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Review article

BIOTECHNOLOGICAL APPLICATION OF PROTEOLYTIC ENZYMES IN POST COCOON TECHNOLOGY

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ABSTRACT:
Sericulture which is not only a tradition but also a living culture is a farm based, labour intensive and commercially attractive economic activity falling under the cottage and small scale sector. Reeling process is a vital link in converting the cocoons into an industrial output yarn. Various chemicals like washing soda, sodium sulphite, sodium silicate, sodium lauryl sulphate, hydrogen peroxide *etc.* are used during the reeling process for drawing the silk thread from the cocoons. However these chemical methods reduce the quality of silk thread and are hazardous to the health of the workers and also cause environmental pollution. Eco friendly proteolytic enzymes, which can hydrolytically cleave the peptide bonds of sericin without destroying the fibroin, is used as a replacement of the harsh and energy demanding chemical treatments. Novel use of proteolytic enzymes in silk industry can be divided into two stages *viz.* during cocoon cooking/reeling and the degumming process. Majority of enzyme is used in degumming which is a silk refining process of the drawn silk fibre or yarn. The number of enzymes used in silk industry is limited as compared to other textile industry. There is an urgent need for scientific studies for potential application of proteolytic enzymes in cocoon cooking and 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.

KEYWORDS: Protease, Fibroin, Sericin, Silk Reeling, Degumming

INTRODUCTION

The history of silk is as long as that of civilization itself. Silk is named in legends, fable and folklore. From its origin in China in about 2200 B.C., the silk industry has had an adventurous course of evolution, becoming established from time to time in other parts of the world. The superiority of silk as a textile fibre has been recognised from time immemorial; the luxurious look, sleek feel and lustre of silk fabric are unquestionably inimitable. Nowadays, varieties of fabrics are available in the market however silk continues to be the "queen of fabrics" due to comfort qualities and eco-friendly nature of the fibre. The natural silks are broadly classified as mulberry (obtained from cocoons of *Bombyx mori* L. silkworm) and non-mulberry *viz* tropical and temperate tasar, eri, muga, and anaphe. Nearly 95% of the global production of non-mulberry silks is tasar. Other varieties *i.e.* fagara, coan, mussel and spider silks are of limited interest and are not used for commercial production. Mulberry silk forms the bulk of the commercial silk produced in the world.

The process of drawing silk thread from cocoon spun by the silkworm is known as reeling**.** As a prerequisite to reeling, cocoon cooking process has to be performed *i.e*. the cocoon has to be softened by decomposing or partially solubilising the sericin component, the proteinaceous silk gum, which binds the protein fibroin strands from which the silk thread is reeled. The silk fibre produced by silkworm is a composite material formed by fibroin protein surrounded by sericin protein which accounts around 67-75 and 22-25%, respectively (Mahmoodi *et al*., 2010). The quantity and quality of sericin varies from one species to other and also across different breeds of the same species. The silk proteins differ considerably in their chemical composition *e.g.* in mulberry proteins, fibroin has roughly 76 mol% of amino acids having non-polar side chain**,** the main among these being glycine and alanine**,** and only about 21 mol% of amino acids having polar groups**.** In sericin**,** however**,** the ratio is the other way round**,** with about 25 mol% of amino acids having non-polar groups and about 75 mol% of amino acids having polar side chains mainly serine**,** aspartic acid**,** glycine and threonine (Komatsu**,**1985)**.** This difference in the composition makes sericin more water soluble than fibroin and it serves as the basis of removal of sericin from the cocoons or from the silk thread. Mulberry cocoons can be easily reeled after boiling in hot water however, due to irregular nature of the shell of non-mulberry cocoons and presence of other components like natural waxes, colouring components, mineral matters *etc*., a number of workers has reported the use of various chemicals like washing soda, sodium sulphite, sodium silicate, sodium lauryl sulphate, hydrogen peroxide *etc.* for cooking the non-mulberry

cocoons (Tikoo and Goel, 1987; Moon *et al*., 1996). However these chemical methods tend to reduce the quality of silk thread so alternative cocoon cooking methods using enzymes have been studied and developed (Singh *et al*., 2003; Nakpathom *et al*., 2009; Mahmoodi *et al*., 2010; Pandey *et al*., 2011).

Enzymes are biological catalyst which accelerates the rates of wide variety of chemical reactions. Different enzymes may cause hydrolysis, reduction, oxidation, coagulation and decomposition reaction. Proteolytic enzymes or proteases are those enzymes which hydrolytically cleave the peptide bond that links amino acids together in the polypeptide chains forming the proteins degrading the protein into small molecules such as peptones, peptides and amino acids. With biotechnological processes involving proteolytic enzymes finding wider applications in medicines and industries, the use of various proteolytic enzymes has increased enormously in recent years. One such use is in textile industry, mostly in textile processing and finishing. The use of enzymes in textile industry has fewer shares in the total enzyme usage in comparison to other industries. However, the usage of enzymes in textile industries is increasing day by day but the use of enzymes in silk industry is very limited as compared to other textiles like cotton industry. Of all the total enzyme consumption in silk industries, majority is used in degumming process and fewer uses of enzymes are reported for cocoon cooking method (mostly in case of non-mulberry cocoons).

Enzymes in cocoon cooking

The usage of enzyme for removal of sericin can be attributed to the discovery of cocoonase. Cocoonases are a group of proteinases produced by several sericigenous insects for the purpose of softening the end of the cocoon by attacking the sericin binding the silk strand together to permit the escape of the adult moth (Kafatos and Williams, 1964). When stored in the dry state at 4°C**,** cocoonases are stable indefinitely**.** Frozen solutions at pH 7-8 are stable for several months**.** At low pH (below pH 4)**,** solutions lose activity rapidly**.** Three cocoonases from (*Antheraea polyphemus***,** *Antheraea pernyi* and *Antheraea mori* have been extensively investigated and are found to behave as trypsin like enzymes (Hruska and Law**,** 1970). Very recently Pandey *et al*., 2011 studied the possible-efficacy of use of *Antheraea mylitta* cocoonase in cocoon-cooking. The 35-40°C temperature and 8.5-9.0 pH range were found to be better for cooking of tasar cocoon in cocoonase. Initial boiling of cocoon in plain water for 30 min followed by cooking in cocoonase (1:5) at 35-40°C has enhanced cooking efficiency and resulted 50-55% silk recovery. It was observed that the yarn obtained from the cocoons cooked in cocoonase preserves the natural beautiful unique tasar silk colour, softness and lustre.

Papain, a serine protease obtained from papaya is an effective cocoon cooking enzyme and is most commonly used for cooking the cocoons (Sinha *et. al*., 1989, Borah and Baruah, 2009). Papain exhibits a wide specificity in its action towards polypeptides. Peptides bonds formed by the carboxyl groups of arginine and lysine residues are highly susceptible to attack by papain. Maximum silk recovery was obtained when the tasar cocoons which was initially boiled for 30 min in a solution containing 0**.**1% washing soda and 0**.**1% soap and steamed for 1 hr were then soaked overnight in raw papaya extract (200 gm of fresh raw and immature papaya fruit homogenized with 1 litre of water) in combination with sodium sulphite (5 gm/ litre) and washing soda (1 gm/ litre) at initial temperature of 50℃**,** pH of the bath being maintained at 8**.**0 (Sinha *et. al*., 1989)**.** Borah and Baruah, (2009) observed that cooking of muga cocoon with pure papain of concentration 0.05 % and Na_2CO_3 (0.20 %) showed highest breaking load while cocoon treated with latex of concentration 0.05 % extracted from fresh green papaya and Na_2CO_3 (0.15 %) produced yarn of highest breaking load.

Another plant protein, Bromelain (a cysteine protease) which is obtained from pineapple is also used for softening of the cocoons (Devi *et al*., 2011). Bromelain prefers to cleave at Lys-, Ala-, Tyr-, Gly- bonds. It is activated by cysteine, bisulfite salt, NaCN, H_2S, Na_2S, and benzoate, however, bromelain is usually sufficiently active without the addition of activators (Silverstein *et al.,* 1975). Singh *et al*. (2004) characterised the bromelain enzyme present in pineapple fruit for potential application in tasar cocoon cooking. It was observed by Devi *et al.*(2011) that cocoons cooked by a standardized pineapple extract procedure involving initial 45 min pressure cooking followed by 1 hr soaking in pineapple extract at 60℃ gave a very high reeling performance. The pineapple extract was prepared by homogenising 150 gm of the fruit pulp with 1 litre of distilled water and the resulting homogenate was strained through a coarse cotton cloth.

Commercial preparations of proteolytic enzymes such as Anilozyme-P, Biopril-50, Trypsin and Pepsin are also used to soften the cocoons (Goel and Rao, 2004) . However, these commercial enzymes are expensive and are not readily accessible to the common silk reelers and weavers. The application of enzymes in cooking process is far less than enzymes used in degumming process (treatment of fibre/yarn).

Enzymes in Degumming

Degumming is removal of sericin and other particles from drawn silk fibre / yarn /fabric. It is a silk refining process consisting of removal of i) sericin ii) the reagents added by the throwster in soaking raw silk and iii) any incidental dirt picked up in any of the operations, throwing or knitting (Gulranjani, 1992). Removal of natural wax, some colouring components and mineral matter is also achieved during degumming process. Since all natural and acquired impurities except sericin constitute only a very small fraction and are comparatively easily removed, the degumming process may be considered primarily as one of cleavage of peptide bonds of sericin, either by hydrolytic or enzymatic methods, and its subsequent removal from fibroin by solubilisation or dispersion in water (Gulranjani, 1992).Degumming results in a silk yarn or fabric with soft handle and enhanced luster. The enzymes trypsin, papain, and bacterial enzymes were the main ones used for

degumming process. It is only during the past few decades that the enzymatic degumming of silk has been developed along scientific lines (Johnny *et al*., 2012). However nowadays a number of enzymes used for silk degumming have been studied and patented day by day.

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 sericine 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 (Gulranjani, 1992).

Papain, the only plant protease that has been extensively investigated for degumming of silk, is a sulphydryl 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 and Speakman, 2004). Nakpathom *et al.* (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.

The enzyme Bromelain, a plant protease, isolated from pineapple, has also been tried and found suitable for silk degumming (Iida, 1972, Devi *et al*., 2012).

A bacterial enzyme Alkalase 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 (Lee *et al*., 1986, Nalankilli, 1992). Very recently process for degumming of silk with fungal protease enzyme has been standardized and found to be economically viable without chemical hazardous. Other alkali stable proteases found suitable for degumming of silk and already patented includes Degummase, Thermodegummase, Esperase, Sausinase, Proteinase, Proteolytic enzyme S114, Lipase, Alcalase, Cellulase, Protosol, Protease A. N. M., Pepsin, *etc* (Gulranjani, 1992).

Advantages of enzymatic method

Enzymatic cooking and degumming has advantages over other chemical methods. As enzymes are highly specific in action, it give minimum damage to fibroin during cooking or degumming process. It is considered to have a mild action on the fibres and is claimed to produce uniformly degummed material with a soft handle (Gulranjani, 1992). 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 and Speakman, 2004). Enzyme treatment is an environmentally friendly process because enzymes are readily biodegradable in nature.

Enzymatic methods also have some economic disadvantage. It needs some pre-treatment processes, since the gum must be swollen before the enzyme bath and it is a very slow reaction compared to alkaline soap method and is time consuming (Sonthisombat and Speakman, 2004).

CONCLUSION AND FUTURE WORK STUDY

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 standardised. 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. Moreover, as the application of proteolytic enzymes in cocoon cooking process is far less than enzymes used in degumming process, more intensive studies regarding enzymatic treatments for cooking the cocoons needs to be undertaken. 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.

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