Some material characteristics of spider silk

E. Van Nimmen*, P. Kiekens* and J. Mertens†

*Ghent University – Department of Textiles, Technologiepark-Zwijnaarde 9, B – 9052, Ghent, Belgium.
E-mail: els.vannimmen@rug.ac.be
E-mail: paul.kiekens@rug.ac.be

†Ghent University – Department of Biology, K.L. Ledeganckstraat 35, B – 9000, Ghent, Belgium.
E-mail: johan.mertens@rug.ac.be

Abstract: Today, spider silk tantalizes a range of researchers who focus on the unusual properties of spider silk. Investigators are developing ways to produce dragline silk in quantities that are applicable in several fields, including medical textiles. Spiders indeed produce a wide range of silks with surprisingly different properties that are thoroughly being examined. To conclude, an experimental section presents the results obtained from experiments at Ghent University. SEM has shown that draglines are composed of several thicker and thinner fibres that stick together, whereas in cocoon silk this composite structure is not observed. The effect of time and the effect of natural weathering conditions are investigated for respectively dragline and cocoon silk. Tenacity and elongation at break are not influenced by time for dragline silk. Exposure of cocoon silk to UV-light and humidity results in a less elastic, a less strong fibre, and appears to lower the stiffness a little bit.

Keywords: spider silk, composition, crystallinity, mechanical properties, silk production, solubility, supercontraction.


Biographical notes: Els Van Nimmen graduated in 1994 at Ghent University in Belgium as a civil engineer in textiles. After her studies, she started her career at the Department of Textiles of the Faculty of Applied Sciences of Ghent University. For a period of six years she worked as a research staff member and was involved with projects, educational as well as research, concerning spinning of high-tech fibres, optimization of processes, automation in textiles, etc. In 1999, she became teaching and research assistant. At the moment she intends to make a PhD on the physico-chemical characterization of spider silk.

Paul Kiekens graduated in chemistry at Ghent University in Belgium in 1977. He obtained his PhD in Chemistry in 1982. In 1998 he became a Professor at Ghent University and head of the Department of Textiles of the Faculty of Applied Sciences. Moreover, he received several titles of Doctor honoris causa (Czech Republik – 1997, Romania – 1999, Poland – 2000). He is the executive coordinator of AUTEX (Association of Universities for Textiles) and is a member of several national and international organizations. He disposes of over 100 publications in national and international journals and on international symposia and conferences. Moreover, he has written several chapters in scientific books.
Johan Mertens graduated in Zoology in 1971 and started in the Laboratory of Ecology as a research staff member. For the first three years, he was active in the limnology and aquaculture section. In 1973, he founded the new pedobiology section within the laboratory. Research interests were centred on population dynamics and chemical communication in soil-dwellers. He received his PhD from Ghent University in 1978. Additional research interests from 1987 onwards are on systematics and ecology of Euphyllopoda (Crustacea), integrated aquaculture and eutrophication of freshwaters. In 1991, he became Professor at Ghent University. He is author of several publications in national and international journals.

1 Introduction

Spider invented silk four hundred million years ago for forming cocoons; the building of bull’s-eye orb webs followed about two hundred million years later. Such webs are highly adapted to absorb insect’s kinetic energy efficiently and cheaply. They also glue and retain the prey, yet remain functional under all sorts of environmental stresses. One important ingredient of these webs is the silk, a marvellous material in its own right. The silk fibres (also those of the egg sacs etc.) are composed completely of silk proteins, which have made an irreversible transition from a soluble silk protein solution inside the spider into an insoluble fibre outside the spider.

Nowadays, spiders are often milked, or ‘silked’ by researchers around the world in an attempt to discover the secret of the high strength and flexibility of the material. Modern interest in silk started in the late 1960s with the first extensive mechanical property examination by Zemlin in 1968. It was the unique balance of material properties, good tensile strength, elasticity, and resistance to fracture that was the driving mechanism behind the continuing interest in silks.

Today, spider silk tantalizes a range of researchers, including geneticists, protein biochemists, and materials scientists [1, 2]. They aim at understanding and replicating the spider silk’s unusual properties or synthesizing them for large-scale production. Spider silk has the strength of steel, yet it also has the ability to billow out on a breeze. It can stretch to 40% of its normal length, yet shorten back with great resiliency. Spider silk easily withstands low temperatures.

Investigators are now developing ways to produce dragline silk in quantities sufficient for applications such as bullet-proof vests, impact sensitive composite systems, parachute cords, surgical sutures, and replacement ligaments. The spider silk’s biocompatibility, biodegradability and mechanical response offer the possibility for high tensile strength sutures, prosthetic devices, artificial tendons, blood vessels, and also skin grafts could greatly benefit from the mechanical response of silks.

2 Silk production

Spiders are able to produce all different kinds of silks, which are totally optimized for their task. Spiders secrete as many as seven separate silks for many different purposes: for the different parts of the web; for the production of egg sacs; for
wrapping in their prey; as a lifeline when jumping or dropping to escape; for transferring semen from the abdomen to the male palp; in draglines marked with pheromones; as a shelter in which they can retreat. Accordingly, spiders have evolved a wide range of silks with surprisingly different mechanical properties ranging from lycra-like elastic fibres to Kevlar-like super fibres.

All 36,000 described species of spiders produce silk in specialized glands located in their abdomens. The silk is produced by the silk glands in the form of a liquid with a molecular weight of about 30,000. The synthesis of the silk protein occurs in specialized cells at the tail of the gland and the silk protein is then secreted into the lumen of the gland, where it is stored in its soluble form. The secretions subsequently leave the gland and travel through lengthy convoluted ducts (spinning tubules), a valve and a distal duct to come out the spigots as water-insoluble fibre. The spinneret’s spigots are the organs that release the silk (Figure 1 [3]).

Recently the Danish scientist, Fritz Vollrath has discovered that a spider uses a method of processing its fibre that is similar to that of manufacturing industrial fibres such as nylon. The spiders harden their silk by acidifying it [4].

3 Spider silk properties

3.1 Chemical composition

There are several varieties of spider silk but the general structure of spider silk, like that of all protein fibres, is substituted nylon 2 [5]. Proteins are polymers assembled from a set of 20 amino acids. Amino acids consist of a single carbon in the middle plus a carboxyl group (−COOH), an amino group (−NH₃) and a side chain (‘R’ group), as is shown in Figure 2 [6]. Different ‘R’ groups give different amino acids different properties. For many years scientists have known that of the 20 natural amino acids, only 7, namely analine and glycine, and with lesser amounts of glutamine, leucine, arginine, tyrosine, and serine, often serve as silk’s primary constituents. Cells ‘stick’ the carboxyl group of one amino acid to the amino group of another to form a peptide bond. The composition, and thus the mole percentages

Figure 1 Two types of silk releasing tubes [3].
and sequence of amino acids, of spider dragline silk are dependent on the spider, diet, weather and other factors [5].

3.2 Solubility

Silk fibres are insoluble in water, dilute acids and alkali, chaotropic agents, such as urea and guanidine hydrochloride, and most organic solvents, and are resistant to most proteolythic enzymes [7].

Only in very harsh solvents, such as LiBr, LiClO₄ or 88% formic acid the spider silk is partly or completely soluble. Once dissolved, the protein precipitates if dialysed or if diluted with typical buffers [8].

3.3 Crystallinity

The crystallinity of spider silk is less than the 40% characteristic of *Bombyx mori* silk [5]. The crystallite size is quite small, in the order of 2 nm normal to the fibre axis and 5 nm along the fibre axis. The fibres do not seem to be overly fibrillar and the dragline silk fibres can be tied in knots without showing evidence of shear band formation on the compressive side.

X-ray studies on silks have established the general crystal structure as pseudo-orthorhombic containing β-pleated sheets.

Grubb and co-workers [9] concluded from a detailed X-ray analysis that two different types of crystals are segregated in individual groups in the structure: a well-oriented crystalline component which makes up only 12% of the material and a less oriented component that makes up one third of the material. The remainder is amorphous and isotropic.

3.4 Thermal properties

The thermal behaviour of spider silk is difficult to interpret. Differential scanning calorimetry and dynamic thermal analysis studies show one or more temperature transitions at about −70 °C to −90 °C, with a sharp drop in modulus [5]. Spider silk is still elastic at −40 °C and only becomes brittle at very low temperatures.

The thermal properties of the spider silk protein suggest that the functional performance of the fibres can be retained up to 180 °C without loss of modulus, while decomposition occurs in excess of 250 °C [7].
3.5 The mechanical properties of spider silk

As mentioned before, spiders produce up to seven types of silk with the spinnerets on their abdomen. Their mechanical properties range from Kevlar-like to rubber-like elastomer fibres. The diameter of most of them is several μm. Various studies on physical and mechanical properties of the silks have shown that the mechanical properties of spider silk depend on the moisture content and the strain rate.

It is fair to say that spider major ampullate (MA) silk is amongst the stiffest and strongest polymeric biomaterials known [10]. A typical modulus $E$ at 50% relative humidity at 100%/min tensile strain rate is about 10–50 GPa. The elongation to break of dragline silks has been variously reported to be 9–30%, and the tensile strength is about 0.8–1.7 GPa.

Viscid silk (form the spiral, capturing part of the web) is also a truly remarkable material and the combination of high strength and extensibility gives it a toughness virtually identical to that of MA silk fibres. The average diameter of a single strand was measured to be 1.5 μm. Viscid silk also shows high mechanical hysteresis and contributes therefore to energy dissipation within the web [10].

The force-elongation behaviour of major and minor ampullate fibres at normal room temperature is visco-elastic [11].

3.6 Supercontraction

Major ampullate silk undergoes a process termed supercontraction [12] by shrinking to as little as 60% of its original length in water but not in organic solvents. Supercontraction of dragline fibres is accompanied by swelling of the fibres to 2–4 μm and a drop of the initial stiffness about a 1000-fold. There is a decrease in tensile strength and a considerable increase in elongation before breaking. Fibres recover their original mechanical properties when dried.

4 Experimental

4.1 Materials and methods

4.1.1 Spiders and silk

The dragline threads of two spider species were studied: Araneus diadematus and Araneus quadratus, two species that occur frequently in Belgium. For the spider species Araneus diadematus the silk of the egg cocoons was investigated too.

4.1.2 Collection of the material

The dragline silk used in this work was withdrawn from the spiders by forced silking. A cork with four toothpicks pierced in it and a drill attached to it, were used for the collection of the material. In this way, 30 samples of dragline silk were made for the two spider species.

To see how the properties of the draglines change in time, the remaining samples of the dragline silks were stored for one and a half year in the laboratory. After this period the fibres were tested again.
The egg cocoon silk for the tests in this work was taken from egg cocoons made by *A. diadematus* spiders found in free nature. As a first step in the preparation of the samples, the eggs were removed from the cocoons. After this, the threads were drawn bit by bit from the remaining silk-cluster under a magnifying glass and hung in the holders of a single fibre tensile tester to be tested.

To get an idea of how these cocoon silk fibres change in time, an accelerated weathering test was performed on the cocoons. And then again, the mechanical properties of the cocoon silk were determined and compared with those before the Xenon test.

### 4.1.3 Microscopy

The morphology of the fibres was studied with a scanning electron microscope (SEM). The raster electron microscope shows objects, which are normally invisible for our eyes. It is used for studying the surfaces of solid specimens that require higher magnifications and greater depths of field than can be obtained optically. The smallest object that can be seen with the electron microscope is approximately 0.3 nm ($= 0.3 \times 10^{-9}$ m).

The principle of a raster electron microscope is based on a ray of primary electrons that is sent to the object and causes a ray of secondary electrons. These secondary electrons are then caught by a detector and processed into a picture.

To get a good image, the samples have to be prepared. This involves that the fibres are laid on a small, round sample holder that is covered with sticky tape and are subsequently covered with a very thin gold layer, which is done with the help of high-voltage.

### 4.1.4 Stress-strain measurements

The stress-strain measurements have been performed with an automatic single fibre tester, the FAVIMAT-ROBOT. This instrument measures simultaneously the fineness (dtex) that allows determining the tenacity (cN/tex), an often used parameter for evaluating the strength of fibres. Because of the high variability of fineness for spider silk, relative values are required in order to allow comparisons.

The measurement of the linear density is based on the vibration method at constant fibre tension and fibre length and variable stimulating frequency.

Tensile tests with the Favimat are carried out according to the constant rate of extension principle.

All tests were done at a controlled environmental condition of $20 \pm 2^\circ$C and $65 \pm 2$ rH.

### 4.1.5 Xenon tester

The Xenon test is a test that is used to measure the light- and weathering fastness of materials. This test is especially used when good agreement with outdoor testing is the major goal. In the test, the natural weathering process is accelerated by exposing the sample under prescribed conditions to artificial sunlight for a certain time period.
4.2 Results and discussion

4.2.1 Morphology of spider silk

From the SEM images (see Figure 3), it was very obvious that the draglines were composed of several thicker (several μm) and thinner fibres (a fraction of a μm) that stick together and form the dragline. The diameter of the dragline was quite different for the different samples and also to a lower degree between the two spider species, although both species showed the same ‘composite’ structure.

The cocoon silk of the spider species *Araneus diadematus* was also examined with the SEM (Figure 4). In contrast with the dragline silk, the cocoon fibre had no visible ‘composite’ structure. The fibres looked all the same in thickness and were perfectly round. So one could guess that the spider uses only one spinneret to make the cocoon silk. It was quite surprising and interesting to see, though, that the cocoon silk did have a structure on its surface. On the surface there are some longitudinal superficial cavities with a length of 1 μm or more. But also on places where there are no cavities, some kind of relief can be seen. It appears to be a very fine (order 0.1 μm) longitudinal structure right beneath the surface. It can be interpreted as being a very

![Figure 3 SEM-images of fresh drag line silk of Araneus diadematus magnified respectively 500 and 5000 times.](image1)

![Figure 4 SEM Images of cocoon silk of Araneus diadematus magnified respectively 500 and 8000 times.](image2)
4.2.2 Effect of time on stress-strain measurements for dragline silk

The effect of time is investigated on dragline silk of two spider species of *Araneus quadratus* and *Araneus diadematus*. Ten repetitions were made for each of the 30 samples, however not all tests succeeded. The stress-strain measurements were determined for fresh and 1.5 year old dragline silk.

Testing parameters for the tensile test were: a gauge length of 10 mm, a test speed of 20 mm/min and a pretension of 0.50 cN/tex.

Because of the unusual morphology of dragline silk (composite fibre), it was not possible on the FAVIMAT to determine the fineness. By means of image analysis on microscopic images, the average diameter of the tested draglines was measured to be 0.27 dtex (5.16 μm) for fresh, respectively 0.12 dtex (3.46 μm) for 1.5 year old *A. diadematus* dragline silk and 0.65 dtex (8.00 μm) for fresh, respectively 0.26 dtex (5.09 μm) for 1.5 year old *A. quadratus* dragline silk. It can be concluded that dragline silk of the spider species *A. diadematus* is much finer than that of *A. quadratus*. The obtained dtex-values are used for the calculation of tenacity and modulus in cN/dtex.

A t-test is performed on the results to evaluate the effect on the mechanical properties. The measured tensile properties are given in Table 1, a significant difference (p < 0.05) is indicated in bold.

The high elasticity (± 30%) found in literature for dragline silk is hereby confirmed.

The tenacity of the fresh dragline silk was 7.65 cN/dtex (tensile strength = 0.99 GPa) for *Araneus diadematus* and 4.48 cN/dtex (tensile strength = 0.58 GPa) for *Araneus quadratus*. The draglines of *quadratus* are in fact of the same strength as the silk of the silk worm, while the dragline silk of *Araneus diadematus* is stronger than all natural fibres that are used in textiles. Also the stiffness, based on the modulus at 10%, appears to be lower for *A. quadratus*.

**Table 1** Tensile properties for fresh dragline silk and 1.5 year old (+ 1.5 Y) with ε elongation at break (%), F force to break (cN), W work to rupture (cN*cm), f tenacity (cN/dtex), E modulus at 10% (cN/dtex) (bold values indicate a significant difference p < 0.05).

<table>
<thead>
<tr>
<th></th>
<th>A. diadematus (25 samples)</th>
<th>A. quadratus (22 samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fresh (+1.5 Y)</td>
<td>fresh (+1.5 Y)</td>
</tr>
<tr>
<td>ε (mean)</td>
<td>29.47</td>
<td>30.47</td>
</tr>
<tr>
<td>ε (CV)</td>
<td>19.11</td>
<td>23.45</td>
</tr>
<tr>
<td>F (mean)</td>
<td>2.07</td>
<td><strong>0.88</strong></td>
</tr>
<tr>
<td>F (CV)</td>
<td>32.22</td>
<td>31.33</td>
</tr>
<tr>
<td>W (mean)</td>
<td>0.72</td>
<td><strong>0.13</strong></td>
</tr>
<tr>
<td>W (CV)</td>
<td>54.57</td>
<td>36.78</td>
</tr>
<tr>
<td>f (mean)</td>
<td>7.65</td>
<td>7.36</td>
</tr>
<tr>
<td>f (CV)</td>
<td>32.22</td>
<td>31.33</td>
</tr>
<tr>
<td>E (mean)</td>
<td>25.43</td>
<td><strong>18.54</strong></td>
</tr>
<tr>
<td>E (CV)</td>
<td>37.70</td>
<td>41.42</td>
</tr>
</tbody>
</table>
It is astonishing to see how neither the elongation at break, nor the tenacity of the draglines for both spider species has changed in time. So one could conclude that the fibres are not influenced nor degraded by the conditions in the laboratory (20 °C and 65% rH).

With respect to modulus, it is difficult to draw conclusions since the decrease for *A. diadematus* is significant, while for *A. quadratus* no significant difference could be proved. However, the decrease is restricted to 10–30%. Here it should be remarked that the incorrectness on the fibre diameter possibly has an influence. The diameter measurement is not repeated for each of the 30 samples.

Work to rupture or the energy required to break the dragline silk, appears to lower significantly (± 80%) by time.

### 4.2.3 Effect of UV-light on stress-strain measurements for cocoon silk

The effect of UV-light is investigated by using the Xenon-test on 5 egg cocoons of the spider species *Araneus diadematus*. For each egg cocoon 50 repetitions were made, however not all tests succeeded. The stress-strain measurements were determined before and after the Xenon-test.

Testing parameters for the tensile test were: a gauge length of 20 mm, a test speed of 20 mm/min and a pretension of 0.50 cN/tex. For the linear density test, a test speed of 5 mm/min and a pretension of 0.80 cN/tex were used.

A t-test is performed on the results to evaluate the effect on the mechanical properties. The results of the tensile tests are shown in Table 2, a significant difference (p < 0.05) is indicated in bold.

A general trend for all the cocoons is the very strong significant decrease (40–70%) in the elongation at break. So it is definitely permitted to conclude that

### Table 2  Tensile properties and linear density of the 5 egg cocoons before (Bxen) and after (Axen) the Xenon test with ε elongation at break (%), F force to break (cN), W work to rupture (cN*cm), f tenacity (cN/dtex), E modulus at 2% (cN/dtex) (bold values indicate a significant difference p < 0.05).

| Cocoon  | Bxen | Axen | Bxen | Axen | Bxen | Axen | Bxen | Axen | Bxen | Axen | Bxen | Axen | Bxen | Axen | Bxen | Axen | Bxen | Axen | Bxen | Axen | Bxen | Axen |
|---------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| ε (mean)| 25.77| 15.37| 37.51| 14.00| 28.07| 7.78 | 29.37| 16.51| 22.72| 6.31 | 28.93| 12.03|      |      |      |      |      |      |      |      |      |      |      |      |
| ε (CV)  | 50.58| 71.12| 56.82| 82.38| 54.17| 45.08| 63.44| 65.68| 60.14| 86.64| 60.74| 82.58|      |      |      |      |      |      |      |      |      |      |      |      |
| F (mean)| 1.56 | 1.36 | 2.25 | 2.08 | 1.67 | 1.35 | 1.82 | 2.00 | 1.69 | 1.31 | 1.81 | 1.63 |      |      |      |      |      |      |      |      |      |      |      |      |
| F (CV)  | 23.45| 35.54| 27.84| 28.94| 27.49| 24.94| 36.77| 20.46| 20.88| 29.11| 31.73| 34.87|      |      |      |      |      |      |      |      |      |      |      |      |
| W (mean)| 0.80 | 0.48 | 1.70 | 0.73 | 0.97 | 0.21 | 1.13 | 0.68 | 0.74 | 0.27 | 1.08 | 0.50 |      |      |      |      |      |      |      |      |      |      |      |      |
| W (CV)  | 50.00| 62.50| 51.76| 71.23| 57.73| 47.62| 63.72| 69.12| 62.16| 51.85| 67.05| 84.04|      |      |      |      |      |      |      |      |      |      |      |      |
| f (mean)| 2.22 | 1.86 | 2.19 | 1.86 | 2.09 | 1.82 | 2.05 | 2.05 | 1.73 | 1.38 | 2.05 | 1.80 |      |      |      |      |      |      |      |      |      |      |      |      |
| f (CV)  | 17.32| 32.35| 22.91| 27.73| 19.43| 18.62| 27.77| 17.36| 22.32| 27.93| 23.91| 27.76|      |      |      |      |      |      |      |      |      |      |      |      |
| E (mean)| 60.18| 59.05| 55.00| 57.68| 56.15| 61.47| 54.48| 61.53| 49.09| 50.00| 54.89| 58.12|      |      |      |      |      |      |      |      |      |      |      |      |
| E (CV)  | 7.94 | 8.94 | 10.23| 9.16 | 12.28| 11.29| 11.42| 7.32 | 10.18| 21.69| 13.04| 13.73|      |      |      |      |      |      |      |      |      |      |      |      |
| dtex (mean)| 0.71 | 0.75 | 1.03 | 1.11 | 0.80 | 0.74 | 0.89 | 0.97 | 0.98 | 0.97 | 0.89 | 0.91 |      |      |      |      |      |      |      |      |      |      |      |
| dtex (CV)| 19.01| 18.87| 14.73| 4.84 | 17.59| 16.33| 24.67| 8.10 | 13.99| 18.41| 21.29| 19.62|      |      |      |      |      |      |      |      |      |      |      |      |
cocoon silk becomes less elastic due to the exposure to UV-light. In comparison to dragline silk, it can be concluded that the elongation is similar (28% for dragline silk [13]).

On the other hand, for force to break it is difficult to draw a conclusion. For most cocoons (except cocoon 4) there is a significant decrease (10–20%). For cocoon 4, the force to break is 10% higher after the Xenon test.

For the influence on work to rupture it can be concluded that the exposure to UV-light results in a significant decrease (40–80%) of work to rupture, in other words, less energy is required to break the spider silk. It should be remarked that the difference is very variable between the different cocoons.

The tenacity of fibres takes into account the linear density (which is also measured by the FAVIMAT instrument). For this parameter, for all cocoons, except cocoon 4, the tenacity decreases significantly (10–20%). For cocoon 4, there is no difference measured before and after the Xenon-test. The absolute tenacity amounts to 1.5–2 cN/dtex that is considerably lower (4 times) than the value for dragline silk (+8 cN/dtex [13]).

For the effect of UV-light on the modulus, no clear conclusion can be drawn. For the cocoons 1, 2 and 5, no significant difference is observed whereas for the cocoons 3 and 4, there is a significant increase (10–15%). So, it appears that the stiffness of spider silk will rather increase than decrease when exposed to UV-light. The absolute value of the modulus is comparable to data found in literature for dragline silk (6.90 GPa or 53 cN/dtex [13]).

Since the FAVIMAT instrument also allows measuring the fineness in dtex, this parameter is evaluated. For the linear density, it appears that UV-light does not have much influence, which could be expected. The differences observed in terms of percentage amount to less than 9%. As can be seen, the absolute fineness of the cocoon silk amounts to approximately 0.9 dtex. Cocoon silk has the same fineness as dragline silk (3.0 μm or 0.9 dtex [10]).

5 Conclusion

Research on spider silk is focused on the explanation of the unusual properties of spider silk and on artificial spinning of these fibres in order to be able to produce ‘synthetic’ spider silk in mass. Literature on spider cocoon silk with respect to their characteristics does not exist. In this work, the morphology, the influence of time and the influence of weathering conditions are investigated on spider dragline and cocoon silk.

It is shown that dragline silk shows a ‘composite’ structure, composed of several thicker and thinner fibres, whereas this is not the case for cocoon silk. Cocoon silk fibres are uniform in fineness and perfectly round. Moreover, longitudinal superficial cavities with a length of 1 μm or more are observed on the surface of the cocoon fibres.

With respect to the tensile properties, first of all, it should be remarked that the high variability for dragline silk is also observed in the properties of egg cocoons [13]. This variability will be further investigated for other spider species.

In comparison to dragline silk [13], it can be concluded that the elongation and modulus are similar while the tenacity is considerably lower for cocoon silk. Further
research on the microstructure of both types of silk is required to explain this difference.

The time dependence of dragline silk is evaluated on fresh draglines and on 1.5 year old draglines. It is shown that the tenacity and elongation at break are not changed in time. The stiffness of the dragline fibre appears to decrease slightly whereas the energy to break the fibre is significantly lower after 1.5 years.

To investigate the influence of UV-light and humidity on the properties of cocoon silk, the cocoon silk was subjected to a Xenon test. It can be concluded that exposure to UV-light will result in a less elastic, a less strong, and probably a little more stiff spider silk. The work to break of the treated spider silk is significantly lower. As could be expected, UV-light has no influence on fineness.

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References

