CHAPTER I

INTRODUCTION

1.1 Rationale

Silk protein produced from cocoons of domesticated silkworm, *Bombyx mori*, can be applied in many fields such as textiles, degradable biomaterials, tissue engineering, biomembrane materials, and cosmetics. In silk industry, tons of silk waste are produced annually which arise from damaged cocoons or from cocoons which are difficult to unreel, together with waste fibre from the processes preparatory to spinning.

Like silk, major components of silk waste are fibroin and sericin proteins. Fibroin consitutes the core fibers which are encased in the gummy layers of sericin coat. In silk textile processing, sericin is removed, resulting in fine silk fibres with enhanced textural properties and wettability. The resulting fibroin fibres can be used to make fabrics or further hydrolyzd to obtain silk fibroin solution or further processed into particles used in various applications. The sericin which was once considered waste of the degumming process has nowadays been realized for a wide range of applications.

Conventionally, degumming is achieved by acid or alkali hydrolysis. However, toxic chemicals and severe conditions used make the process unfavorable. The degummed silk must also be thoroughly rinsed with large amount of water. The development of an effective degumming process based on enzymes as active agents would entail savings in terms of water, energy, chemicals, effluent treatment. The surface of silk fibre degummed with enzymes is smoother than soap and alkali degummed fibre (Gulrajani et al., 1996). However, the higher cost of enzymes themselves has so far limited the development of industrial processes.

Like sericin, fibroin fibres can also be made into solution used in various applications. This is done through similar processes as those used for degumming. Alternatively, mixtures of aqueous solution of salts and organic solvents such as $CaCl_2/H_2O/EtOH$ may be used to dissolve fibroin. Although these solvents can dissolve silk fibres quickly, the disadvantage of this method is that it requires time consuming steps to remove the solvent impurities such as salt and toxic solvent, particularly when it is to be used in biomedical and pharmaceutical applications.

Recent studies demonstrated the use of water at near and above critical condition (T= 374.2 °C, P= 22.05 MPa) as environmentally friendly reaction media (Barner et al., 1992, and Goto et al., 2004). The reason is that at these conditions, 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 field changes from ionic reaction to radical reaction. In general, sub-or supercriticai water has been exploited in two major types of reactions: oxidation and hydrolysis. Supercritical water (above the critical conditions) is mostly used for decomposing municipal organic waste (Goto et al., 2004) and harmful substances such as polychlorinated biphenyls (PCBs) and sodium sulfate (Rogak et al., 1999). For milder reactions in subcritical water, Yoshida et al. (1999) studied the hydrolysis of fish meat without oxidants and Kang and Chan (2004) investigated the decomposition of commercial silk fibroin powder to amino acids in near-critical water. In addition, We conducted a study on hydrothermal decomposition of yeast cells for the production of proteins and amino acids and found that subcritical water could be used to potentially hydrolyze spent brewer's yeast cells, organic waste from brewing industries into more valuable proteins and amino acids. The amount of protein produced increases with an increase in temperature (Appendix D). All these studies demonstrate the potential use of water as reaction media for silk protein hydrolysis.

The aim of this study was to examine a new alternative for subcritical water hydrolysis of silk waste of the iocal silkworm to produce sericin and fibroin solution. The optimal conditions for degumming sericin as well as for hydrolsis of fibroin will first be determined. The protein solutions will further be formed into particles which will then be characterized. Based on these results, suggestion for specific applications can be made.

1.2 Objectives

1.2.1 To investigate the appropriate conditions for hydrolysis of sericin and fibroin derived from silk waste with subcritical water.

1.2.2 To determine the characteristics of silk sericin and silk fibroin solution obtained.

1.2.3 To determine the characteristics of sericin and fibroin particles.

1.3 Working scopes

1.3.1 Experimentation of subcritical water hydrolysis of silk sericin in a batch reactor at various temperatures between 120 and 160 °C, reaction times between 10 and 60 minutes, and the ratio of silk sample to water between 1:20 and 1:100.

1.3.2 Experimentation of subcritical water hydrolysis of silk fibroin in a batch reactor at various temperatures between 160 and 220 °C, reaction times between 10 and 60 minutes, and the ratio of silk sample to water between 1:20 and 1:100.

1.3.3 Investigatigation the molecular size of silk sericin and fibroin solution after processing with subcritical water at various conditions using SDS-PAGE and determinnation the protein and amino acids yields using Lowry's method and Ninhydrin's assay, respectively.

1.3.4 determination the reaction kinetics of the hydrolysis reactions in a batch reactor.

1.3.5 Formation silk sericin and fibroin particles from the solutions obtained by subcritical water hydrolysis and investigatation of the conformation and structural characteristics of the particles obtained with the optimal conditions using particle analyzer, differential scanning calorimetry (DSC), fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). The morphology of fibre and silk sericin and fibroin particles was also observed by scanning electron microscopy (SEM).

1.3.6 Suggestion of possible applications for these products.

1.4 Expected benefits

1.4.1. This study provides an environment friendly alternative for silk degumming and for preparing sericin and fibroin solution by subcritical water hydrolysis to obtain products with high yield, and quality.

1.4.2. This study suggests fundamental information useful for large-scale hydrolysis process for the production of silk protein microparticles used in various applications.