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Production of Water-Soluble Silk Powder from *Bombyx mori* Linn. (Nang-Noi Srisakate 1)

Pilanee Vaithanomsat* and Chidchai Punyasawon[1]

ABSTRACT

The process for production of water-soluble silk powder from *Bombyx mori* Linn. (Nang-Noi Srisakate 1) was optimized. The silk cocoon was cut, dissolved in the solvent mixture at 100 °C until the homogeneity was observed. After that the solution was dialysed and centrifuged at 6,870 × g. The purified silk solution was then freeze-dried and ground into fine powder. The results showed that the mixture of $MgCl_2$/ethanol/H_2O at mole ratio of 0.8:2:8 was the most suitable solvent to completely dissolve silk fiber. The ratio of silk over dissolving solution at 10:100 (by weight) was the most appropriate ratio. Characterization of the product showed similarity to the commercial silk powder in that they were fine yellowish powder with similar amino acid profiles. However, silk powder from laboratory showed higher total nitrogen content with less percentage of ash and moisture when compared to those of the commercial product.

Key words: silk powder, water-soluble, production

INTRODUCTION

The important ingredients of silk are 70-80% fibroin, 20-30% sericin and trace of others including wax, carbohydrate, pigment and inorganic matters. Fibroin is a protein similar in compositions to the sericin surrounding it, however, its closer packing molecules confer great strength on the silk filament. Sericin is a globular protein representing as a tube outside the silk fibroin with its molecular weight distributed between 10 and 300 kDa. It adheres to the outer surface of fibroin in the character like the animal glue. It protects and glues up the fibroin and the unit fibroins are glued up to become cocoon filament. Naturally fibroin is insoluble in water but is soluble in hot acid or alkaline solution. In contrast, sericin can be dissolved in hot water and easily degraded when dissolved in acid or alkaline solution. Silk combines a high strength and flexibility with good moisture absorption, softness and warmth, excellent wearability and a luxurious appearance. Silk is cool and comfortable in underwear or summer clothes, therefore, it has been utilized a lot in textile industry. Many attempts have been emphasized on application of silk for other purposes, for example industry of cosmetics, foods and nutrition according to its characteristics including moisturizing and UV protection.

As silk fiber consists of highly ordered arrangements of fibroin chains linked together by intermolecular hydrogen bonds, for a dissolution process to be effective, swelling of the compact

Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI), Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: p_vaithanomsat@yahoo.com, oplv@ku.ac.th

fibrous structure and breaking of the hydrogen bond network leading to complete dispersion of the individual fibroin molecules are required. Although silk fiber dissolves in several solvent systems, there are still some problems associated with. Marsano *et al*. (2005) dispersed silk fiber in saturated aqueous LiBr (9-10 M) at 60 °C for 3 h. Hofman *et al*. (2006) prepared silk fibroin solution for controlled drug delivery from *Bombyx mori* cocoon by boiling 2 times in an aqueous solution of 0.02 M Na_2CO_3. Then, the cocoon were rinsed with water, solubilized in 9 M aqueous LiBr solution and dialysed against water. Likewise, Xie *et al*. (2006) dissolved degummed silk fiber in a 9.3 M LiBr aqueous solution at ambient temperature to obtain short-chain polypeptides. However, ways of silk fiber dissolution should be studied more in order to provide alternative methods. Therefore, this experiment was intensively concentrated on optimization of dissolving silk cocoon from *Bombyx mori* Linn. (Nang-Noi Srisakate 1) using the solvent mixture under certain conditions to obtain silk protein solution.

MATERIALS AND METHODS

1. Raw material

The silk cocoon used in the experiment was *Bombyx mori* Linn. (Nang-Noi Srisakate 1) from the Research Institute of Silk, Nakornrachsrima Province, Thailand. The sample was chemically analyzed for total nitrogen, ash, loss on drying as described in AOAC (2000) and total amino acids using High Performance Liquid Chromatography connecting with AccQ • Tag column.

2. Preparation of water-soluble silk protein

The method was adapted from Chen *et al*. (2001) and Yamada *et al*. (2001). The silk cocoon was cut into small pieces and dissolved into homogeneous silk solution. The condition used for dissolution process was in the mixture of salt/ethanol/H_2O with a certain ratio of cocoon over the mixture at temperature 100 °C. After that, the homogeneous solution was dialysed against distilled water for 2 times at 4 °C and centrifuged at 6,870 × g for 15 minutes to eliminate impurities. This purified silk protein was subject to freeze-drying for overnight to obtain water-soluble silk powder which was afterwards ground into fine particles.

Dissolving solution for water-soluble silk protein production

The experiment was carried out with Completely Randomize Design (CRD) using 4 different dissolving solutions as 4 treatments and the data was analysed using Analysis of Variance or ANOVA. Following the production procedure in previous section but using 4 different protein dissolving solutions $Ca(NO_3)_2$/ethanol/H_2O (adapted from Chen *et al*., 2001), $CaCl_2$/ethanol/H_2O (adapted from Chen *et al*., 2001; Yamada *et al*., 2001), $MgCl_2$/ethanol/H_2O (adapted from Otoi and Horikawa, 1980) and $ZnCl_2$/ethanol/H_2O (adapted from Otoi and Horikawa, 1980) at the mole ratio of salt:ethanol:water = 1:2:8. The solution with the shortest dissolving period and the largest %protein yield was selected as the most appropriate protein dissolving solution and was used for further experiments.

Mole ratio of dissolving solution for water-soluble silk protein production

The experiment was carried out with Completely Randomize Design (CRD) using 4 concentrations of protein dissolving solution as 4 treatments. The analysis was performed using t-test analysis. Following the production procedure in previous section using the selected protein dissolving solution from previous section but the mole ratios of dissolving solution were varied to 0.4:2:8, 0.6:2:8, 0.8:2:8 and 1:2:8. The mole ratio of dissolving solution giving maximum %protein yield was selected as the most appropriate mole ratio and was used for further experiments.

Ratio of cocoon over dissolving solution for water-soluble silk protein production

The experiment was carried out with Completely Randomize Design (CRD) using 5 ratios of cocoon over the dissolving mixture as 5 treatments. The analysis was done using Analysis of Variance or ANOVA. Following the production procedure in previous section, the selected protein dissolving solution and appropriate mole ratio of dissolving solution from previous section were used but the ratios of cocoon over the dissolving mixture were varied at 2:100, 4:100, 6:100, 8:100 and 10:100 by weight. The ratio of cocoon over the dissolving mixture giving maximum %protein yield was selected and was used for further experiments.

3. Characterisation of product

The silk protein product obtained from the experiment was characterised for total nitrogen, loss on drying, ash, pH, water solubility, total plate count, *Escherichia coli* according to AOAC (2000) and total amino acid using High Performance Liquid Chromatography connecting with AccQ • Tag column.

RESULTS AND DISCUSSION

1. Raw material

Chemical analysis of silk cocoon from *Bombyx mori* Linn. (Nang-Noi Srisakate 1) indicated the content of loss on drying 8.43 %, ash 0.81 %, total nitrogen 14.64 % with the profile of total amino acids as shown in Table 1. It was noticed that the major components of silk protein were glycine, alanine, serine, tyrosine and aspartic acid as also demonstrated for the same strain of silk by Hemachantorn (1987). The congeniality

Table 1 Amino acid profiles of *Bombyx mori* Linn. (Nang-Noi Srisakate 1) silk cocoon.

Amino acid	(g/100 g)[1/]	(g/100 g)[2/]
Aspartic acid	6.57	4.61
Serine	17.18	16.14
Glutamic acid	2.92	2.28
Glycine	**27.68**	**32.14**
Histidine	not detected	0.45
Arginine	1.86	2.07
Threonine	2.96	2.35
Alanine	**21.49**	**21.89**
Proline	0.56	0.16
Cystine	not determined	0.05
Tyrosine	7.82	9.04
Valine	2.95	2.67
Methionine	not determined	not detected
Lysine	1.02	1.30
Isoleucine	0.70	0.41
Leucine	0.84	0.48
Phenylalanine	1.01	0.54

[1/] Nang-Noi Srisakate 1

[2/] Hemachantorn, 1987

of silk towards water depends on the difference of structure at the end of side chain group of the amino acid and the congeniality of amino acid towards water could be, thus, strong or weak. From Table 1, the side chain group end points of glycine and alanine (major components) were either hydrogen or methyl groups indicating high hydrophobic property, therefore, resulting in natural insolubility of silk in water.

2. Preparation of water-soluble silk protein

As mentioned above, the silk was water-insoluble but dissolvable in solvent, especially in alkali metal salts and alkaline earth metal salts such as LiCl, LiBr, NaI, an aqueous solution of chloride, nitrate or thiocyanate of Ca, Mg, Zn (Ohtomo and Horikawa, 1980; Lock, 1993; Freddi *et al*., 1999). When treated with these kinds of solvents, the β-sheet structure of silk fiber would mainly change to random coil due to the highly hydration of the concentrated solution, and the ability of alkali ions in binding with tyrosine residues in fibroin resulting in disruption of the van der'Waals force and hydrogen bonds in fibroin molecules. This would cause fibroin to swell and then dissolve (Wang Zhang *et al.*, 2001). However, an aqueous solution of chloride or nitrate of Ca or Mg are most preferably used because of their low cost and convenience (Otoi and Horikawa, 1980; Hirabayashi *et al*., 2002). Furthermore, combination of alcohol such as, ethanol or methanol, and aqueous salt solution could improve the solubility of fibroin (Ohtomo and Horikawa, 1980). Thus, an aqueous solution of chloride and nitrate of Ca, Mg, Zn in combination with ethanol were selected as silk dissolving solution for the next experiment due to their low cost and availability.

Dissolving solution for water-soluble silk protein production

Figure 1 shows that every type of salt solution used in this experiment ($Ca(NO_3)_2$, $CaCl_2$, $MgCl_2$ and $ZnCl_2$) can make silk protein solubilized and does not significantly affect silk protein yield. This was consistent with those reports of Otoi and Horikawa (1980) and Chen *et al*. (2001) that fibroin from *Bombyx mori* could be dissolved in $CaCl_2$, $MgCl_2$, $ZnCl_2$ and $Ca(NO_3)_2$. However, when dissolving periods were compared, it indicated that the shortest period (25 minutes) to completely dissolve silk cocoon was obtained from $ZnCl_2$ and $MgCl_2$ whereas $Ca(NO_3)_2$ and $CaCl_2$ required longer periods of 80 and 120 minutes, respectively. Comparison between %yields of silk protein resulted from $ZnCl_2$ and $MgCl_2$ solubilization indicated that less %yield of silk protein was obtained from $ZnCl_2$ solubilization as some of the protein was precipitated during dialysis. The solubility of obtained silk proteins was also observed (data not shown) and demonstrated the best water-solubility of silk protein from $MgCl_2$. In addition, the dissolving solution containing $MgCl_2$ was selected for further experiments due to its low of cost.

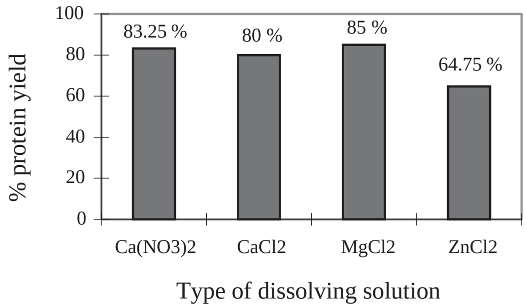

Figure 1 Effects of protein dissolving solution on yield of silk protein powder.

Mole ratio of $MgCl_2$ dissolving solution for water-soluble silk protein production

Figure 2 shows %yield of silk proteins and dissolving periods when ratios of $MgCl_2$ are varied at 0.4, 0.6, 0.8 and 1 per 2 moles ethanol and 8 moles water. It was clear that the ratio of salt significantly affect the solubility of silk cocoon. At 0.4 and 0.6 moles of $MgCl_2$, the silk cocoon could not be dissolved into silk homogeneous solution whereas dissolving solution 0.8 and 1.0 moles of $MgCl_2$ were capable of completely dissolving silk cocoon with no significantly different %yield of silk protein, 84.0% and 85.0%, respectively. This was consistent with the experiments carried out by Ohtomo and Horikawa (1980) who reported that the most appropriate ratios of salts for fibroin dissolving ranging from 20% to 70%. Therefore dissolving solution with 0.8 moles of $MgCl_2$ was used for further experiment due to its low cost.

Ratio of cocoon over dissolving solution for water-soluble silk protein production

Figure 3 shows the influence of ratio between silk cocoon and dissolving solution (2:100, 4:100, 6:100, 8:100 and 10:100 by weight) on %yield of silk proteins and dissolving periods. It was demonstrated that every ratio could be dissolved with selected solution however, dissolving period increased as the ratio was raised whereas the yield of silk powder was not significantly different. Furthermore, the color of resulting silk powder was getting darker as the silk cocoon ratio increased. Therefore the ratio at 10:100 was selected for further experiments.

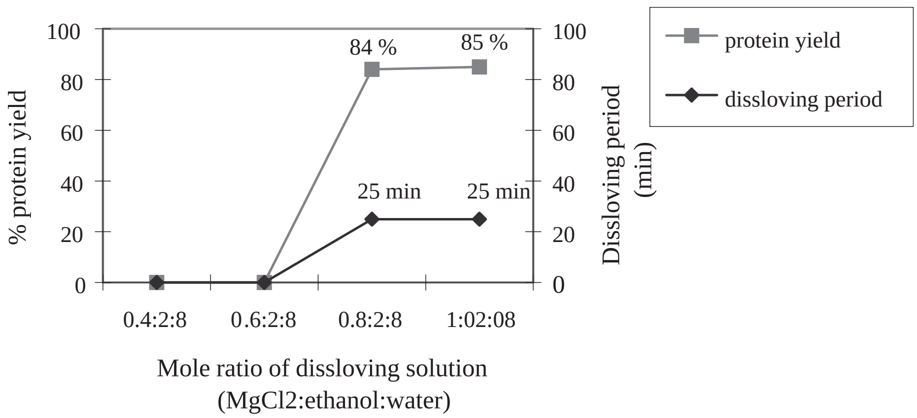

Figure 2 Effects of protein dissolving solution ratio on dissolving period and yield of silk protein powder.

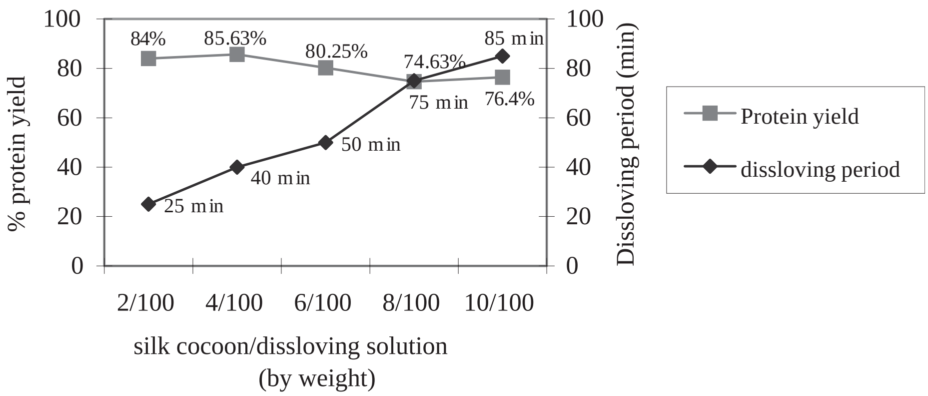

Figure 3 Effects of ratio between cocoon and dissolving mixture on dissolving period and yield of silk protein powder.

3. Characterisation of product

The silk protein powder obtained was fine yellow powder with the contents of total nitrogen, moisture, ash, pH, water solubility, total plate count and *E. coli* of 16.57, 4.06, 1.37, 5.53, 100 %, < 30 CFU/g and negative, respectively as shown in Table 2. When compared with the commercial silk protein powder, it indicated that the silk powder from laboratory contained higher total nitrogen and less moisture and ash than those of the commercial product.

The silk protein powder was also studied for its amino acid profile as shown in Table 3. They showed similarity in amino acid profile in that the majority of them were glycine, alanine, serine and tyrosine. However, differences were observed in

Table 2 Characteristics of silk powder from *Bombyx mori* Linn. (Nang-Noi Srisakate 1) and Commercial product.

Quality	Laboratory product "Nang-Noi Srisakate 1"	Commercial product "Promois®"
Total nitrogen	16.57 %	13.0 %
Loss on drying	4.06 %	10.0 %
Ash	1.37 %	3.0 %
pH	5.53	5.5 – 7.5
Water solubility	100 %	Soluble
Total plate count	< 30 CFU/g	not reported
E. coli	negative	not reported

Table 3 Amino acid profiles of silk powder from *Bombyx mori* Linn. (Nang-Noi Srisakate 1) and commercial product.

Amino acid	(mole %)[1/]	(mole %)[2/]
Aspartic acid	4.66	2.10
Serine	13.87	9.70
Glutamic acid	1.92	1.50
Glycine	41.90	42.90
Histidine	Not detected	Trace
Arginine	1.06	0.10
Threonine	2.04	0.90
Alanine	24.53	30.60
Proline	0.44	0.30
Cystine	Not determined	Trace
Tyrosine	4.51	4.90
Valine	2.55	2.60
Methionine	Not determined	Trace
Lysine	0.67	0.50
Isoleucine	0.56	1.00
Leucine	0.63	0.60
Phenylalanine	1.07	2.30

[1/] Silk powder (Nang-Noi Srisakate 1)
[2/] Commercial product

that the glycine and alanine contents of laboratory silk powder were rather smaller whereas the contents of other amino acids were much higher than those from the commercial product. This might be because the amino acid compositions of raw materials for silk powder production were affected by species of silk. The result was consistent with the experiment done by Hemachantorn (1987) that the chemical compositions of silk protein could be influenced by silk species and feeds and this, therefore, would be able to indicate the amino acid pattern of silk products.

CONCLUSIONS

Silk cocoon could be converted into fine particle with 100% water solubility by using the mixture of solvents. Among the solvent mixtures, the mixture containing $MgCl_2$/ethanol/H_2O at mole ratio of 0.8:2:8 showed the best for completely solubilizing silk cocoon due to its capability of penetrating into silk fiber and then resulting in their structure change. Comparison of resulting silk powder with the commercial "Promois®" silk powder clearly indicated their similar properties, therefore, it might be able to substitute as ingredient in the products containing the commercial silk protein. However, its moisture adsorption, UV protection as well as other related properties should be further evaluated.

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