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Thermal, structural and mechanical characterization of *Nephila clavipes* spider silk in southwest Colombia

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ABSTRACT

Some physical properties of spider silks, including mechanical strength and toughness, have been studied in many laboratories worldwide. Given that this silk is organic in nature, composed of protein, and has similar properties to metal wires or polymers, it has the potential for application in medicine, nanoelectronics, and other related areas. In this study, we worked on spider silk from the *Nephila clavipes* species collected from the wild and kept it in the nursery of the Autonomous University of the West, Cali, Colombia, to determine its physical, thermal, and mechanical properties, seeking possible applications in the medical and industrial sectors and comparing the material properties of the silk from the species from southwestern Colombia with those of the previously studied species from other regions. The mechanical characterization of the material was performed using a universal testing machine; thermal behavior was captured by a thermogravimetric analysis, differential scanning calorimetry, and mass spectrometry; and structural characterization was performed using diffraction X-rays. The results of the thermal characterization demonstrate that the spider silk loses 10 % of water content at 150 °C with significant changes at 400 °C, while the mechanical characterization indicates that the spider silk is much tougher than Kevlar 49 and Nylon 6 since it is capable of absorbing more energy before rupture.

1. Introduction

The spider silk of the *Nephila* Clavipes species is composed of various proteins and up to seven different types of silks formed using seven glands. For example, the mooring line of the silk (dragline silk) is composed of proteins grouped into two main spidroins: mannose-associated serine protease 1 (MaSp1) and mannose-binding protein serine protease 2 (MaSp2), which are composed of crystalline networks in which several amino acids, mainly alanine and glycine, are found (Simmons et al., 1996; Xu and Lewis, 1990; Hauptmann et al., 2013). Lahens et al. (2017) analyzed 28 *Nephila* spidroins, which represent all known types of orb-weaver spidroins and identified 394 coding variations and high-order repetitive structures unique to specific spidroins. Dos Santos-Pint et al. (2018) found that the long protein sequence of flagelliform silk has 45 hydroxylated proline residues, which can help explain the mechanoelastic property of spider silk.

Several studies have determined that the properties of the spider silk largely depend on the geographical location where the *Nephila clavipes* species is found and on the temperature, degree of hydration, form of extraction, and rate of deformation of its threads (Du et al., 2006; Hinman et al., 2000; Cunniff et al., 1994). This variability of factors that influences the material's properties presents a clear evolutionary advantage since it means that the material can adapt to its immediate needs (Cunniff et al., 1994; Saravanan, 2006; Hauptmann et al., 2013). Pertinently, Bartoleti et al. (2018) studied 320 *Nephila clavipes* spiders from 49 sites in South America and sequenced a mitochondrial region of their DNA and two nuclear regions located in Colombia and Brazil.

The elastic deformation and mechanical resistance of the spider silks of several species have recently captured the attention of several researchers owing to their great capacity for absorbing energy before rupture, high degree of deformation, and being five times more resistant than steel and eight times stronger than nylon (Cunniff et al., 1994; Hinman et al., 2000; Vollrath, 2000; Xu and Lewis, 1990; Soler, 2013). Moreover, the properties of spider silks have been studied in terms of where certain external agents reach resonance conditions (Alencastre et al., 2016). These properties are difficult to characterize owing to their production mechanisms, with researchers attempting to artificially reproduce them using various means and methods. Herein, as Alencatre

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(2015) found using his conceptual model that describes an approximate form of the dynamic behavior of a structure made using a spider silk, the properties cannot be 100 % reproduced. In addition, Borja et al. (2015) attempted to recreate the properties of the sedative, whereas, Bowen et al. (2018) obtained specific synthetic spidroin fibers and were the first to fully replicate the mechanical performance of natural fibers.

Swanson et al. (2006) suggested that the spectrum of the dragline silk sequences and material properties those have been produced over time provides a rich resource for the design of biomimetic silk fibers.

The applications of the abovementioned silks for industrial purposes and medicine are diverse. For example, at the clinical level, they are used in the regenerative medicine related to the nervous system (sciatic nerve, peripheral nerves, tibial nerve, Schwann cells) and in bone-system engineering, in wound healing, and in various dermatological procedures (repair and regeneration of the skin). Since spiders produce only small amounts of silk, researchers have investigated processes for the mass production of the proteins that make up natural silks via genetic methods that incorporate the use of bacteria, plants, and mammals (e.g., Gomes et al., 2011; Saravanan, 2006).

This study focuses on the mechanical, thermal, morphological, and structural properties of the mooring silk produced by the *Nephila clavipes* spider from a region of southwestern Colombia, examining its possible applications in the academic, medical, and industrial fields and comparing its characteristics with the corresponding results obtained by researchers from other parts of the world.

2. Methodology

The spider silk used in this study was produced by members of the *Nephila clavipes* species collected from their natural habitat and kept in the nursery of the Autónoma de Occidente University of the West in Cali, Colombia. This study aimed to examine the properties of the silk, with various threads with and without the natural glue analyzed. To examine the glueless threads, they were submerged in water at temperatures of between 30 °C and 35 °C.

The spider thread study was consulted and approved with the animal ethics committee of the Autónoma de Occidente University and the work permit in biological diversity was carried out with the Corporación Autonoma Regional del Valle del Cauca, CVC, under resolution 0710 No 0711 from 2011.

To explore the mechanical properties of the spider silk, it was necessary to use a universal testing machine (Instron/Model 3366/software series IX/s) to measure the tensile strength of the silk in relation to the ASTM E8 Standard. Due to the small diameter of the wires (2–3 μm, approximately one tenth of a human hair) and the small forces involved in the tests (of the order of 10^{-1} N), adaptations of the equipment had to be adapted to achieve a good grip between the jaws and silks. The operation parameters were as follows: rate, 150 mm/min; temperature, 25 °C; and moisture, 51 %.

For the analysis of the morphological properties, an optical microscope was used to determine the number of monofilaments contained in a thread along with their respective diameters.

The structural properties of the glued and glueless silks were examined via X-ray diffraction using a PANalytical diffractometer featuring a high-resolution horizontal goniometer, Bragg geometry, and flush angle geometry.

A high-resolution SEM equipment (FEG-MEV) was used to examine the morphology of the surface of the threads and way in which the surface glue adheres.

The thermal properties were continuously measured as a function of temperature, with a differential scanning calorimeter (DSC Q2000, TA Instruments) used in a range of 50 °C–200 °C at a rate of 10 °C/min to quantify the energetic changes associated with the first-order phase transitions in the material at low and high temperatures. A thermogravimetric analyzer (TGA Q500, TA Instruments) was also used to quantify the change in weight of the spider silk as it was subjected to temperature changes ranging from ambient temperature to 400 °C with rate of 10 °C/min andunder an inert atmosphere of nitrogen. Finally, a mass spectrometer (MS Discovery, TA Instruments) was used alongside thermogravimetric analysis (TGA) to identify the gases detached in the thermogravimetric analyzer. Each measure was repeated five times to achieve the highest possible precision.

3. Results and discussion

The analyzed silks were directly collected from the natural habitat of the *Nephila clavipes* species of spider, as shown in Figure 1.

The spider thread was collected in rectangular frames directly from the habitat located in the nursery of the Autonomous University of the West and was subsequently cleaned in the laboratory to eliminate all the agents impregnated by the glue as much as possible. The thermal analysis was performed using wire samples with and without glue.

(1a)

(1b)

Figure 1. (a) Natural habitat in the nursery of the Autonomous University of the West and (b) female spider of the *Nephila clavipes* species. Source: The authors.

3.1. Thermogravimetric analysis (TGA)

The TGA results shown in Figure 2 indicate that the yarn is thermally stable up to a temperature of 250 °C, presenting appreciable weight variations around 140 °C, which is attributed due to the release of water molecules incorporated within its structure, at 280 °C due to the reduction of the biopolymer, and at 360 °C due to the carbonization process, these. These results are similar to those reported by Cunniff et al. (1994), who determined that the decomposition began at around 233 °C and that the carbonization began at around 437 °C after analyzing *Nephila clavipes* yarns with diameters very similar to those reported in this study. The difference between these results can be attributed to the different environmental conditions in which the silks were produced. Given the results, the silk can potentially be used within a temperature range of up to 280 °C, since within this range, the yarn does not go through the casting process and does not degrade until reaching 40 % of its initial weight.

3.2. Differential scanning calorimetry (DSC)

Figure 3 shows that the mooring silk underwent the first transition at low temperatures of around −23.82 °C, which was presumed to be a structural change. Meanwhile, the second transition occurred at around 30 °C, which is and can be attributed to the release of the glue to capture prey. Both transitions were reported by Saravanan (2006), albeit without an explanation of the results. Then, a prolonged dehydration phase was observed from 40 °C to 143 °C, which involved both structural and compositional water release.

3.3. Mass spectrometry (MS)

Figure 4 shows the mass spectrometry (MS) results. Herein, we can observe the release of several elements making up the gases released during the TGA by the spider silk, with water accounting for the greatest proportion (H_2O, $m/e = 18$), followed by oxygen (O_2, $m/e = 32$), hydroxyl groups ($OH-$, $m/e = 17$), cyclopropene (C_3H_4, $m/e = 40$), and lastly, methane (CH_4, $m/e = 16$), which is a gas released in the absence of oxygen.

In terms of the quantities in which the aforementioned elements are released, it can be held that within the temperature range measured, the yarn tends to dehydrate, maintaining its physical properties in greater proportions.

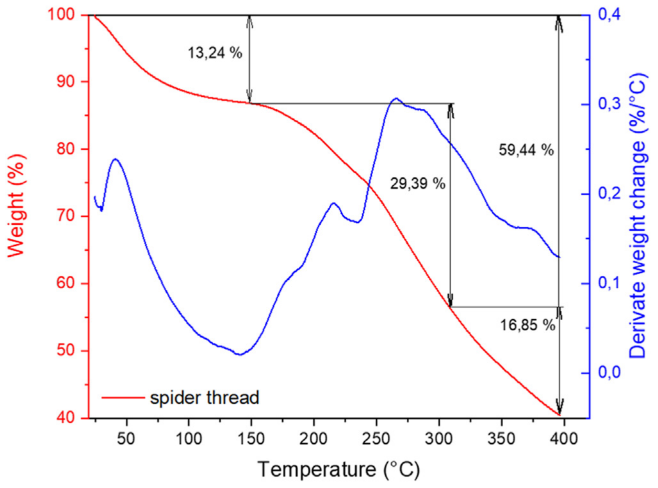


Figure 2. Percentage of loss of weight of the thread from the *Nephila clavipes* spiders under temperatures ranging from 25 °C–400 °C (black curve) and the respective derivative of the weight as a function of the temperature (gray curve). Source: The authors.

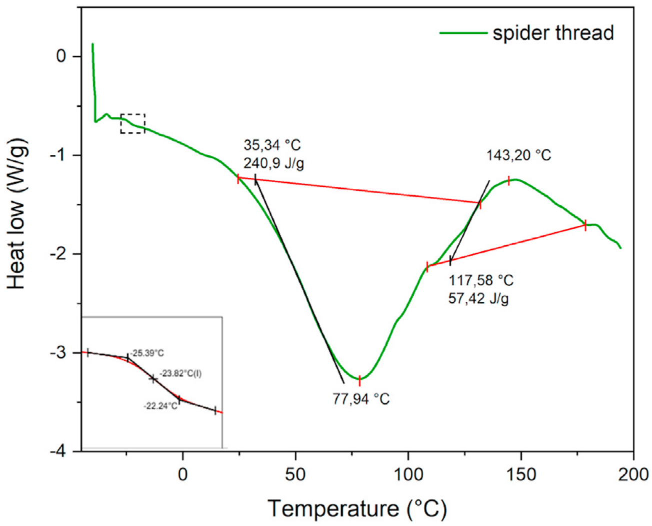


Figure 3. Variation in heat flow with the temperature changes obtained by differential scanning calorimetry (DSC) of the Nephila Clavipes spider thread in a temperature range between -50 °C < T < 200 °C. Source: The authors.

3.4. Optical microscopy (MO)

An optical microscope was used to ascertain the diameter of a single sample wire since it was necessary to also identify the number of monofilaments contained in what initially appears to be a single silk when observed with the human eye. As Figure 5a shows, this apparent single silk actually contains a group of monofilaments that constitute a single monofilament, as shown in Figure 5b. The diameter obtained for this silk was approximately 0.028 mm.

3.5. X-ray diffraction

To obtain the diffractograms shown in Figure 6, several fibers of the spider silk were used to increase the signal obtained (due to the diffraction source that was used). It was not possible to obtain a pattern diffraction of a single fiber since the diffracted radiation was of the order of a noise signal, which would result in producing a diffractogram that is not clear. In addition, the diffracted radiation of several fibers was not advantageous in this case, since the results were averaged in terms of the

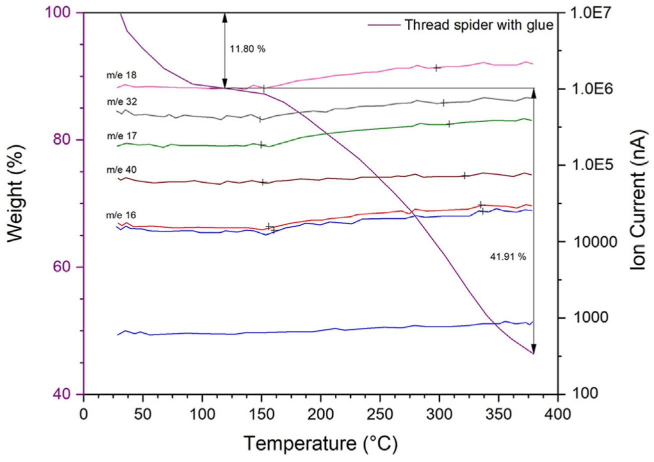
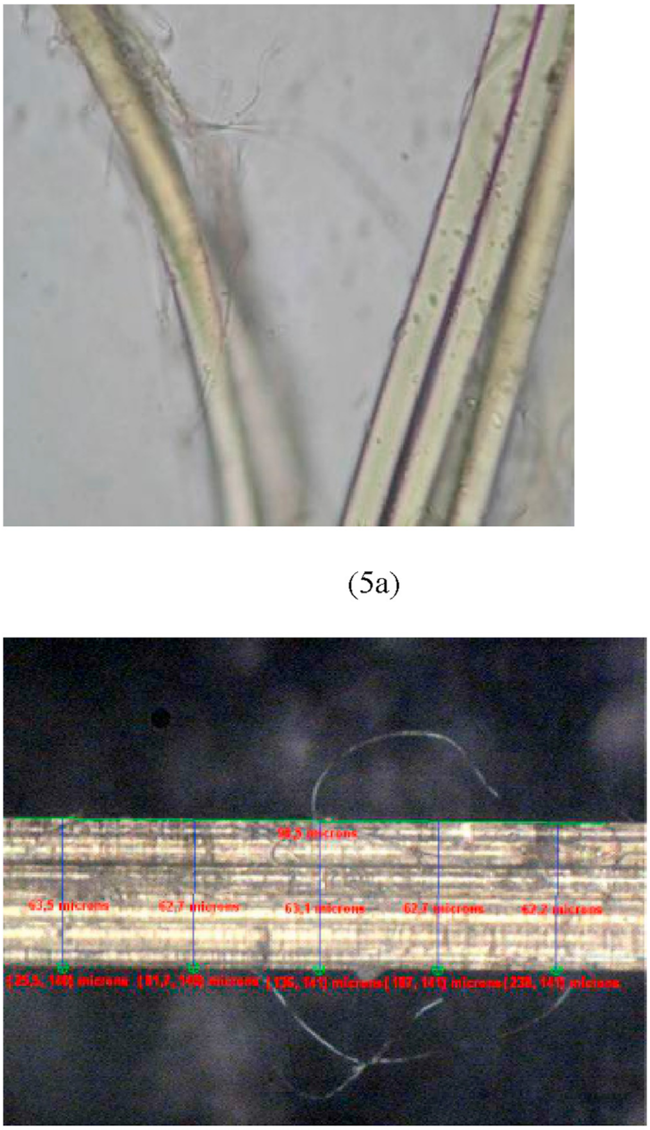


Figure 4. Variation of the weight as a function of temperature (continuous curve, left axis) and mass spectrometry of the spider thread from the *Nephila clavipes* species (right axis, m/e ratios) within a temperature range of 25 °C < T < 400 °C. Source: The authors.



(5a)

(5b)

Figure 5. Microscopic views of the spider thread—(a) expanded 500 times and (b) expanded 100 times. Source: The authors.

structure of all the fibers, and their interpretation was far more confusing, which is generally the case with materials with such great intrinsic variability (Perea et al., 2010).

Figure 6 shows that the diffractograms of the glued and non-glued silk were very similar, which was due to the fact that the same proteins appear in the composition of these silks. However, the glued silk did exhibit a greater bulge to the baseline than the non-glued silk, which was due to the greater contribution of the amorphous phase associated with the greater proportion of glycine in the glued yarn (the amount of glycine in the support yarn is 37 % of the total composition, while in the glue, it was found to be 14 % by Xu and Lewis (1990).

The results of the X-ray diffraction indicate that washing the spider silk to remove the glue to facilitate the handling does not result in a change to the physical properties.

The results shown in Figure 6 exhibit peaks at 2θ angles equal to 7°, 20° and 22°, which are similar to those reported by Du et al. (2006), where the spectra also exhibited peaks at 2° angles of 7°, 17° and 20° corresponding to the indexes (100), (200) and (120), suggesting that the structure of the silk does not depend on the tropical region of the arachnid species.

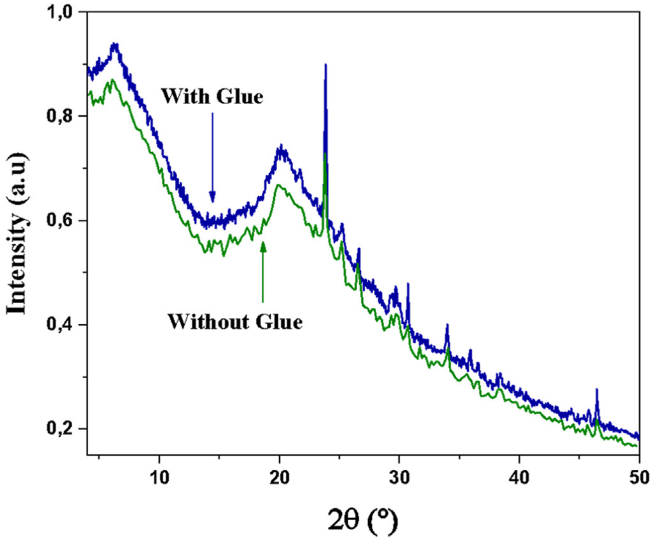


Figure 6. X-ray diffractograms of the thread of the *Nephila clavipes* spiders. The upper spectrum corresponds to the thread with glue and the lower spectrum corresponds to the thread without glue. Source: The authors.

As noted above, the amorphousness of both spectra was due to the amount of glycine contained in the yarns, which contributes to the improvement in its elastic properties, while the alanine contributes to the crystalline phase by improving the properties related to mechanical resistivity. Therefore, while it has not yet been possible to characterize in detail how crystalline zones, rich in alanine (microcrystals) are combined, and or the zones of more flexible conformation rich in glycine are combined, it is clear that such combinations are precisely the origin of the unique properties of spider silk, i.e., the high resistance to stress alongside a high elasticity.

3.6. *Mechanical properties*

An evaluation of specific mechanical properties of the spider silk was conducted using the universal testing machine to determine the material's elastic and mechanical resistance properties. Traction measurements were performed on a single silk with a thickness of 18.4 μm and on a combination of three, five, and six silks, with the results shown in Figures 7 and 8.

The elastic constants shown in Figure 7 (quotient between applied force and elongation) could be obtained through calculating the slope of the first region, known as the elastic zone. For the single silk, an elastic constant value of the order of 17.2 N/m was obtained, while for the three silks, it was 55.2 N/m; for the five silks, it was 98.5 N/m; and for the six silks, it was 107,7 N/m, which was consistent with the dynamic analysis since the force that supports a single silk can be distributed in two, three, and many more silks of the same nature, presenting more resistance to deformation. The fall presented in the figures around the maximum strength was due to the fact that the fiber did not suffer a total break, while some monofilaments of the silk took a little longer to break. This means that the silks presented percentages of deformation of between 12 % and 20 %, which is in agreement with the results obtained by Cunniff et al. (1994), who obtained a percentage of deformation of 16–18 % for silks of the same arachnid species.

Figure 8 shows the relation between the applied force per unit area (effort) and the deformation of the measured yarn quantities. In all tests, the curves fell on the same region, which is the area under the curve from the elastic zone to the plastic zone. The value corresponds to the tenacity

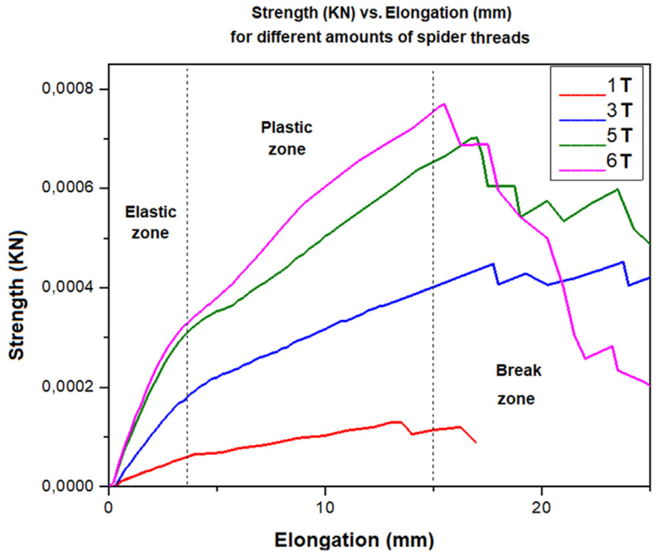


Figure 7. Relationship between applied force and elongation for one, three, five, and six spider threads. Source: The authors.

of the silk, a natural mechanical characteristic of the material. In Table 1, the average of the mechanical parameters obtained for the measurements made is summarized and compared with the values obtained for Nylon 6, Kevlar 49, and the mooring silk analyzed by Cunniff et al. (1994). Table 1 shows the linear density of the analyzed yarn was of the order of 0.289 *tex*, where *tex* is the unit of linear density of the fiber mass. The silk exhibited an elastomer-like behavior, as shown in Figures 7 and 8, which is also the case with Kevlar 49 and Nylon 6 fibers. The silk also had a very low modulus of elasticity and high deformation up to the point of breakage. It should be noted that while there are many types of nylon fibers with different values for the supplied parameters, Nylon 6 was chosen here since it has properties and applications similar to those of spider silk. From Figure 8, it can be concluded that the spider silk is quite tenacious and that, according to Table 1, it is more tenacious than both Kevlar and Nylon 6, while it is also more tenacious than steel, which has a tenacity of the order of 6 MPa. This is due to the fact that the silk absorbs more energy before breaking.

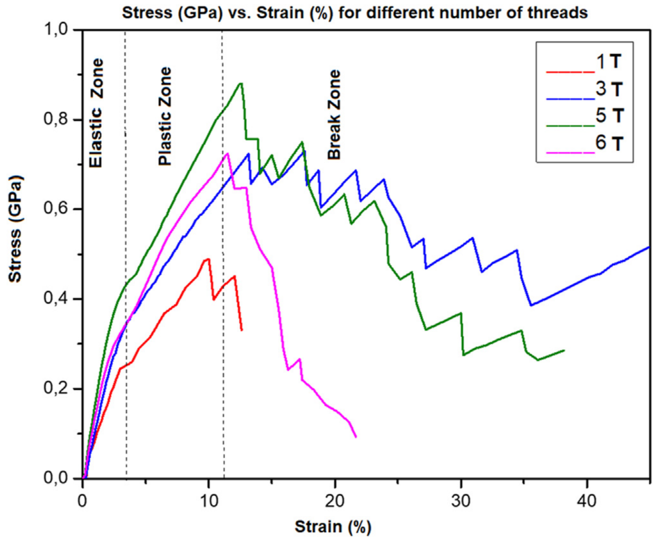


Figure 8. Relationship between effort (force applied per unit area) and deformation for one, three, five, and six spider threads. Source: The authors.

3.7. Surface morphology

Figure 9 shows the SEM micrograph of a spider thread in a space of 100 µm. Herein, what appears to the human eye to be a single thread is actually a bundle of intertwined nanofilaments, which results in the high resistance of spider silk.

Meanwhile, Figure 10 shows the micrograph of the thread impregnated with glue. Here, it can be observed that the glue is like a rubber that covers the threads, which is important for capturing prey.

4. Conclusions

The spider silk of the *Nephila clavipes* species from the southwestern Colombian region exhibits good thermal stability up to 150 °C, which is followed by a weight loss of around 50 % up to 400 °C. This weight loss is

Figure 9. SEM micrograph of a visible thread.

Table 1. Mechanical parameters for spider threads Nephila Clavipes compared with Kevlar 49 and Nylon 6.

Linear density: 0,289 tex; Initial length: 135 mm; Sampling frequency: 0,5 pto/s; crosshead speed: 0,5 mm/min; Temperature: 22 °C; Wet: 62 %.

Material	Strain (%)	Tenacity (MPa)	Young module (GPa)
Silk Néphila Clavipes analyzed	17,0	595	**15,99**
Silk Néphila Clavipes, Cunniff et al. (1994)	16,7	600	**10,9**
Nylon 6	14–22	80	3,2
Kevlar 49	2,4	50	80

Source: The authors.

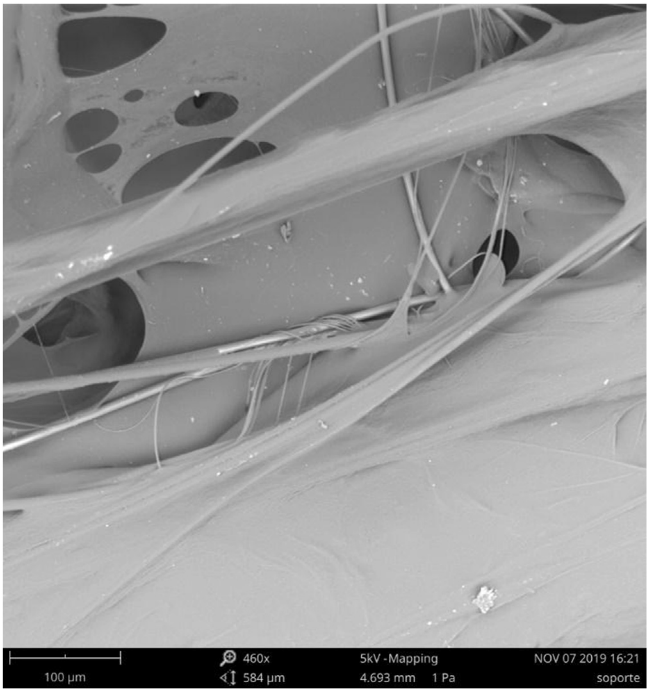


Figure 10. SEM micrograph of spider silk with glue.

due to the release of gases, such as water vapor, oxygen, hydroxyl groups, cyclopropane, and, to a lesser extent, methane, demonstrating that some of the silk's structural properties are maintained up to a temperature of 400 °C, without the emergence of the carbonization process.

The release of the natural glue from the spider silk was observed at a temperature of around 30 °C, without considerable changes in the structural properties, which means that the silk is viable for certain industrial applications where asepsis is required, such as in the textile, electrical, and medical industries. A prolonged endothermic transition between 40 °C and 143 °C (DSC) is also observed, which coincided with the dehydration phase observed in the TGA.

Due to the mechanical properties found in this study, the spider thread in question can be regarded as a highly tenacious material, one that is able to absorb more energy before reaching breaking point. This is due to the fact that it undergoes large elongations since it has a low elasticity constant.

The properties of the spider silk biopolymer at a mechanical, thermal, and structural level relate to science, engineering, and biomedicine, making it an exceptional candidate for numerous applications both at the industrial and medical level, potentially resolving problems that arise when choosing materials with high microelasticity and high mechanical resistance that are compatible with the human body, which is the case here since the silk is mainly composed of proteins and amino acids such as glycine and alanine.

Declarations

Author contribution statement

Gladis Miriam Aparicio-Rojas, Giovanni Medina-Vargas & Edgar Díaz-Puentes: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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References

Alencastre, J., Olarte, C., Rivera, R., Muñoz, J., 2016. Estudio Dinámico del Sistema Araña-Tela de Araña en condiciones de Resonancia. Inf. Tecnol. 27 (4), 139–144.

Alencatre, J., 2015. *Caracterización de las propiedades dinámicas de la seda de araña* (Tesis doctoral). Universidad politécnica de Madrid, España.

Bartoleti, F., Peres, E., Fuentes, F., Da silva, M., Solferini, V., 2018. Phylogeography of the widespread spider Nephila clavipes (Araneae: Araneidae) in South America indicates geologically and climatically driven lineage diversification. *J. Biogeogr.* 45 (6), 1246–1260.

Borja, A., Gonzales, I., Eceiza, A., 2015. Hacia la mímesis de la seda de araña a partir de poliuretanos con segmentos cortos de unidades rígidas y semiflexibles. Revista lationoamericana de metalurgia y materiales 35 (1), 39–48.

Bowen, C., Dai, B., Sargent, C., Bai, W., Ladiwala, P., Feng, H., Huang, W., Kaplan, D., Galazka, J., Zhang, F., 2018. Recombinant spidroins fully replicate primary mechanical properties of natural spider silk. Biomacromolecules 19 (9), 3853–3860.

Cunniff, P.M., Fossey, S.A., Auerbach, M.A., Song, J.W., Kaplan, D.L., Adams, W.W., Eby, R.K., Mahoney, D., Vezie, D.L., 1994. Mechanical and thermal properties of dragline silk from the spider Nephila clavipes. Polym. Adv. Technol. 5 (8), 401–410.

Dos santos-Pint, J., Arcuri, A., Esteves, F., Palma, S., Lubec, G., 2018. Spider silk proteome provides insight into the structural characterization of Nephila Clavipes flagelliform spidroin. Sci. Rep. 8 (1), 14674.

Du, N., Liu, X.Y., Narayanan, J., Li, L., Lim, M.M., Li, D., 2006. Design of superior spider silk: from nanostructure to mechanical properties. Biophys. J. 91 (12), 4528–4535.

Gomes, S., Gallego-Llamas, J., Leonor, I.B., Mano, J.F., Reis, R.L., Kaplan, D.L., 2011. Biological responses to spider silk–antibiotic fusion protein. J. Tissue Eng. Regen. Med. 6 (5), 356–368.

Hauptmann, V., Weichert, N., Rakhimova, M., Conrad, U., 2013. Spider silks from plants-a challenge to create native-sized spidroins. Biotechnol. J. 8, 1183–1192.

Hinman, M.B., Jones, J.A., Lewis, R.V., 2000. Synthetic spider silk: a modular fiber. Trends Biotechnol. 18 (9), 374–379.

Perea, G.B., Pérez-Rigueiro, J., Plaza, G.R., Guinea, G.V., Elices, M., 2010. Efecto de la longitud de onda de la radiación UV sobre la seda de araña. Anales de Mecánica de la Fractura 27, 41–43.

Saravanan, D., 2006. Spider silk-structure, properties and spinning. J. Text. Appar. Technol. Manag. 5 (1), 1–20.

Simmons, A.H., Michal, C.A., Jelenski, L.W., 1996. Molecular orientation and two-component nature of the crystalline fraction of spider dragline silk. Science 271 (5245), 84–87.

Soler, A., 2013. Análisis de la topología de la tela de araña en su comportamiento frente a impacto. (Tesis de pregrado). Universidad Carlos III de Madrid. Leganés, España.

Swanson, B.O., Blackledge, T.A., Beltrán, J., Hayashi, C.Y., 2006. Variation in the material properties of spider dragline silk across species. Appl. Phys. A 82, 213–218.

Vollrath, F., 2000. Strength and structure of spiders' silks. Rev. Mol. Biotechnol. 74 (2), 67–83.

Xu, M., Lewis, R.V., 1990. Structure of a protein superfiber: spider dragline silk. Proc. Natl. Acad. Sci. U.S.A. 87 (18), 7120–7124.