Eco-Friendly Degumming of Silk Fabric and Evaluation of Antimicrobial Properties

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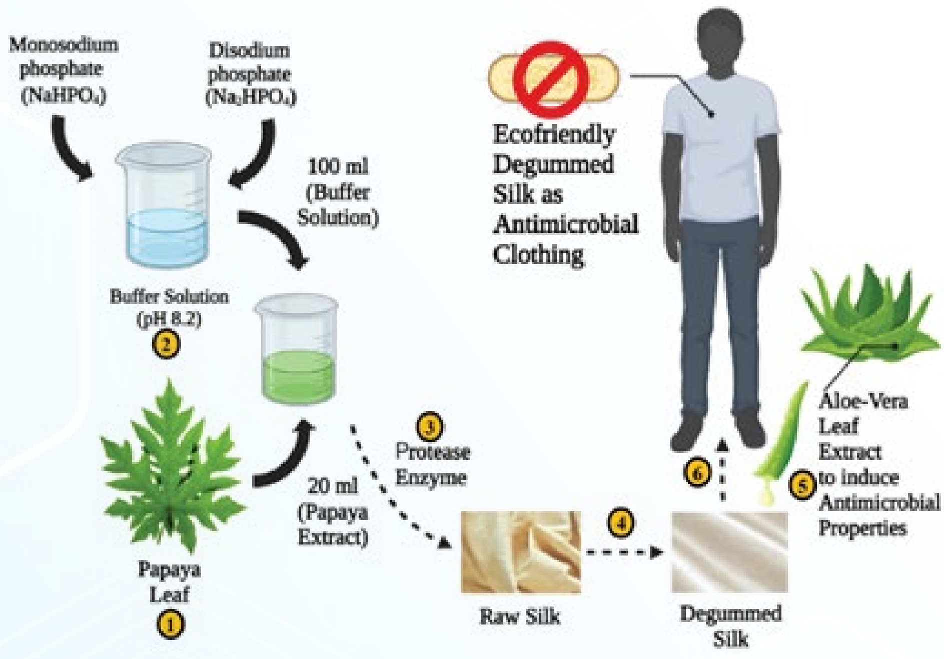
Abstract

The degumming of silk eliminates the cementing sericin layer from the surface, improving its color, texture, luster, absorbency and drape while keeping the fibrous component, fibroin, intact. To transform raw silk into clothing, the process of degumming silk is obligatory; however, this process is usually followed by harmful chemicals. This work aims to perform eco-friendly degumming by optimizing the removal of the sericin content from silk fully or partially, as residual sericin on silk can be used as an antimicrobial agent. This paper also studied the effect of the eco-friendly degumming process using papain-extracted protease enzyme and citric acid from lime on spun silk fabric in different ratios. Subsequently, a comparative study is also made with the conventional alkaline/soap degumming process. In order to evaluate the degumming percentages, weight loss values supported by water vapor permeability and water absorbency tests were performed. Though conventional alkali/soap treatment showed better results on degumming (23% wt. loss) over enzymatic degumming (10.82% wt. loss), there remains a question on the effect on the environment as well as on antimicrobial properties. To increase the antimicrobial properties of the fabric, naturally extracted Aloe vera was used in various processes, including dip-pad-dry coating and spin coating. Agar well diffusion technique was used to evaluate the antimicrobial activity of the specimens with gram-positive bacteria *E.coli* and gram-negative bacteria *S.aureus*. Aloe vera gel applied by dip-pad-dry method without binder showed maximum resistance to both types of bacteria. The antimicrobial properties of treated fabrics were also compared against raw and degummed silk fabric. The result showed better water absorbency and stronger antimicrobial resistance in Aloe vera treated degummed silk fabric.



(Graphical Abstract)

Keywords— Silk, degumming, aloe-vera, protease enzyme, antimicrobial

1. Introduction

In the modern civilized world, textile materials are being considered not just as clothing but emerged as a symbol of growing interest owing to their contribution to multifunctional fields [1], [2]. The genre of textiles is explored from space to water, households to war fields and far beyond. However, textile materials are susceptible to the expansion of microorganisms like bacteria, mold, fungi, pathogenic bacteria and odor. These may cause the wearer's skin infection and create odor [3]. Moreover, because of the widespread pollution brought on by several harmful substances used during textile processing, a huge issue impacting both human existence and the environment has evolved in the modern period. Consumers worldwide consider environmental issues when selecting consumer goods, particularly textiles. Antimicrobial agents help destroy microorganisms' expansion and resist their effects [4], [5]. These agents can always be achieved by applying suitable antimicrobial agents which concern water consumption and leave more chemicals in the environment. Many natural materials, from cotton, wool, silk and linen, have been used for centuries to produce clothing [6]. Since its discovery, silk has been referred to as the "Queen of fibers" [7]. The sheen, lightweight, outstanding mechanical performance, fine and silky texture, efficient moisture transfer and excellent draping quality of silk are only a few of the sumptuous characteristics of its fabric. Moreover, it can naturally inhibit the growth of microorganisms up to a certain extent [8]. A cementing layer of sericin holds together two fibroin filaments, which make up the silk thread produced by the silkworm (Bombyx mori) [9]. About 75 percent of the raw silk is fibroin and 25 percent is Sericin [10]. Sericin, a byproduct of silk manufacturing, is advantageous because it is oxidation-resistant, antimicrobial and UV-resistant and readily absorbs and releases moisture [11]. Sericin has moisturizing, UV-resistant, anti-cancer and antibacterial effects. However, apart from the advantageous features of sericin, the fiber becomes much stiffer due to its presence. As a result, we cannot gain usable fiber after collecting it from its source. For transforming the raw silk into comfortable clothing, this cementing layer of sericin is required to remove by the degumming process [12]. Degumming, a crucial step in processing silk, involves completely removing the sericin [13]. As a result, the materials exhibit improved soft handling, a lustrous appearance and an attractive drape that consumers highly value. Usual methods for degumming silk include chemical (aqueous solutions with organic acids or soap, alkali and synthetic detergents), biochemical (with proteolytic enzymes), or water-alone under pressure methods [14]. Acid degumming is comparatively less used as the alkaline treatment is much safer for fibroin than acids [15]. However, in both cases, whether it is acid or alkaline.

Various recent studies have shown that sericin is often effectively removed by using enzymes [16]. Among the many enzymes, proteolytic enzymes have been extensively studied recently. Papain is a sulfhydryl enzyme isolated from papyrus latex which is most active between pH 5 to 7.5 at 70^{0}C and nucleophilic cysteine thiol in their structure. It requires activation by a sulfhydryl reagent. This method significantly reduces the whole process's prices regarding water, energy, chemical consumption and effluent treatment. However, if not adequately regulated, both the acid and alkaline treatment trigger severe irritants on silk fibroin, resulting in uneven degumming, loss of lustrous appearance, decrease in tensile strength and ultimately deteriorating performance in dyeing [17], [18]. But most importantly, the environmental effect is beyond comparable. The green process of silk degumming is of primary importance in addressing these issues.

This paper proposes to study the effect of the proteolytic enzyme (Protease) degumming on silk fabric. The enzymatic treatments were administered using four different proteases: one neutral, two alkalis and one acidic. Though silk fabric contains antimicrobial properties, we applied Aloe vera gel as an additional agent to strengthen the antimicrobial properties and shield the wearer and the fabric. It is observed that applying Alo vera along with green synthesis significantly improves the antimicrobial properties. Although the degumming percentage is still less than that of the conventional chemical process, green synthesis can act as a substitute for conventional degumming for the sustainability issue.

2. Materials and methods

2.1. Materials

For this work, raw silk fabric was collected from Bangladesh Sericulture Research and Training Institute, Rajshahi- 6207.

2.2. Degumming

The degumming process of silk fabric was done in three ways in this study, (1) Degumming of spun silk fabric with enzyme, (2) Degumming with citric acid and lime and (3) Conventional way of degumming with soda (Na_2CO_3).

2.2.1. Buffer solution preparation

The degumming process was initiated by making a buffer solution to extract and activate the enzyme from the papaya leaf. In order to prepare this buffer solution, at first, two separate beakers containing 7.1g of Disodium phosphate (Na_2HPO_4; MW : 268 g/mol) and 6g of Monosodium phosphate ($NaHPO_4$; MW : 138 g/mol) were taken. Then, 100 mL of distilled water is added to make a solution for each solute. After that, to initiate the dilution process, 16 mL of Disodium phosphate solution was diluted in 100 mL of water; similarly, 6 mL of Monosodium phosphate solution was diluted in 100 mL of water. Finally, to get the buffer solution, an alkali diluted solution of Monosodium phosphate is kept pouring and mixed into a diluted solution of disodium phosphate to maintain pH 8.2.

2.2.2. Protease enzyme extraction

For protease enzyme extraction, we have selected papaya leaves as our source. 2-3 papaya leaves were collected from a nearby orchard and cleaned with distilled water. Later, the leaves are blended and papaya syrup is extracted using mesh fabric. 20 mL of the syrup was separated and poured into 100 mL of buffer solution and the mixture was covered in a darker place at room temperature (27°C) for 24 hours, maintaining a pH of–8.2. The optimum pH is adjusted using Na_2CO_3 or HCl when necessary. After 24 hours, the solution becomes cloudy and white agglomeration is observed, indicating the presence of protease enzyme[19].Total liquor is then filtered with a mesh fabric and the enzyme solution is ready to be used in the degumming process.

2.2.3. Degumming by enzyme

The degumming process was performed using a sample-dyeing machine. After keeping the buffer solution with papaya extract overnight for the formation of the enzyme, it was filtered and taken out in a clean beaker. Next, the raw silk fabric (0.5 g) was rinsed with distilled water. After that, the enzyme solution was moved into a sample dying tube and the raw silk was immersed into the enzyme solution. This process was operated for 1.5 hours at 55°C (M:L =1:20). The treated silk fabric was removed from the dye tube and washed thoroughly with hot and cold water. It was followed by weighing the sample fabric once dried. Finally, the weight of the degummed silk fabric was noted for further comparison.

2.2.4. Degumming by citric acid and lime

Processing of degumming by citric acid and lime follows the same process with the help of a sample dyeing machine. The ratio was kept on M: L=1:20. Here, 0.05 g of raw silk was put in a mixture of 0.5 g/L of detergent and (15-30)% citric acid solution. Then the tube was placed on a sample dyeing machine and run for 60 minutes at 98 °C. After that, the degummed sample is dried and weight is noted.

2.2.5. Degumming by the conventional method

Following the previous process, the ratio was kept on M: L=1:20. Here, 0.05 g of raw silk was put in a mixture solution of 1 g/L of Soda (Na_2CO_3), 0.5g /L of SDS and 5 g/L of Hydrogen peroxide (H_2O_2). Then the tube was placed on a sample dyeing machine and ran for 60 minutes at 80°C. After that, the degummed sample is dried and weight is noted.

2.3. Extraction of Aloe vera gel, solution preparation and impregnation with silk

Aloe vera leaves were trimmed and cleaned using distilled water. Next, they were removed from the gel and dried in an air dryer for three hours at a temperature of 50°C. Methanol was used to immerse the dried gel for a week and it was then filtered via a mesh material. After that, the methanol was distilled using a rotary evaporator. The extracted Aloe vera gel solution was then obtained.

Aloe vera solution and 1% acrylic binder solution were taken for dip coating with a binder, spin coating and dye bath and 70 mL Aloe vera solution was taken for dip coating without binder. Both were combined with the aid of a stirrer. The solution was stirred in an ultrasonicator at 50°C for 20 minutes to ensure adequate and uniform mixing.

2.3.1. Spin coating

The amount of mixed Aloe vera and binder solution was applied to the cloth one drop at a time while the circular plate rotated at 1500 rpm for 30 seconds, according to the weight of the fabric. After that, the specimen was dried for 3 minutes at 80°C in a drying machine. Then, it was finally cured for 2 minutes at 100°C.

2.3.2. Dip coating (with binder)

The mixed solution was prepared by the ultrasonicator and then taken in a beaker. The fabric sample was then immersed in a solution made of Aloe vera and acrylic binder for five minutes. Subsequently, the treated fabric sample was padded by using a padder machine. It was then dried in a drying machine for three minutes at a temperature of 80°C. At last, it was heated to 110°C for 2 minutes to cure it.

2.3.3. Dip coating (without binder)

The solution prepared by the ultrasonicator was taken in a beaker. Then, the fabric sample was dipped in an Aloe vera solution for five minutes. Later, the treated fabric sample was padded by using a padder machine. After that, the specimen was dried for 3 minutes at 80°C in a drying machine. At last, it was heated to 110 °C for 2 minutes for curing.

2.3.4. Using dye bath

The ultrasonicator-prepared solution was poured into a dye pot. After that, an Aloe vera and binder solution were applied to the fabric sample using the same bath. Next, it was processed for 20 minutes at 40°C in the sample dyeing machine. Later, the treated fabric sample was padded using a padder machine to extract the residual liquor from the sample. Then it was dried in a drying machine at 80°C for 3 minutes. Finally, it was cured at 110°C for 2 minutes.

3. Results and discussion

3.1. Degumming performance

The following Eq. (1) was used to evaluate the degumming performance,

$$\text{Degumming Performance (\%)} = \frac{(W - W_1)}{W} \times 100\% \quad \text{(i)}$$

W= Fabric weight before degumming; W_1= Fabric weight after degumming

After degumming with naturally extracted protease enzymes, it was found that it worked better on alkaline pH than acidic pH, in which degum (%) is 10%. In other methods, naturally extracted citric acid from lime had given degum (%) of 13%. However, conventional and chemical-based methods have given better percentages of degumming, but there remains the question of eco-friendly steps. The comparative degumming (%) is depicted in Fig. 1.

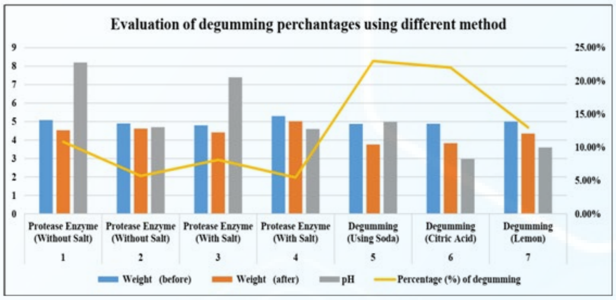


Fig.1 Evaluation of degumming performance (%) using different methods

3.2. Antimicrobial test using disk diffusion method

The antimicrobial assessment is performed using the disk diffusion method. The disc diffusion method was used to determine the antibacterial activity of all samples produced using those procedures. Selected bacteria were subcultured from stock by maintaining them in the incubator for an entire day or overnight. It was necessary to create nutrient broth and agar media following the requirements. Nutrient broth media were prepared in 2 conical flasks (50 mL/conical flask) & 300 mL agar media were prepared in a separate conical flask. Conical flasks containing nutrient broth were incubated for 24 hours. By putting bacteria in an incubator for a night, bacteria from human skin and fingertips were cultured. A colony of human skin bacteria was put into the test tubes and cultured for 3 to 4 hours. 14 Dewater test tubes (9 mL/test tube) were used for serial dilution. All test tubes should be closed with foil paper or cotton plug. 2 bacteria colonies were added to the nutrient broth conical flask media. After that, bacteria-containing nutrient broth conical flasks were serially diluted up to 107 times.

Then, the agar solution was to be poured into 12 circular plates. Once they reached a semi-solid state, 1 cc of the bacteria solution was added to each plate and spread out using a spreader. In this way, samples were examined for three different types of microorganisms. Samples were made utilizing six different methods received treatment; Degumming method, dip coating with a binder, dip coating without a binder, spin coating method & dye bath. The untreated and treated fabrics for each kind and method were examined and conditioned overnight in an incubator at 37°C. The outcome was then determined by measuring the region surrounding the fabric resistant to bacterial attack. By using 12 pectin plates, 72 samples were examined in this manner. The whole procedure and the antimicrobial analysis is reflected in Fig. 2, Fig. 3 and Fig. 4.

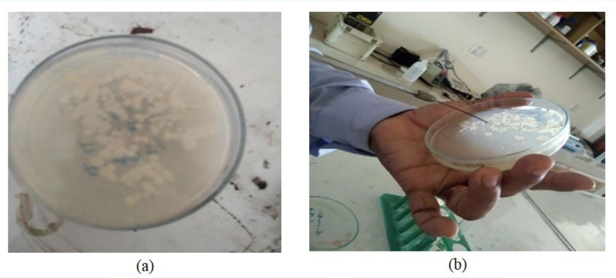


Fig. 2 Bacteria collection from finger

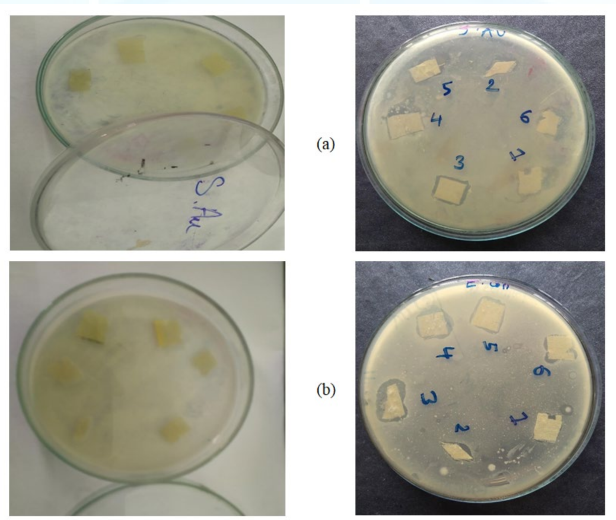


Fig. 3 Zone of inhibition screening using disk diffusion method against (a) S.aureus; (b) E. coli

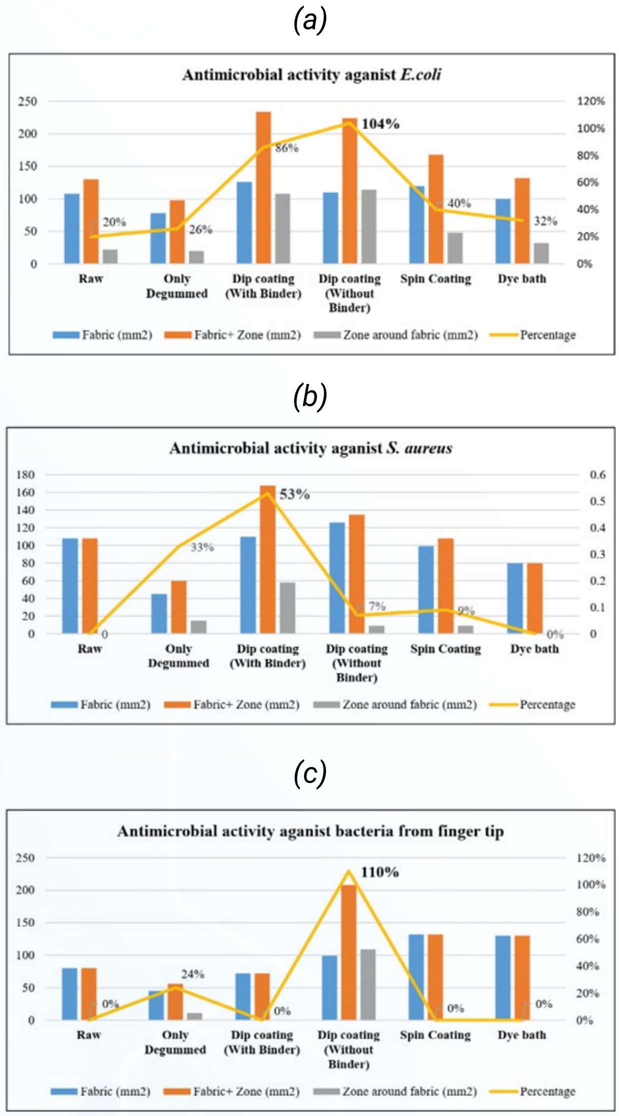


Fig. 4 Antimicrobial activity of various bacterial source (a) E. Coli; (b) S.aureus and (c) finger tip

After testing with a neutral agar diffusion disk, it has been found that raw silk (no finishing done on the surface) is 20% resistant to *E.coli* and not resistant to other bacteria. Degummed silk: slight resistance to all bacteria. Silk coated by a dip-pad-dry method with Aloe vera gel exhibited maximum resistance against all the bacteria.

3.3. Spray rating test

A Spray rating test was performed using GESTER GT-C31. The spray rating test shows (Fig. 5) that the conventional degumming process performed better than others, which the degumming percentages can also validate.

However, degummed silk using protease enzyme performed better than raw silk because of improved absorbance of the degummed silk fabric.

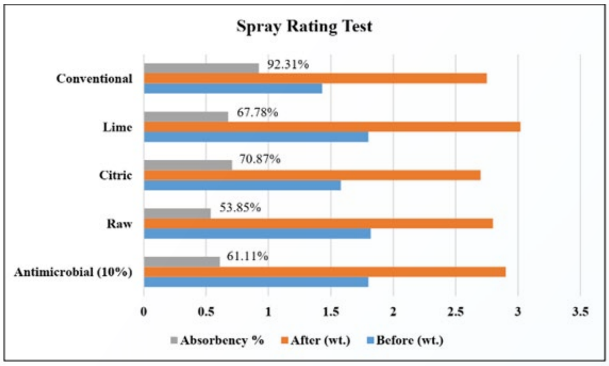


Fig. 5 Assessment of spray rating (%) performance

3.4. Water vapor permeability test

The water vapor permeability test was examined using the Water Vapor Transmission Tester (WVTR-E96-M). From this test, it is noticed that, although there was a coating of antimicrobial substance (Table 1). However, coated silk is still better than raw silk regarding water-vapor permeability due to less sericin presence.

Table 1: Water-Vapor Permeability Test

Sample	Before Wt.	After Wt.	Vapor Transmission rate
Degummed Silk	188.584	188.373	1.97566
Raw Silk	189.511	189.43	0.75843
Degummed and Antimicrobial Coated Silk	187.681	187.553	1.1985

4. Conclusion

In this research work, we have performed an eco-friendly degumming using enzymatic action on Silk fabric. Although our primary focus was on degumming, evaluating the antimicrobial activity is one of the key objectives. The degummed fabric using protease enzymes exhibited moderate (Degum%, 10.82%) feedback, while conventional degum% was 23%. Nevertheless, the antimicrobial activity of these fabrics is significant. For this test, *E.coli*, *S. aureus* and bacteria from human skin were observed; in all cases, the degummed and antimicrobial-coated fabric showed better performance. Antimicrobial activity against *E.coli*. was about 104%, antimicrobial activity against *S. aureus* was about 53% and antimicrobial activity against bacteria from finger tip was about 110%. With the advent of technological advancements, environmental issues are often ignored. Current research will create a pathway toward healthier and eco-friendly clothing processing.

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Conflict of interest

The authors have no conflicts of interest to disclose.

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