CHAPTER III
EXPERIMENTAL

3.1 Materials

Materials used in the present study are as follows:

3.1.1 Waste Cocoons

Waste cocoon used in this study were

- Polyvoltine waste cocoon, Nang Noi
- Polyvoltine x Bivoltine waste cocoon, Dok Bua
- Bivoltine waste cocoon, Jul

3.1.2 Nylon Fiber

Nylon fiber used in this study was kindly supplied by Asia Fiber Public Company Limited.

3.1.3 Polyester Fiber

Rajamangala Institute of Technology kindly supplied polyester fiber used in this study.

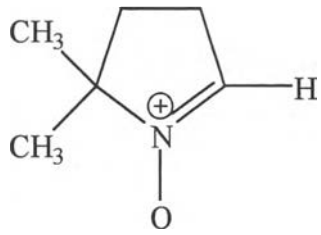
3.1.4 Sericin Powder

Sericin powder of Jul , Dok Bua and Nang Noi were kindly supplied by Office of Atomic for Peace.

3.1.5 Potato Dextrose Agar (PDA) and Nutrient Agar (NA)

PDA, a fungal media used for antifungus testing, and NA, a bacterial media used for antibacterial testing, were kindly supplied by Office of Atomic for Peace.

3.1.6 5,5-Dimethyl-1-pyrroline-N-oxide (DMPO)



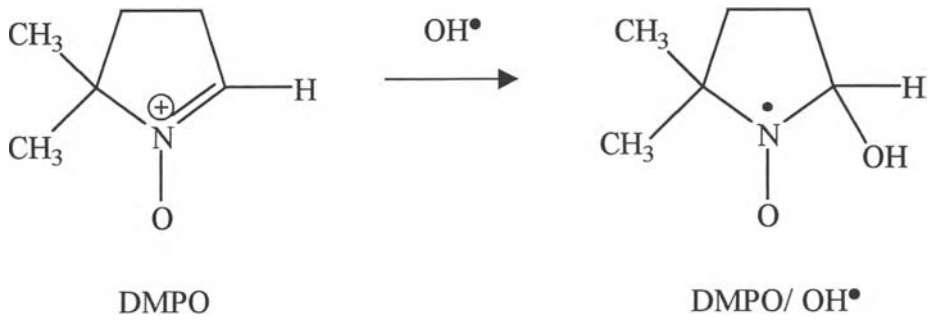
Scheme 2 5,5-Dimethyl-1-pyrroline-N-oxide (DMPO) structure.

DMPO (scheme 2) of purum grade of Fluka was used as spin trapping of hydroxyl radicals. Hydroxyl radicals were formed by Fenton reaction (Scheme 3)

$$Fe^{2+}\text{-EDTA} + H_2O_2 \longrightarrow Fe^{3+}\text{-EDTA} + {}^{\bullet}OH + OH^-$$

Scheme 3 Fenton reaction.

Which were trapped by DMPO and formed DMPO-OH spin-adduct (Scheme 4) and could be directly measured by electron spin resonance.



Scheme 4 Reaction between DMPO and hydroxyl radical.

3.2 Experimental Procedure

3.2.1 Sericin Solution Preparation

Sericin solution was prepared for coating nylon and polyester fiber. Sericin solutions were prepared at concentrations of 20 wt %, 15 wt% and 10 wt% of sericin in distilled water. The waste cocoon was cut into pieces about 1 cm^2 and was boiled in boiling distilled water for 1 hour. The waste cocoon was then filtered out to obtain the sericin solution.

3.2.2 Nylon Fiber and Polyester Fiber Coating

Nylon fiber and polyester fiber were immersed in sericin solution at different concentrations for 1 minutes. Then the fibers were placed in the oven at 60°C for 1 hour. The morphology of the coated fibers and the coated thickness were determined using Scanning Electron Microscopy (SEM).

3.2.3 Antifungus and Antibacterial Testing

Filter papers were cut in a circle with an area of 0.822 cm^2. The filter papers were immersed in the designated sericin solution for 5 minutes. The immersed papers were put in the PDA plate for antifungus testing and NA plate for antibacterial testing. The plates were exposed to the air for 30 minutes. Two days later the clear zone between sample and fungus was measured on the PDA plates and the bacterial colonies within an area of 2.672 cm^2 were counted and compared to the number within an area of 1.850 cm^2 for the NA plates.

3.2.4 Sample Solution for Antioxidant Testing

The hydroxyl radical was prepared from the method of Fenton reaction system. The sample solution was prepared by adding 1mM $FeSO_4$, sericin sample (10% and 20%), 0.92 M DMPO, and 0.1 mM H_2O_2. Sixty seconds after mixing, the sample solution was placed in the sample cell.

3.3 Equipment

3.3.1 Wide Angle X-ray Diffractometer (WAXD)

The WAXD is a technique used to determine the structure of sericin. WAXD was performed at room temperature by a Rigaku Model D/MAX 2000 diffractometer. The CuKα radiation source was operated at 40 kV and 30 mA. Patterns were recorded by monitoring the diffraction appeared in the 2θ range from 2° to 90° with scan speed 5 degrees/minute and scan step 0.02 degree.

3.3.2 Thermogravimetric Analyzer (TGA)

Using a DuPont 2950 thermogravimetric analyzer, characterization of the sericin was performed by heating 10 mg of each sample up to 800 °C at a heating rate of 10 °C/min under nitrogen atmosphere. The mass change with increasing temperature was monitored and recorded.

3.3.3 Fourier-Transform Infared Spectroscopy (FTIR)

FTIR spectra were recorded on a EQUINOx55 BRUKER spectrometer within the wavenumber range 4000-400 cm^{-1} using a deuterated triglycinesulfate detector (DTGS) with a specific detectivity, D of $1x10^{9}cm.Hz^{0.5}.w^{-1}$. Spectra grade KBr (Carlo Erba) was used as a background. KBr was pelletized by using a hydraulic valve press. The sample was pressed under 8 kg/cm^2 for 2 minutes. Then it was transferred to the FTIR chamber. After the background spectrum was obtained, the sample was mixed with the dried KBr. The mixture was ground and pelletized into pellet shape by using hydraulic press and transferred to the sample holder in the chamber. The diameter of the sample was 1 cm and the thickness was about 0.005 cm.

3.3.4 Scanning Electron Microscope (SEM)

Scanning Electron Microscope (JOEL 5200) was used to study the morphology of the coated fibers and determine the coated thickness of various sericin concentrations. SEM digitized photographs were obtained with magnification between 1000-1500 times using an accelerator voltage of 25 kV. The sample was placed on the brass-stub using adhesive tape. The prepared samples were coated with a thin layer of Au via vacuum evaporation and placed into the specimen stage inside the microscope chamber.

3.3.5 Electron Spin Resonance Spectrometer (ESR)

Electron Spin Resonance Spectrometer (JEOL, JAPAN, model JES-RE2X) was used to study the scavenging activity on hydroxyl radical of sericin. The DMPO-OH spin adduct peak area were obtained by using the power of 10.00 mW, micro frequency 9.412 gHz, center field 335.006 mT, sweep width 5.0x1 mT, modulation width 0.63x0.1 mT, amplitude 200, time constant 0.03, modulation frequency 100 kHz and slow sweep time 30 second.