Real Silk Degumming Wastewater Remediation: Simple Applicable Recovery of Sericin Micro and Nano powders

Zahra Mohassel and Babak Noroozi

***Abstract*— in this study, an effectual ecofriendly technique for recycling of sericin has been developed. Pure sericin powders in the range of micro to nano scale were prepared by processing of real silk industry wastewater. Sericin was extracted through different procedures using urea and ethanol incorporating by spray drying and rotary extraction to acquire the desired powders. The obtained powders were evaluated using UV-vis spectrophotometer and Field Emission Scanning Electron Microscope to choose the best in the aspect of size and morphology. Fourier transform infrared spectroscopy spectra of sericin showed an absorption band at 1650 cm^{-1} (amide I), indicating random coil conformation. The structural properties of the produced micro and nano sericin powders were investigated using X-ray diffraction and differential scanning calorimetry analysis, which revealed that chemical precipitation can affect the secondary structure of sericin may arise from the intermolecular hydrogen bond changing.**

***Keywords*: degumming, recovery, sericin, textile, wastewater**

Nomenclature:

DMSO: dimethyl sulfoxide

DNA: deoxyribonucleic acid

DSC: differential scanning calorimetry

FESEM: field emission scanning electron microscope

FTIR: fourier transform infrared spectroscopy

MWCO: molecular weight cut off

SCF: supercritical fluid

XRD: x-ray diffraction

I. Introduction

Domesticated silkworm, *Bombyxmori* is an important economic insect that produces large amount of silk proteins. The major components of silk fibers are fibroin and sericin [1,2]. Silk sericin, a water-soluble globular protein, envelops the silk fibroin fiber with sticky layers that support the formation of a cocoon. This biopolymer constitutes about 20–30% of the total cocoon weight [3,4]. Degumming is the main process to provide silk fiber from dry cocoons. However, large amounts of discarded sericin in wastewater causes serious environmental problems [5,6].

Investigations showed that sericin possesses moistening, UV-absorbent, antioxidant [3], and to some extent tumor-suppressing properties [7]. This biopolymer has been processed into different forms including hydrogels [5,8], nanofibers [9-12], nano-powders [5], nanoparticles and scaffolds [13,14]. Applications of sericin cover a vast range from cosmetics to biomedical products, including anticancer drugs, anticoagulants, and cell culture additives because of precious antioxidant properties [1]. Consumption of sericin enhances bio-availability of Zn, Fe, Mg, and Ca suggesting sericin as a valuable natural ingredient for food industry. Filters composed of polyamide or polyester fibers coated by sericin provide anti-oxidation and antimicrobial activity enhancing their efficiency in polluted air filtration. It is possible to provide cross-linked, copolymerized, or blended sericin with other polymers to produce a new range of biodegradable materials with its own enhanced properties [15]. Use of sericin as a finishing agent for natural and synthetic textiles improves their moisture absorption, anti-static, softness, comfort, anti-bacterial, print ability and dye ability [15,16]. Sericin has been used effectively in preparation of silver nanoparticles as a capping agent preventing coagulation of the silver nanoparticles and keeping them in a moderately stabilized condition in an aqueous medium [17]. Ultrafiltration of heavy metal ions (Pb^{2+}, Co^{2+}, Ni^{2+},Cu^{2+}, Zn^{2+}) has been successfully done from aqueous solutions using sericin [18]. Also the capacity of sericin as a substratum for cell growth has been investigated. In particular, human corneal limbal epithelial cells were assessed for the first time on sericin substrate with an aim of promoting the use of sericin in tissue-engineered constructs for ocular surface restoration [4]. Sericin can also be used as biodegradable glues. Sericin was recommended as a transporter for Deoxyribonucleic acid (DNA) delivery in gene therapy, as an insulin conjugate increasing hormone half-life, and in sulfated form as an anticoagulant. Sericin scaffolds employed as a matrix in fabrication of biomaterials. Sericin-coated films are used on the surface of refrigeration equipment because of anti-frosting properties. Moreover use of the coated films on roads and roofs prevent frost damage [19].

Over the last decade, various methods have been explored to extract sericin from degumming wastewater. All methods are based on adsorption, precipitation, coagulation, evaporation, chromatography, and ultrafiltration. The recovery and recycling of the protein considerably reduces the pollution load in the wastewater

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where significant economic and social benefits are concerned [15].

Protein powders such as sericin are produced through several techniques including desolvation, coacervation, emulsification and electrospray drying [20]. The other techniques with the aim of protein drying leading to formation of desirable powder form with appropriate characteristics are known as freeze-drying, spray drying and supercritical fluid (SCF) drying. Freeze-drying or lyophilisation is a batch drying method that is preferred to dehydrate proteins. Today, the utmost of dried protein products on the market are freeze-dried. However, freeze-drying is recognized as an expensive technology, because of requiring much energy and longtime of processing [21]. Spray drying is an economic continuous tri-step process involving atomization followed by solvent removal through evaporation and subsequent powder collection [22]. SCF drying is a relatively new drying method employed in the pharmaceutical field. Most experience is based on laboratory equipment. The method is based on anti-solvent properties of certain SCF for protein precipitation and water extraction from composition [23,24].

Cho *et al.* [25] reported the successful preparation of sericin nanoparticles conjugated by poly (ethylene glycol). Oh *et al.* [26] prepared sericin beads from ethanol-precipitated sericin using LiCl/DMSO as solvent. Genç *et al.* [27] produced sericin powder with a particle size in the range of 1–20 μm using spray drying. Optimized condition for preparation of sericin nano particles by nano suspension techniques and the produced sericin nanoparticles for cosmetic applications has been reported. Sericin nanoparticles have been prepared using water in silicone emulsification technique and $CaCl_2$ as a cross-link agent. Wu *et al.* [6] prepared sericin powder from silk industry wastewater using spray drying and freeze drying. The size of the powders was not in the nano scales. Agrawal *et al.* [5] precipitated pure sericin powder by urea adding to a silk industry wastewater. However, it is clear that urea has inappropriate impacts on environment.

The aim of the current study was to produce sericin powder in micro and nano scale through different procedures from a real degumming wastewater. Characterization of the obtained sericin powders have been done to evaluate a practical way to extract desirable sericin powder having proper applicable potential.

II. Experimental

A. Materials

Silk industry wastewater containing sericin was prepared from Kiashahr Silk Company, Rasht, Iran. All of the chemicals were analytical grade and was purchased from Merck Company. All other chemicals were reagent-grade products obtained commercially.

B. Structure Characterization

Fourier transform infrared spectroscopy (FTIR) spectrums were taken by KBr pellets at the wave number ranging from 400–4000 cm^{-1} using Nicolet 760 spectrophotometer. UV-vis spectroscopy was carried out with the help of Cintra 10 UV-vis double beam spectrophotometer to determine the absorption of sericin solution. 3 mL of (0.2% W/V) sericin solution in a quartz cell was placed in the sample holder to get the absorption spectrum from 200 nm to 400 nm wavelength to confirm the maximum absorption of sericin protein.

To characterize the shape and size of the obtained powder, SEM analyses were conducted using a high resolution field emission scanning electron microscope (FESEM) (HITACHI S-4160).

X-ray diffraction (XRD) analysis on the powder samples were carried out using UNISANTIS XMD-300 diffractometer equipped with Cu Kα radiation (λ = 1.54 Å) at 45 kV, 0.8 A. Scattering angles ranged from 10º to 80º with a scanning speed at measuring time of 2° min^{-1}.

Differential scanning calorimetry (DSC) measurements were done using DSC302 BAHR thermo analyze. The equipment was provided with a LAUDA proline rp855 cool accessory. The DSC curves achieved using sample mass of about 3 mg and heating rates of 5 ºC min^{-1}. Accurately weighed samples were placed into an aluminum pan and were sealed. Alumina was used as reference and the runs were made by heating the samples from 25 ºC up to 400 ºC.

C. Preparation of Sericin Powder

The procedures for the preparation of sericin powder consisted of the following ways. At the first, degumming wastewater was filtered and purified by centrifuging at 3000 rpm for 15 min to remove all insoluble residues. Then the wastewater was concentrated at about 70 ºC by vaporizing water content using rotary drum evaporator (IKA RV 05 basic). The concentrate was lyophilized by freeze dryer (CHRIST ALPHA 1-2 LD) at -45 ºC and pressure of 20 mbar for 8 h. In the next step, pure ethanol was gradually added to the sericin solution at constant stirring speed to reach a final ethanol concentration of 75% (v/v). The obtained mixture was then subjected to ultra-sonication and then kept at room temperature until sericin precipitated.

In ethanol-induced precipitation, sericin and ethanol both compete to create hydrogen bond with water molecules. Ethanol is fully miscible in water, whereas sericin is immiscible. Precipitation of sericin in water observed when the solution was left at room temperature for few minutes. Therefore, ethanol overcame this competition resulting in dehydration of sericin and increasing the protein–protein interactions. As a result, sericin precipitates by the addition of ethanol [28]. After decanting the ethanol in the supernatant, sericin precipitate was lyophilized by freeze drying to obtain a powder form product.

In a different extraction method, certain amount of urea was added to 100 ml of concentrated sericin solution to make the final concentration of urea to 8 M. The solution was agitated continuously at 60 ^{o}C for one hour. After cooling at room temperature, the solution was dialyzed using dialysis tubing (MWCO: 14 kDa) against distilled water for 48 h. The distilled water was changed every 3 h, to remove urea completely. The solution was then

subjected to lyophilizing by freeze dryer to acquire sericin in powder form.

In the final method, sericin powders obtained from the first step of the procedure were dissolved in distilled water to get 25% wt/v solution and spray dried by Büchi B-191 instrument.

III. Results and Discussions

A. UV-vis Absorption Study

The absorption of sericin solution was explored at a wavelength region of 200 nm to 400 nm using UV-vis spectrophotometer. The maximum absorption of extracted protein is at about 270 nm (Fig. 1) which is in accordance with the silk degumming wastewater and sericin protein. It is believed that peptide bonds are the major absorbing groups for sericin in the ultraviolet region [17].

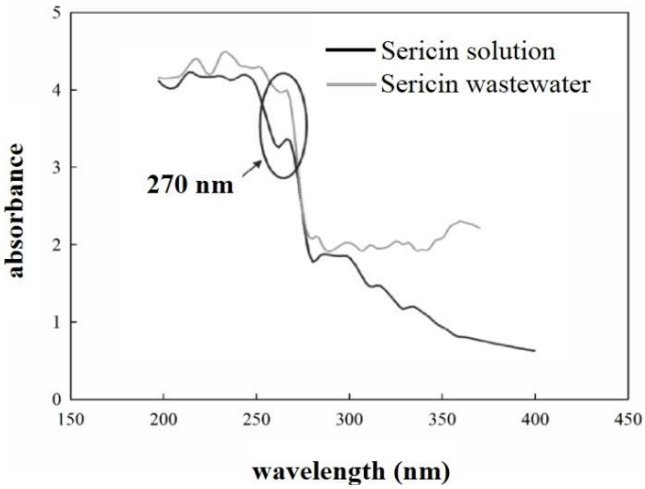

Fig 1. UV-vis spectra of sericin extracted from wastewater.

B. FTIR Analysis

FTIR spectra of all sericin powders obtained from different methods are shown in Fig. 2. All of the samples show a peak around 1650 cm^{-1} confirming the stretching vibration of the C=O bond of the amide I. The peak at 1580–1510 cm^{-1} confirms the presence of the in-plane N−H bending. N−H stretching and O−H stretching band are at 3450-3420 cm^{-1} peak and O−H stretching band overlaps with N−H stretching vibration peak at 3500-3000 cm^{-1}. C=O symmetry stretching is observed at about 1400 cm^{-1}. The FTIR peaks of the sericin powders prepared in this study are similar to those of sericin powder obtained by other researchers [15,29]. The protein conformation can be found by three distinguishable vibration peaks by identifying the peak positions of amide I, II and III [30]. Since the spectrum of single bands (each narrow band is characteristic for a secondary structure) is broadened, the bands overlap cannot be distinguished in the amide envelope. Consequently, the individual component bands that represent different structural elements, such as α-helices, β-sheets, turns and irregular structures are often not resolved and are difficult to identify in the broad amide band. Nevertheless, it is shown in Fig. 2 that the FTIR spectrum of sericin powder changed by addition of chemicals such as ethanol and urea in comparison to the other techniques. In particular, the different shape of peaks for amide I and amide II implies different secondary structure of the sericin protein. It can be corresponded to the fact that denaturation of proteins involves the disruption and possible destruction of both the secondary and tertiary structures. Since denaturation reactions are not strong enough to break the peptide bonds, the primary structure (sequence of amino acids) remains the same after a denaturation process. Denaturation may disrupt the normal alpha-helix and beta sheets in a protein and uncoils it into a random shape. Denaturation occurs because the bonding interactions responsible for the secondary structure (hydrogen bonds to amides) and tertiary structure are disrupted. In tertiary structure there

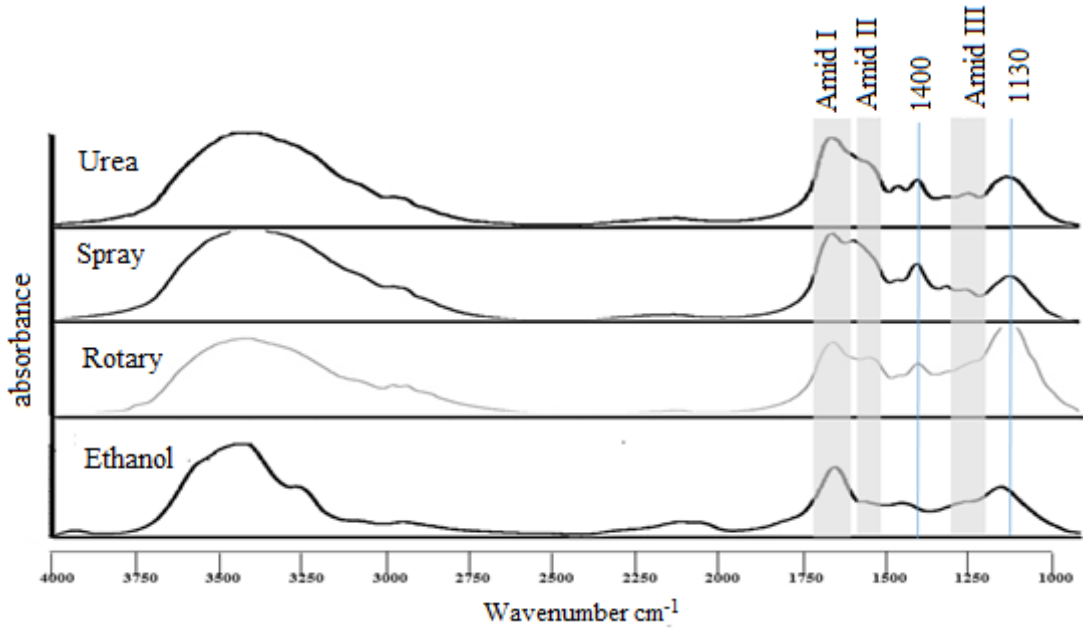

Fig 2. FTIR spectra of sericin powder extracted from wastewater obtained by different methods.

are four types of bonding interactions between "side chains" including: hydrogen bonding, salt bridges, disulfide bonds, and non-polar hydrophobic interactions which may be disrupted. Therefore, a variety of reagents and conditions can cause denaturation [31,32] . The most common observation in the denaturation process is the precipitation or coagulation of the protein which is the aim of recovery in the current study.

C. Surface Morphology

The surface morphology of the recovered sericin powder was examined under the field emission scanning electron microscope at different magnification (Fig. 3). Shape of the particles obtained by urea method, Fig. 3(a), are polygonal with the average size of 500 nm. The particles produced by spray drying, Fig. 3(b), are rather spherical; however, the average sizes of these fine particles are in the micron range (1.4 μm). Lyophilized sericin, Fig. 3(c), shows sponge-like structure with the average size region of 40-70 nm. It is seen in Fig. 3(d) that the sericin powder precipitated by ethanol, generally create an agglomerated structure possibly formed by the hydrophilic properties of the sericin. The sericin exposed to atmosphere picks up moisture immediately and gets agglomerated [15] which can be occurred for two reasons: Van der Waals forces in ultra-fine particles and granulation by high moisture content.

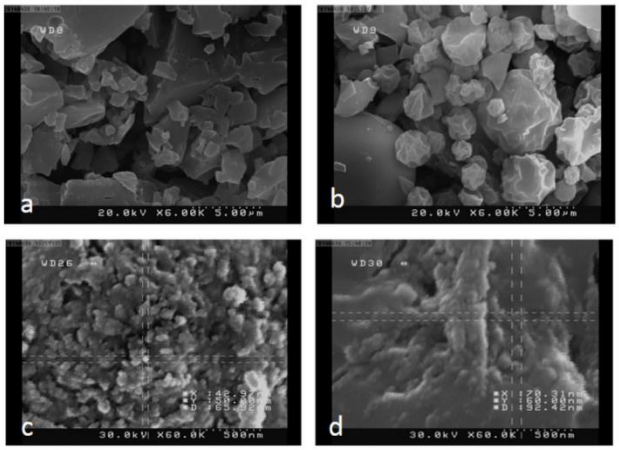

Fig 3. SEM images of sericin powder extracted from wastewater obtained by: a) urea, b) spray drying, c) rotary and d) ethanol method.

D. Thermal Properties

DSC patterns of sericin closely represent the molecular structure and physical properties including crystallinity and solubility [33]. DSC curves of different sericin powder samples are shown in Fig.4.

It is found that different method products have different degradation peaks. However, the exothermic degradation, T_D, of sericin powder obtained from spray drying technique appear in lower temperatures differ from degradation temperatures of samples obtained from other processes. The exothermic peak of sericin obtained by rotary method indicates thermal decomposition of sericin powder at around 370 ºC. The observed degradation temperatures , 360 ºC and 380 ºC for ethanol and urea methods respectively, declares that the use of chemicals during the extraction process influences the thermal stability of sericin [34]. In the urea method sample, the peak has shifted to 380 ºC signifying the enhanced thermal stability. On the other hand, the increased melting point proposes the requirement of higher amount of energy for breaking the bonds in comparison with unprocessed samples. This can be attributed to the strong intermolecular hydrogen bonding formation in serine and threonine residues of adjacent β-sheets in hydrated conditions. These oriented β-sheet aggregates, form the fibrous structure of sericin, which is somehow crystalline. Since melting occurs in crystalline regions, sericin with a higher melting point, which was obtained from ethanol precipitation, reveals increasing of crystallinity and enhanced β-sheets formation [35].

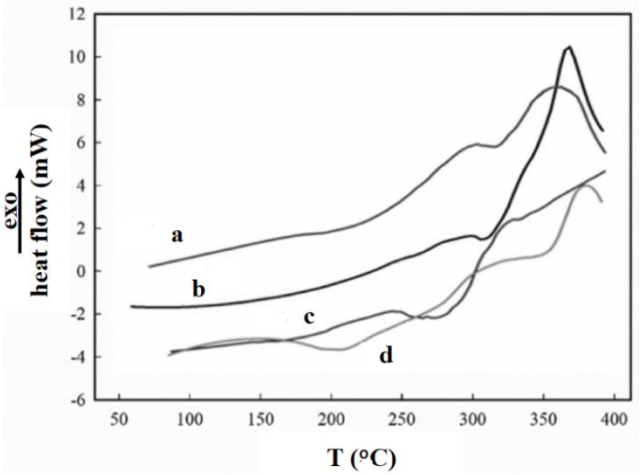

Fig 4. DSC thermograms of sericin powder extracted from wastewater obtained by: a) ethanol precipitation, b) rotary, c) spray drying and d) urea precipitation.

E. XRD Analysis

XRD curves of the produced sericin by different procedures are shown in Fig. 5. Although It has been reported in the literature that sericin is an amorphous macromolecule, it is known to have a characteristic XRD peak at 2θ=20° and a shoulder peak at near 2θ=30º [36]. These two peaks are present in all of the obtained samples but in slightly different forms, indicating a minor difference in the microstructure of sericin. On treatment by ethanol and urea, sericin powder showed an increased peak intensity at 2θ=19.8° and 29.8°. Researchers confirmed that sericin contains both random coils and β-sheets representing amorphous and crystalline regions, respectively. Treating by chemicals generate a transformation from random coil to β-sheet where the aggregated β-sheet structure attributes to intermolecular hydrogen bonding. This leads to an increase in the amount of crystallinity and showing of sharp peaks in the XRD curve [35]. Variations in crystal structure can be remarked during chemical precipitation of the current study. These transitions are indicated by the more intense and narrow bands for 2θ in the range of 20^o and 30^o (Fig. 5). Without chemical treatment, the patterns showed broader bands around the two mentioned diffraction angles.

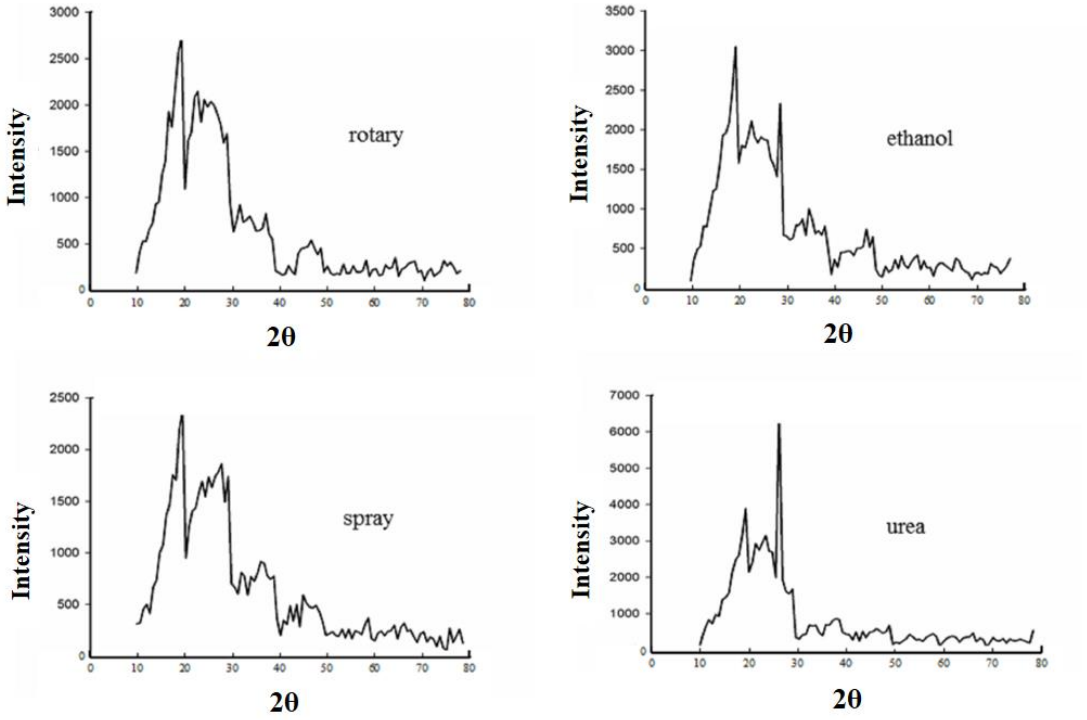

Fig 5. XRD patterns of sericin obtained by different methods.

The Scherrer equation [37] relates the size of crystallites in a solid to the broadening of a peak in a diffraction pattern. This was used in the determination of the size of particles of crystals in the form of sericin powders. The Scherrer equation can be written as:

$$t = \frac{k\lambda}{\beta Cos\theta} \quad (1)$$

where, t is the mean size of the crystallites; k is a dimensionless shape factor with a value close to unity. The shape factor has a typical value of about 0.9, but varies with the actual shape of the crystallite; λ is the X-ray wavelength; β is the line broadening at half the maximum intensity (FWHM) after subtracting the instrumental line broadening, in radians; θ is the Bragg angle (in degrees). The average size of crystallites for the prepared sericin powders were calculated, which is shown in Table I. These values give us an average crystal size; however, they say nothing about the crystal size distribution or spatial arrangement.

TABLE I
MEAN SIZE OF THE CRYSTALLITES OF THE PREPARED SERICIN POWDERS BY DIFFERENT METHODS

sericin sample (recovery method)	rotary		spray drying		ethanol		urea	
2θ (degree)	19.8	29.6	19.7	29.9	19.8	29.8	19.8	29.8
t (Å)	5.6	2.7	6.7	3.3	6.5	4.0	6.5	4.0

It can be seen that the crystallites are marginally longer on the planes regarding to scattering at 2θ=29.8°. As a result, it can be found that treating with chemicals such as alcohol and urea for recovery of sericin may cause an alteration in secondary structure of the protein due to the new arrangement of intermolecular hydrogen bonding. Also, this resulted in slightly proliferation in the amount of crystallinity which showed by sharper peaks of X-ray diffraction curve in the chemical treated samples. These results are consistent with the DSC results.

IV. CONCLUSION

The physiochemical and structural properties of sericin obtained by different procedures were studied using FTIR, SEM, XRD and DSC analysis. FTIR spectrum of sericin showed an absorption band at 1650 cm^{-1} (amide I), indicating random coil conformation. The FTIR results revealed that the extraction process of sericin didn't affect the chemical structure of sericin. But, among the extraction methods used here, extraction with urea and ethanol had some impact on sericin conformation. Thermal properties of the sericin produced by techniques of this research were interesting. The produced sericin showed high degradation temperature comparable to previous reported techniques. This thermal stability may be an added advantage to exploit it as a potential biomaterial. The thermal stability of sericin was influenced by the use of chemicals during the extraction process. XRD pattern spectra affirmed that the intermolecular bonding and conformation of sericin may be influenced by different extraction methods. In conclusion, according to the achievement of the current

research it is claimed that some uncomplicated techniques with the lowest range of used chemicals can be applied for the sericin recovery from the most precious silk industry wastewater.

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