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Effect of silk protein surfactant on silk degumming and its properties

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ABSTRACT

The silk protein surfactant (SPS) first used as a silk degumming agent in this study is an amino acid-type anionic surfactant that was synthesized using silk fibroin amino acids and lauroyl chloride. We studied it systematically in comparison with the traditional degumming methods such as sodium carbonate (Na_2CO_3) and neutral soap (NS). The experimental results showed that the sericin can be completely removed from the silk fibroin fiber after boiling the fibers three times for 30 min and using a bath ratio of 1:80 (g/mL) and a concentration of 0.2% SPS in an aqueous solution. The results of the tensile properties, thermal analysis, and SEM all show that SPS is similar to the NS, far superior to Na_2CO_3. In short, SPS may be used as an environmentally friendly silk degumming/refining agent in the silk textile industry and in the manufacture of silk floss quilts.

1. Introduction

Silk is composed of sericin and fibroin. It is believed that sericin and fibroin account for 20–25% and 70–75% of silk, respectively, and the silk also contains 5% of other impurities. The composition of silk from different types of silkworms varies. Sericin is present on the outside of the silk fiber, and serves as an adhesive to strengthen the cocoon. Both components are important for the development of silkworms during metamorphosis and later stages.

It is known that sericin dissolves well in hot water, especially in alkaline hot water. In the past, Chinese people have used hot water, alkaline or not, to boil cocoons and then reel the silk fibers to get raw silk. However, in the textile industry, residual sericin around the raw silk affects the luster, handling and dyeing quality of silk. Therefore, the production of silk textile fibers or biomedical materials involves the removal of the sericin that surrounds the natural fibroin fibers. In short, the separation of silk fibroin and sericin is an important process and is known as degumming or refining/scouring.

The degummed silk is made by heating the silk fibers in an aqueous alkaline solution followed by a wash with water. Using an alkaline degumming agent is a necessity for the modern silk production process for applications such as the degumming, spinning and production of medical biological materials. Degumming using sodium carbonate (Na_2CO_3) or neutral soap (NS) or both is the most commonly used methods. Nevertheless industrial silk production generates quantities of alkaline wastewater containing sericin, which can cause severe environmental pollution and is a huge waste of biological resources that are difficult to recover [1,2].

The silk degumming methods used in the laboratory involve treatment with highly concentrated urea [3], NS [4,5] or Na_2CO_3. By using the NS-degumming method, researchers can obtain a complete peptide chain of fibroin fiber. The disadvantage of this method is that it is time-consuming because the degumming must be repeated several times to completely remove sericin. The most widely used degumming method at the bench scale is boiling for 30 min in 0.2–0.5% (W/V) Na_2CO_3 or $NaHCO_3$. Howbeit, one issue with this method is that peptides and free amino acids are difficult to separate and purify from hydrolyzates containing sericin. Currently, separating and recovering sericin from a large amount of silk degumming alkaline waste are very difficult due to the numerous alkaline substances used in degumming. For example, bleach and softening agents are necessary for scouring raw silk, silk fabrics and silk floss for the manufacture of silk quilts. Degumming under high temperature and pressure conditions [6] is a low-cost method, but it only removes the outer layer of sericin, leaving the inner layer [7] of sericin adjacent to the fibroin fibers. Although enzymatic degumming can completely remove the sericin by hydrolysis [8,9], it is unsuitable for large-scale use because of its low efficiency. It has also been reported that organic acids are used as silk degumming solvents [10–12]. However, these methods have a poor effect on the mechanical properties of silk fibers. Therefore, developing a new environmentally friendly silk degumming agent is very important for the recovery and utilization of sericin and sericin-containing materials.

Amino acid-based surfactants are preferable for food, pharmaceutical and cosmetic applications. They have low toxicity and can be rapidly degraded, similar to surfactants prepared for biotechnology and chemical methods and the use of renewable raw materials, including waste and vegetable oils [13–19].

We recently developed a new silk degumming agent of strongly alkaline electrolyzed water (pH ≥ 11.5) [20]. It has good degumming

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abilities. Experimental results show that it can completely remove sericin from around silk fibroin, and the properties of the degummed fiber are superior to those of Na_2CO_3- or NS-degummed fibers. However, there are some disadvantages, including the relatively poor stability of the preparation of the electrolyzed water, which makes large-scale commercial applications of the electrolyzed water difficult. It is known that surface-active compounds may have adverse effects on aquatic life. Biodegradability and surfactant compatibility have become very important, not only to consumers but also to the surfactant industry itself, as it is very important for the surfactants' functional performance. Therefore, the development of efficient and novel surfactants that are biodegradable and biocompatible is critical. The compounds from a combination of silk protein hydrolyzates (the hydrophilic moiety) have been produced in this work along with the non-polar and long-chain compound (the hydrophobic moiety) for building up an amphiphilic structure to include environmentally friendly molecules with a high surface activity [21–24]. Amino acid-based surfactants have been made commercially with silk hydrolyzates and lauroyl chloride for many years by the Japanese Kawaken Fine Chemicals Co., Ltd. The amino acid-based surfactant has been widely used in cosmetics and household detergents. However, it has not been applied to the degumming of silk fibers as an environmentally safe degumming agent.

Recently, our laboratory processed waste with silk sericin recovered from a synthetic amino acid-type anionic surfactant [14]. We have synthesized the silk protein surfactant (SPS) with amino acids of silk fibroin and lauroyl chloride using a similar method. SPS, a new type of anionic surfactant, was used as a novel silk degumming agent, and the mechanical and thermal properties of the degummed silk fibers were analyzed as described below.

2. Materials and methods

2.1. Experimental materials

A 20% amino acid solution of silk fibroin was provided by Xintiansi Biotechnology Co. Ltd., Huzhou, P.R. China. The silkworm strains characterized by sex-identified fluorescent cocoons were the yellow male cocoon and the purple female cocoon of *Bombyx mori* YingSu × YingXiao, which fluoresce at 365 nm ultraviolet light [25], and were kindly provided by Professor Xiao-Hua Yu, Sericulture Institute of Soochow University. The lauroyl chloride was purchased from Sharon Chemical Co., Ltd. (Huzhou, P.R. China).

2.2. Synthesis of SPS

SPS was synthesized with silk amino acids and lauroyl chloride as previously described by Wu and Zhang [14], with slight modifications. The silk amino acids were loaded into a four-necked RV620-2 reactor (Shanghai Ya-Rong Biochemical Instrument Factory, Shanghai, P.R. China) equipped with an electronic stirrer and a programmable temperature controller (BILON-W-2003T, Tianjin Bilon Lab Equipment Co., Ltd, Tianjin, P.R. China). Approximately 4.0 L of acetone was added to the reactor, followed by 1.1 L of the lauroyl chloride and 2.0 M NaOH solution. The reaction occurred at room temperature for 4 h until the mixture became reddish brown and viscous. Then, 2 M HCl was added to the reaction to achieve pH 1–2 in order to induce the precipitation of the product. Next, the reddish brown precipitate was washed with water and petroleum ether sequentially and crystallized and recrystallized twice in ethyl alcohol to yield a red product. An alcoholic NaOH solution was added to adjust the pH of the mixture to 7–8, and the solution was then stirred at room temperature for 30 min. The organic solvent and the product were separated; the organic solvent was recycled, and the product (1.5 kg) was dissolved in water to obtain the reddish brown product, N-lauroyl sericin amino acid-based surfactant.

2.3. Na_2CO_3 degumming method

Clean cocoon shells were added at a known weight to 0.5% (w/v) Na_2CO_3 at a ratio of 1:100 (w/v), and then boiled for 30 min. The degummed silk fibroin was washed with 40 °C deionized water three times, and these washes were saved and pooled. The resulting degummed silk was boiled for 30 min in 0.5% Na_2CO_3. After multiple washes, the degummed silk fibroin fiber was air-dried at 80 °C for 24 h and then weighed. This procedure was performed in triplicate, and the degumming rate was calculated as:

$$\text{Degumming rate} = [(\text{cocoon shell weight} - \text{degummed silk weight})/\text{cocoon shell weight}] \times 100\%.$$

2.4. NS degumming method

A known weight of clean cocoon shells was immersed in a 0.2% (w/v) NS solution at a ratio of 1:100 (w/v) and then heated at 100 °C for 30 min. The NS solution was replaced with a fresh solution and heated again. This step was repeated two times. The degummed silk fibroin was washed with 40 °C deionized water three times, and these washes were saved and pooled. The degummed silk fibroin fiber was also washed repeatedly and then air-dried at 80 °C for 2 h. This procedure was performed in triplicate, and the degumming rate was calculated as described in the above section.

2.5. SPS degumming method

SPS was used for degumming cocoons, and the degumming rate was calculated at varying concentrations, temperatures, times and bath ratios. The best combination of conditions was chosen and compared to the other methods in terms of the advantages and disadvantages. This procedure was performed in triplicate, and the degumming rate was calculated as described above in the section "Na_2CO_3 degumming method".

2.6. SDS-PAGE

The degummed silk fibers were added to 20 times 9.3 M LiBr (v/w) and incubated with shaking at 25 °C until dissolved [27]. After the silk fibroin/salt solution was filtered with deionized water or centrifuged at 8000 rpm for 10 min, the filtered solution or supernatant was dialyzed continuously for 48 h with running water to remove $CaCl_2$, smaller molecules and impurities using a cellulose semi-permeable membrane (<5 kDa). The resulting supernatant was referred to as the regenerated silk fibroin for subsequent experiments.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine the molecular mass range of the silk protein. The molecular mass range of the regenerated silk fibroin was determined by SDS-PAGE as described previously by Wang and Zhang [27].

2.7. SEM observation

The silk fibroin fibers degummed by the Na_2CO_3, SPS and NS were observed using a scanning electron microscope (SEM; Hitachi S-4700 cold field emission microscope) at a magnification of 5000×.

2.8. Tensile tests of single filaments

Tensile tests of a silk single filament were carried out as previously described by Cao et al. [26]. Silk fibers produced by the mature silkworm were 1000 m long, and their thickness was not uniform. Each cocoon was hand-reeled in water at 70 °C, thus reducing errors in the