Optimization of Recombinant Spider Silk Protein Purification from Goat Milk

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Background:

Over millions of years, spiders have evolved to produce a biocompatible material known as spider silk. *Nephila clavipes*, more commonly known as the Golden Orb Weaving Spider, is one of the most studied. Orb weaving spiders produce 7 different types of silk with unique functions and properties. These are:

- **Major Ampullate gland**- Dragline and web structural support
- **Minor Ampullate gland**- Auxiliary Spiral
- **Aciniform gland**- Silk for catching prey and soft inner lining of the egg sac
- **Piriform gland**- Glue for joints and attachments
- **Flagelliform gland**- Core fibers of the catching spiral
- **Aggregate gland**- Aqueous coating
- **Tubuliform gland**- Tough outer silk to the egg sac

Of the 7 silks produced; Major Ampullate silk is the strongest natural fiber known to man. Dragline spider silk is unique because of its strength, stretch and biocompatibility. These properties could allow it to be used to create diverse materials such as stronger cables, ultra fine sutures for eye and nerve surgeries, fire-proof clothing, etc. In order to manufacture these materials, one must be able to produce and purify the synthetic spider silk proteins that make up dragline silk, called Masp 1 (M4) and Masp 2 (M5).
Dr. Lewis's lab at USU has made multiple advancements in producing spider silk proteins. Some methods include fermenting *E. coli*, growing genetically modified silkworms, and raising alfalfa. One of the best ways the Lewis lab produces synthetic spider silk is in the mammary (milk) of dairy goats. The transgenic goats produce more protein than the other alternative synthetic production methods and it is the most effective and inexpensive purification method using a Tangential Flow Filtration (TFF) process. The problem is that the TFF system is less efficient than needed. In order to make spider silk economically viable, the system of recovery needs to be optimized. Our current production method yields sufficient protein amounts for research but not for prototype development. This poster will describe our research and our progress toward optimizing production.

Current Production Method:

Production of spider silk protein is accomplished using a tangential flow filtration system (TFF). The initial TFF was composed of one 750 kDa and one 50 kDa column. Each column had one pump designated to control flux rates and flow rates. The first step in this process is removing as much fat as possible from the transgenic goat milk. From the reduced fat milk, we separate fat from whey in the 750 kDa column and simultaneously separate goat milk proteins from the spider silk proteins in the 50 kDa column. In past years, 15 liters of defatted milk was the maximum that could be passed through the TFF system per milk run. This was done in a controlled cold temperature environment. Separation of the proteins took 24 hours, while the entire milk run took approximately one week to complete. After the purification process, each run produced on average 0.2 g/L for the M5 type protein.

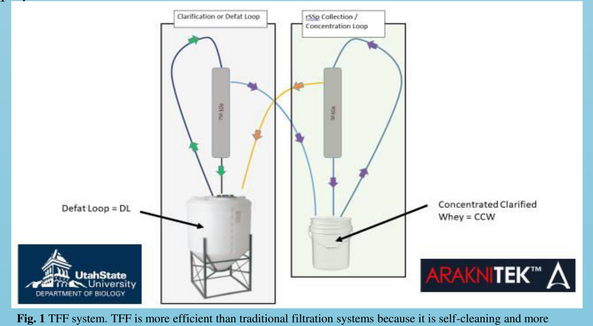

Fig. 1 TFF system. TFF is more efficient than traditional filtration systems because it is self-cleaning and more reliable. Water and particles in it move tangential to the pores in the filter. This perpendicular flow helps to disrupt buildup of the larger particles on the filter itself. If this system is further improved, it has the potential to greatly improve the manufacturing process of synthetic spider silk proteins.

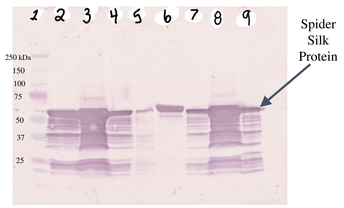

Fig. 2 Two M4 goat samples (160912-M4-222 and 160913-M4-222). Lanes: 1. Ladder 2. Defat (DF) 3. Whey Loop Final (WLF) 4. Fat Loop Final (FLF) 5. Pellet 6. DF 7. Fat Loop Final (FLF) 8. FLF 9. Pellet . Representative purification of the old TFF system.

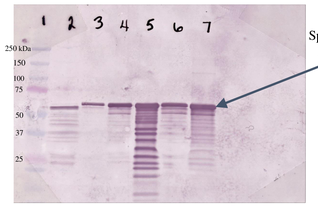

Fig. 3 Two M5 goat samples (161010-M5-323 and 161013-M5-419). Lanes: 1. Ladder 2. DF 3. FLF 4. WLF 5. Pellet 6. DF 7. Pellet
Representative of the old TFF system.

Optimizing Production Methods:

Through several experiments, we began to assess if the following modifications would improve protein yield:

- **Temperature during purification**
- **Upgrading the capacity of the TFF system**
- **A more efficient cleaning process of the TFF system**

Purifications were performed at cold room temperature (4 deg C) and the other at room temperature (20 deg C). Modifications were made to the TFF allowing the system to increase the volume of milk purified by 5x . Adjustments were made to the structural support of the columns and the power source was replaced to eliminate power loss. New pumps with much higher flow rates were attached; one pump for the 50 columns and two bigger ones for the 750 columns. This allowed higher flux rates and flow rates. Two additional columns were added to improve the separation during crossflow. A fan was included to prevent overheating the circuits, making the system more reliable.

In the past, purification runs had 15 liters of milk. We started running 40-80 liters of milk through the TFF system to see if the improved system could handle that volume. With the new TFF system we should expect to purify more protein than the old TFF system within the same amount of time. The final concentrated clarified whey was treated with ammonium sulfate causing the spider protein to precipitate, making it easier to separate the protein from the whey via centrifugation. To improve cleaning methods we introduced an autoclaving step followed by water floss treatment. This should help remove any buildup from the pores such as any leftover impurities from previous runs.

Results:

The temperature change during the purifications showed improvements compared to previous results (Fig. 2 and Fig. 5). Our data indicate that when the TFF was run at cooler temperatures, the process was less efficient. After completing the runs at room temperature, our protein yield improved gradually and showed no signs of protein degradation in the western blots (Fig. 5). It would take about one week to purify 10-15 liters of milk but with the new TFF system, purification time was reduced by 50% and purification yields increased. The new pumps facilitated protein recovery by allowing us to achieve a higher shear rate (1 L/cm^2 vs 20 L/cm^2). The combination of autoclave and water floss treatment to the columns have helped remove old residue, which has allowed better recovery of the proteins (no data shown). Over all, physical adjustments made to the TFF cart improved efficiency of the process. Occasionally, we found that certain purification runs had contaminations. This resulted in extreme amounts of acidic spider silk and goat milk protein (Fig. 8). We found contaminations could be eliminated by better cleaning of the columns, the last three points in Fig. 8 demonstrate the effectiveness of the new cleaning process. After further purification and testing, the final M5 type protein was recovered at ~0.5 g/L (2.5x increase), demonstrating that our protein yields have improved with all new adjustments.

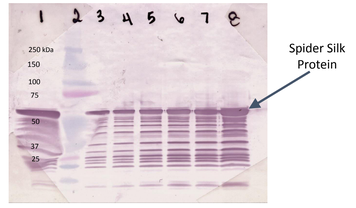

Fig. 4 M4 Goat sample (170515-M4-122). Lanes: 1. DF Initial 2. Ladder 3. 5L 4. 10L 5. 13L at 3:00 6. 12L at 3:20 7. 12L 8. CCW
Shows amount of protein per liter during run.
Representative of the new TFF system.

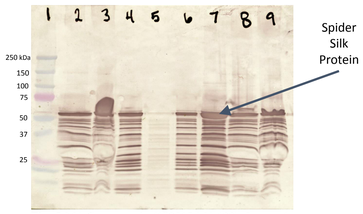

Fig. 5 M4 Goat sample (170515-M4-122). Lanes: 1. Ladder 2. DF Initial 3. DF Final 4. Flux Initial 5. Flux 1 6. Flux Final 7. CCW Final 8. DF I 9. DFF
Representative of the new TFF system.

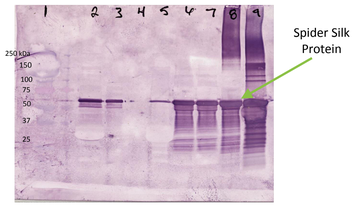

Fig. 6 M5 Goat sample (170719-M5-323). Lanes: 1. Ladder 2. DF 3. CCW Initial 4. 50 Permeate 5. FLF 6. CCW Final 7. CCW Final 8. Pellet 9. M5 Common
Representative of the new TFF system.

Fig 7. M5 Goat sample (170808-M5-208). Lanes: 1. Ladder 2. DF 3. CW Initial 4. During FL 5. CW During 6. FLF 7. CCW 8. Super
9. Supernatant 10. Pellet
Representative of the new TFF system.

Conclusion:

Of the several major changes that have been included in the process of purification, these are the most influential innovations:

- **Temperature effects on purification/recovery of spider silk protein**
- **Increased processing capacity**
- **Improved cleaning treatment process**

Higher temperatures improved protein yield. Milk fats in cold temperatures clump, causing the spider silk proteins to become trapped within them. When placed in room temperature, the milk fat does not clump allowing the system to perform with higher efficiency. The new TFF system produced 5x the amount of protein as the old system in the same amount of processing time. The updated cleaning process of the new TFF system (autoclaving and water treatment) is more thorough. Clean columns are essential for better filtration and our new system has shown better results because of it.
Protein yield has increased 250% compared to the last system while keeping the protein more pure. The data from the old TFF system proves that we were only obtaining a fraction of the pure amount of spider silk protein in goat milk. This means we were losing most of the protein during purification processes and leads us to believe that we can optimize the TFF system further.

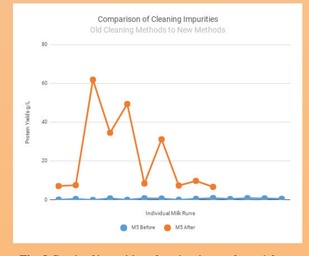

Fig. 8 Graph of impurities after cleaning performed from separate runs.

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