Processig-Structure-Property Relationships of Meat and Bone Meal Derived Bioplastics

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PROCESSING-STRUCTURE-PROPERTY RELATIONSHIPS
OF MEAT AND BONE MEAL DERIVED BIOPLASTICS

A Dissertation
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy
Chemical Engineering

by
Sam Lukubira
August 2014

Accepted by:
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ABSTRACT

Sustainability concerns arising from over-use of fossil-derived materials have prompted renewed interest in development and use of products from renewable biomass. Agricultural materials like soy, starches, cellulose esters and co-products like lignin, soybean meal, feather meal, blood meal and others are being investigated for bioplastic applications. Unlike fossil-based plastics, most of these materials are biodegradable and obtained from renewable precursors, hence sustainable.

This research was focused on the use meat and bone meal (MBM), which is derived from animal tissue parts not utilized for food by humans, as a bio-based raw material in the production of bioplastics for potential geo-structural applications. The MBM precursor was suitably modified to enable its processing using scalable plastics manufacturing techniques such as melt compounding, extrusion, calendering, and vacuum thermoforming. Based on literature studies and previous studies in our lab, glycerol was utilized as a processing aid (plasticizer) at 30 wt%. However, glycerol plasticized sheets did not possess adequate mechanical strength (only about 3% that of synthetic plastics like polyethylene). They also suffered from rapid aging as the plasticizer was lost over time because of its weak physicochemical interactions with the base MBM. Therefore, three approaches were investigated to modify the base MBM material: (i) modification of glycerol plasticized MBM with calcium hydroxide (CH), (ii) physical blending of MBM with linear low density polyethylene (LLDPE) to form MBM polymer composites (MBMPCs) and, (iii) modification with resins from reaction of glycerol with maleic (MA) and phthalic (PtAH) anhydrides.
In the first approach, calcium hydroxide (0, 3, 7 and 10 wt% CH) was initially mixed with glycerol to form a paste. The paste and MBM (with a glycerol to MBM mass ratio of 3:7) were then compounded in a batch mixer at 100°C, 60 rpm for 15-30 minutes followed by thermal compaction at 140°C to produce sheets. CH content of up to 10 wt% increased the tensile strength (TS) of the sheets to 4 MPa and the modulus to 340 MPa, which were 5 and 8 times greater than that of the unmodified MBM bioplastics. Fourier transform infrared (FTIR) analysis showed that the observed increase in TS and TM was attributed to ionic cross-links of calcium ions with the protein residues containing negatively charged oxygen of glutamate and aspartate. However, CH did not significantly improve the water resistance of MBM bioplastic sheets.

As a physical approach to enhance water-resistance and shape integrity, un-plasticized MBM was blended with linear low density polyethylene (LLDPE) as the minor component to consolidate and encapsulate MBM. Results indicated that a minimum of 15 wt% LLDPE content was required to form a nominally continuous binder phase that enabled calendering of MBMPC sheets. Tensile analysis of water soaked samples and water vapor permeability (WVP) measurements revealed that the MBMPCS had significantly better water resistance when compared to that of pure MBM bioplastics.

Finally, as the third approach, MBM was modified by the addition of glycerol-anhydride resins to improve water resistance while retaining biodegradability, unlike MBMPCs that contained non-biodegradable LLDPE. The anhydride resins that retained flow properties
were prepared by controlled reaction of maleic and phthalic anhydride with glycerol. The anhydride modified bioplastics had improved water resistance especially those modified with phthalic anhydride that retained structural integrity even after being soaked in water for more than 24 hours, whereas the pure MBM bioplastics disintegrate in less than an hour. Importantly, at temperatures above 90°C, the modified bioplastics displayed sufficient ductility as revealed from elongation viscosity measurements and were successfully vacuum thermoformed into a three dimensional (cup-shaped) object about 25 mm deep. The vacuum formed cup was tested as a seed growth planter, and was observed to have dimensional stability even with watering through the seedling germination period.

In summary, this research successfully established the development of sustainable bioplastics from MBM animal co-product using scalable polymer processing routes like extrusion, calendering, and vacuum thermoforming. These high-volume manufacturing processes indicate the potential of modified-MBM to be used as a cost-effective bioplastic given that their properties were comparable to those obtained from expensive high protein fractions (> 60%) such as soy protein isolates and corn zein. Going forward, it would be interesting to study how well plants grown in anhydride modified MBM seed planters perform as compared to other seed planters. Also a cradle-to-grave life cycle assessment of MBM-based bioplastics would facilitate their use in commercial applications.
ACKNOWLEDGEMENTS

I would like to extend my sincere appreciation to all individuals who helped me to successfully complete this research and dissertation.
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CHAPTER 1
INTRODUCTION

1.1 Overview

Plastics play an important role in every aspect of our lives. Plastics are used to manufacture everyday products for long term use such as vehicles, appliances, electronics housing and furniture as well as short term applications such as diapers, trash bags, utensils, medical devices and beverage containers. Majority (over 98%) of plastics currently used are derived from fossil-based resins such as polyethylene, polypropylene, polystyrene, polyethylene terephthalate, PMMA, PVC etc.¹ Fossil-based plastics have displaced many traditional materials, such as wood, metal, glass, and paper because of their relative low cost, low weight, high versatility and imperviousness to water.² However, since the turn of the 21ˢᵗ century, there has been growing interest in the use and development of articles from renewable bio-based agricultural materials. This is being driven by mainly two factors: (i) sustainability concerns over the use of fossil resources and (ii) the opportunity to add value to underutilized agricultural materials.

Despite the advantages of fossil-based plastics, there is growing evidence and concern about their sustainability, both economically and environmentally. Because of the growing world population, studies indicate that fossil resources are dwindling rapidly. In addition, petroleum and natural gas is occasionally used as a political weapon, which causes price volatility of products derived from such sources. Furthermore, with respect to the environment, there is a major problem of plastic disposal, because of plastic
persistence in the environment as well as effect on the carbon footprint. The U.S. Environmental Protection Agency (EPA) data for 2012, showed that 12.7% (32 million tons) of total USA municipal solid waste (MSW) in 2012 came from plastics. This is also a major concern as the plastics reduce the amount of arable land and cause marine pollution. The issue of disposal is being tackled through recycling efforts. However, the volume of the plastics being recycled is still very small compared to the amount going in landfills with an estimate of about only 8% (2.7 million tons) recycled in 2012. Recycling efforts are also undermined by various problems, such as separation of plastic additives and fillers from the resin, and the deteriorated properties of recycled plastics.

1.2 Biodegradable Bioplastics

Due to the growing awareness of the unsustainability of fossil-based plastics, the use and development of bioplastics is growing, with an estimated global use of 0.85 million metric tons in 2011 (BCC research) and is expected to increase up to 3.7 million metric tons by 2016, a compound annual growth rate (CAGR) of 34%. The search for alternatives to traditional fossil-based plastics is not limited to just the source but also the downstream consequences of disposal inform of biodegradable plastics. Thus, a bioplastic may be a plastic based on renewable resources (biopolymers) and/or biodegradable polymers including those sourced from fossil resources, these topics are discussed next. Figure 1.1 provides the categorization of biodegradable bioplastics based on the source and some examples.
Figure 1.1. Categorization of biodegradable bioplastics based on source.
1.2.1 Bioplastics from Fossil Resources

Synthetic polymers, such as polycaprolactone (PCL) and polybutylene succinate (PBS), polybutylene adipate terephthalate (PBAT), polyvinyl alcohol (PVOH) and polyethylene oxide (PEO) are biodegrade.\(^5\) According to ASTM D-5488-94d, a biodegradable material is one capable of undergoing decomposition into carbon dioxide, methane, water and inorganic compounds or new biomass by predominantly enzymatic action of microorganisms. These plastics can play an important role in solving the problem of waste disposal and marine pollution, but these are still derived from non-renewable resources.

1.2.2 Bioplastics from Renewable Resources (Biopolymers)

Bioplastics from renewable resources include those derived from biomass conversion such as polylactic acid (PLA)\(^6\) that is polymerized from lactic acid derived from fermentation of corn sugars and polyhydroxyalkanoates (PHAs)\(^7\) purified from microbial fermentation. The other category is of those obtained directly from biopolymers, including polysaccharides such as starch, starch derivatives and cellulose derivatives, lipids and proteins, which are sourced from agricultural products.\(^8\)-\(^10\) The major advantage of agricultural-based bioplastics is their origin from renewable resources (biopolymers) and their inherent biodegradability in the environment if cautious engineering is adopted, which would enable them to mitigate the problem of waste disposal. In this context, production and use of bioplastics derived from these materials is the feasible solution. Currently commercialized bio-based bioplastics are mostly those from cellulosic esters, starch derivatives (TPS), polyhydroxybutyrate (PHB) and polylactic acids (PLA).\(^6,7,11,12\)

Application fields range from uses in the pharmaceutical and biomedical area as potential
biocompatible materials for artificial prostheses, for sutures, and as a medium for controlled drug release; in the field of packaging, including food and shopping bags; and in the field of agriculture as mulching films.\textsuperscript{12}

Since this research was directed towards bioplastics from proteinaceous biomass, the remainder of this Chapter focuses on proteins, protein structures, techniques employed in their conversion into bioplastics sheets/films, and the properties of such bioplastics.

\section*{1.3 Proteins}
Proteins are essential constituents of all living organisms and make up all the body tissues of animals. Higher concentrations of proteins are found in body and muscle tissues. Some proteins are very large macromolecules and very insoluble in water (structural proteins), e.g., collagens found in skin, bone, and connective tissue, and the keratins that give strength to wool, hair, nails, beaks and horns. Others, such as albumins and globulins found in plasma are very soluble in water.\textsuperscript{13-15} In plants, higher concentrations exist as storage proteins in legume grains (peas and soybeans), cereals (wheat, maize, rice, and sorghum), oil seeds (sunflower and cotton seed), and in root vegetables like potato and cassava.\textsuperscript{16}

\subsection*{1.3.1 Protein Structure}
Proteins are heteropolymers consisting of primary, secondary and tertiary structures. The monomeric units are known as amino acids. There 20 basic amino acids responsible for formation of the primary protein structure. The amino acids are characterized by an alpha carbon attached to two terminal functional groups (a basic amine group [-NH\textsubscript{2}] and an
acid carboxyl group [-COOH]) as well as a hydrogen proton and a characteristic substituent (R) group that distinguishes one amino group from the other (Figure 1.2). Because of their multi-monomeric chains, they are different from synthetic amide polymers such as the nylons, which are made up of a single repeating unit.\textsuperscript{14,17}

![Figure 1.2. Schematic showing the functionality of an amino acid molecule](image)

**Primary structures**

The protein primary chain is formed from linkages of amino acids via amide/peptide bonds resulting from the poly-condensation of the amine and carboxylic acid functional groups of adjacent amino acid groups as shown in Figure 1.3. For each peptide bond formed, a water molecule is released. The order of placement of the amino acid residues has the importance of determining the functionality of a particular protein. Both the number of amino acids and the sequence in the primary chain are genetically determined.\textsuperscript{13,17}

![Figure 1.3. Poly-condensation of two amino acids to form a peptide](image)
Secondary structures

Protein secondary structures are made of regularly folded polypeptide chains determined by sterically possible conformations. These molecular structures can be determined by Fourier transform infrared spectroscopy (FTIR). Amide I and II bands arise from bonds that link the amino acids of the protein. Amide I is due to carbonyl stretching in the region of 1600 – 1700 cm$^{-1}$ whereas amide II is due to N-H bending vibrations in the region 1500 – 1560 cm$^{-1}$. The most important regular structures are the alpha helix ($\alpha$-helix) and the beta sheet ($\beta$-sheet) displayed in Figure 1.4. These motifs optimize long range interactions, especially hydrogen bonding. The alpha helix, Figure 1.4(a), is a compact structure with a right hand screw configuration made of 3.6 amino acid residues per turn. The helix is configured in such a way that linear hydrogen bonds are formed between the carbonyl oxygen and the hydrogen atom of an amide four residues further down the chain as illustrated in Figure 1.4(a). For every turn, the helix extends by 0.54 nm, which is referred to as the pitch of the helix. Most $\alpha$-helices are made with hydrophobic residues.
Figure 1.4. Schematic of secondary protein structures (a) the alpha helix structure and (b) the antiparallel beta-sheet (adapted from reference 19)

The beta sheet, Figure 1.4(b), consists of individual β-strands stacked side by side forming a sheet-like structure. The strands are almost fully extended helices, and, therefore, cannot hydrogen bond with neighboring residues of the same strand. They are, however, placed to interact with neighboring chain residues having a similar secondary structure. The strands may be oriented in the same (parallel) or opposite (antiparallel) directions. In the illustration shown in Figure 1.4(b) two antiparallel strands line up edge to edge to form a highly stable sheet with multiple hydrogen bonds. 14,19
Tertiary and quaternary structures

Tertiary structures are characterized by the complex folding of peptide chains of the same protein in such a way as to bury the hydrophobic side chains while the polar side chains are exposed on the surface. This structure is more thermodynamically stable in the aqueous solution and is typical of globular proteins. In structural proteins, further stability is provided by disulfide crosslinks resulting from oxidation of cysteine residues close in space. In quaternary structure, separate protein chains associate to form a cohesive multimeric structure. Tertiary and quaternary structures are stabilized by van der Waals interactions, hydrophobic interactions when in polar solutions, hydrogen and ionic interactions as well as disulfide crosslinks in structural proteins.13,14,17

1.3.2 Protein-Based Bioplastics

Literature indicates that material application of proteins dates back to the 1850s when composite material of saw dust and blood were used to make plaques.20 However, with the advent of fossil derived plastics, the research and use of protein materials came to a halt. Other early commercialized domestic products were made from casein proteins in the early 1900s and in the 1930s Henry Ford filed a patent for a soy-based bioplastic hardened with formaldehyde for use as car body panels.20 Many of the isolate protein bioplastics currently studied come from crops like corn, soybean, cotton, and sunflower seeds. Of these crops, soybean isolate proteins are the most widely studied in the making of bioplastic articles and films, although there also studies on animal isolate proteins such as milk caseins and whey, pig skin gelatin, and fish myofibrillar. Table 1.1(a)
summarizes some of the isolate proteins and their sources in literature studies for potential applications in bioplastics, and a brief description follows.

1.3.3 Proteinceous Animal and Plant Co-products

In contrast to agricultural materials previously outlined, co-products have mainly been limited to animal feed applications. However, in search for ‘green’ alternatives, research in their non-feed applications is increasing significantly and recently a company in New Zealand hopes to commercialize plastics derived from blood meal by 2016.\textsuperscript{21} The use of co-products in bioplastic application has attracted attention, because they are cheaper than staple protein isolates, and do not pose direct competition to human-consumed food. The other driving factor is the need for value addition in the form of alternative non-feed applications.

Table 1.1(b) summarizes some of the co-products and their protein weight fraction composition under consideration for bioplastic processing. Co-products containing protein content of less than 60 wt% have mainly been investigated as fillers and blends with biodegradable plastics such as polycaprolactone and other conventional plastics like nylon and polyethylene.\textsuperscript{22-24} However, in this dissertation we show that co-products with lower fractions of proteins, viz. meat and bone meal (MBM), can also be converted into bioplastics.
Table 1.1. (a) Agricultural source materials and related isolate protein for bioplastic formation reported in various literature studies (b) Protein composition of co-products for bioplastic formation reported in literature studies.

<table>
<thead>
<tr>
<th>(a)</th>
<th>Plant Proteins</th>
<th>Animal Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>Type</td>
<td>Source</td>
</tr>
<tr>
<td>Corn</td>
<td>Zein 25</td>
<td>Milk</td>
</tr>
<tr>
<td>Soy beans</td>
<td>Soy proteins (β-conglycinin and glycinin) 27</td>
<td>Milk after casein removal</td>
</tr>
<tr>
<td>Wheat and corn</td>
<td>Gluten 29</td>
<td>Eggs</td>
</tr>
<tr>
<td>Cotton seeds</td>
<td>Cotton seed proteins (Albumin &amp; Globulin) 31</td>
<td>Fish and beef meat</td>
</tr>
<tr>
<td>Peanuts</td>
<td>Peanut protein (Arachin) 33</td>
<td>Ligaments, tendons, bones</td>
</tr>
<tr>
<td>Sorghum</td>
<td>Kafirin 35</td>
<td>Feathers, hooves, hair</td>
</tr>
<tr>
<td>Rice</td>
<td>Rice bran protein 37</td>
<td>Skin (pigs and cows)</td>
</tr>
</tbody>
</table>
1.3.4 Meat and Bone Meal (MBM)

MBM is a product of the rendering industry with an annual production of 2.5 million metric tons. In the rendering process illustrated in Figure 1.5, the residual animal tissue parts not utilized in human food (with exception of blood, hair, hooves, horns, hides, stomach and ruminal contents) are ground up and cooked with steam at temperatures of 115°C to 145°C for 40 to 90 minutes to deactivate micro-organisms and melt away fat. After cooking, the melted fat is separated from the protein/bone solids using a screw press. Also a large portion of moisture is removed within this step. The remaining components that include proteins, minerals and some residual fats are further processed by additional moisture removal and grinding before being packed for storage and shipment.43

The average composition on a dry basis of US based MBM is approximately 50% crude protein, 10% fat, and 28% ash, which is typically used as animal feed ingredient.43

<table>
<thead>
<tr>
<th>Plant co-products</th>
<th>Protein fraction</th>
<th>Animals co-products</th>
<th>Protein fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distillers Dried Grains with solubles (DDGS)</td>
<td>30%</td>
<td>Feather meal 99</td>
<td>80%</td>
</tr>
<tr>
<td>Soy meal 40</td>
<td>40%</td>
<td>Meat and bone meal 41</td>
<td>50%</td>
</tr>
<tr>
<td>Corn gluten meal 23</td>
<td>60%</td>
<td>Blood meal 42</td>
<td>90%</td>
</tr>
<tr>
<td>Sun flower meal 40</td>
<td>32%</td>
<td>Fish meal 11</td>
<td>60%</td>
</tr>
</tbody>
</table>
Unfortunately, due to its association with bovine spongiform encephalopathy (BSE) “mad-cow” disease, Europe and North America regulations have increasingly restricted its feed application, which has left a large source of low-value biomass.\textsuperscript{44,45} Efforts are under way for alternative non-feed applications of this material in value-added products.\textsuperscript{46-48} Prior studies have reported potential application of MBM as a fuel and adhesive.\textsuperscript{46,47} Because of its protein content, use of MBM as a protein concentrate source and bioplastic has also been reported.\textsuperscript{47,48} In addition, MBM is derived from a renewable source and is biodegradable, making its use in biodegradable bioplastic applications attractive as it can mitigate some of the environmental concerns associated with fossil-based synthetic plastics. Therefore, in this dissertation, the processing of MBM into a bioplastics for non-food uses is reported.

Figure 1.5. A schematic of the rendering process (adapted from Reference 43)
1.4 Methods Used in Protein-Based Bioplastic Formation

Because of their multifunctional macromolecular nature, proteins have numerous interactions depending on the amino-acid residues. They adopt folded native structures stabilized by numerous interactions including hydrophobic, electrostatic, van der waals, and hydrogen bonding, which is the major interaction. Cysteine crosslinks are also found abundantly in structural proteins such as keratin and collagen.\textsuperscript{15} For film formation, extended structures formed by unfolding of protein molecules are required through denaturation. This is achieved through application of heat and or chemical denaturants like urea, guanidine, and sodium sulfite that help break the numerous protein interactions.\textsuperscript{49,50} In addition to denaturants, plasticizers consisting of low molecular weight, non-volatile molecules (e.g., glycerol and sorbitol) are added to lower the protein glass transition temperature (Tg) to enable processing below decomposition temperature. These methods are broadly divided into two categories, wet/ solvent processing and dry/ thermoplastic processing, and are described below.

1.4.1 Wet/Solvent Processing

In solvent processing, the proteins are dissolved in a suitable solvent and then the solution is cast into a film by evaporating the solvent. It is important to have an idea of the intermolecular interactions of the proteins before attempting to solubilize proteins because their solubility is variable based on the amino acid content and native structure. For example, solubilizing of keratin requires addition of disruptive agents to break down cysteine disulfide bonds to obtain a homogeneous solution.\textsuperscript{51,52}
Based on the chemical structure of the protein, a suitable solvent is chosen in which to disperse the proteins. The commonly used solvents are water and aqueous ethanol. Most proteins are soluble in water with exception of corn zein, wheat gluten, sorghum kafirin, and keratin. These water insoluble proteins usually have low content of ionized polar amino acids or numerous disulfide crosslinks. In some cases, to enhance protein dispersion, the solvent system pH is modified by adding acids (e.g., lactic, acetic, or hydrochloric acid), or bases (e.g., ammonium, sodium, or potassium hydroxide) or by adjusting the solvent ionic strength by adding electrolytes. In addition, disruptive agents such as sodium sulfite, cysteine, mercaptoethanol, sodium borohydride may be added along with anti-microbials. The protein solution can then be formed into a film by spreading or casting it on a flat surface and allowing the solvent to evaporate. To speed up the process of solvent removal and subsequent plastic hardening, heat is applied along with controlled forced convection. Most early work on protein films used this technique and many proteins including soy protein isolate, corn zein, wheat gluten, fish myofibrillar and others were successfully processed into films.

However this technique of protein plastic formation has numerous drawbacks that include:

i) Some solvents may be expensive or toxic e.g., 2-mercaptop ethanol and triethanolamine

ii) Large quantities of heat are required to evaporate the solvent

iii) Plastic film thickness is limited and may be difficult to control

iv) Articles of complicated designs cannot be made

v) Specialized equipment need for solvent casting
vi) It is a time consuming process

Generally this process is not economically suitable for large scale production.  

1.4.2 Dry/Thermoplastic Processing

Unlike the solvent casting process, where the protein is solubilized in a solvent, thermoplastic processing is achieved under low hydration conditions. This is the method of choice for industrial processing of synthetic polymers that can be melted e.g., polyolefins. During thermoplastic processing, the material is heated above its softening or melting temperature in addition to application of mechanical energy for mixing of additives, consolidation and shaping. The softening (glass transition) temperature of protein materials, similar to synthetic polymers, is affected by molecular weight, chain rigidity, size and polarity of side residues, presence of intermolecular bonds or crystalline zones, and plasticizer type and concentration. However, unlike synthetic polymers that can be dry processed without plasticizers, all proteins necessarily require addition of plasticizers. Dry processing of protein based materials generally follows the following steps that are summarized in Figure 1.6 by Cuq and co-workers:

i) Plasticizer addition

ii) Heating the plasticized material above Tg

iii) Mechanical energy input for homogenizing, consolidation and shaping

iv) Cooling the rubbery material to ambient temperature into a vitreous material with a more rigid structure.

This method was investigated in this research because it is relatively inexpensive, does not require the use of potentially harmful volatile solvents, and may facilitate the
commercialization of protein bioplastics by utilizing established, cost-competitive processes for high-volume production of synthetic thermoplastics.

Figure 1.6. Schematic representation of the thermoplastic process for processing bioplastics from proteins in relation to the glass transition temperature (adapted from Reference 57)

1.5 Properties of Protein-Based Bioplastics

For packaging or geo-structural protein material applications, they must have suitable physical and mechanical properties. This section reviews the tensile and the water barrier properties of protein-based plastics reported in literature.
1.5.1 Mechanical Properties

Mechanical properties of protein bioplastics films are largely dependent on the method of preparations, plasticizer type and content as well as the temperature and moisture content of the bioplastic at test conditions. Therefore, the reported values fall in a wide range. Table 1.2 summarizes the tensile strength (TS), tensile modulus (TM) and strain-to-failure (STF) of some protein and proteinaceous co-products reported in literature as compared to some synthetic counterparts. All these bioplastics were prepared by thermoplastic processing. Their TS ranges from 2-17 MPa, and the STF values range from 1 to 276% with the smallest strains observed for bioplastics from co-products, which is attributed to inhomogeneity of the raw materials. Some protein bioplastics, especially those from animal based proteins, have TS comparable to that of LDPE; however their STF are significantly inferior. It is also observed that those films that are ductile tend to have poor TS and vice versa. In general, tensile properties of protein bioplastics are inferior to those of synthetic plastics, and this difference becomes more drastic as the bioplastics age. Plastics aged for only 1 month were reported to have a 40% decrease in TS.
Table 1.2. Tensile properties of some protein bioplastics produced by thermal processing as compared to synthetic plastics

<table>
<thead>
<tr>
<th>Material</th>
<th>TS (MPa)</th>
<th>TM (MPa)</th>
<th>STF (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPI-30% Gly</td>
<td>1.7</td>
<td>44</td>
<td>79</td>
<td>Pol et al., 2002</td>
</tr>
<tr>
<td>SPI-25% Gly</td>
<td>7.3</td>
<td>-</td>
<td>262</td>
<td>Jane et al., 1996</td>
</tr>
<tr>
<td>Wheat gluten-28% Gly</td>
<td>2.6</td>
<td>-</td>
<td>276</td>
<td>Gennadios, 1993</td>
</tr>
<tr>
<td>Corn Zein</td>
<td>3.1</td>
<td>101</td>
<td>120</td>
<td>Wang &amp; Padua, 2003</td>
</tr>
<tr>
<td>Pig Gelatin-16% Gly</td>
<td>17.3</td>
<td>490</td>
<td>216</td>
<td>Park et al., 2006</td>
</tr>
<tr>
<td>Myofibrillar proteins</td>
<td>17.1</td>
<td>-</td>
<td>23</td>
<td>Cuq et al., 1995</td>
</tr>
<tr>
<td><strong>Co-products</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feather meal</td>
<td>9.6</td>
<td>220</td>
<td>1.4</td>
<td>Sharma, 2008</td>
</tr>
<tr>
<td>Blood meal-3% SS</td>
<td>9.6</td>
<td>534</td>
<td>12</td>
<td>Pickering, 2010</td>
</tr>
<tr>
<td>Duck weed-25% Gly</td>
<td>1.74</td>
<td>84</td>
<td>3.4</td>
<td>Zeller et al., 2012</td>
</tr>
<tr>
<td><strong>Synthetic Plastics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDPE</td>
<td>8-23</td>
<td>200-400</td>
<td>300-1000</td>
<td>Ed. Baillie, 2004, pp 188</td>
</tr>
</tbody>
</table>

SPI – soy protein isolate, Gly – glycerol, SS – sodium sulfite, LDPE – linear low density polyethylene
1.5.2 Water Barrier Properties

The barrier properties of protein bioplastics depend on the proportion and distribution of non-polar amino acids relative to polar amino acids of the protein. Generally, for protein-based bioplastics, most free hydrophilic groups are able to interact with water vapor and to permit water transfer phenomenon, to the detriment of hydrophobic gas transfers, (e.g., nitrogen, oxygen). Proteins of corn zein and fish myofibrillar that have been determined to contain more hydrophobic residues have lower WVP than say soy protein isolate based films. Protein-based bioplastics generally have much higher water vapor permeability than synthetic plastics, over two to four orders of magnitude higher when compared to say low density polyethylene (LDPE) as displayed in Table 1.3 (data from Reference 50 pp. 20). Water vapor permeation through protein films is further facilitated by the systematic presence of hydrophilic plasticizers, which promote water molecule adsorption. Strategies to decrease WVP of protein films include adding of lipid compounds e.g., beeswax, paraffin and blending the bioplastic with other polymers that have good water barrier properties.
Table 1.3. Water vapor permeability of some protein bioplastics processed by thermal processing and that of LDPE (data from Reference 50, pg. 20)

<table>
<thead>
<tr>
<th>Protein</th>
<th>WVP (ng.m/m².s.Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin-16% Gly</td>
<td>1.42</td>
</tr>
<tr>
<td>Soy protein isolate 30% Gly</td>
<td>2.02</td>
</tr>
<tr>
<td>Whey protein concentrate -33% Gly</td>
<td>2.95</td>
</tr>
<tr>
<td>Calcium caseinate- 30% Gly</td>
<td>2.2</td>
</tr>
<tr>
<td>Corn zein-17% Gly</td>
<td>0.11</td>
</tr>
<tr>
<td>Fish myofibrillar -30% Gly</td>
<td>0.07</td>
</tr>
<tr>
<td>LDPE</td>
<td>$3.6 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

1.6 Treatments to Enhance Physical and Mechanical Properties of Protein-Based Bioplastics

As previously noted, the performance and properties of protein bioplastics are poor when compared to those of commercial synthetic plastics. Therefore, there is continued need to improve the properties of protein bioplastics while using cost-competitive manufacturing routes. Therefore, in addition to plasticizer type and processing parameters (temperature, residence time, pressure etc.), numerous modifications are being investigated in literature studies. These modifications include physical, chemical and enzymatic treatments, and are generally aimed at promoting crosslinking within the film structure to enhance tensile and water barrier properties of the bioplastics; these treatments are summarized below.

49
Heat curing

In this treatment step, preformed protein films of soy protein\(^{49,58}\), wheat gluten \(^{59}\), whey protein\(^{60}\) and peanut protein\(^{61}\) have been thermally treated at temperatures between 55 to 140\(^\circ\)C from a few minutes up to 24 hrs. The treated films showed a minimal improvement in tensile strength (TS) and moisture resistance than the native films, which were further enhanced as the heating time and temperature were increased. These changes are attributed to formation of intramolecular and intermolecular crosslinks, which mainly involve lysine and cysteine amino acid residues. Thermal treatment also helps in the degradation of hydroxyl groups, which also helps improve the water resistance of the films. Generally, thermally treated protein films, were found to have better TS and moisture resistance, lower elongation to break, and water solubility.

Enzymatic treatments

Enzymatic treatments of protein forming solutions have been reported in the literature, with more success achieved with the transglutaminase (TG) enzyme\(^{62,63}\). The enzyme catalyzes the formation of \(\varepsilon-(\gamma\text{-glutamyl}) - \text{lysyl cross-links}\) in protein and is isolated from cattle blood plasma and guinea pig liver\(^{62,63}\). The TG enzymatic cross-linked films of casein, egg white, gelatin and deaminated wheat gluten were reported to have improved TS and moisture resistance\(^{30,64,65}\).

\[
\text{Protein-}\text{Glu(\gamma)}-\text{CONH}_2 + \text{H}_2\text{N(}\varepsilon\text{-Lys-protein) } \xrightarrow{\text{TG}} \text{protein-Glu(\gamma)-CO-HN(\varepsilon)-Lys-protein}
\]
**Irradiation**

Studies have indicated that the aromatic amino acids, such as tyrosine, tryptophan and phenylalanine, can absorb UV radiation and recombine to form covalent crosslinks in proteins. Therefore, proteins with high composition of aromatic amino residues, such as soy protein isolate, sodium caseinate, and egg white when treated with UV irradiation at wavelength of 253.7 nm, were found to have increased TS and lower STF compared to non-treated films.

**Composite films**

Blends of proteins with other biopolymers including starch and lipids have been investigated. Results indicate that bioplastics made from the blend of proteins and lipids had significantly lower water vapor permeability (WVP) than the neat protein films. Some examples of protein-lipid bioplastics reported in literature include proteins of sodium caseinate and wheat gluten with bees wax, soy protein isolate, egg white protein and fatty acids. Also in an attempt to overcome hydrophilicity and improve processability of the soy proteins, they were blended with biodegradable polyesters using maleic anhydride as a compatibilizer. Similarly, blends of corn gluten meal, DDGS and meat meal with synthetic polymers that offer better water resistance such as the olefins have been produced.

**Chemical treatments**

Due to their multi-monomeric and chemical structure functionality, proteins offer extensive sites for chemical modification. Chemical modification of proteins using
crosslinking agents were utilized as early as the 1900s, where casein and soy proteins were cross-linked using formaldehydes, which helped reduce their water absorption by ~25%.\textsuperscript{20} In recent studies, wheat gluten proteins treated with formaldehyde displayed a four times increase in tensile modulus (TM) and TS but a decrease in elongation.\textsuperscript{68} The films also had lower solubility but the water vapor permeability remained unchanged from that of the unmodified films. Similar effects on mechanical properties were reported on studies of soy, pea and gelatin proteins treated with glutaraldehyde but with improved water resistance.\textsuperscript{33,69,70} Other chemicals, including, furfural, succinate anhydride, maleic anhydride, phthalic anhydride, and metal ions such as calcium ions have been investigated for numerous proteins.\textsuperscript{41,71-73} Anhydrides have extensively been studied as grafting agents in the reactive extrusion blending of soy proteins with synthetic polymers and with starch.\textsuperscript{74-76} In this dissertation, processing and properties of MBM bioplastics modified with maleic and phthalic anhydrides are reported.

1.7 Objectives

The literature studies reviewed above indicate that significant research is dedicated to bioplastics from expensive isolated agricultural proteins and co-products of high protein fractions (> 60%). However, there are limited studies on the use of low-value co-products containing lower protein content in the processing of biodegradable bioplastics. Therefore, the overall goal of this research was to process meat and bone meal (MBM) proteaceous co-product (protein content ~50%) into bioplastics using scalable polymer processing routes for non-feed applications. Specifically, the studies were aimed at:
1. Assessing the thermal processing and properties of bioplastics produced from modification of MBM with glycerol and calcium hydroxide.

2. Evaluating the continuous processing and properties of composites from blending of MBM with minor fractions of linear low density polyethylene (LLDPE).

3. Characterizing of resins from reaction of glycerol with anhydrides and their application in processing of thermoformable MBM bioplastics and their related properties.

The remainder of the dissertation is organized as follows. The three main topics (objectives) are documented in Chapters 2, 3, and 4. Finally, a summary of the major findings and recommendations for future work are presented in Chapter 5.

In Chapter 2, suitable plasticizer content (glycerol) and thermal processing conditions of MBM bioplastics were established using dynamic mechanical analysis and thermal analysis. Calcium hydroxide (CH) contents of 3-10 wt% were used to modify plasticized MBM and improve mechanical and barrier properties of the bioplastics. FTIR analysis was used to study the effect of calcium ions on the chemical structure of MBM and how they affect properties of the bioplastics. The bulk of the results presented in this Chapter are based on our published paper (Reference 42).

In Chapter 3, as a strategy to improve water resistance of the bioplastics, MBM was consolidated and calendered with minor fractions of LLDPE to form MBM-polymer composites (MBMPCs). It was hypothesized that since LLDPE has good water resistance
and has low melt temperature, at sufficiently low content, it can form a continuous binder phase that would encapsulate MBM and reduce its interaction with water and thus enhance water resistance of the composite. Processing of MBMPCS and their mechanical and water barrier properties are reported and compared to those of pure MBM and the LLDPE matrix.\textsuperscript{77} The Chapter is primarily based on our published paper. (Reference 77)

\textbf{Chapter 4} presents results on the processing of MBM bioplastics using resins from controlled reaction of maleic (MA) and phthalic (PtAH) anhydride with glycerol. It was hypothesized that if chemical agents that can react with both the glycerol plasticizer and MBM proteins were used in MBM bioplastics processing, they would improve mechanical and water resistance properties of MBM through covalent cross-links. Moreover, this would prevent glycerol from leaching out of the bioplastic. Chemorheology of the resin formation was followed using dynamic time sweep and the resin were characterized using Thermogravimetric analysis (TGA) and viscosity DMA. Mechanical, thermal and water absorption properties of the bioplastics are reported as well as the effect of aging on mechanical properties as compared to bioplastics with glycerol as the only modifier. Unlike, the use of LLDPE, the strategy here was to retain complete biodegradability of MBM derived bioplastics.

Finally \textbf{Chapter 5} provides the major conclusions drawn from the research and also provides recommendations for future research.
1.8 References


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CHAPTER 2
THERMAL PROCESSING AND PROPERTIES OF MEAT AND BONE MEAL

BIOPLASTIC SHEETS MODIFIED WITH GLYCEROL AND CALCIUM HYDROXIDE

2.1 Introduction

Studies on biodegradable materials made from blood meal, feather keratin, gelatin, feather meal, soy and zein proteins have been reported in various literature studies as reviewed in Chapter 1. Processing of protein-based films and sheets by thermal processing requires plasticizers, which intersperse among and within polymer chains, and disrupt hydrogen bonds thereby spreading the chains apart. Polyols (e.g., glycerol and sorbitol) increases molecular mobility, which decreases stiffness and increases ductility of the biomaterial by lowering the glass transition temperature (Tg). Furthermore, non-feed applications like bioplastic sheets require denaturation of proteins to occur, and are aided by the presence of a small amount of water (~ 5%).

In addition to plasticizers and water, other reagents such as chemical denaturants, reducing agents and crosslinking agents have also been added to modify protein configuration and improve properties of resulting bioplastics. Calcium ions are known for influencing mechanical properties of biological materials. In solvent processing, addition of calcium chloride to soy protein and wheat gluten protein solutions has been shown to have significant effects on the mechanical and water sorption properties of resulting films. The tensile strength of such films is reported to range...
from about 1 to 17 MPa at 25°C, and 50% RH. The water vapor permeability was reported to range from 13 to 262 g.mm/m².day.kpa.¹¹

Literature studies indicate that the mechanical properties of polymers are determined by chemical bonding and molecular structure.¹⁸ Secondary molecular structure of protein can be determined by Fourier transform infrared spectroscopy (FTIR). Amide I and amide II bands arise from bonds that link the amino acids of the protein.¹⁹ Amide I is due to carbonyl stretching in the region of 1600 – 1700 cm⁻¹ whereas amide II is due to N-H bending vibrations in the region 1500 – 1560 cm⁻¹.²⁰ Literature studies on the chemical modification of soy flour for adhesive preparation showed that FTIR can be used to follow chemical structure changes of the protein and reveal properties of the final adhesive.²¹ FTIR analysis of amide I band of thermoplastic processed blood meal, showed a decrease in β-content hence decreased molecular order with addition of urea.²

The films reported in the above studies were produced primarily from protein isolates, concentrates or extracts with protein content greater than 70%. However, such purified forms are also expensive. In contrast, MBM is a low-cost biomaterial with a protein content of about 50%, and has a potential as a film precursor. Literature studies on the use of MBM as a bioplastic have explored blending the MBM with expensive sodium caseinate and extruding the mixture into a dog chew toy.²² Also investigations of effect of calcium ions on protein film formation have been limited to solution casting process.¹⁶ Therefore, the present study was directed towards a cost-competitive, thermoplastic processing route of MBM-based sheets. This chapter presents results for studies
conducted on MBM modification without the use of synthetic polymers or expensive additives. The specific objectives were to (i) evaluate the effect of chemical modification of MBM by glycerol and calcium hydroxide on sheet formability, and (ii) characterize the mechanical properties and microstructure of the resulting bioplastic sheets for potential geo-structural applications.

2.2 Experimental

2.2.1 Materials and Processing

Meat and bone meal (MBM) protein (Darling International, Inc.) was used throughout this study. It is an animal co-product with an approximate composition of 50% protein, 8-12% fat, 4-7% moisture, and 35% ash. Because as-received MBM contains large bone particles, it was milled for further processing. The milled MBM sample was sieved through two sieve plates to obtain (a) a coarse grade that was the bottom product of sieving through a 16 mesh sieve (1 mm opening) for a 97 ± 2% yield, and (b) a fine grade that was the bottom product of sieving grade (a) further through a 60 mesh (250 µm opening) that resulted in a yield of 65 ± 2%. Initial studies were conducted on the coarse grade, but most of later studies focused on the fine grade. Milled MBM composition as determined in the laboratory was approximately 53% crude protein, 11% fat, 6% moisture and 30% ash.

Glycerol (SIGMA- Aldrich) was added to the MBM as plasticizer at 15%, 30% and 45 wt% compositions. Following the determination of plasticizer content, the next step was the calcium hydroxide (CH) modification of MBM at 0%, 3%, 7% and 10 wt%. Calcium
hydroxide (VWR International) was initially dissolved in glycerol to form a viscous white paste that was then manually mixed with MBM to form a dough (MBM to glycerol mass ratio of 7:3 was maintained). The dough was intensively compounded in a Haake Rheomix 600 batch mixer at temperatures ranging between 80 and 120°C for a mixing time of 15 to 30 minutes forming consolidated rubbery chunks. Blending and compounding was also done with the fine MBM to study the influence of particle size on the sheet formation and mechanical properties.

The rubbery chunks (12.5 g) were thermally compacted in an open mold 100 mm x 100 mm x 0.6 mm using a press (Carver Model 389.4PR1B00) at 6 MPa and 140°C. A holding time of 2 minutes was allowed for heat transfer to the mold and subsequent softening of the blend. The final load was applied for 2 additional minutes and the sample was subsequently cooled to nominal ambient conditions (40°C) under pressure before removing the sheet from the press. Optical microscopy (Olympus BX60) and scanning electron microscopy (SEM – Hitachi S4800) were used to analyze the texture and surface characteristics of the sheets.

2.2.2 Thermal Analysis

Thermogravimetric analysis (TGA) was conducted using a PerkinElmer Pyris 1 instrument. The samples were heated in an aluminum pan under air atmosphere from 26°C to 400°C at a heating rate of 10°C/min. Differential scanning calorimetry (DSC) was performed with a TA instrument MDSC 2920 from -100°C to 240°C at a heating rate of 20°C/min under a nitrogen atmosphere. Sample weight was nominally 5 mg.
2.2.3 FT-IR Analysis

Fourier-transform infrared (FT-IR) analysis was conducted using a Nexus 870 FT-IR ESP, Nicolet and OMNIC version 5.1 analysis software. The spectra of all the sheets, MBM powder, and glycerol were obtained using a Germanium ATR (attenuated total reflectance) accessory over a spectra range of 4000 - 600 cm\(^{-1}\). For each composition, spectra were obtained at three different points of the sheet and 32 scans with 8 cm\(^{-1}\) resolution were averaged.

2.2.4 Tensile Properties

Static Tensile Test

Tensile tests were conducted following the ASTM D638-10 procedure except that rectangular strips were used rather than dog-bone specimens. The samples were nominally 0.6 mm thick, 1.3 cm wide and 11.4 cm long, and the gauge length was set at 5.7 cm. Mean thickness of the samples was obtained from five points with a Nikon Digimicro, MF-5-01. Mechanical testing of the sheets was performed at a cross-head speed of 0.25 cm/min (Applied Test Systems Inc., Series 900). A minimum of five replicates for each composition were tested to calculate average values. Samples were conditioned for 24 hours at RH levels of 20%, and 50%. Conditioning was also done in a vacuum oven at 50°C (~100kPa vacuum) for 24 hours to obtain almost dry sheets.

Dynamic Tensile Test

TA Instruments RSA III rheometer was used for the dynamic mechanical testing using an initial length of 28 mm, a width of 12.7 mm wide, and a thickness of 0.6 mm thick. An
auto-tension adjustment was applied to the samples during testing. They were preconditioned in an oven under 100 kPa of vacuum at 50°C for 24 hours. The dynamic frequency measurements were obtained at 0.05% strain and 25°C with the frequency varied from 0.1 to 20 rad s\(^{-1}\). The dynamic temperature ramp measurements were conducted at 0.05% strain and a frequency of 6.3 rad s\(^{-1}\).

**2.3 Results and Discussion**

2.3.1 Processing, Thermomechanical and Optical Analysis

Sheet formation from protein material requires the addition of plasticizers, which lower the glass transition temperature of the protein and enable them to be processed at temperatures below the decomposition temperature.\(^{23}\) Figure 2.1(a) displays the thermograms for as-received MBM powder and plasticized MBM (30 wt% glycerol). For the MBM powder, the thermogram shows two mass loss steps: one below 100°C attributed to water evaporation, and a second in 200 to 300°C range due to degradation of proteins and evaporation of low molecular weight components. This behavior is consistent with that reported by other researchers.\(^{24,25}\) Plasticized MBM showed initial mass loss just under 100°C due to water evaporation followed by a continuous mass loss in 120 - 175°C range with a significant loss observed starting at about 175°C, which is the vaporization temperature for glycerol. Therefore, compounding and thermal compaction of plasticized MBM was conducted at, a temperature below 150°C.

Figure 2.1(b) displays the DSC thermograms of as-received MBM and that of sheets plasticized with 30 wt% and 45 wt% glycerol. All thermograms showed thermal
transitions between -2°C and 50°C and a large endothermic peak between 50°C and 200°C. MBM powder showed an endothermic peak at about 0°C from the melting of frozen water in the sample. It also shows a weak glass transition (Tg) between 30°C and 40°C. Plasticized MBM samples also showed a small endotherm at 0°C followed by a Tg between 35°C and 50°C. The large endothermic peak observed between 50°C and 200°C for MBM powder and the plasticized MBM is a combined effect of water loss and protein denaturation. There is a shift of the water loss-protein denaturation peak to higher temperature from 106°C (observed for MBM powder) to 145 and 158°C for 45 and 30 wt% glycerol content, respectively. Similar observations have been made by other researchers on these transitions that result from proteins that have denatured (unfolded) during the compounding and thermal compaction steps.6,26 The low Tg observed for MBM is largely attributed to the higher moisture content of the MBM powder.

Figure 2.1(c) displays the dynamic storage and loss moduli of MBM sheets plasticized with 30 and 45 wt% glycerol. As expected, both samples showed a decrease in the dynamic moduli with increasing temperature, but the storage modulus remained higher than the loss modulus. For the 30 wt% glycerol sheet, the storage modulus was 1855 MPa at -20°C, which is more than an order of magnitude higher than a value of 124 MPa displayed by 45 wt% glycerol sample. This significant difference is observed over the entire test temperature range. Therefore, a plasticizer content significantly greater than 30wt% produced soft, weak sheets while that much less than 30 wt% (i.e., 15 wt%) was found to result in insufficient consolidation. Recent work by Zeller et al also found DMA as a useful technique to establish optimal plasticizer content.27 Thus, based on the
consolidation level, microscopy, and DMA of consolidated sheets, a 30 wt% glycerol composition was established as being adequate. Further, the DSC thermograms had revealed that MBM sheets plasticized with 30 wt% glycerol had a denaturation temperature of about 160°C. Thus, a plasticization level of 30 wt% (glycerol) allowed sufficient motor torque and specific energy input for protein interaction while allowing processability below 150°C, and was used throughout the rest of the study.
Figure 2.1. (a) Thermogravimetric analysis of MBM powder and MBM sheet containing 30 wt% glycerol conducted in air atmosphere at a heating rate of 10°C/min. (b) DSC thermograms at a heating rate of 20°C/min under a nitrogen atmosphere for (1) as-received MBM, (2) MBM sheet plasticized with 30wt% and (3) MBM sheet plasticized with 45 wt% glycerol. (c) Dynamic storage and loss moduli as a function of temperature for 30% and 45 wt% glycerol plasticized MBM sheets.

Initial processing experiments conducted on as-received MBM produced sheets that contained numerous holes. Therefore, milling and sieving was conducted (as described in the experimental section) to obtain coarse and fine grades of the milled sample. Figure 2.2 shows the optical micrographs over a 4 mm² area of MBM-glycerol sheets produced from as-received, coarse, and fine MBM. The regions identified by circles illustrate intense transmitted light due to presence of pin-holes in the sheet. For the given area, as-received MBM sheets had numerous holes (~7) some as large as 200 µm in diameter. However, as the MBM particle size was reduced, the number and size of holes reduced
such that sheets from fine MBM did not show regions of significant intense light transmission. It is evident that as the MBM particle size became smaller, uniform and better consolidated sheets were formed. Thus, the fine grade of milled MBM was used throughout of the remainder of the study (unless otherwise specified).
Figure 2.2. Transmitted light micrographs of MBM-glycerol (70-30) sheets 0.6 mm thick and made from (a) as-received MBM, (b) milled, coarse MBM, and (c) milled, fine MBM.
Another processing variable that affected sheet quality was the environmental humidity during processing. It was observed that, despite the raw MBM having similar initial moisture content of 6.9% (dry basis), different plasticized blends were obtained in different humidity conditions during compounding. The raw MBM powder compounded at a relative humidity of 30% produced powdery material incapable of producing sheets. At a relative humidity of about 50%, rubbery chunks were produced, due to the denaturation effect of moisture. This is consistent with observations reported in prior literature studies that showed that an optimal amount of water is needed, in addition to heat for film formation from proteins. However, it was observed that high humidity levels (greater than 65%) resulted in excessive denaturation, which led to a sticky material that was not suitable for sheet formation. Thus, sheet formation from MBM protein by thermoplastic processing requires attention to plasticizer content, particle size, and environmental humidity. However, the well-consolidated sheets of fine MBM plasticized with 30 wt% glycerol still possessed a tensile strength of only 0.6 ± 0.1 MPa. This is much lower than tensile strength displayed by synthetic polymers like LLDPE (~30 MPa).

2.3.2 FTIR Analysis of MBM Bioplastics

In an attempt to improve mechanical properties, enhancement of molecular interactions using calcium hydroxide (CH) as a chemical modifier was investigated next. FTIR analysis was used to analyze the change in chemical structure of sheets with increasing CH content. The FTIR spectrum of milled MBM is shown first in Figure 2.3. MBM, being a multicomponent material shows numerous peaks consistent with results from
prior literature studies. It has a broadened peak at 3280 cm\(^{-1}\) because of the hydrophilic nature of MBM; the peak is attributed to O-H stretching from the moisture content of MBM. In the same region, there is an overlap of N-H stretching from proteins as well. The intense peaks at 2918 cm\(^{-1}\) and 2850 cm\(^{-1}\) are due to C-H stretching. The peaks at 1455 cm\(^{-1}\) and 1402 cm\(^{-1}\) are also due to C-H deformations. The protein component of MBM is shown by the 1643 cm\(^{-1}\) peak, which represents the amide I band (C=O) stretching in the protein secondary structure. The 1532 cm\(^{-1}\) peak is the amide II band due to N-H bending and C-N stretching. The fat portion of MBM is represented by C=O vibration peak at 1742 cm\(^{-1}\) and C-O vibration peak at 1029 cm\(^{-1}\) from ester linkage of triglycerides.

Figure 2.3. FTIR spectrum of MBM powder showing the amide I and amide II peaks and other peaks
Figure 2.4 displays the FT-IR spectra of a bioplastic sheet containing 7 wt% CH; milled MBM powder and glycerol (plasticizer) are shown for comparison. It was observed that the consolidated sheet has a broadened amide II band whereas the MBM powder has distinct amide I and amide II bands. The broadening is attributed to the interaction of various secondary protein structures (alpha and beta sheets) forming ionic crosslinks with Ca$^{2+}$ via negatively charged oxygen atoms from side residues like glutamate and aspartate.\textsuperscript{29} MBM is reported to contain about 6% glutamic acid and 4% aspartic acid.\textsuperscript{30} It is noted that the peak at 1742 cm$^{-1}$ observed for MBM powder is absent for the processed sheet spectrum. This shows that at a CH concentration of 7 wt%, the fats present in MBM are hydrolyzed and saponified. Previous research on fat saponification has shown similar effects.\textsuperscript{31} The saponification process results in formation of ionized carbonyls which do not absorb in the same region as the non-ionized carbonyl of fats because of resonance effects of the formed carboxylate.\textsuperscript{32} The distinct glycerol peaks between 950 and 1150 cm$^{-1}$ still appear in the processed sheet, which confirm the plasticization presence of glycerol.
In Figure 2.5, FTIR spectra for sheets containing different CH composition (0%, 3%, 7% and 10 wt%) are displayed. A comparison of peaks between 1750 and 1100 cm$^{-1}$, shows that at 3 wt% CH modification, no significant change in chemical structure is observed. Significant chemical changes are observed at CH concentration of 7 wt% or higher. Clearly, as explained previously, the 1740 cm$^{-1}$ peak attributed to the C=O of fat triglycerides disappears. Because there is increased interaction between the protein chains, broadening is observed in the amide II through amide III region. It can therefore be inferred that addition of CH content greater than 7 wt% increases protein chain interaction.
Figure 2.5. FTIR spectra comparing the molecular structure of MBM plastic sheets modified with different calcium hydroxide composition: (a) spectra over a wider range of wave numbers, and (b) zoomed-in spectra of fat and protein characteristic bands
2.3.3 Mechanical Properties

Table 2.1 displays the tensile modulus (TM), tensile strength (TS) and strain-to-failure (STF) as a function of calcium hydroxide (CH) concentration of the plasticized sheets obtained from the fine grade of milled MBM (properties of coarse MBM are presented later for comparison purposes). As the CH concentration increased, the tensile strength initially decreased for 3 wt% CH modification to half the value for non-modified sheets (0.8 ± 0.1 MPa). However, for higher CH content, TS increased by a factor of about four, to 3.2 ± 0.4 MPa for 7 wt% CH. Increase of CH to 10% resulted in sheets with TS ~ 5 times that of sheets with 0 wt% CH. Strain to failure generally decreased as the CH content was increased from 0% to 10 wt%. Sheets with 3 and 7 wt% CH, had their strain to failure decrease by about 40% while that of 10 wt% CH sheets, decreased by 75% compared to unmodified sheets. Similar to the TS, TM of the sheets first decreased when 3 wt% CH was added, but increased by a factor of 3 and 8, when 7% and 10 wt% CH were, added respectively.
Table 2.4. Comparison of tensile strength (TS), strain-to-failure (STF), and apparent tensile modulus (TM) of sheets made from fine MBM with different CH contents.

<table>
<thead>
<tr>
<th>Composition</th>
<th>TS (MPa)</th>
<th>STF (%)</th>
<th>TM (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% CH</td>
<td>0.8 ± 0.1</td>
<td>8.9 ± 1.2</td>
<td>40.2 ± 2.9</td>
</tr>
<tr>
<td>3% CH</td>
<td>0.5 ± 0.06</td>
<td>5.0 ± 0.5</td>
<td>34.8 ± 2.2</td>
</tr>
<tr>
<td>7% CH</td>
<td>3.2 ± 0.4</td>
<td>6.5 ± 2.4</td>
<td>139 ± 20</td>
</tr>
<tr>
<td>10% CH</td>
<td>4.0 ± 0.6</td>
<td>2.1 ± 0.4</td>
<td>341 ± 51</td>
</tr>
</tbody>
</table>

Test samples were pre-conditioned in a 100 kPa vacuum at 50°C for 24 h (n = 5).

For comparison purposes, MBM sheets produced from coarse MBM and modified with 0%, 3%, 7% and 10 wt% CH were also tested, and found to have TS values of 0.6 ± 0.01, 0.3 ± 0.02, 1.6 ± 0.06, and 2.3 ± 0.5 MPa, respectively. Corresponding values for MBM sheets from fine MBM were 0.8 ± 0.1, 0.5 ± 0.06, 3.2 ± 0.4, 4.0 ± 0.6, respectively. In general, sheets made from fine MBM had higher TS than those from coarse MBM. For sheets modified with 7 wt% CH, the fine MBM sheets had a tensile strength of 3.2 MPa and an apparent modulus of 140 MPa, which were twice those of the sheets made from coarse MBM. The STF was found to be about half (6.5%) that of the sheet made with the coarse MBM. Figure 2.6 displays the corresponding SEM micrographs illustrating the difference in surface microstructure of the CH modified sheets from fine and coarse MBM. Higher TS and TM are observed with smaller particle MBM because of better consolidation through increased protein chain interactions. On the other hand, coarse MBM has an effect close to that observed in discontinuous fillers that typically reduce tensile strength of composites.33
Figure 2.6. SEM plane surface images of MBM sheets with 7 wt% CH: (a) sheet from coarse MBM, and (b) sheet from fine MBM.

Figure 2.7 presents stress-strain plots for 7 wt% CH modified MBM sheet showing the effect of environment humidity on the mechanical properties. The samples were preconditioned at 0%, 20% and 50% percent relative humidity (RH). At 0% RH, the samples showed glassy behavior with a high modulus of 140 MPa and low strain to break of about 10%. When the RH was increased to 20%, the sheets demonstrated a rubbery behavior with the modulus decreasing by almost an order of magnitude to 17 MPa while the STF increased by a factor of 3 to 30%. Increase in the relative humidity to 50% decreased the modulus further to 13 MPa and also decreased the strain to failure to values close to those of samples conditioned at ~ 0% RH.

At very low humidity levels, the intermolecular bonds of the sheets are strong and lead to high TM and TS but lower strain to failure. Because MBM protein and glycerol are hydrophilic, moisture is readily absorbed by the sheets. As water molecules diffuse
within the protein chains, they weaken the protein bonds and crosslinks, which lead to lower TM and TS, while the strain to failure increases. However, if excessive moisture is absorbed, the protein chain network is largely destroyed resulting in a decline of the strain as well. Therefore, mechanical properties of MBM sheets can be tailored by adjusting moisture content depending on the desired application.

Figure 2.7. Stress-strain plot showing the effect of environment humidity on the tensile properties of 7 wt% CH modified sheets from fine MBM at 25°C. Test sample were preconditioned for 24 h at each humidity level

Figures 2.8 (a) and (b) display dynamic tensile moduli as a function of frequency for 0%, 3%, 7% and 10 wt% CH modified MBM (fine, milled). For all compositions, as the frequency increased, the dynamic TM increased and solid behavior was observed over
the entire frequency range with the storage modulus being an order of ~3 higher than the loss modulus. From Figure 2.8 (a), addition of CH of 3 wt% resulted in a lower dynamic TM, which agrees well with the observation for the static modulus. Further increase of CH content to 7 wt% and 10 wt% resulted in an increase in the dynamic TM of 5-fold and 10-fold, respectively. These observations are consistent with literature studies where the presence of metal ions like Ca\(^{2+}\) ions resulted in increased modulus of biological gels like alginate.\(^{15}\)
Figure 2.8. Plots of dynamic tensile moduli as a function of frequency for 0%, 3%, 7% and 10% calcium hydroxide modified fine-MBM sheets: (a) storage modulus ($E'$), and (b) loss modulus ($E''$)
Figures 2.9 (a) and (b) display the dynamic TM of 0%, 3%, 7% and 10% calcium hydroxide modified fine-MBM sheets as a function of test temperature. From 25 to 50°C, as expected, all the samples showed a decrease in the dynamic TM. However, solid behavior was maintained over the given temperature range with the dynamic storage modulus (E') remaining about three times higher than the loss modulus (E''). Samples containing 0% and 3 wt% CH had the modulus decrease by approximately a factor of 4 whereas sheets containing 7 wt% and 10 wt% CH had a decrease of approximately a factor of 2 and 3, respectively. MBM sheets with 3 wt% CH content showed a lower dynamic TM than those without CH over the entire test temperature range, whereas sheets containing 7 wt% and 10 wt% had significantly higher dynamic TM in comparison with that for samples containing no CH. At the highest test temperature of 50°C, for samples containing 7 wt% and 10 wt% CH, dynamic TM was approximately an order of magnitude higher than that of unmodified samples. Sheets modified with CH content greater than 7 wt% had significantly higher thermal stability than those with no CH.

The increase in TS and TM of the sheets is primarily attributed to two factors: (i) enhanced denaturation that results in extended conformations hence more chain-like orientation, and (ii) the increased interaction of protein chains through crosslinks with calcium ions via negatively charged oxygen atoms from side residues like glutamate and aspartate. The crosslinks may also be from uncharged oxygen atoms of the main chain carbonyl groups with side chain oxygen atoms from glutamine and asparagine similar to that shown in prior literature studies. The FTIR results discussed in the previous section demonstrated this effect of calcium hydroxide on the chemical structure for CH content.
greater than 7 wt%. Similar observations were reported in literature studies where soy films from calcium chloride treated solution had TS twice that of films from the untreated solution.\textsuperscript{16}
Figure 2.9. Plots of dynamic tensile moduli as function of temperature for 0%, 3%, 7% and 10 wt% calcium hydroxide modified fine-MBM sheets: (a) dynamic storage modulus ($E'$), and (b) dynamic loss modulus ($E''$).
2.4 Conclusions

MBM was successfully processed by thermal compaction into bioplastic sheets using glycerol plasticizer. The processability of MBM was influenced by MBM particle size and environmental humidity. Well-consolidated sheets were produced from fine MBM at ~50% RH, but these sheets possess poor mechanical properties (about 2% of the TS of a synthetic polymer like LLDPE). Chemical modification with calcium hydroxide (7-10 wt%) led to an increase in tensile strength and tensile modulus of MBM sheets by a factor of 4 and 5, respectively. The mechanical properties of the sheets were also affected by MBM particle size and the environmental humidity. The samples from fine MBM had a TS of 4 MPa, which was twice that of samples from coarse MBM. The FTIR spectroscopic analysis demonstrated an increase in protein interaction in samples with CH content greater than 7 wt% as inferred from the broadening of the amide II region (1500 – 1560 cm⁻¹). The improvements of the mechanical properties are attributed to crosslinking effect of calcium ions between the negatively charged oxygen atoms of protein side residues.
2.5 References


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CHAPTER 3
CALENDERED LINEAR LOW DENSITY POLYETHYLENE CONSOLIDATED MEAT
AND BONE MEAL COMPOSITES

3.1 Introduction
In Chapter 2, it was established that MBM is thermally processable into plastic-like sheets when modified with hydrophilic low molecular weight plasticizers (e.g., glycerol). However, the mechanical properties of such bioplastics are significantly lower when compared to those of commonly used polyolefins. Further modification with calcium hydroxide (CH) resulted in bioplastics with improved tensile strength (TS) that was about four times higher for sheets containing up to 7 wt% CH. However, the moisture uptake of the bioplastics was still quite high and accelerated by the presence of the hydrophilic plasticizer. Thus, the mechanical properties were observed to rapidly deteriorate with increasing environmental humidity.

A strategy to prevent this performance deterioration and enhance mechanical properties is the use of synthetic polymers as binders during the processing of bioplastic sheets to produce MBM-polymer composites (MBMPCs) with MBM as the major content (>50 wt%). Synthetic polymers [e.g., linear low density polyethylene (LLDPE)] have excellent mechanical and barrier properties and are easy to process. However, they are derived from fossil resources and are by themselves non-compostable and non-biodegradable. MBMPCs are attractive because they can easily be integrated with industrial polymer processing routes, and yet reduce the content of synthetic polymers, which addresses
sustainability concerns associated with the use of petroleum based plastics. Thus, composites consisting of renewable biomaterial particulates and synthetic polymers are of topical interest from an environmental sustainability perspective.\textsuperscript{6-9}

Composites such as particle boards, where the particulates make up more than 50 wt% of the composite, have been investigated in literature studies. The particulates are mainly cellulosic materials, e.g., wood flour, wheat stalk, sugar cane bagasse and cornhusks.\textsuperscript{8-10} The adhesives/binders used for such composites are mainly thermosetting resins that include urea-formaldehyde, phenol formaldehyde, melamine formaldehyde, and diphenyl diisocyanate.\textsuperscript{11} However, use of such thermosets leads to undesired issues of formaldehyde emissions and limited recyclability.\textsuperscript{11}

Other bio-particulates of non-cellulosic origin (egg shells, chicken feathers, seed weed, and waste shell fish) have been incorporated as fillers into synthetic polymers at fractions usually less than 30 wt%.\textsuperscript{6,12-14} Studies have been conducted with a low concentration of MBM as a filler in high density polyethylene (HDPE).\textsuperscript{15} However, studies on processing high volume fractions of MBM with synthetic polymers using conventional thermoplastic processing routes have not been reported in literature. Therefore, meat and bone meal particulates were consolidated using a thermoplastic LLDPE as the minor phase. Specific objectives of the studies reported in this chapter were to: (i) examine the microstructure of MBMPCs as a function of different MBM contents and (ii) characterize the mechanical and transport properties (water vapor permeability and water absorption) of MBMPCs produced by calendering, which is an industrially relevant process. While not a
strategy that completely eliminates synthetic polymers, the one discussed here minimizes synthetic content significantly and adds value to MBM material.

3.2 Experimental

3.2.1 Materials
Meat and bone meal (MBM) (Darling International, Inc.) was used throughout this study. It is a rendered animal co-product with an approximate composition of 50% protein, 8-12% fat, 4-7% moisture, and 35% ash according to the manufacturer. Because as-received MBM contains large bone particles, it was milled and sieved through a 60 mesh sieve (250 µm opening) to obtain a bottom product that was used in further processing. Linear low density polyethylene (Dowlex 2045 LLDPE) with a MFI (190°C/2.16 kg) of 1.0 g/min and density of 0.92 g/cc (Dow chemical company) was used throughout the study.

3.2.2 Processing MBM-Polyethylene Composites
Milled and sieved MBM was intensively blended with 5, 10, 15, 30, 40 and 60 wt% linear low density polyethylene (LLDPE) using a Haake Rheomix 600 batch mixer at 140°C for 15 mins and 60 rpm mixing speed. The different mixed compositions of MBM-LLDPE were formed into MBMPC sheets using a Collin calender roll mill (model W 100T) with two counter-rotating rolls. Calendering was conducted at 135°C and 3 – 15 rpm with the gap between the rolls set to 0.25 mm as shown in Figure 3.1; the illustrated calendered sheet is about 10 cm wide.
3.2.3 Thermal Analysis and Scanning Electron Microscopy (SEM)

Differential scanning (DSC) analyses of the composites were performed using a Perkin-Elmer Pyris DSC from 30°C to 145°C at 10°C/min in nitrogen atmosphere. Each sample was exposed to two heating and two cooling scans. Thermal gravimetric analysis (TGA) was conducted at a heating rate of 10°C/min from 30°C to 500°C.

SEM-Hitachi S4800 was used to analyze the microstructure of cryogenically fractured cross-section surfaces of the calendered MBMPCs.
3.2.4 Tensile and Flexural Properties

Tensile tests were conducted following the ASTM D638-10 procedure using dog-bone specimen (type V) die-cut from the calendered sheets both in the longitudinal and transverse direction. Mechanical testing of the sheets was performed at a cross-head speed of 0.25 cm/min (Applied Test Systems Inc., Series 900). The flexural modulus was obtained from three point dynamic strain sweep using RSA 3 TA Instruments rheometer at 25°C, 0.002% strain and 6.28 rad/s frequency. The test specimens were nominally 2 mm thick, 12.5 mm wide and 50 mm in length. The three point bend fixture used had a span of 40 mm. A minimum of four replicates for each composition were tested. Samples of MBMPCs were conditioned at 50% RH and 25°C for 48 hrs. Analysis showed that there was no significant difference in tensile properties for specimen tested either in longitudinal or transverse direction. Therefore, results presented herein are for longitudinally cut specimen. In addition, tensile tests on samples of MBMPCS containing 10, 15 and 30 wt% LLDPE were performed on specimens soaked in water for 1 and 3 days.

3.2.5 Water Vapor Permeability (WVP) and Water Absorption

The tests were carried out following the ASTM E 96-05 [Standard Test Methods for Water Vapor Transmission of Materials]. Two replicate circular discs, each having an area of 28.3 cm², were placed on the testing cups each containing 15 ml of distilled water. The cups were tightened by screws, leaving an exposed area of 19.6 cm². The cups were placed in the WVP testing chamber (Model 506A Electro-tech Systems Inc.) maintained
at controlled relative humidity (RH) and temperature. Within the chamber, a Denver instrument Model # P-603-D balance was used to obtain mass as a function of time.

The temperature and humidity were stabilized for 24 hours before testing began. Measurements were taken at 1 hour intervals for the first 12 hours, and then every 5 hours. From a linear regression of the mass versus time curve, water vapor transmission (WVT) in g/m$^2$/s was calculated as: $WVT= \text{slope/\text{Area}} \times 1\text{hr}/3600\text{s}$

Then, water vapor permeability (WVP) in g/m.s.Pa, was calculated as:

$$WVP= \frac{(WVT) \times T}{\text{SVP} (RH_1 - RH_2)}$$

where SVP = saturation vapor pressure (Pa) = 3.166x10$^3$ at 25°C, T (m) = average thickness of the test specimen, RH$_1$ = relative humidity in the test cup $\approx$ 100%, RH$_2$ = relative humidity of the chamber = 50%

The water absorption of calendered MBMPC sheets was determined by using circular discs of 2 mm thickness and 25 mm diameter. Three specimen of each composition were initially dried in a vacuum oven (~ 100 kPa vacuum) at 50°C for 48 hours. The specimens were then placed in separate glass beakers filled with distilled water (200 ml). The samples were withdrawn at intervals of 2 hours for the first 10 hours and less frequently thereafter to record their mass gain. The samples were lightly wiped with a paper towel to remove surface water before being weighed. The mass of the samples were recorded for up to 72 hours.
### 3.3 Results and Discussion

#### 3.3.1 Thermal Analysis

Figure 3.2 (a) displays the thermograms of milled MBM, pure LLDPE and MBMPCS containing 10 and 30 wt% LLDPE content. For the milled MBM powder, the initial mass loss below 100°C is due to water evaporation, while the significant decomposition of its components starts at about 175°C consistent with that reported in previous studies.\(^1,\)\(^16\) Therefore, thermal processing of MBM must be below 175°C. In contrast LLDPE’s threshold decomposition is observed at \(~400°C\), which is about 230°C above that of MBM signifying its relative higher thermal stability. The composites display similar thermograms as that of pure MBM until about 325°C, with only variability observed in the relative mass loss related to the LLDPE content. This is because they were processed at 145°C that was below the thermal decomposition of MBM as displayed in Figure 3.2 (a).

Figure 3.2 (b) displays the first and second heating thermograms of MBMPCS containing 10 and 30 wt% LLDPE compared to pure LLDPE. It is observed that both the first and second heating thermograms of LLDPE are similar and have a flat baseline after the melting peaks. In contrast, those of the composites are variable, with the first heating baselines being wavy after 125°C. This is an indication of the thermal sensitivity of composites containing biomass (MBM) in the given temperature range. The first heating thermograms of composites containing 10 wt % LLDPE do not display a sharp endothermic peak, although there is a broad endotherm from about 60°C to 125°C. When the ratio of MBM to PE in the composite is reduced from 9 (10 wt% PE) to 2.3 (30 wt% PE),
PE), two endothermic peaks are observed in addition to the broad endotherm. The observed broad endotherm, and the characteristic difference of MBMPC thermograms from the first heating thermograms, is due to water evaporation and protein denaturation in combination with PE melting.

Previous DSC studies on thermal processing of MBM have shown that it displays a broad endothermic peak between 50°C and 200°C [Figure 3.2 (b) inset] despite prior thermal treatments. The endotherm has been observed in other protein studies and is related to water loss and protein denaturation (unfolding).\textsuperscript{1,17,18} Therefore, because of these endothermic events, the sharp melting transitions of LLDPE in the first heating are masked. However, once the composites were reheated to 145°C, the protein transitions disappeared and the second thermograms displayed sharp melting peaks between 108°C and 122°C with flat baselines. The irreversibility of protein transitions in DSC measurements may be attributed to complete denaturation of most of the ordered secondary structures that are part of the molten globule state (compact intermediate conformation) proteins formed during prior thermal treatments.\textsuperscript{19} Therefore, compounding and calendering of MBMPCs was done at temperatures ranging between 135 and 150°C that are sufficiently above melting of the LLDPE phase (122°C) but well below 200°C, where significant MBM decomposition is observed in Figure 2 (a).
Figure 3.2. (a) TGA thermograms of milled MBM, pure LLDPE (PE) and MBMPCs containing 10 wt% and 30 wt% PE (b) First and second heating DSC thermograms of MBMPCs containing 10 wt% and 30 wt% PE compared to pure PE. The first heating is indicated by continuous lines while the second heating is represented by discontinuous lines. The inset is a thermogram of MBM showing a large endotherm between 50°C and 200°C and other transitions.
3.3.2 Microstructure

Figure 3.3 displays SEM micrographs of cryogenically fractured cross-sections of pure LLDPE and MBMPCs containing different LLDPE contents; MBM powder is also shown for comparison. The representative MBM micrograph displays a wide range of particulate sizes ranging from ~10 µm to 200 µm nominal diameter highlighted within dashed circles, which may actually be agglomerated smaller particles. In the MBMPC micrographs, the lighter phase is the LLDPE matrix, whereas MBM shows up as dark irregular agglomerates (some highlighted within circles) varying from ~100 µm down to < 1 µm. At 10 wt% LLDPE content, the MBM agglomerates were barely encapsulated by the polyethylene, and MBM agglomerates (> 100 µm) appear to touch one another. At 15 wt% LLDPE content, MBM agglomerates were still observed although most appear to be surrounded by the LLDPE matrix. Increase in the LLDPE content to 40 wt% resulted in significant improvement in the encapsulation of MBM by LLDPE matrix, with an accompanying decrease in average nominal agglomerate size of 40±16 µm. At 60 wt% LLDPE content, its existence as a continuous phase is very clear with the average nominal MBM agglomerate size reduced to 25±9 µm.

Furthermore, the SEM micrographs revealed that there was a small preferential axial orientation (white arrows) of MBM agglomerates especially apparent in composites containing more than 10 wt% LLDPE content, as indicated by the slightly elongated shape. Particle orientation in the calendered composites occurs because of elongation deformation as the blend is nipped through the counter-rotating rolls.20 Although textural orientation in the MBM phase was observed, the LLDPE phase did not show significant
orientation, due to the low calendering speed and the slow cooling that allowed molecular relaxation within the LLDPE phase.

The SEM images indicate that MBM exists as irregular agglomerates in the MBMPC sheets, because it is largely hydrophilic and incompatible with the hydrophobic LLDPE binder. This incompatibility results in phase separation similar to what has been observed in composites of starch and polyethylene. However, when sufficient mechanical energy is transferred from LLDPE to MBM during mixing, shearing action causes the agglomerates to break down to smaller sizes. These smaller domains are encapsulated by the polymer and are held tight on cooling because of the higher thermal expansion coefficient of LLDPE \((200 \times 10^{-6}/K)\) consistent with literature studies on other polymer composites.
Figure 3.3. Representative SEM micrographs of MBM composite sheets consolidated with different LLDPE content compared to pure LLDPE and pure MBM powder. The white arrows indicate the longitudinal axis of calendering.
3.3.3 Mechanical Properties

For the various compositions of MBMPCs evaluated, the tensile strength (TS), strain-to-failure (STF), tensile modulus (TM) and flexural modulus (FM) are summarized in Table 3.1. The TS and STF of MBMPC sheets increased with increasing LLDPE content. The TS for 10 and 60 wt% LLDPE content ranged between 0.7 ± 0.1 MPa and 6.3 ± 0.2 MPa, and STF ranged from 2.3 ± 0.3% to 108 ± 59%. This behavior is consistent with particle-filled composites with poor adhesion between the particulates and polymer matrix. The TS and STF of MBMPCs increases with increasing LLDPE content because it forms a continuous phase that has superior load-carrying properties relative to that of MBM.

Figure 3.4 displays the normalized tensile TS and STF of MBMPCs together with the Nielsen model predictions. The predicated values were calculated using component weight fractions to facilitate comparison with experimental value on the graphs. The volume fractions needed for model calculations, were calculated using LLDPE density of 0.92 g/cc and that of MBM measured as 1.3 ± 0.2 g/cc. The Nielsen model for TS for a composite with no adhesion between filler and polymer matrix is displayed in eq. (3.1) and that of STF assuming good adhesion between filler and matrix is as in eq. (3.2):  

\[
\frac{\sigma_c}{\sigma_m} \approx \left( 1 - \varphi_f^{2/3} \right) S \tag{3.1}
\]

\[
\frac{\varepsilon_c}{\varepsilon_m} \approx \left( 1 - \varphi_f^{1/3} \right) \tag{3.2}
\]

where $\sigma_c$ is the composite TS, $\sigma_m$ is the matrix (LLDPE) TS, $\varepsilon_c$ is the composite strain to failure, $\varepsilon_m$ is the matrix strain to failure, $\varphi_f$ is the volume fraction of MBM and $S$ is a stress concentration function with a limiting value of 1 when there is no stress
concentration. The function \((S)\) accounts for weaknesses in the structure and stress-field caused by the discontinuities at the particle/matrix interface. The STF model assumes that the polymer in the composite breaks at the same elongation as the bulk unfilled polymer.

As observed in Figure 3.4, the Nielsen TS model with \(S =1\) grossly over-predicts the TS of the MBMPCs. This may be attributed to the failure of the model to account for the poor interfacial strength of MBM and the LLDPE matrix. The STF model indicates that presence of small fractions of particulates rapidly decreases the STF followed by a gradual decrease. The disagreement between experimental data and model predication is largely attributed to poor adhesion between MBM and LLDPE. In addition, the models do not account for size and shape of the particulates, which also affect the TS and STF of the composites.

MBMPCs containing LLDPE content of 15-60 wt% displayed a 40 to 73% higher TM compared to base LLDPE (282 ± 45 MPa). However, the composite containing 10 wt% LLDPE displayed a significantly lower TM (139 ± 1 MPa) than that of LLDPE. Tensile moduli of MBMPCs containing 15 to 60 wt% LLDPE content were not statistically different from each other even though the trend of the average TM was to increase with decreasing LLDPE content. It was observed that the flexural moduli of MBMPCs initially increased with LLDPE content of up to 30 wt% and then decreased with higher LLDPE contents of 40 wt% and above. Composites containing 30 wt% LLDPE displayed the highest flexural modulus of 633 ± 23 MPa, which was more than three times that of LLDPE. Furthermore, the flexural moduli of the composites as well as pure LLDPE were
found to be statistically not different from the respective tensile moduli. The lower TM of MBMPCs containing 10 wt% LLDPE content may be attributed to the unconsolidated MBM particles observed in Fig. 3.3 where the MBM agglomerates were not adequately encapsulated by the LLDPE phase. LLDPE content of about 15 wt % or higher was required to form a continuous LLDPE phase. Beyond that content, the FM and TM of the composite surpasses that of the matrix.\(^{25}\)

Figure 3.5 displays a comparison of the normalized tensile modulus of MBMPCs to that of the predictions by the simple rule-of-mixtures displayed in equation 3.3:\(^ {26,27}\)

\[
\frac{E_c}{E_m} = (1 - \varphi_f) + \varphi_f \frac{E_f}{E_m}
\]  

(3.3)

where \(E_c\), \(E_m\), and \(E_f\) are the composite, matrix (LLDPE), and filler (MBM particulates) tensile moduli respectively, and \(\varphi_f\) is the volume fraction of MBM. For the purpose of model prediction, the tensile modulus of MBM was assumed to be equivalent to the measured FM of 530 MPa. The simple additive model generally provided good prediction with the exception of composites containing less than 15 wt% LLDPE. The model works well because the modulus of MBM (530 MPa) does not vary widely from that of LLDPE (280 MPa). Also, the differential thermal shrinkage of the polymer matrix when the composite is cooled (from melt to ambient temperature) causes the polymer to mechanically bind around the MBM solid particles. Overall, increase in the MBM content that has a higher TM than LLDPE increases the modulus of the composites until such fractions where the LLDPE does not form a continuous network, viz. at 10 wt% LLDPE content.
Table 3.1. Summary of tensile strength (TS), Strain to failure (STF), Tensile Modulus (TM), and flexural modulus (FM) of MBMPCs with different composition of LLDPE

<table>
<thead>
<tr>
<th>LLDPE wt% (vol%)</th>
<th>TS (MPa)</th>
<th>STF (%)</th>
<th>TM (MPa)</th>
<th>FM (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 (14)</td>
<td>0.7 ± 0.1</td>
<td>2.3 ± 0.3</td>
<td>139 ± 1.1</td>
<td>165 ± 5.5</td>
</tr>
<tr>
<td>15 (21)</td>
<td>1.4 ± 0.1</td>
<td>7.1 ± 0.9</td>
<td>398 ± 80</td>
<td>298 ± 11</td>
</tr>
<tr>
<td>20 (28)</td>
<td>2.0 ± 0.03</td>
<td>7.2 ± 2.6</td>
<td>476 ± 47</td>
<td>394 ± 19</td>
</tr>
<tr>
<td>30 (39)</td>
<td>2.8 ± 0.3</td>
<td>19.0 ± 1.6</td>
<td>487 ± 127</td>
<td>633 ± 23</td>
</tr>
<tr>
<td>40 (50)</td>
<td>4.0 ± 0.1</td>
<td>45.4 ± 6.7</td>
<td>451 ± 95</td>
<td>507 ± 20.6</td>
</tr>
<tr>
<td>60 (70)</td>
<td>6.3 ± 0.2</td>
<td>108 ± 58</td>
<td>394 ± 40</td>
<td>401 ± 13</td>
</tr>
<tr>
<td>100 (100)</td>
<td>32 ± 1.0</td>
<td>726 ± 32</td>
<td>282 ± 45</td>
<td>238 ± 16</td>
</tr>
</tbody>
</table>

Test samples were pre-conditioned in 50% RH at 25°C for 24 hours (n = 5).

Flexural modulus of pure MBM (0%LLDPE) was measured as 529 ± 97 MPa.
Figure 3.4. Normalized tensile strength (TS) and strain to failure (STF) of MBMPCs as a function of MBM volume fraction compared to theoretical models of Nielsen.

Figure 3.5. Normalized tensile modulus of MBMPCs as a function of MBM volume fraction compared to the simple rule-of-mixing model predictions.
3.3.4 Water Vapor Permeability and Water Resistance of MBMPCs

The water vapor permeability for MBMPCs containing 10, 20, 30, and 40 wt% LLDPE content was measured as 1.34 ± 0.20, 0.95 ± 0.05, 0.77 ± 0.10, and 0.15 ± 0.01 ng/m².s.Pa respectively. The WVP for MBM plasticized with glycerol, but containing no LLDPE, was reported as 2.98 ± 0.02 ng/m².s.Pa. As expected, the WVP of the composites decreased with increasing LLDPE content, and was a whole order of magnitude smaller for the MBMPC containing 40 wt% LLDPE relative to that of glycerol-plasticized MBM. As the LLDPE content in the composite was increased, more MBM particles were encapsulated as observed in the SEM micrographs (Figure 3.3). Moreover, larger polymer content reduces voids, and thus lowers permeability of the composites. The MBMPCs still retain a hydrophilic nature as their WVP was still much larger than that of pure LLDPE (3x10⁻⁵ ng/m².s.Pa).

Figure 3.6 displays the water absorption of MBMPCs containing 10-40 wt% LLDPE content compared to that of pure LLDPE. For each composition, mass of water absorbed gradually increased with time until it plateaued after about 24 hours for composites containing less than 30 wt% LLDPE content. As the amount of LLDPE in the composite was increased, the amount of water absorbed decreased whereas the time to reach equilibrium water concentration increased due to reduced water absorption rate. For example, the maximum amount of water absorbed by composites containing 10 and 40 wt% LLDPE was about 39 ± 0.1 and 11 ± 1.4 wt% after soaking for 22 and 125 hours, respectively. Therefore, consistent with the relatively higher WVP, the MBMPCs retained their hydrophilic nature such that even composites containing as much as 40
wt% LLDPE content absorbed over 10 wt% water content compared to nearly zero absorption for the pure LLDPE matrix.

Figure 3.6. Water absorption of MBMPCs containing different weight fractions of LLDPE (PE) as a function of time. Lines are drawn for visual comparison purpose only.

Figure 3.7 displays the tensile properties of water-soaked MBMPCs containing 10, 15 and 30 wt% LLDPE as measured over duration of 3 days (72 hours). Both the TS and TM for all the composites decreased after one day of soaking, but remained about the same on day three. The TS of MBMPCs containing 10, 15 and 30 wt% LLDPE content decreased to 0.7 ± 0.1, 1.4 ± 0.1 and 2.8 ± 0.3 MPa, whereas the TM sharply decreased by over an order of magnitude to 9 ± 1, 34 ± 3 and 65 ± 10 MPa, respectively. In contrast, the STF of those composites increased several fold to 8 ± 1, 43 ± 6 and 83 ± 5% after being soaked in water for one day, and thereafter remained fairly constant. The observed trend of tensile properties changing after day one and thereafter equilibrating are consistent
with the observed pattern of water absorption of MBMPCs containing LLDPE contents of 10-30 wt% displayed in Figure 3.6.

The decrease in the TS and TM as well as increase in the STF of the composites is due to water absorption by hydrophilic MBM in the composites. In addition, some components of MBM not encapsulated by LLDPE diffuse out of the matrix, which causes additional void formation in the structure and leads to a decrease in composite TS. This is consistent with prior observations where MBM plastic sheets processed with glycerol showed a drastic decrease in TS and TM when they were exposed to high humidity conditions.\textsuperscript{1,28} However, it is important to note that although MBMPCs displayed a decrease in TS and TM, the overall sample integrity was maintained, especially in samples containing 15 wt% and greater LLDPE content. In contrast, pure MBM sheets disintegrate in less than an hour as was reported in previous studies.\textsuperscript{1,28} Therefore, the use of LLDPE as a binder leads to MBMPCs with good water permeability and environment stability that is important in potential semi-durable geo-structural applications such as silt-fencing.
Figure 3.7. Plots showing tensile properties of MBMPCs soaked in water as a function of time: (I) Tensile strength, (II) tensile modulus, and (III) strain to failure.
3.4. Conclusions

Meat and bone meal animal co-product was calendered into bio-composite sheets with LLDPE serving as a binder. Analysis of water-soaked specimens showed that a minimum of 15 wt% LLDPE content was required to form a nominally continuous matrix phase. Such composites possessed good processability and environmental stability. These sheets retained a tensile strength of 1 ± 0.1 MPa, a tensile modulus of 34 ± 3 MPa and a strain-to-failure of 40 ± 3 % after being soaked in water for three days. As evidenced from water vapor permeability and water absorption measurements, MBMPCS displayed enhanced water resistance when compared with pure MBM bioplastics. Because of the enhanced water stability of these composites, relative to pure MBM, they have potential use in semi-durable geo-structural applications where water permeation and limited stability are of importance.
3.5 References


CHAPTER 4

THERMOFORMABLE ANHYDRIDE-GLYCEROL MODIFIED MEAT AND BONE MEAL BIOPLASTICS

4.1 Introduction

In Chapter 2, successful thermoplastic processing of MBM using glycerol as the plasticizer was reported.\textsuperscript{1,2} However, similar to other protein-based materials, the plasticized bioplastics have high moisture sensitivity that rapidly deteriorates their mechanical properties. In Chapter 3, as a strategy to increase water resistance of MBM, bio-based composites of MBM with minor fractions of LLDPE were processed by calendering. The composites had improved water resistance with composites containing only 15 wt\% LLDPE content observed to have a third the water vapor permeability of plasticized MBM bioplastics. While a strategy that significantly reduces the synthetic material while adding value to MBM, it does not fully address the sustainability concerns related to fossil-based plastics because the LLDPE phase is still non-biodegradable. Therefore, this chapter discusses a different approach to improve water resistance of MBM bioplastics while retaining biodegradability.

Because of renewed interest in replacing fossil-based plastics with sustainable alternatives, there is significant need to enhance properties of protein-based bioplastics by the use of alternative plasticizers, heat and UV curing, crosslinking agents, surface active additives, and composite processing.\textsuperscript{3-9} Chemical modifications using crosslinking agents have long been studied for casein and soy based plastics in the 1900s utilizing
formaldehydes, which reduced water absorption by about 25%.\textsuperscript{10} Other chemicals including glutaraldehyde, furfural and metal ions like calcium have been investigated for numerous proteins.\textsuperscript{11-13} In contrast, anhydrides, because of their chemical reactivity, have been largely investigated as grafting agents to introduce functionality in blending natural polymers with synthetic polymers.\textsuperscript{14,15} For instance, polyethylene-grafted maleic anhydride was used in processing of soy-flour based plastics with improved environmental stability.\textsuperscript{14} In other studies, the anhydride monomers were simply added to the protein including the plasticizer and processed by reactive extrusion.\textsuperscript{16-18} It was hypothesized that, at optimal temperatures and extrusion residence time, the anhydride reacts with the reactive hydroxyl and amine protein groups to form a stable three dimensional network.\textsuperscript{19}

As noted above, anhydride monomers have been used in protein-based bioplastic processing to improve mechanical and barrier properties. Similarly, the chemistry of anhydrides and polyols is discussed in literature studies as the basis of formation of thermoplastic polyesters that utilize di-functional alcohols.\textsuperscript{20} However, there are no systematic studies reported in the literature utilizing the reaction of the anhydrides with glycerol to form resins that can interact physically and chemically with MBM and yet lend themselves to thermal processability. Therefore, glycerol was used for dual-purpose, i.e., both as a plasticizer and as a tri-functional alcohol capable of crosslinking with the anhydride, which also can interact covalently with protein residues.\textsuperscript{21,22} The primary objectives of the results discussed in this chapter were to (i) obtain resins derived from reaction of glycerol and anhydrides of maleic and phthalic, (ii) process MBM co-product
with the modified resins into a thermoformable material, and (iii) characterize the thermomechanical and water resistance properties of the modified-MBM bioplastics.

4.2 Experimental

4.2.1 Materials

Meat and bone meal (MBM) consisted of about 50% protein, 8-12% fat, 4-7% moisture, and 35% ash according to the producer (Darling International Inc.). For the current studies, the as-received MBM was milled and sieved through a 60 mesh (250 µm opening). Maleic anhydride (MA) was obtained from Alfa Aesar, phthalic anhydride (PtAH) was purchased from ACROS Organic, and glycerol was bought from SIGMA-Aldrich. Milled MBM composition as determined in the laboratory was approximately 53% crude protein, 11% fat, 6% moisture and 30% ash.

4.2.2 Processing

Two modified resins were synthesized by reacting MA and PtAH with glycerol in a glass reactor at 250°C in a mole ratio of 2:1 to obtain g-MA and g-PtAH resins, respectively, collectively referred to as g-anhydride resins. For g-MA, the reaction time was approximately 90 s and that of g-PtAH was ~ 480 s. Control of reaction time is important to ensure that the resin maintains flow properties instead of forming a cross-linked gel. Next, 60 wt% of MBM was blended with the molten resins in a Rheomix intensive batch mixer at 100°C, 60 rpm and five minutes to form MBM-gMA and MBM-gPtAH consolidated blends, collectively referred to as mod-MBM. The glycerol-MBM blend
(gMBM) was prepared by compounding MBM with 30 wt% glycerol content. The consolidated blends were then formed into mod-MBM and gMBM bioplastic sheets by compression molding using a Carver press (Model 389.4PR1B00) at 95°C and 66.8 KN. A holding time of two minutes was allowed for heat transfer to the mold and subsequent softening of the blend. The final load was applied for two additional minutes and the sample was subsequently cooled to ambient conditions under pressure before removing the sheets from the press.

4.2.3 Thermomechanical Analysis

Thermogravimetric analysis (TGA) of the g-anhydride resins and mod-MBM bioplastics was conducted using a PerkinElmer Pyris 1 instrument. The samples were heated in an aluminum pan under nitrogen atmosphere from 25°C to 500°C at a heating rate of 10°C/min.

Chemorheology of glycerol-anhydride reaction was conducted using the ARES rheometer using the parallel plate fixture. A dynamic time sweep was performed on a mixture of glycerol with anhydride at 250°C using a frequency of 0.1 rad/s and 10% strain. Also a dynamic temperature ramp from ambient room temperature to 140°C at 5°C/min, 1 rad/s and 10% strain was conducted on the synthesized resins to determine the appropriate blend temperature.

Extensional viscosity ($\eta_E(t)$) of mod-MBM bioplastics was also measured using the ARES rheometer equipped with the extension viscosity fixture (EVF) at 95°C and 125°C.
An extension rate (\(\dot{\varepsilon}\)) of 0.1s\(^{-1}\) was used. The samples were nominally 13 mm wide, 1.5 mm thick and 20 mm long. At a constant extension rate (\(\dot{\varepsilon}_o\)), the \(\eta_E(t)\) is given as:

\[
\eta_E(t) = \frac{F(t)}{\dot{\varepsilon}_o A(t)} = \frac{\sigma_E(t)}{\dot{\varepsilon}_o},
\]

where \(F(t)\) is the instantaneous extension force, \(A(t)\) is an instantaneous cross-section area of the sample under test and \(\sigma_E(t)\) is the transient extension stress. \(A(t) = A_0 e^{-\varepsilon_H}\), where \(A_0\) is the cross-section area of the un-stretched sample and \(\varepsilon_H = \dot{\varepsilon}_o t\) is the Hencky strain. These measurements can help quantify the feasibility of processing mod-MBM into 3-dimensional objects by a rapid, low cost manufacturing technique such as vacuum thermoforming. Unlike die extrusion, the plastic flow is extensional (rather than shearing).

Dynamic mechanical analysis (DMA) of MBM bioplastics was performed using TA instruments RSA3 solid analyzer. The dynamic temperature step was performed in the tensile mode from 25°C to 100°C at 5°C/min and 6.3 rad/s frequency. The samples were nominally 12.5 mm wide, 1.5 mm thick and 40 mm long. The strain used varied depending on the temperature range as determined from the dynamic strain sweep measurements. MBM-gMA samples were tested at 1x10\(^{-4}\) % strain whereas MBM-gPtAH specimens were tested at 1x10\(^{-3}\) % strain from 25°C to 50°C. From 50°C to 100°C the strains were increased to 0.01% for both bioplastics.

Tensile tests were conducted following the ASTM D638-10[Standard Test Method for Tensile Properties of Plastics] procedure except that rectangular strips were used rather than dog-bone specimens. The samples were nominally 1.5 mm thick, 13 mm wide and 114 mm long, and the gauge length was set at 57 mm. Mechanical testing of the sheets
was performed at a cross-head speed of 2.5 mm/min (Applied Test Systems Inc., Series 900). A minimum of four replicates for each composition were tested. Un-aged samples were conditioned in a vacuum oven at 50°C (~100 kPa vacuum) for 24 hours to obtain almost dry sheets. After drying, the samples for aging studies were placed in a polyethylene bag and stored at ambient conditions for about 5 months, and tested without any further conditioning.

4.3 Results and Discussion

Figure 4.1 displays the change of storage and loss moduli during the reaction of MA and PtAH with glycerol. For both reactions, the initial moduli overlap below 1 Pa before eventually increasing rapidly with the magnitude of the storage modulus (G') exceeding that of the loss modulus (G'') at the gel point. The crossover represents increase in molecular weight as the reactants crosslink into a three dimensional network. Based on the shorter gel time for MA-glycerol (120 s) that reaction was found to be about four times faster than for PtAH-glycerol (gel time of 510 s). Therefore, to obtain thermoplastic (lightly cross-linked) resins used in MBM bioplastic processing, the reactions were stopped 30 s short of the respective gel time.
Figure 4.1. Change of Storage (G') and loss (G'') moduli during reaction of glycerol with MA (g-MA) and PtAH (g-PtAH) under isothermal conditions (250°C).

Figure 4.2 displays the complex viscosity of g-MA and g-PtAH resins as a function of temperature compared to that of glycerol. At 30°C, glycerol had a viscosity of 0.6 Pa.s that steadily decreased with increasing temperature to 8x10^{-3} Pa.s at 120°C. Similarly, g-anhydride resins viscosity decreased with increasing temperature. At 30°C, g-PtAH resin had a viscosity of 1.7 x10^6 Pa.s that was three orders of magnitude higher than that of g-MA. Viscosity of g-PtAH resin remained significantly higher than that of g-MA as the temperature increased until about 120°C where the viscosity of both resins was the same at about 1 Pa.s, which approaches that of glycerol at room temperature. Compared to glycerol that is a liquid at room temperature, both g-anhydride resins are solid with significantly higher viscosities in the range of polymer melts. The g-anhydride resins
displayed molten thermoplastic properties with the viscosity decreasing as the temperature is increased consistent with lightly cross-linked polymers.\textsuperscript{21}

Figure 4.2. Complex viscosity of g-MA, g-PtAH resins and that of glycerol as a function of temperature.

4.3.1 Thermal Stability

Figure 4.3 displays the thermograms of pure glycerol, g-MA and g-PtAH resins. Glycerol showed a single mass loss step between 140°C and 230°C similar to other low molecular weight compounds due to thermal decomposition into volatiles.\textsuperscript{23} Resins of g-anhydride showed two mass loss peaks, an initial mass loss in a similar range as glycerol (140°C – 275°C) and the second between 300°C and 430°C. The final decomposition for g-PtAH (380°C) was about 50°C lower than that of g-MA resin. Moreover, g-MA forms a char after 430°C while g-PtAH resin is completely volatilized at 380°C. The first mass loss
step of g-anhydride resins is related to decomposition of unreacted monomers of glycerol and anhydrides, whereas the second weight loss step is attributed to the decomposition of the higher molecular weight ester chains of the resins. The better thermal stability displayed by g-MA relative to g-PtAH is attributed to significant cross-linking that results in a char, which is normally observed in cross-linked thermosets due to formation of large molecules that are not easily volatilized. Therefore, the mixing of g-anhydride (g-MA and gPtAH) resins with MBM must be done at temperatures above 100°C (because of low viscosity as displayed in Figure 4.2) but less than 140°C, which is the threshold of the resins decomposition, as displayed in Figure 4.3.

![Thermal Gravimetric Analysis](image)

Figure 4.3. Thermal gravimetric analysis of g-MA and g-PtAH resins compared to glycerol conducted in nitrogen atmosphere at 10°C/min.

Figure 4.4 displays the thermograms of milled MBM together with those of mod-MBMs, i.e., bioplastics containing g-MA and g-PtAH resins. MBM displayed two mass loss
peaks, one below 100°C followed by a continuous loss step from about 140°C to 500°C with major degradation at 310°C. This thermal behavior has been reported in previous MBM studies.\textsuperscript{13,24,25} Mod-MBM bioplastics showed two distinct mass loss steps. The first one between 140°C and 270°C, similar to one observed in the g-anhydride resins (Figure 4.3), is attributed to decomposition of unreacted glycerol and anhydride monomers. The second broad step starts at 270°C, with the peak derivative TGA temperature at 319°C and 295°C for MBM-gMA and MBM-gPtAH bioplastic, respectively. This peak is attributed to degradation of organic polymeric matter such as proteins, carbohydrates and the esters of the g-anhydride resins. As observed with g-MA resins, MBM-gMA bioplastics displayed better thermal stability than MBM-gPtAH bioplastics.

Figure 4.4. Thermal gravimetric analysis of MBM-gMA and MBM-gPtAH bioplastics compared to milled MBM at 10°C/min in a nitrogen environment.
Figure 4.5 displays a plot of storage modulus and tan delta (tan $\delta$) as a function of temperature for mod-MBM bioplastics. The storage modulus decreased with increasing temperature and there was no significant difference in the storage modulus of MBM-gMA and that of MBM-gPtAH bioplastics over the temperature range of 25 to 100ºC. However, for MBM-gPtAH bioplastics, the data reveals a clear secondary transition (Tg) at about 65°C indicated by the maximum in the tan $\delta$ peak. MBM-gMA bioplastics did not show a clear maximum in the tan $\delta$, but rather a broad plateau starting at about 51°C. This is similar to the literature observations on DMA of cross-linked polymers, e.g., UV-cured acrylate polymer. The maximum and broadening in the tan $\delta$ peak represents the softening point of MBM-gPtAH and MBM-gMA bioplastics, respectively, due to cooperative motion of several molecular segments. The higher glass transition of MBM-gPtAH bioplastics is attributed to the increase in polymer chain stiffness due to presence of aromatic groups. Literature studies indicate that as aromatic groups in the main chain increase, the stiffness of the polymer increases and so does the Tg. Because mod-MBM bioplastics display a clear glass transition temperature, they can be thermally molded into desired shapes when heated above the Tg.
Figure 4.5. Dynamic tensile storage and tan δ of MBM-gMA and MBM-gPtAH bioplastics as a function of temperature.

Figure 4.6 displays the transient extensional viscosity of MBM-gMA and MBM-gPtAH bioplastics at two different temperatures (95°C and 125°C) tested at 0.1 s⁻¹ extensional rate (̇). Generally, for both temperatures and compositions, the transient extensional viscosity increased with time and approached nominal steady state (before sample failure). At both temperatures, MBM-gMA displayed a higher maximum extensional viscosity than MBM-gPtAH bioplastics. At 95°C, the maximum extensional viscosity for MBM-gMA (4.32x10⁵ Pa.s) was 1.5 times higher but its maximum Hencky strain before failure (0.35) was a half that of MBM-gPtAH. Similarly, at 125°C, MBM-gMA possessed a maximum extensional viscosity of 1.09x10⁵ Pa.s that was about twice that of
MBM-gPtAH; however, the maximum Hencky strain was only 0.27. The higher extensional viscosity and the generally low max Hencky strains of MBM-gMA bioplastics is attributed to greater cross-linking as indicted from the higher thermal stability discussed in Figure 4.4. Based on the extensional rheology studies, the lower temperature of 95°C was determined as a suitable vacuum thermoforming temperature for MBM-gPtAH bioplastics because of sufficient strength/extension before rupturing of the softened sheet whereas the higher temperature of 125°C was favorable for MBM-gMA bioplastics.

Figure 4.6. Transient extensional viscosity data at 95°C and 125°C for mod-MBM bioplastics at an extensional rate of 0.1 s⁻¹. The inset is an equivalent plot of transient extension viscosity as a function of Hencky strain (\(\varepsilon_H\)) (\(\varepsilon_H = \dot{\varepsilon}t\))
4.3.2 Tensile Properties

Table 4.1 displays the tensile strength (TS), tensile modulus (TM), and strain-to-failure (STF) for mod-MBM bioplastics of un-aged samples and that of samples aged for 5 months. For comparison, tensile data for bioplastics plasticized with glycerol (gMBM) is also shown. For the un-aged mod-MBM bioplastics, TS and TM were significantly greater than those of gMBM sheet (by a factor of ~ 4 and 10) although the STF was ~7 times lower. Similar results have been reported in literature studies on soy proteins modified with maleic anhydride although their reported improvements were smaller. Compared to each other, MBM-gPtAH bioplastics displayed a 24% higher TS (3.7±0.2 MPa) and a 37% higher TM (582±74 MPa) than that of MBM-gMA bioplastics, but about a 20% (1.2 ± 0.2%) lower STF. The higher TS and TM of mod-MBM bioplastics is attributed to the covalently polymerized resins and their chemical interactions with MBM protein residues especially those of basic amine groups such lysine and histidine. In contrast, pure glycerol only acts as a plasticizer with only weak physicochemical interactions, thus the observed low TS and TM but higher STF of gMBM bioplastics.

Compared to the un-aged samples, the TS and TM of aged mod-MBM bioplastics nominally decreased by 60%, whereas those of gMBM bioplastics decreased by ~ 80%. In contrast, the STF of MBM-gMA and MBM-gPtAH bioplastics increased 18 and 8 fold whereas that of gMBM decreased by a factor of about three, which was now significantly lower than that of mod-MBM bioplastics. Thus, the decline in TS and TM due to ageing is highest in gMBM bioplastics. Rapid decline in the mechanical properties of aged
protein films plasticized with glycerol was also observed in soy films and was attributed to the leaching out of the plasticizer and the increased moisture absorption of the films.\textsuperscript{7,28} These factors are slowed down in the modified bioplastics because of the polymerization of glycerol with the anhydrides coupled with chemical interaction with protein residues.

Table 4.1. Tensile strength (TS), strain-to-failure (STF) and tensile modulus (TM) of un-aged and five months aged mod-MBM bioplastics. For comparison, data for gMBM sheets plasticized with 30 wt% glycerol is also displayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Un-aged</th>
<th>Aged for five months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TS (MPa)</td>
<td>STF (%)</td>
</tr>
<tr>
<td>MBM-gMA bioplastic</td>
<td>3.0 ± 0.1</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>MBM-gPtAH bioplastic</td>
<td>3.7 ± 0.2</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>gMBM bioplastic</td>
<td>0.8 ± 0.1</td>
<td>8.9 ± 1.2</td>
</tr>
</tbody>
</table>
Figure 4.7 displays the water absorption of gMBM, MBM-gMA and MBM-gPtAH bioplastics as a function of time. Three specimens of each composition (50 mm x 38 mm x 1.5 mm) were initially dried in a vacuum oven at 50°C to a constant weight. The specimens were then placed in distilled water at room temperature in separate containers. At specific time intervals, the samples were drawn, gently blotted with a paper towel to remove surface water, and then weighed. Results indicate that most water absorption occurred in the first hour of soaking, followed by a gradual increase. For the first hour of soaking, MBM-gPtAH absorbed 8% water whereas MBM-gMA and MBM-g bioplastics absorbed three and four times more water, respectively. The gMBM and MBM-gMA sheets started disintegrating before 1-h and 4-h measurements, respectively, while MBM-gPtAH remained intact for the entire test period of 24 hours.

These results, demonstrating improved water resistance, are consistent with those of other protein bioplastics formed from anhydride modified formulations reported in literature.\textsuperscript{14,29} Because both MBM and glycerol are hydrophilic and the interactions are weak, gMBM bioplastics degrade in a matter of minutes as components dissolve in the water. Better water resistance is observed in mod-MBM bioplastics because of the polymerized resins in addition to chemical cross-links between components. The significantly higher water resistance of MBM-gPtAH bioplastics is attributed to the bulky aromatic groups of the polymeric ester resin that limits penetration and interaction of water in the sheet.\textsuperscript{20} Because of their thermofomability, good mechanical and water resistance properties, anhydride modified MBM bioplastics can be used in semi-durable geo-structural applications such as seed planters.
Figure 4.7. Water absorption of mod-MBM bioplastics over time compared to that of gMBM bioplastics. Lines drawn for visual purpose only.

4.3.3 Potential Applications

As an illustration of a potential application of mod-MBM bioplastics using low-cost, industrially-relevant, rapid techniques, Figure 4.8 (a) displays a 3-dimensional thermoformed cup-shaped prototype from mod-MBM bioplastic. The prototype was vacuum-thermoformed using a Centroform EZFORN SV 1217 tabletop vacuum-forming machine from a 2 mm thick and 150 mm diameter sheet. The feed sheet was initially heated in a convection oven at 105°C and rapidly transferred on to a mold in the vacuum forming unit. The illustrated article was ~ 25 mm deep.

Because of the good water resistance and mechanical properties of mod-MBM as discussed in the results section of Chapter 3, it was successfully tested as a seed growth
planter as shown in Figure 4.8 (b). Over the 15 days needed for seed germination, the prototype bioplastic cup retained dimensional stability even when it was watered daily.

Figure 4.8. (a) Vacuum thermoformed cup (prototype) from MBM-gPtAH bioplastic sheet. (b) Germinated grass seedling planted in the prototype bioplastic (after 15 days)
4.4 Conclusions

Controlled reaction of glycerol with anhydrides (maleic and phthalic) produces resins that have thermoplastic properties and better thermal stability than that exhibited by glycerol as revealed from rheology and thermal analysis. These resins retained thermoplastic properties and were used in the processing of MBM to produce mod-MBM bioplastics. Mod-MBM bioplastics possessed moderate stiffness and a glass transition temperature above 50ºC as well better tensile strength (4 times) than gMBM bioplastics. In addition, they had better water resistance especially bioplastics modified with PtAH that retained structural integrity after more than 24 hours of water soaking whereas gMBM plastics disintegrated in less than an hour. Importantly, when the bioplastics were heated above the glass transition, they displayed sufficient ductility to be molded into 3-dimensional articles using industrially relevant techniques (e.g., vacuum thermoforming). Thus, MBM-gPtAH bioplastic had elongation viscosity of $2.9 \times 10^5$ Pa.s and were successfully vacuum thermoformed into a cup-shaped object about 25 mm deep using a vacuum thermoforming unit. These results demonstrate the potential for new application of inexpensive bio-products, in addition to addressing sustainability concerns related to overreliance on fossil resources.
4.5. References


22. Schwenke, D., Klaus In Enzyme and Chemical Modification of Proteins ; Damodaran, S., Paraf, A., Eds.; Food Proteins and Their Applications; Marcel Dekker, Inc.: New York, USA, 1997; pp 393-423.


CHAPTER 5
CONCLUSIONS AND FUTURE WORK

5.1 Conclusions

The overall goal of this research was to utilize meat and bone meal (MBM) proteinaceous animal co-product in the processing of bioplastics using scalable industrial processing routes for potential geo-structural applications. The goal was successfully accomplished and the specific findings are summarized below.

In Chapter 2, it was established that MBM, similar to other protein materials, was thermally processable into bioplastics using glycerol as a plasticizer at 30 wt% glycerol content. Apart from plasticizer content, MBM processability and properties were optimized with regards to compounding conditions (temperature, pressure, time and relative humidity) and thermal compaction temperature and pressure. Other factors included particle size and moisture content of raw MBM. To further enhance the mechanical properties of MBM bioplastics, calcium hydroxide (CH) at 3-10 wt%, was investigated as a modifier. This modification resulted in a nominally four-fold increase in the tensile strength and modulus at 7-10 wt% CH content compared to that of the unmodified bioplastic Analysis of the bioplastics, using Fourier transform infrared (FTIR) spectroscopy, indicated that the increase in tensile properties was due to ionic cross-links between calcium ions and protein residues with negatively charged oxygen. However, the moisture resistance of CH-modified MBM was not significantly improved (relative to that of hydrophilic MBM)
In Chapter 3, as a strategy to improve water resistance of MBM, while still utilizing thermal plastic processing routes, consolidation and calendering of MBM with a minor fraction of linear low density polyethylene (LLDPE) was investigated. Analysis of the composite sheets after being soaked in water showed that a minimum of 15 wt% LLDPE content was required to form a nominally continuous matrix phase for sheets with good environmental stability. Unlike calcium hydroxide modified and pure MBM bioplastics, the composite sheets (15 wt% LLDPE) retained structural integrity with a tensile strength of ~1 MPa, tensile modulus of ~34 MPa and a strain-to-failure of about 40% even after being soaked in water for three days.

Finally in Chapter 4, unlike the addition of LLDPE that is non-biodegradable, resins from controlled reaction of maleic (MA) and phthalic (PtAH) with glycerol were used to process biodegradable modified MBM (mod-MBM) bioplastics. The mod-MBM bioplastics possessed significantly better water resistance, especially those modified with PtAH that retained structural integrity after being soaked in water for over 24 hours. In contrast the unmodified MBM bioplastic disintegrated in less than an hour. Moreover, the un-soaked sheets possessed moderate stiffness (~350 MPa) and a glass transition above 50 ºC as well as a tensile strength of four times that of unmodified MBM. The increased water stability of mod-MBM bioplastics is attributed to the polymerization of the anhydrides with glycerol and covalent interactions with proteins resulting in semi-cross-linked bioplastics. However, because of the controlled reaction, the bioplastics retained thermoplastic behavior and were subsequently vacuum thermoformed into 3-dimensional
articles. In summary, the modified MBM material offers a sustainable alternative to fossil-derived plastics while adding value to underutilized MBM animal co-product.

5.2 Future Work

Despite the improvements of mechanical and water barrier properties of MBM bioplastics achieved with modifications in the current studies, the properties are not yet optimal for the proposed geo-structural applications. Therefore, as discussed in Chapter 1, assessment of other modifications, such as UV irradiation, heat curing, and blends with lipids, wax, and polysaccharides may be researched further. Also studies with defatted MBM are recommended because preliminary studies indicated the potential for improvement of properties. For instance; carbon dioxide defatted MBM (coarse grade) modified with 7 wt% CH displayed a two and four factor increase in tensile strength and tensile modulus, respectively, but a five-fold decrease in the strain-to-failure. Similarly, fine defatted MBM modified with anhydrides displayed a two-fold increase in TS and TM with no significant effect on the STF. A systematic study needs to be performed to assess other physical and aging characteristics of such defatted MBM grades.

In Chapter 4, preliminary studies on glycerol-anhydride modified MBM bioplastics tested as seed-growth planters showed that they retained dimensional stability over the seed germination period. A study of how the chemical structure of the bioplastics relates to the physical properties is recommended. Also a systematic study on how well plants grown in these planters perform, as compared to other planters, will help in assessing potential applications of the bioplastics.
As part of consumer acceptance for such bioplastics, the aesthetics of the bioplastics, such as color and odor, have to be taken into account. One pitfall of these animal based bioplastics is their susceptibility to microbial attack causing foul odors. Therefore, future studies into the addition of antimicrobials and antioxidants are recommended.

Because sustainability is the major motivation for development and use of bioplastics from animal co-products, a cradle-to-grave life cycle assessment (LCA) of the MBM bioplastics is important. An objective analysis of the amount of energy and material use through the life time of MBM bioplastics would facilitate their commercial adoption.
A.1 Differential Scanning Calorimetric (DSC) Analysis of MBMPCs

In chapter two, DSC analysis of MBMPCs was discussed. The melting temperatures ($T_m$), heat of melting ($\Delta H_m$) and crystallinity from the second heating and cooling are summarized in Table A.1.1

Table A.1.1 Summary of Thermal properties of MBMPCs obtained from DSC analysis for the second heating scan at 10°C/min

<table>
<thead>
<tr>
<th>LLDPE (wt%)</th>
<th>$T_{c1}$ (°C)</th>
<th>$T_{c2}$ (°C)</th>
<th>$T_{m1}$ (°C)</th>
<th>$T_{m2}$ (°C)</th>
<th>$\Delta H_m$ (J/g)</th>
<th>$X$ (%)</th>
<th>$\Delta H_m$ (J/g LLDPE)</th>
<th>$X$ (%)/g LLDPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>111.4</td>
<td>98.3</td>
<td>107.5</td>
<td>120.7</td>
<td>8.0 ± 0.5</td>
<td>2.8 ± 0.2</td>
<td>80.1 ± 5.4</td>
<td>27.8 ± 1.9</td>
</tr>
<tr>
<td>20</td>
<td>111.7</td>
<td>99.7</td>
<td>108.0</td>
<td>121.0</td>
<td>19.8 ± 2.8</td>
<td>6.7 ± 0.9</td>
<td>98.8 ± 13.8</td>
<td>34.3 ± 4.8</td>
</tr>
<tr>
<td>30</td>
<td>111.7</td>
<td>100.3</td>
<td>108.5</td>
<td>121.2</td>
<td>28.9 ± 4.6</td>
<td>9.9 ± 1.6</td>
<td>96.5 ± 15.3</td>
<td>33.5 ± 5.3</td>
</tr>
<tr>
<td>40</td>
<td>111.6</td>
<td>100.2</td>
<td>109.0</td>
<td>121.3</td>
<td>41.4 ± 5.5</td>
<td>14.1 ± 1.9</td>
<td>103.6 ± 13.8</td>
<td>36.0 ± 4.8</td>
</tr>
<tr>
<td>60</td>
<td>111.4</td>
<td>100.1</td>
<td>109.8</td>
<td>122.0</td>
<td>58.8 ± 5.2</td>
<td>20.1 ± 1.8</td>
<td>98.1 ± 8.7</td>
<td>34.0 ± 3.0</td>
</tr>
<tr>
<td>100</td>
<td>105.6</td>
<td>–</td>
<td>108.2</td>
<td>118.9</td>
<td>94.3 ± 12.7</td>
<td>32.7 ± 4.3</td>
<td>94.3 ± 12.7</td>
<td>32.7 ± 4.4</td>
</tr>
</tbody>
</table>

First and second cooling thermograms of MBMPCs containing 10 wt% and 30 wt% LLDPE compared to LLDPE are displayed in Fig. A.1.1. LLDPE showed one crystallization peak at 105.6°C with a long tail, whereas the composites showed two
peaks, a sharp peak at 111.4-111.7°C and a broad peak at 98.3-100.3 °C. Apart from prevalence of a second low crystallization broad peak, it was observed that presence of MBM resulted in higher temperature of crystallization of LLDPE (from 105°C to 111°C). This has been observed by other researchers, when solid components (fibers or particulates) are introduced in polyethylene.\(^1\) As reported in Table A.1.1, although the enthalpy of melting and % crystallinity of MBMPCs increased with increasing LLDPE content, since MBM does not crystallize, the normalized enthalpy of melting was fairly independent of MBM content. Thus, the overall percent crystallinity of LLDPE phase was measured nominally at 30%. Thus, similar to what previous researchers have observed in semi-crystalline polymers filled with inorganic fillers of limited compatibility, bio-particulates (MBM) also do not influence the overall crystallinity of LLDPE.\(^1,2\)

![Figure A.1.1. First and second cooling scans of MBMPCs containing 10 wt% and 30 wt% LLDPE (PE) compared to pure LLDPE.](image)

Figure A.1.1. First and second cooling scans of MBMPCs containing 10 wt% and 30 wt% LLDPE (PE) compared to pure LLDPE.
A.2 Microstructure of MBMPCs

In Chapter 3 section 3.3.2, it was concluded from the SEM analysis that MBM exists as irregular agglomerates in the composites. However, because MBM is a soft material with the rigid components being the bone fragments, further analysis of the observed solids was conducted using energy dispersive x-ray spectroscopy (EDS). EDS is an analytical technique used for elemental analysis or chemical characterization of a sample. Elemental mapping, which shows the concentration of a specific element as a function of position on the specimen was conducted on cross-sections of composites containing 10 and 15 wt% LLDPE. The Hitachi SU6600 variable pressure SEM was used.

Figure A 2.1 (a) displays the SEM/EDS mapping of MBMPCs containing 10 wt% LLDPE (i) Calcium EDS mapping layered over the SEM image, (ii) Carbon EDS mapping and (iii) Phosphorous EDS mapping. In Figure A.2.1 (a)-(i), a large solid (highlighted by the circle) about 200 µm in diameter is observed similar that in Chapter 3 and some regions containing calcium glowing in yellow. The carbon mapping is all red while the phosphorous map glows blue in only some parts of the cross-section. The phosphorous map is observed to overlap that of calcium EDS layered over the SEM. The SEM/EDS analysis indicates that the entire composite section contains carbon that is why it all glows red, however calcium and phosphorous are found in only specific regions. This is because the only component of the composite with significant amounts of calcium and phosphorous are the bone fragments of MBM. Bones are made of crystals of hydroxyapatite \([\text{Ca}_5(\text{PO}_4)_3(\text{OH})]\) embedded in a collagen matrix.\(^3\) Therefore, since the
large highlighted solid in the cross-section is not all colored yellow or blue it implies that it is not a bone fragment but rather MBM agglomerate composed of mainly the proteins and fats. Thus, the observed solids in the composites are of MBM agglomerates and bone fragments. Similar observations and inferences were drawn from SEM/EDS analysis of MBMPCs containing 15 wt% LLDPE displayed in figure A.2.1 (b).

Figure A 2.1 (a) SEM/EDS mapping of MBMPCs containing 10 wt% LLDPE (i) Calcium EDS mapping layered over the SEM image, (ii) Carbon EDS mapping and (iii) Phosphorous EDS mapping.

Figure A 2.1 (a) SEM/EDS mapping of MBMPCs containing 10 wt% LLDPE (i) Calcium EDS mapping layered over the SEM image, (ii) Carbon EDS mapping and (iii) Phosphorous EDS mapping.
Figure A 2.1 (b) SEM/EDS mapping of MBMPCs containing 15 wt% LLDPE showing calcium EDS mapping layered over the SEM image, carbon EDS mapping and the phosphorous EDS mapping.
A.3 Extensional Viscosity Analysis with EVF

Extensional Viscosity is a measure of transient stress growth of a molten material as it is deformed. In Chapter 4, the extensional viscosity of MBM bioplastic measured using the Extensional viscosity fixture (EVF) attached to the ARES Rheometer (Figure A.3.1) was reported. The following fundamental equations relate the measured variables to the calculated values. $^4,5$ A detailed sample preparation and testing is also detailed below.

Hencky rate $\dot{\varepsilon} = \frac{2\Omega(t)R_A}{L_0}$

Hencky Strain $\varepsilon_H = \dot{\varepsilon}t$

Extension Force $F_E = \frac{M(t)}{R_T}$

Extensional Stress $\sigma_E = \frac{F_E(t)}{A(t)}$

For a constant Hencky strain rate experiment

Sample Area $A_t = A_0 \left(\frac{\rho_{solid}}{\rho_{melt}}\right)^{\frac{2}{3}} e^{-\varepsilon_H}$

Extensional viscosity $\eta_E(t) = \frac{F(t)}{\dot{\varepsilon}_o A(t)} = \frac{\sigma_E(t)}{\dot{\varepsilon}_o}$

$R_A =$ Radius of drum attached to the actuator = 10.3 mm

$R_T =$ Radius of drum attached to the transducer = 10.3 mm

$M(t) =$ Measured torque, $\Omega(t) =$ Measured angular velocity

$A(t) =$ instantataneous cross-section area, $F(t) =$ instantataneous etension Force

$\varepsilon_H =$ Hencky strain, $L_0 =$Length of sample = length between clips
Note that these equations provide nominal values; the orchestrator software has additional parameters to correct for machine inertia, thickness variability during stretching etc.

The optimum sample size is recommended as follows:
Length = 18 mm, Width = 10 mm, Thickness = 0.7 mm

**Instrument and Test Preparation**

After turning on the ARES rheometer and installing the upper and lower fixtures correctly, follow these steps to prepare the instrument for extensional viscosity measurements:

1. Close the ARES oven completely and latch it.

2. Select **Utilities/Service/Instrument Configuration** to display the **Setup Instrument Options** dialog.

3. Select “Mode 3. RAA Oven Air Temp” as the **Temperature Loop Control**.

4. Access the **Instrument Control Panel** dialog. Select **On** for the **Environmental Controller** option and enter the desired **Temperature** to be used for your experiment.

While waiting, set up test parameters:

5. Click the green arrow **Start** button. (a) **Edit/Start Instrument Test** dialog is displayed. Enter the desired **Title, Folder, Operator, and Test Notes**.
(b). Find/create your directory for where the file is to be saved. Check **AutoSave Experiment at end of test**, if desired.
(C). under sample geometry, select **Predefined Geometries** and choose “ARES Extensional Fixture” from the **Geometry** drop-down list. Measure the sample dimensions (width and thickness). Click the **Edit Geometry** button. Enter the dimensions in the appropriate field. Click **OK**.

(d). Click the radio button, **Predefined Test Setups** and select the “Extensional Viscosity Test.”

**NOTE:** The EVF tool can only be used with the Extensional Viscosity Test.

(e). Click the **Edit Test** button to display the **Extensional Viscosity Test** dialog; enter test parameters (**Temperature**, **Extensional Rate**, **Extension Zone Time** in the Zone 1 field. This value is normally 3.5 to 4.0/Extensional Rate, **Solid Density** at room temperature and the **Melt Density** at testing temperature etc.)

(f). Click **OK** to exit the **Extensional Viscosity Test** dialog and return to the **Edit/Start Instrument Test** dialog.

6. Once temperature has stabilized, select **Control/Gap Control Panel**. Dialog is displayed.

(a). Click **offset Torque to Zero; offset Normal Force to Zero, Zero Fixture** buttons.

(b). Enter 0.5000 as the commanded gap.

7. Open oven and confirm the position of the two samples clips then pull the clips out a little to accommodate sample loading after the instrument has been fully prepared ( be fast).

8. Turn on the motor.

**Sample Loading and Testing**

9. When oven is at desired testing temperature, open the oven carefully.
10. Thread the rectangular sample from the right side to the left side through the two opened sample clips.

11. Close the left clip just enough to touch the sample. Do not press it in too tightly and compress the sample end.

12. Close the right clip using the same technique. Do not compress the sample.

13. Close the oven

14. Click begin test (green arrow start button)

Save the data when the test has been completed, if you did not select AutoSave.

15. Turn off the motor.

16. Open the oven and remove the remains of the test sample. (Note: It is easier to peel off the sample when cooled to ambient temperature)
A.4 References


