NC STATE College of Textiles

JTATM
Journal of Textile and Apparel, Technology and Management

Volume 9, Issue 3, 2015

Tussar Silk: Scope for Improving Quality by Enzyme Technology

C. Vigneswaran, D. Ridhu Nivethinni, K. Shilpaa, and Sheetal Dhanapal
Department of Fashion Technology,
PSG college of Technology,
Coimbatore, India

ABSTRACT

Tussar silk is a natural protein fiber which composed mainly of fibroin and produced by certain insect larvae to form cocoons. Saturated wild silks are of three types: Tussar, Muga, and Eri. The tussar silk production and usage of tussar silk products have reached to an appreciable extent in global market for variety of diversity products in Textile and Apparel sectors. This has led to the search of improved methods of tussar processing, giving attention to both its quality and handle properties. This article revolves around the advance methods of tussar silk processing which are more eco-friendly when compared to the other normal methods.

Keywords: Tussar silk, Enzyme technology, Value addition, Silk quality, Bioprocess

1. INTRODUCTION

Tussar Silk is also known by its Sanskrit name *'Kosa Silk'* and produced mainly in Jharkhand state of India (Nakpathom et al 2009; Pilanee Vaithanomsat et al 2008). Tussar is valued for its rich texture and natural deep gold color. It is obtained from silk worms that do not breed on mulberry trees but breed on local trees like Sal, Arjun and Saja, and that is why they are also called as *'Wild Silk'*. Tussar Silk is less expensive and durable as compared to cultivated silk because of its short fiber length. The precisely finished and designer garments produced from Tussar Silk are world famous and are exported to various countries worldwide including Europe, Gulf and the United States (Table 1).

The tasar silkworms are of two categories- first the Indian tropical tasar, *Antheraea mylitta*, which feeds on the leaves of *Terminalia arjuna*, *Terminalia tomantosa*, and *Shorea robusta*, and second the Chinese temperate oak tasar, *Antheraea pernyi*, which feeds on the leaves of *Quercus* spp. and *Philosamia* spp.

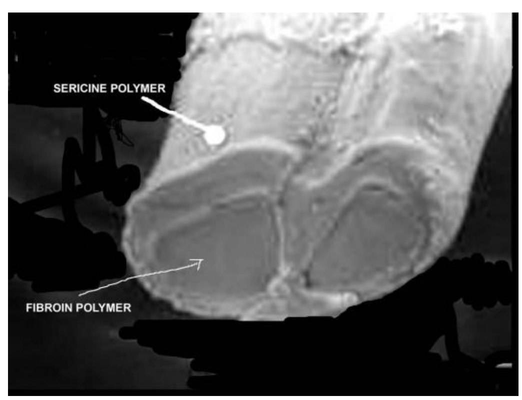

Figure 1. Cross-sectional view of silk

Table 1. Commercially exploited sericigenous insects of the world

Common Name	Scientific Name	Origin
Mulberry silkworm	Bombyx mori	China
Tropical Tassar Silkworm	Antheraea Mylitta	India
Oak Tasar Silkworm	Antheraea proylel	India
Eri Silkworm	Philosamia ricini	India
Muga Silkworm	Antheraea assama	India
Oak Tasar Silkworm	Antheraea yamamal	Japan
Oak Tasar Silkworm	Antheraea pernyi	China

Indian tropical tasar (Tussah) is copperish color, coarse silk mainly used for furnishings and interiors (Figure 1). It is less lustrous than mulberry silk, but has its own feel and appeal. Oak tussar is a finer variety of tussar silk (Vepari et al 2007). The characteristic of tussar silk has rich coarse texture, light and airy. It gives a cool comfort feeling and also delicate and stiff in nature. It has different natural shades (Wang et al 2006; Yang Cao et al 2009). The maintenance of tussar silk is important for example dry cleaning is the safest option for Tussar. Dry cleaned Tussar should not be wrapped in plastic. Silks need to breathe. In case you want to hand wash your tussar fabric, use cold water and a mild liquid soap meant for delicate clothes. Do not wring excess water out. Dry fabric in shade as sun may cause the fabric to fade. Wash dark colors separately and never use chemical bleach (Mahendran et al 2005; Mauney et al 2007).

2. END USES OF TUSSAR SILK

The apparel is the most important Tussar silk produce although it is also used as the base material for handicrafts, furnishing fabrics, and stitched apparel. With the introduction of chemical dyes, the range of available colors has increased significantly. There are fashion designers who use tussar silk in their creations. The precisely finished and designer garments produced from Tussar Silk are known globally and are exported to various countries including Europe, the Persian Gulf and the United States.

Silk's absorbency makes it comfortable to wear in warm weather and while active. Its low conductivity keeps warm air close to the skin during cold weather. It is often used for clothing such as shirts, ties, blouses, formal dresses, high fashion clothes, lining, lingerie, pajamas, robes, dress suits, sun dresses and Eastern folk costumes. Silk's attractive luster and drape makes it suitable for many furnishing applications. It is used for upholstery, wall coverings, window treatments (if blended with another fiber), rugs, bedding and wall hangings. While on the decline now, due to artificial fibers, silk has had many industrial and commercial uses, such as in parachutes, bicycle tires, comforter filling and artillery gunpowder bags.

3. PRODUCTION OF TUSSAR SILK

China and India are the largest producer of raw silk producer in world and the biggest consumer of raw silk and silk fabrics. An analysis of trends in international silk production suggests that sericulture has better prospects for growth in the developing countries rather than in the advanced countries. Silk production in temperate countries like Japan, South Korea, USSR etc., is declining steadily not only because of the high cost of labor and heavy industrialization in these countries, but also due to climatic restrictions imposed on mulberry leaf availability that allows only two cocoon crops per annum. Thus, India has a distinct advantage of practicing sericulture all through the year, yielding a stream of about 4 – 6 crops as a result of its tropical climate.

In China and India, sericulture is not only a tradition but also a living culture. It is a farm-based, labor intensive and commercially attractive economic activity falling under the cottage and small-scale sector. It particularly suits rural-based farmers, entrepreneurs and artisans, as it requires low investment but, with potential for relatively higher returns. It provides income and employment to the rural poor especially farmers with small land-holdings and the marginalized and weaker sections of the society. Several socio-economic studies have affirmed that the benefit-cost ratio in sericulture is highest among comparable agricultural crops (Table 2).

Currently, the domestic demand for silk, considering all varieties, is nearly 35,000 MTs, of which only around 28,475 MTs (2013-14) is getting produced in the country and the rest being imported mainly from China (Yu-Qing Zhang 1998). Indian domestic silk market has over the years been basically driven by multivoltine mulberry silk. Due to inferior quality of the silk produced, India could not meet the international quality standard. Though, R&D efforts have been made to improve the quality of multivoltine silk, even the best of multivoltine silk produced could not match the bivoltine silk in quality. Therefore, it is essential to enlarge the production base and improve current productivity levels of bivoltine silk to meet the international standards and quality demands of the power loom sector. Steps need to be taken to ensure that export units have state of the art automatic weaving machinery (Figure 2).

Table 2. Cost benefit analysis of mulberry sericulture and other competing crops

Item	Mulberry sericulture	Sugarcane	Turmeric
Total input costs	48,659	30,575	29,610
Gross returns	96,132	60,200	55,317
Net returns	47,476	29,625	25,707
CB ratio	1:1.98	1:1.97	1:1.02
Crop period	1 year	1 year	4 – 5 months

Note: Data in Rs/acre/annum

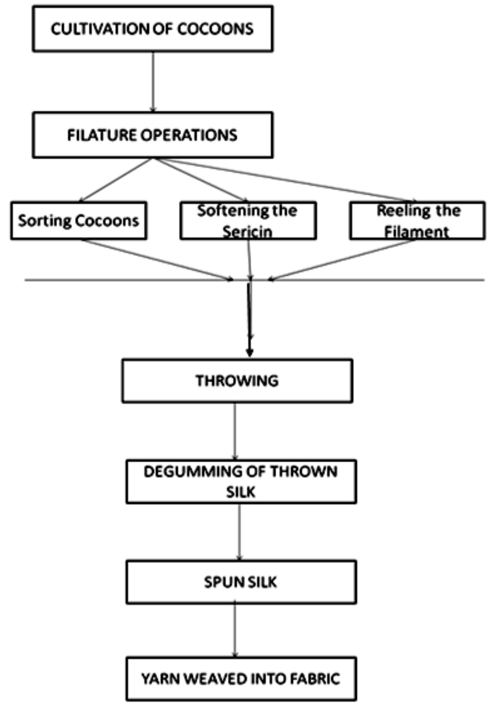

Figure 2. Process flow of Tussar silk

4. ADVANCED METHOD OF TUSSAR SILK PROCESSING

4.1 Ecofriendly Chemical Method

Soapnuts are probably the most sustainable cleaning product for removing low volatile protein substances present in the silk fiber. Soapnuts are an all-natural, plant-based product (just a nut from a tree), easy to grow organic and require very little processing or packaging. They are naturally hypoallergenic, odorless and do no damage to surfaces or fabrics; and there are countless ideas on how to use them, making them sustainable and versatile. The soapnut comes from the soapnut tree (*Sapindus mukorossi*). The trees produce the small black berry, approximately one inch (2-2.5 cm) in diameter, that are deseeded and the shell is dried prior to use.

The shells used for detergent contain something called saponin, which works as a natural surfactant. Surfactants reduce the surface tension of the water, essentially making it wetter and easier to penetrate into soiled tussar silk fabrics. Soapnuts are all-natural and have been used for centuries around the world.

4.2 Enzyme Method

Enzymatic treatment reduces facing up, which significantly improves the pilling performance of garments and increases softness. The main advantage of biopolishing is the prevention of pilling. Cellulases hydrolyze Proteases are also used to treat silk. Threads of raw silk must be degummed to remove sericin, a proteinaceous substance protruding from the surface of yarn that covers the silk fiber. Traditionally, degumming is carried out using an alkaline solution containing soap. This weakens the silk fibrion polymer and hydrolyzed. However, the use of proteolytic enzymes is a better method because they remove the sericin without attacking the fibrin. Tests with high concentrations of enzymes show that there is no fiber damage and the silk threads are stronger than traditional treatments.

The conventional degumming methods like extraction with water, boiling off in soap, Degumming with alkali and 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 which can be solved with enzymatic degumming.

4.2.1 Enzymes

Two types of enzymes are used in this research for the degradation of fibroin to present in the tussar silk to make it soft. They are:

- Alkaline protease
- Lipase

4.2.1.1 Proteases

Proteases are enzymes that hydrolyze proteins via the addition of water across peptide bonds and catalyze peptide synthesis in organic solvents and in solvents with low water content. The hydrolysis of peptide bonds by proteases is termed as proteolysis; the products of proteolysis are protein and peptide fragments, and free amino acids. Proteolytic enzymes are ubiquitous in occurrence, found in all living organisms, and are essential for cell growth and differentiation. 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 (Gowda et al 2007; Gulrajani 1992). Proteases represent one of the three largest groups of industrial enzymes and have traditionally held the predominant share of the industrial enzyme market accounting for about 60% of total worldwide sale of enzymes.

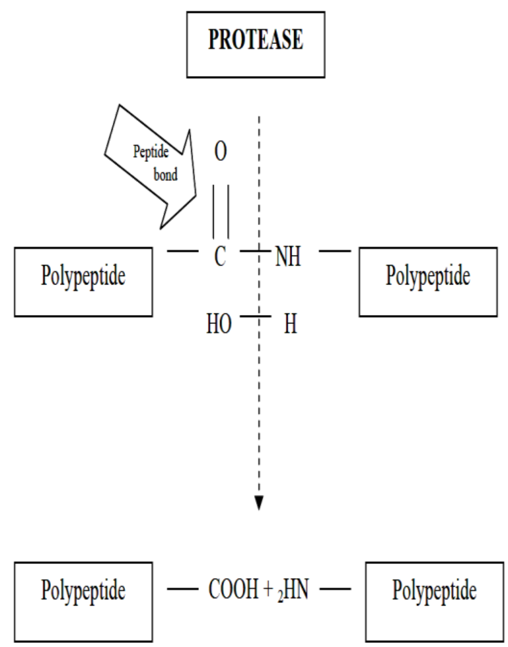

Figure 3. Structure of protease

This dominance of proteases in the industrial market is expected to increase further by the year 2015. Proteases of commercial importance are produced from microbial, animal and plant sources. They constitute a very large and complex group of enzymes with different properties of substrate specificity, active site and catalytic mechanism, pH and temperature activity and stability profiles (Figure 3). Industrial proteases have application in a range of process taking advantage of the unique physical and catalytic properties of individual proteolytic enzyme types. This vast diversity of proteases, in contrast to the specificity of their action has attracted worldwide attention in attempts to exploit their physiological and biotechnological applications (Li et al 2003; Mahendran et al 2006). Proteases of commercial importance are produced from microbial, animal and plant sources. They constitute a very large and complex group of enzymes with different properties of substrate specificity, active site and catalytic mechanism, pH and temperature activity and stability profiles. Industrial proteases have application in a range of process taking advantage of the unique physical and catalytic properties of individual proteolytic enzyme types. This vast diversity of proteases, in contrast to the specificity of their action has attracted worldwide attention in attempts to exploit their physiological and biotechnological applications.

Alkaline proteases account for a major share of the enzyme market all over the world. Alkaline proteases from bacteria find numerous applications in various industrial sectors and different companies worldwide have successfully launched several products based on alkaline proteases (Aramwit et al. 2012). The success of detergent enzymes has led to the discovery of a series of detergent proteases with specific uses. Alkazym (Novodan, Copenhagen, Denmark) is an important enzyme for the cleaning of membrane systems. Other enzymes used for membrane cleaning are Tergazyme (Alconox, New York, USA), Ultrasil (Henkel, Dusseldorf, Germany) and P3-pardigm (Henkel-Ecolab, Dusseldorf, Germany). Pronod 153L, a protease enzyme-based cleaner is used to clean surgical instruments fouled by blood proteins. Subtilopeptidase A is an enzyme-based optical cleaner, presently marketed in India (Kumar et al. 1998). Sakiyama et al. (1998) have reported the use of a protease solution for cleaning the proteinous substance deposited on steel beads. In addition to these major applications, alkaline proteases are also used to a lesser extent for other applications, such as contact lens cleaning (Nakagawa 1994), molecular biology for the isolation of nucleic acid (Kyon et al. 1994), pest control (Kim et al. 1996), and degumming of silk (Kanehisa 2000; Puri 2002).

4.2.1.2 Lipases

Today lipases stand amongst the most important biocatalysts carrying out novel reactions in both aqueous and non-aqueous media. This is primarily due to their ability to utilize a wide spectrum of substrates, high stability towards extremes of temperature, Ph, organic solvents, and chemo-, region- and enantio-selectivity (Chitrangada et al 2008; Chopra et al 1997). More recently, the determination of their three-dimensional structure has thrown light into their unique structural function relationship. Among lipases of plant, both animal and microbial origin, it is the microbial lipases that find the immense applications (Fini et al 2005; Freddi et al 1999). This is because microbes can be easily cultivated and their lipases can catalyze a wide variety of hydrolytic and synthetic reactions. Lipase enzymes find their use in fibroin degradation of silk. Lipids are insoluble in water and need to be broken down extracellular into their more polar components to facilitate absorption if they are to function as nutrients for the cell. Therefore majority of the lipases are secreted extracellularly. Lipases have been purified from animal, plant, fungal and bacterial sources by different methods involving ammonium sulphate precipitation, gel filtraion, and ion excahnge chromatography. In recent years, affinity chromatographic techniques have come into use as this technique decreases the number of steps necessary for lipase purification as well as increases specificity. Currently reversed-micellar and two phase systems mambrane processes and immuno-purification are being used for purification of lipases.

4.2.2 Synergistic Action of Protease-Lipase on Silk Degumming

Proteases and lipases are normally used in combination for degumming and removing others impurities such as waxes, fats, mineral salts and pigments. Waxes and fats as well as colorants and mineral components occur exclusively in silk gum layer (sericin). 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.g-1 fabric combined with a lipase (Lipolase Ultra 50T) at a constant activity of 50 U.g-1 fabric for 30, 60, 90 min. Furthermore, buffer treated and conventional degummed silk fabrics were used as controls. Enzymatic degumming is emerging as an eco-friendly fiber-gentle process where proteolytic enzymes that are effective under alkaline, neutral as well as acidic conditions are being used (Fabiani et al. 1996). With the local availability of the enzymes at a reasonable price this process has a commercial potential in India. Being large molecules, enzymes do not penetrate into the interstices of the fabric and hence are suitable for yarn degumming only. A critical control of the pH and temperature is required to realize the full potential of the enzymes requiring use of sophisticated machinery. Since most of the enzymes are effective at a comparatively low temperature of about 60°C, they are less energy-intensive. The degumming waste liquor that is rich in sericin content is being used as a raw material for the production of sericin powder (Vaithanomsat and Kitpreechavanich 2008). The sericin powder is being used in the cosmetic industry as moisturizer, in hair-care products and also as a sustainable natural textile finishes. Removal of sericin from the waste degumming liquor also substantially reduces the effluent (Acharya et al 2008; Amanda et al 2009).

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 (Freddi et al. 2003). 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 (Long et al. 2008; Chopra et al. 1996). 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 fibers gain the typical shiny aspect, soft handle, and elegant drape highly appreciated by the consumers.

4.2.3 Silk Degumming Process – Comparative Study

A crêpe silk fabric was treated with different alkaline (3374-L, GC 897-H), neutral (3273-C), and acid (EC 3.4 23.18) proteases with the aim to study their effectiveness as degumming agents. Proteases were used under optimum conditions of pH and temperature, while enzyme dosage (0.05–2 U/g fabric) and treatment time (5–240 min) were changed in order to study the kinetics of sericin removal. Degumming loss with soap and alkali was 27 wt%. The maximum amount of sericin removed in 1 h was 17.6, 24, and 19 wt.% for 3374-L (2 U/g fabric), GC 897-H (1 U/g fabric), and 3273-C (0.1 U/g fabric), respectively. Under the experimental conditions adopted, EC 3.4 23.18 was almost ineffective as a degumming agent. Degumming loss increased as a function of the treatment time, reaching a value of 25 wt% with 1 U/g fabric of 3374-L. The feasibility of degumming Persian silk with alcalase, savinase, and mixtures of these enzymes with different alcalase/savinase weight ratios (0/1, 0.25/0.75, 0.5/0.5, 0.75/0.25, and 1/0 g/L) was investigated (Mokhtar et al. 2007). The enzymatic degumming process was performed at 55°C with an operation time of approximately 30 min, whereas the soap degumming process was carried out around the boiling point in 120 min. The evaluation of the data was carried out through the measurement of the weight loss, strength, and elongation of the samples. The optimum amount of sericin removed was 21.52 wt % for alcalase in 30 min, 20.08 wt % for savinase in 60 min, and 22.58 wt % for soap in 120 min. Also, the enzymatic treatment improved properties of the silk yarn such as the strength (33.76 cN/tex for alcalase and 32.03 cN/tex for savinase) and elongation (20.08% for alcalase and 18.42% for savinase). The obtained values were better than the strength (29.90cN/tex) and elongation (18.59%) from the soap degumming method. Through the use of an enzyme mixture (0.5/0.5 g/L), good weight loss (22.43%), strength (33.22 cN/tex), and elongation (17.74%) were achieved in 30 min.

5. CONCLUSION

Enzymes can be used in order to develop environmentally friendly alternatives to chemical processes in almost all steps of textile fiber processing (Yuksek et al 2012). There are already some commercially successful applications, such as amylases for desizing, cellulases and laccases for denim finishing, and proteases incorporated in detergent formulations. Further research is required for the implementation of commercial enzyme-based processes for the biomodification of synthetic and natural fibers. An active field of research is the search for new enzyme-producing micro-organisms and enzymes extracted from extremophilic micro-organisms (Zhang 2002). There is still considerable potential for new and improved enzyme applications in future textile processing (Gulrajani et al 2000; Hino et al 2003).

The application of soapnut and enzymes which are rich in protein degradation on the tussar fabric are studied which is nothing but an eco-friendly process of silk degumming (Kundu et al 2008; Li et al 2012). The fabric treated with the soapnut and enzymes (protease and lipase) are tested for various handle properties using the Kawabatta system of textile testing and the results are studied. Several alkaline, acidic and neutral proteases have been studied as degumming agents since they can dissolve sericin, but are unable to affect silk fiber protein. Alkaline proteases seem to be the best for removing sericin and improving silk surface properties like handle, shine and smoothness (Freddi et al. 2003; Arami et al. 2007).

6. REFERENCES

1. Acharya, C., Ghosh, S.K., Kundu, S.C, Silk fibroin protein from mulberry and non-mulberry silkworms: cytotoxicity, biocompatibility and kinetics of L929 murine fibroblast adhesion, J Mater Sci Mater Med, 19, 2008, 2827–2836.
2. Amanda, R., Murphy, S., and David, L.K., Biomedical applications of chemically-modified silk fibroin, J Mater Chem, 19(36), 2009, 6443–6450.
3. Arami, M., Rahimi, S., Mivehie, L., Mazaheri, F., Mahmoodi, N.M., Degumming of Persian Silk with Mixed Proteolytic Enzymes, J Applied Polymer Science, 106, 2007, 267–275.
4. Arami, M., Rahimi, S., Mivehie, L., Mazaheri, F., Mahmoodi, N.M., Degumming of Persian silk with mixed proteolytic enzymes, J Appl Polym Sci, 106, 2007, 267–275.
5. Aramwit, P., Siritientong, T., Srichana, T., Potential applications of silk sericin, a natural protein from textile industry by-products, Waste Management and Res, 30(3), 2012, 217–224.

6. Chitrangada, A., Boris, H., Subhas, C.K., The effect of lactose-conjugated silk biomaterials on the development of fibrogenic fibroblasts, Biomaterials, 29, 2008, 4665–75.
7. Chopra, S., Chattopadhyay, R., and Gulrajani, M.L., Low Stress Mechanical Properties of Silk Fabric Degummed by Different Methods, J Text Inst, 87(3), 1997, 245–52.
8. Chopra, S., Chattopadhyay, R., Gulrajani, M.L., Low stress mechanical properties of silk fabric degummed by different methods, J Text Inst, 87, 1996, 542–553.
9. Fabiani, C., Pizzichini, M., Spadoni, M., Zeddita, G., Treatment of waste water from silk degumming processes for protein recovery and water reuse, Desalination, 105, 1996, 1–9.
10. Fini, M., Motta, A., Torricelli, P., Giavaresi, G., Aldini, N.N., Tschon, M., Giardino, R., Migliaresi, C., The healing of confined critical size cancellous defects in the presence of silk fibroin hydrogel, Biomaterials, 26, 2005, 3527–36.
11. Freddi, G., Mossotti, R., and Innocenti, R., Degumming of silk fabric with several proteases, J Biotech, 106 (1), 2003, 101–112.
12. Freddi, G., Pessina, G., Tsukada, M., Swelling and dissolution of silk fibroin (Bombyx mori) in N-methyl morpholine N-oxide, Int J Biological Macromolecules, 24(2), 1999, 251–263.
13. Gowda, K.N., Ninge P.N., Sudhakar, R., Subramani, R., Eco-friendly preparatory process for silk: Degumming by protease enzyme, Man-Made Textiles in India, 50(1), 2007, 28.
14. Gulrajani, M.L., Degumming of silk, Review of Progress in Coloration, 22, 1992, 79–89.
15. Gulrajani, M.L., Agarwal, R., Chand, S., Degumming of silk with fungal protease, Indian J Fibre and Text Res, 2000, 25, 138–142.
16. Gulrajani, M.L., Agarwal, R., Grover, A., Suri, M., Degumming of silk with lipase and protease, Indian J Fibre and Text Res, 25, 2000, 69-74.
17. Gulrajani, M.L., Gupta, S.V., Gupta, A., Suri, M., Degumming of silk with different protease enzymes, Indian J Fibre and Text Res', 21, 1996, 270–275.
18. Hino, T., Tanimoto, M., Shimabayashi, S., Change in secondary structure of silk fibroin during preparation of its microspheres by spray-drying and exposure to humid atmosphere, J Colloid Interface Sci, 266, 2003, 68–73.
19. Kim, H.K., Hoe, H.S, Suh, D.S, Kang, S.C, Hwang, C., Kwon, S.T, Gene structure and expression of the ene from Beauveria basiana encoding bassiasin, an insect cuticle-degrading serine protease, Biotechnol Lett, 21, 1996, 777–83.
20. Kumar, C.G, Malik, R.K., Tiwari, M.P, Novel enzyme-based detergents: an Indian perspective, Curr Sci, 75, 1998, 1312–18.
21. Kundu, J., Dewan, M., Ghoshal, S., Kundu, S.C, Mulberry non-engineered silk gland protein visa-vis silk cocoon protein engineered by silkworms as biomaterial matrices, J Mater Sci Mater Med, 19, 2008, 2679–89.
22. Kundu, S.C., Dash, B.C., Dash, R., Kaplan, D.L., Natural protective glue protein, sericin bioengineered by silkworms: Potential for biomedical and biotechnological applications, Progress in Polymer Science, 33, 2008, 998–1012.
23. Kyon, Y.T., Kim, J.O., Moon, S.Y., Lee, H.H., Rho, H.M, Extracellular alkaline proteases from alkalophilic Vibrio metschnikovii strain RH530, Biotechnol Lett, 16, 1994, 413–418.
24. Li, G., Liu, H., Li, T.D., Wang, J., Surface modification and functionalization of silk fibroin fibers/fabric toward high performance applications, Materials Science and Engineering, 32, 2012, 627–636.
25. Li, M., Ogiso, M., Minoura, N., Enzymatic degradation behavior of porous silk fibroin sheets, Biomaterials, 24, 2003, 357–365.

26. Long, J.J., Wang, H.W., Lu, T.Q., Tang, R.C., Zhu, Y.W., Application of Low-Pressure Plasma Pretreatment in Silk Fabric Degumming Process, Plasma Chemistry and Plasma Processing, 28, 2008, 701–713.
27. Mahendran, B., Acharya, C., Dash, R., Ghosh, S.K., Kundu, S.C., Repetitive DNA in tropical tasar silkworm Antheraea mylitta, Gene, 370, 2006, 51–57.
28. Mahendran, B., Padhi, B.K., Ghosh, S.K., Kundu, S.C., Genetic variation in ecoraces of tropical tasar silkworm, Antheraea mylitta D using RFLP technique, Current Science, 90, 2005, 100–103.
29. Mauney, J.R., Nguyen, T., Gillen, K., Kirker-Head, C., Gimble, J.M., Kaplan, D.L., Engineering adipose-like tissue in vitro and in vivo utilizing human bone marrow and adipose-derived mesenchymal stem cells with silk fibroin 3D scaffolds, Biomaterials, 28, 2007, 5280–90.
30. Mokhtar Arami, Sharam Rahimi, Leila Mivehie, Firoozmehr Mazaheri, Niyaz Mohammad Mahmoodi, Degumming of Persian silk with mixed proteolytic enzymes, J Applied Poly Sci, 106 (1), 2007, 267–275.
31. Nakpathom, M., Somboon, B., Narumol, N, Papain Enzymatic Degumming of Thai Bombyx mori Silk Fibers, J Microscopy Society of Thailand, 23(1), 2009, 142–146.
32. Pilanee Vaithanomsat, Taweesiri Malapant and Waraporn Apiwattanapiwat, Silk Degumming Solution as Substrate for Microbial Protease Production, Kasetsart J. (Nat. Sci.), 42, 2008, 543–551.
33. Puri, S., Beg, Q.K., Gupta, R., Optimization of alkaline protease production from Bacillus sp. using response surface methodology, Curr Microbiol, 44, 2002, 286–90.
34. Rajasekhar, A., Ravi, V., Reddy, M.N.., Rao, K.R.S.S, Thermostable Bacterial Protease – A New Way for Quality Silk Production, Int J Bio-Science and Bio-Tech, 3(2), 2011, 43–58.
35. Sakiyama, T., Toyomasu, T., Nagata, A., Imamura, K., Takahashi, T., Nagai, T., Nakanishi, K, Performance of protease as a cleaning agent for stainless steel surfaces fouled with protein, J Ferment Bioeng, 55, 1998, 297–30.1
36. Santin, M., Motta, A., Freddi, G., Cannas, M., In vitro evaluation of the inflammatory potential of the silk fibroin, J Biomed Mater Res, 46, 1999, 382–389.
37. Shao, Z.Z., Vollrath, F., Surprising strength of silkworm silk, Nature, 418, 2002, 741–43.
38. Svilokos Bianchi, A., Colonna, G.M., Developments in the degumming of silk, Melliand Text, 73, 1992, 68–75.
39. Vaithanomsat, P., Kitpreechavanich, V., Sericin separation from silk degumming wastewater, Separation and Purification Tech, 59, 2008, 129–133.
40. Vepari, C., Kaplan, D.L., Silk as a biomaterial, Progress in Polymer Science, 32, 2007, 991–1007.
41. Wang, Y., Kim, H.J., Vunjak-Novakovic, G., Kaplan, D.L., Stem cell-based tissue engineering with silk biomaterials, Biomaterials, 27, 2006, 6064–82.
42. Yang Cao, and Bochu Wang, Biodegradation of Silk Biomaterials, Int J Mol Sci, 10, 2009, 1514–24.
43. 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, 6(2), 2012, 801–8.
44. Yu-Qing Zhang, Natural silk fibroin as a support for enzyme immobilization, Biotechnology Advances, 16, 1998, 961–971.
45. Zhang, Y.Q., Applications of natural silk protein sericin in biomaterials, Biotech. Adv, 20(2), 2002, 91–95.