loaded with different concentrations of TEO (Fig. 5). The morphology of the CS/PEO/TEO electrospun fiber mats was smooth, uniform, and bead-free. The results indicated that the incorporation of TEO in the CS/PEO solution did not affect the fiber morphology.

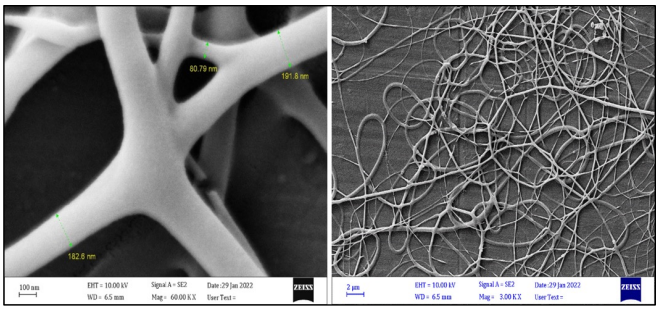


Fig. 2. SEM images of CS/PEO electrospun mat at the ratio of 20/80.

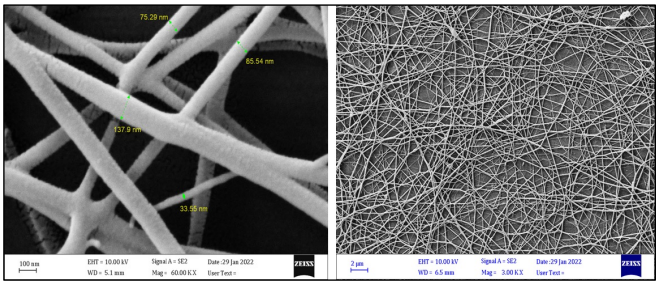


Fig. 3. SEM images of CS/PEO electrospun mat at the ratio of 40/60.

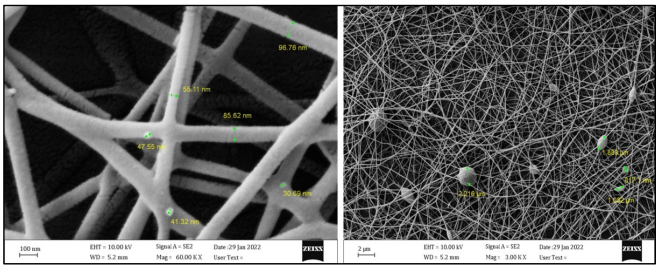


Fig. 4. SEM images of CS/PEO electrospun mat at the ratio of 50/50.

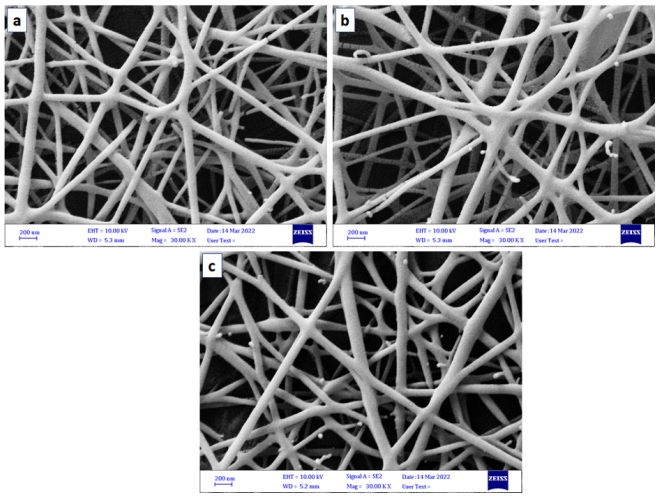


Fig. 5. CS/PEO/TEO nanofibers at concentrations of a) 0.5, b) 1 and c) 1.5% of essential oil.

Fig. 6 shows the successful coating of a nanofibrous mat with poly(MMA-co-MAA). Dip-coating of electrospun nanofibers with copolymer changed the fibrous structure to a porous structure (Fig. 6).

The average fiber diameter was specified by measuring the diameters of randomly selected fibers on SEM images. As it can be observed in Fig. 7, the incorporation of TEO in the CS/PEO mats significantly reduced the fiber diameter from 100 nm (CS/PEO) to 86 nm (CS/PEO/TEO (1.5%)). This could be due to the plasticizer effect of the essential oil (Mori et al., 2015). In this regard, TEO might be acting as a plasticizer, altering the order between single polymer chains and resulting in a decrease in solution viscosity. Further, the addition of TEO may increase the solution conductivity, which decreases the fiber diameter. Nanofiber diameter decreased with the increase of TEO concentrations. The average diameters of CS/PEO/TEO nanofibers decreased from 95 ± 16.17 nm to 94 ± 15.9 nm and 86 ± 12.32 nm for 0.5%, 1%, and 1.5% TEO concentration, respectively. However, the difference in fiber size between CS/PEO nanofibers containing various concentrations of TEO was not statistically significant, proposing that TEO concentration did not touch the fiber diameter (Table 1). A relatively small increase in fiber diameter was attributed to the presence of poly (MMA-co-MAA) coatings (Fig. 7). Nonsignificant changes in the diameter of coated and uncoated nanofibers demonstrated that the coating of electrospun mats with poly (MMA-co-MAA) did not affect the fiber size (Table 1).

3.2. FTIR results

FTIR spectra for CS/PEO and CS/PEO/TEO with different concentrations of essential oil were illustrated in Fig. 8 in a range of 400-4000 cm^{-1}. Patterns of CS/PEO hybrid mats showed the peaks at 1146, 1101 and 1062 cm^{-1} representative of the C–O–C stretching vibrations. Our result demonstrated the maximum peak of C–O–C stretching vibrations at 1101 cm^{-1}, which is in agreement with the previous researches (Rakkapao et al., 2011; Stie et al., 2020). Peaks at 847 and 968 cm^{-1} assigned to CH_2 wagging and CH_2 twisting respectively (Sadri & Arab Sorkhi, 2017). The peaks at 1359 and 1342 cm^{-1} were resulted from C–H deformation of CH_3 groups. C–H bending was appeared at the peak of 1467 cm^{-1}. The C–H stretching was noted at 2879 cm^{-1} and the bands within 3600–2800 cm^{-1} region were associated to hydrogen bonding.

The FTIR spectrum was changed in some regions with adding different concentrations of TEO to CS/PEO electrospun mats. All CS/PEO/TEO compositions showed the new peak at 1740 cm^{-1} due to the C–C bonds of the aromatic rings of TEO.

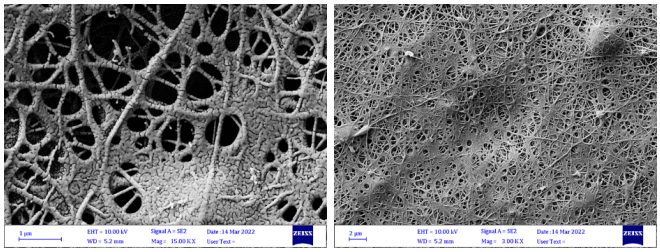


Fig. 6. CS/PEO/TEO nanofibers coated with poly (MMA-co-MAA).

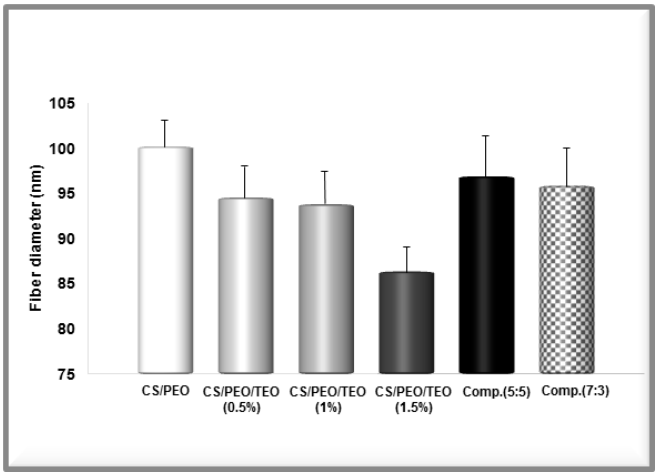


Fig. 7. Fiber Diameter for CS/PEO and CS/PEO/TEO electrospun mats (coated and uncoated).

CS/PEO nanofibers blending with all concentrations of TEO had sharper peak at 2927cm^{-1} in comparison of CS/PEO sample, indicating the increase in C–H bonding. More concentration of TEO leads to an increase in the band intensity in the range of 2900-2800 cm^{-1} that can be attributed to addition of C–H bonds (Fleming, 2011; Rupiasih et al., 2015; Sadri et al., 2016). Moreover, the more bands located at the spectra of 3000 onwards originated from O–H bond in the carvacrol and thymol, indicating TEO existence in nanofibrous mats. The intense band at 1728 cm^{-1} is attributed to the carbonyl group (O–C–O) of carboxylic acid (Farahmand et al., 2015; Ramesh et al., 2007). It was clearly appeared in nanofibers coated with poly (MAA-co-MAA), which include -COOH functional groups. The marked peak (1728 cm^{-1}) became broader as the concentration of the MAA monomer in compositions increased. This is a well-appointed proof for-COOCH3 replacement with carboxyl groups, engendered from MMA and MAA segments respectively.

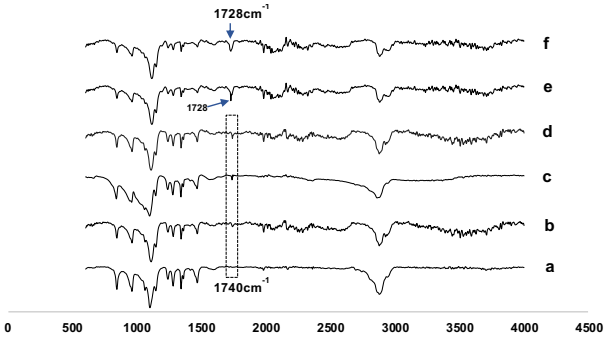
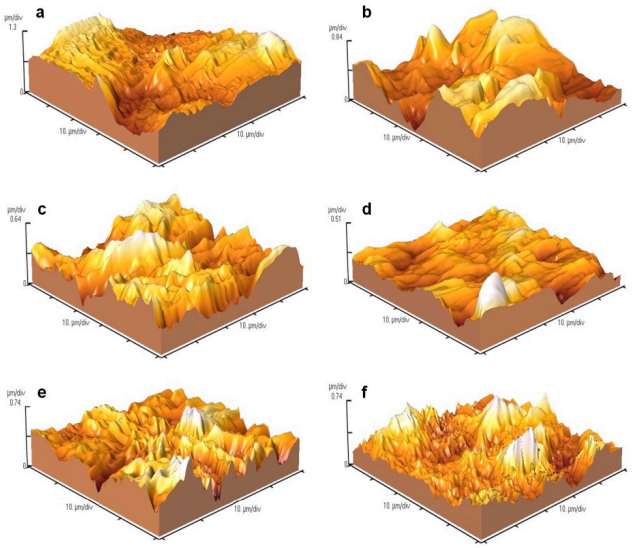


Fig. 8. FTIR spectra recorded for a) CS/PEO, b) CS/PEO/TEO (0.5%), c) CS/PEO/TEO (1%), d) CS/PEO/TEO (1.5%) and %), e and f) coated nanofibers with poly (MMA-co-MAA); comp.(7:3) and comp.(5:5) respectively.

3.3. AFM results

Atomic force microscopy (AFM) was used to evaluate the surface morphology of CS/PEO/TEO blends. CS/PEO fibrous mat without TEO was also investigated for comparison. The topography of the surface is often characterized by the root-mean-square (RMS) roughness, which represents the height fluctuations averaged over

the entire AFM scan size (Choukourov et al., 2017). As it can be observed from Fig. 9 a-d, the RMS surface roughness decreased from 383.3 nm for the pristine CS/PEO mat to 100.7 nm for the CS/PEO/TEO (1.5%) fiber mats (bottom, table). This phenomenon is likely due to the liquid state of the essential oil, leading to a reduction in viscosity of the spinning solution (Liu et al., 2018). A decrease in viscosity leads to a decrease in roughness. Incorporating the essential oil into the fibrous matrices resulted in less roughness of CS/PEO nanofibers. Decrease in surface roughness is bound up with the decrease in fiber diameter, which is consistent with our SEM results (section 3.1, Fig. 7). The coated electrospun mat showed a smoother surface in comparison to the uncoated nanofibrous mats (Fig. 9). Introduction of -COOH functional groups into the structure of electrospun mats apparently reduced the surface roughness. Only CS/PEO/TEO (1.5%) fiber mats resulted in lower surface roughness compared to the coated mats. It should be noted that the electrospun CS/PEO mat incorporated with 1% TEO was coated with poly(MMA-co-MAA). The coating of comp.(5:5) with the high concentration of –COOH groups, however, resulted in higher surface roughness than coating with comp.(7:3) (Fig. 9, e-f). In that sense, it can be explained that comp.(5:5) is a gel-like material by nature, swelling willingly in water due to the foremost number of carboxyl groups (Hosseini et al., 2015).



Composition	Root mean-square roughness (nm)
CS/PEO	383.3
CS/PEO/TEO (0.5%)	354.9
CS/PEO/TEO (1%)	207
CS/PEO/TEO (1.5%)	100.7
Comp.(7:3)	167.6
Comp.(5:5)	186

Fig. 9. AFM images of CS/PEO/TEO nanofibers at concentrations of a) 0, b) 0.5, c) 1 and d) 1.5% of essential oil, e and f) polymer coated nanofibers with comp. (7:3) and comp. (5:5) respectively.

3.4. *WCA results*

Based on the findings in this study, the addition of TEO slightly decreased the average contact angle (Fig. 10). This result might be due to the chemical structure of TEO that includes an oxygenated group, which can interact with the H_2O molecule. In a study by Jin et al. (2013), PCL nanofibers incorporated with four different essential oil extracts, namely Indigofera aspalathoides (IA), Azadirachta indica (AI), Memecylon edule (ME), and Myristica andamanica (MA) were fabricated. Their results indicated that the addition of essential oil decreased the contact angle value compare to pure PCL (Jin et al., 2013), which is in agreement with our results. Considerably lower WCA detected for coated CS/PEO/TEO mats with poly (MMA-co-MAA). It can be as a result of being naturally hydrophilic –COOH groups generated from poly (MMA-co-MAA). There was not recorded any water contact angle for polymer coated nanofibers with comp.(5:5). Coating nanofibrous mats with comp.(5:5), carrying the high concentration of surface –COOH groups, added the extreme hydrophilicity to the electrospun mats.

By using the statistical analysis of variance (ANOVA) and Tukey's test, it was observed that the contact angle of the coated electrospun mat at comp.(7:3) is significantly different from the pure electrospun CS/PEO nanofibers. A statistically significant difference was also recognized between the two groups of CS/PEO and CS/PEO mat containing 1.5% TEO (Table 1). It means that the introduction of TEO and carboxylic groups to the CS/PEO mats increases the wettability of the surface. No significant change was observed in the contact angles of uncoated CS/PEO/TEO mats and the coated ones with comp.(7:3), suggesting that the presence of this number of –COOH groups in comp.(7:3), did not affect the general wettability of the CS/PEO/TEO nanofibers. (Table 1).

3.5. *Antibacterial activity*

Antimicrobial efficacy of electrospun mats was investigated using the zone of inhibition assay (Table 2). Incorporating TEO into CS/PEO electrospun mats resulted in the inhibitory zone around the mat cuts. In our experiment, no inhibition area was displayed around CS/PEO mats for the both tested bacteria. It can be as a result of not migration of chitosan as an antimicrobial agent through the agar media in the agar diffusion test (Pecarski et al., 2014; Pranoto et al., 2005). Therefore, inhibitory effect of chitosan is just limited to bacteria in direct contact with the active sites of chitosan (Coma et al. 2002). Measuring antimicrobial effect via agar diffusion assay is based on the diffusion of antimicrobial agents through the agar gel, resulting in a clear zone around the discs (electrospun mats) (Esmaeili et al., 2021).

The higher concentration of essential oil contributed to a greater inhibitory effect, as expected (Table 2). CS/PEO nanofibrous mats containing 1.5% thyme essential oil exhibited larger inhibition zones against the bacterial strains tested than two other electrospun mats loaded with 0.5% and 1% of TEO. TEO containing phenolic substances expresses remarkable antimicrobial activities via structural and functional damage to the bacterial cell membrane (Celikel & Kavas, 2008).

The electrospun mats incorporated with TEO showed more antibacterial effect against *S.aureus* than *E.coli.* Gram-negative bacteria are more resistant to the antimicrobial agents due to complexity of their cell wall structure. The lipopolysaccharide outer membrane in Gram-negative bacteria surrounds the thin peptidoglycan cell wall, resulting in reduced effect of the antibacterial agents (Nychas, 1995). The inhibition zone diameter values for the *E. coli* dramatically increased from 13.7 mm to 20.4

Table 1. Results of the fiber diameter and water contract angle characteristics of electrospun mats.

Parameter	Nanofiber type					
	CS/PEO	CS/PEO/TEO (0.5%)	CS/PEO/TEO (1%)	CS/PEO/TEO (1.5%)	Comp. (5:5)	Comp. (7:3)
Fiber Diameter (nm)	100.1 ± 19.16 [a*]	94.5 ± 16.17 [a,b]	93.8 ± 15.9 [a,b]	86.3 ± 12.32 [b]	96.9 ± 25.15 [a]	95.8 ± 19.73 [a]
WCA	44.3 ± 3.32 [a]	42.3 ± 1.93 [a,b]	41.5 ± 1.98 [a,b]	39.01 ± 3.84 [b]	0 [c]	39.2 ± 3.46 [b]

* Indicates in each row, there is significant difference between averages with the different superscript letters ($p < 0.05$).

Table 2. Antibacterial activity of CS/PEO/TEO electrospun mats (coated and uncoated).

Bacteria	Inhibition zone diameter (mm)					
	CS/PEO	CS/PEO/TEO (0.5%)	CS/PEO/TEO (1%)	CS/PEO/TEO (1.5%)	Comp. (5:5)	Comp. (7:3)
S. aureus	0 [a*]	14.9 ± 0.65[b]	16.9 ± 1.19 [b]	29.2 ± 1.59 [c]	9.4 ± 1.2 [d]	30.76 ± 1.1 [c]
E. coli	0 [a]	13.7 ± 1.31 [b]	15.1 ± 0.91[b]	20.4 ± 1.41 [c]	8.1 ± 1.04 [d]	22.03 ± 1.34 [c]

* Indicates in each row with different superscript letters are significantly different ($p < 0.05$).

mm for CS/PEO electrospun mats incorporated with 0.5% and 1.5% TEO, respectively. In the case of *S. aureus*, inhibitory zone diameter rose dramatically from 14.9 mm to 29.2 mm with TEO accretion from 0.5 to 1.5%. However, there was no statistically significant difference between CS/PEO electrospun mats loaded with 0.5 and 1% concentration of TEO ($p > 0.05$). This result was somehow expected as both electrospun mats showed approximately the same fiber diameter and wetting characteristics, obtained from fiber diameter calcution and WCA analysis.

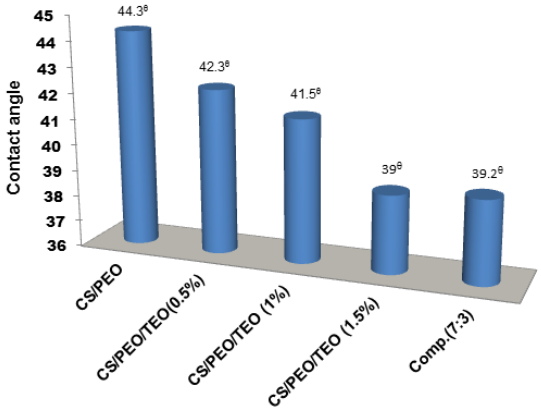


Fig. 10. Water contact angle for CS/PEO, CS/PEO/TEO nanofibers at different concentrations and polymer coated nanofibers with comp.(7:3).

Supreme inhibitory zone was detected for electrospun CS/PEO/TEO fibers coated by comp.(7:3) (Table 2). Such significant bacterial inhibitory effect could be a direct function of –COOH groups. The existence of –COOH groups assists the binding of nanofibers onto the bacterial cell wall, through hydrogen bonding. Hydrogen bonding has been known the favored interaction for carboxylated surfaces (Hosseini et.al., 2015). This attachment along TEO activity accelerates the apoptosis of bacterial cells. Furthermore, the surface of comp.(7:3) showed the higher degree of roughness in comparison to CS/PEO/TEO (1.5%) mats (Section 3.3). Higher roughness and higher surface area lead to more efficient bacteria attachment, resulting in more antibacterial activity.

CS/PEO/TEO nanofibrous mats coated with comp.(5:5), however, resulted in the smallest inhibition zones against the tested bacteria. The interstitial spaces might be covered by polymer coating, resulting in less available surface area and a lower chance of interaction. Moreover, extreme surface hydrophilicity (refer to WAC results, section 3.4) could reduce the bacteria attachment. Comp.(5:5) offers high concentrations of carboxyl groups, but not necessarily the optimum amount. The lower antimicrobial performance of this composition can also be attributed to the steric repulsion effect caused by overly functionalized surfaces (Hosseini et al., 2015).

4. Conclusion

Chitosan-based nanofibers blended with PEO were produced by the electrospinning method with the main application in food packaging sector. TEO, as an antibacterial agent, was introduced to the CS/PEO hybrid solution due to its high antibacterial activity against a broad range of bacteria. Electrospun CS/PEO/TEO mats were functionalized by adding -COOH groups through dip-coating into the poly(MMA-co-MAA) solution. New biopolymer-based electrospun mats resulted in smooth, uniform, and bead-free nanofibers. FTIR confirmed the presence of relevant functional groups of the components in the electrospun nanofibrous mats. Prepared nanofibers also revealed good antibacterial activity against Gram-positive *S. aureus* and Gram-negative *E. coli* in the agar

diffusion method. Use of the copolymer in the optimum ratio of MMA:MAA monomers allowed for higher antibacterial activity, with a larger inhibition zone than uncoated electrospun fibrous mats. The presence of carboxyl groups in the optimum amounts along with TEO, led to a substantial antibacterial effect. Coating electrospun nanofibers with poly(MMA-co-MAA) have promising potential for development in food packaging and preservation systems. Overall, our new electrospun mats based on biopolymers and natural antibacterial agents can be considered as ideal antimicrobial packaging in the food industry.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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