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Preparation and characterization of sericin powder extracted from silk industry wastewater

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Abstract

In this study, we developed a new effective technology for the extraction of sericin from silk wastewater. Sericin was extracted with 75% (v/v) ethanol to obtain crude powder. The chemical composition of sericin powder, including protein, sugar, ash, and amino acid, was assayed in detail. The molecular weight distribution of sericin was also investigated by gel filtration chromatography, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), and high performance liquid chromatography (HPLC) analytical methods. The results suggested that sericin represented a family of proteins with wide-ranging molecular weight distribution. The conformation changed in the course of ethanol precipitation was studied by circular dichroism (CD) analysis. Data implied that the major conformation of sericin protein was random coil, which decreased slightly after being treated with ethanol, and the appearance of β-Turn conformation maybe associated with the packing of molecular chains induced by ethanol. Furthermore, sericin was found to inhibit tyrosinase activity when chlorogenic acid was used as a substrate, and had obvious radical scavenging effects with the 2.2-diphenyl-1-picryl-hydrazil (DPPH) assay. Result suggested that sericin might be a valuable ingredient for food.

Keywords: Sericin; Molecular weight; Circular dichroism; Tyrosinase; Radical scavenging activity; IC_{50}

1. Introduction

Silk sericin is a kind of water-soluble globular protein derived from silkworm *Bombyx mori*, and represents a family of proteins whose molecular mass ranges from 10 to 310 kDa (Wei, Li, & Xie, 2005). Sericin envelops the fibroin fiber with successive sticky layers that help in the formation of a cocoon, constitutes about 20–30% of the total cocoon weight (Masahiro, Hideyuki, & Norihisa, 2000). Sericin consists of 18 kinds of amino acids most of which have strong polar side groups such as hydroxyl, carboxyl, and amino groups (Wei et al., 2005). Sericin is especially rich in aspartic acid (~19%) as well as serine (~32%) (Kwang et al., 2003), which has a high content of the hydroxyl group. The chemical characterization of sericin influences its many physical and biological properties.

To date, sericin has been found to possess various biological functions. Masahiro et al. (2000) reported that consumption of sericin enhances bioavailability of Zn, Fe, Mg and Ca in rats, and suggested that sericin is a valuable natural ingredient for the food industry. Kato et al. (1998) found that sericin could suppress lipid peroxidation, inhibit tyrosinase (polyphenol oxidase) activity *in vitro* and contributes its antioxidant activities to hydroxyl group chelation with trace elements such as copper and iron. The result implied that sericin is a valuable ingredient for cosmetics because it can inhibit tyrosinase activity and this enzyme is responsible for biosynthesis of skin melanin. In addition, Siqin, Noriyuki, Masahiro, Hiromitsu, and Norihisa (2003) found that sericin exerts inhibitory activity on ultraviolet radiation induced acute damage and tumor promotion by

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reducing oxidative stress in the skin of hairless mouse. Masakazu, Kazuhisa, Hideyuki, Hiroshi, and Shigeru (2003) found that sericin possesses the biological activity of preventing cell death and promoting cellular growth after acute serum deprivation. Moreover, sericin has been shown to be useful as a degradable biomaterial, biomedical material and polymers for forming articles, functional membranes, fibers and fabrics (Zhang, 2002). Thus, because of its properties, sericin can be used in food, cosmetics and pharmaceutical products as well as for biomaterials manufacture. So, sericin is considered in our study as a new kind of valuable protein source. Processing of raw silk cocoon produces about 50,000 tons of sericin worldwide each year, however sericin is mostly discarded in silk processing wastewater (Zhang, 2002), and results in a high COD (Chemical Oxygen Demand) level of the degumming wastewater (Fabiani, Pizzichini, Spadoni, & Zeddita, 1996). So, the wastewater released by silk industry easily leads to environmental contamination. Therefore, if this protein by-product is recovered and recycled, it can represent a significant economic and social benefit.

In the present study, in order to recover it from silk industry wastewater and further apply to food production, we focused on investigating the physiochemical, structural, and biological properties of sericin as well as investigating a practical and effective way to extract sericin from wastewater.

2. Materials and methods

2.1. Materials

Silk industry wastewater material containing sericin produced by exploding cocoon shells with high temperature and pressure degumming technique, was obtained from Si-Juan Silk Ltd. (Jiang Su Province, China).

All other chemicals were reagent-grade products obtained commercially.

2.2. Preparation of sericin powder

The degumming wastewater was concentrated at about 40 °C by vaporizing water under steam heating, and recycling the condensate three times. The concentrate was spray-dried as flours. Next, the flours were dissolved in distilled water at the ratio of 1:10 (w/v), and the solution obtained was chilled close to 4 °C. Pure ethanol chilled to −18 °C previously was slowly added to sericin solution with constant stirring to obtain a final ethanol concentration of 75% (v/v). The obtained mixture was then kept at −18 °C overnight. Sericin powder was obtained by centrifuging the sericin solution at 3500 rpm for 20 min, followed by alcohol evaporation under reduced pressure (reached 0.1 MPa) at 40 °C and lyophilized by freeze drying. Sericin extraction yield was calculated by weight method, as shown in the following formula:

$$Y_{\text{Extraction}}\ (\%) = \left(\frac{W_{\text{sericin}}}{W_{\text{flour}}}\right) \times 100, \quad (1)$$

where $Y_{\text{Extration}}$ refers to extraction yield, W_{sericin} refers to sericin powder dry weight, W_{flour} refers to spray-dried flour dry weight.

2.3. Analytical methods

Nitrogen content of sericin powder was determined by the Kjeldahl method (AACC, 1990) and converted to protein content by a factor of 6.25. Total sugar content was determined according to Dubois, Gilles, Hamilton, Rebers, and Smith (1956). Ash content was determined according to AACC Method (AACC, 1990). Nitrogen solubility index (NSI) was determined according to Adler-Nissen (1986) with a slight modification. Four percent spray-dried flour dispersion in distilled water was stirred at 25 °C for 30 min, followed by centrifugation at 3500 rpm/min for 10 min. The supernatant was analyzed for soluble nitrogen content by the Kjeldahl procedure, and NSI was calculated according to Eq. (2)

$$\text{NSI}\ (\%) = \left(\frac{N_{\text{soluble}}}{N_{\text{total}}}\right) \times 100, \quad (2)$$

where NSI is the nitrogen solubility index, N_{soluble} is the soluble nitrogen, and N_{total} is the total nitrogen.

2.4. Quantification of amino acid

Sericin powder was hydrolyzed with 6 M hydrochloric acid at 110 °C for 24 h under vacuum. Tryptophan was determined following alkaline hydrolysis. The hydrolysate was submitted to automated online derivatisation with *o*-phthalaldehyde and reversed phase high performance liquid chromatography (RP-HPLC) analysis in Agilent 1100 (Agilent Technology, Palo Alto, CA, UAS) assembly system using a Zorbax 80A C_{18}column (4.6 i.d. × 180 mm), running at 0.5 ml/min. The results acquired were analyzed with the aid of ChemStation for LC 3 D software (Agilent Technology, Palo Alto, CA, USA).

2.5. Gel filtration on Sepharose CL-6B column

Sericin powder solution (20 mg/ml) was dialyzed against 20 mM, pH 7.0 sodium phosphate buffer solutions and centrifuged at 10,000 rpm for 10 min. Five milliliters of the supernatant was loaded onto a Sepharose CL-6B (1.6 id × 100 cm, Amersham Pharmacia Biotech, Sweden) column. The column was equilibrated and eluted with 20 mM, pH 7.0 sodium phosphate buffer solutions at a flow rate of 0.3 ml/min. Fractions (4.5 ml each) were collected throughout the elution, and each fraction was analyzed for its absorbance at a wavelength of 220 nm, as well as being diluted when absorbance value over 2.000. The materials employed for the calibration of Sepharose CL-6B column were Blue Dextran 2000 (2,000,000 Da), Ferritin

(440,000 Da) and Aldolase (158,000 Da) while absorbance was monitored at 280 nm. With the help of elution volumes of the calibration materials, the following linear regression equation (3) was obtained for the calculation of molecular weight (MW)

$$\text{Log MW} = 1.2841\,K_{av} + 6.3576 \quad (R^2 = 0.9647), \tag{3}$$

where K_{av} = (elution volume − outer volume)/(total column volume − outer volume).

2.6. SDS–PAGE analysis

The SDS–PAGE analysis of sericin was performed according to Laemmli (1970). The resolving gel was 7.5% while the stacking and casting gels were 12.5% and 4%, respectively. The system was run at 100 V using a Dual Mini Slab Kit (Bio-RAD, Mini-PROTEAN 3 Cell). Gels were stained with silver stain. Standard proteins consisting of phosphorylase B (97 kDa), bovine serum albumin (66 kDa), ovalbumin (43 kDa), carbonic anhydrase (31 kDa), soy trypsin inhibitor (22 kDa), and lysozyme (14 kDa), were used as molecular weight markers (Low Molecular Weight Marker, Shanghai Institute of Biochemistry, China).

2.7. High performance liquid chromatography (HPLC) analysis

Sericin powder was dissolved in a mobile phase containing 45% acetonitrile and 0.1% trifluoroacetic acid (TFA) in HPLC grade water and centrifuged at 10,000 rpm for 10 min. The supernatant obtained was analyzed for low molecular weight distribution by using a Waters™ 650E Advanced Protein Purification System (Waters Corporation, Milford, MA, USA) assembly running 10 μl sample at 0.5 ml/min. TSK gel 2000 SWXL (7.8 i.d. × 300 mm) column regulated at 30 °C. A calibration curve was obtained with bovine carbonic anhydrase (29,000 Da), horse heart cytochrome C (12,500 Da), aprotinin (6500 Da), bacitracin (1450 Da), gly-gly-tyr-arg (451 Da) and gly-gly-gly (189 Da). With the help of elution time of calibration materials, the linear regression equation (4) was obtained for the calculation of molecular weight (MW)

$$\text{Log MW} = -0.0242T + 7.27 \quad (R^2 = 0.9905), \tag{4}$$

where T is elution time.

The results were processed with Millennium^{32} Version 3.05 Copyright©1998 (Waters Corporation, Milford, MA, USA).

2.8. Circular dichroism (CD) spectroscopy

Circular dichroism measurements were made in a Jasco J-715 spectropolarimeter (Jasco Inc., Japan) using quartz cuvette, thermostated at 25 °C. Spectra were measured from 250 to 185 nm, with five scans at 100 nm/min, response time constant was 0.25 s, bandwidth was 1 nm, and slit width was 500 μm. Two hundred microliter aliquots of each protein were added to 1 cm pathlength cuvette. Spectra were background corrected, smoothed, and data were expressed in terms of $[\theta]$ using analysis function built into the Jasco software.

2.9. Measurement of inhibitory effect on tyrosinase activity

The inhibitory effect of sericin on tyrosinase activity was assayed by a colorimetric method described by Selim, Mireille, and Alan (1993) and Oda et al. (1989) with a slight modification. The enzyme reaction mixtures had final volumes of 3 ml containing 0.2 ml of 167units/ml mushroom tyrosinase (EC 1.14.18.1, Sigma, USA) solution, 2.3 ml of 5.0 mM chlorogenic acid solution, and with or without 8% (w/v) sericin solution dissolved in pH 7.0, 20 mM sodium phosphate buffer. The enzyme reaction was initiated by the addition of the tyrosinase to the temperature-equilibrated reaction mixture at 25 °C for 10 min, and followed assay enzyme activity was measured by the increase in absorbance at 420 nm using UV-1100 spectrophotometer (Beijing RuiLi analytic apparatus Co., China). One unit of the enzyme activity was defined as the amount of enzyme that caused a change in absorbance of 0.001 per min (OD/min).

2.10. Measurement of free radical scavenging activity (DPPH assay)

The free radical scavenging activity using the 2.2-diphenyl-1-picryl-hydrazil (DPPH) reagent was measured by a method described by Amarowicz, Pegg, Rahimi-Moghaddam, Barl, and Weil (2004) with a slight modification. To 0.5 ml of sericin dissolved in distilled water at gradually increasing concentrations of 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml, 60 mg/ml, 3.5 ml of freshly prepared DPPH radical in a methanol solution (1.0×10^{-4} mol/l) was added and vortexed. After reaction for 25 min at room temperature (25 °C) in the dark, reaction mixtures were centrifuged at 6400 rpm for 5 min. The decolourizing result of the supernatant was assayed at 517 nm and compared with a blank control containing the sericin solution and pure methanol instead of DPPH. In addition, a blind control containing DPPH and distilled water instead of sericin solution was also assayed. Eq. (5) was used for the calculation of free radical scavenging activity

$$\text{Scavenging activity } (\%) = \left(1 - \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{blind}}}\right) \times 100, \tag{5}$$

where scavenging activity refers to the free radical scavenging percentages, $\text{OD}_{\text{sample}}$ refers to the absorbance of the sample, OD_{blind} refers to the absorbance of the blind control.

2.11. Statistical treatment of data

All the data were treated for significance by the One-Way analysis of variance (ANOVA) at $p < 0.05$ with the

aid of SPSS 13.0 for Windows® software (SPSS Inc. Chicago, IL, USA). Post-hoc analysis for significance was done using Tukey's Honestly Significant Difference (HSD) test. All the curves were fitted with Microsoft® Office Excel (Copyright© 1985–2003 Microsoft Corporation, USA) and the y-axis error bars shown at $p < 0.05$.

3. Results and discussion

3.1. Extraction yield of sericin by ethanol precipitation

Natural silk is composed of two kinds of proteins, namely the crystalline fibroin (inside the silk thread) and the amorphous sericin (as a tube outside the thread) (Fabiani et al., 1996). The degumming process is used to eliminate the external sericin prior to dyeing in the silk industry. In this study, cocoon shells were swelled with water and exploded under high temperature and pressure, thus external sericin was easily removed from fibroin. Because fibroin is a water-insoluble fibrous protein, while sericin is a water-soluble globular protein, we determined the nitrogen solubility index (NSI) to character the protein solubility of spray-dried flour. The NSI of the spray-dried flour was found to be $95.3 \pm 1.28\%$ ($n = 3$). This data denoted that the protein composition of the spray-dried flour recovered from the silk industry wastewater was sericin, accounting for about 95%, with little or no contamination by fibroin. Next the spray-dried flour was used as materials to recovering pure sericin by removing the non-protein components.

Silk sericin is a kind of water-soluble natural protein (Wei et al., 2005), maintained in solution by surface hydrophilic groups with the water solvent. So, adding ethanol that is less polar than water could reduce the solvent polarity and sericin tended to become less soluble, thus we could separate sericin from silk wastewater by centrifugation. In addition, to minimise denaturation of the protein in ethanol, the precipitation was conducted at a chilled temperature below 4 °C. In this way we developed a novel and easy approach for preparation of sericin powder from silk industry wastewater. The results about the extraction yield with different concentration ethanol were shown in Table 1.

Table 1
Extraction yield for sericin precipitated by different ethanol concentrations

Ethanol concentration (%) (v/v)[A]	Extration yield (%) (w/w)[B]
30	46.1 ± 0.4^{a}
50	$52.1 \pm 2.8^{a,b}$
70	$61.2 \pm 1.9^{b,c}$
75	$63.6 \pm 2.6^{b,c}$
90	71.3 ± 1.6^{c}

[a–c] Values with the same letter superscript denote no significant difference ($p < 0.05$).
[A] Values expressed as ratio percent of ethanol volume vs. total mixture volume.
[B] Values expressed as ratio percent of sericin powder weight vs. spray dried flour weight (mean of three replicates ± standard deviation).

It can be seen from Table 1 that the yield was gradually enhanced with the increase of ethanol concentration, and extraction yield reached 71% when using 90% ethanol. However, economic considerations being a major factor in industrial extraction, the concentration of ethanol used in this study was 70–80%. We found that 75% ethanol concentration was preferable to 90%, and whereby the yield recovery for sericin powder with 75% ethanol reached 64%.

3.2. Chemical composition of sericin powder

The chemical composition of sericin powder was presented in Table 2. It can be seen from these results that the main composition of sericin powder was protein, with a concentration above 90%. Sugar concentration only accounted for 0.9% although Hyogo and Yoshiko (1967) reported that sericin is a glycoprotein containing glucosamine, galactosamine, mannose and galactose. Ash concentration accounting for 4.2% suggested sericin powder from wastewater possibly contained a little salt.

The amino acid composition of sericin is given in Table 3, and the results show that it contained a high serine content accounting for about 27.3% of the 18 kinds of amino acids. Serine has strongly polar hydroxyl groups, and is possibly related to the functional and physiochemical properties of sericin (Masahiro et al., 2000). It was also found that contents of aspartic acid and glycine were greater than other amino acids except for serine, accounting for 18.8% and 10.7% of the 18 kinds of amino acids, respectively, which indicated that aspartic acid and glycine were also important amino acids attributed to the functions of sericin. In addition, the hydrophilic amino acid amounts up to 70% of the18 kinds of amino acids, which could account for the good solubility and water absorbability of sericin. It was also found that the amount of aromatic amino acids in sericin was very low (accounting for 6.6% of the 18 kinds of amino acids) compared with other proteins, and this was confirmed by the ultraviolet absorption spectrum obtained by wavelength scanning from 190 nm to 300 nm (Fig. 1). As shown in Fig. 1, the maximal absorption wavelength was at 214 nm, not at 280 nm assigned to aromatic amino acids absorption wavelength, and this result indicated that peptide bonds were the major absorbing group for sericin in the ultraviolet region.

Table 2
The chemical composition of sericin

Composition	Concentration (%)
Total nitrogen	14.65 ± 0.04^{a}
Protein	91.6 ± 0.2^{b}
Sugar	0.93 ± 0.04^{c}
Ash	4.20 ± 0.01^{d}

Values expressed as percentage of dry sericin powder (mean of three replicates ± standard deviation).
[a–d]Values with the same letter superscript denote no significant difference ($p < 0.05$).

Table 3
Amino acid composition of sericin

Amino acid	g amino acid/100 g protein	Percentage of total amino acid (%)[a]
Ser	20.21	27.3
Asp	13.93	18.8
Gly	7.91	10.7
Thr	5.57	7.5
Glu	5.29	7.2
Arg	3.64	4.9
Tyr	3.41	4.6
Ala	3.17	4.3
Val	2.82	3.8
Lys	1.54	2.1
His	1.27	1.7
Leu	1.25	1.7
Phe	1.17	1.6
Ile	0.94	1.3
Pro	0.90	1.2
Met	0.36	0.5
Cys	0.24	0.3
Trp	0.32	0.4
Total	73.94	
Hydrophilic	51.69	70%
Hydrophobic	21.93	30%
Aromatic	4.90	6.6%

[a] Percentage of total amino acid (%) expressed as a ratio of a kind of amino acid amount vs. total amino acid amount (only including 18 kinds of amino acids).

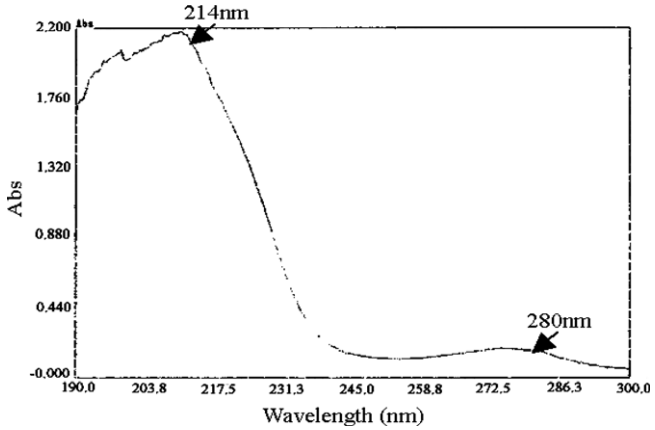

Fig. 1. UV absorption spectrum of sericin. Condition: 3 ml sample (0.25 mg/ml) assay on a UV–VIS spectrophotometer (UV-1100, Beijing RuiLi analytic apparatus Co.) with 1.0 cm quartz cell.

Table 3 shows that sericin powder had a high content of Ser (27.3%), Asp (18.8%), Glu (7.2%) and Thr (7.5%) of the 18 kinds of amino acids. However, the content of Tyr and Ala was very low, only accounting for 4.6% and 4.3%, respectively. This characterization of amino acid composition is a token of sericin according to the report described by Paulownia-village (1962), so amino acid composition analysis results showed that sericin powder obtained by the procedure developed in our study had a high degree of sericin purity, with little or no contamination by fibroin.

3.3. Molecular weight determinations of sericin powder

Previous study findings on sericin indicated that its molecular weight ranged widely from about 10 to over 300 kDa (Zhang, 2002). An accurate determination of the molecular weight distribution of sericin is not reported to date because of the complex components of sericin undergoing different extraction and process condition. To view the molecular weight profile of sericin powder prepared in a novel approach by ethanol precipitation, we used gel filtration chromatography, SDS–PAGE, and HPLC analytic methods to assess different molecular weight distribution ranges of sericin powder.

The chromatogram of sericin on Sepharose CL-6B column was shown in Fig. 2, and two different separated peaks appeared in the elution profile. A polymeric protein fraction (P_1) of high molecular weight was eluted at the exclusion volume and the other protein fraction (P_2) of low molecular weight was eluted as a large peak, and the ratio of P_2 eluted volume vs. P_1 eluted volume was 15:4. The elution volume of P_1 from 76 ml to 90 ml, and P2 from 135 ml to 198 ml, according to the linear regression equation (3), the molecular weight range of P_1 was about from 1,625 kDa to 1,209 kDa, and P_2 was about from 467 kDa to 124 kDa. The result suggested that the P_2 with larger peak area fraction was partially in agreement with the report by others (Zhang, 2002), and we think that the P_1 fraction might be due to the protein conglomeration under the elution conditions.

The protein composition of the molecular weight below 100 kDa was analyzed using non-reducing SDS–PAGE. As shown in Fig. 3, electrophoresis results indicated that sericin appeared in a continuous distribution between 97 kDa and 14 kDa, and there were some bands above 97 kDa and also some bands below 14 kDa. SDS–PAGE results in conjunction with those results obtained from gel filtration chromatography suggested that sericin represents a family of proteins with diverse molecular weight distribution.

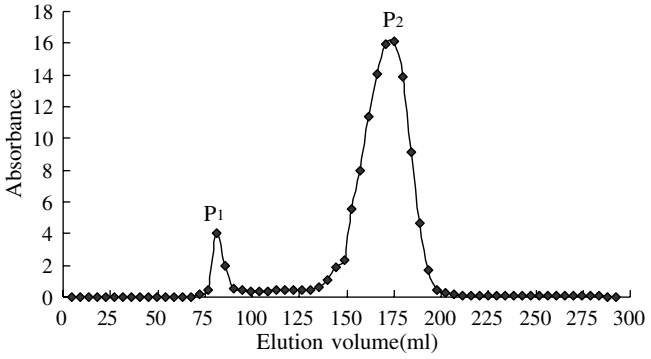

Fig. 2. Chromatogram of sericin on Sepharose CL-6B column. Chromatogram of sericin on Sepharose CL-6B column (1.6 i.d. × 100 cm, separate range: 10–4000 kDa) using 20 mM, pH 7.0 sodium phosphate buffer as the eluent. Other conditions: detection, UV absorbance at 220 nm; flow rate, 0.3 ml/min; each collected fraction volume, 4.5 ml.

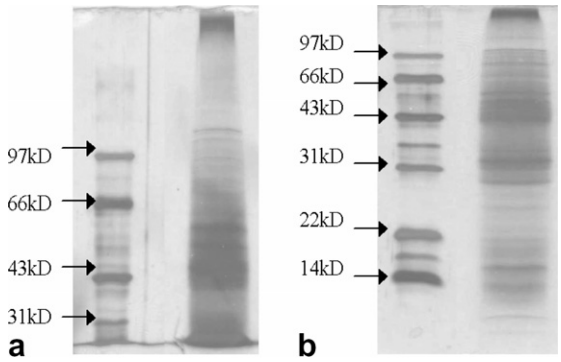

Fig. 3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) of sericin by silver stain. A: 7.5% resolving gel, 4% stacking gel, B: 12.5% resolving gel, 4% stacking gel. Markers consisted of phosphorylase B (97 kDa), bovine serum albumin (66 kDa), ovalbumin (43 kDa), carbonic anhydrase (31 kDa), soy trypsin inhibitor (22 kDa), lysozyme (14 kDa).

The profile of the molecular weight below 10 kDa was shown in Fig. 4. The result shows that the low molecular weight fraction of sericin was mostly distributed around 6 kDa when determined according to the linear regression equation (4). According to the previous research findings that the molecular weight of sericin was over 10 kDa, our data denoted that sericin might be somewhat degraded into lower molecular weight species during the preparation.

Our data show that sericin composition was complex because of its diversity of molecular weight distribution. This characterization of sericin implies that sericin might be rich in biological functions resulting from its various protein components.

3.4. CD analysis on sericin secondary structure

The CD spectrum of sericin solution was assessed in order to determine the conformation change in the course of ethanol precipitation. Fig. 5 shows the spray-dried flour (b curve, solid line) and sericin powder (a curve, broken line) CD curves. As can be seen in Fig. 5, spray dried flour

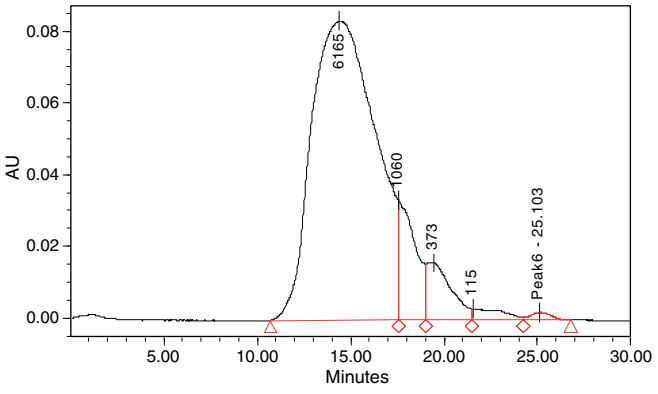

Fig. 4. TSK gel 2000 SWXL-high performance liquid chromatography (HPLC) low molecular weight distribution profile of sericin. *x*-axis was retention time, *y*-axis was UV absorbance at 220 nm.

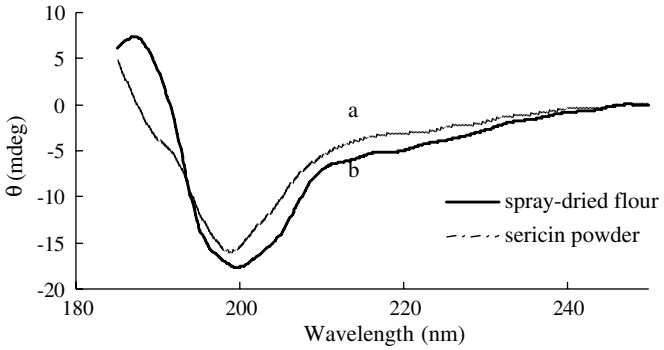

Fig. 5. Circular dichroism (CD) spectrum of sericin. (a) Curve was sericin powder, (b) curve was spray-dried flour.

CD curve approximately agreed with the sericin powder CD curve, and both curves showed strong negative bands at 198 nm assigned to the random coil conformation and weak negative bands at 218 nm assigned to the β-structure. These results seemed to be in good agreement with the results described by Masuhiro, Tadashi, and Tohru (1979). The molar ratio percent of the four possible conformations of sericin were calculated using analysis function built into the Jasco software according to the method proposed by Yang, Wu, and Martinez (1986), and the result was given in Table 4. As shown in Table 4, compared to the spray-dried flour the molar ratio percent of random coil of sericin powder decreased slightly. In addition, similar to the spray-dried flour, sericin powder had no α-Helix conformation, which differs from the report by Masuhiro et al. (1979). It should be noted, however, that the β-Sheet conformation of sericin powder compared to spray-dried flour greatly decreased. In the case of β-Turn conformation, spray-dried flour did not show any mol%. However, sericin powder showed this kind of protein conformation, reached 17 mol%. The β-Turn is comprised of four residues, forms a hydrogen bond between the carbonyl group of residue *i*th and the NH group of residue $i+3$th. This conformation is frequently found at the globular protein surface, and as a common structural element in globular proteins that facilitates folding by reversing the direction of the polypeptide chain (Shin, Ting, & Schultz, 1997), thus the β-Turn can act as one of the key elements in dictating the folded structures of native proteins (Chang et al., 2002). The appearance of the β-Turn conformation for sericin protein treated with ethanol implied that some change for sericin protein molecular structure, and the change was probably associated with molecular aggregation of the pro-

Table 4
Calculated conformations (in mol%) of spray-dried flour and sericin powder

Conformation	Spray-dried flour	Sericin powder
Random coil	56.8	55.4
β-Sheet	43.2	27.6
β-Turn	0.0	17.0
α-Helix	0.0	0.0

tein induced by ethanol according to Lee et al. (2003). Lee et al. (2003) reported that the major conformation of sericin was random coil regardless of the addition of methyl alcohol, and the molecular states of sericin were more densely packed effected by methyl alcohol. Our result seemed to be in good agreement with this conclusion, the major conformations of sericin protein was the random coil (accounting for approximately 55%), and decreased slightly after being treated with ethanol. Because when globular proteins fold tightly into the compact shape, its polypeptide chain often reverses direction, and makes a β-Turn, we assumed that the appearance of the β-Turn conformation maybe associated with the packing of molecular chain induced by ethanol.

3.5. Inhibition of the activity of tyrosinase by sericin

Enzymatic browning in fruits and vegetables is predominantly catalyzed by a copper-containing enzyme, tyrosinase (EC.1.14.18.1, known as catecholase, diphenol oxidase) (Mayer, 1995). This enzyme exists widely in nature and catalyzes the oxidation of *o*-diphenols to *o*-quinones. Kato et al. (1998) reported that sericin could inhibit the activity of tyrosinase. So we investigated the inhibition of sericin on enzymatic browning in fruits and vegetables. Because the mode of inhibition depends on the structures of both the substrate and the inhibitor, chlorogenic acid, which is one of the major phenolic compounds found in fruits and vegetables, was selected as the substrate. Tyrosinase activity with or without 8% sericin was reported in Fig. 6. The data in Fig. 6 shows that the enzymatic activity with sericin was reduced to 23% compared to the activity of control sample without 8% sericin after 600 s. The results suggest that sericin has a strong inhibitory effect on the activity of mushroom tyrosinase, and it is a potential inhibitor for enzymatic browning in fruits and vegetables.

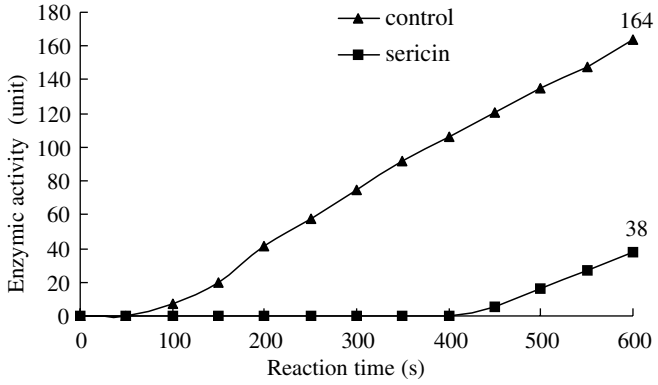

Fig. 6. Progress curve for the inhibition of mushroom tyrosinase by sericin using chlorogenic acid as substrate. The means of duplicate measurements are shown. Tyrosinase activity was measured by determining the increase in absorbance (OD) at 420 nm using UV–VIS spectrophotometer (UV-1100, Beijing RuiLi analytic apparatus Co.).

3.6. Antioxidant activity measured using the DPPH assay

DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging effects of sericin. As antioxidants donate protons to this radical, the absorption decreases. The decrease in absorption was applied to assess of the extent of radical scavenging according to Eq. (6). Fig. 7(a) shows that radical scavenging activity was dose-dependent increasing with an increase in sericin concentration. However, the regression curve was a quadratic equation (Eq. (6)),

$$Y = -0.0077x^2 + 1.8337x + 0.7807, \quad (6)$$

which was transformed to a linear regression equation (Eq. (7))

$$Y = 0.4461x + 0.0057 \quad (7)$$

by plotting the inverse values $1/y$ vs. $1/x$ to obtain a linear regression curve was shown in Fig. 7(b), which showed a good coefficient of correlation ($R^2 = 0.9758$). From the linear regression equation (7), the IC_{50} (the concentrations of

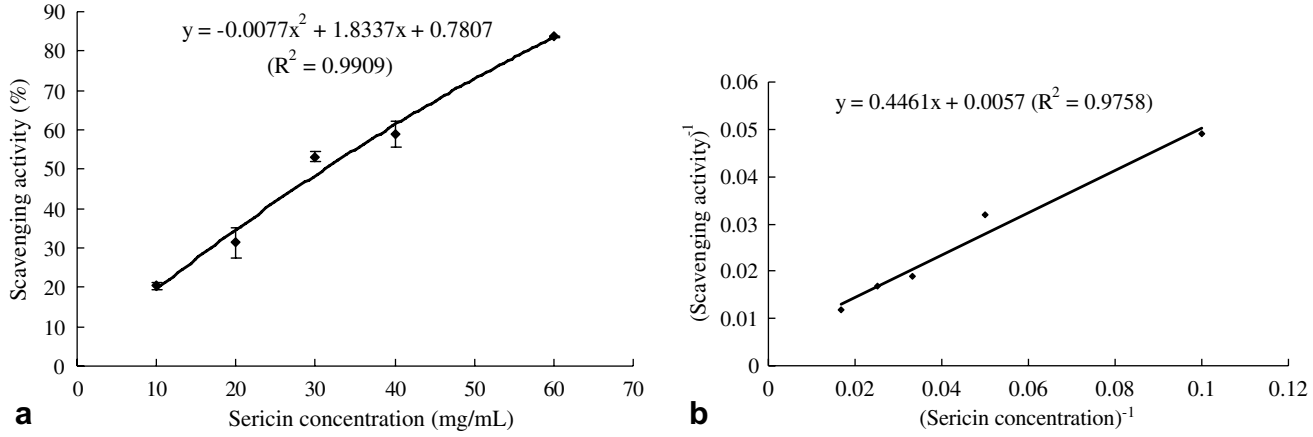

Fig. 7. Scavenging activity of sericin on the 2.2-diphenyl-1-picryl-hydrazil (DPPH) radical. The data shown are means ± standard deviation from triplicate determinations. (a) plot of sericin concentration vs. scavenging activity, (b) plot of inverse values in (a) which was used to estimate IC_{50} (the concentrations of sericin leading to 50% of DPPH radical scavenged).

sericin leading to 50% of DPPH radical scavenged) value was calculated as 31 mg/ml of sericin. Kato et al. (1998) reported that 0.3% sericin could completely inhibit lipid peroxidation. In this study, we provided another evidence for the antioxidation activity of sericin by DPPH assay. At present we are processing sericin through enzymatic hydrolysis, which may yield peptides with better antioxidation and other functional as well as bioactive properties.

4. Conclusion

In this work, we developed a novel extraction procedure to recover sericin from silk industry wastewater by using a chilled ethanol precipitation method, which was an effective and simple method. We focused on studying the physiochemical, structural, and biological properties of sericin obtained by this method. The physiochemical analysis results suggested that sericin power contained high degree purity of sericin, and this protein represented a family of proteins with wide-ranging molecular weight distribution. CD analysis results suggested that its major conformation was random coil that decreased slightly after being treated with ethanol, and the appearance of β-Turn conformation maybe associated with the packing of molecular chains induced by ethanol. In this study, we also found the biological activities of sericin powder, including antioxidant activity and tyrosinase inhibitory activity, which functions are important for potential application of sericin to food manufacture. In the future we are interested in studying enzymatic hydrolysis of sericin to produce small bioactive peptides, which will be more suitable for the development of functional foods than native sericin.

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