We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Chapter 9

Physichochemical and Low Stress Mechanical Properties of Silk Fabrics Degummed by Enzymes

Styliani Kalantzi, Diomi Mamma and Dimitris Kekos

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/53730

1. Introduction

1.1. The silk road

Sericulture or silk production has a long and colorful history unknown to most people. For centuries the West knew very little about silk and the people who made it. For more than two thousand years the Chinese kept the secret of silk altogether to themselves. It was the most zealously guarded secret in history. According to Chinese tradition, the history of silk begins in the 27th century BCE. Its use was confined to China until the Silk Road opened at some point during the latter half of the first millennium BCE.

The writings of Confucius and Chinese tradition recount that in the 27th century BCE a silk worm's cocoon fell into the tea cup of the empress Leizu. Wishing to extract it from her drink, the young girl of fourteen began to unroll the thread of the cocoon. She then had the idea to weave it. Having observed the life of the silk worm on the recommendation of her husband, the Yellow Emperor, she began to instruct her entourage the art of raising silk worms, sericulture. From this point on, the girl became the goddess of silk in Chinese mythology [1].

Though silk was exported to foreign countries in great amounts, sericulture remained a secret that the Chinese guarded carefully. Consequently, other peoples invented wildly varying accounts of the source of the incredible fabric. In classical antiquity, most Romans, great admirers of the cloth, were convinced that the Chinese took the fabric from tree leaves. This belief was affirmed by Seneca the Younger in his Phaedra and by Virgil in his Georgics. Notably, Pliny the Elder knew better. Speaking of the bombyx or silk moth, he wrote in his Natural History "They weave webs, like spiders, that become a luxurious clothing material for women, called silk".

INTECH
open science | open minds

© 2013 Kalantzi et al.; licensee InTech. This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The earliest evidence of silk was found at the sites of Yangshao culture in Xia County, Shanxi, where a silk cocoon was found cut in half by a sharp knife, dating back to between 4000 and 3000 BCE. The species was identified as bombyx mori, the domesticated silkworm. Fragments of primitive loom can also be seen from the sites of Hemudu culture in Yuyao, Zhejiang, dated to about 4000 BCE. Scraps of silk were found in a Liangzhu culture site at Qianshanyang in Huzhou, Zhejiang, dating back to 2700 BCE. Other fragments have been recovered from royal tombs in the Shang Dynasty (c. 1600 - c. 1046 BCE).

During the later epoch, the Chinese lost their secret to the Koreans, the Japanese, and later the Indians, as they discovered how to make silk. Allusions to the fabric in the Old Testament show that it was known in western Asia in biblical times. Scholars believe that starting in the 2nd century BCE the Chinese established a commercial network aimed at exporting silk to the West. Silk was used, for example, by the Persian court and its king, Darius III, when Alexander the Great conquered the empire. Even though silk spread rapidly across Eurasia, with the possible exception of Japan its production remained exclusively Chinese for three millennia.

According to FAO estimates, the world raw silk production for the year 2010 was 164971 tonnes [2]. Approximately 98% of the world's production is in Asia and especially in Eastern Asia (Figure 1). China is the leader in raw silk production followed by India (Table 1).

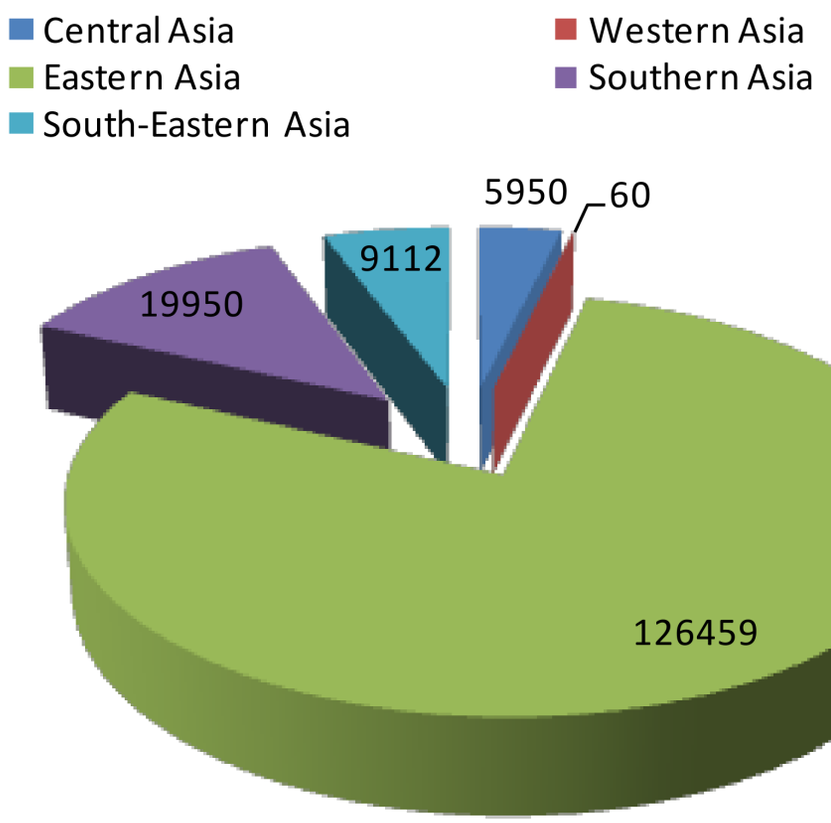


Figure 1. Production of raw silk across Asia (FAO, 2010)

Country	Production (tonnes)	Country	Production (tonnes)
China	126001	Japan	105
India	19000	Afghanistan	50
Viet Nam	7367	Kyrgyzstan	50
Turkmenistan	4500	Turkey	50
Thailand	1600	Cambodia	25
Brazil	1300	Italy	12
Uzbekistan	1200	Lebanon	10
Iran (Islamic Republic of)	900	Bulgaria	5
Democratic People's Republic of Korea	350	Greece	5
Tajikistan	200	Egypt	3
Indonesia	120		

Table 1. Raw silk producing countries [2].

1.2. Types of silk

Silk-producing insects have been classified on the basis of morphological clues, such as follicular imprints on the chorine egg, arrangement of tubercular setae on the larvae, and karyotyping data [3-4]. Classification based on phenotypic attributes is sometimes misleading because morphological features may vary with the environment [5]. Molecular marker-based analysis has been developed to distinguish genetic diversity among silkworm species [6-9]. Most commercially exploited silk moths belong to either the family Bombycidae or Saturniidae, in the order lepidoptera. Silkworms can be divided in three groups: (a) univoltine breed (one generation per year) which is usually found in Europe where due to the cold climate the eggs are dormant in winter and they are hatched in spring, (b) bivoltine breed (two generations per year) usually found in Japan China and Korea, where the climate is suitable for developing two life cycle per year and (c) multivoltine breed (up to eight generations per year) usually found in tropical zone.

The finest quality raw silk and the highest fiber production are obtained from the commonly domesticated silkworm, *Bombyx mori*, which feeds on the leaves of the mulberry plant, *Morus* spp. Other than the domesticated *B. mori*, silk fiber production is reported from the wild non-mulberry saturniid variety of silkworms. Saturniid silks are of three types: tasar, muga, and eri (Table 2).

Common Name	Scientific Name	Origin	Primary Food Plant(s)
Mulberry Silkworm	*Bombyx mori*	China	Morus indica, M. alba M.multicaulis, M.bombycis
Tropical Tasar Silkworm	*Antheraea mylitta*	India	Shorea robusta, Terminalia tomentosa T. arjuna
Oak Tasar Silkworm	*Antheraea proylei*	India	Quercus incana, Q. serrate Q. himalayana, Q. leuco tricophora Q. semicarpifolia, Q. grifithi
Oak Tasar Silkworm	*Antheraea frithi*	India	*Q. dealdata*
Oak Tasar Silkworm	*Antheraea compta*	India	*Q. dealdata*
Oak Tasar Silkworm	*Antheraea pernyi*	China	*Q. dendata*
Oak Tasar Silkworm	*Antheraea yamamai*	Japan	*Q. acutissima*
Muga Silkworm	*Antheraea assama*	India	Litsea polyantha, L. citrate Machilus bombycine
Eri Silkworm	*Philosamia ricini*	India	Ricinus communis, Manihot utilisma Evodia fragrance

Table 2. Commercially exploited sericigenous insects of the world and their food plants [10].

The tasar silkworms are of two categories-Indian tropical tasar, *Antheraea mylitta*, which feeds on the leaves of *Terminalia arjuna*, *Terminalia tomantosa*, and *Shorea robusta*, and the Chinese temperate oak tasar, *Antheraea pernyi*, which feeds on the leaves of *Quercus* spp. and *Philosamia* spp. Indian tropical tasar (Tussah) is copperish colour, coarse silk mainly used for furnishings and interiors. It is less lustrous than mulberry silk, but has its own feel and appeal. Oak tasar is a finer variety of tasar silk [11].

Muga silk is produced by the multivoltine silkworm, *Antheraea assamensis* (also called *A. assama*), which feeds mainly on *Machilus* spp (Table 2). Muga is a golden yellow colour silk. Muga culture is specific to the state of Assam (India) and an integral part of the tradition and culture of that state. The muga silk, a high value product is used in products like sarees, mekhalas, chaddars, etc. [10]. Eri silk is produced by *Philosamia* spp. (*Samia* spp.), whose pri-

mary host plant is the castor (*Ricinus* spp.) (Table 2) [11]. The luster and regularity of *B. mori* silk makes it superior to the silk produced by the non mulberry saturniid silkworms, although non-mulberry silk fibers are also used commercially due to their higher tensile strength and larger cocoon sizes. Spider also produced silk fibers that are strong and fine, but have not been utilized in the textile industries [11].

1.3. Structure of the silk fibre

The cocoons of the mulberry silkworm *B. mori* are composed of two major types of proteins: fibroins and sericin. Fibroin, the 'core' protein constitutes over 70% of the cocoon and is a hydrophobic glycoprotein [12] secreted from the posterior part of the silk gland (PSG) [13].

The fibroin, rich in glycine (43.7%), alanine (28.8%) and serine (11.9%), is composed of a heavy chain (~325 kDa), a light chain (~25 kDa) and a glycoprotein, P25, with molar ration of 6:6:1. The heavy and light chains are linked by a disulfide bond. P25 associates with disulfide-linked heavy and light chains primarily by non-covalently hydrophobic interactions, and plays an important role in maintaining integrity of the complex [14]. The light chain has a non-repetitive sequence and plays only a marginal role in the fiber. The heavy chain contains very long stretches of Gly-X repeats (with residue X being Ala in 64%, Ser in 22%, Tyr in 10%, Val in 3%, and Thr in 1.3%) that consist of 12 repetitive domains (R01–R12) separated by short linkers. It is an antiparallel, hydrogen bonded β-sheet and yields the X-ray diffracting structure called the "crystalline" component of silk fibroin [15]. Silk is a typical representative of β-sheet. Each domain consists of sub-domain hexapeptides including: GAGAGS, GAGAGY, GAGAGA or GAGTGA (G is glycine, A is alanine, S is serine and Y is tyrosine) [16]. In contrast, the 151 residues of the N-terminal, 50 residues of the C-terminal, and the 42-43 residues separating the 12 domains are non-repetitive and "amorphous" [17]. Silk fibroin can exist as three structural morphologies termed silk I, II, and III where silk I is a water soluble form and silk II is an insoluble form consisting of extended β-sheets. The silk III structure is helical and is observed at the air-water interface. In the silk II form, the 12 repetitive domains form anti-parallel b-sheets stabilized by hydrogen bonding [18]. Due to the highly oriented and crystalline structure of Silk II, silk fibroin fiber is hydrophobic and has impressive mechanical properties. When controllably spun, its mechanical property may be nearly as impressive as spider dragline silk [19].

Sericins, the 'glue' proteins constitute 20–30% of the cocoon, and are hot water-soluble glycoproteins that hold the fibers (fibroin) together to form the environmentally stable fibroin–sericin composite cocoon structure [20-22]. Sericin, secreted in the mid-region of the silk gland, comprises different polypeptides ranging in weight from 24 to 400 kDa depending on gene coding and post-translational modifications and are characterized by unusually high serine content (40%) along with significant amounts of glycine (16%), [23-24]. Three major fractions of sericin have been isolated from the cocoon, with molecular weights 150, 250, and 400 kDa [24]. Sericin remains in a partially unfolded state, with 35% β-sheet and 63% random coil, and with no α-helical content [18].

The amino acid compositions of fibroin and sericin have been published, with somewhat differences from paper to paper for some specific amino acid contents [16, 25-26].

The chemical composition of raw silk obtained from the silk worm *Bombyx mori* is presented on Table 3. Silk is produced in several countries and the fibres from different regions contain different amounts of sericin which exhibits diverse chemical and physical properties [27].

Component	
Fibroin	70-80
Sericin	20-30
Wax	0.4-0.8
Carbohydrate	1.2-1.6
Inogranic matter	0.7
Pigments	0.2

Table 3. Composition of raw silk from the silk worm *Bombyx mori* [28-29].

1.4. Silk degumming

Silk processing from cocoons to the finished clothing articles consists of a series of steps which include: reeling, weaving, degumming, dyeing or printing, and finishing. Degumming is a key process during which sericin is totally removed and silk fibres gain the typical shiny aspect, soft handle, and elegant drape highly appreciated by the consumers. In addition, the existence of sericin prevents the penetration of dye liquor and other solutions during wet processing of silk. Also, it is the main cause of adverse problems with biocompatibility and hypersensitivity to silk [17]. Furthermore, to prepare pure silk fibroin solution for silk-based biomaterials, separation of silk fibroin fiber from the sericin glue, is a critical step, since (a) residual sericin causes inflammatory responses and (b) non-degummed fibers are resistant to solubilization [30].

The industrial process takes advantage of the different chemical and physical properties of the two silk components, fibroin and sericin. While the former is water-insoluble owing to its highly oriented and crystalline fibrous structure, the latter is readily solubilized by boiling aqueous solutions containing soap [27], alkali [31], synthetic detergents [32]. However, the higher temperature (95°C) and an alkaline pH (8-9) in the presence of harsh chemicals in the treatment bath impose a markedly unnatural environment on the silk, and thus cause partial degradation of fibroin. Fibre degradation often appears as loss of aesthetic and physical properties, such as dull appearance, surface fibrillation, poor handle, drop of tensile strength, as well as uneven dyestuff absorption during subsequent dyeing and printing [33]. More importantly, the large consumption of water and energy contribute to environmental pollution. These costs have fueled interest in developing a new, effective degumming method which minimizes these adverse effects (Table 4).

Degumming method	References
Traditional (alkali, soap, synthetic detergents)	[27, 31-32]
Microwave irradiation	[42]
Plasma method	[41]
Organic acids	[34-37]
Ultrasound method	[43-44]
Enzymatic method	[33, 46-52]

Table 4. Degumming methods of raw silk

Many researches have been performed on degumming and finishing of silk fiber using acid agents for enhancing the physical properties of silk [34-36]. It has been pointed out that the action of organic acids is generally milder and less aggressive than the action of alkali. Khan et al. [37] investigated silk degumming using citric acid. The surface morphology of silk fiber degummed with citric acid was very smooth and fine, showed perfect degumming (almost complete removal of sericin) like traditional soap-alkali method and the tensile strength of silk fiber was increased after degumming. Tartaric and succinic acids demonstrate efficient sericin removal while retaining the intrinsic properties of the fiber. Freddi et al. [34] studied on the degumming of silk fabrics with tartaric acid and showed the excellent performances of tartaric acid, both in terms of silk sericin removal efficiency and of intrinsic physico-mechanical characteristics of silk fibers. The degummed silk fabric with tartaric acid exhibited a good luster and a 'scroopier' handle in compared with soap degummed fabric. They also demonstrated that dyeability with acid dyes and comfort properties (such as wicking, wettability, water retention and permeability) are also enhanced and concluded that the acid degumming process shows potential for possible industrial application. However, there is a tendency toward a gradual decrease of tenacity and elongation values with increasing tartaric acid in the bath.

Low-temperature plasma treatment has been well studied at research level in textile in the last years due to its rapid, water and chemical free process, as well as resource conservation, though it is not yet really used and well established in industry [38-40]. Long et al., [41] reported that degumming efficiency and properties of silk fabric after low-pressure argon plasma treatment were comparable to the conventional wet-chemical treatment process. Unfortunately, plasma methods result in a notable etching effect from physical bombardments and chemical reactions by excited plasma species on sericin layers.

Microwave irradiation and ultrasound are techniques that have been investigated for their performance as degumming agents by several researchers. Microwave treatment of silk resulted in increased weight loss followed by a decrease in strength of the filaments, whereas the elongation increases. This can be explained by the fact that sericin is acting as an adhesive and working as a coating and wrapping material around the fibroin [42].

Ultrasound has been widely used in chemistry and the dyeing, finishing, and cleaning industries because of its obvious advantages in particle treatment, including dispersion and agglomeration effects. Ultrasonic method combined also with natural soaps (olive oil, turpentine and daphne soaps) or proteolytic enzymes (alcalase and savinase) enables an effective clearance in the degumming process, it facilitates the removal of the substances existing on the raw silk like dirt and sericin and yields positive results in terms of weight loss, whiteness degree and mechanical properties [43-44].

The increasing awareness of legislators and citizens for the ecological sustainability of industrial processes has recently stimulated the interest of scientists and technologists for the application of biotechnology to textile processing [45]. In recent years, various studies have dealt with the removal of sericin by using proteolytic enzymes since they can operate under mild conditions and low temperatures which save energy in comparison to the traditional method. Enzymes act selectively and can attack only specific parts of sericin to cause proteolytic degradation. So the pattern of soluble sericin peptides obtained by degumming silk changes as a function of the kinds of enzyme used, attributing to the different target cleavage of the enzymes.

Several acidic, neutral, and alkaline proteases have been used on silk yarn as degumming agents. Alkaline proteases performed better than acidic and neutral ones in terms of complete and uniform sericin removal, retention of tensile properties, and improvement of surface smoothness, handle, and lustre of silk [46-48]. Enzyme degummed silk fabric displayed a higher degree of surface whiteness, but higher shear and bending rigidity, lower fullness, and softness of handle than soap and alkali degummed fabric, owing to residual sericin remaining at the cross over points between warp and weft yarns [49]. Freddi et al. [33] applied acidic, neutral, and alkaline proteases to silk degumming and found that alkaline and neutral proteases performed better than acidic proteases in terms of complete sericin removal. After complete sericin removal with proteolytic methods, the quality of appearance and retention of tensile properties is expected to be superior to those silks degummed through traditional methods due to less chemical and physical stress applied to the silk during enzymatic processing. Nakpathom et al., [50] degummed Thai *Bombyx mori* silk fibers with papain enzyme and alkaline/soap and reported that the former exhibited less tensile strength drop and gave higher color depth after natural lac dyeing, especially when degumming occurred at room temperature condition. Alcalase, savinase, (two commercial proteolytic preparations) and their mixtures also proved to be feasible for degumming applications [51]. Gulrajani et al., [52] degummed silk with the combination of protease and lipase enzymes, and obtained efficient de-waxing and degumming effects, while maintaining favorable wettability of silk fibers.

Silk degumming is a high resource consuming process as far as water and energy are concerned. Moreover, it is ecologically questionable for the high environmental impact of effluents. 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. This could be made possible by the milder treatment conditions, the recycling of processing water, the recovery of valuable by-products such as sericin peptides, and the lower environ-

mental impact of effluents [33]. However, the limitations of higher cost of enzymes compared to chemicals and the necessary continuous use of enzymes may limit the development of industrial processes using proteolytic degumming methods [41, 49].

1.5. Potential applications of sericin

Sericin is at present an unutilized by-product of the textile industry and the discarded degumming wastewater also ultimately leads to environmental contamination due to the high oxygen demand for its degradation by microbes [53]. It is estimated that out of the 1 million tons (fresh weight) of cocoon production worldwide, or about 400 000 tons of dry cocoon, approximately 50 000 tons of sericin could be recovered from the waste solution [54]. If sericin was recovered, perhaps it could be used as a 'value added' product for many sericin-derived products and purposes [55] and this would also be beneficial in terms of the economy and the environment.

Limitations on devising specific applications are caused by its ability to exist in many forms that depend on its method of extraction and purification, etc. Each specific application requires a particular form so it will be necessary to devise and understand how to prepare consistent products suitable for each. Non-textile applications of sericin range from cosmetics to biomedical products, which include its use in anticancer drugs, anticoagulants, and cell culture additives, for its antioxidant properties [11, 56]. Furthemore, its ability to form crosslink or blends with other polymers to produce more effective films that can be used for new drug delivery methods with reduced immunogenicity and increased drug stability or even new food packaging materials worth further investigation [56].

2. Materials & methods

2.1. Silk fabric

The treatments were carried out on a 100% raw silk fabric (crêpe). Construction parameters are listed on Table 5.

	Wrap yarn	Weft yarn
Number of ends (cm^{-1})	46	110
Fabric weight ($g \cdot m^{-2}$)	89.27	

Table 5. Construction properties of silk fabric

2.2. Enzymes

Esperase® 8.0L and Lipolase® Ultra 50T were kindly provided from Novo Nordisk Co. (Bagsvaerd, Denmark). Papain was purchased from Sigma. All other chemicals were laboratory-grade (analytical reagents, Sigma).

2.3. Enzyme activities

The proteolytic activity was assayed spectrophotometrically with azocasein as a substrate [57]. One unit of activity was defined as the amount of enzyme required to produce a 0.1 increase in absorbance at 440 nm under the assay conditions (50°C and pH 8.0).

Lipase activity was determined against p-nitrophenyl- propionate (pNPP) at pH 7.0 and 25°C [58]. The release of p-nitrophenol was monitored spectrophotometrically at 410 nm with the aid of a microplate reader (Molecular Devices Corporation, Sunnuvale, USA). The reaction was initiated by adding 10 μL of properly diluted enzyme to 190 μL of substrate solution (0.4 mM). Control reactions with inactivated lipase were used to correct nonenzymatic pNPP hydrolysis. One unit of activity was defined as the amount of enzyme which released 1 μmol of product per minute under the conditions described.

2.4. Soap degumming (conventional method)

Silk fabrics were soaked overnight in a solution of 5 $g \cdot l^{-1}$ Marseille soap at pH 9.5 at a liquor-to-fabric ratio 40:1. Next day the silk fabrics were degummed in a boiled alkaline solution containing 10 $g \cdot l^{-1}$ Marseille soap and 1 $g \cdot l^{-1}$ sodium carbonate for 2 h at a liquor-to-fabric ratio 40:1 and pH 9.5. Degummed silk fabrics were first rinsed at 50°C with 1 $ml \cdot l^{-1}$ ammonia and consequently two times at 40°C with 1 $ml \cdot l^{-1}$ ammonia. Finally the fabrics were rinsed with cool water.

2.5. Enzymatic degumming

A fabric sample of approximate weight 3.0 g was immersed in Tris-HCl buffer (50 mM) at pH 8.0. A non-ionic wetting agent (Sadopane SF 0.1% w/v) and the appropriate amount of enzyme(s) supplemented the solution. The liquor-to-fabric ratio was adjusted to 40:1, and the mixture was incubated at 50°C and 50 rpm. Blank samples were obtained by treating silk with buffer alone, without enzyme. Enzyme dosage and treatment time were changed. Inactivation of the enzyme(s) was carried out in hot distilled water for 10 min. At the end of the treatment, silk fabrics were rinsed with distilled water and dried at room temperature. All degumming tests were performed in duplicate.

2.6. Bleaching of degummed silk fabrics

The enzymatic and conventional degummed silk fabrics were bleached using: 0.4% (o.w.g) bleaching agent (Belphor BH); 0.5% (o.w.g) stabilizator (Sifa FL); 20 $ml \cdot l^{-1}$ H_2O_2; 3 $g \cdot l^{-1}$ Na_2CO_3; liquor-to-fabric ratio (30:1); wetting agent Clariant Sandoclean PC-FL (1 $g \cdot l^{-1}$).

The bleaching agent was mixed with the stabilizator, H_2O_2 and the Na_2CO_3. The mixture was stirred and heated at 40°C. The silk fabrics were soaked in the mixture and stirred until the temperature reached 90°C. The fabrics were left for 100 min. The fabrics were removed and rinsed initially with hot tap water and finally they were immersed in water with 2-3 drops of formic acid.

2.7. Weight loss and degumming efficiency determination

Fabric weight loss was recorded as dried sample weight loss. The drying conditions were 105°C in an air-circulated oven for 1 h. The samples were weighed, after cooling in a desiccator. The following equation (Eq. (1)) was used to calculate the weight loss (wt%):

$$Wt\% = \left(\frac{W1 - W2}{W1}\right) \times 100 \tag{1}$$

where, *W1* and *W2* are the weights of the fabric before and after treatment, respectively [59].

The efficiency of the degumming was calculated through a comparison of the enzyme process for silk fabrics with the standard method (degumming using Marseille soap), using the following equation (Eq. (2)):

$$DegumEff = \frac{W_E}{W_{MST}} \tag{2}$$

where, *DegumEff* is the Degumming Efficiency (%), W_E is the percentage of weight loss by the enzyme treatment and W_{MTS} is the percentage of weight loss by the Marseille soap treatment [51].

The degumming with Marseilles soap was taken as the standard 100% weight loss.

2.8. Wettability: Drop test

Wettability of the fabric was measured, by means of the ''drop test" before and after the degumming process. The dried samples at room temperature were tested using AATCC Test Method 39-1980 (evaluation of wettability) [60]. The time period (in sec) between the contact of the water drop with the fabric and the disappearance of the water drop into the fabric was counted as the wetting time. The time of drop disappearance was averaged from measurements in different points of the fabric sample. Wetting times equal or less than 1 sec were considered as indication of adequate absorbency of the fabrics [61]. All measurements were performed in triplicate.

2.9. Whiteness

The whiteness index (Berger degree) of the fabrics was determined using a reflectance measuring Datacolor apparatus at standard illuminant D65 (LAV/Spec. Excl.,d/8, D65/10o) [62].

2.10. Crystallinity index (CrI)

An X-ray diffractometer (Siemens D5000), was used in order to determine the Crystallinity Index, using copper Ka radiation. The angles scanned were 10–30° at 0.01°/s. The Crystallinity Index was determined according to the empirical method of Segal et al. [63] applying Eq. (3)

$$CrI = \left(\frac{I_{002} - I_{am}}{I_{002}}\right) \times 100 \quad (3)$$

where I_{002} is the peak intensity from the lattice plane, and I_{am} is the peak intensity of amorphous phases.

Triplicate sets of data were used to establish the relative error associated with the X-ray diffraction method [63].

2.11. The Kawabata evaluation system

Basic mechanical properties namely tensile, bending and shearing were measured by the KES-FB system under high sensitivity conditions. The temperature was 20 ± 0.5°C, and relative humidity is 65% ± 5. The properties measured were shear rigidity (*G*, gf·cm·degree^{-1}), bending rigidity per unit length (*B*, gf·cm^2·cm^{-1}) and extensibility (*EMT*, %) at 500 gf·cm^{-1}. All measurements were performed in triplicate.

3. Results and discussion

3.1. Characteristics of the enzymes used for degumming

The commercial enzyme preparations used for degumming of the silk fabric are listed in Table 6. Novozymes launched Lipolase® in 1988, the first commercial lipase developed for the detergent Industry. Lipolase® was the first lipase produced by recombinant DNA technology. This lipase, originating from *Thermomyces lanuginosus*, formerly *Humicola lanuginosa*, was expressed in *Aspergillus oryzae*. This enzyme is widely used in detergent formulations to remove fat-containing stains and it also has a broad range of substrate specificity. Furthermore is stable in proteolytic wash solutions. Novozymes launched two variants of Lipolase® issued from rational protein design: Lipolase® Ultra and LipoPrime™. These variants were also expressed in *A. oryzae* [64]. The enzyme preparation used in the present study (Lipolase® Ultra 50T) exhibited optimal pH and temperature of 9.0 and 50°C respectively. Furthermore, it was stable at temperatures 30-50°C and pH values of 7.0-10.0 (the remaining activity was measured after incubation for 24 h at the above mentioned temperatures and the pH values).

Esperase® 8.0L is also a product of Novozymes. It is a bacterial serine type alkaline protease produced by *Bacillus* sp. Esperase® is characterized by excellent perfomance at elevated temperature and pH. It exhibited optimal pH and temperature of 10.0 and 70°C respectively. The enzyme preparation was stable (>90% of its original activity) at pH values of 7.0 and 8.0 and temperatures of 30-50°C (the remaining activity was measured after incubation for 24 h at the above mentioned temperatures and the pH values).

Papain is a cysteine protease, isolated from Papaya (*Carica papaya*) Latex. Papain consists of a single polypeptide chain with three disulfide bridges and a sulfhydryl group necessary for

http://dx.doi.org/10.5772/53730

activity of the enzyme. The pH optimum of papain was found 8.0 while temperature optimum at 50°C. Papain was stable at pH values of 7.0 and 8.0 and temperatures of 30-50°C (the remaining activity was measured after incubation for 24 h at the above mentioned temperatures and the pH values).

Enzyme	Origin	Characteristics	Activity[a]	pH[a]	T (oC)[a]
Papain	*Carica papaya*	Cysteine protease	1.15 U·mg^{-1} dry matter	7.0-9.0 (8.0)	30-50 (50)
Esperase® 8.0L	*Bacillus* sp.	Serine-type protease	19.3 U·ml^{-1}	7.0-8.0 (10.0)	30-50 (70)
Lipolase® Ultra 50T	Aspergillus oryzae (genetically modified)	Lipase	6.1 U·ml^{-1}	7.0-9.0 (9.0)	30-50 (50)

[a] Optimum pH and temperature values in parentheses.

Table 6. Enzymes used for silk degumming

3.2. Protease treatment of silk fabrics: effect of enzyme dosage

Raw silk fabrics were degummed with Papain and Esperase® 8.0L using different enzyme loadings (expressed as Units of protease per g of silk fabric, U·g$^{-1}_{fabric}$) and the physicochemical (weight loss, crystallinity index, whiteness, whiteness after bleaching) and low-stress mechanical properties (Kawabata evaluation system) of the preterated fabrics were assessed. Untreated and conventionally degummed (using Marseille soap) silk fabrics were used as controls.

3.2.1. Weight loss

The weight loss (or degumming loss) represents a quantitative evaluation of the degumming efficiency after standard or enzymatic degumming. The effect of enzyme dosage on the extent of sericin removal was studied by treating silk fabric samples for 60 min with different amounts of the two proteases. The results are depicted in Figure 2 both as weight loss (Fig. 2a) and degumming efficiency (Fig. 2b).

Degumming loss increased linearly as the amount of papain increased, attaining a value of 10.2 % (w/w) (degumming efficiency 50%). Esperase® 8.0L was more efficient in sericin removal as judged by the weight loss (Fig. 2a). Maximum value (20.1%, w/w, degumming efficiency nearly 99%) was obtained at enzyme loading of 75 U·g$^{-1}_{fabric}$. Degumming loss of silk fabrics treated with Marseille soap attained a value of 20.4% (w/w).

Without enzymes, the degumming loss was negligible, owing to the low treatment temperature. In fact, it is well known that sericin can be removed by using water alone, but high temperature is needed to attain complete degumming (110-120°C, under pressure).

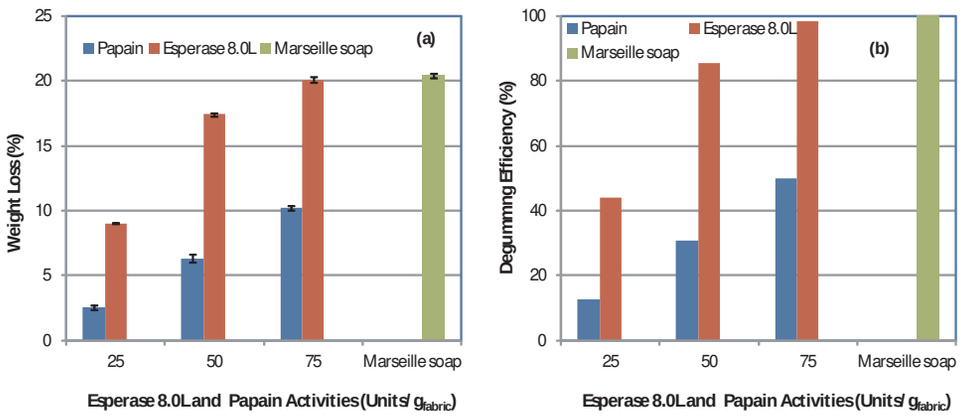


Figure 2. a) weight loss of silk fabrics treated with different types of proteases at enzyme dosages ranged from 25 to 74 $U \cdot g^{-1}_{fabric}$ and (b) degumming efficiency of the enzymatic process

The amount of sericin in raw silk measured in terms of weight loss varies between 17and 38%. Some of the good Chinese and Japanese varieties show about 17 to 17.5%, while yellow Italian silk has about 23% sericin and some Thai varieties have as high as 38% [27].

3.2.2. Wettability and crystallinity

Wettability was a function of both enzyme dosage and type of protease. Silk fabrics treated with Esperase® 8.0L at 75 $U \cdot g^{-1}_{fabric}$ showed adequate water absorbency (<1 sec). On the other hand silk fabrics treated with papain showed higher wetting times namely more hydrophobic fabrics. Although the implementation of higher papain activities formed silk fabrics with lower wetting times (Table 7) these values were higher than 1 sec, which is the maximum wetting time required for efficient dyeing and finishing. The lowest wetting time achieved using papain was 3.65 sec at the highest papain activity (75 $U \cdot g^{-1}_{fabric}$).

Treatment	Wetting time (sec)	Crystallinity Index (%)
Papain ($U \cdot g^{-1}_{fabric}$)		
25	6.12 ± 0.25	61.73 ± 1.48
50	4.16 ± 0.16	62.27 ± 0.98
75	3.65 ± 0.19	63.90 ± 0.89
Esperase® 8.0L ($U \cdot g^{-1}_{fabric}$)		
25	1.80 ± 0.20	63.90 ± 1.30
50	1.36 ± 0.15	66.61 ± 1.10
75	<1	66.72 ± 1.20
Marseille soap	<1	66.48 ± 1.35
No enzyme	6.85 ± 0.20	63.90 ± 1.27

Table 7. Wetting time and Crystallinity Index of silk fabrics treated conventionally (Marseille soap) or enzymatically.

The microstructure of cotton fabrics was investigated by X-ray diffraction. The results are presented on Table 7. The diffraction curves of all silk fibers exhibit the typical pattern of a silk II crystal with high crystallinity [19].

Silk fabrics treated with Esperase® 8.0L exhibited higher Crystallinity Index values compared to papain treated ones. Furthermore, at higher Esperase® 8.0L the crystallinity of the fabric was superior to that treated conventionally (Marseille soap).

3.2.3. Whiteness and whiteness after bleaching with H_2O_2

Natural coloring matters present in silk are associated mainly with sericin and hence are eliminated during degumming. The natural colouring matter of silk can be roughly divided into yellow, green and brown pigments. However the residual pigments are adsorbed by fibroin and hence silk fabrics made from yellow raw silk after degumming are not white but have a cream colour [65]. Lustre is one of the most important properties of silk, hence the method of degumming is significant.

A slight increase in whiteness was observed after treatment with the two different proteases compared to the fabric treated in the absence of enzyme. Esperase® 8.0L exhibited better results compared to papain. However, whiteness of silk fabrics treated with Marseille soap was superior to that of enzymatically treated fabrics (Figure 3a).

The results are in contrast to those reported by Chopra et al., [49] who demonstrated that enzyme treated samples rate marginally better than the soap-treated samples.

Bleaching of silk is for white and pastel shades only. Degummed silk fabrics present a slightly off-white in colour, because of some sericin, which is stubbornly stuck to the fibrin [65]. After enzymatic and conventional degumming the silk fabrics were bleached with H_2O_2. The results indicated that the whiteness after bleaching of all Esperase® 8.0L treated fabrics were the highest (Figure 3b).

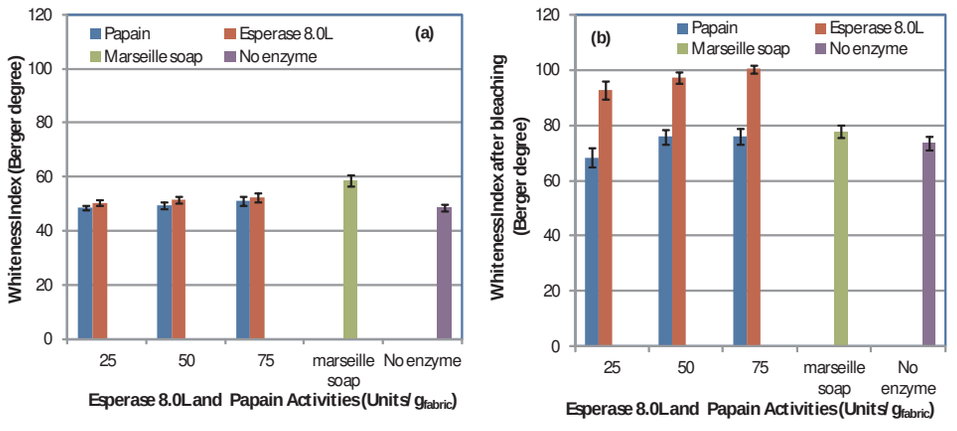


Figure 3. a) Whiteness Index and (b) Whiteness Index after bleaching with H_2O_2 of degummed silk fabrics using Marseille soap (conventional treatment) or papain and Esperase® 8.0L at different loadings.

3.2.4. Bending property

Fabric bending property is apparently a function of the bending property of its constituent yarns. *B* is bending rigidity, a measure of a fabric's ability to resist bending deformation. In other words, it reflects the difficulty with which a fabric can be deformed by bending.This parameter is particularly critical in the tailoring of lightweight fabrics. The higher the bending rigidity, the higher a fabric's ability to resist when it is bent by external force like what happens during fabric manipulation in spreading and sewing.

Enzymatically treated silk fabrics exhibited higher *B* values compared to silk fabrics treated only with buffer solution (no enzyme) (Table 8). Bending rigidity was affected by both type of protease and enzyme concentration. Increasing enzyme concentration resulted in higher *B* values, namely in more stiff fabrics. Silk fabrics treated with papain exhibited 25-116% increase in *B* values while silk fabrics treated with Esperase® 8.0L showed higher increase namely 44-137% in comparison with the buffer treated silk fabrics. Enzymatically treated silk fabrics are less rigid, softer compared to conventionally degummed fabrics.

Chopra et al., [49] reported that enzyme treated silk fabrics exhibited increased bending rigidity compared to soap treated ones. In the present study, even though an increase in bending rigidity was observed through the different experimental conditions, fabric treated with Marseille soap exhibited the highest value of this property.

3.2.5. Shear property

Shear rigidity *G* provides a measure of the resistance to rotational movement of the warp and weft threads within a fabric when subjected to low levels of shear deformation. The lower the value of *G*, the more readily the fabric will conform to three-dimensional curvatures.

Enzymatically treated silk fabrics showed lower *G* values compared to untreated and conventionally treated silk fabrics. Shear rigidity (*G*) of biotreated fabrics, for a given protease, decreased, when enzyme loading was increased (Table 8). The silk fabrics treated with papain showed 5-63% decrease in *G* values for the different enzyme activities, while the fabrics treated with Esperase® 8.0L showed a higher decrease in *G* values which ranged between 65-75%. Lower *G* values, of the enzymatically treated silk fabrics is "translated" in softer fabrics with better drape (Table 8).

3.2.6. Tensile property

The tensile behaviour of fabric is closely related to the inter-fiber friction effect, the ease of crimp removal and the load-extension properties of the yarns themselves. Through the KES apparatus the EMT parameter was determined which reflects fabric's extensibility, a measure of a fabric's ability to be stretched under tensile load. The larger the EMT, the more extensible the fabric.

Type of treatment	Bending rigidity (*B*) ($gf \cdot cm^2 \cdot cm^{-1}$)			Shear stiffness (*G*) ($gf \cdot cm^{-1} \cdot degree^{-1}$)		
	warp	weft	*mean*	warp	weft	*mean*
No enzyme	0.0205±0.0005	0.0117±0.0001	*0.0161*	1.80±0.02	1.60±0.01	*1.70*
P 25 $U \cdot g^{-1}_{fabric}$	0.0256±0.0007	0.0147±0.0003	*0.0202*	1.70±0.03	1.53±0.02	*1.62*
P 50 $U \cdot g^{-1}_{fabric}$	0.0356±0.0009	0.0185±0.0004	*0.0271*	1.30±0.01	1.25±0.01	*1.27*
P 75 $U \cdot g^{-1}_{fabric}$	0.0463±0.0006	0.0234±0.0002	*0.0349*	0.75±0.01	0.50±0.01	*0.63*
E 25 $U \cdot g^{-1}_{fabric}$	0.0337±0.0004	0.0126±0.0003	*0.0232*	0.85± 0.01	0.35±0.01	*0.60*
E 50 $U \cdot g^{-1}_{fabric}$	0.0321±0.0006	0.0283±0.0002	*0.0302*	0.55±0.01	0.35±0.01	*0.45*
E 75 $U \cdot g^{-1}_{fabric}$	0.0479±0.0007	0.0283±0.0005	*0.0381*	0.50±0.01	0.35±0.01	*0.43*
Marseille soap	0.0537±0.0006	0.0264±0.0003	*0.0401*	1.80±0.03	1.60±0.01	*1.70*

P: Papain, E: Esperase® 8.0L

Table 8. Comparison of micromechanical properties of silk fabrics treated for 60 min with various levels of papain and Esperase® 8.0L, with reference and conventionally degummed materials

Tensile property increased when enzyme concentration increased for both protease used (Table 9). Treatment with Esperase® 8.0L and particularly with high concentrations resulted in silk fabrics more elastic compared to those treated with Marseille soap (Table 9).

Type of treatment	Tensile (%)		
	warp	weft	*mean*
No enzyme	5.15 ± 0.2	1.63 ± 0.07	*3.39*
P 25 $U \cdot g^{-1}_{fabric}$	4.09 ± 0.3	1.24 ± 0.04	*2.67*
P 50 $U \cdot g^{-1}_{fabric}$	3.97 ± 0.2	0.94 ± 0.02	*2.53*
P 75 $U \cdot g^{-1}_{fabric}$	4.53 ± 0.3	1.33 ± 0.04	*2.93*
E 25 $U \cdot g^{-1}_{fabric}$	4.51 ± 0.3	1.05 ± 0.04	*2.78*
E 50 $U \cdot g^{-1}_{fabric}$	5.37 ± 0.1	1.49 ± 0.02	*3.43*
E 75 $U \cdot g^{-1}_{fabric}$	6.32 ± 0.3	1.15 ± 0.01	*3.74*
Marseille soap	4.29 ± 0.2	1.42 ± 0.07	*2.86*

P: Papain, E: Esperase® 8.0L

Table 9. Comparison of micromechanical properties of silk fabrics treated for 60 min with various levels of papain and Esperase® 8.0L, with reference and conventionally degummed materials

3.3. Synergistic action of protease-lipase on silk degumming: Effect of treatment time

Proteases and lipases are normally used in combination for degumming and removing others impurities such as waxes, fats, mineral salts and pigments [49]. Waxes and fats as well as colorants and mineral components occur exclusively in silk gum layer (sericin) [66]. The combined effect of enzyme activity (expressed as Units of protease per g of silk fabric) and treatment time on physicochemical and low-stress mechanical properties of cotton fabric was investigated. Raw silk fabrics were treated with two different proteases namely papain and Esperase® 8.0L at an enzyme activity of 50 and 75 $U{\cdot}g^{-1}_{fabric}$ combined with a lipase (Lipolase® Ultra 50T) at a constant activity of 50 $U{\cdot}g^{-1}_{fabric\ ic}$ for 30, 60, 90 min. Furthermore, buffer treated and conventional degummed silk fabrics were used as controls.

3.3.1. Weight loss

The results are depicted in Figure 4 both as weight loss (Figure 4a) and degumming efficiency (Figure 4b). Without enzymes, the weight loss was negligible.

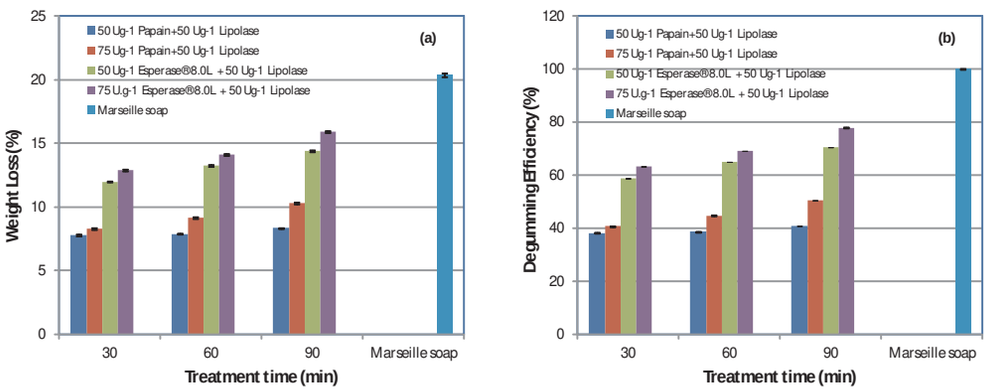


Figure 4. a) weight loss of silk fabrics treated with mixtures of proteases/lipase for 30, 60 and 90 min and (b) degumming efficiency of the enzymatic process

The degree of fabric's degradation was found to be affected by both enzyme concentration and treatment time. Increasing enzyme concentration and treatment time resulted in increased weight loss values. The combination of Esperase® 8.0L with Lipolase® Ultra 50T was found superior to that of papain. The highest weight loss (15.9%, w/w) was achieved when fabrics were treated with 75 $U{\cdot}g^{-1}_{fabric}$ Esperase® 8.0L + 50 $U{\cdot}g^{-1}_{fabric}$ Lipolase® Ultra 50T for 90min, while weight loss during conventional degumming was higher (20.4 %, w/w).

Degumming efficiency did not exceed 78% (Figure 4b). The proteases and lipase combination did not improve the results obtained when the fabrics degummed only with proteases.

3.3.2. Wettability and crystallinity index

Wettability seemed to depend more on the enzymes used for degumming and less on treatment time. (Table 10). The most effective combination is that of Esperase® 8.0L with Lipo-

http://dx.doi.org/10.5772/53730

lase® Ultra 50T. This combination at the highest enzyme loading used, exhibited adequate water absorbency (<1 sec) after 60 min of treatment. The mixture of papain with Lipolase® Ultra 50T did not improve the wettability of the silk fabrics compared to those treated only with papain, even at higher treatment times.

Crystallinity index increased with treatment time for all enzyme combination tested (Table 10). The combination of Esperase® 8.0L with lipase was more effective compared to that of papain. However, the addition of lipase did not seem to improve Crystallinity Index in any condition tested, compared to the action of single protease.

Treatment	Wetting time (sec)	Crystallinity Index (%)
50 $U \cdot g^{-1}_{fabric}$ Papain + 50 $U \cdot g^{-1}_{fabric}$ Lipolase® Ultra 50T		
30 min	5.90 ± 0.18	62.80 ± 1.30
60 min	5.80 ± 0.12	63.20 ± 1.24
90 min	5.60 ± 0.10	63.32 ± 1.31
75 $U \cdot g^{-1}_{fabric}$ Papain + 50 $U \cdot g^{-1}_{fabric}$ Lipolase® Ultra 50T		
30 min	4.72 ± 0.20	62.90 ± 1.36
60 min	3.60 ± 0.15	63.20 ± 1.40
90 min	3.30 ± 0.30	63.32 ± 1.26
50 $U \cdot g^{-1}_{fabric}$ Esperase® 8.0L + 50 $U \cdot g^{-1}_{fabric}$ Lipolase® Ultra 50T		
30 min	2.20 ± 0.20	64.80 ± 1.60
60 min	2.00 ± 0.19	65.00 ± 1.80
90 min	1.50 ± 0.18	65.10 ± 1.10
75 $U \cdot g^{-1}_{fabric}$ Esperase® 8.0L + 50 $U \cdot g^{-1}_{fabric}$ Lipolase® Ultra 50T		
30 min	1.47 ± 0.09	65.20 ± 1.50
60 min	<1	65.40 ± 1.40
90 min	<1	66.00 ± 1.10
Marseille soap	<1	66.48 ± 1.35
No enzyme	6.85 ± 0.20	63.90 ± 1.27

Table 10. Wetting time and Crystallinity Index of silk fabrics treated conventionally (Marseille soap) or enzymatically.

3.3.3. Whiteness and whiteness after bleaching with H_2O_2

The synergistic action of protease-lipase improved the whiteness of the silk fabrics (Figure 5a). Increasing enzyme concentration and treatment time resulted in increased whiteness values. The highest whiteness values for each combination were observed at highest enzyme loading and after 90 min of treatment. Those values were 55.2 and 59.8 Berger degree for papain+ Lipolase® Ultra 50T and Esperase® 8.0L+ Lipolase® Ultra 50T, respectively. It should

be noted that the conventional degumming resulted in a lower whiteness value (58.8 Berger degree) (Figure 5a). The addition of lipase improved the whiteness of the silk fabrics compared to results obtained by the use of protease only. For example the whiteness of the silk fabrics treated with 75 $U \cdot g^{-1}_{fabric}$ Esperase® 8.0L for 60 min was found 52.2 Berger degree, while the corresponding value of the fabrics treated at the same conditions adding 50 $U \cdot g^{-1}_{fabric}$ Lipolase® Ultra 50T was 57.3 Berger degree.

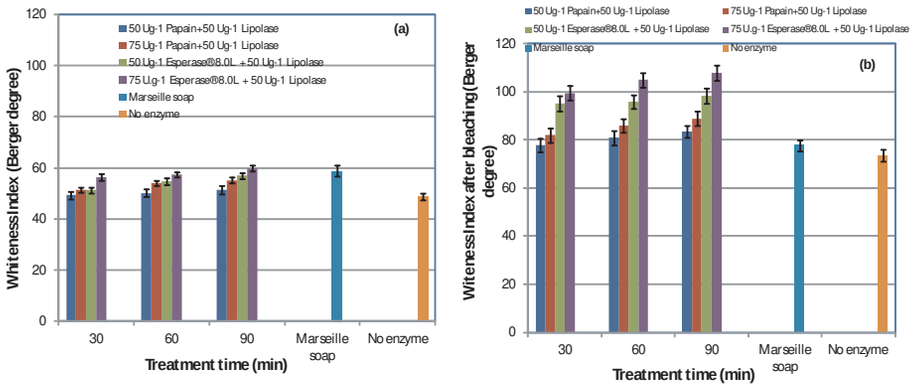


Figure 5. a) Whiteness Index and (b) Whiteness Index after bleaching with H_2O_2 of degummed silk fabrics using Marseille soap (conventional treatment) or mixture of papain and Esperase® 8.0L with Lipolase® Ultra 50T at different loadings and treatment times.

The synergistic action of protease-lipase improved the bleaching effect of the silk fabrics (Figure 5b). Increasing enzyme concentration and treatment time resulted in increased whiteness values after bleaching. The whiteness after bleaching of the silk fabrics treated with papain and Lipolase® Ultra 50T ranged between 78.0-89.0 Berger degree, while the corresponding values for the fabrics treated only with papain were found between 68.4-76.0 Berger degree. Higher whiteness values were observed for the fabrics treated with the mixture of Esperase® 8.0L with Lipolase® Ultra 50T. The whiteness ranged between 95.23-108 Berger degree. The silk fabrics treated with Esperase® 8.0L + Lipolase® Ultra 50T exhibited 40% increase in whiteness in comparison with those treated with the conventional method with Marseille soap, while the corresponding increase in whiteness for the fabrics treated with papain and Lipolase® Ultra 50T was 15%.

3.3.4. Bending property

Enzymatically and soap treated silk fabrics exhibited higher *B* values compared to untreated silk fabrics. Bending rigidity of the enzymatically treated silk fabrics was affected by enzyme concentration and treatment time. Increasing enzyme concentration and treatment time resulted in lower *B* values, namely in less rigid fabrics compared to conventionally treated ones (Table 11).

One interesting aspect is that addition of Lipolase® Ultra 50T caused further decrease in *B* values compared to those obtained when silk fabrics were treated only with protease. For example, silk fabrics treated with 75 $U \cdot g^{-1}_{fabric}$ Esperase® 8.0L exhibited a *B* value of 0.0381 ($gf \cdot cm^2 \cdot cm^{-1}$) after 60

min (Table 8). Addition of 50 $U \cdot g^{-1}_{fabric}$ Lipolase® Ultra 50T resulted in 43% decrease in *B* value (0.0291, $gf \cdot cm^2 \cdot cm^{-1}$). The same pattern was observed for all conditions tested.

Type of treatment	Bending rigidity (*B*) ($gf \cdot cm^2 \cdot cm^{-1}$)			Shear stiffness (*G*) ($gf \cdot cm^{-1} \cdot degree^{-1}$)		
	warp	weft	*mean*	warp	weft	*mean*
No enzyme	0.0205±0.0005	0.0117±0.0001	*0.0161*	1.80±0.02	1.60±0.01	*1.70*
50 Ug^{-1} Papain + 50 Ug^{-1} Lipolase® Ultra 50T						
30 min	0.0300±0.0003	0.0200±0.0004	*0.0250*	1.50±0.02	1.34±0.01	*1.42*
60 min	0.0250±0.0005	0.0170±0.0002	*0.0210*	1.44±0.03	1.30±0.03	*1.37*
90 min	0.0200±0.0002	0.0160±0.0002	*0.0180*	1.28±0.02	1.22±0.02	*1.25*
75 Ug^{-1} Papain + 50 Ug^{-1} Lipolase® Ultra 50T						
30 min	0.0220±0.0002	0.0180±0.0001	*0.0200*	1.34±0.02	1.30±0.01	*1.32*
60 min	0.0170±0.0003	0.0140±0.0002	*0.0155*	1.29±0.03	1.25±0.01	*1.27*
90 min	0.0160±0.0002	0.0130±0.0003	*0.0145*	1.26±0.01	1.23±0.02	*1.25*
50 Ug^{-1} Esperase® 8.0L + 50 Ug^{-1} Lipolase® Ultra 50T						
30 min	0.0424±0.0009	0.0233±0.0002	*0.0329*	1.85±0.04	1.50±0.05	*1.68*
60 min	0.0341±0.0005	0.0200±0.0001	*0.0271*	1.80±0.04	1.45±0.05	*1.63*
90 min	0.0330±0.0007	0.0187±0.0003	*0.0259*	1.60±0.06	1.40±0.05	*1.50*
75 Ug^{-1} Esperase® 8.0L + 50 Ug^{-1} Lipolase® Ultra 50T						
30 min	0.0491±0.0005	0.0231±0.0002	*0.0361*	1.90±0.06	1.20±0.04	*1.55*
60 min	0.0242±0.0003	0.0195±0.0005	*0.0219*	1.58±0.03	0.90±0.03	*1.24*
90 min	0.0214±0.0007	0.0170±0.0003	*0.0192*	1.53±0.02	0.10±0.02	*0.81*
Marseille soap	0.0537±0.0006	0.0264±0.0003	*0.0401*	1.80±0.03	1.60±0.01	*1.70*

Table 11. Comparison of micromechanical properties of silk fabrics treated at varying durations with various levels of Papain and Esperase® 8.0L combined with constant level of Lipolase® Ultra 50T, with reference and conventionally degummed materials

3.3.5. Shear property

Combined action of proteases with Lipolase resulted in silk fabrics with lower *G* values compared to untreated and conventional treated silk fabrics (Table 11). Increasing protease concentration and treatment time resulted in silk fabrics less rigid with better drape (lower *G* values) compared to those treated with Marseille soap.

Shear rigidity was also affected by the addition of Lipolase® Ultra 50T. For example, silk fabrics treated with 75 $U \cdot g^{-1}_{fabric}$ Esperase® 8.0L exhibited a *G* value of 0.43 ($gf \cdot cm^{-1} \cdot degree^{-1}$) after 60 min (Table 8). Addition of 50 U/g Lipolase® Ultra 50T resulted in 65% increase in *G* value (1.24, $gf \cdot cm^{-1} \cdot degree^{-1}$). The same pattern was observed for all conditions tested.

3.3.6. Tensile property

The elasticity, (tensile strength) of the silk fabrics treated with papain and Lipolase® Ultra 50T was higher than the elasticity of the untreated and soap treated silk fabrics and seemed to be dependent on protease dosage and treatment time (Table 12). Increasing papain dosage and treatment time resulted in an increase elasticity of the enzymatically treated of silk fabrics. At highest papain dosage and for treatment times 60 min and more, the elasticity was superior to that of Marseille soap treated silk.

Opposite trend was observed for the fabrics treated with Esperase® 8.0L + Lipolase® Ultra 50T combination. Increasing protease dosage and treatment time the silk fabrics became more anelastic (Table 12).

Type of treatment	Tensile (%)		
	warp	**weft**	***mean***
No enzyme	5.15 ± 0.2	1.63 ± 0.07	*3.39*
50 $U \cdot g^{-1}_{fabric}$ Papain + 50 $U \cdot g^{-1}_{fabric}$ Lipolase® Ultra 50T			
30 min	3.30 ± 0.10	2.70 ± 0.20	*3.00*
60 min	3.60 ± 0.10	3.00 ± 0.10	*3.30*
90 min	3.90 ± 0.20	3.10 ± 0.10	*3.50*
75 $U \cdot g^{-1}_{fabric}$ Papain + 50 $U \cdot g^{-1}_{fabric}$ Lipolase® Ultra 50T			
30 min	4.10 ± 0.20	3.30 ± 0.20	*3.70*
60 min	4.20 ± 0.20	3.40 ± 0.30	*3.80*
90 min	4.40 ± 0.40	3.50 ± 0.20	*3.95*
50 $U \cdot g^{-1}_{fabric}$ Esperase® 8.0L + 50 $U \cdot g^{-1}_{fabric}$ Lipolase® Ultra 50T			
30 min	4.85 ± 0.10	1.74 ± 0.05	*3.30*
60 min	4.75 ± 0.20	1.65 ± 0.02	*3.20*
90 min	4.60 ± 0.20	1.50 ± 0.03	*3.05*
75 $U \cdot g^{-1}_{fabric}$ Esperase® 8.0L + $U \cdot g^{-1}_{fabric}$ Lipolase® Ultra 50T			
30 min	4.96 ± 0.10	1.87 ± 0.09	*3.42*
60 min	4.62 ± 0.40	1.55 ± 0.05	*3.09*
90 min	4.51 ± 0.30	1.36 ± 0.04	*2.94*
Marseille soap	4.29 ± 0.20	1.42 ± 0.02	*2.86*

Table 12. Comparison of micromechanical properties of silk fabrics treated for different times with various levels of papain and Esperase® 8.0L combined with constant level of Lipolase® Ultra 50T, with reference and conventionally degummed materials

http://dx.doi.org/10.5772/53730

Addition of Lipolase® Ultra 50T in papain degumming mixture produces more elastic fabrics compared to those obtained by single papain treatement. For example silk fabrics treated with papain at 75 $U \cdot g^{-1}_{fabric}$ fabric for 60 min exhibited a tensile strength value of 2.93%. Addition of 50 $U \cdot g^{-1}_{fabric}$ Lipolase® Ultra 50T increases this value at 3.80%. On the other hand the use of Esperase® 8.0L + Lipolase® Ultra 50T mixture causes a decrease in the fabric's elasticity.

4. Conclusions

Enzymatic degumming of silk fabric using proteolytic enzymes (papain, Esperase® 8.0L) and mixtures of thereof with a lipolytic (Lipolase® Ultra 50T) enzyme under mild conditions was investigated. The results obtained are encouraging in comparison with those of the conventional method of degumming (Marseille soap).

Silk fabrics treated with Esperase® 8.0L (an alkaline protease) at high concentration (75 U/g fabric) for 60 min exhibited degumming efficiency nearly 99%, which indicate almost complete removal of sericin from the surface of the fabric. Esperase® 8.0L treatment resulted in fabrics with adequate wettability, higher CrI and whitenees after bleaching compared to those treated conventionally (Marseille soap).

On the other hand papain was not so effective in the degumming process. This could probably be attributed to the different substrate specificity of the proteases, that is, the chemical structure of the target cleavage site. The silk fabrics treated with both proteolytic enzymes exhibited low bending and shear rigidity and higher elasticity compared to Marseille soap treated silk fabrics. This means that the silk fabrics after enzymatic degumming were softer, less rigid, with better drape and higher elasticity compared to those treated with Marseille soap.

Since the silk of *Bombyx mori* apart of the proteins fibroin and sericin, also contains fats, wax etc., the combined effect of proteolytic enzymes with a lipolytic one was investigated. Combined action of protease with lipase (Lipolase® Ultra 50T) resulted in lower degumming efficiency compared (under the same conditions) with protease treatment only, but generated silk fabrics with significant improvement in whiteness after bleaching. As far as the properties measured in the Kawabata evaluation system the combination of proteolytic with lipolytic enzymes resulted in silk fabrics with extremely low bending rigidity, reduced shear stiffness and with higher elasticity compared to Marseille soap treated. This means that the fabrics were softer, less rigid with better drape compared to conventionally treated. Addition of lipolase caused decrease in bending and increase in shear rigidity and elasticity of the silk fabrics compared to those treated only with protease.

Silk degumming is a high resource consuming process as far as water and energy are concerned. Moreover, it is ecologically questionable for the high environmental impact of effluents. 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. This could be made possible by the milder treatment conditions, the recycling of processing water, the recovery of valuable by-products such as sericin peptides, and the lower environmental impact of effluents.

Author details

Styliani Kalantzi, Diomi Mamma and Dimitris Kekos*

*Address all correspondence to: kekos@chemeng.ntua.gr

Biotechnology Technology, School of Chemical Engineering, National Technical University of Athens, Zografou Campus, Athens, Greece

References

[1] The Silk Association of Great Britain. http://www.silk.org.uk/history.php (accessed 30 March 2012)

[2] FAO: Food and Agriculture Organization of the United Nations. www.fao.org (accessed 25 March 2012)

[3] Jolly MS, Sen SK. Patterns of follicular imprints in egg shell-a species-specific character in Antheraea. Bull Entomol 1969;10 32-38.

[4] Jolly MS,Narasimhanna MN,Wartkar VB. Arrangement of tubercular setae in Antheraea species (Lepidoptera: Saturniidae). Entomologist 1970;103 18-23.

[5] Souche YS, Patole M. Sequence analysis of mitochondrial 16S ribosomal RNA gene fragment from seven mosquito species. Journal of Biosciences 2000;25 61-66.

[6] Tan YD, Wan C, Zhu Y, Lu C, Xiang Z, Deng HW. An amplified fragment length polymorphism map of the silkworm. Genetics 2001;157 1227-1284.

[7] Mahendran B, Padhi BK, Ghosh SK, Kundu SC. Genetic variation in ecoraces of tropical tasar silkworm, Antheraea mylitta D. using RFLP technique. Current Science 2005;90 100-103.

[8] Mahendran B, Acharya C, Dash R, Ghosh SK, Kundu SC. Repetitive DNA in tropical tasar silkworm Antheraea mylitta. Gene 2006;370 51-57.

[9] Li A, Zhao Q, Tang S, Zhang Z, Pan S, Shen G. Molecular phylogeny of the domesticated silkworm, Bombyx mori, based on the sequences of mitochondrial cytochrome b genes. Journal of Genetics 2005;84 137-142.

[10] http://www.csb.gov.in/silk-sericulture/silk/ (accessed 30 March 2012)

[11] Kundu SC, Dash BC, Dash R, Kaplan DL. Natural protective glue protein, sericin bioengineered by silkworms: Potential for biomedical and biotechnological applications. Progress in Polymer Science 2008;33 998-1012.

[12] Sinohara H, Asano Y, Fukui A. Glycopeptides from silk fibroin of Bombyx mori. Biochimica et Biophysica Acta 1971;237 273-279.

[13] Prudhomme J-C, Couble P., Garel J-P. and Daillie J. Silk synthesis. In: Kerkut GA, Gilbert LJ. (eds) Comprehensive Insect Physiology, Biochemistry and Pharmacology. Oxford: Pergamon Press; 1985. Vol 10 p571-594.

[14] Inoue S, Tanaka K, Arisaka F, Kimura S, Ohtomo K, Mizuno S. Silk fibroin of Bombyx mori is secreted, assembling a high molecular mass elementary unit consisting of H-chain, L-chain, and P25, with a 6:6:1 molar ratio. Journal of Biological Chemistry 2000;275(51) 40517-40528.

[15] Zhou CZ, Confalonieri F, Jacquet M, Perasso R, Li ZG, Janin J. Silk fibroin: structural implications of a remarkable amino acid sequence. Proteins:Structure, Function and Genetics 2001;44 119-122.

[16] Vepari C, Kaplan DL. Silk as a biomaterial. Progress in Polymer Science 2007;32 991-1007.

[17] Li G, Liu H, Li TD, Wang J. Surface modification and functionalization of silk fibroin fibers/fabric toward high performance applications. Materials Science and Engineering C 2012;32 627-636.

[18] Teramoto H, Kakazu A, Asakura T. Native structure and degradation pattern of silk sericin studied by 13C NMR spectroscopy. Macromolecules 2006;39 6-8.

[19] Shao ZZ, Vollrath F. Surprising strength of silkworm silk. Nature 2002;418 741.

[20] Jin HJ, Kaplan DL. Mechanism of silk processing in insects and spiders. Nature 2003;424 1057-1061.

[21] Sinohara H. Glycopeptides isolated from sericin of the silkworm, Bombyx mori. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 1979;63(1) 87-91.

[22] Vollrath F, Knight DP. Liquid crystalline spinning of spider silk. Nature 2001;410 541-548.

[23] Sprague KU. Bombyx mori silk proteins: Characterization of large polypeptides. Biochemistry 1975;14 925-931.

[24] Takasu Y, Yamada H, Tsubouchi K. Isolation of three main sericin components from the cocoon of the silkworm, Bombyx mori. Bioscience Biotechnology and Biochemistry 2002;66 2715-2718.

[25] Colomban P., Dinh HM, Riand J, Prinsloo LC, Mauchamp B. Nanomechanics of single silkworm and spider fibres: a Raman and micro-mechanical in situ study of the conformation change with stress. Journal of Raman Spectroscopy 2008;39(12) 1749-1764.

[26] Freddi G, Pessina G, Tsukada M. Swelling and dissolution of silk fibroin (Bombyx mori) in N-methyl morpholine N-oxide. International Journal of Biological Macromolecules 1999;24(2-3) 251-263.

[27] Gulrajani ML. Degumming of silk. Review of Progress in Coloration 1992;22 79-89.

[28] Rajasekhar A, Ravi V, Reddy MN, Rao KRSS. Thermostable Bacterial Protease - A New Way for Quality Silk Production. International Journal of Bio-Science and Bio-Technology 2011;3(2) 43-58.

[29] Uddin K, Hossain S. A comparative study on silk dyeing with acid dye and reactive dye. International Journal of Engineering & Technology IJET-IJENS 2010;10(6) 21-26.

[30] Wray LS, Hu X, Gallego J, Georgakoudi I, Omenetto FG, Schmidt D, Kaplan DL. Effect of processing on silk-based biomaterials: Reproducibility and biocompatibility. Journal of Biomedical Materials Research B: Applied Biomaterials 2011;99B(1) 89-101.

[31] Rajkhowa R, Wang LJ, Kanwar JR, Wang XG. Molecular weight and secondary structure change in eri silk during alkali degumming and powdering. Journal of Applied Polymer Science 2011;119 1339-1347

[32] Svilokos Bianchi A, Colonna GM. Developments in the degumming of silk. Melliand Textilberichte 1992;73 68-75.

[33] Freddi G, Mossotti R, Innocenti R. Degumming of silk fabric with several proteases. Journal of Biotechnology 2003;106(1) 101-112.

[34] Freddi G, Allara G, Candiani G. Degumming of silk with tartaric acid. Journal of the Society of Dyers and Colourists 1996;112 191-195.

[35] Gulrajani ML, Chatterjee A. Degumming of silk with oxalic acid. Indian Journal of Fibre and Textile Research 1992;17 1.

[36] Gulrajani ML, Sethi S, Guptha S. Some studies in degumming of silk with organic acids. Journal of the Society of Dyers and Colourists 1992;108(2) 79-86.

[37] Khan MR, Tsukada M, Gotoh Y, Morikawa H, Freddi G, Shiozaki H. Physical properties and dyeability of silk fibers degummed with citric acid. Bioresource Technology 2010;101 8439-8445.

[38] Vohrer U, Müller H, Oehr C. Glow-discharge treatment for the modification of textiles. Surface and Coatings Technology 1998;98(1-3) 1128-1131.

[39] Borcia G, Anderson CA, Brown NMD. Surface treatment of natural and synthetic textiles using a dielectric barrier discharge. Surface and Coatings Technology 2006;201 3074-3081.

[40] Yip J, Chan K, Sin KM, Lau KS. Low temperature plasma-treated nylon fabrics. Journal of Materials Processing Technology 2002;123(1) 5-12.

[41] Long JJ, Wang HW, Lu TQ, Tang RC, Zhu YW. Application of Low-Pressure Plasma Pretreatment in Silk Fabric Degumming Process. Plasma Chemistry and Plasma Processing 2008:28 701-713.

[42] Mahmoodi NM, Moghimi F, Arami M, Mazaheri F. Silk Degumming Using Microwave Irradiation as an Environmentally Friendly Surface Modification Method. Fibers and Polymers 2010;11(2) 234-240.

[43] Mahmoodi NM, Arami M, Mazaheri F, Rahimi S. Degradation of sericin (degumming) of Persian silk by ultrasound and enzymes as a cleaner and environmentally friendly process. Journal of Cleaner Production 2010;18 146-151.

[44] Yuksek M, Kocak D, Beyit A, Merdan N. Effect of Degumming Performed with Different Type Natural Soaps and Through Ultrasonic Method on the Properties of Silk Fiber. Advances in Environmental Biology 2012;6(2) 801-808.

[45] Gübitz GM, Cavaco-Paulo A. Editorial: Biotechnology in the textile industry – perspectives for the new millennium. Journal of Biotechnology 2001;89 89-90.

[46] Gulrajani ML, Gupta SV, Gupta A, Suri M. Degumming of silk with different protease enzymes. Indian Journal of Fibre and Textile Research 1996;21 270-275.

[47] Gulrajani ML, Sen S, Soria A, Suri M. Efficacy of proteases on degumming of dupion silk. Indian Journal of Fibre and Textile Research 1998;23, 52-58.

[48] Gulrajani ML, Agarwal R, Chand S. Degumming of silk with fungal protease. Indian Journal of Fibre and Textile Research 2000b;25 138-142.

[49] Chopra S, Chattopadhyay R, Gulrajani ML. Low stress mechanical properties of silk fabric degummed by different methods. Journal of the Textile Institute 1996;87 542-553.

[50] Nakpathom M, Somboon B, Narumol N. Papain Enzymatic Degumming of Thai Bombyx mori Silk Fibers. Journal of Microscopy Society of Thailand 2009;23(1): 142-146.

[51] Arami M, Rahimi S, Mivehie L, Mazaheri F, Mahmoodi NM. Degumming of Persian Silk with Mixed Proteolytic Enzymes. Journal of Applied Polymer Science 2007;106 267–275.

[52] Gulrajani ML, Agarwal R, Grover A, Suri M. Degumming of silk with lipase and protease. Indian Journal of Fibre and Textile Research 2000a;25 69-74.

[53] Fabiani C, Pizzichini M, Spadoni M, Zeddita G. Treatment of waste water from silk degumming processes for protein recovery and water reuse. Desalination 1996;105 1-9.

[54] Zhang YQ. Applications of natural silk protein sericin in biomaterials. Biotechnology Advances 2002;20 91-100.

[55] Vaithanomsat P, Kitpreechavanich V. Sericin separation from silk degumming wastewater. Separation and Purification Technology 2008;59 129-133.

[56] Aramwit P, Siritientong T, Srichana T. Potential applications of silk sericin, a natural protein from textile industry by-products. Waste Management and Research 2012;30(3) 217-224.

[57] Kocabiyik S, Erdem B. Intracellular alkaline proteases produced by thermoacidophiles: detection of protease heterogeneity by gelatin zymography and polymerase chain reaction (PCR). Bioresource Technology 2002;84 29-33.

[58] Bastida A, Sabuquillo P, Armisen, P, Fernández-Lafuente R, Huguet J, Guisán JM. A single step purification, immobilization, and hyperactivation of lipases via interfacial adsorption on strongly hydrophobic supports. Biotechnology and Bioengineering 1998;58(5) 486-493.

[59] Kalantzi S, Mamma D, Christakopoulos P, Kekos D. Effect of pectate lyase bioscouring on physical, chemical and low-stress mechanical properties of cotton fabrics. Bioresource Technology 2008;99(17) 8185-8192.

[60] AATCC Technical Manual. AATCC: Test Method 39 1980;55 286

[61] Li Y, Hardin IR. Enzymatic scouring of cotton: surfactants, agitation, and selection of enzymes. Textile Chemist and Colorist 1998;30(9) 23-29.

[62] Tzanov T, Calafell M, Guenitz, GM, Cavaco-Paulo A. Bio-preparation of cotton fabrics. Enzyme and Microbial Technology 2001;29 357-362.

[63] Segal L, Creely JJ, Martin AE, Conrad C.M. An empirical method for estimation the degree of crystallinity of native cellulose using the X-ray diffractometer. Textile Research Journal 1959;29(10) 786-794.

[64] Houde A, Kademi A, Leblanc D. Lipases and their industrial applications. Applied Biochemistry and Biotechnology 2004;118 155-170.

[65] Sonthisombat A, Speakman PT. Silk: Queen of Fibres -the Concise Story 2004 1-28. (available at http://www.en.rmutt.ac.th/prd/Journal/Silk_with_figuresnew.pdf)

[66] Zahn H, Krasowski A. Silk. In: Ullmann's Encyclopedia of Industrial Chemistry 2002