Spider silks mechanics: Predicting humidity and temperature effects

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A B S T R A C T

We deduce a microstructure inspired model for humidity and temperature effects on the mechanical response of spider silks, modeled as a composite material with a hard crystalline and a soft amorphous region. Water molecules decrease the percentage of crosslinks in the softer region inducing a variation of natural length of the macromolecules. The resulting kinematic incompatibility between the regions crucially influences the final mechanical response. We demonstrate the predictivity of the model by quantitatively reproducing the experimentally observed behavior.

1. Introduction

Due to their extraordinary properties, spider silks represent one of the most intensively studied materials, also in the spirit of biomimetics (Zhao et al., 2014). The availability of more and more sophisticated experimental techniques let in the last decades a deeper understanding – both from a chemical and structural point of view – of the complex multiscale, hierarchical material structure at the base of their notable mechanical behavior. Nevertheless, due to the complexity of its behavior, many important phenomena regulating its loading history dependence, rate, temperature, and humidity effects, remain unclear (Pérez-Rigueiro et al., 2021), especially when multiscale effects are taken in consideration. In this paper we propose a model for describing and predicting humidity and temperature effects and with a special focus on a crucial phenomenon known as supercontraction effect. Firstly addressed in Work (1977), it consists in a shrinkage of the fiber up to 50% of its initial length, when immersed in water or in high humidity environment strongly modifying the mechanical performances. As we show, the comprehension of such striking behavior let us deduce a predictive model for the experimental thermohygromechanical behavior of spider silks, starting from a description at the molecular scale.

Spider silks are composed by an amorphous matrix of oligopeptide chains and by pseudo-crystalline regions made up principally of polyalanine \( \beta \)-sheets (Elices et al., 2011; Sponner et al., 2007) with dimensions between 1 and 10 nm (Keten and Buehler, 2010), mostly oriented in the fiber direction (Jenkins et al., 2013). The cross section of the fiber is highly organized in the radial direction (Li et al., 1994; Eisoldt et al., 2011; Sponner et al., 2007). Moreover, the chemical and structural composition varies according with the different silks produced by the different glands (Cranford et al., 2014) and of course the different species. Here, to fix the ideas, we focus on the most performant case of dragline silk.

More in detail, the thread is covered by a skin, with a chemical and physical protection function, that does not play a role of the complex multiscale, hierarchical material structure at the base of their notable mechanical behavior. The internal part, here referred as soft region, is instead mainly constituted by proteins with a proline content preventing the formation of \( \beta \)-sheet structures (Major ampullate Spidroin 2, MaSp2, (94), Brown et al. (2011)) organized into \( \beta \)-pleated sheets. We refer to this fraction as hard region. The internal part, here referred as soft region, is instead mainly constituted by proteins with a proline content preventing the formation of \( \beta \)-sheet structures (Major ampullate Spidroin 1, MaSp1, Li et al.

![Fig. 1. Cartoon of the silk fiber and its microstructure (a). The outer hard region characterized by crystals of \( \beta \)-sheets is represented by black chains, the soft region molecules are drafted in blue, whereas the embedding elastic matrix is gray. In (b) we schematize the incremental effects of water molecules (added as RH increases) disrupting crystal domains (green dots) and inducing entropic recoiling of chains.](image-url)
Sponner et al. (2007)). This fraction has a significantly lower crystallinity and macromolecules with weaker crystal domains, typically in the form of $\alpha$-helix and $\beta$-turns (Sponner et al., 2007; Nova et al., 2010). The different crystallinity is also due to the shear stress at the spinning duct wall inducing the formation of harder crystal domains in form of $\beta$-sheets mainly in the outer region (Giesa et al., 2016; Brown et al., 2011).

Based on the previous description and referred literature, we model the silk fiber as a composite material with a hard external fraction of crystalline chains and a soft internal fraction of amorphous chains. Moreover, by following the classical approach for polymeric and biopolymeric materials (Flory and Erman, 1982), we suppose that the hard and soft fractions of macromolecules aligned with the fiber axis are embedded in a tridimensional elastic matrix, describing the complex macromolecular network with inner and intrachains connections.

Due to the different crystalline composition, the humidity affects differently the hard and soft fractions. Water hardly breaks the H-bonds of the compact $\beta$-sheet domains in the hard fraction (Yazawa et al., 2019). On the other hand, here, we may observe a misalignment of the crystals with respect to the fiber direction that increases as the relative humidity (RH) grows (Elices and Michal, 2004). Since the material stiffness grows with the alignment of the crystals (Du et al., 2006), water induces a progressive damage in this fraction that we introduce in our model. On the contrary, water content strongly influences the crystal percentage in the soft internal core (Du et al., 2006; Elices et al., 2005, 2011), because $\alpha$-helices and $\beta$-turns are much more easily broken by water molecules. In particular, the experiments exhibit a non uniform variation of the silk properties with a localized transition at a critical value of RH, hereon indicated as RH$_c$, known as supercontraction threshold (Fu et al., 2009).

Finally, an important effect in the evolution of the natural chain length is induced from the stretch history (see Sponner et al., 2006). To consider the disruption process of H-bonds induced by hydration (Du et al., 2006), let us introduce the function $m = m(RH)$ assigning the number of links in the generic humidity state, with $m(0) = m_0$ and $m(100) = m_f$ (initial and permanent number of H-bonds) (Vollrath and Porter, 2006). To the knowledge of the authors, no direct measurement of $m$ is available, so that we consider a Gaussian probability density of rupture events with expected value coinciding with RH$_c$ (see Fig. 2)

$$\frac{d}{dR} = m(RH) - m_f = \int_{m_0}^{m_c} e^{rac{1}{2}(m - m_0)^2} \frac{m}{\sigma^2} dR.$$

2. Model

According with classical Statistical Mechanics results (Rubinstein and Colby, 2003), the expectation value of the end-to-end distance for ideal chains is

$$L_n = \langle r \rangle_0 = b n^{1/2},$$

where $n$ is the number of Kuhn segments with length $\beta$. We refer to the end-to-end distance as natural length for the reason that it is the length that the chain naturally assumes when no force is applied. As we will detail later $n$ depends on humidity in the soft fraction, whereas it depends on the maximum attained stretch in the hard fraction. As a result the natural lengths of these fractions, assumed identical after spinning, vary depending on the external force and humidity fields, inducing a kinematic incompatibility as detailed in the following. We remark that in the recent paper (Cohen et al., 2021), based on this observation, the authors proposed a model with a material undergoing a hard—soft transition reproducing a mutation from glassy to rubbery state induced by RH variations, efficiently describing the variations of stiffness and supercontraction length in correspondence of the supercontraction threshold.

![Fig. 2.](image1.png)

Fig. 2. (a) Damage parameter $d$ representing the percentage of broken links as a function of humidity. (b) Influence of the relative humidity on the number of H-bonds with respect to the initial number of H-bond. Here RH$_c$=80%, $\varepsilon$=5.5 and $m_0/m_f=0.4$.

![Fig. 3.](image2.png)

Fig. 3. Cartoon of a chain of the hard region undergoing a folded → unfolded transition when subjected to a force.

2.1. Soft region

To consider the disruption process of H-bonds induced by hydration (Du et al., 2006), let us introduce the function $m = m(RH)$ assigning the number of links in the generic humidity state, with $m(0) = m_0$ and $m(100) = m_f$ (initial and permanent number of H-bonds) (Vollrath and Porter, 2006). To the knowledge of the authors, no direct measurement of $m$ is available, so that we consider a Gaussian probability density of rupture events with expected value coinciding with RH$_c$ (see Fig. 2)

$$m = m(RH) - m_f = \int_{m_0}^{m_c} e^{rac{1}{2}(m - m_0)^2} \frac{m}{\sigma^2} dR.$$
Here \( d \in (0, 1) \) is a ‘damage’ type parameter, measuring the percentage of broken links and we indicate by \( s^2 \) the standard deviation of RH.

To obtain the corresponding variation of the natural length based on (1), assume that \( n' \) is the (mean) number of chain’s free monomers when the silk is spun. Here and in the following we indicate by the apexes \( s, h, m, \) and \( t \) the soft, hard, matrix and homogenized (total) quantities. If we formally identify the number of H-bonds with the number of domains in which the chain is divided (see the scheme Fig. 1(b)), the mean number of free monomers in each domain is \( n' = n^h(RH) = n^h_s/\hat{n}(RH) \) corresponding to a (humidity dependent) natural length

\[
L_{sn} = \hat{n}(RH)\sqrt{n^h_s/\hat{n}(RH)} b^s = \sqrt{n^h_s \hat{n}(RH)} b^s.
\]

We obtain in this way an analytic measure of the shrinkage chain effect induced by humidity. Observe that instead the contour length is fixed: \( L_c = n' b^c \).

### 2.2. Hard region

As anticipated, following (Du et al., 2006) we assume that the elastic modulus of the crystalline region decreases with RH by considering a (phenomenological) damage function in the macroscopic model deduced in Section 2.3. On the other hand, while \( \beta \)-sheet crystals are affected only in their orientation by humidity, large strain can induce important unraveling effects as fully described in Puglisi et al. (2017) with conformational transitions inside the secondary structure (Cranford et al., 2014; Giesa et al., 2016) from a coiled configuration (Yarger et al., 2018) to an unfolded state (Cranford et al., 2014; Puglisi et al., 2017) (see the scheme in Fig. 3). Thus the mean number \( n'_s \) of available free monomers depends on the maximum attained value of the end-to-end distance so that we assume the existence of a function \( n' = n'(L_{max}) \). Observe that for simplicity we assume that the unfolding is irreversible with \( n' \) monotonically increasing with \( L_{max} \). More general hypotheses could be introduced (De Tommasi et al., 2010) and a discussion of this function will be given later.

As a result for the hard fraction the natural and contour lengths are assigned as

\[
L_s^b = \sqrt{(L_{max}) b^b}, \quad L_{sc} = \hat{n}(L_{max}) b^b.
\]

### 2.3. From single chain to macro laws

Both in the amorphous and crystalline region we adopt a Worm Like Chain (WLC) energy density (per unit chain contour length \( L_c \)) in the form proposed in De Tommasi et al. (2013) \( \Psi_1 = \Psi_1(L, L_c) = \kappa l_c^{-\beta} L^{-\gamma} \), where \( \kappa = \kappa_b^2 \gamma \), \( T \) is the temperature, \( k_b \) the Boltzmann constant and \( L_c \) the persistence length measuring the flexibility of the chain (see e.g. Rubinstein and Colby (2003) for details). This energy respects the limit extensibility condition, \( \lim_{\lambda \to \infty} \Psi_1(L, L) = +\infty \), and allows for explicit calculations. Moreover, following (Trentadue et al., 2021), we extend this function to consider that, as described above, the end-to-end distance \( L \) can be decomposed in a variable (zero force) natural length measured by (1) and the remaining length measuring the elastic elongation \( L_e = L - L_n \). Thus we assume an energy and a force–elongation law for a single chain

\[
\Psi_1 = \kappa \frac{L_e}{L_c} L_c^{2\gamma} \sigma \quad \Rightarrow \quad \lambda = \left[ \frac{L_e}{L_c} \right]^{2\gamma} \quad \Psi_1 = \Psi(L_c, L_e) = \kappa \frac{L_e}{L_c} L_c^{2\gamma} \sigma.
\]

\[
f = \partial \Psi_1 = \kappa \frac{L_e}{L_c} L_c^{2\gamma} \sigma.
\]
with the force decreasing to zero as the length attains its (history dependent) natural length ($L = L_o$ or $L = 0$).

Notice that the total energy considering all the chains of the network can be determined as $\Phi = N \epsilon_i L_i = N \epsilon_i n_i L_i = N \epsilon_i m_i$ where for the sake of simplicity we consider $N/L_i = 1$, with $N, N_a, N_i$ the number of chains per unit volume, area, length respectively.

We remark that the proposed model can be inscribed in the theory of Thermodynamics with internal variables (Coleman and Gurtin, 1967) in the simple case when there is a single external variable $L$ and a single internal variable $L_{\text{max}}$. In our simple setting of the positivity of the dissipation rate $\Gamma = f L - \phi(L, L_{\text{max}}) \geq 0$. Since at given RH the only material fraction involved in the dissipation isothermal processes, to verify the thermodynamic consistency of the model we consider the Clausius–Duhem inequality, requiring

energy dissipation rate reduces to $Q(L_{\text{max}}) = -\partial_{L_{\text{max}}} \phi(L, L_{\text{max}})$: Thus, in view of (5), we obtain $\Gamma = Q(L_{\text{max}}) L_{\text{max}} \geq 0$ that is the hard one, undergoing unfolding effects and variations of the natural length regulated by $L_{\text{max}}$ according to (4), the internal satisfied under our assumption that $\sigma$ is increasing.

Eventually, to obtain the macroscopic behavior of the thread we consider, following (Grubb and Ji, 1999), the classical affinity hypothesis (Rubinstein and Colby, 2003) that identifies the macroscopic stretches with the macromolecular ones. We then first evaluate the following stretch measures of the different fractions

$$\lambda_i = \frac{L_i}{L_{\text{io}}} \text{ total stretch,}$$

$$\lambda_{ie} = L_{\text{io}} \text{ elastic stretch,}$$

$$\lambda_{im} = L_{\text{io}} \text{ permanent stretch,}$$

$$\lambda_{ic} = L_{\text{io}} \text{ contour stretch,}$$

with $L_{\text{io}} = b_i^{1} \sqrt{n_{\text{io}}^{1}}$ denoting the initial natural length of the chains. In particular, the permanent stretch measures the variation of the natural length, having the same role of plastic stretch in classical non linear plasticity theories (see De Tommasi et al. (2015) for a detailed theoretical discussion).

For the soft region, it can be deduced using Eqs. (3) and (6),

$$\lambda_{im} = \frac{\sqrt{\frac{n_{\text{io}}^{1} \gamma_i (R,H)}}{n_{\text{io}}^{1} n_{\text{io}}^{1} b_i^{1}} = \frac{\gamma_i (R,H)}{m_i} \text{ (7)}$$

If we consider a Gaussian distribution for the breaking rate of the bonds as in Eq. (2) we get the following evolution of the permanent stretch as RH varies $\gamma$

$$\lambda_{im} = 1 + a(R,H) \text{ (8)}$$

A typical variation of the damage function and number of domains under our Gaussian probability choice is represented in Fig. 2, whereas the variation of the permanent stretch of the soft fraction is reported in Fig. 4. We remark that the parameters needed to compute the variation of the permanent stretch as a function of humidity are $m_i/m_o$, together with RH, and $\gamma$ assigning the Gaussian distribution function. On the other hand, the corresponding expression for the contour length is $L_{ci} = m s \sigma \epsilon_i b_i = m s b_i$, so that the contour stretch of the amorphous part is constant $\gamma$

$$\lambda_{ic} = L_{\text{io}} = \frac{n_{\text{io}}^{1} b_i^{1}}{n_{\text{io}}^{1} M_o b_i^{1}} = \frac{m_o}{M_o} \text{ (9)}$$

Under an additive assumption and given the number of chains per unitary reference area $N \epsilon$, the (Piola, engineering) stress using Eqs. (5) and (6) is given by

$$\sigma = \frac{[\lambda_{im} \lambda_{ic} - \lambda_{im} \gamma_i (R,H)]^2}{\lambda_{im} - \lambda_{io}} - 1 \text{ (10)}$$
where the permanent and contour stretches are given by Eqs. (8) and (9), whereas $E_s = N_s \kappa_s$ is the elastic modulus of the soft fraction.

For the hard region, the contour and permanent stretches are calculated by using Eqs. (4) and (6). Let $n_{ho}$ be the initial number of available free monomers, the initial natural length $L^h = \sqrt{n^h_0 b^h}$ can be used to calculate the contour stretch as

$$L_c^h = \frac{n_{ho} b^h}{L} = \sqrt{n^h_0 b^h} \sqrt{n^h_0} = \sqrt{n^h_0}$$

and the permanent stretch as

$$L_p^h = \frac{n^h_0 b^h}{L} = \sqrt{n^h_0 b^h} \sqrt{n^h_0} = \sqrt{n^h_0}$$

(12)

Since the effective stretch-induced unfolding depends on the unknown size and strength crystals distribution, following (Trentadue et al., 2021) we assume a simple power law

$$\lambda_c = \lambda_c(\lambda_{max}) \cdot c.$$  

(13)

On the other hand, since by Eq. (4) the permanent and contour stretches are related by $\lambda_c^h = (\lambda_c^p / \lambda_c^o)^{1/2}$, where $\lambda_c^o = c_1$ is the initial contour stretch, by Eq. (13) the permanent stretch is given by

$$\lambda_p^h = (\lambda_{max}) \cdot c.$$  

(14)

It is important to stress that, thanks to previously described microstructure based analysis of the permanent and contour stretches, they are analytically related, so that in this fraction the permanent stretches are directly related to damage, both measured by the unknown function $\eta^h$.

If then, as anticipated, we consider a damage taking care of the described humidity-induced crystal misalignment, the stress–stretch relation for the hard part by using Eqs. (5) and (6) is

$$\sigma^h = E^h \frac{L_c^h}{L} (\hat{\lambda}_c(\lambda_{max}) - \hat{\lambda}_c(\lambda_{max}^o)) \frac{1}{2} - 1$$

(15)

with

$$\hat{\lambda}_c^h = (1 - \alpha \text{RH}) E^h.$$  

(16)

where $E^h = N_s \kappa_s$ is the modulus in the dry condition and $\alpha$ measures the humidity-induced damage rate.

This macroscopic constitutive damage assumption is based on the experimental observation, recalled above, of a crystal misalignment induced by humidity. Indeed, the Wide-Angle X-ray Scattering (WAXS) measurements reported in Fig. 5(a) and reproduced from (Yazawa et al., 2020), indicate a linear dependence of the Full Width at Half Maximum (the statistical measure of the orientation of crystalline $\beta$-sheets adopted in Yazawa et al. (2020)) from RH, indicating a lower orientation of the nano crystals at higher RH values. Such misalignment affects the elastic modulus as described in Du et al. (2006). The resulting modulus variation assumed in (16) is represented in Fig. 5(b).

Eventually, by following (Flory and Erman, 1982), we consider a matrix embedding hard and soft fractions describing the complex macromolecular network composing the spider thread, with inter and intrachains connections. The total free energy is then calculated as the sum of the energy of ideally isolated hard and soft chains described above and elongated along the fiber direction plus an energy term taking care of chains interactions. Under a simple Neo-Hookean assumption (corresponding at the molecular scale to harmonic network chains (Rubinstein and Colby, 2003)) for incompressible material, the matrix stress for a uniaxial extension is

$$\sigma = \mu \frac{\lambda - 1}{(\lambda - 1)^2}.$$  

(17)
with $\mu$ the material shear modulus. Observe that we assume that the matrix natural length coincides with the hard fraction initial one, so that $\lambda^m = \lambda^h$.

We are now in the position of deducing, based on all the microstructure based assumptions and the affinity hypothesis, the macroscopic behavior of the spider thread. Our deduction is based on the main assumption that the spun initial length of the fractions are the same: $n^h b^h = n^s b^s$ (initial kinematic compatibility). After exposition to humidity the soft region chains reduce their natural length (see Fig. 6). The kinematic compatibility then imposes that the different regions undergo the same stretch $\lambda^c$ at zero overall initial thread force. On the other hand, when the fiber is subjected to a force $F > 0$, it undergoes a stretch $\lambda^c$, starting from the new natural configuration $\lambda^c$. Observe also that $\lambda^c$ represents the experimentally measured overall thread stretch. As a final result, the stretches for the soft region (that varies its permanent stretch according to humidity), hard region and matrix starting from the spun initial length (see the scheme in Fig. 7) are given by $\lambda^s = \lambda^h = \lambda^m = \lambda^c$, where $\lambda^c$ represents the experimentally measured stretch.
The overall stress–stretch relation is

$$\sigma(\lambda) = \Theta(\lambda - \lambda_0)(1 - \sigma_{\text{RH}}) E_{\text{h}} \left( \lambda - \lambda_{\text{sh}} \right) \left( \lambda_{\text{oh}} - \lambda_{\text{sh}} \right) + E_{\text{sh}} \left( \lambda_{\text{sh}} - \lambda_{\text{sn}} \right) - 1 + \mu \lambda \lambda_{\text{oh}} - \left( \lambda_{\text{sh}} \lambda_{\text{oh}} \right)$$

(18)

where $\Theta$ is the step function considering that the hard fraction chains are not able to sustain any compressive force ($\sigma = 0$ if $\lambda \leq \lambda_{\text{sh}}$). Observe that from this equation at $\lambda = 1$ and $\sigma = 0$ we determine $\lambda_{\text{sh}}$.

In Fig. 8 we describe the behavior of the model here proposed, representing the stress–stretch curves for different humidity conditions (RH = 0%, 70%, 85%, 90%, 100%). In the dry condition (RH = 0%) the natural length of the hard and soft fraction coincide ($\lambda_{\text{oh}} = \lambda_{\text{sh}}$) and the hard fraction participates to the mechanical response from the beginning. Consequently the force is carried mainly by the much stiffer hard fraction for all the elongation set. The behavior is similar for RH < RH, where a lower stiffness is due to the hard domain disorder inducing a damage according with Fig. 5 and Eq. (12) (see the curve RH = 70%). Once RH > RH, the role played by the amorphous fraction becomes much more relevant because its natural length $\lambda_{\text{oh}}$ undergoes a significant decrease (see Fig. 4). This leads to a consequent substantial decrease of the initial (zero force) length of the total fiber $\lambda_{\text{oh}}$. Thus, as long as the fibrils of the hard region do not reach again their natural length, the mechanical response is given only by the amorphous phase and the matrix (see e.g. point $P_1$ in Fig. 8). Then, as soon as the hard region starts to be stretched (point $P_2$) the force starts again to be mainly sustained by the stiffer crystalline phase (see e.g. point $P_3$). We point out that in this way we give a theoretical interpretation of the "localised" material hardening observed in spider threads (see Fig. 9). The behavior is similar also for higher values of the humidity (RH = 90%), with different values of $\lambda$ distinguishing the two regimes. At extreme humidity conditions (last curve at RH = 100%) the mechanical behavior may be given by the only amorphous phase and matrix if the ultimate stretch of the fiber is lower than the transition threshold.

Even though in this paper we are focused on humidity effects on monotonic stress–stretch curves, in Fig. 8 we also show through dashed lines the system behavior when subjected to unloading. This figure let us show that, based on the microstructure interpretation, the proposed model is able to describe not only the fundamental macroscopic damage effect, but also the experimentally observed presence of residual stretches (Vehoff et al., 2007). Interestingly, permanent deformations are not deduced as usually independently from damage, e.g. through the introduction of new variables, whereas both damage and residual stretches descend from the unfolding of the hard domains.

A comment about a second important aspect of the variable mechanical behavior is now in order: temperature effects. Indeed, when the temperature at fixed RH is increased, the silk undergoes an effect of link scissions as described for humidity (Plaza et al., 2006). Moreover, also temperature growth is accompanied by fiber contraction (Glišović and Salditt, 2007) and again the experiments show the existence of a critical value where such effects of link scission and length variation are strongly localized. In analogy with polymer mechanics this value is indicated as glass transition temperature $T_g$. In particular, in Fu et al. (2009) the authors obtained an experimental linear relation between $T_g$ and RH. Of course such a relation would ask a theoretical description that by itself appears to be very interesting, but it is out of the aims of this paper. Instead, to show that our model can reproduce also the experimental temperature effects, we phenomenologically assume a Gaussian dependence of the number of links from temperature in Eq. (2) (where RH is substituted by $T_g$) and then we modify correspondingly the constitutive equation Eq. (18).

Accordingly RH is substituted by $T_g$. The efficacy of these assumptions are well supported by the experimental comparison considered in the following section.

The final aspect of the model regards the humidity and temperature dependence of the limit stretch: to this hand we need a fracture criterion. Based on the considerations in Yazawa et al. (2020) we here assume that the fracture is regulated by the hard fraction and in particular that the fracture condition is $\lambda = \lambda_{\text{sh}}$, where $\lambda_{\text{sh}}$ is a given constitutive parameter. As we show in the following section, this criterion is successful with the exception of the fully dry condition where the breakage is known to be induced by localized damage defects (Yazawa et al., 2020).

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Fig. 8. Theoretical stress–stretch curves at different humidity conditions (continuous lines correspond to loading and dashed to unloading curves). Three points of the curve RH = 85% are marked to illustrate different regimes schematically illustrated on the right: $RH$ hard phase is unloaded, $\rho$ hard phase in its natural configuration, $\rho$ hard phase in traction. Here $E_h = 2.16$ GPa, $G = 18$ MPa, $\mu = 0.14$ MPa, $\alpha = 1.33$, $c_0 = 0.75$, $a = 0.0099$, $m_1/m_2 = 0.3$, $s = 6.5$, $\lambda = 1.65$, $RH = 80\%$, $\lambda_{\text{sh}} = 1.34$. 

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3. Experimental validation

In this final section we verify the effectiveness of the proposed model in predicting the mechanical behavior of spider silks by quantitatively comparing the main experimental effects induced by humidity and temperature variations on different silks with the theoretical behavior.

Consider first the tensile response under variable RH for a highly stretchable silk (Argiope trifasciata fibers, reproduced by Elices et al. (2005)). As shown in Fig. 9 this silk exhibits a remarkable dependence of the mechanical response on humidity. We may observe two different regimes in accordance with the silk experimental response: for RH < RHc the behavior is almost linear and this is due in our model to the dominance of the hard fraction; for RH > RHc we have two regimes. Initially the silk is highly stretchable, with high deformations at very low forces. In this regime the numerical simulations show that the hard region is shorter than its natural length, so it does not contribute to the fiber stiffness. When this length is attained, the fiber exhibits a sudden hardening. In Fig. 9 it is possible also to verify the efficacy of the introduced fracture criterion. Indeed, we calibrated the hard fraction limit stretch to reproduce the experimental limit at RH = 70% and then we predicted the RH = 90% and 100% cases with errors of only 0.17% and 1.49%, respectively. As anticipated the prediction is less accurate for very low humidities. Observe the possibility of predicting damage, hardening localization and fracture strain at very different values of the humidity with fixed parameters.

To further test the efficiency of the model, we show the possibility of predicting the influence of humidity and temperature on other important material parameters, such as elastic modulus, supercontraction stretch of unrestrained fibers and limit stretches (Fig. 10). It is important to remark, regarding the predictivity properties of the model, that in this prediction of the experiments we fixed the material parameters and changed only RHc at different temperatures using the experimental values in Plaza et al. (2006). In Fig. 10 we also reproduce the limit stretch for the available testing temperature (55°C).
Furthermore, we consider the effects induced by variable temperature at fixed RH in Plaza et al. (2006). We evaluated $T_o$ at given RH=50% using the relation reported in the same paper. The results exhibited in Fig. 11 show again an accurate reproduction of the experiments. We remark that a comparison of the values of $E'$ at RH=50% and different temperatures lead to different values of the elastic moduli estimated by Figs. 10 and 11, thus showing that the data refers to different silks so that different parameters have been used in the two figures.

In addition to the Argiope trifasciata spider fibers (Fig. 9), we consider tensile tests performed on a Nephila clavata spider fiber under various RHs (0%, 75%, 97%) reproduced from (Yazawa et al., 2020). In Fig. 12 we report the comparison between the experimental results and the theoretical model. Despite this silk shows a remarkably different response to the humidity variations, the proposed model is once again significantly successful in quantitatively predicting the observed experimental behavior. In Fig. 12 we also test the above described rupture hypothesis for the Nephila clavata fibers by using the value corresponding to the experimental break at RH = 75% to predict the breaking strain at RH = 97% with an error of 0.22%. This remarkable small error confirms the plausibility of the proposed rupture criterion. Observe anyway that this hypothesis in this silk cannot be applied to the fully dry case where the breakage is typically induced by localized defects (Yazawa et al., 2020).

As a last comparison with experimental results, in Fig. 13 we show the possibility of predicting with remarkable accuracy the complex mechanical response of a dragline silk in dry condition (reproduced from (Gosline et al., 1999)). Notice that here, the thread is produced by a third species of spider, the Araneus diadematus.

4. Discussion

A comment is in order. As anticipated previously, in the recent paper (Cohen et al., 2021) the authors proposed a different model well reproducing with 8 parameters the experiments in Fig. 10(a),(b). Our model, being based on a more detailed description of the microstructure, considering the two different fractions and stretch induced unfolding effects, ends up with 10 parameters. On the other hand, the proposed model is able to reproduce the whole stress–strain curves as function of both temperature and humidity, with unloading, increasing damage, fracture and residual stretches. It is important to observe that the most diffuse models efficiently describing damage and residual stretches in soft polymeric materials (without any temperature and humidity effects) adopt 7–8 parameters (see De Tommasi et al. (2019) and references therein).
Fig. 13. Theoretical (continuous lines) vs experimental (dots) stress–stretch curves for Araneus diadematus spider fibers (reproduced from (Gosline et al., 1999)). Here $E_o=6.75$ GPa, $E=13.6$ MPa, $\mu=1$ MPa, $c_1=1.005$, $c_2=1.979$, $a=0$, $m_r/m_o=0.13$, $s'=4.5$, $A_r=1.25$, RH$_o=84\%$.

5. Conclusions

The described ability of the proposed model of predicting the experimental behavior of different mechanical properties make us confident that it well reproduces the humidity and temperature effects at the molecular scales. This is supported even more by predicting the behavior of different silks and environmental conditions. We strongly believe that this is a consequence of our microstructure deduction of the material response function. The physical meaning of all the adopted (microscopic) parameters opens up the possibility of applying the proposed model not only to other protein materials with similar structures (Puglisi et al., 2017), but also in the design of bioinspired materials employing chosen specific proteins (Greco et al., 2021; Liu et al., 2019).

CRediT authorship contribution statement

Vincenzo Fazio: Conceptualization, Methodology, Software, Writing. Domenico De Tommasi: Conceptualization, Methodology, Supervision. Nicola Maria Pugno: Conceptualization, Methodology, Supervision. Giuseppe Puglisi: Conceptualization, Methodology, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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