Investigating The Rheological Properties Of Sericin

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Introduction

Sericin is a by-product of silk, spun by the Bombyx Mori Silk worm for metamorphosis, shown in Figure 1. Each cocoon contains roughly 900 metres of raw silk. The silk strands are made from 2 fibroin fibres coated with sericin as seen in Figure 2. The silk industry uses the fibroin to create silk, and discards 50,000 tonnes of Sericin into rivers and lakes every year [3].

Figure 1: Bombyx Mori Silkworm [1]

Sericin contains 18 amino acids including essential amino acids. Essential means that humans don't produce these amino acids naturally, instead they must be obtained through diet or skincare. Sericin's variety of amino acids provides it with a range of beneficial properties. For example, Serine and Aspartic acid give sericin it's hydrophilic nature. Therefore sericin contains a vast amount of moisture. As sericin has a structure similar to human skin, it is readily absorbed by the skin. This allows it to penetrate deeply, bringing moisture with it to hydrate through the epidermis layer. This reduces dryness which alleviates the symptoms of skin conditions such as atopic dermatitis (eczema). Figure 3 lists some of sericin's beneficial applications. This moisture also facilitates a range of processes such as healing and UV protection. Furthermore sericin has potential for the skincare and medical industry: it is an anticoagulant which thins the blood, and also can reduce and inhibit the growth of tumours. Sericin is cleansing and encourages cell proliferation making it an excellent ingredient for wound healing gels. This, alongside sericin being an antioxidant, reduces the signs of ageing. Finally sericin is an antityrosinase and so inhibits the production of melanin, where melanin is the chemical responsible for blackheads, age spots and skin condition such as melasma [4].

Figure 2: Sericin and Fibroin [2]

Sericin's wide range of properties, particularly with regards to the treatment and management of a broad spectrum of skin conditions, highlights why it is such a favourable natural active ingredient in skincare formulations. Characterising its physical properties is therefore an important step in incorporating sericin into formulations so that its stability, efficacy and manufacture can be optimised. This project aims to investigate whether sericin can be frozen for long term storage, as well as benchmarking its properties for quality control.

Figure 3: Sericin's Properties

Rheology

Rheology is a measure of deformation and the flow behaviour of a material. A rheometer uses oscillatory motion to deform a material and measures the torque produced by a material to determine its viscosity, storage modulus and loss modulus. The storage modulus measures the energy stored as elastic deformation [5]. The loss modulus measures the energy dissipated as heat, representing the viscous properties of the material. An analogy of this is dropping a bouncy ball. The stored elastic energy is the height it reaches after bouncing and the energy lost as heat is its reduction in height from the position it was originally dropped from. Double concentric gap geometry, depicted in Figure 4, was used for all experiments due to its high sensitivity and precise stress control.

Measurements - Oscillation, Frequency and Strain Sweeps

This set of experiments was conducted on an additive gel containing sericin. An oscillation time test was used to capture the rheological properties of the additive during gelation whilst the temperature was held constant. A low strain and frequency of 0.1% and 0.5 Hz respectively were employed to prevent damage of the forming gel structure, especially in the early stages of the test. The sample was prepared by heating to 85°C in a small beaker. This reduced the viscosity, allowing the additive to be pipetted into the geometry before starting the test. The next experiment was a frequency sweep which was conducted immediately after the oscillation test. The geometry was made to oscillate with a strain of 0.1% and the frequency was increased from 0.1 to 10 Hz. This enabled the measurement of the viscoelastic properties after gelation. Immediately afterwards, a strain sweep was conducted. This increased the strain from 0.01% to 30% in steps at a constant frequency of 1 Hz. This enabled the critical strain to be determined for the formed gel. Beyond the critical strain, permanent structural damage occurred.

Figure 4: Double Concentric Gap Geometry

Temperature Ramp

Fresh,1 month frozen and 4 months frozen samples of the sericin additive were used. The samples were prepared using the same procedure outlined above, by heating each sample to 85°C before pipetting it into the geometry. However, each sample was held at 5°C for 24 hours before starting the experiment. This allowed the additive to form a strong gel within the geometry. After the wait, the temperature was increased at 1°C /minute from 5°C to 90°C whilst oscillating the sample at 0.1% strain and a frequency of 0.5 Hz.

Viscosity Flow Curves

The final aim of the project was to measure the viscosity of a face serum product containing sericin. Flow curves were conducted to determine the materials' viscosity over a range of shear rates from 0.0001-10,000/s. As shown in Figure 5, this determines if products can undergo manufacturing processes such as levelling, mixing and spraying, which have a shear rate of 1000-10,000/s. Furthermore a material's flow curve can be used as another benchmark for quality control.

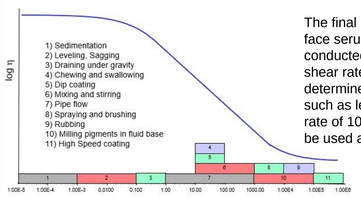

Figure 5: Manufacturing Processes - Shear Rate and Viscosity [6]

Results Fresh Vs Frozen Sericin Additive

Figure 6 shows an oscillation sweep comparing the fresh (red), 1 month frozen (orange) and 4 months frozen (pink) samples tested at 20º C. Despite having lower initial values for the loss and storage modulus, the 4 months frozen samples reached 1600 Pa after 1000 minutes, almost matching the fresh sample's storage moduli. This suggests that after gelation the fresh and 4 months frozen samples have the same rheological properties. This is supported by the frequency sweep in Figure 7, as the fresh and 4 months old samples produce the same storage and loss moduli across all frequencies. Figure 8 shows a strain sweep, which measures the material's response during deformation. The fresh sample's structure broke down at a strain rate of 1.78%, shown by the rapid decrease in its storage modulus. The 4 month frozen sample had a higher critical strain of 2.85% before it started to deform. Consistently the 1 month sample exhibits lower storage and loss moduli than the fresh and 4 month frozen samples. This may be due to biological variation between the samples, as a slight genetic mutation in silkworms could have a considerable impact on the quality of the sericin they produce. Overall the results for the fresh and 4 month samples suggest that freezing sericin has little impact on its rheological properties.

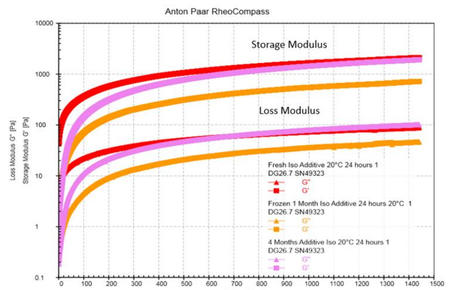

Figure 6: Oscillation Sweep for Fresh, 1 Month and 4 Month Samples

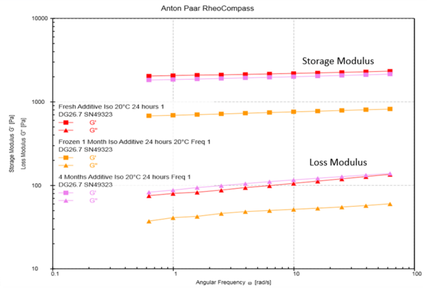

Figure 7: Frequency Sweep for Fresh, 1 Month and 4 Month Samples

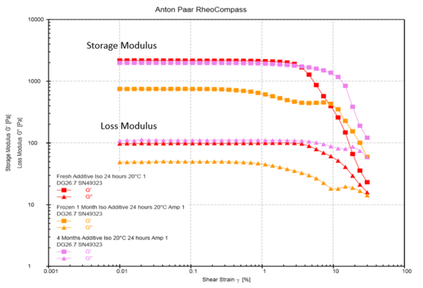

Figure 8: Strain Sweep for Fresh, 1 Month and 4 Month Samples

Visual Observations

Although the above results suggest there is only a negligible difference between the 4 months frozen and fresh samples, visually there is a large difference. Before freezing, the sericin additive formed a gel at room temperature, which held its form whilst inverted, (Figure 9a). When defrosted, the frozen samples did not form a gel, instead thawing into a liquid with high amounts of sedimentation. This high turbidity, shown in Figure 9b, suggests that the structure of the sericin proteins has been damaged. Whilst heating to 85°C reduced this sedimentation, remaining floating clumps suggested the structure has been damaged. Interestingly, after being heated and cooled to room temperature, the frozen samples formed a weak gel. This gel could be inverted and maintain its form. However, if shaken, it would become a viscous liquid that would take several hours to reform into a gel. Therefore, the rheological properties must have changed.

(a) (b)

Figure 9: Fresh and Thawed Out Frozen Sample

Temperature Ramp

Figure 10 depicts the temperature sweeps for the fresh (cyan), 1 month frozen (pink) and the 4 months frozen (purple) samples. The fresh sample undergoes an early transition at 20ºC, shown by its reduction in storage modulus. This is novel as it is not mentioned in existing literature. Freezing the sericin delays this early transition to 25ºC and 45ºC respectively for the 1 month and 4 month frozen samples. Furthermore, freezing the sericin reduces its initial storage modulus from 3170 Pa for the fresh sample to 1169 Pa for the 4 month frozen sample. These changes are critical and highlight the missing structure visually observed by the frozen samples turbidity in Figure 9b. This lost structure may contain the essential amino acids which are responsible for sericin's beneficial properties. Thus the quality of frozen sericin cannot be guaranteed. All samples exhibit a similar secondary melt at around 75ºC. This part of the structure, which has not been damaged due to freezing, is likely to be caused by the stabilising ingredients which maintains the gel's consistency. At 82ºC, the fresh sample's storage modulus rapidly increases. This may occur due to water evaporation from the sample at elevated temperature, rendering the sample more viscous. The 1 month frozen sample's storage modulus also starts to increase, but at a higher temperature of 94ºC. This suggests frozen sericin may be more hydrophilic as the 1 month sample was more efficient at holding onto the water.

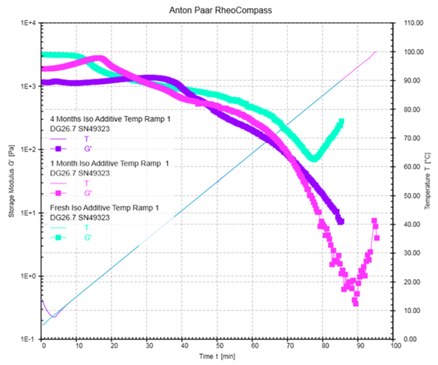

Figure 10: Temperature Ramp 10°C/minute 0.1% Strain 0.5Hz

Modelling: Knee Function

A knee function can be used to describe the rate of the transitions for the temperature ramps [7]. This models the asymptotic power law behaviour of each transition and can be quantitatively compared to future temperature ramps conducted on sericin.

$$Predicted\ G' = G'_L + \frac{\Delta G'}{1 + \left(\frac{T}{T_g}\right)^n}$$

$\Delta G'$ is the range of the storage moduli, calculated by $G'_U - G'_L$, where G'_L and G'_U are the lower and upper storage moduli of the selected transition. T_g is the glass transition temperature, which corresponds to the middle of the transition. The parameter n is calculated from the data.

Figures 11 and 12 show the knee function is an excellent model for both the fresh and 4 months old samples' transitions. The predicted G' values from the model (shown in orange) match the experimental results (shown in blue). As the time stored in a freezer increases, the parameter n increases from 8.13 to 21.59. The glass transition temperature T_g also increases from 19.6°C to 45.1°C. Both the upper and lower storage moduli values (G'_U and G'_L) decrease between the fresh to 4 months stored in a freezer. The knee function models each transition, allowing easy comparison between this data and future studies on sericin.

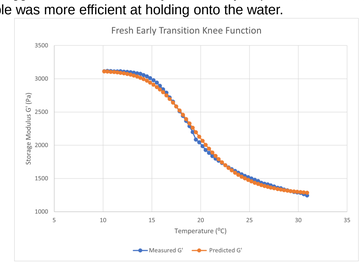

Figure 11: Fresh Early 20°C Transition Knee Function

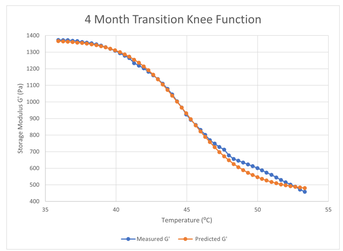

Figure 12: 4 Month 45°C Transition Knee Function

Face Serum Flow Curves and the Herschel-Bulkley Model

Figure 13 shows the flow curves for the sericin face serum conducted at a temperature of 35ºC. As shear rate increases, from an initial value of 0.002/s, the viscosity of the initial flow curve (light purple) decreases. This shear thinning behaviour is expected for sericin. The rerun flow curve (dark purple) has a significantly lower maximum viscosity of 0.828 Pas at 0.001/s compared to the initial run's maximum viscosity of 42.434 Pas. This reduction in viscosity suggests the structure of the sericin has been damaged by the high shear rates of 10,000/s. In the rerun flow curve, the remaining viscosity is imparted to the face serum by stabilisers used in the product. These stabilisers have not been damaged, thus the viscosity is maintained for shear rates above 100/s.

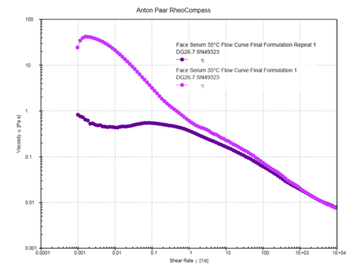

Figure 13: Face Serum Flow Curve 35°C Initial and Rerun

The Herschel-Bulkley function can be used to describe the flow curves for future quantitative comparison. The Herschel-Bulkley model [8] is a model which describes a non-Newtonian fluids relationship with stress and strain as it yields.

$$\tau = \tau_0 + k\dot{\gamma}^n$$

Where τ_0 is the estimated yield stress of the material, $\dot{\gamma}$ is the shear rate (/s) and parameters k and n are to be calculated from the data. The stress τ was calculated for each data point by multiplying the measured torque with the shearing geometry's stress constant, K_σ =12035, for the double concentric gap geometry used.

By manipulating the equation, the Herschel-Bulkley model can be rewritten as:

$$\ln(\tau - \tau_0) = \ln(k) + n\ln(\dot{\gamma})$$

Therefore after estimating a value for τ_0 , the parameters k and n can be numerically evaluated by plotting $\ln(\tau - \tau_0)$ against $\ln(\dot{\gamma})$. $\ln(k)$ will be the y-intercept and n is the gradient.

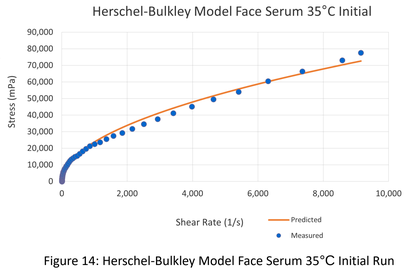

Figure 14: Herschel-Bulkley Model Face Serum 35°C Initial Run

τ_0 was estimated to be 0, k and n were calculated to be 768.8 and 0.4972 respectively. These parameters give a predicted curve which can be compared to the actual data, shown in Figure 14. The predicted curve is very similar to the measured data. This method can also be used for the repeated flow curves that are conducted after 10 minutes of holding. This data is important for quality control in future mass production.

Conclusions

Storing the sericin for time periods of up to 4 months in a freezer impacts its rheological properties. Whilst initially exhibiting a lower storage and loss moduli before gelation in the oscillation sweep, the moduli later matched the fresh values. After gelation, the 4 months frozen frequency sweep was consistent with the fresh samples, shown in both the frequency and strain sweep. On the other hand, the 1 month old sample continually exhibited lower storage and loss moduli than the fresh samples. This was justified by biological variation between sets of samples. By visually comparing the fresh gel to the more turbid frozen 4 months samples, it can be seen that freezing the sericin had altered its structure. This was supported by the temperature ramp. The fresh sericin displayed an early transition at 20°C which is not mentioned in existing literature. Freezing the sericin for 1 month delayed this to 25°C, and freezing for 4 months delayed it to 45°C. Freezing the sericin also reduced the initial storage modulus. This lost structure may contain the essential amino acids, which are responsible for sericin's beneficial properties, or sericin's ability to gel at a rapid rate. Therefore the quality of products containing frozen additive cannot be guaranteed. These transitions were further analysed using a knee function, which was used for quantitative comparison. Overall storing sericin for long time periods of more than 1 month at sub-zero temperatures significantly impacts its rheological properties.

The Face Serum flow curve provides a valuable benchmark for skincare formulations containing sericin for future quality control. The formula shear thinned supporting the literature on sericin. After shearing at a rate of 10,000/s, the sericin structure of the face serum has been damaged, shown by the rerun flow curve not matching the initial values of viscosity. The Herschel-Bulkley model can be used to describe each flow curve. The parameters of the function can be used to quantitatively compare these benchmark tests for future quality control.

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