CHARACTERIZATION AND DETERMINATION OF BIOFILM FORMING POTENTIAL OF A WASTE SERICIN-RICH PROTEIN MIXTURE

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ABSTRACT: A sericin-rich silk protein mixture (S_R) recovered from silk wastewaters was obtained in powder form and characterized in terms of molecular weight, elemental composition, pH, moisture and ash contents. The biofilm forming potential of the protein powder for making packaging materials was determined. Films made of protein mixture were quite fragile, hence protein mixture was blended with polyvinyl alcohol (PVA) at 5/100 ratio. Films were cast on glass sheets where glycerol was used as a plasticizer. These films were characterized in terms of oxygen permeability and mechanical properties. Although PVA films were found to be impermeable to oxygen, addition of sericin-rich protein powder resulted in higher oxygen permeability of films. The mechanical properties were found suitable for packaging purposes. These results revealed that sericin-rich protein powder can be used as an additive in making biofilms for packaging purposes where oxygen transmission is not a problem.

1. INTRODUCTION

Sericin is a valuable silk protein, which is currently discarded as a waste in textile industry in Turkey. Sericin has a variety of end-uses in industries such as cosmetics and pharmaceuticals, as well as production of biomaterials and membranes [Mori and Tsukada, 2000; Zhang, 2002]. In recent years, interest has grown for sericin recovery from silk wastewaters in view of promising research findings related to its useful properties such as antioxidation, UV resistance, moisture absorption and biocompatibility [Mondal *et al.*, 2007]. The aim of this study is to determine the characteristics and biofilm forming potential of a sericin-rich protein mixture recovered from silk wastewaters in an attempt to contribute to the value-added utilization of waste sericin.

2. RESULTS AND DISCUSSION

2.1. Characterization of recovered powder material

Sericin was recovered from wastewater by a two-stage process; nanofiltration plus ethanol-induced precipitation, where the precipitate was converted to powder form by freeze-drying [Capar *et al.*, 2008; Capar, 2012]. The pictures of pure sericin standard obtained commercially (S_C) and sericin-rich mixture (S_R) recovered from wastewater are depicted in Fig. 1. The LC–MS analysis of recovered protein mixture revealed that the powder contains

92.9% sericin and 7.1% actin cytoplasmic A3 protein, where the iso-electric point (pI) of sericin is 5.9 [Capar, 2012].

(a) (b)
Figure 1: Powder sericin; (a) commercial (b) recovered from wastewater.

Sericin is a family of proteins with a wide range of molecular weights (MW), i.e., 6-467 kDa. This is due to the fact that MW of sericin is affected by factors such as pH, temperature and processing time. In this work, the MW of commercial and recovered sericin samples were determined as 138 kDa and 90 kDa, respectively. Recovered sericin can be classified as high-MW sericin (>20 kDa), which is suitable for making biomaterials [Zhang, 2002]. As seen from Table 1, elemental compositions of commercial and recovered samples are similar, although C, H and N contents of S_R are slightly lower. The differences were attributed to the fact that commercial powder is pure sericin, whereas recovered powder contains 7% impurity.

Table 1: The elemental composition of sericin-rich powder (dry basis)

	S_C	S_R
C%	42.5	37.9
H%	6.4	5.8
N%	13.9	10.2
S%	0.3	0.4
Total (%)	64.9	54.3

As seen from Table 2, pH of S_C was acidic whereas pH of S_R was near neutral, which is attributed to the possible differences in methods of recovery. Recovery method of S_R is unknown, but it seems to be applied at acidic conditions. On the other hand, sericin was recovered under neutral pH in this work. Moisture content of S_C was higher than that of S_R. On the other hand, ash content of S_C was lower than that of S_R, which means that the organic content of recovered sericin was lower. This was attributed to the likely presence of some inorganic matter such as salts in the recovered sample as well as the presence of another protein in the mixture.

Table 2: pH, moisture and ash contents

	S_C	S_R
pH	3.9	7.7
Moisture, %	7.4	3.1
Ash, %	2.7	14.4

2.2. Biofilm forming potential of recovered material

The biofilm forming potential of recovered sericin-rich protein mixture was determined in order to evaluate its compatibility as a packaging material. Films made of pure sericin are known to be too fragile, therefore it is generally blended with other materials. In this work, sericin-rich mixture was blended with polyvinyl alcohol (PVA) (hot water soluble, average molecular weight 70 kDa, viscosity 11-14 cP). It has been reported that a hydrogel obtained by the mixture of sericin and PVA shows perfect elasticity and moisture absorption-desorption properties (Yoshii *et al.*, 2000). Glycerol was used as plasticizer.

PVA solutions of 10% (w/w) were prepared by stirring and heating at 80 ºC for 3 h. Sericin solutions of 5% (w/w) were prepared in a similar way by stirring and heating at 60 ºC for about 3 h. The pH of the sericin solutions was adjusted to 10 by NaOH (0.5 M) to enhance its solubility. The glycerol solution was prepared by mixing glycerol with water and stirring (40% v/v). Sericin/PVA blend ratio of 5/100 was used. The blend solutions were stirred and heated at 80 ºC for at least 1 h and then degassed for 1 h. After adjusting the blade thickness to 250 μm, and setting up the casting machine, 5-10 mL of solution was poured into the reservoir of the blade, which was then cast on glass. Then, the films were left overnight for drying at 30º C, followed by heat treatment at 75 ºC for 30 min to improve cross-linking. After the films were removed from glass, they were labeled and stored at room conditions (Figure 2). The specifications of films are given in Table 3. Films were characterized by oxygen permeation, SEM and mechanical properties.

Figure 2: Biofilm made of PVA and recovered sericin-rich protein powder

Table 3: Specifications of films

Film type	Sericin/PVA	Glycerol (%)
PVA	0/100	0
PVA+Gly	0/100	1
PVA+Gly+S_C	5/100	1
PVA+Gly+S_R	5/100	1

As seen from Table 4, the permeances of pure PVA films with and without glycerol are almost the same and very low, which indicate that PVA films are impermeable to oxygen. On the other hand, preliminary studies had shown that films including sericin have good oxygen permabilities (data not shown). Therefore, films including sericin were subjected to permeability tests (Table 5).

Table 4. Permeances of pure PVA films

Film type	Test duration (min)*	Permeance* (mol $m^{-2}s^{-1}$ Pa^{-1})
PVA	1268	5.9×10^{-17}
PVA+Gly	1265	6.0×10^{-17}

*average of two samples from the same film

Table 5. Permeability of sericin films

Film type	Permeability (Barrer) (cm3.cm)/(s.cm^2.cmHg) $x10^{-10}$
PVA+Gly+S_C	3258 ± 93
PVA+Gly+S_R	1371 ± 88

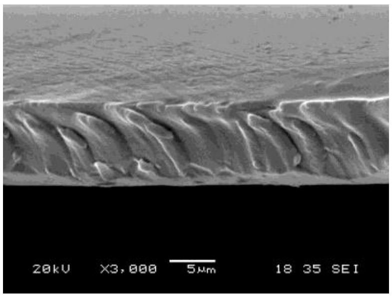

Figure 3: SEM analysis of pure PVA film

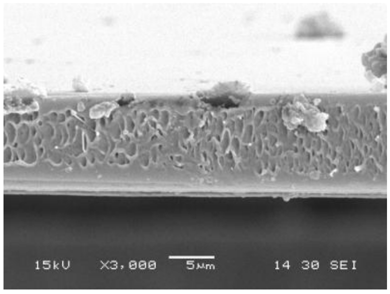

Figure 4: SEM analysis of sericin film (PVA+Gly+S_R)

As seen from Figure 3, films made of pure PVA are dense, symmetric and nonporous, which supports the results of very low oxygen permeance. However, when sericin is added into the films, a porous structure is observed, which is not uniform throughout the cross-section. (Figure 4). These results show that addition of sericin increases the oxygen permeability of films significantly, due to the formation of porous structure.

The mechanical properties of films were also determined. Tensile strength of a material is the maximum stress it can withstand while being stretched or pulled before failing or breaking. Hence, it is a measure of film integrity. Tensile strength of films (1) PVA, (2) PVA + Gly, (3) PVA + Gly + S_C and (4) PVA + Gly + S_R were determined as 12.1 MPa, 12.7 MPa, 12.6 MPa and 20.5 MPa, respectively (Figure 5). These results show that addition glycerol and commercial sericin did not improve tensile strength, whereas addition of sericin-rich protein powder significantly increased tensile strength. This may be due to differences in recovery methods of sericin samples. The recovery method of commercial sericin is unknown, but its pH is 3.9, which implies that it was recovered at acidic conditions. On the other hand, sericin-rich protein powder was recovered via membrane processes plus ethanol precipitation and has a pH of 7.7. Furthermore, S_R contains another protein, actin cytoplasmic A3. The ash contents were also different, where S_R has much higher inorganic content as compared to S_C. All these factors might have contributed to the improved tensile strength of the film containing S_R. However, more research is needed to verify these results.

For comparison, it is useful to note that tensile strengths of some materials such as human skin, high-density polyethylene and steel are 20 MPa, 37 MPa and 400 MPa, respectively [Wikipedia, 2013]. The tensile strength of a biodegradable film made of wheat gluten (protein) was reported as 8 MPa, where addition of xylan (polysaccharide) reduced the tensile strength down to 2 MPa depending on its type and ratio of addition. These films were found acceptable [Kayserilioglu *et al.*, 2003]. It can be inferred from these information that recovered sericin-rich powder is mechanically compatible as a biofilm additive.

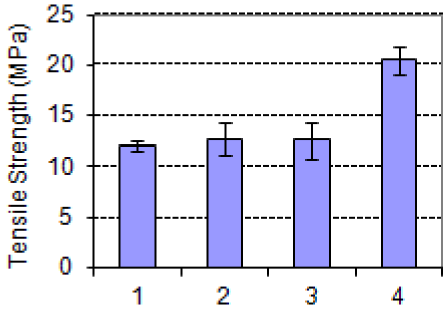

Figure 5: Tensile strength
(1) PVA (2) PVA + Gly (3) PVA + Gly + S_C (5/100) (4) PVA + Gly + S_R (5/100) (average of 2 duplicate films, where 5 samples from each film were measured)

Young's modulus is a measure of film stiffness and measures the resistance to elastic deformation under load. Young's Modulus for films (1) PVA, (2) PVA + Gly, (3) PVA + Gly + S_C and (4) PVA + Gly + S_R were determined as 352 MPa, 182 MPa, 234 MPa and 392 MPa, respectively (Figure 6). It is clear that addition of glycerol as a plasticizer resulted in decreased Young's modulus, i.e., decreased stiffness. This is expected as the addition of plasticizer aimed to increase flexibility of films. However, addition of recovered sericin increased stiffness of films. For comparison, some examples of Young's modulus are given: 800 MPa for high density polyethylene, 200.000 MPa for steel, 10-100 MPa for rubber and 500 MPa for teflon.

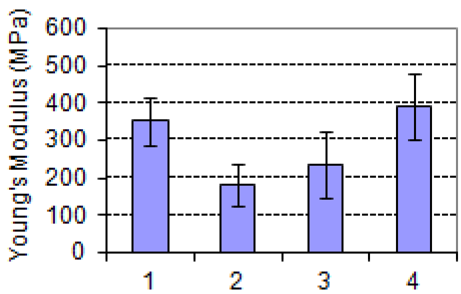

Figure 6: Young's modulus (Conditions are same as in Figure 5)

Elongation at break, which is a measure of film stretching was determined as 82%, 138%, 115% and 108% for (1)

PVA, (2) PVA + Gly, (3) PVA + Gly + S_C and (4) PVA + Gly + S_R, respectively (Figure 7). As expected, addition of plasticizer resulted in a significant increase of film stretching. On the other hand, presence of sericin caused some reduction in stretching property, but it was still higher than that of pure PVA films.

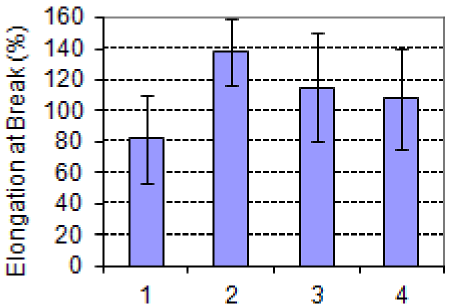

Figure 7: Elongation at break (Conditions are same as in Figure 5)

3. CONCLUSIONS

A biofilm was prepared using sericin-rich protein powder recovered from silk wastewaters by membrane processes.

The recovered sericin-rich powder had a molecular weight of 90 kDa, which is classified as high-molecular weight.

The pH, moisture and ash contents of recovered sericin differed from those of commercial sericin, which was attributed to possible variations of recovery methods. The C, H and N contents of recovered sericin was slightly lower than that of commercial sericin. This was attributed to the fact that recovered powder was a mixture of two proteins rather than pure sericin.

PVA films were dense, nonporous and impermeable to oxygen whereas sericin films were porous and permeable to oxygen.
The mechanial properties of films were satisfactory; it was revealed that sericin can be used as an additive of packaging materials for those applications where oxygen transmission is not a problem.

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