



ISSN: 0975-833X

REVIEW ARTICLE

PROTEASES FOR DEGUMMING: A NOVEL, GREEN AND ECOFRIENDLY WAY TO QUALITY SILK PRODUCTION

*Snehal V. More

Division of Biochemical Sciences, National Chemical Laboratory, Pune, India

ARTICLE INFO

Article History:

Received 24th August, 2015

Received in revised form

15th September, 2015

Accepted 17th October, 2015

Published online 30th November, 2015

Key words:

Silk, protease,
Conventional degumming,
Sericin, Enzymatic degumming.

ABSTRACT

The textile industry is the largest industry in terms of value, production and also in effluent generation. With the increasingly important requirement for textile manufacturers to reduce pollution in textile production, the use of enzymes in the chemical processing of fibers and textiles is rapidly gaining wider recognition because of their non-toxic and eco-friendly characteristics. As far as textiles are concerned, researchers' emphasis on reduction of the use of harsh chemicals and reuse of effluent waste water. Majority of enzyme is used in degumming which is a silk refining process of the drawn silk fibre or yarn. The conventional degumming methods like extraction with water, boiling off in soap, degumming with alkali/acidic solutions have certain disadvantages like, removal of the sericin with low percentages, the surface hardening and damage of the filaments, lack of stringent control over process conditions and more time duration. Substituting proteolytic enzymes in silk degumming process for harsh chemicals such as alkalis, acids or soaps and conditions should improve the quality and preserve the physical properties of silk fibroin, to bring uniform removal of sericin and to reduce the pollution levels. Use of proteolytic enzymes in silk degumming resulted in complete sericin removal and retaining tensile properties, while improving surface smoothness and luster. There is an urgent need for scientific studies for potential application of proteolytic enzymes in silk degumming process as enzymatic method is known to be eco friendly reduces energy cost and enhances the productivity and quality of silk as compared to the chemical methods

Copyright © 2015 Snehal V. More. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Citation: Snehal V. More, 2015. "Proteases for degumming: A novel, green and ecofriendly way to quality silk production", *International Journal of Current Research*, 7, (11), 23032-23038.

INTRODUCTION

Enzymes are essential for all metabolic processes and are responsible for many essential biochemical reactions in all living organisms. They are natural protein molecules that act as highly efficient catalysts in biochemical reactions, that is, they help a chemical reaction take place quickly and efficiently and are also biodegradable. Proteases belong to the class of hydrolyses, which degrade proteins into small peptides and amino acids by catalyzing the reaction involved addition of water to cleave the peptide bond. They are ubiquitous and widely distributed in plants, animals and microbes. Proteases are among the most important hydrolytic enzymes and have been studied extensively since the advent of enzymology. There is renewed interest in the study of proteolytic enzymes, mainly due to the recognition that these enzymes not only play an important role in the cellular metabolic processes but have also gained considerable attention in the industrial community (Gupta, 2002).

Proteases account for approximately 40% of the total enzyme sales in various industrial market sectors, such as detergent, food, pharmaceutical, textile, leather, diagnostics, waste management and silver recovery. Proteases are also important from a physiological point of view, as they are involved in many cellular processes like protein turn over, digestion and fungal morphogenesis, spore formation and spore germination etc. Currently, the largest share of the enzyme market has been held by detergent proteases which are active and stable at alkaline pH (Rao, 1998). Yet, there is a continued search for proteases having novel properties and newer applications for existing proteases.

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 (Arami *et al.*, 2007). It is recognized for its characters such as luster, water absorption, heat retention, smooth feeling, and comfort etc. because of its sheen and luster it is known as the Queen of all

*Corresponding author: Snehal V. More

Division of Biochemical Sciences, National Chemical Laboratory,
Pune, India

fibers. Silk fiber consists of the two fibrous proteins, fibroin and a gummed amorphous protein named sericin, which cements the fibroin fibers together. Fibroin and sericin proteins are present in about 75 and 25% of total weight respectively. Fibroin protein is a high molecular weight polypeptide (~ 350KDa), insoluble in hot water and composed of glycine, alanine and serine in molecular ratio 3:2:1, with a six residue repetition of -(Gly-Ser-Gly-Ala-Gly-Ala)_n (Zhou *et al.*, 2000). This highly regular sequence is involved in the formation of antiparallel β -pleated sheet structure, which characterizes the crystalline regions in the spun filament. 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.

Degumming is a process where sericin is totally removed from the fibroin wall to obtain shine, smoothness and other properties (Freddi *et al.*, 2003). A series of steps are involved in the silk processing: reeling, weaving, degumming, dyeing/printing and finishing. After degumming, the silk fiber becomes shiny and its elasticity improves. The post degumming condition of silk fiber, such as handling, luster and rubbing behavior is greatly dependant on the quantity of sericin remaining on the silk fibroin (Das, 2013). In the traditional methods of degumming the raw silk, fibers are treated with alkali and soap at 95°C–100°C or are boiled at elevated temperature and/or pressure for 1–2 h. Disadvantages associated with these methods are uneven degumming, strength loss of fibers and high resources consumption with respect to water and energy as well as high output of effluents with polluting substances (Gulrajani, 2000a).

Enzymatic degumming is gaining lot of attention in recent years, as it is a milder process with negligible input of hazardous chemicals and recovery of valuable byproducts such as sericin is possible. However, the use of enzymes in the silk industry is relatively unexplored and it has generated a lot of interest only in the last twenty years (More *et al.*, 2013). Microbial proteases used for degumming are mainly from *Bacillus* species, though few fungal proteases are also used (Arami *et al.*, 2007; Freddi *et al.*, 2003; Das *et al.*, 2013; Anghileri *et al.*, 2007; Gulrajani *et al.*, 1996 and Gulrajani *et al.*, 2000b) and one recent report using actinomycete protease from our group (More *et al.*, 2013). However, most of these studies are carried out with commercial enzymes such as alcalase, degummase, savinase. This review aims to analyze and update the information available on various aspects and impacts of use of proteases in silk degumming in comparison with conventional degumming.

Proteases

Proteases are grossly subdivided into two major groups, i.e., exopeptidases which cleave the peptide bond proximal to the amino or carboxy termini of the substrate and endopeptidases, which cleave peptide bonds distant from the termini of the substrate. Based on the functional group present at the active site, proteases are further classified into four prominent groups, i.e., serine proteases, aspartic proteases, cysteine proteases, and metalloproteases. Proteases are also classified into acid, neutral and alkaline proteases on the basis of pH range in which their

activity is optimum. They constitute a very large and complex group of hydrolytic enzymes that degrade proteins into small peptides and amino acids. Proteases catalyze the addition of water across amide (and ester) bonds to cleave using a reaction involving nucleophilic attack on the carbonyl carbon of the scissile bond. They differ widely in their properties such as substrate specificity, active site and catalytic mechanism and possess different profiles for mechanical stress, chemical environment, pH and temperature for stability and activity. Because of their broad substrate specificity, proteases have a wide range of applications such as in leather processing, detergent formulations, baking, brewing, meat tenderization, peptide synthesis, cheese manufacture, soysauce production, protein hydrolysate, pharmaceutical industry, waste treatment, silk industry, organic synthesis, recovery of silver from waste photographic film, as well as analytical tools in basic research and have high commercial value.

Most of the commercial proteases are of bacterial origin and fungal proteases are gaining attention in recent years. Natural microorganisms have over the years been a great source of enzyme diversity. Although, proteases are produced by variety of bacteria such as *Pseudomonas aeruginosa* (Oh *et al.*, 2000), *Flavobacterium*, *Staphylococcus aureus*, *Achromobacter* and species belonging to *Streptomyces* (Lazim *et al.*, 2009 and Azeredo *et al.*, 2004) bacteria are the most dominant group of alkaline protease producers and genus *Bacillus* being the most prominent source exploited for industrially important proteases (Lazim *et al.*, 2009 and Deng *et al.*, 2010). However, in recent years there is interest in fungal proteases as seen from the large number of publications related to fungal proteases. Fungi elaborate a wider variety of enzymes.

Moreover, enzymes of fungal origin are advantageous due to the ease of cell removal during downstream processing. In addition, fungi can effectively secrete various hydrolytic enzymes in submerged as well as solid state fermentation. Several reports are available on production of proteases by fungi belonging to the genera *Aspergillus* (Charles *et al.*, 2008; Vishwanatha *et al.*, 2010a and Vishwanatha *et al.*, 2010b) *Penicillium* (Sindhu *et al.*, 2009 and Zhu *et al.*, 2009), *Rhizopus* (Kumar *et al.*, 2005), *Humicola* (Aleksieva *et al.*, 2000), *Mucor* (Zheng, 2009) etc. Other fungi are also known to produce extracellular alkaline proteases, for example *Conidiobolus coronatus* (Laxman, 2005), *Beauveria* sp. (Shankar, 2011).

Silk

The silk fiber is a natural animal fibre. Silk contains a very small amount of sulphur, unlike wool. There are two types of the silkworms. The first type is the 'Mulberry silk' (*Bombyx mori*) also called cultivated silk and 'Wild silk' of which Tussah silk is the most important representative. Silkworm larvae cultivated in provided habitats and fed with fleshy picked mulberry leaves produce Mulberry silk. Cultivated silk is different from Tussah silk- native to China and India-- in that Tussah silk worms are fed only oak leaves. Cultivated silk are fine, almost white when (degummed) and soft filaments with luster. Nearly 80-85% of the World Silk Production consists of cultivated silks. White wild silks are

never as white as the cultivated silk filament; they are coarser, more irregular and brownish in appearance. The silk fiber has two main parts called sericin and fibroin which come out as a consequence of this spinning process. Sericin is also known as the silk gum. It is a minor component of the fiber (i.e. 25% of the weight of raw silk) and it has more impurities such as waxes, fats and pigments. Sericin is a yellow, brittle and inelastic substance. It conceals the unique luster of fibroin and acts as an adhesive for the twin fibroin filaments. Sericin is dissolved in a hot soap solution and is known as an amorphous structure.

Fibroin is the principal water insoluble protein (i.e. 78% of the weight of raw silk). Fibroin has a highly oriented and crystalline structure. Amino acid compositions of silk fibroin and sericin are presented in table 1 and 2 (Sambaditya Raj, 2012). 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 (Arami *et al.*, 2007). 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.

Silk degumming

The removal of sericin from the raw silk is a key step in silk processing to obtain an ideal fiber for textile industry and known as 'degumming'. Removal of natural wax, some colouring components and mineral matter is also achieved during degumming process. Textile industry is under considerable environmental pressure owing to its large energy and water consumption and subsequent environmental pollution. Degumming of silk involves the cleavage of peptide bonds of sericin, either by hydrolytic or enzymatic methods, and the subsequent removal of sericin from the silk fibroin.

Table 1. Amino acid compositions of silk fibroins (residues / 1000 residues)

Amino Acids	B. Mori fibre	Tussah A. permyi fibre
Glycerine	446.0	265.0
Alanine	294.0	441.0
Valine	22.0	7.0
Leucine	5.3	8.0
Isoleucine	6.6	—
Serine	121.0	118.0
Threonine	9.1	1.1
Aspartic Acid	13.0	47.0
Glutamic Acid	10.2	8.0
Lysine	3.2	1.0
Arginine	4.7	26.0
Histidine	1.4	8.0
Tyrosine	51.7	49.0
Phenylalanine	6.3	6.0
Proline	3.6	3.0
Tryptophan	1.1	11.0
Methionine	1.0	—
(Cysteine) ₂	2.0	—
Gly > Ala	Gly > Ala	

Principle of silk degumming process is increasing the silk gum solubility by breaking the peptide linkage of sericin structure

into small molecule such as amino acid and its oligomer with hydrolysis reaction. It is an important step and the fibers glue should be totally removed in order to prepare the fiber to subsequent mechanical process and achieve a level dyeing and increase the softness, absorbency, and luster.

Table 2: Amino Acid composition of Silk Sericins (residues / 1000 residues)

Amino Acids	B. mori cocoon	Tussah A. permyi cocoon
Glycine	127.0	149.9
Alanine	55.1	27.8
Valine	26.8	11.9
Leucine	7.2	9.9
Isoleucine	5.5	8.0
Serine	319.7	226.0
Threonine	82.5	149.6
Aspartic acid	138.4	122.5
Glutamic acid	58.0	67.4
Lysine	32.6	14.7
Arginine	28.6	54.5
Histidine	13.0	25.0
Tyrosine	34.0	49.2
Phenylalanine	4.3	6.0
Proline	5.7	19.1
Tryptophan	—	—
Methionine	0.5	1.3
(Cysteine) ₂	1.4	1.8

Degumming is an expensive process and it causes about 20-25% weight loss, decreases the yellowness and strength. Consequently, the whiteness of the fibers can be increased due to the sericin removal. To preventing the fiber damages during the treatment, a number of parameters such as temperature, time and pH should be controlled (Talebpour *et al.*, 2013).

Conventional degumming

Conventionally, removal of sericin is achieved in boiling at high temperatures with degumming solution containing soap or addition of chemical agents like acid, alkali or detergent. Weakly alkaline solution like sodium bicarbonate, ammonia or acidic solution like tartaric, citric acid, succinic acid, dichloro, trichloro acetic acids have been used for degumming of silk.

Khan *et al.* (2010) 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.* (1996) 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.

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 (Mahmoodi *et al.*, 2010). 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 (Yukse, 2012).

Low-temperature plasma treatment has been well studied. Long *et al* (Long, 2008), 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. 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. Soap makes sericin swell, then emulsifies it in the degumming bath, and removes it from the filaments. The presence of soap and alkalis in the wastewater makes this method a nonecofriendly process (Gulrajani, 1990). As far as the environment is concerned, the utilization of chemicals by most of the aforementioned methods introduces serious pollution to the receiving waters. Arami *et al.* (2003) reported conventional degumming with Marseille soap (5g/L) keeping the ratio of solid to liquid as 1:30 for 60 min. They observed that 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) 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).

Similar degumming of the raw silk yarn was performed in an aqueous solution containing 10 g L⁻¹ Marseille soap and 1 g L⁻¹ sodium carbonate, at 98°C, for 1 h by Anghileri *et al* (2007) and they found enzymatic degumming similar or even better than soap degummed silk. Moreover, enzymatic degumming can be carried out in relatively short time at lower temperature than that required in industrial silk degumming plants becoming a real strategy of energy saving. Similar results were reported by (2012). The conventional detergents are not the choice for silk degumming as these chemical will interfere the basic physical and chemical properties of silk leading to the complication in the quality of silk. Though chemical based detergents have been used for long time but same time silk produced by these methods always has shorter shelf life and various other complications like less tensile strength, more hygroscopic etc (Rajasekhar, 2011).

Enzymatic degumming

Enzyme degumming involves the proteolytic degradation of sericin, using the specific proteins with minimum effect on fibroin. Enzymes 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. Enzymes are ecofriendly products, operate under mild conditions and low temperatures, and so consume less energy than other methods (Freddi, 1996 and Gulrajani, 1990). Enzymes act selectively and can attack only specific parts of the substrate to destroy the unwanted sections. Proteolytic enzymes do not readily attack fibroin in fibrous form apparently because the protein chains in silk are densely packed without bulky side chains.

It has a lesser risk of over degumming than alkaline soap degumming moreover weight loss can be easily modified by adjusting the concentration of enzyme, the reaction time and the use of optimum pH and temperature. With enzymatic method, silk is treated at low temperature which not only reducing energy costs but also prevents fibre weakness (Sonthisombat, 2004). Trypsin, a proteolytic enzyme secreted by the pancreas catalyses the hydrolysis of the peptide bond between the carboxyl group of lysine, or the carboxyl group of arginine and amino groups of adjacent amino acids. Trypsin is most active in the pH range of 7-8 and temperature of 37° C. Since sericin is a polar, less crystalline protein with a relatively high lysine and arginine content, it is easily hydrolysed by trypsin. On the other hand, fibroin is not affected by this enzyme, due to lower proportion of the lysine and arginine present in its structure (Gulrajani, 1992).

Anghileri *et al* (2009) reported that among the proteases tested, they found that biodegumase (a commercial enzyme preparation for degumming) was best followed by papain and trypsin in that order. Pepsin which is an acidic protease was not very good. Low degumming efficiency with a commercial acidic protease was also observed. Degumming of mulberry silk with trypsin (pH 7.8-8; 50°C; 1h) and pepsin (pH 1.5-2; 60°C; 1h) was compared with soap method (pH 10.5; 98°C; 2-4 h). Gum loss with trypsin varied from 15.85 to 18.9% while it was lower for pepsin (14.93 to 16.92%) compared to 15.39% gum loss for soap method (Krishnaveni, 2010). They found that degumming with proteases showed better dye uptake, colour strength, luster and softness of fabric compared to soap. Papain, the only plant protease that has been extensively investigated for degumming of silk, is a sulphhydryl enzyme isolated from papyrus latex.

The enzyme is most active in the pH range of 5-7.5 and temperature of 70-90° C (Sonthisombat, 2004). Nakpathom *et al* (Nakpathom, 2009) compared Thai *Bombyx mori* silk fibers degummed with papain enzyme and degummed with alkaline/soap. It was observed 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. However, percent weight loss of all degumming processes was within the same range. (Duran *et al.*, 2007) also reported enzymatic degumming of silk fibers using papain. Degumming of silk with papain resulted in more dye uptake, in the case of reactive dyes The enzyme Bromelain, a plant protease, isolated from pineapple, has also been tried and found suitable for silk degumming (Devi *et al.*, 2011). A bacterial enzyme Alcalase marketed by Novo has been found to be very effective in hydrolysing sericin. It has been observed that this enzyme to be more effective than

trypsin and papain (Nalankilli *et al.*, 1992). Chopra and Gulrajani (Chopra *et al.*, 1994) reported degumming with alcalase to cause 100%, 92% and 60% degumming with Chinese silk, Musheerabad and Bangalore silks respectively. Gum removal in Chinese silk was accompanied by strength loss. The silk-degumming efficiency of an alkaline protease from *Bacillus* sp. RGR-14 was studied and results were analyzed gravimetrically (fiber weight reduction) and by scanning electron microscopy (SEM) of treated silk fiber.

After 5 h of incubation of silk fiber with protease from *Bacillus* sp., the weight loss of silk fiber was 7.5% (Puri, 2001). Arami *et al* (2007) investigated the effect of mixtures of commercially available enzymes like Alcalase and Savinase as well as they compared the results with conventional degumming with Marseilles soap. They observed that 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. On the other hand Marseilles soap treatment resulted 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. 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. Đurašević *et al.* (2008) compared the dyeing behavior of enzymatically (treated with alcalase) degummed silk fibers to that of the conventionally degummed fibers.

They found certain advantages of enzymatic over soap degumming which included greater degumming efficiency and energy save, achieved through lower process temperature. Enzymatically degummed fibers showed no changes after dyeing due to milder treatment conditions under which the fibres were processed which prevented fibrillation and dusting i.e. fiber damage while damage to the soap degummed fibres was enhanced in dyeing process. Krishnaveni and RajKumar (Krishnaveni, 2008) studied effect of enzymatic degumming on dyeing of silk. Degumming was carried out with proteolytic enzymes (Biodegumming, Papain, Trypsin and Pepsin) in the concentration range of 5 to 10% after which the fabric was dyed. They found that degumming with proteases showed higher gum loss (21%) compared with soap (16%), better dye uptake, colour strength, luster and softness of fabric compared to soap. Degumming with biodegumming was best followed by papain and trypsin. Johnny and Chinnammal (Johnny, 2012) reported degumming of mulberry silk with Marseille's soap and soda, purified commercial protease and crude protease from *Bacillus* Species.

They observed even with high concentration of enzyme, there was no fiber damage and the silk threads were stronger than the conventional treatment. Talebpour *et al* (Talebpour *et al.*, 2013) employed three degumming agents, i.e. alkali, enzyme, and Seidlitzia Rosmarinus (Kelyab). They observed that all three effectively removed the sericin and resulted in about 17–25% weight loss. The strength of the treated samples decreased with the severity of the degumming treatments. The weight loss trends for all three agents were similar but the samples

treated with alkali at higher treatment time showed lowest values for strength. Pookajorm *et al.* (2013) studied silk degumming with crude protease produced by a bacterial strain, CRC_6NB, isolated from an area of silk industrial waste in Thailand. They compared silk degumming efficiency of their enzyme, a commercial enzyme Alcalase and with conventional method of boiling in a synthetic detergent (SILKOBLANC BN[®]), and according to scanning electron micrographs and staining tests, the enzyme showed similar result in sericin removal from silk yarn without pre-treatment.

The degumming results were also better than a commercial enzyme (Alcalase[®]) in terms of the remaining sericin residues and fibroin fibrillation under the same enzyme doses and conditions. Raw mulberry silk was degummed with fungal alkaline protease where the enzyme degummed the silk uniformly without strength loss. The weight loss with enzyme loading of 3ml/g and treatment time of 3 h was gave 19.8% that was comparable with soap degumming for 1.5 h which gave weight loss of 20.4% (Laxman, 2012). Other alkali stable proteases found suitable for degumming of silk and already patented includes Degumming, Thermodegumming, Esperase, Sausinase, Proteinase, Proteolytic enzyme S114, Lipase, Alcalase, Cellulase, Protosol, Protease A. N. M., Pepsin, etc (Gulrajani, 1992) Crude protease from isolate SM1 (*Bacillus thuringensis*) was able to perform degumming of raw silk fabric in significant amount.

After the enzymatic treatment, texture of the fabric became shiny and the volume of the yarn increased. The other properties of the fabric like tensile strength, yarn count, colour fastness to water either improved or remained unchanged after the enzymatic treatment in comparison with untreated sample (Das, 2013). Six indigenous microbial proteases were evaluated for degumming of Chinese bivoltine silk by our group in National Chemical Laboratory. Among the proteases tested, two fungal and two actinomycete proteases were promising, which showed weight loss similar to conventional method (19.58% to 21.78%). This is the first report of silk degumming using actinomycete protease with low enzyme dose and shorter duration of time. The fibers showed no significant differences in tensile strength or elongation at break by enzymatic degumming indicating no strength loss when compared with conventional degumming (More, 2013).

Conclusion

Sericulture being a major agro based industry playing an important role in the rural economy in India, the health hazards of the workers and also concerns about the environment and pollution associated with the use of chemicals in silk reeling industries needs to be taken into consideration. The alternative use of eco-friendly enzymatic technologies needs to be developed and standardized. Enzyme treatment is an environmentally friendly process because enzymes are readily biodegradable in nature. There are very limited reports available in the literature till date on proteases in silk degumming. Protease based silk degumming has been shown higher efficiency, expressed over removal of sericin and energy save, achieved through lower process temperature.

Milder treatment conditions under which the fibers were processed during enzymatic degumming prevented fibrillation and dusting i.e. fiber damage. As commercial enzymatic preparations are expensive and not readily accessible to the reelers and weavers, there is an urgent need for scientific studies for potential application of proteolytic enzymes isolated from common cheaper sources. The use of proteolytic enzymes will help to strengthen and promote sericulture industry by enhancing productivity, saving resources like energy and chemicals and improving quality of silk.

REFERENCES

- Aleksieva, P. and Peeva, L. 2000. Investigation of acid proteinase biosynthesis by the fungus *Humicola lutea* in an airlift bioreactor, *Enzyme Microb Technol* 26:402-405.
- Anghileri, A., Freddi, G., Mossotti, R., Innocenti, R. 2007. Mechanical Properties of Silk Yarn Degummed with Several Proteases. *Journal of Natural Fibers* 2007; 4(1) 13-23
- Arami, M., S. Rahimi., L. Mivehie., and F. Mazaheri, 2007. Degumming of Persian silk with mixed proteolytic enzymes. *J. Appl. Poly. Sci.* 106: 267–27
- Azeredo, L.A.I., Freire, D.M.G., Soares, R.M.A., Leite, S.G.F. and Coelho, R.R.R. 2004. *Enzyme Microb Technol*, 34: 354-358.
- Charles, P., Devanathan, V., Anbu, P., Ponnuswamy, M.N., Kalaichelvan, P.T. and Hur, B. K. 2008. Purification , characterization and crystallization of an extracellular alkaline protease from *Aspergillus nidulans* HA-10, *J Basic Microbiol*, 48: 347-352
- Chopra, S. and M. L. Gulrajani, 1994. Comparative Evaluation of the Various Methods of Degumming Silk. *Indian J. Fibre and Textile Res*, 19: 76-83.
- Das, S., Mathummal, S., Thakur, R. A RayChaudhuri, S. 2013. *American Journal of Biochemistry and Biotechnology*, 9 (1): 12-18
- Deng, A., Wu,J., Zhang, Y., Zhang, G. And Wen T. 2010. *Bioresource Technol*, 101:7100–7106.
- Devi, Y. R., Singh, L. R. and Devi, S.K. 2011. Pineapplebromelain: An effective oak tasar cocoon cooking. *International Journal of Current Research and Review*,3(10): 90-92.
- Duran, K., T. M. D. Özdemir., and A. G. E. S. Namligöz., 2007. The enzymatic degumming of silk fibers. *TEKSTİL ve KONFEKSİYON* 3: 182-186.
- Đurašević, VW, Machnowski, and A. Kotlinska, 2008. Dyeing behaviour of differently degummed silk fibers. In “4th International Textile, Clothing and Design Conference – Magic World of Textiles
- Freddi, G., Allara, G., Candiani, G. 1996. Degumming of silk with tartaric acid. *Journal of the Society of Dyers and Colourists* 112 191-195.
- Freddi, G., R. Mossotti., and R. Innocenti., R., 2003. Degumming fabric with several protease *J. Biotechnol.* 106: 101-112.
- Gulrajani ML, R. Agarwal., A. Grover., and M. Suri., 2000b. Degumming of silk with lipase and protease. *Indian Journal of Fibre and Textile Res.* 25: 69-74.
- Gulrajani, M. L., Das, S., Sethi, S. 1990, *Indian J Fibre Text Res* 15, 173.
- Gulrajani, M. L., R. Agarwal, and S. Chand. 2000a. Degumming of silk fungal protease. *Indian Journal of Fibre and Textile Research* 25: 138–142.
- Gulrajani, M.L. 1992. Degumming of silk *Rev Prog Coloration*, 22: 79-89.
- Gulrajani, M.L., S.V.Gupta., A. Gupta., and M. Suri, 1996. Degumming of Silk with different Protease Enzymes, *Indian J. Fibre Textile Res.* 21: 270–275
- Gupta, R., Beg, Q. K. and Lorenz, P. 2002. *Appl Microbiol Biotechnol*, 59: 15-32.
- Johnny, R.V. A. and Chinnammal, S. K. 2012. Degumming of silk using protease enzyme from *Bacillus* species, *International Journal Of Science and Nature*, 3(1), 51-59.
- Khan, M.R., Tsukada, M., Gotoh, Y., Morikawa, H., Freddi, G., Shiozaki, H. 2010. Physical properties and dyeability of silk fibers degummed with citric acid. *Bioresource Technology* 2010;101 8439-8445.
- Krishnaveni, V., and G. RajKumar, 2010. Study on effect of proteolytic enzyme degumming on dyeing of silk. *Colourage* ISSN 0010-1826, 57: 61-68.
- Kumar, S., Sharma, N. S., Saharanb, M. R. and Singh, R. 2005. Extracellular acid protease from *Rhizopus oryzae*: purification and characterization, *Proc Biochem*, 40: 1701–1705.
- Laxman, R. S., 2012. In “Biotechnology of Microbial Enzymes” Ed. Vijai Kumar Gupta and Manimaran Ayyachamy, Nova Science Publishers, 277-295
- Laxman, R.S., Sonawane, A. P., More, S. V., Rao, B. S., Rele, M.V., Jogdand, V. V., Deshpande, V. V. and Rao, M. B. 2005. Optimization and scale up of production of alkaline protease from *Conidiobolus coronatus*, *Proc Biochem*, 40: 3152–3158.
- Lazim, H., Mankai, H., Slama, N., Barkallah, I. and Limam, F. 2009. *J Ind Microbiol Biotechnol*, 36:531-537
- Long, J.J., Wang, H.W., Lu, T.Q., Tang, R.C., Zhu, Y.W. 2008. Application of Low-Pressure Plasma Pretreatment in Silk Fabric Degumming Process. *Plasma Chemistry and Plasma Processing* : 28 701-713.
- Mahmoodi, N.M., Moghimi, F., Arami, M., Mazaheri, F. 2010. Silk Degumming Using Microwave Irradiation as an Environmentally Friendly Surface Modification Method. *Fibers and Polymers*; 11(2) 234-240.
- More, S.V., Khandelwal, H.B., Joseph, M.A. and Laxman, R.S. 2013. Enzymatic Degumming of Silk with Microbial Proteases, *Journal of Natural Fibers*, 10:2, 98-111
- Nakpathom, M., B. Somboon and N. Narumol, 2009. Papain enzymatic degumming of thai *Bombyx mori* silk fibers. *J. Micr. Soc. Thailand*, 23: 142-146.
- Nalankilli, G. 1992. *The Indian Textile Journal*, 103(3): 110-114.
- Oh, Y.S., Shihb, I.L., Tzeng, Y.M. and Wang, S.L. 2000. *Enzyme Microb Technol*, 17:3-10.
- Pookajorm, S. Uyen, U., Wongsangchantra, P.Y. 2013. Enzymatic Silk Degumming Using a Proteas from Bacterial Strain CRC_6NB. Pure and applied chemistry international conference 2013.(PACCON 2013)
- Puri, S. 2001. An alkaline protease from a *Bacillus* sp.: Production and potential applications in detergent formulation and degumming of silk. MSc thesis, University of Delhi, New Delhi.

- Rajasekhar, A., Vallurupalli, R.2., Reddy, M. N., Sambasiva, Rao. K.R. S 2011. Thermostable Bacterial Protease - A New Way for Quality Silk Production *International Journal of Bio-Science and Bio-Technology* Vol. 3, No. 4 43-58
- Rao, M. B., Tanksale, A. M., Ghatge, M. S. and Deshpande, V. V. 1998. *Microbiol Mol Biol Rev*, 62: 597-635
- Sambaditya Raj 2012. Silk Enzymatic Degumming, www.fibertofashion.com Published On Thursday, May 24, 2012
- Shankar, S., Rao, M. and Laxman, R. S. 2011, Purification and characterization of an alkaline protease by a new strain of *Beauveria sp.*, *Proc Biochem* 46: 579-585.
- Sindhu, R., Suprabha, G.N. and Shashidhar, S. 2009. Optimization of process parameters for the production of alkaline protease from *Penicillium godlewskii* SBSS 25 and its application in detergent industry, *Afr Journal Microbiol Res*, 3: 515-522.
- Sonthisombat, A. and Speakman, P. T. 2004 Silk : Queen of Fibres - The Concise Story. www.en.rmutt.ac.th/prd/Journal/Silk.
- Talebpour, F., Veysian, S.M., Golfazani, M.E.H 2013. Degumming of Silk Yarn Using Alkali, Enzyme and *Seidlitzia Rosmarinus* *J. OF Textiles Polymers*, 1 (2): 60-64
- Vishwanatha K S, Appu Rao A G and Singh S A (2010b), Acid protease production by solid-state fermentation using *Aspergillus oryzae* MTCC 5341: optimization of process parameters. *J Ind Microbiol Biotechnol*, 37:129–138.
- Vishwanatha, K.S., Appu Rao, A.G and Singh, S.A. 2010a, Production and characterization of a milk-clotting enzyme from *Aspergillus oryzae* MTCC 5341. *Appl Microbiol Biotechnol*, 85: 1849-1859.
- Yukse, M., Kocak, D., Beyit, A., Merdan, N. 2012. 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.
- Zheng, X. T. and Zhao, X. H. 2009. Optimization of fermentation conditions for proteases produced by *Mucor*. *Microbiol*, 36: 193-197.
- Zhou, C.Z., F. Confalonieri, N. Medina, Y. Zivanovic and C. Esnault *et al.*, 2000. Fine organization of *Bombyx mori* fibroin heavy chain gene. *Nucl. Acids Res.*, 28: 2413-2419.
- Zhu, H. Y., Tian, Y., Hou, Y. H. and Wang, T. 2009. Purification and characterization of the cold active alkaline protease from marine cold adaptive *Penicillium chrysogenum* FS010, *Mol Biol Rep*, 36: 2169-2174.
