Production and Identification of Polypeptide from Silks by Hydrothermal Method

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Abstract. There are two kinds of proteins in silk, sericin and silk fibroin. Polypeptide compounds from silk sericin and silk fibroin were prepared by hydrothermal method. The process of silk dissolution was investigated under different solid-liquid ratio, reaction temperature and reaction time. By controlling the operating parameters of hydrothermal method, the temperature, material ratio and time were further optimized, and the best experimental results were obtained, the expected decomposition of silks occurred when the ratio of silks to waters was selected as 1 to 10, at 140 degree in 30 min. The molecular weight of polypeptide was detected by SDS-PAGE electrophoresis and analysed by MALDI-TOF-MS. The results showed that the molecular weight of the polypeptide obtained from silks was about 6000-8000Da. After literature research, the polypeptide with such molecular weight could have better performance for some functional additives.

1. Introduction
Silk is a natural polymer material produced by silkworm. Its main components are sericin and silk fibroin. Silk fibroin is the main component of silk, accounting for about 70-75%, sericin accounts for about 20-25%, and about 5% of wax, carbohydrates, pigments and impurities. The proportion of different components of silkworm varieties is also different, and the percentage of sericin is also different. In terms of silk utilization, sericin is mostly separated as waste, which reduces the overall utilization rate of silk. In the production of high-grade silk garment products, because the existence of sericin will affect the luster, hand feel and printing and dyeing processing of silk products, the separation of silk fibroin and sericin has become the primary problem to be solved in the application of silk materials. At the same time, in terms of silk utilization, sericin is mostly separated as waste, which reduces the overall utilization rate of silk.

Silk fibroin mainly exists in the form of silk fibroin and silk peptide. Silk fibroin can be hydrolyzed to obtain silk peptide. Due to different degree of hydrolysis, its molecular weight also varies greatly, ranging from hundreds to thousands. Different from silk fibroin, silk peptide is soluble in water and soluble with common surfactants. Due to the characteristics of more hydrophilic groups and small molecular weight in the side chain of silk peptide, silk peptide has good moisturizing effect, strong permeability, can combine with epithelial cells through the stratum corneum, and participate in and improve the metabolism of epithelial cells.

Sericin accounts for about 20% - 30% of the cocoon mass and is synthesized and secreted by the central silk gland of silkworm. Different from silk fibroin, sericin is a structurally oriented globular protein. Sericin is easily soluble in water, but the solubility of different levels of sericin is very different. The amino acid composition of sericin protein is also very similar to that of natural moisturizing factor, which makes sericin have good moisturizing and film-forming properties.
addition, many polar amino acid molecules on the polypeptide chain of sericin protein are on the surface, which can combine with the cells in the stratum corneum after water is transmitted to the stratum corneum, so as to keep the skin moist and moisturize.\textsuperscript{5}

When pure water dissolves silk at high temperature (hydrothermal method), sericin basically falls off gradually from the outside and inside, that is, the sericin protein dissolved first is mainly macromolecular components, and then small molecular components. Furthermore, silk fibroin will be dissolved and destroyed. Macromolecular sericin and silk fibroin proteins are unstable.\textsuperscript{6} After hydrolysis, they form oligopeptides or peptides with small molecular weight and relatively stable, which is conducive to the further application of silk.

A simple degumming method of high temperature dissolution in pure water will be used to obtain mixed proteins with different molecular weights. Using different degumming conditions, the molecular weight of protein will change greatly and degrade to different degrees. For Sericin and silk fibroin obtained from degumming, a research scheme to obtain proteins (peptides) with specific molecular weight range under specific experimental conditions was designed.\textsuperscript{7} Find out the corresponding relationship between different separation conditions and the molecular weight of protein fragments (peptides), and realize "customized separation and purification of molecular weight" protein.\textsuperscript{8}

2. Experiments and Scheme Design

2.1. Scheme Design

The orthogonal experiment was used to control the variables to design the experiment. The independent variables are: temperature, time, feed ratio and silkworm species. The temperature is set as high temperature group and low temperature group. The temperature of high temperature group is 200 °C, 180 °C and 160 °C, and the temperature of low temperature group is 140 °C, 120 °C and 100 °C. Time setting: 30min, 60min, 80min, 90min, 120min. Material ratio: 40:1, 25:1, 10:1.

2.2. Experiments

The experimental operation is carried out according to the following steps:\textsuperscript{9}

Cut the cocoon shell into blocks of about 1cm\textsuperscript{2} and weigh it for standby.

Transfer the weighed cocoon shell to the reactor, weigh the corresponding volume of deionized water with a measuring cylinder and pour it into the reactor. Seal and mark.

Put the treated kettle into the heating box, and start timing when the heating box rises to the set temperature.

According to the designed heating time, take out the small kettle in time and cool it.

After the kettle cools down, the mixture is pumped and filtered.

Suck out part of the liquid with a rubber tipped dropper for sample retention.
Collect the solid in the Petri dish and put it into the drying oven for drying.

Table 1. Weight change of solid products obtained from silks after hydrothermal treatment at higher temperatures.

| Time (min) | Ratio of materials | Temperature (degree) | Weight of raw material (g) | Weight of solid (g) |
|------------|--------------------|----------------------|---------------------------|--------------------|
| 30         | 25                 | 200                  | 2.403                     | 1.333              |
| 80         | 40                 | 200                  | 1.499                     | 0.284              |
| 120        | 10                 | 200                  | 6.009                     | 0.273              |
| 120        | 25                 | 180                  | 4.794                     | 2.877              |
| 30         | 40                 | 180                  | 0.755                     | 0.523              |
| 80         | 10                 | 180                  | 6.000                     | 3.976              |
| 30         | 10                 | 160                  | 6.002                     | 4.385              |
| 80         | 25                 | 160                  | 2.403                     | 1.647              |
| 120        | 40                 | 160                  | 1.501                     | 0.980              |
The solid-liquid mixed protein is filtered by a vacuum suction device. The viscous liquid is sericin and the solid is silk fibroin.

Take some samples of the liquid with a pipette for retention. Dry the solid in the oven and weigh it. The molecular weight of the liquid protein was measured by SDS-PAGE and MALDI-TOF-MS spectrometry.

The experimental results were listed in Table 1 and Table 2. And the analytical results were shown in Figure 1-3.

Table 2. Weight change of solid products obtained from silks after hydrothermal treatment at lower temperatures.

| Time (min) | Ratio of materials | Temperature (degree) | Weight of raw material (g) | Weight of solid (g) |
|------------|--------------------|----------------------|---------------------------|---------------------|
| 30         | 10                 | 100                  | 12.036                    | 11.238              |
| 60         | 25                 | 100                  | 4.809                     | 4.462               |
| 90         | 40                 | 100                  | 3.000                     | 2.771               |
| 30         | 25                 | 120                  | 2.400                     | 2.339               |
| 60         | 40                 | 120                  | 1.500                     | 1.432               |
| 90         | 10                 | 120                  | 6.001                     | 5.032               |
| 30         | 40                 | 140                  | 1.499                     | 1.426               |
| 60         | 10                 | 140                  | 6.000                     | 4.730               |
| 90         | 25                 | 140                  | 2.400                     | 1.754               |

3. Results and Discussion

3.1. Effect of Temperature
The solution was analyzed using SDS-PAGE to determine the molecular weight range of the hydrolyzed protein. The content of total protein and total amino acids in soluble products was analyzed. In the results of temperature (100 - 140 °C) in this study, it is found that the molecular weight of the obtained protein is higher, and the molecular weight of the polypeptide obtained in a short time is greater than 40000 Da. In the results of high temperature (160 - 200 °C), it is found that the molecular weight of the obtained polypeptide is small, and even polypeptide molecules higher than 2000 Da can not be found in Figure 1-3.

3.2. Residual Solid after Hydrothermal Treatment
The conditions originally used in degumming experiment were used to investigate the hydrolysis of silk fibroin. In the high temperature part of the experiment, silk fibroin was also largely dissolved in water, and the proportion of residual solids decreased. After 200 degrees and 120 minutes, the proportion of residual solids decreased to 15% in Table 1 and in Figure 4.

3.3. Effect of Reaction Time
Silk fibroin was carried out at different reaction time, 30 to 120 minutes. After each reaction, the content of the remaining silk residue was determined by weight, and the protein and polypeptides content of the soluble analytical product were determined. The results are summarized in Table 1 and Table 2. It revealed that the hydrolysis of silks could carried out almost with long time.

3.4. Effect of Ratio of Materials
The ratio of silk fibroin to water was different (1:10, 1:25 and 1:40), for the hydrothermal decomposition of sericin, the reaction is carried out at different temperatures. The weight is shown in Table 1 and Table 2. The ratio of silk to water is 1:25, the weight results of silk residue show that with the polypeptide decreases. the remaining amount of silk is that the conversion rate of raw silk is 60
percent, and the substance of all sericin is transformed into soluble products, because the total amount of sericin in raw silk fiber is about 20-30%. And partial fibroin was dissolved into the solution.

Figure 1. Spectrum of MALDI-TOF of the product obtained by treatment of silks at 100 degree within 60 min.

Figure 2. Spectrum of MALDI-TOF of the product obtained by treatment of silks at 120 degree within 30 min.

Figure 3. Spectrum of MALDI-TOF of the product obtained by treatment of silks at 140 degree within 30 min.
Figure 4. Photos of residual silks after several treatments as described in experimental part.

3.5. Optimum Molecular Weight
It can be seen from table 1 that the high-temperature group degums completely, but there will also be dissolution of silk fibroin, and the silk fibroin is poorly formed, and the protein is inactivated and burnt due to high temperature. The yield of 6000-8000Da polypeptide is low and can not produce the desired product. Therefore, high-temperature hydrothermal decomposition of silk is not suitable for silk treatment. Incomplete sericin separation will occur when the temperature is low. No more target product polypeptides can be produced.

3.6. Application of Polypeptides
The hydrolyzed silk fibroin polypeptide also has good flexibility, tensile strength, air and moisture permeability, slow-release and so on. The hydrolyzed sericin polypeptide has good flexibility, antioxidant, cell adhesion and value-added activity. The high-quality material physiological properties provide a wide application prospect for the application research of cocoon protein hydrolysis, which can be applied in the fields of food, medicine, materials, cosmetics, biosensors, cell culture and so on. Sericin and silk fibroin were separated and purified, the operation steps were simplified, and high-quality peptides were obtained to play their different functions.

4. Conclusions
In this project, cocoon protein is heated with high-temperature pure water under different conditions. Change the temperature, heating time, material ratio and other independent variables, observe the silk fibroin quality after degumming, record and calculate the silk fibroin yield, and measure the molecular weight of sericin. The high temperature group degummed completely, but the silk fibroin formation was poor, the high temperature protein inactivated burnt paste, and the silk fibroin yield was low. High temperature sericin breaks into small molecular peptides or amino acids, which can not be identified by molecular mass spectrometry. Low temperature sericin can estimate the molecular weight range. The molecular weight of low temperature group is about 6000da and 8000da. Silk fibroin can promote skin tissue regeneration and wound healing. At the same time, it has strong air permeability and moisture retention, easy plastic shape, safety and non-toxic. Silk fibroin can be processed into medical products such as band aids for hemostasis and healing. Sericin is similar to human skin in structure and contains amino acids required by human beings. It can be combined with silk fibroin and related drugs to make spray hemostasis spray.
5. Acknowledgements
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6. References
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