e-Polymers, 2019; 19: 154–167 **DE GRUYTER**

Research Article **Open Access**

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The fabrication and characterization of casein/PEO nanofibrous yarn via electrospinning

https://doi.org/10.1515/epoly-2019-0017
Received July 10, 2018; accepted October 09, 2018.

Abstract: Casein, a natural protein, has been used as a biopolymer-based resource for producing fiber. This fiber provides high comfort properties with a pH close to human skin. This study focused on evaluating the production feasibility of casein nanofiber twisted yarn with the highest protein content in the hybrid yarn, obtaining suitable spinning conditions and desirable properties of the produced yarn. The desirable yarn achieved by composing 90% casein and 10% polyethylene oxide. The yarn strengthened using several types of cross-linking methods and the best technique was obtained to spin an optimized engineered yarn. Consequently, the biodegrading test conducted on the optimized yarn (90% casein and 10% polyethylene oxide blend) in the presence of 40% di-isocyanate as strengthener additive. Observations showed that after ten days, the sample mostly degraded in the solvent and its strength massively reduced so it could be considered as an environmentally friendly, biodegradable fiber.

Keywords: casein; polyethylene oxide; strengthening; electrospinning; rupture morphology

1 Introduction

Casein rooted from the Latin word *Caseus* which means “the cheese”. Casein is extracted from either animal or vegetable proteins, including eighty percent of the cow’s milk protein. casein-based products are rendering different advantages that make them suitable for different applications such as plastic, color, medical and dentinal goods, glue, and fibers industry (1). Some of these advantageous properties are including UV resistance (2), high moisture transferring ability, good resistant against tiny insects and fungi (increasing the fabric lifetime), high moisture absorption due to the presence of amide and carboxylic groups, fast and simple colorant adsorption during dying process, suitable performance in contact with the skin especially for humans with allergenic problems due to the pH equality (3). Natural antibacterial effect of casein fibers also, make it possible to have several hygienic features (4).

Casein fibers first introduced in 1930 and since then there are several investigations for improving the mechanical properties of fibers and also pursuing eco-friendly production passways. Casein fibers production is principally possible through several methods including extruding, cross-linking and electrospinning (1). In the extruding process, casein powder is dissolved in sodium hydroxide to obtain a solution with suitable concentration. Consequently, the resultant solution extruded into a coagulation bath containing sodium sulfate and acetic acid. Although the produced fibers have similar properties to that of wool, they are more sensitive to the alkali condition and would swell in water. Moreover, these fibers are wrinkled quickly and need to be controlled and hanged out. Aralac (5), caslen, Cargan, Tiolan, Lactofil, Fibrolane, and Silkool were commercial name of casein fibers produced in different countries (6-8). There are reports of production casein fibers based on new patents in Germany (9-11). Due to low tensile strength and their high solubility in water, cross-linking of casein fiber is necessary (12,13). Crosslinking agents like glutaraldehyde, formaldehyde and toluene diisocyanate used for casein crosslinking but they all have toxicity problems. Yang and reddy produced casein fibers through wet spinning and in after treatment crosslinking carried out using citric acid. But there is still a cytotoxic effect because of using crosslinker (14).

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Electrospinning is a simple and effective method for producing superfine and continuous fibers with a diameter of about several micrometers to a few ten nanometers and also can apply for producing the nanocomposites, hallow, and core-shell fibers (15,16). This method relies on concepts of electromagnetic forces, and its main idea refers to more than 70 years ago, but from the 1990s when nanotechnology gained more attention, electrospinning as a simple production method for nanofibers gets more attention (17,18). more than a hundred types of different polymers either in solution or melted form have been successfully spun into the superfine fibers by using this method (19,20). Producing yarns from nanofibers is a new investigation area which could bring valuable properties of nanostructure polymers to the field of yarn application. Nanostructure yarn could be used in sutures and medical applications. Few methods introduced for producing nano yarns (21-23).

Casein nanofibers have also produced by Xie and Hsieh with the help of additive polymers during the electrospinning process. They mixed casein with two types of polymers including polyethylene oxide (PEO) and polyvinyl alcohol (PVA), to reach the possibility of the casein electrospinning process. Functional differences between the two selected polymers were also investigated by considering different concentrations and several fractions (24). Bier et al. produced casein fibers having glycerol and paraffin oil for overcoming the brittle structure of produced fibers and using nontoxic ingredients, but their fibers still have high water solubility (25,26). Biranje et al. produced an electrospun nanofibrous mat of PVA-casein for wound dressing application. They reported that the spinnable PVA/casein ratio is 50/50 to 80/20. In vitro biodegradation in phosphate buffer containing proteinase enzyme shows a degradation time of 24 h (27). Grothe et al also produced a nanofibrous mat containing 92% casein and 8% PEO through needleless electrospinning (28).

What has been done until now is referred to Nano-web production of casein by the help of two polymers, i.e. polyvinyl alcohol (PVA) and polyethylene oxide (PEO). The produced nanofibers contain maximum 92% casein with diameters ranging from 100-500 nm. Current research is aiming to investigate the possibility of producing nanofibrous yarns containing a higher amount of casein directly for different applications such as a suture. Nanofibrous yarn contains a higher number of fibers per specific cross section of yarn which results in the higher absorption and drainage ability. In addition, usage of nanofibrous yarn is more convenient and has higher potential application than nanofibers per se. Since the researchers normally face numerous difficulties during the production of nanofiber containing the higher extent of casein component, achieving this product is considered as the main novelty of this work. Through the experimental procedure, optimized conditions regarding the nanofiber yarn production method would be achieved and the mechanical properties of the produced sample yarns would be determined. Therefore, through this research, the following objectives would be traced in general:

1. Studying the production possibility of nanofiber yarn containing 90% casein component;
2. Investigating different kinds of strengthening methods and comparing them;
3. Evaluating the strength of samples by considering different fractions of casein component, before and after strengthening;
4. Investigating the biodegradability characteristics of the samples.

2 Experimental

2.1 Materials

All chemical components including poly-ethylene-oxide (PEO) (Ave. Mw of 300000 g/mol Aldrich), Casein (477.55 g/mol, Quelab, UK), triethanolamine (at concentration of 98%, Merck) and toluene-diisocyanate (174.16 g/mol, Merk) as a crosslinking agent were used as received without any purification. In order to obtain buffer solutions, phosphate-buffer-saline (PBS, Aldrich), was also employed.

2.2 Electrospinning setup

The basic setup used in this study for electrospinning of Nanofiber yarn has been illustrated in Figure 1. As the schematic shows, the basic setup contains a high voltage producer connected to the syringe needle (prepared solution is fed by the syringe needle), a twister that rotates neutral substrate and then twists the Nanofiber to form the desired yarn, and a take-up roller or collector to collect the twisted yarn. The Syringe Pump was STC-527, Terumo, Japan.

2.2.1 Yarn electrospinning conditions

The optimized condition for electrospinning of two desired samples was as follows: 12.5 kV voltage for power supply, feeding rate of 0.2 ml/h, 3000 TPM (twist per meter) for

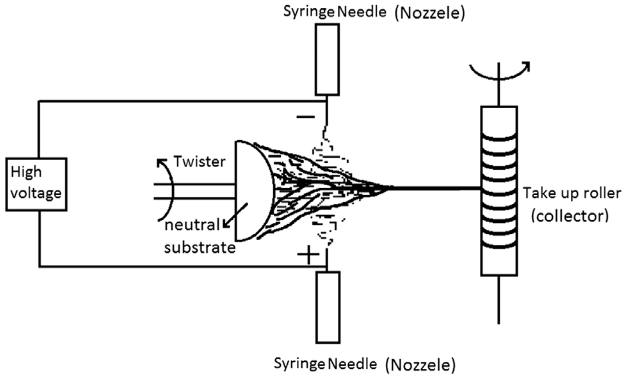


Figure 1: Schematic of yarn electrospinning setup used in this study.

nanofiber yarn twist, distance of 26 cm adjusted between tips of the two nozzles, distance of 22 cm adjusted between the neutral substrate and the collector and distance of 5 cm adjusted between the center of electromagnetic shield and the neutral substrate. Humidity was kept constant throughout the electrospinning at 65% humidity.

2.3 Determining the optimized concentration of polymeric solutions

The concentration of the polymeric solution is considered as an important and essential parameter in order to achieve the optimized condition for the electrospinning process and the yarn production. It should be noted that inappropriate concentration would influence the electrospinning efficiency. At very low concentrations, the possibility of bead formation would increase due to the lower viscosity of the solutions. The main reason for this phenomenon is attributed to a disturbance of the polymeric flow throughout the Taylor cone zone during spinning. This phenomenon which could be known as solution-drop spraying (bead formation) is an important reason for decreasing the strength of produced nanofiber web layers by providing some stress concentration points within the structure. On the other hand, the lower ability of highly concentrated solutions for overcoming the capillary forces would also result in the non-uniform production of yarn.

2.4 Optimizing concentration of casein solution, using tri-ethanol-amine (TEA) as the solvent (weight-volume ratio)

Casein is a natural polymer which is available in both water-soluble and water-insoluble forms. The casein used in this study was of the insoluble form; so that the original tri-ethanol-amine (TEA) with 98% concentration was used as the casein solvent. Before adding the casein to the solvent, first, the concentration of TEA must be reduced to 5% volume-volume ratio by using deionized water. For obtaining the casein solution with suitable concentration, two weights of casein powder (5% and 10%) were separately added to the solvent (TEA 5%). For this aim, two prepared solutions were applied through electrospinning with the help of auxiliary polymer (PEO) and the produced yarns were compared. For comparing the effect of casein concentration on the solution behavior during spinning, the concentration of auxiliary polymers must be constant. Primary experiments showed that using the casein with 5% concentration results in unusually attached yarn formation. This problem could be solved by increasing the casein concentration to 10%, Increasing the viscosity of casein solution up to an optimum point leads to the formation of uniform yarn by electrospinning. Through the following experimental procedures of this work, the 10% concentration was selected.

2.5 Optimizing concentration of PEO/water (weight-volume ratio)

Prior to the electrospinning process, a suitable concentration of PEO solution must be determined to do so, different solutions of PEO/water ranging from 4% to 8% were prepared in order to obtain the optimized concentration. For producing PEO solution, a certain amount of PEO powder was weighted at first, then gradually solved into the deionized water which was stirred using a bath sonicator at room temperature. In the next step, the casein solution was added to the PEO-water solution and then stirred for 3-4 h until a homogenized, white-colored solution was achieved.

By increasing the percentage of casein component in the total solution (PEO/water and Casein/TEA), the viscosity of total solution decreases and as a result the concentration of PEO component should be increased in order to gain an optimized viscosity for the solution (29). The optimum concentration of PEO was obtained at 26% for future experiments.

2.6 Yarn strengthening process via crosslinking

Preliminary experiments showed that all the produced nanofiber yarns have low strength (Table 1), so different procedures were applied to increase yarn strength. Among several methods and functional materials, in this research, toluene di-isocyanate was used as one of the crosslinking agents during the yarn strengthening process. Based on the literature, two different concentrations of 20% and 40% w/w of TDI/polymer solution was chosen. Di-isocyanate crosslinking agent in its liquid form was slowly added to the prepared solutions in 30 min and was stirred for 24 h. Consequently, the prepared solution was used for electrospinning and the Nanofiber yarn was produced. The collected yarns from electrospinning were kept at 40°C in ambient pressure for 1 h (21).

2.7 Determining mechanical properties of produced yarns

Mechanical properties of nanofiber yarns were tested by Zwick Universal tensile testing machine (model 1446-60; Germany) according to ASTM D3822 testing method. Applying tensile loads to the specimens via displacement of crosshead connected to a load cell was adjusted to be at a constant rate of elongation (CRE). The tensile test was performed on specimens with 25 mm length at a constant crosshead speed of 15 mm/min. Tensile properties of the experimental samples are summarized in Table 1.

2.8 Investigating biodegradation properties of the produced yarns

For investigating biodegradation properties of the produced nanofiber yarns, a simulator environment with the ability to simulate the human's body conditions having pH = 7.4 at 37^{0}C according to ASTM F1635-04A was used (30).

Phosphate buffered saline (PBS) was used to provide pH 7.4. This buffer solution was poured into several small-sized glass container and the best-chosen samples were immersed in the solution. Consequently, the prepared samples of similar length were located in the simulator for different time intervals of 2, 6, 10, 14 and 18 days. After the specified time, the samples were removed from the solution of phosphate buffer saline, rinsed with deionized water and dried in an oven at 105°C for 1 h. Samples, before and after biodegradation process was weighted up to four decimal digits and weight loss percentage of the yarn samples was calculated from Eq. 1:

$$\text{Weight loss}\left(\%\right)=\frac{W_n - W_t}{W_n}\times 100 \tag{1}$$

where W_n is the initial weight of the dry sample before biodegradation test and W_t is the weight of the dried sample after testing.

2.9 Chemical structural analysis of nanofibrous yarn by FTIR

Investigation of crosslinking reaction carried out by FTIR spectroscopy of samples in the range of 400 to 4000 wavenumber using BOMEM FTIR MB-series, MB-100 (Hartmann & Braun, Canada). For this mean, all samples prepared in form of KBr discs and FTIR run in resolution of 16 for 32 scans. Spectras of triethanolamine, toluene diisocyanate, casein, mixture of casein in triethanolamine, casein/PEO/TDI mixture after and before crosslinking recorded for comparison.

2.10 Fiber morphology study by SEM

Scanning electron microscopy (SEM) (EM-3200, KYKY) used for the study of the surface morphology of fibers and yarns. Fibers produced in spinning triangle studied for achieving the optimum combination of polymers and an average diameter of produced fibers. After that, SEM of produced yarn studied in case of morphology and arrangement. Cross section of produced yarn also investigated by SEM to see the effect of crosslinking on yarn structure and morphology.

3 Results and discussion

3.1 Fiber morphology in spinning triangle

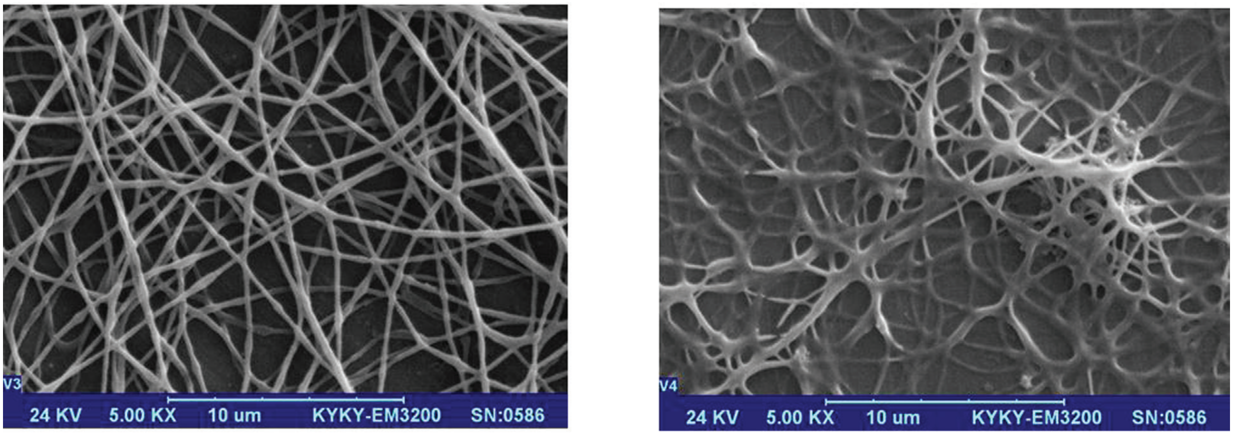
PEO/casein nanofiber micrographs in a spinning triangle were recorded using a Scanning electron microscope

Table 1: Tensile properties of nanofiber yarns produced in this study.

Yarn sample	Yarn count (*Tex*)	Specific stress in Fmax (*cN/Tex*)	Elongation in F_{max} (%)	Work of rupture (*N.mm*)	Elastic modulus (*cN/Tex*)
PEO (15%)/Casein (10%) 20:80	12 $_{(CV\% = 3.50)}$	0.93 $_{(CV\% = 24.36)}$	21.9 $_{(CV\% = 14.13)}$	0.47 $_{(CV\% = 0.51)}$	56.78 $_{(CV\% = 10.17)}$
PEO (26%)/Casein (10%) 10:90	14 $_{(CV\% = 0.05)}$	0.59 $_{(CV\% = 23.42)}$	156.3 $_{(CV\% = 16.20)}$	1.92 $_{(CV\% = 33.03)}$	4.20 $_{(CV\% = 20.33)}$

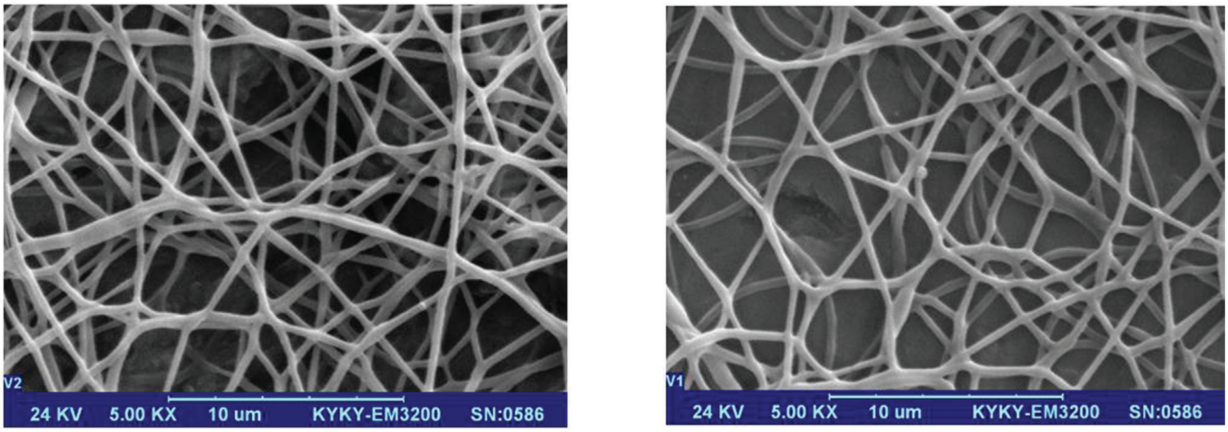
(SEM) (EM-3200, KYKY) and illustrated in Figure 2. From this figure, the mean values of fiber diameters were determined using digimizer (Belgium 4.1) software by measuring the diameter of 100 random nanofibers. These results are listed in Table 2. It can be seen that the diameter of PEO nanofibers from 7% PEO solution is 264.1 nm and by addition of 5% casein, the uniformity of nanofibers has lost due to decreased viscosity of the solution related to the nature of casein. Casein is a protein with a globular structure and lots of polar side groups which lead to its high elasticity and therefore it is not electrospinable (24). Therefore, for electrospinning, a 10% casein solution, the percentage of high viscosity PEO solution was increased up to 26%. Table 2 shows that the addition of 15% and 26% PEO solution to 10% casein solution resulted in nanofibers formation with 358 and 359 nm diameters, respectively. In fact, while the concentration of two PEO samples of 3 and 4 in Table 2 are different, applying 20:80 and 10:90 volume fractions of the PEO and casein solutions give the almost equal amount of these polymers in final electrospinning solution. That is why the nanofiber diameters from solution 3 and 4 are similar and only their diameter distributions are different (Figures 3a and 3b).

Figure 2a shows the SEM image of a continuous nanofiber web of 7% PEO solution (without Casein). Figure 2b refers to the nanofiber web produced by adding 30% casein with 5% concentration to the PEO solution.



(a)
PEO (7%) : Casein (0%)
Ratio of 100:0

(b)
PEO (7%) : Casein (5%)
Ratio of 70:30



(c)
PEO (15%) : Casein (10%)
Ratio of 20:80

(d)
PEO (26%) : Casein (10%)
Ratio of 10:90

Figure 2: SEM images of nanofibers: the effect of PEO concentration on fiber uniformity in spinning triangle zone during yarn production.

The presence of beads and fiber non-uniformity is obvious for this sample. The main reason for this phenomenon is attributed to the low concentrations of two polymers. Moreover, it should be noted that inserting casein into the polymeric solution decreases the viscosity (24). For compensating the reduced viscosity due to the presence of casein component in the solution, increasing the percentage of casein solution would result in increasing the concentration of PEO which in turn leads to the production of nanofibers with better quality. The improved condition of electrospinning by increasing the ratio of casein component can be clearly observed from Figures 2c and 2d in which the PEO/casein ratios are 20:80 and 10:90, respectively. Figures 3a and 3b show fiber diameter distribution of PEO/Casein 20:80% and 10:90% respectively. Diameter distribution is more uniform by increasing the ratio of casein component.

3.2 Yarn morphology

After optimizing the electrospinning of the nanofibers using different ration of PEO/Casein, those rations were used to produce nanofibrous yarn according to Figure 1. SEM images of nanofiber yarn produced from PEO/casein solution are illustrated in Figure 4 with two different magnifications. From Figures 4a-c, it can be seen that the fiber arrangement within the yarn structure and the surface uniformity of constitutive fibers improve by increasing the PEO concentration. Since nanofibers produced from 7% PEO solution without casein are relatively uneven with low strength, the resultant nanofiber yarn was also weak and as a result, they were ignored for further analysis including SEM image. Incorporating 5% casein to the 7% PEO solution also lead to the formation of uneven nanofibers with a flattened structure containing beads as illustrated in Figures 4a and 2b. SEM images in Figures 4b and 4c clearly shows that increasing the PEO component up to 26% would significantly enhance the formation of uniform nanofibers even in the presence of 10% casein. By increasing the ratio of PEO/Casein to 10/90 (Figures 4c and 2d), the number of beads decreased and more uniform and oriented nanofiber yarns were produced. This result shows that for producing a uniform nanofiber yarn containing 90% casein, it is necessary to increase the concentration of PEO simultaneously. This result supports the finding of other researchers reports on producing nanofibers containing 80:20 casein PEO (24). The result of the present study also confirms that these nanofibers have enough strength to produce nanofiber yarns directly.

Table 2: Mean value of nanofiber diameters produced from PEO/casein solutions.

	Nanofibers sample (volume fraction of the components)	Fiber diameter *(nm)*
1	PEO (7%)/Casein (0%) 100:0	264 (CV% = 20.53)
2	PEO (7%)/Casein (5%) 70:30	Uncountable
3	PEO (15%)/Casein (10%) 20:80	358 (CV% = 16.58)
4	PEO (26%)/Casein (10%) 10:90	359 (CV% = 16.15)

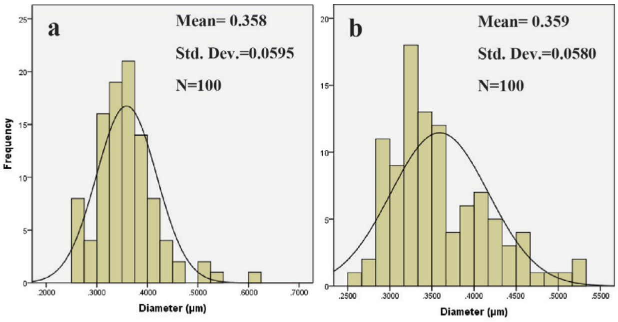


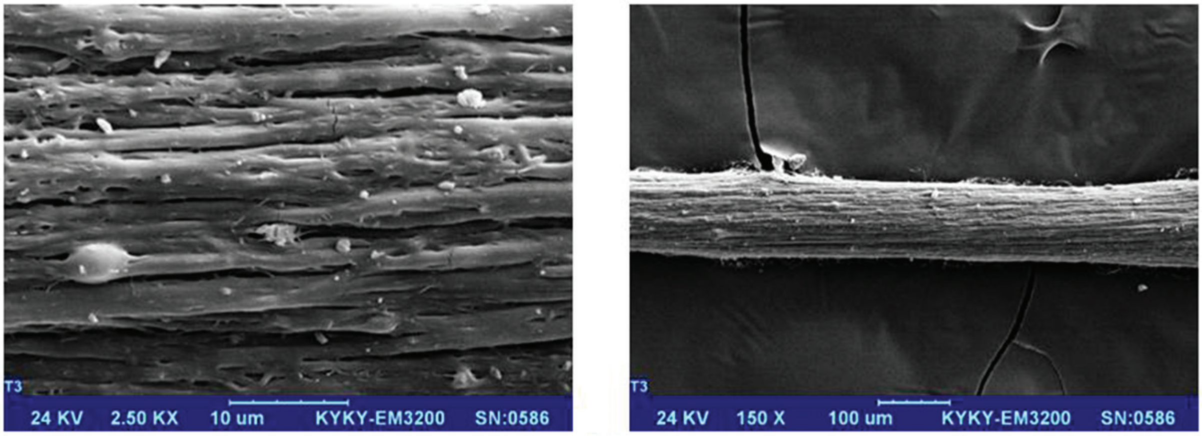
Figure 3: Fiber diameter distribution of (a: PEO (15%)/Casein (10%) 20:80%) and (b: PEO (26%)/Casein (10%) 10:90%).

3.3 Mechanical properties of produced yarns

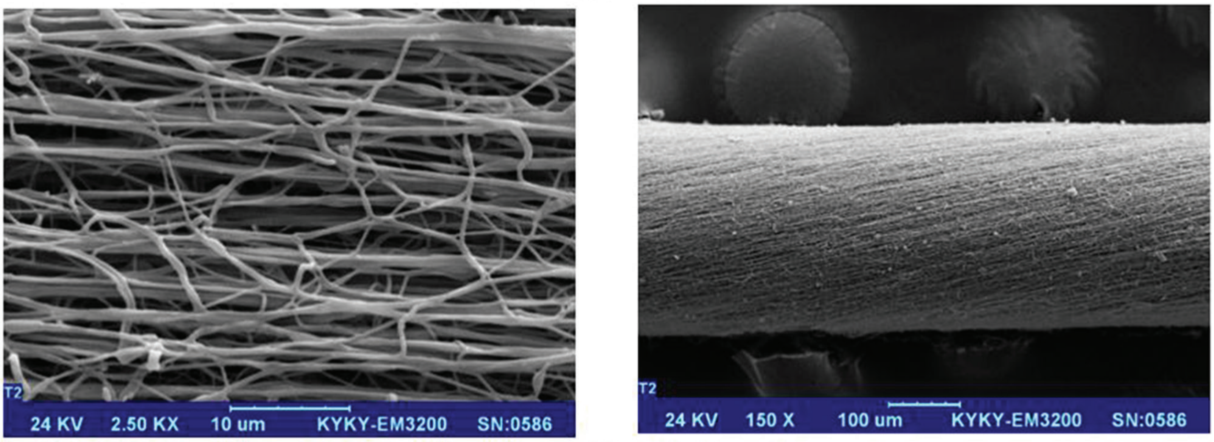
The best-produced PEO/casein nanofiber yarns based on their uniformity illustrated in the SEM images were chosen for mechanical testing. For investigating the effect of crosslinking on the strengthening of the produced nanofiber yarn, cross-linked yarn with two different concentrations of 20% and 40% w/w of toluene diisocyanate per polymer solution, were tested as well. Table 3 gives the results of the tensile testing of PEO/casein crosslinked and non-cross-linked yarn. The given values are the average of ten measurements (see Figure 5).

Figure 6 shows the variation trend of the yarns tensile characteristics in terms of specific tension, breaking elongation, work of rupture and elastic modulus. Detailed reports achieved through ANOVA statistical tests and LSD ($p<0.05$) analyses.

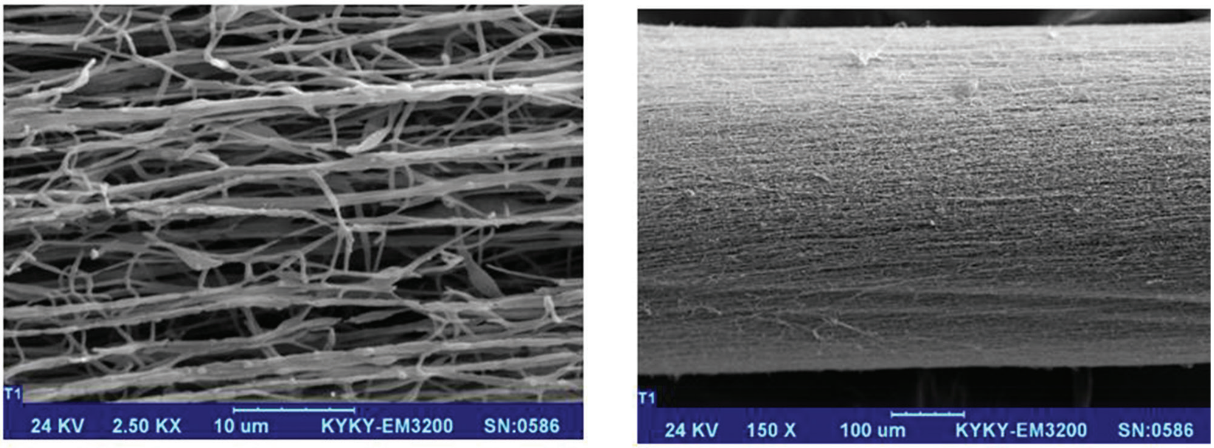
The data in Table 3 and Figure 6 shows that produced cross-linked nanofibers have higher mechanical strength in addition to an increased brittleness. Increasing the constitutive di-isocyanate component up to 20% results in the increasing of the yarn elongation at break due to numerous elastin links formation. Further increase of di-isocyanate up to 40% leads to the formation of a strong network containing higher linkage between the chemical



(a)
PEO (7%) : Casein (5%)
Ratio of 70:30



(b)
PEO (15%) : Casein (10%)
Ratio of 20:80



(c)
PEO (26%) : Casein (10%)
Ratio of 10:90

Figure 4: SEM images of nanofiber yarn produced from PEO/casein solution.

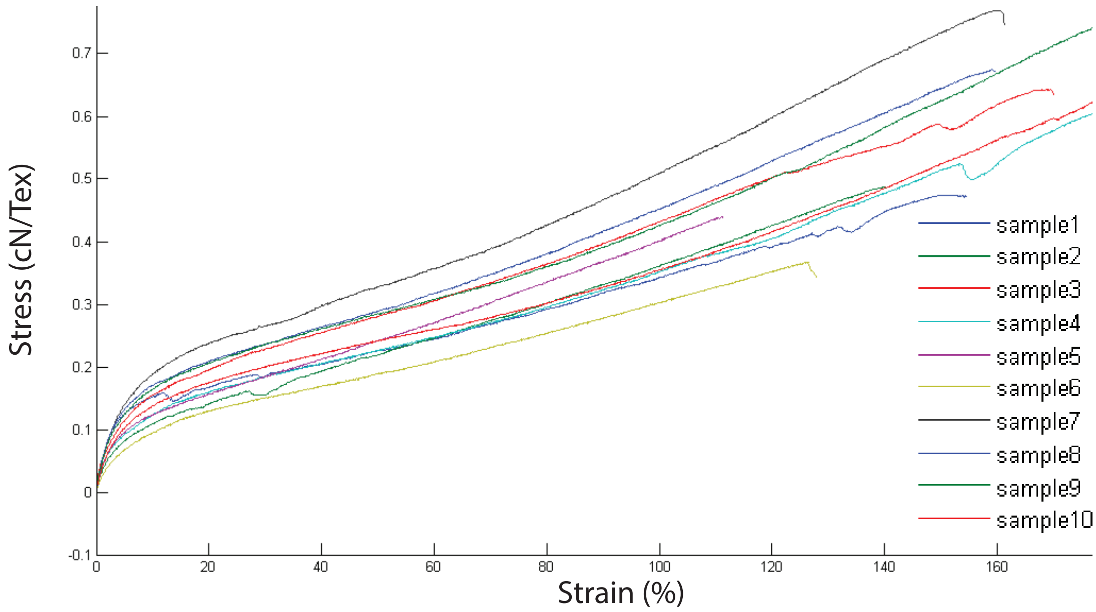


Figure 5: Ten stress-strain curves of PEO (26%)/Casein (10%) 10:90 sample.

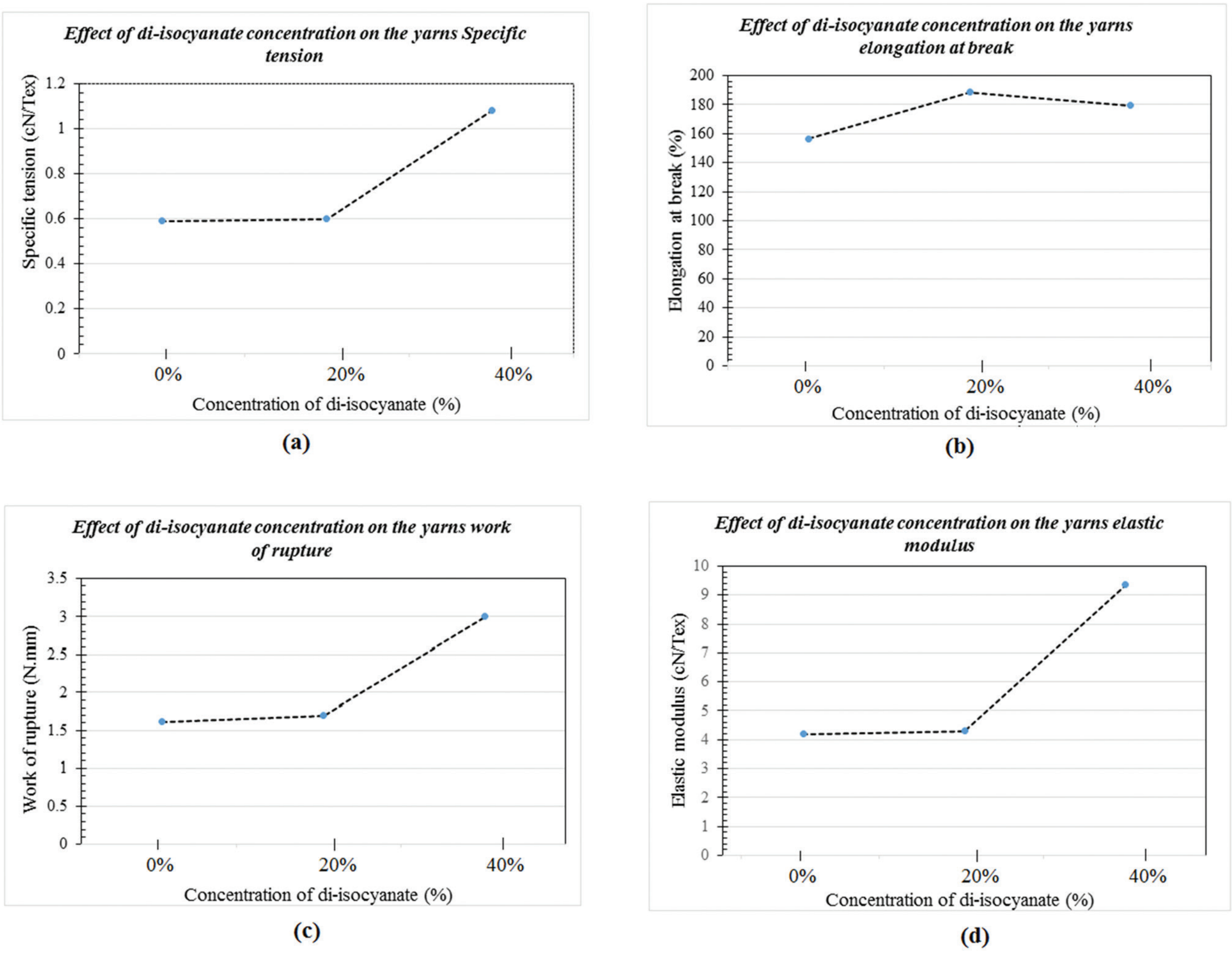


Figure 6: Variations of mechanical characteristics of nano-yarns in relation to the concentration of di-isocyanate as the crosslinking agent.

Table 3: Tensile characterizations of nanofiber yarn in relation with di-isocyanate concentration.

Di-isocyanate concentration (%)	Yarn count (*Tex*)	Specific tension in F_{max} (*cN/tex*)	Elongation in F_{max} (%)	Work of rupture (*N.mm*)	Elastic modulus (*cN/Tex*)
0	14 (CV% = 5.20)	0.59 (CV% = 2.42)	156.3 (CV% = 16.2)	1.92 (CV% = 3.03)	4.2 (CV% = 2.33)
20	14 (CV% = 6.70)	0.60 (CV% = 11.22)	188.5 (CV% = 15.40)	1.70 (CV% = 13.50)	4.30 (CV% = 2.80)
40	14 (CV% = 3.00)	1.08 (CV% = 23.00)	179.3 (CV% = 37.45)	3.00 (CV% = 6.60)	9.35 (CV% = 1.31)

groups of polymers which lead to the lower elongation of produced yarn as well as higher brittleness nature.

In order to study the mechanism of rupture during tensile testing on the Zwick instrument, deformed shapes of the cross sections of broken nanofiber yarns were observed using an optical microscope by the magnification of 1000 times. Figure 7 shows a complete broken cross-section of crosslinked yarn (Figure 7b) in comparison to the less brittle shape of broken non-crosslinked yarn. The yarn cross section was also investigated by scanning electron microscopy. Figure 8 shows that the cross-linked nanofibers have higher nanofiber diameter and seems that swelling happened in nanofibers after cross-linking. It is possible that due to the presence of excess amount of active solvents and diisocyanate, a foam-like polyurethane has formed which has led to the increased diameter, yarn strength, and elongation. In the crosslinking reaction, a different hypothesis is considered and will be discussed.

3.4 Investigating the results of infrared spectrum

Caseins are in the form of globular proteins and contain different amino acids with polar side groups including 15% NH_2 and 15% OH group, in addition to the 26% COOH group. Among them, NH_2 and OH groups have the ability to react with isocyanate group to form a covalent linkage. It should be noted that water in PEO solution can react with diisocyanate to form water-born polyurethane as well. At first, the OH group of water will react with the toluene diisocyanate (TDI) to form unstable carbamic acid which in turn decomposes to amine and carbon dioxide. The resultant amine group from the previous reaction or the amine group of the casein will be participated in another reaction with TDI to form urea linkage (Figure 9) (31).

On the other hand, the OH group of casein has the possibility to react with isocyanate group in the basic condition of the casein solution due to the presence of triethanolamine. Although PEO has two OH reactive end groups, the possibility of the crosslinking between PEO and toluene diisocyanate is expected far less than casein. By increasing the amount of toluene diisocyanate, the probability of the crosslinking of casein chain with TDI, its amine product or another casein chain will increase as illustrated in Figure 10.

In order to study the possibility of a cross-linking reaction, FTIR spectra of triethanolamine, TDI, casein, casein in triethanolamine, the mixture of casein/PEO/TDI before and after crosslinking recorded and compared to each other. Figure 11a shows the FTIR spectra of TDI as crosslinker and Triethanolamine as solvent. TDI has a characteristic strong peak at 2270 cm^{-1} related to the isocyanate(–N=C=O) functional group and main peaks of triethanolamine are located at 1072, 1036, 907, 882 and 1458 cm^{-1}. The spectrum of pure casein, casein in ethanolamine and casein-PEO- ethanolamine are given in Figure 11b. Casein has a broad peak at 3400 cm^{-1}, attributed to the OH and N-H stretching of the amine groups in casein and its broadening is related to the inter and intra hydrogen bond formation. The bands at 1656, 1530, 1447 and 1235 cm^{-1} correspond to C=O stretching, C-N stretching, C-H deformation and N-H deformation vibrations of the peptide linkage in the casein, respectively (32,33). The spectrum of the final polymer solution of PEO-casein in triethanolamine (Figure 11b) shows that the major peaks of solvent in the region of 800-1400 cm^{-1} are dominated and have covered the peaks of PEO polymer. However, the carbonyl group and -N-H- bending of amide band of the casein with a slight shift at 1648 cm^{-1} and 1458 cm^{-1}, respectively, are visible. The characteristic peak of C-N group at 1530 cm^{-1} cm has completely disappeared due to the interaction of casein with solvent.

Figure 11c shows the FTIR spectra of pure casein and cross-linked produced nanofiber. As it shows, the presence of the main peaks of casein and PEO and disappearance of the peaks of triethanolamine at 907, 882 and 1151 cm^{-1} in the spectrum of produced nanofiber in Figure 11c could be related to the removal of solvent from nanofiber after washing process.

Regarding crosslinking process, it is well known that the reaction between isocyanate functional groups with hydroxyl and amine groups in casein and water, as it is shown in Figures 11 and 10, leads to the formation of

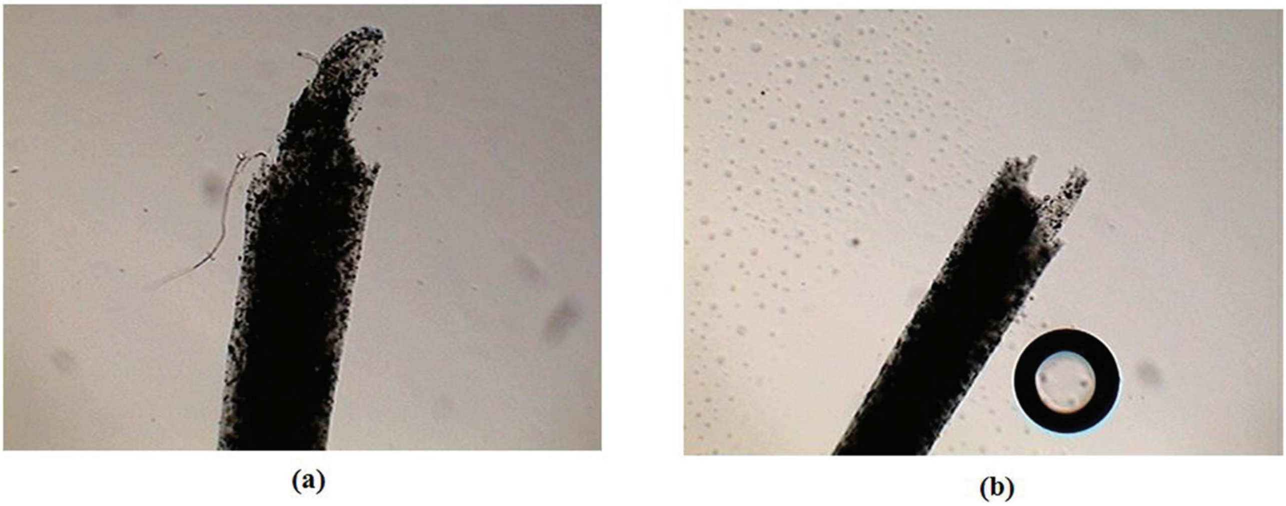


Figure 7: Optical microscope images of nanofiber yarn cross-section; (a) without di-isocyante and (b) containing 40% di-isocyante.

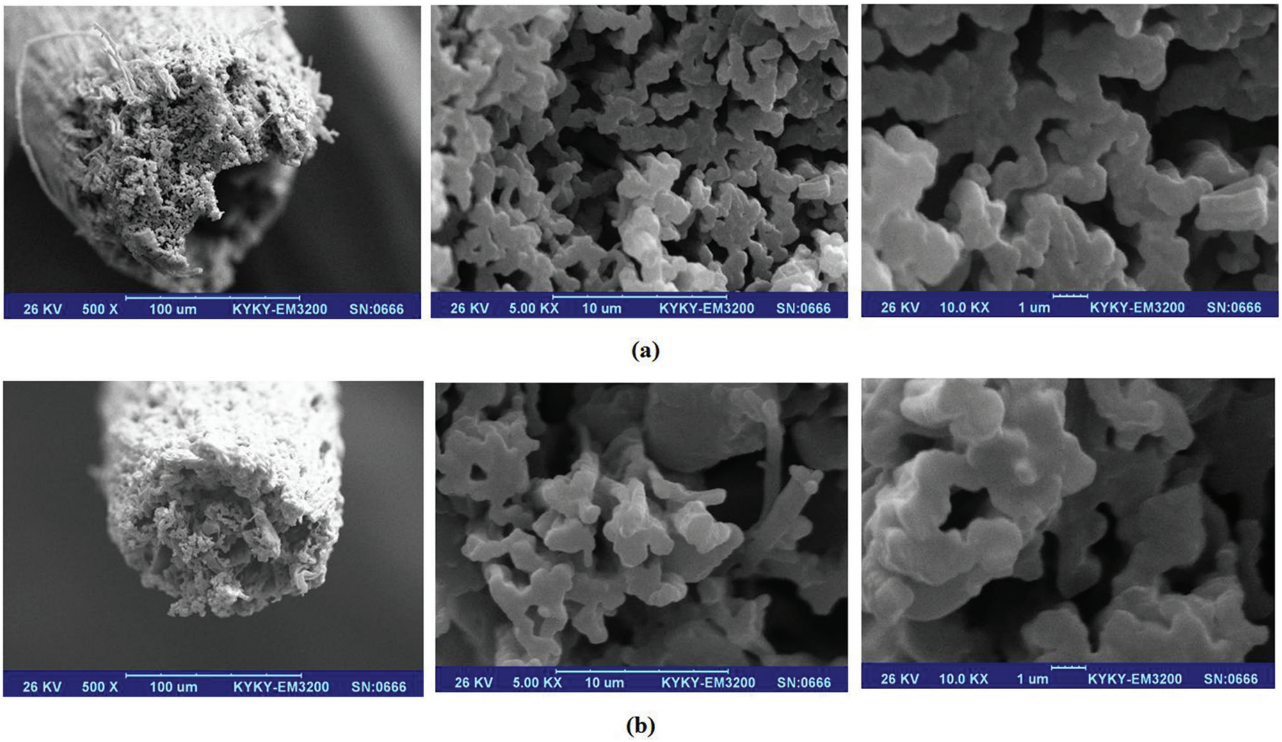


Figure 8: SEM images of nanofiber yarn cross-section; (a) without di-isocyanate, and (b) containing 40% di-isocyanate.

urethane and urea linkage. It is suggested that formation of a new peak at 3150 cm^{-1} which could be attributed to the new -NH- bonds, in addition to the small peak at 1739 cm^{-1} may confirm the urethane formation (33). Sharpening of the peak around 3355 cm^{-1} shows that there is less hydrogen bonding which can further confirm the usage of free OH and NH_2 side groups of casein due to urethane formation. If the crosslinking process happens between casein polymer chains and crosslinker, the protein structure will change which results in FTIR band variation. In addition, it is being reported that in the presence of basic ethanolamine, casein chain can be hydrolyzed partially, causing the reduced intensity of amide II band. The spectrum of cross-linked nanofiber shows that the bands of C=O stretching of amide I and C-N stretching of amide II at 1653 cm^{-1} and 1540 cm^{-1}, respectively, has sharpened and the ratio of amide I/amide--II band has changed which are the evidence of structural change in the casein. It is believed that participation of

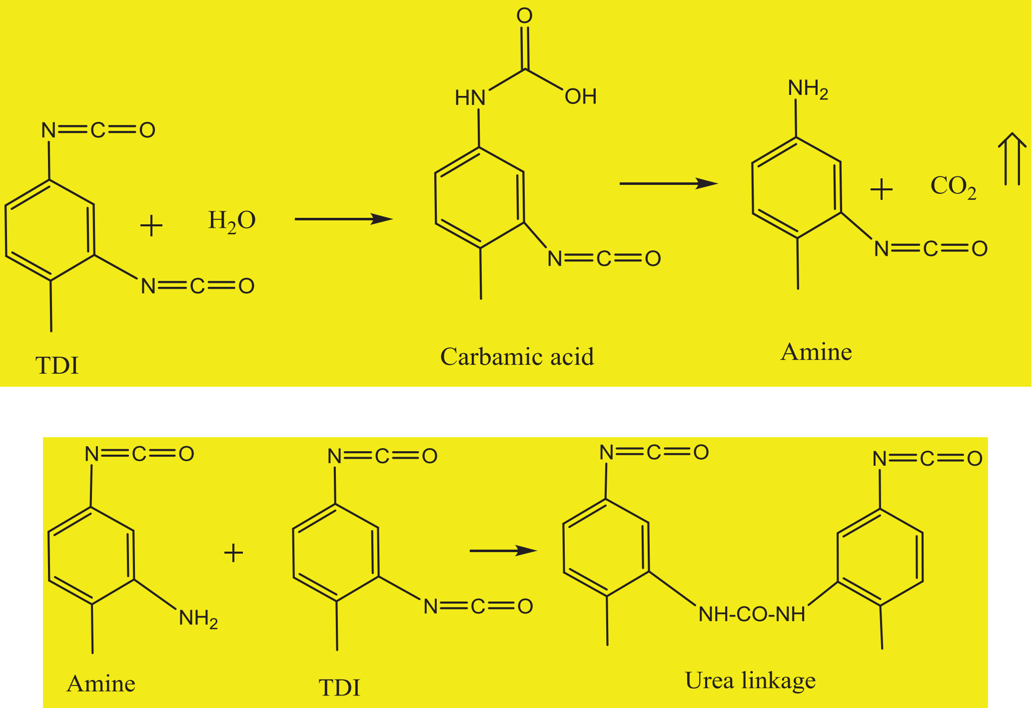


Figure 9: Formation of urea linkage in TDI in the presence of water.

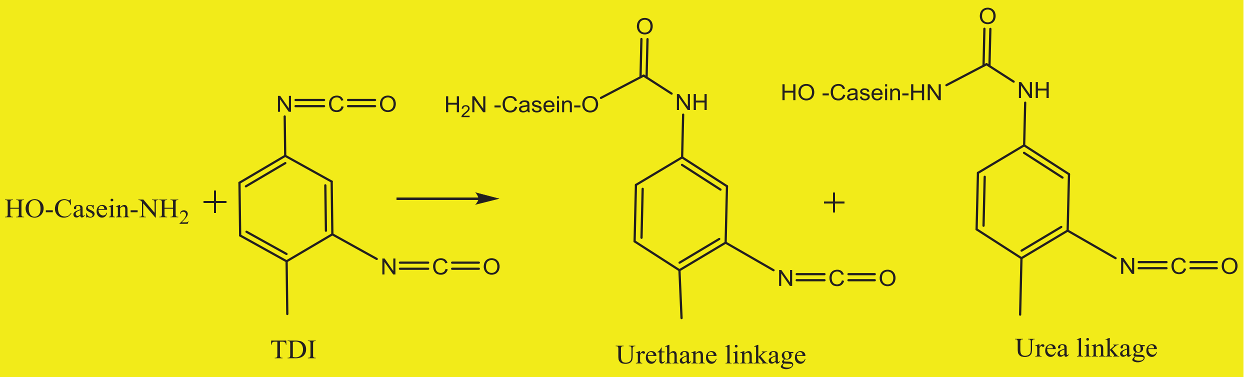


Figure 10: Possible cross-linking reaction between casein and TDI.

hydroxyl and amine groups of casein in the crosslinking process has reduced their availability for hydrogen bonding formation. It can be seen in Figures 11b and 11c that after addition of diisocyanate, peptide bands at 1530, 1447 and 1236 cm^{-1}, have shifted toward higher wave numbers of 1540, 1459 and 1248 cm^{-1}, respectively, as another sign of changes in the chemical structure of the protein.

At the same time, the appearance of three peaks at 1096, 1077 and 1033 cm^{-1} could be assigned to -C-O-C- peaks of PEO, and also the formation of polyurethane or polyurea linkage. Two small peaks at 1341 and 1360 cm^{-1} are claimed to be related to the -CH_2- group of PEO in their crystalline form (31). Moreover, loss of peak at 2272 in cross-linked nanofiber yarn in Figure 11c confirms the absence of toxic agent within the nanofiber yarn structures, although more precise analytical techniques for measuring the trace amount of diisocyanate in the nanofiber yarn is necessary.

3.5 Effect of biodegradation on nanofiber yarns strength

According to ASTM D 5488 94d, the term "biodegradable" is defined as "the ability to undergo decomposition into carbon dioxide, methane, water, inorganic compounds, and biomass". Since casein is principally classified as a green-biopolymer, the biodegradation ability of

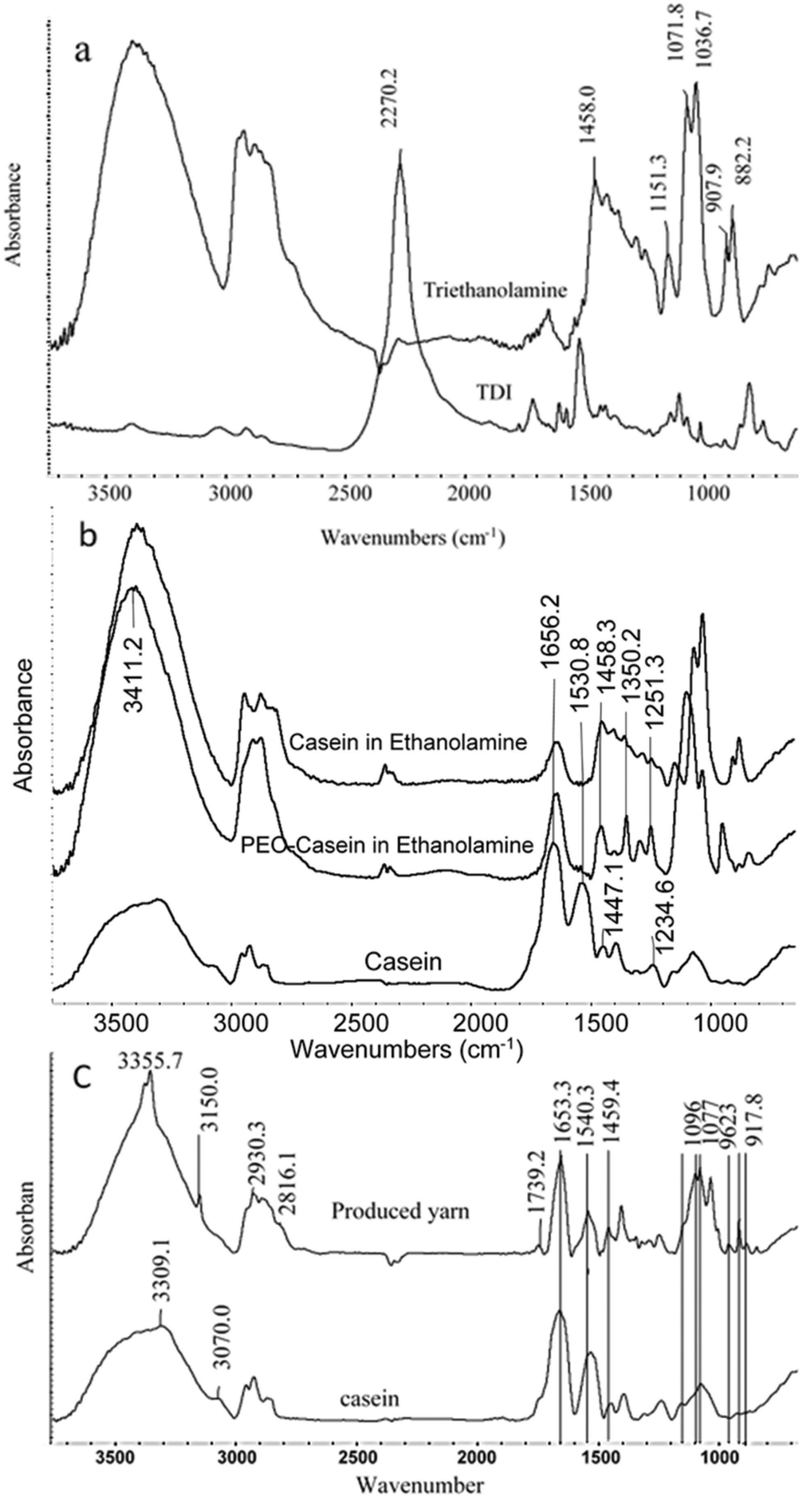


Figure 11: FTIR results of; (a) 100% di-isocyanate and triethanolamine, (b) pure casein, casein in ethanolamine and peo/casein in ethanolamine c) casein and crosslinked nanofiber with diisocyanate.

Table 4: Biodegradation results of nanofiber yarns.

Samples	Number of exposing days	Number of measure-ments	Average of differences between initial and secondary weight
Series 1	2	5	40.60 %
Series 2	6	5	43.40 %
Series 3	10	5	58.35 %
Series 4	14	1	80.00 %
Series 5	18	0	Not available

the hybrid nanofiber produced in this study is of great importance.

Degradation results of the yarns (PEO (26%): Casein (10%)) 10:90%, exposed to the PBS solution for 2, 6, 10, 14, and 18 days, are given in Table 4. The results indicate that after 18 days' exposure, the nanofiber yarns are totally degraded. It could be related to water solubility nature of PEO. In fact, after 18 days the PEO component will be dissolved and as a result, the PEO/casein nanoweb will degrade. This could further confirm that crosslinking did not occur between PEO and casein functional groups and only casein chains were cross-linked together. Weight reduction of polymer composite in washing process might be related to the solubility of non-cross linked PEO polymer.

4 Conclusion

Production of hybrid nanofiber yarn made of casein and poly-ethylene-oxide is the main aim of this research through which by applying different testing method, optimum conditions for spinning the nanofiber yarn containing 90% casein and 10% PEO could be achieved. Important optimized conditions regarding the electrospinning parameters, i.e., input voltage, feeding rate, twist, the distance between the two nozzles, the distance between the neutral substrate and the collector, the distance between the center of the electromagnetic shield and the neutral substrate were determined. The nanofiber yarn contacting PEO with 26% concentration and casein with 10% yarn possess unsuitable strength. Increasing di-isocyanate crosslinking agent at 40 wt%, would significantly enhance the nanofiber yarn strength and work of rupture. Statistical analysis showed that increasing di-isocyanate component within the PEO/casein solution up to 20% significantly affect the nanofiber yarn tensile characteristics in term of elongation at break due to the higher amount of network structures formation. Further increase in di-isocyanate content decreases the yarn elongation, which could be attributed to the formation of many more network structures between the chemical constitutive components. The increasing rate of elastic modulus as a result of adding 20% di-isocyanate crosslinking agent into the PEO/casein solution, is not statistically significant. But there are significant differences between the elastic modulus of strengthened and unreinforced PEO/casein yarns by increasing the amount of di-isocyanate component up to 40%. Considering the images of cross section morphology, the nanofiber yarn strengthened with 40% di-isocyanate would exhibit brittleness nature compared with unreinforced yarns. The results of biodegradation testing showed that samples exposed to the buffer solution would lose a greater portion of their strength after 10 days, while the total degradation of the nanofiber yarn would happen after 18 days of exposure.

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