Kefiran/poly(vinyl alcohol)/poly(vinyl pyrrolidone) composite nanofibers: fabrication, characterization and consideration of effective parameters in electrospinning

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Abstract

The kefir/polyvinyl alcohol and polyvinyl pyrrolidone nanofibers were effectively fabricated for the first time using electrospinning of poly(vinyl alcohol), poly(vinyl pyrrolidone) and kefir blend solution. The effect of solution parameters, such as kefir concentration and poly(vinyl alcohol)/poly(vinyl pyrrolidone) mixing ratio, were considered. The parameters' effects, such as voltage, nozzle-to-collector distance and feeding rate on nanofibers morphology, were also checked by the scanning electron microscopy images. After changing the parameters on different steps, designed by the chemo metrics software, the finest nanofibers were produced in the following conditions: 2% kefir concentration, 30/70 kefir/polymer mixing ratio, 12 kV voltage, 200 mm needle-to-collector distance and 0.5 (mL/h) polymer injection rate. The nanofibers produced in these conditions were uniform without knots or adhesion in the lowest diameter of 156 nm. Kefir concentration and kefir/polymer mixing ratio were found as the most effective parameter in the morphology and diameter size of the nanofibers. The attenuated total reflectance (ATR-Ft-IR), atomic force spectroscopy, differential scanning calorimeters and thermal gravimetric analysis were used to investigate molecular structures, three-dimensional morphology and heating properties of the nanofibers, respectively.

Keywords Poly(vinyl alcohol) · Poly(vinyl pyrrolidone) · Kefiran · Electrospinning · Composite nanofiber

1 Introduction

Over the last decade, the electrospinning technique has been developed quickly for the fabrication of nanofibers because it is a simple, versatile, inexpensive and efficient technology for manufacturing sub-micron and industrial-scale nanofibers. The broad range of applications for electrospun nanofibers shows their importance, for example in drug delivery [1], wound dressing and tissue scaffolds [2–4], surgical implants [5], composite materials [6], filtration [7], chemical catalysis [8] and electronic devices [9]. Polymeric nanofibers, which have been obtained from

the electrospinning method, can produce a large variety of polymers such as commercial, conducting, biological, inorganic, composite, and liquid crystalline polymers [10].

Kefiran (Kf) is an exopolysaccharide from kefir micro flora, which is one of the best edible polymers used in the food industry and packaging material as a texturing and gelling agent [11–13]. It acts against the pathogenic genera *Salmonella*, *Helicobacter*, *Shigella*, *Staphylococcus*, and *Escherichia coli* because it has some anti-inflammatory features [14]. Also, it has been reported that kefir has some antifungal [15], antitumor [16] and anti cholesterolic [17] characteristics and can modulate the immune

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activities [18] and modulate the gut immune system [19]. Kefiran is a water-soluble and branched gluconolactone that is capable of forming an extremely thin film [20] with low opacity and weak mechanical properties; therefore, it needs some modifications for different applications. In order to improve the performance of kefir, the blending of two or more polymers has been recently suggested [21–24].

One of the processes to get new material with multiplicity properties is the solution blending diverse polymers. These properties are related to the characteristics of the parent polymers and the blend composition [25]. There are many reports about blending of polymers to improve the solubility, chemical and physical properties. To overcome some limitation of poly(vinyl alcohol) (PVA) such as high hydrophilicity, poor stability in water, insufficient elasticity and rigid structure numerous studies reported the blending with poly(vinyl pyrrolidone) (PVP) which led to the preparation of hydrogel or polymers with better properties [26]. PVA have good hetero polymer interaction with PVP due to the sufficient hydrogen bonding between arranged hydroxyl groups along the chain of PVA as a proton-donor with electronegative oxygen and tertiary amides of PVP as a good proton-acceptor [27]. These polymers have been used in fabrication of nanofibers using electrospinning method due to their non-toxic, biodegradable and biocompatible properties [10]. Gökmeşe et al. have prepared and characterized PVA/PVP nanofibers as promising materials for wound dressing [28]. Shankhwar et al. have fabricated electrospun PVA/PVP nanofibrous membranes and have approved that mechanical property of the PVA membranes improved significantly with PVP addition. Furthermore PVP improved hydrophilicity and sustainable degradation of the membranes. This study proposed PVA-PVP nanofibrous membranes as promising interactive antibiotic-eluting wound dressing materials [29]. Aytimur and coworkers have investigated that the addition of PVP, to PVA/poly(acrylic acid) (PAA) structure increased the thermal stability of the nanofibers and decrease the fiber diameter [30].

Nano-structures such as nano films and nano composites from PVA and kefiran polymers mixtures have been synthesized [31, 32]. Faridi and his colleagues studied the electrospinning of kefiran nanofibers and effective parameters on its morphology [33]. Their study shows that increasing kefiran concentration up to 4.00 wt% resulted in the formation of bead-free and uniform fibers. Also, the diameter of kefiran nanofibers increased with enhancing the solution concentrations, the applied voltage and the injection rate. In total it is quite evident that all kefiran nanofibers produced in a variety of conditions in the mentioned study had the diameters over 200 nm. Unfortunately, kefiran alone lacks the necessary stability

in thermal and chemical conditions. It is well dissolved in water and is degraded under wet conditions. Moreover, the rheological study of kefiran solutions showed that kefiran solutions did not show important extensional properties, displaying a behavior close to the Newtonian at low Hencky strains [9]. Thus, it seems that because of the importance of kefir as an additive in the food products, the addition of another polymer into the formulation is necessary to achieve higher viscoelastic properties. To improve the physical characteristic of kefiran nanofibers, our research group has studied the effects of PVA addition on kefiran nanofibers in previous research [34]. The results of our study revealed that the produced nanofibers were more resistance than kefiran nanofibers. Nevertheless, the PVA/kefiran nanofibers had higher diameters than kefiran nanofibers. Besides, the diameter of PVA/kefiran nanofibers experienced an increase from 475 to 766 nm as the proportion of kefiran increased.

Therefore, in this study we decided to use PVA/PVP to be blended with kefiran and considered the effects of PVP addition on the morphology and diameter of kefiran nanofibers. As synthetic polymers, PVA and PVP were used for the preparation of composite nanofibers with kefiran because of their biocompatibility and biodegradability on the one hand and their good electrospinning ability and high mechanical and chemical resistance during the electrospinning process on the other. Using water as a green solvent together with all polymers is another advantage of the composite solution.

Then, after electrospinning and characterization with different analyses, the best parameters for the fabrication of nanofibers were determined using SEM images.

2 Experimental

2.1 Kefir grains activation

Kefir grains were prepared from a home seller. Then, 100 g of kefir were cultivated in milk for one month to activate the seeds. Each day, the seeds were poured into 800 mL of low-fat milk in a glass container and then wrapped with foil to prevent light contact at room temperature. After 24 h, the fermented milk of kefir was removed from the seeds. Then, the seeds were washed slowly with water and again cultured in fresh milk. This process continued for one month to activate and increase the volume of seeds.

2.2 Extraction of kefiran polysaccharide from kefir seeds

A certain mass of kefir beans was mixed with distilled water in a 1:10 proportion for 40 min using a magnetic

stirrer. The resulting mixture was poured in a falcon and was centrifuged at 10,000 rpm for 20 min at 20 °C. The polysaccharide contained in the supernatant (fluid in the falcon after centrifugation) was precipitated by adding 96% cold ethanol in twice the volume of the supernatant and keeping it for 20 min in a freezer. After 24 h, the resulting mixture was centrifuged for 20 min at 4 °C and dissolved in warm water; the sedimentation process was then carried out twice as described above. The final sediment was placed in an oven at 60 °C for 48 h to form kefiran polymer.

2.3 Preparation of kefiran solution

As a polymer, 3.62 g of kefiran were added to the water at 60 °C for 3 h. Afterward, to completely dissolve the polymer, the temperature was adjusted to 100 °C in order to prepare a uniform and milky solution. All kefiran solutions with 2, 6 and 10%wt were prepared by the same method.

2.4 Preparation of PVA/PVP/kefiran solution

PVA powder ($M_w=88,000 \text{ g mol}^{-1}$, 88% hydrolyzed) was purchased from Sigma. Eight grams of PVA powder were dissolved in 100 ml distilled water (8% W/W) at 60 °C and were stirred to obtain a homogeneous solution. The PVP (K 90) powder was purchased from Sigma. The PVP solution (8% W/W) was prepared by the same method at room temperature. The mixture of 50:50 V/V of PVA and PVP were mixed with Kf solutions (in different concentrations) slowly using magnetic stirrer at 100 °C for 3 h in different proportions (70:30, 50:50 and 30:70). Moreover, a 4% W/W Tween80 surfactant was added to these mixtures.

2.5 Electrospinning process

The main machine used in the fabrication of nanofibers was Electroris (Fanavar Nano Meghyas Ltd., Co., Tehran, Iran). This device works based on the electrostatic method using a high voltage as a power supply system with an output power of 0–35 kV (DC) and amperage of 0–2 mA with maximum volatility of 2%. In addition, a syringe pump with a precision of 10 $\mu\text{L/h}$ for injection rate and a cylinder rotary drum in 8 cm diameter as a collector with a controllable speed up to 3500 rpm were used on this device. A typical syringe (5 mL) was used to inject a polymer solution and the needle of the 18-gauge type was used as a nozzle. After filling the syringe with a polymer solution, the syringe was placed in its own place and the needle was connected to the high-voltage supply system. To fabricate nanofibers during the electrospinning process, the required high voltage was applied between the nozzle and collector that were coated with aluminum foil. The polymeric solutions were placed in the syringe and

then the syringe was placed in its position at the device. The solution was pumped through the needle using the syringe pump. When we adjusted the variable parameters on the device, we saw the state of the exit of the polymer from the nozzle and how to form the Taylor jet when we switched on the voltage. We performed this process for all solutions with different concentrations.

2.6 Characterization

Nanofibers obtained from the top of an aluminum foil mounted on a rotating drum were used for further analysis. The coated sheets were cut with scissors in small pieces of $3 \times 3 \text{ mm}$ and the pieces were placed with carbon adhesive on the sample holder. After coating with sputter coater Bal tech (005 SCD), the obtained nanofibers morphology was examined by scanning electron microscopy (SEM, Philips XL30 model). The Fourier transform infrared (FT-IR) spectra of the samples were recorded by Shimadzu 8400 s, Japan. The nanofiber and KBr pressed mixture were used as a clear disk. All FT-IR spectra were collected in the range of $400\text{--}4000 \text{ cm}^{-1}$. The differential scanning calorimeter (DSC) analysis was performed under the nitrogen atmosphere by NETZSCH Differential Scanning Calorimeter. At least 5 mg of each sample was weighed, sealed in aluminum pans, and heated from 25.5 to 350 °C by a heating rate of 10 °C/min. The reference material was an empty pan. The area of the melting peak for the sample was calculated by the instrument's software. Atomic force microscopy (AFM) was used for the surface analysis using AFM device NT-MDT Model TD150, Russia. The effects of different parameters on the mean diameter of nanofibers were analyzed by one-way analysis of variance (ANOVA) by the SPSS 16.0 software. The experimental design matrix and data analyses were conducted by the STATGRAPHICS plus 5.1 software. The fiber diameter distribution histogram and average fiber diameters were obtained via SEM image analysis using the Microstructure Measurement and Origin software.

3 Result and discussion

3.1 Electrospinning of Kf/PVA/PVP solution

Kefiran is an exopolysaccharide produced by microorganisms during the fermentation of kefir grain in milk. This biopolymer has different biological effects that make it highly desirable for food packaging and other application. To improve the physical properties and limitations of this biopolymer, the combination of two biopolymers, such as starch and whey protein, has been used by several researchers [19, 35]. Herein we used PVA/PVP blend

polymers for preparation of composite nanofibers with kefir. According to previous studies, fine and smooth nanofibers of PVA and PVP are gained in 6–10% (w/v) and 8% (w/v) can produce the thinnest nanofibers possible [36]. PVA and PVP solutions were prepared 8% (w/v) in water as solvents. To examine the effects of kefir concentration on nanofibers' morphology, 2%, 6% and 10% (w/v) of kefir were prepared in water. Tween80 surfactant was added to these mixtures in order to gain a homogenous solution, to decrease the surface tension of obtained mixtures, to have a continuous electrospinning process and to create fine and smooth fibers.

3.2 Optimization of electrospinning parameters

Since several responses were assessed in an experimental design, the optimum points attained individually for each factor did not concur in all cases. There are many statistical methods for resolving multiple response difficulties such as overlaying the contours plot for each response, constrained optimization problems and the desirability approach. However, the most important methodology of the desirability function described is commonly utilized for multi-response optimization. In this study, in order to achieve optimum conditions of electrospinning, a chemometric design methodology based on a box-Behnken design (BBD) was applied for the optimization of the effective factors on the electrospinning of Kf/PVA/PVP nanofibers.

Several factors were studied in this research: the kefir concentration and kefir/polymer ratio as solution effective parameters as well as voltage, nozzle-to-collector distance, and injection rate as device effective parameters on the electrospinning process. All five parameters were changed in three level at their low, mid-range, and high values. The effects of these factors on the morphology, appearance and the diameters of nanofibers were studied by the analysis of the SEM images of the nanofibers. For this purpose, 25 experiments were designed using a chemometric design. In this design, kefir concentrations were 2, 6 or 10 wt%. The Kf/polymer ratios were changed in 70/30, 50/50 or 30/70. Twelve, 18 and 25 kV were selected for the voltage variation. Also, the nozzle-to-collector distances ranged from 100, 150 to 200 mm. All the designed experiments were done in conditions shown in Table 1. The SEM images of the experiments are shown in Fig. 1.

3.3 Effect of parameters on nanofiber diameter

To achieve the proper concentration of kefir for electrospinning, different concentrations of kefir (2, 6 and 10%wt) were dissolved in deionized water, and then PVP/

PVA solution was prepared at the constant concentration of 8% in water. As can be seen in Fig. 1, the best morphology of nanofibers was in 2% wt of kefir solutions (Fig. 1e, f, g). These images show a smooth, bead less and nanofibrous morphology. In another study, Faridi and his colleagues studied the electrospinning of kefir alone without any copolymers [33]. They found that 2% kefir could not form nanofibers during the electrospinning process. Beadless and uniform fibers were formed by increasing kefir concentration up to 4.00 wt% [33]. They used 6% kefir as the best concentration to consider the effects of other parameters on kefir morphology. But in this study, the addition of PVA/PVP to 6% and 10% kefir was not successful in nanofiber fabrication. This phenomenon can be a result of an increase in the viscosity and enhancement of polymer chain entanglement that can decrease the jet path from tip-to-collector distance [37, 38]. The previous literature also confirms that an increase in concentration can increase the mean diameter of nanofibers [40, 41]. In the addition of PVA/PVP to 2% kefir, the mixing ratio of polymers with kefir was very important. The fine, beadless and smooth nanofibers were fabricated when the mixing ratio of kefir with other polymers was 30:70; yet, in kefir/polymer ratio of 2% kefir (a, b, c and d), a high volume of knots were observed. As observed in Fig. 1, the optimum condition for the electrospinning of Kf/PVA/PVP was 2% kefir with the mixing ratio of 30/70 for Kf/polymer proportion (see Fig. 1e, f and g). The distributions of fibers' diameters were determined using the measurement software for 20 nanofibers and drawn on the origin software. The distributions histogram of E, F and G are shown in Fig. 2a–c) The mean diameter of E, F and G were 226, 173 and 156, respectively. As a result, the optimum voltage, nozzle-to-collector distance and injection rate were 12 kV, 200 mm and 0.5 mL/h, respectively with the diameter of 156 nm for Kf/PVA/PVP nanofibers. In order to investigate the effects of voltage on morphology, the applied voltage was increased from 12 to 25 kV according to the chemometric Box-Behnken design as shown in Table 1. Among different voltages, the lowest voltage (12 kV) showed the best result in Fig. 1.G. The literature also confirms that decreasing applied voltage decreases the diameter of nanofibers. The quicker movement of solution from the nozzle to the collector, shorter jet flight time and less time for being stretched in the high applied voltages could lead to thicker and larger nanofiber diameters [42]. When the applied voltage of 6.00 wt% kefir solution was raised from 10 to 20 kV, the mean nanofiber diameter increased from 193 to 273 nm [33]. The electrospinning of Kf/PVA/PVP led to thinner nanofibers (156 nm) than kefir nanofibers (193 nm) at 10 kV.

Nozzle-to-collector distances were changed from 100 to 200 mm in Table 1 to consider the effects of

Table 1 Experiments conducted for electrospinning of Kf/PVA/PVP

Experiment	Kefiran concentration (wt%)	Kefiran/polymer ratio%	Voltage	Nozzell-to-collector distance (mm)	Injection rate (mL/h)
A	2.0	70.0	18.0	200.0	0.5
B	2.0	70.0	12.0	200.0	2.0
C	2.0	70.0	25.0	100.0	2.0
D	2.0	50.0	18	150.0	1
E	2.0	30.0	25.0	100.0	2.0
F	2.0	30.0	12.0	100.0	0.5
G	2.0	30.0	12.0	200.0	0.5
H	2.0	70.0	25	200.0	0.5
I	6.0	50.0	25.0	150.0	1
J	6.0	50.0	18	100.0	1
K	6.0	50.0	18	150.0	1
L	6.0	50.0	18	150.0	2.0
M	6.0	50.0	18	150.0	1
N	6.0	50.0	18	200.0	1
O	6.0	50.0	12.0	150.0	1
P	6.0	50.0	18	150.0	0.5
Q	6.0	70.0	18	150.0	1
R	6.0	30.0	18	150.0	1
S	10.0	70.0	25.0	100.0	0.5
T	10.0	70.0	12.0	100.0	0.5
U	10.0	70.0	12.0	200.0	2.0
V	10.0	50.0	18	150.0	1
W	10.0	30.0	25.0	200.0	0.5
X	10.0	30.0	25.0	200.0	2.0
Y	10.0	30.0	12.0	100.0	2.0

nozzle-to-collector distances on the nanofiber diameter. It was observed that the highest distance with 200 mm resulted in the finest Kf/PVA/PVP nanofibers. The comparison of Fig. 1f and g shows that changing distance from 100 to 200 mm, other conditions being constant, slightly changed the diameters of nanofibers from 173 nm to 156 nm. The literature has also pointed out that the effects of nozzle-to-collector distances on nanofiber diameters are not as significant as other parameters [43]. The injection rates of polymer from the nozzle were chosen as 0.5, 1 and 2 mL/h (Table 1). As can be seen, the optimum condition of Kf/PVA/PVP nanofibers was attained when the injection rate was 0.5 mL/h. Increasing the injection rate and the high voltage at Fig. 2a (Table 1g) lead to the thickest nanofibers with 226 nm diameter. These results completely agree with the literature in that an increase in the feeding rate can increase the diameter of nanofibers. Bigger Taylor cone has been produced at the needle in a high injection rate and attained finer nanofibers [44]. However, a very low injection rate could not supply a sufficient amount of

solution at the tip of the needle because an excessive increase in the flow rate can result in nanofibers without sufficient solvent evaporation and with flattened, beaded web-like appearance [45]. Therefore, the finest nanofibers are determined by the balance between these two effects. According to our previous study, the finest Kf/PVA nanofibers were fabricated when the 50:50 proportion of Kf:PVA solution was electrospun at the applied voltage and the tip-to-collector distance, injection rate and temperature were selected at 15 kV, 150 mm, 2 mL/h and 35 °C, respectively. The mean diameters of fibers in PVA and Kf/PVA nanofibers were about 254 nm and 538 nm, respectively, at mentioned electrospinning conditions [34], while the electrospinning of kefiran alone could produce nanofibers with 193 nm diameters [33]. The blending of PVA and kefiran during the electrospinning process almost doubled the diameter of nanofibers. Therefore, according to the findings, the addition of PVA/PVP to kefiran could decrease the mean diameter of nanofibers (156 nm) in contrast to PVA (254 nm), kefiran (193 nm) and Kf/PVA (538 nm) nanofibers.

Fig. 1 SEM image of nanofibers

Fig. 1 (continued)

Fig. 1 (continued)

Fig. 1 (continued)

3.4 Characterization

After the determination of optimum conditions for electrospinning by the SEM analysis, the characterization of nanofibers was done by FT-IR, DSC and AFM analyses.

FT-IR analysis of PVA nanofiber, kefiran nanofiber, Kf/PVA nanofiber and Kf/PVA/PVP nanofiber were shown in Fig. 3. The pure PVA nanofibers exhibited various transmittance peaks at 813, 1226, 1353, 1535, 1724, 2875 and 3614 cm^{-1} which were attributed to the (C–C), (C–O), (CH), (CH–OAC) and (C=O) residual from primary vinyl acetate, (CH₂) and free (OH) resonance, respectively, which is completely agree well with previous studies [46, 47]. In kefiran nanofiber spectra, peak at 1645 cm^{-1} are related to bound water [48]. The sharp peak at 1020 cm^{-1} and finger

Fig. 2 Diameter distribution of Kf/PVA/PVP with proportion of 30/70 in condition of **a** sample E with 25 kV voltage and 100 cm distance **b** sample F with 12 kV voltage and 100 cm distance **c** sample G with 12 kV voltage and 200 cm distance

Fig. 3 FT-IR spectra of **a** PVA, **b** kefiran, **c** Kf/PVA nanofiber, **d** Kf/PVA/PVP nanofiber

print region at $1200\text{--}900\text{ cm}^{-1}$ is characteristic of saccharide structure and stretching modes of carbohydrate [49]. The absorption peaks of Kf/PVA nanofibers appear at 2921, 2850, 1730, 1645, 1440, 1320, 1242, 1095, and 850 cm^{-1} , which were attributed to the (CH₂), (CH), (C=O) residual from primary vinyl acetate, OH bending, (CH–OH), (CH–OAC), (CH), (C–O), and (C–C) [34]. The absorption bands at 3434, 2955, 1661, 1424, 1291 and 1018 cm^{-1} related to OH stretching, aliphatic CH, amide C=O, CH₂ and C–N respectively, are the characteristic peaks of PVP [50].

The FT-IR spectra of Kf/PVA/PVP nanofibers are shown in Fig. 3. d. As shown in the Fig. 3, absorptions peaks appear at 3444, 3419, 3000, 2924, 2360, 1735, 1642, 1462, 1440, 1425, 1379, 1292, 1078, 669 and 651 cm^{-1} , which were attributed to the OH, CH stretching, O–C=N, C=O, CH₂ and CH vibration, C–O–C, C–O, C–N and C–C. The fingerprint region ranged from $900\text{ to }1200\text{ cm}^{-1}$ is related to the stretching mode of carbohydrate rings and side groups (C–O–C, C–OH and C–H) in Kf/PVA/PVP nanofibers. The broad peaks near 1020, 1095, 1078 cm^{-1} are characteristic of the saccharide structure in Kf, Kf/PVA and Kf/PVA/PVP respectively. Furthermore the C–N group peak of PVP (1018 cm^{-1} in PVP) appears as shoulder of 1078 cm^{-1} peak. The O–H bending mode of bound water also resulted in a peak at 1614 cm^{-1} . The appearance of absorption peaks in 2360, 1642 and 1078 cm^{-1} approved the presence of PVP. These peaks slightly shifted to higher wave number in contrast to pure PVP ($2320, 1661, 1018\text{ cm}^{-1}$). Likewise, the peaks at 1735 cm^{-1} and the peaks in the region of $1300\text{--}1800\text{ cm}^{-1}$ corresponded to the bending and stretching vibrations of the PVA. Due to comparison of the previous spectra the hydroxyl group of PVA nanofiber and kefiran nanofiber appear at 3614 and 3000 cm^{-1} , however the Kf/PVA nanofibers shows these two peaks together with. The PVP have a characteristic peaks for O–H group

of absorbed water at 3434 cm^{-1} [50]. The appearance of this peak in Kf/PVA/PVP nanofiber led to broad peak at $3000\text{--}3600\text{ cm}^{-1}$ which was indicative of different hydroxyl groups.

Figure 4 shows the DSC thermogram of electrospun Kf/PVA nanofibers and Kf/PVA/PVP nanofibers. It can be seen from the thermogram of Kf/PVA nanofibers that the melting peak appears around 185°C , but there is no clear melting peak in the thermogram of PVA nanofibers [34]. However, two endothermic peaks were observed in the thermogram of Kf/PVA/PVP nanofibers. These peaks appeared in 276°C and 304°C with $\Delta H = -47.6734\text{ J/g}$ and $\Delta H = -43.0745\text{ J/g}$, respectively. These peaks appeared in temperatures higher than 205°C and 185°C , attributable to the PVA powder and the melting point of Kf/PVA nanofibers, respectively. The melting point of PVA increased with the addition of Kf/PVP blend despite the addition of kefiran alone. The degradation temperature of Kf/PVA nanofibers was measured at 266°C , while the degradation of Kf/PVA/PVP was not observed until 350°C .

The thermogram analyses showed that the Kf/PVA/PVP nanofibers were more thermal resistance than Kf/PVA nanofibers. This may be due to the resistant three-dimensional structure of PVP molecules and the good connectivity of blend polymers to each other by a hydrogen bond. Consequently, a strong crystal structure was formed in electrospun Kf/PVA/PVP nanofibers. The higher thermal tolerance of Kf/PVA/PVP composite nanofibers in contrast to kefiran nanofibers and Kf/PVA composite nanofibers makes it a good candidate to be used in many applications such as catalyst, food industry, etc.

The effect of heating on the Kf/PVA/PVP was evaluated by thermal gravimetric analysis (TGA). Figure 5 shows the TGA and DTA curves of the Kf/PVA/PVP nanofibers. The first weight loss at about $100\text{--}200^\circ\text{C}$ with endothermic peak is

Fig. 4 DSC thermograms of **a** Kf/PVA nanofiber. **b** Kf/PVA/PVP nanofiber

Fig. 5 TGA and DTA analysis of Kf/PVA/PVP nanofibers

Fig. 6 AFM analysis of Kf/PVA/PVP nanofibers

due to the Loss of adsorbed water. The second and principal weight loss in the range of 250–280 °C in TGA curve can be attributed to the degradation of PVA and Kf and Kf/PVA from the nanofiber. The degradation of Kf/PVA/PVP nanofiber was not observed until 350 °C.

The AFM image of Kf/PVA/PVP nanofibers is shown in Fig. 6. In the AFM image, it is possible to capture images from the surface of biodegradable samples with

a precision nanometer inline X, Y and an angstrom in Z-direction approving three-dimensional structure and size. The AFM image can provide information on the properties of material surfaces such as hardness and surface adhesion by measuring the forces between the probe and the sample as a function of their mutual separation in AFM analysis that this method is sometimes referred as atomic force spectroscopy (AFS) [51]. As demonstrated in Fig. 5, the surface topography of nanofibers as a hard material approves the presence of a continuous and smooth nanofibrous structure. It also shows the good porous structure of Kf/PVA/PVP composite nanofibers without adhesion or beads. The high-density fibrous nature of the mats with uniform fibers is in good agreement with the SEM results.

4 Conclusion

The aim of this study was the fabrication of Kf/PVA/PVP nanofibers by the electrospinning method. The effects of different parameters such as kefir/polymer weight ratio, kefir concentration, voltage, nozzle-to-collector distance, injection on morphology and an average diameter of nanofibers were studied. Results revealed that the most important parameters were Kf/polymers weight ratio and kefir concentration. The finest nanofibers were fabricated when Kf concentration and Kf/polymers

weight ratios were 2% and 30/70, respectively. The reduction of the applied voltage decreased the diameter of nanofibers. The highest nozzle-to-collector distance with 200 mm could produce the finest Kf/PVA/PVP nanofibers. Moreover, increasing the feeding rate led to an increase in the diameter of nanofibers. Hence, optimum voltage, nozzle-to-collector distance and injection rate were 12 kV, 200 mm and 0.5 mL/h, respectively, with a diameter of 156 nm for Kf/PVA/PVP nanofibers. The characterization of nanofibers was done by FT-IR, DSC and AFM analyses. The DSC analysis showed a high thermal tolerance for Kf/PVA/PVP composite nanofibers. The FT-IR analysis agreed well with the molecular structure of PVA, PVP and kefir. The AFM analysis approved the three-dimensional structure of nanofibers.

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Compliance with ethical standards

Conflict of interest We have no conflict of interest.

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