Effect of Extraction Process Parameters on Silk Fibroin Attributes

Jeffery Roshko, BME Graduate Student, ENV 170 Fall 2018, December 16th 2018

Introduction

Despite recent advances in the development of composite materials such as carbon fiber, many composites remain resource intensive to produce, require harsh chemicals to process, and are hazardous to work with.[1] As global population grows, development of new materials that can fulfill a variety of needs from transportation to healthcare while minimizing impact on human and environmental health is paramount. Looking to nature for guidance, we find many naturally occurring composites which have been refined through evolutionary processes. These include an array of functional structures such as limpet teeth, mollusk shell, and arthropod exoskeleton, which are commonly composed of a fibrous phase embedded in a protein matrix, analogous to engineered composites such as fiber reinforced polymers.[2] . As the understanding of composites continues to expand, properties and performance of composites containing naturally-derived materials are being investigated.[3-5]

One natural material available in large quantities due to thousands of years of cultivation, while exhibiting impressive properties is Silk. It is also biocompatible, resilient, and suitable for processing without the use of hazardous solvents.[3] This is favorable in comparison to other high-performance composites, and makes silk a strong candidate for use in composites. While silk appears well-poised for use in this application, variability inherent in the raw material must be well understood before embarking on its use in more complex structures. Our lab works with a silk processing protocol developed here at Tufts to prepare the material for engineering applications, but the protocol has been modified for increased quantities required for macro-scale constructs. This study will attempt to explore the variation created by our lab's process, and identify aspects that could be improved to achieve increase control over important silk properties.

Background

Silk from cocoons of the Bombyx mori silkworms exhibits impressive mechanical properties, including ultimate tensile strength up to 4.5 GPa (vs 7 GPa for carbon fiber and 1 GPa for stainless steel for reference).[6-8] Silk has been tested in a multitude of composites with varying success. Studies of silk fiber reinforced with traditional polymer matrices suggest that while silk may outperform other natural fibers when utilized in the same matrix, it underperforms synthetic fiber equivalents, but results also suggest that performance is greatly influenced by microstructure of silk fibroin.[3] For example, silk fiber reinforced epoxy composite tubes exhibited lower energy absorption when compared to carbon fiber reinforced epoxy composites under axial compression despite having higher fiber toughness.[4] The mechanism of failure here is not well understood; incompatibility of silk fiber in epoxy matrices might be partially responsible. In another study, chitin, a polysaccharide in arthropod exoskeletons, and silk fibroin, the main component of silkworm silk, were cast into films.[5] Stiffness was higher for composite films than films made with either individual component. However, chitin-silk films exhibited the same nanostructure as chitin-only films, whereas silk-only films did not exhibit a nanofibrous structure. This is possibly due to processing conditions used, which can result in reduction of protein molecular weight and disruption of the formation of secondary structure of the silk protein, and ultimately a reduction in performance.[10] Based on the results of these studies, there appears to be opportunity to explore use of silk in composites if better control over molecular structure could be achieved.

One technique to control molecular structure of silk fibroin is directed self-assembly. Using this technique, various structures can be produced by precisely controlling water evaporation from silk fibroin solutions, functionality thought to be related to the natural process by which arthropods create silk.[11] As an added benefit, structures produced with directed assembly can be doped with additional functional molecules while maintaining or even exploiting the unique structures formed.[12] Applying rigorous control in the production of silk-matrix composites using directed self-assembly may drastically influence nano, micro, and macro structure of composites produced, resulting in materials with improved material properties, and may lead to unique combinations of chemical, biological, and mechanical functionality.

As the precursor to experimentation with these composites, as well as to a host of experiments performed by researchers around the world, we require consistent raw material as a starting point. In my lab, we have started to characterize a silk fibroin extraction process developed by a partner lab here at Tufts to determine inherent variability in the material, as well as this process. Numerous experiments were conducted to investigate baseline variability. Heating, rinsing, and dialysis were measured, optimized, and tested. Several analytical techniques were used, including Scanning Electron Microscopy and FT-IR Spectroscopy to quantify microstructure, viscometry to understand flow behavior, and SDS-PAGE to determine molecular weight distribution. Initial results seem to fall within expected ranges for each attribute as observed by other researchers, but there also seems to be variability in viscosity and molecular weight. There are also significant gaps in information due various researchers completing varying degrees of testing in different timeframes, so this data needs to be cleaned and sorted. As multiple techniques and types of equipment that were used to conduct the process, as well as operator error, these additional sources of error need to be understood to determine how best to proceed (automation, training, equipment selection, etc.).

Methods

Data is initially sorted from multiple excel files to one single data sheet for each analytical test. After compiling each test, those with incomplete array of tests were removed from the analysis. A scatter plot is then produced for each analytical test, and outliers examined, comparing to notes for each batch of material to ensure testing was performed as defined in each protocol. In RStudio Version 1.1.456, linear regressions [lm()] were examined for numerical responses, and categorical logistic regressions [glm()] were examined be prepared for the main variables of interest. Categorical factors were tested for interactions as well, and ANOVA used to determine whether these regressions are significant between the different groups defined by these categorical conditions/variables.

Conclusions

While sorting through data for outliers, it was determined that a significant portion of data was missing, and imputing via mean, median, or mode affected distributions significantly. As such, only a fraction of the dataset remained for analysis. Of the remaining data, several relationships were extracted. As expected, concentration and viscosity are closely related. Additionally, user-error in preparation also appeared to contribute to differences in concentration, although categorical regression analysis did not yield statistical significance, possibly because the sample size was quite small. One potential positive here is that a lack of significance of factors such as vessel and date suggest that aspects of the processing of this raw material are robust, which is what we are trying to determine at this point. In the future, after initial process stability is determined, we intend to intentionally vary inputs and determine effects, if any.

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Silk fibroin regeneration process