การเตรียมโปรตีนไหมเซริซินและคุณลักษณะของผงไหม
Silk Protein Sericin Preparation and Its Characterization

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บทคัดย่อ

ในการวิจัยครั้งนี้มีวัตถุประสงค์เพื่อหาวิธีการสกัดเซริซินที่เหมาะสมจากรังไหมพันธุ์ UB1xUB5 เพื่อให้ได้ปริมาณผงไหมที่มากและมีขนาดอนุภาคเหมาะสำหรับการประยุกต์ใช้ในการเตรียมผลิตภัณฑ์เครื่องสำอางโดยเปรียบเทียบการสกัดเซริซินจากรังไหมด้วยการต้มในด่างและน้ำร้อน พบว่าการสกัดด้วยวิธีการต้มน้ำร้อนที่อุณภูมิ 100 องศาเซลเซียส นาน 9 ชั่วโมง ให้ปริมาณผงเซริซินมากที่สุดคือ 21.67% โดยน้ำหนัก มวลโมเลกุลของเซริซินที่วิเคราะห์ด้วย SDS-PAGE พบเป็นแถบกว้างอยู่ในช่วง 37 kDa ถึง 250 kDa ส่วนเซริซินที่สกัดโดยการต้มด้วยด่างและเซริซินที่ถูกไฮโดรไลท์ด้วย 1.0 N โซเดียมไฮดรอกไซด์ พบมวลโมเลกุลมีขนาดเล็กกว่า 37 kDa สำหรับการศึกษากระบวนการทำให้แห้งด้วยเทคนิคการทำแห้งแบบพ่นฝอย (Spray drying) ให้ปริมาณผง เซริซินน้อยกว่าเทคนิคการทำแห้งแบบเยือกแข็งภายใต้สภาวะสุญญากาศ (Lyophilization) แต่เทคนิคการทำแห้งแบบพ่นฝอยจะให้ผงเซริซินที่มีขนาดอนุภาคเล็กกว่าและมีขนาดค่อนข้างสม่ำเสมอ จากผลการวิเคราะห์ผงเซริซินด้วยเทคนิคทางสเปคโทรสโคปีพบว่า เซริซินทั้งหมดมี UV และ IR สเปคตรัมที่คล้ายกัน เนื่องจาก ผลหมู่ฟังก์ชันในกรดอะมิโนที่เป็นส่วนประกอบในโครงสร้างของเซริซินเป็นแบบเดียวกัน แต่ผลของลักษณะทางกายภาพผง เซริซินที่สกัดและทำให้แห้งด้วยวิธีที่แตกต่างกันจะให้รูปร่างของอนุภาคที่แตกต่างกันด้วย เมื่อส่องด้วยกล้องจุลทรรศน์แบบส่องกราด (Scanning electron microscope)

Abstract

The objective of this study was to optimize the appropriate method for the preparation of sericin powder in order to obtain the highest yield and a suitable particle size for cosmetic applications. We investigated the methods for sericin extraction from silkworm cocoon (UB1xUB5 strain) by alkaline and hot water degumming. The highest yield of sericin powder was 21.67 % w/w by hot water degumming, at 100°C for 9 hrs with the molecular weight range observed being between 37 kDa to 250 kDa by SDS-PAGE. For alkaline degumming and hydrolyzed sericin by 1.0 N NaOH, we observed the molecular weight to be less than 37 kDa. In the drying process, spray drying yielded less than lyophilization but provided more homogeneous and smaller sericin powder than lyophilization. All sericin powder from the different extraction methods

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showed similar physical properties on UV and IR spectra due to the same functional groups on their amino acids. However, the morphology of sericin powder obtained from different extraction methods and different drying processes showed different shapes by SEM.

คำสำคัญ: โปรตีนไหม, เซริซิน, คุณลักษณะของผงไหม
Keywords: silk protein, sericin, characterization

Introduction

In nature, silk fiber is composed of two proteins, silk fibroin and silk sericin, which are produced by the silkworm (*Bombyx mori*). Sericin envelops the fibroin fiber with successive sticky layers that help in the formation of a cocoon. Sericin constituent is about 20-30 % of the total cocoon weight and it is a macromolecular protein which has molecular weight ranging widely from about 10 to over 310 kDa (Wu et al., 2006). Sericin consists of 18 amino acids, most of which have strongly polar side groups such as hydroxyl, carboxyl, and amino groups (Sarovart et al., 2003). Sericin is also especially rich in aspartic acid (~19%) as well as serine (~32%) (Wu et al., 2006). Sericin is active as an antibacterial (Sarovart et al., 2003), antioxidant, tyrosinase inhibitor (Kato et al., 1998; Wu et al., 2007; Zhang, 2002), UV protector (Zhaorigetu et al., 2003) and skin moisturizer (Padamwa et al., 2005). Natural sericin can be recovered from the silk cocoon by various degumming processes such as hot water degumming.

Sericin was extracted from cocoons by boiling in water at 95°C for 120 min (Kato et al., 1998). The other degumming methods include: extraction by water under pressure with autoclave at 110-120 °C for different periods of time (Cho et al., 2003; Lee et al., 2003; Yamada et al., 2001; Zhang, 2002; Zhang et al., 2004); alkaline or soap degumming in which sericin was extracted by 1.0 M NaOH solution, 0.02 M Na2CO3 or Marseille soap (Yamada et al., 2001; Zhang et al., 2006; Dash et al., 2008; Ki et al., 2007; Sargunamani et al., 2006); acid degumming in which silk cocoons are boiled in acid solution such as citric, tartaric or succinic acid for 30 min (Kurioka et al., 2004); and enzyme degumming in which raw silks were immersed in buffer solution containing different amounts of enzyme and then inactivate proteases at 85 °C for 10 min (Freddi et al., 2003). After extraction, the sericin powder could be obtained by lyophilization or spray drying.

In the process of degumming, sericin can be degraded or hydrolyzed into a smaller protein or peptide to obtain the low molecular weight sericin of less than 20 kDa. The low molecular weight sericin can be dissolved in water and is useful for cosmetic applications including skin care and hair care products, health products and medications. (Zhang, 2002). Sericin which has high molecular weight between 20 and 310 kDa can be dissolved in boiling water but it is poorly soluble in cold water (Zhang, et al., 2004). The high molecular weight sericin is mostly used as a cell growth promoter, for its antibacterial or moisture-absorbing and desorbing properties in medical biomaterials, as a degradable biomaterial, as a compound polymer, in functional biomembranes, hydrogels and functional fibers and fabrics (Zhang, 2002).

In this research, we intended to optimize the appropriate method for the preparation of sericin

powder from the silkworm cocoons (UB1xUB5 strain) in order to obtain the highest yield and a suitable particle size. The method of extraction such as hot water or alkaline degumming and the method of drying such as lyophilization or spray drying were investigated. The molecular weight, characteristics of sericin powder such as UV and IR absorption, particle size and morphology of sericin powder by Scanning Electron Microscope (SEM) were also studied and are reported in this paper.

Materials and Method

1. Sample, chemicals and equipments

The silkworm cocoon used in this experiment was *Bombyx mori,* UB1xUB5 strain, which was obtained from the Queen Sirikit Sericulture Center, Khon Kaen Province, Thailand. Dialysis tube for desalting of sericin solution was 1200-1400 molecular cut off cellulose tubing (Cellu Sep.). Freeze dryer (Flexi-Dry™ μP) and spray dryer (niro A/S-Gladsaxevej, Denmark) were used for the drying process. SDS-PAGE analysis (Bio-RAD, Mini-PROTEAN 3 Cell) was used for molecular weight analysis. UV-VIS spectrophotometer (UV-1700, Shimadzu) and IR spectrophotometer (Perkin Elmer Spectrum One FTIR) were used for spectrophotometric analysis. Particle size was measured by Mastersizer 2000, Malvern. The morphological study was done by scanning electron microscope (SEM), Hitachi S-3000N.

2. Preparation of sericin powder

For alkaline degumming (adapted from Yamada et al., 2001), silk cocoons were cut into small pieces and then boiled in aqueous solution of 0.05% Na_2CO_3 (1g of cocoons per 30 ml of water) at a temperature of 100 °C for 60 min, repeated twice. Then the filtrate was concentrated by rotary evaporator and adjusted to pH 7.0. The residue was dialyzed in water for 3 days. Sericin powder was obtained from lyophilization. For hot water degumming (adapted from Yamada et al., 2001 and Zhang et al., 2004), silk cocoons were immersed in water (1g cocoon per 30 ml of water) overnight and then boiled at 100°C for 1 hr, repeated twice. The filtrate was lyophilized to obtain sericin powder. Other boiling periods of 1.5, 3.0, 6.0 and 9.0 hr, were also studied. The effects of the drying process, such as lyophilization and spray drying, was investigated from sericin powder obtained by hot water degumming at 100°C for 9 hr.

To prepare hydrolyzed sericin (adapted from Zhang et al., 2006), The silk cocoons were immersed in water (1g of cocoons per 30 ml of water) overnight and then boiled at 100°C for 6 hr, repeated twice. The filtrate was adjusted to pH 10.0 by 1.0 N NaOH solution and then boiled for 2.0, 4.0 and 6.0 hr. The residue was neutralized by 1.0 N HCl solution and then centrifuged at 5000 rpm for 30 min, repeated twice. The supernatant was lyophilized to provide hydrolyzed sericin powder.

3. Characterization study of sericin powder

The molecular weight distribution of silk sericin was determined by SDS-PAGE with 12.0% acrylamide gel and 4.0% condensing gel. The proteins were stained with Coomasie Blue R250. The aqueous solution of sericin (0.25 mg/ml) was measured for maximum absorption by UV-VIS spectrophotometer and sericin powder was measured by IR spectrophotometer. The particle sizes of sericin powders and their morphology were analyzed by Mastersizer 2000 and SEM, respectively.

Results and Discussion

1. Yields of sericin powder

By alkaline degumming, 12.13% by weight of fine yellowish powder was obtained from silkworm UB1xUB5 strain. From hot water degumming at 100°C, for 1.5, 3.0, 6.0 and 9.0 hr, the yields were 9.98%, 13.58%, 18.89% and 21.67% by weight, respectively (Figure 1). The yield of sericin powder increased according to the period of boiling. The long period of hot water degumming might cause contamination by fibroin so hot water degumming at 100 °C, for 9.0 hr gave the highest yield of sericin powder.

During the drying process, lyophilization and spray drying were used and we could obtain sericin powder at 20.28% and 13.17% by weight, respectively (Figure 2). The spray drying process yielded less than lyophilization because we lost some powder in the spray drying container. The spray drying process can provide smaller particle size (5.81 μm on average) and more homogeneously round particles than lyophilization. In addition, the spray drying process takes only a few hours while lyophilization takes more than overnight to obtain dry sericin powder.

2. Characterization study of sericin powder

By alkaline degumming, the molecular weight of sericin from silkworm UB1xUB5 strain was less than 37 kDa on SDS-PAGE gel (Figure 3, Lane 1). On the other hand by hot water degumming, sericin powder from silkworm cocoons (UB1xUB5 strain) was between 37 kDa to 250 kDa (Figure 3, Lanes 2-5) on SDS-PAGE gel obtained from hot water degumming for 6 hours and further hydrolyzed by 1.0 N NaOH. The molecular weight of hydrolyzed Sericin was less than 37 kDa on SDS-PAGE gel (Figure 3, Lanes 6-8).

Sericin contains high serine content at about 33.4% and aspartic acid content of about 16.7%. Most of the amino acids in sericin have strongly polar side groups such as hydroxyl, carboxyl and amino groups. From UV-VIS spectrometry in Figure 4, two maximal absorption wavelengths were observed from all peptide bonds and aromatic acids at 206 nm and 275 nm, respectively.

IR spectroscopy was also performed to determine the conformation index of silk protein. Protein conformation was determined by identifying the peak positions of amide I and II corresponding to C=O and N-H (Ki et al., 2007). The IR spectra of sericin obtained from silkworm cocoons (UB1xUB5 strain) by various methods are shown in Figure 5, i.e. hot water degumming with spray drying (Figure 5A), hot water degumming with lyophilization (Figure 5B), hydrolyzed sericin by 1.0 N NaOH after hot water degumming with lyophilization (Figure 5C) and alkaline degumming with lyophilization (Figure 5D). All these IR spectra showed the same pattern of N-H stretching band at 3316 $cm^{-1}$and peak at 1541 cm^{-1}, C=O stretching peak at 1654 cm^{-1} and C=O symmetry stretching peak at 1400 cm^{-1}. These peaks were those of β-sheet and random coil, amide I, and amide II conformations, respectively (Lamoolphak et al., 2008). All IR spectra confirmed that all sericin obtained from the different extraction methods were the same compound and had the same conformation of sericin.

For particle size analysis, sericin obtained from alkaline degumming, hot water degumming by spray drying, lyophilization and hydrolyzed sericin by 1.0 N NaOH showed average sizes of about 0.268 μm, 2.670 μm 28.860 μm and 5.610 μm,

respectively. The results show that spray drying provides a smaller particle size of sericin than lyophilization.

From Figure 6, SEM photographs show the different morphologic patterns of sericin powder obtained from the different preparation methods such as lumpish or agglomerate solid obtained from alkaline degumming with lyophylization (Figure 6a) and hydrolyzed sericin (Figure 6d), the spherical shape obtained from hot water degumming with spray drying (Figure 6c) and the tabular shape obtained from hot water degumming with lyophilization (Figure 6b). Differences in morphologic pattern of sericin may depend on the different drying process, either lyophylization or spray drying.

Conclusion

We could obtain sericin powder from silkworm UB1xUB5 strain cocoons, at 12.13% (w/w) by alkaline degumming and 21.67% (w/w) from hot water degumming at 100°C for 9 hours. From the experiment, the yield of hot water degumming was similar to hot water degumming under autoclave at 110°c for 30 min by Kurioka et al. (2004) which provided 21.0 %w/w of sericin. For the other degumming methods, alkaline degumming gave 25.7%w/w of sericin, acid degumming with boiling in citric acid gave 27.8%w/w of sericin (Kurioka et al., 2004) and urea degumming by Dash et al. (2006) gave 5%w/w of sericin. Sargunamani and Selvakumar (2006) found that sericin contents were 25.14% and 22.8% from enzyme degumming and soap degumming, respectively.

For a comparison of drying processes, spray drying gave less yield of sericin powder than lyophilization, however the spray drying provided the smaller and more homogeneous particles of sericin. The SDS-PAGE gel showed a wide range of sericin molecular weights and that was due to protein fragmentation during preparation steps. All preparation methods for sericin powder gave similar UV and IR spectra because all sericin powders contain similar functional groups on their amino acids. The morphology of sericin powder obtained by SEM showed different characteristics depending on the different preparation methods. However, the properties of sericin powder such as the particle shape, the particle size and its physical properties should be considered in order to prepare the appropriate raw material for cosmetic or other applications. The other methods for sericin powder and the other studies on physical properties will be further performed in order to find the optimal sericin product for cosmetic and other applications in the near future.

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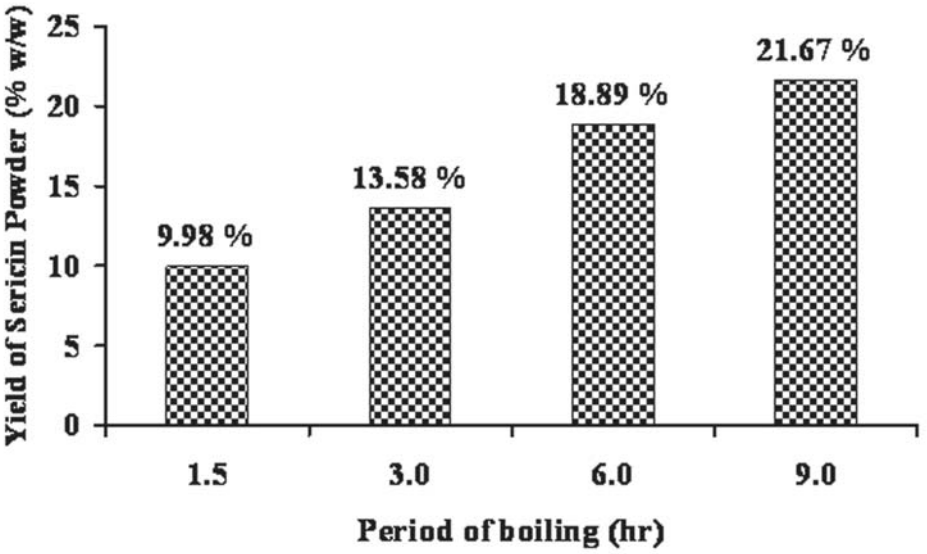

Figure 1. Yield of sericin powder (%w/w) obtained from silkworm cocoons (UB1xUB5 strain) by hot water degumming for 1.5, 3.0, 6.0 and 9.0 hr.

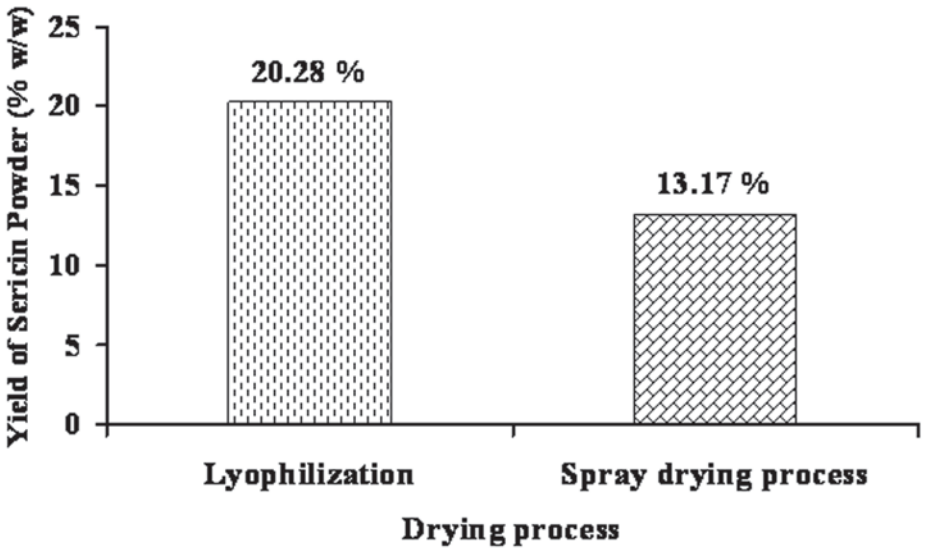

Figure 2. Yield of sericin powder (%w/w) obtained from silkworm cocoons (UB1xUB5 strain) by lyophilization and spray drying.

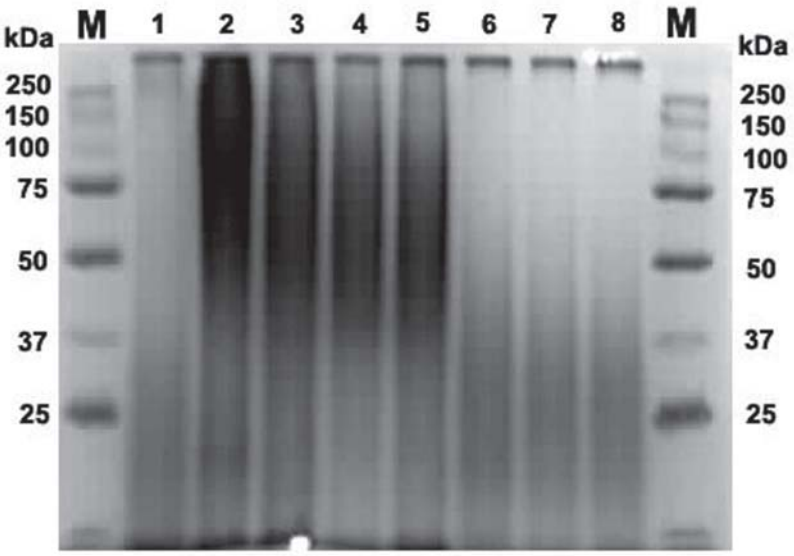

Figure 3. SDS-PAGE of sericin obtained from silkworm cocoons (UB1xUB5 strain) by alkaline degumming (Lane 1), hot water degumming for 1.5 (Lane 2), 3.0 (Lane 3), 6.0 (Lane 4) and 9.0 (Lane 5) hr and sericin after hydrolysis by 1.0 N NaOH for 2.0 (Lane 6), 4.0 (Lane 7) and 6.0 (Lane 8) hr.

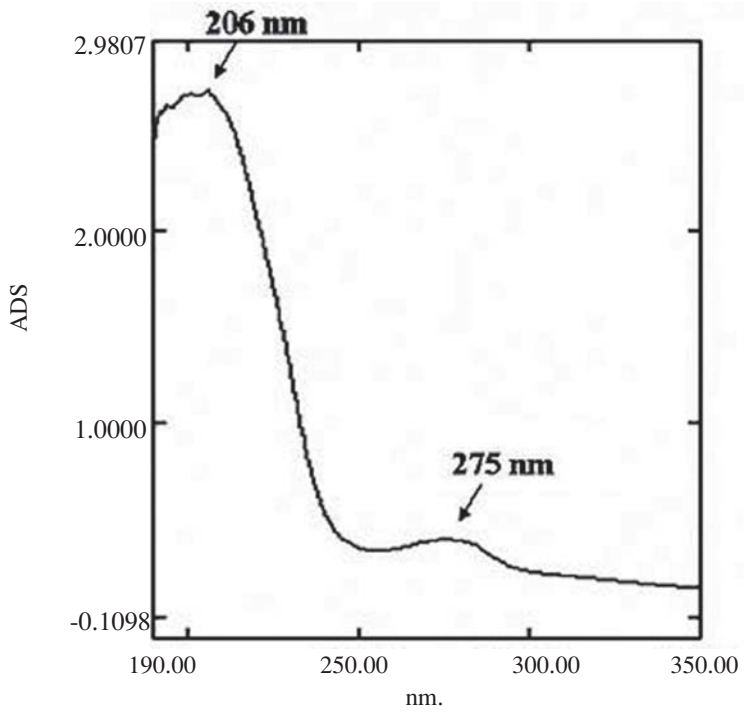

Figure 4. UV absorption spectrum of sericin solution.

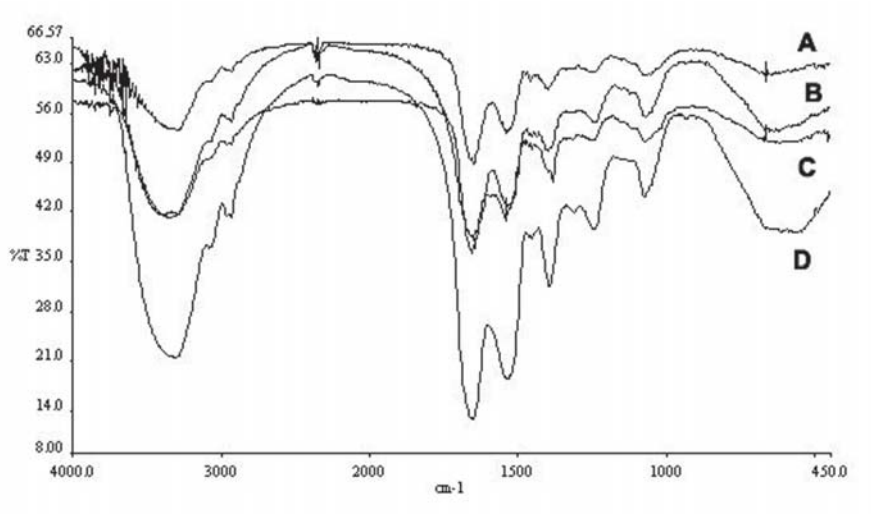

Figure 5. IR Spectrum of sericin powder obtained from silkworm cocoons, UB1xUB5 strain by hot water degumming with spray drying (A) and with lyophilization (B), hydrolyzed sericin by 1.0 N NaOH (C) and alkaline degumming sericin (D).

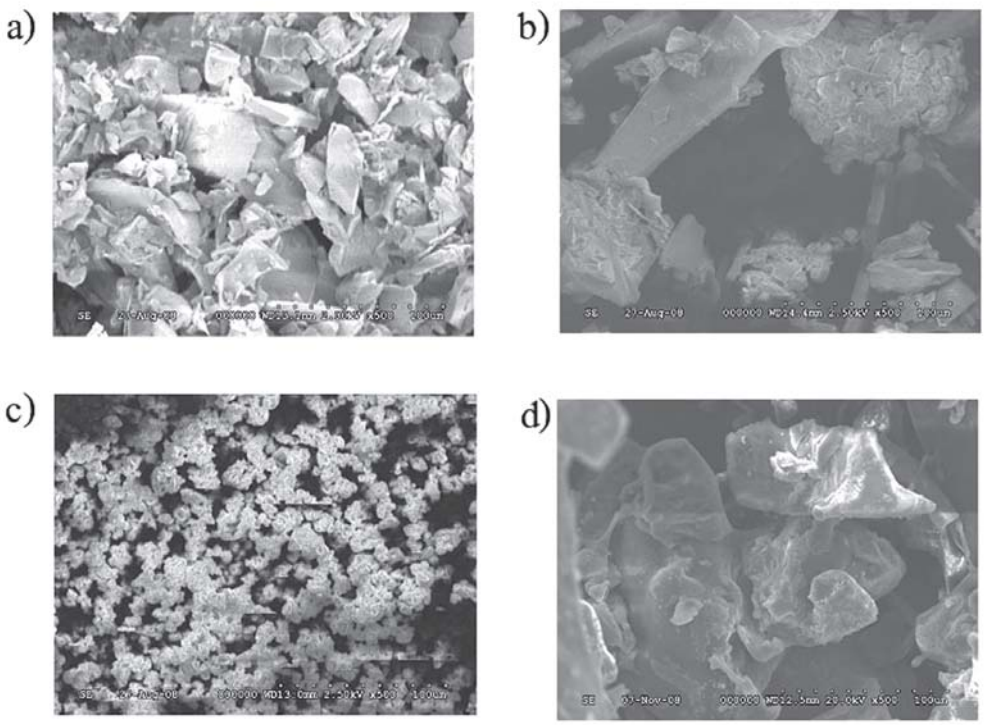

Figure. 6 SEM photographs of sericin powder obtained from silkworm cocoons by alkaline degumming with lyophylization (a), hot water degumming with lyophilization (b) and spray drying (c) and hydrolyzed sericin by 1.0 N NaOH (d).