Degumming *Gonometa postica* cocoons using environmentally conscious methods

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Degumming *Gonometa postica* cocoons using environmentally conscious methods

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Declaration

"I declare that this dissertation, which I hereby submit for the degree Philosophiae Doctor at the University of the Free State, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution. I further more cede copyright of the thesis in favour of the University of the Free State."

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Ismari van der Merwe

2 February 2015

The establishment of a sustainable wild silk industry in Africa could pave the way for similar Africa-unique projects to capture the true spirit of the continent. That spirit that determines her worth and echoes in her truths: "Every morning in Africa, a gazelle wakes up. It knows it must run faster than the fastest lion or it will be killed. Every morning a lion wakes up. It knows it must outrun the slowest gazelle or it will starve to death. It doesn't matter whether you are a lion or a gazelle… when the sun comes up, you'd better be running."

(Author: Unknown)

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Chapter 1

GENERAL INTRODUCTION

1.1 Introduction

Silkworm silk has been a commodity for over 3 000 years. Its use is justified, both by an exceptional combination of mechanical properties and thermal stability. Furthermore, its biodegradability and biocompatibility offer many opportunities for new applications (Zhang, 2002).

In the developing world, people seek sustainable and environmentally friendly sources of income. Wild silkworm farming is a unique industry with a great potential for employment generation, artisanal development and export earnings (Mbahin *et al.,* 2008). Strong silk of high commercial value is provided by the African species of silk moths (Fening *et al.,* 2010; Mbahin *et al.,* 2008).

During the 1980's, wild silk from Southern Africa appeared the first time on the European markets. Interest in the products, as another source of wild silk, was immediately shown. Italian manufacturers requested tests to be done on the silk and the results compared with Chinese silk from *Bombyx mori* and Asian wild silk species. Patterson (2002) reported that, in quality, 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 *Gonometa* silk, when treated under the same conditions, was easier to bleach and was easier dyeable with all the chief classes of dye.

The *Gonometa postica* silk worm is endemic to the Kalahari and Namibia regions of Southern Africa. It is also known as the Molopo worm and lives on *Acacia* species, predominantly on *Acacia erioloba* (camel thorn) and *Acacia mellifera* (blackthorn). The cocoons produced by the *G. postica* silk worm look very similar to the pods of the *Acacia* tree (Figure 1.1), but the silk is indigestible and gathers in the rumen of multiple-stomach animals, causing starvation. Numerous cattle, sheep and even game are lost annually in Namibia, due to the ingestion of *G. postica* cocoons (Veldtman, 2005).

Figure 1.1: *Gonometa postica* cocoons in the trees looking very similar to the pods of the *Acacia* tree (Dreyer, 2013).

At first, rural communities have collected wild silk cocoons to prevent ingestion by livestock, especially during dry spells. Unaware that they were wasting a valuable natural resource, they changed the cocoons into traditional leg rattles (Nyoni, 2009). 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 represented an opportunity to move back to nature, as a source for fibre, job creation, whilst protecting the health of the animals (Veldtman, 2005).

Silk fibre is made of two different proteins – the core structural protein called fibroin and the gummy sheath protein called sericin. Degumming of the sericin disclose the fibroin fibre which has properties favourable for the development and production of a variety of different products. Methods currently used to degum the silk are, extraction with water (Sargunamani & Selvakumar, 2006), boil-off in soap (Chopra & Gulrajani, 1994), using alkalis (Taddei *et al.,* 2003), enzymatic degumming (Raval & Banaerjee, 2003), acidic solutions (Gulrajani & Chatterjee, 1992) and ultrasound (Mahmoodi *et al.,* 2010), especially for the silk of *B. mori*. The silk of *G. postica* is, however, more difficult to degum because it contains more sericin and calcium compounds (Sharma *et al.,* 1999). Scanning electron microscopy (SEM) images of undegummed fibres of *G. postica* showed that the fibres were cemented together with sericin unlike that of the *B. mori* fibres (Mhuka *et al*., 2013). Harsh degumming processes can, however, damage the fibre and/or pollute the environment.

1.2 Problem statement

A major concern of the textile industry is the need to make the most efficient use of natural fibres (Nabieva *et al.,* 2004). The trend in the textile industry is at present towards eco-friendly processes and minimising the adverse ecological effects of production (Raval & Banerjee, 2003). Silk degumming is a high resource-consuming process, as far as water and energy are concerned (Freddi *et al.,* 2003). Moreover, it is ecologically questionable, because of the high environmental impact of effluents. The development of an effective degumming process would mean saving water and energy, recovery of valuable by-products such as sericin peptides, and lower environmental impact of effluents (Freddi *et al.,* 2003; Raval & Banerjee, 2003).

1.3 Aim

The main aim of this study was to develop and evaluate environmentally conscious degumming methods that could discriminate between sericin and fibroin of the *Gonometa postica* cocoon, without harming the fibroin.

1.4 Objectives

The principle objectives of the study were:

- To calculate the degumming efficiency of the chemical versus the biological degumming methods, on weight loss of *G. postica* cocoons.
- To investigate the effect of the chemical versus the biological degumming methods, by making use of scanning electron microscope (SEM) images.
- To determine the effect of degumming of *G. postica* cocoons with chemical and biological degumming methods on the tensile strength of the silk fibres.
- To determine the effect of the chemical and biological degumming methods on the fibroin degradation in the cocoons of *G. postica.*
- To determine the microbial composition of silk fibres after different degumming methods.

1.5 Structure of the dissertation

The first chapter of the study consists of a general introduction with the motivation for and aim of the study. A detailed literature survey on various topics relevant to this work is presented in chapter 2. This includes all the information needed to understand and interpret the problem of degumming. Chapter three, deals with the

methods used for the degumming processes as well as the methods used to determine the effects of the degumming processes on the quality of the fibre in terms of physical, chemical and microbial properties. Chapter four includes the results and discussion of the different degumming methods. Chapter five concludes with recommendations and further research possibilities.

Chapter 2

LITERATURE REVIEW

2.1 Introduction

There are four types of natural silk which are commercially known and produced in the world under controlled circumstances (sericulture). Among them mulberry silk is the most important and contributes as much as 99% of world production. The term "silk" in general therefore refers to the silk of the mulberry silkworm. Three other commercially important types fall into the category of non-mulberry silks namely: Tasar silk, Eri silk and Muga silk. There are also other types of uncontrolled, non-mulberry silk, which are mostly wild and exploited in Africa and Asia. They are Anaphae silk, Fagara silk, Coan silk, Mussel silk, and Spider silk (International Sericultural commission, 2013).

The Kalahari wild silk is produced by the larvae of *Gonometa postica.* Kalahari wild silk production is not controlled. The cocoons are harvested by people from the area after the moth has matured and left the cocoon. The successful production of the silk is regarded as an important tool for economic development of the country as it is a labour intensive and high income generating industry that delivers products of economic importance. Not only does it give the people an opportunity for job creation and food on the table, but also a means to earn foreign exchange. The production forms part of a community project by Gina Olivier (Olivier, 2007).

Furthermore, it represents an opportunity to move back to nature as a source for fibre and work. The source is available, waiting for the development of an effective and environment friendly degumming method. This also has a benefit of promoting sustainability and environment awareness (Thiry, 2004).

More pressure is placed on the natural resources of developed and developing countries. It is therefore important that the managing of resources takes place in a sustainable manner. In the case of the *Gonometa* species, the natural populations are the capital and overexploitation of this capital could result in extinction of the local populations (Veldtman, 2005). Knowledge of the biology of *Gonometa postica* is therefore very important as not to overexploit the natural resource and keep the process economically viable (Veldtman, 2005).

A detailed literature survey on various topics relevant to this work will now be presented. This includes all the information needed to understand and interpret the problem of degumming *G. postica* silk cocoons as an important tool for economic development.

2.2 Silkworm varieties

The best known domesticated silkworm is the mulberry variety, *Bombyx mori* L. (Figures 2.1 & 2.2), which feeds on the leaves of the

mulberry tree (*Morus alba*) (Kadolph, 2010). They are found in China, South Africa, Zimbabwe, Japan, Korea and Vietnam (Xia *et al*., 2004).

Figure 2.1: Mulberry silkworm (International Sericultural commission, 2013).

Figure 2.2: Mulberry silk cocoons (International Sericultural commission, 2013).

Another domesticated silkworm is *Philosamia ricini,* which feeds on the leaves of the castor tree (*Ricinus communis)* and produces Eri (Endi or Errandi) silk which is of a good quality. Furthermore it is almost as white in colour as *B. mori* silk. Even though Eri silk (Figures 2.3 & 2.4) is spun from the cocoon of domesticated silkworm, it is a "peace" silk because silk caterpillars are not destroyed in the cocoon,

but are allowed to emerge as moths and live a full life cycle (Kundu *et al.,* 2008).

Figure 2.3: Eri silk worm (International Sericultural commission, 2013).

(a) **(b)**

Figure 2.4: Eri silkworm cocoon (a) and silk (b) (International Sericultural commission, 2013).

A semi-domesticated multi-voltine silkworm is *Antheraea assamensis*, which feeds on the aromatic leaves of the Som (*Machilus bomycine*) and Soale (*Litsaea polyantha*) plants. This silk is known as Muga silk (Figures 2.5 & 2.6) and is of considerable interest to the silk industry (Kundu *et al.,* 2008), as an almost 5 cm long cocoon is

produced. Muga is renowned for its glossy fine texture, durability and natural golden amber glow (Mahendran *et al.,* 2006).

Figure 2.5: Muga silk worm (International Sericultural commission, 2013).

(a) **(b)**

Figure 2.6: Muga silk cocoon and silk (International Sericultural commission, 2013).

Wild silk is a variety of silk obtained from the cocoons of different caterpillars that have not been domesticated (Ngoka *et al.,* 2008). These caterpillars grow principally on wild foliage and complete their life cycle naturally.

Tasar silk (Figures 2.7 & 2.8), the most popular and available type of wild silk, can be obtained from the genus *Antheria* or *Attacus*. Tasar is a corruption of the Hindi word, *tasar*, which means "shuttle", perhaps alluding to the shape of the cocoon. Tasar refers to a fibre, not a fabric. The designation actually covers different species of related moths and biologists often use the term to refer to the whole genus (Mahendran *et al*., 2006). Indian tasar silks are obtained from the cocoons of the silk moth *Antheria mylitta* (tropical tasar). Chinese wild silk can be obtained from *Antheria pernyi* and Japanese silk from *Antheria yamamai.* The silk of *Antheria yamamai* was formerly exclusively used by Japanese royalty. The green caterpillar feeds on oak leaves and the cocoon is large and bright greenish (Mahendran *et al.,* 2006).

Figure 2.7: Tasar silk worm (International Sericultural commission, 2013).

(a) **(b)**

Figure 2.8: Tasar silk cocoons (a) and silk (b) (International Sericultural commission, 2013).

The silk of southern and central Africa is produced by silkworms of the genus Anaphae (Figure 2.9 & 2.10): *A. moloneyi; A. panda, A. reticulate; A. venata,* and *A. infracta.* They spin cocoons in communes, all enclosed by a thin layer. The tribal people collect them from the forest and spin the fluff into a raw silk that is soft and fairly lustrous. The silk obtained from A. infracta is known locally as "Book"' and those from *A. moloneyi* as Trisnian-tsamia and "koko" (Tt). The fabric is elastic and stronger than that of mulberry silk. Anaphae silk is used, for example, in velvet and plush (International Sericultural commission, 2013).

Figure 2.9: Anaphae silk worm (International Sericultural commission, 2013).

(a) **(b)**

Figure 2.10: Anaphae silk cocoon (a) and silk (b) (International Sericultural commission, 2013).

Fagara silk (Figure 2.11) is obtained from the giant silk moth *Attacus atlas* L. and a few other related species or races inhabiting the Indo-Australian bio-geographic region, China and Sudan.

(a) **(b)** **(c)**

Figure 2.11: Fagara silk worm (a); Fagara silk cocoon (b) and silk (c) (International Sericultural commission, 2013).

They spin light-brown cocoons nearly 6 cm long with peduncles of varying lengths (2 – 10 cm) (International Sericultural commission, 2013).

Spider silk – a non-insect variety – is soft and fine, but also strong and elastic (Figure 2.12). Due to the high cost of production, spider silk is not used in the textile industry; however, durability and resistance to extreme temperature and humidity make it indispensable for cross hairs in optical instruments (International Sericultural commission, 2013).

Figure 2.12: Spider silk (International Sericultural commission, 2013).

Mussel silk (Figure 2.13) is obtained from a bivalve, *Pinna squamosa* found in the shallow waters along the Italian and Dalmatian shores of the Adriatic. Its production is largely confined to Taranto, Italy (International Sericultural commission, 2013).

Figure 2.13: Mussel silk worm (International Sericultural commission, 2013).

Coan silk (Figure 2.14) is obtained from the larvae of *Pachypasa atus* D., from the Mediterranean bio-geographic region (Southern Italy, Greece, Romania and Turkey). Commercial production came to an end long ago because of the limited output and the emergence of superior varieties of silk (International Sericultural commission, 2013).

Figure 2.14: Coan silk worm (International Sericultural commission, 2013).

In Africa, including Southern Africa, the species, *Gonometa postica* and *Gonometa rufobrunnea,* are found and utilised for silk production (Kundu *et al.,* 2008; Mahendran *et al.,* 2006). They are

known to produce high quality silk, comparable to that of the domesticated silk moth *B. mori* L. *Gonometa postica* is polyphagous and feeds on the leaves of *Acacia erioloba, A. tortillis, A. mellifera, Burkea africana, Brachystegia* spp. and the alien, *Prosopis glandulosa. Gonometa rufobrunnea* feeds only on the mopane trees (*Colophospermum mopane)* (Delport *et al.,* 2005). Generally, *Gonometa* species is difficult to rear domestically. The reason is that they can only survive on *Acacia* leaves and the leaves cannot be harvested, as the leaves wilt and become inedible to the larvae as soon as it is removed from the tree. Recent studies, in the Nguni and Kamaguti in eastern and western Kenya respectively, showed that semi-domestic rearing of *Gonometa* spp. is possible through the use of net sleeve cages on tree branches of *A. elatior* Brendan. The moths, however, have to be caught in the wild in order to lay their eggs in the laboratory, but once they are hatched, the larvae are released back in the wild to feed on *Acacia* leaves until they spin their cocoons. Attempts are now being made to develop an artificial diet for laboratory rearing (Ngoka *et al.,* 2008; Fening *et al.,* 2008). Sericulture is labour-intensive. About 1 million workers are employed in the silk sector in China. The silk industry provides employment to 7.6 million people in India, and 20,000 weaving families in Thailand. China is the world's single biggest producer and chief supplier of silk

to the world markets (Table 2.1). India is the world's second largest producer (International Sericultural commission, 2013).

Table 2.1: Global Silk Production (International Sericultural commission, 2013).

Country	2008 (in metric ton)	2009 (in metric ton)	2010 (in metric ton)	2011 (in metric ton)	2012 (in metric ton)
Brazil	1177	811	770	558	614
Bulgaria	7.5	6.3	9.4	6	8.5
China	**98 620**	**84 000**	**115 000**	**104 000**	**126 000**
Colombia	0.6	0.6	0.6	0.6	0.6
Egypt	3	3	0.3	0.7	0.7
India	18 370	19 690	21 005	23 060	23 679
Indonesia	37	19	20	20	20
Iran	180	82	75	120	123
Japan	96	72	54	42	30
North Korea				300	300
South Korea	3	3	3	3	1.5
Philippines	1	1	1	1	0.89
Syria	0.4	0.6	0.6	0.5	0.5
Thailand	1 100	665	655	655	655
Tunisia	0.08	0.04	0.12	3	3.95
Turkey	15	20	18	22	22
Uzbekistan	770.5	780	940	940	940
Vietnam			550	500	450
Madagascar	15	16	16	16	18
TOTAL	**120 396**	**106 169**	**139 115**	**129 684**	**152 868**

Sericulture can help keep the rural population employed and prevent migration to big cities and securing remunerative employment; it requires small investments while providing raw material for textile industries (International Sericultural Commission, 2013).

Silk is a natural protein fibre derived from domesticated, semi-domesticated or wild silkworms. The major silk producing countries in the world are; China, India, Uzbekistan, Brazil, Japan, Republic of Korea, Thailand, Vietnam, DPR Korea, and Iran. Few other countries are also engaged in the production of cocoons and raw silk in negligible quantities; Kenya, Botswana, Nigeria, Zambia, Zimbabwe, Bangladesh, Colombia, Egypt, Japan, Nepal, Bulgaria, Turkey, Uganda, Malaysia, Romania, and Bolivia (International Sericultural Commission, 2013).

Even though silk has a small percentage of the global textile market - less than 0.2% - its production base is spread over 60 countries in the world. While the major producers are in Asia (90% of mulberry production and almost 100% of non-mulberry silk), sericulture industries have been lately established in Brazil, Bulgaria, Egypt and Madagascar as well (International Sericultural Commission, 2013).

2.3 The life-cycle and ecology of the silkworm

The life cycle of the silkworm consists of four stages: egg; larvae; pupae and adult stages. The duration of the life cycle varies according to the species and the climatic conditions or seasons (Hartland-Rowe, 1992). The egg stage (Figure 2.15) lasts between 10–11 days (Fening *et al.,* 2010). The eggs are distinctive almost spherical in shape, about 1 mm in diameter and white with a dark grey micropyle.

Figure 2.15: Moth of *G. postica* laying eggs (Maclean, 2013).

The eggs are randomly laid in clusters of 2–25 eggs on various substrates (Veldtman *et al.,* 2007; Veldtman, 2005; Veldtman *et al.,* 2002; Hartland-Rowe, 1992). Research done by Fening *et al*. (2010) found that the total number of eggs laid by a female moth ranged from 42–532 and 71–426 for the first and second generation moths, respectively. This has supported earlier findings by Kioko (1998) and Ngoka *et al.* (2008). Egg-laying can extend from 2–13 days, with the most eggs laid in a period of 4–5 days (Fening *et al.,* 2010). The moths' ovi-position is bimodal (Ngoka *et al.,* 2008).

The eggs hatch into 3 mg, hairy larvae (Figure 2.16), with an appetite needed to grow 34 times in size, moulting about five times (tetra-moulters) during this period. This is part of a longer life-cycle and causes the silk that is produced, to be of a thicker fibre (Dingle, *et al.,* 2005).

Figure 2.16: Little black pillars with white hair (Maclean, 2013).

The interval between two moultings is called a stadium and the larvae, at each stage, are called an instar (Dingle, *et al.,* 2005). Temperature and light will influence the moulting process; at a higher temperature, larvae enter into moulting earlier (Rao *et al.,* 1998). The larvae are 10–15 cm long and become as thick as a man's finger. Six larval instar stages are reached in approximately five weeks. The first and second instar stages (Figure 2.17) last 4–6 days. *Gonometa postica* larvae are sociable up to the end of the third instar (Figure 2.18). Colour variations occur between the first and second instar stages.

Figure 2.17: First moult (Instar 2). Slightly larger pillars with an orange blush (Maclean, 2013).

Figure 2.18: Second moult (instar 3). Much larger pillars with bright orange bristles and a big moustache (Maclean, 2013).

The larvae, being black with some white markings, aggregate in groups of up to 10 individuals, near the tips of slender leafless woody branches. *Gonometa postica* feed on certain species of *Acacia* and other plants, but reject mopane trees. They rest during the day and feed exclusively at night. Large larvae wander widely and may travel up to 20 m in a single night, to feed (Hartland-Rowe, 1992). The head capsule (cast after each moult) measurement reveals gradual increments from the first to the sixth instar stage (Ngoka *et al.,* 2008). After the first moult, larvae acquire a mixture of white and black hairs, with much longer hairs on the lateral sides (Figure 2.19).

Figure 2.19: Third moult (instar 4). Larvae of *G. postica* acquire a mixture of white and black hairs (Bhekisisa, 2013).

Larvae are also equipped with sharp black and brown pointed setae, which can snap off when they pierce the human skin, causing a painful rash. The fully grown 'worm' or caterpillar is armoured with poisonous spikes (Ngoka *et al.,* 2007).

After the worm has reached the limits of its growth, it ceases to eat, diminishes in weight, changes colour and starts to spin a cocoon. The silk glands are structured like tubes consisting of a posterior, middle and anterior section. The anterior is extremely thin, leading to the spinneret in the head of the larvae from which the silk is excreted. Fibroin is secreted in the posterior and transferred by peristalsis to the middle section, which acts as a reservoir. Here it is stored as a viscous aqueous solution until required for spinning. The majority of the sericin is created within the walls of the middle

section. The fibroin is stored in a weak gel state and when it is spun it changes into a sol state with liquid-crystalline order (Inoue *et al*., 2003). The fibroin and sericin are reserved side by side in the middle section without mixing one into the other (Nirmala, *et al*. 2001; Lee, 1999).

The Fillips glands discharge a liquid protein. These silk glands (Figure 2.20) consist of two long thick-walled sacs, running along the sides of the body, and open in a common orifice – the spinneret or seripositor - on the under lip of the larvae. This spinning process starts when the silk worm draws out the thread of liquid protein.

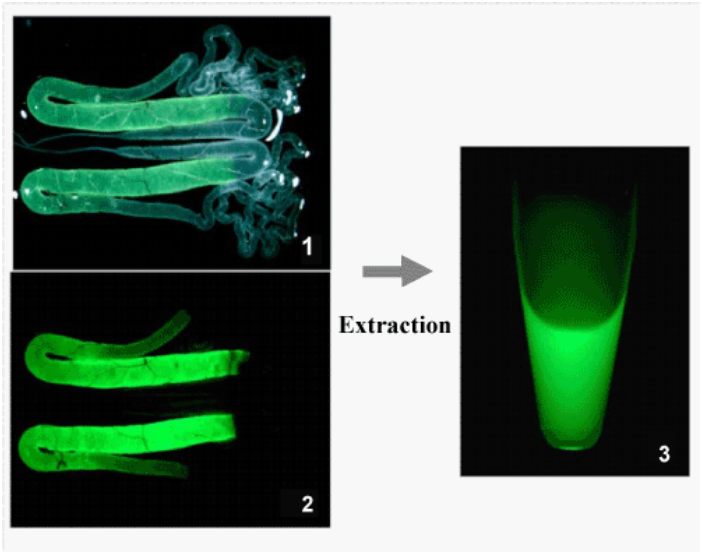

Figure 2.20: Silk gland of *G. postica* silkworm. It consists of two long thick-walled sacs, running along the sides of the body (Tatemastu *et al*., 2012).

The worm makes multiple movements, back and forth, with its head, in the form of a figure eight, to spin the cocoon, which is

eventually built up of many layers of silk. The fluids, hardened on contact with air, form a composite thread (Altman *et al.,* 2003). The silk worm constructs a complete cocoon within approximately three days. The shell is made of a single continuous silk strand with a length in the range of 1000–1500 m and conglutinated by sericin (Zhao *et al*., 2005).

The cocoon's surface (Figure 2.21) is armoured with poisonous spikes, similar to that on the worm's body. The needle like spines and hairs are important structures for protecting the larvae and cocoons against natural enemies such as predating birds (Teshome *et al*., 2011; Zhang *et al*., 2002). The cocoon also protects the moth pupa against microbial degradation and desiccation during metamorphosis (Zhao *et al*., 2005).

Figure 2.21: The cocoon of *G. postica* is armoured with poisonous spikes, similar to that on the worm's body (Holland, 2012).

After the larvae have spun the cocoons, they transform to the pupa stage or chrysalis by moulting a final time. The silken cocoon shell is comfortable and protective, allowing the pupa (Figures 2.22 and 2.23) in it to evolve into a silkworm moth. The ellipsoidal cocoon has the smallest thickness at its two ends so that the moths can break through it after the metamorphosis from pupa to moth (Zhao *et al*., 2005).

Figure 2.22: The pupa of *G. postica* inside the cocoon (Rebelo, 2012).

Figure 2.23: The pupa of *G. postica* outside the cocoon (Rebelo, 2012).

A variable proportion of these pupae give rise to a second generation of moths in January and February, whose offspring eventually produce cocoons at the end of March and the beginning of April. Due to the strength of the silk, the pupas can diapause for years (Veldtman, 2005).

In southern Africa, *G. postica* has two generations annually, one with and another without diapause (Veldtman *et al.,* 2002). The diapause silk worms strains produce ~250–500 mg/cocoon shell, three to four times that of the non-diapause (~80–120 mg/cocoon shell) strains (Zhao *et al.*, 2011). Veldtman *et al*. (2002) also observed an intermediate generation of *G. postica* in mid-summer (December to January), with pupation occurring in early autumn (March to April). This is advantageous to farmers, since it allows them to have two harvests of cocoons per year.

A moth develops in about two weeks, if the pupa is not destroyed. It secrets an alkaline solution which so weakens the fibres that they are easily broken and the moth can push its way out at the bottom of the cocoon, leaving an opening in one end of the cocoon (Figure 2.24).

The *G. postica* is an eggar moth with brown fore wings (Figure 2.25). It has a defence system in the form of a spread of needle-sharp poisonous hair.

The moths' show a distinct sexual dimorphism in that the female is corpulent and twice the size of the male.

Figure 2.24: The *G. postica* moth emerging from the cocoon (Holland, 2012).

Figure 2.25: A moth (female) of *G. postica* (Holland, 2012).

The moths are nocturnal and emerge without functional feeding mouthparts. They have a brief life, usually 3–5 days, with a maximum of nine days (Ngoka *et al.,* 2008; Hartland-Rowe, 1992).

2.4 The cocoon

The *G. postica* cocoon consists of polymeric composite materials which possess excellent mechanical properties. The cocoon shells, as typical protective structures, exhibit extensive variation to meet the specific needs of its species (Teshome *et al*., 2011; Vollrath & Porter, 2009). *Gonometa postica* have short white hairs interwoven throughout the cocoon layers while the brown needle-like spines with sharp buds on their surface are attached to the outer cocoon surface. Empty cocoons (Figure 2.26) are presently being collected from natural populations of *G. postica* in the North-West Province of South-Africa (Dreyer, 2013). Cocoons vary in quality, shape and colour. Some cocoons are of perfect quality, but others are internally and externally stained, have holes or are mouldy (Musayev, 2005).

Figure 2.26: Cocoons of *G. postica* (Dreyer, 2013).

Cocoons of *Gonometa* species have a white deposit on the surface. The Fourier-Transform Infrared (FTIR) spectra peaks around 1312–712/cm for outer surfaces, which indicates the presence of calcium oxalate crystals on the cocoons (Teshome *et al.,* 2011). Cocoon surfaces also show great cross bindings, wrinkles and a networking of twisting filaments in different shapes and forms conferring rough outer surfaces (Kebede *et al.,* 2013; Teshome *et al*., 2011). The surface of the cocoon, according to Teshome and co-workers (2011), has fibres held together in pairs by sericin, other secretions and impurities.

The arrangement of the fibres in all the cocoon shells lacks uniformity throughout the outer surface. The cocoon has many wrinkles on its outer surface that form due to non-uniform shrinking during drying (Zhao *et al*., 2005). Teshome and co-workers (2011) also found that the inner walls were smooth and uniform and the fibres are tightly bound together by a large amount of sericin making them appear more solid and intact.

The shape of the cocoon is specific to the specific species (Rahman *et al.,* 2004). The Japanese species are peanut-shaped, the Chinese and the European, elliptical and the poly-voltine species, spindle-like (Musayev, 2005). Elliptical shaped cocoons have uniform shell thickness throughout the cocoon layers. Peanut shaped cocoons have uneven shell thickness, in that both sides are thick and the

middle is thin (Sangappa, 2003). The cocoon shell thickness of *G. postica* can differ between 0.536–0.222 mm and is single compact layered cocoons (Teshome *et al*., 2011).

The colour of the cocoons is also specific to the species. Pigments in the sericin layers determine the colour and colours are limited to white, yellow, yellowish green and golden yellow (Lee, 1999). The yellow-green colour of the cocoons of Japanese oak silkworms is attributed to flavone pigments; the light brown colour of the cocoons of Chinese oak silkworms, muga silkworms and Eri silkworms are due to tannin. The colour, which seems to be depending, to some extent, upon the source of food, is not confined to the sericin but is distributed throughout the whole fibre. Colouration protects the progeny from natural enemies such as parasitoids (Diptera: *Tachnidae and Hymenoptera* species) and predators (birds) (Veldtman, 2005).

2.5 Cocoon processing

The first step in the processing of the cocoon is the boiling process. Uniform cooking improves raw silk recovery and quality (Zhao *et al.,* 2005; Sen & Babu, 2004). The compactness is linked to silkworm variety, shell thickness, silk filament thickness and sericin quantity. Cocoons with better compactness and uniform shell thickness, assist in achieving uniform cooking which, in turn, results

in better raw silk recovery and quality raw silk. Humidity will play a role during the cooking process; high humidity during mounting results in hard cocoons, while low humidity will make the cocoon layer soft. This factor will influence the air and water permeability during the boiling of the cocoons. A hard shell reduces reelability, while a soft shell may multiply defects, therefore suggesting a moderate humidity for the best results (Sangappa, 2003).

Loose, fluffy silk filaments cover the cocoons. These filaments are flat and tend to stick together, which, coupled with the sericin, makes fully automated mechanical reeling very difficult. Wild silk cocoons, harvested after the moth has matured, cannot be reeled (Good *et al.,* 2008). These cocoons can be degummed, cleaned, carded and spun into spun silk yarn, known as 'spun silk' (Zhang *et al.,* 2008). Two types of spun silk are produced. The first is called 'Schappe silk', which is obtained from outer and inner parts of cocoons or pierced cocoons. The second type is 'bourette silk' or 'silk noils', obtained from the waste in picking, carding, combing and spinning Schappe silk.

The workload, rate of production, evenness of silk threads and even dynamometric properties of spun silk are largely determined by the filament length of the cocoon. In multi-voltine species, the filament length is between 500–600 m and in bi-voltine races between 700–1500 m (Zhao *et al.,* 2005; Vandaveer, 2001). Ten silk

stands (cocoons) are needed to make one silk thread (Vandaveer, 2001).

For the production of uniform, finer denier raw silk, longer and finer cocoons would be needed. Again, silkworm species and cocoon spinning conditions will influence filament denier. 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 amount of cocoons required therefore to spin 1 kg of *G. postica* silk is 2 326–4 762 cocoons (Kioko, 1998).

Reelability influences raw silk yield, productivity and raw silk quality. Although cocoon properties influence reelability, temperature and humidity are more significant and must be maintained during cocoon spinning (Sangappa, 2003). The reelability of the cocoons will also have an influence on the non-broken filament length. Non-broken filament length will be high if reelability is better, but low if reelability is poor. The number of castings per minute under a given reeling speed is determined by the non-broken filament length (Sangappa, 2003). The position of the filament in the cocoon shell will cause the size of the filament to vary. Silk is finer in the inner layers and coarser in the outer layers, ranging between 25% and 40% respectively, depending on the species. The more the size variation, the bigger the increase in size deviation, maximum deviation and evenness variation of the raw silk will be (Sangappa, 2003).

On the surface of the cocoons are many wrinkles, due to non-uniform shrinking during drying (Zhao *et al.,* 2005). The wrinkles are coarser on the outer layer than within the inner layer. The outline varies according to the species and breeding conditions. High temperature and low humidity settings render fine wrinkles and a more cotton-like texture to the cocoon layers. The more coarsely wrinkled the cocoons are, the more poorly it reels (Lee, 1999).

The same tendency as above applies to the grain. Reelability is good if the grains are uniform. If the grains are irregular and have an uneven density, the reelability is poor. *Gonometa postica* cocoon shells have short, white hairs interwoven throughout the cocoon layers, while the brown needle-like spines with sharp buds on their surface, are found attached to the outer surface. The sharpness of the buds decreases towards the base of the spines. The presence of a large number of voids in the cross-section of wild silk fibres was reported by Narumi *et al*. (1993). The cocoons of *G. postica* have a unique feature, i.e. the presence of a well-formed peduncle. The purpose of the peduncles on cocoons is to connect the cocoons to twigs of host plants, by forming a strong ring. The tensile strength of the peduncle is very high, holds the cocoon and provides protection from predators and other environmental hazards (Teshome *et al.,* 2011; Dash *et al.,* 2006).

The cocoon-shell ratio influences the quality of the raw material. The better the ratio, the better the yield and quality of the silk. The shell ratio also has an influence on reeling cost; therefore reelers would prefer cocoons with high shell ratios. It is noted that silkworm species with high cocoon shell ratios have less resistance to diseases. A balance between ratio and resistance of silkworm species must, therefore, be maintained (Sangappa, 2003).

2.5.1 Chemical degumming of wild silk

Due to the sericin on the surface of silk fibres (Freddi *et al.,* 2003), they are rigid and stiff. Degumming is the key process to remove and allow silk fibres to gain its typical lustre, soft feel and elegant drapability, highly appreciated by consumers (Freddi *et al.,* 2003). During degumming, other impurities that affect the lustre and softness are also removed. The methods for degumming can be classified into four main groups: soap, alkalis, acidic solutions; and degumming by enzymatic methods (Ravikumar, 2007).

2.5.1.1 Alkali degumming

The mechanism of sericin removal by chemical degumming, using alkalis and acids affects the dispersion, solubilisation and hydrolysis of the different sericin polypeptides (Freddi *et al.,* 1999a). Hydrolysis prevails when strong alkaline compounds are added to the

degumming bath. Suitable procedures for controlling parameters, such as temperature, time, pH and alkalinity, must be implemented on an industrial scale in order to attain effective sericin removal, without triggering the hydrolytic degradation of fibroin and thus the fibres, which can be caused by harsh chemicals in the treatment bath. Fibre degradation 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. The alkalis used for degumming are sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), sodium hydrogen carbonate ($NaHCO_3$) or sodium phosphate (Na_3PO_4). Silk is boiled in this medium for 30–120 min (Freddi *et al.,* 1999a).

2.5.1.1.1 Orvus paste

This degumming solution is made of two chemicals: an alkali and a surfactant (Seves *et al*., 1998). Orvus paste is a pure anionic detergent consisting of 100% sodium lauryl sulphate ($NaC_{12}H_{25}SO_4$), with a neutral pH and is completely biodegradable. Anionic surfactants have negative charges (Lin *et al.,* 2008). As a pure detergent, Orvus paste does not contain bleaches, enzymes, sulphates, fillers, brighteners or other chemicals that might affect textiles. Anionic detergents are inexpensive, high foaming and can be powerful cleaners. However, anionic detergents are inactivated by too

hard water, because the carboxyl group of the molecule will ionize in hard water and react with the calcium (Ca) and magnesium (Mg) ions. Orvus paste should be used with caution, as it can irritate the skin and cause allergic reactions such as dermatitis and eczema. Sodium lauryl sulphate ($NaC_{12}H_{25}SO_4$), can burn the eyes or damaged skin (Anon, 2008). Due to the above facts and seeing that Orvus paste as a soft detergent which would prevent harming the silk fibres; it was used as the control chemical degumming method during this research work.

2.5.1.1.2 Other alkali methods

Robson (1999) used a standard soap/soda ash method for degumming. The bath contained 2 g/l Marseille soap and 0.8 g/l soda ash (Na_2CO_3). The silk was gently agitated and boiled at 98°C for 60 min. The samples were then rinsed in distilled water for 10 min, again with gentle agitation, followed by further degumming in 1.5 g/l soda ash. Again the sample was rinsed, first in hot distilled water and then in cold distilled water.

Sargunamani & Selvakumar (2003) applied 12 g/l soap at pH 10–11 for 45 min at 85±5°C, using a liquor ratio of 60:1. Rajkhowa *et al*. (2000) used 0.5% Na_2CO_3 and 0.5% Na_2O_3Si with a material-to-liquor ratio of 1:20, at 90°C for 30 min to degum Tasar cocoons. However, Seves *et al*. (1998) reported that the use of Na_2CO_3

damages the fibre. A material-to-liquor ratio of 1:25, for 120 min, was recommended.

According to Das *et al.* (2005), and Sen & Murugesh (2003), Tasar cocoons have hard and compact shells that prohibits normal cooking procedures. Hence, they used water containing 10% ethylenediamine, at 80°C, for 50 min to soften the shell. Reddy & Yang (2010) treated the cocoons with chloroform at room temperature to remove any waxes on the surface. The fibres were then washed using 1% $NaC_{12}H_{25}SO_4$ to remove impurities. Degumming was done by using a 10% $C_2H_4(NH_2)_2$ and 0.5% Na_2CO_3 solution at 80°C for 50 minutes with a cocoon-to-solution ratio of 1:20. The degummed silk was washed in warm water (Reddy & Yang, 2010). Normal degumming was done by using laboratory grade 2 g/l sodium carbonate and 0.6 g/l sodium dodecyl sulphate at 100°C with a material-to-liquor ratio of 1:25. After degumming, the cocoons were thoroughly washed in warm distilled water followed by washing in cold distilled water (Acharya *et al.,* 2009; Rajkhowa *et al.,* 2009).

2.5.1.2 Acid degumming

Alkali reactions at pH>8.5 favour rapid removal of sericin. In a similar manner, an acidic pH<3.0 also removes sericin (Prasong *et al.,* 2009). Cao *et al.* (2013) found that efficient degumming could be achieved at a pH of 1.5–2.0, using hydrochloric acid (HCl), oxalic acid

($H_2C_2O_4$) or tartaric acid ($C_4H_6O_6$). Research done by Gulrajani & Chatterjee (1992) proved that treatment times between 30–120 min have little effect, compared to temperature and acid concentration. All three acidic processes, however, minimise weight loss. Weight loss increases with higher acid concentration and temperature. A maximum weight loss close to 28% has been reported at 100°C and close to the highest acid concentration of 13.5 g/l. It is clear that 85% of weight loss takes place within the first 20 min. After that, there is a slow increase in weight loss, with increase in time (Gulrajani & Chatterjee, 1992).

A poor correlation between influencing factors and the tenacity of yarns was found. It was also apparent that with an increase in acid concentration and temperature, the elongation-at-break decreases. It seems that the sericin and the little amount of wax in the silk act as lubricant, the removal of which reduces the elongation-at-break. An increase in weight loss causes the denier of the yarn to reduce, consequently making the yarn flexible and thus decreasing flexural rigidity. From tests it is clear that fibres degummed under optimum conditions (100°C; 30 min; 6.75 g/l acid; 3 g/l surfactant) are clean, free from sericin and surface damage. As the acid concentration and treatment time are increased, fibrillation starts (Gulrajani & Chatterjee, 1992).

2.5.1.3 Enzymatic degumming

In recent years, studies (Rajasekhar *et al.,* 2011; Freddi *et al.,* 2003) have dealt with degumming using proteolytic enzymes. Proteolytic enzymes like trypsin, pepsin, chymotrypsin and papain can be used for silk degumming. Trypsin and papain are recommended for degumming, because of their different effects on fibroin and sericin. These enzymes hydrolyse peptide bonds, formed by carboxyl groups of lysine (Lys) and arginine (Arg). The Lys is more abundant in the sericin than in the fibroin (Chopra *et al.,* 1996). For degumming, trypsin requires a weak alkaline medium (pH = 8) at 40–50°C, while papain requires a weak acid medium (pH = 5.2) at 70°C. Being large molecules, enzymes do not penetrate into the interstices of the fabric and hence are suitable for yarn degumming only (Rajasekhar *et al.*, 2011).

Singh *et al*. (2003) used pineapple extract in silk cocoon degumming and reeling. A proteinase-assay mixture was prepared by mixing 1.0 ml of pineapple extract with 0.2 ml of 0.3 (w/v) azocasein at 30°C. He found that the cocoon extract neither caused inhibition of the activity nor enhanced its time-dependent loss by incubation at 60°C. However, it caused an enhanced time-dependent loss of the activity by incubation at 60°C with sodium carbonate.

Several other acidic, neutral and alkaline proteases have been used for degumming silk. Alkaline proteases performed better than

acidic and neutral ones in complete and uniform sericin removal, the retention of tensile properties and the improvement of surface smoothness, handling and preserving lustre of silk (Gulrajani & Agarwal, 2000; Gulrajani *et al.,* 2000a; 1998). The combination of a lipase and a protease resulted in effective de-waxing and degumming, with positive effects on the wettability of silk fibres (Gulrajani *et al.,* 2000a).

2.5.1.4 Quality of the water

The quality of the water, as the main medium in the degumming process, plays an important role. Salt in the water enhances the degrading of silk, due to the distribution of ions in the internal and external phases. The quality of the water will differ from place to place and thus, the degumming process cannot be standardised (Cao *et al.,* 2013). Cao *et al*. (2013) used hard and distilled water with different concentrations of an industrial grade detergent, based on alpha olefin sulphate. An optimum quantity for the detergent was determined and water with different levels of hardness was used. Degumming loss, tenacity and elongation were tested.

Degumming with hard water requires large quantities of detergent. This can be minimised by using soft water with a total hardness of 50–100 ppm, making the degumming process economical. The use of lower concentrations of detergents could

result in reducing pollutants in the effluent. Moreover, degumming is effective at lower levels of hardness because hard water reacts with the detergent to precipitate Ca and Mg ions. Precipitation deposits on the fibre surface inhibit penetration of the degumming solution and hinder the whole washing process (Cao *et al.,* 2013).

Silk degumming is a high resource consuming process as far as water and energy are concerned. Moreover, it is ecologically questionable, due to the high environmental impact of effluents. The development of an effective degumming process, based on enzymes as active agents, could mean saving water, energy and eventually chemical and effluent treatment. Degumming in an ecological friendly way makes milder treatment conditions possible, as well as the recycling of processing water, the recovery of valuable by-products such as sericin peptides and the lowering of the environmental impact of effluents (Freddi *et al.,* 2003).

2.5.1.5 Acceleration of the degumming process

Non-traditional techniques for reducing processing time and energy consumption, and for improving product quality are being investigated today. For example, Fakin *et al*. (2005) reported on the use of ultrasound. Ultrasound may be broadly divided into power ultrasound and diagnostic ultrasound. Fakin *et al*. (2005) showed that the introduction of ultrasonic energy into the processing bath significantly accelerated the physical and chemical processes, mainly

due to the phenomenon known as cavitation. Cavitations are the growth and explosive collapse of microscopic bubbles. As sound waves pass through liquids, the sonic vibration generates a local pressure wave in addition to the ambient hydrostatic pressure, giving rise to cycles of compression and rarefaction (negative pressure). The microscopic bubbles form during rarefaction cycles and are crushed during the next compression cycle. The sudden, explosive collapse of these bubbles can generate hot spots, i.e. localized high temperature, high pressure shock waves and a severe shear force capable of breaking chemical bonds.

Ultrasound applications doubled the impurity removal from fibres during alkaline and acidic scouring and did not increase the weight loss during bio-scouring. It enhanced the efficiency of the bleaching process, since the weight loss was about 3% more after bleaching with ultrasound than the corresponding treating and bleaching without ultrasound. Furthermore, ultrasound did not decrease polymerisation. The application of this technique in the bleaching bath increased the whiteness of the fibres (Fakin *et al.,* 2005).

Thus, ultrasound can be regarded as an appropriate method for accelerating degumming processes. It is especially effective if used together with Marseille soap, tartaric acid and papain. It does, however, induce a significant increase in weight loss at certain

temperatures and times. The positive conclusion is that if ultrasound is used for degumming, lower temperatures than the conventional are required (Fakin *et al.,* 2005).

2.5.2 Biological degumming of wild silk

Through the literature provided in 2.5.1., it is implied that the methods of degumming are classified according to the degumming agents employed.

This section looks at a few methods, not yet considered as possible methods of degumming *G. postica* cocoons. It gives a background on the possible environment conscious agents, such as vermicompost, distilled water, catholyte, and *Eucalyptus* oil, which will now be discussed in detail.

2.5.2.1 Vermicompost

Vermicompost is prepared from organic materials using earthworms, as a low cost and eco-friendly technological system. Millions of tons of animal, agriculture and kitchen waste are produced annually, creating smell and pollution problems. Release of unprocessed animal manure into agricultural fields contaminates ground waters causing a strong public health risk, by means of nutrient and microbial contamination (Aira & Domínguez, 2009). Microorganisms 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 opportunities for self-employment for rural people, by utilizing the available agricultural resources (Rajendran *et al.,* 2008). There are several reports regarding the potential utilization of composting epigeic earthworms for organic waste management (Amaravathi & Reddy, 2014; Birunda *et al*., 2013; Huang *et al.,* 2013; Mehta & Karnwal, 2013; Vig *et al.,* 2011; Prakash & Karmegam, 2010; Benitez *et al*., 2005; Garg & Kaushik, 2005). Microorganisms and earthworms help nature to maintain nutrient flows from one system to another and to minimise environmental degradation (Gupta & Garg, 2008; Garg *et al*., 2006). The vermicomposting procedure changes the chemical composition of the substrates that are subjected to the earthworm activities. The ash content of cattle, swine and sheep manure vermicompost increase compared to the initial substrates (Nourbakhsh, 2007), whereas the fine granular peat-like end product which is produced, contains elevated levels of nitrogen, phosphorus and potassium (NPK) in available form, micronutrients, micro-flora, enzymes and growth regulators (Ansari, 2011; Arancon *et al*., 2008) and pH values that significantly decrease (Nourbakhsh, 2007).

Vermicomposting is a mesophilic, aerobic, bi-oxidation (Munnoli *et al*., 2010; Tang *et al.,* 2006) and non-thermophilic process (Bentíze *et al.,* 2000; Eivira *et al.,* 1998) in which earthworms

(*Eisenia fetida* and *Eudrilus eugenae)* interact intensively with microorganisms and soil invertebrates within the decomposer community. They strongly affect the decomposition processes, accelerating the stabilization of organic matter, and greatly modifying its physical and biochemical properties (Aira & Domínguez, 2008). The microorganisms and earthworms are active at 10–32°C (Nagavallema *et al.,* 2004).

The microorganisms produce the enzymes that cause the biochemical decomposition of the organic material (animal, agriculture and kitchen waste), but the earthworms are the crucial drivers of the process (Suthar, 2008; Aira *et al.,* 2002). They are involved in the indirect stimulation of the microbial population, through fragmentation and ingestion of fresh organic matter. This results in a greater surface area available for microbial colonization, thus dramatically increasing microbiological activity. The earthworms modify the microbial biomass and activity through stimulation, digestion and dispersion in the casts and interact closely with other biological components of the vermicomposting systems, thereby affecting the structure of the micro-flora and micro-fauna communities (Nath *et al*., 2009; Lores *et al.,* 2006). The decaying organic matter in the vermicomposting systems is a spatially and temporally heterogeneous matrix of organic resources with contrasting qualities that result from the different rates of

degradation that occur during decomposition (Prakash & Karmegam, 2010; Yasir *et al.,* 2009; Elvira *et al.,* 1998).

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 = ±7 (Munnoli *et al.,* 2010). The action of the earthworms changes the composition of humid substances in the organic matter both quantitatively and qualitatively (Petrussi *et al.,* 1988). This process enhances waste conversion (Nagavallema *et al.,* 2004). High population densities of earthworms in vermicomposting systems result in a rapid turnover of the organic matter into earthworm casts (Aira *et al.,* 2003).

High rates of mineralization occur in the organic matter-rich earthworm casts, which greatly enhances the availability of inorganic nutrients, particularly ammonium and nitrates, but also phosphorus (P), potassium (K), Ca and Mg. Vermicompost also contain plant growth hormones produced by microorganisms and plant growth regulators such as humates, in the production of which microorganisms also play a role. Munnoli *et al*. (2010) reported the diversity of eight bacterial groups from the fresh soil gut and cast of the earthworms as α-proteobacteria, β-proteobacteria, χ-proteobacteria, δ-proteobacteria, Bacteroidetes, Verrucomicrobia, Planctomycetes and Firmicutes. Bacteroidetes, α-proteobacteria and

β-proteobacteria were predominant in the soil and worm cast samples of *Eisenia fetida*.

Earthworms are red or purple (Figure 2.27), 10–15 cm long, and live for only 28 months. These worms consume 10% soil and 90% organic waste materials. They can tolerate temperatures ranging from 0–40°C, but they regenerate the best at temperatures between 25°C–30°C and a moisture level of 40–45% (Nagavallema *et al.,* 2004).

Figure 2.27: *Eisenia fetida* worms (Pienaar, 2009a).

Earthworms, of which there are nearly 4 000 species, are classified into two classes: the burrowing and the non-burrowing species (Pienaar, 2009a). The non-burrowing species *E. fetida* and *E. eugenae,* live in the upper layer of the soil surface (Pandit *et al.,* 2012).

Vermicompost can be produced in pits below the ground, heaps and tanks above the ground and in cement rings or a commercial

compost maker. The key to a successful vermicomposting system is to provide the earthworms with an ideal environment for growth. The basic requirements are: oxygen, moisture, and moderate digester temperatures. The pH, ammonia, and salt concentrations in the bin are important variables to control and maintain during operation. Suitable bedding; adequate food sources and good aeration are also important (Nagavallema *et al.,* 2004).

Nedunchezhiyan *et al*. (2011) found that a mixture of cotton waste, with cattle manure in the ratio 1:5 gave the best results. Other residues, however, may also be used, such as groundnut husks, soybean residues, vegetable waste, municipal solid wastes or biogas sludge. Organic wastes can be ingested by earthworms and egested as a peat-like material (Figure 2.28).

Figure 2.28: Vermicompost (Pienaar, 2009a).

According to Prakash & Karmegam (2010) vermicomposting has been used as an efficient and low cost means of composting organic

waste such as paper-pulp and sludge from paper mills and dairy plants (Kaushik & Garg, 2004). Compared to chemical fertilisers, the cost of vermicompost production is insignificant, as a 1 g worm could convert 4 g of activated sludge in 5 days (Anon, 2006).

Vermicompost can also be produced on farms. It is a product that is rich in chelating- and phyto-hormonal elements and need no further processing before being used (Garcia *et al*., 1995). The worms, as such, become economically viable products if sold to fishery, poultry, dairy and pharmaceutical industries (Sinha *et al.,* 2009). The unavailability of land and public consciousness has made dumps and landfills expensive and impractical (Kaushik & Garg, 2004).

Furthermore, vermicompost can replace expensive and impractical landfills as well as expensive chemical processes (Pienaar, 2009b).

A few bacterial species, mostly Gram-negatives, were found to attach themselves and grow on silk buried in soil. Seves *et al.* (1998) found that these bacteria use sericin rather than fibroin for growth. It was this fact and also preliminary work done on the cocoons in the Department of Consumer Science, UFS, which led to the inclusion of vermicompost as a possible environmentally friendly degumming method in this study.

2.5.2.2 Distilled water

Distillation involves the conversion of untreated water into water vapour, which is then condensed back into liquid form (Figure 2.29). Most of the contaminants are left behind in the boiling chamber, with the condensed water being virtually contaminant-free. Several different types of distillation systems are available, the system chosen generally depending on the quantity of water required.

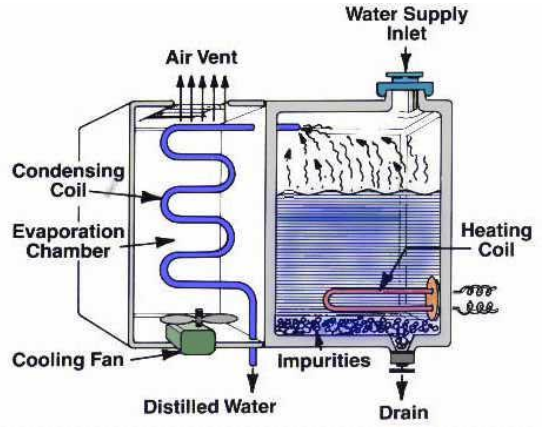

Figure 2.29: Modern point-of-use distillation system (Anon, 2013).

Sericin is water-soluble (Wu *et al*., 2006; Zhang *et al*., 2004; Sarovart *et al.,* 2003). In the present study, a combination of water and the possibility of the presence of protein degrading microorganisms were identified as a method to degum the cocoons. Degumming with distilled water is simple and low cost (Wu *et al*., 2006; Zhang *et al*., 2004; Sarovart *et al.,* 2003).

2.5.2.3 Catholyte

The basic principles of the electrochemical activation technology were discovered in 1972, by the Russian engineer, Vitold M. Bakhir (Tomilov, 2002). Today it is used in a wide range of applications including medicine, agriculture (Lobyshev, 2007), microbiology and the food industry (Khrapenkov *et al.,* 2002). It is further also used in water purification and decontamination and as an environmentally friendly anti-microbial and washing media (Bakhir, 2005).

The process includes a physical and chemical process combined with electrochemical and electro-physical actions (Lobyshev, 2007; Tomilov, 2002). The result of the process is the altering of the molecular state of the water from stable into metastable (activated) aqueous media (Bakhir, 2005; Khrapenkov *et al.,* 2002). Electrochemical activation causes a purposeful change of the acid-base and oxidative-reductive properties of the water (Bakhir, 2005).

Fresh water and distilled water are suitable for electrochemical activation, but requires a high voltage, which results in an unnecessary high consumption of electricity. Therefore, water and salt is used as the raw products for the electrochemical activation mechanism (Marais & Brözel, 1999). Salt is dissolved in the water causing a higher content of ions and thus lower voltage (Tomilov, 2002). Once the water is passed through the electrochemical anode and cathode cells, it is electrochemically activated and are

characterised by metastability (Lobyshev, 2007). This means that the water exhibits unnatural values of its physical and chemical parameters (Khrapenkov *et al.,* 2002). The parameters that differ from the original water use include the activity of electrons (redox potential) in the water, the electric conductivity and the pH (Tomilov, 2002).

Catholyte will remain in its state of metastability for a couple of days. Catholyte water is a highly alkaline surfactant detergent with reduced redox potential which can give up electrons (Forostyan *et al.,* 1987) and is negatively charged. Catholyte is non-toxic to the environment (humans and animals), is easy to handle and can serve as a non-foaming detergent. It increases the cleaning abilities of detergents when added. Through precipitation, it removes bio-film, protein, fat and heavy metals from water (Gidarakos et al., 2009). It can be safely disposed of in sewage systems and can be used in all stages of cleaning (Bakhir, 1997).

The primary components in a catholyte solution are: hydrogen (H_2) hydroxyl ions (OH-) from sodium hydroxide, a small amount of hydrogen peroxide (H_2O_2) and sodium hydroxide (NaOH). According to Lobyshev (2007) the H_2O_2 of catholyte has a concentration of 10^{-7} M. It also has high Na and K concentrations in comparison with the initial water (Forostyan *et al.,* 1987). Catholyte has a pH of 11.6–12.5 and is thus a strong reducing solution, with high adsorption

enhanced wetting properties, serving as an effective surfactant (Annandale *et al.,* 2008). The alkali concentration in catholyte is proportional to the mineralization of the water and the electricity consumption during the process when synthesized (Bakhir, 1997).

The use of catholyte, as an environmentally friendly anti-microbial and washing media (Bakhir, 2005) is well known. Theoretically it can serve as an effective surfactant in the degumming process of the cocoons, which was the reason for including this method in this study.

2.5.2.4 *Eucalyptus* oil

Southern Africa is a major producer of *Eucalyptus* oil. While most of it is being produced in South-Africa, significant quantities come from Swaziland. Several species of *Eucalyptus* (family Myrtaceae) contain volatile oils, but fewer than 20 species of them have been exploited commercially for oil production. *Eucalyptus smithii* grow particularly well in South-Africa and Swaziland. The trees are evergreen with alternate, broadly lancelet, unevenly stalked leaves, 7–17 cm in length and 3–5 cm in width (Yarosh *et al.,* 2001). The trees produce large amounts of leaf biomass, leading to *E. smithii* being preferred for oil production.

Oil is isolated by hydro-distillation from the *Eucalyptus* leaves (Assareh *et al.*, 2007). The aromas are characteristic of a particular species (Ugalde & Pérez, 2001). The major active ingredient is

cineole (60%) or eucalyptol (Yarosh *et al.*, 2001). Doran & Brophy (1990) distinguished up to 41 other compounds in the oil. Cineole is an environmentally friendly chemical compound and can replace ozone-depleting solvents, currently phased out due to the Montreal Protocol. The *Eucalyptus* trees can sequester CO_2 and thus combat the greenhouse effect (Soh & Stachowiak, 2002). According to Reynolds (1989), medicinal *Eucalyptus* oil contains 70% (w/w) cineole; it also contains pinene and other terpenes, and may contain small quantities of phellandrene. The source and purity of the oil will influence the composition.

The chemical name of *Eucalyptus* oil is 1, 3, 3-trimethyl-2-oxabicyclo-{2.2.2.}-octane. The molecular formula for cineole is $C_{10}H_{10}O$ and its molecular weight is 154.25 (Budavari, 1996). It should be stored at temperatures not exceeding 25°C in well filled containers, and must be protected from light (Reynolds, 1989). The physical properties of *Eucalyptus* oil is summarised in Table 2.2.

Eucalyptus oil is used as a decongestant, often in combination with other volatile substances. It can be used orally for catarrh and coughs, and be applied as a rubefacient for better blood circulation or as flavouring (Reynolds, 1989). Newell *et al.* (1996), recommended *Eucalyptus* oil as a cleaning solvent, a fragrance, an antiseptic, a febrifuge that reduces fever and an expectorant in herbal medicine. The oil has a cooling and deodorizing effect on the body, helping with

fevers, migraine and malaria. It is also very effective against bacteria, especially staphylococci (Nagata *et al*., 2008).

Table 2.2: Physical properties of *Eucalyptus* oil (Yarosh *et al*., 2001)

Colour	Colourless to pale yellow liquid.
Form	Liquid oil.
Odour	Camphoraceous odour.
Taste	Pungent, spicy, cooling taste.
Solubility	Insoluble in water. Soluble in 70% alcohol. Miscible in alcohol (90%), dehydrated alcohol, oils, date and paraffin. Miscible in ether, chloroform, glacial acetic acid.
Boiling point	176°C–177°C
Density	0.921–0.923

Sericin envelops the fibroin fibre with successive sticky layers that help in the formation of the cocoons. It is a hydrophilic glue-like protein that holds the hydrophobic fibroin monofilaments together to form the spun silk fibre. In previous tests by the Consumer Science Department, UFS, *Eucalyptus* oil was successfully used as a cleaning solvent of the cocoons. It is a natural oil and also environmentally friendly. It was therefore included in this study as a possible method to degum the glue-like sericin of the cocoons.

2.6 Determination of Silk Quality

2.6.1 Size and weight of the cocoon

In evaluating the quality of the raw material, size and weight are critical characteristics. Silkworm variety, rearing season and harvesting conditions will have an influence on the size. Male and female cocoons are very different in size and weight, and the ranges of the two do not overlap. Male cocoons weigh an average of 3 g, while female cocoons, and weigh an average of 5.5 g. Thus, female cocoons are approximately twice as large as male cocoons and yield more silk fibre. Sex ratios in natural populations will, therefore, be important when harvesting cocoons (Veldtman *et al.,* 2002).

Weight is the most significant commercial feature, because cocoons are sold by weight (Sangappa, 2003). The weight index signals the quantity of the raw silk that can be reeled. Fening *et al*. (2010b), separated the cocoons into males and females, before measurements. The weight of the cocoons was positively correlated with the amount of raw silk produced which was used to determine the quality of silk fibre production, which also had a bearing on the size of the cocoon. They also found that there was not a significant ($p>0.05$) difference in the weight of the cocoons due to food plants and season (Kioko *et al.,* 2007; Veldtman *et al.,* 2002).

The weight of the cocoon ranges from 1.5–2.2 g for male pure breeds and 1.8–2.5 g for female hybrid breeds. In nature, the weight

of the cocoon does not remain stable and diminishes until the pupae transforms into a moth and emerges from the cocoon (Table 2.3). This gradual process takes place due to moisture evaporation from the body of the pupae and fat consumption during the metamorphosis process (Lee, 1999).

Table 2.3: Daily loss in weight of fresh *G. postica* cocoons (Lee, 1999).

Days after mounting	6	7	8	9	10	11	12	13
Days after pupation	2	3	4	5	6	7	8	9
Index of fresh cocoon weight	100	99.4	98.8	98.3	97.7	97.0	96.1	95.1

Gonometa postica is also sexually dimorphic in cocoon length, width and shape. Cocoon length is a suitable alternative measure of occupied cocoon mass and may be used as a rough estimate of silk yield (Veldtman *et al.,* 2002). Veldtman *et al*. (2002) like Kioko *et al*. (2007) found that the cocoon sizes differ significantly between species, sexes and localities, but not between generations and host-plant specific populations (Table 2.4). The differences between the males and females differed significantly ($p<0.01$).

Table 2.4: Mean cocoon mass, length and width of male and female cocoons of *G. postica* (Veldtman *et al.,* 2002).

Sex	**n**	**Mass (g)**	**Length (mm)**	**Width (mm)**
Male	248	2.85±0.02	36.00±0.11	16.41±0.05
Female	227	6.81±0.06	45.87±0.17	21.34±0.08

The cocoons can be uni-voltine, bi-voltine or multivoltine, which refer to the number of hatchings of the silkworms per year. Most wild silkworms are multivoltine; however, their cocoon size is smaller, which results in a shorter bave length and a lower commercial value (Dingle *et al.,* 2005).

Another negative observation for the silk yield is that dwarfs (significantly smaller than average cocoons) are found in *G. postica* populations. A study done by Veldtman (2005) found the frequency of dwarfism low and in most cases it occurred in approximately 1.5% of the sampled population. Dwarf cocoons ranges between 15.13–27.31 mm in length (n = 32). When dwarfs occur at such low frequencies, they should have no effect on the average silk yield per cocoon of harvested natural populations. The cause of dwarfism in *G. postica* or the sex of these individuals is presently unknown (Veldtman *et al.,* 2002).

Bi-voltinism in *G. postica* may result in cocoon size differences between generations (Veldtman, 2005). Bivoltine species range

between 60–100 cocoons/l while the amount is much higher for multivoltine species (Lee, 1999). The size of the cocoon should not vary too much and if it should be the case, it would lead to a variation in shell ratio, filament size and length (Sangappa, 2003).

Finally, the thickness of the cocoon shell is important. The cocoon shell is a natural structure, which protects the silkworms from hot and cold weather and filters harmful light (Musayev, 2005; Zhao *et al.,* 2005). The cocoon body reflects and absorbs light in specific wavelength ranges (Musayev, 2005). The thickness of the cocoon is not constant and changes according to its three sections. The thickest segment is the central constricted part, while the dimensions of the expanded portions of the head are 80–90% that of the central part (Zhao *et al.,* 2005).

The thinnest part of the cocoon is at the two ends, allowing the moth to break through it when moving from the pupa to the moth stage (Zhao *et al.,* 2005). The measure of thickness determines the raw silk yield and is, therefore, a consequential factor. The thickness is also influenced by the silkworm varieties and the technology used for rearing and mounting. Univoltine and bivoltine species will produce a thicker shell than multivoltine species. The thickness and hardness of the cocoons not only differ among the individual cocoons, but also in the head, trunk part and the caudal part of the same cocoon (Zhao *et al.,* 2005; Lee, 1999).

2.6.2 Morphological structure of the fibre

Wild silk varies greatly as to the 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 are damaged (Figure 2.30) by the moth's 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).

Figure 2.30: Damaged cocoons – after the moths' emergence (Dreyer, 2013).

The fibres of *G. postica* are relatively smooth with occasional grooves and ridges. They are ribbon-like, twisted with uneven width and their surface shows numerous irregularities, such as fissures. The irregular surface structure displays traverse fissures, creases, folds

and uneven lumps. Furthermore, the fibres are broad and show distinct longitudinal striations, peculiar flattened markings, usually running across the fibre. These markings can be due to reeling operations, when the soft gum is slipped or broken in the crossing (Kadolph, 2010). *Gonometa postica* fibres have globular and triangular cross-sections, with rounded corners (Kadolph, 2010).

These fibres also have longitudinal striations and are porous (Das *et al.,* 2005). A longitudinal view of the silk fibre shows a very irregular surface structure, covered by a sericin layer (Figure 2.31).

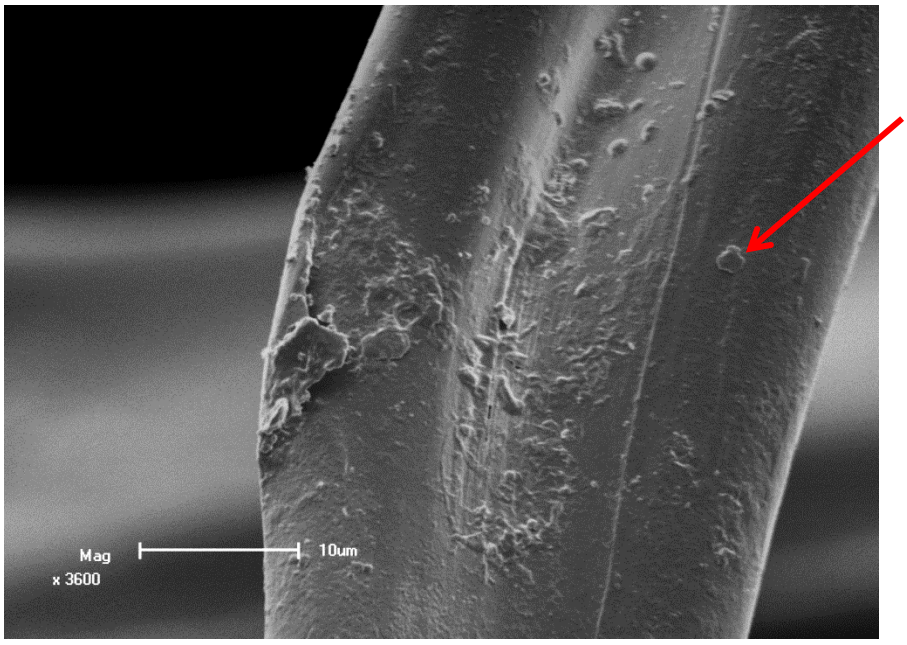

Figure 2.31: A longitudinal view of the silk fibre shows a very irregular surface structure, covered by a sericin layer (own picture).

A cross-sectional view (Figure 2.32) of the fibre shows that it is roughly elliptical, with two triangular brins completely surrounded by

sericin, normally facing each other on the flat side of the triangle. Each single filament is called a brin, and the double filament is known as a bave (Kadolph, 2010). The diameter of the fibres is 11–12 μm. These nanofibrils are oriented parallel to the axis of the fibre and interact strongly with each other. The nanofibrils will assemble into bundles, called micro-fibrils (Foo *et al.,* 2006; Tanaka *et al.,* 2001).

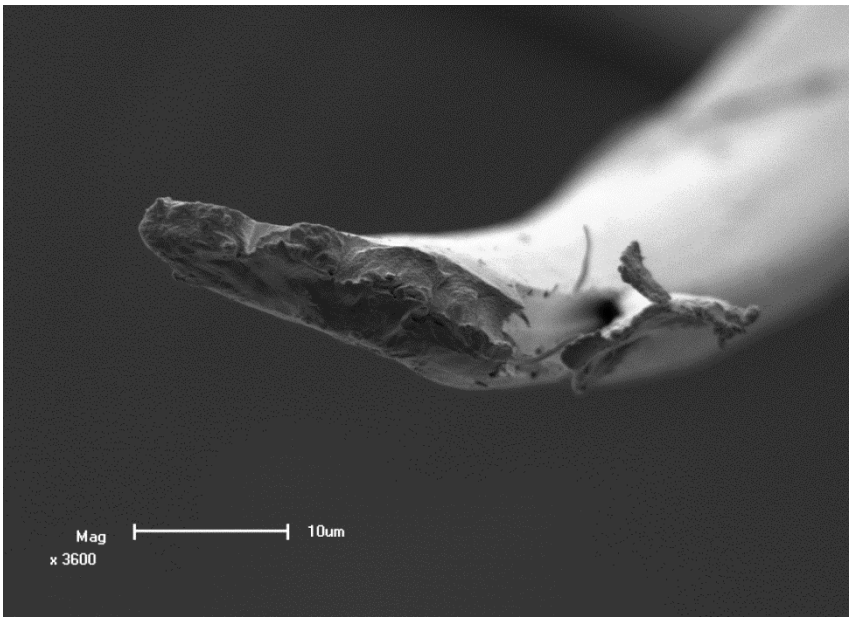

Figure 2.32: A cross-sectional view of the fibre shows that it is elliptical (own picture).

2.6.3 Physical properties of the silk

A cross-section of a silk fibre shows that it is roughly elliptical. The triangular twin filaments, which normally face each other, are completely covered by sericin. After degumming, the two triangular fibroin filaments (brins) separate into individual filaments, giving different fibre geometry than the raw silk, i.e. a finer and more lustrous fibre (Khan *et al.,* 2008; Li *et al.,* 2003). Silk filaments are approximately 900–1 700 m long. The diameter of the silk is from 9–11 μm. In 1978, Tortora reported that the triangular cross-sections of silk resulted in lower covering power and reduced lustre which has not yet been contradicted by other authors. Although the surface of the fibre is smooth, the roughly triangular shape changes the pattern of the light (Kadolph, 2010). The reason is that the silk filament is usually slightly twisted around itself and will cause the change in the light reflection.

Silk, before degumming, does not possess high lustre (Mather & Wardman, 2011). After degumming the lustre is soft and high with an occasional sparkle, but not so bright as manufactured fibres with round cross sections (Kadolph, 2010). A soft, subdued lustre is the result of the broken intensity of the reflected light (Cai & Qui, 2003). 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

silk worm feeds, thus *G. postica* having a rich, tawny colour (Mather & Wardman, 2011).

The gravity of silk is 1.25 (for pure degummed silk) (ASTM Test Method D 276-12). Sericin has a slightly higher gravity than fibroin. Lightweight fabric can be made of silk, because of the fine diameter of the fibre and its high tenacity.

Silk can absorb a great deal of moisture (30%) and still have a dry feeling. The moisture regain is 11%, but after degumming the regain is only 9% (Cai & Qui, 2003). Silk is a poor conductor of electricity. It accumulates a static charge with friction, which renders it difficult to handle in the manufacturing process. The charge can be dissipated by high humidity (Kadolph, 2010).

Silk can be heated to 140°C without danger of decomposition, but at 170°C it rapidly disintegrates. Degummed fibres burn in the air and produce an odour like burning wool or hair, leaving a black crisp and easily crushable ash. Silk ceases to burn when it is removed from the flame (Kadolph, 2010).

2.6.4 Mechanical properties

Silk, as a natural fibre, offers a unique combination of strength of 2.8–5.2 g/den and ductility unrivalled by any other man-made fibre (Kadolph, 2010; Poza *et al.,* 2002). Mather & Wardman (2011) stated that silk has the same strength as an iron wire of the same

diameter. This strength is due to the high orientation of silk at the molecular and supra-molecular levels (Kadolph, 2010), which gives it a large load-bearing capability, together with a damage tolerant response.

A 1 mm silk yarn will support a weight of 45 kg. Its wet strength is slightly less (80–85%) than its dry strength, because the hydrogen bonds between protein polymers are broken by moisture (Mather & Wardman, 2011). The tenacity within a cocoon increases from the outer to the inner layers along the filament length. This is true for all the silk varieties (Kushal & Murugesh, 2004).

Silk has a moderately high modulus, meaning that it will resist an initial tensile force and will not stretch easily (Wynne, 1997). The elongation of silk fibres before breaking is about half that of wool, but considerably higher than cotton. It has fairly good recovery if stretched only slightly, but will not recover completely from high extensions (Wynne, 1997). At 2% elongation the fibre has an elastic recovery of 92%. The breaking elongation is 20% (Kadolph, 2010). The elements of silk (C, H, O and N) are joined together in wavy molecular chains. This structure with its single chains gives silk an elasticity of 15–20% (Kadolph, 2010).

2.6.5 Chemical composition of the filament

Natural silk fibre (Figure 2.33) is one of the strongest and toughest materials mainly because of the dominance of well orientated β-sheet structures of protein chains (Matsumoto *et al.*, 2008; Liu *et al.,* 2004). Fibroin and sericin are the two major proteins of silkworm cocoons. They differ considerably in their chemical composition as well as in their accessibility (Chopra & Gulrajani, 1994). Other minor components include proteins, lipids and carbohydrates (Gauthier *et al.,* 2004).

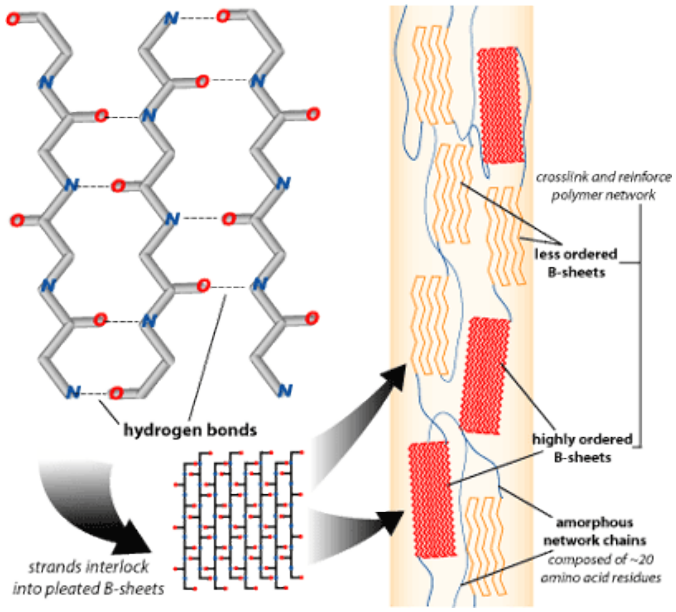

Figure 2.33: The structure of a strand of silk (Kennedy, 2013).

2.6.5.1 Sericin

Sericin is a water-soluble macromolecular globular protein (Wu *et al*., 2006; Zhang *et al*., 2004; Sarovart *et al.,* 2003). When it is dissolved in a polar solvent, hydrolysed in acid or alkaline solutions, or degraded by a protease, the size of the resulting sericin molecules depends on factors such as temperature, pH, and the processing time. Lower molecular weight sericin peptides (<20 kDa) or sericin hydrolysate are used in cosmetics, health products and medications. High-molecular weight sericin peptides (>20 kDa), is soluble in boiling water but poorly soluble in cold water and are mostly used as medical biomaterial, degradable biomaterials, compound polymers, functional bio-membranes, hydrogels, and functional fibres and fabrics (Zhang *et al*., 2004; Zhang, 2002).

Sericin envelops the fibroin fibre with successive sticky layers (Figure 2.34) that help in the formation of the cocoons. It is, therefore, a family of hydrophilic glue-like proteins (Alam *et al.,* 2007; Jin & Kaplan, 2003; Vollrath & Knight, 2001), that holds the hydrophobic fibroin monofilaments together to form the spun silk fibre (Kadolph, 2010). Sericin contributes about 25–30% of the total cocoon weight (Dash *et al.,* 2006; Zhang, 2002).

Sericin is a natural macromolecular and an albuminoidal protein (Dash *et al.,* 2006; Zhang *et al.,* 2004). The sericin protein is made up of 18 amino acids (Dube *et al*., 2006; Takasu *et al.,* 2002; Li *et*

al., 2000), most of which have strong polar side groups such as hydroxyl, carboxyl and amino groups (Sarovart *et al.,* 2003; Zhang, 2002). Sericin can be broken down into various amino acids, of which at least 12 have been isolated. Predominant amino acids, identified by researchers, are serine (Ser), glycine (Gly), glutamic acid (Glu) and tyrosine (Tyr). The percentage of serine and tyrosine, both bulky amino acids, is lower (Dash *et al.,* 2007).

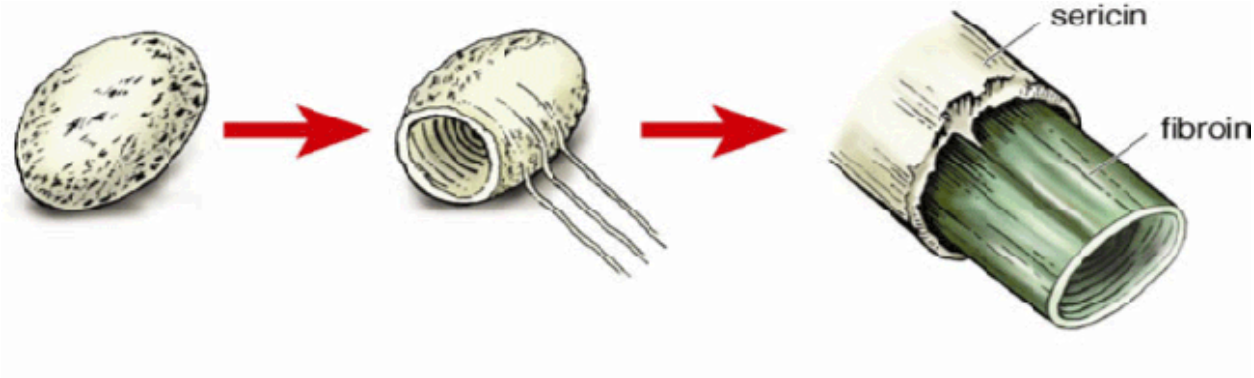

Figure 2.34: Protein components of silk (Sobajo *et al*., 2008).

The sericin of the cocoon shells can be separated into two layers: α-sericin, which is present in the outer layer of the cocoon shell; and β-sericin which is found in the inner layer (Robson, 1999). The α-sericin contains less carbon and hydrogen, and more nitrogen and oxygen than the β-sericin (Trivedy *et al.,* 2008). The α-sericin is more soluble in boiling water than the β-sericin. Mondal *et al*. (2007) reported that the amino acid composition of sericin is species specific.

Sericin ($C_{15}H_{25}N_5O_8$) consists mainly of a random coil or β-structure (Zhang, 2002). According to Tsukada & Bertholon (1981), sericin remains in a partially unfolded state, with 35% β-sheet and 63% random coil and no α-helical content. Sericin can be cross-linked, co-polymerised and blended with other macromolecular materials, especially synthetic polymers, to produce materials with improved properties (Zhang, 2002).

Sericin represents a family of proteins having a molecular mass of 10–310 kDa (Sarovart *et al.,* 2003; Takasu *et al.,* 2002; Kato *et al.,* 1998). Wild silk cocoons contain three prominent bands. The lowest band is 70 kDa, whereas the higher molecular weight sericin (200 kDa) is from the peduncle of the cocoon. According to Aramwit and co-workers (2011), heat and acid extraction result in sericin with a molecular weight of 35–150 kDa, whereas sericin extracted by alkaline solution, has a molecular weight of 15–75 kDa. Sericin with a low molecular weight, commonly <20 kDa, is soluble in cold water (Aramwit *et al.,* 2011; Takasu *et al.,* 2002), which can be recovered during the early stages of raw silk production. Higher molecular weight sericin is soluble in hot water and can be obtained from the later stages of the degumming process. Rigano *et al*. (2005) reported that five principal fractions of sericin have been isolated, with glycoprotein of different molecular weight (65–400 kDa). Sericin between 10 and >225 kDa can be obtained by urea extraction.

Sericin is soluble in different temperatures of water and gels on cooling (Wang & Zhang, 2011). The structure (Figure 2.35) is mainly amorphous without any crystallization, even though it is subjected to similar stress levels during spinning (Jiang *et al.,* 2006; Zhao *et al.,* 2005). Sericin is divided into the outer, middle and inner sericin layers. Differences in amino acid composition among the three layers are significant (Wang & Zhang, 2011).

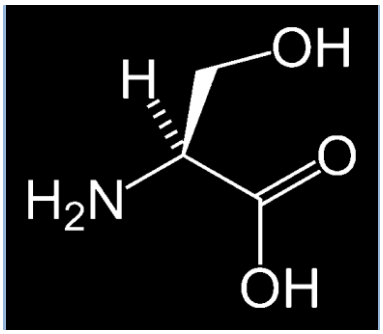

Figure 2.35: Primary structure of sericin (Jiang *et al*., 2006; Zhao *et al*., 2005).

Besides the functions of covering, adhesion and protection of the fibroin, sericin lubricates and promotes the wrapping of the filament in the construction of the cocoon. It serves as a bivalent cation donor and acceptor of water molecules that free themselves from the fibroin crystalline region (Rigano *et al.,* 2005).

Wild silk sericin is relatively insoluble compared to the silk of *B. mori*, due to the chemical interaction between it and inorganic minor components of tannins, contained originally in wild silk (Asakura *et*

al., 2004). The amino acid composition of the sericin of wild silk is characterised by more alanine (Ala), aspartic acid (Asp) and arginine (Arg) contents and less glycine (Gly). It has an Arg-Gly-Asp tri-peptide sequence (Mhuka *et al.,* 2013). This composition is related to the abundance of $(Ala)_n$-sequence, which favours α-helix formation (Li *et al.,* 2003).

2.6.5.2 Fibroin

Fibroin is an insoluble protein created by the larvae of *B. mori*, other moth genera such as *Antheraea, Cricula, Samia* and *Gonometa*, numerous other insects and spiders. Fibroin is secreted from the posterior silk gland (PSG) of the mature silkworm larva during spinning and remains distinct in the extruded thread. It is a delicate, fibrous, hydrophobic glycoprotein twin filament (two polypeptides) (Ki *et al.,* 2007; Wu *et al.,* 2006; Poza *et al.,* 2002), that exhibits many impressive physicochemical properties (Marsano *et al*., 2005; Zhang *et al*., 2005). The fibroin fibre itself is a bundle of several fibrils with a diameter of 1 μm. A fibril contains 15 nm wide micro-fibrils. Micro-fibrils are packed together to form the fibril bundle and several fibril bundles produce a single strand. The protein is predominantly hetero-dimeric, and consists of heavy and light chains, bonded together by a single disulphide bond (Zhou *et al.,* 2000; Tanaka *et al.,* 2001). Fibroin has an amorphous region of about one-third and a crystalline portion (Figure 2.36), of about two-thirds, in which there are two

crystalline forms, silk I and silk II, reported as the dimorphs of silk fibroin, on the basis of extensive investigations by a variety of analytical methods.

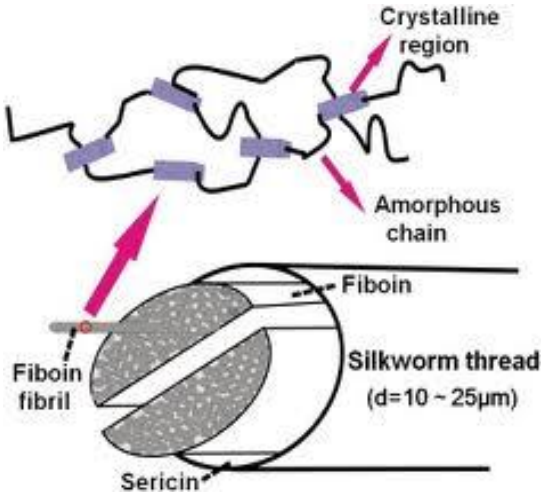

Figure 2.36: Crystalline and amorphous regions of a fibroin fibril (Zhou *et al.,* 2000; Tanaka *et al.,* 2001).

Silk II, the structure of the fibre after spinning, is mainly an anti-parallel β-sheet (Wang & Zhang, 2013).

The amino acid composition of the silk is important as it has an influence on the sequence of the protein chains which then determines protein orientation and conformation. It, therefore, has a direct impact on the physical and chemical properties of the silks. Table 2.5 summarizes the amino acid composition of the fibroin protein from the *G. postica* cocoons (Mhuka *et al.,* 2013).

Glycine, Alanine and Serine are predominant in *G. postica* fibroin (Figure 2.37), accounting for more than 70% of the total amino acid content. These three amino acids have the smallest side chains of all the amino acids allowing for regular packing in the formation of the β-sheet structure of fibroin.

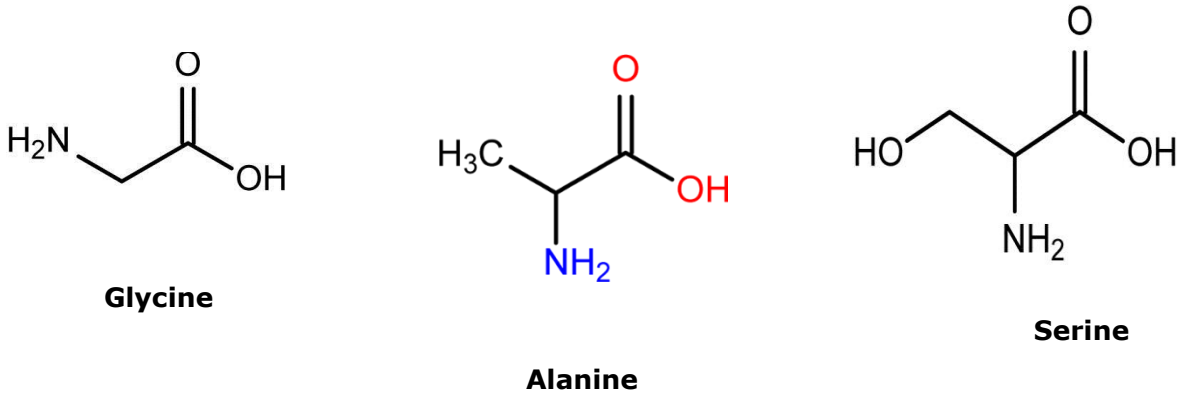

Figure 2.37: The three predominant amino acids in *G. postica* silk (Sashina *et al.*, 2006).

Glycine is one of the simplest amino acids; a peptide-group building block with R=H which makes it a very flexible group that can adopt a wide range of folded structures. Alanine with R=CH_3 and serine with R=OH confer hydrophobicity and polarity (Rauscher *et al.*, 2006).

Table 2.5: Amino acids composition of *G. postica* silk fibroin (Mhuka *et al*., 2013).

Amino acid	*G. postica*
Non-polar (%):	
Glycine	36.89
Alanine	28.14
Proline	2.54
Valine	1.60
Leucine	1.47
Isoleucine	0.82
Methionine	0.31
Polar (%):	
Serine	12.10
Threonine	0.87
Cysteine	0.08
Acidic (%):	
Aspartic acid	4.22
Glutamic acid	1.16
Basic (%):	
Arganine	6.54
Lysine	1.06
Histidine	0.23
Aromatic (%):	
Tyrosine	8.29
Phenylalanine	0.77
Tryptophan	-

Fibroin is characterised by highly repetitive hydrophobic sequences such as GAGAGS, GAGAGY and GAGAGVGY (Figure 2.38).

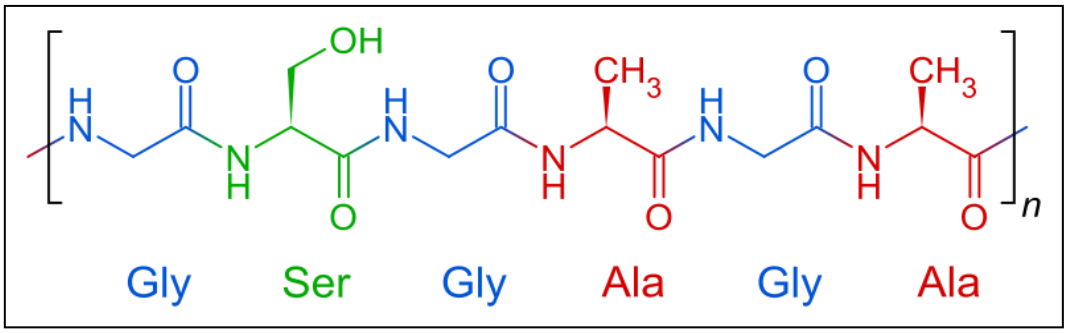

Figure 2.38: Primary structure of fibroin (Dyakonov *et al.,* 2012).

Researchers (Fedic, 2003; Zurovec & Sehnal, 2002; Zhou *et al.,* 2001) suggested that the Gly-rich domains and the repeat (Figure 2.38), motifs (Gly-Ala) may have a bearing on the quality of silk produced. The fibroin contains 16 different peptide units that lack side-chain amide groups and sulphur-containing units, thus having covalent cross-links. Hydrolysis shows that over 90% of the peptide units are derived from four amino acids; Gly, Ala, Ser and Tyr and about 75% from two amino acids; Gly and Ala (Mhuka *et al.,* 2013; Zhao *et al.,* 2005*)*.

The ratio of Gly:Ala for *G. postica* is 1:31 (Table 2.5), whereas that of *B. mori* is 1:54. This suggests a difference in the primary structure and/or organization of residues in the fibroin of the two species (Mhuka *et al.,* 2013).

Due to its flexible, amphiphilic molecular motif, with predominantly hydrophobic blocks with hydrophilic ends (Jin & Kaplan, 2003; Zhou *et al.,* 2000), fibroin exhibits micelle formation and a liquid crystalline structure. The latter is responsible for long-

range orientation; effective dehydration and control of the viscosity (shear) sensitivity (Jin & Kaplan, 2003).

Three fibroin formations are distinguished: silk I, with a helical conformation; silk II, with an anti-parallel β-sheet conformation; and thirdly, a random coil without definite order (Zhang *et al.,* 2002). The wild silk fibroin contains polyalanine repeat sequences of the β-type.

Fibroin has two phases: a highly crystalline β-sheeted phase; and a lesser or non-crystalline phase (Jin & Kaplan, 2003). The crystalline regions tend to be oriented along the fibre axis, because the fibre is drawn as it is extruded from the spinnerets of the silkworm (Asakura *et al.,* 2004). The β-sheet formation, along with its morphological crystalline orientation, contributes to the stability and some of the unique mechanical features of the spun silk fibres. All the side chains are arranged in an anti-parallel and orderly manner (Zhang *et al.,* 2002).

Padamwar & Pawar (2004) reported that the filament is made of both crystalline and amorphous domains. The crystalline region constitutes about 20–25% of the silk and is entirely composed of Gly and Ala residues. The amino acids Tyr, Lys and Arg, are present in the amorphous region, due to their larger side groups. In the crystalline regions of fibroin, the sequence of Gly alternates with Ala or Ser and is stretched to full extent. Hence, a structure may be formed in which anti-parallel chains are linked together as formed

sheets. In the H-atom form, the Gly residues protrude from one side of the sheet and hydroxymethyl and methyl groups protrude from the other side. Hydrogen bonds are generally formed between CO- and NH-groups from adjacent chains. The arrangement of the chains can be described as a pleated sheet (Horan *et al.,* 2005). The high proportion of Gly, a small amino acid, allows tight packing and the fibres are strong and resistant to breaking.

Fibroin is only solubilised in concentrated salt solutions (Datta *et al.,* 2001). Fibroin has a molecular mass of around 400 kDa (Tamura & Sakate, 1988).

2.7 Conclusions

Silk, a well-known natural fibre produced by *Gonometa postica*, is composed of two kinds of protein; fibroin forms the thread core and the glue-like sericin surrounds the fibroin fibres and cements them together. Fibroin comprises both heavy and light chains and has an amorphous region of about one-third and a crystalline portion of about two-thirds. The physical and mechanical properties of the silk fibroin depend on the conformation of the molecular chain and the crystal structure. In the traditional process of changing raw silk into textile fibres, sericin is removed partially or completely to make silk textiles.

Silk worm fibres are influenced by processing conditions. Silk degumming and fibre dissolution induce breakage of the peptide chain to various degrees and they then directly affect the structure and properties of the silk fibres.

Reported degumming methods, such as the use of Na_2CO_3 solution, neutral soap-alkali and highly concentrated urea buffer, focus on degumming efficiency and the features of sericin peptide and hydrolysates. Degumming with acid, alkali, enzymes and ultrasound might impact the surface characteristics and mechanical properties of silk fibroin.

The successful production of the silk of *G. postica* is regarded as an important tool for economic development of the country as it is a labour intensive and high income generating industry that delivers products of economic importance. It gives the people an opportunity for job creation and food on the table, but also a means to earn foreign exchange.

Chapter 3

MATERIALS AND METHODS

3.1 Cocoons

Gonometa postica cocoons were obtained from a farm on the border (19°11'6.67''S, -24°13'3.33''E) between Namibia, Botswana and South Africa. The cocoons consisted of a high quality wild silk (Figure 3.1).

Figure 3.1: Silkworm cocoons from *Gonometa postica.*

All the cocoons of the sample of *G. postica* have hatched. The silk around the hole was brittle because of the enzyme (cocoonase) that the moth secreted when it emerged from the cocoon. The cocoons were cut open, and the skin-remains and other matter were removed by the collectors on the farm. It was transported to the laboratory of the Consumer Science Department, University of the Free State, where it was trimmed at the top, cleaned out by hand and the brittle silk removed with a scissor. The cocoon samples 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 ±5 cocoons, depending on the size of the cocoons.

3.2 Preparation of degumming liquors

3.2.1 Orvus paste

The Orvus solution for the degumming bath was a mixture of 38 g washing soda and 38 g Orvus paste (Proctor & Gamble; Figure 3.2) in 3.8 l of distilled water prepared in a 4 l glass bottle.

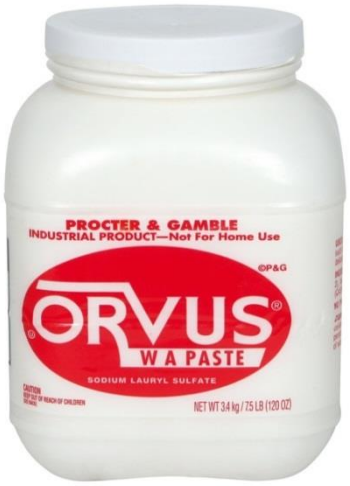

Figure 3.2: Orvus paste.

3.2.2 Vermicompost

The product used was obtained from the late Prof. G. van Tonder (Figure 3.3). According to Van Tonder (Personal communication, 2011), vermicompost contains the highest grade of humus available. It adds beneficial micro-organisms, nutrients and minerals to the soil that sustain healthy plant life. The high number of micro-organisms is good for plant and soil health and it will prevent harmful micro-organisms from spreading in the soil. He further stated that the casts of the red compost worms (*Eisenia fetida*) are rich in humic acids, have a perfect pH balance, and contain plant growth factors similar to those found in seaweed.

Figure 3.3: Vermicompost.

Vermicompost was made by using cow dung and plant materials. The cow dung ensures good nutrition and natural food for the earthworms. Because the manure is partially decomposed, it is

consumable by the worms (Garg *et al*., 2008). The green-matter piles were hosed down with nutrient-rich water to aid decomposition. The piles were left for 45 d to achieve a ratio of carbon (C): nitrogen (N) of 20:1. Once the pile reached the peak stage of decomposition, it was aerated by a turner. This stage is essential as harmful NH_3 is released from the compost and microbial activity is increased. The earthworms were removed and the vermicompost was then sieved by use of a 1 mm sieve. Vermicompost is odourless.

3.2.3 Catholyte

Water was electrochemically activated, by passing a water solution with a 5% NaCl concentration through a water electrolyser (Fig. 3.4; 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 l/min. The catholyte had a pH of 12–13 and was used within 90 min of preparation (Annandale *et al*., 2008; Lobyshev, 2007).

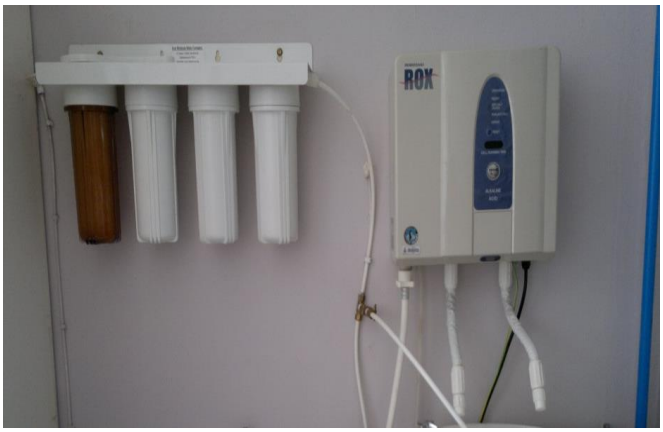

Figure 3.4: The water electrolyser unit (Water Electrolyser Instruction Manual, Hoshizaki) in the Consumer Science laboratory, UFS).

The composition of the electrochemically activated water is depicted in Table 3.1. The analysis was done by the Institute for Groundwater Studies, University of the Free State.

Table 3.1: The composition of the catholyte used for degumming as determined by the Institute of Groundwater Studies, University of the Free State.

Determinant	Units	Value
Electrical conductivity*	mS/m	16 177
Ca	mg/l	2.5
Na	mg/l	22 482
K	mg/l	8.68
Cl	mg/l	26 506
PO_4	mg/l	4.74
SO_4	mg/l	1 034
Al	mg/l	0.063
Cu	mg/l	0.073
Fe	mg/l	0.105
Mg	mg/l	<0.01
Ni	mg/l	<0.1
Zn	mg/l	0.042

*(where electrical conductance is calculated in mS/m which is EC x 10^{-3}; Ca (calcium); Mg (magnesium); Na (sodium); K (potassium); Cl (chloride); PO_4 (phosphate); SO_4 (sulphate); Al (Aluminium); Cu (copper); Fe (iron); Ni (nickel); Zn (zinc).

3.2.4 Distilled water

Tap water was passed through a Fistreem water still apparatus (Fisons Scientific equipment). The distilled water was used for the experimental degumming work.

3.2.5 *Eucalyptus* oil

Purified (100%) *Eucalyptus* oil BV 397 (Act 101/1965) (Allied Drug Company [Pty] Ltd.) for medicinal use was used for the experimental degumming.

3.3 Degumming methods

3.3.1 Orvus paste

This method was considered the control method. Eighteen samples of ±5 g conditioned cocoons (see section 3.1) in glass bottles with lids were covered with 100 ml Orvus paste solution and kept at 32°C in an incubator, for 10 days. As fibroin is sensitive to hot alkalis (Taddei *et al.*, 2003), degumming conditions for it were carefully controlled (32°C) in order to limit the occurrence of degradation by peptide bond cleavage. After 5 days, the first three samples were removed for further treatment as follows: a) cocoons were rinsed three times in a sieve with distilled H_2O at 30°C (Figure 3.5); b) subsequent rinsing with a mild citric acid solution (1 ml/2 l cold distilled H_2O) to get rid of any fatty residues; c) final rinsing with

a fabric conditioner (15 ml/l of cold distilled H_2O; Figure 3.6) to loosen the fibres; d) drying with filter paper at room temperature (Robinson, 2001); e) conditioning for 48 hours at 21±1°C and 65±2% RH; f) weighing of the conditioned cocoons. This whole process was repeated on days 6, 7, 8, 9 and 10, with the remaining samples.

Figure 3.5: Rinsing of the degummed cocoons.

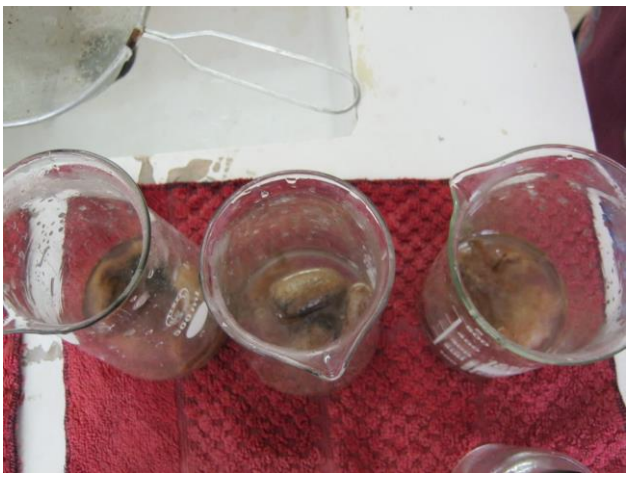

Figure 3.6: Cocoons in fabric softener solution (15 ml/l of cold distilled H_2O).

3.3.2 Vermicompost

Eighteen samples, each containing ±5 g of conditioned cocoons (see section 3.1) were wrapped in hessian pieces of 20 cm x 20 cm. This was done 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 (Figure 3.7).

Figure 3.7: Cocoons in vermicompost in containers at 32°C.

After 5 days, the first three samples were removed from the vermicompost and unwrapped. The cocoons were pasteurized at 72°C for 16 seconds due to the possible presence of pathogenic micro-organisms. The same treatment procedures as described in section 3.3.1 were followed. This procedure was repeated on days 6, 7, 8, 9 and 10 day with the remaining samples.

3.3.3 Catholyte

Eighteen samples of ±5 g conditioned cocoons (section 3.1) were placed in glass bottles with lids and covered with 100 ml catholyte (pH = 12.85). The cocoons 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 in section 3.3.1. This procedure was repeated on days 6, 7, 8, 9 and 10 day with the remaining samples.

3.3.4 Distilled water

Eighteen samples of ±5 g conditioned cocoons (section 3.1) were placed in glass bottles with lids and covered with 100 ml distilled H_2O. They 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 with the same procedures as in section 3.3.1. This procedure was repeated on days 6, 7, 8, 9 and 10 day with the remaining samples.

3.3.5 *Eucalyptus* oil and distilled water

Eighteen samples of ±5 g conditioned cocoons (section 3.1) were covered with a solution consisting of 10 ml *Eucalyptus* oil and 90 ml of distilled H_2O, and kept at 32°C in an incubator for 10 days. After 5 days, the first three samples were removed and the same

procedure described in section 3.3.1 was applied. This procedure was repeated on days 6, 7, 8, 9 and 10 day with the remaining samples.

3.3.6 *Eucalyptus* oil and catholyte

Eighteen samples of ±5 g conditioned cocoons (section 3.1) were placed in glass bottles with lids and covered with a solution consisting of 10 ml *Eucalyptus* oil and 90 ml catholyte (pH = 12.85). The cocoons were kept at 32°C in an incubator for 10 days. After 5 days, the first three samples were removed and the same procedure described in section 3.3.1, were applied. This procedure was repeated on days 6, 7, 8, 9 and 10 day with the remaining samples.

3.3.7 *Eucalyptus oil* and Orvus paste

Eighteen samples of ±5 g conditioned cocoons (section 3.1) were place in glass bottles with lids and covered with a solution consisting of 10 ml *Eucalyptus* oil and 90 ml Orvus paste. The samples were kept at 32°C in an incubator for 10 days. After 5 days, the first three samples were removed and the same procedures described in section 3.3.1 were applied. This procedure was repeated on days 6, 7, 8, 9 and 10 day with the remaining samples.

3.4 Physical fibre property analysis after different degumming methods

3.4.1 Weight loss

The % weight loss of the silk fibres (Figure 3.8) after degumming was calculated by the equation:

$$\% \text{ weight loss} = [\frac{W_o - W_t}{W_o}] \times 100$$

where W_o is the weight of the silk fibre samples before and W_t the weight of the silk fibre samples after degumming (Nakpathom *et al*., 2009).

Figure 3.8: Silk fibres used for fibre property analysis.

3.4.2 Degumming efficiency

Degumming efficiency assessments can be done by a gravimetric method, by staining with some dyes that distinguish between fibroin and sericin, by the determination of the viscosity of the degumming solution and by SEM (Arami *et al.,* 2007). In this study though, the efficiency of the degumming was calculated by comparing the weight loss of the cocoons of each of the methods with the weight loss of the cocoons in the Orvus-paste control method.

The efficiency was calculated with the following formula:

$$\text{Degumming efficiency} = \frac{Wt_E}{Wt_S}$$

where Wt_E is the % weight loss by the other treatments and Wt_S is the % weight loss by the Orvus-paste treatment (Teli & Rane, 2011; Nakpathom *et al.*, 2009).

3.4.3 Morphology of silk fibre analysis

A morphological characterisation of the silk fibres from each degumming method was performed by scanning electron microscopy (SEM). The fibres were cut and glued to the stubs by metal glue and left overnight to dry. It was then vacuum sputter-coated with a

5 angstrom coating of gold (60 nm) to make the fibres electrically conductive (Zhang *et al.,* 2012; Good *et al.,* 2008). The 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. It was photographed at a voltage of 15 kV at room temperature.

3.5 Mechanical fibre property analysis

3.5.1 Tensile strength

The tensile strength tests were conducted with the Instron Instrument (Mecmesin Limited, United Kingdom) and the standard test method for tensile properties of single textile fibres (ASTM D3822). The degummed samples were conditioned for a minimum of 48 hours at 21±1°C and 65±2% relative humidity before the tests were conducted. Samples were randomly selected from a bundle of fibres and 20 contiguous experimental samples were used from each of the selected fibre bundles. Single fibre tenacity was measured to determine the level of damage caused by the different degumming methods. The tensile strength tests were performed to compare the mechanical properties of the silk fibres. The Instron test determines the maximum force before the fibre breaks and the elongation at maximum force.

The silk fibre samples were cut gently into 30 mm short fibre fractions in the length in order to make sure that the fibre was not stressed plastically during the process. A total of ten samples per degumming method were prepared. Every chopped fibre was mounted and taped across a hole, which is 10 mm long, of a rectangular cardboard (Figure 3.9).

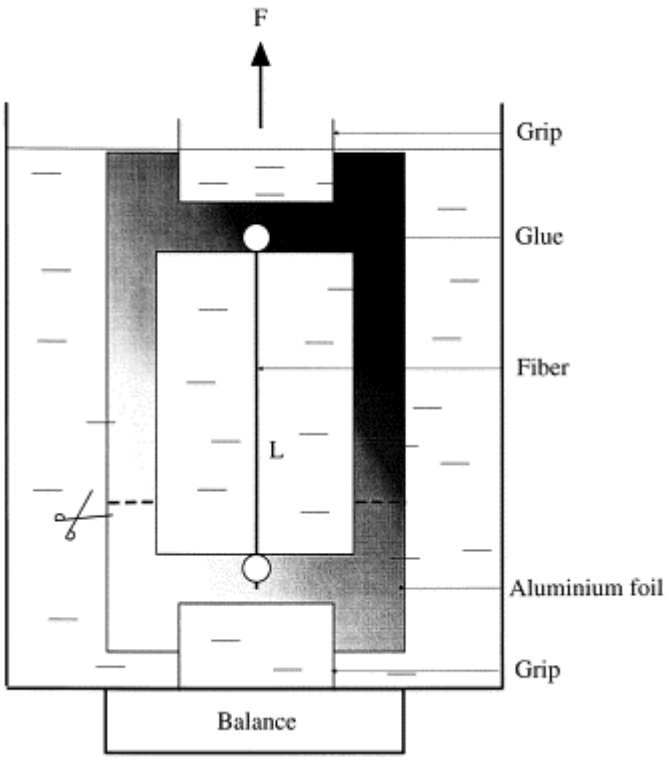

Figure 3.9: Experimental set up for tensile strength test of *G. postica* silk fibres (Pérez-Rigueiro *et al.,* 2000).

The cardboard was then fixed in an Instron Instrument. The fibre gauge length was 30 mm between the two grips of the machine. The cardboard was then cut along the dotted lines and separated into

two parts to ensure tensile loading was completely transmitted to the fibre during tests as shown in Figure 3.9.

All tests were conducted at a rate of 1 mm/min under ambient conditions of 21±1°C and 65±2% relative humidity. The gauge length of the Instron was set at 100 mm ± 1 mm and the rate of extension at 100 mm/min. The ramp rate of the apparatus was 20 kN/min. The samples were placed in clamps at zero force (pretension mounting) with the middle of the sample in line with the centre point of the jaw edges. The moveable clap was then extended until the fibre ruptured. The (a) maximum load (or force) in Newton and (b) the displacement (extension) in millimetres were recorded. The test is performed uni-axially, preventing any torque being introduced.

3.6 Chemical fibre analysis

3.6.1 Silk fibre solution preparation

Fibre samples from each degumming process were dissolved in 10 ml of 10 M lithium thiocyanate (Sigma Aldrich; 308374) at room temperature for 24 h, with a material to liquor ratio of 1 (g):10 (ml). Any undissolved parts in the solution were removed using a centrifuge (Gemmy Universal K Harmonic PLC 024), operating at 4000 rpm at room temperature for 10 min. The solution was filtered and the filtrate dialyzed for 6 h at room temperature against deionized water, using dialysis sacs (molecular weight CO 12 000 Da,

Sigma Aldrich). Water was changed every 2 h, because solutions from fibres formed gels during long hours of dialysis. The final concentration of the silk solution varied between 2% and 4% (w/v). The actual concentration was determined by weighing the remaining solid after drying overnight at 60°C. The concentration of silk aqueous solution was adjusted by diluting with deionized water to 1% (w/v).

3.6.2 One-dimensional SDS-PAGE

Molecular weight of the samples were determined using precast Tris-Glycine 4–20% gradient gels (NuSep; NB12-420) by sodium dodecyl sulphate–polyacrylamide gel electrophoreses (SDS-PAGE) without any reducing agent, using the Mini-PROTEAN 3 Cell system and sample buffer (Nu Sep; BG-145), Tris-HEPES-SDS running buffer and molecular weight standards (Bio-Rad; 161-0317) following the manufacturer's recommended standard protocols. A total of 30 μl of 1% (w/v) silk solution as determined by weighing the solid after drying were loaded into each well. It was run at a constant voltage of 200 V for 50 min. Gels were removed according to manufacturer's instructions and stained in 25 ml Coomassie NuBlu Express Stain (NuSep) solution for 30 min and scanned.

3.7 Microbial analysis and identification of silk fibres after different degumming methods

3.7.1 Microbial analysis

For microbial analysis, the degumming solution after degumming was evaluated in duplicate. One millilitre of degumming solution was pipetted into 9 ml phosphate buffer (pH 7.2). Further dilutions were made up to 10^{-8} in phosphate buffer. One ml of each diluent was pipetted into separate petri dishes and standard plate count agar (SPCA; Oxoid) pour plates were made. Incubation was done at 32^{o}C, for 48 h. After incubation the colonies were enumerated by means of a colony counter (Harrigan, 1998).

3.7.2 Microbial identification

Different colonies from the SPCA plates based on colony morphology and Gram staining were purified. Representative isolates were selected (one for each group of similar colonies) and identified/profiled using the Biolog Gen II identification system (Biolog, Inc., Hayward, California) according to the manufacturer's instructions. Both Gram-negative and Gram-positive protocols were followed, depending on the isolates' initial screening reactions: GN or GP; oxidase test; catalase test and growth on MacConkey agar and TSI slants.

3.8 Statistical Analysis

3.8.1 Degumming data set

For each study day, treatment and replicate, the ratio ($\frac{A}{B}$) of "after" and "before" weights was formed. Separately for each study day, 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:

$$\text{Degumming efficiency} = \left(\frac{R_C}{R_T}\right) = \frac{\left(\frac{A_c}{B_c}\right)}{\left(\frac{A_T}{B_T}\right)}$$

where

- A_C is the geometric mean weight "after" for the control treatment
- B_C is the geometric mean weight "before" for the control treatment
- A_T is the geometric mean weight "after" for the test treatment in question
- B_T is the geometric mean weight "before" for the test treatment in question

3.8.2 Maximum load data set

Separately for each study day, the variables maximum load and displacement at maximum load were 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 (SAS Version 9.2, Proc GLM).

Furthermore, the mean values for all treatments were calculated, as well as the pairwise difference of the mean values and associated 95% confidence intervals and P-values for the difference.

Chapter 4

RESULTS AND DISCUSSION

4.1 Physical fibre properties after different degumming methods

4.1.1 Weight loss

The chemical composition of silk by weight are, in general, 75–83% of silk fibroin, 17–25% of sericin, 1.5% of wax and about 1–2% of others such as hydrocarbon (Lee *et al.,* 2005). Other studies indicated that sericin may even contribute to about 25–30% of the total cocoon weight (Dash *et al.,* 2006; Zhang, 2002). Degumming of silk fibres by hot alkaline solutions allow removal of the soluble protein components (sericin), leaving the fibroin core in an almost pure form (Taddei *et al.,* 2003). The removal of the sericin component of the composite silk fibre, is usually expressed as weight loss and is an important factor affecting the quality of a silk (Vishuprasad, 2004). The weight loss, which represents a quantitative evaluation of the degumming efficiency, indicates the level of degumming of the cocoons after the different methods were applied (Sah & Pramanik, 2010).

Degumming resulted in disintegration of the cocoons with considerable weight loss and formation of loose fibres (Table 4.1) which was in accordance with Reddy (2009). The quantity and nature of sericin are fundamental characteristics in conferring distinctive traits to the fibres in the cocoon (Mondal *et al.,* 2007). The sericin

gives a callous and stiff feeling to the fibre and hides the rich lustre of the silk fibre (Arami *et al.,* 2007). Degumming weight loss increased linearly as the number of days increased for each method, attaining a value between respectively 7% for distilled water and *Eucalyptus* oil to 31% for catholyte, on day 5 (Table 4.1). Weight loss for catholyte on days 5 and 6 was already more than 30%.

On day 6 the result of degumming with Orvus paste and *Eucalyptus* oil was 31%. On day 7 it was only three of the methods, namely catholyte and *Eucalyptus* oil, distilled water and *Eucalyptus* oil and vermicompost that did not have a degumming weight loss of more than 30%. By day 8 the combination of catholyte and *Eucalyptus* oil and the combination of distilled water and *Eucalyptus* oil respectively caused 29% and 20% degumming weight loss.

Table 4.1: Average weight loss of *Gonometa postica* cocoons over 10 days.

DAY	TREATMENT	Average weight loss (g)	Average weight loss (%)
		Original weight (±5–6 g)	
5	Orvus paste	1.567	28
	Catholyte	1.673	31
	Catholyte and *Eucalyptus* oil	1.247	22
	Distilled water	1.387	26
	Distilled water and *Eucalyptus* oil	0.380	7
	Orvus paste and *Eucalyptus* oil	1.476	28
	Vermicompost	1.444	26
6	Orvus paste	1.536	29
	Catholyte	1.650	32
	Catholyte and *Eucalyptus* oil	1.436	28
	Distilled water	1.430	27
	Distilled water and *Eucalyptus* oil	0.673	13
	Orvus paste and *Eucalyptus* oil	1.633	31
	Vermicompost	1.430	27
7	Orvus paste	1.700	31
	Catholyte	1.670	34
	Catholyte and *Eucalyptus* oil	1.527	29
	Distilled water	1.737	31
	Distilled water and *Eucalyptus* oil	0.943	19
	Orvus paste and *Eucalyptus* oil	1.830	34
	Vermicompost	1.487	27
8	Orvus paste	1.733	34
	Catholyte	1.964	37
	Catholyte and *Eucalyptus* oil	1.473	29
	Distilled water	1.656	32
	Distilled water and *Eucalyptus* oil	1.090	20
	Orvus paste and *Eucalyptus* oil	1.893	35
	Vermicompost	1.680	30
9	Orvus paste	2.013	36
	Catholyte	1.906	37
	Catholyte and *Eucalyptus* oil	1.643	31
	Distilled water	1.777	34
	Distilled water and *Eucalyptus* oil	1.410	26
	Orvus paste and *Eucalyptus* oil	2.006	37
	Vermicompost	1.753	32
10	Orvus paste	1.993	36
	Catholyte	1.906	37
	Catholyte and *Eucalyptus* oil	1.643	31
	Distilled water	1.943	35
	Distilled water and *Eucalyptus* oil	1.540	27
	Orvus paste and *Eucalyptus* oil	2.187	41
	Vermicompost	1.690	33

The influence of the Orvus paste, catholyte and the combination of catholyte and *Eucalyptus oil* are depicted in Figure 4.1. The standard was taken as the maximum degumming weight loss of 30% as was suggested by Wray *et al.* (2011). The catholyte method of degumming already degummed the silk fibres by more than 30% on day 5. There was a steep rise in the degumming process up to day 5 and then a steady rise up to day 9, when it started to stabilize. It seemed that the *Eucalyptus* oil rather hindered than improved the degumming process.

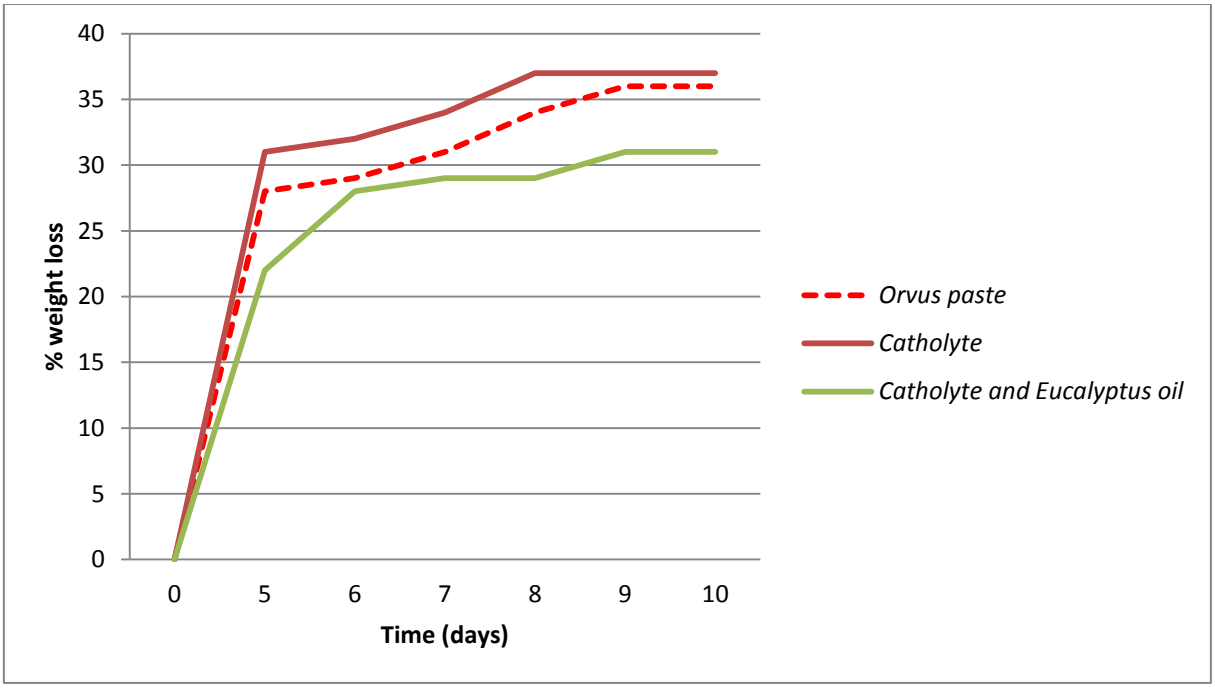

Figure 4.1: Percentage weight loss over 10 days for the Orvus paste, catholyte and catholyte and *Eucalyptus* oil degumming methods.

The two methods, distilled water and distilled water and *Eucalyptus* oil are showed in Figure 4.2. The distilled water reacted much in the same way as the Orvus paste (control). There was a steep rise in degumming up to day 5 and then again from day 5 to day 10. At day 10, the degumming process again seemed to stabilize.

Interesting was the big difference between the distilled water and distilled water and *Eucalyptus* oil added to the process. In the case of distilled water and *Eucalyptus* oil, the weight loss did not even reach the 30% mark. This indicated that the *Eucalyptus* oil hindered the degumming process.

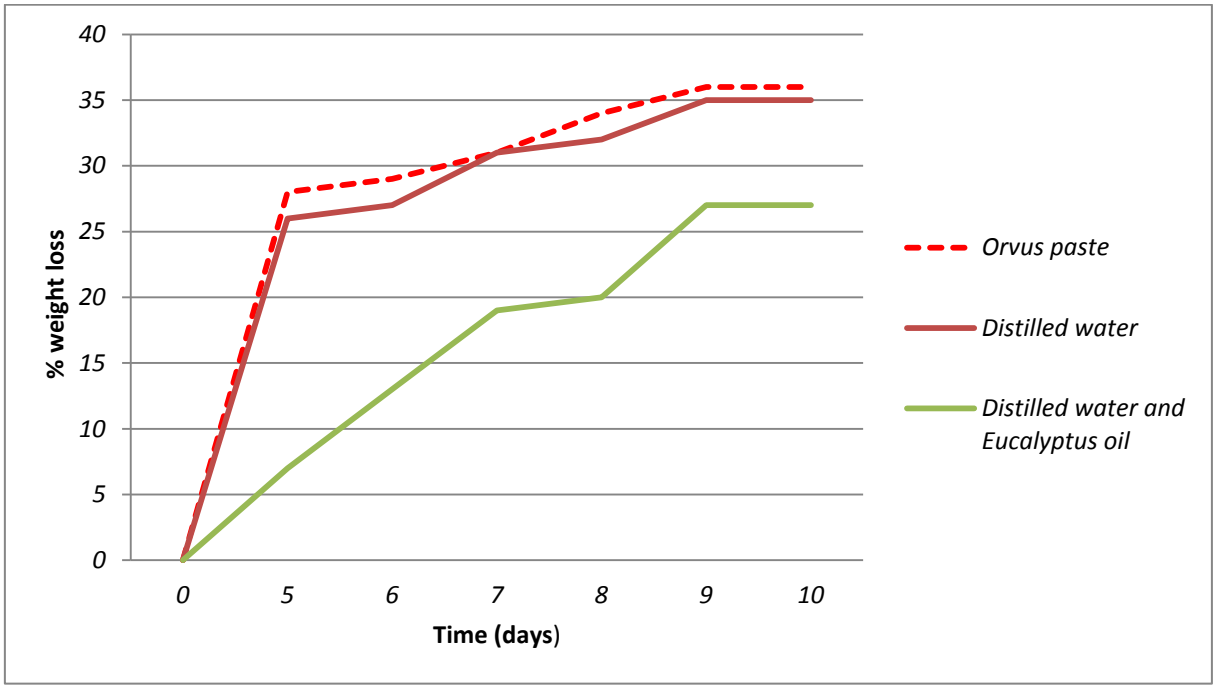

Figure 4.2: Percentage weight loss over 10 days for the Orvus paste; distilled water and distilled water and *Eucalyptus* oil degumming methods.

In contrast with this the results of Orvus paste, the combination Orvus paste and *Eucalyptus* oil and vermicompost showed different results. The addition of *Eucalyptus* oil to Orvus paste clearly showed a better result than that of the Orvus paste on its own (Figure 4.3). Orvus paste stabilized at around 36% weight loss on day 10, whereas the combination of Orvus paste and *Eucalyptus* oil carried on with the degumming process up to 41% after 10 days.

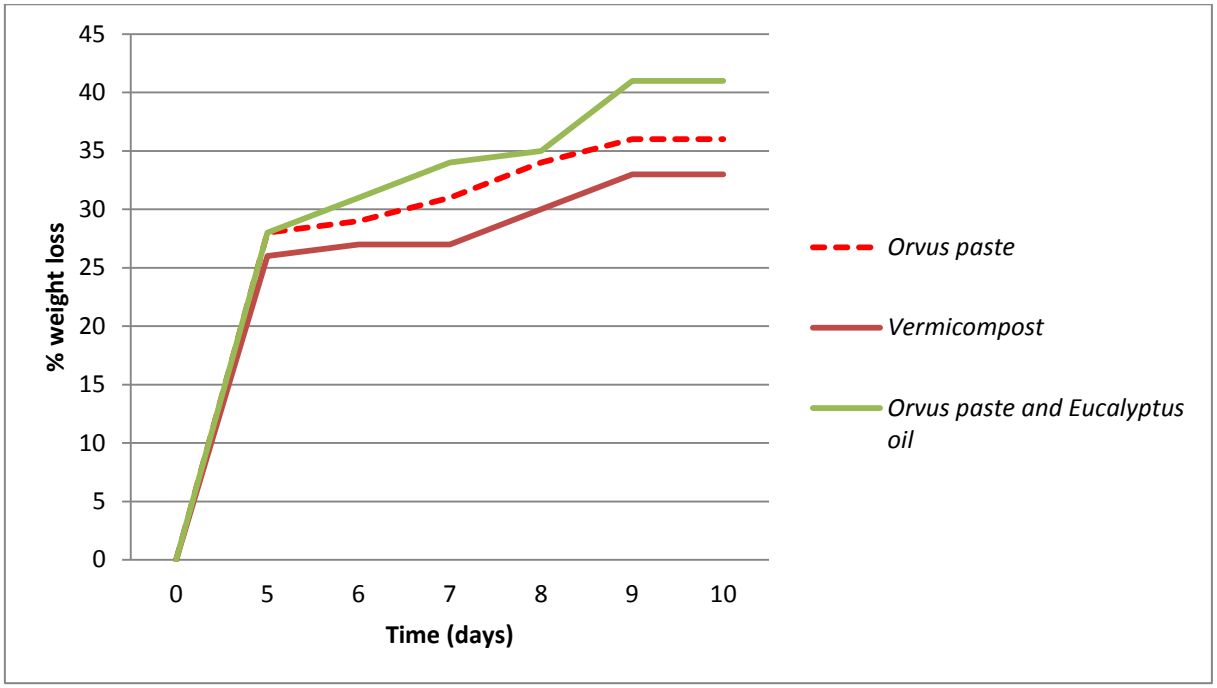

Figure 4.3: Percentage weight loss over 10 days for the Orvus paste, vermicompost and Orvus paste and *Eucalyptus* oil degumming methods.

When looking at a combination of all the methods, catholyte and the combination of Orvus paste and *Eucalyptus* oil gave better degumming weight loss than the conventional alkaline Orvus paste (Figure 4.4). As the days increased, the weight loss reached 36% on

day 10 with Orvus paste; 27% with distilled water and *Eucalyptus* oil; 41% with the combination Orvus paste and *Eucalyptus* oil; 37% with catholyte; 31% with the combination catholyte and *Eucalyptus* oil and 33% with vermicompost.

Only distilled water and *Eucalyptus* oil remained at 27% loss which indicated that, at this point, the degumming started to stabilize at a specific weight loss. It was clear that in this study, the weight loss of *Gonometa postica* fibres ranged from 27 to 41% over a time period of 10 days. This was in contrast with previous reports that the sericin content of *Gonometa postica* silk is less than the mulberry silk and as low as 12–16% (Prasong *et al.,* 2009; Cavaco-Paulo & Gubitz, 2003). The results are, however, similar to the results of Teshome *et al.* (2011) who found the sericin content to be between 23–56.8%.

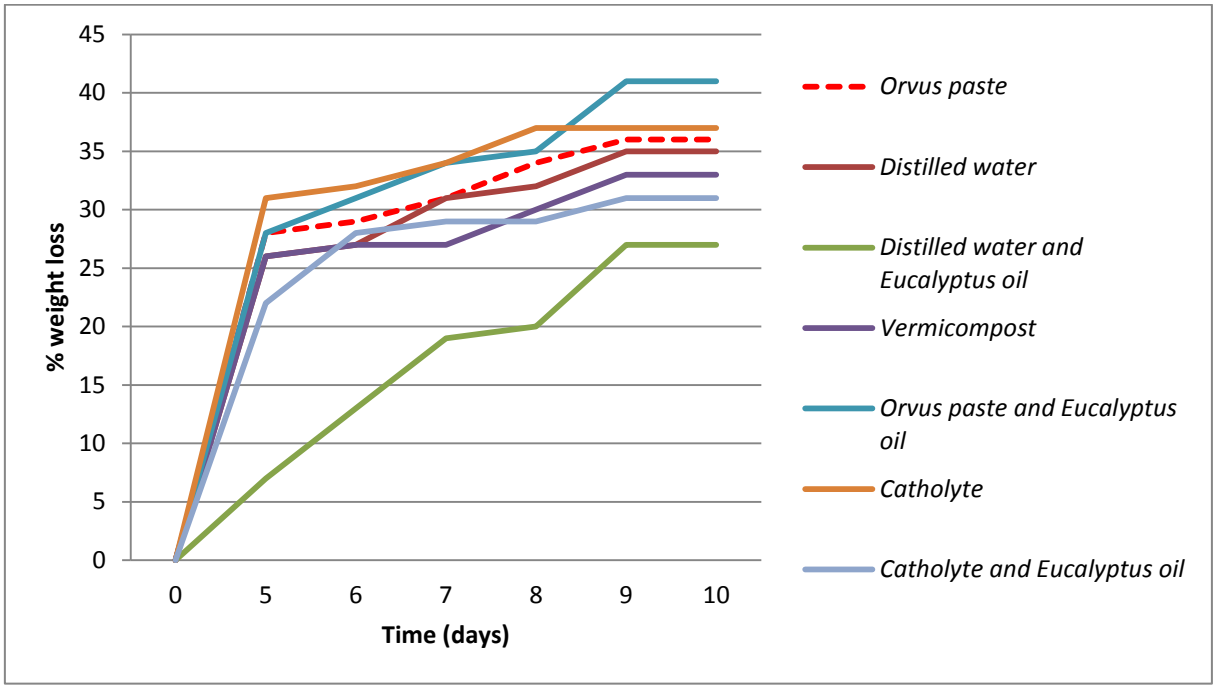

Figure 4.4: Percentage weight loss over 10 days for all the degumming methods.

4.1.2 Degumming efficiency

The efficiency of the degumming was calculated by again comparing each of the methods with the Orvus-paste control method.

The data depicted in Table 4.2 clearly indicated that within 5 days of the degumming, it was only catholyte ($p = 0.022$) and the combination of distilled water and *Eucalyptus* oil ($p = 0.0002$) that significantly differed from the control, Orvus paste. Catholyte already reached a point of 114.6% efficiency relative to Orvus paste while distilled water and *Eucalyptus* oil only had a 76.8% degumming efficiency. The rest of the data did not include catholyte, since it already reached a point above the 100% mark.

Over a time period of 6 days the efficiency did not change much. Orvus paste and *Eucalyptus* oil showed an efficiency of 103.6% relative to Orvus paste and distilled water and *Eucalyptus* oil an efficiency of 81.9% (Table 4.2). The last mentioned method differed significantly ($p = 0.017$) from the Orvus paste (control).

After 7, 8 and 9 days, distilled water reached an efficiency level of 96.1%; Orvus paste and *Eucalyptus* oil an efficiency level of 100.9% and vermicompost an efficiency level of 93.3% (Table 4.2). Distilled water and *Eucalyptus* oil reached the 86.6% level and still was the only method that differed significantly ($p = 0.0006$) from the control (Table 4.2).

As the time of exposure increased, it was only distilled water and *Eucalyptus* oil that further differed significantly from the control, Orvus paste (Table 4.2). After 10 days there was a good comparison between the different environmentally conscious methods and the control, Orvus paste. It was evident that the combination Orvus paste and *Eucalyptus* oil and catholyte methods were more efficient than the Orvus paste method. The values of distilled water and *Eucalyptus* oil were between 60% and 80%.

Table 4.2: Influence of different degumming methods on the degumming efficiency of *Gonometa postica* silk fibres.

DAY	TREATMENT	Efficiency	p-Value
		(Relative to Orvus paste = control)	
5	Catholyte	114.6	0.022[i]
	Catholyte and *Eucalyptus* oil	104.3	0.433
	Distilled water	92.8	0.177
	Distilled water and *Eucalyptus* oil	76.8	0.0002*
	Orvus paste and *Eucalyptus* oil	98,7	0.798
	Vermicompost	97.9	0.685
6	Catholyte	-	-
	Catholyte and *Eucalyptus* oil	-	-
	Distilled water	90.9	0.198
	Distilled water and *Eucalyptus* oil	81.9	0.017*
	Orvus paste and *Eucalyptus* oil	103.6	0.622
	Vermicompost	97.2	0.687
7	Catholyte	-	-
	Catholyte and *Eucalyptus* oil	-	-
	Distilled water	99.2	0.886
	Distilled water and *Eucalyptus* oil	84.7	0.011*
	Orvus paste and *Eucalyptus* oil	104.5	0.423
	Vermicompost	94.8	0.336
8	Catholyte	-	-
	Catholyte and *Eucalyptus* oil	-	-
	Distilled water	96.9	0.427
	Distilled water and *Eucalyptus* oil	83.2	0.0006*
	Orvus paste and *Eucalyptus* oil	102	0.589
	Vermicompost	94.8	0.187
9	Catholyte	-	-
	Catholyte and *Eucalyptus* oil	-	-
	Distilled water	96.1	0.443
	Distilled water and *Eucalyptus* oil	86.6	0.0171*
	Orvus paste and *Eucalyptus* oil	100.9	0.8547
	Vermicompost	93.3	0.2036
10	Catholyte	-	-
	Catholyte and *Eucalyptus* oil	-	-
	Distilled water	97.3	0.578
	Distilled water and *Eucalyptus* oil	85.4	0.008*
	Orvus paste and *Eucalyptus* oil	107.6	0.1576
	Vermicompost	95.2	0.326

4.1.3 Morphology of silk fibres

The surface morphology of silk fibres degummed by different degumming methods was investigated by SEM. The surface morphology of the silk fibre before degumming is shown in Figure 4.5. The surface characteristic of the *G. postica* silk fibre was fairly rough 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.

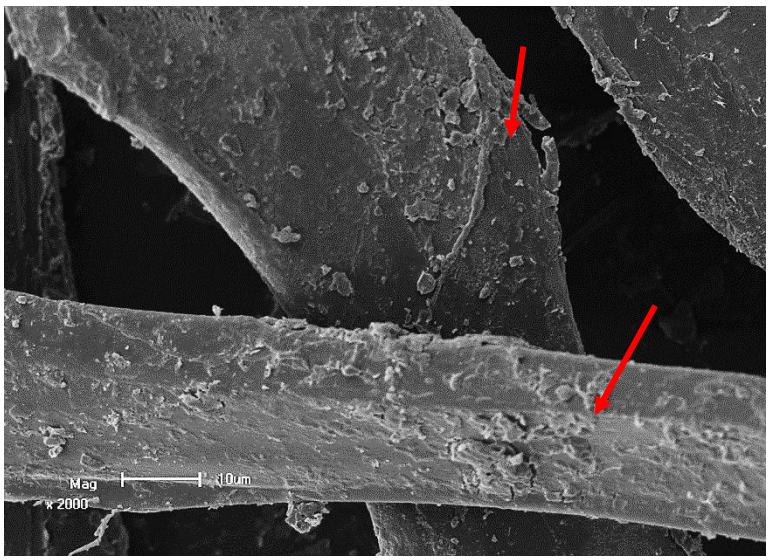

Figure 4.5: The *G. postica* silk fibres before degumming. Sericin is indicated by the arrows.

The silk fibres of *G. postica* have many longitudinal striations (Figure 4.5 – bottom arrow) on their surface and are porous which make them lighter than mulberry silk (Teshome *et al.,* 2011). These fibres are flattened, ribbon-like filaments of much larger diameter

than mulberry silk (Teshome *et al.,* 2011). In Figure 4.5, the flat triangular shape is evident and the fibre is not circular in cross section. *Gonometa postica* have fibre diameters of 18–33 μm (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 stiffness (Chattopadhyay, 2008).

Orvus paste was used as the control degumming method. After 5 days of exposure to Orvus paste (Figure 4.6), the degumming weight loss was 28%. The SEM micrograph indicated large amounts of sericin still present on the fibres.

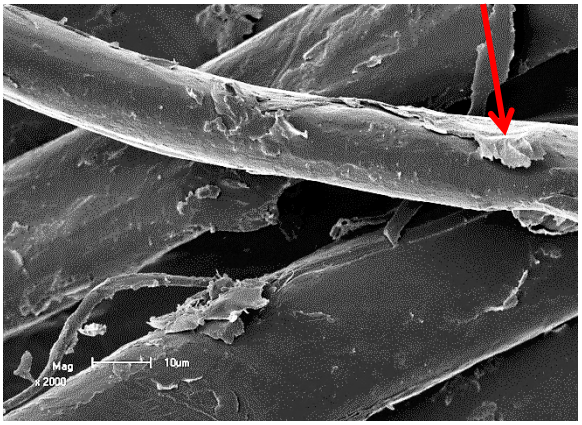

Figure 4.6: *Gonometa postica* silk fibres after 5 days of exposure to Orvus paste (degumming weight loss of 28%).

Ten days of exposure of the silk fibres to Orvus paste resulted in a degumming weight loss of 36% (Figure 4.7). The SEM

micrograph indicated some sericin remnants still present on the fibres.

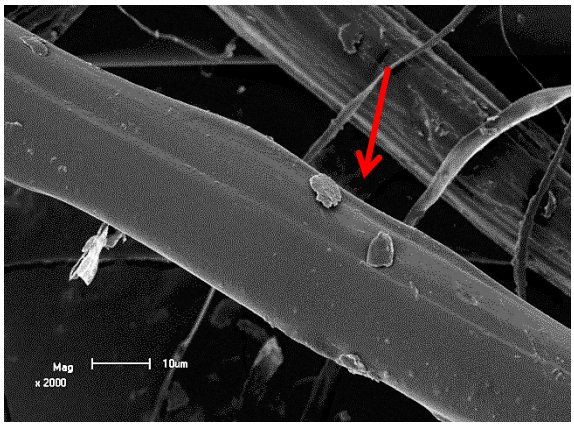

Figure 4.7: *Gonometa postica* silk fibres after 10 days of exposure to Orvus paste (degumming weight loss of 36%).

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 4.8). Sericin remnants were still present.

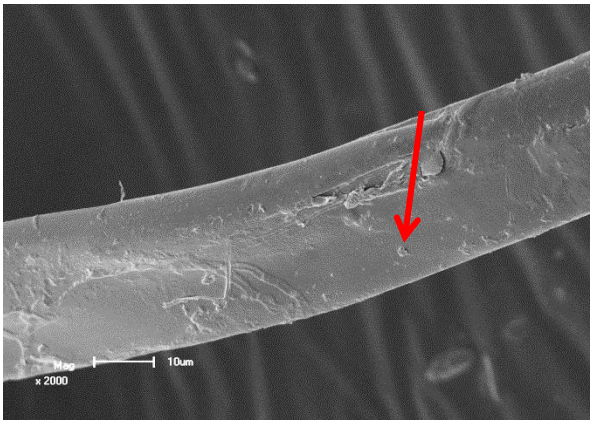

Figure 4.8: *Gonometa postica* silk fibres after 5 days of exposure to Orvus paste and *Eucalyptus* oil (degumming weight loss of 28%).

Exposure of 10 days to the combination method of Orvus paste and *Eucalyptus* oil resulted in a degumming weight loss of 41% (Figure 4.9). The SEM micrograph showed a clean smooth fibre, but there were still some remnants present on the fibre.

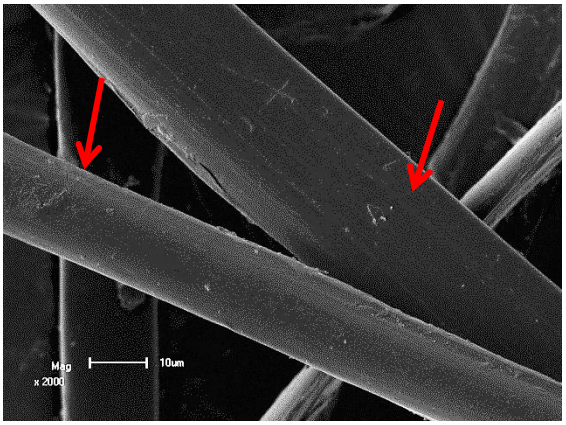

Figure 4.9: *Gonometa postica* silk fibres after 10 days of exposure to Orvus paste and *Eucalyptus* oil (degumming weight loss of 41%).

Sericin remnants were observed to be present on the fibre sample after 5 days of exposure to catholyte (Figure 4.10). The amount of sericin on the fibroin was already less than on the untreated sample (Figure 4.5).

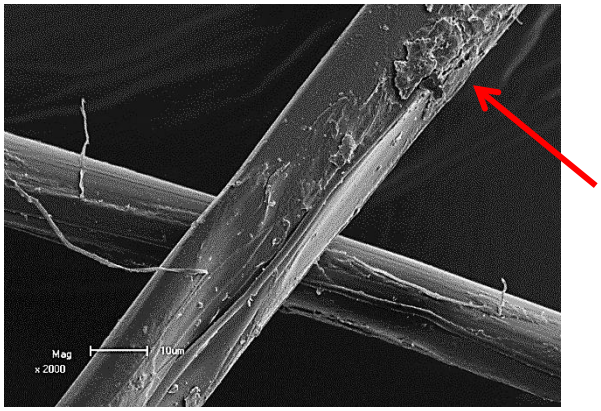

Figure 4.10: *Gonometa postica* silk fibres after 5 days of exposure to catholyte (degumming weight loss of 31%).

After exposure of 10 days to catholyte, sericin was observed to be effectively removed, as indicated by the smooth silk fibres (Figure 4.11).

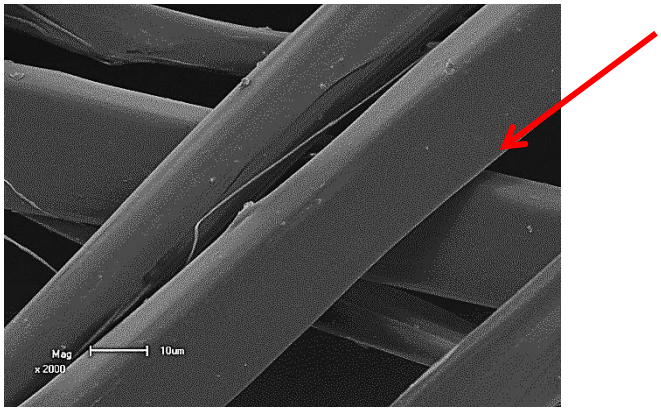

Figure 4.11: *Gonometa postica* silk fibres after 10 days of exposure to catholyte (degumming weight loss of 37%).

Exposure of the fibres to the combination degumming solution of catholyte and *Eucalyptus* oil resulted in a low degumming loss of 22% (Figure 4.12). Degumming seemed to be much slower with this method.

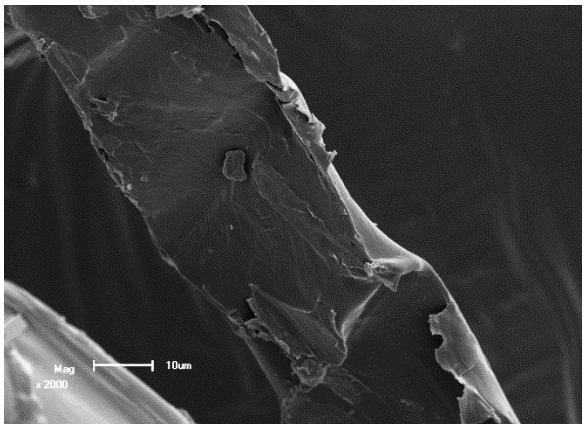

Figure 4.12: *Gonometa postica* silk fibres after 5 days of exposure to catholyte and *Eucalyptus* oil (degumming weight loss of 22%).

After 10 days of exposure to catholyte and *Eucalyptus* oil, the fibres were partially cleaned and a smooth surface was evident. Remnant of sericin was still present in small amounts (Figure 4.13).

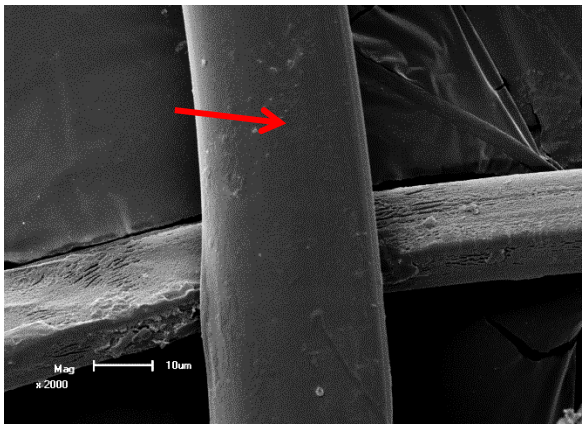

Figure 4.13: *Gonometa postica* silk fibres after 10 days of exposure to catholyte and *Eucalyptus* oil (degumming weight loss of 31%).

After exposure of 5 days of the silk fibres to distilled water, sericin was still present all over the fibres (Figure 4.14).

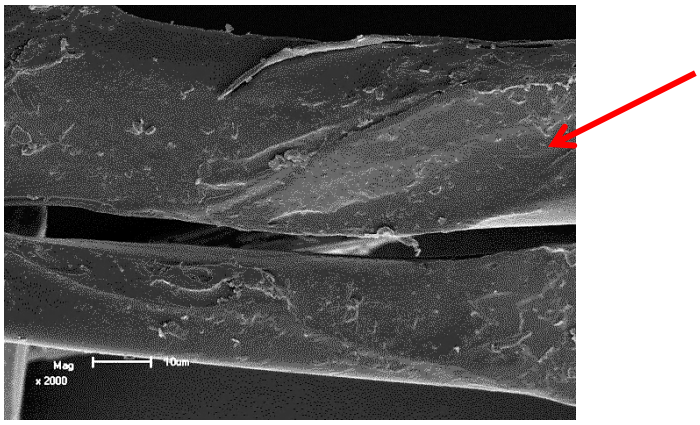

Figure 4.14: *Gonometa postica* silk fibres after 5 days of exposure to distilled water (degumming weight loss of 26%).

After exposure of 10 days to distilled water, the result was clear, smooth surfaces, but there were still some evidence of remaining sericin (indicated by the arrow in Figure 4.15). This indicated that with this method, the degumming process will take longer than 10 days to complete the degumming.

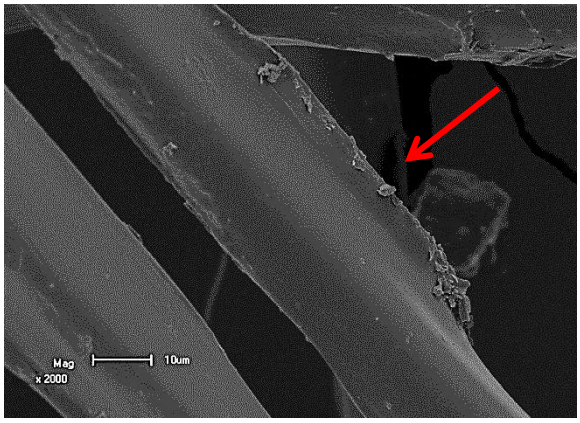

Figure 4.15: *Gonometa postica* silk fibres after 10 days of exposure to distilled water (degumming weight loss of 35%).

Distilled water and *Eucalyptus* oil had a degumming weight loss of only 7% after 5 days of exposure (Figure 4.16). The large amount of sericin was evident on the SEM micrograph.

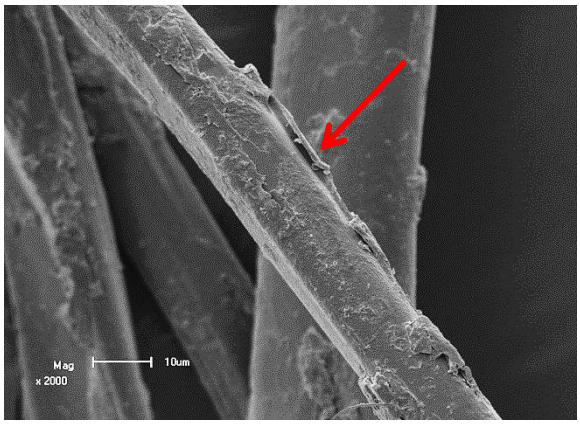

Figure 4.16: *Gonometa postica* silk fibres after 5 days of exposure to distilled water and *Eucalyptus* oil (degumming weight loss of 7%).

After 10 days of exposure to the combined method of distilled water and *Eucalyptus* oil, sericin remnants were still observed (Figure 4.17). The degumming weight loss was only 27%. This method also, therefore, needed more time to complete the degumming process.

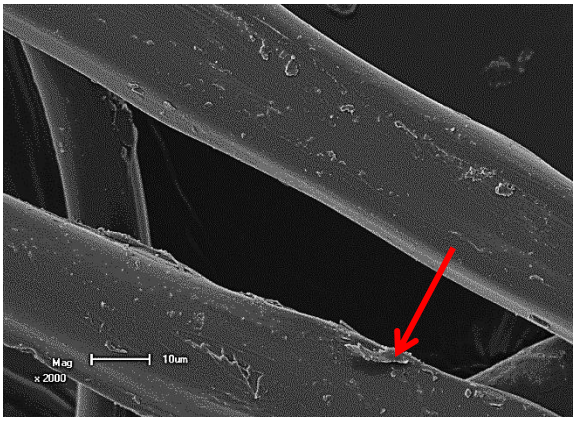

Figure 4.17: *Gonometa postica* silk fibres after 10 days of exposure to distilled water and *Eucalyptus* oil (degumming weight loss of 27%).

An exposure of 5 days to vermicompost resulted in a degumming loss of 26% (Figure 4.18). The rough surface of the *Gonometa postica* silk fibres indicated large amounts of sericin.

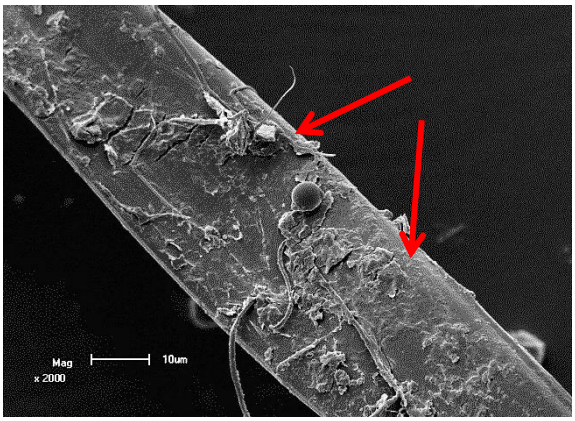

Figure 4.18: *Gonometa postica* silk fibres after 5 days of exposure to vermicompost (degumming weight loss of 26%).

After 10 days of exposure to vermicompost, the *G. postica* silk fibres still showed sericin remnants all over the fibre (Figure 4.19). The time period for this method clearly was not sufficient. The parts of the fibre that were degummed showed clean, smooth surfaces.

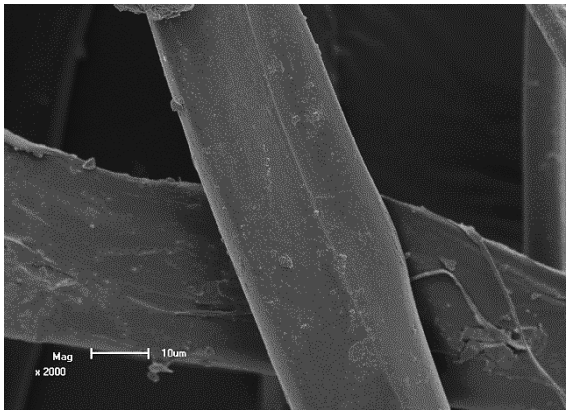

Figure 4.19: *Gonometa postica* silk fibres after 10 days of exposure to vermicompost (degumming weight loss of 33%).

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 showed good to moderately good degumming results after an exposure time 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.

The average weight loss, after 10 days, varied between 27% and 41%. 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) (Teh *et al*., 2010).

4.2 Mechanical fibre properties after different degumming methods

4.2.1 Maximum load

The effect of the different degumming methods on the mechanical properties of the fibres was investigated. Strength is the ability to resist stress and is expressed as tensile strength (Kadolph, 2010) or as tenacity. It is the strength of the fibres under tension. It,

therefore, measures the resistance of a fibre to stretching in one specific direction (Collier & Tortora, 2001). The tensile properties of a fibre, and specifically the tensile strength, are an important characteristic in determining the performance of a fibre. An example of a broken *G. postica* silk fibre is shown in Figure 4.20.

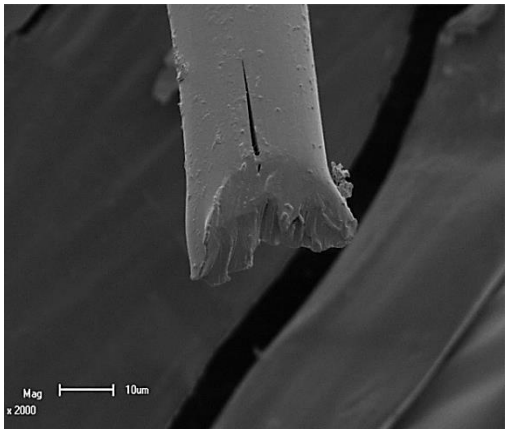

Figure 4.20: A broken *G. postica* silk fibre.

The tensile strength of a fibre is expressed as the breaking force, or maximum load, which is the force, required rupturing or breaking the fibre (Kadolph, 2010). The maximum load in this study was expressed in Newton (N) as measured by the Instron Instrument. The results are given in Table 4.3.

From the results it was clear that there was a significant ($p < 0.001$) difference between the tensile strength of the fibres treated with catholyte, and the combination of Orvus paste and *Eucalyptus* oil after 5 days of exposure. The mean maximum load for the two methods was 0.415 N and 0.513 N, respectively. When comparing

the combination of catholyte and *Eucalyptus* oil, distilled water and Orvus paste (control) after 6 days of exposure, no significant difference between these three methods were evident, this could be indicative of a decrease in strength. Results in previous research (Teli & Rane, 2011) indicated that with an increase in the severity of degumming, the tensile strength decreased progressively. Their results clearly indicated a reduction in the breaking load, from the mildest to most severe conditions of degumming (Teli & Rane, 2011).

After an exposure of 8 days a comparison between the combination catholyte and *Eucalyptus* oil, and vermicompost methods was made. A significant ($p < 0.001$) difference was found between these two methods, with mean maximum loads of 0.464 N and 0.280 N respectively.

The degree of crystallinity is quite high in silk fibres (Zhou *et al.,* 2000; Tanaka *et al.,* 1999) and crystallites are expected to reinforce the structure of fibres (Wang & Zhang, 2013). However, the differences in crystalline content in the different fibres from this study are not substantial, but the differences in orientation are quite substantial (Table 4.3). This, on application of the load, will cause the more compliant amorphous phase to deform first. It is the presence of randomly oriented molecules in the amorphous phase of *G. postica*, which, according to Rajkhowa *et al.* (2000), gives them their

Table 4.3: Influence of different degumming methods on the maximum load of silk fibres.

DAY	TREATMENT	Mean max load (N)	Difference between means	p-Value
		(Relative to Orvus paste = control)		
10	Catholyte	0.332	0.064	0.0104*
	Distilled water	0.339	0.071	0.0047*
	Distilled water and *Eucalyptus* oil	0.329	0.061	0.0139*
	Orvus paste and *Eucalyptus* oil	0.410	-0.141	<0.001*
	Vermicompost	0.408	-0.140	<0.001*
		(Relative to Catholyte)		
10	Orvus paste	0.268	0.064	0.0104*
	Distilled water	0.339	-0.007	0.7689
	Distilled water and *Eucalyptus* oil	0.329	0.003	0.9121
	Orvus paste and *Eucalyptus* oil	0.410	-0.077	0.0023*
	Vermicompost	0.408	-0.076	0.0026*
		(Relative to distilled water)		
10	Catholyte	0.332	0.003	0.7689
	Orvus paste	0.268	0.071	0.0047*
	Distilled water and *Eucalyptus* oil	0.329	0.009	0.6862
	Orvus paste and *Eucalyptus* oil	0.410	-0.070	0.0052
	Vermicompost	0.408	-0.069	0.0060
		(Relative to distilled water and *Eucalyptus* oil)		
10	Catholyte	0.332	0.003	0.9121
	Distilled water	0.339	0.009	0.6862
	Orvus paste	0.268	0.061	0.0139*
	Orvus paste and *Eucalyptus* oil	0.410	-0.080	0.0016*
	Vermicompost	0.408	-0.079	0.0019*
		(Relative to Orvus paste and *Eucalyptus* oil)		
10	Catholyte	0.332	-0.077	0.0023*
	Distilled water	0.339	-0.070	0.0052*
	Distilled water and *Eucalyptus* oil	0.329	-0.080	0.0016*
	Orvus paste	0.268	-0.141	<0.0001*
	Vermicompost	0.408	0.001	0.9604
		(Relative to vermicompost)		
10	Catholyte	0.332	-0.076	0.0026*
	Distilled water	0.339	-0.069	0.0060*
	Distilled water and *Eucalyptus* oil	0.329	-0.079	0.0019*
	Orvus paste and *Eucalyptus* oil	0.410	0.001	0.9604
	Orvus paste	0.268	-0.140	<0.0001*

relatively higher extensibility. As extension continues, the load is progressively transferred to the crystalline regions.

From the results obtained (Table 4.3), it was clear that there was a significant difference between the maximum and average loads that the fibres could withstand after 10 days of processing using different degumming methods. The mean maximum load for the silk fibre exposed for 10 days to Orvus paste, was 0.268 N. The rest of the methods differed significantly (Table 4.3), from the Orvus paste treatment. After 10 days of exposure, the mean maximum load for catholyte was 0.332 N ($p = 0.0104$), for distilled water 0.339 N ($p = 0.0047$), for the combination distilled water and *Eucalyptus* oil 0.329 N ($p = 0.0139$), for Orvus paste and *Eucalyptus* oil 0.410 N ($p < 0.001$) and for vermicompost 0.408 N ($p < 0.001$).

The difference between the mean maximum load of the different environmentally friendly methods when compared to the control (Orvus paste) differed significantly on day 10, indicating that the fibres were much stronger when degummed with the environmentally friendly methods than with the Orvus paste method (Table 4.3; Figure 4.21).

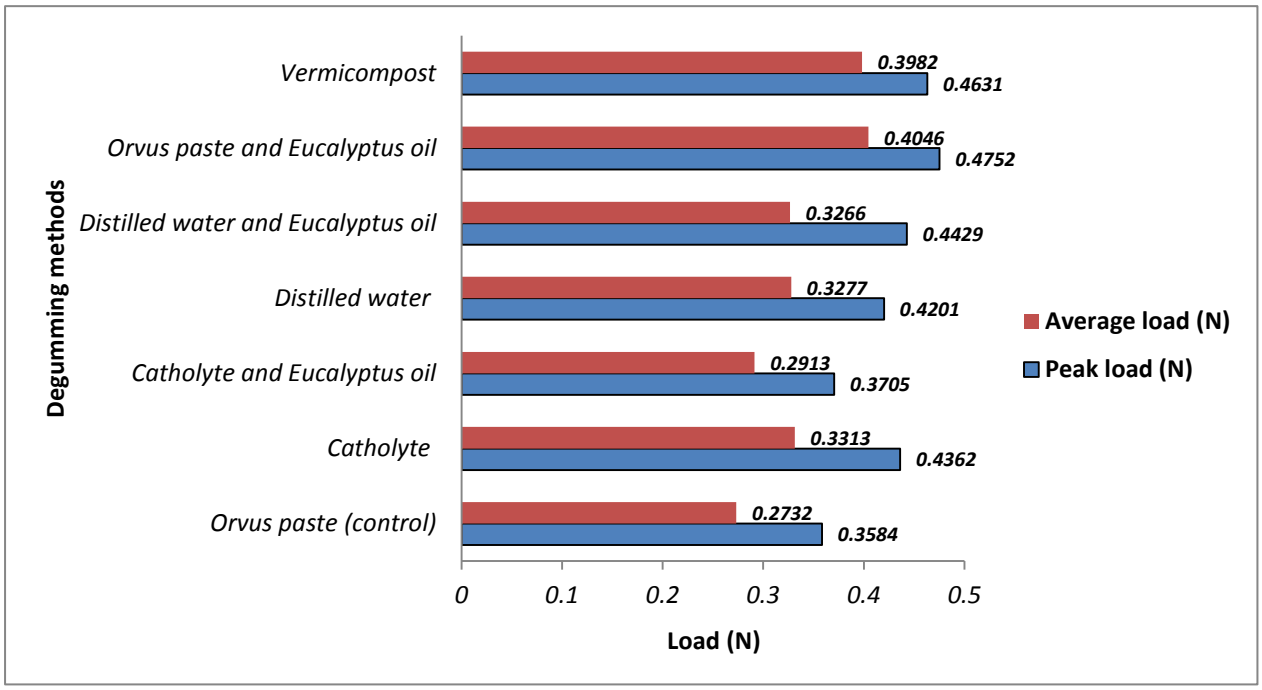

Figure 4.21: Peak and average load of silk fibres degummed with Orvus paste and different environmentally conscious degumming methods after 10 days.

The mean maximum load of the catholyte method compared well with the mean maximum loads of distilled water, and the combination distilled water and *Eucalyptus* oil with no significant differences. Catholyte, however, differed significantly from Orvus paste ($p = 0.0104$), Orvus paste and *Eucalyptus* oil ($p = 0.0023$) and Vermicompost ($p = 0.0026$) (Table 4.3).

The mean maximum load of distilled water was only significantly different ($p = 0.0047$) from the mean maximum load of Orvus paste (Table 4.3).

The mean maximum load of distilled water and *Eucalyptus* oil (Table 4.3) resulted in a good comparison to catholyte and distilled

water, but differed significantly with the mean maximum loads of Orvus paste ($p = 0.0139$), Orvus paste and *Eucalyptus* oil ($p = 0.016$) and vermicompost ($p = 0.0019$), indicating that it is not a very efficient degumming method.

Although the mean maximum load of the Orvus paste and *Eucalyptus* oil combination was significantly different from the mean maximum loads of catholyte ($p = 0.0023$), distilled water ($p = 0.0052$) and the distilled water and *Eucalyptus* oil combination ($p = 0.0016$), it was most significant ($p<0.001$) for Orvus paste (Table 4.3).

The mean maximum load of the vermicompost method was significantly different from the mean maximum loads of the catholyte ($p = 0.0026$), distilled water ($p = 0.0060$), the combination of distilled water and *Eucalyptus* oil ($p = 0.0019$) and the most significantly ($p < 0.001$) different from the Orvus paste method (Table 4.3). The fibres of these methods were therefore, significantly stronger than fibres from the other methods tested. This was also found in a study by Sen & Babu (2004), when they indicated that the silk fibres of *G. postica* have great mechanical properties and average breaking extension values.

The vermicompost degumming method resulted in fibres with a mean maximum load of 0.408 N, which delivered the best results of all the methods, compared to the Orvus paste treatment (0.268 N)

(Table 4.3), though the fact that the process of degumming was not completed should be considered.

4.2.2 Displacement

Displacement is a vector quantity that refers to "how far out of place an object is"; it is the object's overall change in position. The displacement of fibres is the difference between the initial position of the fibre and any later position. The displacement of the fibres before breakage was correlated to the different methods used to degum the fibres. Figure 4.22 illustrates the influence of the different degumming methods on the displacement percentage at maximum load of the fibres.

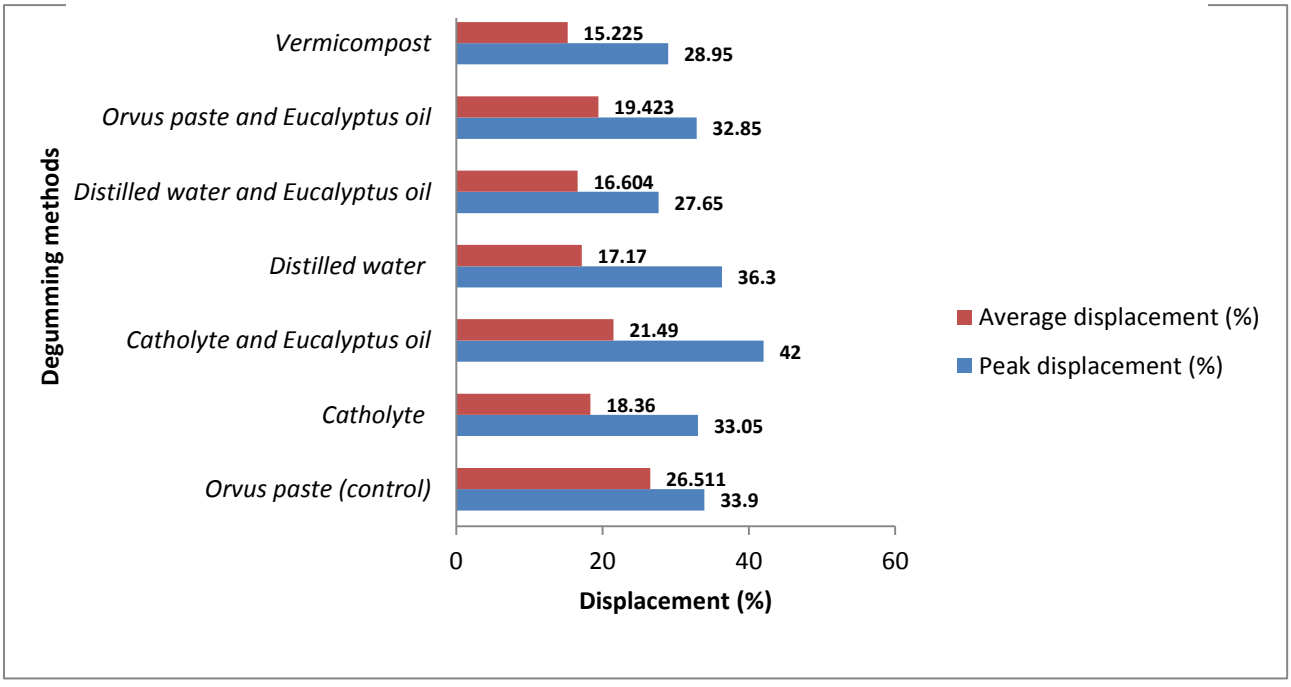

Figure 4.22: Peak and average displacement of silk fibres degummed with Orvus paste and different environmentally conscious degumming methods.

From the data depicted in Table 4.4, it was clear that all the environmentally conscious methods differed significantly ($p < 0.001$) from the control, Orvus paste, which had a displacement mean of 5.147 mm, after 8 days of degumming treatment. All the other methods showed much shorter displacement means and differed from 2.693 mm for vermicompost to 3.616 mm for the combination method of Orvus paste and *Eucalyptus* oil.

Table 4.4: Influence of different degumming methods on the displacement of silk fibres.

DAY	TREATMENT	Mean Displacement (mm)	Difference between means	p-Value
		(Relative to Orvus paste = control)		
10	Catholyte	3.089	-2.058	<0.001*
	Distilled water	2.935	-2.212	<0.001*
	Distilled water and *Eucalyptus* oil	3.342	-1.805	0.0001*
	Orvus paste and *Eucalyptus* oil	3.616	1.531	0.0010*
	Vermicompost	2.693	2.454	<0.001*
		(Relative to Catholyte)		
10	Orvus paste	5.147	-2.058	<0.0001*
	Distilled water	2.935	0.154	0.7271
	Distilled water and *Eucalyptus* oil	3.342	-0.253	0.5668
	Orvus paste and *Eucalyptus* oil	3.616	-0.527	0.2352
	Vermicompost	2.693	0.396	0.3710
		(Relative to distilled water)		
10	Catholyte	3.089	0.154	0.7271
	Orvus paste	5.147	-2.212	<0.001*
	Distilled water and *Eucalyptus* oil	3.342	-0.407	0.3580
	Orvus paste and *Eucalyptus* oil	3.616	-0.681	0.1267
	Vermicompost	2.693	0.242	0.5837
		(Relative to distilled water and *Eucalyptus* oil)		
10	Catholyte	3.089	-0.253	0.5668
	Distilled water	2.935	-0.407	0.3580
	Orvus paste	5.147	-1.805	0.0001*
	Orvus paste and *Eucalyptus* oil	3.616	-0.274	0.5352
	Vermicompost	2.693	0.649	0.1451
		(Relative to Orvus paste and *Eucalyptus* oil)		
10	Catholyte	3.089	-0.527	0.2352
	Distilled water	2.935	-0.681	0.1267
	Distilled water and *Eucalyptus* oil	3.342	-0.274	0.5352
	Orvus paste	5.147	1.531	0.0010*
	Vermicompost	2.693	0.923	0.0402
		(Relative to vermicompost)		
10	Catholyte	3.089	0.396	0.3710
	Distilled water	2.935	0.242	0.5837
	Distilled water and *Eucalyptus* oil	3.342	0.649	0.1451
	Orvus paste and *Eucalyptus* oil	3.616	0.923	0.0402
	Orvus paste	5.147	2.454	<0.001*

In the case of the fibres from the Catholyte method, it was clear that this method compared well to all the other methods, except for Orvus paste. There was a significant ($p < 0.0001$) difference between the displacement mean for Catholyte and Orvus paste.

When distilled water was used to degum the fibres, it resulted in a good comparison to the other degumming methods. Again, there was only a significant ($p < 0.001$) difference with the Orvus paste treatment, with the rest of the methods being in the same range.

When comparing the combination methods, namely distilled water and *Eucalyptus* oil, and Orvus paste and *Eucalyptus* oil with the vermicompost treatment, no significant differences were found. These methods only differed significantly from Orvus paste ($p = 0.0001$; $p = 0.0010$ and $p < 0.001$, respectively).

In summary, Table 4.5 depicts the maximum load means, displacement means at maximum load and the tensile strain at maximum load for the fibres degummed with the different methods.

Table 4.5: The impact of degumming methods on the mechanical properties of silk fibre.

Degumming methods	Maximum load (N)	Displacement at maximum load (mm)	Tensile strain at maximum load (%)
Orvus paste (control)	0.268	5.147	27.085
Catholyte	0.332	3.089	15.445
Distilled water	0.339	2.935	14.675
Distilled water and *Eucalyptus* oil	0.329	3.342	16.710
Orvus paste and *Eucalyptus* oil	0.410	3.616	18.080
Vermicompost	0.408	2.693	13.465

At maximum load the displacement for the fibres ranged from 2.693–5.147 mm, while the tensile strain at maximum load ranged from 13.465–27.085%. In Figure 4.23 it is clear that the two Orvus paste methods had the biggest displacement, as well as tensile stain percentage.

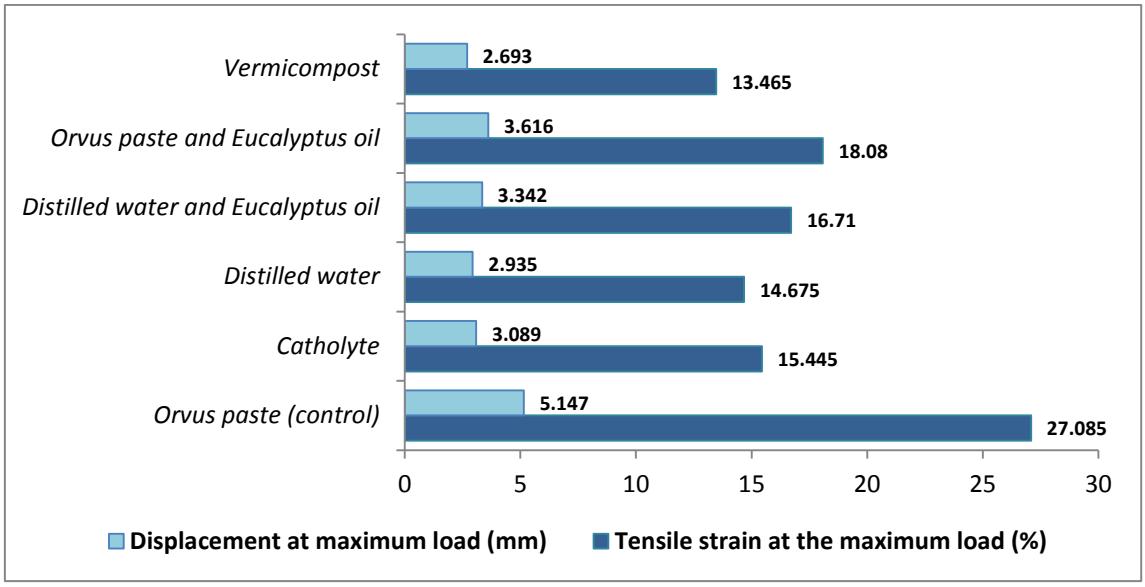

Figure 4.23: Correlation between displacement at maximum load (mm) and tensile strain at maximum load (%) for the different methods used for degumming *G. postica* fibres.

Second was the combination of distilled water and *Eucalyptus* oil, with Catholyte, distilled water and vermicompost next in line. Elongation at break of silk fibres was between 18–25% under normal conditions (Nguku *et al.,* 2007). In this study on *Gonometa postica* silk, elongation at break was, however, between 13–27%.

4.3 Chemical fibre properties after different degumming methods

Silkworm cocoons are composed of two kinds of silk protein; silk sericin, which makes up the membrane of the fibre, and silk fibroin, which makes up the inner portion of the fibre. It is expected that silk fibroins, with high alanine (Ala) and glycine (Gly) contents,

are heavy chains (H-chains) that show migration on gels during SDS-PAGE (Žurovec & Sehnal, 2002; Zhou *et al.,* 2000). Therefore, SDS-PAGE can be useful to reveal qualitative and quantitative changes in molecular weight during degumming. Various studies indicated that at 26 kDa, there is a light chain (L-chain). The high and light chains are linked by disulphide bond(s) and about 30 kDa make up a P25 glycoprotein that associates with the H-L complex primarily by hydrophobic interactions. The H-chain is fibrous protein due to the presence of Gly, Ala and serine (Ser). On the other hand, the L-chain is non-fibrous and contains relatively high amounts of leucine (Leu), isoleucine (Ile), valine (Val) and acidic amino acids (Zhou *et al.,* 2000).

The lithium thiocyanate aqueous solution is known as the least degrading method for silk fibre dissolution (Wang & Zhang, 2013; Chen *et al.,* 2001). During dissolution of degummed silk fibre in lithium thiocyanate, the amide bonds of the fibroin molecular chain might be cleaved into different lengths resulting in easy solubilisation in water (Tao *et al.*, 2007; Takasu *et al.,* 2002). These water soluble silk fibroin is called regenerated silk fibroin (Zhang *et al.,* 2005; Kim *et al.,* 2004), and are easily denatured and separated on a gel.

The *G. postica* silk fibres in this study, after degumming with different methods, were subjected to SDS-PAGE and the results are indicated in Figure 4.24. In Figure 4.24A, lanes 1 and 2, and in

Figure 4.24B, lanes 11 and 12, represent two different protein standards. The *G. postica* silk fibroin degummed with different methods is found in lanes 3–9 (Figure 4.24A) and in lanes 13–19 (Figure 4.24B), and showed molecular weight ranges from 6 kDa to more than 200 kDa.

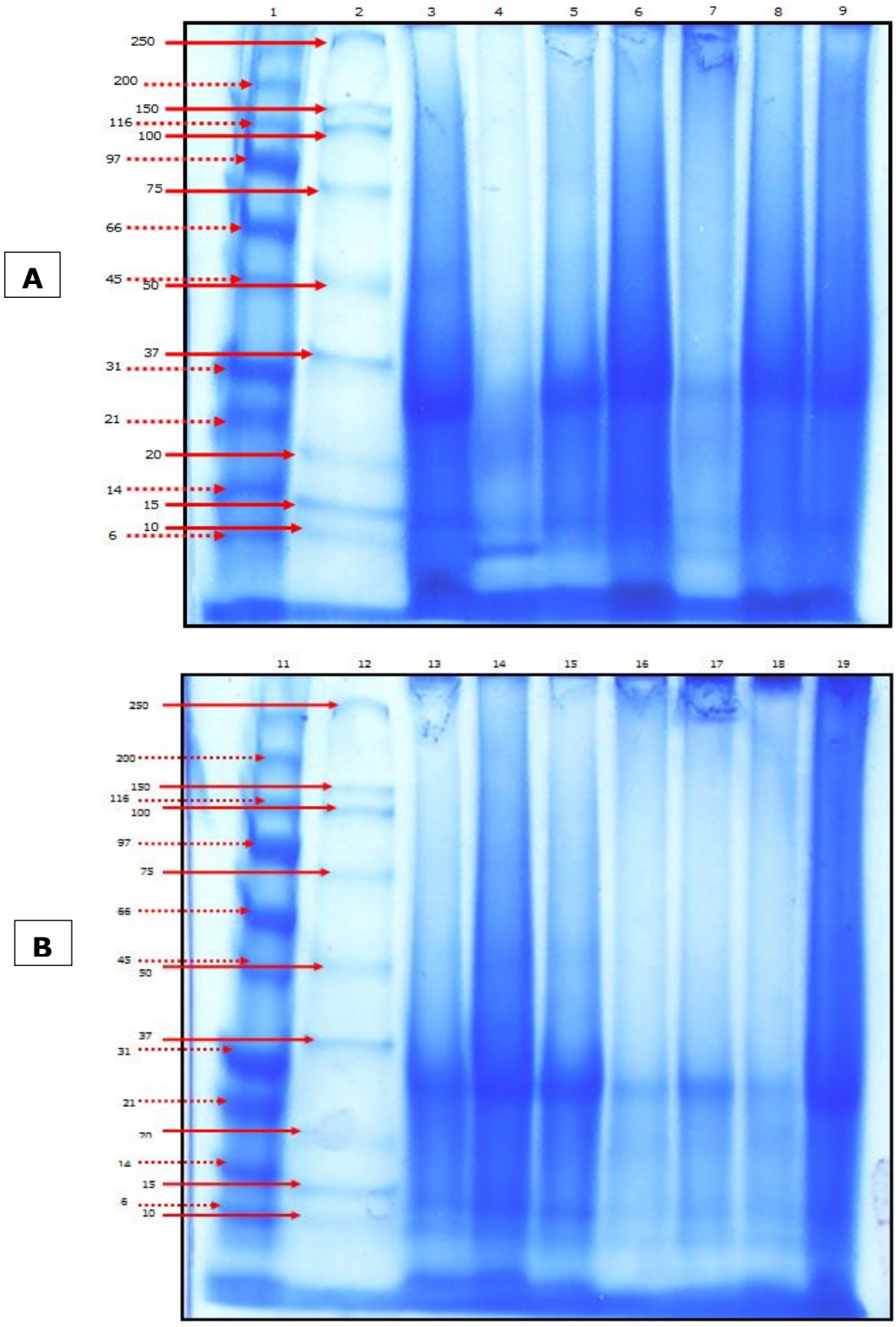

Figure 4.24: SDS-PAGE of silk fibres subjected to various degumming methods and stained with Coomassie blue. Lanes 1 and 11: high molecular weight protein standard: Lanes 2 and 12: standard; Lane 3: Catholyte (5 days); Lane 4: Orvus paste (90°C); Lane 5: Distilled water and *Eucalyptus* oil (10 days); Lane 6: Vermicompost (8 days); Lane 7: Orvus paste and *Eucalyptus* oil (10 days); Lane 8: Vermicompost (10 days); Lane 9: Distilled water (10 days); lane 13: Distilled water (6 days); Lane 14: Catholyte and *Eucalyptus* oil (9 days); Lane 15: Catholyte and *Eucalyptus* oil (8 days); Lane 16: Orvus paste (6 days); Lane 17: Orvus paste (10 days); Lane 18: Orvus paste and *Eucalyptus* oil (5 days); Lane 19: Catholyte (9 days).

The fibroin as visualized by SDS-PAGE and which was in accordance with the findings by Wray *et al*. (2011). Lanes 3 and 19 represent the catholyte degumming method of the silk fibres. The cocoons were exposed for 5 and 9 days, respectively. The presence of smeared patterns in lane 3 suggested some degradation, whereas the total smear in lane 19 represent definite degradation as degumming time (days) increased (Teh *at al*., 2010).

Orvus paste (control) is represented in lanes 16 and 17 (Figure 4.24B). The cocoons were exposed for 6 and 10 days, respectively. SDS-PAGE showed both these samples had visible bands around 25 kDa, 20 kDa, 15 kDa and even as low as 16 kDa. The ~25 kDa band, which is probably the L-chain (Sah & Pramanik, 2011), appears regardless of the absence of reducing agent, suggesting that the L-chains were separated from the H-chains during degumming.

Lanes 6 and 8 represent the vermicompost method, with an exposure of 8 and 10 days, respectively (Figure 4.24A). These two lanes showed heavily smeared patterns, with visible bands at 25 kDa and again at 15 kDa and 6 kDa. According to the literature (Tao *et al.,* 2007; Yamada *et al.,* 2001) wild silk fibres showed heavily smeared bonds on SDS-PAGE. This again showed that increased degumming time resulted in a decrease in the silk fibroin average molecular weight. This was evident by the migration of the smear further down the gel.

The combination distilled water and *Eucalyptus* oil is represented in lane 5. The exposure time for this sample was 10 days. A light smear turned into a heavier smear further down the gel. Clear bands were visible at 25 kDa, 15 kDa and 6 kDa (Figure 4.24 A).

Orvus paste and *Eucalyptus* oil were applied to samples represented in lanes 7 and 18. The exposure times were 10 and 5 days, respectively. The 5 days exposure in lane 18 showed clear bands at 25 kDa, 20 kDa, 15 kDa and 6 kDa (Figure 4.24 B). More smeared patterns were evident on day 10 of exposure (Figure 4.24 A).

In lane 9 (Figure 4.24A) and lane 13 (Figure 4.24B), samples were degummed using distilled water for an exposure time of 10 and 6 days, respectively. Exposure time of 6 days clearly resulted in a decrease in the silk fibroin's average molecular weight as evident by migration of the smear down the gel. With an exposure of 10 days, degradation was suggested, as clearly evident from the dark smeared pattern in lane 9.

Lanes 14 and 15 represent the combination degumming method – catholyte and *Eucalyptus* oil (Figure 4.24B). The exposure times were 9 and 8 days, respectively. Nine days suggested degradation with an increase of degumming time. Visible bands formed at 25 kDa, 20 kDa, 15 kDa and 6 kDa, indicating that the silk

fibroin protein was being degraded into lower molecular weight fragments (Yamada *et al.,* 2001), again suggesting that the L-chains were separated from the H-chains during degumming.

The presence of broad bands below the 6 kDa mark in lanes 3–9 possibly indicated that the dialysis membrane could retain some short chains during the short dialysis time. The smear might be degradation products of H-chains (350 kDa) obtained due to cleavage of amide bonds of raw silk protein formed by degumming and dissolution, while the band at 25 kDa corresponded to the L-chain of raw silk protein (Zhang *et al.*, 2012; Wray *et al.*, 2011).

The 25 kDa band appeared with all the degumming methods, regardless of the absence of a reducing agent. This suggested that the L-chains were separated from the H-chains during degumming (Rajkhowa *et al*., 2009). A portion of covalent bonds, besides the secondary bonds between silk fibroin molecules such as hydrogen bonds and hydrophobic bonds, are obviously destroyed during the process of degumming *G. postica* silk fibres under different conditions and dissolving them by lithium thiocyanate solution. The dissolution process caused the silk fibroin to degrade to some extent (Tao *et al.*, 2007). The results further indicated that the lower molecular weight fragments were still intact, which means that the fibroin protein was not fully degraded.

Results indicated that SDS-PAGE can be successfully applied to indicate the different influences that the environmentally friendly degumming methods had on the silk fibres.

4.4 Microbial analysis and identification of silk fibres after different degumming methods

A few bacterial species were found in the degumming solutions after 10 days. Evaluation of the degumming solutions for each method resulted in four bacterial species being identified with *Bacillus mycoides* isolated most frequently (Table 4.6).

Table 4.6: Different micro-organisms identified in degumming solutions.

Degumming Method	**Organism**	**BIOLOG Probability**
Orvus paste	*Bacillus mycoides*	100
Orvus paste and *Eucalyptus* oil	*Pseudomonas syringae* pv *tagetis*	100
Catholyte	*Bacillus mycoides*	100
Catholyte and *Eucalyptus* oil	*Enterobacter sakazakii*	100
Distilled water	*Bacillus mycoides*	100
Distilled water and *Eucalyptus* oil	*Bacillus mycoides*	100
Vermicompost	*Bacillus cereus/thuringiensis* C *Bacillus mycoides*	97 100

Bacillus mycoides has large cells of ≥3 μm. Other characteristics include chains of cells, formation of acid from glucose, hydrolysis of starch and being non-motile (Di Franco *et al.,* 2002).

Bacillus mycoides are found in soil and common pesticides, and have no negative effects on humans or the environment.

Pseudomonas syringae pv ***tagetis*** (PST) is a phytopathogenic bacterium. It is rod-shaped, Gram-negative, with an aerobic metabolism and polar flagella. It is a plant pathogen (Zhang *et al.,* 2007) and causes bacterial leaf spot, which are circular necrotic lesions on leaves and petioles, with dark purple margins. ***Pseudomonas syringae*** pv ***tagetis*** can also cause apical chlorosis on sunflowers and sunflower seeds (Horst, 2008).

Enterobacter sakazakii *is* an opportunistic pathogen that has been associated with sporadic cases and outbreaks causing meningitis, necrotizing enterocolitis and sepsis especially in neonates. It is a bacterium that was recently reclassified into eight distinct taxa of a new genus *Cronobacter* (Iversen *et al.,* 2008). It is Gram-negative, motile, rod-shaped, and non-spore-forming and will grow in aerobic and anaerobic conditions. It is considered an opportunistic pathogen and dry infant formula serves as the mode of transmission (Al-Holy *et al.,* 2010). Enterotoxin-like compounds are produced by some strains (Beuchat *et al.,* 2009). It measures 3 by 1 μm, is highly flagellated and can produce a protective biofilm. It produces a yellow culture. The temperature range for growth of this organism is 5.5–45°C, with an optimum growth temperature of 39.4°C (Kandhai *et*

al., 2006). It has been recovered from cerebrospinal fluid, blood, sputum, throat, nose, stool, gut, wounds, bone marrow, eye, ear, stomach aspirates, anal swabs, breast abscess, flies, rodents (Mramba *et al.,* 2006), water, dust, soil, plant materials, mud and vacuum cleaners (Healy *et al.,* 2010).

Bacillus cereus/thuringiensis **(Bt)** is a Gram-positive, soil-dwelling, spore-forming, rod-shaped bacterium, being 5 μm in length and 1 μm in width. It grows at 37°C and produces a diamond-shaped crystal form from its crystal proteins (Cry proteins). *Bacillus thuringiensis* (Bt) is highly specific and effective against target pests and demonstrates the potential to be successfully produced by continuous production technology. It is, therefore, the most common environmentally-friendly insecticide used and is the basis of over 90% of the pesticides available in the market today (Cherif *et al.,* 2007). *Bacillus thuringiensis* (Bt) pesticide S-layer (where the Cry protein and toxins lie) has no known negative effects on humans, vertebrates, or plants (Bravo *et al.,* 2007).

From this information, it was clear that the most probable source of these four bacteria was the soil and/or plant material surrounding the silk worm cocoons before harvesting.

Chapter 5

General conclusions and recommendations

5.1 General conclusion

The aim of this study was to evaluate environmentally conscious degumming methods that could discriminate between sericin and fibroin, without harming the fibroin and compare that against standard Orvus paste method.

The efficiency of catholyte, vermicompost and distilled water as alternatives to chemical Orvus paste as degumming method for *Gonometa postica* wild silk was compared over 10 days of incubation at a constant temperature of 32°C.

Degumming is the key process during which sericin is removed by thermo-chemical treatment of the cocoon. Since degumming imposes a relatively harsh environment on the silk fibroin, the possibility of changes occurring in fibre microstructure and mechanical properties, or even fibroin degradation, must be considered (Jiang *et al.,* 2006).

The following conclusions were made based upon the objectives set for this study and the results obtained:

The first objective was to calculate the degumming efficiency of the chemical versus the biological degumming methods, on the degumming weight loss of *G. postica* cocoons. In this study, the degumming weight loss of the silk fibres ranged from 7% to 41% and is inconsistent with previous reports that the sericin content of wild

silk fibres is less than the mulberry silk and is as low as 12 – 16% (Prasong *et al.*, 2009).

The second objective included the use of scanning electron microscope (SEM) images. After the 5 days of exposure to the different degumming methods, all the methods still showed residual sericin on the SEM micrographs. After an exposure of 10 days the results looked different. Fibres degummed with catholyte, the combination Orvus paste and *Eucalyptus* oil, Orvus paste, vermicompost and the combination catholyte and *Eucalyptus* oil all showed cleaned and smooth surfaces. On the remaining micrographs, small amounts of residual sericin were still present. This indicated that distilled water on its own would take more than 10 days to reach 100% degummed status. After the degumming was complete the fibres looked shiny and had a rich straw colour.

The third objective was to determine the effect of degumming of *G. postica* cocoons with chemical and biological degumming methods on the tensile strength of the silk fibres. Silk features exceptional mechanical properties such as high tensile strength and great extensibility, making it one of the toughest materials known. The exceptional strength of silkworm silks, exceeding that of steel, arises from β-sheet nano-crystals consisting of highly conserved poly-(Gly-Ala) and poly-Ala domains (Keten *et al.*, 2010). Hydrogen bonding is readily dissociated when the fibres are swollen in water.

The results indicated that the difference between the different methods when compared with the control (Orvus paste) did differ significantly ($p<0.001$), indicating that the fibres were much stronger when degummed with the environmentally friendly methods than with the Orvus paste method. Therefore, it can be concluded the fibre mechanical properties, when applying the different degumming methods, decrease in the order: Orvus paste and *Eucalyptus* oil > vermicompost > distilled water > catholyte > distilled water and *Eucalyptus* oil > Orvus paste.

The fourth objective of the study was to determine the effect of the chemical and biological degumming methods on the fibroin degradation in the cocoons of *G. postica.* Results from the different methods showed that with all the methods used, there was some or other molecular chain rupture, indicated by the smearing bands on the SDS-PAGE gels. The presence of the 25 kDa band further suggested that the light chains were separated from the heavy chains during degumming. The presence of broad bands below the 6 kDa mark could indicate that the dialysis membrane could retain some short chains during the short dialysis time.

Disposal of industrial sludge is a serious problem. If vermicomposting is adopted, a waste product is converted into a value-added product and the disposal of an industrial pollutant in open dumps and sanitary landfills can be reduced (Sangwan *et al*.

2010). The organic recycling company, Turfnet, sees huge potential for vermicompost in South Africa (Farthing, 2009). This is organic compost that is believed to be the cleanest form of organics in the world. The recycling of green matter back into soil is a huge part of preventing global warming. One ton of vermicompost was already sold for R 2 000 in 2009; however, one handful of vermicompost can be spread over a large area because of the high nutrient content (Farthing, 2009).

A combination of the degumming and the vermicomposting can bring about a full circle in any farming community. It can mean saving of water and electricity and a lot of job opportunities for communities.

The fifth objective was to do the microbial analysis and identification of the silk fibres degummed with the different methods. The presence of the *Bacillus* and *Pseudomonas* species in the degumming solution might have an influence on the tensile strain at the maximum load (%). This correlates with results of Szostak-Kotowa (2004) who found extensive degrading of silk in the presence of *Bacillus, Pseudomonas, Serratia* and *Streptomyces* species.

Taking everything into consideration it can be concluded that the methods used make it possible to produce good quality silk fibres even from the cocoons of *G. postica,* using environmentally friendly methods.

5.2 Recommendation

Further work should include the demineralizing (Gheysens *et al.*, 2011) 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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ABSTRACT

The trend in the textile industry is at present towards eco-friendly processes and minimising the adverse ecological effects of production. Silk degumming is a high resource-consuming process, as far as water and energy are concerned. It further is ecologically questionable, because of the high environmental impact of effluents. The development of an effective degumming process would mean saving water and energy, recovery of valuable by-products such as sericin peptides, and lower environmental impact of effluents. The aim of this study was to develop and evaluate environmentally conscious degumming methods that could discriminate between sericin and fibroin, without harming the fibroin.

The methods used and evaluated as environmentally conscious methods were tested against Orvus paste and all the samples were exposed to the method at a constant temperature of 32°C and a period of 10 days.

Results indicated that an increased degumming time resulted in a decrease in the silk fibroin average molecular weight. A clear band at 25 kDa appeared with all the methods, indicating that the light chains were separated from the heavy chains during degumming.

The degumming weight loss ranged from 27 to 41% over a time period of 10 days. Two of the methods namely the combination Orvus paste and *Eucalyptus* oil and catholyte were more efficient than Orvus paste.

The SEM micrographs showed no fibrillations. This indicated further that the degumming methods were successful; the sericin was removed without damage done to the surfaces of the fibres.

The maximum load and displacement means of the fibres differed with the different degumming methods applied. The strongest fibres were obtained from the control method. The weakest fibres came from the Vermicompost method.

The results demonstrated that the environmentally conscious methods allow efficient, low cost degumming of sericin.

OPSOMMING

Die tendense op die oomblik in die tekstielindustrie is ‘n beweging na ekologies vriendelike prossesse en om die onvriendelike ekologiese effekte van die produksie te minimaliseer. Die ontgomming van sy, behels ‘n hoë verbruik van hulpbronne, veral wat betref die verbruik van water en energie. Dit word verder ekologies bevraagteken as gevolg van die hoë impak van die afvalwater. Die ontwikkeling van ‘n effektiewe ontgommingsproses sal die spaar van water en energie beteken, die herwinning van kosbare byprodukte soos serisien peptiede en die verlaging in die ekologiese impak van die afvalwater.

Die doelwit van die studie was om ekologiese verantwoordelike ontgommingsmetodes te ontwikkel en te evalueer, wat tussen serisien en fibroin kan diskrimineer, sonder om die fibroin te beskadig.

Die metodes wat gebruik en geëvalueer is as ekologiese verantwoordelike metodes, is teen *Orvus paste* (kontrole) getoets en al die monsters is blootgestel aan ‘n konstante temperatuur van 32°C vir ‘n periode van 10 dae.

Resultate het aangedui dat ‘n toename in die ontgommingstyd, ‘n afname in die sy fibroin se gemiddelde molekulêre gewig tot gevolg gehad het. ‘n Duidelike band verskyn by 25 kDa vir al die metodes, wat aandui dat die ligte kettings geskei het van die swaarder kettings gedurende die ontgommingsproses.

Die ontgommingsgewigsverlies lê tussen 27 en 41% oor ‘n tydperk van 10 dae. Twee van die metodes, naamlik die kombinasie *Orvus paste* en *Eucalyptus* olie en katolyte was meer effektief as *Orvus paste.*

Die SEM mikrofoto’s het geen fibrillasies aangetoon nie. Dit het verder op die sukses van die ontgommingsmetodes gedui; die serisien was verwyder sonder enige skade aan die veseloppervlaktes.

Die maksimum gewig en verplasing gemiddeldes verskil met die toepassing van verskillende ontgommingsmetodes. Die sterkste vesels was verkry met die kontrole metode. Die swakste vesels kom van die Vermikompos metode.

Die resultate demonstreer dat die ekologiese verantwoordelik metodes, effektiewe, lae loste ontgomming van serisien tot gevolg gehad het.