DECLARATION AND APPROVAL

This work is my original work and has not been presented for an award of degree in any other university.

Name: Addis Teshome Kebede **Registration No.: I80/80201/11**

Signature: _____ **Date: _____**

APPROVAL BY SUPERVISORS

This thesis has been submitted to the University of Nairobi with our approval as supervisors.

Dr. Jacques M. Kabaru
University of Nairobi, School of Biological Sciences,
P. O. Box: 30197-00100, Nairobi, Kenya

Signature _____ Date _____

Dr. John M. Onyari
University of Nairobi, Department of Chemistry,
P. O. Box: 30197-00100, Nairobi, Kenya

Signature _____ Date _____

Prof. Suresh K. Raina
icipe- African Insect Science for Food and Health,
Commercial Insects Programme,
P.O. Box: 30772-00100, Nairobi, Kenya

Signature _____ Date _____

Professor Fritz Vollrath
University of Oxford, Department of Zoology,
South Parks Road, Oxford OX1 3PS, UK.

Signature _____ Date _____

DEDICATOIN

TO

MY BELOVED FAMILY AND FRIENDS

ACKNOWLEDGENTS

I wish to acknowledge and express my gratitude to several people and institutions that assisted and encouraged me in one or another way throughout this study. I could not have completed this study without them.

My sincere appreciation goes to Drs. J. M. Kabaru and J. M. Onyari, both from University of Nairobi, for accepting to serve as my university supervisors and for their great input, critical criticism, supply of literature and numerous valuable suggestions that have shaped this thesis and the publications so far. They tirelessly and in the shortest time possible reviewed, corrected and brought in critical decisions into the drafts of this thesis.

It is my pleasure to thank Professor Suresh K. Raina, who went well beyond the call of duty as ICIPE (International Centre of Insect Physiology and Ecology) supervisor for giving me a chance at the Commercial Insects Program, for his endless optimism and being cheerful and encouraging throughout it all. His constant moral support, guidance and encouragement are highly appreciated.

Many thanks to Professor Fritz Vollrath, from University of Oxford, and the Oxford Silk Research Group members for providing me with excellent research facilities and patience in familiarizing me with laboratory equipments and analyses software. All my supervisors contributed a lot through their valuable comments and expert suggestions throughout the research work and the preparation of this thesis.

I wish to express my profound gratitude to all colleagues at the Commercial Insect Program for their support and friendship. I also would like to thank my fellow scholars and staff of ICIPE, who were always helping and encouraging me in social interactions and research undertakings. Conversations and mutual support from them not only stimulated new insights, but also provided necessary encouragement along the way. The time I spent with them will be precious and unforgettable.

Many thanks are due to German Academic Exchange Service (DAAD) for providing the scholarship and to International Fund for Agricultural Development (IFAD) for funding the research project. Thanks are also due to ICIPE and the African Regional Postgraduate

Program in Insect Science (ARPPIS) for providing logistical and research support. Many thanks are also to Capacity Building and Institutional Development (CBID) staff especially Mrs Lillian Igweta, Lisa Omondi and Margaret Ochenda for their constant support.

Finally, I want to appreciate my family and friends for their care, unfailing encouragement, and loving support with their priceless prayers, tireless assistance and encouraging words during my whole life. It is not possible to thank everybody, but I want to thank all people who contributed to this thesis through their support and invaluable friendship. Above all, this thesis will not be complete without giving thanks and adoration to the Almighty God for his divine guidance throughout my life.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
DNA	deoxyribonucleic Acid
FAO	United Nations Food and Agriculture Organization
FTIR	Fourier Transform Infrared Spectroscopy
GLM	Generalized Linear Model
H-chain	Heavy chain fibroin
HPLC	High Performance Liquid Chromatography
ICIPE	International Center of Insect Physiology and Ecology
L-chain	Light chain fibroin
LSD	Least Significant Difference
RH	Relative Humidity
rpm	rotations per minute
SEM	Scanning Electron Microscope
TGA	Thermogravimetric Analysis
UVB	Ultraviolet B
WHO	World Health Organization

ABSTRACT

Wild silk production is a unique eco-friendly agro-practice with enormous potential for income generation and additional benefit of compatibility with conservation goals. African wild silkmoths occur in three major families; *Saturniidae, Lasiocampidae* and *Thaumetopoeidae* and have significant economic importance mainly due to their exceptional quality fibers and aesthetic values. Silk cocoon shells from these insects are non-woven structures majorly composed of two proteins, fibroin and sericin, and depending on their origin, exhibit extensive variation in structure, property and composition directly influencing their ecological adaptive functions. Hence, this study was aimed at investigating the extent of variability in structure, composition and properties of silk cocoon shells and fibers from the major African wild silkmoth species: *Gonometa postica, Anaphe panda, Argema mimosae* and *Epiphora bauhiniae*. Scanning Electron Microscope (SEM) was used to study the surface and cross sections of the cocoon shells and degummed fibers. The components on cocoon shell surfaces were determined by Fourier Transform Infrared spectroscopy (FTIR). The composition of major fibroin amino acids was established with High Pressure Liquid Chromatography (HPLC). Moisture regain and weight loss of fibers and cocoons were determined by oven dry method. The dissolution properties were studied using 9M aqueous solutions of Cacium chloride ($CaCl_2$), Lithium bromide (LiBr) and Sodium thiocynate (NaSCN). Mechanical properties were measured with Instron tensile testing machine. Thermal decomposition behaviours were determined using Thermogravimetric Analyzer (TGA) at temperature range of 25 - 800 and 25 - 900°C for fibers and cocoon shells, respectively.

SEM micrographs showed that *G. postica* and *A. panda* cocoons had thorny spines and hairs on their surface, while *A. mimosae* cocoons were highly perforated. The FTIR spectra peaks around 1312 and 712cm^{-1} for outer surfaces revealed the presence of calcium oxalate crystals on *G. postica* cocoons. Cocoon surfaces also showed great cross bindings, wrinkles and networking of twisting filaments in different shapes and forms conferring rough outer surfaces. Fiber surfaces were irregular with several fibriliar sub-structures running along the fiber axis in *A. mimosae* and *E. bauhiniae*. The cross sectional view of degummed fibers revealed elongated, rectangular, triangular, globular and wedge shaped fibers with variable number and size of voids. HPLC results showed that African silk fibroins were characterized by the presence of high proportions of the three amino acids, glycine, alanine, and serine. These amino acids represented 71-74 and 82% of the total amino acids present in wild and *Bombyx mori* silk fibroins, respectively. No significant difference ($P < 0.05$) was observed among the moisture regain of the wild silk degummed fibers. However, there were significant differences ($P > 0.05$) in weight loss and moisture regain among cocoons as well as shell layers. *E. bauhiniae* cocoons had the lowest weight loss and moisture regain of 23.2 and 5.6%, respectively while *G. postica* and *A. mimosae* had the highest weight loss and moisture regain, 56.8 and 9.1%, respectively. In both *A. panda* and *E. bauhiniae*, the outer cocoon layers had the lowest moisture regain and highest weight loss while the inner layer of *E. bauhiniae* and middle layer of *A. panda* lost the least weight. African wild silk fibers and cocoons also showed significant variability ($P > 0.05$) in their dissolution behaviours. Degummed fibers were more readily soluble than the cocoon shells. *B. mori* had higher solubility than the wild silk cocoon shells (51.5%) and fibers (59.3%). Among the wild

species, *G. postica* cocoons and degummed fibers had the highest solubility (37.3 and 51.7%, respectively). LiBr was the most effective dissolving agent for both the cocoons and fibers (41.2 and 84.5%, respectively).

The tensile test measurements showed that *G. postica* and *A. mimosae* cocoons had lower breaking stress and breaking energy and higher breaking strain than *B. mori*. *Gonometa postica* fibers had the highest breaking strain (41.3%), while *E. bauhiniae* had the lowest breaking stress (237MPa). Wild silk fibers had breaking energy ranging between 34.5-76.4J/cm^3. The TGA curves showed *A. mimomsae* fibers and cocoons had higher temperature for water loss (113 and 154°C, respectively). *G. postica* cocoons underwent multistep decomposition between 296-413, 483-506, and 710-730°C. *B. mori* had the highest total weight loss for degummed fibers (93.4%), while *E. bauhiniae* cocoons lost the highest total weight (97.2%). The overall results demonstrated the extensive variability present in structure, composition and properties among silks of the four African wild silkmoths. This could be associated with the selection pressure for environmental adaptation experienced by each species and the difference in molecular organization and composition of silk spun by each species. The physical structure and chemical composition of the cocoon shells and fibers have contributed towards the variations observed in the properties and these features have commercial and industrial implications. Hence, the value of silks when compared across taxa should be based on suitability of the fiber properties for specific application as well as potential silk products.

CHAPTER ONE

GENERAL INTRODUCTION

1.1. Background information

Silks are fibrous proteins containing repetitive sequences of amino acids that are stored as a liquid but configure into solid filaments when spun upon secretion (Craig, 1997). Silks, from domesticated and wild silkworms and spiders, represent a unique and important class of polymeric composite materials in nature providing a wide range of evolutionary and ecological functions with an optimum microstructure and properties. Owing to its high qualities of strength, elasticity, softness, absorbency and affinity for dyes, silk has been used for over 5000 years and is still considered a unique textile material in the world (Li *et al.,* 2006). Silk, specifically from *Bombyx mori* L., is a premium priced textile commodity, although its sheer volume is less than 1 percent of the market for natural textile fibers. In addition to applications in textile, silk fibers have gained a great deal of attention because of their potential use as multifunctional materials in various fields, such as biotechnological, biomedical, tissue engineering, cosmetics, ink additives, resin composites and paints (Wang *et al.,* 2007; Kim *et al.,* 2008; Fang *et al.,* 2009; Nagai *et al.,* 2009). Many indigenous wild silkmoth species around the world have also been utilized locally and regionally for many years.

Wild silkmoths, sometimes referred to as non-mulberry silkmoths, generally defined as those that are not reared in captivity but present in the wild on their natural food plants. Craig

(2008) described "wild" silk as any type of silk other than that spun by *B. mori*, and it is produced all over the world by different species of silkmoths across wide range of ecologies. African wild silkmoths are known to be found distributed in most regions of Africa and occur mainly in three major families, Saturniidae (*Ephiphora* and *Argema* species), Lasiocampidae (*Gonometa* species) and Thaumetopoeidae (*Anaphe, Epanaphe* and *Hyposoides* species) with significant economic importance due to their exceptional quality fibers and aesthetic values (Kioko, 1998; Delport, 2006). The potential of these African indigenous species for wild silk production has been documented in Nigeria (Ashiru, 1988), Botswana (Hartland-rowe, 1992), Zimbabwe (Chikwenhere, 1992), Uganda (Gowdey, 1953), South Africa (Veldtman, 2005), Kenya (Raina, 2004), and Madagascar (Peigler, 1993; Razafimanantosoa *et al*., 2006) among others. With the rich diversity of wild silkmoths recorded in Africa, wild silk farming has the potential to provide economic incentives for communities (Raina *et al*., 2009). For example, Mbahin (2008) reported processing of *Anaphe panda* silk cocoons increased the net income of farmers twenty four times. Wild Lepidoptera silks also provide a tool for sustainable development and conservation of biodiversity (Akai, 2000; Thangavelu, 2002). Wild silks have advantage over mulberry silk in that they generally come in natural colours and are not only user friendly but also healthy owing to their porous texture and thermal properties (Arunkumar *et al.,* 2006). However, despite rich biodiversity and suitable environmental, social and economic conditions, silks from the African wild silkmoths have largely escaped the notice of the modern commercial and scientific world until recently.

Silk cocoon shells from silkmoths are non-woven structures and exhibit extensive variation in composition, structure, and properties, which impose direct influence on their adaptive functions. The urge to characterize and understand silks has been driven by variability in their exceptionally useful properties such as lightweight, high modulus, high strength, high elongation and energy to break and thermal stability depending on their origin (Kaplan *et al.,* 1991; Amiraliyan *et al.,* 2009). These properties, however, are influenced by the individual silk fiber components, hierarchical structure, spinning conditions and the degumming process (Chen *et al*., 2001; Shao and Vollrath, 2002; Pe´rez-Rigueiro *et al.,* 2002; Zhang *et al.,* 2010a). The amino acid composition of silk produced by arthropods is also highly variable and is known to influence physical properties of the fibers (Lucas *et al*., 1958). The protein composition, together with spinning speed and the carving of the fiber's surfaces when it passes through the larval spinnerets, result in different biomechanical and surface properties of the silk fibers.

Ecological adaptations also play a significant role in effectively modifying the structure and properties of the silk fibers and construction of variable cocoons. Specifically, absorbed moisture and temperature are likely to determine the mechanical and physical properties of both the sericin 'gum' coating and the integral structural proteins (fibroin) of the silks (Fut *et al.,* 2009). The importance of cocoon shells in temperature regulation, water loss reduction from the pupae (Rosner and Führer, 1996) and acquisition of heat (Danks, 2004) has been reported. The thermal tolerance of organisms also plays significant role as performance in nature mainly depends on native adaptability to varied environmental conditions (Manjunatha

et al., 2010). Thus, proper understanding of such natural polymer materials is an indispensable step toward clarifying their structure- property- function relationships.

1.2. Statement of the problem

Silk cocoon characteristics of many silk producing insects vary widely in size, structure, properties, as well as in other features such as orientation, attachment to the substrate and amino acid composition of the individual components. In some species, these properties also vary with season and location. Most of the research works in this regard has been focused on spiders and mulberry silkworms in an attempt to comprehend and improve their properties, and hence broaden their application. So far, there is little comprehensive information available on the diversity of different structures and properties of the silks produced by African wild silkmoths. Only data about their biology, host plant distribution and ecology is available. Hence, detailed study of their fiber and cocoon shell structures and properties is required to gain a complete view of their nature and design their wider application.

Although the African wild silks have many important advantages over the Asiatic wild silks, it has not also been possible hitherto to introduce it in substantial quantity to the textile or any other industry. One possible reason for this might be limited data on their properties and the underlying rationale for the variations, if any. There has also been limited instructive study on the structure and properties of the silk fibers that are grown and produced in a wide range of environmental conditions to establish whether the correlations have practical validity. As the

micro and macrostructure of cocoon shells and fibers affect properties of the silk such as strength, stiffness and resilience, the relation between such mechanical properties and the protein components has not yet been established for African wild silk fibers.

1.3. Justification

With the current increased quest for development of excellent biomaterials and other biological organic products, silk appeared as one of the best candidates. The demand for silk is constantly increasing in the world market and this provides excellent opportunities for any producer country to diversify and optimize any source of production. The ever growing evidence on the existence of diverse properties of silks from various silk producing arthropods instigated the search for new silk sources and design ways to exploit them effectively in the desired fields of application. There is also an urgent need to accumulate information on silk structure and properties in relation to product requirements. The African wild silkmoths are among such insects known to produce silk of economic importance and with enormous potential to be exploited. Therefore, understanding of the different properties of these silks (and silkworms and host plants) allows innovative conservation and poverty alleviation programs thereof to better leverage their success.

A complete comprehension of the diversity of silk fibers from African wild silkmoths and their structure and properties is also required to develop management plans for the conservation and sustainable utilization. This comes about by disseminating information to

the many potential users, many of whom do not as yet even know the importance of the silk, especially the African wild silks. This is also a key factor for the successful application of silk fibers in various fields and helps in selection of good quality cocoons for silkworm breeding and processing programmes. This information will also be useful to select species of wild silkmoths that could have an optimum economic and conservation effect with fibers that exhibit tailored combinations of properties under specified environments. The knowledge on the structures and properties of cocoon shells and fibers will give confirmation and develop quality standards that could be of interest for the design and tailoring of advanced silk materials. This will give silk producers, processors and traders additional confidence to effectively compete in the special silk markets. The study is also important to highlight the potential of Africa to introduce completely new forms of wild silk fibers to the world and exploration of new opportunities of how they can be utilized in the future. More importantly, this study serves as a comprehensive academic and research reference regarding silks produced by African wild silkmoths.

1.4. Hypotheses

1. The surface structure and cross sectional shape and size of the African wild silkmoths' cocoon shells and fibers do not show variability.

2. African wild silkmoths produce silk cocoon shells and fibers that do not exihibit variability with regard to their amino acid composition, solubility, weight loss and moisture regain properties.

3. There is no variation in mechanical (tensile) and thermal degradation properties of degummed silk fibers and cocoon shells from the African wild silkmoths.

1.5. Objectives

1.5.1. General objective

To determine the extent of variability in structure, composition and properties of African wild silk cocoon shells and fibers from three major Lepidopteran (*Saturniidae, Lasiocampidae* and *Thaumetopoeidae*) silkmoths.

1.5.2. Specific objectives

1. To study the surface structures and cross sectional properties of cocoon shells and degummed fibers from African wild silkmoths
2. To determine the amino acid composition of fibroins from African wild silk fibers
3. To determine the sericin content and moisture regain properties of African wild silk cocoon shells, degummed fibers and cocoon shell layers
4. To study the dissolution properties of African wild silk cocoon shells and degummed fibers in different solvent systems
5. To determine the tensile properties (stress-strain responses) and thermal degradation behaviour of African wild silk cocoon shells and degummed fibers

CHAPTER TWO

LITERATURE REVIEW

2.1. Seri-biodiversity

Seri-biodiversity refers to the variability in sericigenous (silk producing) insects and their host plants, which are economically and ecologically important biodiversity and largely, forest based (Srivastava and Thangavelu, 2005). The word silk is most often associated with the filaments produced by the domesticated silkworm *Bombyx mori*, but the fact is, a number of classical animals such as lepidoptera, hymenoptera, diptera, neuroptera, acarids and araneids have evolved to produce a variety of silks with different properties and for varying purposes (Craig, 1997; Hardy and Scheibel, 2009; Vollrath and Porter, 2006). The most important functions of silk include web construction, shelter building, leaf tying, construction of pupal cocoons, and as a safety line when dislodged from a substrate (Common, 1990).

Craig (2008) described “wild” silk as any type of silk other than that spun by domesticated silkworm, *B. mori* and it is produced all over the world by different species of silkmoths across wide range of ecologies. Across the arthropods, silk displays a diversity of material properties and chemical constituents and is produced from glands with different evolutionary origins (Craig, 1997). There are several domesticated and wild sericigenous insects and their host plants, which are abundant in different regions and parts of the world. However, only a few types of insects are commercially exploited and there remains a great scope for producing novel silk from other lesser known species (Mbahin, 2008).

2.1.1 Silkworms

In total, there must be well over 200,000 different silks with either major or minor sequence differences (Craig, 1997). Though accurate data are not available on the silkworm germplasm in different countries of the world, approximate information indicate that there are 4310 silkworm germplasm accessions available in different countries (FAO, 2003). There are around 3000 genotypes of *B. mori* at the global level, which includes mutants, parthenoclones, polyploids and geographical races (Nagaraju *et al.,* 2001).

There are 400-500 species of silk-producing moths in the world, but only nine species sucha as *Bombyx mori, Philosomia ricini, Antheraea mylitta* and *Anthereae assama* are commercially cultivated (Dingle *et al.,* 2005). Jolly *et al.* (1975) reported about 80 wild silk species occurring in Asia and Africa to produce silk of economic value. In Kenya, Uganda and Tanzania, 58 wild silk moth species were found to occur in Saturnidae (19 species in six Genera), Lasiocampidae (33 species in 17 Genera) and Thaumetopoeidae (six species in one genus) Families (Kioko, 1998). Nassig *et al*. (1996) reported that the Family Saturniidae comprises of about 1200-1500 species all over the world making it the largest Family of Bombycoidea containing about 1861 species in 162 Genera and 9 subfamilies (Lemaire and Minet, 1998). They include some of the largest and most spectacular species of all Lepidoptera which are univoltine to multivoltine depending upon the climatic conditions and are distributed in both temperate and tropical region (Regier *et al.,* 2008). Kakati and Chutia (2009) recorded fourteen species of sericigenous insects belonging to eight Genera from the

state of Nagaland, India, comprising species of *Antheraea, Actias, Samia* and *Loepa*. Silkworms produce cocoon shells from silk-protein-based fibers as a means of protection during their metamorphosis into moths. The cocoon is firm to resist mechanical damage, durable to withstand harsh weather conditions, non-palatable to resist attacks by animals, and immune to microbial degradation.

2.1.2. Silk from spiders

Currently, 40,700 spider species belonging to 3,733 Genera and 109 Families (Platnick, 2009) are known and more than 40,000 species use dragline silk for a variety of functions such as lifelines and the frames of webs (Blackledge *et al.*, 2009) and further, more than 130 different shapes of spider webs are known (Römer and Scheibel, 2008). Among the most studied webs are the so-called orb webs which consist of several different types of silk (Vollrath, 2000). Orb-weaving spiders (Araneidae) such as *Nephila clavipes* can produce up to seven different types of silk that originate from different abdominal glands (Lewis, 2006) to capture prey, protect their offspring/prey and as lifelines to escape from predators (Römer and Scheibel, 2008; Hardy and Scheibel, 2009). A number of taxonomically diverse species of araneoid spiders adorn their orb-webs with conspicuous silk structures, called decorations or stabilimenta. The function of these decorations remains controversial and several explanations have been suggested including stabilizing and strengthening the web, hiding and concealing the spider from predators, preventing web damage by larger animals, such as birds, increasing foraging success and providing a sunshield (Herberstein *et al.*, 2000).

2.1.3. Other silk producing arthropods

The ability to produce silk proteins has evolved multiple times in the arthropods. The fibrous proteins, constituting the silk, are produced in different glands (colleterial, salivary, dermal glands, and Malpighian tubules) in both adult and larvae (Rudall and Kenchington, 1971). Weijers *et al.* (2008) reported the green lacewing (*Mallada signata*, Neuroptera) produces two distinct classes of silk, the larval lacewing cocoon silk and the adult lacewing egg-stalk silk. Caddis flies, *Limnephilus decipiens,* use silk rather sparsely for stitching portable larval cases from the pieces of plants, sand, or other foreign materials and larvae of *Hydropsyche angustipennis* produce large amounts of silk to spin food-collecting nets and retreat tunnels (Yonemura *et al.*, 2009). From an early larval stage, the caterpillars of *Galleria mellonella (*Pyralidae), which develop in bee colonies, spin silky tubes that protect them against the detection and killing by the bees. Larvae of the Mediterranean flour moth, *Ephestia kuehniella*, which typically attack ground cereal products, spend most of their life in silk tubes that probably provide some protection against parasitoids and reduce water loss (Fedič *et al*., 2003). Larvae of Siphonaptera (fleas) produce silk from the labial glands to construct nests, pupation cases and individual and group cocoons (Sehnal and Sutherland, 2008). The glowworm (*Arachnocampa luminosa:* Mycetophilidae) larvae spin silken nests on the roof of caves or overhangs and suspend silken snares from this nest to trap prey. Mayflies (Ephomeroptera) attach themselves to surfaces using suckers, claws and small silk filaments (Cave, 1998). *Theriodopteryx ephemeraeformis* (bagworms) has been reported to produce remarkably different ultrafine silk fibers (Reddy and Yang, 2010a).

Cocoon silks produced from the labial glands of Hymenoptera (sawflies, ants and bees) has also been reported (Sehnal and Sutherland, 2008). The larvae of social wasps and hornets (Hymenoptera:Vespinae) also spin a silk weave around their body before metamorphosing to a pupal stage (Spradbery, 1973). *Tetranychus urticae* is a ubiquitous phytophagous mite with abundant silk production continuously while walking for protection against biotic and abiotic agents and as sex pheromone (Clotuche *et al.*, 2009). The silk gland of last instar *Diatraea saccharalis* produces large amount of silk to protect the insect (Victoriano *et al*., 2007). Weaver ants, *Oecophylla*, use silk from larvae approaching metamorphosis to bind the leaves of their nest together and Psocoptera have two labial glands, one pair produces saliva and the other, silk (Chapman, 1998). Edgerly and Rooks (2004) documented the diversity of *Antipaluria urichi* (Embiidina :Clothodidae) spinning behaviour and use of silk, implications for maternal care, short and long term costs of spinning, and how the variable reliance on silk as a building material relates to colonial behaviour. In Neuroptera and few Coleoptera, a secretion from the malpighian tubules flows into the hindgut and is extruded from the anus to spin the cocoon (Rudall and Kenchington, 1971). Hard cocoons consisting of particles of earth, dung fibers or any other suitable material cemented together by a liquid that dries to a smooth, glistening, translucent substance on the inside of the cocoon were made by all species of Musciidae other than *Passeromyia* and *Philornis*, that form white froth, thick, soft coating around the puparium, usually without objects deliberately cemented into it (Ferrar, 1980). Scorpions, mygalomorphs and ctenizids use silk to line burrows and construct trip-lines (Vollrath and Selden, 2007).

Unusual silk occur in the cocoon of Chrysopid larvae, which are composed of a cuticulin like materials and of *Ptinus tactus* larvae which are formed from an unbroken chitaneous thread of peritrophic membrane extruded from the anus (Richards and Davies, 1977). Other filamentous proteins, such as the widely studied byssus of molluscus, have a silk like structure, but are not included in the definition of silk (Gellynck, 2006). Another silk or silk like filament is the biopolymer composed of at least ten proteins secreted by the salivary glands of the bloodworm *Chironnomous* (Case and Wieslander, 1992). Male myriapoda secrets fibrous proteins from their accessory glands to produce sperm webs, sperm stalks, and mating threads while the female Diplopoda and Symphyla use them for molting, egg cocoons, defence and communication (Craig, 2003).

2.2. Composition of silk

The cocoons of mulberry and non-mulberry silkworms contains two major proteins, a double strand of fibroin, which is held together by a gummy substance, sericin. Silk also contains several low molecular weight polypeptides, waxes and fats. The silk fiber has a triangular cross section and composed of two protein mono filaments (known as brins) lie with the flat sides of the triangles together, encircled by a sericin coating (Altman *et al.*, 2003). The brins are fibroin filaments made up of bundles of nano-fibrils, approximately 5nm in diameter, with a bundle diameter of around 100nm (Poza *et al.*, 2002). The fiber is smooth and transparent and has a rod-like shape, occasional swelling or irregularities along its length and with a diameter of 9–11µm (Li *et al.*, 2006).

2.2.1 Fibroin

Fibroin accounts for about 70-80% of the raw silk thread depending on the silkworm origin. The fibroin consist of three proteins: heavy chain fibroin (H-fib), light chain fibroin (L-fib) and P_{25} (fibrohexamerin protein) (Kojima *et al*., 2007). *B. mori* H-fib is a large protein (~390 kDa) and L-fib is lighter (25-30kDa) (Inoue *et al*., 2000). The H- chain consists of three domains: an N-terminal domain (contributing to the solubility of the H-Chain), a repeated region (contributing to the physical properties of fibroin fiber) and a C-terminal domain (containing cysteine residues that form intra- and inter-molecular disulfide bonds). P_{25} is a glycoprotein containing asparagin-linked oligosaccharide chains and forms a compact structure due to intra-molecular disulfide linkages but associates with the H-L complex by non-covalent interactions (Tanaka *et al.,* 1999). Its central role is maintaining the structure of elementary unit. However, these compositions might not always exist in all kinds of silk fibers. For example, silks of caddis flies contain H-fibroin and L-fibroin but not P_{25} component (Yonemura *et al*., 2009) while the specialized moth Family Saturniidae has lost both L-fibroin and P_{25} (Tanaka and Mizuno, 2001). The composition of amino acid residues in the fibroin is 45.9% Glycine, 30.3% Alanine, 12.1% sericin, 5.3% Tyrosine, 1.8 % Valine, and only 4.7% of the other amino acid residues (Zhou *et al.,* 2000). On the other hand, the L-chain is non-fibrous and contains relatively high amounts of leucine, isoleucine, valine, and acidic amino acids (Shimnra, 1983). The major constituent of fibroin repetitive region is a hexapeptide $(Gly\text{-}Ala\text{-}Gly\text{-}Ala\text{-}Gly\text{-}Ser)_n$ in *B. mori* while in wild silk and *Galleria*

mellonella the repetitive region is poly-alanine and has higher amino acids with long side chains than *B. mori* silk (Fedicˇ *et al.*, 2003).

2.2.2. Sericin

Sericin is sticky protein, which glues together fibroin threads and constitutes about 25–30% of silk protein and 20 – 30% of the cocoon weight (Zhang, 2002). The greatest sericin content is present in the outer layer of cocoon whereas the least sericin proportion is present in the innermost layer (Mondal *et al*., 2007a). Sericin is a mixture of proteins with a large number of amino acids containing hydroxyl groups and is therefore more soluble in water. It majorly contains serine (32%) and large proportion of several polar amino acids that confers it with high hydrophilicity and adhesion (Gamo *et al.,* 1977; Padamawar and Pawar, 2004). The sericin amino acids can be categorized into three types: neutral (glycine, alanine, leucine, isolucine, proline phenylalanine, tryptphan, cystine, valine, serine, theonine and tyrosine), alkaline (arginine, histidine and lysine) and acidic (Methionine, aspartic acid, and glutamic acid) (Datta and Nanavaty, 2007). Komatsu (1975) and Robson (1985) reported sericin might be separated into sericin I, II, III and IV depending on their hot water solubility. Sericin I is more soluble than the others and sericin IV being harder to dissolve. The wild silk sericin is relatively insoluble compared to *B. mori*, mainly due to chemical interaction between silk sericin and inorganic minor components or tannins contained originally in wild silk (Mondal *et al*., 2007a).

2.2.3. Other components of silk

Silk also contains several low molecular weight polypeptides and a small percentage of waxes and fats (~1.5%), carotenoids pigents and mineral components (~2%), ash (0.3- 2.0%) and small amounts of polysaccharides which occur exclusively in the sericin layer (Voegeli *et al*., 1993; Aruga, 1994; Schoeser, 2007). Several proteins that are smaller than the main fibroin and sericin components appear to serve defensive anti-bacterial and anti-fungal roles (Akai, 1977). Green cocoons are already known to contain a compound that is a typical polyphenolic substance flavonol, with clear antioxidant and antibacterial activities (Mase *et al.*, 2008).

2.2.4. Amino acid composition

Silks are fibrous proteins made up of repetitive sequence of amino acids. These amino acids are stored in the animal as a liquid and configure into fibers when sheared at secretion (Craig, 2003). Amino acid composition of mulberry and non-mulberry silk varies and is used to establish the first classification of silks (Lucas *et al*., 1955). The specific ratios of amino acids are dependent on rearing conditions, processing, use, and age (Miller and Reagan, 1989). Glycine, alanine, and serine form the major components of mulberry and non-mulberry silk although large differences have been found in the relative frequency of each amino acid (Dhavalikar, 1962; Pe´rez-Rigueiro e*t al*., 2000). The composition of these three amino acids was the highest in Eri silk (84.26%) followed by mulberry (82.8%), Tasar (72.06%) and

Muga (67.77%). Nadiger *et al.* (1985), however, reported higher percentage (91%) for mulberry silk than other wild silks (77 - 82%). In General, higher relative content of alanine than glycine was reported in wild silkworm silk than *B. mori* (Gary and Gary, 2008).

Alanine and glycine are dominant in cocoon shell of *Rondotia menciana* and *B. mandarina*, (Ito *et al*., 1992). Most of the silk proteins of ants, bees and wasps have few glycine residues and high content of acidic residues (Rudall, 1962). In ichneumonoids, serine, alanine and glycine can comprise up to 80% of the amino acid residues (Craig and Riekel, 2002). *Pycnopsyche guttifer* (Walk.) silk have dominantly glycine, serine and Arginine (Engster, 1976). These results show that the amino acid composition the cocoon is species specific. A summary of the amino acid composition of various silks is presented in Table 1.

Table 1: Percentage amino acid composition of silks from selected arthropods

Amino Acids	**Species**						
	B. mori	*R. Menciana*	*B. mandarina*	*Nephila clavipes* (residue/100 total residue)	*Argiope aurantia* (residue/100 total residue)	*Anaphe*	*S.c. ricini*
Glycine	42.9 -45.5	41.15	41.88	37.1	34.7	32.3	33.2
Alanine	30-30.9	37.17	37.48	21.1	22.2	59.1	48.4
Serine	10.7-12.2	7.68	9.71	4.5	5.1	2.4	5.5
Tyrosine	4.25-4.8	2.5	3.95	2.9	3.8	0.8	4.5
Valine	2.16-2.5	1.05	1.80	1.8	1.5	0.4	0.4
Aspartic acid	1.43-1.9	1.92	1.18	2.5	1.6	1.7	2.7
Glutamic acid	1.07-1.7	3.48	1.03	9.2	1.6	0.9	0.7
Threonine	0.83-0.9	0.68	0.70	1.7	0.8	0.3	0.5
Lysine	0.36-0.4	0.72	0.24	0.5	0.5	0.1	0.2
Phenylalanine	0.61-0.7	0.31	0.61			0.1	0.2
Isoleucine	0.54-0.6	0.56	0.39	0.9	0.8	0.2	0.4
Arginine	0.46-0.5	1.98	0.3	7.6	2.9	-	1.7
Leucine	0.41-0.6	0.60	0.33	3.8	4.2	0.6	0.3
Proline	0.36-0.5	0.13	0.12	4.3	6.4	0.6	0.4
Histidine	0.20	0.06	0.19			0.5	1.0
Methionine	0.07-0.1			0.4	0.3	0.02	0.01
Cystine	0.03-0.14			0.1	0.3	-	0.01
Tryptophan							0.3

Data from; Lombardi and Kaplan, 1990; Ito *et al*., 1992; and Tanaka *et al*., 2008

2.3. Properties of silk

2.3.1. Tensile properties

The tensile strength of a material is the maximum amount of tensile stress that it can be subjected to before failure. Tensile strength is measured in units of force per unit area. It has an important application in engineering, especially in the fields of material science, mechanical engineering and structural engineering. High tensile strength is the major mechanical requirement imposed on the cocoon silk. *Bombyx mori* cocoon has a tensile strength of about 0.5GPa, with a breaking elongation of 15% and a breaking energy (toughness) of 6 x10^4 J/kg (Shao and Vollrath, 2002; Pérez-rigueiro *et al.*, 1998). Michalak *et al*. (2005) showed that the specific strength of fibers produced by caddis-flies is 5.7×109N/m^2. Typical *Nephila* spider dragline silk has strength of 1.3GPa, with an elongation of 40% and a toughness of 6 x10^4J/kg (Madsen *et al*., 1999; Vollrath *et al*., 2001). Tussah silk fiber has a relatively high extensibility as compared to *B. mori* silk fiber and other natural fibers (Cheung, 2009). These mechanical measures show considerable inter- and even intra-individual variability. For example, the tensile properties of silk collected from the spider web shows an intrinsic variability, even among fibers from a single web (Work, 1976). The large variability of the tensile properties of spider silk was interpreted in biological terms as a contribution to the survival ability of the spider (Madsen *et al*., 1999).

The mechanical properties of silk fibers appear to be determined by the amino acid sequence of the heavy-fibroin, which is for the most part composed from repeats (Fedicˇ *et al*., 2003)

as well as the conditions under which the protein is produced (Kerkam *et al.,* 1991; Holland *et al*., 2006). The extraordinary toughness of silk is assumed to be encompassed by the strong and stiff crystalline units, taking up the mechanical load in stretched as stiffness attracts force and thereby protects against failure (Xio *et al*., 2009), while the interspersed "amorphous" regions confer the elasticity of the silk (Fedicˇ *et al*., 2003). Shao and Vollrath (2002) indicated the mechanical properties of *Bombyx* silk, like those of spider silk, depend crucially on spinning conditions.

2.3.2. Solubility and thermal properties

The solubility of silk fibroin in certain solvents has been studied in recent years and several different solvent systems including Lithium bromide (Alessandrino *et al*., 2008), N-methylmorpholine-N-oxide (NMMO) hydrate (Plaza *et al*., 2008), Phosphoric acid/formic acid mixture (Ki *et al*., 2007a), Calcium nitrate (Kweon *et al*. 2001), and $CaCl_2$ (Miyaguchi and Hu, 2005) have been recommended. These solvents can be classified into two as acidic solvents (Formic acid) and high ionic-strength aqueous salt solutions. Acidic solvents tend to be harsh and have poor solution stability, while basic solvents make removal of the inorganic salts from the solutions difficult (Wang *et al.,* 2006). The spider silk protein is partially soluble in mineral acids and alkaline hydroxides and completely soluble in concentrated solutions of Lithium bromide, Lithium thiocynate or Calcium chloride, and ethyl alcohol (Saravanan, 2006).

Thermal decomposition of *B. mori* silk occurs above 250°C and the fibers are thermally stable up to 230°C (Kaplan, 1998). Prasong *et al.* (2009) found thermal decomposition of *B. mori* silk occurs at 350°C and 360°C for silk with and without sericin, respectively, while Eri silk underwent three thermal decomposition stages at 200-300°C , 300-350°C and 350-400°C however, the silk did not completely decomposed even at 1000°C. Zhang *et al*. (2002) reported 9% and 8.2% initial weight loss from cocoon shell and silk fiber, respectively attributed to the evaporation of water 105°C. They also reported only 1% and 0.9% of the original cocoon shell and silk fiber weight remains as ash after heat treatment at 550°C. Dragline silk from the spider, *N. clavipes* has a thermal stability to about 230°C (Cunniff *et al.*, 1994).

2.4. Applications of silk

2.4.1. Dietary and medicinal applications

B. mori has high protein content, reasonable nutrient compositions and ample contents, a short lifespan, easy breeding method, small growth room, and little odour and waste water produced (Yang *et al.*, 2009). The proximate compositions of silkworm pupae is in the range of 12 to 16% total protein, 11 to 20% total fat, 1.2 to 1.8% carbohydrates, 65 to 70% moisture and 0.8 to 1.4% ash which can be easily digested and absorbed by human body, promote the physiological functions of the gastrointestinal tract and plays an excellent role in lowering blood-glucose levels (Mishira *et al*., 2003). *B. mori* pupae is very rich in protein, oil, carbohydrate and minerals and can be utilized as a high potential raw material for various industries including pharmaceuticals, paints, varnishes, soaps, candles, bio-diesel and plastic

industries (Trivedy *et al*., 2008). The protein of silkworm pupae is a new available source of high quality protein that contains all the amino acids needed by the human body with appropriate proportions in line with FAO/WHO standards (Xia and Zhao, 2003; Zhou and Han, 2006). More than 30% of pupae oil is linolenic acid, which is the raw material for human docosahexaenoic acid (DHA) exerting an important effect on human intellect and memory improvement, sight-protection and is a precaution chemical against hyper-lipoidemia (Lu *et al*., 1998). In fact, the protein of pupae is better than the protein of soybean, fish or beef (Trivedy *et al*., 2008). Sericin contains amino acids that can be easily absorbed by human body and over 17% of which are eight kinds of essential amino acids higher than that found in common food (Zhang *et al.,* 2000; Yang *et al.,* 2009). Intake of sericin containing food relieves constipation, suppresses development of bowel cancer and accelerates the absorption of minerals (Padamawar and Pawar, 2004).

2.4.2. Cosmetic application

The silk fibroin peptides are used in cosmetics due to their glossy, flexible, elastic coating power, easy spreading and adhesion characters (Kumaresan *et al.,* 2007). The extracts of silk protein like hydrolyzed silk, silk amino acids, silk powder and raw silk are used in soap making, personal care and cosmetic products (Gulrajani, 2006). Powdered silk fibroin is considered to be effective as an additive for cosmetic and pharmaceutical preparations because of its moderate moisture absorption and retention properties and its high affinity for the human skin (Takeshita *et al.,* 2000). Sericin alone or in combination with silk fibroin has been used in skin, hair and nail cosmetics. Sericin when used in the form of lotion, cream and

ointment shows increased skin elasticity, anti-wrinkle, and anti-aging effects (Li *et al.,* 2009.). Nail cosmetics, containing 0.02 - 20% sericin are reported to prevent nail from chapping, brittleness, and imparting the inherent gross to nails (Yamada *et al.,* 2001). Creams containing 0.001- 30% (w/w) of sericin improves cleansing properties with less skin irritation (Sakamoto and Yamakishi, 2000). Sweat and sebum absorbing type of cosmetics containing cellulose impregnated with fibroin dispersion and aqueous sericin solution are also reported (Miyashita, 1999). Chinese oak silkmoth, *Antheraea pernyi*, is commercially cultivated mainly for silk production and presently it is mostly used as a source of insect food (larva, pupa, and moth) and for cosmetics (Li *et al.*, 2009).

2.4.3. Applications in pharmaceuticals, biomaterials and biomedical materials

2.4.3.1. Applications of fibroin

Among the natural biodegradable polymers like collagen, gelatine and chitosan, silk fibroin provides an important set of material options for biomaterials and scaffolds in biomedical applications because of its high tensile strength, controllable biodegradability, haemostatic properties, non-cytotoxicity, low antigenicity and non inflammatory characteristics (Stitzel *et al*., 2006). This protein is biocompatible, degrades slowly in the body, and is readily modified into a variety of formats and Generates mechanically robust materials (Altman *et al*., 2003). Fibroin has been used particularly as a suture material in tendon repairs for many years (Lovett *et al*., 2007) as it does not cause inflammatory reactions and absorbed after wounds heal (Reddy, 2009). Fibroin is also used to produce vascular grafts (Sakabe *et al.,* 1989),

membrane frames that allowed oxygen flow for contact lens (Minoura *et al.,* 1990) and artificial skin for use in healing fire burned wound as fibroin had high evaporation rate (Liu *et al.,* 2010). Moreover, fibroin is appropriate for producing artificial ligament tissues as fibroin absorbs calcium ions in a great deal and is very flexible (Fang *et al.,* 2009). Fibroin also helps drug delivery process which could control drug release (Tsukada *et al.,* 1994) and regularly used as healing wound pad as it allows cell adhesion and cell growth (Min *et al.*, 2004).

In order to function as a potential biomaterial, biocompatibility is obviously an important prerequisite, and *in vivo* studies have shown that both *B. mori* and spider silk are biodegradable and evoke a comparable defence reaction as materials routinely used in surgery (Vollrath *et al*., 2002; Gellynck *et al.,* 2008). Spider silk have good clotting properties due to the fine size of its threads and have anti-bactericidal properties (Vollrath and Knight, 2001). MacIntosh *et al*. (2006) demonstrated *Nephila edulis* egg case silks support the attachment, proliferation and re-differentiation of bovine articular chondrocytes *in vitro*. Kim *et al.* (2008) showed aqueous-derived porous silk fibroin scaffolds could be applied in bone tissue engineering. The hexapeptides derived from fibroin not only enhance insulin-stimulated glucose uptake but also block the development of insulin resistance in cells exposed to chronic insulin (Kim *et al.*, 2009). The silk fibroin peptides are also used in the biomedical field with a novel bio-mimetic design of silk fibroin-nerve guidance conduit (SF-NGC) used for peripheral nerve reGeneration (Reddy, 2009). Fibroin proteins from *Antheraea mylitta* and *B. mori* are immunologically inert and invoke minimal immune response (Acharya *et al*., 2008). The fibroin powder is known for wound dressing by

maintaining a moist environment and absorbing excess exudates from the wound (Teramoto *et al*., 2008). The silk fibroin could also be applied as an alternative material for removing/filtration of particulate matters in indoor air (Triped *et al*., 2009).

2.4.3.2. Applications of sericin

Sericin solutions has the potential to promote wound healing and wound-size reduction in rat eyes, probably as a result of increased cell movement and proliferation (Nagai *et al*., 2009). Sericin has also been identified as a potent antioxidant and photo-protective agent against ultraviolet B (UVB) irradiation in mouse skin model (Dash *et al*., 2008). Sericin enhances the attachment and growth of fibroblasts (Tsubouchi *et al.*, 2005). Terada *et al.* (2002) found growth promotion in several human cell lines and mouse hybridomas when sericin was added to the culture media. Tsubouchi *et al*. (2002) reported wounds covered with sericin powder exhibited accelerated wound healing in dogs. Sericin was recommended as vehicle for DNA delivery in the gene therapy (Yanagihara *et al*., 2006) and as insulin conjugate that would extend the hormone half-life in treated patients (Zhang *et al*., 2006). The other proven applications include hernia repair, tissue wall reconstruction and organ support as bladder slings (Sehnal and Sutherland, 2008), scaffolds for tissue engineering (MacIntosh *et al*., 2006) and as controlled release drug delivery vehicles (Wang *et al*., 2007; Hardy and Scheibel, 2009).

CHAPTER THREE

GENERAL MATERIALS AND METHODS

3.1. Silkmoths and cocoon shells

Cocoon shells of the four African wild silk species were collected from four different ecosystems in Kenya namely; the coastal forest of Arabuko Sokoke (*A. mimosae* Biosduval), the semi-arid Mwingi district acacia forest (*G. postica* Walker), the arid and semi-arid West Pokot region (*E. bauhiniae* Guer) and the tropical rain forest of Kakamega (*A. panda* Biosduval) (Fig. 1a-d). *B. mori* cocoons (race ICIPE II) were obtained from the Commercial Insect Program of International Centre of Insect Physiology and Ecology (ICIPE) (Fig 1e).

3.2. Cleaning of cocoon shells

B. mori cocoon shells were harvested seven days after the completion of spinning, and left in the sun for two days to kill the pupae. Wild silk cocoon shells were collected after the adult moth had emerged. All the cocoon shells were cut open by a sharp razor blade and dead pupae and/or remaining debris of the pupae were removed. The inside and surface of the cocoons were scrubbed to remove any impurities and other foreign materials such as leaves and branches. Stained and deformed cocoons shells were rejected.

3.3. Degumming of cocoon shells

Degumming (process of removing the gum (sericin) component of silk fibers usually through boiling with alkaline chemicals) solution was prepared by adding 10g Na_2CO_3 (commonly known as Magadi soda) in a beaker with 1500ml of deionized water and stirred with a magnetic stirrer for 10 minutes at room temperature. Then the volume was then adjusted by adding distilled water to 2000ml in a volumetric flask. Cleaned cocoons were enclosed in wire mesh cages with a volume of 717cm^3 and boiled with the degumming solution for one hour for *B. mori*, 1.5 hours for *G. postica*, 3 hours for *A. mimosa* and *E. bauhiniae* and 5 hours for *A. panda*. Boiled cocoons were soaked in liquid detergent solution of 50ml/L of distilled water for 3 minutes and washed with hot and cold distilled water twice. Excess water was removed by filter paper.

3.4. Chemicals

All reagents used were of analytical grade. Deionized water (made by Elix Milipore Darmstadt®) was used throughout the experiment.

3.5. Data analysis

All percentage data were subjected to appropriate transformation procedures to stabilize the variance before the final statistical analysis. Means were analyzed using one-way and two-

way analysis of variance (ANOVA) with PROC ANOVA procedure (SAS Institute, 2010). Least Significance Difference (LSD) tests (α=0.05) were used to separate means.

(a) *Argema mimosae*

(b) *Gonometa postica*

(c) *Epiphora bauhiniae*

(d) *Anaphe panda*

(e) *Bombyx mori*

Figure 1: Cocoon shells of the four African wild and *Bombyx mori* silkmoths

CHAPTER FOUR

MICROSTRUCTURES OF COCOON SHELLS AND DEGUMMED FIBERS

4.1. INTRODUCTION

Silk, from both domesticated and wild silkworms and spiders, represents a unique and important class of polymeric composite materials in nature providing a wide range of evolutionary and ecological functions with optimum microstructures and properties (Zhao *et al.*, 2005; Cheung *et al.*, 2008). Larvae of silkmoths shoot out silk threads and form cocoon shells to enwrap themselves completely prior to metamorphosis. These cocoon shells are non-woven structures majorly composed of two proteins, fibroin and sericin. They exhibit remarkable variation in structure, property and composition which imposes direct influence on their adaptive functions. The functional significance of these proteinaceous structures includes crypsis, provision of barricade from predators, pathogens and parasitoids and assisting the pupae to complete their metamorphosis by improving thermal and/or moisture conditions (Lyon and Cartar, 1996). The multitude of variations in diet, breed and climate resulted in considerable diversity in the type and properties of the cocoons and fibers produced by silk producing insects of different origins and within the same species. Thus, species- specific fiber mechanical, chemical and physical properties reflect the ecology and behaviour of cocoon-spinning insects (Danks, 2004; Reddy and Yang, 2010a) and can

significantly influence the quality, quantity and efficiency of the reeling process in commercial varieties (Vasumathi *et al.*, 2004).

Studies have shown the existence of macro- and microscopic level structure and property variations among cocoon shells and fibers from mulberry and wild silkworms. Narumi *et al.* (1993) revealed the presence of a large number of voids in the cross sections of wild silk fibers in contrast to mulberry silk. The silk fibers from the wild silkmoths have many longitudinal striations on their surface and are porous which make them lighter than mulberry silk (Sen and Babu, 2004a). Wild silk fibers also consist of flattened, ribbon-like filaments of much greater diameter than mulberry silk (Das, 1992). Although the potential of African wild silkmoths for production of large cocoons with economic importance has been documented. However, the microscopic structures and properties of these fibers and cocoon shells have not yet been studied in detail. Comprehensive studies of such structures will lead to better understanding of the cocoon spinning behaviours of the silkworms and how silk fibers are arranged in the cocoon shells to suit their designed purposes. This information would be of immense value in the prudent evaluation of the potential of the African wild silk fibers and cocoon shells for commercial application and further investigation on the structure-property-function relations.

4.2. MATERIALS AND METHODS

4.2.1. Surface and cross sectional study of cocoon shells

Cocoon shell discs (5mm diameter) were pressed out of the middle section of the cocoons with a sharp hole-punch for external and internal cocoon surface studies. Cocoon shells were also cut into slices by using a sharp blade for cross sectional observation. Spines and hairs from *G. postica* and *A. panda* cocoons were collected using forceps. All samples were separately mounted onto copper stubs using double sided sticking tape and were then sputter-coated with gold for three minutes to minimise charging under the electron beam. The samples were then studied with SEM ((Jeol Neoscope, JCM-5000 (Nikon®, UK)) under an accelerating voltage of 10kV with a beam current of 0.1nA. Ten cocoons were pressed out five times at random locations with a sharp hole-punch and the thicknesses of the discs were measured using a digital micrometer (Mitutoyo, IP 65).

4.2.2. Longitudinal and cross-sectional study of degummed fibers

Degummed fibers were placed in 70% ethanol for three days to remove remnant sericin and fatty materials and were then dried at room temperature. Longitudinal and cross sections were prepared by cutting fibers mounted in plastic tubes using a new razor blade. The slices were mounted onto copper stubs using double-sided sticking tape and were coated with gold on their cut surface to be observed by SEM.

4.2.3. Fourier Transform Infrared Spectroscopy (FTIR) study

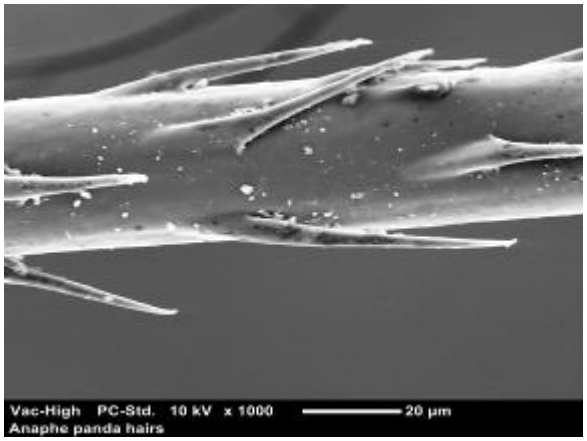
Three cocoon discs of each species were sampled three times on each side (inside and outside of the disc) and all the spectra were averaged. Spectra were acquired at 4cm^{-1} resolution from 7000 to 400cm^{-1} and average of 64 scans. All spectral operations were performed using OMNIC version 7.3 (Thermo Scientific, Madison, WI).

4.3. RESULTS

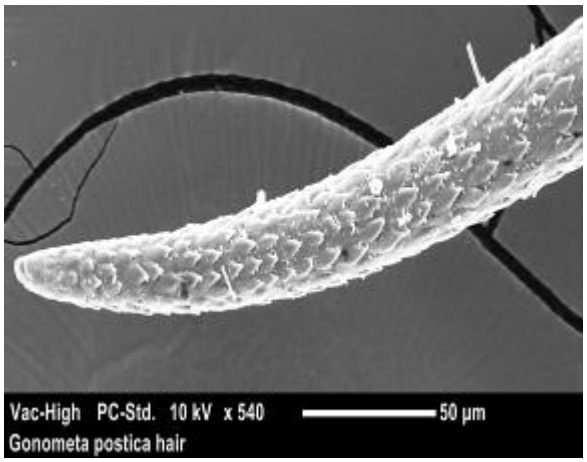
4.3.1. Surface and cross sections of cocoon shells

Being a typical protective structure, surfaces of cocoon shells exhibited extensive variation to meet the specific needs of a given species. *A. panda* and *G. postica* cocoon shells had dense white and brown hairs and spines, respectively embedded from the body of the larvae during spinning (Fig. 2a-c). The average diameter of *A. panda* hairs was 34µm and had thorny spines (6-8µm in diameter) arranged alternatively over the surface. *Gonometa* cocoons had short white hairs interwoven throughout the cocoon layers while the brown needles like spines with sharp buds on their surface were found attached to the outer cocoon surface (Fig 2b). The sharpness of the buds decreased towards the base of the spines (Fig. 2c).

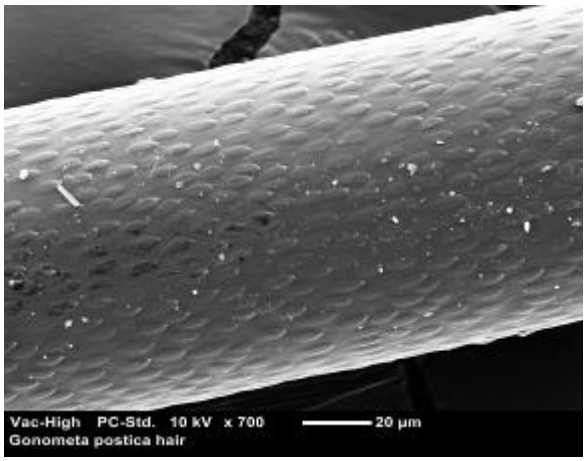
E. bauhiniae cocoon shells had strong peduncle to attach on the twig of a plant. It was composed of highly cross-linked fibers embedded with mass of sericin gum (Fig. 3a and b). In the rest of the species, cocoons were sessile and directly attached along their full length to twigs and forked branches. The attachment surfaces for *A. mimosae* and *G.postica* were smooth and plastered with massive amount of gum (Fig. 4a and b). Unlike the other cocoon surfaces, the crystals in *G. postica* were found covered with the gum material at the attachment surface.



(a) *Anaphe panda* cocoon hair

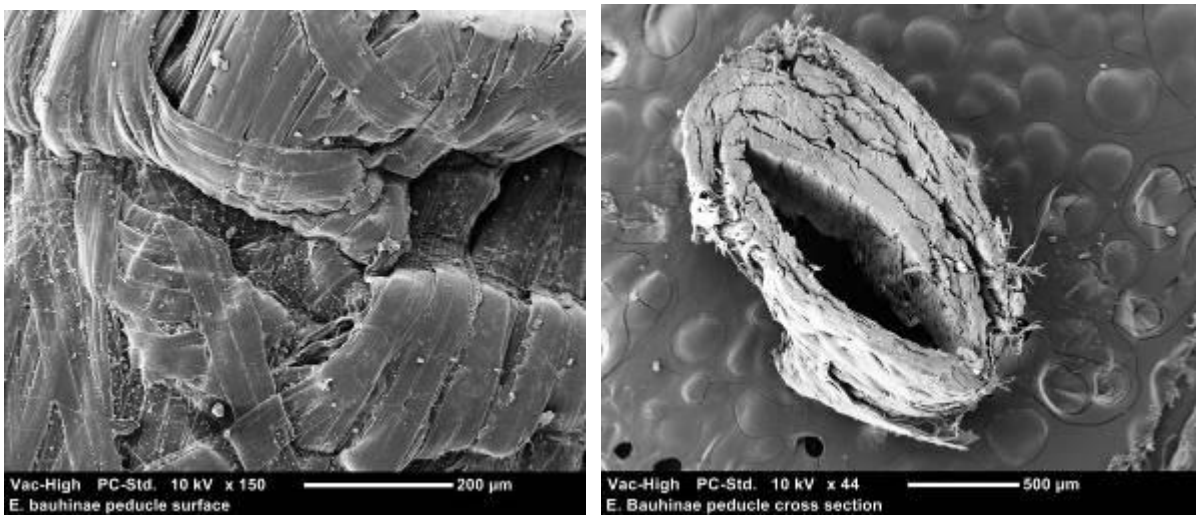


(b) *Gonometa postica* spines (tip)



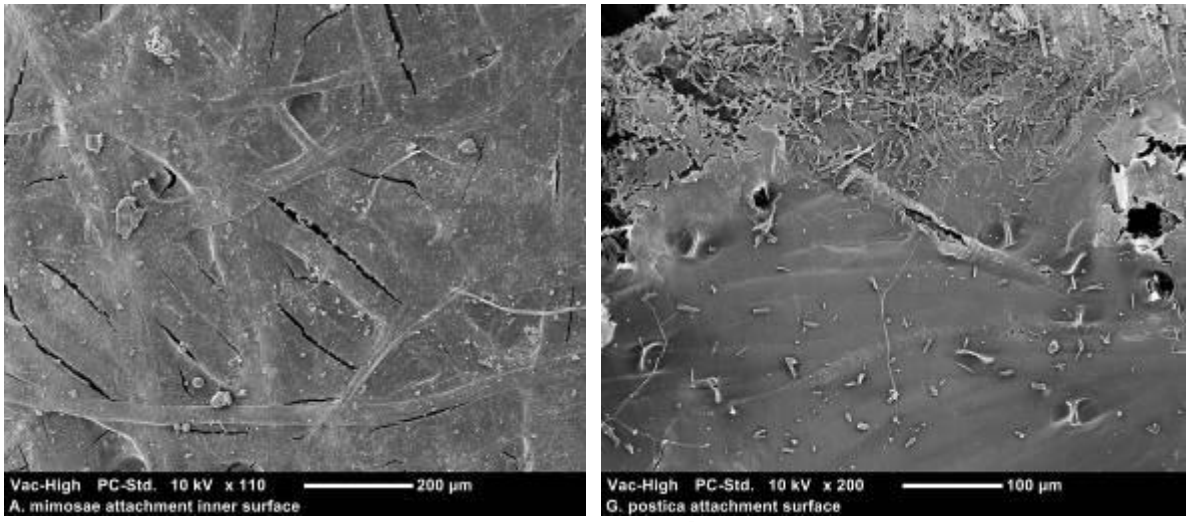
(c) *Gonometa postica* spines (base)

Figure 2: SEM micrographs of hairs and spines from the surfaces of *Anaphe panda* and *Gonometa postica* cocoon shells



(a) Surface (b) Cross section

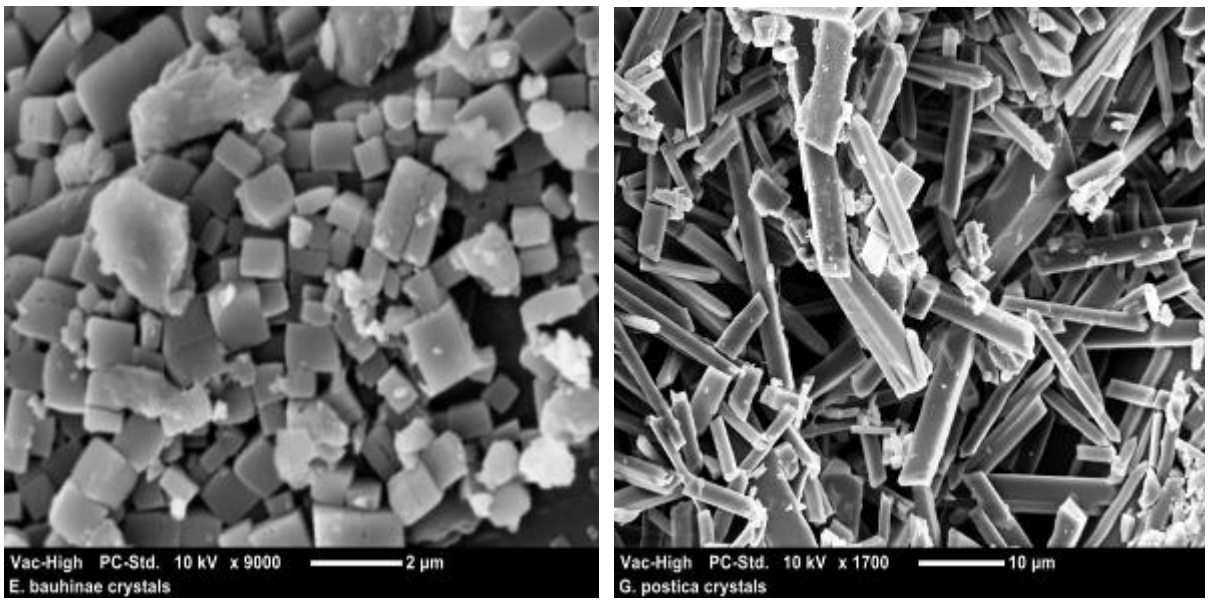
Figure 3: SEM micrographs of surface *(a)* and cross section *(b)* of *Epiphora bauhiniae* cocoon peduncle



(a) Argema mimosae *(b) Gonometa postica*

Figure 4: SEM micrographs of attachment surfaces of cocoon shells of *Argema mimosae* and *Gonometa postica*

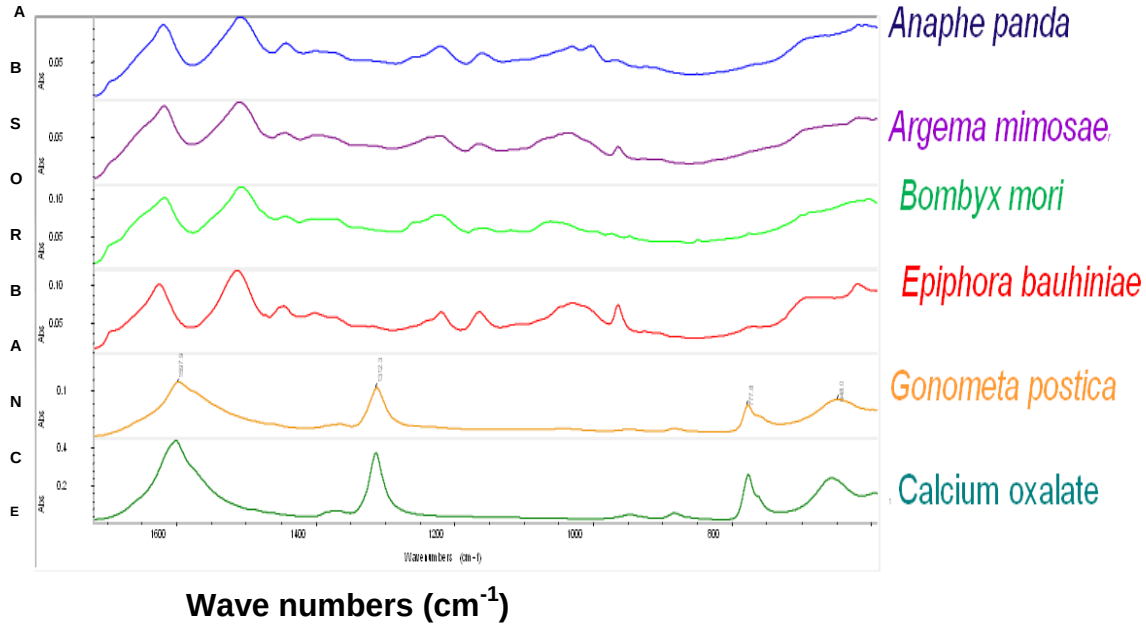
G. postica and *E. bauhiniae* cocoon shells were covered with whitish rectangular and cubic shaped granular crystalline materials, respectively throughout the surface except areas of fiber intrusion and attachment surfaces for the former (Fig. 5a and b). FTIR spectra for the outer cocoon shell surface of *G. postica* showed the peaks at 1312cm^{-1} and 777cm^{-1} were attributed to Calcium Oxalate crystal monohydrate (Siga) (Fig. 6*i*). However, the FTIR spectra could not clearly identify *E. bauhiniae* crystals as calcium oxalate, which might be due to their smaller size and quantity or difference in the chemical composition of the crystals. *G. postica* cocoons had prominently higher level of the crystals than *E. bauhiniae*. *G. postica* had 110-130µm thick calcium oxalate crystal layer deposit. The inner surfaces of all the cocoon shells had no calcium oxalate crystal layers (Fig. 6*ii*).

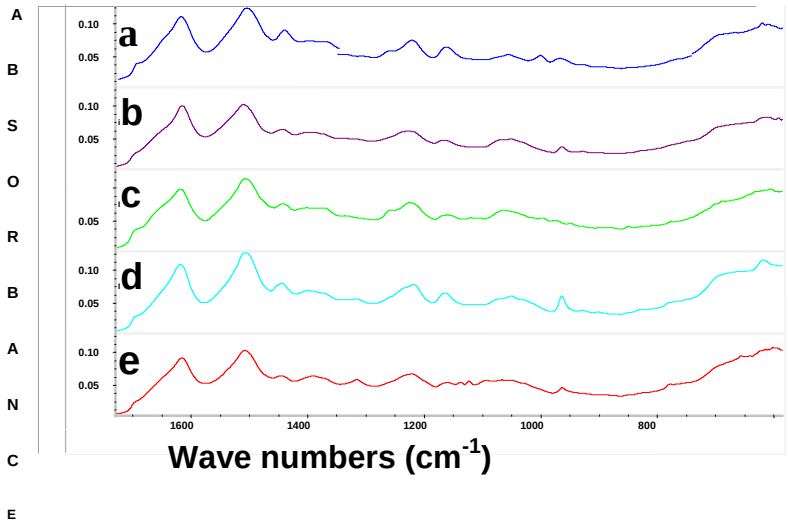


(a) *Epiphora bauhiniae* (b) *Gonometa postica*

Figure 5: SEM micrographs of cocoon shell crystals of *Epiphora bauhiniae* and *Gonometa postica*

(*i*) Outer surface

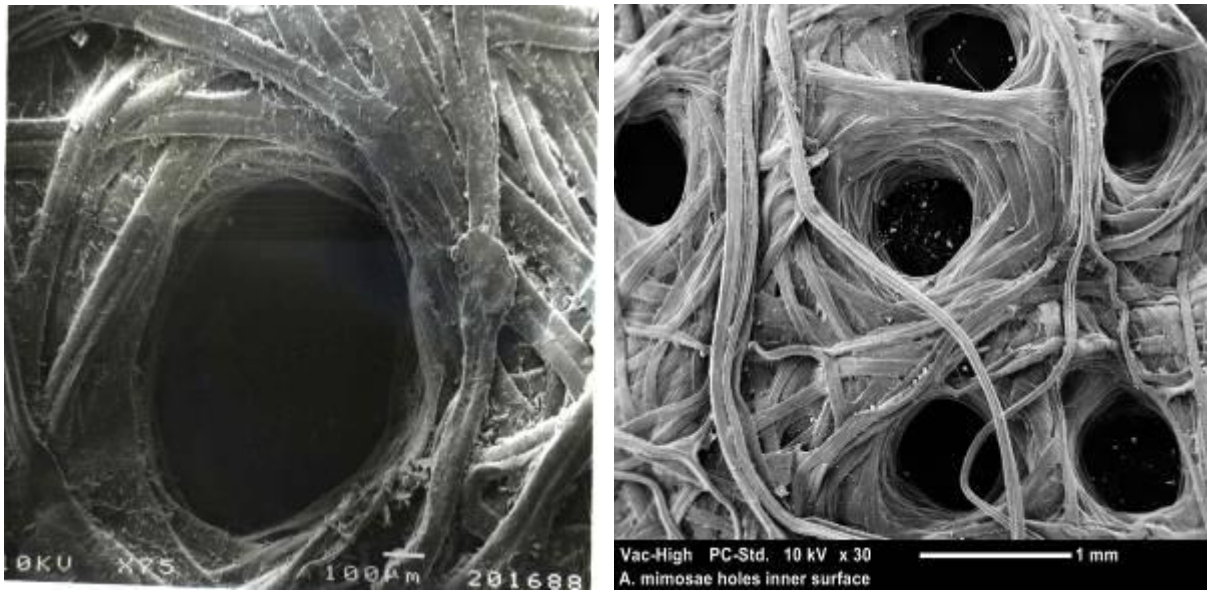




(*ii*) Inner surface

Figure 6: FTIR spectroscopy of outer (*i*) and inner (*ii*) cocoon shell surfaces of (a) *Anaphe panda*, (b) *Argema mimosa*, (c) *Bombyx mori*, (d) *Epiphora bauhiniae*, (e) *Gonometa postica*, (f) Calcium oxalate hydrate

Unlike others, *A. mimosae* cocoons had considerable amount of holes formed as a ring like pattern (Fig. 7a and b). The width of the holes ranged between 448–512µm. However, the number, size, distribution and structure of the holes varied considerably with the position on the cocoon, the highest number being found at the emerging tips and near the attachment areas while larger holes were located at the middle section of the cocoons.



(a) Outer surface (b) Inner surface

Figure 7: SEM micrographs of outer (a) and inner (b) surfaces of hole/perforations of *Argema mimosae* cocoon shells

The cocoon shells also showed variation in thickness and color. *G. postica* and *A. mimosae* had the largest and smallest cocoon shell thickness of 0.536 and 0.234mm, respectively (Table 2). Color of the cocoon shells also ranged from whitish grey to silver.

Table 2: Cocoon shells thickness of African wild and *Bombyx mori* silkmoths (Mean ±SE)

Species	**Thickness of cocoon shell (mm)**	**Cocoon colour**	**Host plant**
Bombyx mori	0.409 ± 0.02^{b}	white	Mulberry plant (*Morus alba*)
Argema mimosae	0.234 ± 0.01^{d}	silver	*Sclerocarya birrea*
Anaphe panda	0.408 ± 0.018^{b}	Brown/grey	*Bridelia micranth*
Epiphora bauhiniae	0.319 ± 0.08^{c}	White grey	*Zizyphus mucronata*, *Z. Mauritania*
Gonometa postica	0.536 ± 0.08^{a}	Whitish brown	Acacia species

Means followed by the same letter in a column are not statistically significant ($P < 0.05$) using the least significant difference test (LSD)

African wild silkmoth cocoon shells were intact with highly cross-linked fibers and the space among the fiber strands and layers were smaller than *B. mori* cocoon shells granting better rigidity and compactness (Fig. 8a-e). The surface of the cocoons had fibers held together in

pairs by sericin gum, other secretions and impurities. The arrangement of the fibers in all the cocoon shells missed uniformity throughout the surface and showed great cross bindings, wrinkles and networking of twisting filaments in different shapes and forms. Such random twisting and bending of fibers imparted rough outer surface and rigidity for the cocoon shells. Fibers of *B. mori* and outer layer of *A. panda* cocoon shells appeared flossy and irregularly arranged. *G. postica, A. mimosae* and *E. bauhiniae* had smooth and uniformly plastered inner walls and the fibers were tightly bound together by the sericin gum making them appear more solid and intact (Fig. 8f-j).

All the cocoon shells were multilayered structures of fibers packaged in different fashion (Fig. 8k-o). However, depending on their cross sectional layers the cocoon shells were divided into two groups; single and double layered cocoons. The first group composed of *A. mimosae, G. postica* and *B. mori*, had single compact cocoon layer, while the second group composed of *E. bauhiniae* and *A. panda* had two hard layers (outer and inner most layers) joined by a middle flossy layer of silk fibers. Cocoon shells could also be devided in two groups depending on the presence of exit holes. *B. mori* and *G. postica* had closed cocoons while, *A. panda, E. bauhiniae* and *A. mimosae* had cocoon openings for the emerging adults left by the spinning larvae. The fiber layers of *B. mori* were arranged in more or less orderly but slack manner with wide space. Fibers of *A. mimosae* and *G. postica* were deeply encrusted and were slightly visible making the cocoons more compact and rigid. However, the thin outer layer of *A. panda* and *E. bauhiniae* were composed of loosely packed and visible fibers.

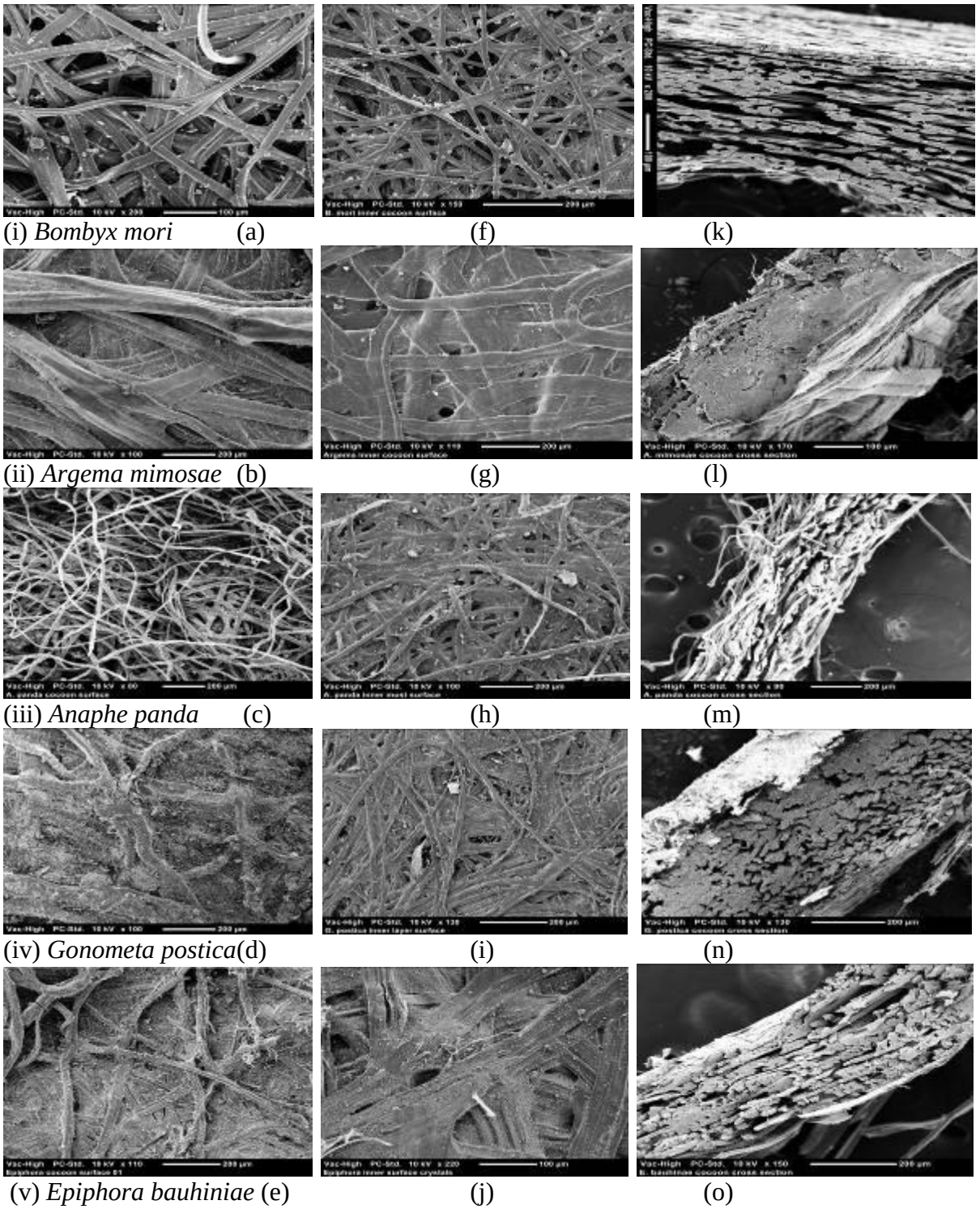
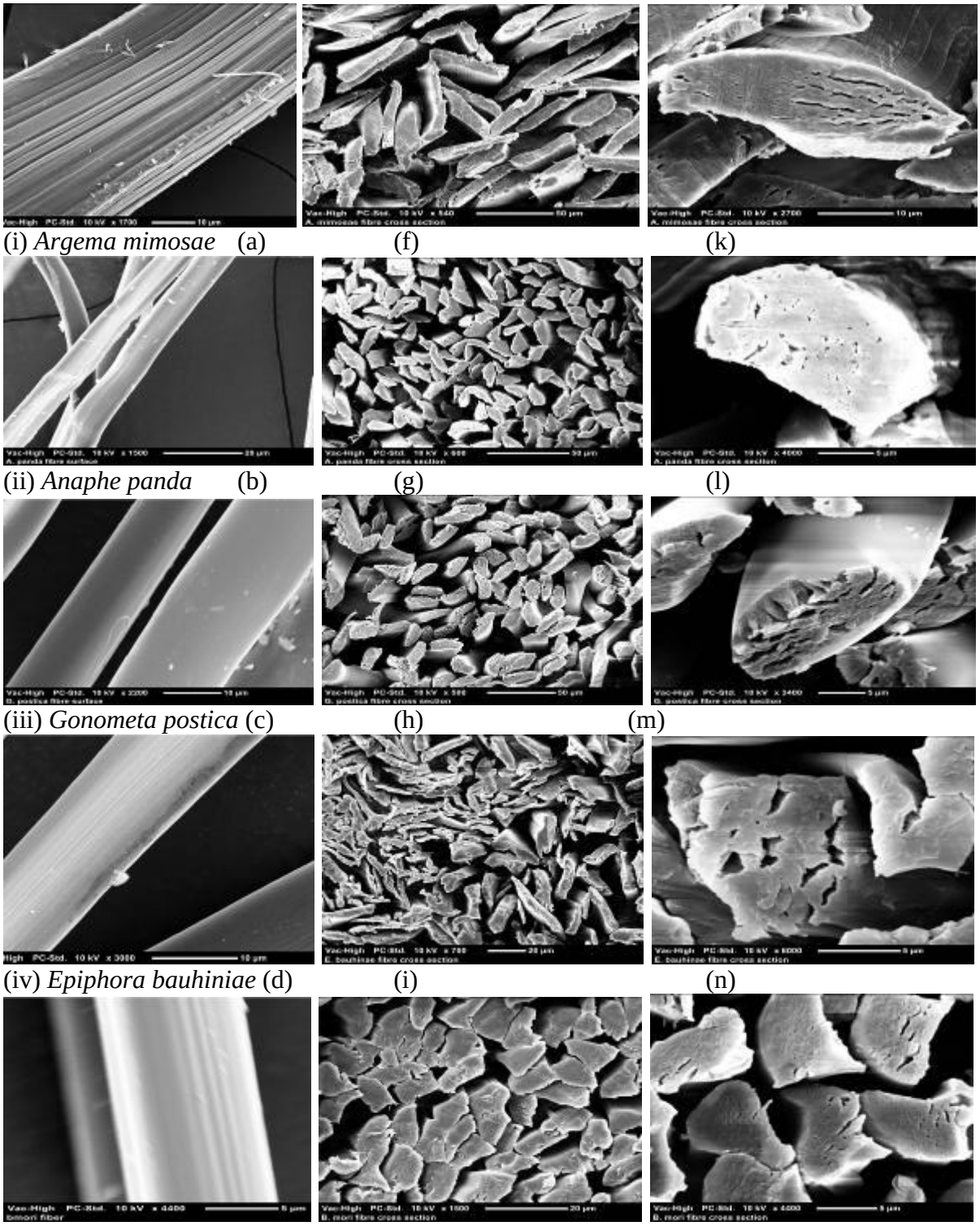


Figure 8**:** SEM micrographs of the outer (a-e), inner (f-j) surfaces and cross sections (k-o) of cocoon shells of African wild and *Bombyx mori*

4.3.2. Fiber surfaces, cross sections and voids

After degumming, the bave were separated into individual brins and compact cocoons became flosses of fibers. *A. mimosae* and *B. mori* had the thickest and smallest brin with a diameter of 49 and 10.5µm, respectively. Figure 9 (a and d) showed the existence of several longitudinal striations and fibrillar structures in *A. mimosae* and *E. bauhiniae* fibers, respectively. The fibers of *A. panda* were unique from other species with their bamboo-like appearance with jointed nods at intervals (Fig. 9b). *G. postica* and *B. mori* fibers had relatively smooth fiber surfaces with occasional groves and ridges. The other wild silk fibers formed ribbon like twisting with uneven width along the fiber and their surfaces showed many irregularities such as fissure.

The silk fibers also showed tremendous variabilities, even between fibers of the same species, in shape and size of their cross sections. *A. mimosae* and *E. bauhiniae* fibers had mostly flattened, elongated and rectangular cross sections (Fig. 9 f and i), while fibers of *G. postica* and *B. mori* had globular and triangular cross sections. The silk filaments spun by *A. panda* had mostly crescent-shaped, triangular or round cross-sections. The fibers also showed great variability in the shape, size and number of voids present. *B. mori* silk fibers had fewer and smaller voids compared to the wild silk fibers. Some voids of *G. postica, A. mimosae* and *E. bauhiniae* were large enough to reveal the microfibrilar structures (Fig. 9k-o).



(i) *Argema mimosae* (a) (f) (k)

(ii) *Anaphe panda* (b) (g) (l)

(iii) *Gonometa postica* (c) (h) (m)

(iv) *Epiphora bauhiniae* (d) (i) (n)

(v) *Bombyx mori* (e) (j) (o)

Figure 9: SEM micrographs of the surface (a-e), cross section (f-j) and macrovoids (k-o) of African wild and *Bombyx mori* silk fibers

4.4. DISCUSSION

From the cocoon macro and microstructures, it is evident that silkworms larvae of each species ensure the pupae are well protected from natural enemies and environmental calamities by spinning hard and compact cocoon shells, which fits into their respective ecology. The needle-like spines and hairs in *G. postica* and *A. panda* are important structures for protecting the larvae and cocoons against natural enemies such as predating birds. Peigler (2004) also reported that the Genera *Gonometa* and *Borocera* embed what were previously protective body spines of the larvae in the cocoon surface hence protecting the developing chrysalis. These spines and hairs can sometimes be irritating on contact and along with other cocoon chemicals and dusts might be reasons for eye and skin irritations that make working with the cocoons of *Anaphe* and *Gonometa* uncomfortable, and causing workers' allergies in some cases.

The surface and interstices between fibers and layers of the cocoon shell of *G. postica* and *E. bauhiniae* cocoons are also packed with calcium oxalate crystals, defecated during spinning, which confer lower risk of bird attack and hardening of the cocoon shells (Veldtman, 2005). The functional adaptations of these crystals and the colour of cocoons range from camouflage to heat absorption or light transmission (Peigler, 1993). Thus, the crystals are perfect ecological adaptations for the arid and semi-arid environments where these two species are found. Other wild silk cocoons, such as *A. assama*, also showed the presence of calcium oxalate crystal deposits on their surface (Freddi *et al.*, 1994). The difference in size and shape of crystals suggests the role they might have on influencing the texture of the cocoon surface.

However, the extensive infiltration of these crystals between the silk fibers makes *Gonometa,* and apparently other wild silk cocoon shells, extremely hard and difficult to reel (Akai and Nagashima, 2003; Gheysens *et al.,* 2011).

The larvae of the wild silkmoths also utilised different mechanisms to ensure firm and secure attachment of the cocoons to a substrate before pupating. This helps the cocoons to avoid falling over to the ground and being taken away by wind and water, and to prevent attack from predators like birds. The silken petiole (peduncle) of *E. bauhiniae* varied in fiber arrangement and compactness from the cocoon shells indicating that there might also be a difference in the chemical composition. Such variations have been reported in *A. mylitta,* where the peduncle sericin has higher molecular weight and slight difference in amino acid composition than the cocoon shells (Dash *et al.,* 2006). The other wild silk cocoons were sessile; however, they have high sericin gum at the attachment surface, which confers strong binding to the attachment substrates. The cocoon shell holes of *A. mimosae* have adaptive significance by allowing fresh air exchange with inner cocoon atmosphere where the pupae reside. These holes provide great ecological adjustment mechanism against the humid and hot coastal rain forest climate (Arabuko Sokoke) for *A. mimosae*. Reddy and Yang (2010b) also reported *Copaxa multifenestrata* (Saturniidae) have perforated construction with considerable amounts of holes on the surface of the cocoons.

Wild silk cocoon shells exhibited cross-bindings and junction of filaments forming an intricate network and irregularly shaped structures with more cementing gum between the

filaments. Such structures of cocoon shells have also been reported in *A. mylitta* (Shamitha and Rao, 2006). *B. mori* shells are more loosely arranged and less compact than their wild counterparts. Such loose arrangement together with comparatively uncomplicated sericin removal procedure might be an added advantage to its success as a major textile fiber by allowing easy reeling with minimum breaking percentage. The wrinkles on outer cocoon surfaces might have been formed due to non-uniform shrinking during drying (Zhao *et al.,* 2005). The multilayered and porous structure of the cocoon shells serves as a mechanical barrier and offers a better energy absorbing property due to the wave impedance matching of different plies (Tsai and Prakash, 2005). The dimension of the fiber spaces (compactness) varied with the species mainly due to the spinning behaviour and amount of silk materials (sericin and fibroin) avialable to construct the cocoons. These porous structures can also be viewed as a trade-off between optimising the use of a limited amount of silk materials from the silk gland and construction of efficiently large cocoon structures, which can withstand possible attacks from the outside. Tagawa and Sato (2009) also emphasised the balance between economy and robustness of cocoons is important for cocoon-spinning insects.

The variability in fiber size, surface and cross sectional structures might be due to wide variation in cocoon size (Komatsu, 1980). Mbahin (2008) and Nel (2007) reported the size of *A. panda* and *G. postica* silk fiber is about 20 and 15-25μm, respectively. The cross sectional view of the brins appeared largely as elongated rectangular, wedge-shaped, triangular, or globular in wild silk fibers. This is in agreement with reports for other wild silk species (Buschle-Diller, 2010; Zhang *et al.,* 2010b). Freddi *et al.* (1993) reported that silk threads

spun by *G. rufobrunnae* are variable in cross-sectional dimension and can be round, triangular or even flat and ribbon like. Fibers of *A. mimosae* and *E. bauhiniae* exhibited a distinctly flattened surface with longitudinal micro fibrils similar to *A. assama* silk fibers (Freddi *et al.*, 1994). Thus, it can be concluded that these fibers are made up of bundles of microfibrils oriented along the fiber axis. The larval body movement, speed of spinning, body twisting and bending during the cocoon forming process and elasticity of the fibers when emerging from the spinnerets might force the fibers to acquire different shapes and sizes. The cross-markings and twisting of the wild silk fibers might also be caused by the overlapping of one fiber on the other before the substance of the fiber has completely hardened, in consequence of which such areas are flattened out. The resulting cross sectional size and shape of fibers have been reported to have a direct relationship with the yarn's lustre, friction and mechanical properties (Narumi and Kobayashi, 1995).

The cocoon filaments of various wild silkmoth species were also found to have voids that were absent in *B. mori* silk fibers (Narumi *et al.*, 1993; Lin *et al.*, 1993). However, in this study, voids were observed in *B. mori* fibers. Narumi *et al.* (1992) and Mondal *et al*. (2007b) also reported the rare voids in cocoon filaments of *B. mori* and *B. mandarina*. Silk fibers are also known to have micro-voids (8–11nm) which differ in their number, size and distribution between species with larger volume fraction and number in *B. mori* than tussah silk (Kawahara and Shioya, 1999). Akai *et al.* (1991) revealed that the voids in *Antherea* originate from the droplets in liquid silk material present in the posterior silk gland and stretched out with the protein masses during spinning. Akai and Nagashima (1999) noted voids in *A. panda*

fibers are traces of the rhythmical movement of liquid silk, which reflects the spinning behaviour and speed. African wild silk fibers have larger and numerous voids which could be an advantage for chemical modifications as these voids play a significant role in the penetration of water and other chemicals to the fiber resulting in marked increase in cross section.

CHAPTER FIVE

AMINO ACID COMPOSITION OF SILK FIBERS

5.1. INTRODUCTION

Silk represent a Family of high-quality animal fibers composed of amino acids which vary significantly in relative composition, structure, and functional properties (Prasong *et al.*, 2009). It is majorly composed of two proteins; fibroin and sericin, together with small quantities of other proteins, waxes, fats, salts, and ash (Schoeser, 2007). Although both fibroin and sericin consist of proteins, they strongly differ in the related building blocks (amino acids). Fibroin is a single protein with roughly 76% of its amino acids having non-polar side chains, the main ones being glycine (43.7%) and alanine (28.8%). On the other hand, sericin is a mixture of proteins and contains a large number of amino acids with hydroxyl groups (Arami *et al.*, 2007). The main amino acids are serine (33.4%), aspartic acid (16.7%), glycine (13.5%), and threonine (9.7%) (Chopra and Gulrajani, 1994). The amino acid sequences of these major silk proteins have been determined for a number of silk types and species, revealing extensive variation. This variation in sequence is hypothesized to confer different material properties in different silks (Swanson *et al*., 2006).

Common to insect silks are high levels of glycine, alanine, and serine. Together, these three small amino acids take up to about 80 to 85% of the total amino acids in *B. mori* and *A. pernyi* silks. Such a high concentration of these amino acids having small and simple side

groups in silk fibroins permits the arrangement of polypeptide molecules into an orderly and crystalline manner and is responsible for the desirable mechanical, physical and chemical properties of the silk fibers (Kaplan *et al.,* 1994). Glycine and alanine represent 50% of the total number of amino acids in silk, although large differences have been found in the relative frequency of each amino acid (Pérez-Rigueiro *et al.,* 2001).

Except for few dominant amino acid residues, wide variations in composition have been observed in silks of different origin (Prasong *et al.,* 2009). Such variation can further be influenced by the dietary intake of the insect and the environmental conditions during the spinning process (Shao and Vollrath, 2002). Warwicker (1960) showed that silks produced by over 70 species in the classes Insecta and Arachnida have similar crystalline structure, basic chain axis and hydrogen bonding dimensions, while differences arise in the inter-sheet spacing due to the different amino acid compositions and sequences of the fibroins. Some investigators have proposed that the diverse amino acid composition of silks may be the result of relaxed selection on proteins used outside of a cellular environment (Lucas and Rudall, 1968). It has also been argued that amino acid composition of silks evolved in response to selection for specific functional properties such as prey capture, egg and pupae protection (Craig, 1997; Gosline *et al.,* 1984). Based on such structural considerations, attempts have been made to relate the chemical constitution of silk fibers to their outstanding mechanical, physical and chemical properties. Lucas *et al.* (1955) showed that the amino acid composition of mulberry and non-mulberry silk varieties is different and influenced the physical properties. However, there is insufficient information to make a definitive

correlation between chemical composition and structural properties for the African wild silk fibers. Thus, the composition and properties of these silk fibers in terms of basic amino acids is still uncertain.

5.2. MATERIALS AND METHODS

5.2.1. Sample preparation

About 100g of cocoons were cut in pieces and gently washed in 100ml of warm water at 65°C for three minutes. To eliminate the pigents and inorganic compounds, the pieces were immersed into 70% methanol at 25°C for ten days. There after cocoons were degummed with standard procedure for each species and rinsed by warm deionized water repeatedly in order to remove remnant sericin.

5.2.2. Hydrolysis of silk

Approximately 1mg of silk was lyophilized and crushed using liquid nitrogen, dried in an oven to remove moisture at 105°C before acid hydrolysis. Hydrochloric acid (6N) (2ml) containing 1% of phenol was added to the dry silk in a vial, sealed under nitrogen and heated in an oven at 110°C for 24 hours. The hydrolysate was then centrifuged at 13000 *g* for five minutes and filtered through glass wool. The supernatant was diluted 20 times with 50% methanol before analysis.

5.2.3. Amino acid analysis

Multiple analyses were carried out on HPLC (Prominence LGE-UV Low Pressure Gradient HPLC system-Shimadzu, Japan: Column Gemini C4, Length 250mm, ID 4.6mm, Run time 25minutes) under the following gradient system (Table 3). The resulting peaks were identified by running authentic standards of the amino acid**.**

Table 3: Sample running conditions for HPLC amino acid analysis of African wild and *Bombyx mori* silk fibroins

Time(min)	Module	action	value	Solvent
0.01	Pumps	B.Conc	5	Solvent A: dH_2O (5% HCOOH),
3	Pumps	B.Conc	10	Solvent B: Methanol
13	Pumps	B.Conc	30	
25	Controller	Stop		

5.3. RESULTS AND DISCUSSION

The percentage composition of the major amino acids in the wild and *B. mori* silk fibroins was presented in Table 4. The results showed that there were variations in quantities of the amino acids present in the fibroins, although all the fibroins examined so far were Generally characterized by the presence of high proportions of the three simple amino acids, glycine, alanine, and serine. The three amino acids represented about 71-74 and 82% of the total amino acids present in wild and *B. mori* silk fibroins, respectively. In all the wild species, alanine was the dominant amino acid (34-36%) followed by glycine and serine. However, in *B. mori,* glycine was predominant (43%). Other amino acids including glutamic acid, asparatic acid, valine and methionine were also found to be incorporated in various amounts depending on the origin of the fibroin. Though the content of alanine was higher for the African wild fibroins compared to *B. mori,* it was lower than other non-mulberry silk fibroins particularly for Muga (44%) (Freddi *et al.,* 1994). However, the proportion reported here was comparable with eri, tasar and muga (34-36%) as reported in Sen and Babu (2004a). However, alanine content as high as 52-59% has been reported for the outer layer of *Anaphe reticulate* and the sum of alanine and glycine content accounted for more than 90% of the total amino acids (Perepelkin, 2007; Tanaka *et al*., 2008). The composition of some of the amino acids such as tryptophan, cystine, asparagine, glutamine and isoluecine could not be included in this study because their analysis and degradation products were hampered by their instability in oxidative or strongly acidic media, deamination, slow cleavage and/or light.

Table 4: Percentage composition of major amino acids in the African wild and *Bombyx mori* silk fibroins

Amino acids	***Argema mimosae***	***Epiphora bauhiniae***	***Anaphe panda***	***Gonometa postica***	***Bombyx mori***
Aspartic acid	4.34	4.19	4.78	4.68	1.52
Glutamic acid	1.31	1.11	1.12	1.02	1.73
Serine	8.85	9.65	8.17	8.56	10.34
Glycine	27.84	27.64	29.62	29.51	43.45
Threonine	0.23	0.03	0.04	0.02	0.82
Alanine	34.72	34.52	36.53	36.43	28.04
valine	1.42	1.22	1.23	1.13	2.57
Methionine	0.28	0.25	0.26	0.16	0.11
leucine	0.68	0.48	0.49	0.39	0.75
lysine	0.19	0.12	0.13	0.03	0.25

Table 5 showed the ratios of different amino acids categorized into groups which have significant correlation with the properties of the fibroins. The ratio of bulky to non-bulky amino acids was higher for wild silk fibroins ranging from 0.10 to 0.12. Similarly, the ratio of hydrophilic to hydrophobic was also higher for all wild silks analysed (0.22-0.24) than *B. mori* (0.19). In General, the large portion of the *B. mori* fibroin was made up of simple amino acids such as glycine and alanine. The glycine/alanine ratio, therefore, was higher for *B, mori* (1.5) than wild fibroins (0.8). Freddi *et al.* (1993) reported *Gonometa rufobrunnae* silk fibroin contains a large amount of glycine and alanine, with glycine/alanine ratio of 1.5 similar to that of *B. mori* silk fibroin. However, all the wild silk fibroins in this study had similar glycine/alanine ratios. These two amino acids are known to form the major part of the crystalline structure of the fibroin and thus definitely lead to a different crystallographic arrangement. Simmons *et al.* (1996) reported that the tensile strength of silk is attributed to the crystalline regions, whereas elasticity is believed to reside in the amorphous domains.

No General correlation could be made between the biological classification of the wild silk moths and the amino acid composition of the fibroin they produce. Some fibroins from biologically unrelated species (*A. panda* and *G. postica*) have closely similar amino acid compositions. *A. mimosae* and *E. bauhine* (Saturniidae) exhibited fair similarity at least in the three major amino acids. Some authors argue that such diversity and evolution of silk is related to selection for specific purpose (Craig, 1997) which could also be true for the African wild silkmoths as they produce similar type of silks for common purpose during a limited period in their lives. The observed heterogeneity in composition might rely on the selective

protein expression and host plants used among the species. Craig *et al*. (1999) also suggested that differences in dietary energy versus dietary diversity may be important to silk expression in herbivores lepidopterans and the high alanine, serine and glycine composition of silks produced by the larvae might reflect their predictable and energy rich diet.

Table 5: Various Amino Acid Ratios

Ratio[a]	***Bombyx mori***	***Gonometa postica***	***Anaphe panda***	***Argema mimosae***	***Epiphora bauhiniae***
Hydrophilic/hydrophobic	0.19	0.21	0.20	0.23	0.22
Bulky/non-bulky side groups	0.08	0.10	0.11	0.11	0.11
Glycine/alanine	1.54	0.81	0.81	0.80	0.80

[a]**Hydrophilic amino acids:** serine, threonine, aspartic acid, glutamic acid, tyrosine, arginine, lysine, histidine

Hydrophobic amino acids: glycine, alanine, cystine, proline, tryptophan, valine, isoleucine, phenylalanine, methionine, tryptophan, leucine

Amino acids with bulky side groups: aspartic acid; glutamic acid, tyrosine, phenylalanine, leucine, isoleucine, valine, arginine, cystine, methionine, lysine, tryptophan

Amino acids with non-bulky side groups: glycine, alanine, serine, threonine, proline, histidine

CHAPTER SIX

WEIGHT LOSS AND MOISTURE REGAIN OF COCOON SHELLS AND DEGUMMED FIBERS

6.1. INTRODUCTION

Silks are natural polymers, which have been gaining wide use in variety of applications such as degradable biomaterials, biomedical and functional bio-membrane materials and fibers besides their traditional use as a textile raw material (Zhang, 2002; Mondal *et al.,* 2007a, Wilaiwan *et al.,* 2010). The use of such silks can be justified both by the combination of mechanical properties, non-toxicity, biocompatibility and biodegradability depending on their origin and treatment.

Moisture regain is the percentage of moisture that a dry fiber will absorb from air under standard conditions of temperature and moisture (Hollen and Saddler, 1973). Specifically, absorbed moisture is likely to determine the mechanical and physical properties of both the sericin 'gum' coating and the integral structural proteins of the silks (Fut *et al.,* 2009). Some silks can indeed be hygroscopic with a moisture regain of 11% at 65% relative humidity (RH) and 27°C (Ranganathaian, 2003; Sonthisombat and Speakman, 2004) which is larger than many other commercial fibers and more in line with wool (Salamone, 1999). This interaction of moisture and fiber has technical as well as commercial consequences. For example, in *B. mori* silk, the absorption of water causes 1.6% fiber swelling in the longitudinal crosswise

(Schoeser, 2007). This apparently small degree of swelling can influence the rate of heat and moisture vapour transfer through the textile fiber (Fuzek, 1985). Moreover, the weight change resulting from moisture absorption can affect the commercial weight of the fibers as well as affecting properties such as dying, finishing and apparent comfort of the product (Salamone, 1999).

However, most silk contains not only the fiber matrix protein but in addition, have a coating of sericin or similar compounds. This sericin component of the composite silk fiber (typically of two fiber brins held together into the bave by the sericinaceous 'gum'), is usually expressed as weight loss and is an important additional factor affecting the quality of a silk (Vishuprasad, 2004). The weight loss from a raw silk filament depends on the water solubility and higher alkali sensitivity of the sericin as compared to the fibroin (Choundhury, 2006). Quantity and nature of sericin are fundamental characteristics in conferring distinctive traits to the cocoon (Sadov *et al.*, 1987). Sericin coating has important biological properties such as oxidation and UV resistance as well as anti-bacterial and moisture control (Mondal *et al.*, 2007b). However, the sericin coating also gives a callous and stiff feeling to the fiber and hides the rich luster and whiteness of the silk brins in addition to preventing the penetration of dye (Arami *et al.*, 2007). These necessitate its removal by process of boiling (degumming) to obtain an ideal fiber for the textile industry and any other specific applications. Because of these effects, understanding the amount of sericin present in silk bave as wells as their water absorption properties of fibers and cocoon shells has considerable technical and economic implications for a silk as a commercial proposition.

6.2. MATERIALS AND METHODS

6.2.1. Determination of weight loss from cocoon shells and cocoon shell layers

Cleaned cocoon shells (20g) of all the wild silkmoths and cocoon shell layers of *A. panda* and *E. bauhiniae* were used. These two species were selected because of the clear distinction in the physical structure of the three shell layers in their cocoons (double cocoons); a leathery outer envelope, a loose and flossy intermediate layer and thick walled and rigid inner layer. These layers were designated as outer, middle and inner, respectively and were separated manually. All cocoon shells and shell layers were degummed separately. Boiled cocoons and layers were soaked in liquid deteregent solution of 50ml/L of distilled water for 3 minutes and washed with hot and cold distilled water twice. Excess water was removed by filter paper and ccocoon filaments were dried for 24 hours in oven at 110°C. Dried fibers were weighed using sensitive balance and the quantity of sericin (gum) removed from the cocoon shells and shell layers, expressed in percentage, was obtained using following equation **(**Nakpathom *et al.*, 2009):

$$\text{Weightloss}\,(\%) = \frac{Wi - Wf}{Wi} \times 100$$

Where, Wi - mass of fibers before degumming (g) and

Wf - mass of fibers after degumming (g)

6.2.2. Determination of moisture regain of cocoon shells, degummed fibers and cocoon shell layers

Moisture regain was determined by the oven drying procedure. Cocoon shells, shell layers and degummed fibers were placed in an oven at 110°C for 24 hours for uniform drying. Oven-dried samples (20g) were placed at laboratory bench for 72 hours at room conditions (23 ± 2°C and 71 ± 3% RH). Samples were then weighed using sensitive balance and the moisture regain percentage was obtained using the formula by (Nawaz *et al.*, 2002):

$$\text{Moisture Regain}(\%) = \frac{Wf - Wi}{Wi} \times 100$$

Where, W_i= weight of oven dried sample (g) and

W_f = final weight of sample (g)

All the experiments were replicated four times. Percentage data was log transformed to bring the data to normal distribution. One-way ANOVA was used to analyze the moisture regain and weight loss of cocoons, layers and degummed fibers with PROC ANOVA procedure (SAS Institute, 2010). LSD test (α=0.05) was used to separate the means.

6.3. RESULTS

6.3.1. Weight loss and moisture regain of cocoon shells and degummed fibers

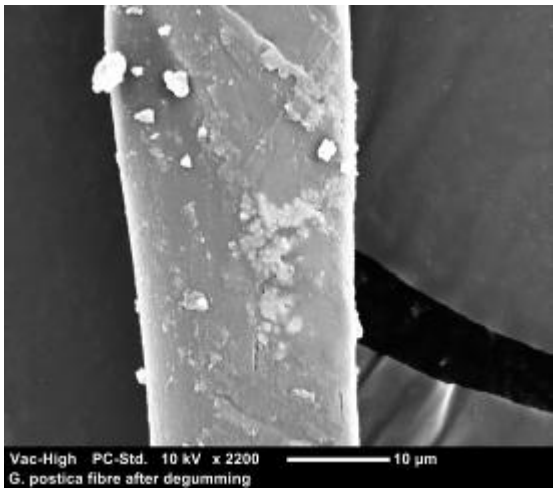
The effect of degumming of four African wild and *B. mori* silk cocoons shells on percentage weight loss and moisture regain was presented in Table 6. There was a highly significant difference ($P > 0.05$) in weight loss between the cocoon shells. *G. postica* and *E. bauhiniae* cocoons had the highest and lowest weight losses (57 and 23%, respectively). *B. mori* and *A. mimosae* cocoons had comparable weight losses (29 and 31%, respectively) which were marginally higher than *A. panda* (26%). The SEM micrographs revealed that the fibers were covered with different quantities of sericin gum after the degumming treatment indicating that all the sericin layer was not removed from the fiber matrix (Fig. 10).

There were also significant differences ($P > 0.05$) in the moisture regain percentage between cocoon shells and degummed fibers. *A. mimosae* and *E. bauhiniae* cocoon shells had the highest and lowest moisture regain percentages (9 and 5.6%, respectively). *B. mori* and *A. panda* cocoons gained marginally higher (statistically non-significant ($P < 0.05$) moisture (7.9 and 8.0%, respectively) than *G. postica* cocoon shells (7.1%) (Table 6). In contrast to the cocoon shells, the moisture regain of degummed wild silk fibers was not significantly different among species with *E. bauhiniae, G. postica* and *A. panda* gaining 10, 9.9 and 9.9%, respectively. However, *B. mori* had the lowest value (8.5%) which was significantly different ($P > 0.05$) from the other wild fibers.

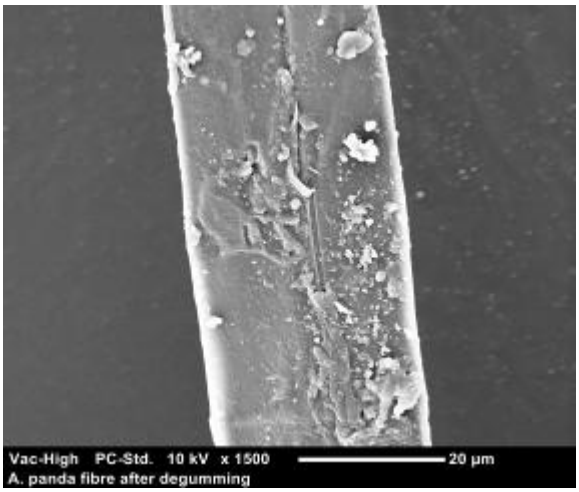
Table 6: Percentage weight loss and moisture regain of African wild and *Bombyx mori* cocoon shells and degummed fibers (Mean ± SE)

Species	Weight loss (%)	Moisture regain (%)	
		Cocoon shells	Degummed fibers
Bombyx mori	29.35± 0.18^{bc}	7.93 ± 0.35^{ab}	8.53 ± 0.32^{b}
Gonometa postica	56.84± 0.32^{a}	7.11 ± 0.53^{b}	9.95 ± 0.46^{a}
Epiphora bauhiniae	23.19± 0.45^{d}	5.64 ± 0.62^{c}	10.42 ± 0.34^{a}
Argema mimosae	31.39± 0.97^{b}	9.05 ± 0.52^{a}	9.75 ± 0.39^{a}
Anaphe panda	25.55± 3.63^{cd}	8.02± 0.35^{ab}	9.91 ± 0.22^{a}

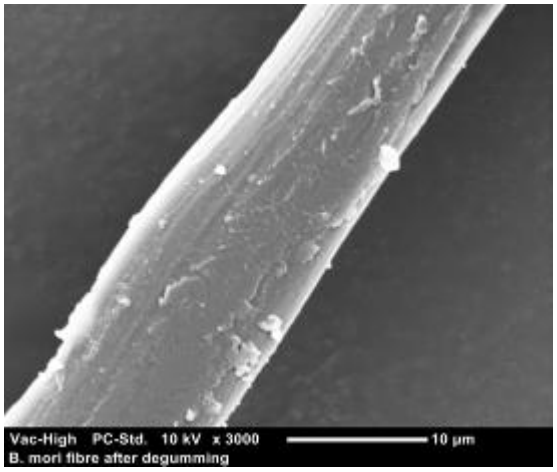
Means followed by the same letter in a column are not statistically significant ($P < 0.05$) using the least significant difference test (LSD)



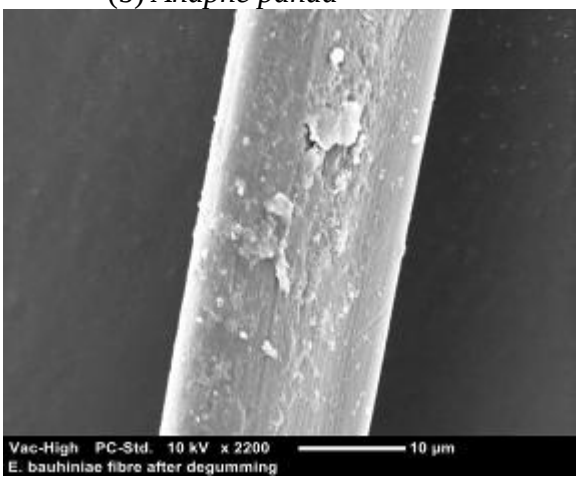
(a) *Gonometa postica*



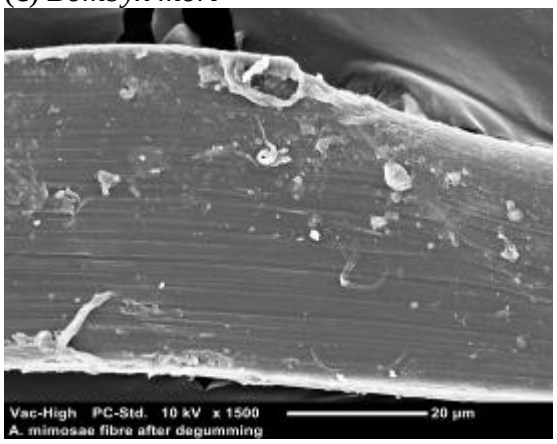
(b) *Anaphe panda*



(c) *Bombyx mori*



(d) *Epiphora bauhiniae*



(f) *Argema mimosae*

Figure 10: SEM micrographs of remnant sericin coating on African wild and *Bombyx mori* fibers after degumming

6.3.2. Moisture regain and weight loss of cocoon shell layers

Moisture regain of layers of *E. bauhiniae* and *A. panda* cocoon shells was shown in (Table 7). There was significant difference ($P > 0.05$) in moisture regain among the cocoon shell layers of both species. The inner hard layers had the highest regain (6.8 and 8.6% for *E. bauhiniae* and *A.panda*, respectively). However, there was no significant difference ($P < 0.05$) between the regain percentages of degummed shell layer fibers of *E. bauhiniae* while, *A. panda* degummed layer fibers showed significant difference ($P > 0.05$) with the inner layer regaining more moisture (9.4%). The moisture regain progressively increased towards the inner layer for *A. panda*.

However, there was a highly significant difference ($P > 0.05$) in weight loss percentage from the cocoon shells in both species (Fig. 11). The outer skin like layers had the highest weight losses of 30.5 and 44.6% for *E. bauhiniae* and *A.panda,* respectively and the weight loss significantly decreased to the inner layer in *E. bauhiniae.*

Table 7: Percentage moisture regain of layers of *Epiphora bauhiniae* and *Anaphe panda* cocoon shells (Mean ± S.E.)

Species	Layers	Moisture regain (%)	
		Cocoon shells	**Degummed fibers**
Epiphora bauhiniae	Outer	5.27 ± 0.35^{c}	8.5 ± 0.23^{a}
	Middle	5.0 ± 0.49^{c}	8.63 ± 0.72^{a}
	Inner	6.83 ± 0.35^{b}	8.37 ± 0.22^{a}
Anaphe panda	Outer	6.96 ± 0.56^{b}	8.85 ± 0.42^{b}
	Middle	7.46 ± 0.03^{ab}	8.43 ± 0.06^{ab}
	Inner	8.63 ± 0.45^{a}	$9.49 \pm .08^{a}$

Means followed by the same letter in a column are not statistically significant ($P < 0.05$) using the least significant difference test (LSD)

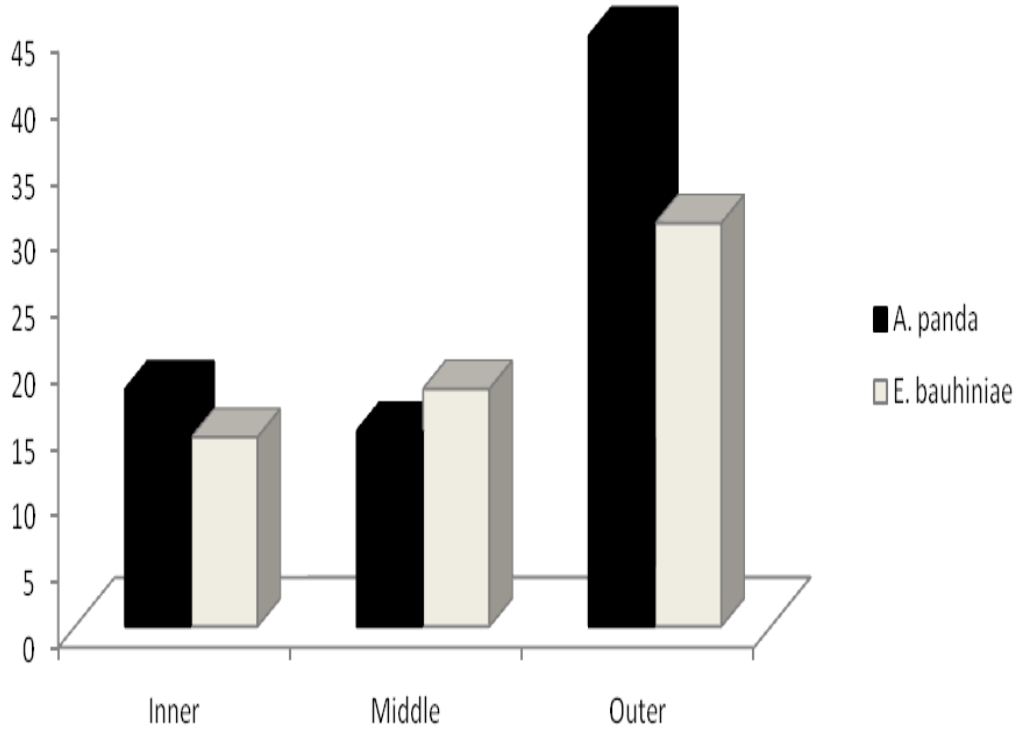


Figure 11: Percentage weight loss of layers of *A. panda* and *E. bauhiniae* cocoon shells

6.4. DISCUSSION

Weight loss and moisture regain varied among the African wild silk cocoon shells and degummed fibers. The weight loss and moisture regain for *B. mori* was well within the limits of 19 to 30% reported in other studies (Arami *et al.,* 2007; Gulrajani, 1988; Sarovart *et al.,* 2003). The weight loss from African wild silk cocoon shells ranged from 23 to 56.8 % and is inconsistent with previous reports that the sericin content of wild silk fibers is less than the mulberry silk and as low as 12 -16% (Cavaco-paulo and Gubitz, 2003; Prasong *et al.,* 2009). Karumar (1999) reported 5 - 15% sericin gum content for tussah silk fibers. The degumming loss in *A. proylei, A. assama, A. pernyi* and *A. yamamai* was in the range of 7 - 13% except for *A. mylitta* and *Philosamia Cynthia ricicni* which showed 3.7 and 4.5% loss, respectively (Kato, 1991). However, higher sericin gum content up to 38 and 68% have been reported for Thai silk, *Rondotia menciana* and *B. mandarina* cocoon shells, respectively (Dhavalikar, 1962; Ito *et al.,* 1992). Despite the higher values, the weight losses reported for the African wild silk cocoon shells might not be still the true reflection of the gum content of the cocoons as verified by the presence of remnant traces of sericin gum observed on the SEM images after degumming.

The variations in the amount of sericin gum and fibroin in cocoons shells might be due to a number of factors including genetic differences in the sericin protein as well as external environmental variables (Shamitha and Rao, 2006) which together can determine the quality of cocoons for commercial purposes (Singhvi and Bose, 1991). According to Kato and Hata

(1998) the cocoon filaments of polyphagous silkworms with coarse filaments have higher values for degumming losses than conventional cocoon filaments. This, together with the presence of calcium oxalate crystals, can be an explanation for the high weight loss of *G. postica* which is a polyphagous silkworm feeding on more than one acacia host plants including *Acacia tortilis, A. elatior, A. nilotica, , A. mellifera, A. mearnsii, A. hockii* and *A. brevispica* (Ngoka, *et al.,* 2007; Fening *et al.,* 2008). Nasreen *et al.* (1999) also reported difference in quality of *B. mori* cocoons from larvae fed on mixed leaves. Chemical composition of sericin also affects its solubility in hot water resulting in considerable variation in weight loss (Komatsu, 1975; Robson, 1985). The presence of certain amount of sericin after degumming shows its important role in processing and application of the fibers.

Physical properties and chemical composition of silk fibers from mulberry and wild silkmoths determine the variability in different parameters (Mondal *et al.,* 2007b; Srisuwan and Srihanam, 2009). Interestingly, there was an increase in moisture regain for all cocoon shells when fibers were degummed. Sen and Babu (2004a) also observed similar results that non-mulberry silk fibers had marginally higher moisture regain values of 10.8%. However, this is in contrast with Snowalker (1993) who reported degummed fibers to have lower moisture regain due to loss of sericin. The difference in moisture regain might be due to variations in compactness of the cocoon shells, change in the quantity, composition and chemistry of sericin gum across layers and surface structure of the fibers. Moisture regain of cocoon shells and fibers could provide information on the extent of areas accessible to water vapour within the fiber. The higher moisture regain for *A. mimosae* cocoon shells might be

due to the presence of holes/perforations on the cocoon surface providing more area for moisture ingress (Fig.7a-b). The presence of higher ratios of hydrophilic versus hydrophobic amino acid residues in the chemical architecture of non-mulberry compared to mulberry silks might also affect in the higher moisture regain of the wild silks (Table 5). The presence of larger voids in the wild silk fibers might also contribute to their higher moisture absorption than *B. mori* (Fig 9k-o).

General trends of decreased weight loss and increased moisture regain from outer to inner shell layers were observed in *A. panda* and *E. bauhinia* cocoons, respectivelye. Nawaz *et al.* (2002) revealed that with change in filament type and size (denier), moisture regain changes significantly due to the difference in structures of fibers as well as changes in liner density and arrangement of monomers in the polymer (Munro, 1987). The presence of foreign matters such as dust and hair like brittles on outer layer of *A. panda* suggested the influence of physical structure in preventing fibers from absorbing moisture. In conclusion, the variation in weight loss and moisture regain among the different cooons and shell layers can be the demonstration of the difference in the amount and composition of sericin present in each layer. Such layer wise variation in cocoon properties down the cocoon layers may confound objectionable fabric patterns either from visual or textural perspective (Das and Ghosh, 2010).

CHAPTER SEVEN

DISSOLUTION PROPERTIES OF COCOON SHELLS AND DEGUMMED FIBERS

7.1. INTRODUCTION

Raw silk consists of essentially two major proteins, fibroin (the core structural protein) and sericin (the surrounding glue gum). Sericin, which covers the periphery of the raw silk fibers, is highly soluble in hot water and can easily be removed in the process of degumming, while fibroin is not soluble in water (Arami *et al*., 2007). The solution behaviour of silk fibroin is of interest due to its novel self-assembly and processing related to fiber spinning in spiders and silkworms, resulting in remarkable mechanical properties (Matsumoto *et al.*, 2008). In recent years, fibroin from the domesticated silkworm, *B. mori* has been the dominant source for silk-based biomaterials and its dissolution properties are often required for non-textile applications. This is mainly due to the difficulty to dissolve silk fibroin in common solvents for obtaining a true solution because of its high molecular weight and crystallinity (Ki *et al.,* 2007a).

The insolubility of silk fibroin in common organic solvents is due to the intermolecular hydrogen bonding existing in the polymer and its hydrophobic nature (Furuhata *et al.,* 1994; Jin and Kaplan, 2003). Fibroin dissolution is further affected by non-uniformity in chemical composition, supra-molecular structure and morphological futures of natural fibers (Sashina

et al., 2006). The environmental factors and the processing conditions used by the silk producing insects also influence the solution behaviour and ultimately the material properties of these fibers (Matsumoto *et al.,* 2008).

Despite these difficulties, the solubility of silk in certain solvents has been studied and several proper solvent systems have been successfully used to dissolve silk fibroin including LiBr (Alessandrino *et al*., 2008), N-methylmorpholine-N-oxide (NMMO) hydrate (Plaza *et al*., 2008), phosphoric acid/formic acid mixture (Ki *et al*., 2007a), calcium nitrate (Kweon *et al*., 2001), and $CaCl_2$ (Miyaguchi and Hu, 2005). Ajisawa (1998) also recommended chaotropic reagents such as lithium thiocyanate *(*LiSCN), LiBr, NaSCN, and $CaCl_2$ as dissolution reagents for silk fibroin. However, the solubility of different silk fibers in these solvent systems was reported to be inconsistent. Srisuwan and Srihanam (2009) found *B. mori* silk is more soluble than Eri silk. *Antherea pernyi* fibroin is also difficult to dissolve due to the strong inter and intra-molecular interactions between its fibroin molecules and chains (Kweon *et al*., 2000; 2001). In this regard, the wild silk from Genus *Antheraea* is mostly studied (Dash *et al*., 2007). The African wild silkworms, which produce commercially important silk are found widely distributed in different geographical regions of Africa. However, little information is available so far on their structure and properties, of which dissolution properties are no exceptions.

7.2. MATERIALS AND METHODS

7.2.1. Determination of dissolution of cocoon shells and degummed fibers

Degummed fibers were dried in oven at 110°C for 24 hours and stored in desiccators prior to use. Analytical grades of $CaCl_2$, NaSCN and LiBr were used. Aqueous solutions (9M) were prepared by dissolving 299.68, 234.48 and 218.84g of $CaCl_2$, LiBr and NaSCN, respectively in 300ml distilled water and stirred with a magnetic stirrer at room temperature for ten minutes. Air dried degummed fibers (0.1g) from each species were dissolved in 10ml of each solvent solution at 70°C for 3 hours with gentle shaking of flasks at 40rpm in an incubator (Sah and Pramanik, 2010). Oven dried cocoon shell discs (1g) were also dissolved the same way. The resulting solutions were allowed to settle overnight. The undissolved silk was filtered through filter paper, washed, dried in a vacuum drying oven at 110°C for 24 hours and weighed. The dry weight remaining was expressed as a percentage relative to the initial weight and solubility was calculated as (Rastogi *et al.*, 2001; Kweon *et al.*, 2001):

$$\text{Solubility}(\%) = \frac{Wi - Wf}{Wi} \times 100$$

Where W_i = initial weight of the sample (g) and

W_f = final weight of the sample (g)

The experiment was replicated four times. Percentage solubility was arcsine transformed to bring the data to normal distribution and analyzed with two-way ANOVA with PROC ANOVA procedure (SAS Institute, 2010). LSD test (α=0.05) was used to separate the means.

7.2.2. Scanning Electron Microscope observation of fibers after dissolution

Dried fibroin supernatant of *A. panda, A. mimosae* and *E. bauhinae* were mounted onto copper stubs using double-sided sticking tape and sputter-coated with gold for three minutes. The samples were then observed with SEM.

7.3. RESULTS

7.3.1. Solubility of cocoon shells

Table 8 showed the percentage solubility of cocoon shells dissolved with 9M aqueous solutions of $CaCl_2$, LiBr and NaSCN. The results confirmed that dissolution property of cocoon shells varied depending on the origin of cocoon shells and the solvents used. The interaction effects of solvents and species was highly significant ($P > 0.05$). *B. mori* had the highest percentage solubility across the solvents used (51.5%) and LiBr had significantly higher dissolving ability (41.2%). There was no significant difference in the solubility of cocoon shells in $CaCl_2$ and NaSCN, even though the former had slightly higher dissolving ability (Table 8). Among the wild silk cocoon shells, *G. postica* had higher solubility. *G. postica* cocoon shells were dissolved completely in LiBr solutions and the supernatant was composed of the calcium oxalate crystals and remnants of cocoon spines and hairs.

Table 8: Percentage solubility of cocoon shells of four African wild silkmoths and *Bombyx mori* (Mean ± SE)

Species	**Solvents**			
	$CaCl_2$	**LiBr**	**NaSCN**	**Mean**
Bombyx mori	29.8±0.9[a]	97.7±0.4[a]	26.8±1.2[a]	51.5±23.1[a]
Anaphe panda	11.2±0.4[c]	21.5±0.8[c]	11.2±0.5[c]	14.6±3.4[c]
Argema mimosae	5.7±0.7[d]	7.7±0.6[d]	3.4±0.9[d]	5.6±1.2[c]
Epiphora bauhiniae	3.1±0.5[e]	3.2±0.4[e]	2.4±0.4[d]	3.1±0.3[e]
Gonometa postica	15.4±0.9[b]	74.7±1.3[b]	21.6±0.9[b]	37.3±18.8[b]
Mean	**13.1±4.7[b]**	**41.2±19.1[a]**	**13.1±4.8[b]**	**22.4±9.3**

Means followed by the same letter in the same column are not statistically significant ($P < 0.05$) according to Least Significant Difference (LSD) test

7.3.2. Solubility of degummed fibers

The dissolution of degummed fibers also showed significant differences ($P > 0.05$) in interaction of species and solvents. Like the cocoon shells, *B. mori* fibers also showed highest solubility in all the soluvents used (59.3%) and LiBr proved the best solvent agent for the fibers (84.5%) (Table 9). *G. postica* had non-significant solubility percentage (97%) as *B. mori* in LiBr. Unlike the cocoon shells, there was a significant difference in dissolution percentage of fibers in $CaCl_2$ and NaSCN (18.2 and 25.4%, respectively). Much of the solid fibers of *A. mimosae*, *A. panda* and *E. bauhiniae* in these solvents were intact after three hours of treatment, which indicate that dissolution of fibers was not complete in these species.

Table 9: Percentage solubility of African wild and *Bombyx mori* degummed silk fibers (Mean ± SE)

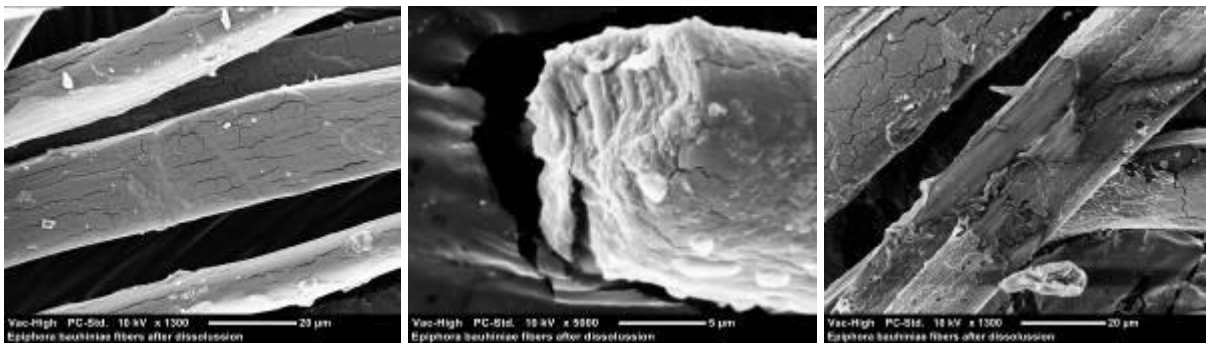
Species	**Solvents**			
	$CaCl_2$	**LiBr**	**NaSCN**	**Mean**
Bombyx mori	29.9±1.2[a]	99.1±0.2[a]	48.8±1.9[a]	59.3±20.6[a]
Anaphe panda	12.5±1.1[c]	84.4±0.6[b]	20.5±1.8[c]	39.1±22.7[c]
Argema mimosae	12.1±1.3[c]	77.2±0.6[c]	11.2±1.5[d]	33.5±21.8[d]
Epiphora bauhiniae	14.1±1.0[c]	64.4±1.0[d]	11.5±1.4[d]	30.1±17.2[e]
Gonometa postica	22.2±0.6[b]	97.5±0.2[a]	35.4±1.7[b]	51.7±23.2[b]
Mean	**18.2±3.4[c]**	**84.5±6.5[a]**	**25.4±7.3[b]**	**42.7±21.0**

Means followed by the same letter in the same column are not statistically significant ($P < 0.05$) according to Least Significant Difference (LSD) test

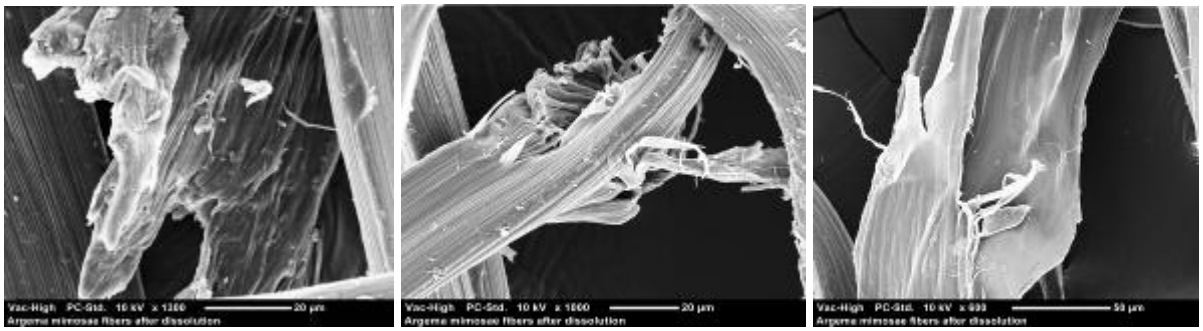
The SEM micrographs for these fibers after treatment with LiBr highlighted the differences in the mechanism of dissolution (Figure 12). It was noted that part of the fibers of *A. panda* in LiBr showed a strong tendency of gelling and were difficult to handle (Figure 12a). This might be an indication for the need for longer time of exposure for complete dissolution. On

the other hand, *E. bauhiniae* fibers were cracked and signs of disintegration and fractures were observed on the surface (Fig. 12b), while fibers of *A. mimosae* were broken in to pieces exposing the individual fibrils (Fig. 12c).

(a) *Anaphe panda*



(b) *Epiphora bauhiniae*



(c) *Argema mimosae*

Figure 12: SEM micrographs of *Anaphe panda*, *Epiphora bauhiniae* and *Argema mimosae* fibers after dissolution with 9M LiBr

7.4. DISCUSSION

The study clearly showed that African wild silk cocoon shells and degummed fibers have variability in their solubility in different solvent systems. LiBr exhibited higher dissolving ability than the other solvents. However, this is in contrast with Srisuwan and Srihanam (2009) who reported that 9M LiBr had the least ability to dissolve *Philosamia ricini* silk fibroin. The low solubility of *A. panda*, *E. bauhiniae* and *A. mimosae* cocoon shells and degummed fibers in all the solvent systems could be due to the short time of treatment or the solvent systems were not strong enough to produce the desired level of dissolution. The temperature and concentration of the solutions could also be a factor for low solubility of the cocoon shells. Other wild silks, such as *A. pernyi* silk fibroin also require a high concentration of chaotropic salts, high temperature, and longer treatment time (Kweon *et al.*, 2000). Miyaguchi and Hu (2005) also reported that the solubility of *B. mori* silk fibroin increased sharply with higher concentration of $CaCl_2$, addition of ethanol and longer heating periods. The presence of sericin/gum and other impurities on the cocoon shells might also have prevented the penetration of the solvents and resulted in lower solubility than the degummed fibers. The difference in solubility showed the variability existing in chemical structure and composition among the African wild silk cocoon shells and fibers.

The solubility of the African wild silk cocoon shells and fibers was significantly lower than *B. mori* except *G. postica* in LiBr solution demonstrating the presence of clear difference in chemical composition among the silks tested. Solubilisation of non-mulberry cocoons with

LiBr and LiSCN proved also to be less effective and resulted in low yield than *B. mori* (Mandal and Kundu, 2008). Dissolution of a protein was reported to depend on its structure, molecular weight and structure of its macromolecules, and polarity and steric arrangement of the side groups (Sashina *et al.*, 2003). The amino acid sequence of polypeptides also plays a very important role in the solubility and crystallization of silk fibroins (Tanaka *et al.*, 2002).

Fabrication of large amount of fiber wastes produced from the silk industries involves formation of reGenerated fibroin materials such as solution, powder, film, gel, and filament by dissolving in proper solvent systems depending on its preparation conditions and application field (Sashina *et al*., 2006). Active efforts are being made to develop processes involving preparation of working solutions of natural polymers and their conversion into particular, fibers and films. Although the African wild silk is solely utilized as a textile raw material, the recent venture and development of silk in various areas of applications requires further processing such as dissolving in different solvent systems using several methods. Solutions of fibroin which can easily be transferred in to gels, powder or films for biomaterial preparation are required. For this, solution systems, which dissociate the intra-molecular bonding of silk fibers without breaking the polypeptide chains, are essential. In this regard, even though this study is far from this confirmation, it sheds light on the solution properties of the African silk fibers and their potential for subsequent preparation of films, scaffolds and fibers for various applications.

CHAPTER EIGHT

TENSILE PROPERTIES AND THERMAL DEGRADATION BEHAVIOURS OF COCOON SHELLS AND DEGUMMED FIBERS

8.1. INTRODUCTION

Natural fibers from animals and plants have found their way into commercial applications, once again. Silk is a unique class of structural animal fiber and has long been regarded as a superb natural material due to its characteristic high strength, elongation, feel and luster. The variation in composition, structure and properties of silks from different arthropods has led to their considerations in wider applications expanding from more traditional textile industries to fields such as biomedical, biotechnological, and tissue engineering. The usefulness of silk fibers in these and many other applications is associated with its predominant failure mechanism under the conditions of the application and its adaptability to varied environmental conditions facilitated by the silk's molecular composition and hierarchical structure. These, in turn are both affected by the conditions during its production such as spinning speed, relative humidity, temperature, pH, ionic strength, solvent composition and mechanical stress as well as the degumming process (Chen *et al*., 2001; Shao and Vollrath, 2002; Pe´rez-Rigueiro *et al.,* 2002; Zhang *et al.,* 2010b; Manjunatha *et al.,* 2010).

The mechanical properties of silk fibers also depend on the structure of the silk constituent proteins (sericin and fibroin) before and after silk fiber formation (Lin *et al.,* 2009). For

example, sericin coating adds to the tensile properties of the silk fiber that are primarily determined by the fibroin structure (Jiang *et al*., 2006). Sericin could also induce the transition of silk fibroin from the random coil or α-helix to the β-sheet structure, and further improve the mechanical properties of silk fibers (Ki *et al.,* 2007b). High crystallinity confers the silk material greater strength by virtue of the network of hydrogen bonds between and within the proteins (Sutherland *et al.,* 2010).

Temperature and moisture are among the environmental stresses influencing properties and functions of silk cocoons and fibers. Cocoon shells are important in temperature regulation, water loss reduction from the pupae and acquisition of heat (Rosner and Führer, 1996; Danks, 2004). Temperature also influences the amount of water absorbed by silk fibers resulting in alteration of physical properties. Chemical composition and structure of silk fibers determine the thermal tolerance behaviour of the cocoon shells and fibers and the resulting weight loss due to dehydration and/or decomposition. Hence, it is appropriate to deploy Thermal Analysis (TA) to investigate the response of natural materials such as silk, to temperature scans and to explore together with other data, the structure-property-function relations of natural fibers (Wiedemann and McKarns, 1990). Accordingly, a comparative study of the mechanical properties and thermal degradation behaviours of cocoon shells and degummed fibers from four African wild silkmoth was studied.

8.2. MATERIALS AND METHODS

8.2.1. Determination of tensile properties of cocoon shells

Cocoon shells of *G. postica* and *A. mimosae* were used due to their single cocoon shell structure. *B. mori* (Race *icipe* II) cocoon shells were used for comparison. Three full cocoon shell strips (dimensions of 5x15mm) were cut longitudinally from the middle sections of each cocoon and five cocoon shells were used for each species. Instron 5542 tensile testing instrument (load cell 500N) was used for tensile testing. Tensile test was carried out at room temperature with gauge length of 5mm and at a speed of 2mm/min.

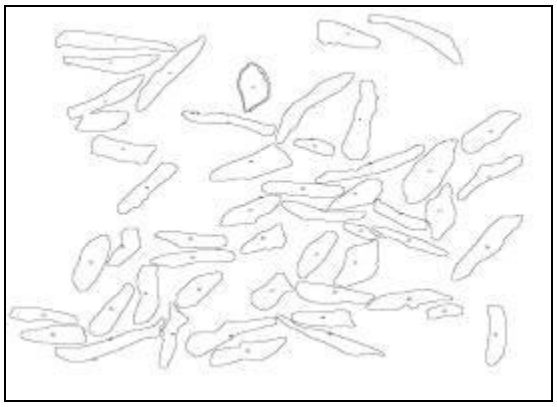
8.2.2. Determination of tensile properties of degummed fibers

The tensile test was carried out in accordance with the American Standard for Test and Measurement (ASTM) D 3379-75. Fibers (100mm long) were obtained by pulling gently after degumming and were left overnight in air before testing. The fibers were then cut gently into 30mm ensuring that the fibers were not stressed plastically during the process. Every fiber was then mounted and taped across a hole, which is 10mm long, of a rectangular cardboard which was then fixed in an Instron 5542 materials testing machine (5N load cell). Before being tested, each specimen was examined under an optical microscope to ensure that only single fibers were used. The gauge length was adjusted at 10mm. The cardboard was then cut on both sides and separated into two parts to ensure tensile loading was completely transmitted to the fiber during test. All tests were conducted at a rate of 0.1mm/sec at 22°C

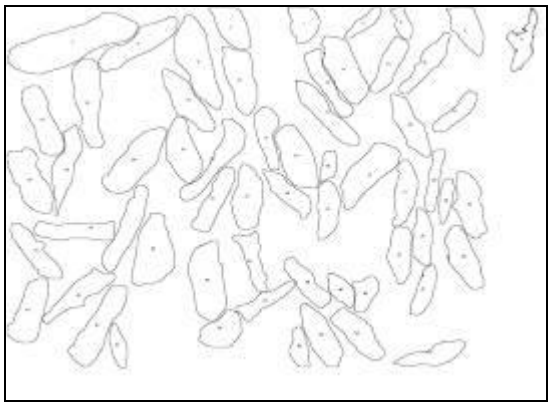
and 55% RH. Ten tests were made for each species to Generate the average tensile stress-strain curve.

8.2.2.1. Measurement of cross sectional area of fibers

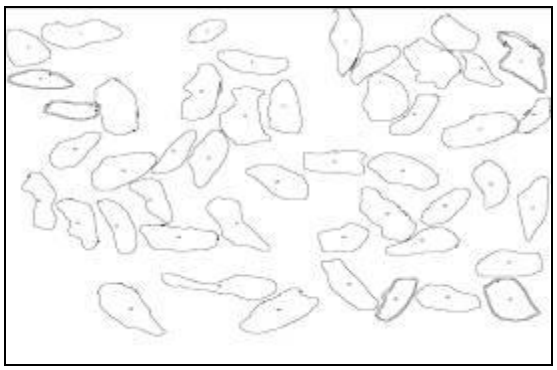
Cross sectional areas of the fibers were prepared by clamping orientated silk threads in a holder and cutting them across with a new razor blade before sputtering them with gold for SEM viewing. Digital images of transverse sections on the SEM micrographs were nalyzed with ImageJ 1.42q software (Fig 13). Normalized cross-sectional areas were obtained by averaging 150 brins for each sample assuming that sample volume will be conserved during the tensile tests (Pe´rez-Rigueiro *et al.*, 1998).



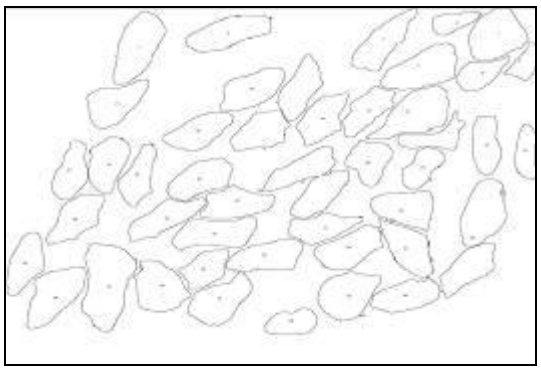
(a) *Argema mimosae*



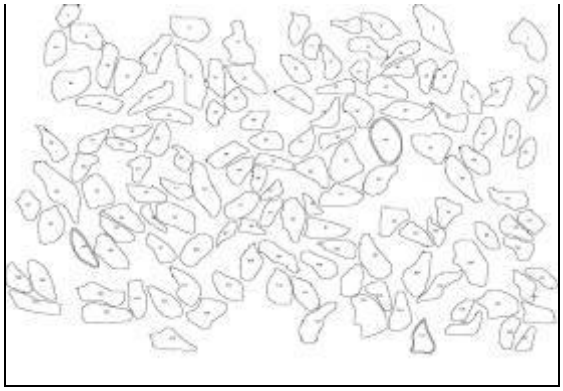
(b) *Epiphora bauhiniae*



(c) *Gonometa postica*



(d) *Bombyx mori*



(e) *Anaphe panda*

Figure 13: Imagej software images of the cross sectional area of African wild and *Bombyx mori* silk fibers

8.2.3. Analysis of fracture behaviour of cocoons and fibers

Cocoon shells and fibers after the tensile failure were used. Tested samples which were broken at the middle were mounted on a copper stub with a double-sided sticking tape and sputter coated with gold and then were examined under SEM to analyze the fracture mechanisms.

8.2.4. Determination of thermal degradation behaviours of cocoon shells and degummed fibers

Discs of cocoon shells and degummed fibers (7mm in diameter) were pressed out from middle section of the cocoon and the compressed degummed fibers floss, respectively with a sharp hole-punch. The samples were loaded into a Q50 TGA from TA instruments. Test conditions included temperature ranges of 25 - 900 and 25 - 800°C for cocoon shells and degummed fibers, respectively, at a heating rate of 20°C/min, N_2 flow of 60mL/min and air cool time of 40 minutes. Samples of cocoon shell discs and degummed fibers after TGA were mounted onto copper stubs, sputter-coated with gold and examined with SEM.

All Instron and TGA data were analyzed with one-way ANOVA with PROC ANOVA procedure (SAS Institute, 2010). LSD test (α=0.05) was used to separate the means. The tensile parameters were calculated with a home designed program in Excel (Tensile Import v2.0) (developed by University of Oxford Silk Research Group). Stress-strain curves were plotted using Origin Pro 8© programme.

8.3. RESULTS

8.3.1. Tensile properties of cocoon shells

The measured data of the tensile properties of cocoon shells was presented in Table 10, and the average stress-strain curves were presented in Figure 14. The results clearly demonstrated that the tensile properties of silk cocoon shells differed from one species to another. Modulus reduced as strain increased and the binding points between fibers were observed to break progressively in the cocoon shells. After a peak stress, there was rapid fall in stress, which showed the continuous fiber bonding present in the cocoon shells was broken and replaced by simple entangled fibers. *G. postica* and *A. mimosae* had lower breaking stress and breaking energy and higher breaking strain and initial modulus than *B. mori* (Table 10). However, the strain at maximum stress and the modulus were not statistically significant for the cocoons tested.

The SEM micrographs of cocoon shells fracture surfaces after failure were observed (Fig. 15a-c). It was clear that the surrounding gum of the cocoon shells was still intact connecting the cocoon fibers together in a bonded but open network. Fiber debonding was observed in all the species tested. Some of the silk fibers within the fracture area were broken and pulled out from one part of the cocoon specimen. The micrographs also clearly showed fiber pull-out and debonding predominated the fracture mechanism with fairly clean and recognizable fiber surface without matrix adherence. In addition, crack initiation sites and propagation through

the matrix were observed. The clear fracture surfaces showed poor fiber interfacial bonding in cocoon shells.

Table 10: Tensile properties of *Gonometa postica*, *Argema mimosae* and *Bombyx mori* cocoon shells (Mean ± SE)

Species	**Initial modulus (MPa)**	**Maximum stress (MPa)**	**Strain at Max stress (%)**	**Breaking energy (J/cm^3)**
Argema mimosae	404.6±59.8[a]	69.6±7.0[c]	21.2±1.0[a]	13.8±1.9[b]
Gonometa postica	397.4±63.4[a]	47.2±1.7[b]	23.3±1.2[a]	11.4±0.6[b]
Bombyx mori	334.5±28.3[a]	101.1±4.2[a]	20.8±1.2[a]	20.1±0.7[a]

Means followed by the same letter in the same column are not statistically significant ($P < 0.05$) according to Least Significant Difference (LSD) test

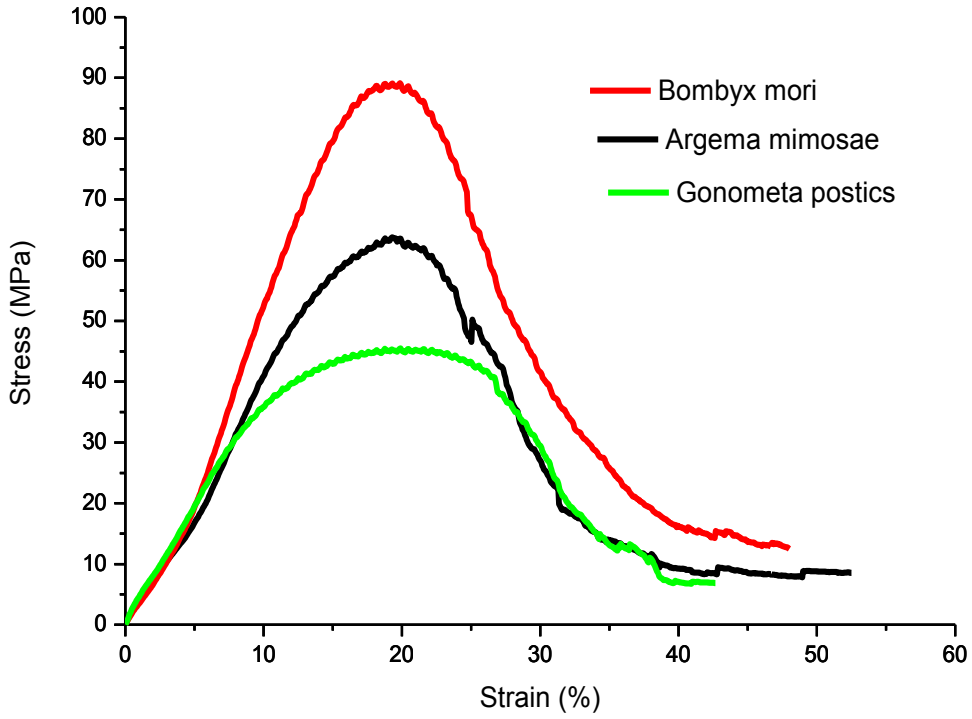
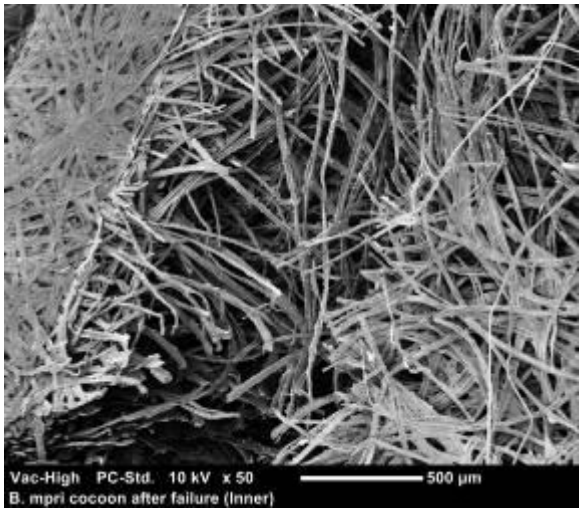
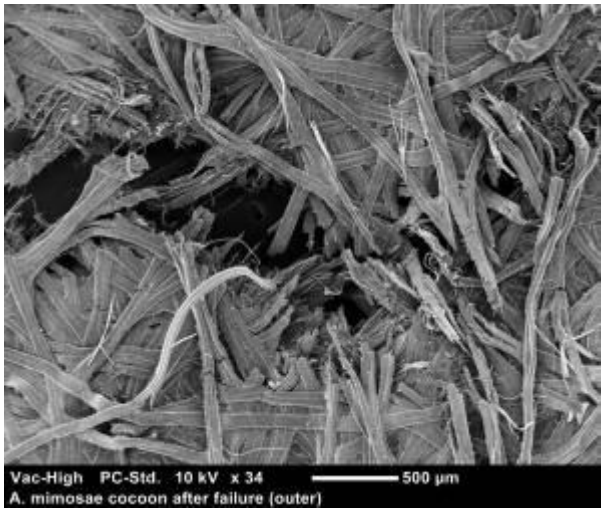


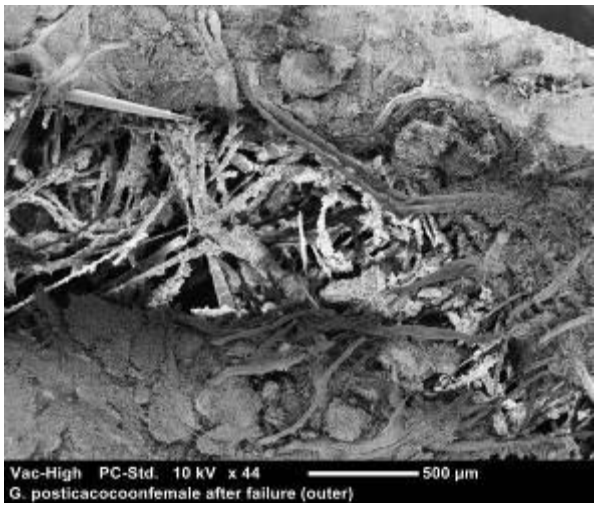
Figure 14: Stress-strain curves for *Gonometa postica*, *Argema mimosae* and *Bombyx mori* cocoon shells



(a) *Bombyx mori*



(b) *Argema mimosae*



(c) *Gonometa postica*

Figure 15: SEM micrographs of fracture surfaces of *Gonometa postica*, *Argema mimosae* and *Bombyx mori* cocoon shells after tensile failure

8.3.2. Tensile properties of degummed fibers

The tensile properties of degummed fibers of African wild silks also showed significant variability ($P > 0.05$) as shown in Table 11. *G. postica* fibers had the highest breaking strain (41.3%) and breaking energy (76J/cm^3). *E. bauhinae* had the lowest breaking stress (247MPa). All of the other wild silk fibers had breaking energy between 34-52J/cm^3 that was lower than *B. mori* (66J/cm^3) (Table 11). However, *B. mori* had the highest breaking stress (427MPa), which was not statistically significant with *A. panda* and *A. mimosae*. The stress-strain curves of *A. mimosae*, *E. bauhiniae* and *G. postica* fibers showed sigoidal shape and three regions were distinguished: an initial linear elastic region, a yield region, and a hardening region (Figure 16). However, *B. mori* and *A. panda* fiber started with an elastic region followed directly by strain hardening where the stress increases non-linearly with strain. Despite the average cross sectional area used to calculate the tensile properties, wild silk fibers showed noticeably higher standard deviation than *B. mori* indicating the considerable variation in the diameter of the fibers along their length and between fibers (Table 12).

Table 11: Tensile properties of single African wild and *Bombyx mori* silk fibers (Mean ± SE)

Species	**Initial modulus (MPa)**	**Ultimate Tensile Strength (MPa)**	**Ultimate Tensile Strain (%)**	**Breaking energy (J/cm^3)**
Anaphe panda	6344.4±798.9^{b}	365.1±51.1^{ab}	17.7±0.7^{c}	43.3±7.2^{cd}
Argema mimosae	8326.5±742.0^{a}	363.4±6.5^{ab}	20.7±1.0^{bc}	51.6±2.9^{bc}
Epiphora bauhiniae	7497±331.7^{ab}	247.7±6.3^{c}	20.8±1.3^{b}	34.5±2.4^{d}
Gonometa postica	7153.9±544.6^{ab}	310±37.1^{b}	41.3±1.4^{a}	76.4±8.2^{a}
Bombyx mori	8787±555.1^{a}	427.6±10.6^{a}	21.8±0.5^{b}	66±2.8^{ab}

Means followed by the same letter in the same column are not statistically significant ($P < 0.05$) according to Least Significant Difference (LSD) test

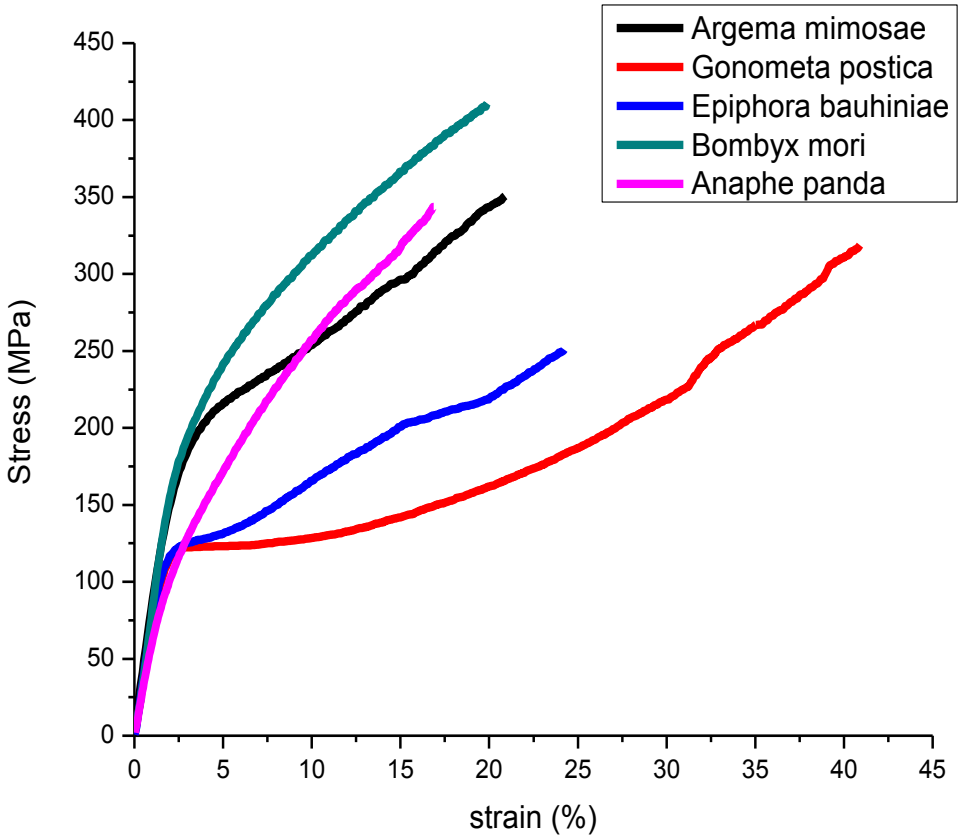


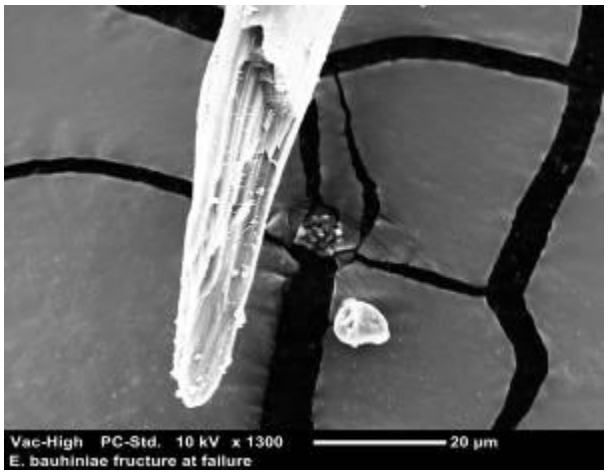
Figure 16: Stress- strain curves for African wild and *Bombyx mori* degummed single silk fibers

Table 12: Cross sectional area of African wild and *Bombyx mori* silk fibers (brin) (Mean ±SE)

Silk fibers	**Area (µm^2)**	***n***
Anaphe panda	96.7± 5.7^c	150
Argema mimosae	388.5± 14.5^a	150
Epiphora bauhiniae	100.4± 5.3^c	150
Gonometa postica	161.4± 8.2^b	150
Bombyx mori	59.9± 2.5^d	150

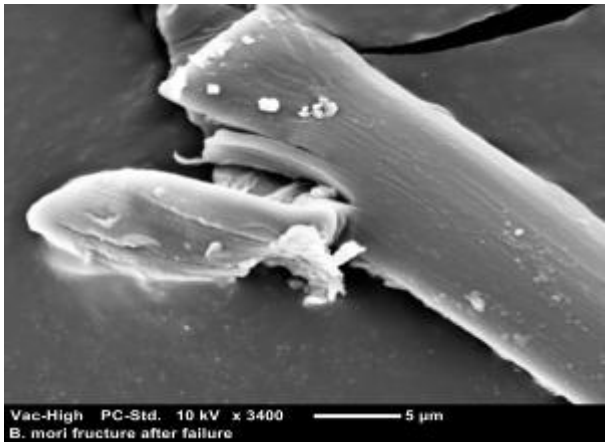
Means followed by the same letter in the same column are not statistically significant ($P < 0.05$) according to Least Significant Difference (LSD) test

The fractures of fibers after failure were shown in Fig. 17 (a -e). It was not possible to Generalize the fracture behaviours for the fibers tested. However, *B. mori* and *A. panda* fibers seemed to store the stress by stretching and then fiber pull-out occurred. *G. postica* fibers showed fiber crack or split during failure. However, *A. mimosae* and *E. bauhiniae* had ripped smooth failure surfaces.

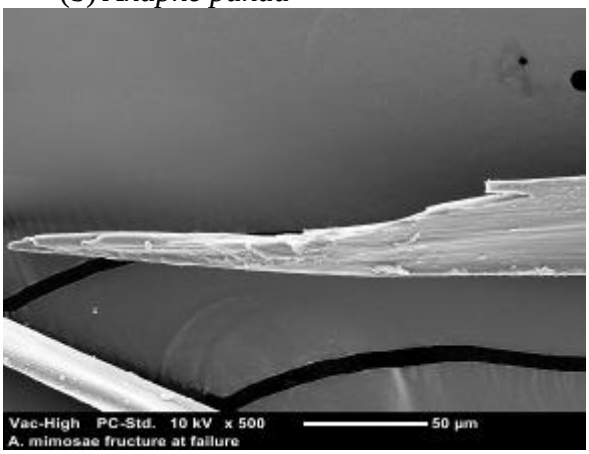


(a) *Epiphora bauhinae*

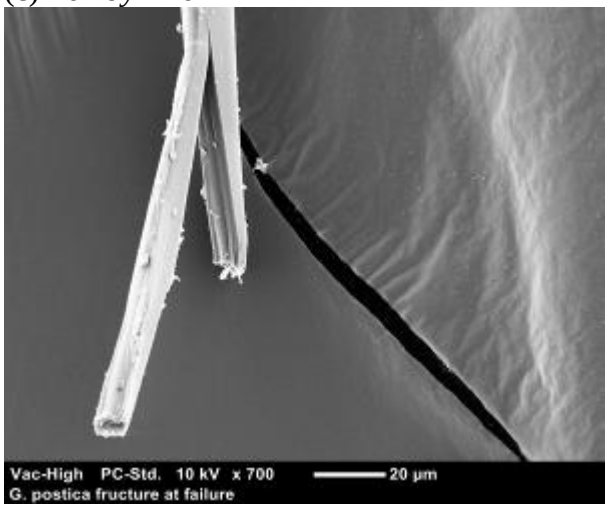
(b) *Anaphe panda*



(c) *Bombyx mori*



(d) *Argema mimosae*



(e) *Gonometa postica*

Figure 17: SEM micrographs of fracture surfaces of degummed fibers after tensile failure

8.3.3. Thermal degradation behaviour of cocoon shells

The dehydration loss from cocoon shells was highest for *A. mimosae* (11.9%) and lowest for *B. mori* (9.8%) (Table 13). However, the weight losses from the other wild silk cooons were non-significant ($P < 0.05$). Figure 18 illustrated the thermal decomposition behaviours of cocoon shells. The TG curves showed that weight loss due to dehydration of absorbed water molecules associated in cocoon shells continued rapidly up to 101, 120, 125, 154, and 127°C for *B. mori, G. postica E. bauhiniae, A. mimoae,* and *A. panda,* respectively.Substantial weight losses followed the stable or gradual decrease in weight as heating temperature increased. Rapid weight losses begun at 300, 306, 302, 313, and 296°C for *B. mori, E. bauhiniae, A. mimosae, A. panda* and *G. postica,* cocoons, respectively. *B. mori* cocoons had relatively inferior thermal resistance than most of the wild silk cocoon shells. The thermal decomposition of *G. postica* cocoons occurred in multiple steps than the rest of the species with additional decompositions occurring at temperature ranges of 483-506 and 710-730°C. In *A. mimosae,* weight loss became stable and constant after the rapid weight loss while other species continued to lose weight gradually to the end. *E. bauhinae* and *G. postica* cocoon shells had the highest and lowest total weight losses of 97.4 and 75.5%, respectively.

The SEM images in Figure 19(a-e) showed the cocoon shells after heat exposure. *A. mimosa*e, *A. panda* and *E. bauhiniae* decomposed cocoon discs partially maintained the fiber appearance though decomposition was significant, while *B. mori* and *G. postica* cocoon discs were completely decomposed and lost their integrity in the process.

Table 13: Weight loss of African wild and *Bombyx mori* degummed silk fibers and cocoon shells after TG analysis (Mean ± SE)

Species	**Dehydration (%)**		**Decomposition (%)**		**Total weight loss (%)**	
	Cocoon shells	**Fibers**	**Cocoon shells**	**Fibers**	**Cocoon shells**	**Fibers**
Anaphe panda	9.9±0.5^{b}	11.1±0.4^{b}	52.0±0.1^{b}	51.5±0.3^{b}	91.3±0.1^{b}	85.9±4.0^{a}
Argema mimosae	11.9±0.1^{a}	12.8±0.0^{a}	61.2±0.5^{a}	59.2±1.9^{a}	74.9±0.5^{c}	71.8±1.0^{b}
Epiphora bauhiniae	11.8±0.1^{a}	11.9±0.0^{b}	46.5±0.3^{d}	47.2±1.2^{c}	97.2±0.1^{a}	87.3±2.3^{a}
Gonometa postica	11.5±0.8^{a}	13.3±0.4^{a}	50.4±1.1^{c}	53.1±1.4^{b}	71.2±1.5^{d}	77.3±3.4^{b}
Bobmyx mori	9.8±0.1^{b}	8.5±0.3^{c}	49.5±0.2^{c}	54.6±0.3^{b}	95.1±0.4^{a}	93.4±2.4^{a}

Means followed by the same letter in the same column are not statistically significant ($P < 0.05$) according to Least Significant Difference (LSD) test

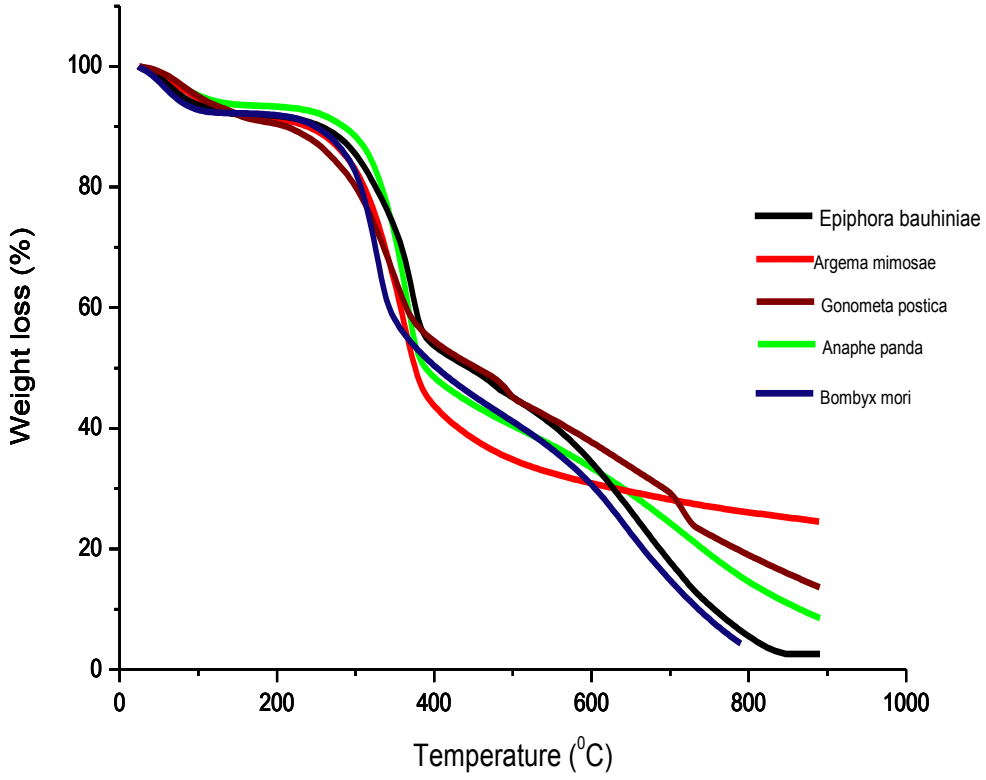
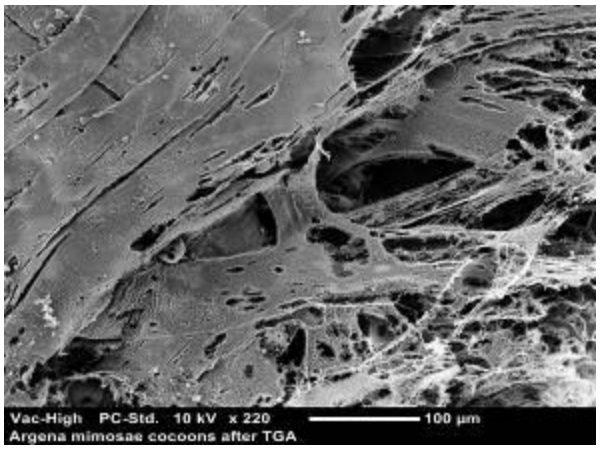
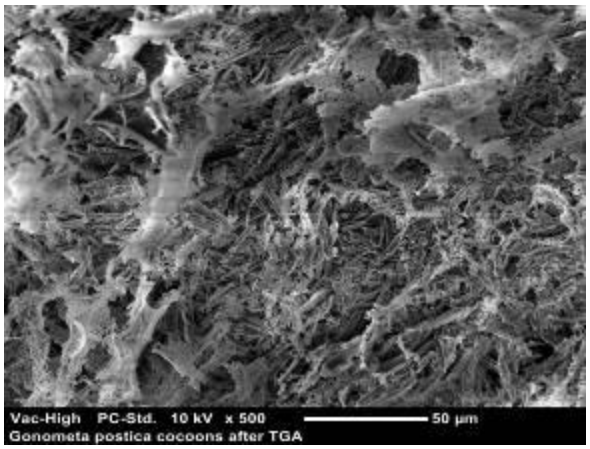


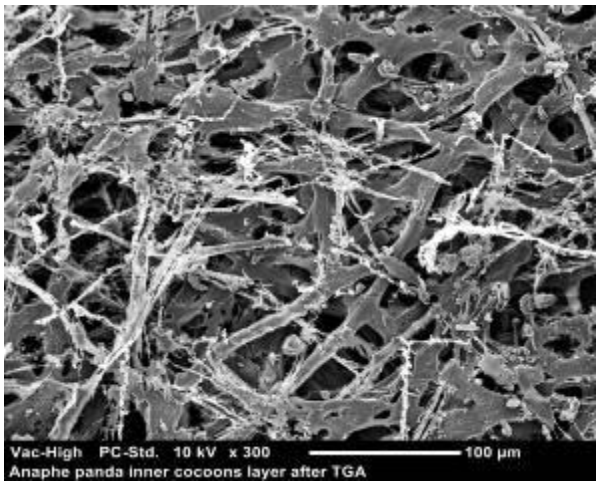
Figure 18: TGA curves for cocoon shells of African wild and *Bombyx mori* silkmoths



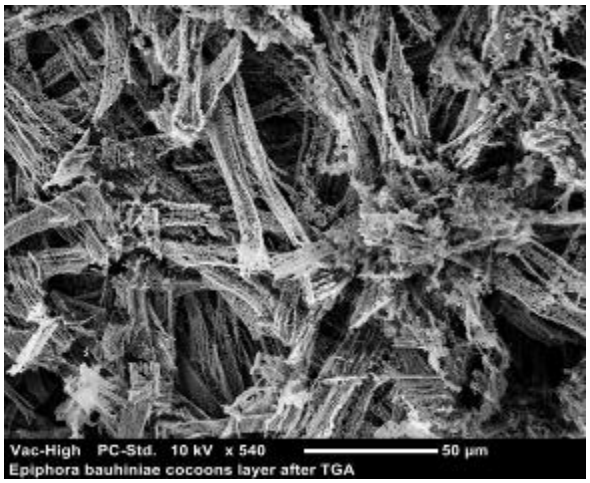
a) *Argema mimosae*



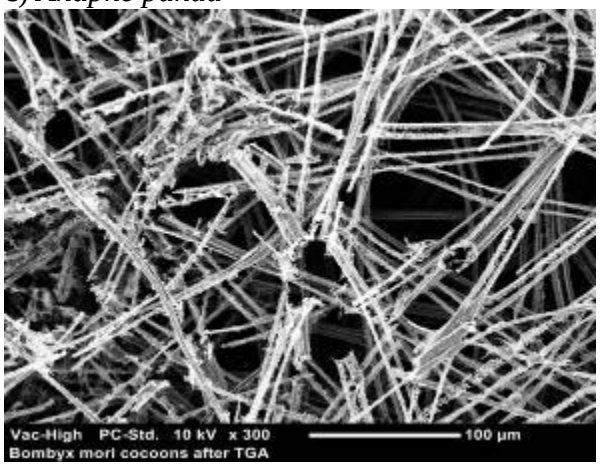
b) *Gonometa postica*



c) *Anaphe panda*



d) *Epiphora bauhiniae*



e) *Bombyx mori*

Figure 19: SEM micrographs of cocoon shells discs of African wild and *Bombyx mori* silkmoths after TG analysis

8.3.4. Thermal degradation behaviour of degummed fibers

The TGA curves in Figure (20) showed the weight losses from degummed fibers. Initial weight losses due to dehydration of the fibers occurred rapidly up to 100, 107, 112, 100, and 107°C for *B. mori, E. bauhiniae, A. mimosae, A. panda* and *G. postica*, respectively. The dehydration loss was the highest for *G. postica* (13.3%) and the lowest for *B. mori* fibers (8.5%) (Table 13). Major decomposition of degummed fibers commenced at 303, 301, 292, 312 and 290°C for *B. mori, E. bauhiniae, A. mimosae, A. panda* and *G. postica*, respectively. Unlike the cocoons, *B. mori* fibers were slightly more thermally stable than most of the wild silk fibers. The weight loss after the rapid decomposition was the hieghest in *A. mimosae* (59.2%) and the lowest in *E. bauhiniae* (47.2%) (Table 13). The multistep thermal degradation of *G. postica* cocoon shells could not be observed in degummed fibers. *B. mori* fibers had the highest total weight loss (93.4%), though it is not significantly different from other species except *A. mimosae* and *G. postica*.

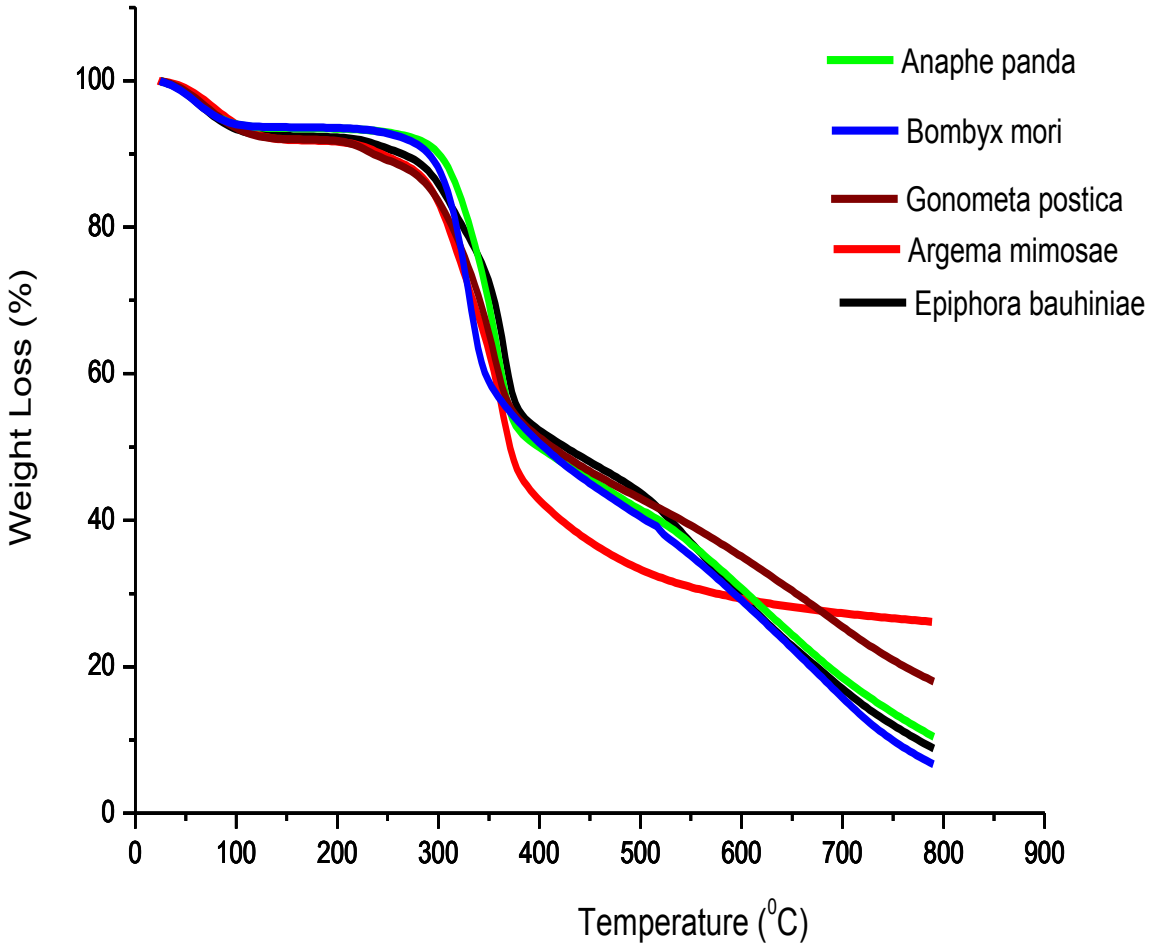
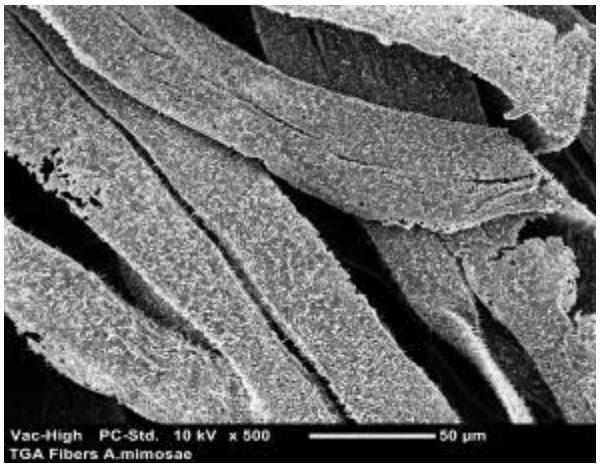
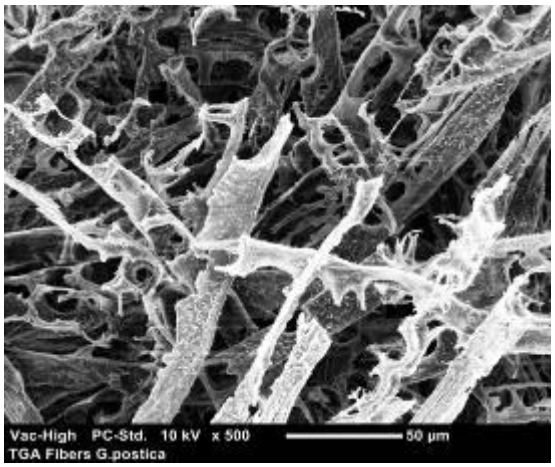


Figure 20: TGA curves of African wild and *Bombyx mori* degummed silk fibers

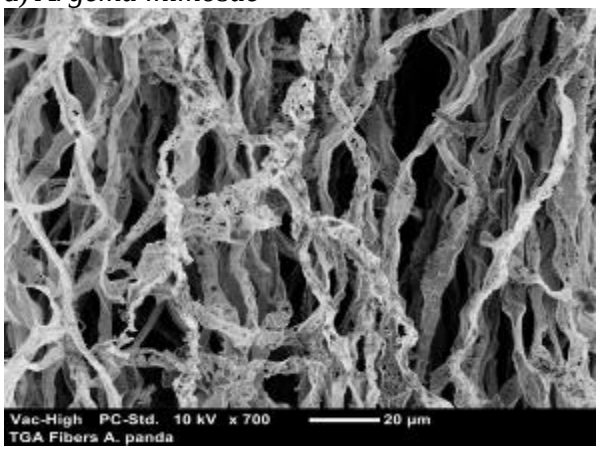
The SEM images in Figure 21(a-e) showed degummed fibers maintained their shape in *A. mimosa*e, *A. panda* and *E. bauhiniae* while *B. mori* and *G. postica* fibers were completely decomposed and lost their integrity in the process. In *A. mimosae* and *E. bauhiniae*, the broken individual fibrils became more exposed and were easily seen after the heat treatment (Fig 22a-b). Rapid decomposition occurred at slightly higher temperature for the cocoon shells than the degummed fibers in all the African wild silks except for *B. mori* (Fig 23).



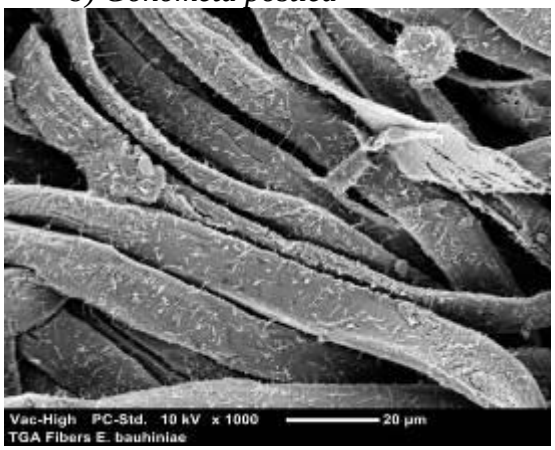
a) *Argema mimosae*



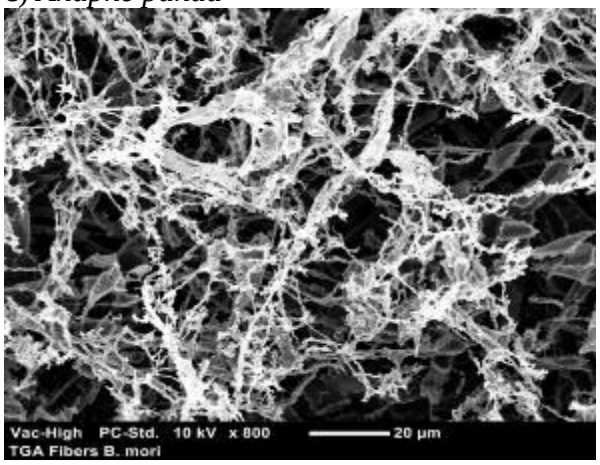
b) Gonometa postica



c) *Anaphe panda*

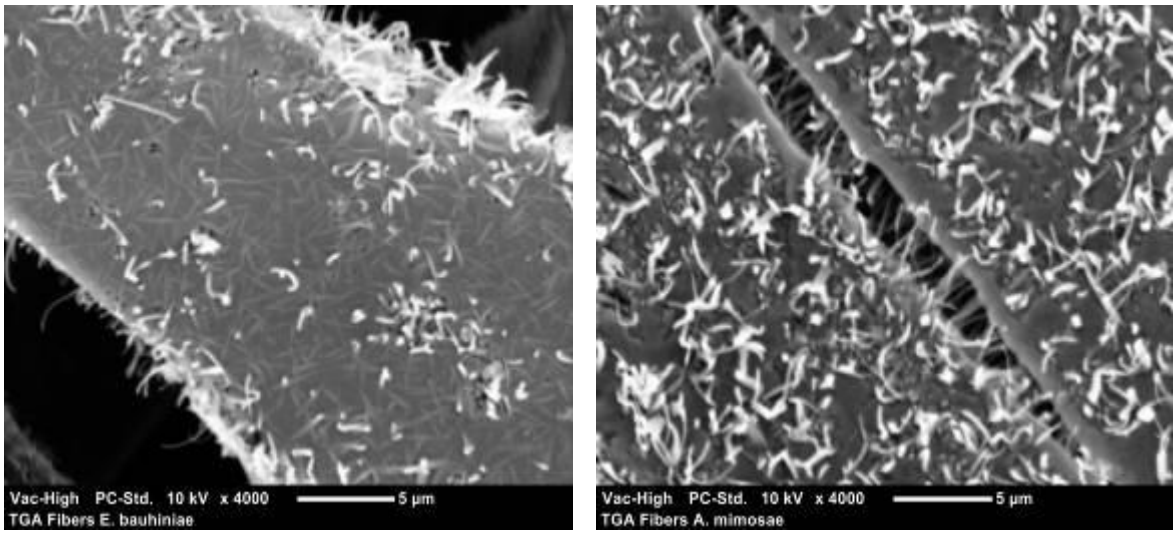


d) *Epiphora bauhiniae*



e) *Bombyx mori*

Figure 21: SEM micrographs of African wild and *Bombyx mori* degummed silk fibers after TG analysis



(a) *Epiphora bauhiniae* (b) *Argema mimosae*

Figure 22: High magnification SEM micrographs of *Argema mimosae* and *Epiphora bauhiniae* fibers after TG analysis

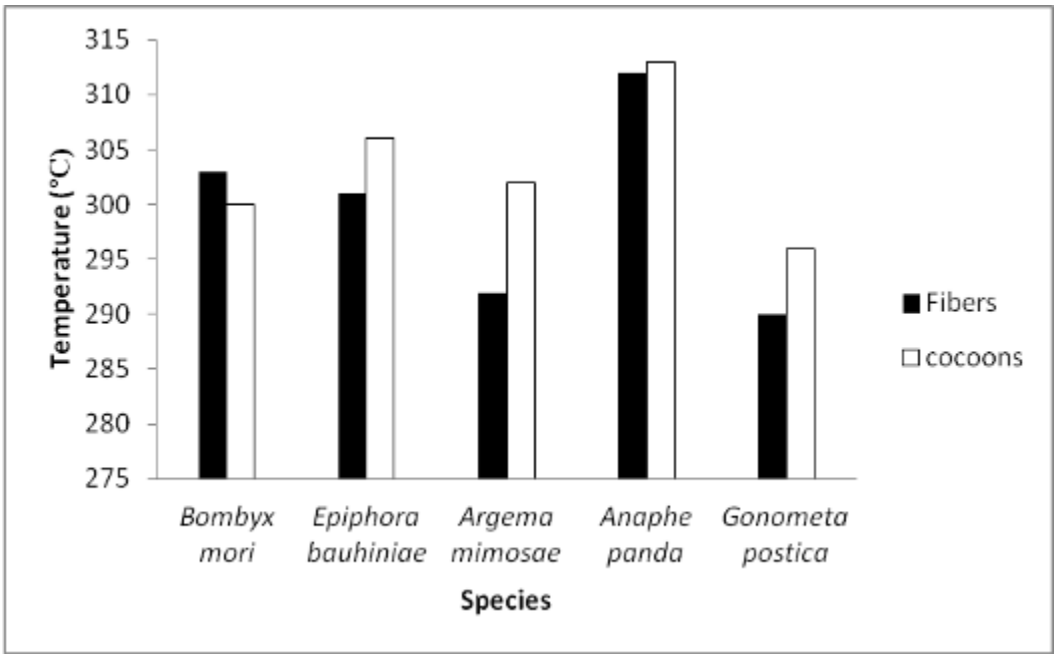


Figure 23: Decomposition on-set temperatures for cocoon shells and degummed fibers of African wild and *Bombyx mori* silks

8.4. DISCUSSION

The results demonstrated the existence of distinct variations in the mechanical properties and thermal degradation behaviours among the four Africa wild silk cocoon shells and degummed fibers. The stress-strain curves, which relate the applied stress to the resulting strain, revealed that each species has its own unique stress-strain response. This has also been reported for other mulberry and non-mulberry varieties (Das *et al.,* 2005; Rajkhowa *et al.*, 2000). *B. mori* and *A. panda* fibers showed absence of a well-defined yielding point and strain-hardening region suggesting the presence of well-developed crystalline regions that are responsible for smaller and gradual elongation with increasing stress (Sen and Babu, 2004b). Other studies reported higher tensile modulus and tensile strength for the normal compact cocoons (0.66GPa and 53.98MPa, respectively) which is inconsistent with the findings here (Huang *et al.,* 2008). However, Zhao *et al.* (2005) reported comparable tensile modulus and breaking strain for *B. mori* compact cocoon shells tested along longitudinal directions. It was not possible to correlate the results of the tensile properties for the African wild cocoon shells with other findings. However, the variations in the measured data for these cocoon shells might be attributed to several reasons. The irregular silk fiber arrangement and wrinkles throughout the cocoon shells that are related to the environmental conditions during cocoon formation and drying play a significant role in determining the cocoon propeties. In addition, the variation in cocoon thickness, and the entire spinning process might also have contributed to the observed differences. The main reason for poor mechanical properties in cocoon shells of *G. postica* might be the presence of calcium oxalate crystals combined with the high gum

contents leading to easy rapture and weak bonding between the fibers as can be evidenced from the SEM micrographs of the fractured surfaces.

The study also revealed that *A. panda* and *E. bauhiniae* silk fibers have comparable mechanical properties to the commercial standard, *B. mori*. The typical silk from *B. mori* cocoon has a tensile strength of about 0.5GPa, breaking strain of 15–22%, and tensile elastic modulus 7–17GPa (Gosline *et al.,* 1999; Hakimi *et al.,* 2007). Thus, for *B. mori,* the present findings match consistently with previous results. In most of the results, the wild silk fibers showed non-significant difference in the measured tensile properties. Sen and Babu (2004b) reported that average breaking extension values are greater for non-mulberry varieties (21-24%, 23-30% and 17-24% for Muga, Eri and Tasar silks, respectively) than *B. mori* (12 - 15%). However, in this study most of the wild silk fibers had lesser breaking elongation than *B. mori*. Lucas *et al.,* 1955 reported that *Saturniidae* silks have lower values of the elastic modulus and larger extensibility when compared with the *Thaumetopoeidae,* which is also found to be true in this study. The morphological defects such as partial silk fibroin rupture observed in *G. postica* fibers can also be a possible cause for the drop of the tensile strength. Mechanical properties are also found to be influenced by the fiber denier (Iizuka *et al.*, 1993). Longer period of boiling the cocoons (1.5-5 hours) with alkaline solution might have also affected the mechanical properties of the African wild silk fibers. Reduced proportion of the simple amino acids found in wild silk fibroins which is related to the crystalline fraction, is likely to lead to a more compliant material, and the larger amorphous fraction explains the increase in extensibility. The fracture properties of silk fibers are poorly reproducible and

governed by microstructural defects rather than by intrinsic limitations of the silk polymer (Pe´rez-Rigueiro *et al.,* 2000).

The study further showed that permanent decomposition of cocoon shells started very early for *B. mori* making the African wild cocoons thermally relatively more stable. The wild cocoon shells had higher temperature for dehydration, which suggests the presence of highly adsorbed water molecules (intrinsic structural water) and the variation in chemical composition of constituent proteins. The high heat stability of the cocoon shells contributes as self-thermoregulation structure and helps to avoid severe dehydration except under extreme environmental conditions. In previous studies, similar results were reported for *B. mori* cocoon shells exhibiting dehydration and decomposition losses at 98 and 280°C, respectively (Zhang *et al.,* 2002). They also suggested that the substantial weight decrease is due to the oxidation and cleavage reaction of the -OH chain of the silk fibers. The multistep decomposition of *G. postica* cocoon shells could be due to the presence of calcium oxalate crystals on their surface. *A. pernyi* silk fibers and other silks belonging to the Family *Saturniidae* also undergo several steps of decomposition due to the difference in polymorphs of crystalline structure and amino acid composition (Kweon *et al.,* 2000). The absence of change in structure of *A. mimosae* and *E. bauhinae* fibers after thermal treatment might be due to the compactness of the fiber discs during the sample preparation process. In conclusion, silks produced by African wild silkmoths revealed properties that have considerable promise for commercial applications.

CONCLUSIONS AND RECOMMENDATIONS

GENERAL CONCLUSIONS

The main focus of the study was to determine the extent of variability in structure, composition and properties of silk cocoon shells and fibers produced by four African wild silkmoths with a view to understand and maximize their ecological and economic benefits. The following conclusions can be made from the results of the study.

1. The macro- and microscopic structures of the cocoons and fibers of the African wild silkmoths vary considerably. The cocoons are endowed with structures such as spines and hairs (*A. panda* and *G postica*), holes/perforations (*A. mimosae*), calcium oxalate crystals (*G. postica* and *E. bauhiniae*) and peduncle (*E. bauhiniae*), which aid them in adopting and overcoming specific environmental and biological constraints.

2. All the cocoon shell surfaces are rough and intact with highly cross-linked fibers held together in pairs by sericin gum, other components (tannins) and impurities. The arrangement of the fibers in all the cocoon shells lack uniformity throughout the outer surface and show great cross bindings and networking of twisting filaments in different shapes and forms. The African wild silk fibers have larger and numerous voids that play a significant role in chemical modifications and result in marked increase in cross section. Such physical properties and structures of cocoons affect the methods and ease of processing, and hence, determine the value of the cocoons as source of textiles fibers as well as use in other applications.

4. Fibroins have high proportions of the amino acids, glycine, alanine, and serine representing more than 71 and 82% of the total amino acids present in wild and *B. mori* silks, respectively. The African wild silk fibroins have higher amounts of amino acids with hydrophilic side groups, which are correlated with their high moisture regain properties than *B. mori*.

5. There is a significant difference in weight loss and moisture regain of cocoon shells and shell layers after boiling-off. However, the moisture regain of degummed wild silk fibers was not significantly different among the species. Degummed fibers have higher moisture regain ability than cocoon shells. Inner cocoon layers have higher moisture regain while outer layers have higher weight loss percentages. The observed variation in the properties of cocoon layers suggests the need for separate degumming of layers in contrast to the usual practise of mass boiling if that were practically possible. The results also suggest the need for selection of custom-built degumming techniques for each type of silk in order to optimize quality and quantity of fibers that can be drawn from the different cocoon shells.

6. There is a significant difference in dissolution properties of cocoons and fibers produced by the African wild silkmoths. Degummed fibers dissolve more readily than cocoons in all the solvents used. African wild silk cocoons and fibers are less soluble than *B. mori*. *Gonometa postica* cocoons and degummed fibers have the highest solubility (37.3 and 51.7%, respectively) among the wild silks. Lithium bromide is the most effective dissolving agent for both cocoons and fibers (41.2 and 84.5%, respectively).

7. African wild cocoons and fibers have significant variations in tensile properties. *A. mimosae* and *G. postica* cocoons have higher initial moduli and tensile strains at maximum stress than *B. mori*. *B. mori* cocoons have significantly higher maximum stress at break (101MPa), and breaking energy (20J/cm^3). Fiber pull-out and breakage (debonding) are the dominant fracture mechanisms in cocoon shells. Degummed fibers of *G. postica* have the highest breaking energy (76.4J/cm^3) and breaking strain (41.3%). *B. mori* has the highest breaking stress than the wild silk fibers (427.6MPa) though it was not statistically significant with *A. panda* and *A. mimosae*. The stress-strain curves of *A. mimosae*, *E. bauhiniae* and *G. postica* fibers show sigoidal shape with an initial linear elastic region, yield region, and hardening regions.

8. The African silks undergo thermal degradation when exposed to heat sources. Permanent degradation starts relatively earlier for *B. mori* making most of the African wild silk cocoons thermally more stable. Degummed fibers of African wild silks also showed comparable heat resistance as *B. mori*. Such relatively higher thermal resistance by the wild fibers make them convenient candidate to use as fiber-insulators up to their decomposition limits.

9. Although *B. mori* and wild silks are both class of natural proteins, their overall properties are different that can be attributed to the selection pressures put forth over time and cocoon shells have maintained these specific and General features to best suit their unique demands placed upon them.

RECOMMENDATIONS

Wild silkmoths produce commercially important silk and are found distributed along wide range of agro-ecologies in many parts of Africa. They feed primarily on variety of readily available food plants in tropical forests and woodlands. They are known to hold promise in contributing for livelihood and improving the socio-economic status of farm families. Wild silk production could diversify rural people's economy base, and therefore, encourage them to be allied with the current world efforts in enhancing conservation-based development. However, lack of information among farmers and policy makers on economic value of wild silk production coupled with absence of quality standards based on proper properties measurements hindered wide scale silk production in Africa. Accordingly, the following recommendations can be made in relation to the results of the study;

1. The existence of information on diversity in properties and structure revealed among African wild silks can further be exploited to design specific economic and conservation projects and enhance the contribution of wild silk production to the development of a sustainable natural environment, which is under threat due to rapid industrialization, population growth and other man made hazards.

2. The information Generated on silk structure and property relationship should be utilized in broadening its application in fields other than textile. Further studies to test the significance and application of the properties identified should also be initiated to introduce African wild silkmoths to the international market. Moreover, the value of silk types when

compared across taxa should be based on suitability of fiber properties as well as silk potential products.

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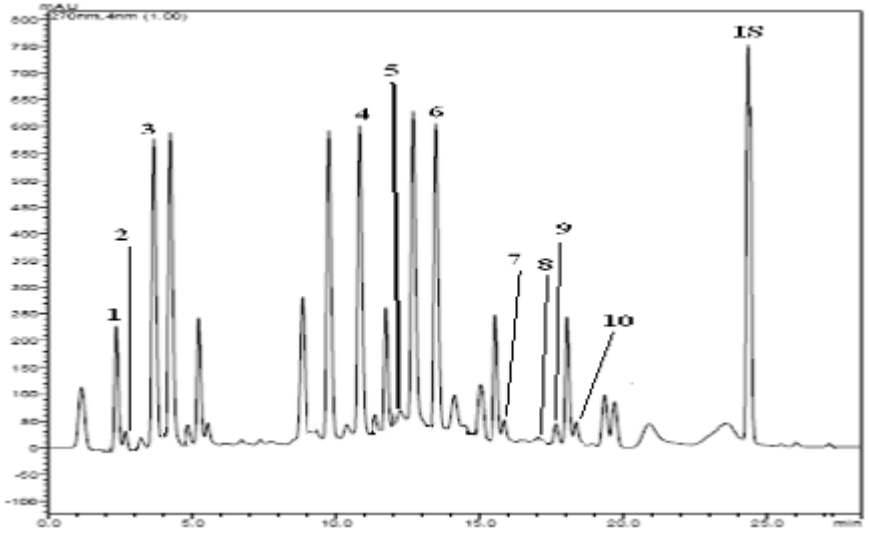
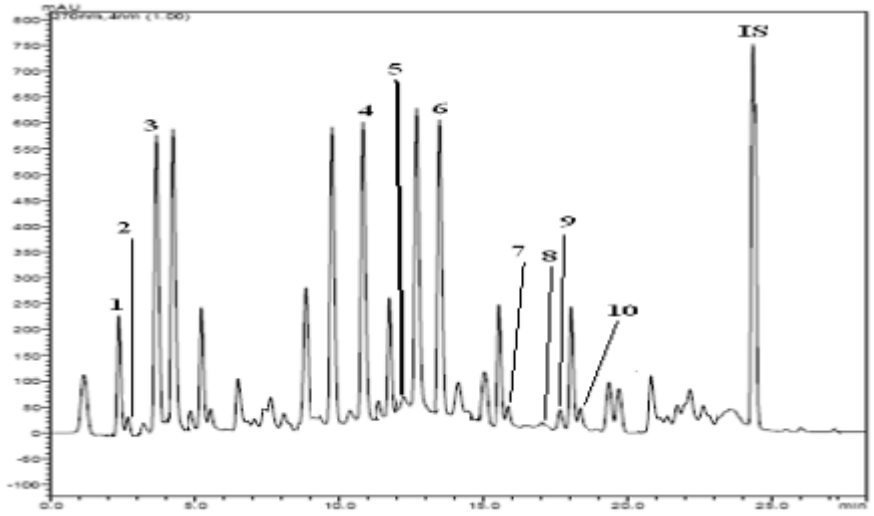
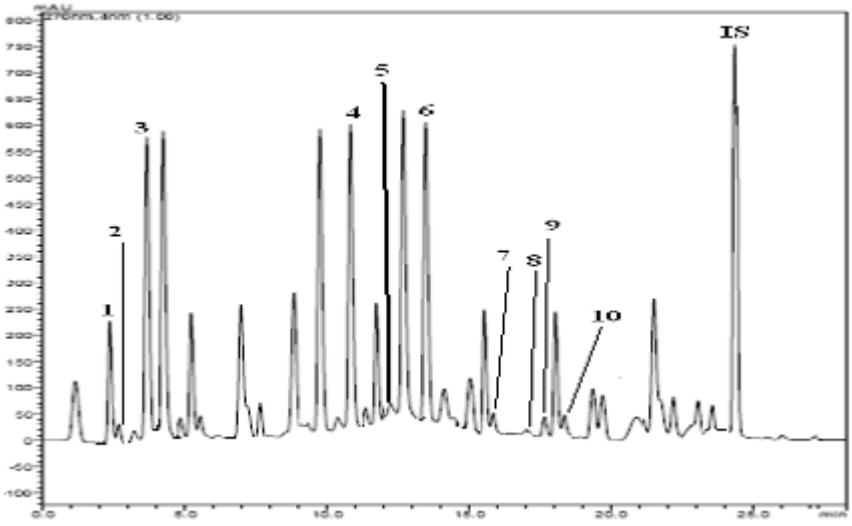
APPENDICES



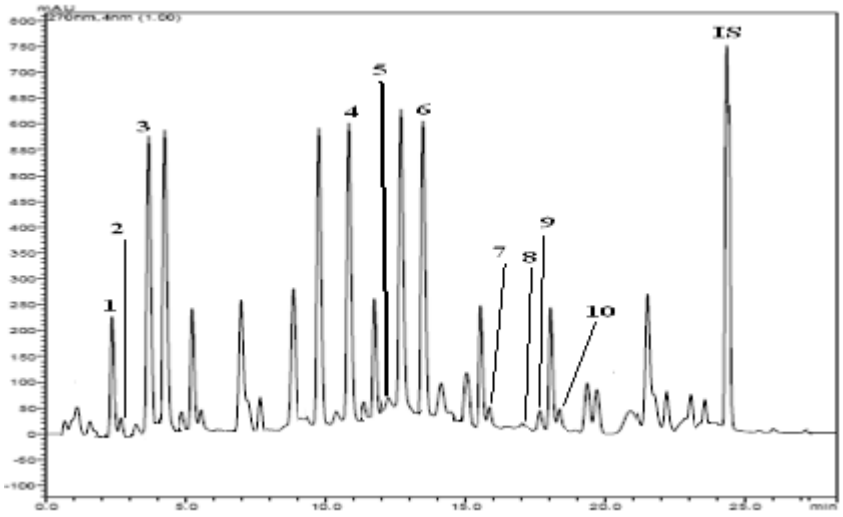
(a) *Argema mimosae*



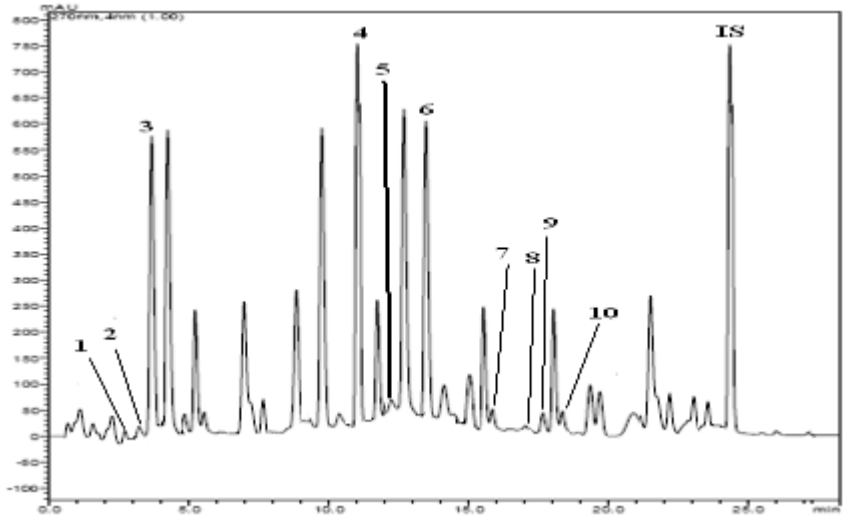
(b) *Epiphora bauhiniae*



(c) *Anaphe panda*



(d) *Gonometa postica*



(e) *Bombyx mori*

Appendix 1: HPLC peaks for the amino acid analysis of African wild and *Bombyx mor*i fibroins

(a) Scanning Electron Microscope (SEM)

(b) Thermogravimetric Analyzer (TGA)

(c) Instron Tensile Testor (5542)

(d) Fourier Transform Infrared spectroscopy

Appendix 2: Some of the Equipments used in the study
(http://users.ox.ac.uk/~abrg/spider_site/machines.html)

List of published journal articles from the thesis

1. Addis T., S. K. Raina, F. Vollrath, J. M. Kabaru, J. Onyari and E. Nguku, 2011. Study on moisture regain and weight loss of African wild silk cocoon shells and degummed fibers, *Journal of Entomology* , 8 (5): 450-458

2. Addis T., F. Vollrath, S. K. Raina, J. M. Kabaru, and J. Onyari, 2012. Study on microstructures of African Wild silk cocoon shells and fibers, *International Journal of Biological Macromolecules*, 50 (1): 63-68

3. Addis T., J. M. Onyari, S. K. Raina, J. M. Kabaru, and F. Vollrath, 2013. Mechanical and thermal degradation properties of silk from African wild silkmoths, *Journal of Applied Polymer Sciences*, 127 (1): 289-297.

Manuscripts submitted related to the thesis

1. Addis T., S. K. Raina and F. Vollrath, 2012. Structure and properties of indoor reared African wild silkmoth, *Gonometa postica*, cocooon shells and degummed fibers, *Journal of Insect Science.*

2. Addis T. and S. K. Raina, 2012. Properties and structures of silk from the African Wild Silkmoth, *Anaphe panda* Boisduval (Lepidoptera: Thaumetopoeidae) *Journal of Natural Fibers*

3. Addis T., S. K. Raina, J. M. Kabaru and J. M. Onyari, 2012. Dissolution properties of silk cocoon shells and degummed fibers from African wild silkmoths, *Journal of Applied Sciences*