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Hydrothermal production and characterization of protein and amino acids from silk waste

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Abstract

Non-catalytic hydrothermal decomposition of sericin and fibroin from silk waste into useful protein and amino acids was examined in a closed batch reactor at various temperatures, reaction times, and silk to water ratios to examine their effects on protein and amino acid yields. For the decomposition of sericin, the highest protein yield was found to be 0.466 mg protein/mg raw silk, obtained after 10 min hydrothermal reaction of silk waste at 1:100 silk to water ratio at 120 °C. The highest amino acid yield was found to be 0.203 mg amino acids/mg raw silk, obtained after 60 min of hydrothermal reaction of silk waste at 1:20 silk to water ratio at 160 °C. For the hydrothermal decomposition of fibroin, the highest protein yield was 0.455 mg protein/mg silk fibroin (1:100, 220 °C, 10 min) and that of amino acids was 0.755 mg amino acids/mg silk fibroin (1:50, 220 °C, 60 min). The rate of silk fibroin decomposition could be described by surface reaction kinetics. The soluble reaction products were freeze-dried to obtain sericin and fibroin particles, whose conformation and crystal structure of the particles were shown to differ from the original silk materials, particularly in the case of fibroin, in which the change from β-sheet conformation to α-helix/random coil was observed.
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1. Introduction

In the silk industry, tons of silk wastes are produced annually from damaged cocoons or from the cocoons that are difficult to unreel. Like silk, major components of silk waste are fibroin and sericin proteins. Fibroin constitutes the core fibers which are encased in the gummy layers of sericin coat. In silk textile processing, sericin is usually removed, resulting in fine silk fibers whose textural properties and wettability are enhanced. The resulted fibroin fibers can be used to make fabrics or can be further hydrolyzed or solubilized to obtain silk fibroin solution. The solution might be processed into particles used in a wide range of applications. The removed sericin, which was once considered waste of silk industry and was generally discarded, has nowadays been realized for various applications in biomaterial, biomedical, and pharmaceutical industries (Zhang, 2002; Wu et al., 2007).

Conventionally, removal of sericin is achieved in boiling water but boiling water alone is ineffective. Alternatively, the process could be catalyzed by the addition of acid or alkali, however, acids or alkali are considered toxic chemicals and the severe conditions used make the process unfavorable. The development of an effective degumming process based on enzymes as active agents would entail savings in terms of water, energy, chemicals, and effluent treatment (Gulrajani et al., 1996). However, the higher cost of enzymes themselves has so far limited the development of industrial processes.

Fibroin fibers can also be made into solution by acid or alkali hydrolysis, similar to that described for sericin removal. Alternatively, mixtures of an aqueous salt solution and an organic solvent such as $CaCl_2/H_2O/EtOH$ may be used to dissolve fibroin (Hino et al., 2003; Nam and Park,

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2001). Although these solvents can dissolve silk fibers quickly, the disadvantage of this method is the requirement of time consuming steps to remove the impurities such as salt and toxic solvent.

In recent years, a number of studies demonstrated that water at near and above critical conditions ($T = 374.2$ °C, $P = 22.05$ MPa) is an environmental friendly medium for various oxidation and hydrolysis reactions (Barner et al., 1992; Goto et al., 2004). At these conditions, the ion product of water, K_w, changes specifically around the critical point. The value of K_w at 25 MPa increases with temperature up to 10^{-11} at 523.2 K and then decreases to 10^{-19} at 673.2 K and 10^{-22} at 773.2 K. Due to the large change of ϵ and K_w with temperature and pressure, the reaction type changes from ionic reaction to radical reaction. Supercritical water (at the conditions above the critical point) is mostly used for decomposing municipal organic waste (Goto et al., 2004) and harmful substances such as polychlorinated biphenyls (PCBs) and sodium sulfate (Rogak and Teshima, 1999). Subcritical water, or pressurized water at the temperatures above boiling but below critical temperature, is generally employed for milder hydrolysis or hydrothermal reactions, which have been demonstrated by several studies to effectively convert cellulosic (Sasaki et al., 1998; Goto et al., 2004) and proteinaceous biomass such as fish meat (Yoshida et al., 1999), silk fibroin powder (Kang and Chun, 2004), and baker's yeast (Lamoolphak et al., 2006), into useful products.

The aim of this study was to investigate the potential use of subcritical water for the hydrothermal conversion of silk waste from a local silk industry into protein and amino acids. The optimal conditions for the hydrothermal conversions of sericin and fibroin were first determined. The molecular size of protein in the soluble products was analyzed using SDS–PAGE and the amount of protein and amino acids yields were analyzed using Lowry's method and the Ninhydrin assay. The kinetics of silk fibroin decomposition was proposed which provided fundamental information useful for a large-scale process. Moreover, the silk sericin and fibroin powder prepared by freeze-drying the sericin and fibroin solutions was characterized.

2. Experimental

2.1. Materials

The silk waste was obtained from local silk farm (Mahasalakham, Thailand). The silk waste was rinsed in cold water to get rid of the contaminants, and was then cut into short fragments having the average length of 100 mm. The silk sample was then soaked in distilled water for 5 min prior to the hydrothermal reaction.

2.2. Hydrothermal conversion of silk sericin

The removal of sericin by hydrothermal reaction was carried out in a 100 ml SUS-316 stainless steel pressure vessel (AKICO, Japan). After loading the sample and deionized water, the vessel was then tightened and heated to a desired temperature by means of a heating jacket. The reaction was allowed to take place for a specified period, after which the reactor was immediately cooled to room temperature by submerging it into a water bath. The reaction products consisted of the aqueous solution and the remaining silk residue, which were separated from the soluble product using a filter paper (Whatman no. 1, Maidstone, England). The sample residue was then dried in a vacuum oven at 60–70 °C and the solution was assayed to determine the molecular weight range using SDS–PAGE. The amount of total protein, and total amino acids in the soluble products were analyzed and the structural conformation of the solid residue was examined. In this study, the effects of temperature (120–160 °C), ratio of sample/deionized water (1:20–1:100), and reaction time (10–60 min) on the product yields were determined. The pressure in the reactor was estimated based on saturated steam to be between 0.1985 and 0.6178 MPa for the temperature range studied.

2.3. Hydrothermal conversion of silk fibroin

The silk fibroin fiber used for the hydrolysis experiment was obtained after degumming the silk waste at the optimal conditions. This was achieved by using a general laboratory autoclave, operated at 120 °C for 30 min, which allowed a large amount of fibroin fiber to be obtained. The hydrothermal conversion of the fibroin sample was carried out in the same manner as that described earlier for sericin conversion. The experiments were however conducted over a higher temperature range of 160–220 °C whose corresponding saturated vapor pressures, based on steam table data, were between 0.62 and 2.79 MPa. The reaction time ranges between 10 and 60 min and the weight ratio of sample to deionized water was between 1:20 and 1:100.

2.4. Analysis

2.4.1. SDS–PAGE

The molecular weight distribution of the constituents of silk sericin and fibroin solution was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) with 12% acrylamide gel and 5% condensing gel, which was stained with 0.25% Coomassie Brilliant Blue R-250 (Aldrich, WI, USA), according to the method described previously by Laemmli (1970).

2.4.2. Protein and amino acid composition

The protein content in the sericin and fibroin solution was assayed by Lowry's method (Lowry et al., 1951) using bovine serum albumin (BSA) as a standard. Amino acid content was analyzed by Ninhydrin assays using L-Alanine solutions as a standard. Briefly, Ninhydrin reagent, containing 1 ml of 1% w/w Ninhydrin solution, 2.4 ml of

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