RECOVERY OF SERICIN PROTEIN FROM SILK PROCESSING WASTEWATERS BY MEMBRANE TECHNOLOGY

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ABSTRACT

RECOVERY OF SERICIN PROTEIN FROM SILK PROCESSING WASTEWATERS BY MEMBRANE TECHNOLOGY

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Cocoon cooking wastewaters (CW) and silk degumming wastewaters (SDW) of silk processing industry were treated by membrane processes for sericin recovery. CW contains only sericin while SDW contains both sericin and soap. Sericin in CW had four molecular weight (MW) fractions; 175-200 kDa (Sericin-1), 70-90 kDa (Sericin-2), 30-40 kDa (Sericin-3) and 10-25 kDa (Sericin-4). Two alternative process trains were developed for CW; 1. centrifugation + microfiltration + nanofiltration + precipitation, 2. centrifugation + microfiltration + nanofiltration + dialysis + precipitation. In the first process, a sericin/silkworm protein mixture was obtained with a sericin content of 39-46%. In the second one, however, a pure sericin product was obtained. The sericin recovery efficiency of the developed process train was found as 76%. Severe flux declines of 70-75% were observed in NF stage in both process trains. However, cleaning with 0.5 M NaOH and 190-200 mg/L free chlorine restored the fluxes by 83-127%.

The MW of sericin in SDW was 110-120 kDa. The soap and sericin were separated in the pre-treatment stage consisting of centrifugation (pH 3.5, 10 min) and gravity settling (4 ^{o}C, 24 h). The ultrafiltration membrane with molecular weight cut-off of 5 kDa achieved 59% sericin recovery at pH 3.5, accompanied by severe flux decline of 88%. Furthermore, clean water flux was restored by only 31% via chemical cleaning.

Keywords: Nanofiltration; Recovery; Sericin; Silk processing wastewater; Ultrafiltration

ÖZ

İPEK İŞLEME ATIKSULARINDAN MEMBRAN TEKNOLOJİSİ İLE SERİSİN PROTEİNİNİN GERİ KAZANILMASI

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İpek endüstrisinin koza pişirme (KA) ve ipek yumuşatma atıksuları (İYA), serisin geri kazanımı için membran prosesleri ile arıtılmıştır. İYA, serisin ve sabun içerirken; KA sadece serisin içermektedir. Koza pişirme atıksuyunda dört ayrı molekül ağırlığına sahip serisin bulunmuştur; 175-200 kDa (Serisin-1), 70-90 kDa (Serisin-2), 30-40 kDa (Serisin-3) ve 10-25 kDa (Serisin-4). Koza atıksuları için iki alternatif süreç geliştirilmiştir; 1. santrifüj + mikrofiltrasyon + nanofiltrasyon + çökeltme, 2. santrifüj + mikrofiltrasyon + nanofiltrasyon + diyaliz + çökeltme. İlk süreçte, %39-46 serisin içeriğine sahip olan serisin/ipekböceği proteini karışımı elde edilmiştir. İkincisinde, ise saf serisin elde edilmiştir. Geliştirilen geri kazanım sürecinin serisini geri

kazanma verimi %76 olarak bulunmuştur. NF aşamasında her iki süreçte %70-75'lik ciddi akı azalmaları gözlenmiştir. Ancak, 0,5 M NaOH ve 190-200 mg/L serbest klorla yapılan yıkama ile akılar %83-127 oranında iyileştirilmiştir.

İYA'daki serisinin molekül ağırlığı 110-120 kDa olarak bulunmuştur. Atıksudaki sabun ve serisin, santrifüj (pH 3,5, 10 dk) ve cazibe ile çökmeyi (4 ^{o}C, 24 s) içeren ön-arıtma aşamasında ayrılmıştır. Molekül ağırlık ayırma sınırı 5 kDa olan ultrafiltrasyon membranı, %88'lik ciddi bir akı azalması ile birlikte, pH 3,5'te %59 oranında serisin geri kazanımı sağlamıştır. Bunun yanı sıra, saf su akısı kimyasal yıkama ile sadece %31 oranında iyileştirilebilmiştir.

Anahtar Sözcükler: Nanofiltrasyon; Geri kazanım; Serisin; İpek işleme atıksuyu; Ultrafiltrasyon;

To My Father,

Secaattin Aygün...

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ABBREVIATIONS

AOAC	: Association of Official Analytical Chemists
APHA	: American Public Health Association
ASABE	: American Society of Agricultural and Biological Engineers
BCA	: Bichinchoninic Acid
BOD	: Biochemical Oxygen Demand
BTTG	: British Textile Technology Group
CFG	: Centrifugation
CMF	: Concentration Mode of Filtration
CP	: Concentration Polarization
COD	: Chemical Oxygen Demand
CW	: Cocoon Cooking Wastewater
Da	: Dalton
EPA	: Environmental Protection Agency
EWA	: European Water Association
FDH	: Frozen-Defrosted-Heated
FPLC	: Fast Performance Liquid Chromatography
GC-MS	: Gas Chromatography-Mass Spectrometry
GPC	: Gel Permeation Chromatography
GS	: Gravity Settling
HPLC	: High Performance Liquid Chromatography
J_{cw}	: Clean Water Flux
J_{ww}	: Wastewater Flux
kDa	: Kilo Dalton
kPa	: Kilo Pascal
MALDI-TOF MS	: Matrix-Assisted Laser Desorption/Ionisation-Time of Flight Mass Spectrometry
MF	: Microfiltration
MW	: Molecular Weight
MWCO	: Molecular Weight Cut-Off

NF	: Nanofiltration
NTU	: Nephelometric Turbidity Unit
Pt-Co	: Platinium Cobalt
PVA	: Polyvinyl Alcohol
RO	: Reverse Osmosis
rpm	: Revolution Per Minute
S_C	: Commercial Sericin
S_N	: Native Sericin
S_R	: Recovered Sericin
SDS-PAGE	: Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
SDW	: Silk Degumming Wastewater
SEM	: Scanning Electron Microscopy
TFC	: Thin Film Composite
TMP	: Trans Membrane Pressure
TOC	: Total Organic Carbon
TRMF	: Total Recycle Mode of Filtration
TS	: Total Solids
UF	: Ultrafiltration
USEPA	: United States Environmental Protection Agency
UV	: Ultraviolet
UVA	: Ultraviolet Absorbance
VRF	: Volume Reduction Factor
WPCR	: Water Pollution Control Regulation

CHAPTER 1

INTRODUCTION

1.1. General Information

Textile industry is one of the most important and largest industrial sectors in the world. It has a high importance in terms of its environmental impact since it is characterized by the consumption of a great variety of chemicals and high volumes of fresh water (Barredo-Damas et al., 2006; Fersi et al., 2005; Tüfekçi et al., 2007). In addition, textile industry has several subcategories, which lead to the generation of effluents with quite different characteristics and flow rates. Hence, it is difficult to apply a general treatment method successfully for all textile effluents. In this regard, each textile subcategory needs to be handled individually.

Silk processing is a subcategory of textile industry, which covers activities such as production of cocoons, spinning of the silk yarn, dyeing and manufacturing of the final products. These activities lead to the generation of cocoon cooking, silk degumming and dyeing wastewaters. In silk processing, firstly, the cocoons are cooked to kill off the insects and unwind the silk fibers. The silk fibers are enveloped by the silk gum, namely sericin, which must be removed prior to dyeing. The degumming process is used to remove the external sericin, which consists of boiling silk fibers in a hot water bath containing soap and sodium carbonate, where the silk fiber loses 25-27% of its original weight, corresponding to the amount of sericin discarded in the wastewater.

Water consumption in silk processing industry can be considered to have a moderate rate, where 50-240 liters of water is used for one kg of product (EWA, 2005). In Turkey, cocoon cooking wastewaters (CW) are generated seasonally with a flow rate of 1875 L/day for 80 days of the year and silk degumming wastewaters

(SDW) are generated with a flow rate of 7500 L/week throughout the year (Akdağ and Sipahioğlu, 2007).

CW and SDW have quite high COD, total solids, color and turbidity. The COD and sericin contents of CW are as high as 11-17 g/L and 5-8 g/L, respectively. On the other hand, SDW contains much higher amounts of organic matter, i.e., 55-63 g/L of COD and 27-34 g/L of sericin (Geçit et al., 2007a). Therefore, they need to be treated properly prior to discharge into the receiving environment.

There are a lot of conventional methods currently available for treating textile wastewaters to meet the discharge limits imposed by legislation: biological treatment, physico-chemical treatment, carbon adsorption and chemical oxidation with oxidizing agents like ozone and hydrogen peroxide. These conventional methods have been used for mainly meeting the discharge standards. In addition, advanced treatment methods have been gaining great attention because of increasing public awareness and the threat on the depletion of earth's resources. In this regard, industrialists and scientists have been forced to consider the recovery and reuse of valuable raw materials from industrial wastes as a method of sustainable development.

Membrane technology has emerged as a promising method for material recovery from industrial effluents in an efficient way. Membrane processes have wide range of applications in the textile industry. They have gradually become an attractive alternative to the conventional separation processes in the treatment of wastewater. They are used widely for protein separation. However, there are some problems related with membranes. One of them is high operational cost. The other problems are membrane fouling and concentration polarization, which occur during membrane processes. Membrane fouling is one of the critical issues in the application of membrane technology for protein purification (Kwon et al., 2008). However, the application of membrane filtration not only enables high removal efficiencies, but also allows recovery of water and some valuable waste constituents for possible end uses (Fersi et al., 2005).

The sericin in silk processing wastewaters is a valuable protein; however, it is currently discarded as a waste. Sericin can be used in food, cosmetics and pharmaceutical products as well as for manufacturing biomaterials because of its unique properties such as moisture absorption/desorption, antibacterial and antioxidant properties, and UV resistance (Fabiani et al., 1996; Rigueiro et al., 2001; Shen et al., 1998; Wu et al., 2007). The commercial value of sericin is high, as evidenced from the price of about 80-90 € per its gram on the market. The cocoon production in the world is about 1 million tons, which is equivalent to 400000 tons of dry cocoon, and processing of the raw silk produces about 50000 tons of sericin (Zhang, 2002). Therefore, the recovery of sericin from cocoon cooking wastewaters would provide economical benefits. It would also significantly reduce the environmental impact of silk production processes (Fersi et al., 2005) and help sustainable development.

1.2. Aim and Scope of the Study

The objective of this thesis is to recover sericin protein from the cocoon cooking wastewaters and silk degumming wastewaters of silk processing industry using membrane technology. The minimization of environmental pollution caused by wasting of sericin protein is also aimed.

In literature, there are a few studies on sericin recovery from silk processing industry (Fabiani et al., 1996; Vaithanomsat et al., 2007; Wu et al., 2007), where silk degumming wastewaters (SDW) were mainly used. However, no literature background seems to exist on the recovery of sericin from cocoon cooking wastewaters (CW), which are generated prior to SDW. The molecular weight (MW) distributions of sericin in CW and SDW are quite different. Sericin in SDW has mainly single MW fraction of 110-120 kDa whereas sericin in CW has a wide range of MW changing from 10 kDa to 200 kDa (Geçit et al., 2007a). Therefore, these wastewaters need to be handled individually to determine the most suitable membrane process for sericin recovery. In Turkey, silk processing has been performed for long years but it has decreased significanly in recent decades. Silk is imported but processed in our country, leading to the generation of wastewaters

containing high amounts of sericin. The recovery of sericin protein would definitely provide economical and environmental benefits for Turkey and other silk producing countries. However, there seems to exist no such study conducted in Turkey. International studies related with sericin are in the form of patents, where generated information and experience cannot be reached. Therefore, developing a method for the recovery of sericin from silk processing wastewaters will definitely contribute to existing literature since CW and SDW produced in Turkey have different properties when compared with silk wastewaters mentioned in literature.

The following studies were performed to achieve the targets of this thesis:

- Characterization of CW and SDW of silk processing industry,
- Determination of the MW distribution of sericin in CW and SDW to be recovered,
- Determination of the most suitable pre-treatment methods to membrane processes for sericin recovery from CW and SDW,
- Determination of the most suitable membrane processes for sericin recovery from CW and SDW,
- Characterization of the recovered sericin and assess its quality via comparison with the properties of commercial and native sericins used as reference.

In the first part of the study, sericin obtained from two sources were characterized in terms of moisture, organic and inorganic contents, elemental compositions, pH and MW in order to use them as reference for evaluating the properties of recovered sericin. The commercially obtained sericin (S_C) and native sericin (S_N) obtained from locally supplied cocoons had similar properties; however, the former had lower solubility in water. Hence, it was decided to use native sericin as a reference for comparison and as a calibration standard in sericin analysis.

In the second part of the study, sericin present in silk processing wastewaters was characterized prior to the selection of the most suitable pre-treatment methods and membrane separation processes for sericin recovery. The sericin concentrations and MW distributions of sericin in cocoon cooking wastewaters and silk degumming

wastewaters were determined. The MW of sericin in CW had four fractions named Sericin-1 (175-200 kDa), Sericin-2 (70-90 kDa), Sericin-3 (30-40 kDa) and Sericin-4 (10-25 kDa). On the other hand, the MW of sericin in SDW was 110-120 kDa. CW was also characterized in terms of the pollution parameters such as COD, total solids, color, turbidity and pH.

In the third part of the study, the most suitable pre-treatment method preceding membrane processes for CW was determined. This part of the research covers the comparison of the fluxes and removal performances of a variety of physico-chemical processes like gravity settling (GS), centrifugation (CFG), and microfiltration (MF) in single and sequential modes. In these alternatives, MF media having pore sizes of 1 μm, 8 μm and 20-25 μm were used. The most suitable pre-treatment method for cocoon cooking wastewaters was found as CFG + MF (1 μm). In this stage, 1-16% of sericin was lost.

In the fourth part of the study, the most appropriate membrane process for CW was determined. This part includes the comparison of the performances of two nanofiltration (NF) and three ultrafiltration (UF) membranes with molecular weight cut-offs (MWCO) of 20 kDa, 5 kDa and 1 kDa. The performances of these membranes were evaluated based on their rejection efficiencies for sericin and pollution parameters. The fouling behavior of selected UF and NF membranes were also evaluated in terms of the extent of flux decline and the efficiency of the membrane cleaning procedures for flux recovery. NF was found to be better than UF for recovery of all sericin fractions, whereas UF was found suitable for fractionation of sericin into different molecular weights. Hence, all fractions of sericin were concentrated by NF-DK and NF-90 membranes. In concentration mode of filtration tests, NF-DK was proved to perform better than NF-90, and selected as the best membrane for sericin recovery from CW.

Concentrated sericin was precipitated using acid and alternatively alcohol in order to determine the most suitable precipitation agent among HCl, HNO_3, H_2SO_4, $C_2H_4O_2$ and C_2H_6O. The recovered sericin samples were characterized and their properties were compared with those of reference sericin samples in terms of their elemental compositions (C, N, S and H), moisture, ash and organic contents. Their solubilities

and their UV scans were also compared. Moreover, to verify that the recovered powder was really sericin, ion exchange chromatography, 2-D gel electrophoresis and MALDI-TOF analyses were done. The comparison with SWISS-PROT and ExPASy protein databases showed that the recovered sericin was compatible with SER1 (O96614) with MW of 9161 Da and SER2 (O96615) with MW of 20302 Da.

The recovered sericin contained another protein originating from silkworm, which could not be separated by any of the membranes tested. Therefore, recovered sericin samples were dialyzed to increase their purities. The molecular weight distributions and elemental compositions of dialyzed samples were also determined. As a result, two alternative process trains were proposed; 1. CFG + MF (1 µm) + NF + precipitation for recovering sericin/silkworm protein mixture with a MW of 90 kDa (called low quality product), 2. CFG + MF (1 µm) + NF + dialysis + precipitation for recovering pure sericin with a MW of 44 kDa and 85 kDa (called high quality product). Possible application areas for both products were mentioned.

In the last part of the study, SDW was characterized in terms of sericin, total protein, MW, COD, total solids, color, turbidity and pH. Then, the most suitable pre-treatment method preceding membrane processes was investigated for prevention of fouling of the post membrane and separation of sericin from soap. In pre-treatment stage, physico-chemical methods such as GS, CFG and pH adjustment were adopted in single and sequential modes in order to choose the best one that minimizes loss of sericin and maximizes the removal of soap and other pollution parameters. The alternatives were evaluated based on sericin rejection performances and separation performances for soap. The best pre-treatment method was found as CFG at acidic pH (3.5) + GS (4 ^{o}C, 24 h). Then, three UF alternatives were tested for sericin recovery. In these alternatives, rejection performances for sericin, separation performances of UF for soap and sericin, removal performances of pollution parameters, and flux declines were investigated. The UF membranes with MWCO of 20 kDa and 5 kDa were used, and neither of them could separate sericin and soap. Hence, the best method for sericin recovery from SDW was found as CFG at acidic pH (3.5) + GS (4 ^{o}C, 24 h) + UF.

The presence of soap in SDW made sericin recovery a difficult task. Furhermore, the quality of recovered product may not be good enough as sericin may contain soap. An alternative degumming technique suggests the use of water vapor which is applied in boilers at a temperature of 120-130 ºC and a pressure of 300-400 kPa (Fabiani et al., 1996). This method was simulated in the laboratory in order to check whether the use of soap could be eliminated, which would cause less adverse environmental impacts. Sericin concentration and molecular weight of this simulated SDW, and soluble fraction of sericin were determined. The results showed that total soluble fraction of sericin was about 24%, which was consistent with the fraction obtained by conventional degumming process, i.e., 25-27%.

CHAPTER 2

SILK PROCESSING WASTEWATERS

2.1. Sericulture

Silk is a natural protein fiber, some forms of which can be woven into textiles. The best-known type of silk is obtained from cocoons made by the larvae of the mulberry silkworm *Bombyx mori*. The life cycle of *Bombyx mori* begins with eggs laid by the adult moth. The larvae emerge from the eggs and feed on mulberry leaves. In the larval stage, the *Bombyx mori* is the caterpillar known as the silkworm. The silkworm spins a protective cocoon around itself so that it can safely transform into a chrysalis. In nature, the chrysalis breaks through the cocoon and emerges as a moth. The moths mate and the female lays 300 to 400 eggs. A few days after emerging from the cocoon, the moths die and the life cycle continues.

Silk production is a seasonal activity and does not need much investment since it is an operation starting with providing of mulberry leaf, which is the only nutrient source of silkworm, and progressing until the production of silk. Generally, one cocoon produces between 300 and 600 m of silk filament, made essentially of two elements. The fiber, called fibroin, makes up between 75% and 90%, and sericin, the gum secreted by the caterpillar to glue the fiber into a cocoon, comprises about 10-25% of silk. Fibroin is insoluble whereas sericin is soluble in water. Other elements include fats, salts, and wax. To make approximately one meter of silk material, about 3000 cocoons are used (How Products are Made, 2008). Silk is one of the strongest natural fibers but loses up to 20% of its strength when wet. It has a good moisture regain of 11%. Its elasticity is moderate to poor: if elongated even a small amount, it remains stretched. It can be weakened if exposed to too much sunlight (Wikipedia, 2008).

Silk was produced by Chinese people 4000 years ago for the first time. Today, in the world, approximately 60 countries produce silk and the silk production is important for 20 of them in terms of economy. China, India, Turkmenistan, Brazil, Uzbekistan, Thailand and Iran are important countries in the world where silkworm is grown. In Turkey, the raising of silkworm has been done since 1500 years. Bursa, placed on the historical Silk Road, is one of the cities which produces important amount of silk for a long period of time. Bilecik, Antalya, Eskişehir and Hatay are the other cities where silk is produced. However, in the last 20 years, there has been a decrease in raising silkworm in Bursa as well as in country-wide (Keskin and Çeliker, 2003).

2.2. Silk Processing

Silk processing from cocoons to the finished articles consists of a series of steps which include reeling, weaving, degumming, dyeing or printing, and finishing (Zahn, 1993; Freddi et al., 2003). Figure 2.1 shows how the silk is produced. The cocoons are processed into silk thread in the filature where the cocoons are sorted by various characteristics, including color and size, so that the finished product can be of uniform quality. The cocoons must then be soaked into hot water to loosen the sericin. At this stage, some of the sericin is removed. Then, the filaments are combined to form silk thread. Silk thread, also called yarn, is formed by throwing, or twisting, the reeled silk. First, the skeins of raw silk are categorized by color, size, and quantity. Next, they are soaked into warm water mixed with oil or soap to soften the sericin. The silk is then dried. To achieve the distinctive softness and shine of silk, the remaining sericin must be removed from the yarn by soaking it in warm soapy water. Degumming decreases the weight of the yarn by as much as 25% (How Products are Made, 2008).

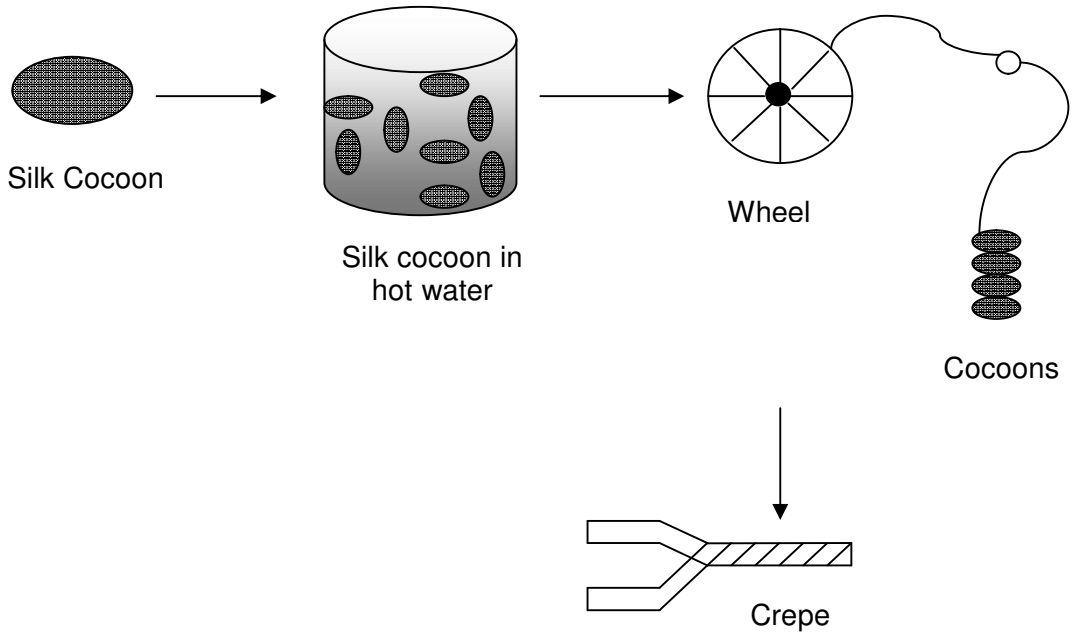


Figure 2.1. Silk production steps

Degumming is a key process during which sericin is totally removed and silk fibers gain the typical shiny aspect, soft handle, and elegant drape highly appreciated by the consumers. The degumming process generally makes use of soaps and soda at pH 10 (Fabiani et al., 1996). Soap is the most important agent in degumming. Pure olive oil soda soaps (Marseilles soaps) with 62% to 65% total fatty acid content are the best for degumming purpose. Possible substitutes for olive oil are sesame, cottonseed and groundnut oils. The amount of soap employed is usually calculated on the weight of material and is as high as 20-50% (Davidsohn, 1953).

The mechanism of sericin removal in chemical degumming is a combination of various effects such as dispersion/solubilization and hydrolysis of different sericin polypeptides (Freddi et al., 1996). Silk can be dyed as yarn before weaving or as woven fabric after degumming. Acid dyes, metal-complex dyes, and reactive dyes can be used to dye silk to a wide range of colors (Britannica, 2008). These dyes

depend on a chemical reaction taking place, under alkaline conditions, in an aqueous solution, making a permanent bond with the fibers called co-valent bonding. The alkali is the fixing agent. These alkaline conditions will damage protein fibers, so this should be a consideration when vat dyeing silk. The alkali should be greatly reduced or replaced with acetic acid, which is sometimes called a painting solution. Gaubers salt can be substituted for common salt. Fixing is done by steaming or the application of a cold fix agent, sodium silicate (Silk Wholesalers, 2008). In dyeing process, the silk is submerged into the hot dyebath for twenty minutes. Then, silk is removed from the dye and rinsed thoroughly with warm water (Helium, 2008). The dyed silk is mainly used for silk carpet manufacturing. Besides, silk is used for several products like dress and finery, yarn of needlework and surgical operation (Keskin and Çeliker, 2003).

Conventionally, removal of sericin is achieved in boiling water or in degumming solution containing soap. However, since boiling water alone is ineffective, the process could be catalyzed by the addition of acid or alkali but acids or alkali are considered as 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. Therefore, Lamoolphak et al. (2008) found that without addition of toxic chemicals, silk waste could be hydrothermally decomposed into protein and amino acids. An alternative degumming technique using water at temperature of 120-130 °C and pressure of 300-400 kPa has also been suggested by Fabiani et al. (1996).

2.3. Characteristics of Silk Processing Wastewaters

Silk processing is a subcategory of textile industry, which is one of the most important and the largest industrial sectors in the world. Textile industry consists of a number of processes employed for converting fibers of natural origin such as cotton, silk and wool, and of synthetic origin such as nylon; first, into fabrics by weaving and knitting and then, into the final products by applying wet processes such as dyeing,

sizing, printing, and finishing (Çapar, 2005). Wet processing produces the most significant amounts of emissions and waste in textile operations (International Finance Corporation, 2007). The amount of water used varies widely in the industry, depending on the specific processes operated at the mill, the equipment used, and the prevailing management philosophy concerning water use. Water consumption in textile industry is quite high with typical rates of 200-500 liters of water per kg of product (Marcucci et al., 2001). In silk processing, however, 50-240 liters of water is used for one kg of product (EWA, 2005). When compared with typical water consumption rates, water consumption in silk processing industry can be considered to have a moderate rate.

All textile wastewaters cause environmental problems. These are mainly caused by discharge of wastewaters. Textile processing employs a variety of chemicals, depending on the nature of the raw material and product. Some of these chemicals are enzymes, detergents, dyes, acids, sodas and salts. Textile wastewaters contain substantial pollution loads in terms of chemical oxygen demand (COD), biochemical oxygen demand (BOD), total solids (TS) and heavy metals. Due to usage of dyes and chemicals, effluents are dark in color, which is aesthetically undesirable. They also increase the turbidity of water body and limit photosynthetic activity. Textile effluents are toxic for human beings and cause a serious threat to ground and surface water recourses (Aslam et al., 2004).

Two types of wastewaters are generated in sericin removal processes; first, the wastewaters from the cocoon cooking process (CW) and second, the wastewaters from the silk degumming process (SDW). These wastewaters contain high COD, color and turbidity. The characteristics of some textile wastewaters are presented in Table 2.1 (BTTG, 1999; Çapar, 2005; Geçit et al., 2007a; Kim Chi, 2005; Vaithanomsat et al., 2008). As seen from this table, cocoon cooking and silk degumming wastewaters have much higher COD, total solids, color and turbidity than the other types of wastewaters have.

Table 2.1. Characteristics of some textile wastewaters

Parameter	Value				
	Cocoon Cooking	**Silk Degumming**	**Woven/Knitted Fabric Finishing**	**Stock and Yarn Dyeing**	**Carpet Mill**
COD (g/L)	6-15	50-60	0.1-1	0.8	0-4
BOD (g/L)	1-2.4	4-5	0.05-0.4	0.25	0.3-1.2
Color (Pt-Co)	4000-8000	20000-30000	-	-	10-700
Turbidity (NTU)	400-1000	4000-4200	-	-	0-70
T. Solids (g/L)	12-13	30-40	-	-	0.6-0.9
T. Protein (g/L)	7-9	40-45	-	-	-
Sericin (g/L)	4-5	30-35	-	-	-
Temp. (°C)	90-100	90-100	30-40	25-50	30-40
pH	5-6	7-9	7-9	5-9	6-8

Regarding water consumption and wastewater generation rates, silk processing can be placed among those textile subcategories having less adverse impact on the environment. However, sericulture is a seasonal activity and carried out by the villagers in villages. Hence, sericulture leads to the generation of dispersed point sources of pollution, which is difficult to handle in terms of treatment. In Turkey, cocoon cooking process is carried out for about 80 days in a year. For example, 14 tons of silk cocoon were processed in Bilecik in 2006. Approximately 125 kg of cocoon were cooked in a day and 300 L of water was used for each 20 kg of cocoon

(Akdağ, 2007), leading to the generation of cocoon cooking wastewaters with a flow rate of 1875 L/day (corresponding to a total wastewater volume of 150 tons in a year). In silk degumming process, 70-80 tons of silk yarns are dyed in a year where 550-600 kg of silk yarn is degummed in 5000 L of water once or twice a week. This leads to the weekly generation of 5000-10000 L of silk degumming wastewater, corresponding to an average flow rate of 7500 L/week. In this process, 50-75 kg of soap and 3 kg of soda are used for degumming of 150-200 kg of silk (Sipahioğlu, 2007).

Since silk processing wastewaters contain high amounts of protein and high COD, adverse results arise in nature due to insufficient treatment. Therefore, they are not recommended for disposal directly to drains because of a possible impact on the environment through depletion of oxygen. Furthermore, silk processing wastewaters contain soap. Soap has also the potential to harm the environment since it has a variety of fragrances and chemicals in it. Some chemicals used in soap fragrances have been proven to cause birth defects and liver damage in animals (Green Living Tips, 2008). Also, surfactants are among the most widely disseminated xenobiotics that may enter waste streams and the aquatic environment (Sigoillot et al., 1992; Margesin et al., 1998; Eichhorn et al., 2001 and 2002). They are harmful to human beings, fishes and vegetation, and they are responsible to cause foams in rivers and effluent treatment plants and reduce the quality of water. Surfactants cause short-term as well as long-term changes in ecosystem (Aboulhassan et al., 2006). Therefore, silk processing wastewaters should be treated by an appropriate method before discharging to the environment. Furthermore, these wastewaters contain a valuable protein named sericin. However, this protein is being discarded as a waste. Hence, valuable raw material should be recovered since it is very important in terms of environment and economics. In parallel to treatment target for suitable discharge, these wastewaters should be assessed for valuable raw material recovery.

2.4. Treatment of Silk Processing Wastewaters

There are a lot of conventional methods currently available for treating textile wastewaters in order to comply with the limits imposed by legislation for discharge. These methods are mainly biological treatment, physico-chemical treatment (precipitation and coagulation followed by flocculation and sedimentation), carbon adsorption and chemical oxidation with oxidizing agents like ozone and hydrogen peroxide. These methods have their own advantages and disadvantages (Table 2.2) (Demmin et al., 1988; Çapar, 2005), and the target to be achieved is important for the selection of the treatment method.

Among conventional treatment methods, biological treatment provides high COD removal but variable color removal. Furthermore, it requires nutrients and long residence times, and many toxic compounds cannot be removed by this process. In chemical precipitation, the dosages of chemicals can be adjusted. However, this process produces large amounts of sludge which pose handling and disposal problems. Adsorption process is usually applied for the removal of dissolved organic material. Activated carbon has been the most widely used adsorbent for removal of recalcitrant organic compounds including the textile dyestuffs from wastewaters. However, the main disadvantages of adsorption process are high cost and difficult adsorbent regeneration. Ozone oxidation can achieve high color removal, reduce the level of organic compounds, improve biodegradability, destroy phenols, and insure disinfection. One of the drawbacks of ozonation is the cost (Gahr et al., 1994). Formation of toxic compounds can also be a disadvantage.

A study revealed that biological treatment could be applied using nitrification-denitrification for the treatment of silk processing wastewaters (Rigoni-Stern et al., 1996). Another study showed that ozone treatment could also be used for color removal (Muthukumar et al., 2005). Fabiani et al. (1996) suggested ultrafiltration process for the treatment of wastewater from silk degumming process for protein recovery and water reuse.

In Table 2.3, the discharge standards for textile wastewaters originating from clean fiber, yarn production and finishing processing set in Water Pollution Control

Regulation (WPCR, 2004) are given. In this study, these criteria were used to evaluate the effluent quality of treated silk processing wastewaters as no specific limits could be found for these wastewaters.

Table 2.2. Conventional technologies for textile wastewater treatment

Technology	Advantages	Disadvantages
Biological Treatment	Low operational costs; high efficiency in COD removal	Long residence times; may require nutrients; very large aeration tanks, lagoons, land areas; many toxic compounds not removed; variable color removal
Chemical Precipitation	Adjustable chemical dosages	Color removal varies with dye class and dyeing process; little information on BOD and COD removal; chemicals handling can be a problem; increased sludge production
Activated Carbon	Removal of dissolved organic material	Expensive capital investment; long residence times; low adsorption capacity; frequent and expensive regeneration; color removal is dye-specific
Ozone Oxidation	High color removal; removal of organic compounds; improves biodegradability; disinfection	Very expensive capital investment; heavy metals and solids require separate treatment; formation of toxic compounds

Table 2.3. Discharge standards for textile wastewaters originating from clean fiber, yarn production and finishing processing

Parameters	Unit	Composite Sample (2 h)	Composite Sample (24 h)
COD	(mg/L)	350	240
Ammonium-N (NH_4-N)	(mg/L)	5	-
Free Chlorine	(mg/L)	0.3	-
Total Chrome	(mg/L)	2	1
Sulfide (S^{-2})	(mg/L)	0.1	-
Sulfite	(mg/L)	1	-
Oil and Grease	(mg/L)	10	-
Fish Bioexperiment	-	4	3
pH	-	6-9	6-9

Recently, water consumption and waste generation have become considerable concerns for textile manufacturers since textile industry uses very high amount of water and a great variety of chemicals (Demmin et al., 1988; Barredo-Damas et al., 2006). Therefore, advanced treatment methods such as membrane processes can be adopted for treating textile effluents in an efficient and cost-effective way. Moreover, the increasing public awareness and the threat on the depletion of earth's resources have been forcing industrialists and scientists to consider the recovery of valuable raw materials from industrial wastes as a method of sustainable development. Sustainable development and cleaner production concepts have aroused in parallel to the need of consuming the earth's depleting resources wisely due to the ever increasing population. Hence, adopting the integrated pollution control approach as an alternative to the conventional treat-discharge approach has become a necessity. For example, in textile industry, various chemicals and dyes are used. These can be recovered from the textile effluents using membrane technology and a large proportion of wastewater can be reused (Naveed et al., 2006). The recovery of these materials is very important since pollution strength of wastewater and cost will also be decreased. In future, many of textile factories will

face the requirement of reusing a significant part of all incoming freshwater because traditionally used methods are insufficient for obtaining the required water quality (Fersi et al., 2005). Therefore, the conventional end-of-pipe treatment strategy needs to be replaced with waste minimization, recovery and reuse options, and an effective management of the environment.

In the treatment of silk degumming wastewaters, the relevant discharge standards have to be met. Furthermore, these wastewaters can be used as a source of sericin, which is currently discarded as a waste. Sericin represents a valuable by-product that can be used in cosmetics and pharmaceutical production (Fabiani et al., 1996). Because of its properties, sericin can be used in food, cosmetics and pharmaceutical products as well as for manufacturing biomaterials (Wu et al., 2007). The commercial value of sericin is high, i.e., about 80-90 € per g of sericin. Processing of the raw silk produces about 50000 tons of sericin. If this sericin protein is recovered and recycled, it can represent a significant economic and social benefit (Zhang et al., 2002). In addition, sericin recovery would significantly reduce the environmental impact of silk production processes (Fersi et al., 2005). Due to the macromolecular nature of the sericin, the most appropriate method for sericin recovery is the membrane technology (Fabiani et al., 1996).

CHAPTER 3

MEMBRANE PROCESSES

3.1. Historical Background

Although the use of membrane processes has increased rapidly in recent years, the application of membranes for water treatment extends back several decades. The first commercial membranes for practical applications were produced in Germany after World War I, in 1920, and used for the filtration of bacteria at laboratory scale (Mulder, 1997). Since 1960s, reverse osmosis (RO) membranes have been used for the desalination of water, with more widespread use of nanofiltration (NF) for softening and the removal of total organic carbon (TOC) dating to the late 1980s. However, the commercialization of backwashable hollow fiber microfiltration (MF) and ultrafiltration (UF) membrane processes for the removal of particulate matter (i.e., turbidity and microorganisms) in the early 1990s has had the most profound impact on the use, acceptance, and regulation of all types of membrane processes for drinking water treatment (EPA, 2005). Membranes suffered from four problems that inhibited their widespread use as a separation process: they were unreliable, slow, unselective and expensive. Solutions to each of these problems have been developed during the last 30 years (Baker, 2004). However, there are still some problems related with membranes. For example, fouling can be a serious problem especially in those cases where biological fluids are handled. The fouling problem manifests itself economically in the form of loss of productivity due to reduced equipment efficiency, increased material cost for cleaning, and contamination problem due to the growth of micro-organisms (Pelegrine et al., 2005). Concentration polarization is also a serious problem but it is a reversible phenomenon unlike fouling. Presently, a lot of studies are carried on to overcome these problems.

Membrane processes have wide range of applications in the textile industry. They have gradually become an attractive alternative to the conventional separation processes in the treatment of wastewater. The application of membrane filtration not only enables high removal efficiencies, but also allows recovery of water and some valuable waste constituents for possible end uses (Fersi et al., 2005).

3.2. Definitions

A membrane can be defined as a barrier, which separates two phases and restricts transport of various chemicals in a selective manner when a driving force is applied across it. A membrane can be homogeneous or heterogeneous, symmetric or asymmetric in structure, solid or liquid, can carry a positive or negative charge or be neutral or bipolar. Transport through a membrane can be affected by convection or by diffusion of individual molecules, induced by an electric field or concentration, pressure or temperature gradient (Srikanth, 2008). Membrane filtration can be used as an alternative for flocculation, sediment purification techniques, adsorption (sand filters and active carbon filters, ion exchangers), extraction and distillation (Lenntech Membrane Technology, 2008).

Membrane filtration systems can be managed in either dead-end flow or cross-flow (Figure 3.1). In dead-end filtration, all the feed is forced through the membrane, which implies that the concentration of rejected components increases and consequently, the quality of the permeate decreases with time (Mulder, 1997). The feed flows to the filter media perpendicularly and this causes the accumulation of the retained particles, leading to the formation of a cake layer at the surface. The cake grows continuously bringing about an increasing pressure drop and/or a decreasing permeate flux. Dead-end filtration is generally suitable for concentrated suspensions, and is not appropriate for the filtration of very fine and dilute suspensions or production of very pure filtrates (Murkes and Carlsson, 1988).

In cross-flow filtration, the feed is recycled under an applied pressure and flows parallel to the filter media. The purpose of this flow is to control the thickness of the cake. This type of filtration provides longer service lives for the filter media.

Membrane filtration is almost always carried out by cross-flow (Murkes and Carlsson, 1988; Mulder, 1997). In cross-flow filtration, the feed stream is divided into two streams, i.e., retentate or concentrate stream and permeate stream, which means that either the concentrate or the permeate is the product (Mulder, 1997). Permeate is the portion of the solution passing through the membrane while retentate is the portion of the feed solution retained on the high-pressure side of the membrane (Figure 3.1).

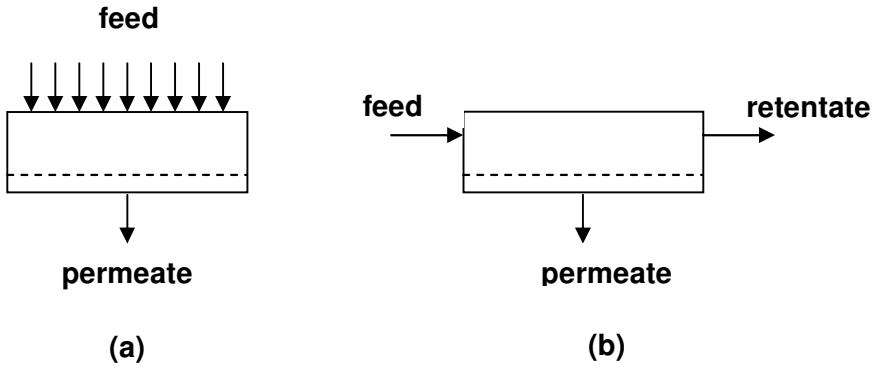


Figure 3.1. Schematic drawing of **(a)** dead-end and **(b)** cross-flow filtration

The performance or efficiency of a given membrane is determined by two parameters: its selectivity and productivity. Selectivity is expressed as a parameter called retention or separation factor which is unitless. Productivity is expressed as a parameter called flux, which is defined as the volume of liquid flowing through the membrane per unit area and unit time (Mulder, 1997).

The membrane performance can change with time and often typical flux-time behavior may be observed: the flux through the membrane decreases over time. This behavior is mainly due to concentration polarization, adsorption, gel layer formation and plugging of the pores. All these factors induce additional resistance

on the feed side to the transport across the membrane (Noble, 1995). Concentration polarization means the accumulation of solutes close to or on the membrane surface. As a result, either the resistance to solvent transport increases or local osmotic pressure increases, both causing decrease in flux and change in sieving characteristics of the membrane. Concentration polarization is a reversible phenomenon (Mulder, 1997). On the other hand, membrane fouling is defined as the process in which solute or particles deposit onto the membrane surface or into membrane pores such that membrane performance is deteriorated. There are various types of foulants namely colloidal (clays, flocs), biological (bacteria, fungi), organic (oils, polyelectrolytes, humics) and scaling (mineral precipitates) (Wikipedia, 2008). Fouling includes adsorption, pore blocking, precipitation and cake formation (Mulder, 1997).

The consequence of concentration polarization and fouling is always a reduction in separation performance. There are methods to improve the performance of separation: pretreatment of feed solution, adjustment or tailoring of membrane properties, membrane cleaning and improvement of operating conditions such as increase of cross-flow velocity (Noble, 1995).

3.3. Pressure-Driven Membrane Processes

Membrane processes may be different in their modes of operation, structures and driving forces used for the transport of different chemical components. Classification of membrane processes according to their driving forces is shown in Table 3.1. In most of the membrane processes used in water and wastewater treatment, the driving force is a pressure difference across the membrane (Mulder, 1997). There are four commonly accepted pressure-driven membrane processes. These are microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO). The hydrodynamic resistance of the membrane and therefore, pressure applied across the membranes increases while the pore size gets smaller (Table 3.2) (Mulder, 1997).

Table 3.1. Classification of membrane processes according to their driving forces

Pressure Difference	Concentration Difference	Temperature Difference	Electrical Potential Difference
MF	Pervaporation	Thermo-osmosis	Electrodialysis
UF	Gas separation	Membrane distillation	Electro-osmosis
NF	Vapour permeation		Membrane electrolysis
RO	Dialysis		
Piezodialysis	Diffusion dialysis		
	Carrier-mediated transport		

Table 3.2. Specifications of pressure-driven membrane processes

Process	Pore Size	Pressure	Separation Mechanism
MF	0.05-10 µm	0.1-2 bar	Sieving
UF	1-100 nm	1-10 bar	Sieving
NF	0.5-5 nm	5-20 bar	Solution-Diffusion
RO	< 1 nm	10-100 bar	Solution-Diffusion

3.3.1. Microfiltration

MF is the membrane process which most closely resembles conventional coarse filtration. Membranes with a pore size of 0.05–10 µm perform microfiltration. MF can be implemented in many different water treatment processes when particles with a diameter greater than 0.1 mm need to be removed from a liquid. MF is a membrane filtration process operating at 0.2-4 bar pressure (generally less than 2 bars) allowing molecules of the size of salts, sugars and proteins to pass through the membrane pores, while molecules of the size of bacteria and fat globules are rejected. The principle of MF is physical separation. MF is often applied in dead-end

filtration mode in the analytical laboratories, which is one of the most important application areas of MF process today. On the other hand, cross-flow application is preferred for larger scale applications to ensure longer media life. Typical MF applications are sterilization in food and pharmaceutical industry, clarification in beverage industry, ultrapure water production in semiconductor industry, water treatment, separation of oil-water emulsions, and cell harvesting in biotechnology (Mulder, 1997).

3.3.2. Ultrafiltration

UF is a membrane process whose nature lies between NF and MF. The pore sizes of the membranes used range from 1 nm (on the NF side) and 100 nm (on the MF side). Ultrafiltration is a membrane filtration process operating at 1-10 bar pressure and allowing molecules of the size of salts and sugars to pass through the membrane pores, while molecules of the size of proteins are rejected (SPX Corporation, 2008). It removes bacteria, viruses, proteins and some sugars from effluents without possibility of re-growth after treatment (Gadani et al., 1996). A wide range of molecular weight cut-offs (MWCO) are available from 1 kDa to 500 kDa (Naveed et al., 2006). UF is used over a wide field of applications involving situations where high molecular weight components like colloidal materials, organic and inorganic polymeric molecules have to be separated from low molecular weight components such as sodium, calcium, magnesium chloride and sulfate. Examples of fields where UF is applied are food, dairy, textile, metallurgy, pharmaceutical, chemical, paper and leather industries. Applications of UF process in food and dairy industries are the concentration of milk and cheese making, recovery of whey proteins, recovery of potato starch and proteins, concentration of egg products, and the clarification of fruit juices and alcoholic beverages (Mulder, 1997).

3.3.3. Nanofiltration

NF is a pressure-driven process applied in the area between the separation capabilities of RO and UF membranes, that is, in the separation of ions from solutes such as small molecules of sugars. The typical pore size of NF membranes is 0.5-5 nm, and the applied pressures are typically 5-20 bars, which are lower than the RO process, but yield higher fluxes (Scott, 1996; Mulder, 1997). NF membranes allow the separation of low molecular weight organic compounds (200-1000 Da) and divalent salts with an appreciable softening effect (Fersi et al., 2005). Its separation characteristics are based on sieving effect, but most commercial NF membranes are also charged. So, ion rejection by NF membranes results from the combination of electrostatic and steric interactions associated with charge shielding, Donnan exclusion and the degree of ion hydration. This membrane technology can achieve higher COD rejection than ultrafiltration (more than 90%) with greater flux than reverse osmosis (RO) and sometimes less fouling problems. In order to obtain good efficiency and to prevent fouling in the NF membranes, a correct pre-treatment has to be considered (Gozàlvez-Zafrilla et al., 2008). NF is mainly applied in the steps of drinking water purification process such as water softening and desalination of brackish water, wastewater treatment, decolouring and micro pollutant removal, organic substances removal such as micro pollutants and multivalent ions. NF membranes have moderate retention for univalent salts (Mulder, 1997). Typical rejections of NF are 60% for NaCl, 80% for calcium bicarbonate and 98% for magnesium sulphate, glucose and sucrose (Scott, 1996).

3.3.4. Reverse Osmosis

Reverse osmosis is a high-efficient technique for dewatering process streams, concentrating/separating low molecular weight substances in solution, or cleaning wastewater. It has the ability to concentrate all dissolved and suspended solids. RO permeate contains a very low concentration of dissolved solids (GEA Filtration, 2008). The typical RO membrane pore size is less than 1 nm and they can essentially separate all solutes with molar masses greater than 300 Da completely from the solution. Water, having a molecular size nearly one tenth of the RO pore

size, can pass through the membrane freely (Matsuura, 1994). In order to overcome the molecular friction between permeates and membrane polymers, during diffusion, large operating pressures are applied in the range of 10-100 bar. RO is principally seen in the processing of aqueous solutions in the areas of desalination of brackish water and sea water, production of pure water for a variety of industries, wastewater treatment, and concentration of solutions of food products, pharmaceutical solutions and chemical streams (Scott, 1996).

3.4. Membrane Fouling and Cleaning

Fouling of membrane diminishes its productivity as a consequence of flux decline. Fouling results mainly from three sources, namely, particles in the feed water, build-up of sparsely soluble minerals and by-products of microorganism growth. Flux decline has a negative influence on the economics of a given membrane operation, and for this reason, measures must be taken to reduce its incidence (Mulder, 1997).

The degree of membrane fouling determines the frequency of cleaning, lifetime of the membrane, and the membrane area needed, and this will have a significant effect on the cost, design and operation of membrane plants (Speth et al., 1998). Membrane cleaning has important economic and environmental implications for the overall performance of membrane process (Chen et al., 2006). However, it is an essential step in maintaining the permeability and selectivity of a membrane process. Cleaning is usually done by physical, chemical and physico-chemical methods. The type of cleaner required depends on the nature of the foulant and the membrane material. Typical membrane cleaning agents are acids, alkalis, chelatants, detergents, formulated products and sterilizers. To avoid fouling and resulting frequent cleaning procedures, an adequate pre-treatment of the feed is required. Pre-treatment needs to be designed to remove suspended solids in the feed. In the simplest form, pre-treatment involves micro-straining with no chemical addition and include pH adjustment, coagulation/precipitation, slow sand filtration, adsorption on activated carbon, and microfiltration (Mulder, 1997; Baker, 2004). Last but not least, the bacteriological conditions of the feed and the plant must be controlled to prevent growth of microorganisms (Water and Wastes Digest, 2008).

Membrane fouling is one of the critical issues in the application of membrane technology for protein purification (Kwon et al., 2008). Protein fouling during membrane process is typically dominated by the deposition of large protein aggregates on the membrane surface (Kelly et al., 1993). Previous studies have revealed that some components in the fouling layer are easy to remove by rinsing while other components require specific cleaning strategies (Chen et al., 2006). Protein fouled membranes almost universally require chemical cleaning to restore membrane performance. Cleaning agents used for protein fouling fall into three broad categories: strong bases, strong oxidizing agents and strong acids (Field et al., 2008). Strong bases such as NaOH cause a dramatic change in pH, which, in addition to chemically attacking the deposits, can increase the electrostatic repulsion between the foulants and the membrane (Sayed Razavi et al., 1996). Increase in electrostatic repulsion reduces the adhesion between membrane and fouling materials and enhances the cleaning efficiency. Strong oxidizing agents such as NaOCl, in part act to hydrolyze the foulant (Kuzmenko et al., 2005). The existence of strong oxidizing agents generally increase the hydrophilicity of foulants. Therefore, oxidation reduces the adhesion of fouling materials to membranes and increases cleaning efficiency. Strong acids such as HCl, nitric acid and citric acid are effective cleaning agents. They are used primarily for removing scales and metal dioxides from fouling layers. When membrane is fouled by iron oxides, citric acid is very effective because it not only dissolves iron oxides precipitates, but also forms complex with iron. In addition, some of organic compounds such as polysaccharides and proteins also hydrolyze (Hong et al., 1997). Wu et al. (2006) showed that proteins could be removed from UF membranes by using chlorine. In cleaning UF membranes, a chemical method using NaOH and NaOCl at moderate temperature was developed by Crawford et al. (1995) and 100% efficiency was obtained with 180-200 ppm NaOCl at 20-60 min.

In summary, it can be stated that chemical cleaning is an undesired process in terms of operational cost and membrane lifetime. However, it is inevitable for the continuity of the membrane filtration processes.

CHAPTER 4

LITERATURE REVIEW ON SERICIN RECOVERY FROM SILK PROCESSING WASTEWATERS

4.1. Characteristics of Sericin

Silk sericin is a natural macromolecular protein derived from silkworm *Bombyx mori.* Sericin is represented by a family of proteins with their molecular weight distributed between 10 and 300 kDa (Fabiani et al., 1996; Zhang, 2002). When subjected to alkaline degumming process, sericin is degraded into sericin peptides or hydrolyzed sericin with molecular weight less than 20 kDa (Zhang et al., 2004; Vaithanomsat et al., 2007).

Wu et al. (2007) showed that the main composition of sericin powder was protein, with a concentration above 90%. Sugar concentration only accounted for 0.9% although Hyogo and Yoshiko (1967) reported that sericin is a glycoprotein containing glucosamine, galactosamine, mannose and galactose. Ash concentration accounting for 4.2% suggested that sericin powder from wastewater possibly contained a little salt.

The sericin solution at room temperature is a partially gelled liquid with high viscosity dependent on temperature and pH. The sericin solution is a non-Newtonian fluid whose viscosity depends on the velocity of the flow (Fabiani et al., 1996). The sericin peptides having molecular weights of less than 60 kDa, commonly less than 5 kDa, are soluble in cold water. These are characterized by excellent moisture absorption and release, and a lot of biological activities such as antioxidation, tyrosinase activity inhibition (Kato et al., 1998), and pharmacological functions such as anticoagulation (Tamada, 1997), anticancer activities (Sasaki et al., 2000; Zhaorigetu et al., 2001), cryoprotection (Kazuhisa et al., 2001) and promotion of digestion (Sasaki et al., 2000). The rest, a higher range of molecular weight ranging

from 60 to more than 300 kDa, is poorly soluble in cold water but soluble in boiling water (Zhang et al., 2006).

Sericin is a highly hydrophilic protein and classified into at least six proteins of different lengths generated by alternatively splicing the primary transcripts of two sericin genes, Ser1 and Ser2. In ExPASy protein databases, sericin is defined as Ser1 and Ser2. The Ser1 gene encodes for various polypeptides which contain several repeats of a 38 amino acid motif with a high content of hydroxyl amino acids, whose composition is very close to the average composition of sericin (Garel et al., 1997). In a study of Anghileri et al. (2007), chemical properties of oxidized sericin were determined by amino acid analysis. The amino acidic pattern of sericin is dominated by the presence of hydroxyl (serine, threonine, tyrosine), acidic (aspartic acid, glutamic acid), and basic (lysine, histidine, arginine) amino acid residues, which totally account for about 73 mol%. Glycine and alanine are minor components, with a total concentration of only 20 mol%. Other amino acids (proline, methionine, isoleucine, leucine, phylalanine, cysteine, valine and tryptophane) are present in very small amounts. Sericin is a globular protein. Its molecular formula (Kim, 2007) is given in Figure 4.1 and the structures of some amino acids of silk sericin (Morrison and Boyd, 1992) are shown in Table 4.1. Serine and threonine are important because both are related to some mechanisms of sericin functionality such as the antioxidant activities and the tyrosinase-inhibitory effect (Kato et al., 1998). In addition, Wu et al. (2008) showed that the amount of the hydrophilic amino acids was up to 76%, and could explain why sericin possesses the water absorbability and good solubility. Some of the amino acid residues of sericin macromolecule have polar side groups whereas others have non-polar side groups. So, sericin macromolecule has both hydrophilic and hydrophobic elements (Wei et al., 2005). The isoelectric point (pI) of sericin purified from the cocoon shell of silkworm and that of silk fiber have been found as 4.3 (Kurioka et al., 2002) and 5 (Mondal et al., 2007).

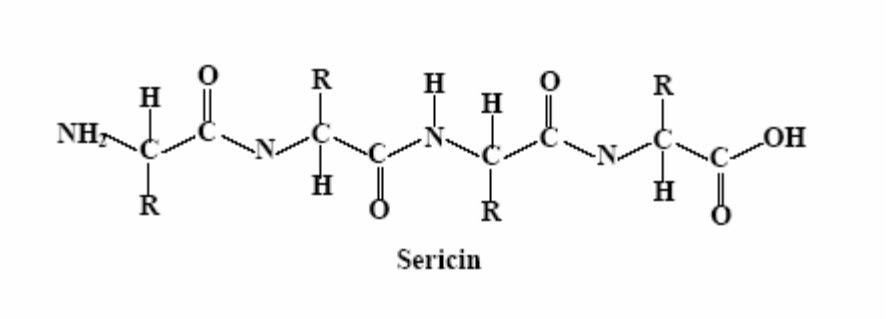


Figure 4.1. Molecular formula of sericin

Table 4.1. Structures of some amino acids of silk sericin

Amino Acid	Structure	Amino Acid	Structure
Aspartic Acid	$HOOCCH_2CHCOOH$ $\vert$ NH_2	Methionine	$CH_3{-}S{-}CH_2CH_2CHCOOH$ $\vert$ NH_2
Threonine	NH_2 $\vert$ $CH_3CHCHCOOH$ $\vert$ OH	Isoleucine	NH_2 $\vert$ $CH_3CH_2CHCHCOOH$ $\vert$ CH_3
Serine	NH_2 $\vert$ $HOCH_2CHCOOH$	Leucine	NH_2 $\vert$ $CH_3CHCH_2CHCOOH$ $\vert$ CH_3
Glutamic Acid	NH_2 $\vert$ $HOOCCH_2CH_2CHCOOH$	Lysine	$H_2NCH_2CH_2CH_2CH_2CHCOOH$ $\vert$ NH_2

Table 4.1. Structures of some amino acids of silk sericin (cont.'d)

Amino Acid	Structure	Amino Acid	Structure
Glycine	NH_2 | CH_2COOH	Arginine	NH_2 | $H_2NCNHCH_2CH_2CH_2CHCOOH$ ‖ NH
Alanine	$CH_3CHCOOH$ | NH_2	Cysteine	NH_2 | $CH_2CHCOOH$ | SH

Sericin can be detected by matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF MS), which is a relatively novel technique where a co-precipitate of an UV-light absorbing matrix and a biomolecule is irradiated by a nanosecond laser pulse. The ionized biomolecules are accelerated in an electric field and enter the flight tube. During the flight in this tube, different molecules are separated according to their mass to charge ratio and reach the detector at different times. In this way, each molecule yields a distinct signal. The method is used for detection and characterization of biomolecules such as proteins, peptides, oligosaccharides and oligonucleotides, with molecular masses between 0.4 and 350 kDa. Protein identification by this technique has the advantage of short measuring time, i.e., a few minutes, and negligible sample consumption, i.e., less than 1 pmol, together with additional information on microheterogeneity (e.g. glycosylation) and presence of by-products (Camp, 2008).

Elemental analysis is also used to characterize sericin. It determines the composition of sericin in terms of carbon (C), nitrogen (N), hydrogen (H) and sulphur

(S). In a study of Whewell (1941), it was found that C, H and N contents of sericin were 42.6%, 5.8% and 16.5%, respectively.

Proteins generally have two absorbance peaks in the UV region, one between 215-230 nm, where peptide bonds absorb, and another at about 280 nm due to light absorption by aromatic amino acids (Biotechnology Project, 2008). The silk sericin shows a peak absorbance at around 280 nm of wavelength (Kim, 2007) (Figure 4.2).

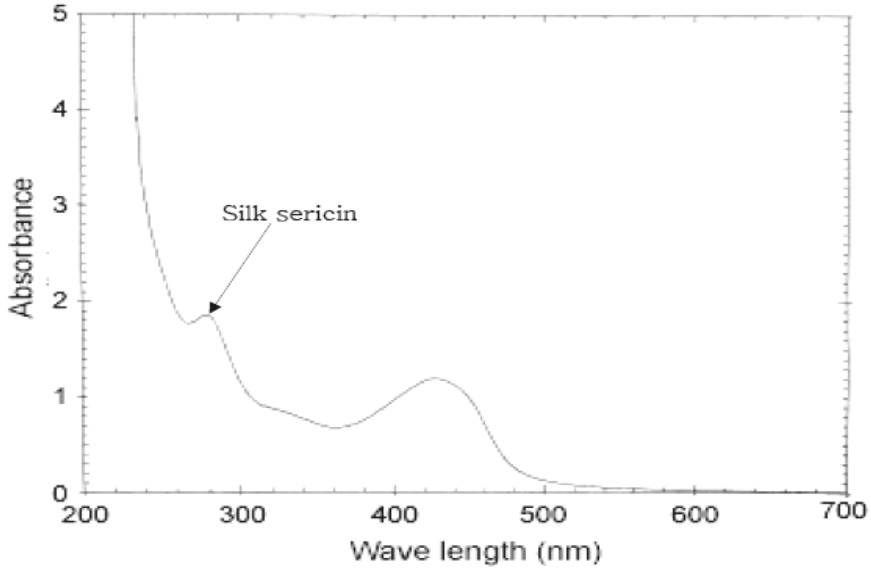


Figure 4.2. Absorption spectrum of silk sericin

The range of molecular weight of sericin can be determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). In Figure 4.3, molecular weight distribution of sericin determined by Kim (2007) is given. As can be seen, molecular weight of sericin changed from 26 to 170 kDa. On the other hand, Wu et al. (2007) found that MW of sericin was 14-467 kDa. These results indicate that MW of sericin is affected by the conditions of applied methods.

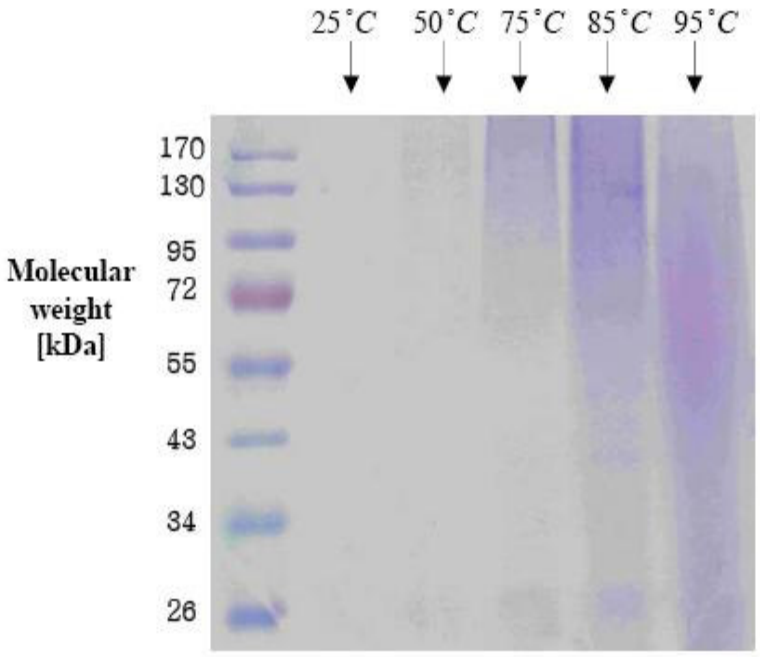


Figure 4.3. SDS-PAGE (10 % gel) of silk sericin

4.2. Uses of Sericin

Sericin protein is useful because of its antioxidative, antibacterial, UV resistance, and moisture-absorbing and -desorbing properties (Shen et al., 1998; Rigueiro et al., 2001). For production of materials with improved properties, sericin can be cross-linked, copolymerized and blended with other macromolecular materials, especially artificial polymers. It is also used as an improving reagent or a coating material for natural and artificial fibers, fabrics, and articles (Zhang, 2002). It can be used in many fields such as cosmetics, medical and polymer materials (Shen et al., 1998; Rigueiro et al., 2001). Sericin, either obtained directly from the cocoons or recovered from degumming wastewater, can be considered as a valuable natural polymer worth of being used for a wide range of applications, including those related to biomaterials (Anghileri et al., 2007).

The application areas of sericin differ with respect to its molecular weight. It has been reported that low MW sericin peptides (less than 20 kDa) or sericin hydrolysates are used in cosmetics including skincare and hair care products, health products, and medications. On the other hand, high molecular weight sericin peptides (greater than 20 kDa) are mostly used as medical biomaterials, degradable biomaterials, compound polymers, functional biomembranes, hydrogels, and functional fibers and fabrics (Zhang, 2002). Furthermore, sericin can be used to make membranes for use in separation processes. A membrane made of sericin can effectively separate alcohol from the mixture of water and alcohol. Mizoguchi et al. (1991) described a cross-linked thin film made of sericin for use as a separating membrane for water and ethanol. In a study of Yoshikawa et al. (2001), the gel material produced by mixing agar or agarose with sericin of 20 kDa average molecular weight can separate ether-alcohol mixtures. Yamada and Fuwa (1993) also prepared a membrane from sericin, which was capable of resolving racemic mixtures. In Kim's study (2007), sericin was blended with polyvinyl alcohol (PVA) to form membranes that are permselective to gases. Due to their hydrophilic properties, a novel water-swollen sericin/PVA membrane was developed for the permeation of carbon dioxide and nitrogen gases.

Sericin can also be used as a coating material on surfaces of refrigeration equipment such as refrigerators, deep freezers, refrigerated trucks and ships because of its antifrosting action. Moreover, use of sericin coated film on roads and roofs can prevent frost damage and ease snow removal. The protective property of sericin has also been useful in preparation of art pigments and protection of article surfaces (Tanaka, 2001).

Sericin has been found to possess various biological functions. Masahiro et al. (2000) reported that consumption of sericin enhances bioavailability of Zn, Fe, Mg and Ca in rats, and suggested that sericin is a valuable natural ingredient for food industry. Kato et al. (1998) found that sericin is a valuable ingredient for cosmetics because it can inhibit tyrosinase activity and this enzyme is responsible for biosynthesis of skin melanin. In addition, Siqin et al. (2003) found that sericin exerts inhibitory activity on ultraviolet radiation induced acute damage and tumor promotion by reducing oxidative stress in the skin of hairless mouse. Masakazu et al. (2003)

found that sericin possesses the biological activity of preventing cell death and promoting cellular growth after acute serum deprivation.

Sericin has been proven to be a very useful protein in biotechnology and biomedical applications. Zhaorigetu et al. (2003a,b) reported data on the protective effect of sericin against both chemical and UV radiation induced tumorogenesis by reduction of oxidative stresses. Takeuchi et al. (2005) showed that high MW sericin films effectively induced hydroxyapatite nucleation under biomimetic conditions. Sericin-coated α-tricalcium phosphate ceramics showed improved durability and desirable bioresorption rate as novel bone repair devices (Miyazaki et al., 2004). Similarly, Tsubouchi et al. (2005) reported that sericin films enhanced attachment of cultured human skin fibroblasts.

Sericin is applicable as an antioxidant in the field of medicines, cosmetics, foods and food additives (Kato et al., 1998; Sarovart et al., 2003). It 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 lines and wound dressing (Yamada et al., 1998; Sarovart et al., 2003). Sericin has a high antioxidant and antibacterial activity, which means that sericin can stop the oxidation reaction of free radical and inhibit microorganisms growth leading to numerous diseases. Sarovart et al. (2003) have used sericin coated on fiber as an air filter to reduce the amount of free radical entering through the body and inhibit microorganisms growing on the air filter media.

In most applications, sericin, which is obtained conventionally from cocoon shell, is used. However, sericin protein can also be recovered from silk effluents in an attempt to reduce the environmental pollution caused by the silk processing wastewaters. Recovery of sericin from silk effluents would also provide economical benefits as well as contribution to cleaner production and sustainable development efforts. In recent years, recovered sericin have found application in cosmetic industry as ingredient of skin and hair care products. It has also been used as finishing agent for natural (Kongdee et al., 2005) or man-made textiles (Lee et al., 2004) with good results in terms of moisture absorption, antistatic properties, softness, and comfort. In a study, Cortez et al. (2007) investigated whether enzymes

like transglutaminases are able to graft silk proteins into wool fibers altering their properties or repairing the damage introduced during earlier processing by using fluorescently labelled silk sericin protein, which was recovered from silk processing effluents and had an average molecular weight of 30 kDa.

Grafting chitosan with sericin peptides may complement the outstanding properties of the polysaccharide (antimicrobial activity) with the new ones brought by sericin (antioxidant, UV-resistant, moisturizing, solubility, etc.). This will result in the production of valuable bio-based polymers from renewable resources under the mild reaction conditions assured by the specificity and selectivity of enzymes. In a study of Anghileri et al. (2007), the kinetics of the enzymatic reaction of *Agaricus bisporus* mushroom tyrosinase with sericin peptides purified from industrial wastewater and other silk-derived model substrates was investigated. Tyrosinase was able to oxidize about 57% of sericin-bound tyrosine residues. Sericin peptides recovered from degumming wastewater were effectively oxidized by tyrosinase. Tyrosinase-oxidized sericin underwent non-enzymatic cross-linking with chitosan.

Similarly, Wu et al. (2008) showed an effective bioprocess for the production of bioactive peptides from recovered sericin, which may be used as valuable ingredients in the food, cosmetic and medicine industries.

In conclusion, the above-mentioned studies clearly show that sericin is a very promising protein for several industrial sectors. Furthermore, sericin recovered from silk processing wastewaters has also proven to be useful in these areas. To this end, studies on the recovery of sericin from silk processing wastewaters deserve great interest.

4.3. Sericin Recovery

The sericin peptides having MW of less than 60 kDa can be recovered at early stages of raw silk production. The rest, a higher range of MW ranging from 60 to more than 300 kDa, can be obtained at the later stages of silk processing or silk degumming (Zhang et al., 2006). In literature, there are few studies on the recovery

of sericin from silk effluents (Fabiani et al., 1996; Vaithanomsat et al., 2007; Wu et al., 2007). In one study, Fabiani et al. (1996) have tested membrane processes for the recovery of sericin from silk degumming wastewaters, where ultrafiltration was chosen due to the simple composition of degumming waste solution and the macromolecular nature of sericin. The recovery of more than 97% of sericin with different UF membranes having molecular weight cut-off of 20-30 kDa was reported. Membrane permeability decreased with time since a protein gel was formed on the membrane surface, as expected. Membranes after UF experiment were cleaned according to a washing procedure so as to recover at least 90% of the initial water permeability of the membrane.

In another study related with the recovery of sericin, Vaithanomsat et al. (2007) tried to recover sericin in addition to improvement of the quality of degumming wastewater for further applications. In order to reduce the treatment costs and to recover valuable sericin protein, membrane filtration and enzymatic hydrolysis of recovered sericin were studied. Results showed that wastewater quality was improved and also an amount of sericin protein with molecular weight of 2427-9863 Da was recovered after membrane filtration process. The recovered sericin was further enzymatically hydrolyzed to obtain sericin hydrolysate having average molecular weight of 1046-2795 Da, which is mostly suitable for cosmetics application. A portion of degumming waste solution was directly dried using freeze-drying and tray-drying methods. Another portion was passed through UF with membrane having MWCO in the 20-80 kDa range in order to obtain the concentrated sericin protein. Results illustrated that UF of degumming waste with a 20-30 kDa membrane allowed the recovery of 2427-9863 Da sericin at an efficiency of 94%. Removal of impurities by membrane filtration could actually improve the quality and yield of sericin.

In addition to membrane studies, Wu et al. (2007) suggested the method of ethanol precipitation for sericin recovery from silk wastewater. Sericin was extracted with 75% (v/v) ethanol to obtain crude powder having MW of 14-467 kDa. This method does not seem to be feasible as far as the environment and the economy are concerned due to the high amounts of ethanol requirement at industrial scale.

In conclusion, silk processing wastewaters contain a valuable protein, namely sericin, which is discarded as a waste product in textile wastewaters. However, extensive research proves that sericin can impart useful and unusual properties to polymer gels, membranes, foams, fibers, and other composite materials. Moreover, discharge of sericin into the receiving environment may cause serious pollution problems due to the very high organic content of silk wastewaters. In addition, wasting sericin leads to an economical loss. Therefore, it is an important issue to recover sericin from silk processing wastewaters.

In silk processing, there are two sources of sericin, the cocoon cooking process and the silk degumming process. In the above-mentioned studies, the source of sericin was mainly silk degumming wastewaters and no literature background seems to exist on the recovery of sericin from cocoon cooking wastewaters, which are generated prior to silk degumming wastewaters. The cocoon production in the world is about 1 million tons, which is equivalent to 400000 tons of dry cocoon (Zhang, 2002). In Turkey, approximately 12 tons of sericin is wasted from these two wastewaters in a year. Therefore, the recovery of sericin from cocoon cooking wastewaters would definitely provide economical benefits for the world and Turkey.

The MW distributions of sericin in cocoon cooking and silk degumming wastewaters are quite different; sericin in silk degumming wastewaters has mainly single MW fraction of 110-120 kDa whereas sericin in cocoon cooking wastewaters has a broad range of MW changing from 10 kDa to 200 kDa (Geçit et al., 2007a). Hence, the membrane processes required for sericin recovery from these wastewaters would be different. UF has been found suitable for silk degumming wastewaters and there is a need to determine the most suitable membrane process for sericin recovery from cocoon cooking wastewaters. Furthermore, silk degumming wastewaters of Turkey contain soap and soda unlike the silk degumming solutions mentioned in literature, which contain only sericin. To this end, the aim of this thesis is to determine the most suitable membrane-based processes for sericin recovery from cocoon cooking wastewaters and silk degumming wastewaters of silk processing industry. The minimization of environmental pollution caused by these wastewaters was also aimed. First, the cocoon cooking and silk degumming wastewaters of silk processing industry were characterized in terms of sericin concentration, molecular

weight distribution of sericin, total protein and environmental quality parameters such as COD, total solids, color and turbidity. Then, the most suitable pre-treatment methods to membrane processes were investigated. In pre-treatment stage for CW, physico-chemical methods such as gravity settling, centrifugation and microfiltration were adopted in single and sequential modes in order to choose the best one that minimizes loss of sericin and maximizes the removal of pollution parameters. Then, the most suitable membrane processes among UF and NF were determined for sericin recovery from CW. The rejection performances and flux declines of membranes were compared for selecting the most suitable membrane process. Finally, the recovered sericin was characterized and compared with the properties of commercial and native sericin used as reference.

In pre-treatment stage for SDW, physico-chemical methods such as gravity settling, centrifugation and pH adjustment were adopted in single and sequential modes in order to choose the best one that minimizes loss of sericin and maximizes the removal of soap and other pollution parameters. Then, the most suitable membrane processes among UF (20 kDa) and UF (5 kDa) were determined for sericin recovery from SDW. The rejection performances for sericin, separation performances of UF for soap and sericin, removal performances of pollution parameters and flux declines were investigated when selecting the most suitable membrane process.

CHAPTER 5

MATERIALS AND METHODS

5.1. Sampling

Since silk yarn production is a seasonal process, cocoon cooking wastewaters are generated in autumn each year. Therefore, in the autumn of year 2006, two cocoon cooking wastewater samples (CW1 and CW2), which were approximately 100 L each, were collected from the cocoon cooking plant in Bilecik. The first sample CW1 was consumed immediately in pre-treatment studies. The second sample CW2 was divided into smaller volumes and frozen at -20 ^{o}C to avoid putrefaction. They were used for membrane filtration experiments after defrosting and heating (Effect of preserving conditions of CW on membrane performance is given in Appendix A). The fractions of CW2 sample were named CW2-A, CW2-B, CW2-C, CW2-D, CW2-E, CW2-F, CW2-G and CW2-H. The CW2 samples were consumed in membrane filtration experiments. In addition to cocoon cooking wastewaters, silk degumming wastewaters were sampled three times from the silk degumming process stream of the silk dye-house located in Bursa. These samples were named SDW1, SDW2 and SDW3, respectively.

All the samples were analyzed according to the parameters given in Table 5.1. In each analysis, at least duplicate samples were used. The samples from CW2-A to CW2-E were used in total recycle mode of filtration (TRMF) experiments. Since these experiments were completed in a short period of time, no protective chemicals were added to the samples. However, in concentration mode of filtration experiments (CMF), which took longer times, 0.02 % NaN_3 was added to the samples CW2-F, CW2-G and CW2-H in order to avoid putrefaction.

As can be seen in Table 5.1, silk degumming and cocoon cooking wastewaters have very high amounts of organic matter. Chemical oxygen demand (COD) is 11-17 g/L

in CW whereas it is 55-63 g/L in SDW. The significant COD difference between these wastewaters is due to two reasons; the presence of soap in SDW, which significantly contributes to COD, and application of three consecutive degumming cycles in the plant, which means generation of a more concentrated wastewater. CW and SDW are both highly colored and turbid, with a range of 4500-44000 Pt-Co for color and 500-7400 NTU for turbidity. Similarly, total solids contents are very high, i.e., around 10 g/L and 40 g/L for CW and SDW, respectively. The amounts of total protein and sericin in CW are 5-10 g/L and 5-8 g/L, respectively. On the other hand, they are almost six times higher in SDW, i.e., 35-47 g/L and 27-34 g/L, respectively. Another important difference between SDW and CW is pH; CW has slightly acidic to neutral pH of 5.8-6.5 while SDW has alkaline pH of 8.6-9.6 due to the presence of soap and Na_2CO_3. Moreover, samples contain 0.3-1.3 g/L of carbohydrates, which originate from sericin structure. In summary, SDW has worse characteristics as compared to CW in terms of pollution parameters but it seems to be an abundant source of sericin as the sericin concentration in SDW is almost six times higher than that of CW.

SDW1 was used for characterization purposes, only. SDW2 and SDW3 were used for determination of the most suitable pre-treatment method and also the application of membrane separation tests.

Table 5.1. Characteristics of CW and SDW samples

Sample	Wastewater Quality							
	Sericin (mg/L)	T. Protein (mg/L)	COD (mg/L)	T. Solids (mg/L)	Color (Pt-Co)	Turbidity (NTU)	Carbohydrate (mg/L)	pH
CW1	-	9443	11600	10238	8650	771	-	5.9
CW2-A	5809	8357	14575	12560	6060	561	355	5.8
CW2-B	-	9883	14920	13130	25000	1050	-	5.8
CW2-C	-	9127	14250	12880	27150	468	-	5.9
CW2-D	5562	7606	15245	12630	5490	559	406	5.9
CW2-E	6354	8151	13075	12460	6200	543	769	6.2
CW2-F	5679	5510	14205	12470	5640	823	289	6.1
CW2-G	7957	7992	17050	14040	5970	985	1290	6.5
CW2-H	5043	7202	13600	11800	4520	745	592	5.9
SDW1	-	35634	62850	40637	43600	7370	-	9.6
SDW2	34002	46747	59150	39900	26050	4134	513	8.6
SDW3	27581	-	55950	-	-	-	-	9.1

5.2. Experimental Methods

5.2.1. Centrifugation

The wastewater samples were centrifuged at 3000-4000 rpm for 10-30 min by using Hettich Universal and Rotofix 32A model centrifuge apparatus.

5.2.2. Microfiltration

In order not to lose sericin in pre-treatment stage, the applicability of dead-end microfiltration (MF) as a physicochemical process was tested for the pre-treatment of cocoon cooking wastewaters. A conventional vacuum filtration apparatus (Millipore) providing dead-end filtration was used. The filter media having a radius of 47 mm and pore sizes of 1 μm, 8 μm and 20 μm were used under a vacuum of 550 mm Hg (gauge). The properties of filter media are given in Table 5.2. The filtrate were collected and analyzed for COD, color, turbidity, total solids, total protein, sericin and carbohydrate. The fluxes were determined by dividing the total volume of the filtrates by the filtration time and filter area.

Table 5.2. Properties of filter media

Filter Name	Type	Material	Effective Area (m^2)	Molecular Weight Cut-off (MWCO) / Pore Size (μm)
Whatman 41	MF	Cellulose	0.0010	20-25 μm
Millipore	MF	Cellulose	0.0010	8 μm
Whatman GF/B	MF	Fiberglass	0.0010	1 μm
Osmonics PW	UF	Polyethersulfone	0.0044	20 kDa
Osmonics PT	UF	Polyethersulfone	0.0044	5 kDa
Osmonics GH	UF	TFC [a]	0.0044	1 kDa
Osmonics DK	NF	TFC [a]	0.0044-0.036	[b]
Dow FilmTec NF90	NF	TFC [a] polyamide	0.0044-0.072	100 Da [c]

[a] Thin film composite
[b] 98 % $MgSO_4$ rejection
[c] 97 % $MgSO_4$ rejection

5.2.3. Ultrafiltration and Nanofiltration

Ultrafiltration (UF) and nanofiltration (NF) membranes were used in TRMF experiments by using Berghof BHT-2 model membrane filtration system while CMF experiments were performed by using a DSS LabStak M20 model plate-and-frame membrane filtration system. Schematic representation of UF and NF systems is shown in Figure 5.1.

In TRMF experiments, both permeate and retentate were recycled back to the feed tank and thus, the feed quality was assumed to be constant since the feed volume did not change throughout the experiment. In this way, UF and NF performances were compared, and thus, the most appropriate membrane was chosen.

In Berghof BHT-2 model system, inlet pressure was adjusted to 2 bars for UF and 5 bars for NF. The wastewater flow rate was adjusted to 30 L/h for both UF and NF. The properties of membranes are given in Table 5.2.

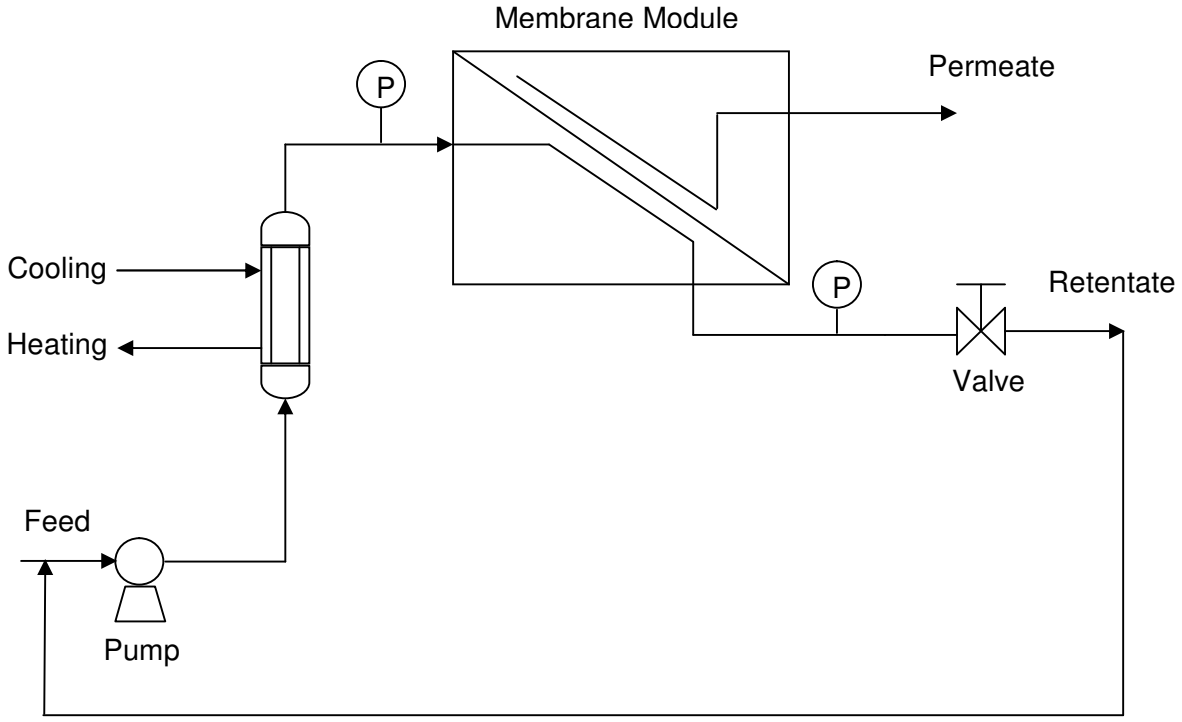


Figure 5.1. Schematic representation of UF and NF systems (P: Pressure gauge)

In CMF experiments, permeate was collected in a separate tank and sericin in wastewater was concentrated by reducing the feed volume with respect to time. In these experiments, volume reduction factor (VRF) was calculated as follows:

$$VRF = \frac{(V_i - V_s)}{V_r}$$

where V_i and V_r are the initial volume of feed and the volume of the retentate, respectively, and V_s is the volume of feed sampled throughout the experiment.

At the end of CMF experiments, VRF was realized as 4.6, 4.2 and 4.2, respectively (Table 5.3). That is, initial volumes of wastewater were reduced from 9.0 L, 9.8 L and 9.2 L to 1.9 L, 2.3 L and 2.1 L, respectively, during 16-21 h of filtration period.

Table 5.3. VRF calculations in CMF experiments

Sample	Wastewater Volume (L)				
	V_i	V_s	V_p*	V_r	VRF
CW2-F	9.0	0.17	6.9	1.9	4.6
CW2-G	9.8	0.20	7.3	2.3	4.2
CW2-H	9.2	0.18	6.9	2.1	4.2

* V_p: Permeate volume

In concentration mode of NF experiments, one pair of NF-DK membrane having an effective area of 0.036 m^2 was used with CW2-F and CW2-G samples. On the other hand, two pairs of NF-90 membranes having a total effective area of 0.072 m^2 were used with CW2-H sample. The inlet pressure was adjusted to 5 bars whereas the pressure on the retentate side was recorded as 4.8 bars. The permeate side was open to atmosphere (0 bar). Thus, the average trans membrane pressure applied on the membrane was determined as 4.9 bars. The wastewater flow rate was adjusted to 372 L/h. Clean water and wastewater fluxes were determined and the experiment was finished when the fluxes stabilized. Permeate stream was regularly sampled as the VRF increased.

At the end of TRMF and CMF experiments, permeates were collected and they were analyzed for their COD, color, turbidity, total solids, total protein, pH and sericin contents.

In TRMF experiments, virgin membranes were used in each run. In CMF experiments, the same membrane was used for more than once after performing chemical cleaning. The fouled membranes were cleaned chemically at the end of each experiment and the clean water fluxes of cleaned membranes were determined. Flux measurements were carried out by collecting the permeate in a graduated cylinder in definite time intervals. The fluxes were calculated via dividing the collected volume by time and effective membrane area.

In order to determine the flux declines, the fluxes were measured in four steps:
1- Initial clean water flux (I): This is the initial clean water flux of clean membrane prior to its first use,
2- Wastewater flux (W): This is the wastewater flux stabilized with respect to time during filtration,
3- Clean water flux of the fouled membrane (F): Clean water flux was measured with the fouled membrane after the filtration of wastewater had been finished,
4- Clean water flux of the cleaned membrane (C): This is the last flux measured with clean water after the membrane had been subjected to chemical cleaning.

The effects of concentration polarization and fouling on flux declines were determined based on calculations given in Table 5.4.

Table 5.4. Flux decline calculations

Formula	Description
(I-W)/I	Total flux decline
(F-W)/F	Flux decline due to concentration polarization
(I-F)/I	Flux decline due to total fouling
(C-F)/C	Flux decline due to reversible fouling
(I-C)/I	Flux decline due to irreversible fouling

5.2.4. Membrane Cleaning

All the membranes used in membrane filtration tests were cleaned after the experiments were run with the wastewaters in order to remove the organic and inorganic precipitates from the surface of the membranes (The effect of chemical cleaning on membrane performance is given in Appendix B). Membranes were taken out of the system and they were soaked into a solution containing 0.5 M NaOH and approximately 200 mg/L free chlorine for 20-30 min. The cleaned membranes were always kept wet in 0.25% sodium bisulfite solution in order to avoid bacterial growth on membranes.

5.2.5. Extraction of Sericin from Cocoon

Sericin was extracted from cocoons and used as a calibration standard. To do this, the cocoons were first cut into small pieces. Then, water was added onto them and they were autoclaved at 120 ^{o}C for 1 hour (The effect of autoclave time on the solubility of sericin was investigated and given in Appendix C). The sericin solution was filtrated through 1.6 µm filter (Whatman GF/A). After filtration, cold ethanol was added slowly into sericin solution until a final ethanol concentration of 75% (v/v) was obtained. The supernatant of ethanol was discarded and the settled sericin was frozen at -80 ^{o}C. Then, it was dried in a lyophilizator to obtain powder sericin (Kurioka et al., 2004; Wu et al., 2007; Vaithanomsat et al., 2008).

5.2.6. Precipitation of Sericin

After the wastewater samples were concentrated by NF process, sericin was precipitated in two ways; first, four types of acid (HNO_3, HCl, H_2SO_4 and $C_2H_4O_2$) (0.3% v/v) were used to decrease pH from 6.1 to 3.8, at which sericin becomes insoluble; second, ethanol (75% v/v) was used. Samples into which acids or ethanol were added, were centrifuged at 3000 rpm for 10-20 min to have the sericin as a pellet. The precipitated sericin was frozen at -80 ^{o}C and then, it was dried in a

lyophilizator to obtain powder sericin. The properties of recovered sericin were compared with those of native sericin and commercially obtained Brazilian sericin.

5.2.7. Dialysis

Recovered sericin samples were dialyzed against pure water in order to increase their purities. The molecular weight cut-off of dialysis sacks was 3.5 kDa (Serva). After sericin solutions were added into the dialysis sacks, the ends of sacks were closed and put into beakers filled with pure water. After that, these beakers were shaken in a water bath at 37 ^{o}C for 1-2 days. At the end of dialysis, sericin solutions in the dialysis sacks were analyzed by using high performance liquid chromatography (HPLC).

5.2.8. Protein Solubility

The solubility of recovered sericin samples at various pH values was determined with the method suggested by Wu et al. (1998). Samples were poured into the centrifugation tubes and adjusted to pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0 with 1 M NaOH and 1 M HCl. The tubes were thoroughly shaken and centrifuged at 4000 rpm for 10 min. Protein contents in the original sample and in the supernatant were determined by the bichinchoninic acid (BCA) protein protocol. In this way, the pH at which sericin solubility was maximum was determined. Protein solubility was calculated using the following equation (Were et al., 1997; Chove et al., 2007):

$$\text{Solubility } (\%) = \frac{(\text{Protein content of the supernatant})}{(\text{Total protein content in the sample})} \text{ x } 100$$

5.2.9. Analytical Methods

In order to make quantitative analysis of sericin and to determine its molecular weight distribution, Shimadzu Prominence Model HPLC system was used. In this system, a gel permeation chromatography (GPC) column (Nucleogel aqua OH-40-8) and a buffer solution containing 0.3 M NaCl and 0.05 M phosphate were used. These analyses were made at 30 ^{o}C and their ultraviolet absorbances (UVA) were read at 230 nm (Ogino et al., 2006). In sericin analysis, the flow rate of mobile phase was adjusted to 1 mL/min and it was decreased to 0.3 mL/min for the determination of molecular weight distribution. All samples were filtered through a 0.45 μm filter (Millipore Millex-HV), and then, injected into the system by means of a syringe having a volume of 20 μL. As calibration standards, sericin obtained from the native silk cocoons was used. In sericin analysis, it was observed that sericin eluted as a broad peak between 6.5 and 11.0 min (Figure 5.2).

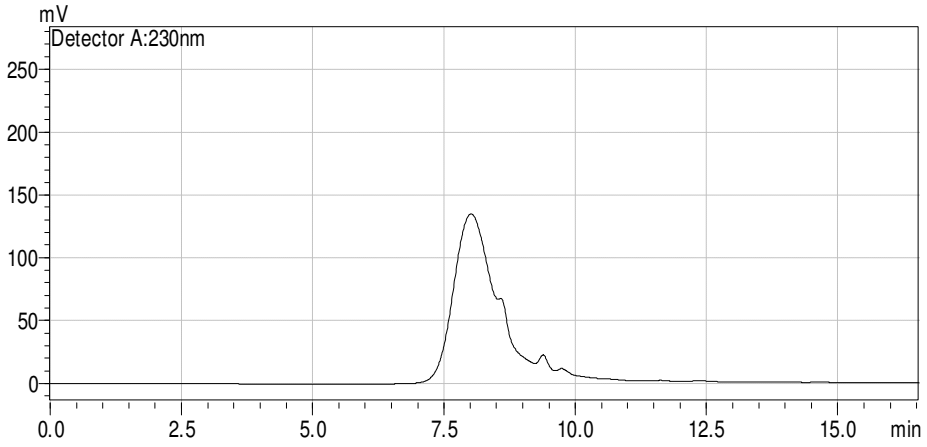


Figure 5.2. HPLC chromatogram of standard sericin solutions

(Concentration=2.5 mg/mL)

In determining the molecular weight distribution of sericin, a standard protein mixture containing cytochrome c monomer (12.4 kDa), myokinase (32 kDa), enolase (67 kDa), lactate dehydrogenase (142 kDa) and glutamate dehydrogenase (290 kDa) (Calbiochem) was used (Figure 5.3). Using the peak times (t) of standard proteins, the following linear regression equation was obtained.

$$\log MW = -0.1262t + 8.46 \qquad (r^2=0.98)$$

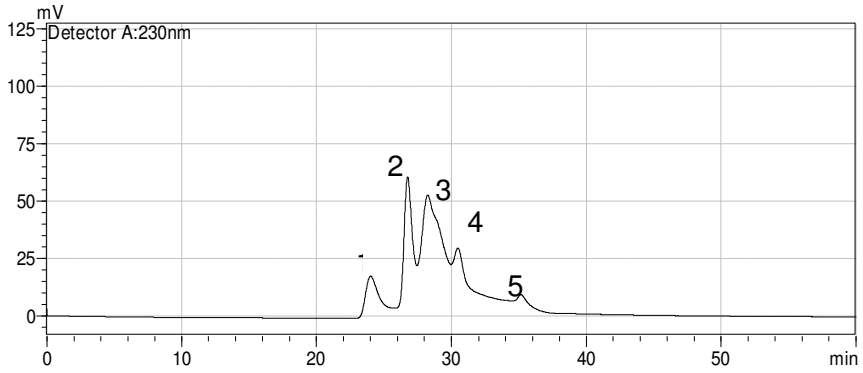


Figure 5.3. HPLC chromatogram of standard protein mixture (1. Cytochrome c monomer: 12.4 kDa, 2. Myokinase: 32 kDa, 3. Enolase: 67 kDa, 4. Lactate dehydrogenase: 142 kDa, 5. Glutamate dehydrogenase: 290 kDa)

Total protein analysis was done by using BCA protocol (Krieg et al., 2005). It is a spectrophotometric method where copper-BCA mixture is added into the samples and these samples are incubated in water bath at 37 ^{o}C for 30 min. Thus, in an alkaline environment, Cu^{2+} ion is reduced to Cu^{+} ion as a result of the reaction of protein and copper. Then, color occurs in the samples with the composition of BCA-Cu^{+}. In total protein analysis, UVA of the samples were read at 562 nm by using Hitachi model spectrophotometer and total protein concentrations were determined by the calibration equation, which was developed using the sericin standard obtained from the native cocoons (Appendix D).

Carbohydrate analyses of the wastewater samples were done by using Dubois method (Dubois et al., 1956). Firstly, stock glucose solution was prepared and the necessary dilutions were done. Then, 50 μL phenol (80 % w/w) and 5 mL sulphuric acid were added into the standards and samples. After waiting for 10 min, samples were thoroughly shaken and they were kept in a water bath at 30-35 ^{o}C for 15 min. Finally, UVA of the samples were read at 490 nm. Carbohydrate concentrations were determined by using the relevant calibration curve (Appendix E).

For the analysis of pollution parameters, standard methods given in Table 5.5 were mostly used (APHA, 1995). The COD values of the samples were determined using the HACH DR-2000 Model spectrophotometer at 620 nm according to the HACH Method 8000 approved by USEPA. Color measurements were done with the same apparatus at 455 nm. UVA measurements were done by using Shimadzu 1601 Model spectrophotometer at 275-290 nm. Turbidities of the samples were measured with HACH 2100N Model turbidimeter. Total solids amount of the samples were determined by gravimetric analysis.

Table 5.5. Analytical methods

Parameter	**Method**
UVA	Standard Method 5910 B
COD	HACH 8000 approved by USEPA
Color	Standard Method 2120 B
Turbidity	Standard Method 2130 B
Total Solids	Standard Method 2540 B
Total Protein	BCA Total Protein Protocol (Krieg et al., 2005)
Sericin	HPLC (Ogino et.al, 2006), GPC
Carbohydrate	Dubois Method (Dubois et.al, 1956)
pH	Standard Method 4500-H^{+} B

5.2.10. Fraction Collection with Fast Performance Liquid Chromatography (FPLC)

Fraction collection was done using FPLC to separately collect the sericin and the foreign substance in CW, which is originated from silkworm. This analysis was done in Middle East Technical University Central Laboratory. Varian ProStar Model FPLC system was used. In this system, Hiprep 16/60 Sephacryl-200 column and a buffer solution containing 0.3 M NaCl and 0.05 M phosphate were used. These analyses were made at room temperature and their UVA values were read at 230 nm. The flow rate of mobile phase was adjusted to 0.7 mL/min. All samples were filtered through a 0.45 μm filter, and then, injected into the system by means of a syringe having a volume of 1-1.2 mL. The sample was collected in 57 tubes, where the tubes 1-41 contained sericin and tubes 42-55 contained the foreign substance originating from silkworm. The contents of the tubes 42-55 were identified using MALDI-TOF at Ankara University Biotechnology Institute.

5.2.11. Characterization of Sericin

The moisture content, elemental composition, ash content and pH of commercial (S_C) and native sericin (S_N) samples were determined. Moisture contents of S_C and S_N samples were found via drying at 100 ^{o}C for 3 h followed by constant weight determination. Elemental compositions were determined in Middle East Technical University Central Laboratory. The weight percents of carbon (C), hydrogen (H), nitrogen (N) and sulfur (S) elements of sericin samples were determined on dry basis using a LECO CHNS-932 elemental analyzer. In this analysis, the instrument is heated to a temperature of 1000 ºC and approximately 1 g of sample is placed inside a silver capsule, which is dropped into the furnace, where it is completely combusted. Infrared detection is used to measure the C, H and S, whereas N is measured using thermal conductivity detection. Moreover, to determine the organic and inorganic contents of sericin, S_C and S_N were ignited at 600 ^{o}C for 1 h.

2-D gel electrophoresis and MALDI-TOF analyses have been done in Ankara University Biotechnology Institute Proteomics Laboratory. Firstly, recovered sericin sample, which was precipitated with ethanol, was passed through the ion exchange columns (anion and cation columns), which provides the separation of biomolecules relative to their charges. After passing through anion and cation columns, 2-D gel electrophoresis was done so that all proteins in recovered sericin sample were separated in the gel according to their pI values and their molecular weights. Then, each spot obtained in the gel was loaded into MALDI-TOF. In MALDI-TOF analysis, a co-precipitate of an UV-light absorbing matrix and sample is irradiated by a nanosecond laser pulse. The ionized sample is accelerated in an electric field and enters the flight tube. During the flight in this tube, different molecules are separated according to their mass to charge ratio and reach the detector at different times. In this way, each molecule yields a distinct signal. The MALDI-TOF spectrums of recovered sericin sample were compared with SWISS-PROT and ExPASy protein databases in order to identify sericin.

5.2.12. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

To detect the fatty acids that originate from soap and to show whether soap exists or not in silk degumming wastewater, GC-MS analysis has been done in Ankara University Biotechnology Institute Instrumental Analysis Unit. Firstly, extraction was applied to samples, where 40 mL of 2% methanolic NaOH was added to 20 g of sample, followed by boiling until saponification occurred. At the end of saponification, 50 mL of 14% BF_3 was added and the sample was boiled for 5 min. Then, 20 mL of n-heptane was added and boiled for another 1 min. After that, 4 mL of saturated NaCl was added. Finally, it was put in separation funnel and phase separation was observed after 5-10 min (AOAC, 1980). Then, 1 µL of extracted sample was injected into GC (Shimadzu GC-MS QP2010 Plus). This injected sample passed through a capillary column (Teknokroma TR-CN 100) by means of He, and came to MS. Here, it was fragmented by electron bombardment and thus, it was separated with respect to its mass/charge (m/z) ratio within quadropoles. Finally, MS spectrums were obtained and the peaks were matched with peaks in WILEY7 and NIST147 libraries.

CHAPTER 6

RESULTS AND DISCUSSION

In this study, sericin from two different sources were used as reference for evaluating the quality of sericin recovered from silk processing wastewaters (S_R). These are commercial sericin (S_C) purchased from a Brazilian company and native sericin (S_N) extracted from cocoon shells obtained from Bilecik. S_N was also used as a calibration standard in total protein and sericin analyses. The reason for using S_N instead of S_C as a calibration standard is explained in Appendix F. The properties of reference sericin samples and the sericin present in silk processing wastewaters were determined prior to the selection of the most suitable pre-treatment methods and membrane separation processes for sericin recovery.

6.1. Characterization of Sericin Used as Reference

The properties of sericin were compared to those reported by others (Zhang et al., 2002; Silk Biochemical Co., Ltd., 2008) and the results are given in Table 6.1. The moisture contents of S_C and S_N were found as 7.4% and 8.6%, respectively. These values are slightly higher than 5% reported by Silk Biochemical Co. but quite close to 9% and 8.2% reported by Zhang et al. (2002) for cocoon and silk yarn, respectively.

The elemental compositions of sericin samples are given in Table 6.1. As seen, carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) contents of S_C and S_N were quite close to each other, with a total element content of 62-63%. The N contents of S_C and S_N, which were determined as 13.9% and 14.9%, respectively, were almost same with 14.0% and 14.7% reported by others (Silk Biochemical Co. Ltd., 2008; Wu et al., 2007). Moreover, S, H and C contents of S_N and S_C were very close, i.e., 0.2-0.3%, 6.2-6.4% and 41.0-42.5%, respectively.

The ash contents of S_C and S_N were determined as 2.7-3.8% (Table 6.1). In a study in which the thermal properties of silk fibers were examined, this content was reported as 0.9% and 1.0% after the ignition of cocoon and silk yarn, respectively, at 550 ^oC for one night (Zhang et al., 2002). The reason that the ash contents of S_C and S_N are slightly higher than the values reported may be the difference in time carried out for the ignition process. Another reason is probably ignition of different materials, that is, pure sericin was ignited in this study whereas cocoon shell made of sericin and fibroin was ignited by Zhang et al. (2002). The ash contents of sericin and fibroin may differ, resulting in different values. On the other hand, the ash contents of S_C and S_N were quite close to those of 4% and 4.2% reported for pure sericin by Wu et al. (2007) and Silk Biochemical Co. Ltd. (2008). Ash concentration accounting for 4.2% suggested that sericin powder from wastewater possibly contained a little salt. This is in agreement with the inorganic content of cocoon, which includes calcium, potassium, sulphur, phosphorus, silicon and magnesium (Zhang et al., 2001; ASABE, 2008). These data revealed that the organic contents of S_C and S_N were 97.2% and 96.2%, respectively. The C, H, N, and S contents were found as 62-63%, and the difference between the total elements and organic contents is attributed to their oxygen contents.

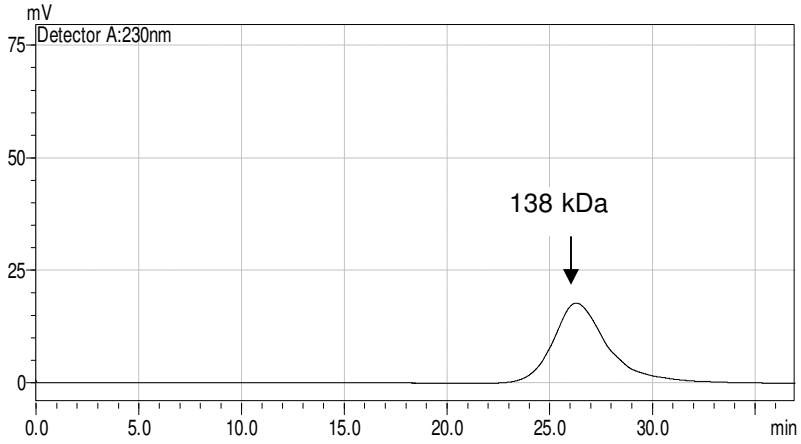
Table 6.1. Properties of sericin

Parameter	Source/Reference				
	This Study		Others		
	Sericin from Brazilian Company (S_C)	Sericin from Native Cocoon (S_N)	Silk Biochemical Co. Ltd. *	Zhang et al. (2002)	Wu et al. (2007)
Moisture (%)	7.4	8.6	≤ 5	8.2-9.0	-
Nitrogen (%)	13.9	14.9	≥ 14	-	14.7
Sulphur (%)	0.3	0.2	-	-	-
Hydrogen (%)	6.4	6.2	-	-	-
Carbon (%)	42.5	41.0	-	-	-
Ash (%)	2.7	3.8	≤ 4	0.9-1.0	4.2
pH	3.9	7.1	5-7	-	~ 7
MW (kDa)	138	124	6-15	10-300	14-467

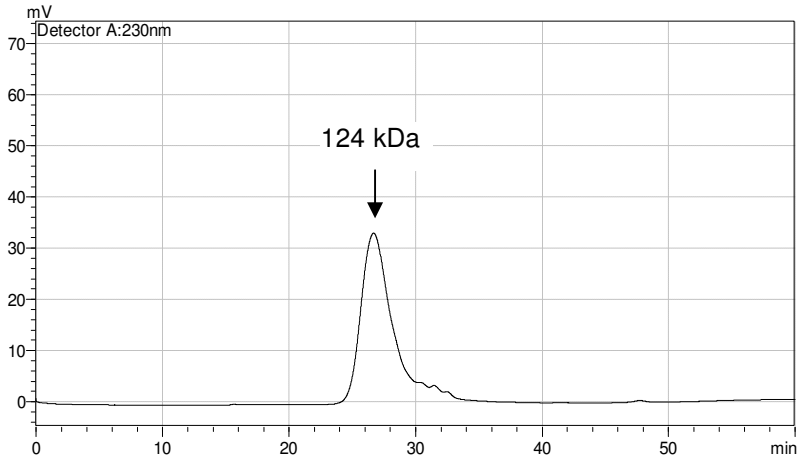
* http://www.alibaba.com/catalog/10624740/Sericin.html

In literature, pH of sericin is given as 5-7 (Wu et al., 2007; Silk Biochemical Co. Ltd., 2008). The pH of S_C and S_N were measured as 3.9 and 7.1, respectively. The pH difference is probably due to the extraction methods applied. Sericin in powder form can be obtained by precipitation using both acid and ethanol (Kurioka, 2004), which influence the pH of sericin solution.

A wide range of molecular weights (MW), that is, 6-467 kDa is reported for sericin (Wu et al., 2007; Silk Biochemical Co. Ltd., 2008). This is due to the fact that molecular weight of sericin is affected by factors such as pH, temperature and processing time (Zhang, 2002). In this study, the molecular weights of S_C and S_N samples were determined as 138 and 124 kDa (Figure 6.1), respectively. As seen, these values are in agreement with the literature.



(a)



(b)

Figure 6.1. HPLC chromatogram for MW of **(a)** S_C **(b)** S_N

Characterization of sericin samples showed that the properties of commercial and native sericin samples are quite similar to those reported in the literature and hence, they can be safely used as reference for evaluating the quality of recovered sericin.

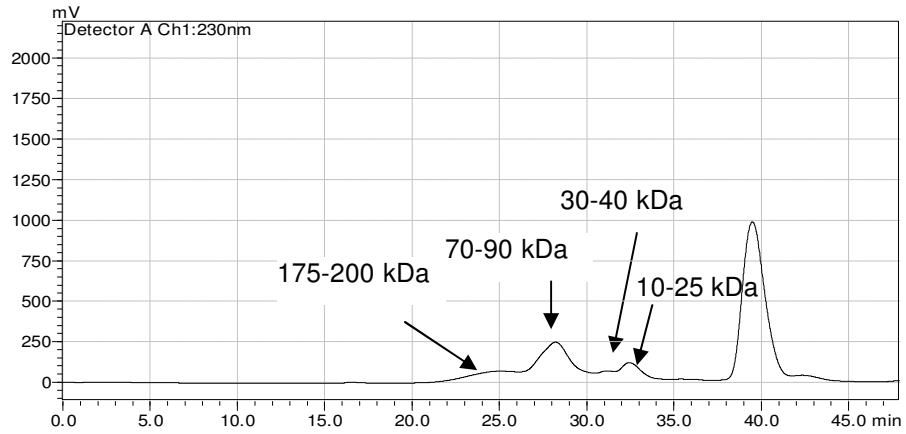
6.2. Characterization of Sericin in Silk Processing Wastewater

Sericin concentrations in CW and SDW were very different from each other. Sericin concentration in CW was 5043-7957 mg/L whereas it was 27581-34002 mg/L in SDW (Table 5.1). Flow rates of CW and SDW are 1875 L/day and 7500 L/week. By simple calculation, it was found that 750-1200 kg of sericin can be recovered from CW and 10000-13300 kg of sericin can be recovered from SDW annually. Since these amounts are really great, it is worth recovering sericin from silk processing wastewaters.

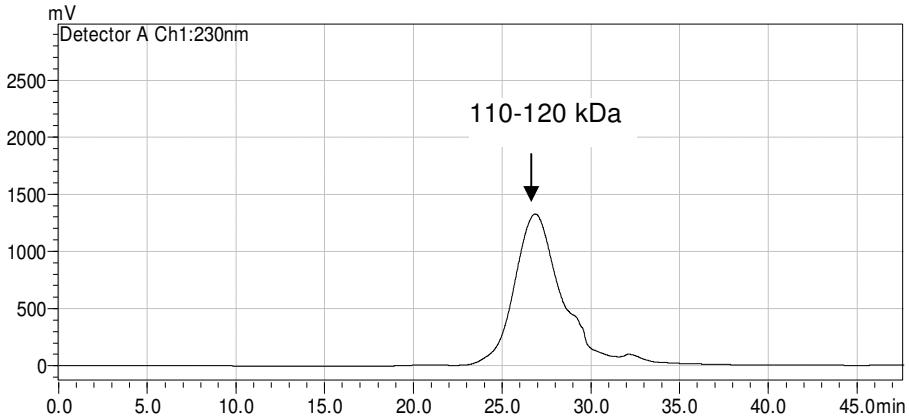
Before determining the most suitable membrane-based processes for sericin recovery, sericin in silk processing wastewaters was characterized. Molecular weight of sericin in CW and SDW was determined by using gel permeation chromatography (GPC) (Figure 6.2). Table 6.2 lists the MW of sericin polypeptides and their percentages in these wastewaters. As seen, there are four sericin fractions in CW; Sericin-1 with MW of 175-200 kDa at a fraction of 5-25%, Sericin-2 with MW of 70-90 kDa at a fraction of 53-69%, Sericin-3 with MW of 30-40 kDa at a fraction of 4-8%, and Sericin-4 with MW of 10-25 kDa at a fraction of 12-22%. Among all fractions, Sericin-2 is abundant and Sericin-3 is scarce in CW. In SDW, there is only one sericin fraction, that is, Sericin-SDW, with a MW of 110-120 kDa.

Table 6.2. MW of sericin in CW and SDW

Name of Sericin Fraction	MW (kDa)	Percentage in Wastewater (%)	
		CW	SDW
Sericin-1	175-200	5-25	-
Sericin-2	70-90	53-69	-
Sericin-3	30-40	4-8	-
Sericin-4	10-25	12-22	-
Sericin-SDW	110-120	-	100



(a)



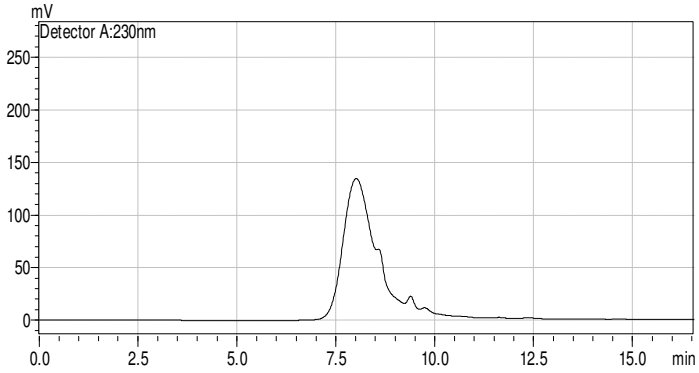
(b)

Figure 6.2. HPLC chromatograms of MW of sericin in **(a)** CW **(b)** SDW

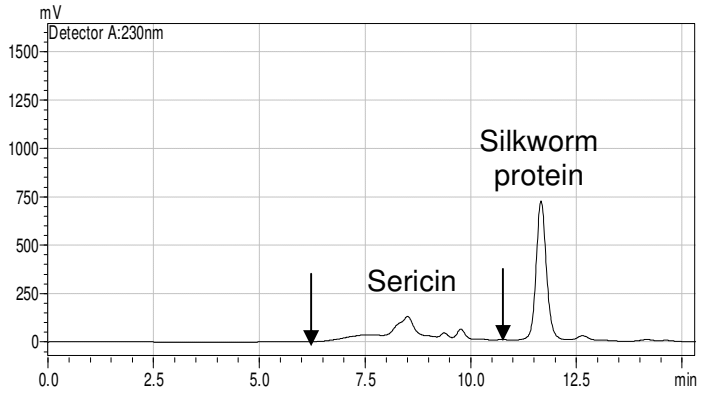
Figure 6.3(a) shows the chromatogram of standard sericin obtained from cocoon whereas Figures 6.3(b) and 6.3(c) represent the peaks of sericin in raw CW and SDW. As seen from these chromatograms, there are more than one peak in CW between 6.0 and 11.0 minutes when sericin exits from the column. This shows that there are a lot of sericin peptides having different molecular weight (MW) in cocoon cooking wastewater. It was observed that the peaks of wastewater samples and standard sericin were different in origin although they exited the column in the same

time interval. The reason for this is that sericin in wastewater and standard sericin obtained from cocoon have different MWs. This is an expected situation since it is known that the MW distribution of sericin in wastewater depends on the method of acquisition of sericin (Zhang et al., 2002). For example, sericin peptides having low MW dissolve in cold water whereas those having high MW dissolve in hot water. Some factors such as water temperature, waiting period in water and pH affect the MW of sericin in water.

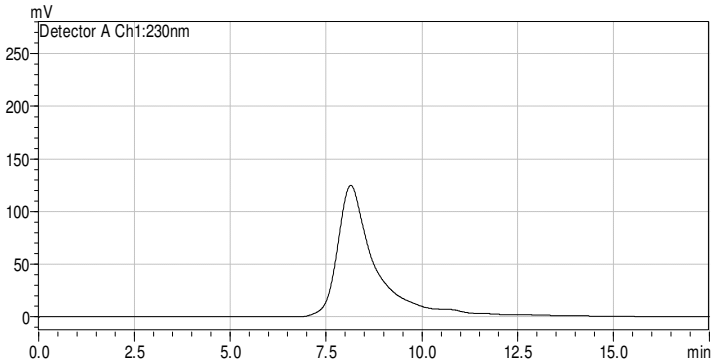
In Figure 6.3(b), it was observed that there was another substance except sericin eluting from the column between 11.5 and 13.5 minutes. The MW calibration formula enabled the calculation of MW of this substance as approximately 3 kDa. The presence of foreign substance was observed only in CW. To determine the origin of this foreign substance, the components of CW were analyzed separately. It was found that this substance was originating from the silkworm (Appendix G). In order to identify this substance, a solution was obtained by autoclaving a few silkworms in 25 mL ultrapure water at 120 ^{o}C for 1 h. Firstly, fraction collection was done with FPLC to collect the sericin and the foreign substance separately. Then, to identify the foreign substance, MALDI-TOF analysis was performed in Ankara University Biotechnology Institute Proteomics Laboratory with the fraction containing foreign substance. When MALDI-TOF spectrums of this substance were compared with SWISS-PROT and ExPASy protein databases (ExPASy, 2008), compatibility with proteins belonging to *Bombyx mori* was found (Table 6.3) The MALDI-TOF analysis proves that the foreign substance in CW is a protein originating from *Bombyx mori*, the silkworm. Further analysis was not performed to determine which protein it was; however, it may be speculated that this protein was Bombyxin B-2 (P26734) as it has a MW of 10 kDa, which is closest to 3 kDa obtained by GPC. This analysis revealed that CW contains a silkworm protein in addition to sericin, which needs to be separated in post membrane process.



(a)



(b)



(c)

Figure 6.3. HPLC chromatograms of **(a)** Standard sericin, S_N (2.5 mg/mL), **(b)** Sericin in raw CW (1/2 dilution), **(c)** Sericin in SDW (1/10 dilution)

Table 6.3. Properties of proteins matched with foreign substance

ExPASy Accession Number	Protein Name	Origin	Molecular Weight (Da)
P07836	Actin, muscle type A1	*Bombyx mori*	41876
P22922	Antitrypsin	*Bombyx mori*	43499
P26734	Bombyxin B-2	*Bombyx mori*	10039
Q17239	5-hydroxytryptamine	*Bombyx mori*	48599
Q2F637	14-3-3 protein zeta	*Bombyx mori*	28097
Q566B1	Bursicon	*Bombyx mori*	17901

6.3. Sericin Recovery from Cocoon Cooking Wastewaters

6.3.1. Selection of the Pre-treatment Process for CW

In membrane separation processes applied for the recovery of sericin protein, it is necessary to find an appropriate pre-treatment method to control membrane fouling. However, there should be no or minimum protein removal in pre-treatment stage in order to maximize the amount of sericin recovered from wastewater. Therefore, three physico-chemical methods were considered in four alternatives for the pre-treatment of CW. These were gravity settling (GS), microfiltration (MF) and centrifugation (CFG) in single and sequential modes as shown in Figure 6.4.

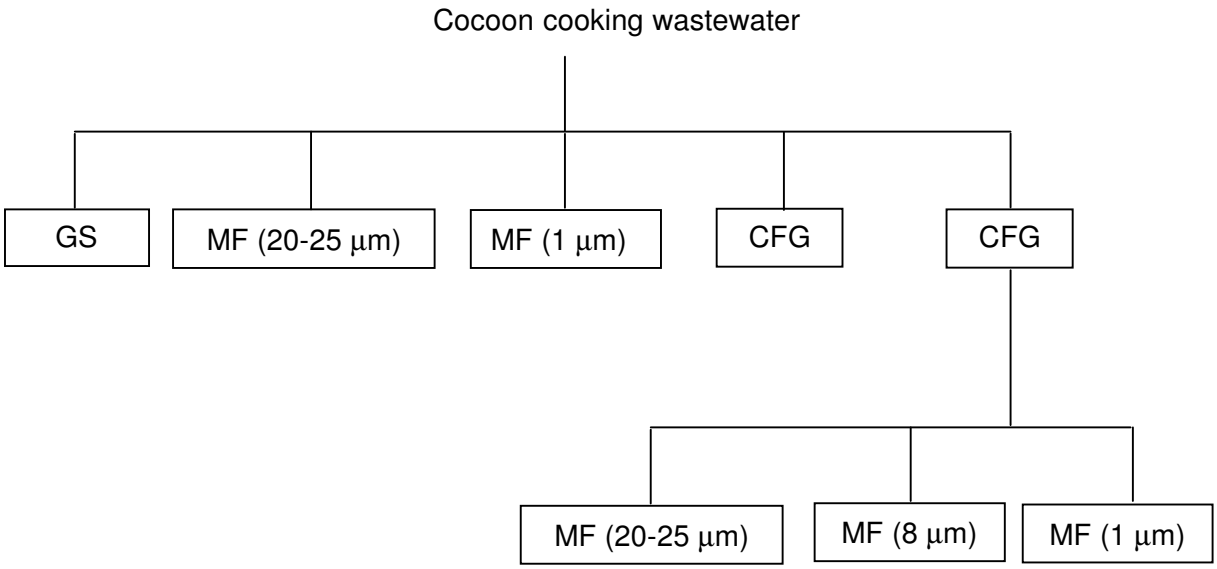


Figure 6.4. Schematic presentation of pre-treatment alternatives

In the first alternative, CW was kept for about 21 h to settle by gravity since this was considered as the most economical pre-treatment method. However, solids settling efficiency was only 34%. Moreover, CW started to decompose, which made it difficult to apply gravity settling for this type of wastewater. In the second alternative, microfiltration was applied where two filter media having pore sizes of 20-25 μm and 1 μm were used. MF (20-25 μm) provided 15%, 8% and 20% removal efficiencies for COD, color and turbidity, respectively, accompanied with a flux of 930 L/h/m^2. Since the removal efficiency of this filter media was low, MF (1 μm) was applied. Although the removal rates for COD, color and turbidity significantly increased to 34%, 79% and 99%, respectively, a very rapid clogging of the filter media was observed (Table 6.4). The clogging of the filter media caused severe flux decline, where the flux of MF (1 μm) was almost five-fold less than that of MF (20-25 μm). These results revealed that dead-end MF alone was not an acceptable pre-treatment method for CW.

Table 6.4. Removal performances of pre-treatment processes

Process	Removal Performance (%)					Flux [a]
	COD	T. Solids	T. Protein	Color	Turbidity	(L/h/m^2)
MF (20-25 μm)	15	-	-	8	20	930
MF (1 μm)	34	-	-	79	99	200
CFG	26	21	22	74	92	-
CFG + MF (20-25 μm)	27	26	24	76	95	32900
CFG + MF (8 μm)	33	25	33	80	96	8500
CFG + MF (1 μm)	35	26	32	80	98	10500

[a] It was found by dividing total volume filtrated by total filtration time and effective membrane area.

In the third alternative, centrifugation was applied at 3000 rpm for 10 min in order to improve removal efficiencies. The removals for COD, total solids and total protein were 26%, 21% and 22%, respectively. Furthermore, color and turbidity removals were 74% and 92% (Table 6.4). These data show that CFG and MF provided similar removal efficiencies, and hence, they would be equally effective in controlling fouling in the post membrane unit. However, it was observed that the centrifuged wastewater contained some floating material such as cocoon pieces. These materials had to be removed in order to recover sericin as pure as possible in post membrane separation unit.

Since the application of CFG and MF in single stages was found insufficient, they were applied sequentially for maximizing the fluxes and improving the removal efficiencies of pollution parameters. Therefore, centrifugation was followed by MF in the fourth alternative. The suspended solids which were not settled in CFG were filtrated through a metal filter not to clog the post filters. As seen in Table 6.4, the removal performances slightly increased by applying post MF (20-25 μm) to CFG. The removals of COD, total solids and total protein increased to 27%, 26% and

24%. Similarly, the removals for color and turbidity increased to 76% and 95%, respectively. On the other hand, flux significantly increased from 930 L/h/m^2 to 32900 L/h/m^2 by applying CFG prior to MF (20-25 µm), which corresponds to 35-fold increase of flux. In CFG + MF (8 µm) and CFG + MF (1 µm) alternatives, the removal efficiencies increased to 33-35%, 25-26% and 32-33% for COD, total solids and total protein, respectively. Similarly, the color and turbidity removals increased to 80% and 96-98%, respectively.

The fluxes of MF (8 µm) and MF (1 µm) media were 8500 and 10500 L/h/m^2, respectively. The change in fluxes with time was monitored. As seen from Figure 6.5, the fluxes of MF (1 µm) and MF (8 µm) were equal to each other after 20 seconds and then, they both decreased rapidly. The maximum flux was observed in MF (20-25 µm), where it was 2.2-2.4 times higher than those of MF media with smaller pore sizes at the end of 50 seconds. Although the highest flux was obtained in CFG + MF (20-25 µm), the best permeate quality was obtained in CFG + MF (1 µm) (Table 6.5).

Table 6.5. Pre-treated wastewater characteristics

Process	Pre-treated Wastewater Quality				
	COD (mg/L)	**T. Solids (mg/L)**	**T. Protein (mg/L)**	**Color (Pt-Co)**	**Turbidity (NTU)**
CFG	8597	8075	7403	2280	60
CFG + MF (1 µm)	7513	7613	6426	1730	18
CFG + MF (8 µm)	7773	7700	6341	1760	33
CFG + MF (20 µm)	8460	7625	7148	2040	38

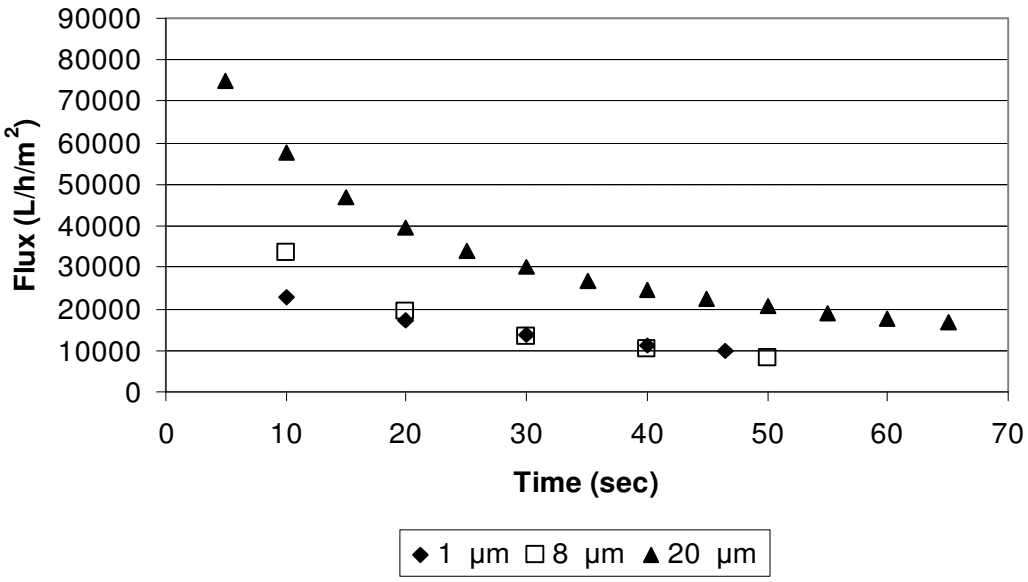


Figure 6.5. Comparison of fluxes in MF

Among the pre-treatment alternatives considered, centrifugation was found very suitable for removing suspended solids. On the other hand, the purity of recovered sericin is an important issue as the primary goal is to recover this protein. In this regard, the pre-treatment process has to ensure that all pollutants are removed and sericin remains in wastewater at highest purity. Centrifugation alone may not be sufficient to reach this goal, and a two-stage pre-treatment method would be necessary, although more costly than a single stage method. To this end, the necessity of the second stage in pre-treatment was investigated. MF (1 µm) seemed to be better than MF (20-25 µm) to follow centrifugation as it provided the best filtrate quality. Hence, it was decided to adopt MF (1 µm) after centrifugation.

The effect of MF (1 µm) on the flux decline of post membrane was also tested by carrying out two sets of experiments; 1. CFG + UF (20 kDa), 2. CFG + MF (1 µm) + UF (20 kDa). In this way, the effect of adopting MF on the removal of pollution parameters and the flux declines of post membrane unit was determined in order to evaluate the advantage of MF in the pre-treatment stage. The permeate qualities

and rejection performances of these two process alternatives are given in Table 6.6. As seen, without MF (1 μm), UF (20 kDa) membrane achieved 66% and 53% rejection for total protein and COD, and 94% and 99% rejection for color and turbidity, respectively. With preceding MF (1 μm), however, UF (20 kDa) membrane achieved higher rejection efficiencies of 87% and 55% for total protein and COD. On the other hand, rejection for color and turbidity remained almost the same, that is, 93% and 99%, respectively.

The effect of adopting MF on the flux decline of post UF was also investigated. It was observed that flux decline decreased from 88% to 80% by adopting MF prior to UF. Two alternative cleaning methods were applied to recover the clean water fluxes. In CFG + UF (20 kDa), both filtration system and membrane were washed with NaOH (pH 10) followed by HNO_3 (pH 3). However, clean water flux was recovered by only 27%. In the second alternative, a mixture of NaOH and chlorine was used as cleaning chemicals (Crawford et al., 1995; Wu et al., 2006). The membranes were soaked into a solution containing 0.5 M NaOH and approximately 200 ppm free chlorine for 40-45 min. Then, clean water fluxes were determined again. The clean water flux recovery increased from 83% to 104% by applying MF in the pre-treatment stage (Table 6.6). The reason for flux recovery greater than 100% may be the increased hydrophilicity of the membrane due to chemical exposure.

The flux decline analyses of UF with and without MF are shown in Table 6.7. As seen, concentration polarization and total fouling were 42% and 79%, respectively, in UF without MF, where 20% of total fouling was reversible and 73% was irreversible. This means 73% of original clean water flux could not be restored by chemical cleaning. By applying MF prior to UF, concentration polarization increased to 67% and total fouling decreased to 40%. Furthermore, clean water flux was completely recovered by chemical cleaning. These results indicated that adopting MF as the second-stage in pre-treatment was beneficial in terms of both rejection performance and flux control. Hence, it was decided to apply MF (1 μm) after centrifugation in the pre-treatment stage.

Table 6.6. Permeate qualities and flux analysis of UF (20 kDa) process (Total r*ejection performances, %*)

Parameter	**CFG + UF (20 kDa) (w/ CW1)**	**CFG + MF (1 µm) + UF (20 kDa) (w/ CW1)**
Sericin (mg/L)	nm	2080
T. Protein (mg/L)	2738 *(66)*	1624 *(87)*
COD (mg/L)	5440 *(53)*	5205 *(55)*
T. Solids (mg/L)	nm	6500 *(37)*
Color (Pt-Co)	540 *(94)*	570 *(93)*
Turbidity (NTU)	4.6 *(99)*	7.2 *(99)*
pH	5.7	5.9
Flux Decline (%)	88	80
Flux Recovery with $NaOH+HNO_3$ (%)	27	68
Flux Recovery with NaOH+Chlorine (%)	83[a]	104[b]

nm: not measured
[a]0.5 M NaOH + 196 ppm free Cl for 45 min
[b]0.5 M NaOH + 240 ppm free Cl for 40 min

After selecting CFG + MF (1 µm) as the most suitable pre-treatment process, it was applied to CW samples before all UF and NF experiments. HPLC chromatogram of pre-treated CW for sericin is given in Figure 6.6. In Tables 6.8 and 6.9, pre-treated CW qualities and their rejection performances are shown. As seen, sericin and protein rejections were low as desired; that is, 1-16% and 3-18%, respectively. COD and total solids removal efficiencies were also low since COD and total solids originate mainly from sericin. The rejections of COD, total solids, color and turbidity were 23-36%, 11-19%, 48-92% and 94-98%, respectively. The carbohydrates were also removed at an efficiency of 12-46%.

Table 6.7. Flux decline analysis of UF

Process	Flux (L/m^2/h) (T=20-24 ºC)				Flux Decline (%)			
	I	W	F	C	C.P.	T.F.	R.F.	I.F.
CFG+UF (20 kDa)	76.0	9.5	16.3	20.4	42	79	20	73
CFG+MF (1 µm)+UF (20 kDa)	67.9	13.6	40.7	70.6	67	40	*	*

I: Clean Water
W: Wastewater
F: Clean water before cleaning
C: Clean water after cleaning
C.P. : Concentration polarization [(F-W)/F]
T.F. : Total fouling [(I-F)/I]
R.F. : Reversible fouling [(C-F)/C]
I.F. : Irreversible fouling [(I-C)/I]
* Could not be calculated since the value of C is greater than the value I.

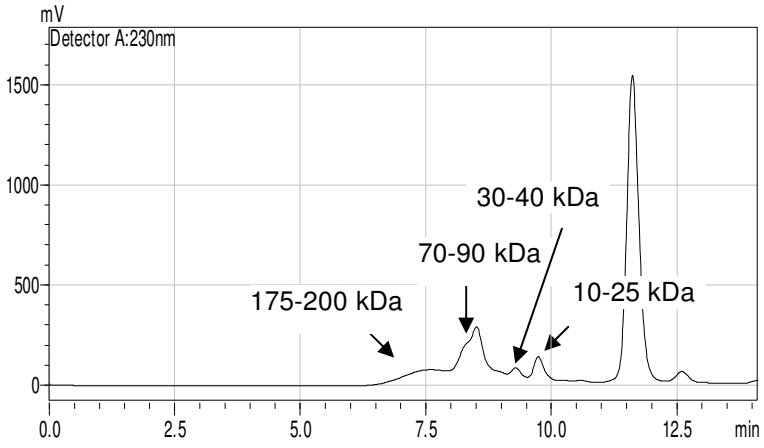


Figure 6.6. HPLC chromatogram of sericin in pre-treated CW

Table 6.8. Pre-treated CW quality (TRMF experiments)

Parameter	Pre-treated CW Quality *(Rejection Performance, %)*				
	UF (20 kDa) (w/ CW2-A)	**UF (5 kDa) (w/ CW2-B)**	**UF (1 kDa) (w/ CW2-C)**	**NF-DK (w/ CW2-D)**	**NF-90 (w/ CW2-E)**
Sericin (mg/L)	5775 *(1)*	9706	8716	6087	5639 *(11)*
Sericin-1	1416	nm	nm	1319	108
Sericin-2	3074			3203	2662
Sericin-3	471			575	397
Sericin-4	814			991	1038
T. Protein (mg/L)	7711 *(8)*	9607 *(3)*	8217 *(10)*	7084 *(7)*	6982 *(14)*
COD (mg/L)	10540 *(28)*	10470 *(30)*	10395 *(27)*	9780 *(36)*	10090 *(23)*
T. Solids (mg/L)	10730 *(15)*	10700 *(19)*	10640 *(17)*	10870 *(14)*	11110 *(11)*
Color (Pt-Co)	2450 *(60)*	2170 *(91)*	2130 *(92)*	2190 *(60)*	2840 *(54)*
Turbidity (NTU)	36 *(94)*	17 *(98)*	14 *(97)*	21 *(96)*	30 *(94)*
Carbohydrate (mg/L)	251 *(29)*	nm	nm	299 *(26)*	634 *(18)*
pH	5.9	5.8	5.9	5.9	6.1

nm: not measured

Table 6.9. Pre-treated CW quality (CMF experiments)

Parameter	Pre-treated CW Quality *(Rejection Performance, %)*		
	NF-DK (w/ CW2-F)	**NF-DK (w/ CW2-G)**	**NF-90 (w/ CW2-H)**
Sericin (mg/L)	4768 *(16)*	7800 *(2)*	4595 *(9)*
Sericin-1	280	1268	226
Sericin-2	4283	3697	3301
Sericin-3	0	892	0
Sericin-4	205	1943	1068
T. Protein (mg/L)	5262 *(5)*	6570 *(18)*	6328 *(12)*
COD (mg/L)	10205 *(28)*	11140 *(35)*	9730 *(28)*
T. Solids (mg/L)	10700 *(14)*	11610 *(17)*	10500 *(11)*
Color (Pt-Co)	2080 *(63)*	3010 *(50)*	2360 *(48)*
Turbidity (NTU)	17 *(98)*	56 *(94)*	18 *(98)*
Carbohydrate (mg/L)	156 *(46)*	1136 *(12)*	425 *(28)*
pH	6.1	6.5	5.9

6.3.2. Selection of the Most Appropriate Membrane Separation Process

In order to determine the most appropriate membrane separation process for sericin recovery from cocoon cooking wastewaters, three UF membranes having MWCO of 20 kDa, 5 kDa and 1 kDa, and two NF membranes (NF-DK and NF-90) were tested in total recycle mode of filtration (Figure 6.7). The performances of these membranes were evaluated by comparing the rejection efficiencies for sericin and pollution parameters as well as monitoring the flux declines. To see the reproducibility of membrane processes, another two UF (5 kDa) membranes were used. The results of these two UF experiments are given in Appendix H. After

selecting the most appropriate membrane, it was used in CMF experiments in order to concentrate sericin in wastewater.

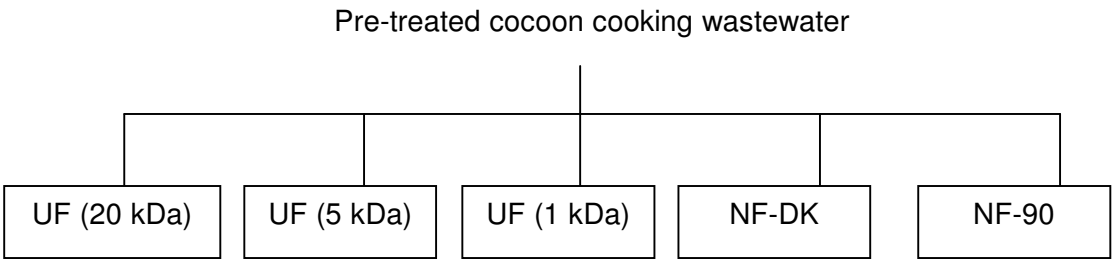


Figure 6.7. Schematic presentation of membrane separation alternatives

6.3.2.1. Rejection Performances of UF and NF Membranes

6.3.2.1.1. Rejection of Sericin

UF and NF permeate qualities and the corresponding rejection performances for sericin are given in Table 6.10. As seen, the performances of UF membranes for sericin rejection were quite low. When the MWCO was decreased from 20 kDa to 5 kDa, sericin rejection efficiency increased from 36% to 52%. Similarly, total protein rejection was realized as 57% and 68% for UF (20 kDa) and UF (5 kDa), respectively. The sericin rejection performances of these membranes were quite low as compared to those reported by Fabiani et al. (1996), where they achieved 97% sericin rejection with a UF membrane having MWCO of 20-30 kDa. One reason for this is probably the differences in MW distributions of sericin in cocoon cooking wastewaters used in this study and the silk degumming wastewater used in their study. Sericin in CW has a broad range of molecular weights due to the long duration of cooking process, where sericin in hot water decomposes into smaller

molecular weight polypeptides (Wu et al., 2007). The differences in membrane properties may be another reason for different performances.

A tight UF was required to increase the sericin rejection efficiency. A UF (1 kDa) membrane was used for this purpose and sericin rejection efficiency slightly increased to 60% whereas total protein rejection remained at 67% (Table 6.10). Despite low rejection performances, the flux declines were quite high, i.e., 88% for UF (20 kDa) and 83% for UF (5 kDa) membranes. These flux declines were very similar to those reported by Fabiani et al. (1996). As compared with former UF membranes, the flux decline of UF (1 kDa) was lower, i.e., 58% (Table 6.10). The flux declines and fouling analysis of membranes are further discussed in Section 6.3.2.2.

These results revealed that 40% of sericin in CW would be lost in permeate and only 60% of sericin would be recovered even with the tightest UF membrane. Therefore, UF was found insufficient to recover all sericin fractions but it could enable fractionation of sericin into different molecular weight components. Moreover, none of the UF membranes could separate sericin from other protein originating from the silkworm in wastewater. As seen from Figures 6.8 and 6.9, the second peak which belongs to the silkworm protein is present both in retentates and permeates of UF membranes. This means that sericin recovered by these UF membranes would contain this protein, and be regarded as a mixture rather than a pure product.

In NF processes, two different NF membranes, namely NF-DK and NF-90 were tested for sericin recovery. Sericin rejections were as high as 94% and 95% and total protein rejections were 84% and 100% for NF-DK and NF-90, respectively (Table 6.10). In NF-90, although sericin was a protein, total protein concentration was found as 0 mg/L. The reason for this was the difference between the sensitivities of the methods used for sericin and total protein analyses. Sericin concentration was determined using HPLC whereas total protein was determined using a spectrophotometric method (BCA protocol), which was less sensitive than HPLC. Flux declines were high in both NF processes, that is, 70% in NF-DK and 75% in NF-90. Further explanations about flux decline analysis will be given in Section 6.3.2.2.

Table 6.10. UF and NF performances for sericin (TRMF experiments)

Parameter	Permeate Quality *(Rejection Performance, %)*				
	UF (20 kDa) (w/ CW2-A)	**UF (5 kDa) (w/ CW2-B)**	**UF (1 kDa) (w/ CW2-C)**	**NF-DK (w/ CW2-D)**	**NF-90 (w/ CW2-E)**
Sericin (mg/L)	3669 *(36)*	4621*(52)*	3488 *(60)*	392 *(94)*	296 *(95)*
Sericin-1	0 *(100)*	nm	nm	0 *(100)*	0 *(100)*
Sericin-2	2719 *(12)*			65 *(98)*	0 *(100)*
Sericin-3	218 *(54)*			221 *(62)*	252 *(37)*
Sericin-4	732 *(10)*			106 *(89)*	44 *(96)*
T. Protein (mg/L)	3287 *(57)*	3108 *(68)*	2715 *(67)*	1119 *(84)*	0 *(100)*
Flux Decline (%)	88	83	58	70	75

nm: not measured

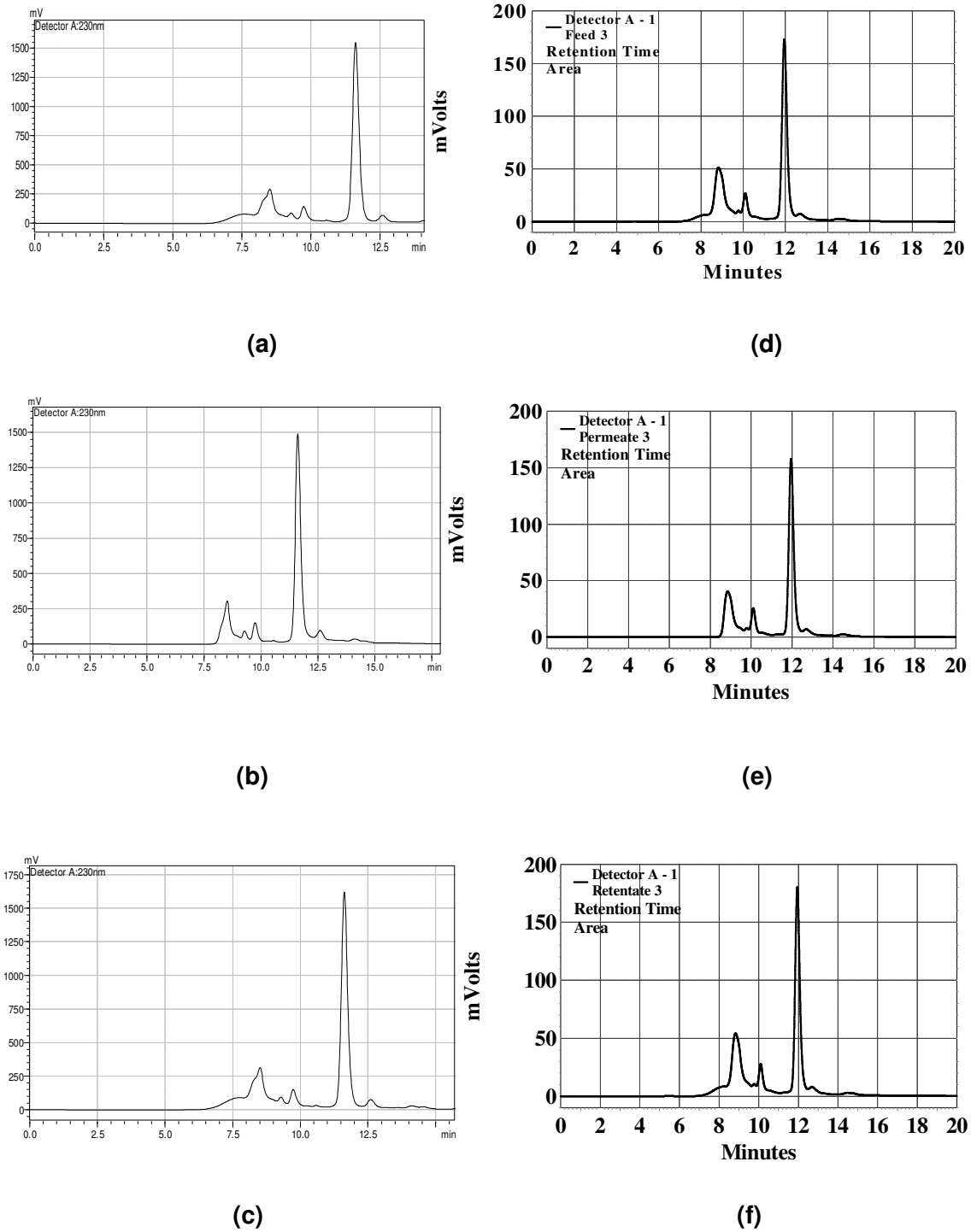
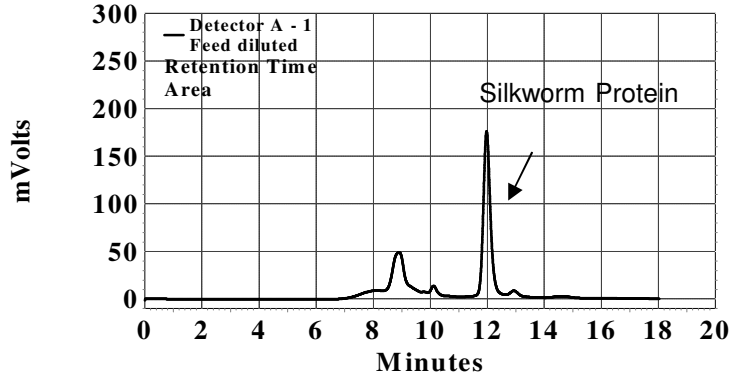
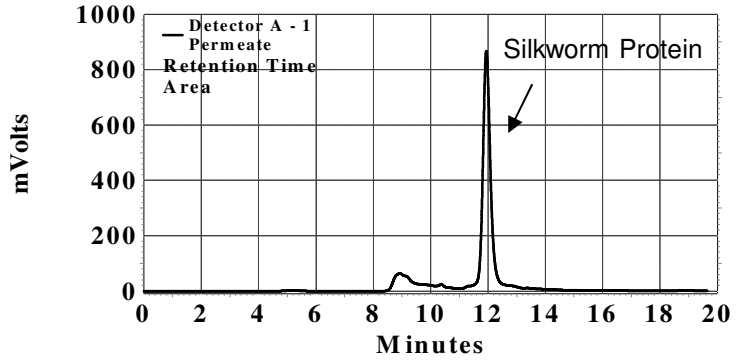


Figure 6.8. HPLC chromatograms **(a)** UF (20 kDa) feed **(b)** UF (20 kDa) permeate **(c)** UF (20 kDa) retentate **(d)** UF (5 kDa) feed (1/10 dilution) **(e)** UF (5 kDa) permeate **(f)** UF (5 kDa) retentate (1/10 dilution)



(a)



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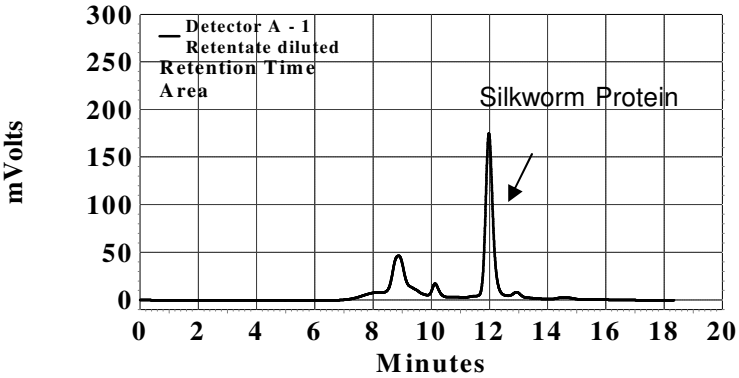


(c)

Figure 6.9. HPLC chromatograms **(a)** UF (1 kDa) feed (1/10 dilution) **(b)** UF (1 kDa) permeate (1/5 dilution) **(c)** UF (1 kDa) retentate (1/10 dilution)

The molecular weight distributions of sericin were determined in the feed and permeate streams of UF and NF membranes (Figure 6.10). In UF (20 kDa) feed, Sericin-1 and Sericin-2 had fractions of 25% and 53%. Sericin-3 had a fraction of 8% and Sericin-4 had the rest of 14%. In UF (20 kDa) permeate, there was no Sericin-1 since it was totally rejected. The fraction of Sericin-2, however, was 74% in the permeate, which was rejected at an efficiency of 12% (Table 6.10). Sericin-3 and Sericin-4, which were rejected at efficiencies of 54% and 10%, were observed at fractions of 6% and 20% in the permeate (Figure 6.10).

In the feed of NF-DK, Sericin-1, Sericin-2, Sericin-3 and Sericin-4 were found at fractions of 22%, 53%, 9% and 16%, respectively. The MW distribution of sericin in the feed of NF-90 was a bit different than that of NF-DK. The fraction of Sericin-1 was only 3% whereas that of Sericin-2 was 63%. Moreover, the fraction of Sericin-3 was 10% and that of Sericin-4 was 24%. These data show that half of the sericin in CW was Sericin-2 (Figure 6.10).

In NF-DK permeate, the highest MW was Sericin-2 and its fraction and rejection efficiency were 17% and 98%, respectively (Table 6.10). Moreover, it was found that 56% of sericin was Sericin-3, which was rejected at 62%. The fraction of Sericin-4 was 27% whereas its rejection efficiency was 89%. However, in NF-90 permeate, sericin having the highest MW was Sericin-3 with a fraction of 85% and a rejection of 37%. The rest of sericin was Sericin-4 at 15%, and it was rejected at an efficiency of 96% (Figure 6.10). As a result, it was observed that NF membranes rejected Sericin-1 and Sericin-2 at most.

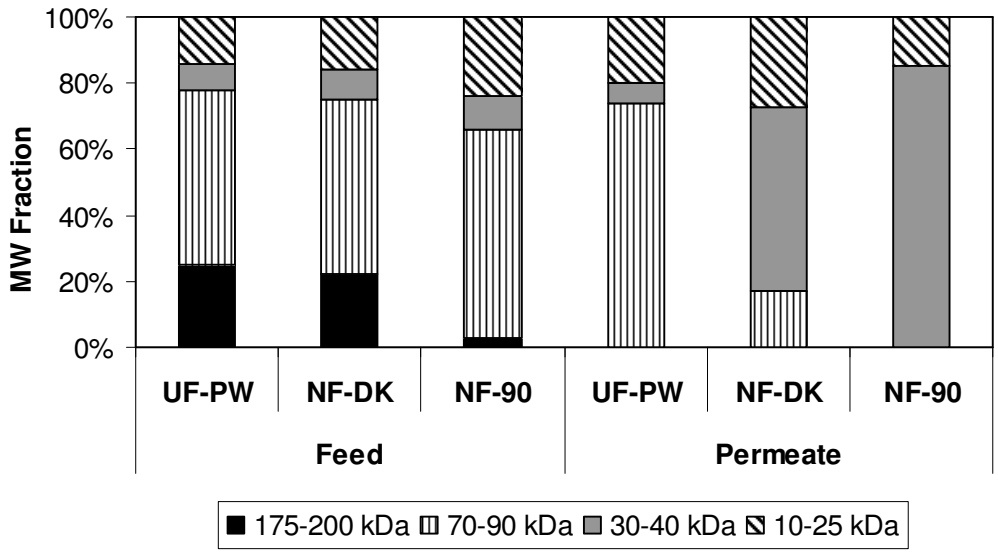


Figure 6.10. MW fractions of sericin in UF and NF

HPLC chromatograms for NF-DK and NF-90 are given in Figure 6.11. As seen, silkworm protein in these chromatograms could not be separated even with NF membranes. The rejection efficiency of NF-90 was higher for silkworm protein since the amount of this protein was lower in the permeate. Therefore, NF-DK performed better than NF-90 in terms of rejection efficiency for the silkworm protein as it was not desired in the feed side.

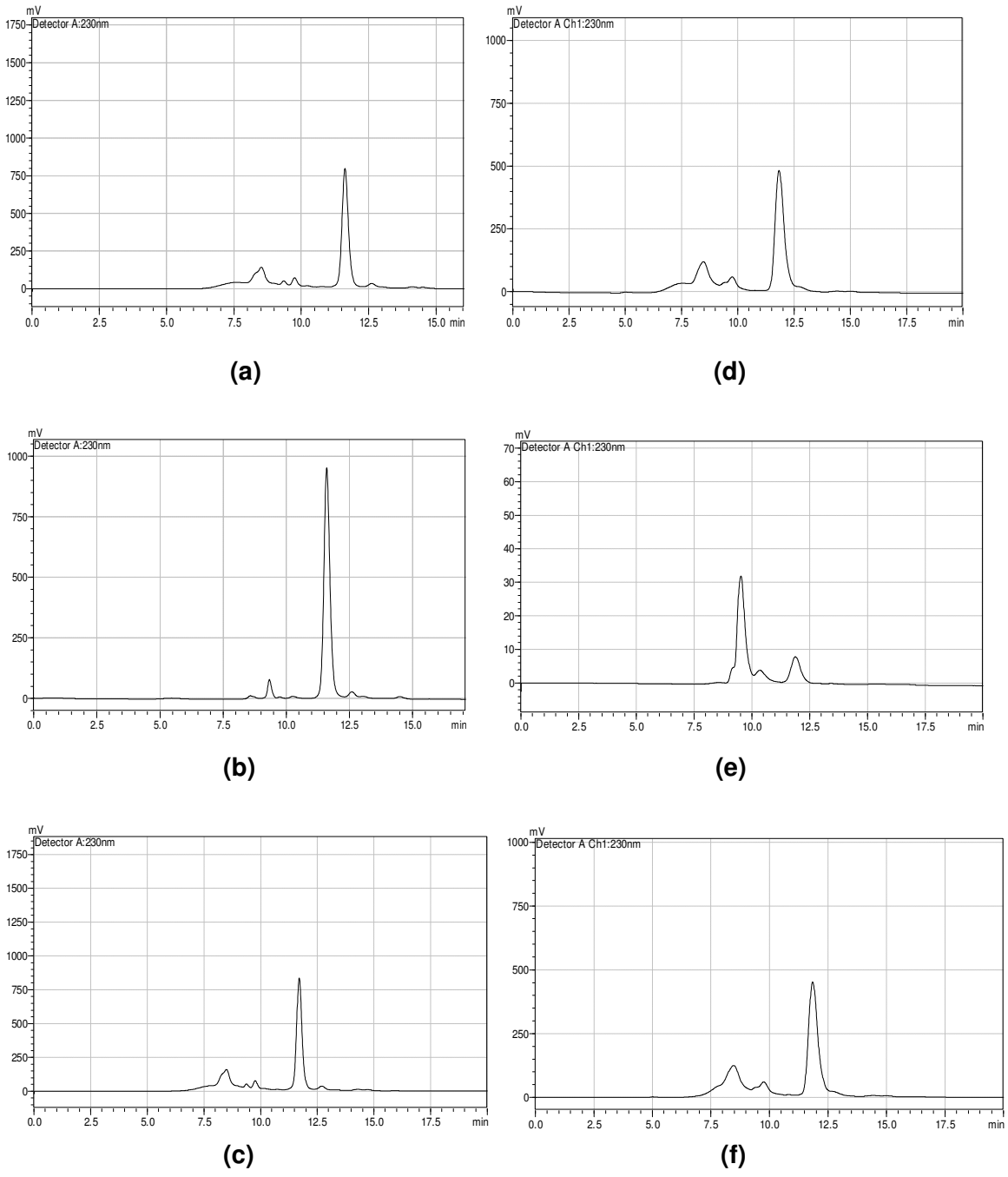


Figure 6.11. HPLC chromatograms **(a)** NF-DK feed (1/2 dilution) **(b)** NF-DK permeate **(c)** NF-DK retentate (1/2 dilution) **(d)** NF-90 feed (1/2 dilution) **(e)** NF-90 permeate **(f)** NF-90 retentate (1/2 dilution)

These results show that UF cannot recover all sericin fractions in CW but rather it would be useful for fractionating sericin into different molecular weights. The area in which recovered sericin will be used is an important factor for the appropriate process selection. Sericin in cocoon cooking wastewater can be classified as high molecular weight sericin since 78-88% of sericin has MW greater than 20 kDa, which is defined as high MW by Zhang (2002). This fraction of sericin can be used in making biomaterials and membrane productions whereas sericin having low MW (peptides smaller than 20 kDa) is appropriate for the cosmetic and skin care products. The UF membranes used in this study could not retain high MW sericin. UF (20 kDa) retained only Sericin-1 completely, which constitutes 25% of sericin in CW. Similarly, UF (5 kDa) and UF (1 kDa) membranes showed low rejection performances for sericin. Therefore, it was concluded that NF was the most appropriate process for the recovery of all sericin fractions considering the sericin rejection efficiencies. The flux decline analysis described in Section 6.3.2.2 was also considered in selecting the most suitable membrane process.

6.3.2.1.2. Removal of Pollution Parameters

Regarding the removal of pollution parameters, all UF membranes had poor performance. The removal efficiencies of UF (20 kDa) and UF (5 kDa) membranes for COD, total solids, color and turbidity were 41-43%, 36-44%, 64-64% and 42-43%, respectively (Table 6.11). The performance of UF (1 kDa) membrane was only slightly higher than the former UF membranes where COD, total solids, color and turbidity removal efficiencies were 52%, 51%, 83% and 53%, respectively. On the other hand, NF membranes showed quite high rejection performances for pollution parameters, as expected. The rejection efficiencies of NF-DK and NF-90 membranes for COD, total solids, color and turbidity were 90-99%, 90-98%, 97-100% and 93-98%, respectively (Table 6.11).

The relevant effluent discharge standard for COD is 350 mg/L (WPCR, 2004). However, in UF (20 kDa) and UF (5 kDa) permeates, COD was 5980 and 6210 mg/L (Table 6.11), which is not suitable for discharge without additional treatment. UF (1 kDa) permeate contained also 5040 mg/L COD, which is well above the

discharge limit. On the other hand, 1020 mg/L and 109 mg/L COD remained in NF permeates, which are quite close to the discharge limit of 350 mg/L. These results revealed that UF alone is not sufficient for treatment of cocoon cooking wastewaters prior to discharge. Similarly, NF-DK permeate needs to be further treated to meet the discharge standard for COD. However, NF-90 permeate quality was excellent and satisfied the discharge criteria.

Table 6.11. UF and NF performances for pollution parameters (TRMF experiments)

Membrane	Permeate Quality *(Rejection Performance, %)*				
	COD (mg/L)	**T. Solids (mg/L)**	**Color (Pt-Co)**	**Turbidity (NTU)**	**pH**
UF (20 kDa) (w/ CW2-A)	5980 *(43)*	6850 *(36)*	890 *(64)*	21 *(42)*	5.7
UF (5 kDa) (w/ CW2-B)	6210 *(41)*	5975 *(44)*	790 *(64)*	10 *(43)*	5.6
UF (1 kDa) (w/ CW2-C)	5040 *(52)*	5175 *(51)*	370 *(83)*	7 *(53)*	6.1
NF-DK (w/ CW2-D)	1020 *(90)*	1125 *(90)*	62 *(97)*	2 *(93)*	6.0
NF-90 (w/ CW2-E)	109 *(99)*	167 *(98)*	12 *(100)*	0.7 *(98)*	6.6

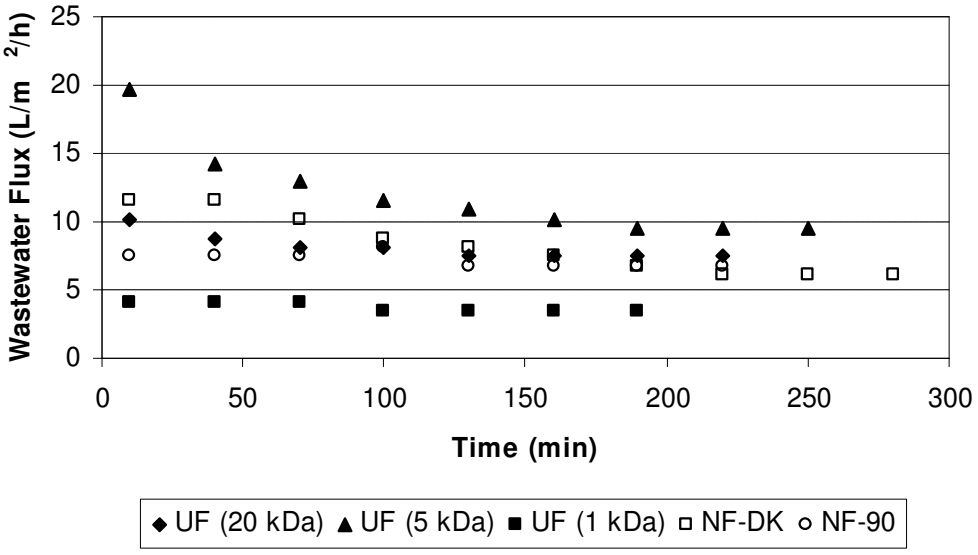
6.3.2.2. Flux Decline Analyses of UF and NF Membranes

Besides the rejection performances, flux decline is also an important parameter for the evaluation of the membrane separation processes. High flux decline means that membrane process should be stopped frequently and the membrane should be

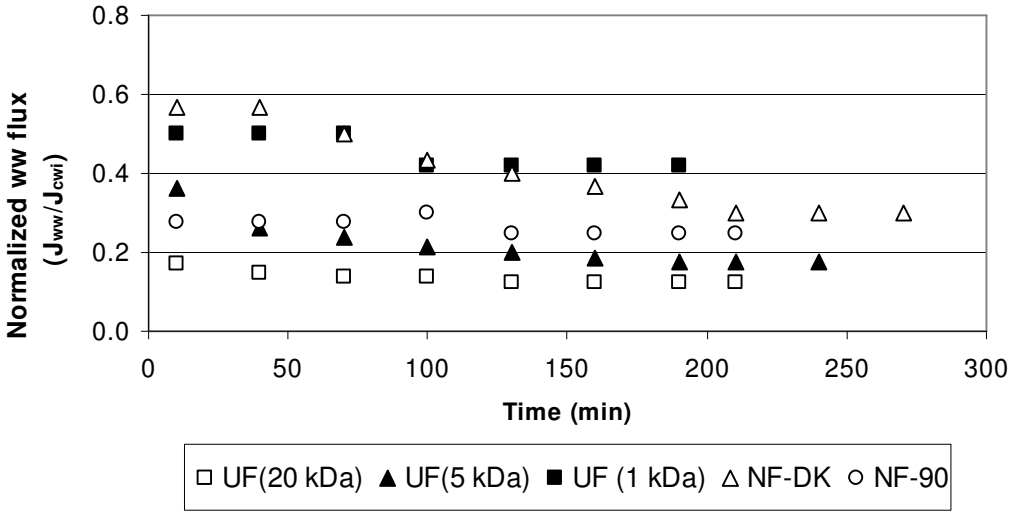
cleaned chemically and/or mechanically. As a result, membrane life decreases and operational cost increases.

In the protein separation applications, high flux declines are often observed since proteins adhere to the membrane surface (Carić et al., 2000). Sericin is a gummy protein. Therefore, it forms a layer on the membrane surface and causes flux declines. In this study, the change of flux decline with time was monitored in membrane separation experiments. Clean water fluxes of virgin membranes were determined as reference and wastewater fluxes were monitored with respect to time during filtration. Figure 6.12 shows unsteady state wastewater fluxes and normalized wastewater fluxes (J_{ww}/J_{cwi}).

Similar to the study of Fabiani et al. (1996), wastewater fluxes decreased considerably compared to clean water fluxes as soon as membrane filtration started, and they remained almost constant during 200-270 min (Figure 6.12(a)). As can be seen in Figure 6.12(b), the normalized fluxes are rather low, that is, the flux declines are rather high. When compared to clean water fluxes, the normalized wastewater fluxes remained at 12% for UF (20 kDa), 17% for UF (5 kDa), 42% for UF (1 kDa), 30% for NF-DK and 25% for NF-90 membranes during 200-270 min of filtration. In other words, in these processes, flux declines became 88%, 83%, 58%, 70% and 75%, respectively. It is an expected situation that flux declines are considerably high due to the adhesion of proteins to membrane surface, thus causing a severe decrease in water flux. The gel layer formation on the membrane surface was displayed by scanning electron microscopy (SEM). The extent of fouling on the surface which causes flux declines is obviously seen in Figure 6.13.



(a)



(b)

Figure 6.12. (a) Unsteady state wastewater fluxes **(b)** Normalized fluxes of UF and NF processes

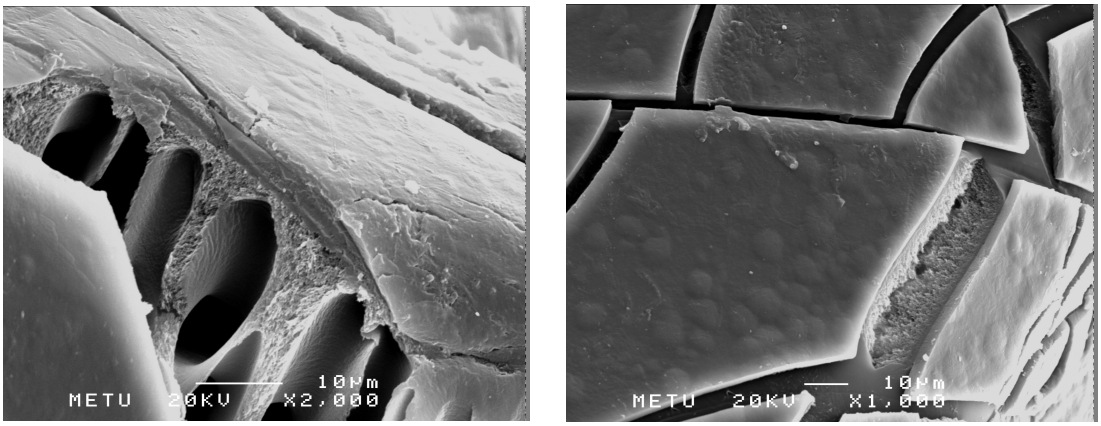


Figure 6.13. SEM photographs of fouled UF (5 kDa) membrane

Although the flux declines were severe, the improvement of fluxes was possible by recirculating the feed at a high flow rate in the membrane separation system. However, the existing system (Berghof BHT-2 model membrane filtration apparatus) was not appropriate for adjusting high flow rates. In this system, pressure and flow rate can be adjusted to limited values. Wastewater flow was adjusted to 30 L/h so that pressures were maintained at 2 bar and 5 bar in UF and NF experiments, respectively. On the other hand, flow rate has been increased to 372 L/h in DSS LabStak M20 model membrane system, which was used in concentration mode of filtration experiments. The change of filtration system enabled the flux decline of NF membrane to decrease from 67% to 31%. Consequently, it was thought that better results in terms of flux declines could be obtained by using different membrane configurations such as spiral wound membrane modules. Further research is needed to investigate the effect of membrane configuration on the flux declines.

6.3.2.3. Flux Recovery Using Chemical Cleaning

To recover the clean water fluxes, chemical cleaning was done. The percentages of the recovered fluxes are given in Table 6.12. As seen, clean water fluxes of clean membranes were recovered by 83-127% with chemical cleaning. The reason for flux

recovery greater than 100% may be that pore sizes of UF and NF membranes were increased after chemical cleaning with chlorine. Moreover, since NF membranes are charged, pores of NF membrane could be opened due to wastewater chemistry.

Table 6.12. Effect of chemical cleaning on flux recovery

Membrane	Flux Decline (%)	Flux Recovery (%)	Cleaning Conditions
UF (20 kDa)	88	105	0.5 M NaOH +193 ppm free Cl for 30 min
UF (5 kDa)	83	85	0.5 M NaOH + 200 ppm free Cl for 25 min
UF (1 kDa)	58	83	0.5 M NaOH + 197 ppm free Cl for 20 min
NF-DK	70	127	0.5 M NaOH + 179 ppm free Cl for 30 min
NF-90	75	95	0.5 M NaOH + 194 ppm free Cl for 30 min

The flux declines in UF and NF are due to concentration polarization and fouling. Concentration polarization is a reversible effect, which is diminished by the release of pressure and replacement of the wastewater by clean water. On the other hand, membrane fouling can be reversible and/or irreversible. Reversible fouling is the result of the gel layer formation on the membrane surface. Since this layer can be removed by chemical or mechanical cleaning, the original clean water flux can be recovered. In irreversible fouling, membrane fouls permanently and the original clean water flux cannot be recovered by cleaning process since the pollutants adhere to the membrane surface and/or clog the pores of the membrane.

As seen in Table 6.12, total flux declines were 58-88% in UF and 70-75% in NF. In Table 6.13, the effects of concentration polarization and fouling are given. It was observed that the effect of concentration polarization on the flux declines was

maximum in UF (20 kDa), that is, 69%. The effect of concentration polarization decreased to 30% and 38% in UF (5 kDa) and UF (1 kDa) membranes, respectively. The fraction of fouling was 59% in UF (20 kDa) and 75% in UF (5 kDa), of which 71% was reversible and 15% was irreversible in the latter. This means 15% of original clean water flux could not be restored by chemical cleaning. The fraction of fouling was as low as 33% in UF (1 kDa), of which 20% was reversible and 17% was irreversible. These results may indicate that the formation of gel layer on the membrane surface was dominant as compared to pore clogging. Increased cleaning time and higher concentration of free chlorine were effective in flux recovery, as evidenced from 105% recovery of UF (20 kDa) flux (Table 6.12).

Table 6.13. Percentages of concentration polarization and fouling

Membrane	Flux (L/m^2/h) (T=19-21 ºC)				Flux Decline (%)			
	I	W	F	C	C.P.	T.F.	R.F.	I.F.
UF (20 kDa)	59.7	7.5	24.4	62.4	69	59	*	*
UF (5 kDa)	54.3	9.5	13.6	46.2	30	75	71	15
UF (1 kDa)	8.1	3.4	5.4	6.8	38	33	20	17
NF-DK	20.4	6.1	16.3	25.8	63	20	*	*
NF-90	27.2	6.8	19.0	25.8	64	30	26	5

I: Clean Water
W: Wastewater
F: Clean water before cleaning
C: Clean water after cleaning
C.P. : Concentration polarization [(F-W)/F]
T.F. : Total fouling [(I-F)/I]
R.F. : Reversible fouling [(C-F)/C]
I.F. : Irreversible fouling [(I-C)/I]
* cannot be calculated since the value of C is greater than the value I.

The fractions of concentration polarization for NF-DK and NF-90 membranes were 63% and 64% whereas fouling was 20% and 30%. Furthermore, the reversible fouling in NF-90 membrane was 26% whereas irreversible fouling was only 5%. These results reveal that NF membranes performed better than UF membranes in terms of fouling and flux recovery.

When assessing all results discussed in Section 6.3.2, it was seen that NF offers the best performance for sericin recovery via rejecting all the sericin peptides having both low and high MW. Also, total fouling is considerably low in NF as compared to UF. Furthermore, the discharge standards was met only by the NF-90 permeate. Section 6.3.3 describes the results of concentration mode of NF experiments implemented in order to concentrate sericin. Both NF-DK and NF-90 membranes were used in CMF tests in order to further evaluate their performances under worsening feed conditions, which would simulate their performances in industrial scale applications.

6.3.3. Concentration of Sericin with NF

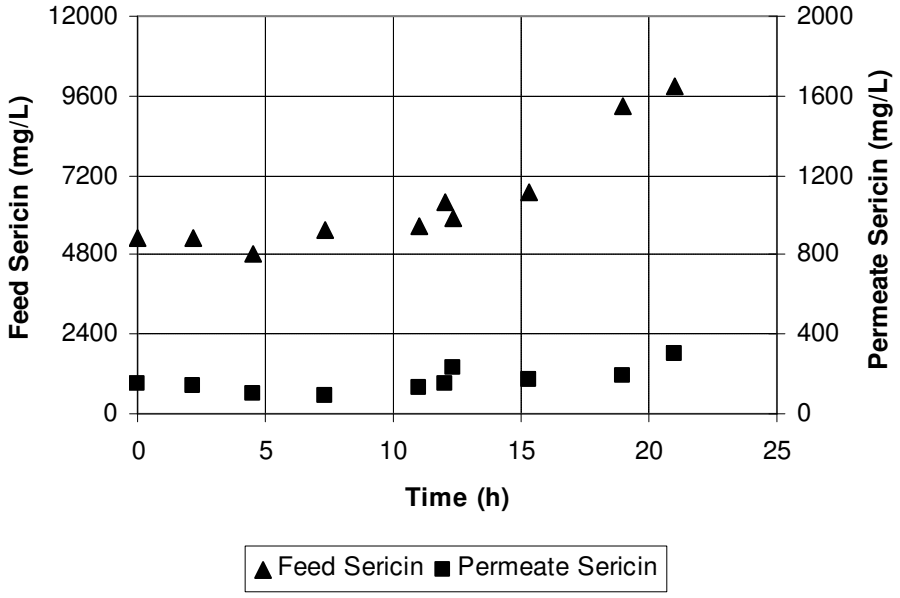
6.3.3.1. Rejection Performance of NF

After NF has been selected as the most appropriate membrane separation process, three sets of experiment were carried out to concentrate sericin in wastewater, where CW2-F, CW2-G and CW2-H samples whose characteristics have been given in Table 5.1 were used. In the first two experiments (NF-1 and NF-2), same NF membranes, i.e., Osmonics NF-DK, were used whereas in the third experiment (NF-3), Dow FilmTec NF-90 membrane was used.

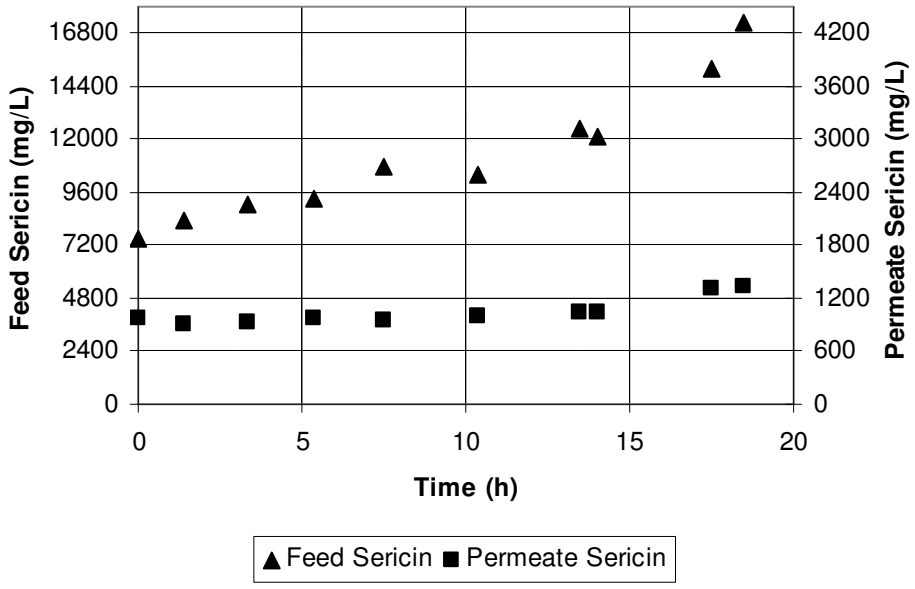
In CMF experiments, the changes of sericin concentration and COD in the feed and permeate with respect to time were monitored. The levels of sericin and COD in NF feed and permeate are given in Figures 6.14 and 6.15. As seen in Figure 6.14(a), sericin concentration of feed in NF-1 increased approximately two times at the end of the 21 h-filtration process. That is, when volume reduction factor (VRF) reached 4.6, sericin concentration of feed increased from 5332 mg/L to 9888 mg/L whereas

that of permeate increased from 151 mg/L to 298 mg/L (Table 6.14). In NF-2 experiment, concentration of sericin in feed, which was 7534 mg/L, increased by approximately 2.3 times, that is, it reached to 17280 mg/L at the end of the 18.5 h-filtration period although permeate quality remained at 977-1332 mg/L (Figure 6.14(b) and Table 6.15). As seen in Figure 6.14(c), in NF-3 experiment, at the end of the 16 h-filtration period, sericin in feed increased from 4350 mg/L to 12307 mg/L. Sericin in permeate, however, started with 89 mg/L and then, reached 309 mg/L (Table 6.16). Sericin rejection efficiencies became 97%, 87-92% and 97-98%, respectively, in NF-1, NF-2 and NF-3 experiments.

The reason for the lower rejection efficiency in NF-2 as compared to NF-1 may be the usage of the same NF membranes in both experiments. These membranes were chemically cleaned three times during and at the end of NF-1 experiment to remove fouling. Hence, the pores might have opened due to chemical exposure, which resulted in lower rejections. NF-DK and NF-90 membranes showed similar rejection performances for the silkworm protein which was found in wastewater as mentioned before. It was observed that this protein was present in both permeate and retentate. In other words, both NF-DK and NF-90 membranes could not completely separate this silkworm protein from sericin. Moreover, the amount of this protein was lower in the permeate of NF-90 membrane due to its higher rejection performance. This was not desired since the amount of impurity would be more if sericin was concentrated with NF-90 membrane. In HPLC chromatograms, the ratio of area of silkworm protein to area of sericin was 0.25 in permeate of NF-90 membrane whereas this ratio was 14.4 in permeate of NF-DK membrane. This means that silkworm protein is easily passed through NF-DK membrane and it is rejected more by NF-90 membrane.


12000
9600
7200
4800
2400
0
2000
1600
1200
800
400
0
0
5
10
15
20
25
Feed Sericin (mg/L)
Permeate Sericin (mg/L)
Time (h)
Feed Sericin
Permeate Sericin

(a)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9600
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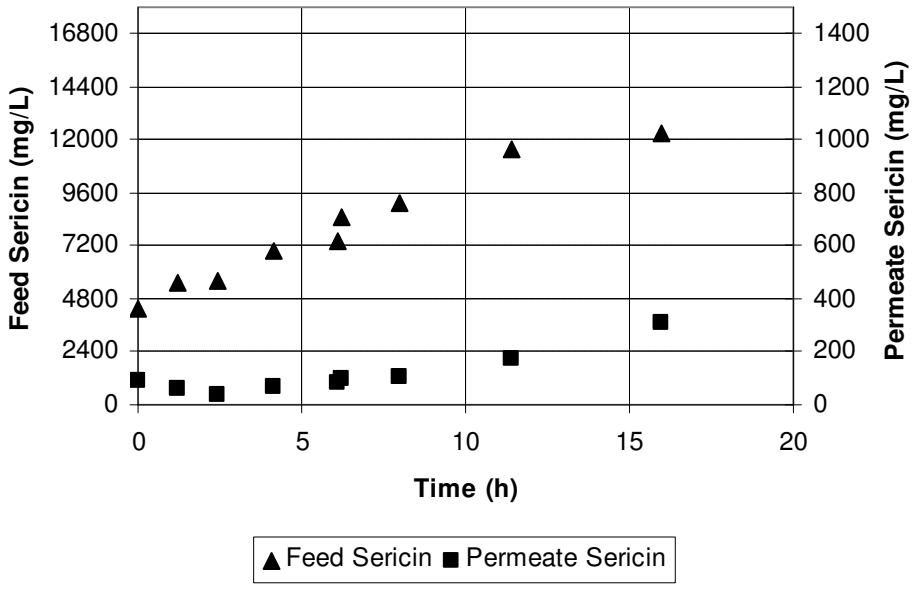
4200
3600
3000
2400
1800
1200
600
0
0
5
10
15
20
Feed Sericin (mg/L)
Permeate Sericin (mg/L)
Time (h)
Feed Sericin
Permeate Sericin

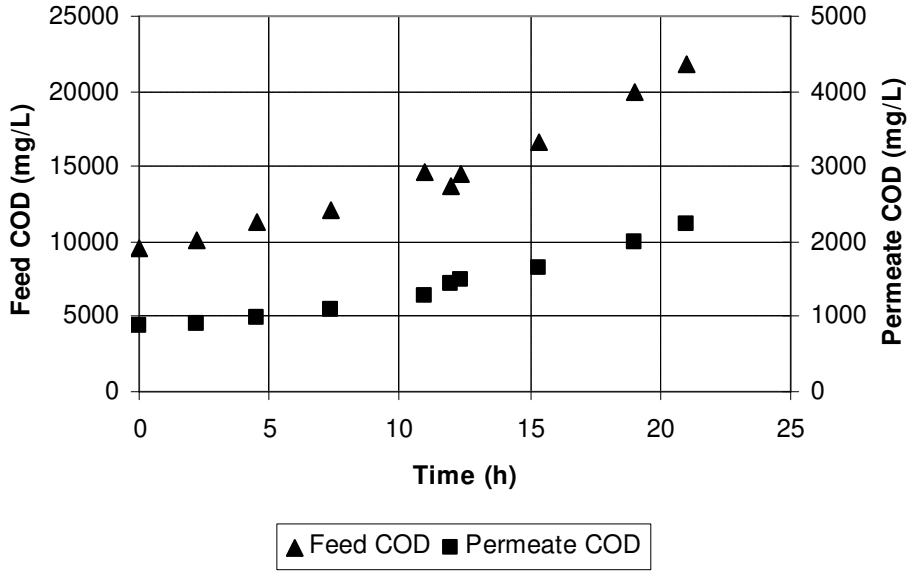
(b)



(c)

Figure 6.14. Change of sericin with respect to time **(a)** NF-1 **(b)** NF-2 **(c)** NF-3 experiments

As seen from Figure 6.15(a), in NF-1, COD of feed increased from 9560 mg/L to 21813 mg/L, that is, approximately 2.3 times at the end of 21 h whereas that of permeate changed from 867 mg/L to 2229 mg/L. In NF-2, however, feed COD was 11395 mg/L at t=0 and 30825 mg/L at t=18.5 h while permeate COD remained at 646-1929 mg/L. In NF-3, although COD of feed increased from 9420 mg/L to 24210 mg/L, that is, approximately 2.6 times, permeate COD changed between 32-470 mg/L at the end of 16 h. These data show that rejection performance of NF-90 membrane was really good.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20000
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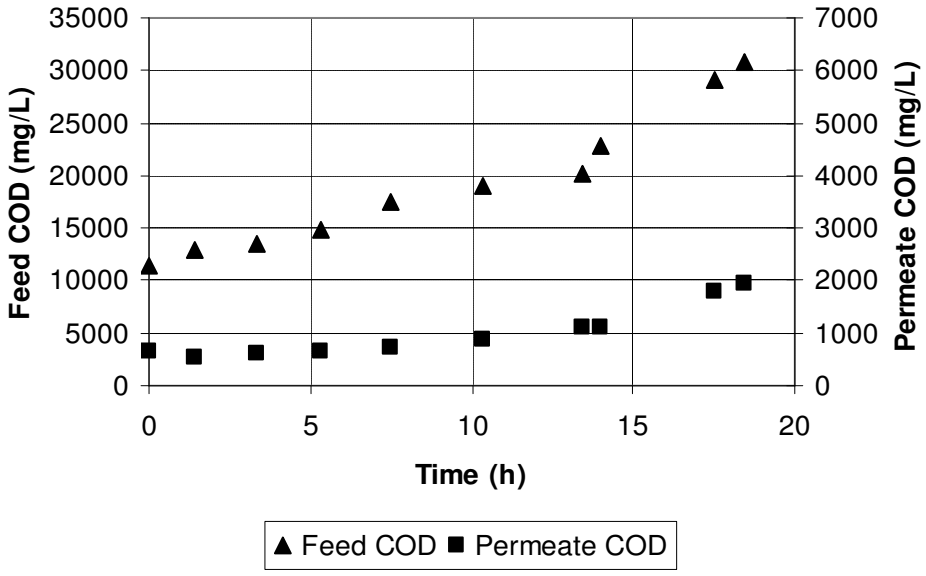
10000
5000
0
Feed COD (mg/L)
5000
4000
3000
2000
1000
0
Permeate COD (mg/L)
0
5
10
15
20
25
Time (h)
Feed COD
Permeate COD

(a)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15000
10000
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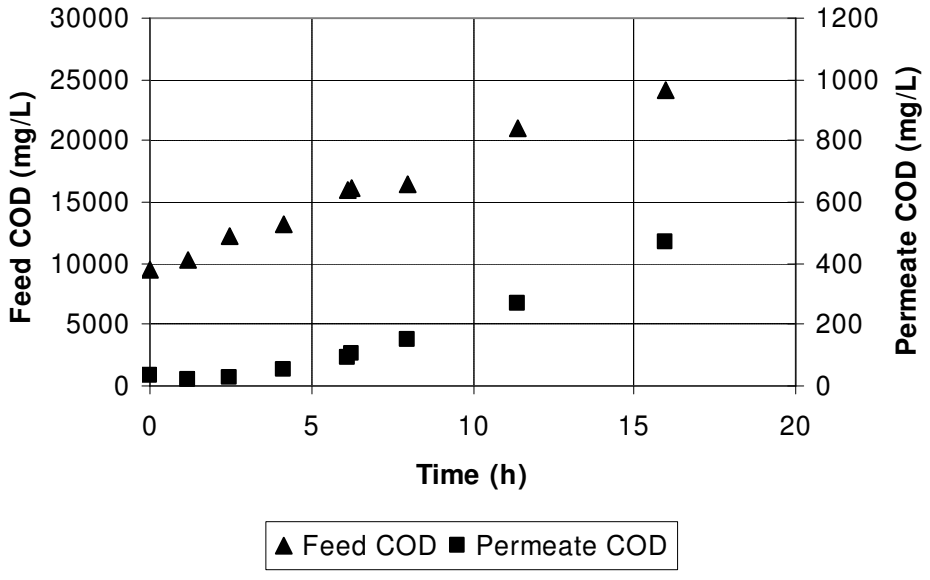
Feed COD (mg/L)
7000
6000
5000
4000
3000
2000
1000
0
Permeate COD (mg/L)
0
5
10
15
20
Time (h)
Feed COD
Permeate COD

(b)



(c)

Figure 6.15. Change of COD with respect to time **(a)** NF-1 **(b)** NF-2 **(c)** NF-3 experiments

All pollution parameters and rejection efficiencies observed in NF-1, NF-2 and NF-3 experiments are given in Tables 6.14-6.16. As seen, the rejection efficiencies were quite high for all parameters even when VRF increased to 4.2-4.6. This is an advantage in terms of membrane performance. In NF-1, NF-2 and NF-3 experiments, total solids, color and turbidity rejections were 87-95%, 99-100% and 100%, respectively. Even when VRF was 4.2-4.6, total solids, color and turbidity amounts was 1267-2670 mg/L, 1-100 Pt-Co and 0.3-0.4 NTU, respectively, in permeate of NF-1, NF-2 and NF-3. These results reveal that NF membranes would have good performances for both sericin rejection and removal of pollution parameters in industrial scale application.

Table 6.14. Wastewater characteristics obtained in NF-1 experiment

Parameter	Measured Value				Rejection Ratio (%)	
	Feed		Permeate			
	t=0 h (VRF=1)	t=21 h (VRF=4.6)	t=0 h (VRF=1)	t=21 h (VRF=4.6)	t=0 h (VRF=1)	t=21 h (VRF=4.6)
Sericin (mg/L)	5332	9888	151	298	97	97
Sericin-1	22	-	-	-	100	-
Sericin-2	4850	8385	20	74	100	99
Sericin-3	324	-	20	167	94	-
Sericin-4	136	1503	111	57	18	96
T.Protein (mg/L)	4248	9990	460	1380	89	86
COD (mg/L)	9560	21813	867	2229	91	90
T.Solids (mg/L)	9250	20800	1033	2670	89	87
Color (Pt-Co)	1520	5000	3	37	100	99
Turbidity (NTU)	15	84	0.5	0.3	97	100
pH	6.1	6.0	5.5	6.1	-	-

Table 6.15. Wastewater characteristics obtained in NF-2 experiment

Parameter	Measured Value				Rejection Ratio (%)	
	Feed		Permeate			
	t=0 h (VRF=1)	t=18.5 h (VRF=4.2)	t=0 h (VRF=1)	t=18.5 h (VRF=4.2)	t=0 h (VRF=1)	t=18.5 h (VRF=4.2)
Sericin (mg/L)	7534	17280	977	1332	87	92
Sericin-1	189	-	-	-	100	-
Sericin-2	4450	11166	79	262	98	98
Sericin-3	801	1376	641	514	20	63
Sericin-4	2094	4738	257	556	88	88
T.Protein (mg/L)	6599	13472	561	1834	91	86
COD (mg/L)	11395	30825	646	1929	94	94
T.Solids (mg/L)	11610	27950	-	2400	-	91
Color (Pt-Co)	3010	8463	28	100	100	100
Turbidity (NTU)	56	177	-	0.4	-	100
pH	6.4	6.4	6.2	6.5	-	-

Table 6.16. Wastewater characteristics obtained in NF-3 experiment

Parameter	Measured Value				Rejection Ratio (%)	
	Feed		Permeate			
	t=0 h (VRF=1)	t=16 h (VRF=4.2)	t=0 h (VRF=1)	t=16 h (VRF=4.2)	t=0 h (VRF=1)	t=16 h (VRF=4.2)
Sericin (mg/L)	4350	12307	89	309	98	97
Sericin-1	74	-	-	-	100	-
Sericin-2	3207	9081	3	76	100	99
Sericin-3	-	-	64	61	-	-
Sericin-4	1069	3226	22	172	98	95
T.Protein (mg/L)	7038	11519	0	236	100	98
COD (mg/L)	9420	24210	32	470	100	98
T.Solids (mg/L)	10500	25667	-	1267	-	95
Color (Pt-Co)	2560	7210	-	1	-	100
Turbidity (NTU)	20	96	0.2	0.4	99	100
pH	5.90	6.05	4.97	5.61	-	-

In CMF experiments, MW distributions of sericin in feed and permeate were also determined (Figure 6.16). The highest MW of sericin in wastewater was Sericin-1 in NF-2 and NF-3 experiments. The fractions of Sericin-1 were 3% and 2% in CW2-G and CW2-H samples, respectively. However, the highest fraction of sericin was possibly accumulated on the membrane surface and Sericin-1 could not be found in NF-1 experiment since CW2-F sample was filtrated in total recycle mode for a few hours before starting the CMF experiment.

The fractions of Sericin-2 did not change so much in feed. In NF-1, their fractions were 91% at the beginning and 85% at the end. However, these fractions were determined as 13% and 25% in permeate. In NF-2, the fractions of Sericin-2 increased from 59% to 65% in the feed while it increased from 8 to 20% in the permeate. In NF-3, however, the percentages of Sericin-2 did not change in feed, that is, remained constant at 74% whereas it increased from 3 to 25% in the permeate from the beginning to the end of the experiment. These results indicate that mainly Sericin-2 was rejected by the NF membranes as observed in TRMF experiments.

In NF-1 experiment, Sericin-3 could not be found in the feed. The reason for this could be the accumulation of Sericin-3 as a gel layer on the membrane surface during total recycle mode of filtration performed in the first few hours of experiment. In NF-2, Sericin-3 became 8-10% in the feed during the experiment whereas it decreased from 66% to 39% in the permeate. It was probably caused by the increase of Sericin-2 and Sericin-4 in the permeate at the end of the experiment. In NF-3, however, Sericin-3 was not found in the feed while it decreased from 72% to 55% in the permeate. It is expected in CMF experiments that membrane performance changes with time because of the change in feed quality. The selectivity of membrane can change since the gel layer formed on the membrane surface acts as a dynamic membrane.

The smallest sericin peptides with 10-25 kDa, namely Sericin-4, were present at low amounts in wastewater. Fractions of these peptides were only 9-15%, 27-28% and 24-26% in the feeds of NF-1, NF-2 and NF-3, respectively. It was observed that these sericin peptides unexpectedly passed through the membranes although they were larger than the pore size of NF membranes. It may be speculated that it was resulted from the structures of protein and/or membrane, or the interactions between them. Sericin-4 was found in the permeate at 74% at the beginning of NF-1 but this fraction decreased to 20% at the end of the experiment since the fractions of Sericin-2 and Sericin-3 increased in the permeate. In NF-2 and NF-3 permeates, the fractions of Sericin-4 were 26-41% and 20-25%, respectively. As discussed above, selectivity of membranes can change with time and so, membrane performance can worsen in CMF experiments. However, in conducted experiments, the rejection ratio

of sericin did not decrease with time and remained over 90% on average during concentration period. Thus, these results also show that NF is a suitable technique for sericin recovery.

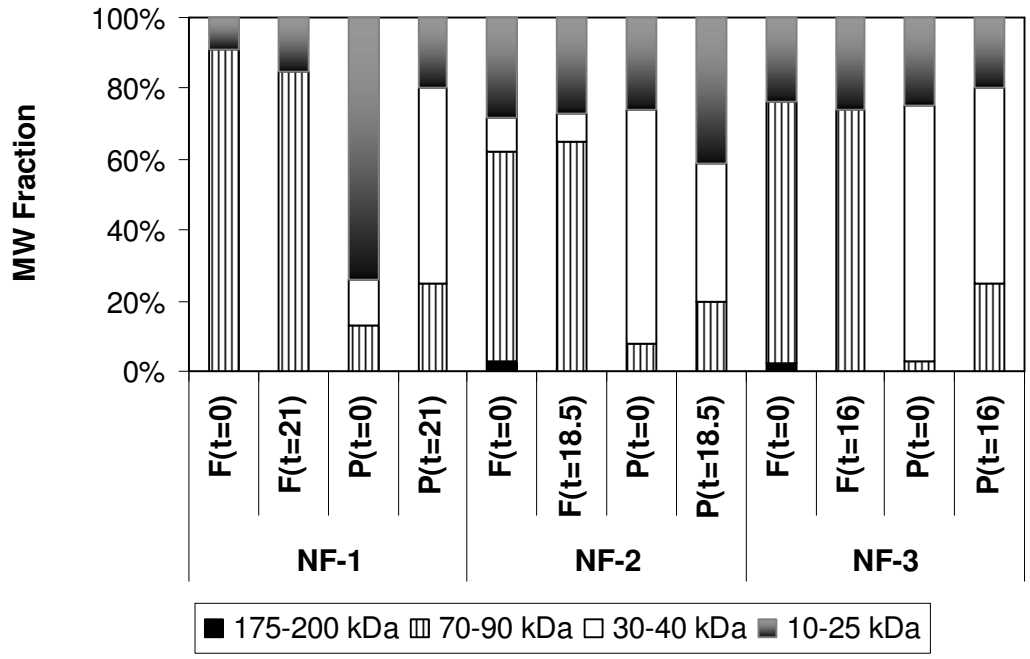


Figure 6.16. Molecular weight distribution of sericin in NF-1, NF-2 and NF-3 experiments

6.3.3.2. Flux Declines in NF Processes

Fluxes observed in NF-1, NF-2 and NF-3 experiments are given in Figure 6.17. As seen, when VRF was 1, the flux started to decrease approximately as much as 30% in NF-1. After a filtration period of 12 h, flux decline rose above 60%. Therefore, the operation was stopped and the first chemical cleaning was done. Flux decline decreased to 44% and after approximately 6 h more filtration, flux decline became 70%. Hence, the second cleaning process was applied and flux decline was

decreased from 66% to 51%. VRF reached 4.6 by doing almost 2 h more filtration. At the point where the experiment was terminated, flux decline of the membrane again reached 62% (Figure 6.17 and Table 6.17). At the end of the experiment, membrane was chemically cleaned once more. The original clean water flux was restored by 118%.

In NF-2, the same membrane was used and at t=14 h when flux decline reached 70%, chemical cleaning was applied once to this membrane. However, obtained flux recovery was not high. At the end of the experiment, flux decline increased to 78% and therefore, chemical cleaning was repeated, providing the recovery of original clean water flux by 81% (Table 6.17).

In NF-3, flux decline became 95% as a result of increasing VRF to 4.2. NF-90 membrane used in this experiment was cleaned once during CMF. However, this cleaning process did not give a good result, that is, wastewater flux did not increase. In addition, clean water flux was recovered as much as 75% by chemical cleaning done at the end of the experiment (Table 6.17). These data obviously show that NF-90 membrane was much more adversely affected by the wastewater chemistry and presence of protein. Hence, it was concluded that NF-DK membrane, which was used in NF-1 and NF-2 experiments, was more suitable for sericin recovery. Therefore, it was decided to recover sericin by NF-DK membrane. The next section describes the precipitation of sericin concentrated with NF-DK membrane.

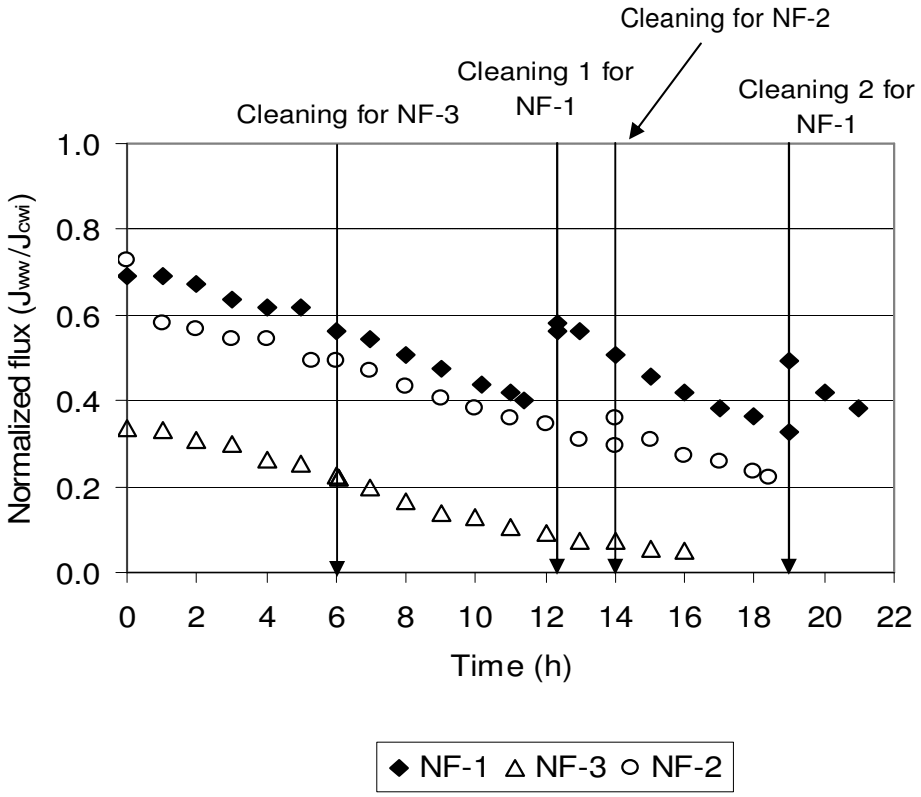


Figure 6.17. Normalized fluxes in NF-1, NF-2 and NF-3 experiments

Table 6.17. Effect of volume reduction on flux declines in CMF experiments

Experiment	Flux (L/m^2/h) at T=19-21 °C			Flux Decline (%), (I-W)/I	Flux Recovery (%), C/I
	Clean Water, I	Wastewater, W	Clean Water After Cleaning, C		
NF-1	18.3	7	21.7	62	118
NF-2	27.0	6	22.0	78	81
NF-3	33.0	1.7	24.7	95	75

6.3.4. Precipitation of Concentrated Sericin

Ethanol is commonly used to precipitate sericin (Takasu et al., 2002; Kurioka et al., 2004; Wu et al., 2007). However, the volume of ethanol required is three times higher than that of sericin solution. Therefore, precipitation with ethanol does not seem to be an environmentally friendly method when the wastewater flow rates that will arise in a real cocoon cooking plant are taken into consideration. However, the problem of ethanol requirement at high volumes can be solved by providing an ethanol recovery unit in the sericin recovery plant that will be constructed at industrial scale. Sericin can also be precipitated using acids since it becomes insoluble at pH 3.8 (Kodama, 1926). It is clear that the amount of acid would be much smaller than ethanol for precipitating sericin, and this would cause less pollution. On the other hand, the quality of recovered sericin has the highest importance, and the most suitable agent has to be preferred. Therefore, concentrated sericin was precipitated using both ethanol and acids in order to determine the most suitable precipitation agent. The quality of recovered sericin was compared to those of standard sericin. The solubilities of recovered sericin samples were also determined.

6.3.4.1. Comparison of Acids and Ethanol for Precipitation

Firstly, nitric acid (HNO_3) was tested. The pH of the concentrated sericin obtained in NF-1 was adjusted to 3.8 with HNO_3 and then, it was centrifuged at 3000 rpm. The amount of acid needed to adjust the pH of the concentrated sericin was a few milliliters for a volume of 200-300 mL. In other words, it was only 0.3% of the concentrated sericin by volume.

Sericin concentration of feed at t=21 h was 9888 mg/L whereas sericin in the supernatant of the precipitate was 3626 mg/L. Thus, the efficiency of the precipitation with acid was found as 63%. To increase the precipitation efficiency, firstly, the speed of centrifugation was raised to 5000 rpm. However, no additional sericin precipitate was observed. Secondly, the pH of the supernatant was decreased down to 1.9 and then, it was centrifuged at 5000 rpm for 10 min. As a

result, again no additional sericin precipitate was found. The precipitation efficiency obtained with HNO_3 was the same with 64% efficiency obtained by Wu et al. (2007) using 75% ethanol (v/v). In the study of Wu et al. (2007), sericin precipitation efficiency was increased just about to 71% even if 90% ethanol (v/v) was used. Therefore, the precipitation efficiency obtained in this study was found acceptable. Sericin precipitate was frozen at -80 ^{o}C and then, dried in lyophilizator to get sericin in powder form.

A solution of recovered sericin which was precipitated with HNO_3 was prepared at a concentration of 0.5 mg/mL and analyzed by HPLC (Figure 6.18). In its HPLC chromatogram, a peak belonging to HNO_3 was observed in the same time interval with sericin. Therefore, it was decided not to use HNO_3 for precipitation since it contaminated sericin.

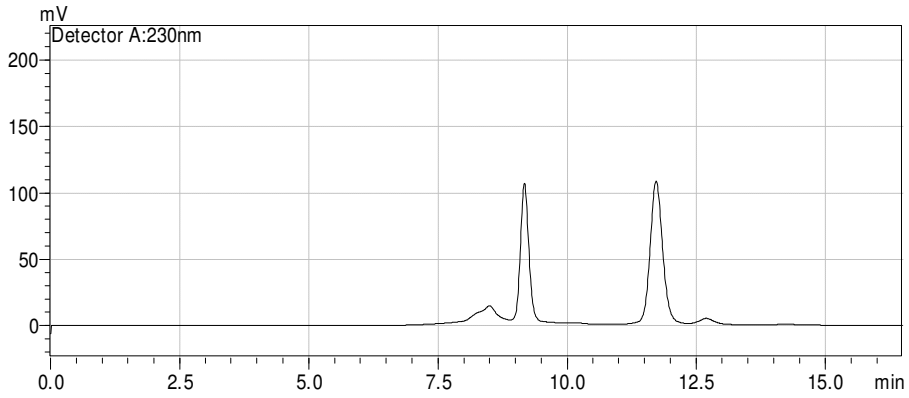


Figure 6.18. HPLC chromatogram of sericin precipitated with HNO_3

To find another acid, which is more suitable than HNO_3 for precipitation of sericin, hydrochloric acid (HCl), sulphuric acid (H_2SO_4) and acetic acid ($C_2H_4O_2$) were tested. The sericin concentrated in NF-2 experiment was divided into equal volumes and their pHs were adjusted to 3.8 by using the acids mentioned above. After the addition of acids, these samples were centrifuged to precipitate sericin. Alternatively,

cold ethanol (C_2H_6O) was added into another concentrated sericin sample at a final ethanol concentration of 75% (v/v). The supernatants of the precipitates were wasted after centrifugation. The precipitates were frozen at -80 ^oC and then, dried by means of a lyophilizator to get their powder forms. Since the cocoon cooking wastewater was dark colored, sericin recovered with HCl was also brown. However, sericin recovered with ethanol was not brown since volume of ethanol was very high so that the color of concentrated sericin became yellow (Figure 6.19). The HPLC chromatograms of the solutions which were prepared from four different sericin powders are shown in Figures 6.20-6.23. It was observed before that among the acids used, only nitric acid gave a distinct peak together with sericin (Figure 6.18). Also, it was understood that acetic acid causes two times more COD than other acids do in supernatant which is wasted after the precipitation of sericin. Hence, it was concluded that acetic acid should not be preferred for sericin precipitation since its supernatant will cause significant pollution. It was understood that HCl, H_2SO_4 and ethanol could be suitable for sericin precipitation.

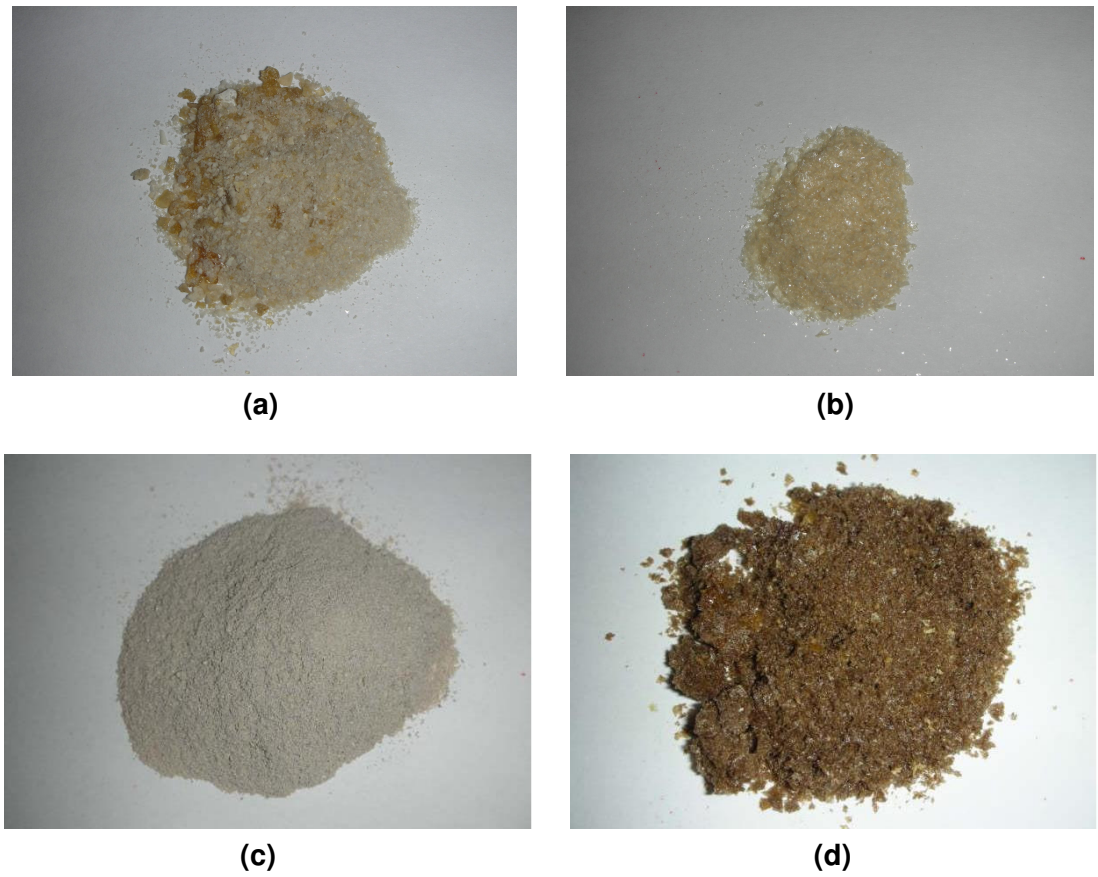


Figure 6.19. Pictures of **(a)** S_N before grinding **(b)** S_C **(c)** Recovered sericin with ethanol **(d)** Recovered sericin with HCl

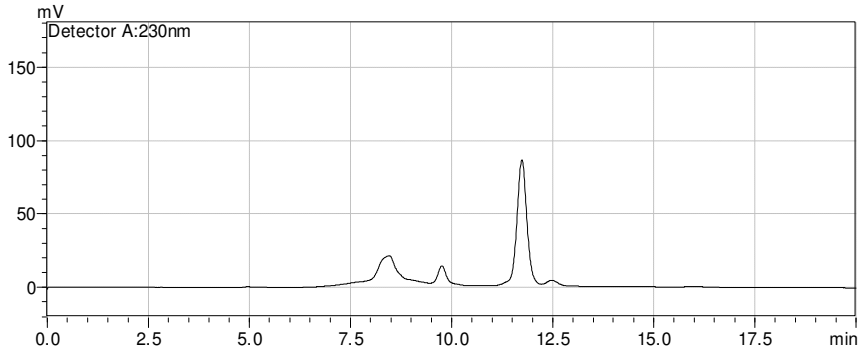


Figure 6.20. HPLC chromatogram of sericin precipitated with HCl

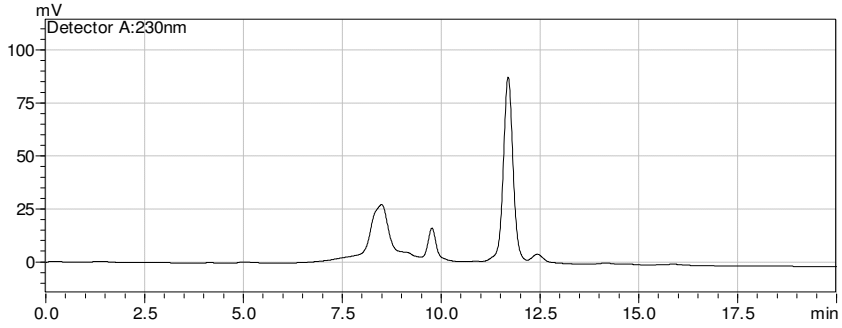


Figure 6.21. HPLC chromatogram of sericin precipitated with H_2SO_4

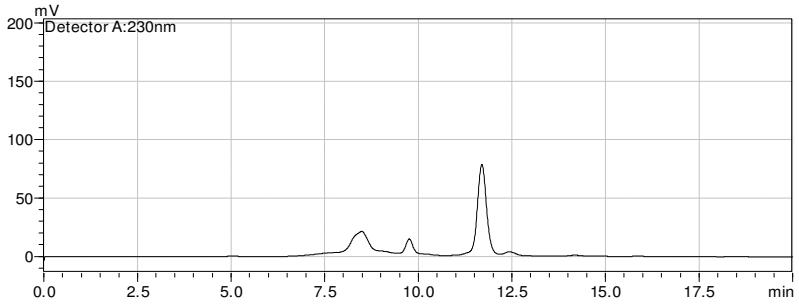


Figure 6.22. HPLC chromatogram of sericin precipitated with $C_2H_4O_2$

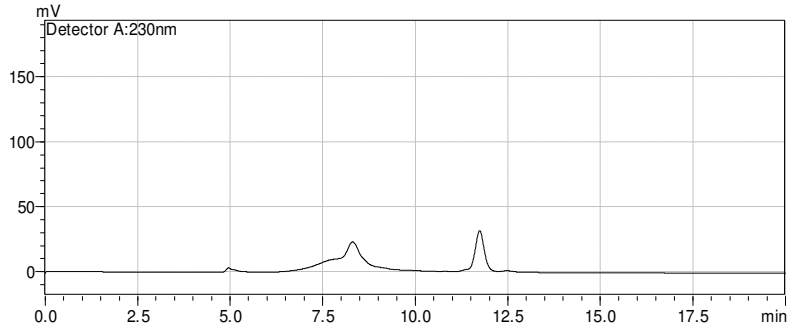


Figure 6.23. HPLC chromatogram of sericin precipitated with C_2H_6O

Recovered sericins were prepared at known concentrations. Then, their sericin concentrations were analyzed by HPLC (Table 6.18). As seen, the measured concentrations of all samples were quite lower than the prepared concentrations. The measured concentration of recovered sericin with HNO_3 was the lowest. The reason for this is that HNO_3 eluted together with sericin and the sericin concentration of this sample could not be measured correctly. Moreover, the difference between the prepared and measured concentrations of all samples was resulted from the silkworm protein. As a result, sericin fraction in recovered powder was found as 39-46%, where the rest was silkworm protein.

Table 6.18. Prepared and measured concentrations of recovered sericins

Sample	Prepared Concentration (mg/L)	Measured Concentration (mg/L)
Sericin with HCl	1000	395
Sericin with H_2SO_4	1000	464
Sericin with $C_2H_4O_2$	1000	403
Sericin with HNO_3	1000	147
Sericin with C_2H_6O	1000	449

6.3.4.2. Comparison of Elemental Compositions of Recovered Sericin Samples

The quality of recovered sericin samples were also evaluated by using elemental analysis. As seen from Table 6.19, commercial sericin purchased from the Brazilian company and native sericin obtained from cocoons by ethanol precipitation have almost the same elemental compositions. However, the compositions of recovered sericin samples were slightly different from each other and from the standard sericin samples. This difference may be resulted from different moisture contents of recovered sericin and standard sericin samples. The C, H and N contents of recovered sericins were lower than those of standard sericins. On the other hand,

their S contents were slightly higher. The C contents of commercial and native sericin were 41-43% whereas it was 36-40% in recovered sericin samples. The highest C content was observed in sericin precipitated with acetic acid, and this was thought to be due to the additional carbon coming from acetic acid. Similarly, N and H contents of recovered sericin samples were lower than those of standards. Commercial sericin and native sericin included 14-15% N whereas recovered sericin samples had 10-13% N. The highest N content belonged to sericin precipitated with HNO_3, where additional N came from nitric acid. The H content of standard sericin was 6.4-6.2% whereas it was 5.4-5.8% in recovered sericin samples. The S content of recovered sericin samples were 0.4-2.9%, with highest content belonging to the sample precipitated with sulphuric acid, as expected. The reason for differences in elemental compositions was thought to be due to the presence of silkworm protein in recovered sericin samples.

In all samples, C/H and C/N ratios were relatively close to each other. However, C/S ratios were very different. These ratios facilitate the determination of the molecular formula of the sample. The elemental analysis results showed that the most representative elemental composition could be obtained in samples precipitated with HCl and C_2H_6O. The elemental composition of samples recovered with HCl and C_2H_6O were quite similar.

The health and environmental hazards of the acids and ethanol were also considered for comparison. According to the evaluation of Australian Government (2008), the least hazardous substance for health and environment is HCl, followed by ethanol with total points of 2.2 and 2.5, respectively (Table 6.20). Hence, the suitability of HCl and ethanol for sericin precipitation was also verified by the hazard ranking.

Table 6.19. Elemental analysis (dry basis) of sericin samples

Sample	C (%)	H (%)	N (%)	S (%)	Total (%)	C/H	C/N	C/S
Commercial Sericin	42.5	6.4	13.9	0.3	63.1	3.1	6.7	157.3
Native Sericin	41.0	6.2	14.9	0.2	62.3	2.8	6.7	241.3
Recovered Sericin								
with HNO_3	36.7	5.4	12.9	0.6	55.6	2.9	6.8	63.2
with HCl	37.7	5.6	11.0	0.7	55.0	3.4	6.8	55.5
with H_2SO_4	37.0	5.5	10.7	2.9	56.1	3.4	6.7	12.9
with $C_2H_4O_2$	40.3	5.8	10.9	0.6	57.6	3.7	6.9	66.0
with C_2H_6O	37.9	5.8	10.2	0.4	54.3	3.7	6.6	88.2

Table 6.20. Health and environmental hazards of acids and ethanol

Substance	Hazard Point		
	Health (out of 3)	Environment (out of 3)	Total (out of 6)
HCl	1.5	0.7	2.2
C_2H_6O	1.2	1.3	2.5
HNO_3	1.8	0.8	2.6
$C_2H_4O_2$	1.7	1.0	2.7
H_2SO_4	2.3	1.3	3.6

Moisture, ash and organic contents of the recovered sericins were also determined (Table 6.21). For determining moisture content, recovered sericin samples were dried at 100 ^oC for 1 hour. For determining ash content, however, samples dried at 105 ^oC were ignited at 600 ^oC for 90 min. As seen from Table 6.21, moisture content

of recovered sericins changes between 2.8-3.9%, which are lower than that of 7.4% and 8.6% obtained for commercial (S_C) and native (S_N) sericins, respectively (Table 6.1). However, this may result just from storing conditions and the difference was not found to be important. The ash contents of recovered sericins, which were 11.3-14.4%, are quite higher than those of S_C and S_N, i.e., 2.7% and 3.8%. This means the organic contents of recovered sericins were lower as compared to standard sericins. The differences in ash contents was also attributed to the presence of silkworm protein in recovered sericin samples.

Table 6.21. Moisture, ash and organic contents of recovered sericins

Sample	Moisture Content (%)	Ash Content (%)	Organic Content (%)
Recovered Sericin			
with HNO_3	3.9	11.3	88.7
with HCl	3.1	12.8	87.2
with H_2SO_4	2.8	13.9	86.1
with $C_2H_4O_2$	2.8	11.6	88.4
with C_2H_6O	3.1	14.4	85.6

6.3.4.3. Solubility of Recovered Sericin Samples

In order to further characterize the recovered sericin samples, the solubility of recovered sericin at varying pH was investigated. For this purpose, pH of prepared sericin solutions was adjusted to values changing between 3 and 11 (Table 6.22). Then, these samples were centrifuged at 4000 rpm for 10 min. Total protein analysis was done with the supernatants of these centrifuged samples. As seen in Table 6.22, solubility of sericin precipitated with acids was only 47-65% at pH 3, which was as low as 59-88% at pH 7. The solubility of sericin increased for all the acids used when pH was increased from 3 to 11, where it reached 89-110% at pH 11. On the

other hand, solubility of sericin precipitated with ethanol was above 90% at all pH values. The reason for the solubility values which are above 100% may be that protein in the original sample could not be dissolved completely and protein concentration in supernatant of the samples measured after pH adjustment and centrifugation might increase.

Table 6.22. Effect of pH on sericin solubility

pH	Solubility (%)				
	Sericin-HNO_3	**Sericin-HCl**	**Sericin-H_2SO_4**	**Sericin-$C_2H_4O_2$**	**Sericin-C_2H_6O**
3	47	53	65	56	92
4	65	58	63	59	99
5	56	52	68	58	98
6	56	66	61	59	108
7	88	66	68	59	105
8	61	70	62	67	98
9	58	70	62	76	95
10	73	83	70	83	103
11	110	95	89	96	101

These results show that ethanol is better than acid for precipitation considering the end-use of recovered sericin. Sericin precipitated with acid would necessitate the adjustment of pH to basic conditions for achieving complete solubility. This would limit the applicability of recovered sericin in several end-use areas. On the other hand, complete solubility of sericin recovered with ethanol at all pH values would provide significant benefits. In addition, a higher sericin precipitation efficiency of 84% was achieved with ethanol, which was calculated based on sericin

concentrations of 17280 mg/L and 2809 mg/L in the NF retentate and supernatant of the precipitate. As a result, it was decided to precipitate sericin using ethanol.

6.3.4.4. UV-Scan Comparison of Sericin Samples

UV-scans of recovered sericin solutions were done at wavelengths between 190 and 800 nm. As seen in Figures 6.24-6.28, same results were obtained for all acids and ethanol. Sericin protein gives a peak between 275 and 290 nm. It was observed that recovered sericins and standard sericin gave peaks at the same wavelength intervals but standard sericin gave a higher and more distinct peak (Figure 6.29). The reason for this may be that standard sericin is more pure than recovered sericin. In recovered sericin, there is also silkworm protein and this protein probably gives a peak together with sericin between 275 and 290 nm. Therefore, the peak of recovered sericin may not be as distinct as that of standard sericin.

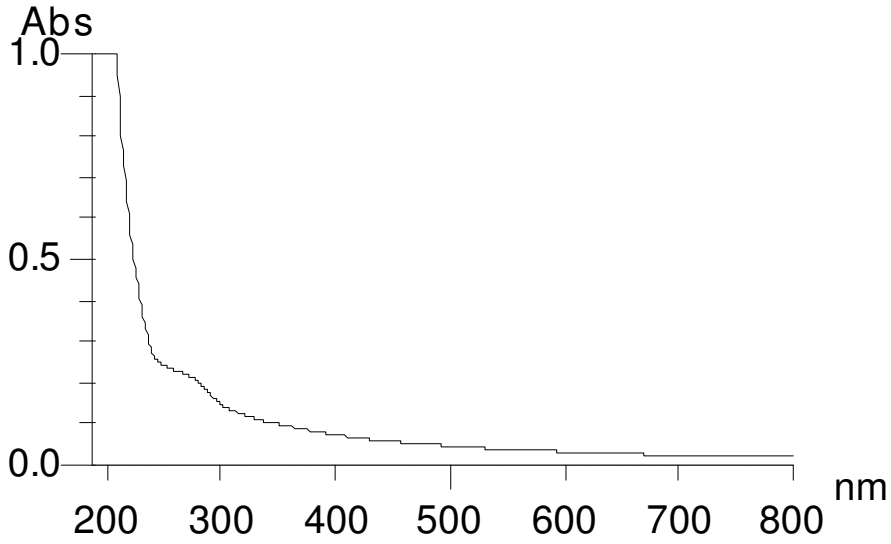


Figure 6.24. UV-scan of recovered sericin with HNO_3

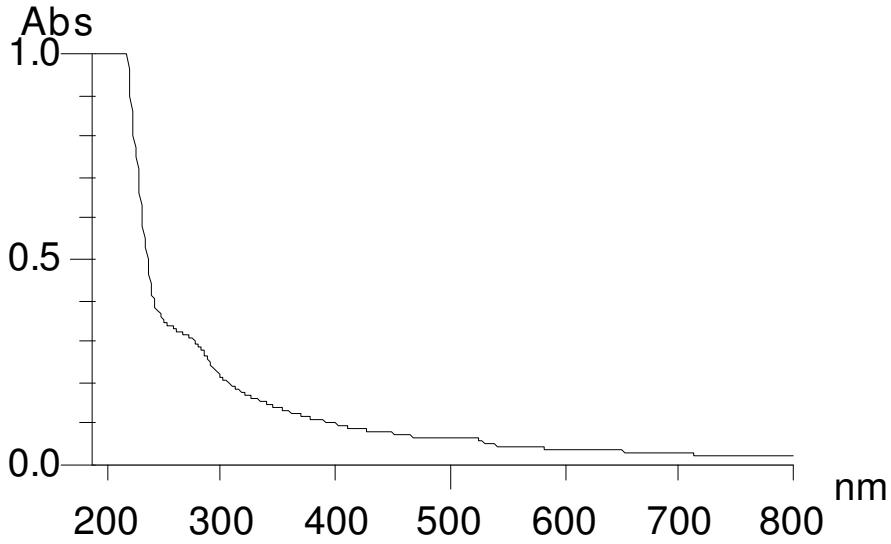


Figure 6.25. UV-scan of recovered sericin with HCl

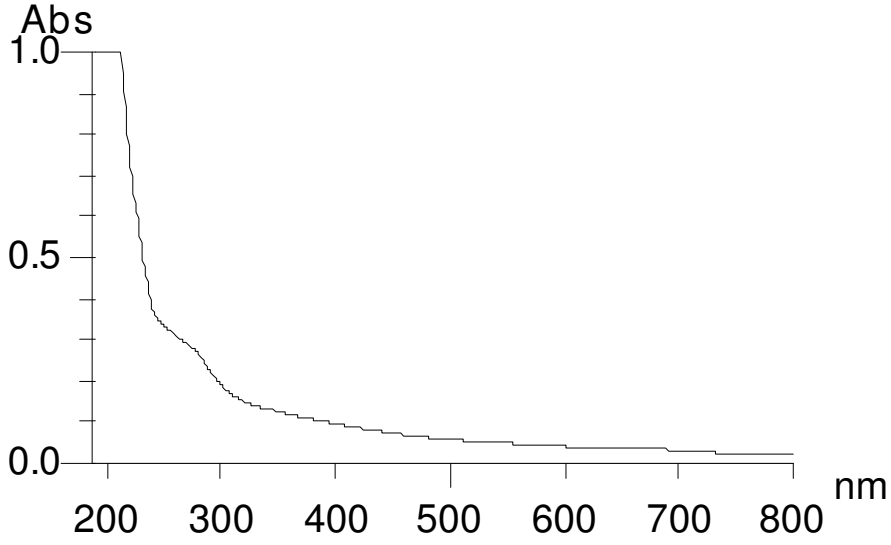


Figure 6.26. UV-scan of recovered sericin with H_2SO_4

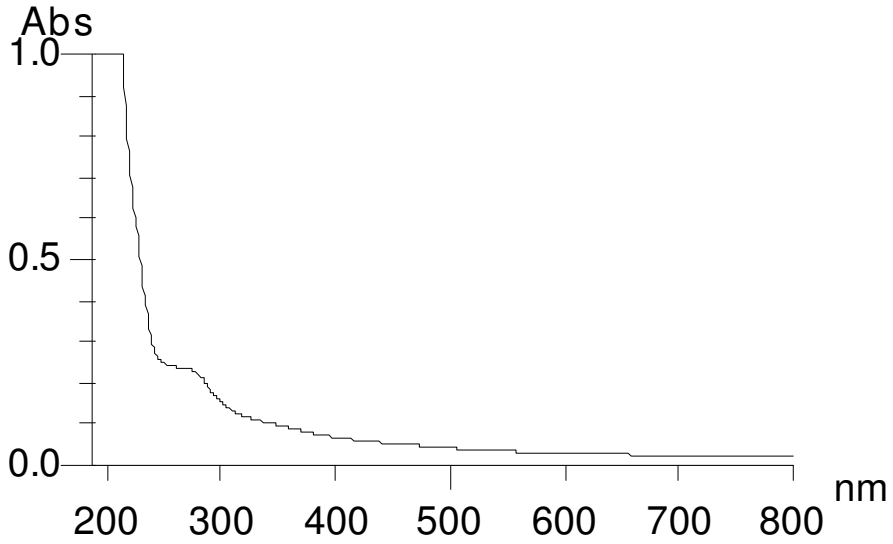


Figure 6.27. UV-scan of recovered sericin with $C_2H_4O_2$

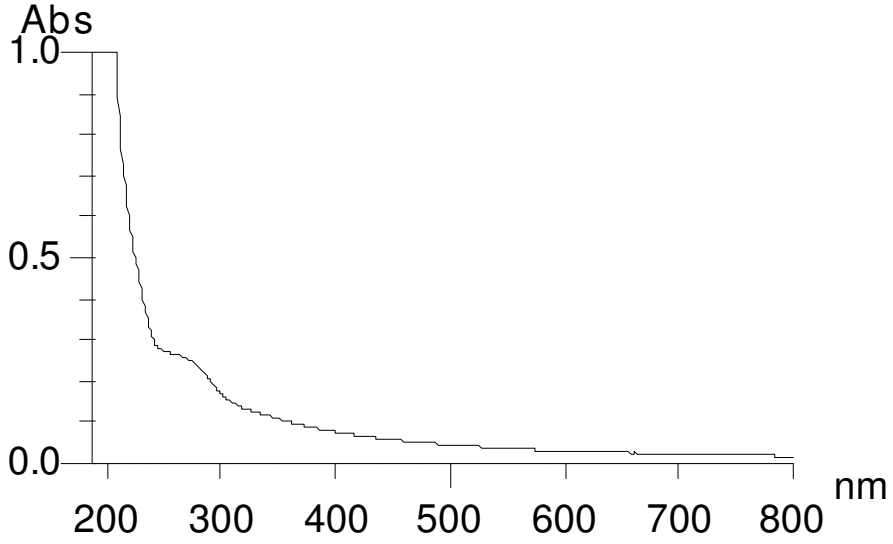


Figure 6.28. UV-scan of recovered sericin with C_2H_6O

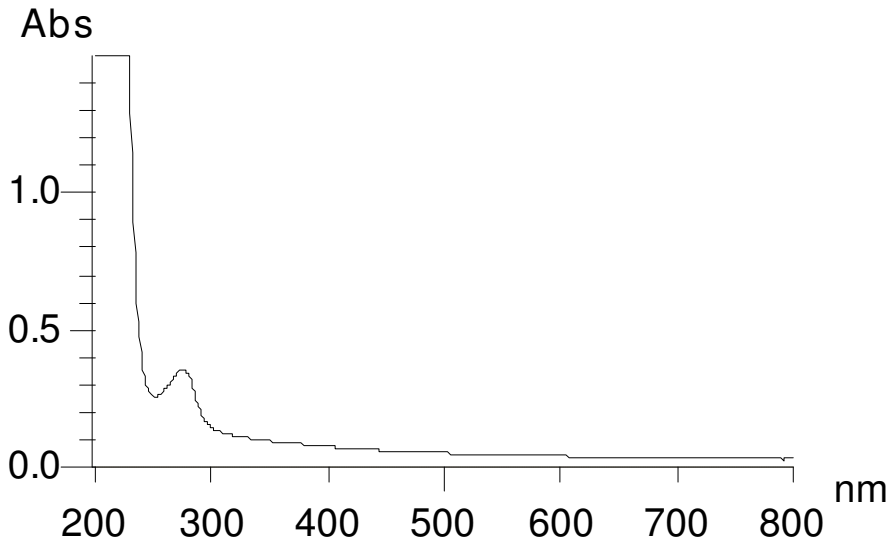


Figure 6.29. UV-scan of standard sericin obtained from native cocoon

6.3.5. Identification of Recovered Sericin

In order to verify that the recovered powder is really sericin, ion exchange chromatography, 2-D gel electrophoresis and MALDI-TOF analyses have been done in Ankara University Biotechnology Institute Proteomics Laboratory. Sericin which was concentrated with NF-DK membrane was precipitated using ethanol and then, it was obtained in powder form after drying by means of a lyophilizator.

Firstly, sericin sample was passed through the ion exchange column, which provides the separation of biomolecules relative to their charges. To find the isoelectric point (pI) of sericin, both anion (Q) and cation (S) columns were used. Ion exchange chromatograms are given in Figures 6.30 and 6.31. These figures show that recovered sericin is an acidic protein and its isoelectric point (pI) is between 5 and 6. In literature, Kurioka et al. (2002) found that protein purified from the cocoon shell of silkworm, *Bombyx mori,* has a pI of 4.3. Also, Mondal et al. (2007) proved that pI of silk fiber is around 5.

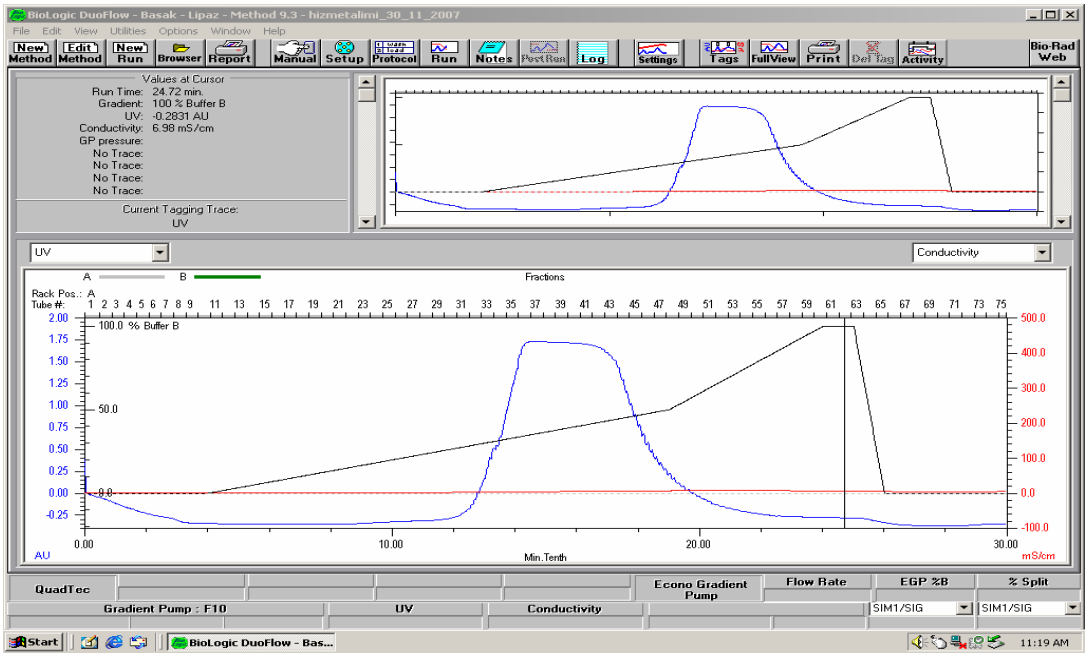


Figure 6.30. Anion (Q) column chromatogram

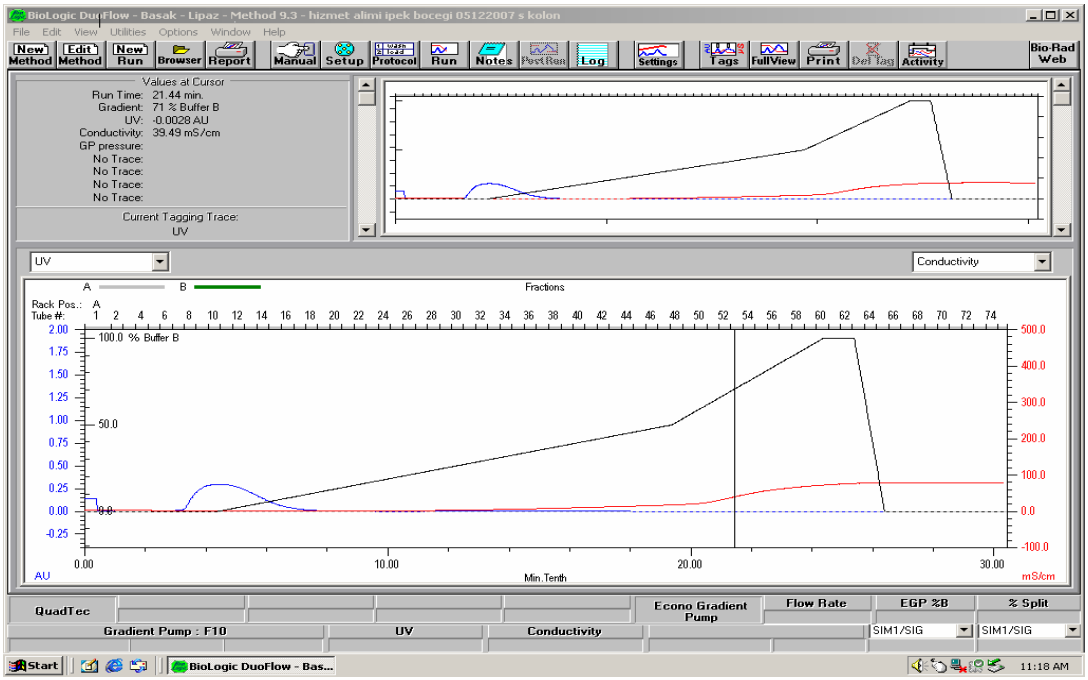


Figure 6.31. Cation (S) column chromatogram

After passing through anion and cation columns, sericin samples were loaded into MALDI-TOF. In Figure 6.32, spectrum of mass to charge (m/z) ratio is given. Figures 6.32(a) and 6.32(b) show the results of duplicate analyses of sericin samples passing through anion column whereas Figures 6.32(c) and 6.32(d) show the results of duplicate analyses of sericin samples passing through cation column. As seen, m/z values belonging to same sample were almost the same. Some peaks were not observed in the sample passing through cation column (Figures 6.32(c) and 6.32(d)). This was attributed to the possible rejection of some proteins in the anion column, which would lead to their disappearance in the exit of the following cation column.

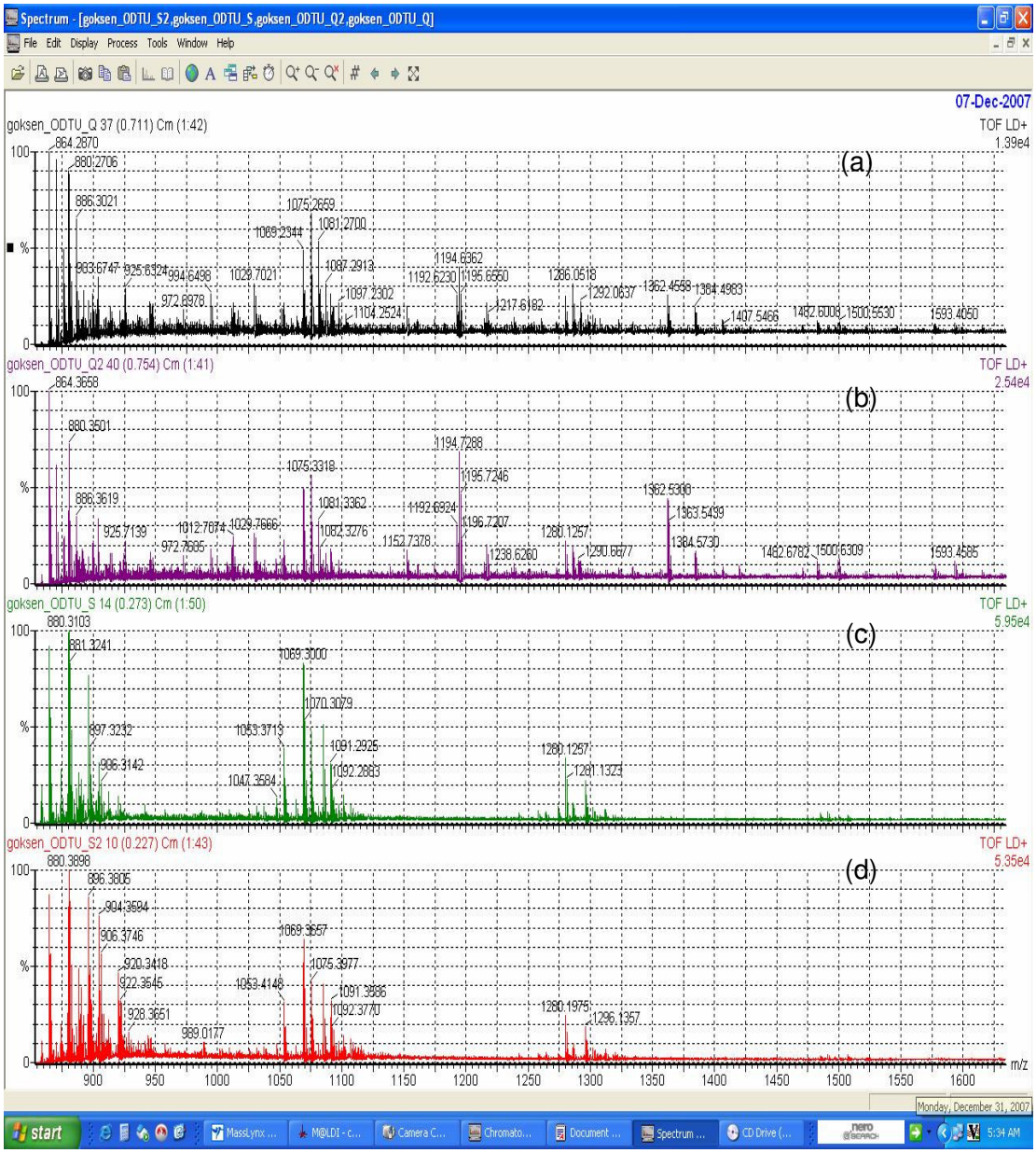


Figure 6.32. MALDI-TOF spectrum of sericin passed through anion and cation columns

When MALDI-TOF spectrums were compared with SWISS-PROT and ExPASy protein databases (ExPASy, 2008), compatibility with proteins belonging to a lot of reptile animals family was found. The results of this analysis were not good enough

and the proteins in the recovered sericin sample were needed to be charged separately into MALDI-TOF. Hence, two dimensional (2-D) gel electrophoresis was done prior to MALDI-TOF and all proteins in sericin sample were separated in the gel according to their pI values and their molecular weights. As a result of electrophoresis, protein spots (Spot 1 and Spot 2) having pH of 4-6.5 were determined (Figure 6.33). Furthermore, a protein spot having pH of 8-9 was observed. In terms of molecular weight distribution, an acidic protein with MW of 9 kDa and a group of acidic protein with MW of 25-40 kDa were observed. MALDI-TOF spectrums of these spots were again compared with SWISS-PROT/TrEMBL databases and it was found that they were compatible with SER1 sericin having the code of O96614 and MW of 9161 Da and SER2 sericin having the code of O96615 and MW of 20302 Da. Except these, a few E.coli and fungus proteins were identified. This may be expected as bacteria/fungus grow rapidly in wastewater. Contamination of the samples from another source was also possible. In conclusion, MALDI-TOF analysis proved that the recovered sample contained sericin.

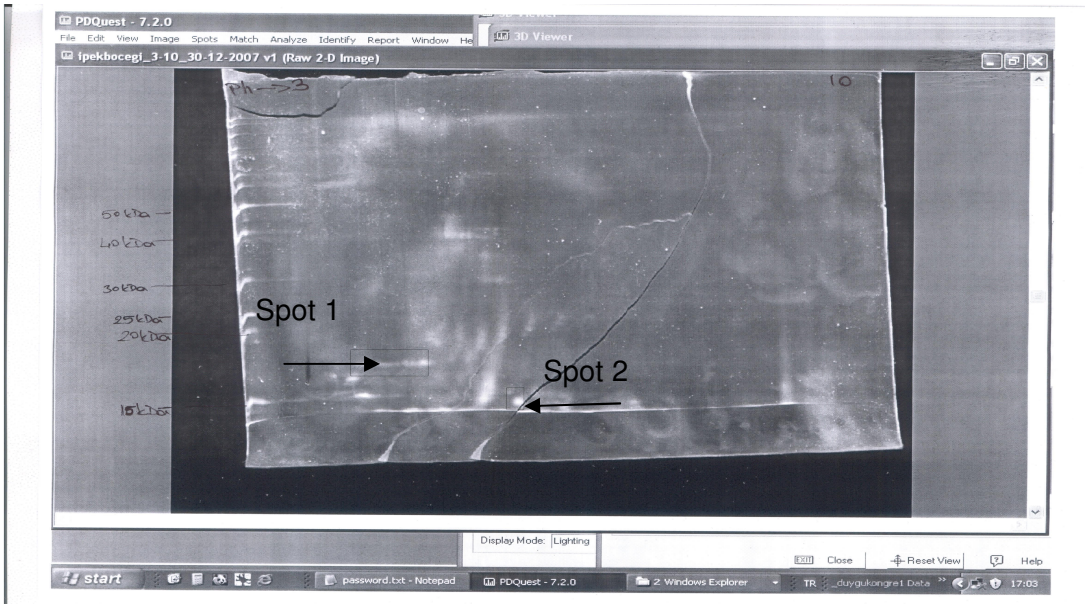


Figure 6.33. Representation of 2-D gel electrophoresis of sericin sample

6.3.6. Purification of Precipitated Sericin by Using Dialysis Process

Since the protein originating from silkworm could not be separated from sericin, it was decided to apply dialysis in order to increase the purity of sericin samples. A dialysis sack having small MWCO (3.5 kDa) was used to prevent the loss of small sericin peptides. HPLC chromatograms of the dialysed sericin solutions are shown in Figures 6.34-6.38. As seen, the HPLC chromatograms obtained after dialysis were very similar to each other. Figure 6.37 shows that HNO_3 was removed from sericin solution which was kept in a dialysis sack having MWCO of 3.5 kDa for 24 h. The silkworm protein was not observed in any of the dialyzed samples.

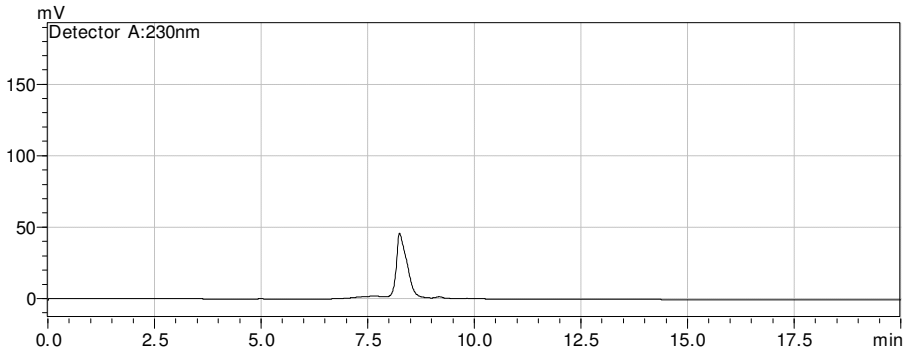


Figure 6.34. Sericin precipitated with HCl and dialyzed (>3.5 kDa)

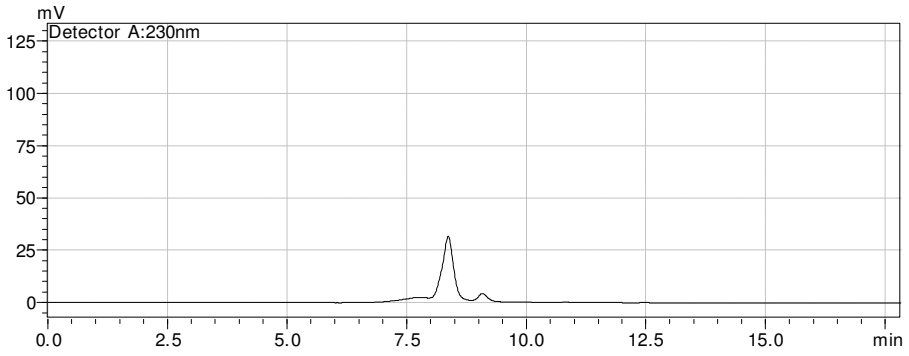


Figure 6.35. Sericin precipitated with H_2SO_4 and dialyzed (>3.5 kDa)

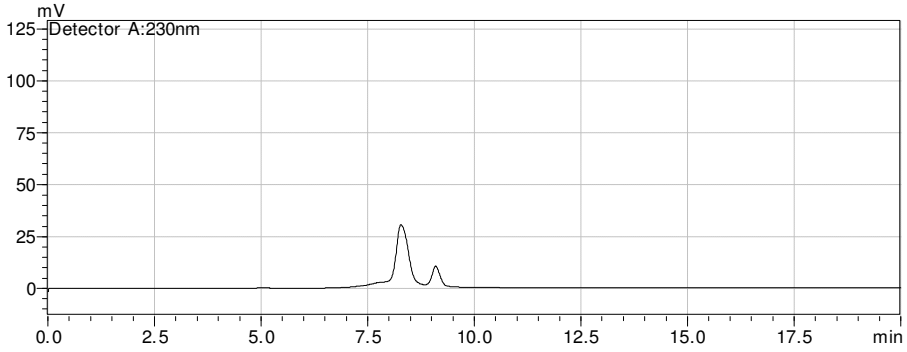


Figure 6.36. Sericin precipitated with $C_2H_4O_2$ and dialyzed (>3.5 kDa)

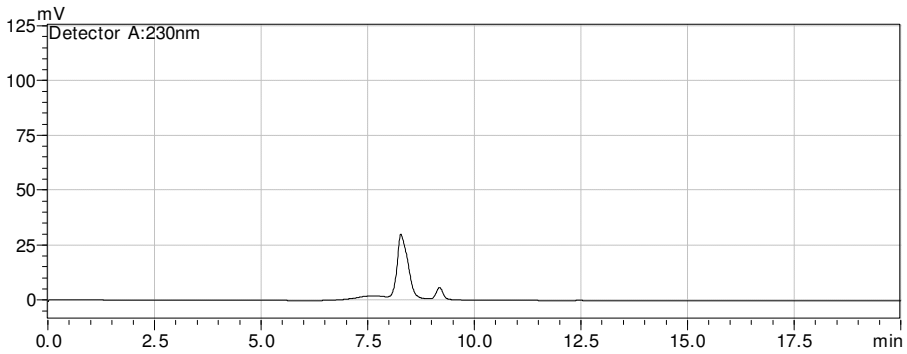


Figure 6.37. Sericin precipitated with HNO_3 and dialyzed (>3.5 kDa)

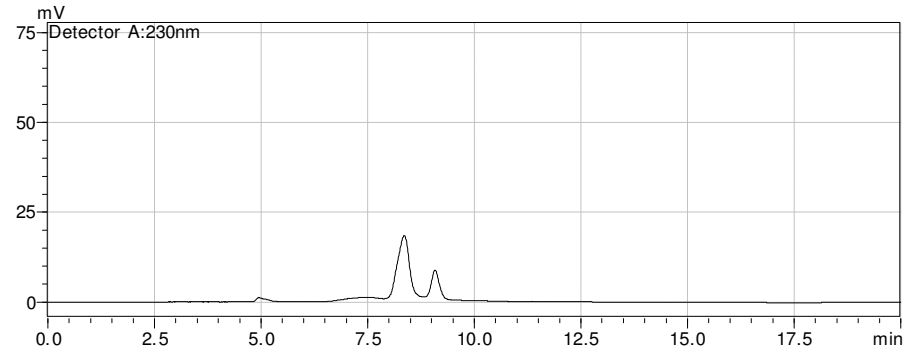


Figure 6.38. Sericin precipitated with C_2H_6O and dialyzed (>3.5 kDa)

MW distributions of dialyzed sericin samples were determined and compared with each other (Table 6.23). Three different molecular weight ranges were found in all samples. These were S-1 (150-180 kDa), S-2 (85-100 kDa) and S-3 (40-45 kDa). As seen, S-2 was present in all samples at the highest ratio of 79-97%. The ratios of other peptides changed between 0% and 20%. All acids and ethanol gave almost same results with respect to MW distribution. In terms of MW, the difference between dialyzed and non-dialyzed sericin samples was the loss of sericin having MW of 10-25 kDa. The reason for this may be speculated that MWCO of the dialysis sack was greater than 3.5 kDa, in fact. Therefore, sericin peptides with MW of 10-25 kDa might have passed to the other side of the dialysis sack.

Table 6.23. MW distribution and amount of sericin after dialysis

Precipitation Agent	**Sericin Remaining After Dialysis**		
	Name	**MW (kDa)**	**Concentration (mg/L)** ***(Percentage, %)***
HCl	S-1	169	8 *(2)*
	S-2	96	323 *(97)*
	S-3	40	3 *(1)*
	TOTAL		334
H_2SO_4	S-1	157	31 *(12)*
	S-2	86	205 *(79)*
	S-3	44	23 *(9)*
	TOTAL		259
$C_2H_4O_2$	S-1	151	2 *(1)*
	S-2	90	270 *(84)*
	S-3	44	48 *(15)*
	TOTAL		320
HNO_3	S-1	176	29 *(11)*
	S-2	92	204 *(80)*
	S-3	40	23 *(9)*
	TOTAL		256
C_2H_6O	S-1	-	-
	S-2	85	154 *(80)*
	S-3	44	42 *(20)*
	TOTAL		220

Elemental compositions of dialyzed recovered sericins were also determined (Table 6.24). Carbon contents of these samples were 40.8-45.3%, which were quite close to 41-43% C contents of reference sericins (Table 6.19). H contents change between 7.3-8.8% which is higher than those of S_C and S_N. Dialyzed recovered sericin samples contained 14-17% N, which were almost the same as 14-15% obtained for commercial and native sericins (Tables 6.19 and 6.24). C/H ratios of these samples were 4.7-5.9% whereas their C/N ratios were 2.4-3.0. These ratios are close to that of S_C and S_N, that is, C/H ratios were 6.6% for both of them whereas C/N ratios were 3.1% and 2.8%, respectively. There was no S in dialyzed recovered sericin samples. The reason for this may be speculated that there might be less sulphur in sericin but more sulphur in silkworm protein, where the latter might have been removed from recovered sericin during dialysis.

Table 6.24. Elemental compositions (dry basis) of dialyzed sericin samples

Dialyzed Sample	C (%)	H (%)	N (%)	S (%)	Total (%)	C/H	C/N
Recovered Sericin							
with HCl	45.3	8.8	16.8	-	70.9	5.2	2.7
with H_2SO_4	40.8	8.7	16.8	-	66.3	4.7	2.4
with $C_2H_4O_2$	44.2	7.9	16.8	-	68.9	5.6	2.6
with HNO_3	43.6	7.9	15.2	-	66.7	5.5	2.9
with C_2H_6O	43.1	7.3	14.4	-	64.8	5.9	3.0

6.3.7. Process Train Developed for Sericin Recovery from Cocoon Cooking Wastewaters

To develop the process train for sericin recovery from cocoon cooking wastewaters, the most suitable pre-treatment and membrane separation process were selected

considering the alternatives given in Figure 6.4 and Figure 6.7. The decision was made based on the highest rejection efficiencies achieved by these processes. To achieve sericin recovery from CW, the best pre-treatment method was determined as CFG + MF (1 μm). For membrane separation process, two alternatives were developed: 1. NF + precipitation of concentrated sericin with ethanol + freeze drying and 2. NF + dialysis + precipitation of concentrated sericin with ethanol + freeze drying. The developed process trains are shown in Figure 6.39 and sericin recovery efficiency is given in Figure 6.40. Accordingly, 76% of high MW sericin can be recovered from cocoon cooking wastewaters by the developed process train.

There are two kinds of sericin which can be obtained from cocoon cooking wastewaters by the developed process train: low quality sericin with a MW of 90 kDa and high quality sericin with a MW of 44 and 85 kDa having the fractions of 20%, and 80%, respectively. Different MWs were due to dialysis process. It may be speculated that in dialysis, sericin could be separated into peptides having different molecular weights since dialysis process was performed at 37 ^{o}C and sericin was affected by temperature. Since low quality sericin contains silkworm protein as an impurity, one possible end-use can be the production of animal food. Moreover, in a study of Cortez et al. (2007), recovered sericin was used to increase yarn strength leading to increased fabric longevity. Therefore, this low quality sericin can also be used in similar jobs. Besides, biofilms can be prepared with this low quality sericin as packaging material. High quality sericin, however, can be used as an antioxidant in the field of medicines, cosmetics, food and food additive (Kato et al., 1998). It can also be 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 (Yamada et al., 1998).

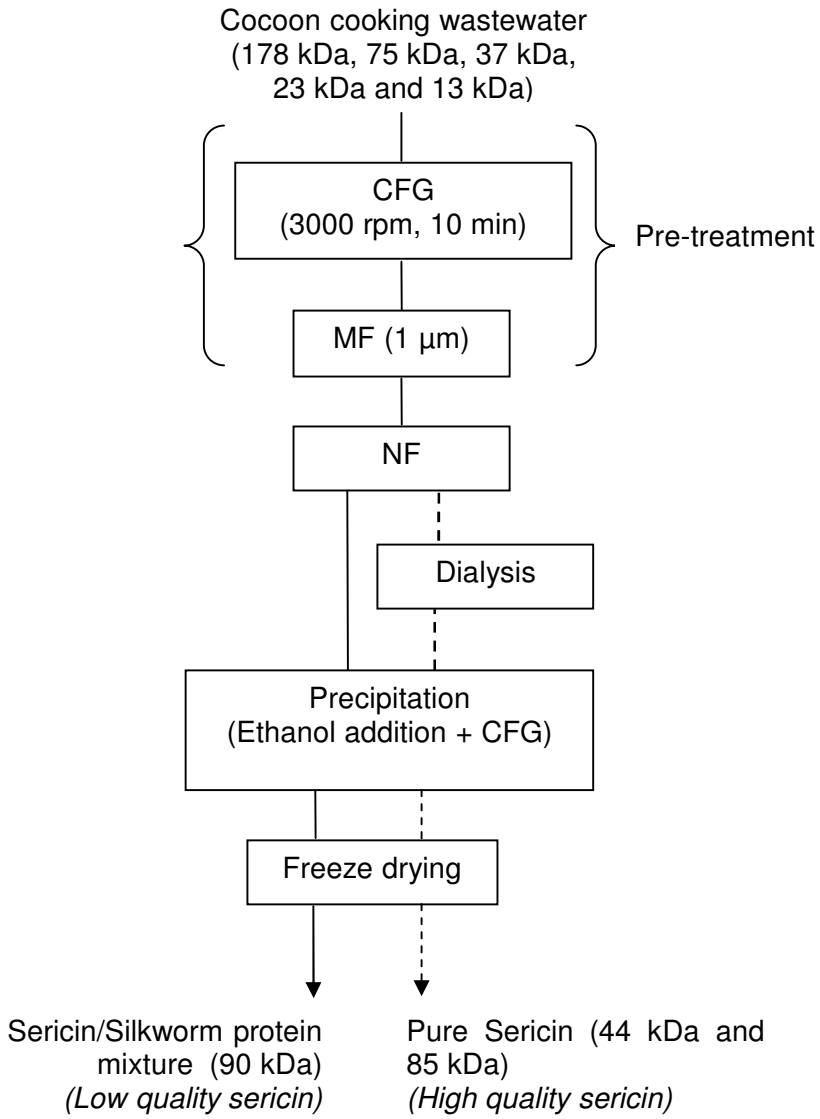


Figure 6.39. Process train developed for sericin recovery from cocoon cooking wastewaters

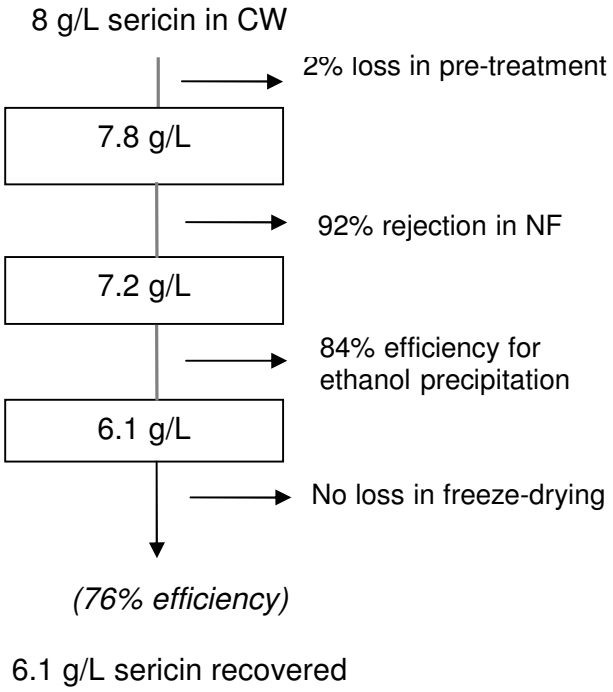


Figure 6.40. Schematic presentation of sericin recovery efficiency

6.4. Sericin Recovery from Silk Degumming Wastewaters

Silk degumming wastewaters (SDW), whose characteristics were given in Table 5.1, were handled as the second source of sericin. The molecular weight of sericin in SDW was determined as 110-120 kDa. In this part of the study, a membrane based sericin recovery method was determined for SDW. This wastewater had a COD of about 60 g/L, of which almost half resulted from sericin concentration. The soap content of SDW could not be determined directly. However, the difference between sericin and COD concentrations was taken as a reference to evaluate the soap removal efficiency. The relationships between sericin, soap and COD were examined. As a result, it was found that 1 g of sericin is almost identical to 1 g of COD and 1 g of soap gives approximately 2.5 g of COD. The results are explained in detail in Appendix I.

GC-MS was also used to detect the fatty acids that originate from soap, and the presence of fatty acid peaks were taken as an indication for the presence of soap in treated samples. In this way, separation efficiency of sericin from soap was determined.

The information on the actual amounts of sericin and soap in SDW was gathered from the plant personnel. For SDW2 sample, 100-120 kg of soap and 15-20 kg of soda were added into 4-4.5 tons of water. In this water, 550-600 kg silk yarn was degummed. Therefore, the expected amount of sericin in wastewater was calculated as 140-156 kg since silk yarn loses 25-27% of its weight. This is almost equal to 35 g/L sericin concentration. Moreover, the expected soap concentration in wastewater was calculated as 22-30 g/L. In the characterization study of SDW, the measured sericin concentration was 34002 mg/L, which perfectly agrees with the expected value. Similarly, total protein content was determined as 46747 mg/L, and COD was found as 59150 mg/L. Since the difference between sericin and COD was taken as the soap concentration, it was found that soap concentration was 25148 mg/L, which agrees with the expected value. For SDW3, sericin concentration was 27581 mg/L and COD was 55950 mg/L (Table 5.1). So, soap concentration was found as 28369 mg/L.

6.4.1. Determination of the Most Suitable Pre-treatment Method for SDW

To prevent fouling of the post membrane and to separate sericin from soap, an appropriate pre-treatment process was investigated. As experienced with CW, a portion of sericin in wastewater was lost in pre-treatment stage. To minimize this loss and not to contaminate sericin with chemicals, physical techniques were considered to be more suitable. For cocoon cooking wastewater, centrifugation and microfiltration were applied. Therefore, for silk degumming wastewaters, firstly, centrifugation was tried. All the pre-treatment alternatives for SDW are depicted in Figure 6.41.

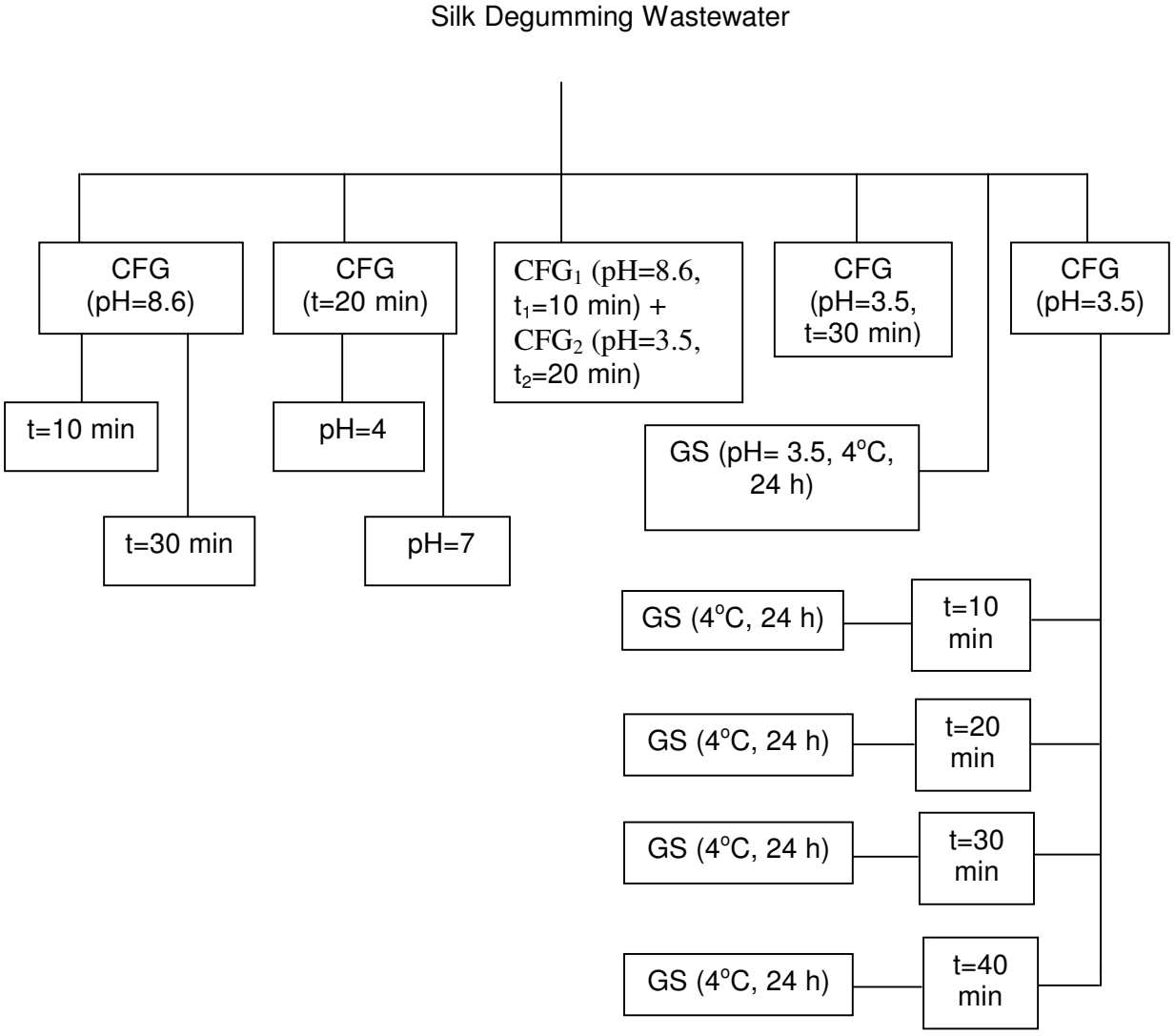


Figure 6.41. Schematic presentation of pre-treatment alternatives for SDW

In the first alternative, CFG was applied at the original pH of SDW and the effect of centrifugation time was investigated. The corresponding removal efficiencies are given in Table 6.25. As seen, sericin and total protein loss were quite low, i.e., 5% and 22% for t=10 min and 2% and 14% for t=30 min, respectively. However, the removal efficiencies for pollution parameters were also low, such that COD and total solids were removed at only 23% and 11% for t=10 min, and 24% and 13% for t=30 min. On the other hand, color and turbidity removals were 51% and 65% for t=10

min, and 67% and 78% for t=30 min, respectively. The centrifuged wastewater for t=10 min had a COD of 38850 mg/L and sericin concentration of 23684 mg/L whereas centrifuged wastewater for t=30 min had a COD of 38375 mg/L and sericin concentration of 24420 mg/L. This means that these centrifuged wastewaters contained high amount of soap, as evidenced from the difference between COD and sericin concentrations.

Table 6.25. Effect of time and pH on centrifugation performance

Parameter	Centrifuged Wastewater Quality *(Removal, %)*					
	CFG					$CFG_1 + CFG_2$
	pH=8.6		pH=7.0	pH=4.0	pH=3.5	pH_1=8.6, pH_2=3.5
	t=10 min	t=30 min	t=20 min	t=20 min	t=30 min	t_1=10 min, t_2=20 min
Sericin (mg/L)	23684	24420	33059	29633	22190	20133
	(5)	*(2)*	*(3)*	*(13)*	*(11)*	*(20)*
T. Protein (mg/L)	20950	23115	54147	47672	23436	20388
	(22)	*(14)*	*(0)*	*(0)*	*(13)*	*(24)*
COD (mg/L)	38850	38375	51000	40050	29150	29438
	(23)	*(24)*	*(14)*	*(32)*	*(42)*	*(42)*
T.Solids (mg/L)	27475	26975	-	-	30475	29400
	(11)	*(13)*			*(1)*	*(5)*
Color (Pt-Co)	22725	15650	23550	35500	94	59
	(51)	*(67)*	*(10)*	*(0)*	*(99)*	*(100)*
Turbidity (NTU)	2845	1798	3190	5555	233	73
	(65)	*(78)*	*(23)*	*(0)*	*(97)*	*(99)*

The reason for low removal efficiencies in centrifugation stage was suspected to be the basic pH of wastewater. In order to investigate the effect of pH on centrifugation performance, the second pre-treatment alternative consisted of reducing the pH of SDW to 7.0 and 4.0 prior to CFG performed for 20 min. As seen from Table 6.25, a significant increase in removal efficiencies was not observed, although some increases occurred for some parameters. When pH was decreased from 8.6 to 7.0 and 4.0, the loss of sericin was 3% and 13%, respectively. COD removal became 14% at pH 7.0 and 32% at pH 4.0. Color and turbidity values, however, increased when pH was decreased to 4.0 (Table 6.25). The centrifuged SDW2 sample contained 51000 mg/L and 40050 mg/L of COD at pH 7.0 and 4.0, where the corresponding sericin concentrations were 33059 mg/L and 29633 mg/L, respectively. These values clearly show that centrifugation at lowered pH values did not help separate soap from sericin.

Since sericin and soap could not be separated in the second alternative, other pre-treatment alternatives were considered. In the third alternative, two-stage centrifugation was applied. SDW, at its original pH, was first centrifuged for 10 min. Then, pH of the supernatant was decreased to 3.5 prior to second stage centrifugation applied for 20 min. As shown in Table 6.25, sericin and total protein were lost at 20%, and 24% whereas COD, total solids, color and turbidity removals were 42%, 5%, 100%, and 99%, respectively. The COD and sericin concentrations in the supernatant were 29438 mg/L and 20133 mg/L, where the difference was due to the presence of soap that could not be settled completely. However, the lowest ratio between COD and sericin was obtained at pH 3.5, which means highest soap removal achieved so far. Furthermore, a very clear supernatant was obtained in the second stage, with color and turbidity as low as 59 Pt-Co and 73 NTU. These results revealed that acidic pH conditions had to be maintained to enhance soap removal. Therefore, it was decided to consider the second stage only as this would achieve separation of sericin and soap in single stage CFG and reduce the costs associated with the application of a two-stage process. As a result, the third alternative was eliminated.

In the fourth alternative, CFG at pH 3.5 was applied for 30 min. As can be seen in Table 6.25, sericin and total protein were removed at 11% and 13%, respectively.

COD, total solids, color and turbidity removals became 42%, 1%, 99% and 97%, respectively. Sericin concentration and COD in the supernatant were 22190 mg/L and 29150 mg/L. The difference between them showed that there was still soap in supernatant. Color and turbidity values were 94 Pt-Co and 233 NTU. In conclusion, it was obviously seen that the results of two-stage CFG and one-stage CFG were very similar. Therefore, it was decided that one-stage CFG could be applied after pH adjustment to 3.5.

It was observed that the supernatant became very clear when the centrifuged sample was kept in the refrigerator overnight. All the soap seemed to have settled. Based on this observation, it was decided to apply gravity settling (GS) following centrifugation in the fifth alternative. Actually, CFG is a quite effective method for settling of particulates. However, in the case of soap removal, it was found insufficient. The reason for this may be due to the physical properties of the soap such as density. The centrifugation time was also optimized. The pH of samples were adjusted to 3.5 first, and then, centrifugation was applied for 10, 20, 30 and 40 min. SDW sample, whose pH was adjusted to 3.5 but not centrifuged, was used as a control. The supernatants of centrifuged samples and the control were left to settle themselves at room temperature for 24 h. However, it was observed that soap accumulated at the top of the wastewater. This result showed that soap could settle at 4 ^{o}C but not at room temperature. Actually, the soap accumulated at the top of the wastewater can be skimmed off (Davidsohn, 1953) and then, the phase below this can be used as pre-treated SDW. However, in laboratory conditions, this was not possible. Therefore, these samples were placed into the refrigerator. Table 6.26 shows the supernatant qualities and sericin contents as well as the removal efficiencies for COD, color and turbidity of GS and CFG + GS effluents. As seen, sericin concentrations were 24080-27450 mg/L and the COD values were between 22688 mg/L and 26000 mg/L, in GS and all CFG + GS effluents. So, sericin and COD values were very close to each other, which means maximum removal of soap was achieved. In terms of color and turbidity, centrifugation for 10 min and 20 min gave the best results. Color and turbidity were 4963 Pt-Co and 21 NTU in the sample centrifuged for 10 min whereas they were 5913 Pt-Co and 37 NTU in the sample centrifuged for 20 min. Also, in GS, color and turbidity removals were close to that in CFG for 10 min and 20 min. However, since slow settling was observed in

GS, it was not chosen as the most suitable pre-treatment process. In CFG (t=30 min) + GS and CFG (t=40 min) + GS processes, color and turbidity were removed at a smaller ratio than in other alternatives with less centrifugation times, i.e., 65-66% and 86-90%, respectively. It may be due to experimental error. Soap was accumulated at the top of the wastewater in CFG (t=30 min) + GS and CFG (t=40 min) + GS processes since they were left to settle themselves at room temperature. When sampling, the soap layer at the top dispersed into the supernatant found at the bottom. Therefore, the COD, color and turbidity values of the sample taken from the bottom increased leading to low removal efficiencies.

All the pre-treatment results revealed that the highest soap removal was achieved in the fifth alternative. Hence, the most suitable pre-treatment method for SDW was determined as CFG at acidic pH (3.5) followed by gravity settling (GS) at 4 ^{o}C for 24 h. The schematic presentation of the most suitable pre-treatment method for SDW is depicted in Figure 6.42.

Table 6.26. Optimization of centrifugation times at pH 3.5

Parameter	**Centrifuged Wastewater Quality *(Removal, %)***				
	(pH=3.5)				
	GS	**CFG (t=10 min) + GS**	**CFG (t=20 min) + GS**	**CFG (t=30 min) + GS**	**CFG (t=40 min) + GS**
Sericin (mg/L)	27450	24080	25533	25842	26509
	(1)	*(13)*	*(7)*	*(6)*	*(4)*
COD (mg/L)	23725	22688	23513	26000	23240
	(58)	*(59)*	*(58)*	*(54)*	*(55)*
Color (Pt-Co)	6175	4963	5913	9025	8950
	(76)	*(81)*	*(77)*	*(65)*	*(66)*
Turbidity (NTU)	396	21	37	567	416
	(90)	*(99)*	*(99)*	*(86)*	*(90)*

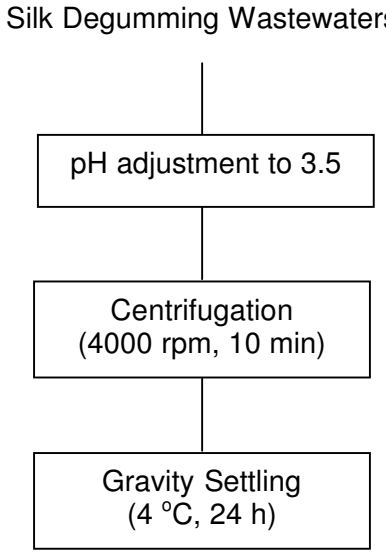


Figure 6.42. Best pre-treatment process for SDW

6.4.2. Ultrafiltration of Silk Degumming Wastewaters

Complete separation of soap and sericin was achieved by centrifugation at pH 3.5 followed by gravity settling at 4 °C for 24 h, which was selected as the most suitable pre-treatment process for SDW. Regarding the recovery of sericin in the post membrane process, UF was found suitable as the MW of sericin in SDW was 110-120 kDa. This wastewater did not contain sericin polypeptides with smaller MW. Hence, it was thought that NF would not be required as in the case of CW, and UF would be sufficient to concentrate sericin at a high efficiency.

Three alternatives were tested for sericin recovery using UF as shown in Figure 6.43. The reason for choosing these alternatives was to investigate the possibility of separating soap and sericin in the simplest process train. As seen from Figure 6.43, the simplest process train is the first one, where UF is preceded by CFG at the original pH of wastewater. In this alternative, the separation performance of UF for soap and sericin was investigated. The second alternative is more complex than the first one but simpler than the third one; it consists of centrifugation at the original pH of wastewater followed by pH adjustment to 3.5 and then UF. This alternative was considered to investigate whether acidic pH conditions would help UF to separate soap and sericin. Finally, the third alternative is the most complex one, where a two-stage pre-treatment process including centrifugation at pH 3.5 followed by gravity settling at 4 °C for 24 h is required before UF. In this alternative, soap and sericin would be separated in the pre-treatment stage and UF would be used for concentrating sericin. The comparison of overall performances of these alternatives provided determination of the most suitable method of sericin recovery from SDW.

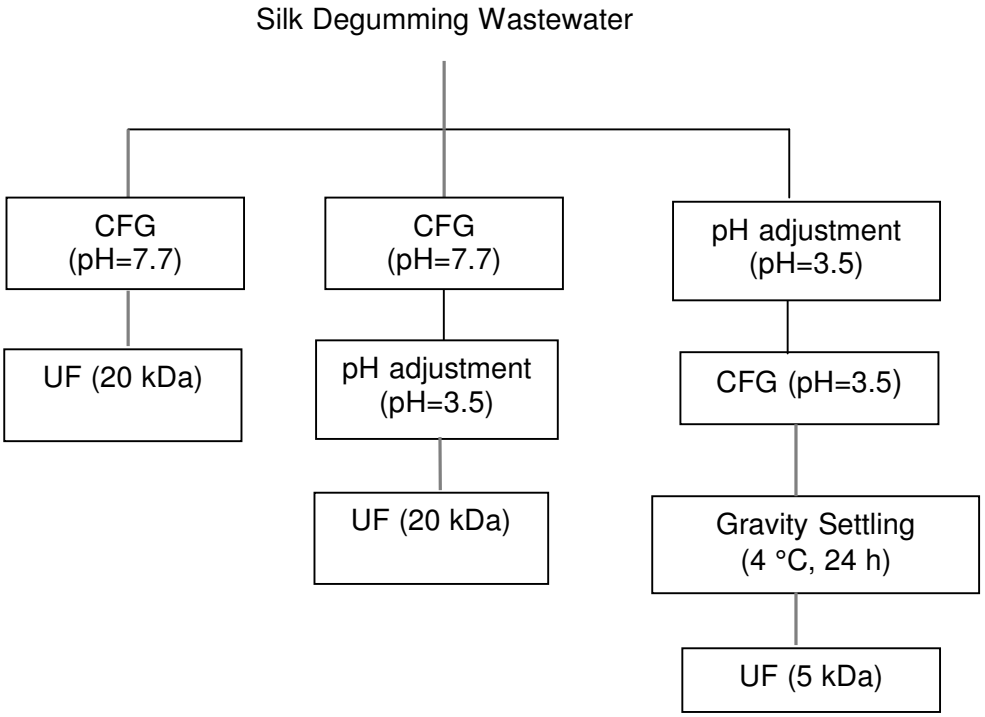


Figure 6.43. Schematic presentation of alternatives tested for sericin recovery

6.4.2.1. Rejection Performances of UF Membranes

The feed and permeate qualities and the rejection performances of the alternative processes are shown in Tables 6.27 and 6.28. As seen, in terms of permeate quality and rejection peformance, the first and second alternative processes provided almost similar results. In the first alternative, the feed and permeate sericin concentrations were 24252 mg/L and 3725 mg/L, corresponding to a sericin rejection efficiency of 85% at the original pH of 7.7. The MW of sericin in the feed and permeate were 119 kDa and 92 kDa, respectively. The feed and permeate COD were 32650 mg/L and 8985 mg/L, corresponding to a COD rejection efficiency of 72%. The difference between the feed sericin and COD was due to the presence of soap in the feed side, where feed COD was 1.3 times higher than the sericin

concentration. This means that the feed COD is not due to the presence of sericin only, and the feed contains considerable amount of soap. These results revealed that sericin and soap could not be separated by the UF (20 kDa) membrane when UF was preceded by CFG at the original pH of 7.7.

The performance of the same membrane was better in the second alternative, where pH was reduced to 3.5 in the UF stage. Sericin concentrations were 20610 mg/L and 314 mg/L in the feed and permeate, providing a rejection efficiency of 98%. MW of feed and permeate were almost same in the first alternative, that is, 119 kDa and 96 kDa. The rejection of COD was slightly lower, i.e., 70%, with feed COD as high as 39500 mg/L and permeate COD of 11820 mg/L. These results indicate that running the UF experiment at acidic pH improved the rejection performance for sericin but worsened the rejection performance for COD. The ratio of COD to sericin in the feed was 1.9, which means that sericin and soap could not be separated by the UF (20 kDa) membrane at pH 3.5.

The rejection performance of UF (20 kDa) membrane was rather high for sericin, total protein, color and turbidity. For COD and total solids, however, rejection performance of UF (20 kDa) membrane was lower. Especially, total solids rejection was very low. The reason for this might be the experimental error since in permeate, there could not be 21300 mg/L total solids. Color and turbidity were completely removed in both processes. The permeates of both first and second alternatives cannot be discharged to the environment since they do not meet the discharge criteria of 350 mg/L for COD.

Sericin and soap could not be separated by the UF (20 kDa) membrane in the first and second alternatives. Hence, the third alternative was considered, where sericin and soap were separated in the pre-treatment stage. In this alternative, the role of UF was to concentrate sericin rather than separate it from soap. In the pre-treatment stage, pH of SDW was adjusted to 3.5 by HCl and centrifugation was applied at 4000 rpm for 10 min prior to settling of soap at +4 ^{o}C. Then, UF (5 kDa) was applied to improve the permeate quality. The feed and permeate qualities and the rejection performances of this process are shown in Tables 6.27 and 6.28. As seen, COD and sericin concentration in the feed were 24188 mg/L and 24274 mg/L, which were

almost equal. This means that COD in the feed totally originates from sericin, and no soap exists in the wastewater. However, it can also be seen that UF (5 kDa) membrane was not able to reject all sericin. It passed almost half of the sericin to permeate side, i.e., sericin concentration in the permeate was 10063 mg/L, corresponding to a rejection efficiency as low as 59%. The reason for low rejection performance of UF (5 kDa) might be the acidic pH of wastewater. So, it may be useful to increase the wastewater pH back to its original value before performing UF (5 kDa) filtration test. Another possibility is to use UF (20 kDa) membrane at pH 3.5 as it achieved almost complete rejection of sericin.

In terms of pollution parameters, color and turbidity were completely removed by UF (5 kDa) membrane (Table 6.29). However, UF (5 kDa) permeate is not suitable for discharge as it contains 10090 mg/L of COD which is much greater than the discharge standard, i.e., 350 mg/L. In order to meet the discharge standards, all sericin must be recovered, which in turn, will provide complete rejection of COD. Hence, one suggestion might be to repeat the UF (5 kDa) filtration process at the original pH of wastewater. Another suggestion might be the application of UF (20 kDa) membrane as mentioned above.

Table 6.27. Feed and permeate qualities of UF processes

Parameter	Feed and Permeate Quality					
	UF (20 kDa) @ pH=7.7		UF (20 kDa) @ pH=3.5		UF (5 kDa) @ pH=3.5	
	Feed	Permeate	Feed	Permeate	Feed	Permeate
Sericin (mg/L)	24252	3725	20610	314	24274	10063
MW of sericin (kDa)	119	92	119	96	110	106
T. Protein (mg/L)	26675	3608	24697	2972	nm	nm
COD (mg/L)	32650	8985	39500	11820	24188	10090
T. Solids (mg/L)	33370	8725	42530	21300	nm	nm
Color (Pt-Co)	3675	85	31900	73	4713	345
Turbidity (NTU)	1780	0.4	4010	0.4	17	3
pH	7.7	8.0	3.5	3.4	3.5	3.4
Flux Decline (%)	85		94		88	
Flux Recovery (%)	89[a]		97[b]		31[c]	

nm: not measured
[a]0.5 M NaOH + 201 ppm free Cl for 20 min
[b]0.5 M NaOH + 207 ppm free Cl for 30 min
[c] 0.5 M NaOH + 192 ppm free Cl for 35 min

Table 6.28. Rejection performances of UF processes

Parameter	Rejection Efficiency (%)		
	UF (20 kDa) @ pH=7.7	**UF (20 kDa) @ pH=3.5**	**UF (5 kDa) @ pH=3.5**
Sericin (mg/L)	85	98	59
T. Protein (mg/L)	86	88	nm
COD (mg/L)	72	70	58
T. Solids (mg/L)	74	50	nm
Color (Pt-Co)	99	100	93
Turbidity (NTU)	100	100	84

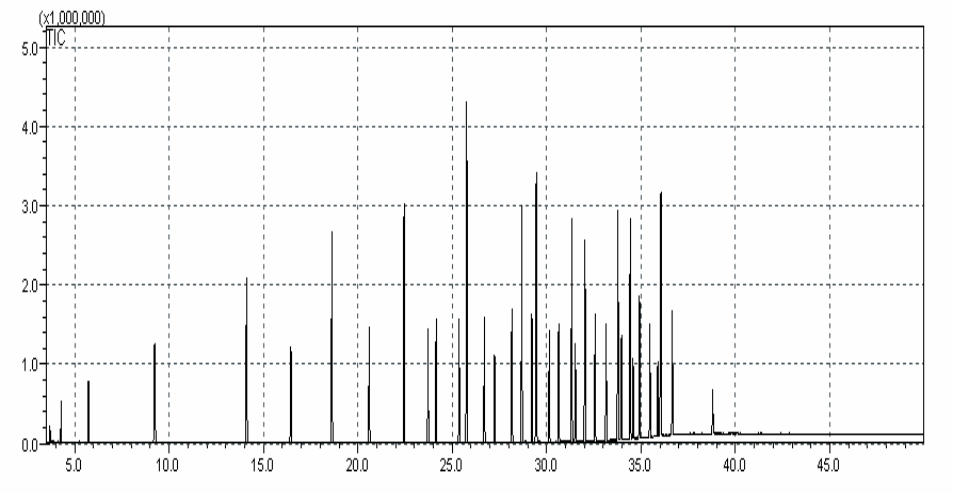
nm: not measured

Table 6.29. Overall removal efficiencies for pollution parameters

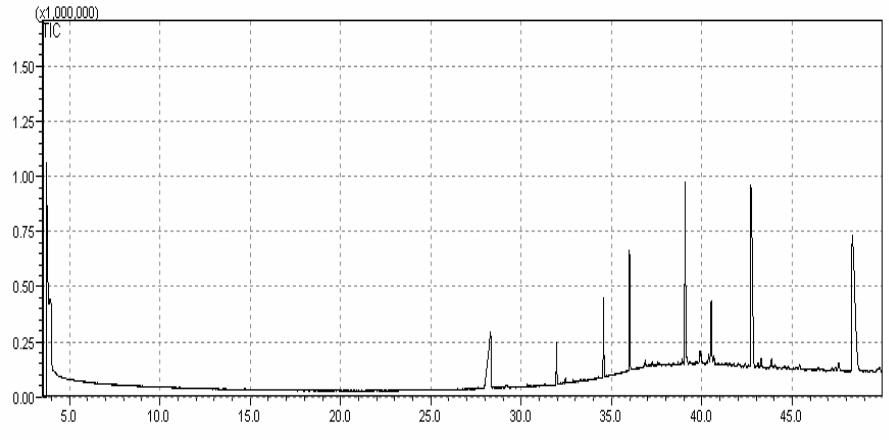
Parameter	Overall Removal Efficiency (%)		
	UF (20 kDa) @ pH=7.7	**UF (20 kDa) @ pH=3.5**	**UF (5 kDa) @ pH=3.5**
COD (mg/L)	81	76	82
T. Solids (mg/L)	75	37	nm
Color (Pt-Co)	100	100	99
Turbidity (NTU)	100	100	100

nm: not measured

To verify that soap was removed from SDW, UF (5 kDa) feed and permeate were analyzed using gas chromatography-mass spectrometry (GC-MS) in Ankara University Biotechnology Institute Instrumental Analysis Unit. Figure 6.44 shows their chromatograms. As seen from Figure 6.44(a) and 6.44(b), when compared with fatty acids, raw SDW contains also fatty acids coming from soap. Figure 6.44(c) and 6.44(d) prove that there were no soap in UF (5 kDa) feed and permeate, and applied pre-treatment method could remove soap completely.



(a)



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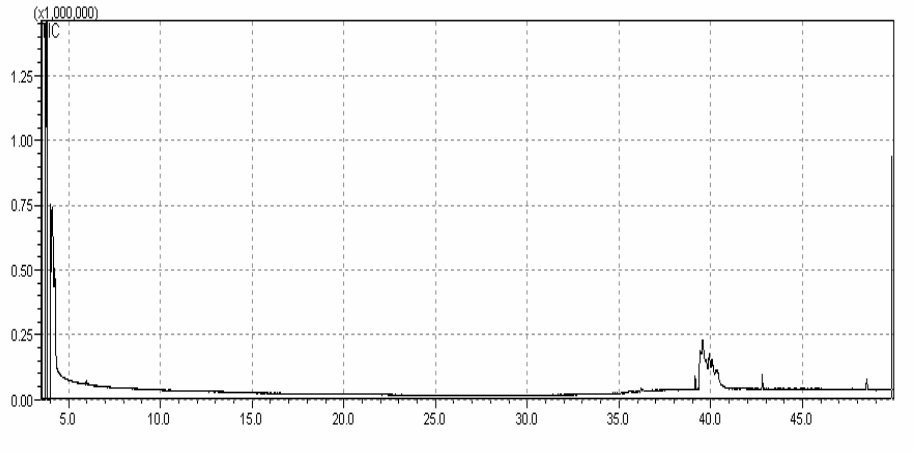
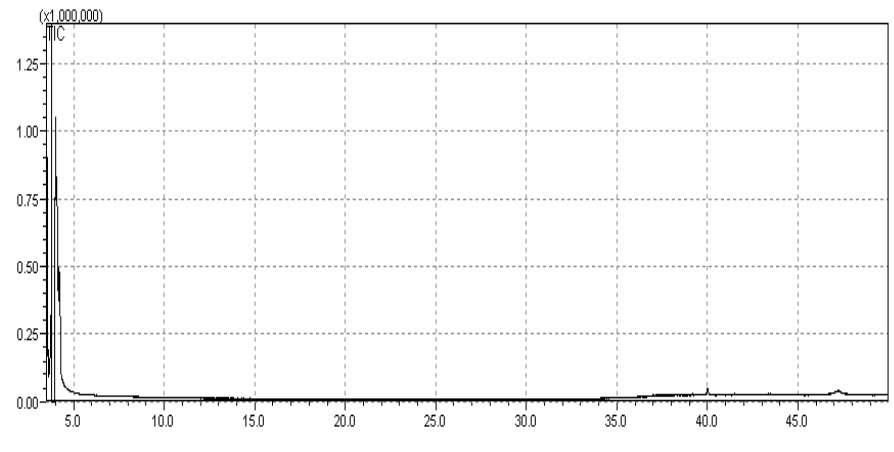


(c)



(d)

Figure 6.44. GC-MS chromatograms **(a)** fatty acid standard **(b)** raw SDW **(c)** UF (5 kDa) feed **(d)** UF (5 kDa) permeate

6.4.2.2. Flux Decline Analyses of UF Membranes

The flux declines were severe in all alternatives, that is, 85% in UF (20 kDa) at pH 7.7, 94% in UF (20 kDa) at pH 3.5, and 88% in UF (5 kDa) at pH 3.5. Although chemical cleaning lead to almost complete flux recovery in UF (20 kDa), the clean water flux could not be recovered in UF (5 kDa). In other words, flux recovery of UF

(20 kDa) membrane was 89-97% while that of UF (5 kDa) was only 31%. The reason for this may be that there occurred significant pore clogging in UF (5 kDa) membrane, which could not be removed by the chemical cleaning procedure. Another reason could be that when there was soap in SDW, sericin might be adhered to it and therefore, its interaction with the membrane was not so adverse. However, after all of the soap was removed from SDW, there remained only sericin in wastewater. Hence, high concentration of sericin might have affected the membrane, that is, caused irreversible fouling, so adversely that chemical cleaning became ineffective.

The flux declines were analyzed in Table 6.30. As seen, at pH 7.7, concentration polarization was higher and fouling was lower than that at acidic pH. At pH 7.7, concentration polarization was 78% whereas 33% and 53% at pH 3.5. Moreover, total fouling was 34% at pH 7.7 and 81-88% at acidic pH. As a result, pH near neutral gives better results in terms of flux decline but it gives worse results in terms of soap removal.

The results obtained so far clearly show that recovery of sericin from silk degumming wastewaters is not an easy task. Severe flux decline is a big disadvantage for the application of the proposed process. Moreover, the wastewater should be freed of soap before sericin recovery. Therefore, another silk degumming technique with less adverse environmental impacts should be applied. If degumming is done without using soap like reported by Fabiani et al. (1996), sericin recovery from this wastewater would be more practical and more economical as there would be less number of unit operations in the process train. This alternative degumming technique was explained in detail in Section 6.4.3.

Table 6.30. Flux decline analysis of UF processes

Process	Flux ($L/m^2/h$) (T=17-24 ºC)			Flux Decline (%)				
	I	W	F	C	C.P	T.F	R.F	I.F
UF (20 kDa) @ pH=7.7	63.3	9.3	41.7	56.7	78	34	27	11
UF (20 kDa) @ pH=3.5	53.3	3.0	6.3	51.7	53	88	88	3
UF (5 kDa) @ pH=3.5	43.4	5.4	8.1	13.6	33	81	40	69

I: Clean Water
W: Wastewater
F: Clean water before cleaning
C: Clean water after cleaning
C.P. : Concentration polarization [(F-W)/F]
T.F. : Total fouling [(I-F)/I]
R.F. : Reversible fouling [(C-F)/C]
I.F. : Irreversible fouling [(I-C)/I]
* cannot be calculated since the value of C is greater than the value I.

6.4.3. Alternative Silk Degumming Technique for Sericin Recovery

The conventional degumming process consists of boiling silk fibers in a hot water bath containing soap and sodium carbonate, where the silk fiber loses 25-27% of its original weight, corresponding to the amount of sericin discarded in the wastewater. A relatively new technique, in which water vapor is applied in boilers at a temperature of 120-130 ºC and a pressure of 300-400 kPa (Fabiani et al., 1996) eliminates the use of soap, causing less adverse environmental impacts. Sericin had been recovered from the alternative silk degumming wastewater by UF (Fabiani et al., 1996). In this study, the alternative degumming technique was simulated in order to determine the soluble fraction of sericin. Some silk yarns were autoclaved at 135 ^{o}C for 3 h. As a result, it was found that total soluble fraction of sericin was about 24%, which was consistent with the fraction obtained by conventional degumming process, i.e., 25-27%. Quantitative analysis and MW distribution of sericin in the simulated silk degumming wastewater were done (Table 6.31 and Figure 6.45).

Table 6.31. Sericin concentration and MW of simulated SDW

Sample	Sericin Concentration (mg/L) *(Percent of Silk Yarn, %)*		MW (kDa)
	Expected	**Determined**	
SDW degummed with alternative technique	2561 *(25-27)*	3323 *(24)*	102

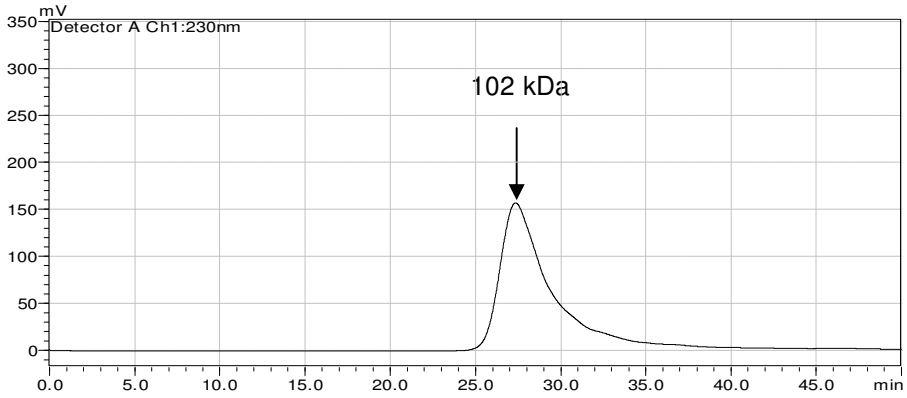


Figure 6.45. MW chromatogram of simulated SDW

As seen from Table 6.31, there is a little difference between expected and determined sericin concentrations. Moreover, MW of simulated SDW was determined as 102 kDa like that of conventional SDW. In conclusion, this relatively new silk degumming technique can be applied for elimination of the use of soap, which would definitely help recovery of sericin by a simpler and more economical method. The pre-treatment method proposed in this study would be partially or completely omitted and UF would achieve sericin recovery. The application of alternative degumming technique would also minimize the environmental pollution caused by the presence of soap in addition to sericin.

CHAPTER 7

CONCLUSIONS

In this study, cocoon cooking wastewaters and silk degumming wastewaters of silk processing industry were treated by membrane processes for sericin recovery. The most suitable process trains were developed for sericin recovery from these wastewaters. The conclusions drawn from the experimental studies are listed as follows:

1. The characterization study of cocoon cooking wastewaters (CW) and silk degumming wastewaters (SDW) revealed that there were four sericin fractions in CW, namely Sericin-1 (175-200 kDa), Sericin-2 (70-90 kDa), Sericin-3 (30-40 kDa) and Sericin-4 (10-25 kDa). These fractions were present in CW at 5-25%, 53-69%, 4-8% and 12-22%, with Sericin-2 as the most abundant fraction. The wide MW distribution of sericin in CW was due to the decomposition of sericin into smaller polypeptides during cocoon cooking process. On the other hand, there was only one sericin in SDW with a MW of 110-120 kDa, namely Sericin-SDW. The sericin in CW and SDW was classified as high MW sericin (≥20 kDa), which is suitable for use in making bio-based materials.

2. The presence of a foreign substance except sericin was observed in CW. By the help of MALDI-TOF analysis, it was identified as a protein originating from silkworm. It was concluded that separation of sericin and the silkworm protein was required for the recovery of sericin from CW.

3. The most suitable pre-treatment process for CW was found out to be centrifugation (CFG) followed by microfiltration (MF) (1 μm) among the alternatives of single and sequential applications of gravity settling (GS), MF

and CFG. The best permeate quality was obtained in CFG + MF (1 μm). In addition, the removal efficiencies achieved for pollution parameters were highest in this process alternative. Adopting MF as the second-stage pre-treatment method improved the flux decline of post membrane, where flux decline of UF (20 kDa) membrane decreased from 88% to 80%. Flux recovery of post UF achieved by chemical cleaning was also increased from 83% to 104% by adopting MF (1 μm). Therefore, it was concluded that the most suitable pre-treatment method for CW was CFG + MF (1 μm).

4. UF (1 kDa, 5 kDa and 20 kDa), and NF (NF-DK and NF-90) membranes were tested for the determination of the most appropriate membrane filtration process for sericin recovery. Sericin rejections in UF were as low as 36-60% and COD removals were 41-52%. UF membranes rejected only Sericin-1 completely, and the other fractions were poorly retained even with the tightest UF membrane having MWCO of 1 kDa. Furthermore, flux declines were as severe as 58-88% due to the formation of a heavy cake layer on the membrane surface. On the other hand, sericin rejection efficiencies of NF membranes were as high as 94-95%. Moreover, COD, total solids, color and turbidity were removed by 90-99%, 90-98%, 97-100% and 93-98% by NF-DK and NF-90 membranes, respectively. NF-90 permeate quality was suitable for discharge without additional treatment. The flux declines in NF were also as high as 70-75%. The effect of concentration polarization (CP) on flux decline of UF varied between 30% and 69%, whereas it was 63-64% for NF. The effect of fouling was also high for UF membranes, i.e., 33-75%. On the other hand, effect of fouling on NF was only 20-30%. Hence, it was concluded that NF performed better than UF for sericin recovery from CW in terms of both rejection efficiency and flux decline. UF was found insufficient as it provided partial rejection of sericin fractions. It was also concluded that UF may be appropriate for fractionation of sericin into different MW components.

5. Despite high flux declines, the original clean water fluxes were successfully restored by chemical cleaning with NaOH and free chlorine. The clean water flux recovery was 83-105% for UF and 95-127% for NF.

6. None of the membranes could separate the silkworm protein from sericin completely. Moreover, the ratio of silkworm protein to sericin was higher in NF-90 retentate than that of NF-DK, which means accumulation of silkworm protein in the feed side of NF-90 at higher extent. This was not desired considering the purity of recovered sericin. However, sericin in CW was concentrated using both NF membranes to compare their performances under worsening feed conditions. A volume reduction factor (VRF) of 4.2-4.6 was achieved with severe flux declines of 62-78% for NF-DK and 95% for NF-90. Furthermore, the original clean water flux of NF-DK was restored by 81-118% whereas that of NF-90 remained at 75%. It was concluded that NF-DK membrane was more suitable for sericin recovery from CW as NF-90 membrane was much more adversely affected by the wastewater chemistry and presence of protein.

7. In precipitating the concentrated sericin, a suitable agent was sought among the alternatives of four acids (HNO_3, HCl, H_2SO_4, $C_2H_4O_2$) and alcohol (C_2H_6O). The quality of recovered sericin samples were evaluated by elemental analysis, solubility analysis at varying pH values and UV-scan comparison. The elemental analysis showed that the most representative elemental composition could be obtained when HCl and C_2H_6O were used. However, the solubilities of samples precipitated with HCl were as low as 53-66% at acidic pH, and solubility increased as pH increased to 11. On the other hand, sericin precipitated with ethanol was completely soluble in water at all pH values changing from 3 to 11. Hence, it was concluded that ethanol was the most suitable precipitation agent for sericin.

8. The HPLC analysis of sericin solutions prepared with recovered samples revealed that sericin fraction in recovered powder was 39-46% and the rest was silkworm protein. Therefore, it was concluded that the developed process train of CFG + MF (1 µm) + NF + precipitation with ethanol achieved the recovery of a sericin/silkworm protein mixture with sericin MW of 90 kDa. This product was named low quality sericin. The sericin recovery efficiency of the developed process train was found as 76%.

9. The recovered sericin/silkworm protein was further analyzed by ion exchange chromatography, 2-D gel electrophoresis and MALDI-TOF in order to verify that the recovered powder really contains sericin. Ion exchange chromatograms revealed that recovered sericin is an acidic protein and its pI is between 5 and 6. In 2-D gel electrophoresis, all proteins in sericin sample were separated in the gel according to their pI values and their molecular weights. As a result, two protein spots having pI of 4-6.5 and 8-9 were determined. In terms of molecular weight distribution, these spots means the presence of an acidic protein with MW of 9 kDa and a group of protein with MW of 25-40 kDa. The analysis of these spots by MALDI-TOF showed that recovered powder was compatible with SER1 sericin (O96614) with MW of 9161 Da and SER2 sericin (O96615) with MW of 20302 Da defined in ExPASy protein database. In conclusion, it was proved that the recovered sample contained sericin.

10. In order to separate the silkworm protein from sericin, dialysis was applied. The dialyzed samples did not contain the silkworm protein, which means a pure product was obtained. Hence, an alternative process train CFG + MF (1 µm) + NF + dialysis + precipitation with ethanol was developed, which achieved the recovery of pure sericin with MW of 44 kDa and 85 kDa with the fractions of 20% and 80%, respectively. This product was named high quality sericin.

11. The most efficient pre-treatment process for SDW was found out to be CFG at pH 3.5 for 10 min followed by GS settling at 4 ^{o}C for 24 h among several alternatives of CFG at alkaline, neutral and acidic pH for time intervals varying between 10 min and 40 min. The separation of sericin and soap was achieved only in CFG (pH 3.5, 10 min) followed by GS (4 ^{o}C, 24 h). Hence, this process was selected as the most suitable pre-treatment method for SDW. Indeed, soap can be separated from sericin by skimming it off from the surface when the acidified wastewater is kept at room temperature instead of 4 ^{o}C, and this seems to be a more convenient method at industrial scale application. However, the latter was applied in this study at laboratory conditions. Hence, it was concluded that two alternatives were possible for

the pre-treatment of SDW; CFG (pH 3.5, 10 min) + GS (4 ^{o}C, 24 h), and CFG (pH 3.5, 10 min) + skimming soap off at room temperature.

12. Three alternatives were tested for sericin recovery from SDW using UF; 1. CFG at pH 7.7 (10 min) + UF (20 kDa) at pH 7.7, 2. CFG at pH 7.7 (10 min) + UF (20 kDa) at pH 3.5, 3. CFG at pH 3.5 (10 min) + GS (4 ^{o}C, 24 h) + UF (5 kDa) at pH 3.5. It was observed that sericin and soap could not be separated in the membrane filtration stage in the first two alternatives. In the third alternative, sericin and soap were separated in the pre-treatment stage and therefore, the role of UF was to concentrate sericin rather than separate it from soap. The rejections of pollution parameters in UF (20 kDa) were better than that in UF (5 kDa). Moreover, it was seen that UF (5 kDa) membrane rejected only 59% of sericin whereas UF (20 kDa) membrane achieved almost complete rejection. The flux declines were severe in all alternatives, that is, 85% in UF (20 kDa) at pH 7.7, 94% in UF (20 kDa) at pH 3.5, and 88% in UF (5 kDa) at pH 3.5. Although chemical cleaning provided almost complete flux recovery in UF (20 kDa), that is, 89-97%, the clean water flux could not be recovered in UF (5 kDa), which remained at 31%. The low recovery of clean water flux in UF (5 kDa) was attributed to the very high concentration of sericin in the feed. In the absence of soap, sericin probably adhered to membrane surface, causing irreversible fouling. Therefore, it was concluded that the most suitable method for sericin recovery from SDW was CFG (pH 3.5, 10 min) + GS (4 ^{o}C, 24 h) + membrane filtration. Further research is required to determine the most appropriate membrane for concentration of sericin in the membrane stage.

13. The complete separation of soap was verified by GC-MS analysis of fatty acids in UF (5 kDa) feed and permeates. No fatty acid peaks were detected in feed and permeate samples, and hence, it was concluded that the developed method successfully separated all the soap from sericin in SDW.

14. The presence of soap in SDW makes sericin recovery a difficult task. Moreover, it causes severe environmental pollution. In an attempt to propose an environmentally friendly degumming technique, an alternative method

(Fabiani et al., 1996) was simulated where sericin was removed at elevated temperature and 300-400 kPa pressure without using soap and soda. It was found that total soluble fraction of sericin obtained with alternative degumming technique was about 24%, which was consistent with the fraction obtained by conventional degumming process, i.e., 25-27%. In conclusion, this new silk degumming technique is suggested for the elimination of soap consumption, which would definitely help sericin recovery by a simpler and more economical method.

15. Sericin recovery with membranes is one of the most problematic applications of membrane technology due to severe flux declines. Although chemical cleaning was effective in restoring the fluxes, frequent cleaning cycles would shorten membrane life and increase operational costs. Hence, development of novel membranes with minimized fouling properties would be a milestone for spreading membrane technology for these types of protein separation applications.

CHAPTER 8

FUTURE WORK

Taking into consideration all the discussions and conclusions of this study, the following future work is suggested:

1. In an attempt to determine the most suitable membrane for sericin recovery from SDW, UF (5 kDa) should be repeated at the original pH of SDW to investigate the effect of pH on separation performance. Alternatively, UF (20 kDa) membrane should be used after the selected pre-treatment stage of CFG at pH 3.5 (10 min) + GS (4 ^{o}C, 24 h) since this membrane achieved almost complete rejection of sericin.

2. Sericin recovered from SDW should be characterized in terms of moisture content, ash content, elemental composition and protein identification. In order to achieve this, the most suitable pre-treatment should be applied. Then, sericin should be concentrated using the most suitable membrane. Finally, recovered sericin in powder form should be obtained by ethanol precipitation followed by freeze-drying.

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APPENDIX A

EFFECT OF PRESERVING CONDITIONS OF CW ON MEMBRANE PERFORMANCE

The cocoon cooking wastewaters cannot be supplied at any time from the silk processing plants since silk yarn production is a seasonal activity performed in autumn. Therefore, the wastewaters collected from the plant in Bilecik were divided into small volumes and frozen at -20 ^{o}C to keep them in laboratory conditions, and these were defrosted when needed. Since it was noticed that total protein, color and turbidity values of frozen-defrosted CW were rather low when compared with original CW; before UF (5 kDa) experiments, frozen-defrosted CW was heated to increase the protein content in it to the original level (Table A.1). It was considered that sericin in frozen wastewater could be crystallized. Since sericin is a protein having both cold water- and hot water-soluble fractions, it was decided to apply heating after defrosting the samples. Heating process adversely affected color and turbidity. These parameters highly increased, that is, color increased from 3180 Pt-Co to 25000 Pt-Co whereas turbidity increased from 122.3 NTU to 1050 NTU. On the other hand, no significant effect was observed for other parameters. However, heating was necessary as evidenced from the high difference between total protein and COD in frozen-defrosted sample. The amount of total protein was measured correctly in the heated sample, which was very close to the original value.

Table A.1. Effect of freezing-defrosting-heating processes on the wastewater characteristics

Parameter	Wastewater Characteristics		
	Original CW	**Frozen-defrosted**	**Heated for 60 min after defrosting**
Total Protein (mg/L)	9443	6208	9883
COD (mg/L)	11600	13220	14920
Total Solids (mg/L)	10238	11700	13130
Color (Pt-Co)	8650	3180	25000
Turbidity (NTU)	771	122.3	1050

In order to further investigate the effect of freezing-defrosting-heating processes on wastewater characteristics and membrane performance, cocoon cooking process was simulated in laboratory conditions. For this, 1 kg of cocoon was boiled in 15 L of water for 45 min. Then, the simulated wastewater (CW-S) was cooled and divided into equal volumes. In Table A.2, the characteristics of simulated wastewaters are given. First sample was centrifuged and then, filtered through 1 μm. After the pre-treatment process, UF (5 kDa) was applied. Second sample, however, was frozen at -20 ^{o}C and after a few days, it was defrosted and heated. The pre-treatment processes were applied to this sample and then, it was filtered through UF (5 kDa). Finally, performances of these two UF membranes were compared.

Table A.2. Characteristics of simulated wastewater

Sample	Simulated Wastewater Quality	
	CW-S	**CW-S (FDH)***
Total Protein (mg/L)	6159	5993
COD (mg/L)	7925	9030
Total Solids (mg/L)	7360	8050
Color (Pt-Co)	5690	3970
Turbidity (NTU)	301	234
pH	7.1	6.7

*FDH: Frozen-Defrosted-Heated

UF (5 kDa) was applied with simulated wastewater before and after FDH processes and the results are given in Table A.3. According to this table, COD, total solids and total protein removals were 50-55% before FDH while these removals were 46-48% after FDH. As seen, these removals are close to each other. Similarly, there is no difference in sericin removal efficiency, which was 33% before FDH whereas it was 27% after FDH. However, this difference may not be resulted from FDH process. Also, when pre-treatment and UF (5 kDa) processes were evaluated together, obtained results were rather close. The quality of permeates obtained by UF (5 kDa) were also close to each other. Consequently, it was decided that there was no drawback of applying FDH processes. Therefore, the experimental studies were carried out with frozen-defrosted-heated CW samples.

Table A.3. Effect of FDH processes on the performance of UF (5 kDa)

Wastewater	Applied Process	Permeate Quality *(Removal, %)*						
		Sericin (mg/L)	**T. Protein (mg/L)**	**COD (mg/L)**	**T. Solids (mg/L)**	**Color (Pt-Co)**	**Turbidity (NTU)**	**pH**
CW-S (before FDH)	1. CFG	-	5531	7655	7190	5380	276	6.5
		(-)	*(10)*	*(3)*	*(2)*	*(5)*	*(8)*	
	2. MF (1 μm)	4872	5285	7320	7110	4480	192	6.6
		(-)	*(4)*	*(4)*	*(1)*	*(17)*	*(31)*	
	3. UF (5 kDa)	3255	2379	3490	3525	1170	17	6.6
		(33)	*(55)*	*(52)*	*(50)*	*(74)*	*(91)*	
	CFG+MF+UF	***(-)***	***(61)***	***(56)***	***(52)***	***(79)***	***(94)***	
CW-S (after FDH)	1. CFG	-	4747	7005	7290	2370	108	6.6
		(-)	*(21)*	*(22)*	*(9)*	*(40)*	*(54)*	
	2. MF (1 μm)	5083	5086	6875	7120	2010	65	6.6
		(-)	*(0)*	*(2)*	*(2)*	*(15)*	*(40)*	
	3. UF (5 kDa)	3722	2737	3565	3700	850	16	6.5
		(27)	*(46)*	*(48)*	*(48)*	*(58)*	*(75)*	
	CFG+MF+UF	***(-)***	***(54)***	***(61)***	***(54)***	***(79)***	***(93)***	

APPENDIX B

EFFECT OF CHEMICAL CLEANING ON MEMBRANE PERFORMANCE

NaOH and free chlorine, which were applied in cleaning process to clean the fouled membrane, are chemicals suggested by a filter manufacturer firm for UF membranes (Wu et al., 2006). It has been reported that chlorine has harmful effects for UF membranes in acidic conditions but it has no harmful effects when applied with NaOH in basic conditions (Liu et.al, 2004). Since chlorine is a very strong oxidant, it was suspected whether it had adverse effects such as wear on membrane surface and pore opening. In order to investigate this effect, UF membranes fouled with wastewater were cleaned with NaOH and chlorine for one time, then, wastewater was filtrated through these membranes for the second time and then, these membranes were cleaned in the same way. The removal performances and the permeate qualities are given in Table B.1 and the effect of the second cleaning process on the flux is shown in Table B.2.

It was observed that there was no considerable change in permeate quality and removal performance at the end of the second cleaning (Table B.1). Flux recovery obtained after second cleaning, i.e., 94%, was as high as that obtained after first cleaning, i.e., 90% (Table B.2). Therefore, it was concluded that the applied cleaning process was appropriate.

Table B.1. Effect of chemical cleaning process on UF performance

Parameter	Permeate Quality *(Removal, %)*			
	CFG + MF (1 µm) + UF (20 kDa)		CFG + MF (1 µm) + UF (5 kDa)	
	Before Cleaning	After Cleaning	Before Cleaning	After Cleaning
Sericin (mg/L)	2080 *(51)*	-	3292 *(52)*	3989 *(49)*
T.Protein (mg/L)	1624 *(77)*	1756 *(62)*	3108 *(68)*	2731 *(68)*
COD (mg/L)	5205 *(29)*	5975 *(28)*	6210 *(41)*	6120 *(44)*
T.Solids (mg/L)	6500 *(14)*	7175 *(28)*	5975 *(44)*	6625 *(36)*
Color (Pt-Co)	570 *(53)*	690 *(55)*	790 *(64)*	800 *(71)*
Turbidity (NTU)	7.2 *(36)*	6.1 *(40)*	9.9 *(43)*	8.0 *(67)*

Table B.2. Effect of chemical cleaning on membrane flux

Process	Flux ($L/m^2/h$)				Flux Decline (%)				Flux Recovery (%)
	I	W	F	C	C.P.	T.F.	R.F.	I.F.	
CFG+ MF(1 µm)+ UF(20 kDa)	54.3	10.9	24.4	48.9	56	55	50	10	90
CFG+ MF(1 µm)+ UF(5 kDa)	46.2	6.1	9.5	43.4	36	79	78	6	94

I: Clean Water
W: Wastewater
F: Clean water before cleaning
C: Clean water after cleaning
C.P. : Concentration polarization [(F-W)/F]
T.F. : Total fouling [(I-F)/I]
R.F. : Reversible fouling [(C-F)/C]
I.F. : Irreversible fouling [(I-C)/I]

APPENDIX C

EFFECT OF AUTOCLAVE TIME ON THE SOLUBILITY OF SERICIN

The effect of autoclave time on the solubility of sericin was investigated to apply the correct autoclave time. For this, three cocoon samples, each 1 g, were prepared. They were dried and their weights were determined. These cocoons were put into water and kept in the autoclave at 120 °C for 1, 2 and 5 h, respectively. As seen from Table C.1, the amount of sericin dissolved in water increased by 4.5% and 7.7% by increasing the autoclave time from 1 h to 2 h and further to 5 h. The amount of increase was found to be insignificant, and hence, 1 h was chosen as the autoclave time.

Table C.1. Effect of autoclave period on the sericin solubility in water and on the yield

Autoclave Time (h)	Solubility in Water (%)	Percent Increase (%)
1	22.2	-
2	23.2	4.5
5	23.9	7.7

APPENDIX D

CALIBRATION CURVE USED FOR PROTEIN ANALYSIS

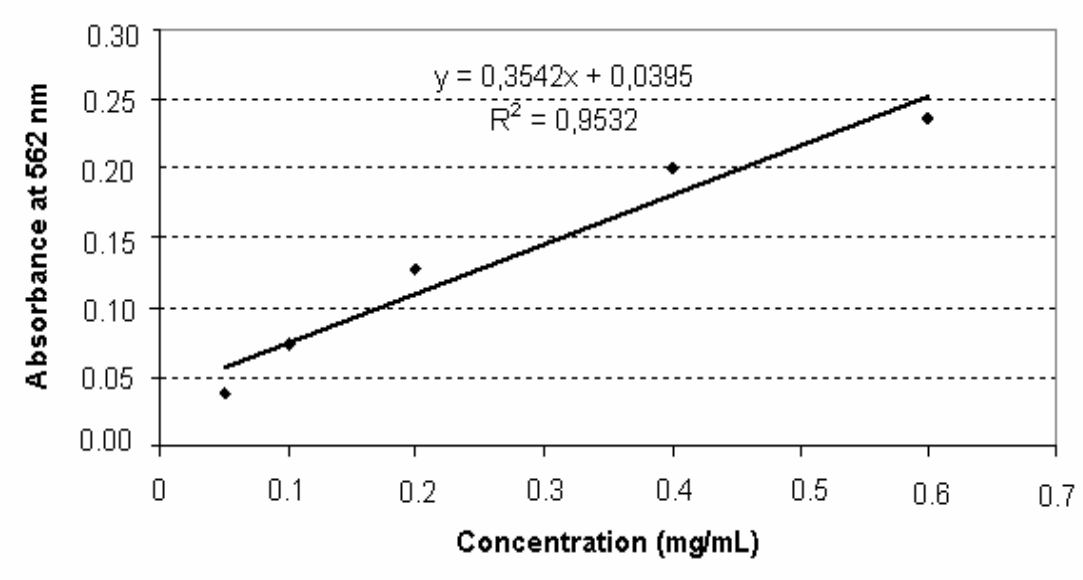


Figure D.1. Calibration curve used for protein analysis

APPENDIX E

CALIBRATION CURVE USED FOR CARBOHYDRATE ANALYSIS

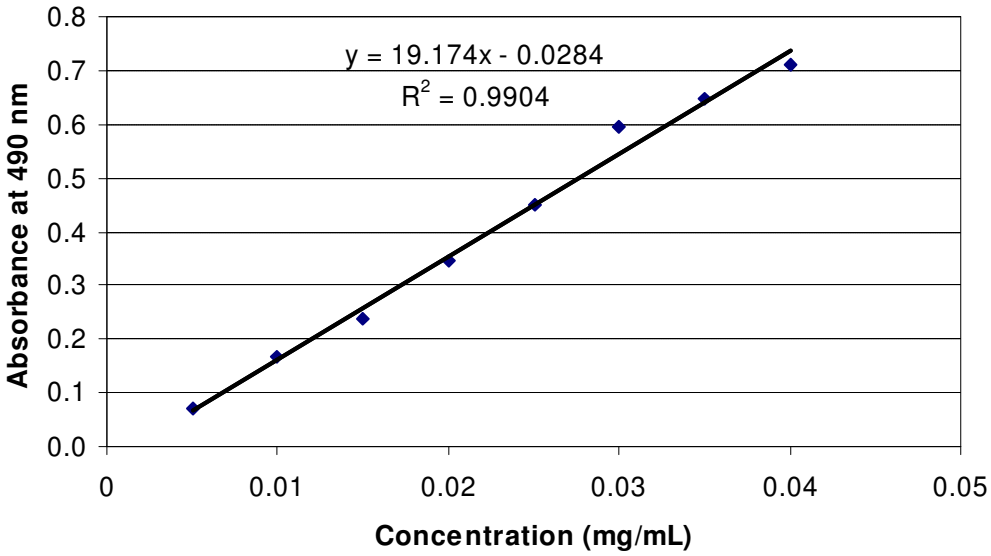
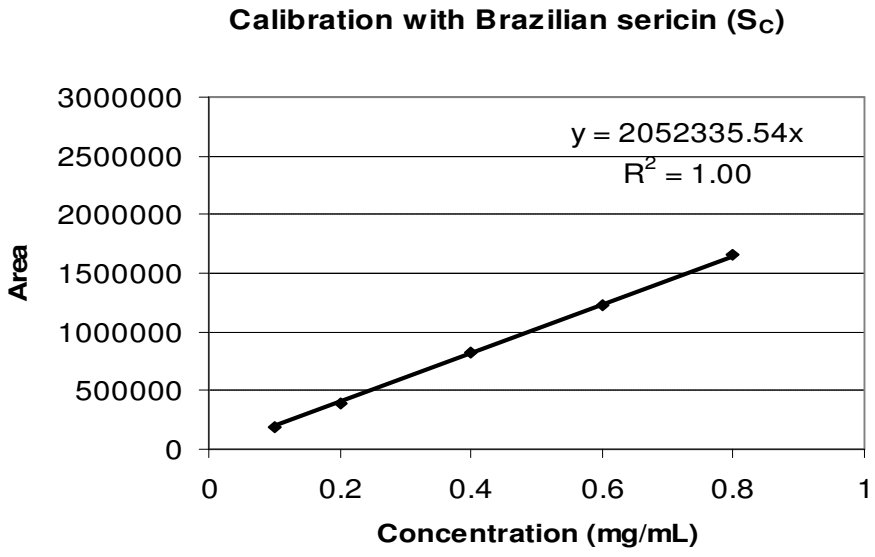


Figure E.1. Calibration curve used for carbohydrate analysis

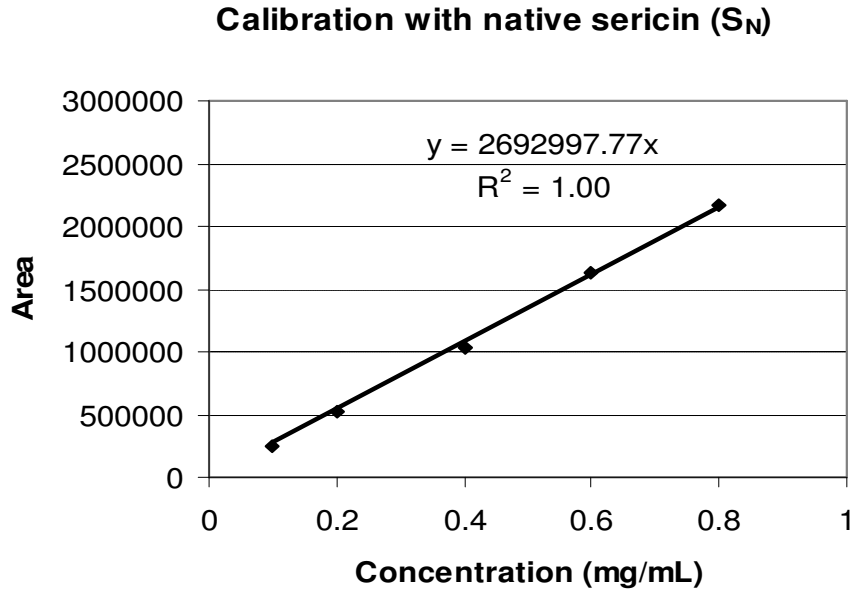
APPENDIX F

SELECTION OF CALIBRATION STANDARD FOR PROTEIN AND SERICIN ANALYSES

It was noticed that in the solutions prepared from Brazilian sericin (S_C) to be used as calibration standard, powder sericin could not be dissolved completely and a precipitate was formed. Also, in the calibration made by using HPLC, smaller areas were read with the sample S_C; in the calibration curve obtained as $y = ax$, the a value obtained with S_C was 76% of the value obtained with S_N. As seen in Figure F.1, the ratio of the area obtained with S_C to the area obtained with S_N is equal to 0.76. This means sericin concentration would be overestimated with the calibration curve prepared with S_C. It is known that sericin solubility is minimum at pH 3.8 and protein is precipitated at this pH (Kodama, 1926). Since the pH of S_C was 3.9, it may not be dissolved completely in water. As a result, in this study, it was decided that native sericin (S_N) would be used as the calibration standard.



(a)



(b)

Figure F.1. Calibration curves obtained with **(a)** S_C **(b)** S_N

APPENDIX G

ANALYSIS OF COMPONENTS OF COCOON COOKING WASTEWATERS

To describe the foreign substance in CW, the components of CW were analyzed separately. Three distinct solutions were prepared at laboratory conditions. The first solution was obtained by autoclaving 3.99 g of dried cocoon shell in 100 mL ultrapure water at 120 ^{o}C for 1 h followed by filtering through a filter media having a pore size of 1.6 μm (Whatman GF/A). For the second solution, the same procedure was applied but 3.73 g of silkworm was used instead of cocoon shell. For the third solution, a total of 8.04 g of cocoon and silkworm were autoclaved together. The first solution represents the sericin standards used in the experiments whereas the third one represents the cocoon cooking wastewater. The second solution was prepared to understand the difference between the structures of standard sericin and sericin in wastewater. COD, total protein and sericin analyses were done for these three samples, and ratios between them were calculated (Table G.1).

For the first solution composed of only cocoon, COD/T.Protein and COD/Sericin ratios were found as 1.0 and 1.1. These ratios were maximum for the second solution formed by only silkworm, that is, 3.6 and 2.5. The higher ratio in the silkworm solution is an indication of the organic nature of the foreign substance. Therefore, it can be said that this substance may be carbohydrate, fat or another protein originating from the dead body of silkworm which exists inside the cocoon. For the third solution, the ratios were 1.6 and 1.5 whereas they were 1.8 and 2.4 for the original wastewater. COD/T.Protein ratio in original wastewater was closer to the ratio in the third solution, as expected. Nevertheless, COD/Sericin ratio in original wastewater was not very close to that in the third solution. The reason for this may be the freezing-defrosting-heating processes applied to the wastewater before use. It was thought that 45 min-heating period might be insufficient for dissolving the frozen sericin completely and the sericin concentration after heating might be measured lower than that in raw wastewater. So, the COD/Sericin ratio became

higher than expected. The analysis of these solutions revealed that the source of sericin in wastewater was not only cocoon shell but also the silkworm itself. The ratio of sericin concentrations in second and third solutions show that about 28% of sericin in cocoon cooking wastewater comes from the dead body of silkworm, whereas 72% come from the cocoon shell. The foreign substance was later found to be a protein originating from silkworm.

Table G.1. Analysis of components of cocoon wastewater (CW)

Sample	Sericin (mg/L)	T. Protein (mg/L)	COD (mg/L)	COD/T. Protein	COD/Sericin
Cocoon (Solution 1)	9609	10733	10180	1.0	1.1
Silkworm (Solution 2)	3791	2632	9440	3.6	2.5
Cocoon + Silkworm (Solution 3)	13705	12616	20570	1.6	1.5
Original CW*	6067	7979	14615	1.8	2.4

* Average values belonging to CW2-A, CW2-B, CW2-C, CW2-D, CW2-E, CW2-F, CW2-G, CW2-H samples were given.

APPENDIX H

REPRODUCIBILITY EXPERIMENTS

To test the reproducibility of membrane processes, two UF (5 kDa) membranes were used. The removal performances and the permeate qualities are given in Table H.1. Sericin and total protein were removed at 36-39% and 65-72% whereas COD, total solids, color and turbidity were removed at 48-49%, 46-48%, 76-77% and 80-82%, respectively. Moreover, flux declines were 86% and 83%, respectively. It was observed that there was no considerable change in permeate qualities and removal performances in both experiments, SET-1 and SET-2. Therefore, it was concluded that membrane processes could be reproduced.

Table H.1. Reproducibility performance of UF (5 kDa) membrane

Parameter	**Permeate Quality *(Removal, %)***	
	SET-1	**SET-2**
Sericin (mg/L)	4552 *(36)*	4401 *(39)*
T.Protein (mg/L)	3201 *(65)*	2439 *(72)*
COD (mg/L)	6210 *(48)*	5905 *(49)*
T.Solids (mg/L)	6200 *(46)*	6000 *(48)*
Color (Pt-Co)	700 *(76)*	680 *(77)*
Turbidity (NTU)	8.6 *(82)*	9.6 *(80)*
Flux Decline (%)	86	83

APPENDIX I

THE RELATIONSHIPS BETWEEN SERICIN, SOAP AND COD

It was determined how much COD was caused by 1 g of sericin using S_N. Three sericin solutions of known concentrations were prepared and then, their COD contents were measured (Table I.1). As a result, it was found that 1 g of sericin was identical to 1 g of COD.

Table I.1. Relationship between sericin and COD

Sericin Concentration (mg/L)	**Corresponding COD (mg/L)**	**COD/Sericin**
180	182	1
360	307	0.9
1200	1361	1.1

The same experiment was done for soap. Four soap solutions with known concentrations were prepared and then, their COD contents were measured (Table I.2). As seen, 1 g of soap was identical to 2.5 g of COD.

Table I.2. Relationship between soap and COD

Soap Concentration (mg/L)	Corresponding COD (mg/L)	COD/Soap
200	499	2.5
400	978	2.4
600	1473	2.5
1000	2460	2.5

Equal amounts of sericin and soap were mixed and their COD contents were also determined to see their synergistic effect (Table I.3). As seen, when they came together, their corresponding COD increased with respect to the COD caused by only sericin and decreased with respect to COD caused by only soap. Sericin and soap (1/1 w/w ratio) were identical to 1.9 g of COD. As seen, measured COD values were a bit higher than the expected COD.

Table I.3. Relationship between sericin, soap and COD

Sericin (mg/L)	Soap (mg/L)	Sericin + Soap (mg/L)	Measured COD (mg/L)	Expected COD (mg/L)
200	200	400	867	700
500	500	1000	2137	1750
800	800	1600	2905	2800