Rev.Adv.Mater.Sci. 5 (2003) 193-198

THE USE OF SERICIN AS AN ANTIOXIDANT AND ANTIMICROBIAL FOR POLLUTED AIR TREATMENT

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Received: June 30, 2003

Abstract. The aim of this study was to enhance the properties of air filters by coating the filter with silk sericin, which is mostly discarded in wastewater during silk processing. The antioxidant activities against hydroxyl radical (OH^-), the antimicrobial activities and the fiber-coated surface were studied in silk sericin from different species of waste cocoon; Polyvoltine waste cocoon (Nang Noi), Polyvoltine x Bivoltine waste cocoon (Dok Bua), and Bivoltine waste cocoon (Jul). The concentration of sericin solution in distilled water ranged from 10 to 20%. Among the different species of waste cocoon, the Jul has the highest antioxidant inhibition of OH^- capacity, while the Dok Bua is the second with the Nang Noi having the lowest inhibition activity. For antifungus activity, the Dok Bua and Jul has comparable effective and Nang Noi the lowest capacity. The Dok Bua has the highest inhibition activity in Micrococcus type of bacteria, with Nang Noi second and Jul the lowest capacity. The coated surfaces of sericin on nylon fibers and PET fibers were smooth. This study demonstrates that sericin from the Dok Bua species has the best properties for coating air filters.

1. INTRODUCTION

In the formation of silk filament, the cocoon shell is composed of two proteins named fibroin, held together by a gum-like protein called “sericin”. Removal of the sericin from silk fibroin is accomplished by a process called “degumming”. Most of the sericin must be removed during raw silk production at the reeling mill and the other stages of silk processing. At present, sericin is mostly discarded in silk processing wastewater. If this sericin protein is recovered and recycled, it could lead to significant economic and social benefits. The amount of sericin ranges from 19 to 28 percent according to the type of cocoon, usually the sericin content of the cocoon shell is at the maximum level at the outside layer 1 becoming progressively lower at the middle layers 2 and 3 and the minimum at the inside layer 4 [1].

Sericin is a macromolecular protein. It’s molecular weight ranges widely from about 10 to over 300 kDa. The sericin protein is made of 18 amino acids most of which have strongly polar side groups such as hydroxyl, carboxyl, and amino groups. This protein can be cross-linked, copolymerized, and blended with other macromolecular materials, especially synthetic polymers, to produce materials with improved property [2]. Sericin is applicable as an antioxidant in the field of medicines, cosmetics, foods, and food additive [3]. The protein is also used as a coating material for natural and artificial fibers, which can prevent abrasive skin injuries, the development of rashes and antibacterial for the products such as diapers, diaper liners, and wound dressing [4].

Filter media can be treated with an antioxidant coating for example the cigarette filter having Thione Antioxidant Complex (TM) to reduce amount of free radical damage incurred by a smoker [5]. Free radi-

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Table 1. Characteristic of the three species of silk cocoon.

Name	*Shape of cocoon*	*Cocoon Color*	*Country of Origin*	*Details*
Nang Noi	Spindle	Yellow	Thai	Polyvoltine
Dok Bua	Oblong	Yellow	Thai + Japan	Polyvoltine x Bivoltine
Jul	Oblong	White	Japan	Bivoltine

cal is a highly reactive species that can drastically attack all biological molecules including lipid, DNA and protein by initiating a free radical chain reaction [6]. Environmental free radicals enter human body through skin, respiration and other means. Toxic radicals are not only in the air, but are also part of some microorganisms. Fungi and bacteria are important factors influencing indoor air quality. A wide range of fungal and bacterial species can be isolated from indoor air. Several health effects have been associated with fungal and bacterial species in the indoor environment. Filter media coated with an antibacterial/antifungi coating will keep the filter from being a potential incubator of fungi and bacteria, which are airborne and would potentially grow on the filter [7].

As sericin has a high antioxidant and antibacterial activity, this means sericin can stop the oxidation reaction of free radical and inhibit microorganisms growth leading to numerous diseases. The application of this study is to use sericin coated on fiber as an air filter to reduce the amount of free radical enter through the body and inhibit microorganisms growing on the air filter media. Different species of silk cocoon have different properties of sericin depending on its amino acid composition and amount of each amino acid. This work studies the different action on antioxidant against hydroxyl radical and antimicrobial of three different cocoon species.

2. EXPERIMENTAL DETAILS

2.2. Materials

Three species of waste cocoon are use in this study. Table 1 summarized the characteristic of the three species of silk cocoon. The sericin powder, PDA, a fungal media used for antifungus testing, and NA, a bacterial media that used for antibacterial testing, were kindly supplied by Office of Atomic for Peace. Nylon fiber and polyester fiber for coated with sericin were kindly supplied from Asia Fiber Public Company Limited and Rajamangala Institute of Technology respectively. 5,5-Dimethyl-1-Pyrroline-N-Oxide (DMPO) of purum grade of Fluka was used as spin trapping of hydroxyl radicals.

2.2. Experimental Procedure

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 [8]. Nylon fiber and polyester fiber were immersed in sericin solution at different concentrations for 1 minute. 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 (JOEL 5200) (SEM). SEM digitized photographs were obtained with magnification between 1000-1500 times using an accelerator voltage of 25 kV.

The functional group and absorbency of the three species of sericin powder were determined using Fourier-Transform Infrared 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.

Electron Spin Resonance Spectrometer (ESR) (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 fre-

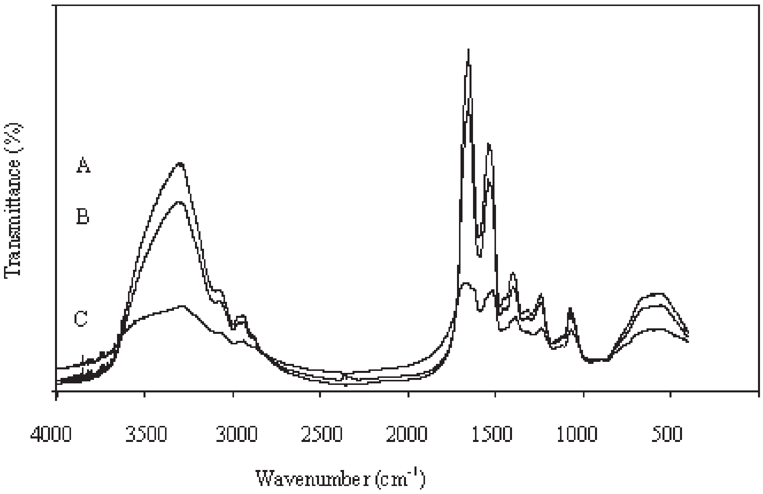
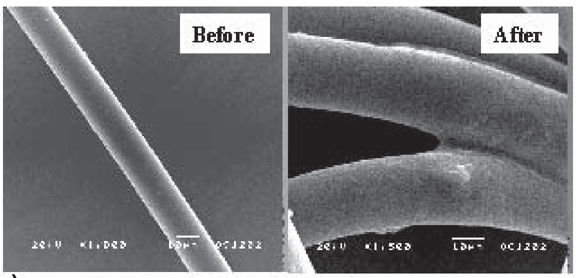


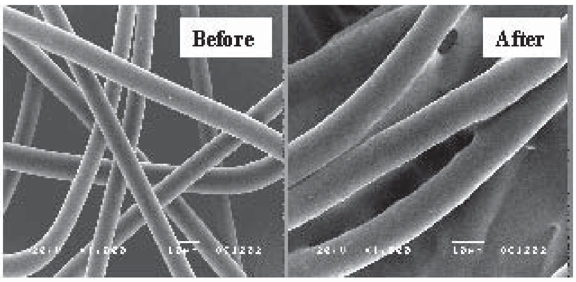
Fig. 1. FT-IR spectra of: (A) sericin Dok Bua, (B) sericin Nang Noi, and (C) sericin Jul.

quency 100 kHz and slow sweep time 30 second. 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 [9].

The filter papers were cut in a circle with area 0.822 cm^2 and immersed in the designated sericin solution for 5 minutes for antimicrobial testing. 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.



a)



b)

Fig. 2. The morphology of: (a) nylon fiber and (b) polyester fiber coated with sericin.

3. RESULT AND DISCUSSION

3.1. FT-IR Characterization

FT-IR spectra of all sericin species are shown in Fig. 1, all of the species shown NH stretching band at 3440-3420 cm^{-1} and 1560-1530 cm^{-1}, OH stretching band at 3600 cm^{-1}, C=O stretching band at 1680-1640 cm^{-1} and C=O symmetry stretching at 1400 cm^{-1}. The interesting point was the different absorbance of each species, sericin Dok Bua has the highest absorbance followed by sericin Nang Noi and sericin Jul has lowest absorbance. This can be explained by the amount of amino acid composition of each sericin. Sericin Dok Bua and sericin Nang Noi have 18 amino acids, sericin Jul has only 14 amino acids.

3.2. Morphological Characterization

The morphology and the fiber diameter of the fiber coated with sericin are shown in Fig. 2 and Fig. 3, respectively. The average fiber diameters of each sericin concentration were calculated from thirty SEM pictures. Fig. 3 demonstrates that the Nylon

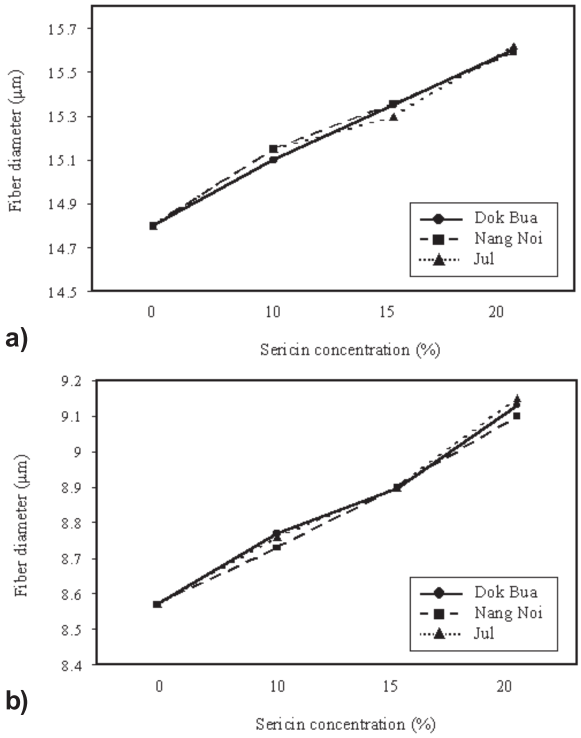


Fig. 3. The fiber diameter of: (a) nylon fiber and (b) polyester fiber coated with sericin.

and PET fiber diameters increased slightly with increased sericin concentration. The coating thickness are 0.3-0.8 μm for Nylon and 0.15-0.6 μm for polyester respectively and are not significantly different for all three sericins.

The morphology of the coated fiber shows that the sericin coating is smooth along the fiber. There are also some sericin connections between two fibers suggesting that it acts like glue. Moreover, the micrographs reveal the fracture of sericin film indicating that sericin film is rather brittle.

3.3. Antioxidant Efficiency Characterization

Fig. 4 is the antioxidant efficiency resulting from peak area of DMPO-OH spin adducts by using ESR. The antioxidant efficiency obtained by using Eq. (1).

$$\%\text{Antioxidation} = \frac{100\%(\text{standart peak area} - \text{sample peak area})}{\text{standart peak area}} \quad (1)$$

The initial concentration of the hydroxyl radical was 0.06 mM. Decreasing in concentration of DMPO-OH spin-adduct indicated that the reaction has low

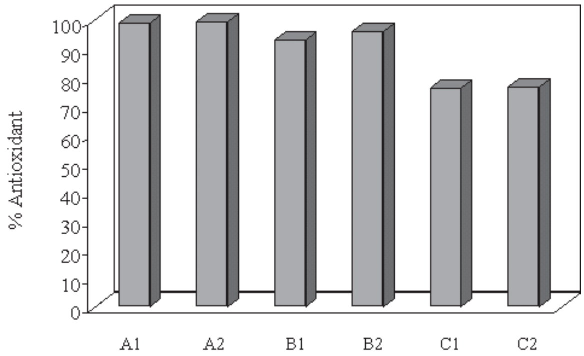


Fig. 4. Antioxidant efficiency of sericin: (A1, A2) sericin Jul 10%, 20%, (B1, B2) sericin Dok Bua 10%, 20% and (C1, C2) sericin Nang Noi 10%, 20%, respectively.

concentration of hydroxyl radicals. Clearly sericin reduces the concentration of the hydroxyl radicals, and hence sericin has antioxidant efficiency. There is slight increase in antioxidant efficiency with increasing sericin concentration. Sericin Jul has highest antioxidant efficiency follow by sericin Dok Bua and sericin Nang Noi has lowest antioxidant efficiency.

3.4. Antifungal Efficiency Characterization

The picture of fungal inhibition zone of sericin Dok Bua is shown in Fig. 5. Antifungal efficiency increases when the concentration of sericin increases as shown in Fig. 6. Furthermore, from the length of

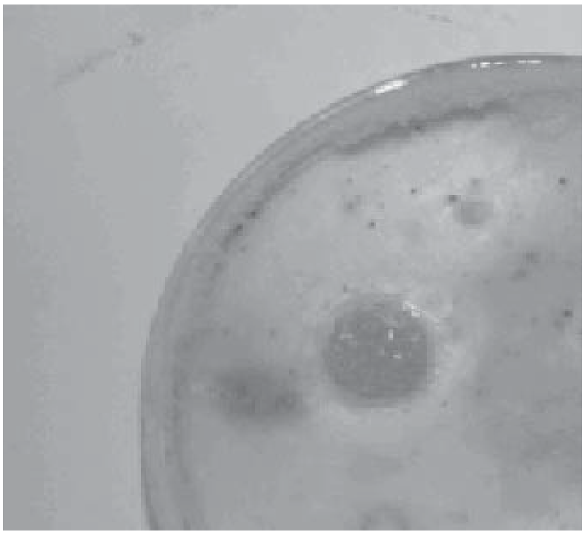


Fig. 5. The fungal inhibition zone of sericin Dok Bua.

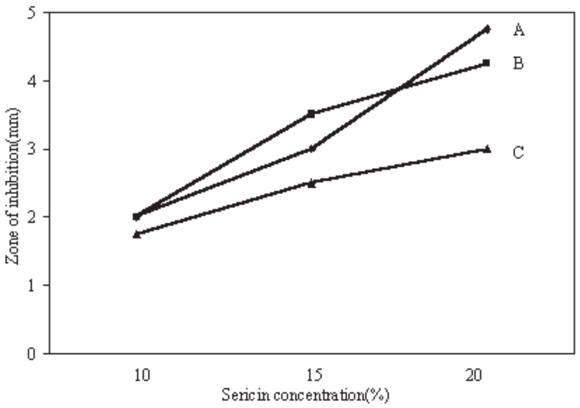


Fig. 6. Zone of fungus inhibition of sericin: (A) sericin Dok Bua, (B) sericin Jul, and (C) sericin Nang Noi.

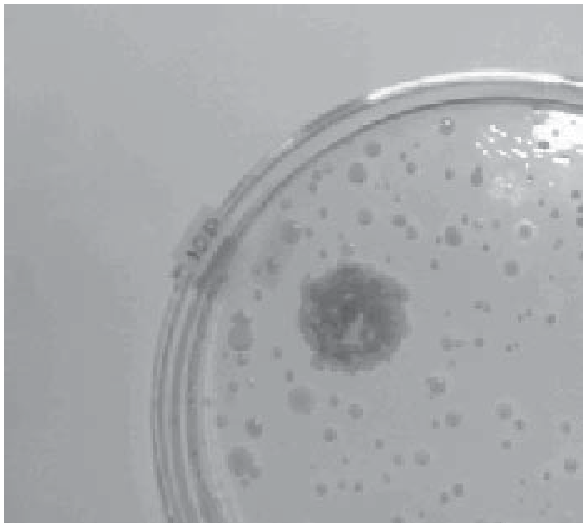


Fig. 7. The bacterial inhibition zone of sericin Dok Bua.

fungus inhibition, sericin Dok Bua and Jul has an equally capacity, and Nang Noi has the lowest capacity.

3.5. Antibacterial Efficiency Characterization

Fig. 7 shows the bacterial inhibition zone of sericin Dok Bua. It appeared from the NA plate that two types of bacteria colony was observed; the white colony (Bacillus type) which appeared around the sericin sample and the yellow colony (Micrococcus type) which slightly appeared around the sericin sample. This can be concluded that all sericin samples had no Bacillus type bacteria inhibition capacity but still had Micrococcus type inhibition capacity. The antibacterial efficiency obtained by using Eq. (2).

$$\%\text{Antibacterial} = \frac{100 - (100 \cdot \text{colony from sample area})}{\text{colony from standart area}} \quad (2)$$

From Fig. 8 the antibacterial efficiency increases when the concentration of sericin increases. Furthermore, from the amount of bacteria colonies, sericin Dok Bua has the highest antibacterial capacity, while Nang Noi is the second and Jul has the lowest capacity.

4. CONCLUSIONS

A new approach to use a natural material, sericin or adhesive silk protein to provide healthy environment is a promising future. Silk sericin could be coated onto nylon and polyester fibers and has a strong potential to be used for indoor air filters to reduce the amount of toxic free radicals, fungi, and Micrococcus type of bacterias. By using a simple coating technique, the sericin waste can increase the value of the air filter.

Among the different species of waste cocoon, Polyvoltine waste cocoon Nang Noi, Polyvoltine x Bivoltine waste cocoon Dok Bua, and Bivoltine waste cocoon, Jul, Jul has the highest antioxidant capacity inhibition of OH^-, while Dok Bua is the second and Nang Noi has the lowest inhibition activity. For antifungal activity, Dok Bua and Jul has equally ca-

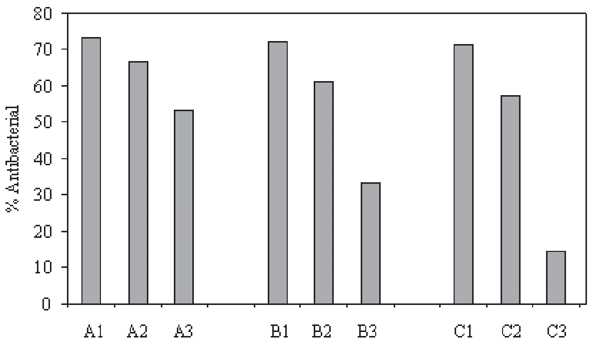


Fig. 8. Antibacterial (micrococcus type) of sericin: (A1, A2, A3) sericin Dok Bua 20%, 15%, 10%, (B1, B2, B3) sericin Nang Noi 20%, 15%, 10%, and (C1, C2, C3) sericin Jul 20%, 15%, 10%, respectively.

pacity, and Nang Noi has the lowest capacity. The inhibition activity in Micrococcus type of bacteria was found that Dok Bua has the highest capacity, while Nang Noi is the second and Jul has the lowest capacity. The coated surfaces of sericin on Nylon fiber and PET fiber were smooth along the fiber. Thus, sericin from Dok Bua has the best properties for coating the air filters.

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