Nanocomposite Film of Recycled Silk Powder and Polypropylene

Shadi Karimi Rabbani, Fatemeh Dadashian, Nahid Hemmati Nejad and Niloofar Eslahi

Abstract—In this study, the possibility of producing silk nanopowder by a commercial proteolytic enzyme in combination with a mechanical treatment is investigated. Different conditions are applied in order to investigate the effects of enzyme concentration and treatment time. The SEM photographs show that the particle size of the produced silk powder is mostly less than 100 nm. The prepared powder is then blended with polypropylene (PP) in different percentages for producing composite films. The SEM micrographs indicate that silk nanoparticles distributed uniformly in the film. Characteristic peaks of both silk and PP are found in FTIR spectra and thus no obvious chemical changes occur during the blending. With the increase in the ratio of the silk powder, there is an evident decrease in the tensile strength and elongation at break. However, silk powder plays an important role in improving the moisture absorption of the films.

Key words: Silk nanopowder, enzymatic treatment, nanocomposite, Polypropylene (PP)

I. INTRODUCTION

Natural fibers such as silk, wool, cotton or hemp are wasted during processing and final usages. Therefore, a new idea of reusing these fibers has large marketing potential because of their excellent intrinsic properties. Meanwhile, not only the textile industry, but many other industries like the bio-medical industries need such valuable bio-compatible materials [1-6]. In recent years, there has been a great interest in investigating fibers based on regenerated protein to develop new textile materials and to reuse redundant protein materials [7–12]. An alternative method to reuse protein fibers is the preparation of keratin solution, which is extracted from these fibers using complex chemical methods and then blended with synthetic polymers to produce protein biomaterials [13-15]. However, keratin solution is prone to decompose in the process of extruding at high temperature and the inherent microstructure of protein is destroyed inevitably. On the other hand, since the protein powder could keep the original properties of the materials without destroying the microstructure, it has been widely applied in modern industries and some hi-tech related fields due to its unique properties [16,17]. The reactivity of protein fibers can be enhanced greatly by converting them into fine powder due to the high surface-to-volume ratio [18]. Superfine powder is defined as all of the powder smaller than 30 μm in diameter. It can be divided into three grades, i.e. micrometer powder (particle diameter bigger than 1 μm), sub-micrometer powder (particle diameter between 1 and 0.3 μm), and nanometer powder (particle diameter ranges from 0.1 to 0.001 μm) [19]. Silk particles are usually produced through mechanical attrition of fibers or regeneration from a silk solution [20-23]. Solution routes have inherent drawbacks in the preparation process, such as long time of dialysis, high production costs, safety and environmental problems. In contrast, mechanical attrition method can avoid these problems. However, silk fibers are viscoelastic and not easy to crush. Therefore, various pre-treatments were used to reduce fiber strength prior to mechanical methods, such as alkali degradation [24], exposure to high temperature [25], radiation [26], or steam explosion [27].

In this paper, silk fibers are hydrolyzed with a commercial proteolytic enzyme followed by milling process to produce silk nanopowder. The powder is then blended with polypropylene (PP) to produce blend films. Both powder and the blend films are characterized with scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) spectroscopy. Moisture absorption of the samples and the mechanical properties of the films are measured as well.

II. EXPERIMENTAL

A. *Preparation of silk powder*

Silk filament waste was provided by East Guilan Silk Incorporation of Guilan Province, Iran. Sodium carbonate, boric acid, acetic acid and borax were of analytical grade and purchased from Merck Co., Germany. At first, silk fibers were washed using 1 g/L nonionic detergent and 1 g/L sodium carbonate at 60 °C for 30 min at L:G=40:1 (liquor to goods ratio), then rinsed thoroughly in lukewarm distilled water and dried at room temperature for 48 h. The washed fibers were cut into short pieces around 1-2 mm before being treated by a commercial proteolytic enzyme (Savinase 16.0LEX (EC.3.4.21.14), provided by Novozymes Inc., Denmark) at 7, 10, 15, 20 and 25 g/L concentration in boric acid-borax buffer (pH=8.5) at 55 °C for 8 h in Linitest machine (Originl Hanua Linitest) to investigate the effect of enzyme concentration on silk fiber diameter reduction. After this step, the samples were treated with 20 g/L enzyme (the most effective concentration according to the previous step) at 55 °C for

S. Karimi Rabbani, F. Dadashian, N. Hemmati Nejad and N. Eslahi are with the Department of Textile Engineering of Amirkabir University of Technology, Tehran, Iran. Correspondence should be addressed to F. Dadashian (e-mail: dadashia@aut.ac.ir).

8, 16, 24, 32, 40, 48 and 56 h in Linitest machine to evaluate the influence of the processing time. After the enzymatic treatment, the bath temperature was raised to 80 °C and a solution of acetic acid was added to reduce the pH of the treatment bath in order to denature the enzyme. The resulting mixture was filtered through a glass fiber filter using a Buchner funnel. The filtered powder was rinsed with several portion of distilled water, and then dried in room temperature for 48 h. The powder was finally ground with a high-energy milling machine (RETSCH PM400) in a wet media (methanol) for 3 min, then oven dried at 70 °C for 30 min. This media provided sufficient shear required to separate silk fibrils and could suppress agglomerations of the particles.

B. Preparation of silk powder/PP blend film

Plasticizer poly (ethylene) glycol (analytical grade, MW 400) was provided by Merck Co., Germany. A fiber grade isotactic polypropylene (granule, PP512) with melt flow rate (MFR) of 24 g/10 min was also purchased from Sabic Co., Saudi Arabia. At first, poly (ethylene) glycol (15% content) was slowly added to silk powder, the mixture was stirred in a blender for 5 min, and then the mixture was blended with PP at various contents. In order to blend sufficiently, the blended silk powder and PP were extruded in a twin screw extruder (Brabender W50, Germany, 2002) at 180 °C for 15 min at 200 rpm and cut into small pellets. The pellets (blends) were sandwiched between two pieces of PET films which were then hot-pressed for 5 min under the molding pressure of 5 MPa and a molding temperature of 160 °C. Finally, the blend films were taken out from the PET films after they cooled down in the air.

C. Characterization of the silk powder and the blend films

Scanning electron microscopy (SEM) analysis was carried out with XL30 Philips, Netherlands, at 25 kV acceleration voltages, after gold coating. The SEM images were also processed by Manual Microstructure Distance Measurement software to measure the particle size.

The FTIR spectra of the samples were studied by a Nicolet, Nexus 670 infrared spectrophotometer with a scanning range from 4000 to 400 cm-1. The moisture regain of the samples was measured by gravimetric method and it was defined as follows:

Moisture regain (%) = (W1 − W2)/W2 × 100

where, W1 and W2 stand for the conditioned and dry weight of the samples, respectively.

Tensile tests were carried out using an Instron 5566 Universal Testing Machine, at a gauge length of 60 mm and strain rate of 1.5 mm/s, the width of the samples was 20 mm, and each sample was tested five times and the results were averaged.

III. RESULTS AND DISCUSSIONS

A. Characterization of the produced silk powder

In order to determine the appropriate enzyme concentration and treatment time, fiber diameter was measured by an optical microscope (Projectina,

Switzerland) after each experiment. Figure 1 shows the effect of enzymatic treatments on the rate of fiber diameter reduction. It can be seen that the enzyme concentration has an optimum limit and the maximum diameter reduction has occurred by using 20 g/L enzyme. Therefore, this concentration was applied for investigating the effect of enzymatic treatment time on the fiber diameter changes. It is obvious that the fiber diameter reduced by increasing hydrolysis time up to 48 h and prolonging the hydrolysis process showed no more decrease in the diameter. Therefore, the sample prepared under the optimum condition (20 g/l enzyme for 48 h) was then subjected to a high-energy milling machine to achieve silk nanoparticles.
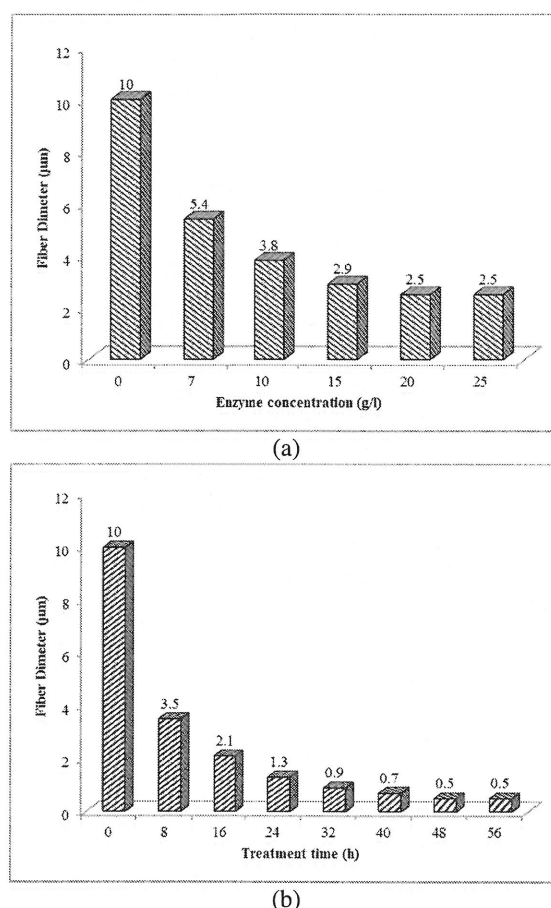

(a)

(b)

Fig. 1. The effect of (a) enzyme concentration and (b) hydrolysis time on silk fiber diameter.

The SEM images of untreated and treated samples are illustrated in Figure 2. The micrograph of silk fibers before enzymatic hydrolysis clearly reveals a relatively smooth surface and their diameter was around 9–11µm (Figure 2a). Following enzymatic hydrolysis, the fibers were fibrillated and separated into much smaller and narrower products and surface etching on the fibers can be clearly seen in Figure 2b. The SEM image of the particles produced after milling process is shown in Figure 2c indicating that the particles have almost spherical shape. However, the actual shapes of the particles may differ in thousands way and there still exist some irregular shapes which lead to the broad particles size distribution.
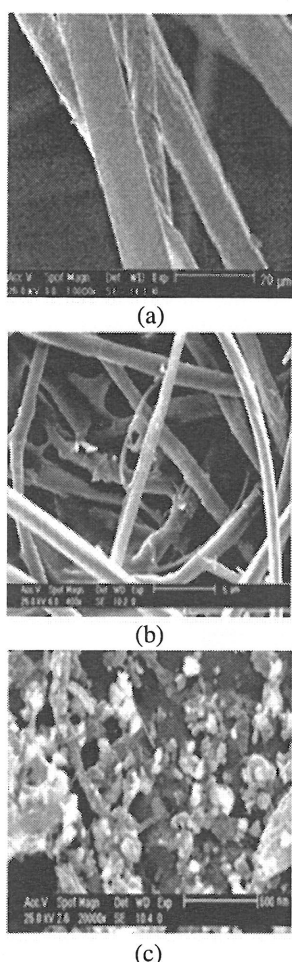
(a)

(b)

(c)

Fig. 2. SEM images of silk fiber (a) raw, (b) enzyme treated under optimum condition, (c) enzyme treated under optimum condition + milling.

The results of particle size distribution measurement by image processing software from the SEM images are illustrated in Figure 3. The size distribution of produced powder shows that most particles are between 60-120 nm.
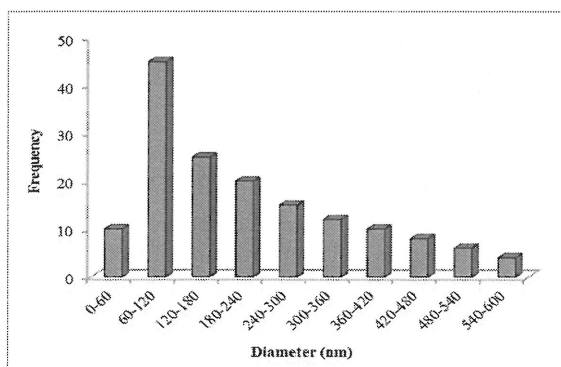

Fig. 3. Silk particle size distribution after milling process.

In order to find the chemical structural changes of treated silk fibers, the FTIR spectra of the samples are shown in Figure 4. The results indicate that Amide I (ν C=O) band appears at ~1638 cm^{-1} with a weak shoulder at around 1700 cm^{-1} in the original silk fibers, and in the silk treated with savinase, it appears around ~1632 cm^{-1} and Amide II (δ NH + νCN) and Amide III (ν CN + δ NH)

bands locate at ~1522 and 1230 cm^{-1}, respectively, while for original silk fibers, they appear at ~1509 cm^{-1} and ~1230 cm^{-1}. The FTIR spectra of silk powder confirm that there are no evident changes in the chemical structure during enzymatic hydrolysis. In addition, the characteristic bands around 1630 and 1520 cm^{-1} represent dominant β-sheet conformation in the enzyme treated sample. The Amide I band of silk treated with Savinase has become narrower and slightly shifts to a lower wave number (from 1638 to 1632 cm^{-1}). The shifts may result either from a change in the spectral components caused by the presence of different conformations in the crystalline and amorphous regions of silk fibers or from a slightly different crystalline environment [27]. Also, there are no significant changes in the chemical structure of the enzyme treated sample after milling. Amide I band appears at ~ 1632 cm^{-1} in the silk powder, and Amide II and Amide III bands locate at ~1515 cm^{-1} and ~ 1230 cm^{-1}, respectively. As can be seen, β-sheet crystalline structure is still remained in the silk powder after milling process.
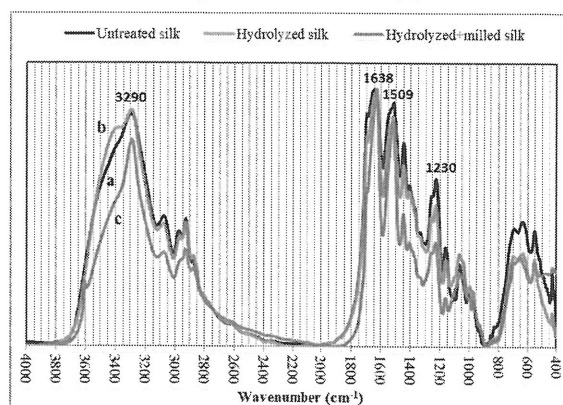

Fig. 4. FTIR spectra of silk fiber: (a) untreated, (b) hydrolyzed, and (c) hydrolyzed and milled.

Crystallinity index can be also calculated as the intensity ratio of 1265 and 1235 cm^{-1} from FTIR spectrum [28,29]. The calculated values of crystallinity are 0.89, 0.93 and 0.91 for the raw, enzyme treated and silk powder, respectively. Thus, the treated samples have a little higher crystalline structure in comparison to the original silk fiber due to the destruction of the amorphous region during enzymatic hydrolysis. It should be noted that crystallinity of the powders decreased slightly in milling process.

Moisture regain of control sample (raw fiber), hydrolyzed and milled samples are shown in Table I. The results indicate that with decreasing silk fiber diameter upon enzymatic treatment and as a consequence increasing the specific surface, moisture regains of both samples (treated with enzyme and milling process) increased.

TABLE I
THE MOISTURE REGAIN OF THE RAW AND TREATED SAMPLES

Sample	Moisture regain (%)
Silk fiber (raw silk)	7±0.4
Silk treated with 20 g/l enzyme for 48 hours	16.95±0.5
Silk powder (treated with 20 g/l enzyme for 48 hours and milling)	20.33±0.5

B. *Characterization of silk powder/PP blend films*

The surface morphologies of silk powder/PP blend films at 10/90 and 30/70 blend ratio are shown in Figure 5. The photograph of blend film in Figure 5a shows a smooth surface. However, when the silk powder increased from 10 to 30 %, some agglomerations have occurred in the blend film. These photographs suggest that the silk powder was blended with PP under the plasticization of polyethylene glycol in the extrusion, and at the low blend ratio (10/90), silk powder distributed uniformly in the film and almost no agglomerations occurred. Probably, PP melted and silk powder dispersed evenly into it, in the process of extrusion.
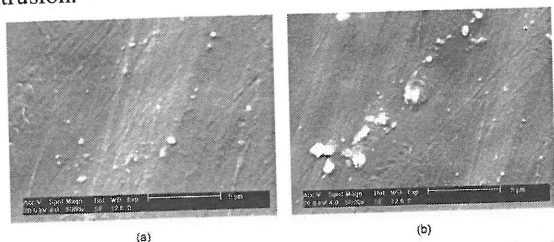

Fig. 5. SEM photographs of silk powder/PP blend films at ratio of (a) 10:90 and (b) 30:70.

The FTIR spectra of blend film (with 30% silk powder) are compared with that of PP and silk powder in Figure 6. The spectra of the blend film shows a strong absorbance in the region of 2800-3100 cm^{-1}, a specific methyl absorbance at 1380 cm^{-1} and a strong absorbance at 1460 cm^{-1} which are the characteristic absorbing bands of PP due to the stretching of C-H, the CH3 symmetrical bending vibration, and CH2 bending vibration, respectively. Besides, the spectra of the blend film exhibits absorbing bands around 3300 (N-H and O-H), 2850 (-CH2), 1630 (Amide I, C=O stretching), 1515 (Amide II, secondary NH bending), and 1233 cm^{-1} (Amide III, C-N stretching), which indicate the characteristic vibration bands of protein in silk.
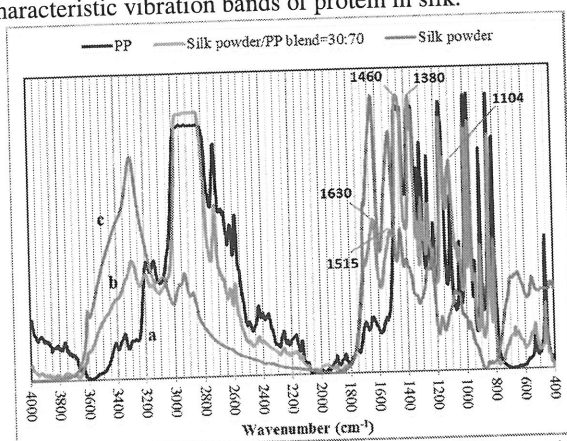

Fig. 6. FTIR spectra of (a) PP, (b) silk powder/PP blend films at ratio of 30:70, and (c) silk powder.

There are also other absorbing bands which show alcoholic group of polyethylene glycol at 1068 (COH), 1127, 1104 cm^{-1} (COC) in the blend film. These bands are due to the addition of polyethylene glycol during preparation of the films. In total, the FTIR spectra of the silk powder/PP blend film shows the characteristic absorbing bands of both silk fiber and PP indicating that blending does not influence their chemical structures.

The results of moisture regain and tensile strength tests are concluded in Table II. It can be seen that with an increase in silk powder content, moisture regain of the blend films enhance. The reason could be the presence of the silk powder with higher absorbance moisture in the blend, and therefore moisture ability of PP could be improved by addition of silk powder.

TABLE II
MOISTURE REGAINS AND TENSILE PROPERTIES OF SILK POWDER/PP BLEND FILMS

Sample	Moisture regains (%)	Breaking load (N)	Elongation at break (mm)
Silk powder	20.33±0.5	—	—
Pure PP film	0.01±0.01	81.11±4.5	3.8±0.4
Silk powder/PP film (10/90)	1.52±0.4	51.37±6.8	2.7±0.5
Silk powder/PP film (30/70)	2.98±0.5	35.37±9.1	1.64±0.8

It is also obvious that with an increase in the powder content, the blend film will become more weakened. Although the silk powder was incorporated sufficiently into PP after extrusion, addition of the powder increased the number of holes in the blend film and brought destruction in the tensile strength. Besides, poor interfacial adhesion between the silk powder and PP matrix tended to decrease the mechanical properties of the blend films.

IV. CONCLUSION

In this study, the possibility of producing silk nanoparticle with a commercial proteolytic enzyme, an environmental friendly method, along with milling process was investigated. The effects of enzyme concentration and hydrolysis time were evaluated to find the optimum process condition. After the enzymatic process, mechanical treatment by a rotary mill was carried out to produce silk nanoparticles. The SEM photographs showed that the particle size of the produced powder was mostly less than 100 nm. The FTIR spectra analysis demonstrated that the enzyme treated fiber had β-sheet crystalline structure and after milling process this conformation was maintained. The moisture regain of the treated samples increased as well.

The silk powder was then blended with PP to produce nanocomposite film. SEM photographs revealed that the superfine silk powder could be evenly blended with PP under the plasticization of polyethylene glycol in the extrusion, the silk powder distributed uniformly in the film and there were almost no agglomerations at low blend ratio. Characteristic peaks of both silk and PP were found in FT-IR spectra and thus there were no substantial changes in their chemical structure upon blending. Moisture regains of the blend films enhanced with increasing powder content. Besides, addition of the silk powder in PP destructed the integrity of PP and no linkage occurred at the interface, thus the mechanical properties of the blend film declined greatly with an increase in the silk powder content.

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