THE PHYSICAL FIBRE PROPERTIES OF *GONOMETA POSTICA* AFTER DEGUMMING THE COCOONS WITH DIFFERENT METHODS

Ismari van der Merwe*, Hester JH Steyn, Celia J Hugo & Robert Schall

ABSTRACT

Silk fibres from *Gonometa postica* were degummed with different, not yet considered, environmentally-conscious methods to conserve energy. The agents used were vermicompost, distilled water, catholyte, and *Eucalyptus* oil. The results were compared to a control, Orvus paste, which is currently in use as a chemical degumming agent. The physical properties of the silk fibres that were evaluated in this study included weight loss determination, degumming efficiency and scanning electron microscopy. The results indicated that the weight loss of *G. postica* fibres ranged from 27 to 41% over a time period of 10 days for the different methods evaluated. Orvus paste and Eucalyptus oil, catholyte and Orvus paste caused the greatest weight loss indicating the best sericin removal. Distilled water and *Eucalyptus* oil were the least successful in sericin removal, delivering a weight loss of less than 30%. The SEM micrographs indicated some sericin remnant still present on the fibres. Catholyte set a better standard for performance. It was concluded that catholyte, vermicompost and distilled water can be recommended as alternatives to the chemical Orvus paste as degumming method for *G. postica* wild silk.

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INTRODUCTION

Wild silkworm farming is a unique industry with a great potential for employment generation, artisanal development and export earnings (Kioko et *al.* 1999). Strong silk of high commercial value is provided by the African species of silk moths (Mbahin *et al.* 2008). During the 1980s, wild silk from Southern Africa appeared on the European markets. Patterson (2002) reported that quality-wise, two closely-related species of the genus *Gonometa rufobrunnea* (brown copper) and *Gonometa postica* (dark copper) could successfully compete with the other non-mulberry silk types.

The *G. postica* silkworm is endemic to the Kalahari and Namibia regions of Southern Africa and lives on acacia tree species, predominantly on *Acacia erioloba* (camel thorn) and *Acacia mellifera* (blackthorn). Cocoons (10 –15 cm) of the *G. postica* caterpillar produce the Kalahari tussah, a traditional leg rattle (Nyoni 2009). At first, rural communities collected wild silk cocoons to prevent ingestion by livestock, especially during dry spells, when it was realised that these cocoons were composed of wild silk, a new industry was pioneered to collect and degum the spent cocoons. This presented an opportunity to use natural products as a source of fibre and work.

FIGURE 1: *GONOMETA POSTICA* COCOONS IN THE TREES (DREYER, 2013)

Wild silk cocoons, harvested after the moth has matured, cannot be reeled. These cocoons can be degummed, cleaned and carded and spun into spun silk yarn, known as 'spun silk' (Good *et al.* 2008). The mean weight of silk from one cocoon is 0.4 ± 0.2 g for females and 0.21 ± 0.1 g for males. The number of cocoons required to spin 1 kg of *G. postica* silk is 2 326 – 4 762 cocoons (Kioko 1998).

Wild silk varies in terms of quality and is, as a whole, coarser, more uneven and more difficult to handle than fibres produced by cultivated worms. Wild silk cocoon strands are shorter, because they emergence from the cocoon (Good *et al.* 2008). The structure of the fibre is irregular, but very strong, which makes these fibres more durable (Dash *et al.* 2006). A cross-section of a silk fibre shows that it is roughly elliptical. These fibres have longitudinal striations and are porous (Das *et al.* 2005). The diameter of the fibres is 18 –33 µm. A longitudinal view of the silk fibre shows a very irregular surface structure, covered by a sericin layer. The silk of *G. postica* is difficult to degum because of these sericin and calcium compounds that cement them together (Mhuka *et al*. 2013*)*.

Sericin is a water-soluble macromolecular globular protein (Zhang *et al*. 2004; Wu *et al*. 2006) and contributes about 25 –30% of the total cocoon weight (Zhang 2002; Dash *et al.* 2006). Silk, before degumming, does not possess high lustre. Wild silks are uneven, brown and slightly less lustrous. The natural colour of the wild silk fibre is due to the type of leaf upon which the silkworm feeds, thus *G. postica* silk having a rich, tawny colour. Silk, as a natural fibre, offers a unique strength of 3 – 5 g per dtex. Silk is resistant to most mineral acids, except for sulphuric acid and strong hydrochloric acid which dissolves it (Sutherland *et al.* 2010). Silk is less sensitive to diluted alkalis, but the lustre of the fibre is somewhat diminished (Vepari & Kaplan 2007). When treated with strong hot caustic alkalis, the fibre dissolves (Rajkhowa *et al.* 2011).

The methods for degumming can be classified into four main groups: soap, alkalis, acidic solutions and degumming by enzymatic methods (Ravikumar 2007). Chemical degumming can cause fibre degradation and often results in a loss of aesthetic and physical properties, causing a dull appearance, surface fibrillation, poor handling and weakening of tensile strength. Fibre degradation will also result in uneven dyestuff absorption during subsequent dyeing and printing. Silk is boiled in alkaline medium for 30 - 120 minutes. Unfortunately, chemical degumming methods are not environmentally friendly.

Orvus paste is a pure anionic detergent consisting of 100% sodium lauryl sulphate (NaC12H25SO4), with a neutral pH and is completely biodegradable. Orvus paste does not

contain bleaches, enzymes, fillers, brighteners. Anionic detergents are inexpensive, high foaming and can be powerful cleaners. Seeing that Orvus paste as a soft detergent would prevent the harming of the silk fibres it becomes a sensible choice to use as the standard chemical method in research. Vermicompost is prepared from organic materials using earthworms, as a low cost and eco-friendly technology system. Millions of tons of animal, agriculture and kitchen waste are produced annually, creating smell and pollution problems (Aira & Domínguez 2009). Micro-organisms and earthworms as organic resources can help solve the problems in an ecologically sound, economically viable and socially acceptable technical way (Devi *et al*. 2009). This technology also provides self-employment for rural people, by utilizing the available agricultural resources (Rajendran *et al.* 2008). Micro-organisms produce enzymes that cause biochemical decomposition of organic material (animal, agriculture and kitchen waste), but earthworms are the crucial drivers of the process (Aira *et al.* 2002; Suthar 2008). The earthworms modify the microbial biomass and activity (Nath *et al*. 2009). Vermicompost has a high porosity and water-holding capacity and a low C: N ratio. The moisture content of castings ranges between 32 – 66% and the pH is ±7 (Munnoli *et al.* 2010). The cost of vermicompost production is insignificant, as a 1 g worm could convert 4 g of activated sludge in 5 days (Anon 2006).

A combination of water and protein degrading micro-organisms (if present) on the wild silk were identified as a possible method to degum cocoons. Degumming with distilled water would also be simple and a low cost option. *Eucalyptus* oil is successfully used as a cleaning solvent. It is a natural oil and also environmentally friendly, which might be an effective method to aid in the degumming of glue-like sericin.

Electrochemical activation technology, discovered in 1972, is used in a wide range of applications (Lobyshev 2007) as environmentally friendly anti- microbial and washing media (Tomilov 2002; Bakhir 2005). Catholyte produced from electrochemical activation, remains stable for a few days. Catholyte is non-toxic to the environment (humans and animals), is easy to handle and can serve as a non-foaming detergent. Theoretically it can serve as an effective surfactant and could be applied to degumming procedures.

The degumming process of cocoons is thus problematic; harsh degumming processes can damage the fibre and pollute the environment. The development of an effective and environmentally conscious degumming method was necessary. Therefore, the aim of this study was to develop and evaluate environmentally conscious degumming methods that could remove sericin without harming the fibroin.

METHODS

Materials

Gonometa postica cocoons were obtained from a farm on the border between Namibia, Botswana and South Africa. The cocoons consisted of a high quality wild silk and had all hatched.

The collectors on the farm cut the cocoons open, and removed the skin-remains as well as other matter. In the laboratory, the cocoons were trimmed at the top, cleaned out by hand and the brittle silk surrounding the emergence hole was removed with a scissor. The cocoons were conditioned at 21 ± 1°C and 65 ± 2% relative humidity (RH), before weighing. Eighteen samples of ±5 g were weighed for each degumming method. A ±5 g sample consisted of approximately five cocoons, depending on the size of the cocoons.

Degumming media

Degumming media included catholyte, Orvus paste (control), vermicompost, distilled water and eucalyptus oil (the *Eucalyptus* oil was combined with each of the other media):

Orvus paste The Orvus solution for the degumming bath was a mixture of 38 g sodium carbonate (Protea Chemicals) and 38 g Orvus paste (Proctor & Gamble) in 3.8 L of distilled water prepared in a 4 L glass bottle. Catholyte water was electrochemically activated by passing a water solution with a 5% NaCl concentration through a water electrolyser (Hoshizaki Electric Co., ROX-10WB-E unit). To ensure adequate softness, tap water was filtered beforehand. The electrolysis was carried out under uniform conditions of a continuous electric current of 12 ampere (A) and pressure of 75 kilo -Pascal (kPa). Catholyte was produced at 1.0-1.2 litre/min. The catholyte had a pH of 12–13 and was used within 90 min of preparation (Annandale *et al*. 2008).

Vermicompost The product used was obtained from a researcher (Prof G van Tonder) at Groundwater studies at the University of the Free State. The vermicompost was made by using cow dung and plant materials. The piles were left for 45 days to achieve a carbon (C): nitrogen (N) ratio of 20:1. Once the pile reached the peak stage of decomposition, it was aerated by a turner to release harmful NH3 and increase microbial activity.

Distilled water Tap water was passed through a Fistreem water still apparatus (Fisons Scientific Equipment).

Eucalyptus oil Purified *Eucalyptus* oil BV 397(Act 101/1965) (Allied Drug Company (Pty) Ltd.) for medicinal use was used for the experimental degumming.

Degumming

Degumming with Orvus paste-method (control) Eighteen samples of ±5 g conditioned cocoons in glass bottles with lids were covered with 100 mL Orvus paste solution and kept at 32 C in an incubator, for 10 days. After 5 days, the first three samples were removed and treated as for vermicompost. This was repeated on days 6, 7, 8, 9 and 10.

Degumming with catholyte Eighteen samples of ±5 g conditioned cocoons were placed in glass bottles with lids and covered with 100 mL catholyte (pH = 12.85). The samples were kept at a constant temperature of 32 C in an incubator for 10 days. After 5 days the first three samples were removed and treated as for vermicompost. This was repeated on days 6, 7, 8, 9 and 10.

Degumming with the vermicompost-method Eighteen samples, each containing 5 g of conditioned cocoons were wrapped in 20 cm x 20 cm hessian pieces to keep the silk clean. The wrapped cocoons were placed into a container (30 x 20 x 15 cm) on a bed of vermicompost. They were covered with a thick layer of vermicompost and left for 10 days at a temperature of 32°C. After 5 days, the first three cocoon samples were removed from the vermicompost, unwrapped and pasteurized at 72°C for 16 seconds. They were rinsed three times in a sieve with distilled H2O at 30 C. The samples were subsequently rinsed with a mild citric acid solution (1 mL/2 L cold distilled H2O) to get rid of any fatty residues, and finally with a fabric softener (Staysoft) solution (15 mL/L of cold distilled H2O) to loosen the fibres. The samples were then dried with filter paper at room temperature (Robinson, 1999). After conditioning for 48 hours at 21 ± 1°C and 65 ± 2% RH, the samples were weighed again. The process was repeated on days 6, 7, 8, 9 and 10 with the remaining samples.

Degumming with distilled water Eighteen samples of ±5 g conditioned cocoons were placed in glass bottles with lids and covered with 100 mL distilled H2O and kept at a constant temperature of 32 C in an incubator for 10 days. After 5 days, the first three samples were removed and treated with the same procedure as for vermicompost. This was repeated on days 6, 7, 8, 9 and 10.

Degumming with Eucalyptus oil mixtures

Eucalyptus oil and distilled water: Eighteen samples of ±5 g conditioned cocoons were covered with a solution consisting of 10 mL of *Eucalyptus* oil (100% pure; Allied Drug Company (Pty) Ltd.) and 90 mL distilled H2O.

Eucalyptus oil and catholyte: Eighteen samples of ±5 g conditioned cocoons were placed in glass bottles with lids and covered with a solution, consisting of 10 mL *Eucalyptus* oil (100% pure; Allied Drug Company (Pty) Ltd.) and 90 mL Catholyte (pH = 12.85).

Eucalyptus oil and Orvus paste: Eighteen samples of ±5 g conditioned cocoons were place in glass bottles with lids and covered with a solution, consisting of 10 mL *Eucalyptus* oil (100% pure; Allied Drug Company (Pty) Ltd.) and 90 mL Orvus paste.

All samples were kept at 32 C in an incubator for 10 days. After 5 days, the first three samples of each were removed and treated as for vermicompost. This was repeated on days 6, 7, 8, 9 and 10.

Fibre property analyses

Weight loss and Degumming efficiency The efficiency of the degumming was calculated by comparing the weight loss of the cocoons in each of the methods with that of the Orvus-paste control method.

Scanning electron microscopy (SEM) analysis A morphological characterisation of the silk fibres from each degumming method was performed by SEM. The fibres were cut

and glued to the stubs by metal glue and left overnight to dry. Fibres were vacuum sputter-coated with a 5 angstrom coating of gold (60 nm) to make them electrically conductive (Good *et al.,* 2008; Zhang *et al.* 2012). A Shimadsu SSX 550 Superscan Scanning electron microscope was used and samples were observed at 15 kV and 20 kV acceleration voltage and 8 –15 mm working distance. Photographs were taken at a voltage of 15 kV at room temperature.

Statistical analyses For each study day, treatment and replicate, the ratio ($\frac{A}{B}$) of "after" and "before" weights was for the (natural) logarithm of these ratios [ln ($\frac{A}{B}$)] was statistically analysed using a one-way analysis of variance (ANOVA) model fitting the factor treatment. An F -test and d associated P-value for treatment was obtained from the ANOVA.

Furthermore, for all treatments the mean values of the log [$\frac{after}{before}$] weight ratio were calculated (that is, the means for each treatment, ln [$\frac{A}{B}$]). The pairwise mean difference "control - treatment" between those mean values, and associated 95% confidence intervals and P-values were also reported. Taking the anti-log of the mean values for each treatment on the logarithmic scale yields the geometric mean R_T = ($\frac{A_T}{B_T}$) of the [$\frac{after}{before}$] weight ratio for each treatment, and thus yields the mean percent weight reduction. Taking the antilog of the pairwise mean difference "control – treatment" yields the geometric mean efficiency of the treatment in question, namely:

Degumming efficiency = ($\frac{R_C}{R_T}$) = $\frac{(\frac{A_C}{B_C})}{(\frac{A_T}{B_T})}$ where

- AC is the geometric mean weight "after" for the control treatment
- BC is the geometric mean weight "before" for the control treatment
- AT is the geometric mean weight "after" for the test treatment in question
- BT is the geometric mean weight "before" for the test treatment in question

RESULTS AND DISCUSSION

Weight loss

Degumming weight loss increased linearly as the number of days increased for each method (Figure 2). On day 5, values of between 7% (distilled water and *Eucalyptus* oil) and 31% (catholyte) were obtained. On days 5 and 6, catholyte had the most weight loss of more than 30%. It was clear that the weight loss of *G. postica* fibres ranged from 27 to 41% over a time period of 10 days. This was inconsistent with previous reports where the sericin content was less in mulberry silk and as low as 12 to 16% in the silk of *G. postica* (Prasong *et al*. 2009). The results of this study were similar to those of Teshome *et al*. (2011) who reported a sericin content of between 23 and 56.8%. No weight loss occurred after day 9. Orvus paste and *Eucalyptus* oil, catholyte, and Orvus paste caused the greatest weight loss indicating the best sericin removal. Distilled water and *Eucalyptus* oil were the least successful in sericin removal, the only method delivering weight loss of less than 30%.

Efficiency of different degumming methods

The comparison of the degumming methods compared to Orvus paste on day 10 is given in Figure 3.

Orvus paste, the standard degumming medium is considered to be 100% efficient. It was clear that catholyte performed the best in degumming (114.6%) followed by the combination of catholyte and Eucalyptus oil (104.3%). The Vermicompost was slightly less effective (97.9%), followed by distilled water (92.8%).

Scanning electron microscopy (SEM) analysis

The silk fibres of *G. postica* have many longitudinal striations on their surface and are porous which make them lighter than mulberry silk (Teshome *et al.* 2011). These fibres are flattened, ribbon-like filaments (fibroin) of much larger diameter than mulberry silk (Teshome *et al.* 2011). The sericin coated fibroin strand itself is a bundle of several fibrils (Mhuka *et al.* 2013). In Figure 4, the flat triangular shape is evident and the fibre is not circular in cross section. *Gonometa postica* have fibre diameters of 18 – 33 m (Mhuka *et al*. 2013). Though silk fibre diameter has not been a point of discussion in technological applications, it is an important characteristic in the textile industry. The diameter of the fibre will influence properties such as abrasion resistance, softness and 17 stiffness (Chattopadhyay 2008). The surface morphology of silk fibres degummed by different degumming methods was investigated by SEM

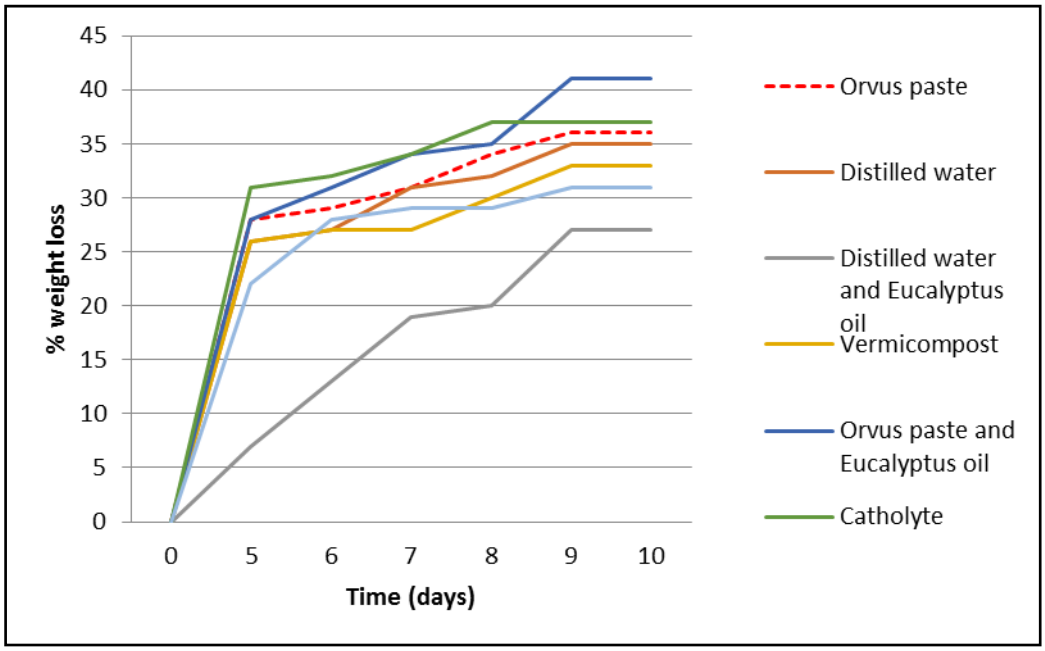


FIGURE 2: PERCENTAGE WEIGHT LOSS OVER TIME PERIOD OF 10 DAYS

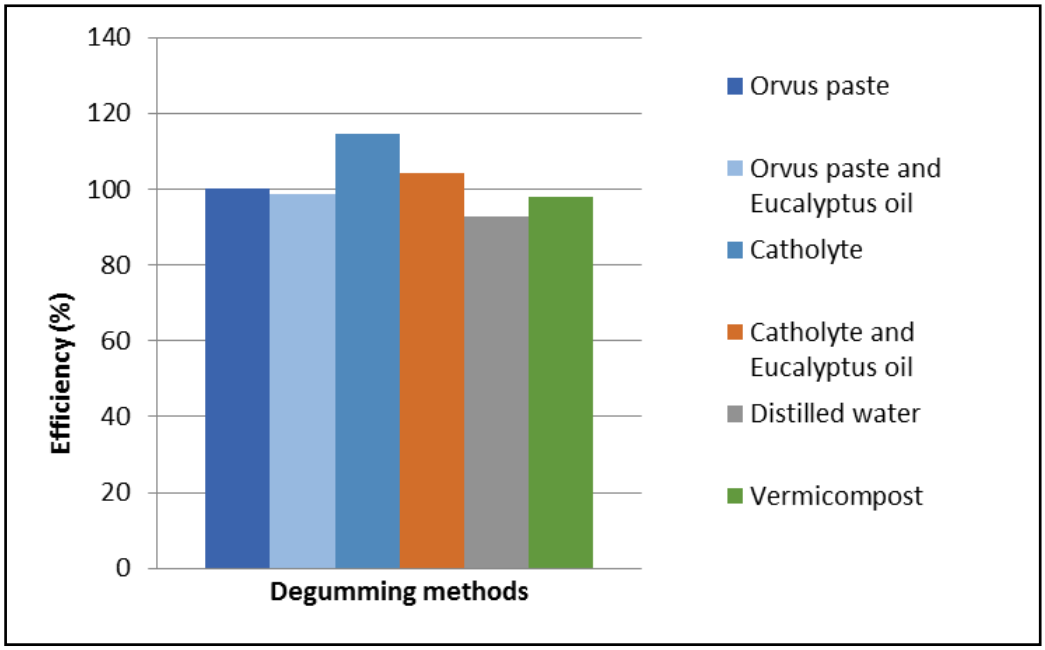
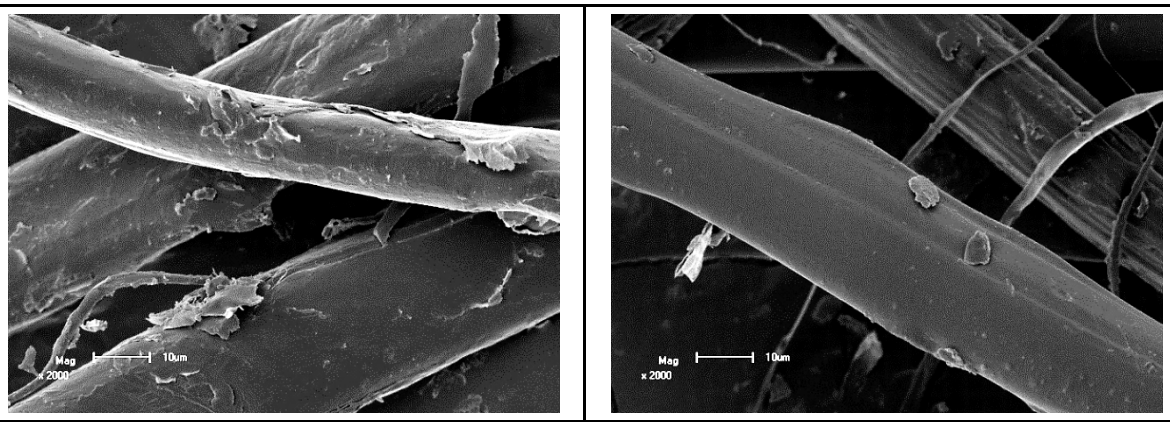


FIGURE 3: THE EFFICIENCY OF DIFFERENT DEGUMMING METHODS COMPARED TO THE STANDARD ORVUS PASTE ON DAY 10

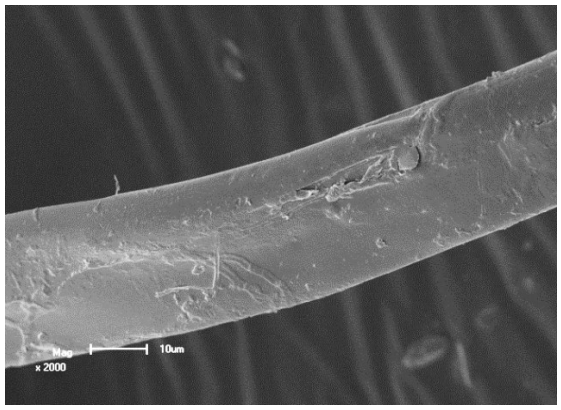
in this study. The surface characteristic of the *G. postica* silk fibre was fairly rough 20 which indicated large amounts of sericin present on the fibre. The sericin appeared as some partially non-uniform coating on the surface of the fibres and various granules and impurity deposits were visible in the vacant spaces in between fibres.

Orvus paste was used as the control degumming method. After 5 days of exposure to Orvus paste (Figure 4a), the degumming weight loss was 28%. The SEM micrograph indicated large amounts of sericin still present on the fibres. Ten days of exposure of the silk fibres to Orvus paste resulted in a degumming weight loss of 36% (Figure 4b). The SEM micrograph indicated some sericin remnants still

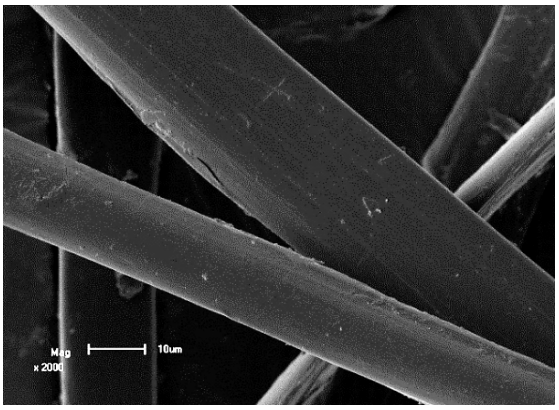


(a) Orvus paste (5 days)

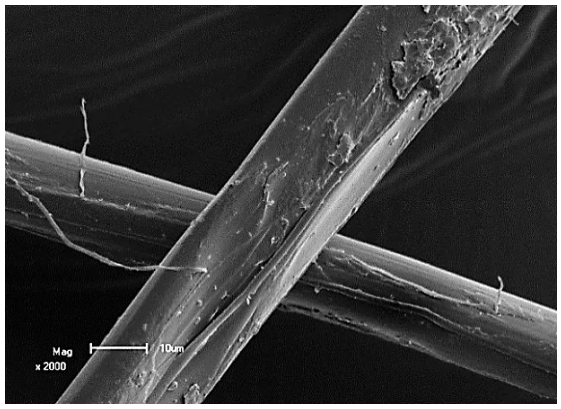
(b) Orvus paste (10 days)



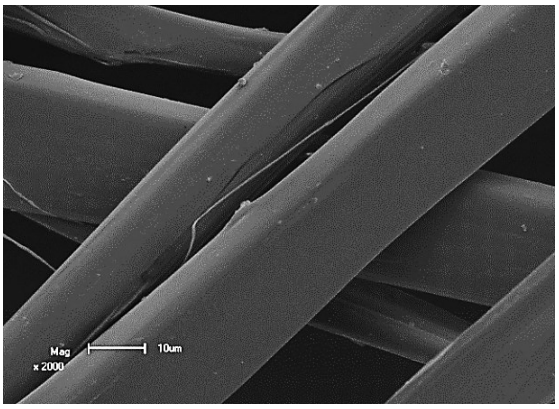
(c) Orvus paste and *Eucalyptus* oil (5 days)



(d) Orvus paste and *Eucalyptus* oil (10 days)



(e) catholyte (5 days)



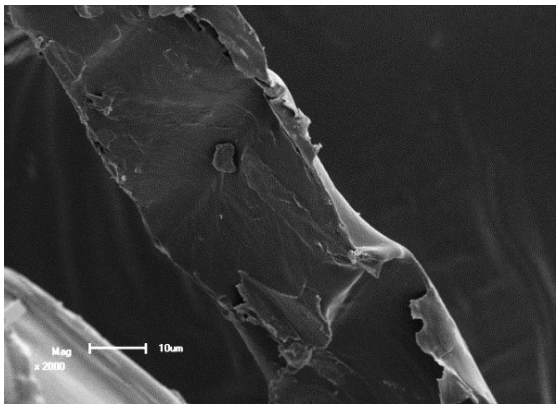
(f) catholyte (10 days)

FIGURE 4: SEM MICROGRAPHS OF SILK FIBRES DEGUMMED WITH DIFFERENT METHODS: (a) ORVUS PASTE (5 DAYS); (b) ORVUS PASTE (10 DAYS); (c) ORVUS PASTE AND *EUCALYPTUS* OIL (5 DAYS); (d) ORVUS PASTE AND *EUCALYPTUS OIL* (10 DAYS); (e) CATHOLYTE (5 DAYS); (f) CATHOLYTE (10 DAYS)

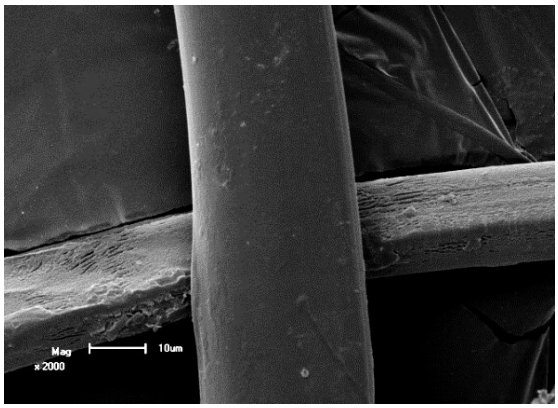
present on the fibres.

After an exposure time of 5 days, Orvus paste and the combination of Orvus paste and *Eucalyptus* oil showed the same degumming loss of 28% (Figure 4.c). Sericin remnants were still present. Exposure of 10 days to the combination method of Orvus paste and *Eucalyptus* oil resulted in a degumming weight loss of 41% (Figure 4d). The SEM micrograph showed a clean smooth fibre, but there were still some remnants present on the fibre.

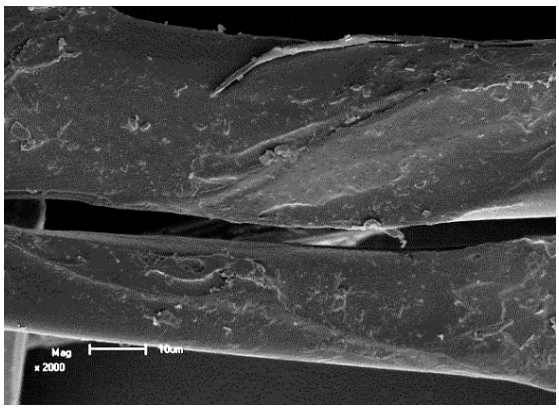
Sericin remnants were observed to be present on the fibre sample after 5 days of exposure to catholyte (Figure 4e). The amount of sericin on the fibroin was already less than in Figure 4a). After exposure of 10 days to catholyte, sericin



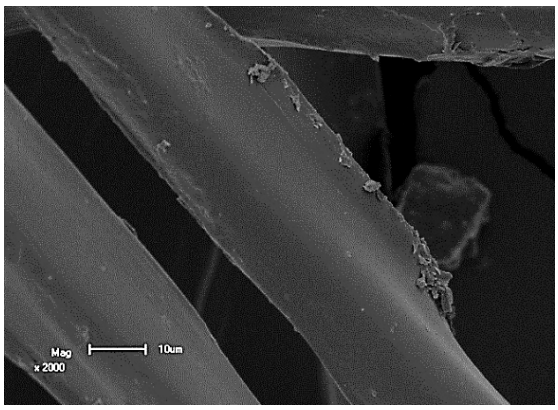
(a) catholyte and *Eucalyptus* oil (5 days)



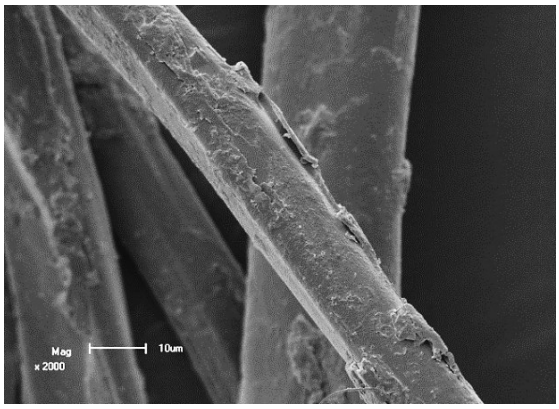
(b) catholyte and *Eucalyptus* oil (10 d)



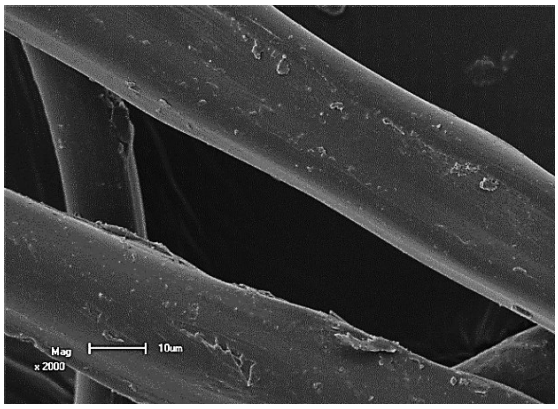
(c) distilled water (5 days)



(d) distilled water (10 days)



(e) distilled water and *Eucalyptus* oil (5 days)

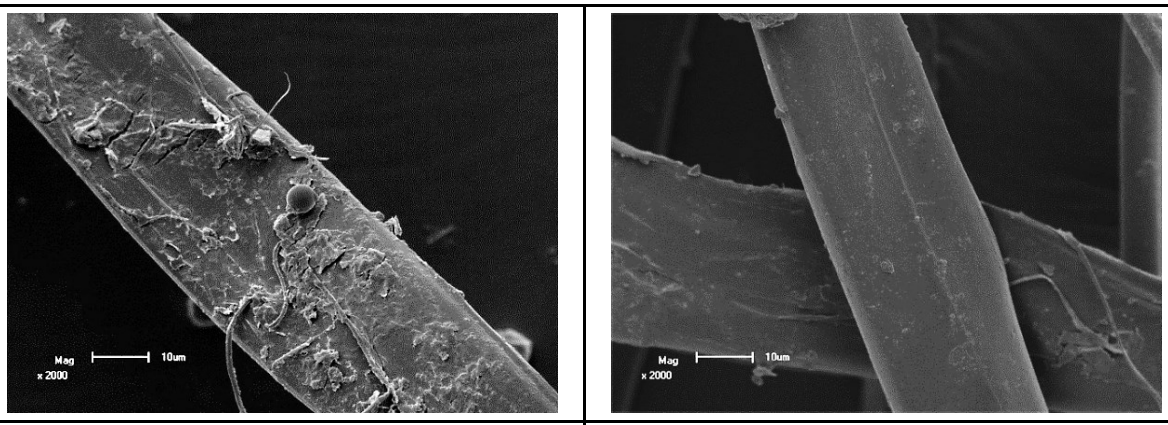


(f) distilled water and *Eucalyptus oil* (10 days)

FIGURE 5: SEM MICROGRAPH OF SILK FIBRES DEGUMMED WITH DIFFERENT METHODS: (a) CATHOLYTE AND *EUCALYPTUS* OIL (5 DAYS); (b) CATHOLYTE AND *EUCALYPTUS* OIL (1O DAYS); (c) DISTILLED WATER (5 DAYS); (d) DISTILLED WATER (10 DAYS); (e) DISTILLED WATER AND *EUCALYPTUS* OIL (5 DAYS); (f) DISTILLED WATER AND *EUCALYPTUS* OIL (10 DAYS)

was observed to be effectively removed, as indicated by the smooth silk fibres (Figure 4f).

Exposure of the fibres to the combination degumming solution of catholyte and *Eucalyptus* oil resulted in a low degumming loss of 22% (Figure 5a). Degumming seemed to be much slower with this method. After 10 days of exposure to catholyte and *Eucalyptus* oil, the fibres were partially cleaned and a smooth surface was evident. Remnants of sericin was still present in small amounts (Figure 5b). After exposure of 5 days of the silk fibres to distilled water, sericin was still present all over the fibres (Figure 5c). After exposure of 10 days to distilled water, the result was clear, smooth surfaces, but



(a) vermicompost (5 days) (b) vermicompost (10 days)

FIGURE 6: SEM MICROGRAPHS OF SILK FIBRES DEGUMMED WITH DIFFERENT METHODS: (a) VERMICOMPOST (5 DAYS); (b) VERMICOMPOST (10 DAYS)

there were still some evidence of remaining sericin. This indicated that with this method, the degumming process will take longer than 10 days to complete.

Distilled water and *Eucalyptus* oil, had a degumming weight loss of only 7% after 5 days of exposure (Figure 5e). The large amount of sericin was evident on the SEM micrograph.

After 10 days of exposure to the combined method of distilled water and *Eucalyptus* oil, sericin remnants were still observed (Figure 5f).The degumming weight loss was only 27%. This method also, therefore, needed more time for completion of the degumming process. An exposure of 5 days to vermicompost resulted in a degumming loss of 26% (Figure 6a). The rough surface of the *G. postica* silk fibres indicated large amounts of sericin.

After 10 days of exposure to vermicompost, the G. *postica* silk fibres still showed sericin remnants all over the fibre (Figure 6b). The time period for this method clearly was not sufficient. The parts of the fibre that were degummed showed clean, smooth surfaces.

Differences in the surface morphology of the degummed silk fibres were observed among the SEM micrographs of the fibres. The micrographs of the different degumming methods samples showed good to moderately good degumming results after an exposure ime of ten days and no signs of destruction or damage on the surface of the silk fibres. The fibre surfaces were smooth, showing only very shallow longitudinal striations attributable to the fibrillar structure of the degummed silk fibres. Based only on the morphological results, the best degumming method in this study was catholyte, catholyte and *Eucalyptus* oil and distilled water. The average weight loss, after 10 days, varied between 27% and 41% for all the applied methods. No fibrillations were observed in the fibroin fibres, indicating no fibroin degradation due to degumming. Fibrillations, as such, are likely to be caused by weakening of at least one type of the non-covalent interactions (hydrogen bonds and van der Waal's forces) (The *et al*. 2010).

CONCLUSION

Silk degumming is a high resource consuming process as far as water and energy are concerned. The development of an effective degumming process would mean saving water and energy, the recovery of valuable by-products such as sericin peptides, and a lower environmental impact of effluents. The application of environmentally conscious degumming methods catholyte, vermicompost and distilled water can be recommended as alternatives to the chemical Orvus paste as degumming method for *G. postica* wild silk. Catholyte was the most effective in degumming. The use of vermicompost and distilled water might improve in performance with extended exposure. Further work should include the demineralizing of the *G. postica* cocoons with ethylenediaminetetraacetic acid (EDTA) and the degumming afterwards with these environmentally conscious methods, to then analyse the results and the influence thereof on the final textile product. A further project with the same methods but different time and temperature indications should also be part of future research.

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