(REFEREED RESEARCH)

SERICIN REMOVAL FROM SILK FIBERS WITH ECO-FRIENDLY ALTERNATIVE METHODS

İPEK LİFLERİNDE ALTERNATİF ÇEVRE DOSTU YÖNTEMLERLE SERİSİN GİDERME

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ABSTRACT

Silk fibers, composed of sericin and fibroin, are spun by the silkworm bombyx mori. Fibroin and sericin account for about 75 wt% and 25 wt% of the raw silk, respectively. Sericin is the natural cement to hold the fibroin fibers together during cocoon production. The conventional method of sericin removal is degumming with Marseille soap at alkaline pH, which leads to generation of high strength wastewaters. In this study, extraction of sericin from silk fibers was performed with alternative methods, i.e., degumming at high temperature-high pressure and enzymatic degumming under different process conditions. The results were compared with conventional method in terms of fiber whiteness, brightness, weight loss, breaking strength and elongation. The conventional process, enzymatic process with 8% savinase and 1100 C in the high temperature process results were comparable, which indicated that alternative methods can be used for degumming of sericin.

Keywords: Silk, eco-friendly, enzyme, protease, degumming, sericin

ÖZET

Serisin ve fibroinden oluşarak kompozit bir yapı oluşturan ipek lifleri Bombyx Mori türü ipek böceği tarafından eğrilmektedir. Fibroin ve fibroini bir arada tutan doğal çimento olan serisin ham ipeğin ağırlıkça sırasıyla % 75 ve % 25' ini oluşturur. Serisin, geleneksel olarak iplikten Marsilya sabunu ile alkali pH ortamında uzaklaştırılır. Bu çalışmada ipek lifinden serisin uzaklaştırma işlemi yüksek sıcaklık-yüksek basınç ve enzimatik yöntemlerle farklı proses şartları altında yapılmıştır. Sonuçlar konvansiyonel proses ile beyazlık, parlaklık, ağırlık kaybı, kopma mukavameti ve uzaması bakımından karşılaştırılmıştır. Konvansiyonel, % 8 savinaz enzimi ile yapılan enzimatik ve 1100 C' de yüksek sıcaklık yöntemleriyle elde edilen sonuçlar birbirlerine benzerdir. Bu sonuçlar, serisin sökmede alternatif yöntemlerin kullanılabileceğini göstermektedir.

Anahtar Kelimeler: İpek, çevre dostu, enzim, proteaz, serisin sökme, serisin

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1. INTRODUCTION

The discovery of "queen of textile=silk" goes back to 2640 B.C. In the story about silk, princess Xi Lin Shi acted a part. While she was drinking tea in the mulberry garden, a cocoon dropped into the cup and it was partially dissolved. She wanted to get rid of this, but the cocoon outer layers continued to unwind as a continuous flament. In this way, first process of reeling was found by the princess (1,2).

Silk was one of the world's most exclusive fibers because of matchless characteristics like luster, sensuousness, comfort and remarkable mechanical properties (high breaking strength, toughness and initial stiffness). This natural fibrous protein polymer was produced by sericigenous insects like silkworms, mites, spiders, scorpions and fleas. These different sourced silks differ in structure, composition, so their properties differ widely and depend on their origin (2, 3, 4, 5).

Silk is classified into two as mulberry (domesticated silk) and non-mulberry (wild silk muga, eri and tasar). Both of them consist of almost 20 different types of amino acids and they are double-thread filaments. The raw silk is a composite fiber composed of fibroin and sericin, which are proteinaceous materials. Brin, known as fibroin core, is a structural protein enveloped by sericin. Sericin is a water-soluble glue, which can be named as gum or natural sizing agent. As the name implies, it bonds fibroins together. Sericin also has small amounts of contaminants like minerals, dust, pigments and waxy materials. Fibroin is composed of glycine, alanine and serine amino acids. Insoluble properties of fibroin are due to the non-polar amino acids. The content of silk obtained from various sources are examined; mulberry silk's fibroin content is 70–80%, for tassar silk it is 85– 90%. Sericin is made up of serine, aspartic acid, glycine and threonine. The sericin content of mulberry is 20–30%, that of tasar is 8–15%. The mulberry and tasar silks have 0.37% and 0.48% wax, respectively. Therefore, each type of silk has its own special properties (6, 7, 8, 9, 10).

The effects of sericin and other impurities on silk fibers show that penetration of chemicals into the fiber is blocked by sericin. Natural sizing agent, also, gives harshness and stiffness to the fiber. Therefore, removing of it is an important and prior step to get a proper material for textile. The name of expelling sericin from raw silk fiber and seperating the individual fibroin flaments is known as degumming. In the degumming process, peptide bonds of sericin are cleavaged in different ways (4, 8, 11, 12). Water, soap, enzymes, acids, alkali, magnetic fields were utilized for degumming. After degumming, silk fabrics showed enhanced soft handle, shiny aspect and elegant drape, so highly appreciated by the consumers (13, 14,15, 16,17). The presence of colouring matters, xanthophyll and tannin, in silk fibers made the colour of mulberry's yellow and for tassar it was varied from beige-green. Thus, bleaching process needs to be compulsory (6, 7, 9). Hydrogen peroxide and potassium permanganate found place as bleaching agents (4, 6, 12, 18).

The silk conventional processing cycle was made up of bathing, boiling, rinsing, and in some cases bleaching, so it required several hours (13). The conventional degumming step was carried out frequently with alkali and soap (10-20 g/L), pH about 10 and boiled at 92-98^0 C for 2-4 h (8). The disadvantage of this process is irregular degumming, loss of fiber strength, high water and energy demand, high strength effluents, dull appearance and surface fibrillation. To obstruct spending high process time requirements and fiber damages, synthetic detergents could be used instead of soap. Enzymes, also, could be utilized to supply further advantages besides time and preventing fiber damages, like strength loss controlling and degumming silk uniformly. Moreover, growing environmental awareness caused enzymes to be used as an eco-friendly technology and necessary for researchers especially in the last twenty years. Nevertheless, using of enzymes for degumming is comparatively not enough discovered. Proteases and lipases were generally used in combination for degumming and removing waxes, fats, mineral salts, respectively. Proteases, which originate from animal, plant, and microbial were reported for removing gum. Especially, *Bacillus* species were used to obtain proteases (8,10, 20).

Except enzymatic and conventional method which was boiling off in Marseille soap or with alkalis, extraction with water under pressure and degumming in boiling acidic solutions are other methods to remove sericin. By acidic degumming, physical properties of silk are enhanced compare with alkali methods because of milder and less agressive action of organic acids, so it is a safer method than alkali method. Generally, tartaric, oxalic, citric, succinic acids are used in degumming bath at 100^0 C for 60 min. Degumming process of cocoon can be done at the high temperature, as water at room temperature doesn't dissolve gum completely. This method is advantageous because it does not have any contamination. However the high process time requirement brings the risk of fibroin damages. The other disadvantage of high temperature treatment is incomplete degumming, so sometimes soap or synthetic detergents are added to prevent it (20, 21, 22, 23, 24).

Within the aforementioned knowledge, in this study *Bombyx Mori* silk fibers gums were removed with enzymatic, high temperature and alkali/soap processes. Effects of these methods were analyzed individually and to make right comments, their results were compared in terms of tensile strength, whiteness, brightness and residual sericin by percent weight of loss calculation. In this way, optimum results for degumming with enzymatic and high temperature processes were obtained, which formed the aim of this study. In the enzymatic process, hydrolysis with protease enzyme, which originates from *Bacillus sp*. at different concentrations and treatment times were employed for extraction of sericin. For 8% of protease solution, the hydrolysis was almost complete in 30 minutes. Like degumming results, the other measured values were same with conventional process outcomes. Similar results were achieved for enzymatic process and high temperature process applied at 110^0 C for 20 minutes. The high temperature processes were run at three different temperatures and process times. These conditions were kept similar with enzymatic process to make an accurate comparison. The results showed that alkali/soap process for degumming was not an indispensable process.

2. EXPERIMENTAL

Raw silk fibers which were provided from *Kirman İplik* with 54.474 Stensby, 51.51 brightness, 1. 67 N/mm^2 breaking strength and 16.66 % max elongation values were used in this study.

The removal of sericin were conducted with three different process: conventional method with Marseille soap, enzymatic process with protease and high temperature-high pressure method.

In conventional method, alkaline (pH 9.5) sericin degumming bath was heated to 98^0 C and process was continued for 45 min at this temperature in *Dyetech Polybath* brand sample dyeing machine. Liquor ratio was applied as 1:30.

As alternative methods, enzymatic and high temperature processes were conducted. Savinase 16L enzyme (Novozymes), which was produced from *Bacillus sp* was used in enzymatic process. Recommended operating conditions were pH 7-11 and 10-60 ^{0}C. This study was performed at 50^0 C at pH 8.5, in 3 different concentrations

(4%, 8%, 12%) for 3 different times (20, 30, 40 minutes). During the experiments, pH and temperature of solvent were kept under control. A nonionic wetting agent (0.1ml/l Nuyasol Nek) was used to increase the penetration of chemicals into the fibers. In the high temperature process neutral medium (pH 7.1) bath was used. Samples were treated at 3 different temperatures (100^0 C, 110^0 C, 120^0 C) and durations (10, 20, 30 minutes) in ATAC brand Sample Dyeing Machine. Liquor ratio was applied as 1:10 for both alternative methods. The other chemicals (Na_2CO_3, CH_3COOH) used were laboratory grade. All experiments were repeated three times.

After degumming processes two hot rinsing (each of them 10 minutes) and neutralization were done to complete the degumming process cycle. Each hot rinsing was performed at 70^0 C. The neutralization process was conducted after conventional and enzymatic process with Albegal set and acetic acid, respectively. In the removal of sericin at high temperature and pressure, just two rinsing process was done with circulation from outside to the inside. Soft water was used in all processes and washings.

After degumming and washing, samples were conditioned for 24 hours at 20-22^0 C temperature and 60% relative humidity. Tests were performed after conditioning.

Whiteness and brightness indexes were measured according to AATCC 110 – 1995 test method with Macbeth Color - Eye MS2020 spectrophotometry. These values were measured ten times for each sample.

Shimadzu AGS-X device was used to measure thebreaking strengths and elongations. Ten measurements were taken from different parts of each sample using 50 N load cell.

3. RESULTS

3.1. Weight loss

In Figure 1, the weight losses for alkaline medium with Marseille soap and enzymatic methods are compared. Average weight loss of conventional method reached almost 21 %. The weight loss of enzymatic process with 8% enzyme concentration applied for at least 30 minutes reached the same value with conventional method. Also, same values were reached with 12% enzyme concentration for all tested time points.

For high temperature studies, the values of the experiment which was performed at 100^0 C wasn't reached to the conventional method value, however at 110^0 C and 120^0 C process results reached and exceeded it for all tested time points (Figure 2).

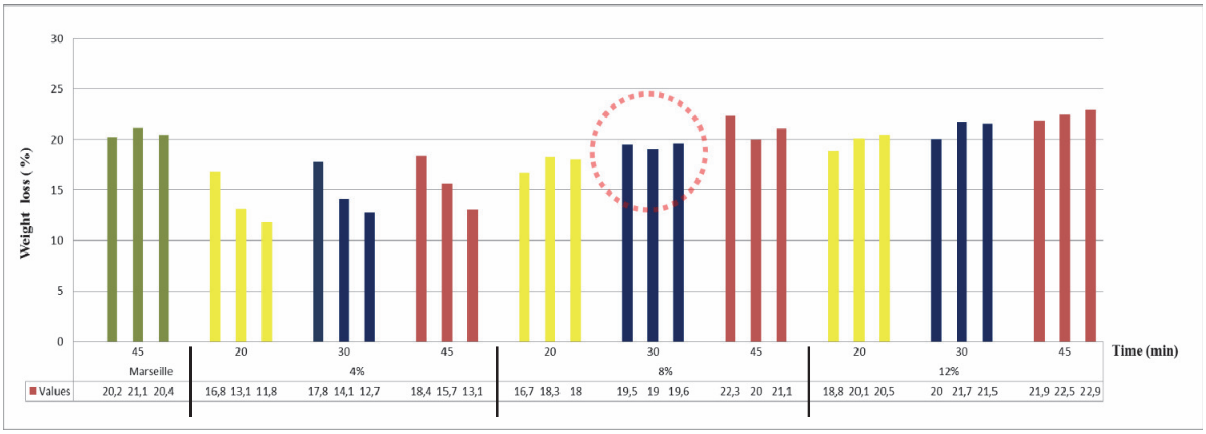

Figure 1. Comparison of weight losses for conventional and enzymatic method

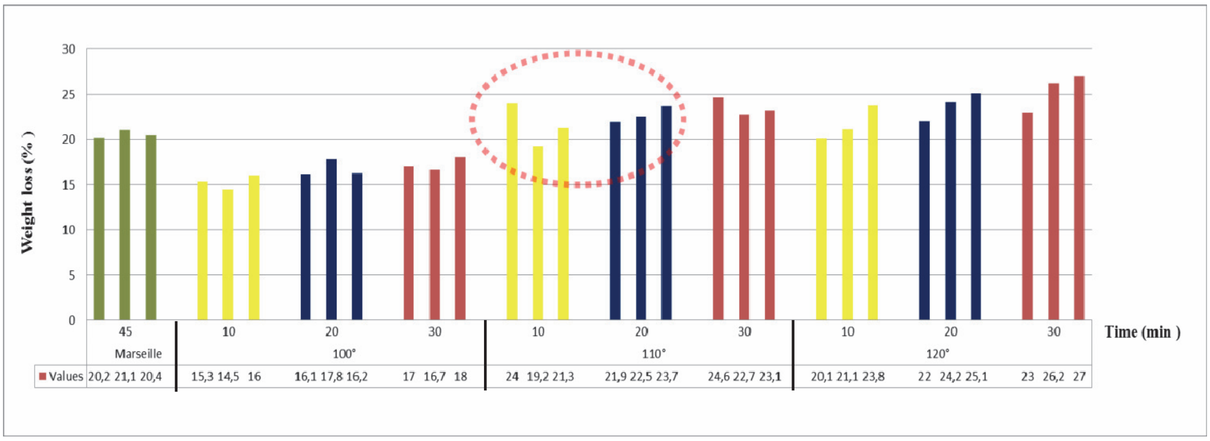

Figure 2. Comparison of weight losses for high temperature and conventional method

3.2. Whiteness and brightness values

Figure 3 and 4 show whiteness values for enzymatic, conventional and high temperature methods. Whiteness of raw fiber was measured as 54.5 Stensby, whereas whiteness of fibers degummed with Marseille soap for 45 minutes was 63 – 69 Stensby, which was 21% higher. In case of enzyme, whiteness values of 64-69 Stensby, 68-71 Stensby and 67-69 Stensby were measured for contact periods of 20 min, 30 min and 45 min, respectively. These values are quite close to those obtained for conventional degumming.

The whiteness values obtained at 100°C for 10, 20 and 30 min were 57-59 Stensby, 55-57 Stensby and 57-60 Stensby, respectively. These values increased slightly at 110°C. On the other hand, there were no significant increase at 120°C, where whiteness values reached to 62-71 Stensby, 69-73 Stensby and 60-69 Stensby for 10, 20 and 30 minutes of contact times.

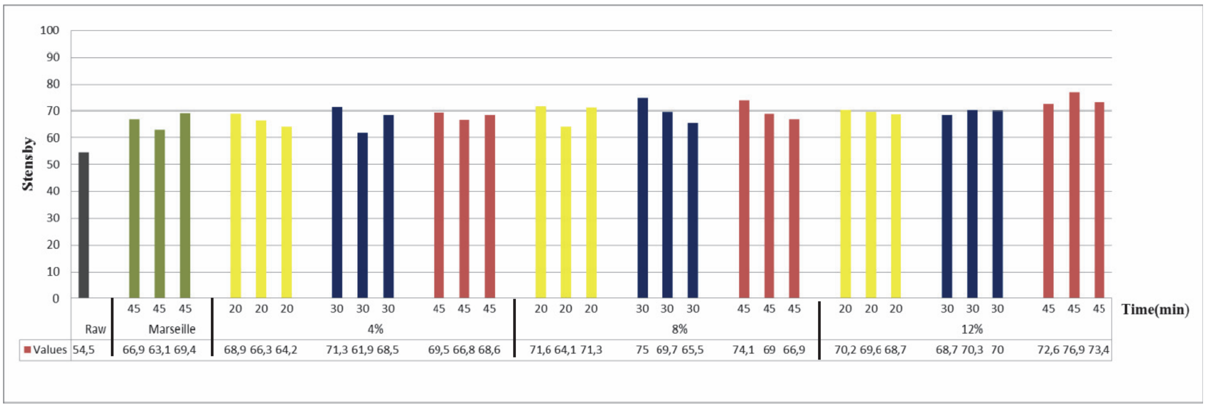

Figure 3. Comparison of whiteness for enzymatic and conventional method

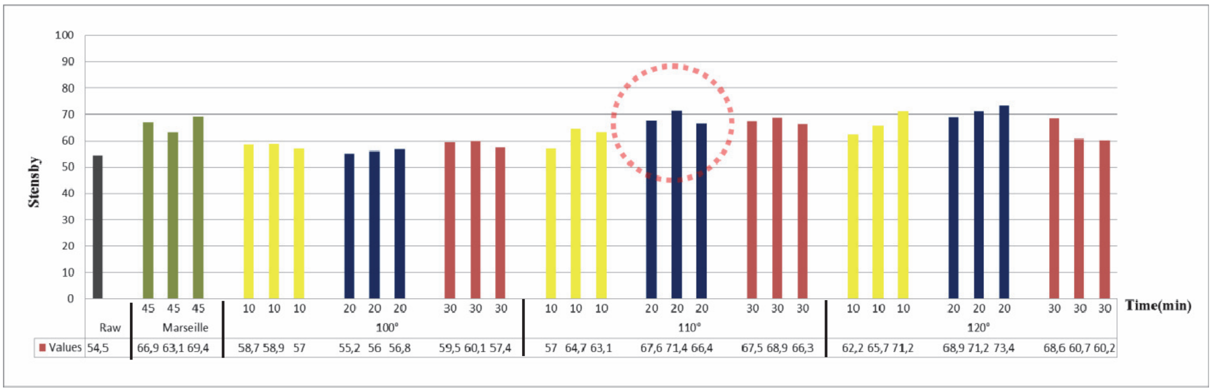

Figure 4. Comparison of whiteness for high temperature and conventional method

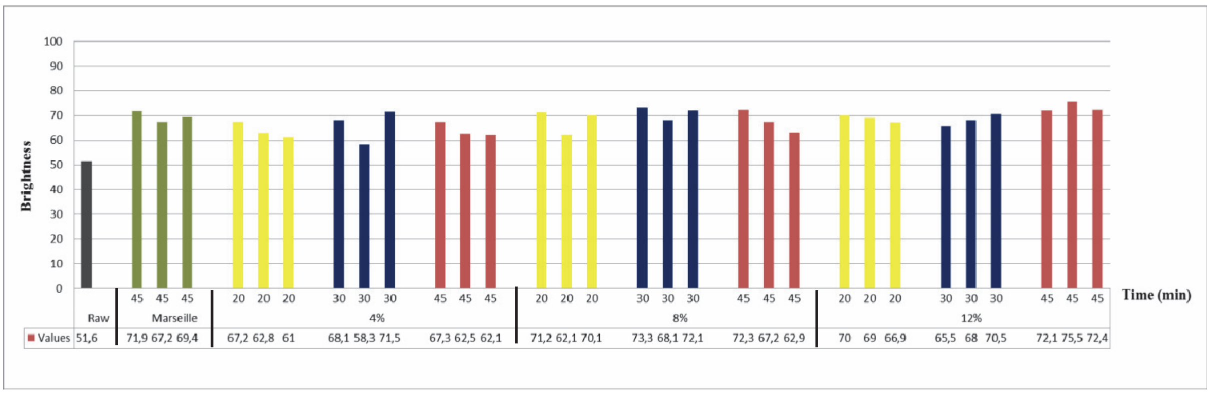

Figure 5. Comparison of brightness for enzymatic and conventional method

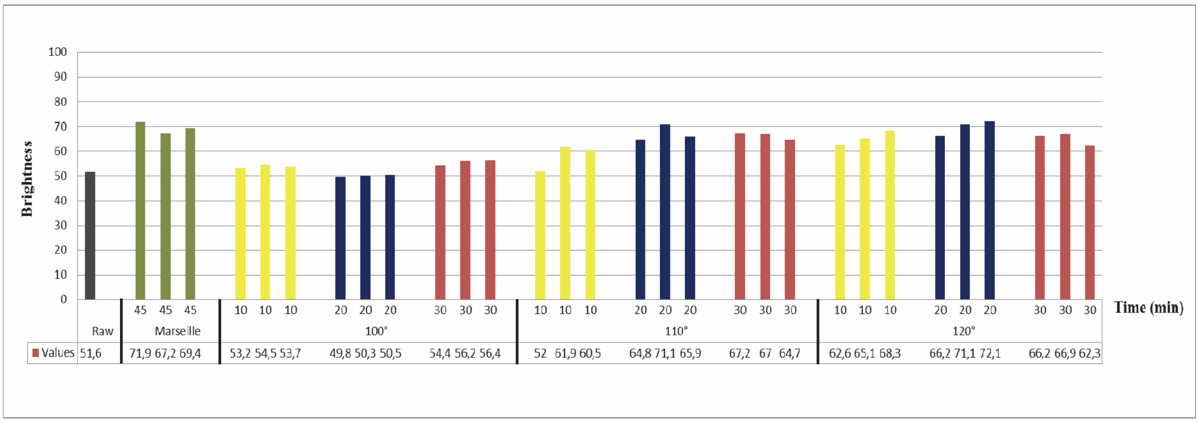

Figure 6. Comparison of brightness for high temperature and conventional method

As seen from Figures 3-6, raw silk fibers had 54.4 Stensby and 51.6 brightness values. Whiteness of 66.3 Stensby and 69.3 brightness values were reached with conventional method. All of the concentrations and time points of enzymatic processes and high temperature processeses exceeded these values. The reason of increase in whiteness was thought to be the destroying of natural coloured materials such as xanthophyll and tanin.

3.3. Loss of breaking strength and elongation

Breaking strength of raw silk fibers was measured as 1.7 N/mm^2. After degumming with Marseille soap strength values decreased to 1.1 N/mm^2, as expected. The breaking strength losses increased when enzyme used at high concentrations and long study periods. Likewise enzymatic processes, for high temperature experiments increasing the process duration and temperature led to higher breaking strength losses. For 120^0 C in high temperature process, it was suspected that not only sericin was removed but the fibroin might have hydrolyzed, as well.

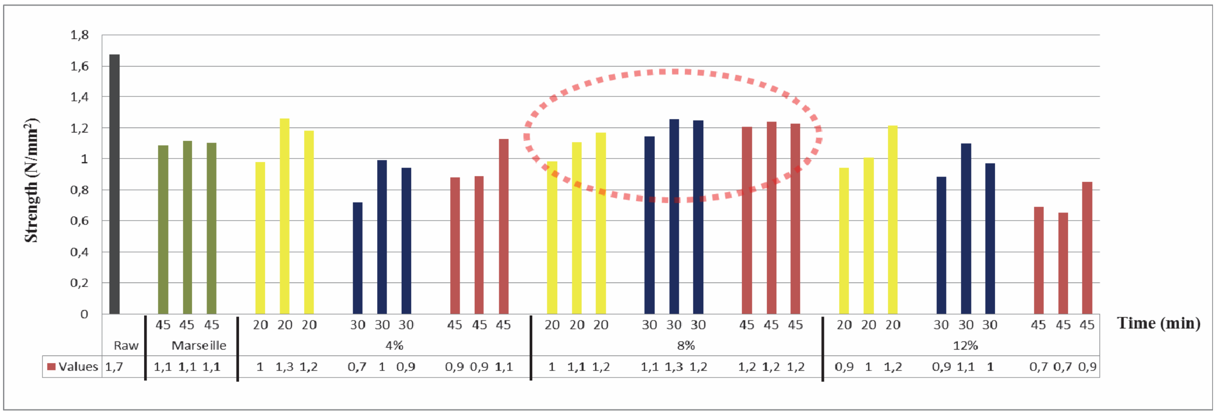

Figure 7. Comparison of breaking strength loss for enzymatic and conventional method

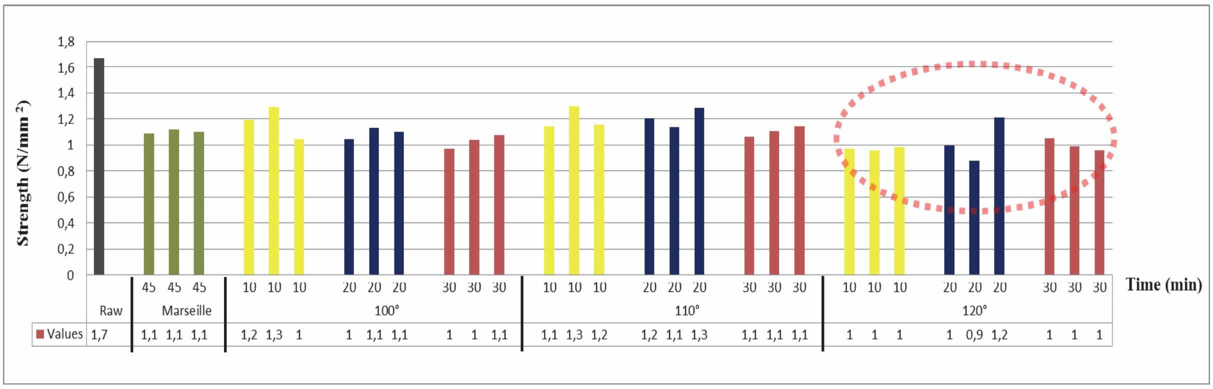

Figure 8. Comparison of breaking strength loss for high temperature and conventional method

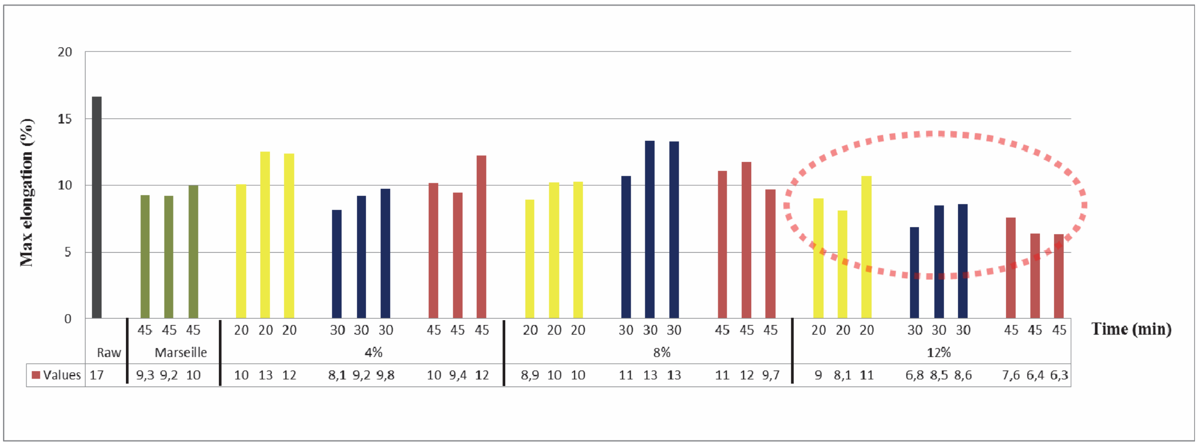

Figure 9. Comparison of elongation for enzymatic and conventional method

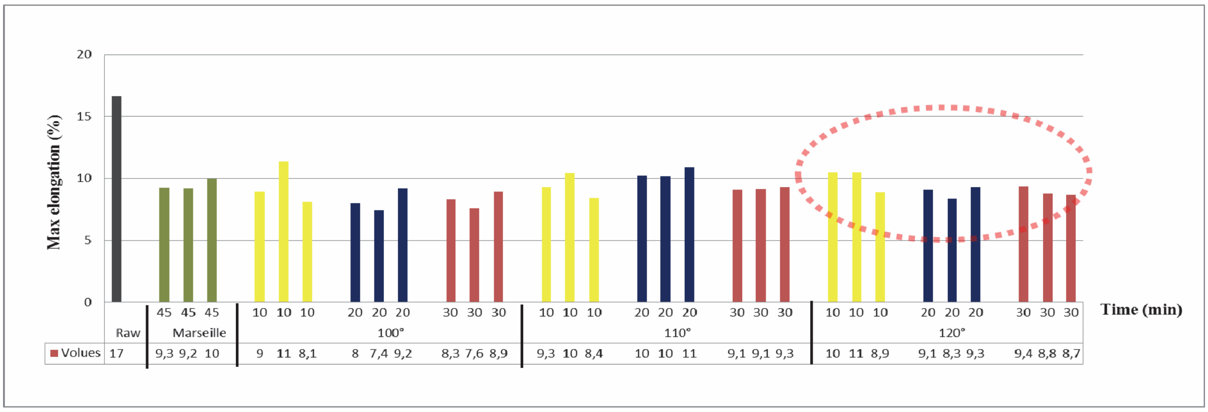

Figure 10. Comparison of elongation for high temperature and conventional method

4. Conclusions

- The weight loss obtained with Savinase 16L enzyme with 8% enzyme concentrations at minimum 30 minutes duration was comparable to the efficiency of conventional method.
- The whiteness and brightness values of enzymatic degumming method for all concentration and time points were similar to the results of conventional method.
- The best breaking strength and elongation values were reached with 8% enzyme concentration and 30 minutes. The strength losses increased when enzyme concentration increased to 12% for all time points.
- When all values were considered, it was concluded that enzymatic process resulted in the best results with 8% enzyme concentration at 30 minutes.
- The values obtained from conventional method results were reached with high temperature process at least at 110^0C for 10 minutes. The weight losses increased up to 25% when high temperature process was performed at 120^0C. It was suspected that both sericin and fibroin were hydrolized.
- In high temperature experiments which were carried out 110^0 in 20 minutes whiteness and brightness values were comparable with conventional method. Likewise, the test which was done at 120^0 yielded the same whiteness and brightness values.
- The breaking strength values in the study which were performed at 100^0 and 110^0 C had similar results with conventional method. However, strength losses for 120° were higher than conventional method.
- The highest breaking elongation values were obtained at 110^0 C and 20 minutes.
- When all values were considered, it was concluded that the best results of high temperature processes were achieved at 110^0 C and 20 minutes.
- When conventional and enzymatic processes were compared in terms of cost it can be said that even though enzyme is more expensive than soap, enzymatic treatments are not overpriced because of lower process times and temperatures. Based on comparison made ecologically, it was an indisputable fact that enzymatic processes were the most environmentally friendly method because of its lower energy requirement and structure of enzyme which was biological molecule. In particular, growing environmental precautions will make enzymes more important.
- As comparison was made between high temperature and conventional methods, the values obtained for high temperature processes were reached with conventional methods. Because of zero chemical needs, the high temperature process is considered to be more environmentally friendly. Degumming of sericin at high

temperature would also provide more economical process trains for sericin recoveryfrom silk effluents.

- It was concluded that both methods of high temperature and enzymatic processes are more eco-friendly than the conventional degumming method.

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