Degumming of Persian Silk with Mixed Proteolytic Enzymes

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ABSTRACT: The feasibility of degumming Persian silk with alcalase, savinase, and mixtures of these enzymes with different alcalase/savinase weight ratios (0/1, 0.25/0.75, 0.5/0.5, 0.75/0.25, and 1/0 g/L) was investigated. The results were compared with those of soap degumming, which is a common silk degumming process. The effectiveness of parameters such as the treatment time, concentration of enzymes, and liquid ratio on degumming was studied. The enzymatic degumming process was performed at 55°C with an operation time of approximately 30 min, whereas the soap degumming process was carried out around the boiling point in 120 min. The evaluation of the data was carried out through the measurement of the weight loss, strength, and elongation of the samples. The optimum amount of sericin removed was 21.52 wt % for alcalase in 30 min, 20.08 wt % for savinase in 60 min, and 22.58 wt % for soap in 120 min. Also, the enzymatic treatment improved properties of the silk yarn such as the strength (33.76 cN/tex for alcalase and 32.03 cN/tex for savinase) and elongation (20.08% for alcalase and 18.42% for savinase). The obtained values were better than the strength (29.90 cN/tex) and elongation (18.59%) from the soap degumming method. Through the use of an enzyme mixture (0.5/0.5 g/L), good weight loss (22.43%), strength (33.22 cN/tex), and elongation (17.74%) were achieved in 30 min. Scanning electron micrographs confirmed and supported the observed data. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 106: 267–275, 2007

Key words: biodegradable; biofibers; biopolymers; enzymes

INTRODUCTION

Persia has been known by the Silk Road, and its civilization is closely attached to magnificent silk carpets with amazing designs and colors.[1] Silk has been used as a textile fiber for over 5000 years. Its many highly desirable physical characteristics, such as good mechanical properties, brightness, drape ability, comfort, softness, and dye ability, and the convenience of reeling long (300–1200 m) continuous fibers from cocoons have certainly contributed to its success as a specific fiber.[2,3]

Natural raw silk is composed mainly of sericin (22–25%), fibroin (62.5–67%), water, and mineral salts. The two proteins differ considerably in their percentages of amino acids and configurations. Fibroin is a single protein that is insoluble in hot water, and roughly 76% of its amino acids have nonpolar side chains, the main ones among these being glycine (43.7%) and alanine (28.8%); only about 21% have polar groups. Sericin is a mixture of proteins and contains a large number of amino acids with hydroxyl groups. It contains about 25% nonpolar groups and about 75% polar side chains made mainly of serine (33.43%), aspartic acid (16.71%), glycine (13.49%), and threonine (9.74%).[4] Because of these differences in the compositions and configurations, fibroin forms a high volume fraction of β-sheet microcrystals, which act as reinforcements and contribute to the strength and stiffness of silk. Sericin is primarily amorphous and acts as a gum binder to maintain the structural integrity of the cocoon, so sericin is more water-soluble than fibroin.[2,4–6] This difference makes the gum easily removable from the filaments through various processes without considerable damage to the filaments.[7,8]

Sericin gives a harsh and stiff feeling to the fiber and hides the rich luster and whiteness of silk. Also, it prevents the penetration of dye liquor and other solutions during wet processing, so silk degumming is an essential process to obtain an ideal fiber for the textile industry.[9] During the degumming process, sericin is hydrolyzed, and the amide bonds of the long protein molecules are broken into smaller fractions, which are dispersed and solubilized in degumming agents and media.[4,9] Silk degumming causes a 20–25% weight loss, which depends on the source and sort of silk. This weight loss recently has been compensated with synthetic materials through coating techniques.[7,10,11]

TABLE I
Degumming Process Conditions

Material		Concentration of the main ingredient (g/L)					Time (min)	LR
Marseille soap		5					30, 60, 90, 120	30 : 1
Alcalase		0.25, 0.50, 0.75, 1.00, 2.00					60	30 : 1
Savinase		0.25, 0.50, 0.75, 1.00, 2.00					60	30 : 1
Alcalase		1					15, 30, 60, 90, 120	30 : 1
Savinase		1					15, 30, 60, 90, 120	30 : 1
Alcalase		1					30	20 : 1, 30 : 1, 40 : 1, 50 : 1
Savinase		1					60	20 : 1, 30 : 1, 40 : 1, 50 : 1
Enzyme mixture	Alcalase	0.00	0.25	0.50	0.75	1.00	30, 60	30 : 1
	Savinase	1.00	0.75	0.50	0.25	0.00		

The degumming processes are grouped into five main groups: extraction with water under pressure at 115°C, boiling off in soap or with alkalis, enzymatic degumming, and degumming in boiling acidic solutions.[4] The recommended standard method of degumming is based on Marseilles soap, which is prepared from olive oil. Marseilles soap is very expensive and has to be imported; therefore, degumming is generally carried out with nonstandard native and home-made soaps based on sodium stearate.[4] Soap makes sericin swell, then emulsifies it in the degumming bath, and removes its from the filaments.[7,8] The presence of soap and alkalis in the wastewater makes this method a nonecofriendly process.[12,13]

Most of the aforementioned degumming processes impose a markedly unnatural environment on the silk; therefore, one should consider the possibility that changes could occur in the fibroin structure and its mechanical properties.[2]

As far as the environment is concerned, the utilization of chemicals by most of the aforementioned methods introduces serious pollution to the receiving waters. Among these methods, only the enzymatic method has the ability to react with specific sites of the sericin, so through a controlled process one can avoid the aforementioned shortcomings.[14] Enzyme degumming involves the proteolytic degradation of sericin, using the specific proteins with minimum effect on fibroin. They are selective and biodegradable, and there is no soap required in the enzymatic degumming process; therefore, uneven dyeing problems caused by metallic soap can be avoided. Silk's affinity to dyes, especially to reactive dyes, is significantly improved by the enzymatic treatment.[3] The application of enzymes in textile industries recently has been increased.[10,15,16] Enzymes are ecofriendly products, operate under mild conditions and low temperatures, and so consume less energy than other methods.[12,13,17,18] Enzymes act selectively and can attack only specific parts of the substrate to destroy the unwanted sections.[19,20]

An enzyme reacts with a substrate as a catalyst, and the substrate molecule fits into the active sites of the enzyme's molecular structure like a key fitting into a lock, forming an enzyme–substrate complex (lock–key theory). This complex then is broken and yields an end product and a reproduced original enzyme molecule. The reproduced enzyme will start a new cycle.[9]

Freddi et al.[6] applied photolytic enzymes (alkaline, neutral, and acidic proteases) to silk degumming and found that alkaline and neutral proteases effectively degummed crepe silk fabric and that the degumming kinetics depended on the enzyme dosage and treatment time. Gulrajani and coworkers[14,18,21,22] performed silk degumming with some protease and lipase enzymes. Various studies have been reported on the applications of enzymes in degumming, but the use of savinase and mixed enzymes has not been reported.[4,6,14,18,21,22]

In this research, for the first time, the degumming of Persian silk was investigated with proteolytic enzymes, namely, alcalase, savinase, and their mixtures, under different conditions. The effects of various parameters such as the time, concentration of the enzyme, and liquid ratio (LR) on the degumming process were examined, and the optimum conditions are reported.

EXPERIMENTAL

Materials

Ten-folded raw Persian silk yarn (63 ± 6.7 Tex and 240 twists per meter) was obtained from Guilan Silk Co. (Rasht, Iran). Alcalase and savinase were obtained from Novo Nordisk Co. (Bagsvaerd, Denmark). A nonionic surfactant, Irgasol NA, was provided by Ciba Co. (Tehran, Iran). All other chemicals were laboratory-grade (analytical reagents, Merck, Tehran, Iran). An Ahiba 1000 dyeing instrument (Denver, Colorado) was used for silk degumming. An Instron 5566 (Applied Science Co., Ithaca, New York) was used for measuring the mechanical prop-

TABLE II
Effect of the Time on the Weight Loss, Strength, and Elongation of Silk Yarn

| Treatment time (min)	Alcalase					Savinase					Soap				
	Weight loss (%)	Strength (cN/tex)	Standard deviation	Elongation (%)	Degumming efficiency (%)	Weight loss (%)	Strength (cN/tex)	Standard deviation	Elongation (%)	Degumming efficiency (%)	Weight loss (%)	Strength (cN/tex)	Standard deviation	Elongation (%)	Degumming efficiency (%)
0	0.00	37.27	0.9165	15.86	0.00	0.00	37.27	0.9165	15.86	0.00	0.00	37.27	0.9165	15.86	0.00
15	18.61	35.61	0.5736	16.94	82.37	16.61	34.22	0.8885	16.94	73.56	—	—	—	—	—
30	21.52	33.76	0.7075	20.08	95.22	18.72	34.84	0.8609	16.83	82.90	13.75	29.52	0.6491	18.36	60.89
60	22.01	33.85	0.6725	20.30	97.43	21.40	32.03	0.8877	18.42	94.77	22.02	29.37	1.0234	17.83	97.52
90	22.93	33.43	0.7788	20.90	101.42	23.57	33.59	0.9764	17.50	104.38	22.23	28.90	1.7351	17.00	98.45
120	23.51	32.98	1.1478	20.76	104.07	23.63	33.95	0.7089	18.17	104.65	22.58	29.90	0.4677	18.59	100
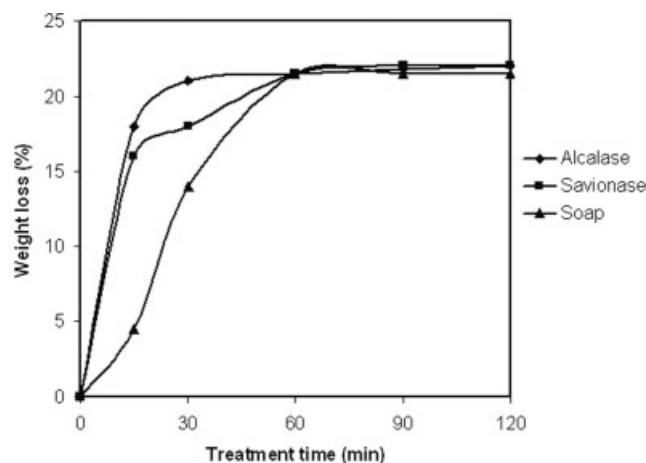
Figure 1 Effect of the treatment time on the weight loss of silk yarn.

erties of the yarn. Scanning electron microscopy (SEM) micrographs of the samples were prepared with a Leo 1455VP scanning electron microscope (Cambridge, England).

Degumming method

Silk degumming was performed in Ahiba 1000 with raw silk yarn. The details of the experiments used for degumming with soap, enzymes, and a mixture of enzymes are given in Table I. Raw silk was used without further treatment for all experiments. Each experiment under the given conditions (Table I) was performed three times. Then, 10 samples from each experiment were selected, and the average data for 30 measurements were reported. Silk yarns were treated with 5 g/L Marseille soap, 5 g/L sodium bicarbonate (pH 8–9), and 5 g/L Irgasol NA at 95°C for 30, 60, 90, and 120 min with an LR value of 30 : 1. The optimum treatment time was obtained (Table I).

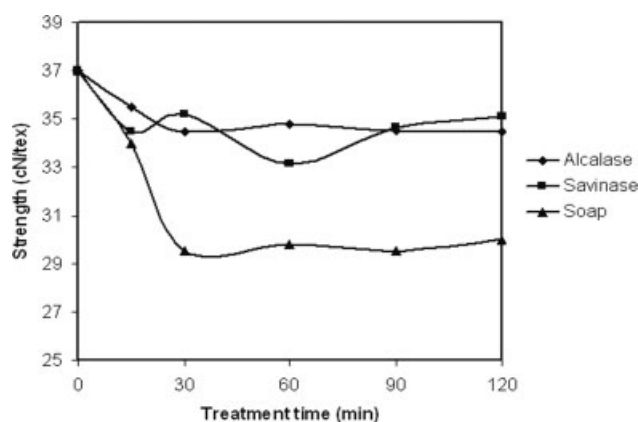For degumming with enzymes in all experiments, 5 g/L sodium bicarbonate and 5 g/L Irgasol NA as

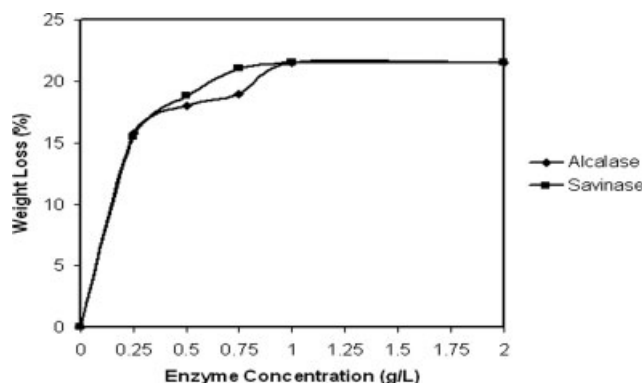**Figure 2** Effect of the treatment time on the strength of silk yarn.

Figure 3 Effect of the enzyme concentration on the weight loss of silk yarn.

a nonionic surfactant were used. The effect of the enzyme concentration on the degumming process was investigated at 55°C for 60 min (LR = 30 : 1). The effect of time on the degumming process was studied at the optimum concentration of the enzyme. Also, the effect of LR on the silk degumming efficiency was studied. The pH in all the experiments was 8–9, which was recommended by Novo Nordisk. Finally, various ratios of alcalase to savinase were used for 30 and 60 min (Table I).

Determination of the weight loss

The weight loss was determined through the measurement of the difference in the weights of the untreated and enzyme-treated samples. After the treatment, the samples were dried in an oven at 95°C to reach a constant weight. The weight loss was calculated and reported as the initial weight percentage.

Determination of the degumming efficiency

There are several methods for assessing the degumming process, such as the gravimetric method, stain-
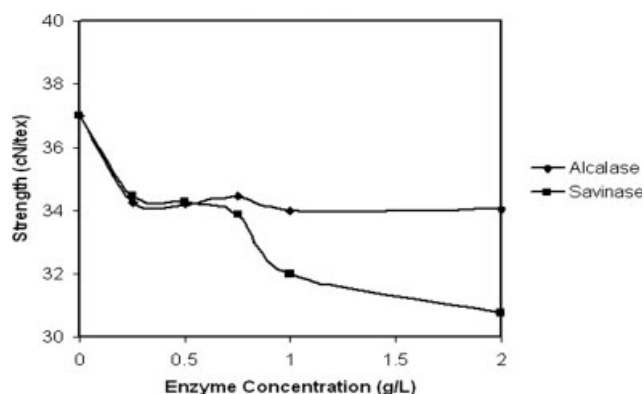

Figure 4 Effect of the enzyme concentration on the strength of silk yarn.
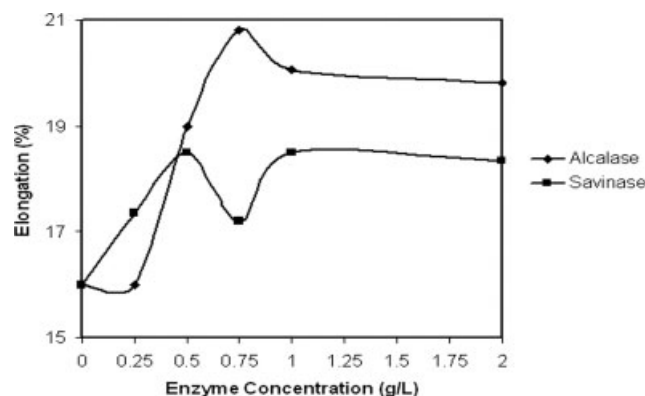
Figure 5 Effect of the enzyme concentration on the elongation of silk yarn.

ing methods with some dyes that distinguish between fibroin and sericin, determination of the viscosity of the degumming solution, and SEM. In this work, the efficiency of the degumming was calculated through a comparison of the enzyme process for silk yarn with the soap process (the standard method). Yarn samples were degummed with Marseilles soap for 120 min. The degumming with Marseilles soap was taken as the standard 100% weight loss. The efficiency of each enzyme concentration at different time intervals was computed with the following formula:

$$\text{Degumming efficiency} = \text{Wt}_E / \text{Wt}_S$$

where Wt_E is the percentage of weight loss by the enzyme treatment and Wt_S is the percentage of weight loss by the soap treatment.[17]

SEM studies

The extent and quality of degumming can be qualitatively assessed by the observation of degummed fibers under a scanning electron microscope. The filaments of samples treated under optimized conditions and untreated samples were scanned. If a degumming process is insufficient and incompatible, residual sericin should appear as deposits on the surface of the filaments, and nonuniformity over the surface of the yarn must be observed.

Physical properties

An Instron 5566 with a 10-cm gauge length and a speed of 50 mm/min was used. The strength and elongation at break were obtained. Because of the extensive number of samples, only the standard deviation (α) for the strength of the samples is reported (α = 0.95).

TABLE III
Effect of the Enzyme Concentration on the Weight Loss, Strength, and Elongation of Silk Yarn

| Enzyme concentration (g/L)	Alcalase					Savinase				
	Weight loss (%)	Strength (cN/tex)	Standard deviation	Elongation (%)	Degumming efficiency (%)	Weight loss (%)	Strength (cN/tex)	Standard deviation	Elongation (%)	Degumming efficiency (%)
0.00	0.00	37.27	0.9165	15.86	0.00	0.00	37.27	0.9165	15.86	0.00
0.25	16.52	34.22	0.7990	15.91	73.16	16.50	34.55	1.1194	17.33	73.07
0.50	18.17	34.43	1.8016	18.89	80.47	19.58	34.43	0.3903	18.25	86.71
0.75	19.46	34.50	1.3551	20.18	86.18	21.40	33.92	3.4404	17.90	94.77
1.00	21.53	33.76	0.7075	20.08	95.35	21.61	31.94	0.7144	18.42	95.70
2.00	21.60	33.50	1.3733	19.75	95.66	418.20	30.84	0.8080	18.20	96.10
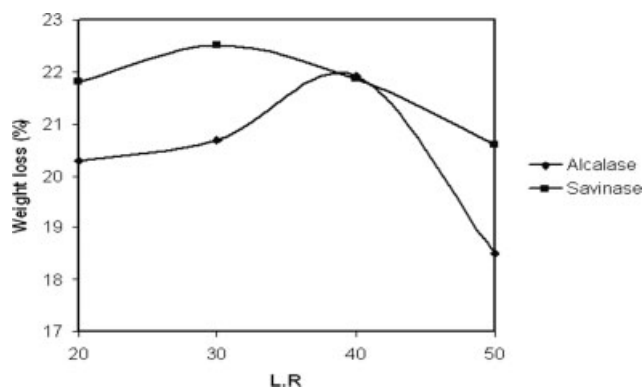
Figure 6 Effect of LR on the weight loss of silk yarn.

RESULTS AND DISCUSSION

Effects of the time and types of enzymes

Table II and Figures 1 and 2 show the effects of the treatment time on the weight loss, strength, and elongation of silk yarn. Degumming with alcalase gives good results at 30 min, but the treatments with soap and savinase require 60 min.

The initial rates of degumming with both enzymes are fast, whereas with soap, it is comparatively slow. At 30 min, the degumming efficiencies are 95.22, 82.90, and 60.89% for alcalase, savinase, and soap, respectively, whereas at 60 min, the efficiencies for all are almost equal. In all methods, after 60 min, sericin removal reaches a smooth plateau of a constant value. The data in Table II show that the strength loss in the alcalase and savinase processes is comparative, but it is more pronounced in the soap process. This can be taken to mean that degumming with alcalase and savinase independently does not significantly damage silk filaments. In the case of the soap treatment, the strength loss can be attributed to the higher temperature of the treatment media, which must be taken into consideration.

In all cases, with an increasing treatment time, the elongation increases, and it is higher in the alcalase
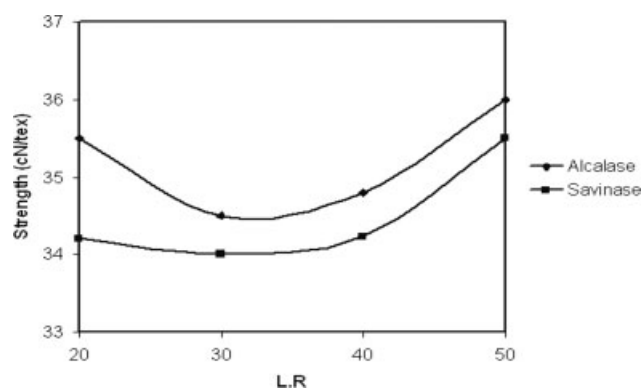

Figure 7 Effect of LR on the strength of silk yarn.

TABLE IV
Effect of LR on the Weight Loss, Strength, and Elongation of Silk Yarn

	Alcalase				Savinase			
LR	Weight loss (%)	Strength (cN/tex)	Standard deviation	Elongation (%)	Weight loss (%)	Strength (cN/tex)	Standard deviation	Elongation (%)
20 : 1	20.34	35.38	1.1330	18.58	21.78	34.25	1.0343	17.53
30 : 1	20.69	34.51	0.9843	17.58	22.67	34.00	0.9173	18.34
40 : 1	22.06	34.97	0.9813	19.17	21.96	34.24	0.9733	17.44
50 : 1	18.54	36.08	0.7778	17.00	20.40	35.52	0.7439	16.91

process than in the other two processes. It is believed that sericin is acting as a binder and sticking to media of the fibroin filament, so we can imagine that sericin is preventing the movement of filaments over one another. As the results show, increasing the treatment time decreases the amount of sericin left over the filament, thus facilitates the easy expansion of the filament, and so increases the elongation of samples.

These observations are in good agreement with those of other researchers.[14,18,22]

Effect of the enzyme concentration

Figure 3 shows that increasing the enzyme concentration causes more weight loss, but it gradually reaches a constant value. The weight loss, elongation, and strength of silk are not significantly different after treatments with 1 or 2 g/L of either enzyme (Figs. 3–5 and Table III), so increasing the enzyme concentration above 1 g/L does not appear useful. For enzyme concentrations below 1 g/L, there is a general increase in the elongation. This may be due to the removal efficiency of the enzymes from increasing the concentration for both enzymes and the mild effect of alcalase on the filament in comparison with savinase. With respect to the strength of the samples, it can be concluded that increasing the enzyme concentration results in a decline in the filament strength. This may be due to the high initial

concentration of sericin at the surface of the filament, which gradually decreases because of hydrolysis and precipitation.

In both cases, an increase in the elongation and a decrease in the strength are results of sericin removal, which acts as an adhesive for filaments. Also, the decline of the strength of the filament might be described by the interaction between the enzyme molecules and fibroin protein. This interaction could result in damage to and hydrolysis of some part of the fibroin filament.

Effect of LR

Water is an invaluable material in the textile industry, and a large volume of effluent is produced during the degumming process and causes serious environmental problems. In this respect, optimizing the LR is an important factor for the silk degumming process, and by optimizing this factor, we can save considerable amounts of energy and chemicals. To study the effect of LR, samples were treated at different LRs, and the results are shown in Figures 6 and 7 and Table IV. Referring to Figure 6 and Table IV, we find that with an increase in LR, the weight-loss efficiency smoothly increases, reaches a maximum, and then declines, so the best LR is 40 : 1 for alcalase and 30:1 for savinase. Further elaboration in Table IV shows that the elongation and strength of the samples are in good agreement with the weight-

TABLE V
Effect of the Enzyme Mixture Concentration on the Weight Loss, Strength, and Elongation of Silk Yarn

| Alcalase (g/L)	Savinase (g/L)	Treatment time (min)	Weight loss (%)	Strength (cN/tex)	Standard deviation	Elongation (%)	Degumming efficiency (%)
0.00	1.00	30	17.19	32.66	1.5660	15.00	76.13
		60	20.40	32.03	0.8877	18.42	90.35
0.25	0.75	30	19.38	32.39	1.0771	15.83	85.83
		60	20.96	35.48	0.6683	18.33	92.82
0.50	0.50	30	22.43	33.22	1.0515	17.74	99.34
		60	22.38	34.77	0.8068	17.42	99.11
0.75	0.25	30	22.40	32.54	1.1330	18.61	99.20
		60	22.22	34.95	0.8195	18.18	98.41
1.00	0.00	30	20.46	33.59	1.0964	19.44	90.61
		60	21.95	33.85	0.8867	20.30	97.21
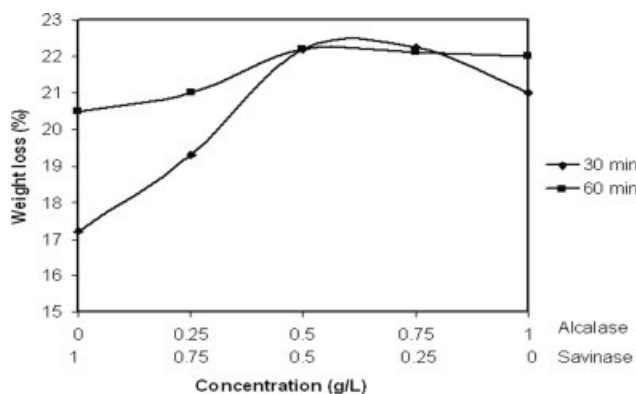
Figure 8 Effect of the enzyme mixture on the weight loss of silk yarn.

loss variations. The low weight-loss yield at low LRs might be the result of saturation of the bulk solution with sericin and consequently possible sericin precipitation on the filaments. However, increasing LR provides a sufficient medium for hydrolysis and removal of the sericin. The decline of the weight-loss efficiency at higher LRs might be attributed to the increase in the number of enzyme molecules, which will provide the environment for an increase in the enzyme–enzyme interactions and possible micelle formation and consequently the reduction of the enzyme activity.

Effect of the enzyme mixture

Silk degumming was carried out with different ratios of enzymes as treatment media, and the results are summarized in Table V and Figure 8. According to the results obtained for the individual enzymes, the optimum time for the removal of sericin is 60 and 30 min for savinase and alcalase, respectively (Table II). With the same amount of each enzyme in a mixture (0.5 g/L), the maximum

sericin removal at 30 and 60 min is the same, and no significant differences can be observed. Figure 8 shows that at 30 min, savinase by itself is not able to remove the optimum amount of sericin, whereas with a decreasing concentration of savinase and an increasing concentration of alcalase, an improvement in the weight-loss efficiency can result. A smoother pattern for alcalase can be observed, and the superiority of alcalase to savinase might be concluded. The results show that savinase and alcalase independently are not effective for degumming, whereas with a mixture of them, the degumming efficiency is significantly increased. Alcalase and savinase appear to be selectively working toward specific sites on the substrate. On the basis of the data, it is possible to say that the best treatment time for degumming with a mixture of the enzymes is 30 min at optimum alcalase/savinase concentration ratios of 0.5/0.5 and 0.75/0.25.

Microscopic studies

SEM micrographs of samples are shown in Figures 9 and 10. In the untreated silk yarn micrographs (Fig. 9), sericin appears as a partially nonuniform coating on the surface of the yarn. However, the micrographs of samples degummed by alcalase, savinase, a mixture of the enzymes, and soap at the optimum concentration show perfect degumming and no sign of destruction and damage to the surface of the yarn (Fig. 10).

CONCLUSIONS

We have attempted to investigate the applicability of enzymes, particularly mixtures of enzymes (alcalase and savinase), to the effective degumming of Persian silk under mild conditions. The obtained results are encouraging in comparison with those of the con-
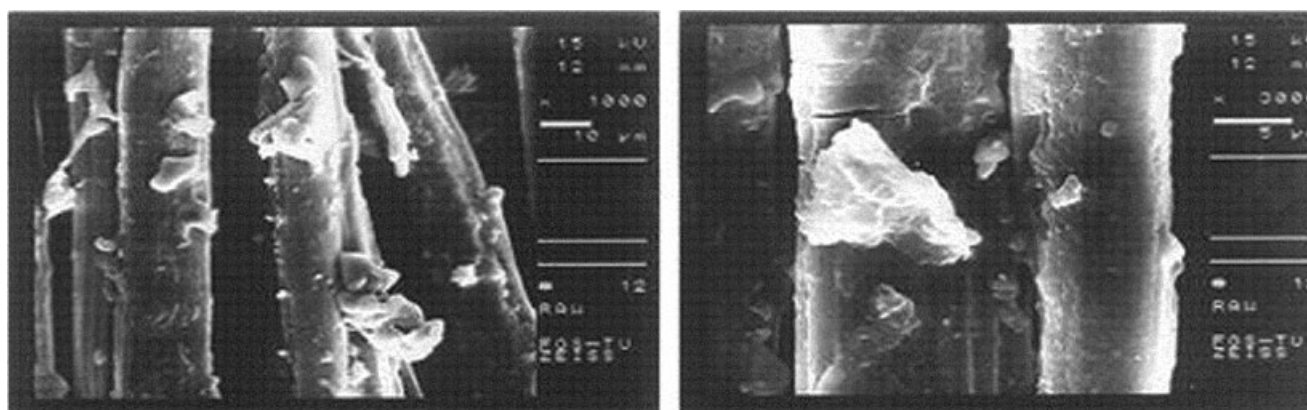

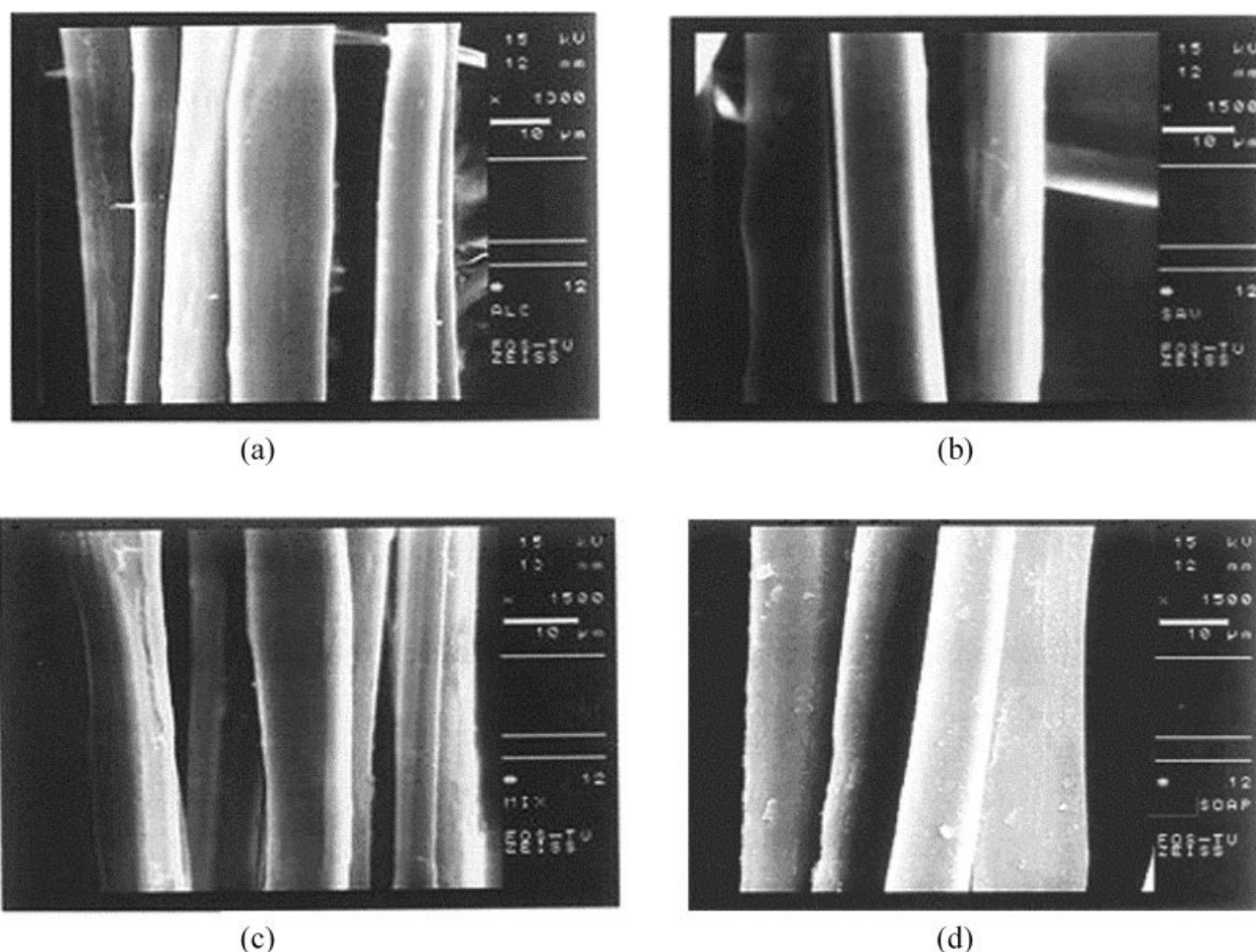**Figure 9** SEM micrographs of the undegummed sample.

Figure 10 SEM micrographs of the degummed samples treated with (a) alcalase, (b) savinase, (c) an enzyme mixture, and (d) soap.

ventional method of degumming (the soap method); that is, alcalase, savinase, and their mixtures effectively degum silk fibers.

The results show that an enzyme concentration of 1 g/L and a treatment time of 30 min for alcalase and 60 min for savinase are sufficient to achieve considerable weight loss without significant damage to fibroin. For a mixture of the enzymes (alcalase and savinase) with a 1 : 1 ratio, the optimum removal of sericin with minimum damage to fibroin with 30 min of treatment was obtained. In general, for all the experiments under the specified conditions, the results show that through the expansion of the treatment time, the amount of sericin remaining over the samples decreased. In other words, as the treatment time increased, more sericin was removed from the raw silk samples. A detailed study of the results in the tables and figures has revealed that the strength and elongation of the samples are justifiable with the weight-loss results; that is, with the increasing weight loss of the samples, the strength of the filaments decreases, whereas the elongation increases. This can be explained by the fact that sericin is act-

ing as an adhesive and working as a coating and wrapping material around the fibroin.

The Marseilles soap treatment results in the complete removal of sericin, but the quantity of soap needed is high, and this makes the method expensive and nonecofriendly. Also, the higher temperature (95°C) at an alkaline pH (8–9) most likely will cause partial degradation of fibroin, and thus the reduction of the strength (21–25%) might be considered the main disadvantage of the soap treatment method (the conventional method). However, the mixed enzymatic treatment is milder (temperature = 55°C) and gives fairly good weight loss in a short time (30 min) with minimum strength loss (9–14%). The SEM micrographs have confirmed the obtained results and are in good agreement with the weight loss, strength, and elongation.

To support the effectiveness of the enzymatic treatment for degumming silk, we are doing further research on dyeing samples, which might provide further information on the effectiveness of the method. Once more, it is important to mention that the advantages of the enzymatic degumming of silk

are minimum environmental damage, less consumption of energy, and a short operation time.

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