# FINAL REPORT
# February 26, 2016

# INTERACTIVE AND INTEGRATIVE ENGINEERING OF RENDERED PROTEINACEOUS MATERIALS BASED THERMOSET BIOCOMPOSITES FOR HIGH-STRENGTH, SUPERIOR-PERFORMANCE APPLICATIONS

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**Project Start Date:** July 1, 2014

**Duration of Project**: 18 months

## Lay Summary:

The project aims to enhance the strength, toughness, durability, as well as temperature stability of polymers by treatment with meat-derived proteins. The chemical structure of the protein will be used to form strong bonds with the polymer matrix to generate a highly crosslinked structure. By the introduction of carefully selected additive molecules, which reacts with the polymer-protein composite, the internal network, as well as the properties, will be improved. The final composites with heightened properties will be employed in state-of-the-art applications in diverse fields such as automobile industry.

## Objective (s):

The overall objective of the study is to develop high-strength, toughened, hydrophobic, odor-free, cross-linked thermosets and composites from proteinaceous materials from rendering industry for performance-oriented applications.

## Project Overview:

The first task of the project was to extract and purify the protein and characterize its chemical composition (**Part 1**). After understanding the chemical architecture of the protein, the project investigated the reinforcement efficiency of the proteinaceous material via two pathways. The trials employing the protein as a filler is mentioned first (**Part 2**) followed by the application as a crosslinker (**Part 3**). The mechanical properties of protein cross-linked epoxy resins were successfully enhanced (up to 700% in case of tensile strength) by introducing other novel materials such as bismaleimide (BMI) and hyperbranched polymers (HBP) or a combination. Finally, preliminary results regarding the fabrication of a highly beneficial bio-friendly polyurethane (PU)-based composite with enhanced mechanical properties is presented (**Part 4**). We envisage our future efforts in this direction will generate a high-value PU-protein composite with immediate applicability in packaging, coating, etc.

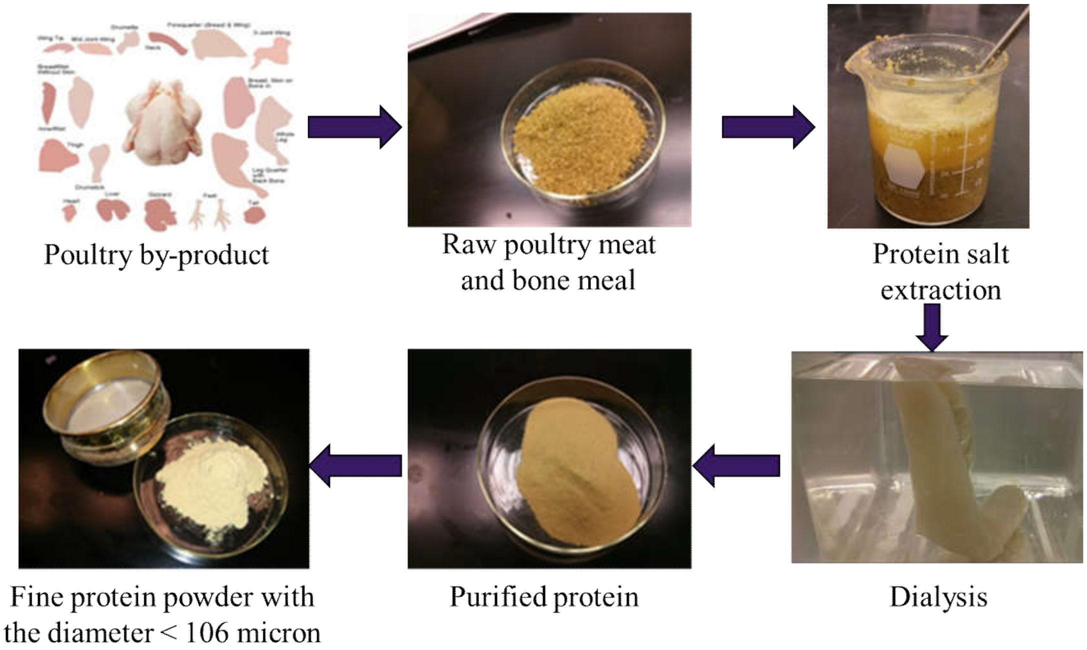


**Figure 1.1 The experimental flow chart to extract protein from poultry samples.**

## 1. Protein extraction:

### 1.1 Method and experiment

The experimental parameters such as the ratio between extraction solution and sample weight, duration of the extraction process, etc. were optimized via repeated experimental procedures. In a typical process, 200g of as-received poultry protein was dissolved in an extraction solution (834ml water, 36g NaCl, 0.5g $MaCl_2$, 8.1g $KH_2PO_4$ and 8.7g $Na_2HPO_4$) and stirred for 30 min. Subsequently, the mixture was centrifuged, and the dissolved salt-coated protein in the supernatant was collected. The obtained supernatant was dialyzed to eliminate the salt which was then dried to get pure protein. The dried pure protein was milled to a powder form for further characterization.

### 1.2 Characterization of protein's chemical architecture: Identifying the functional groups

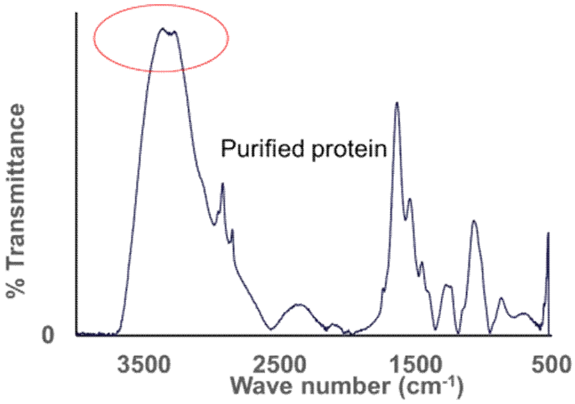


**Figure 1.2 FTIR spectrum of the animal protein sample.**

*Key Observations:*

- The -3100 peaks attributes to $–NH_2$ stretching peaks. The amine group is an active function group to react with epoxy groups.

After identifying the active functional groups ($-NH_2$), the possibility of using the extracted protein as a reinforcement agent in epoxy resin was carried out. First, the use of the protein as a filler was examined, as given in the following part.

## 2. Animal protein as fillers:

Here, the large protein particles were used as fillers to analyze the properties and study the reactivity of animal protein. Figure 2.1 depicts the plausible mechanism of interaction where the amine groups on the protein interact with epoxy and hardener to enhance the properties.

## 2.1 The reaction mechanism

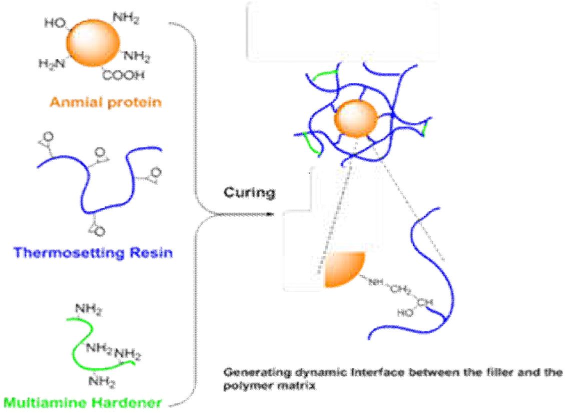


Figure 2.1 The mechanism of protein as fillers.

## 2.2 Method and materials

Purified protein powder

Epoxy resin and hardener

Silicon mold for cast molding

Tensile test specimen

Figure 2.2 The experimental procedure to prepare tensile test specimen by using protein as fillers to the general epoxy matrix.

## 2.3 Mechanical properties

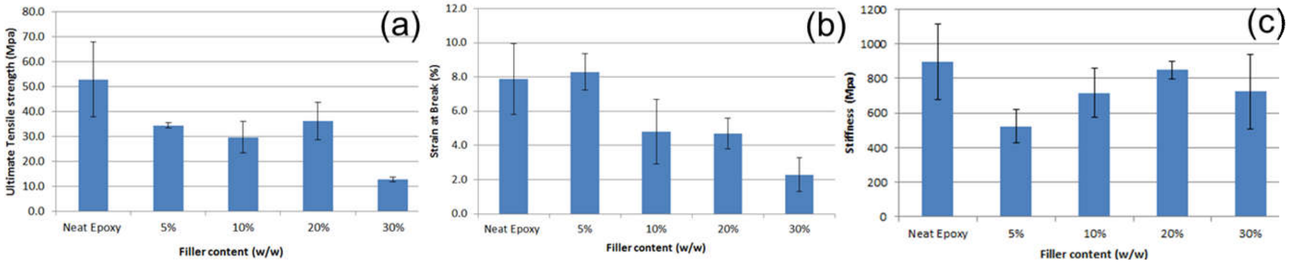


Figure 2.3 The stress-strain curves (a), ultimate tensile strength(b), strain at break(c) and stiffness(d) properties of the Neat Epoxy, 5%, 10%, 20% and 30% protein added.

*Key Observations:*

- The addition of protein as a filler into the widely used epoxy system resulted in the general decrease in mechanical properties, especially the ultimate tensile strength and elongation. Hence, as an alternate, use of protein as cross-linkers I epoxy matrix was investigated, as discussed in the following section.

## 3. Protein as epoxy cross-linker

Here, the extracted protein was used as a crosslinker to take advantage of the inherent amine functional groups (Figure 1.2).

### 3.1 Experimental section:

| | |
|---|---|
| Protein extraction & Purification | • Extract protein from raw materials and purify using dialysis |
| Grinding purified protein | • To decrease the particle size and increase the reaction surface area |
| Vacuum drying | • To remove the moisture in the protein |
| Crosslinking | • To prepare the sample for mechanical and thermal test |
| Structure and property relationship | • Perform thermal and mechanical characterizations |

**Figure 3.1: Schematic of research work.**

### 3.2 Results and discussion:

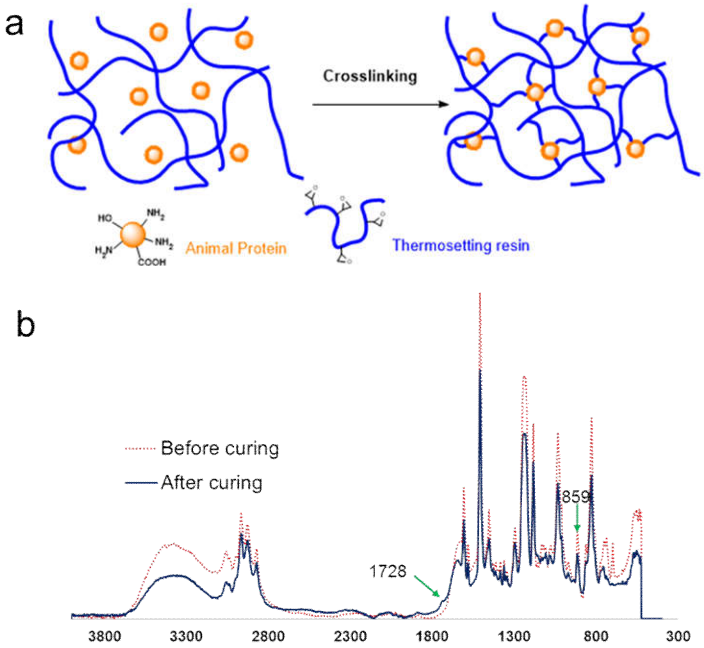


**Figure 3.2: (a) Crosslinking reaction mechanism; (b) FTIR spectra of epoxy and protein mixture before and after curing.**

In this pathway, the active amine groups are expected to react with the epoxy groups on the polymer resin to create a highly crosslinked matrix structure to enhance the mechanical properties (as shown in Figure 3.2 a). Figure 3.2b displays the IR spectrum of the sample before and after the protein-based curing process. The key observations obtained from the plots are:

*Key Observations:*

- The increase in the intensity of the peak at 1728 $cm^{-1}$ corresponding to an ester group indicates that new ester groups are getting formed during the curing process, illustrating the successful crosslinking.
- The simultaneous decrease in intensity of the peak at 859 $cm^{-1}$ (due to an epoxy group) suggests that during the crosslinking process, the epoxy groups are getting converted to the ester groups.

### 3.2.1 Protein ratio optimization

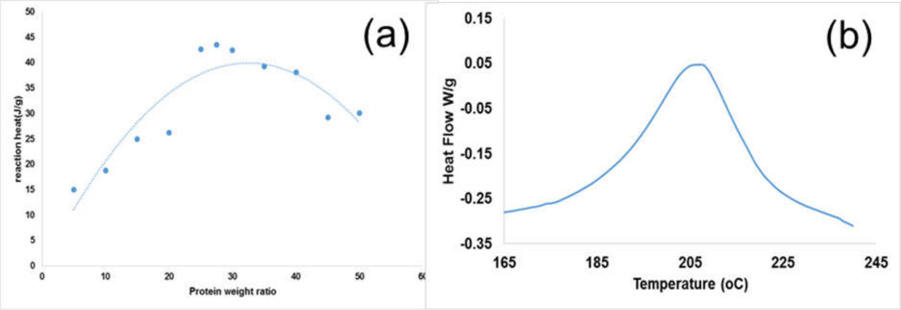


**Figure 3.3: (a) reaction heat at various protein ratios; (b) heat flow thermogram of the optimal (30%) protein ratio-epoxy formulation.**

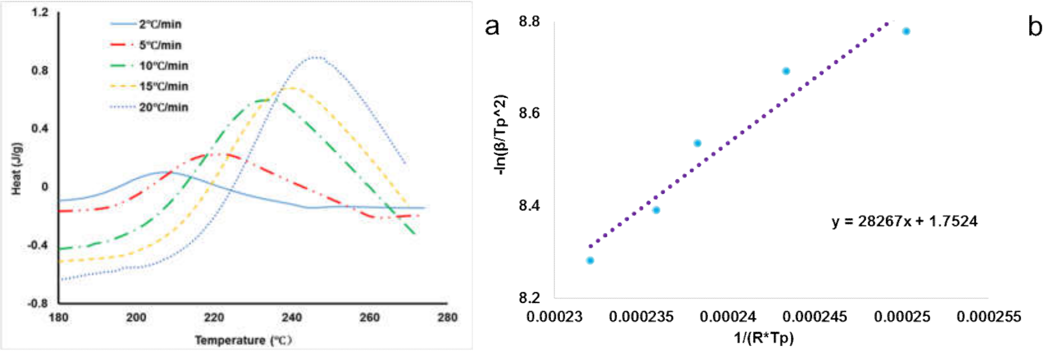
The optimum protein ratio for the curing ratio was identified based on the total enthalpy of the epoxy curing reaction or the heat of reaction. At optimum protein content, the heat of curing reaction will be the highest. Figure 3a and b show the variation of reaction heat and heat flow thermogram with varying protein content in the mixture.

*Key Observations:*

- The effect of protein content on the total enthalpy of the epoxy curing reaction was investigated. Based on the reaction heat, the optimal protein weight ratio was found to be 30wt%.
- At optimal protein ratio, we conducted mechanical tests (see table below). However, as can be observed, the derived properties did not meet the acceptable performance characteristics for application in the automotive field.

| Extension at Maximum Load (mm) | Youngs Modulus (GPa) | Ultimate Tensile Strength (MPa) |
|---|---|---|
| 1.739 | 0.03275 | 2.245 |

### 3.2.2 The cross-linking reaction kinetics



c

| Sample | β℃/min) | $T_i$(K) | $T_p$(K) | Ea(KJ/mol) | A(s-1) |
|---|---|---|---|---|---|
| DGEBA/Protein (30%) | 2 | 460.48 | 480.63 | 28267 | 589 |
| | 5 | 468.3 | 494.28 | | |
| | 10 | 475.86 | 504.79 | | |
| | 15 | 482.3 | 509.89 | | |
| | 20 | 489.11 | 518.21 | | |

d

$$-\ln\left(\frac{\beta}{T_P^2}\right) = \frac{E_a}{RT_P} - \ln\left(\frac{AR}{E_a}\right)$$

Activation energy ($E_a$);
Preexponential factor (A)

**Figure 3.4: (a) DSC curves at various heating ramp rate; (b) Kissinger plots of the optimal (30%) protein ratio-epoxy formulation; (c) DSC data summary; (d) formula used to calculate the reaction activation energy.**

The samples were analyzed by differential scanning calorimetry (DSC) to understand the cross-linking reaction kinetics as well as to calculate the activation energy of the process. Figure 3.4 depicts the different DSC curves and the calculation of activation energy.

*Key Observations:*

- Calculated the reaction activation energy $Ea$ using the slope of the line, which was found to be equal to 28.267 KJ/mol.

Since the protein-crosslinked epoxy samples did not demonstrate the desired levels of properties, we investigated the effect of incorporation of special additives such as bismaleimide (BMI) and hyperbranched polymers (HBP) to the optimal protein-epoxy mixture. The enhanced crosslinking induced by BMI and HBP will potentially improve the strength and toughness of the resulting matrix. The additives were introduced into the protein-epoxy, either individually or as a combination as discussed below.

### 3.2.3 Optimal BMI ratio

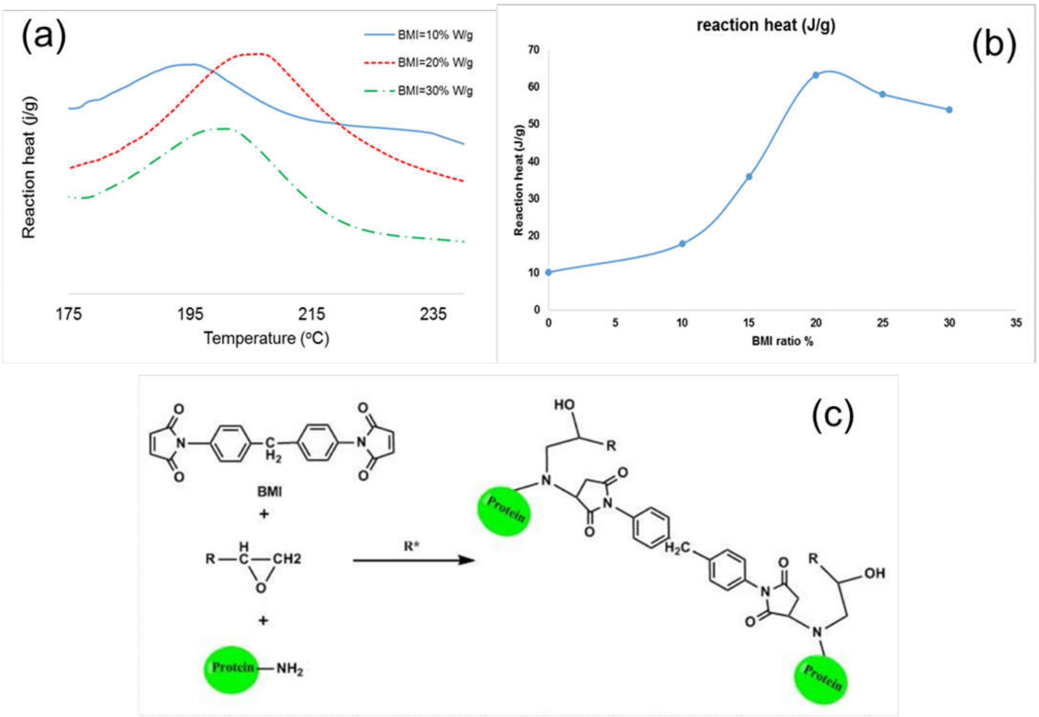


**Figure 3.5: (a) DSC cure curves with different BMI ratios; (b) Reaction efficiency with different BMI ratios; (c) Reaction mechanism using BMI as chain extender**

The effect of the addition of BMI was investigated first. Figure 3.5 illustrates the change in the heat of reaction after addition of different amounts of BMI as well as the proposed mechanism of BMI incorporation into the epoxy matrix, via reacting with both the epoxy as well as the protein.

*Key Observations:*

- The effect of BMI ratio on the curing reaction heat was investigated.
- As shown above, a maximum reaction heat was observed for 20wt% BMI content.

### 3.2.4 Optimal HBP ratio

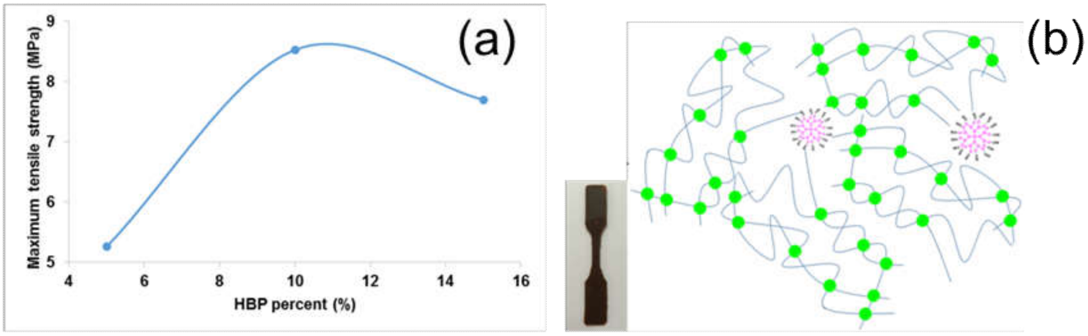


**Figure 3.6: (Left) Tensile strength for different HBP weight ratios; (Right) reaction mechanism.**

The optimal amount of HBP in the mixture was also identified separately as shown in figure 3.6.

*Key Observations:*

- The effect of HBP ratio on the mechanical properties was investigated.
- As shown above, maximum tensile strength was observed for 10wt% HBP content.

### 3.2.5 Effect of both BMI and HBP on the mechanical and thermal stability of epoxy-protein matrix.

Here, the optimized amount of BMI (20 wt%) and HBP (10 wt%) were introduced into the epoxy-protein matrix and the mechanical properties were investigated (Figure 3.7).

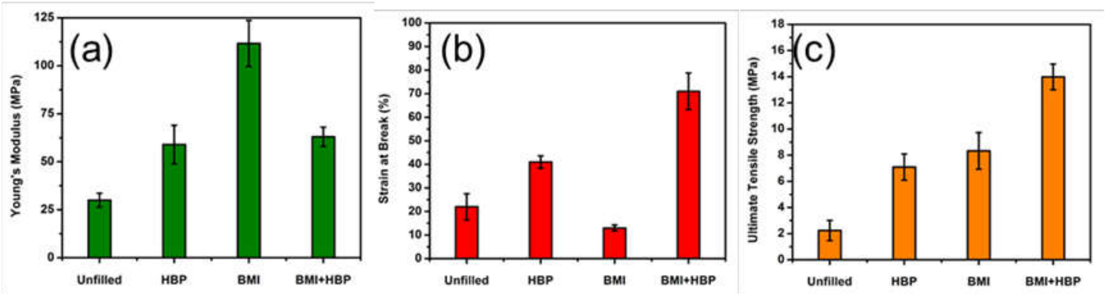


**Figure 3.7: (a) Young's modulus, (b) Strain at break, (c) Ultimate tensile stress values achieved by pure protein-epoxy curing product, HBP added, BMI added, or both HBP and BMI added.**

*Key Observations:*

- The addition of HBP has enhanced the modulus, strength, and strain-at-break of the epoxy-protein thermoset matrix.
- The addition of BMI increased the modulus and strength but compromised on the strain-at-break.
- The simultaneous addition of BMI and HBP has resulted in complimentary properties i.e. superior strength and strain-at-break without compromising much on the Young's modulus.

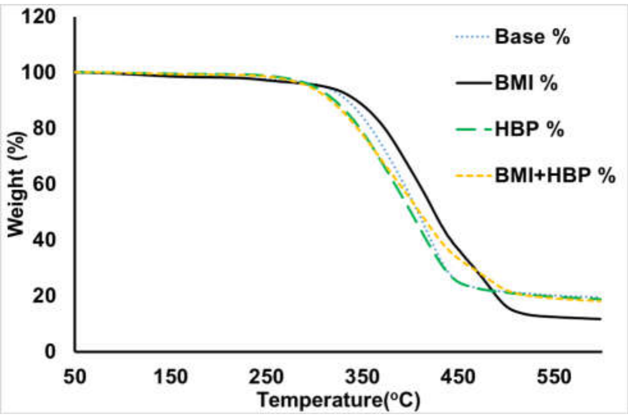


**Figure 3.8: Thermogravimetric analysis curves of pure protein-epoxy curing product, HBP added, BMI added or both HBP and BMI added.**

The thermal stability of the different composites was also analyzed (Figure 3.8).

*Key Observations:*

- Comparison of the onset and 10% weight degradation temperatures in the parent epoxy protein sample and the samples after the addition of BMI and HBP both in combination suggested that the thermal stability of the sample decreased after the introduction of the additives.

## 3.3 Conclusion

The study proved that the present protein with free, active amine groups can be used as a cross-linker for an epoxy matrix. However, the desired mechanical properties (demonstrated by the use of conventional hardeners) were not achieved. The addition of special additives such as BMI and HBP to the reaction system leads to significant improvement in the mechanical properties. However, for proposed applications e.g. in the automotive industry, considerable improvement is necessary via further refining of the reaction scheme. We identified the miscibility difference between the epoxy and the protein to be the most critical problem which limits the reaction between the protein and the epoxy. The presence of hydrophilic surface groups such as carboxylic and amine groups make the protein highly hydrophilic, and epoxy is hydrophobic. This incompatibility between the constituents resulted in a considerable reduction in the cross-linking reaction, leading to reduced mechanical properties of the cured samples. Hence, it is imperative to solve this problem first to improve the properties of protein-based thermoset materials.

Protein being the core part of the study, we identified a new direction for the study by changing the resin part, i.e. changing the hydrophobic epoxy with hydrophilic polyurethane. We plan to employ highly hydrophilic Waterborne Polyurethane (WPU) to have better interaction with protein and generate high-performance plastics as discussed below.

# 4. A highly bio-friendly Waterborne Polyurethane - Protein hybrid polymer

## 4.1 Methods and Experimental:

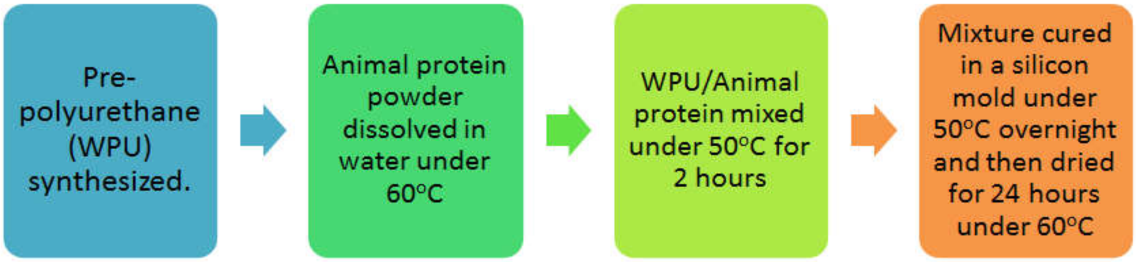


**Figure 4.1: Schematic of research work.**

## 4.2 Results and discussion:

### 4.2.1 Hybrid formation reaction between WPU and animal protein

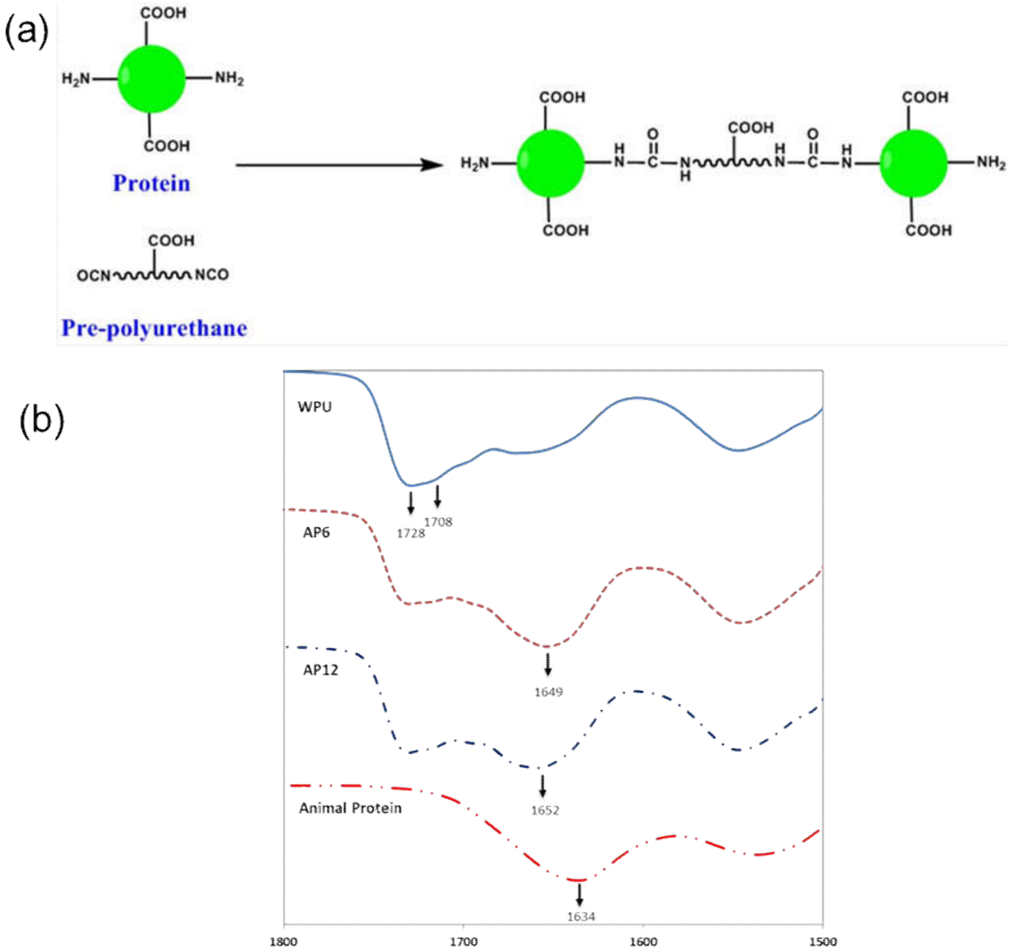


**Figure 4.2: (a) Crosslinking reaction mechanism; (b) FTIR spectra of pure WPU, 6%, and 12% animal protein added, pure animal protein.**

<u>*Key Observations:*</u>

- The region between 1750 and 1700 cm$^{-1}$ corresponding to carbonyl stretching showed two prominent peaks at 1728 cm$^{-1}$ (free –C=O) and 1708 cm$^{-1}$ (free O=C–O-H) in WPU. The higher intensity of the free carbonyl peaks indicated that most urethane carbonyls in WPU are not hydrogen-bonded, which could form an intermolecular hydrogen bond with the amine groups present in the animal proteins as shown in (Figure 4.2 A).
- For animal protein, the characteristic peaks of amide I, attributed to C=O stretching, centered around 1634 cm$^{-1}$ was observed. The band shifted to higher wavelength with the addition of WPU, indicating strong intermolecular interactions between WPU and protein.

### 4.2.2 The optical properties of the composite and miscibility of constituents

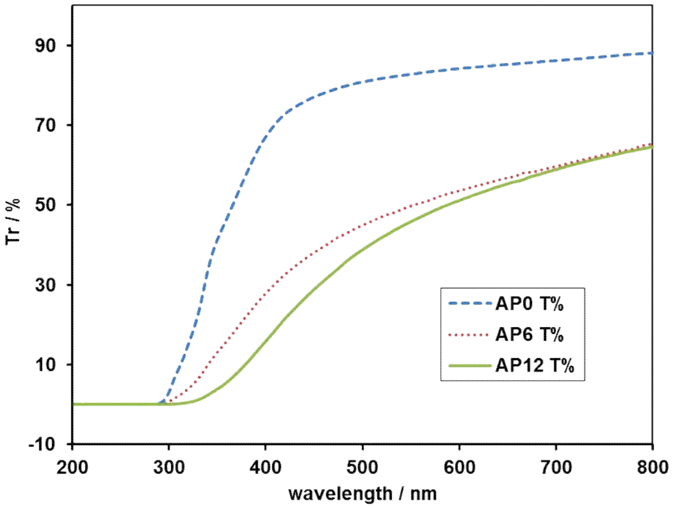


**Figure 4.3: Dependence of the optical transmittance (Tr) on wavelength for different animal protein content of the WPU and WPU/animal protein composite.**

<u>*Key Observations:*</u>

- All the films exhibit the highest transmittance at 800nm and then decreased gradually with shorter wavelength values. At less than 300nm, the samples become mostly opaque.
- The optical transparency of the materials could be regarded as an auxiliary criterion to judge the compatibility of the blends and interfacial interactions. The results indicate a good miscibility of animal protein and WPU hybrid polymer.

### 4.2.3 Structure of the hybrid polymer

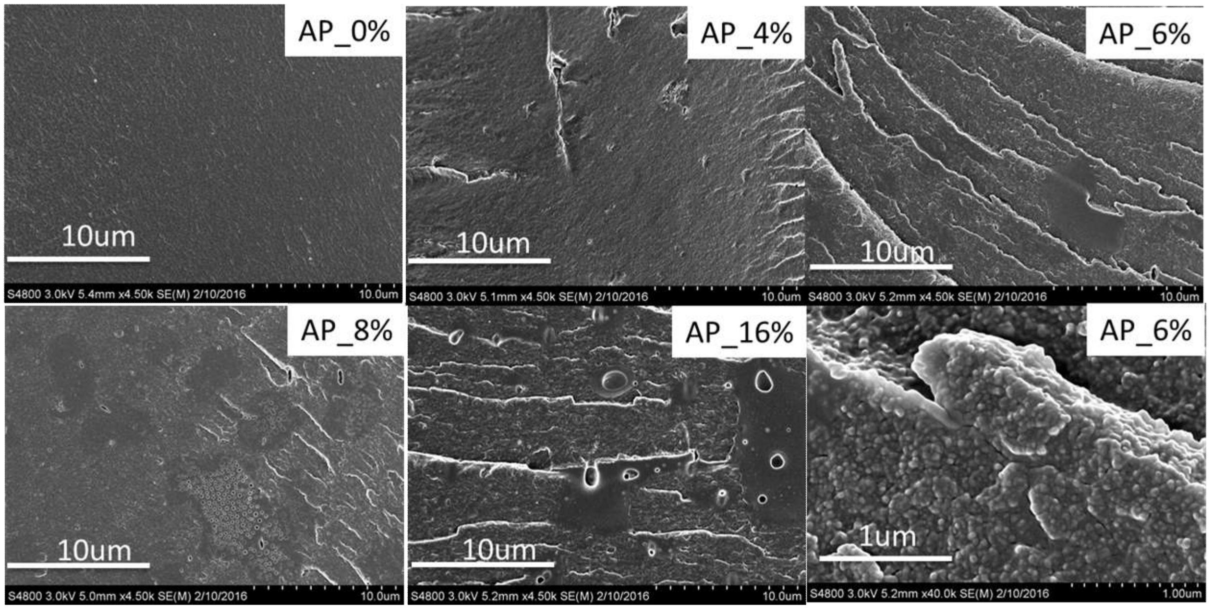
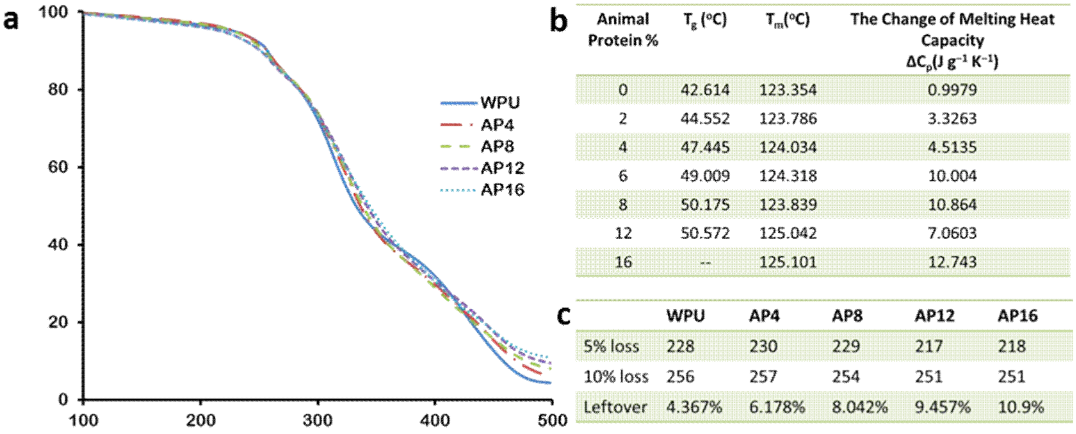


**Figure 4.4: SEM photographs of the cross sections of the pure WPU, 4%, 8% and 16% animal protein added.**

*Key Observations:*

- With less than 8% animal protein, the cross-section of the hybrid polymer seems highly homogeneous, indicating good miscibility between the two components.
- When the protein concentration was higher than 8%, a noticeable interfacial separation of the components was observed. However, refining the fabrication process by modulating the mixing time and force, this is expected to be mitigated.

### 4.2.4 Thermal properties



| Animal Protein % | $T_g$ (°C) | $T_m$(°C) | The Change of Melting Heat Capacity $\Delta C_p$($J\ g^{-1}\ K^{-1}$) |
|---|---|---|---|
| 0 | 42.614 | 123.354 | 0.9979 |
| 2 | 44.552 | 123.786 | 3.3263 |
| 4 | 47.445 | 124.034 | 4.5135 |
| 6 | 49.009 | 124.318 | 10.004 |
| 8 | 50.175 | 123.839 | 10.864 |
| 12 | 50.572 | 125.042 | 7.0603 |
| 16 | -- | 125.101 | 12.743 |

| | WPU | AP4 | AP8 | AP12 | AP16 |
|---|---|---|---|---|---|
| 5% loss | 228 | 230 | 229 | 217 | 218 |
| 10% loss | 256 | 257 | 254 | 251 | 251 |
| Leftover | 4.367% | 6.178% | 8.042% | 9.457% | 10.9% |

**Figure 4.5: TGA curve (A); The Tg and Tm summary of WPU and WPU/animal protein from DSC curves (B) and 5% weight loss, 10% weight loss and leftover from TGA (C).**

*Key Observations:*

- The $T_g$ of pristine WPU is around 42.614. For WPU/Animal Protein cross-linked hybrid polymer, one single Tg shifted to a higher temperature is observed, indicating the successful crosslinking reaction between WPU and animal protein molecules.
- The $T_m$ and change melting heat capacity increase with an increase of animal protein present further proving the successful hybrid formation.
- The TGA data demonstrates that there is no compromise regarding the thermal stability of WPU after the addition of animal protein and formation of the composite.

### 4.2.5 Mechanical properties.

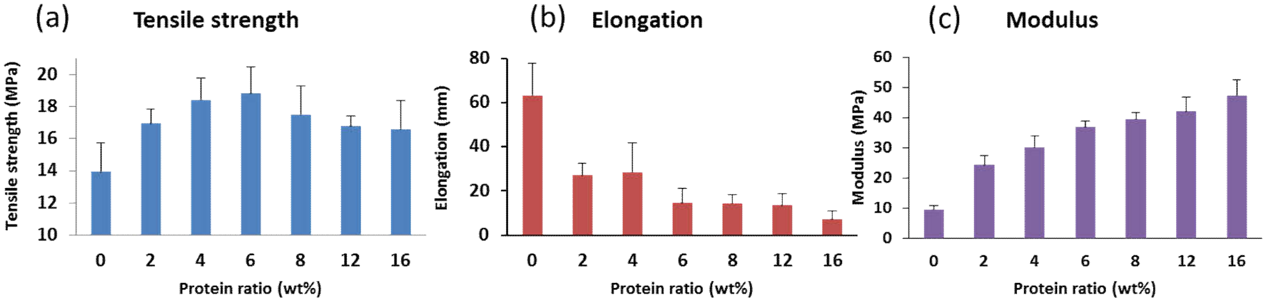


**Figure 4.6: (a) Ultimate tensile stress, (b) Strain at break, (c) Young's modulus values achieved by pure WPU and different weight ratio protein added.**

*Key Observations:*

- The addition of animal protein enhanced the modulus, and strength because of the formation of the cross-linked matrix.
- The addition of protein, however, resulted in a decrease in the strain-at-break.

## 4.3 Conclusion

Elastic WPU/animal protein based hybrid polymer were prepared by adding animal protein to crosslink waterborne polyurethane through casting and evaporation process. The WPU/animal protein hybrid films exhibited excellent compatibility between the constituents. The formation of highly crosslinked matrix resulted in significant improvement in mechanical properties. Moreover, the addition of highly renewable and environmentally friendly materials into WPU matrix, the biocompatibility of the system is expected to improve. Hence, with a suite of attractive properties achieved by the addition of animal protein, the resultant hybrid PU material is supposed to have wide applicability in diverse fields including the automotive industry.

**Impacts and Significance:** The principle scientific benefit of this study is the use of animal proteins in the development of high-strength waterborne polyurethane composites for automotive coating/painting and other applications. Polyurethanes are widely used in the manufacture of cars, offering real benefits in terms of comfort, protection and energy conservation. The new hybrid polymers have better mechanical performance than pure WPU with added recyclability/sustainability. It is well-known that the introduction of polyurethane gives automotive vehicles reduced weight and increased fuel economy. The proposed WPU can be used as interior and exterior coating materials as well. This coating provides a car's exterior with the high gloss, durability, scratch resistance, and corrosion resistance. Their durability and light weight, combined with their strength, make them ideal for cushioning purposes. Moreover, this protein-WPU based composite material has better biocompatibility so that it will provide more applications in different areas. The sales of vehicles are advancing at a rapid pace with more than 150M units to be sold worldwide by 2017. Due to stringent CAFÉ standards (49-61 miles/gal for 2020-2025, respectively), the need for light-weighting is exigent. It is well known that the most drastic light-weighting can be achieved by adopting composite materials. Hence, a lot of R&D is dedicated to the development of composites, especially with multi-functional and structural properties. Based on the extensive application of polyurethane in the automotive industry and the concurrent increased recycle problem, our new protein-WPU hybrid polymer has a large potential consumer market.

**Publications:**

1. Yu, X., Zheng, T., and Pilla, S., "Energy-efficient Processing of Rendered Animal Proteins as Value Added Bio-crosslinkers in High-Strength Thermosets," *SPE-ANTEC Technical Conference*, Indianapolis, IN (May 2016)
2. Yu, X., Zheng, T., and Pilla, S., "Thermal and Mechanical Properties of Waterborne Polyurethane Crosslinked by Rendered Animal Proteins," *SPE-ANTEC Technical Conference*, Indiana polis, IN (May 2016)

**Outside funding:** No outside funding has been pursued at this point. However, given the potentiality of the results, we intend to apply for funding to either National Science Foundation, Environmental Protection Agency or National Institutes of Health.

**Future Work:**

Given the potentiality of animal proteins, as we have observed in our preliminary studies, we intend to continue this research in the following areas.

1. Continuation of WPU-animal protein work with special additives such as BMI and HBPs (similar to our work in sections 3.2.3 and 3.2.4) to further enhance their properties
2. Synthesizing water soluble epoxy and develop epoxy-animal protein hybrid thermoset polymer. Compared to polyurethanes, epoxies have higher mechanical properties so employing the methodology developed on WPU to water soluble epoxy will open new yet high-strength application base for these material systems.
3. Once applicable animal protein based hybrid polymers are synthesized, advanced composites using carbon or glass fibers will be designed and developed which will potentially have far-reaching application.

**Acknowledgments:** My sincere thanks to my graduate students Xiaoyan Yu and Ting Zheng for their diligent efforts in the project. I would also like to thank my research scientist, Dr. Sreeprasad Sreenivasan for his insights on the project. Also, thanks to Kim Ivey for her kind assistance on the FTIR and TGA characterization.