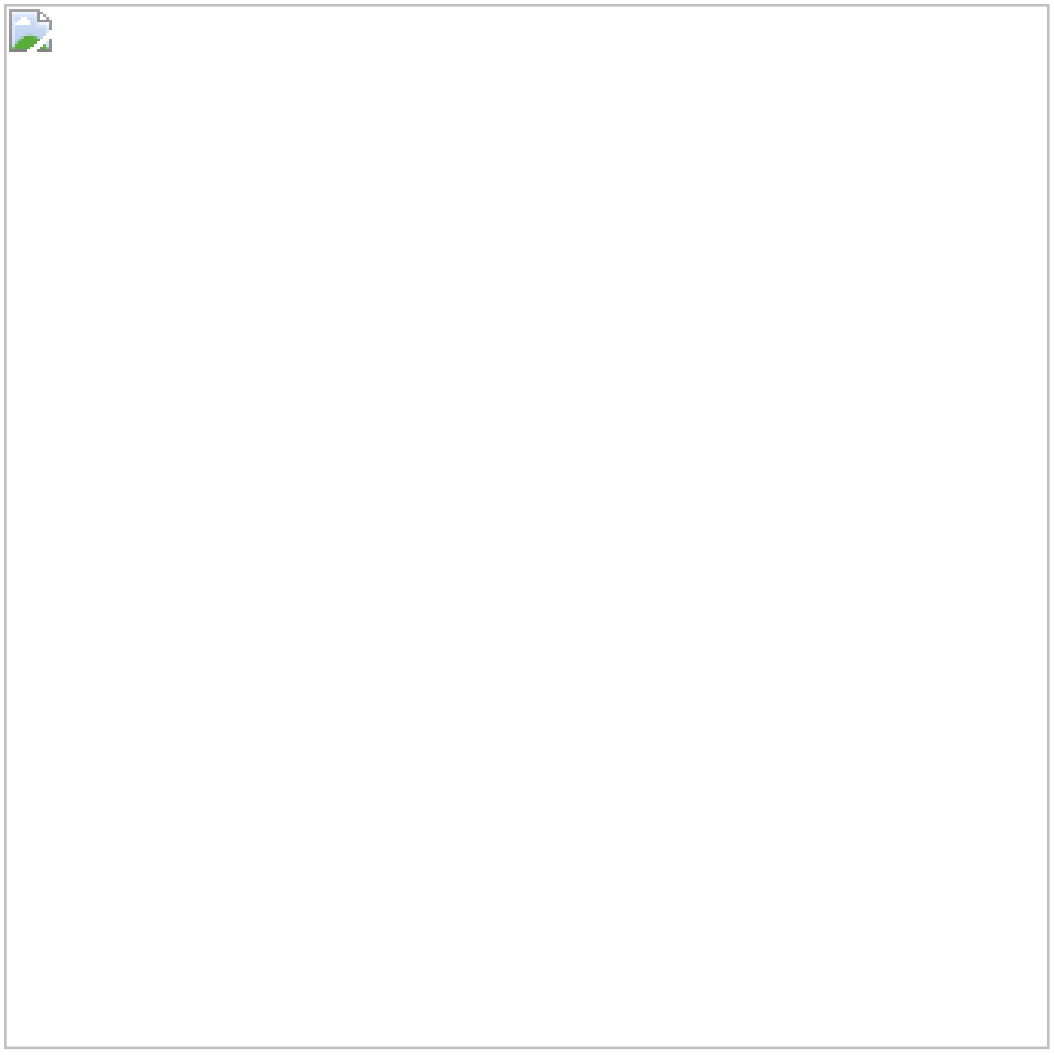

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Structure, Composition, and Properties of Silk from the African Wild Silkmoth, *Anaphe panda* (Boisduval) (Lepidoptera: Thaumetopoeidae)

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ABSTRACT: Silk cocoon nests, as well as the fiber structure, compositions, and properties of the African wild silkmoth, *Anaphe panda*, collected from Kakamega tropical rainforest (western Kenya) were studied using scanning electron microscopy, high-pressureliquid chromatography, tensile tests, and thermogravmetric analysis, and they were compared with the industrial standard, *Bombyx mori*. Cocoon nests are complex structures made up of inner, middle, and outer layers. The inner hard parchment was found to protect a mass of (20–200) individual soft flossy cocoons that enclose the pupae. The outer surface of the cocoon nests was covered with a mass of hair-like bristles. Fibers contained crescent-shaped and globular cross-sections with nods at regular intervals. Alanine (34%) and glycine (28%) were the dominant fibroin amino acids observed. Total weight loss after degumming the cocoon nest was 25.6%. Degummed fibers showed higher moisture regain of 9% when compared with cocoon nests (8%). The fibers had 0.4 GPa breaking stress and 15.4% breaking strain. Total weight loss values after thermogravimetric analysis were 86% and 90% for degummed fibers and cocoon shells, respectively.

KEYWORDS: tensile failure wild silk, degumming, *Anaphe panda*, *Bombyx mori*, conservation

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Introduction

Wild silk-producing insect species are multipurpose insects that produce high-quality silk and include attractive moths valued by collectors. The African species of wild silkmoths include three major families: Saturniidae (*Ephiphora* and *Argema* species); Lasiocampidae (*Gonometa* species); and Thaumetopoeidae (*Anaphe*, *Epanaphe*, and *Hyposoides* species), and all of these have significant economic importance.[1] Many of these wild silkworms have been utilized locally and regionally by the indigenous communities to a modest degree. The *Anaphe* species are widely distributed in the intertropical regions of Africa such as Nigeria, Uganda, Kenya, Cameroon, Congo, and Togo.[2] Important species used in the production of *Anaphe* silk include *A. infracta* (Walsingham), *A. venata* (Butler), *A. panda* (Boisduval), *A. reticulata* (Walker), *A. carteri* (Walsingham), *A. moloneyi* (Druce), and *A. ambrizia* (Butler).[3] *Anaphe panda* has significant potential in many African countries as a source of income for rural communities and conservation of biodiversity through wild silk production because it produces a huge cocoon nest that is communally weaved by 20–200 silkworm larvae.[4,5] Larvae of Anaphe *spp.* have also been reported to be local delicacies in west African countries, such as Nigeria, to meet the protein requirements of communities as a planned part of the diet throughout the year or when they are seasonally available.[6]

In Kenya, *A. panda* is primarily found in the Kakamega rainforest, a mild-altitude tropical rainforest (Western Kenya), and *Bridelia micrantha* and *Pseudolachnostylis maprouneifolia* are the major host plants and include *Bridelia micrantha-maprouneifolia*.[5] *Anaphe* silk cocoon collection and processing is an emerging field for communities around the forest as a source of off-farm income, an activity that safeguards the diverse forest from encroachment. To enhance this community-driven initiative, it is essential to ensure that the communities receive higher benefits and greater reward from harvesting cocoons than from

destroying the forests. However, the success and maintenance of such enterprises depend on the amount of income generated, the production process, and the product quality. Hence, analyzing and testing of raw silk quality and their properties is essential for maintaining both high silk output and value addition to the products. Several studies on the *Anaphe* species ranging from their biology, ecology and host distribution,[3,5] economic importance,[7] structure, properties of cocoons and filaments,[8–10] and the detailed structure and composition of the fibroin[11] have been conducted. More thorough investigations on *Anaphe* cocoon nests and fibers are necessary to locate and extract the fibers needed for further research. However, a comprehensive study on *A. panda* silk has not yet been documented. We report the composition, structure, and properties of the cocoon nests and fibers obtained from *A. panda*.

Materials and Methods

Collection and preparation of cocoon shells and fibers. Dead cocoon nests (after moth emergence) of *A. panda* were collected from the Kakamega rainforest, western Kenya, in May 2011. *Bombyx mori* cocoons (race ICIPE II) were obtained from the Commercial Insect Program of the International Centre of Insect Physiology and Ecology (*icipe*). Cocoons were dissected with a sharp blade to remove dead pupae and other insect exuvia from the inner sections. Leaves, small twigs, and other foreign matters were also removed from the outer surfaces of *A. panda* cocoons before degumming. Cocoons were soaked in hot water for 10 minutes to remove any remaining dust particles and then boiled in an aqueous solution of 5 g/L of sodium carbonate (locally known as magadi soda) for 5 hours and 1 hour for *A. panda* and *B. mori*, respectively. Degummed fibers were washed with a detergent solution comprised of 50 mL/L distilled water for 3 minutes, and 200 mL of distilled water twice. Cocoons were enclosed in wire mesh cages with a volume of 717 cm^3 to ensure homogeneous boiling. Fibers were then immersed in 70% ethanol for 3 days to remove fatty and waxy substances from the surface of fibers. Cross-sectional slices of fibers were produced by pulling the fibers through an empty plastic tube and a clean cut was made using a new razor blade.

Randomly selected cocoon nests were pressed out into discs at the middle section with a sharp hole-punch for surface observation.

Scanning electron microscopy. For scanning electron microscopy (SEM) observation, samples were mounted onto copper stubs using double-sided tape and sputter-coated with gold for 3 minutes. The surface and cross-section of the samples were taken for SEM (Neoscape, JCM-5000; JEOL, Tokyo, Japan) under an accelerating voltage of 10 kV and a beam current of 0.1 nA.

Amino acid analysis. Approximately 1 mg of degummed silk was lyophilized and crushed in liquid nitrogen and then dried in an oven to remove moisture at 105°C before acid hydrolysis. Two milliliters of 6 N hydrochloric acid containing 1.0% of phenol was added to the dry silk in a vial, which was sealed under nitrogen and heated in an oven at 110°C for 24 hours. The hydrolysate was then centrifuged for 5 minutes and filtered through glass wool. The supernatant was diluted 20 times with 50% methanol before analysis. Multiple analyses were carried out using high-performance liquid chromatography (HPLC) (Prominence LGE-UV Low Pressure Gradient HPLC system; Shimadzu, Kyoto, Japan; Column Gemini C4, Length 250 mm, ID 4.6 mm, run time 25 minutes) and the resulting peaks were identified by running authentic standards of the amino acids. Cocoons of *B. mori* were analyzed similarly for comparison.

Determination of weight loss. Twenty grams of cleaned cocoon shells of *A. panda* and *B. mori* were used. The quantity of removed sericin and other cocoon components that resulted due to degumming was obtained using following equation:[12]

$$WL = \frac{Wi - Wf}{Wi} \cdot 100\ [\%] \quad (1)$$

where *WL* is the weight loss (amount of removed sericin) expressed as %, *Wi* is the weight of fibers before degumming (g), and *Wf* is the weight of fibers after degumming (g).

Determination of moisture regain. Moisture regain was determined using the oven drying procedure. Cocoon shells and degummed fibers of *A. panda* and *B. mori* were placed in an oven at 110°C for 24 hours. Twenty grams of oven-dried shells and fibers were then placed on a laboratory bench for 72 hours under room conditions (23°C ± 2°C and 71% ± 3% Relative Humidity (RH)). The moisture regain percentage for the cocoon shells and degummed fibers was obtained using the following equation:[13]

$$\text{Moisture regain } (\%) = \frac{Wf - Wi}{Wi} \cdot 100 \quad (2)$$

where *Wi* is the initial weight of the oven-dried sample, and *Wf* is the final weight of the sample after 72 hours at room temperature.

Tensile testing of fibers. Single degummed fibers (brin) of *A. panda* and *B. mori* were mounted and glued onto a 10 mm slotted rectangular cardboard, which was then fixed onto a tensile testing machine (5542 5 N load cell; Instron, Norwood, MA, USA). Tests were conducted with a gauge length of 10 mm at a rate of 0.1 mm/seconds at room temperature. Ten tests were conducted to generate the average stress–strain data. Cross-sectional areas of the fibers were calculated from the digital images of transverse sections on SEM micrographs and analyzed with ImageJ 1.42 q software (http://imagej.nih.gov/). Normalized cross-sectional areas were obtained by averaging 50 brins for each sample. The tensile parameters were calculated using a designed program designed in Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) by our lab (Tensile Import v.2.0). Stress–strain curves were plotted using Origin Pro 8 (OriginLab Corporation, Northampton, MA, USA).

Thermogravimetric analysis. Cocoon nests and degummed fiber discs (7 mm in diameter) of *A. panda* and *B. mori* were pressed out of the middle section of the cocoon shells and degummed floss, respectively, with a sharp hole-punch. Thermogravimetric analysis was performed on a TA Instruments model Q500 (TA Instruments, New Castle, DE, USA). Three tests were conducted for each sample. Temperature ranges of 25°C–900°C and 25°C–800°C were used for cocoon shells and degummed fibers, respectively, at a heating rate of 20°C/minute, as well as an N_2 flow rate of 60 mL/minute, and air cool time of 40 minutes.

Data analysis. Percentage data were subjected to logarithmic transformation procedures to stabilize the variance before the final statistical analysis. Means were analyzed using one-way analysis of variance with a PROC analysis of variance procedure. Least significant difference tests ($\alpha = 0.05$) were used to separate means.

Results and Discussion

Structures of cocoon nests and degummed fibers. *Anaphe* cocoon nests are sessile and fixed to branches or twigs of a tree along their side by a silk band, and they can be regarded as a large single cocoon consisting of three envelopes of silk that enclose and protect the pupae. These nests are complex cocoon structures that can consist of 20–200 individual cocoon clusters.[5] The outer envelope is composed of very fine, but fairly strong and long fibers (Fig. 1A). Cross-sections of the outer cocoon shell layer were loosely packed with irregularly arranged fibers (Fig. 1B) and had hair-like bristles (34.29 µm in diameter) covering its surface, which is incorporated from larval spines during spinning. The bristles may be used for protecting the larvae from natural enemies such as birds (Fig. 1C). These bristles have tiny spines (diameter of 7.33 µm) arranged alternatively over their surface. These spines along with other chemicals and dusts

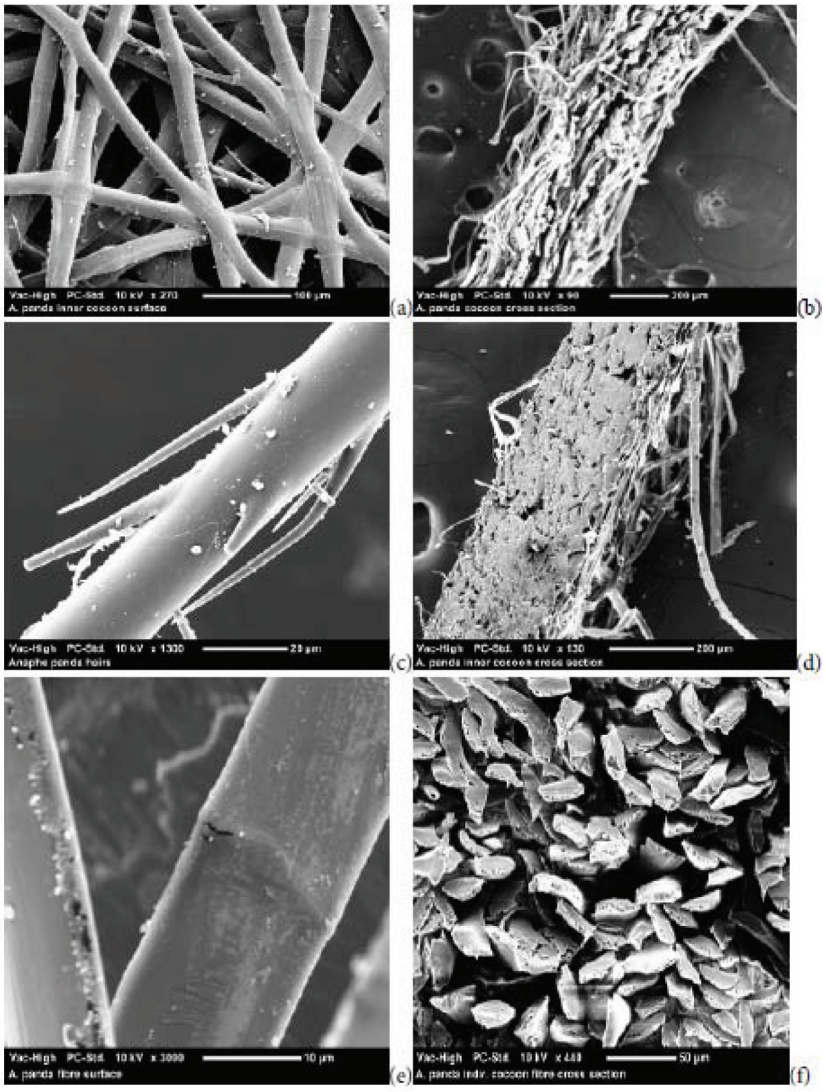

Figure 1. SEM micrographs of *Anaphe panda* cocoon shell and fiber surfaces and cross-sections. (**A**) Surface of outer layer; (**B**) cross-section of outer cocoon layer; (**C**) hairs on cocoon surface; (**D**) cross-section of inner cocoon layer; (**E**) nodes on the fiber outer surface; (**F**) cross-section.

Table 1. Composition of major amino acids in the fibroin of *Anaphe panda* and *Bombyx mori* (ratios mol/g).

AMINO ACIDS	*ANAPHE PANDA*	*BOMBYX MORI*
Aspartic acid	4.78	1.5
Glutamic acid	1.12	1.73
Serine	8.66	10.34
Glycine	27.65	43.45
Threonine	0.04	0.82
Alanine	34.53	28.04
Valine	1.23	25.7
Methionine	0.26	0.11
Leucine	0.49	0.75
Lysine	0.13	0.25

may make working with *Anaphe* cocoons uncomfortable and cause allergies in workers. The middle layer consists of several closely packed sheets of silk. The innermost layer is compact and extremely tough parchment, which is made up of long and fine fibers that are embedded with high sericin gum (Fig. 1D). This provides protection for the cluster of individual cocoons located inside, and makes up to 21% of the total weight of the cocoon. The toughness of the inner layer and compacted clusters of individual cocoons may play a role in reducing the overall incidence of cocoon parasitism.[14,15] Such complicated communal nests may provide a high degree of protection in both the fire-prone regions and in the high rainfall forests, such as the Kakamega, with lower temperatures and long rainy seasons, which would be an evolutionary advantage. Generally, fibers were smooth with few fractures and had bamboo-like structures with nodes at certain intervals (Fig. 1E). The cross-sectional shape of fibers varied from globular to curved (crescent) (Fig. 1F). Double trapdoors (tunnels) constructed by the larvae on the cocoon nests and individual cocoons allow the moth to pass through after emergence. The tunnels may also be used as air exchange windows that condition the inside environment for the larvae and pupae during spinning and dormancy.

Amino acid composition of fibroin. The results showed that both *A. panda* and *B. mori* were characterized by the presence of high proportions of the three amino acids, glycine, alanine, and serine (Table 1). The three amino acids represented approximately 80% and 89% of the total amino acids present in *A. panda* and *B. mori*, respectively. Alanine was the dominant amino acid (34%) followed by glycine and serine in *A. panda*; however, in *B. mori* glycine (43%) was predominant. Other amino acids, including glutamic acid, asparatic acid, valine, and methionine were also found in various amounts. The proportions reported here were comparable with those reported for eri, tasar, and muga (34%–36%).[16] However, the alanine content of *A. panda* was lower than other nonmulberry silk fibroins, particularly for muga (44%).[17] Alanine content as high as 52%–59% has also been reported for the outer layer of *A. reticulate* and the sum of alanine and glycine content accounted for more than 90%.[11,18] The composition of some of the amino acids such as tryptophan, cystine, asparagine, glutamine, and isoluecine could not be included because their analysis and degradation products were inhibited by their instability in oxidative or strongly acidic media, deamination, slow cleavage, and/or light.

Weight loss and moisture regain of cocoon shells and degummed fibers. Table 2 shows the moisture regain and weight loss of the cocoon shells and degummed fibers of *A. panda*. The total weight loss due to degumming was 25.6%; however, the traces of sericin gum observed on the fibers (Fig. 1B) suggested that the degumming process did not completely remove all of the gum present on the fibers. The weight loss reported here was higher than for other wild silks such as *Antheraea proylei*, *A. assama*, *A. pernyi*, and *A. yamamai* (7%–13%).[9] However, *A. panda* had less sericin gum than *Gonometa postica*, which showed more than 50% weight loss.[19] The nature and chemical composition of sericin protein considerably affects its solubility in hot water and contributes to the amount of total weight loss from silk cocoon shells. Degummed fibers showed higher moisture regain (9.9%) than cocoon nests (8%). This may be due to the presence of hairs and other foreign matter on the surface of the cocoon nest, which deny access to moisture absorption. The moisture regain of the *A. panda* cocoon nest and fibers were higher than for *B. mori*, as reported by Sen and Babu.[16] They also reported that moisture regain of nonmulberry silks is higher than that of their domestic counterparts. This may be due to the difference in compactness, as well as the change in quantity and composition of the sericin and fibroin (hydrophilic/hydrophobic amino acids) in the cocoon shells and degummed fibers. *A. panda* contained a higher hydrophilic/hydrophobic

Table 2. Weight loss and moisture regain of *Anaphe panda* and *Bombyx mori* silk fibers and cocoon shells.

SPECIES	WEIGHT LOSS AFTER DEGUMMING (%)	MOISTURE REGAIN (%) (COCOON NESTS)	MOISTURE REGAIN (%) (DEGUMMED FIBERS)
Anaphe panda	25.6 ± 3.6	8.0 ± 0.2	9.9 ± 0.2
Bombyx mori	29.4 ± 0.2	7.9 ± 0.4	8.5 ± 0.3

*Means followed by the same letter in a column are not statistically different ($P < 0.001$, $\alpha = 0.05$).

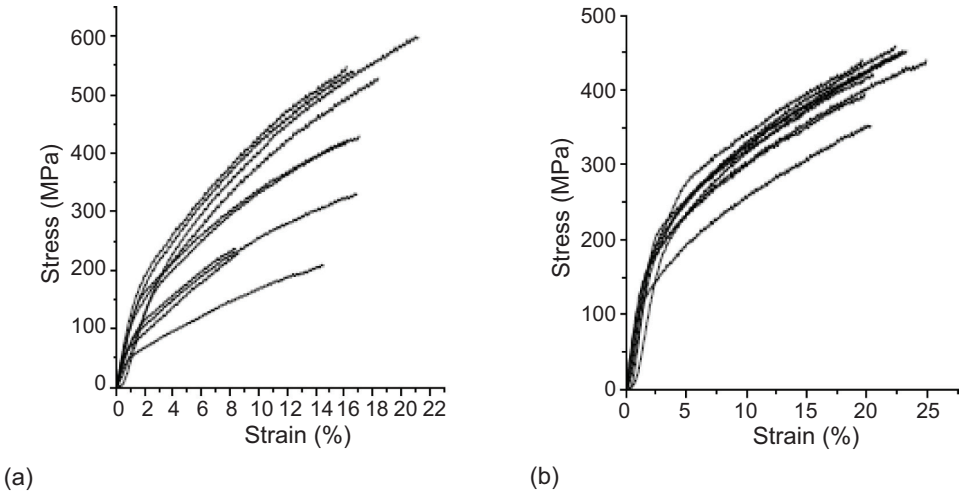

Figure 2. Stress–stress curves of degummed single fibers of (**A**) *Anaphe panda* and (**B**) *Bombyx mori*.

amino acid ratio (0.23) than *B. mori* (0.19) (Table 1), which may have contributed to the higher moisture regain.

Tensile and thermal degradation properties. Figure 2 shows the stress–strain curves of single *A. panda* and *B. mori* fibers. The curves were widely scattered for *A. panda*. Such variation in mechanical properties is inevitable for wild silk fibers due to their irregular cross-sectional area throughout the fiber axis and across the different layers of the cocoon nest. The stress–strain curves of both *A. panda* and *B. mori* showed two regions: an initial elastic region directly, followed by a strain-hardening region. *A. panda* fibers showed a lower breaking strain (15.4%) and comparable breaking stress (0.4 GPa) as *B. mori* (Table 3). In contrast, Sen and Babu[16] reported higher breaking extensions for nonmulberry silk fibers. Gheysens et al[19] also reported higher (37%) breaking strains for *G. postica*. The extended time of boiling for *A. panda* in the alkaline solution coupled with a possible difference in the proportion of the amorphous and crystalline region of the fiber may have contributed to the difference in the tensile properties observed.

Figure 3 shows the thermogravimetric curves of *A. panda* and *B. mori* degummed fibers and cocoon nests. Initial rapid weight loss occurred due to the loss of water from the cocoon shells and fibers followed by a period of steadiness. However, with increased temperature, a rapid decline in weight followed due to decomposition. This occurred at 314°C and 293°C for *A. panda* and *B. mori* cocoon shells, respectively. *B. mori* fibers and cocoon shells had the highest total weight loss (94.8% and 93.5%, respectively) (Table 3). However, *A. panda* cocoon shells showed better heat resistance with both dehydration and decomposition occurring at higher temperatures than *B. mori*. The higher heat stability in the cocoon shells, as compared to the fibers, may indicate a role in thermoregulation. Danks[20] also reported the role that cocoon shells play in the acquisition and conservation of heat. Both cocoon shells and degummed fibers underwent a multistage weight loss scenario, which may be due to the difference in the polymorphs of crystalline structures, and due to the amino acid composition.[20] Zhang et al[22] also reported similar decomposition behavior for *B. mori* cocoon shells, as illustrated by rapid weight losses occurring at 98°C and 280°C.

Conclusion

The African wild silkmoth, *A. panda*, produces silk of potential economic and biological importance. The presence of different layers in cocoon nests suggests the need for a custom-built degumming method to optimize fiber output and quality. Hence, subsequent studies should examine degumming methods, particularly the amount and type of degumming chemicals to be used, their boiling time, and subsequent drying and spelling techniques for each layer. *A. panda* fibers showed comparable tensile properties and thermal resistance with the industrial standard, *B. mori*, and hence holds considerable promise for commercial application.

Table 3. Tensile properties and thermogravimetric weight loss of *Anaphe panda* and *Bombyx mori* degummed fibers

SPECIES	BREAK STRAIN (%)	BREAK STRESS (MPa)	INITIAL MODULUS (MPa)	BREAKING ENERGY (J/cm^3)	TOTAL WEIGHT LOSS (%) (COCOON SHELLS)	TOTAL WEIGHT LOSS (%) (DEGUMMED FIBERS)
Anaphe panda	15.4 ± 0.6^b	406.4 ± 3.8^b	8161.4 ± 15.1^b	43.31 ± 1.5^b	90.2 ± 0.5^a	86.5 ± 3.5^b
Bombyx mori	21.8 ± 0.5^a	427.6 ± 10.6^a	8787 ± 555.1^a	66 ± 2.8^a	93.5 ± 0.3^a	94.8 ± 2.6^a

*Means followed by the same letter in a column are not statistically significant ($\alpha = 0.05$).

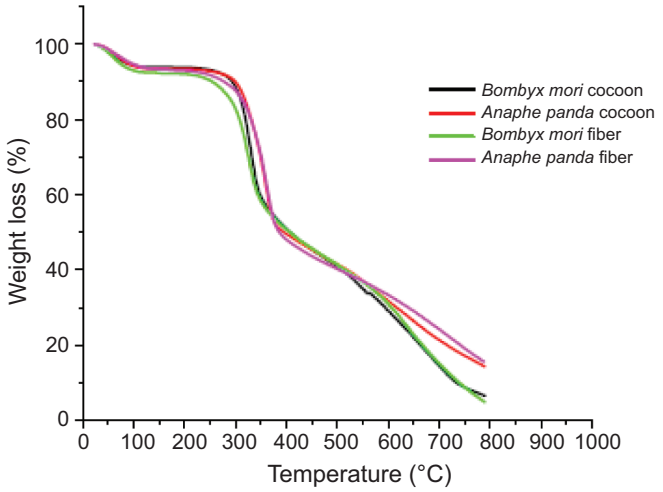

Figure 3. TGA curves of *Anaphe panda* fibers and cocoon shells.

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Author Contributions

Conceived and designed the experiments: AK, JK and SR. Analyzed the data: AK. Wrote the first draft of the manuscript: AK. Contributed to the writing of the manuscript: JK and SR. Agree with manuscript results and conclusions: AK, JK and SR. Jointly developed the structure and arguments for the paper: AK and JK. Made critical revisions and approved final version: JK and SR. All authors reviewed and approved of the final manuscript: AK, JK and SR.

DISCLOSURE AND ETHICS

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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