PROTEIN-BASED PLASTICS AND THEIR POTENTIAL USE IN MEDICAL AND FOOD PACKAGING APPLICATIONS

by

ALEXANDER JONES

(Under the Direction of Suraj Sharma)

ABSTRACT

The use of conventional plastics in medical and food packaging applications is ubiquitous, as the ability of a lightweight material that is able to withstand the stresses of application is paramount. However, the use of these materials comes with many drawbacks, such as the lack of biodegradability of the polymers utilized, as well as the lack of antibacterial properties, causing the potential spread of disease, contamination of food, and the existence of plastics in landfills. In order to address these issues, we have examined proteins that could serve as a substitute to the traditional polymers that are utilized on the market today in medical and food packaging applications. The types of proteins we examined for potential use in such applications were albumin from hen egg white, whey protein isolate, edible soy protein, and zein protein from corn. Through our screening studies, we have identified albumin from hen egg white and zein from corn as two different types of protein that possess the mechanical, thermal, and antimicrobial properties that would be highly useful in these applications. After screening, we then conducted additional tests to determine if the addition of low density polyethylene (LDPE), a traditional polymer, will alter the biodegradation and drug elution properties of the resulting protein-based thermoplastic. After testing for susceptibility to biodegradation, we

determined that plastics that contained higher levels of LDPE were less susceptible to degradation, while the addition of LDPE had no significant effect on the ability of the plastic to release a drug or food preservative. When albumin and zein are compared to LDPE for environmental impact based on a life cycle assessment, we find that the production of the biomass to be converted into plastic possesses a larger environmental impact than LDPE production. Based on the research conducted in these studies, it will be possible to further examine other types of proteins that can be utilized in plastic production, the use of more additives to enhance the properties of the resulting plastic, as well as determining ways to limit the environmental impact of plastic production, use, and disposal.

INDEX WORDS: Bioplastics, Sustainability, Antibacterial, Drug Elution, Thermoplastics, Proteins, Polymers

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DEDICATION

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Literature Review

Common materials used in medical and food packaging plastics

With the constant development of plastics and polymer blends, more medical devices and types of food packaging are being made from different types of polymers. This is due to the advantages posed by plastic materials in medical applications, such as decreased cost and weight of material, as well as the enhanced properties of a given material that lend itself to certain applications, such as packaging film, containers, syringes, tubing, and sutures (McKeen 2014). The type of plastic that can be used in a given application is highly dependent upon the properties that must be possessed by the plastic itself. For instance, in applications such as medical or food product packaging or containers for storage, plastics made from medium to low density polyethylene (MDPE and LDPE) (Fengmei 2000) and polypropylene (Woo 2002) are common in use, with these two polymers the most widely used in food packaging (Marsh 2007). In applications where more stress resistance and flexibility will be required of the plastic, polymers such as ultra-high molecular weight polyethylene (UHMWPE) and polyvinyl chloride (PVC) can be used for applications such as joint replacement materials (Costa 2006) and leak-prevention connectors (Hakkarainen 2003). A more recent advance has been the development of plastics that would be able to elute drugs, as well as bioabsorbable within the body after a given period of time. Polymers such as the biodegradable polylactic acid (PLA) (Ikada 2000) and thermoresponsive hydrogel copolymers (Jones 2008) that have been loaded with drugs can serve

as materials that will have a short residence in the body with minimal invasive procedures to remove the plastic required. The method of incorporating antimicrobial materials in plastics can also utilized for food packaging applications, as the use of compounds such as pediocin (Ming 1997) and nisin (Mauriello 2005) can be utilized in food packaging plastics to prevent bacterial growth. With the use of nanomaterials such as clays and nano-sized sensors, it is possible to both produce a material that has a robust barrier to interactions with the environment, as well as provide information on if a food has been potentially contaminated (Duncan 2011). With the versatility and range of choices of polymer materials to utilize, the use of plastics in medical applications and food packaging has become crucial.

Limitations of materials used today in medical and food packaging applications

There are multiple disadvantages that are caused by the use of traditional plastics in medical and food packaging applications. In medical applications, the usage of plastics has the unintended consequences of causing hospital acquired infections (HAIs) in medical settings. This can be due to the contamination of the plastic, which can occur in multiple ways, such as coming in contact with microorganisms that are growing on the skin of the patient or within the mucous membranes (von Eiff 2005). Once contamination occurs, the process of biofilm formation can occur through the colonization and dispersion of bacteria, as illustrated in **Figure 1.1**. This biofilm formation growth can be aided with the flow of contaminated material in devices such as a catheter tube, causing the gradual formation of macro colonies in the tubing itself. This potential for infection of an individual who has already been admitted for a medical procedure will put the patient at an undue risk of further complication, as well as cause more time to be spent in the hospital. The economic cost of HAIs has been estimated to be 88 billion dollars in 2009 in the United States (Lobell 2012), causing 6% of all fatalities in the US every year (Scott

II 2009). Another major disadvantage possessed by plastics in medical applications is the potential of leachables and extractables gradually leaving the plastic and going into the patient. The material that leaches from the plastic consists of additives that are used in plastic production such as plasticizers and lubricants (Rahman 2004), and can lead to irritation and potential toxicity for the patient (McKeen 2014).

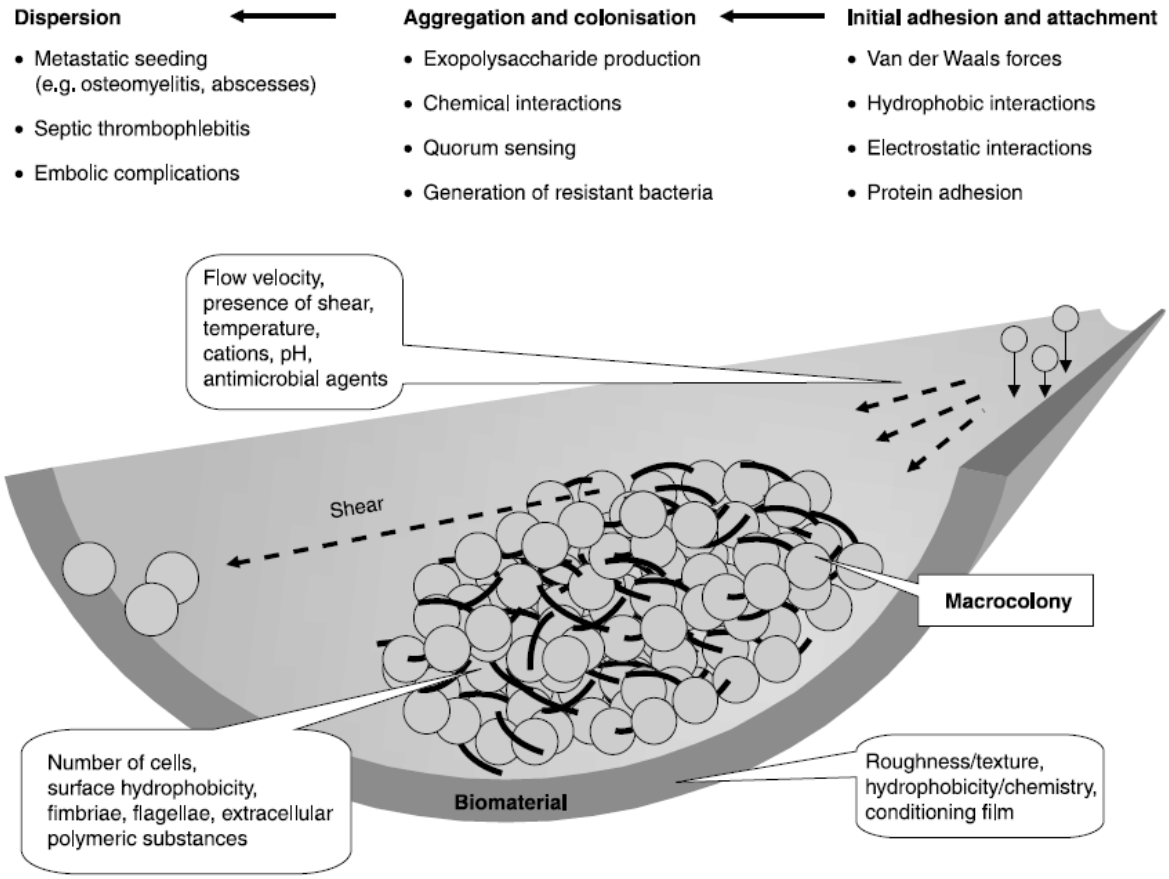


Figure 1.1: Process of biofilm formation on medical plastics, and spread of biofilm growth in catheter tube (von Eiff 2005). Reprinted with permission.

For food packaging applications, a major issue arises when certain conditions are present in the sealed container, such as a high oxygen or moisture content. These conditions will allow any bacteria that are sealed within the plastic container to thrive off of the food contained within the package, causing spoilage (Suppakul 2003). Another source of potential contamination of foods being packaged will come from the packaging material itself, as additives that have been

utilized in the production of the food packaging plastic will gradually leach from the plastic into the food product over time. This contamination begins with the gradual diffusion of the various additives within the polymer, as the rate at which an additive will diffuse out of the plastic is dependent upon Fick's laws of diffusion (Lau 2000). After diffusing within the plastic, the contaminant will then interact with the food product, with more soluble or porous foods being able to take up more of the contaminants (Lau 1996). After the contaminant has interacted with the surface of the food, it will then disperse into the bulk of the food, with the process accelerated when either heat or mixing is applied to the bulk food material (Limm 1995). This spoilage of food will lead to a substantial loss of edible material, as after contamination it will no longer be possible to consume the product. This waste of food has a substantial impact in terms of economics in the United States, as it has been estimated that 55 million metric tons of food will be wasted, which is equivalent to 29% of all food produced in the United States on a yearly basis (Venkat 2011).

One other major drawback for the use of traditional plastics in food packaging is the lack of biodegradability of the polymers that are currently used. While the thermoplastics that are used in food packaging are recyclable, the amount of plastic food packaging material that eventually gets recycled by the consumer is minimal. For instance, of all of the high density polyethylene (HDPE) used in food packaging, only 9.7% ends up getting recycled, while for LDPE the amount is negligible (EPA 2010). This lack of recycling results to large amounts of food packaging plastics being disposed of as landfilling or incineration that have an adverse impact on the environment. In the United States, 13.7 million tons of plastic packaging ends up in municipal solid waste systems per year (Marsh 2007).

Advances in plastics for medical and food packaging applications

To improve the properties of medical and food packaging plastics, multiple areas of research have been examined. For both medical and food packaging applications, one common method of preventing plastics from causing infection or contamination is through the placement of drugs within the plastic itself. In medical devices, this can be done with multiple types of medical devices, such as musculoskeletal and orthopedic-related devices. Infection prevention is possible through loading a drug into materials such as cements (Hanssen 2004) and films (Aviv 2007), which gradually elute from the polymeric material into the human body. When a compound such as gentamicin has been loaded into a material that will promote elution, it is possible to decrease the amount of bacterial colonies that will form on a material over a given period of time, as shown in **Figure 1.2**. When loaded into materials that do not promote the elution of antibacterial compounds, there is a decrease in the inhibition of bacterial growth in food packaging applications. This can be done with the addition of food preservatives such as organic acids and enzymes within the structure of the plastic. Food preservatives such as acetic acid (Ouattara 2000) and lysozyme (Appendini 1997) can help in the prevention of food spoilage when incorporated into food packaging plastics through gradual elution from the material into the food product.

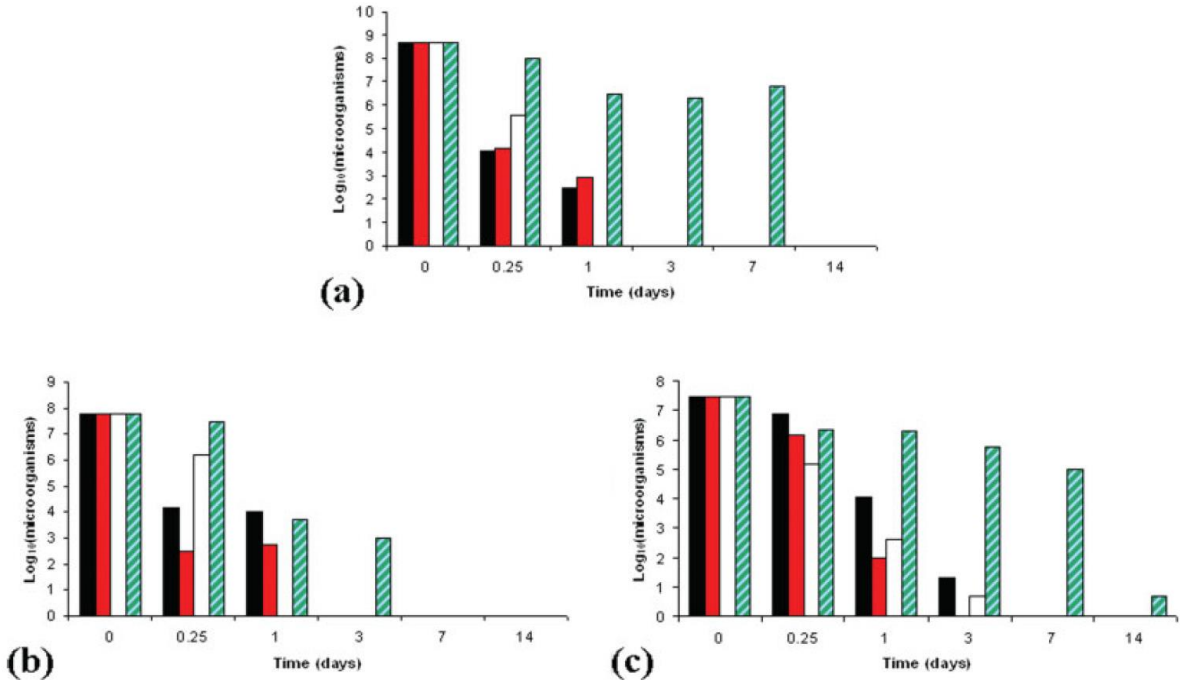


Figure 1.2. Number of colony forming units (CFU) versus time for variation experiments: (a) Pseudomonas aeruginosa, (b) Staphylococcus epidermidis, (c) Staphylococcus aureus. The releasing films are: Black - PLLA film containing 30% (w/w) gentamicin; Red - PLLA film containing 30% (w/w) gentamicin; White - PDLGA film containing 10% (w/w) gentamicin; Green - control - PLLA film without gentamicin (Aviv 2007). Reprinted with permission**.**

Another method in which infection and spoilage due to plastic material can be prevented is through the use of coatings on the surface of the polymer that will prevent bacterial adhesion or growth. A common coating that is used in medical applications utilizes silver nanoparticles, as silver is a known antimicrobial agent that is toxic to bacteria and fungi. When silver nanoparticles are prepared to allow for application to the surface of a plastic through methods such as the sol-gel process (Marini 2007) or through colloidal suspension (Eby 2009), the amount of bacterial growth is limited. For food packaging, the first materials that were designed to be antibacterial were wax coatings that had fungicides incorporated into the wax itself (Appendini 2002). Recent advances in antimicrobial food packaging include the addition of the antibacterial peptide nisin in biodegradable food packages (Jin 2008), and the use of pediocin that is bound on the plastic surface to prevent the spoilage of packaged meat (Gálvez 2014).

The modification of the surface of the plastic to prevent bacterial adhesion has also been examined for medical and food packaging applications. One method of bacterial adhesion prevention is by lubricating the surface of a plastic to the extent that bacteria simply slide off the plastic without any adhesion possible. This surface modification has great potential in the use of medical tubing, as oil-infused polydimethylsiloxane (iPDMS) will prevent inoculated bacteria from adhering to the interior walls of the tubing (MacCallum 2015). As shown in the process of coating tubing in **Figure 1.3**, the application of this iPDMS will result in medical tubing that will prevent the adhesion of water to the surface of the tubing, resulting in tubing that is larger in size and able to pump effluent into a solution without bacteria growing within the tubing. Bacterial adhesion to plastic surfaces can also be prevented through shrink-wrapping a plastic to the extent where the plastic itself becomes superhyrdophobic. As bacterial cultures require some amount of moisture for adhesion to take place, the creation of a structured surface that prevents water adhesion will also prevent bacteria from adhering to shrink-wrapped surfaces that are common in food packaging (Freschauf 2012).

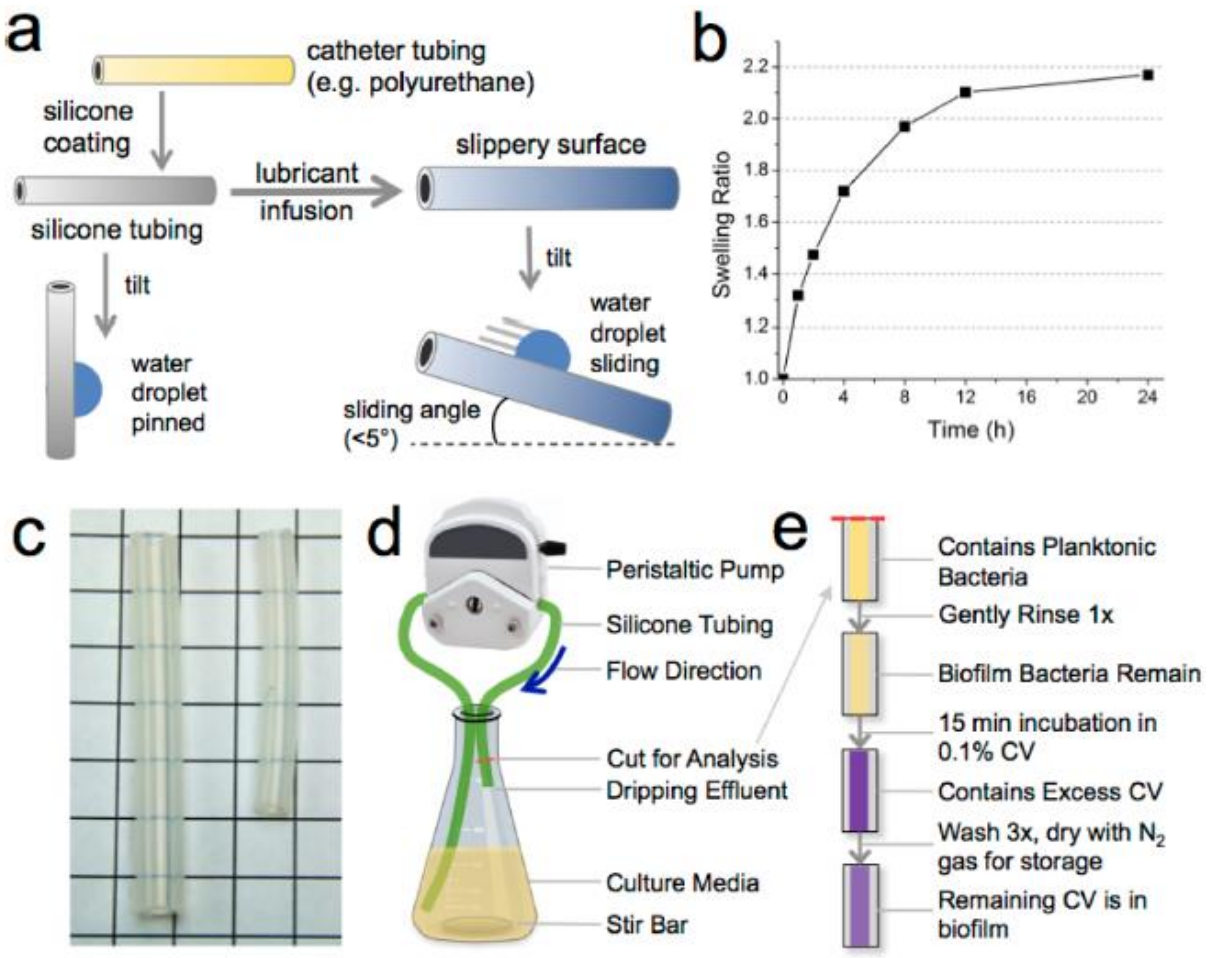


Figure 1.3.: The infusion of a lubricant into a medical plastic by immersion. (a) Slippery surface of plastic in comparison to untreated sample. (b) Swelling ratio of plastic tubing used in study. (c) Larger iPDMS tube (left) compared to untreated tube (right). (d) Process of determining biofilm formation in tubing. (e) Process used to quantify biofilm with Crystal Violet (CV) solution (MacCallum 2015). Reprinted with permission.

The production of bioplastics from biopolymers

An alternative source of material that can be used in the production of plastics are polymers from bio-based sources. It is possible to derive polymers from three different bio-based sources: directly from biomass, such as polysaccharides, proteins, and lipids; the conversion of bio-based monomers into traditional polymers such as polylactates and polyesters; and the production of polymers such as PHA and bacterial celluloses from natural materials that have been genetically modified (Mohanty 2005). We have chosen to utilize proteins as the raw material for plastic production due to its ability to form a plastic through denaturing by

temperature under pressure, as well as properties that can be imparted onto the plastic by the protein itself.

The primary amino acid structure of the protein can serve as a repeat unit for polymerization to produce biopolymers (Kessel 2010). Proteins differ from traditional polymers in that they will also possess a secondary structure. This secondary structure is due to the tendency for proteins to loop or coil together when the protein is in a globular resting state, forming α-helices or β-strands that can be stabilized through hydrogen bonding (Nölting 2005). However, it is the tertiary structure of the protein that will determine the biological properties of the protein, as it is a collection of organized and unorganized sections of secondary structure (Shi 2014). With a repeat structure that has become looped or coiled over time, it will be necessary to place the protein under stress in order to form a plastic.

To produce a plastic from protein biomass, it will be necessary to denature the protein to break all of the secondary bonds except for the primary bonds in the amino acid backbone. To denature the protein, it is possible to use certain chemical agents such as acetic acid (López-Alonso 2010) or urea (Bennion 2003), as well as through the application of heat (Kato 1983). It will also be necessary to apply a certain amount of force during and after the process of denaturing, as this will ensure that a plasticization will occur. If a proper amount of force is not applied, partial plasticization will be the result, as some of the protein will revert to its relaxed/coiled state (Sue 1997). With plasticization complete, it is possible to obtain plastics that possess properties that are similar to the properties contained by the protein itself.

There are beneficial properties of certain proteins that will enhance the properties in the resulting plastic. For instance, microbial resistance has been found in multiple types of proteins, and this would allow for the prevention of contamination of materials that come into contact with

the plastic. Some examples of antimicrobial proteins that have been utilized in plastic production include zein protein derived from corn (Lagaron 2012) and proteins that are found in human blood serum (Patel 2007), as well as proteins that contain enzymes such as albumin derived from chicken egg white (Ünalan 2011). The biodegradation of the protein would also be a potential positive property that the plastic would possess, decreasing the amount of plastics contained in landfills. Plastics that have been made from proteins, such as soy (Paetau 1994) and casein that has been derived from bovine milk (Audic 2003), have been found to naturally biodegrade, reducing the environmental impact of plastic use. As shown in **Table 1.1**, it is possible to produce a material that will possess a similar tensile strength when compared to traditional plastics. However, the bioplastics are limited in the amount of elongation they can withstand before breaking, as they cannot reach the 150-500% elongation at break that is seen in polyethylene or PVC plastics. Another potential use of proteins in the production of plastics is their use in the enhancement of flavor and nutrition when incorporated into food packaging. Proteins such as zein can be utilized to mask undesirable tastes (Windschauer 2012), while proteins such as casein and whey are able to impart an additional amount of protein into food when utilized as packaging (Cutter 2006).

Table 1.1.: Tensile properties of various natural and petroleum-based plastics: NaCAS – Sodium Caseinate; WPI – Whey Protein Isolate (Audic 2003). Reprinted with permission.

Film	Tensile strength[a] (MPa)	Elongation at break (%)
NaCAS/Glycerol (4:1)[b]	17.4–26.7	10.5
NaCAS/Glycerol (2:1)	10.9–11.7	73.7–84.2
NaCAS/PEG (4.54:1)	10.9–16.35	5.3
NaCAS/PEG (1.9:1)	10.9–13.9	25.4
WPI/Glycerol (5.7/1)	29.1	4.1
WPI/Glycerol (2.3/1)	13.9	30.8
WPI/Sorbitol (2.3/1)	14.0	1.6
WPI/sorbitol (1/1)	14.7	8.7
Starch/Glycerol (2.52/1)	17.2	10.8
LDPE	13.0	500
HDPE	26.0	300
Plasticized PVC (wrap film)	15.0–30.0	150–350

The production of plastics through the use of proteins and polymers and their properties

In producing pure protein bioplastics, applications for plastics made out of specific proteins have been determined. One protein that has been examined for potential in the production has been the albumin that is derived from the egg white of a hen. When mixed with glycerol as a plasticizer to aid the production process, its resulting rheological properties make the mixture more easily processed when compared to wheat gluten proteins that have been plasticized with glycerol (Jerez 2007). When analyzed for mechanical properties, the albumin-based plastics have been found suitable enough for packaging applications (Muneer 2014), as well as agricultural and medical materials (Sharma 2012). Proteins from the keratin family have also been used to produce bioplastics, with films that are meant for edible and medical applications. For instance, the fibrous nature of keratin proteins allows for good cell adhesion and proliferation, showing a potential use in biomedical applications (Tanabe 2002), while its

low cost and biodegradability allow for compostable packaging and agricultural films (Bietz 1996). A potential for antibacterial use is possible as well, as illustrated in **Table 1.2**. The use of keratin will reduce the amount of *E. coli* growth witnessed by 20-26% when compared to fibrin. Antibacterial properties of the resulting material can be enhanced with the inclusion of chitosan, which will also result in higher levels of swelling in the resulting material, as shown in **Tables 1.2** and **1.3**. Another protein of note that has been utilized in the production of bioplastics is zein, derived from corn. Like keratin proteins, zein protein can be processed into a plastic that has potential use in food packaging and medical applications. The use of zein-based food packaging is beneficial due to the fact that zein possesses antioxidant properties (Shi 2014). For medical applications it has been found to be a potential material to be used for controlled drug delivery (Suzuki 1989).

Tables 1.2 and 1.3.: Reduction of *E.coli* growth when in contact with keratin-chitosan-based films, and swelling of films in aqueous solutions (Tanabe 2002). Reprinted with permission.

Film	Reduction rate (%)
Fibrin	0 ± 1
Keratin	23 ± 3
Keratin–chitosan (25 : 1)	54 ± 6^{b}
Keratin–chitosan (5 : 1)	61 ± 6^{b}
Keratin–chitosan (5 : 4)	62 ± 4^{b}
Chitosan	82 ± 8^{b}

Film[a]	Swelling ratio (%)[b]		
	pH 4.0	pH 6.3	pH 8.9
Keratin	0 ± 0	0 ± 0	90 ± 6
Keratin–chitosan (20 : 1)	23 ± 3	12 ± 1	51 ± 5
Keratin–chitosan (5 : 1)	87 ± 4	88 ± 6	15 ± 3
Keratin–chitosan (3 : 1)	104 ± 6	126 ± 8	14 ± 3
Chitosan	Dissolved	382 ± 20	0 ± 0

[a] The weight ratio of keratin to chitosan in composite films was shown in the parenthesis. The data represent mean ± SD of three experiments.

[b] Swelling ratio $= (L_{wet} - L_{dry}/L_{dry}) \times 100$, where L_{dry} and L_{wet} are the length of a side of the dry and swollen film, respectively.

However, a major issue with the use of protein plastics in applications that are common for traditional thermoplastics are their diminished properties in comparison, such as modulus and elongation. If these protein-based plastics are unable to possess similar (if not better) properties compared to traditional polymers, then there is no point in the use of these proteins in plastic production. In order to enhance the properties of protein-based plastics, the addition of traditional polymers has been examined. One protein that has been studied extensively when blended with traditional polymers has been soy. Plastics made from this protein possess a modulus that is 50% higher than engineering plastics that are epoxy-based (Wang 1996). Soy protein also possesses water solubility properties that allow for it to be blended with other plastics, and be used in materials such as automobile parts (Ly 1998) and biodegradable packaging (Swain 2004). Kertain protein that has been derived from the feathers of chickens has also been examined for potential grafting with acrylates and methacrylates. The intended application of these materials is aimed for biomedical applications, as it is hypothesized that the materials will be useful for the development of scaffolds for tissue engineering (Reddy 2013). It is also possible to design proteins in the lab that are specifically designed to be utilized as a constituent in a block copolymer for thermoplastic applications. For instance, it is possible to design a cross-linked thermoplastic that possesses a Young's modulus that is higher by a factor of three when prepared in 10% TFE solution, and an increase in elongation that is by a factor of five when prepared in a 10% water solution (Nagapudi 2005).

Gaps of current research and challenges to be addressed

While a considerable foundation has been laid for this research, there are many challenges that must be addressed before a protein-based plastic can be effectively utilized in medical and food packaging applications. The gaps in research that we aim to address with this

research are: the lack of comparative analysis (viscoelastic, antimicrobial, drug elution) that has been conducted on plastics that are made primarily from protein; the lack of comparisons between types of proteins that can be utilized; and the lack of knowledge of how the combination use of plasticizers and additives will alter the antimicrobial and drug elution properties of the resulting protein-based plastic.

There is a significant amount of research that has been conducted on the properties of plastics and films that utilize protein as an additive in the production process of plastics, but not as the main constituent. To address this issue, we have examined four different proteins (albumin from hen egg white, soy edible protein, whey protein isolate, and zein corn protein) that can be utilized as the main constituent to determine if protein-based plastics possess properties suitable for medical and food packaging applications.

In protein plastic research, there has been a general lack of comparison between proteins in plastic production. Certain types of proteins such as soy (Schilling 1995, Sue 1997, Kumar 2002) and casein (Munro 1980, Audic 2003, Cardoso 2010) been heavily studied in isolation for potential uses, but not in direct comparison. With the research conducted, we hope to directly compare types of protein plastics under the same conditions and determine properties, such as surface antimicrobial, mechanical, and viscoelasticity. This knowledge is necessary in deciding what types of proteins can be used to replace traditional polymers in medical and food packaging applications.

There is also a general lack of analysis when comparing the types of additives that can be utilized in the production of protein-based plastics. The use of glycerol as a plasticizer has been extensively examined in protein plastics (Zhang 2001, Gao 2006, Sothornvit 2007), yet there is a general lack of information on other plasticizers, such as water or natural rubber latex. Similarly,

other additives such as food preservatives and drugs have been examined in use of petroleum-based plastics (Dimalo 1994, Queiroz 2001, Vartiainen 2003), but not in protein-based plastics. It will be necessary to address this lack of information, as properties such as biodegradation may allow for more effective drug and food preservative elution properties when compared to petroleum-based plastics.

Justification of proteins chosen for plastic production

In the choice of proteins that will be utilized for plastic production, we have chosen four proteins that are all globular in structure: albumin from hen egg white; soy protein; whey protein isolate; and zein protein from corn. Globular proteins have been chosen for their ability to fold and denature, which determine the end properties of the resulting plastic (Travaglini-Allocatelli 2009). Unlike structural proteins, globular proteins can serve multiple purposes, such as enzymes, messengers, transporters, amino acid stock and regulation of chemical reactions (Creighton 1993). For our study, we have chosen four globular proteins based on their potential use as a biodegradable plastic, as well as their antibacterial activity potential.

For the four proteins chosen for these studies, each have previously been examined for their potential substitute for traditional thermoplastics. For hen egg white albumin-based proteins, it has been found that when processed with plasticizers during plastic production, it is possible to produce a plastic that is suitable for packaging, agricultural, horticulture, and medical applications (Sharma 2012). The albumin-based plastic may also possess antimicrobial properties based on enzymatic substituents that are found in the protein itself, such as lysozyme (Hughey 1989). Soy protein-based plastics have already been found to possess properties that are similar to HDPE and PS (Schilling 1995), as well as antimicrobial potential when produced with additives such as grape seed extract, nisin, and EDTA (Sivarooban 2008). Whey protein-based

edible films are produced for food applications (Banerjee 1995), and can be modified with oregano, rosemary and garlic essential oils to impart antimicrobial properties (Seydim 2006). The protein of zein that is derived from corn has been researched for use in packaging and consumer goods (Lai 1997), and can be utilized in applications that will require antimicrobial properties when blended with chitosan (Torres-Giner 2009).

Objectives of studies conducted

Production of albumin, whey, soy, and zein bioplastics

The first objective for this dissertation dealt with the preliminary screening of proteins to use in developing bioplastics. The main objective of the first part of this dissertation was to determine the thermal, viscoelastic, tensile, and surface antimicrobial properties of bioplastics produced from proteins. Plastics intended for medical or food packaging applications require raw materials that can be easily processed into an end material, mechanical durability and flexibility to withstand the stresses of use, and bacteriostatic/bacteriocidal properties that will limit bacterial growth, if not prevent it. The preparation of bioplastics made from four proteins (albumin, whey, soy, and zein) was accomplished with the use plasticizers (water, glycerol, or natural rubber latex in **Chapters 2-4**).

Sub-Goals

1. Thermal characterization of protein-based bioplastics through use of differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). **(Chapters 2-4)**
2. Determination of mechanical and viscoelastic properties of bioplastics with tensile testing and dynamic mechanical analysis (DMA). **(Chapters 2-4)**
3. Analysis of antimicrobial surface potential of bioplastics through testing of bacterial adhesion onto plastic surface and viable bacteria removal. **(Chapters 3 and 4)**

Production of thermoplastics with the use of albumin or zein blended with LDPE

After screening the proteins for viability in medical and food packaging applications, it was necessary to conduct further analysis on albumin and zein-based plastics. The main objective of this second area of the dissertation was to examine in greater detail the production and potential use of these two proteins in plastics, as well as how said plastics are affected by the addition of a traditional thermoplastic monomer (LDPE). This preparation of bioplastics was achieved through the blending of proteins (albumin or zein) with the thermoplastic monomer at differing ratios, with glycerol utilized as a plasticizer.

Sub-Goals

1. Thermal characterization of protein-based thermoplastics through use of differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). **(Chapter 4)**
2. Determination of mechanical and viscoelastic properties of thermoplastics by tensile testing and dynamic mechanical analysis (DMA). **(Chapter 4)**
3. Evaluation of water absorption and solubility of thermoplastics by immersion in water. **(Chapter 4)**
4. Measurement of susceptibility of plastics to degradation by microbial attack through soil burial. **(Chapter 4)**
5. Analysis of the antimicrobial surface potential of thermoplastics through testing of bacterial adhesion onto plastic surfaces and viable bacteria removal. **(Chapter 4)**
6. Characterization of drug elution capability and kinetics of protein-based plastics loaded with drugs (ampicillin or ciprofloxacin) or food preservatives (sodium benzoate or sodium nitrite). **(Chapter 4)**

7. Study of the environmental impact of production of albumin and zein-based plastics and comparison to LDPE-based thermoplastics through use of life cycle assessment. **(Chapter 5)**

References

Appendini, P., Hotchkiss, J H (2002). "Review of antimicrobial food packaging." Innovative Food Science & Emerging Technologies **3**(2): 113-126.

Appendini, P., Hotchkiss, J. (1997). "Immobilization of lysozyme on food contact polymers as potential antimicrobial films." Packaging Technology and Science **10**(5): 271-279.

Audic, J.-L., Chaufer, B., Daufin, G. (2003). "Non-food applications of milk components and dairy co-products: A review." Lait **83**(6): 417-438.

Aviv, M., Berdicevsky, I., Zilberman, M. (2007). "Gentamicin-loaded bioresorbable films for prevention of bacterial infections associated with orthopedic implants." Journal of Biomedical Materials Research Part A **83A**(1): 10-19.

Banerjee, R., Chen, H. (1995). "Functional properties of edible films using whey protein concentrate." Journal of Dairy Science **78**(8): 1673-1683.
Bennion, B. J., Daggett, V. (2003). "The molecular basis for the chemical denaturation of proteins by urea." Proceedings of the National Academy of Sciences of the United States of America **100**(9): 5142-5147.

Bietz, J. A., Lookhart, G.L. (1996). "Properties and non-food potential of gluten." Cereal Food World **41**: 376-382.

Cardoso, J. C., Albuquerque, R.L.C., Padilha, F.F., Bittencourt, F.O., de Freitas, O., Nunes, P.S., Pereira, N.L., Fonseca, M.J.V., Araujo, A.A.S. (2010). "Effect of the Maillard reaction on properties of casein and casein films." Journal of Thermal Analysis and Calorimetry **104**(1): 249-254.

Costa, L., Bracco, P., del Prever, E.M.B., Kurtz, S.M., Gallinaro, P. (2006). "Oxidation and oxidation potential in contemporary packaging for polyethylene total joint replacement components." Journal of Biomedical Materials Research Part B: Applied Biomaterials **78B**(1): 20-26.

Creighton, T. E. (1993). Proteins: Structures and molecular properties. New York, W.H. Freeman and Company.

Cutter, C. N. (2006). "Opportunities for bio-based packaging technologies to improve the quality and safety of fresh and further processed muscle foods." Meat Science **74**(1): 131-142.

Dimalo, F., O'Halloran, J.J., Quale, J.M. (1994). "In vitro elution of ciprofloxacin from polymethylmethacrylate cement beads." Journal of Orthopaedic Research **12**(1): 79-82.

Eby, D. M., Luckarift, H.R., Johnson, G.R. (2009). "Hybrid Antimicrobial Enzyme and Silver Nanoparticle Coatings for Medical Instruments." ACS Applied Materials & Interfaces **1**(7): 1553-1560.

EPA (2010). Municipal Solid Waste in the United States: 2009 Facts and Figures. Washington, DC, U. S. E. P. Agency.

Fengmei, L., Ying, W., Xiaoguang, L., Baoyu, Y. (2000). "Evaluation of plastic packaging materials used in radiation sterilized medical products and food." Radiation Physics and Chemistry **57**(3-6): 435-439.

Freschauf, L. R., McLane, J., Sharma, H., Khine, M. (2012). "Shrink-induced superhydrophobic and antibacterial surfaces in consumer plastics." PLoS One.

Gálvez, A., López, R.L., Pulido, R.P., Burgos, M.J.G. (2014). Biopreservation of meats and meat products. Food Biopreservation. New York, Springer New York.

Gao, C., Stading, M., Wellner, N., Parker, M.L., Noel, T.R., Clare Mills, E.N., Belton, P.S. (2006). "Plasticization of a protein-based film by glycerol: A spectroscopic, mechanical, and thermal Study." Journal of Agricultural and Food Chemistry **54**(13): 4611-4616.

Hakkarainen, M. (2003). "New PVC materials for medical applications—the release profile of PVC/polycaprolactone–polycarbonate aged in aqueous environments." Polymer Degradation and Stability **80**(3): 451-458.

Hanssen, A. D. (2004). "Prophylactic use of antibiotic bone cement: an emerging standard—in opposition." The Journal of Arthroplasty **19**(4S1): 73-77.

Hughey, V. L., Wilger, P.A., Johnson, E.A. (1989). "Antibacterial Activity of Hen Egg White Lysozyme against Listeria monocytogenes Scott A in Foods." Applied and Environmental Microbiology **55**(3): 631-638.

Ikada, Y., Tsuji, H. (2000). "Biodegradable polyesters for medical and ecological applications." Macromolecular Rapid Communications **21**(3): 117-132.

Jerez, A., Partal, P., Martinez, I., Gallegos, C., Guerrero, A. (2007). "Egg white-based bioplastics developed by thermomechanical processing." Journal of Food Engineering **82**(4): 608-617.

Jin, T., Zhang, H. (2008). "Biodegradable polylactic acid polymer with nisin for use in antimicrobial food packaging." Journal of Food Science **73**(3): 127-134.

Jones, D. S., Lorimer, C.P., McCoy, C.P., Gorman, S.P. (2008). "Characterization of the physicochemical, antimicrobial, and drug release properties of thermoresponsive hydrogel copolymers designed for medical device applications." Journal of Biomedical Materials Research Part B: Applied Biomaterials **85B**(2): 417-426.

Kato, A., Osako, Y., Matsudomi, N., Kobayashi, K. (1983). "Changes in the emulsifying and foaming properties of proteins during heat denaturation." Agricultural and Biological Chemistry **47**(1): 33-37.

Kessel, A., Ben-tal, N. (2010). Introduction to Proteins: Structure, Function, and Motion. Boca Raton, FL, CRC Press.

Kumar, R., Choudhary, V., Mishra, S., Varma, I.K., Mattiason, B. (2002). "Adhesives and plastics based on soy protein products." Industrial Crops and Products **16**(3): 155-172.

Lagaron, J. M., Ocio, M.J., Lopez-Rubio, A. (2012). Antimicrobial packaging polymers. A general introduciton. Antimicrobial Polymers. J. M. Lagaron, Ocio, M.J., Lopez-Rubio, A. Hoboken, NJ, John WIley & Sons.

Lai, H.-M., Padua, G.W. (1997). "Properties and microstructure of plasticized zein film." Cereal Chemistry **74**(6): 771-775.

Lau, O.-W., Wong, S-K (2000). "Contamination in food from packaging material." Journal of Chromatography A **882**(1-2): 255-270.

Lau, O.-W., Wong, S-K. (1996). "The migration of plasticizers from cling film into food during microwave heating—effect of fat content and contact time." Packaging Technology and Science **9**(1): 19-27.

Limm, W., Hollifield, H.C. (1995). "Effects of temperature and mixing on polymer adjuvant migration to corn oil and water." Food Additives & Contaminants **12**(4): 609-624.

Lobell, K. W., Stamou, S., Sanchez, J.A. (2012). "Hospital-acquired infections." Surgical Clinics of North America **92**(1): 65-77.

López-Alonso, J. P., Bruix, M., Font, J., Ribó, M., Vilanova, M., Jiménez, M.A., Santoro, J., González, C., Laurents, D.V. (2010). "NMR spectroscopy reveals that RNase A is chiefly denatured in 40% acetic acid: Implications for oligomer formation by 3D domain swapping." Journal of The American Chemical Society **132**(5): 1621-1630.

Ly, Y. T.-P., Johnson, L.A., Jane, J. (1998). Soy protein as biopolymer. Biopolymers from Renewable Resources. D. L. Kaplan. Berlin, Springer-Verlag Berlin Heidelberg**:** 144-174.

MacCallum, N., Howell, C., Kim, P., Sun, D., Friedlander, R., Ranisau, J., Ahanotu, O., Lin, J.J., Vena, A., Hatton, B., Wong, T-S., Aizenberg, J. (2015). "Liquid-infused silicone as a biofouling-free medical material." ACS Biomaterials Science & Engineering **1**(1): 43-51.

Marini, M., Niederhausern, S.D., Iseppi, R., Bondi, M., Sabia, C., Toselli, M., Pilati, F. (2007). "Antibacterial activity of plastics coated with silver-doped organic-inorganic hybrid coatings prepared by sol-gel processes." Biomacromolecules **8**(4): 1246-1254.

Marsh, K., Bugusu, B. (2007). "Food packaging—roles, materials, and environmental issues." Journal of Food Science **72**(3): R39-55.

McKeen, L. W. (2014). Plastics used in medical devices. Handbook of Polymer Applications in Medicine and Medical Devices S. Ebnesajjad, Modjarrad, K. New York, William Andrew.

Mohanty, A. K., Misra, M., Drzal, L.T. (2005). Natural fibers, biopolymers, and biocomposites. Boca Raton, FL, CRC Press.

Muneer, F. (2014). "Bioplastics from natural polymers." Introductory paper at the Faculty of Landscape Architecture, Horticulture and Crop Production Science **4**: 1-10.

Munro, P. A., Southward, C.R., Elston, P.D. (1980). "The effect of casein manufacturing variables on the properties of rennet casein plastics." New Zealand Journal of Dairy Science and Technology **15**(2): 177-190.

Nagapudi, K., Brinkman, W.T., Leisen, J., Thomas, B.S., Wright, E.R., Haller, C., Wu, X., Apkarian, R.P., Conticello, V.P., Chaikof, E.L. (2005). "Protein-based thermoplastic elastomers." Macromolecules **38**: 345-354.

Nölting, B. (2005). Protein folding kinetics: biophysical methods. Berlin, Springer.

Ouattara, B., Simard, R.E., Piette, G., Begin, A., Holley, R.A. (2000). "Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan." International Journal of Food Microbiology **62**(1-2): 139-148.

Paetau, I., Chen, C-Z, Jane, J (1994). "Biodegradable plastic made from soybean products. 1. Effect of preparation and processing on mechanical properties and water absorption." Ind. Eng. Chem. Res. **33**: 1821-1827.

Patel, J. D., Ebert, M., Ward, R., Anderson, J.M. (2007). "S. epidermidis biofilm formation: Effects of biomaterial surface chemistry and serum proteins." Journal of Biomedical Materials Research Part A **80A**(3): 742-751.

Queiroz, A. C., Santos, J.D., Monteiro, F.J., Gibson, I.R., Knowles, J.C. (2001). "Adsorption and release studies of sodium ampicillin from hydroxyapatite and glass-reinforced hydroxyapatite composites." Biomaterials **22**(11): 1393-1400.

Rahman, M., Brazel, C.S. (2004). "The plasticizer market: an assessment of traditional plasticizers and research trends to meet new challenges." Progress in Polymer Science **29**(12): 1223-1248.

Reddy, N., Jiang, Q., Jin, E., Shi, Z., Hou, X., Yang, Y. (2013). "Bio-thermoplastics from grafted chicken feathers for potentialbiomedical applications." Colloids and Surfaces B: Biointerfaces **110**: 51-58.

Schilling, C. H., Babcock, T., Wang, S., Jane, J. (1995). "Mechanical properties of biodegradable soy-protein plastics." Journal of Materials Research **10**(9): 2197-2202.

Scott II, R. D. (2009). The direct medical costs of healthcare-associated infections in U.S. hospitals and the benefits of prevention. D. A. Pollack. DeKalb County, Georgia, Centers for Disease Control and Prevention.

Seydim, A. C., Sarikus, G. (2006). "Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils." Food Research International **39**(5): 639-644.

Sharma, S., Luzinov, I (2012). "Water aided fabrication of whey and albumin plastics." Journal of Polymers and the Environment **20**: 681-689.

Shi, W., Dumont, M-J. (2014). "Review: bio-based films from zein, keratin, pea, and rapeseed protein feedstocks." Journal of Materials Science **49**(5): 1915-1930.

Sivarooban, T., Hettiarachchy, N S, Johnson, M G (2008). "Physical and antimicrobial properties of grape seed extract, nisin, and EDTA incorporated soy protein edible films." Food Research International **41**(8): 781-785.

Sothornvit, R., Olsen, C.W., McHugh, T.H., Krochta, J.M. (2007). "Tensile properties of compression-molded whey protein sheets: Determination of molding condition and glycerol-content effects and comparison with solution-cast films." Journal of Food Engineering **78**(3): 855-860.

Sue, H. J., Wang, S, Lane, J L (1997). "Morphology and mechanical behaviour of engineering soy plastics." Polymer **38**(20): 5035-5040.

Suppakul, P., Miltz, J., Sonneveld, K., Bigger, S.W. (2003). "Active packaging technologies with an emphasis on antimicrobial packaging and its applications." Journal of Food Science **68**(2): 2003.

Suzuki, T., Sato, E., Matsuda, Y., Tada, H., Unno, K., Kato, T. (1989). "Preparation of zein microspheres conjugated with antitumor drugs available for selective cancer chemotherapy and development of a simple colorimetric determination of drugs in microspheres." Chemical and Pharmaceutical Bulletin **37**(4): 1051-1054.

Swain, S. N., Biswal, S.M., Nanda, P.K., Nayak, P.L. (2004). "Biodegradable soy-based plastics: Opportunities and challenges." Journal of Polymers and the Environment **12**(1): 35-42.

Tanabe, T., Okitsu, N., Tachibana, A., Yamauchi, K. (2002). "Preparation and characterization of keratin–chitosan composite film." Biomaterials **23**(3): 817-825.

Torres-Giner, S., Ocio, M.J., Lagaron, J.M. (2009). "Novel antimicrobial ultrathin structures of zein/chitosan blends obtained by electrospinning." Carbohydrate Polymers **77**(2): 261-266.

Travaglini-Allocatelli, C., Ivarsson, Y., Jemth, P., Gianni, S. (2009). "Folding and stability of globular proteins and implications for function." Current Opinion in Structural Biology **19**(1): 3-7.

Ünalan, I. U., Korel, F., Yemenicioğlu, A. (2011). "Active packaging of ground beef patties by edible zein films incorporated with partially purified lysozyme and Na_2EDTA." International Food Science and Technology **46**(6): 1289-1295.

Vartiainen, J., Skytta, E., Enqvist, J., Ahvenainen, R. (2003). "Properties of antimicrobial plastics containing traditional food preservatives." Packaging Technology and Science **16**(6): 223-229.

Venkat, K. (2011). "The climate change and economic impacts of food waste in the United States." International Journal of Food System Dynamics **2**(4): 431-446.

von Eiff, C., Jansen, B., Kohnen, W., Becker, K. (2005). "Infections associated with medical devices: Pathogenesis, management and prophylaxis." Drugs **65**(2): 179-214.

Wang, S., Sue, H.-J., Jane, J. (1996). "Effects of polyhydric alcohols on the mechanical properties of soy protein plastics." Journal of Macromolecular Science, Part A **33**(5): 557-569.
Windschauer, R. J., Virgallito, T.T. (2012). Taste masking compositions and edible forms thereof U. S. P. a. T. Office. United States, Acme Specialty Products, Llc. **US20120321727 A1**.

Woo, L., Sandford, C.L. (2002). "Comparison of electron beam irradiation with gamma processing for medical packaging materials." Radiation Physics and Chemistry **63**(3-6): 845-850.

Zhang, J., Mungara, P, Jane, J (2001). "Mechanical and thermal properties of extruded soy protein sheets." Polymer **42**(6): 2569-2578.

CHAPTER 2

THERMAL, MECHANICAL, AND MOISTURE ABSORPTION PROPERTIES OF EGG WHITE PROTEIN BIOPLASTICS WITH NATURAL RUBBER AND GLYCEROL[1]

[1] Alexander Jones, Mark Ashton Zeller, and Suraj Sharma. 2013. *Progress in Biomaterials*, **2**:12. Reprinted with permission.

Abstract

Petroleum-based plastics have many drawbacks: the large amount of energy required to produce the plastic, the waste generated as a result of plastic production, and the accumulation of waste due to the slow rate of degradation. It is because of these negative attributes of conventional plastic use that attention is being focused on environmentally friendly plastics from alternative sources. Albumin protein provides one possible source of raw material, with inherent antimicrobial properties that may make it suitable for medical applications. This study was conducted to investigate the various bioplastic properties of the albumin with the use of three plasticizers—water, glycerol, and natural rubber latex. Based on the results, the 75/25 Albumin-Water, the 75/25 Albumin-Glycerol, and the 80/20 Albumin-Natural rubber blends were the best for each plasticizer. A subsequent time study was conducted to determine water stability, with the 80/20 Albumin-Natural rubber blend having possessed the best thermal, tensile, and viscoelastic properties overall.

Keywords: bioplastics, albumin, sustainability, plasticizers

Introduction

Using conventional plastics comes with a multitude of drawbacks: the large amount of energy that is required to produce the plastic, the waste that is a result of plastic production, and the use of materials that do not biodegrade readily. In order to shift the production of plastics towards a more sustainable path, research was conducted to determine the types of renewable bioplastic resources that could be converted into a plastic form. For instance, the polylactide (PLA) biopolymer, one of the few renewable polymers, is naturally produced on a large scale (Mukerjee T 2011). A common theme for various bioplastics that will replace conventional plastics is their tendency to biodegrade, compared to petroleum-based plastics that are resistant

to chemical and biological attacks. According to a review study by Flieger et al., there are three groups of biodegradable polymers that can be utilized in the production of bioplastics: biopolymers by chemical synthesis, biopolymers through fermentation processes by microorganisms, and biopolymers from chemically modified natural products (Flieger M 2003). Under the classification of chemically modified natural products is the use of protein in producing bioplastics. Protein must be modified chemically by the addition of plasticizers and the use of thermal treatments. One of the main initiative for the use of modified natural products in bioplastics is the continual drive to find more uses for agricultural commodities (Flieger M 2003).

It is necessary to determine the thermal and mechanical properties of bioplastics produced from protein, as this will help identify the process by which the bioplastic should be made, as well as what applications the resulting plastic will be suitable for. A study by Sharma et al. determined that the albumin from dried chicken egg white denatures at a temperature of 136.5°C ± 3°C (Sharma S 2008). This indicates that in order to produce a plastic from the dried chicken egg white albumin, the material must be molded at 136.5°C ± 3°C to ensure that the protein will be denatured, and be able to orient and form a bioplastic. When the tensile properties of the protein-based plastics were measured, it was determined that the breaking of hydrophobic interactions and hydrogen bonds of the bioplastics initiated a reversible yield point (Sharma S 2008). This reversal of the yield point allows tensile stress to be placed onto the bioplastic multiple times, as long as the breaking point is not reached. This makes the resulting bioplastic very suitable for situations where low levels of stress are placed upon the bioplastic, such as being implanted into humans for timed drug release.

In order to determine the potential uses of protein-based bioplastics, thermal and mechanical properties must be examined. Since the protein-based bioplastics require a lower processing temperature and possess tensile properties similar to plastics such as high density polyethylene (HDPE), it is possible to manufacture a bioplastic at a lower production cost (Jerez A 2007). However, one potential drawback of using proteins in plastics are their hygroscopic properties The water absorption of various bioplastics ranged from 40 to 320 % (Jerez A 2007). The tendency for bioplastics to absorb water may result in a plastic with lower elasticity, as the moisture content may alter the elastic modulus of the resulting plastic. Another potential drawback that arises when using protein-based (or any polymer) materials is the lack of knowledge about how the materials will react to bacteria in their environment. A study by Hook et al. determined that certain polymers were actually better at preventing microbial growth than either the silicone or silver-hydrogel coatings that are commonly applied to medical devices before being implanted into humans (Hook A 2012). One other potential drawback with bioplastics is the permanent deformation when a stress is applied (Widiastuti 2012). Although researchers have utilized composites to decrease the amount of deformation and creep, research must still be conducted in order to examine both the positive and negative properties of the protein bioplastics in order to determine if they are suitable for certain applications (Dorigato 2012).

Certain constituents of albumin from hen egg white pose the potential advantage of possessing inherent antibacterial properties allowing for potential pharmaceutical and medicinal uses. The albumin possesses this property most notably from its lysozyme enzyme constituent, utilizing a lysis reaction that breaks down the peptidoglycan barrier of bacteria consisting of the glycosidic (1-4) β-linkage between N-acetylglucosamine and N-acetylmuramic acid (Baron F

2007). It is also possible to improve the antimicrobial properties of the lysozyme enzyme through the use of chemical modifications. There are various preservatives, such as nisin and sodium lactate, as well as substances such as ethylenediaminetetraacetic acid (EDTA), butylparaben, and trisodium phosphate, that can be added to the lysozyme to enhance its properties (Cegielska-Radziejewska R 2010). The utilization of albumin in medical plastic production would be an extension on what chicken albumin is being used for today. It is because of inherent antibacterial enzymes and improvement through chemical modification that albumin is already used in various medical applications, such as circulatory support, drug delivery, and the removal of toxins from the body (Peters Jr. 1996). Another area in which albumin plastics could be utilized is drug elution, as it would serve as a material that could release a low dose of antibiotic material over a period of time in the body while limiting the risk of infection (Zilberman 2008). Our objectives when conducting this study were to observe the properties of albumin plastics when plasticized with water, glycerol, and natural rubber latex and to evaluate the thermal, viscoelastic, and mechanical properties of these plastics as time progressed. With the knowledge gained in this study, it would be possible to determine the feasibility of albumin protein plastic use for an intermediate period of time.

Methods

Materials

The albumin from hen egg white (purity of ≥ 99%) utilized in the production of bioplastics was obtained from Sigma Aldrich. The plasticizers used to form the bioplastics were obtained through various sources: the deionized water was obtained through filtering water in the lab; the glycerol was obtained from Sigma Aldrich, with a purity of ≥ 99%; and the natural

rubber latex (NRL) (70% solid, 30% water mixture with a pH of 10.8) was obtained from the Chemionics Corporation.

Preparation of Compression Molded Samples

Moldings of the albumin-based bioplastic blends were performed on a 24-ton bench-top press (Carver Model 3850, Wabash, IN, USA) with electrically-heated and water-cooled platens. The stainless steel molds were custom made to form either dog bone-shaped bioplastics for mechanical analysis (to allow the tensile tester to have a grip on the ends of the plastic during testing), or two small rectangular flex bars for various property analyses. Data presented in this study were generated from compression molded samples using a 5 minute cook time at 120.5 ºC, followed by a 10 minute cooling period, under a pressure of at least 40 MPa, as a certain minimum amount of pressure was required in order to mold a plastic (Sue 1997). The bioplastic blends were prepared in small batches of $\leq$ 6 g by hand mixing the protein and the plasticizer with a stir bar in a glass beaker, with stirring conducted until a consistent blend is obtained. The material is then poured into the molds at a constant weight, with the dynamic mechanical analysis (DMA) flexbars made of 2 g and the dog bones made of 6 g albumin-plasticizer mixture. After the samples were cooled for 10 minutes under pressure, the pressure was released and the samples were removed. The samples were then placed in a conditioning chamber for at least 24 hours, unless otherwise noted. The conditioning chamber was set to 21.1°C and 65% relative humidity.

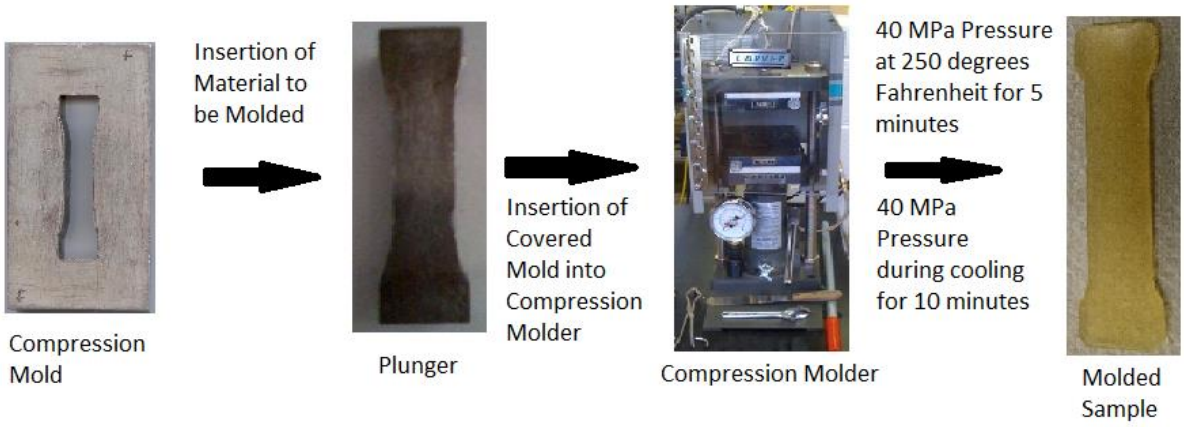


Figure 2.1: Schematic of the Molding of Albumin Plastic Samples

Weight Change and Ambient Moisture Gain/Loss Analysis

The bioplastic samples were placed in the conditioning settings to determine the moisture content over time—initial, 1, 2, 3, 4, 5, 6, 24, 48, 72, and 96 hours after molding. In order to ensure accurate measurements, four DMA flexbars were prepared and analyzed during this process. Moisture content of plastics were analyzed by cryocrushing bioplastics under liquid nitrogen for each blend type (n = 4) and heated at 80 °C for one hour, with ten minutes of cooling afterwards. The equation used to determine the moisture content was

$$MC = [(W_0 - W_{0d})/W_0] \times 100$$

Where W_0 = Initial weight of specimen; W_{0d} = Weight of specimen after drying.

Dynamic Mechanical Analysis

After conditioning, DMA flex bars were analyzed for their viscoelastic properties through the use of dynamic mechanical analysis (Menard 1999) by using a DMA 8000 Dynamic Mechanical Analyzer from Perkin Elmer , starting at a temperature of 25°C and ending at a temperature of 160°C, with a temperature ramp of 2 °C min^{-1}. The settings of the analyzer were set to dimensions of 9×2.5×12.5 mm^3 using a dual-cantilever setup at a frequency of 1 Hz with a displacement of 0.05 mm, within the range of plastic deformation. Each sample type was

analyzed in duplicate (n = 2) to ensure precision. The DMA flex bars were also tested in intervals of immediate, 24 hours, and 5 days after molding in order to determine the viscoelastic properties over time.

Thermal Analysis

Thermal gravimetric analysis (TGA) was performed using a Mettler Toledo TGA/SDTA851e, and sifferential scanning calorimetry (DSC) was performed using a Mettler Toledo DSC821e. TGA was performed from 25°C – 800 °C under a N_2 atmosphere with a heating rate of 10 °C min^{-1}. DSC was performed from -50°C to -250 °C under a N_2 atmosphere with a heating rate of 20 °C min^{-1}. All samples (n = 2) were prepared with weights between 2.0 – 4.0 mg, as the samples were cut from DMA flex bars for each blend. TGA and DSC tests were conducted in intervals of immediate, 24 hours, and 5 days after molding.

Scanning Electron Microscopy (SEM)

Albumin SEM samples (n =2 for each plastic type) were prepared from cryogenic DMA flex bar fracture surfaces after being placed in a conditioning chamber (21.1°C and 65% relative humidity) for at least 24 hours. DMA flex bars were submerged in liquid nitrogen for 20 seconds; after that they were immediately broken. The samples were mounted, then sputter coated for 60 seconds with an Au/Pt mix. SEM images were recorded on a Zeiss 1450EP variable pressure scanning electron microscope. Coated samples were analyzed at 20X, 100X, and 500X for each blend type.

Mechanical Properties

The mechanical properties of the conditioned albumin bioplastics were measured by using the Instron testing system (Model 3343) interfaced with the Blue Hill software. The test was performed according to the standard test method for tensile properties of plastics (ASTM D

638-10, Type I) with a 5 mm min^{-1} crosshead speed, a static load cell of 1000 N, and a gauge length of 4 cm. Samples were run in quintuplicate (n=5) for each blend type in order to ensure precise measurement.

Statistical Methods

Statistical analysis of the data generated in the measurement of moisture content and mechanical properties were done through the use of power analysis. For each plastic type tested, statistical values based on the mean and standard deviation were generated, with p-values (p = 0.05 or less) comparing plastic types based on the properties being tested generated from the Student's T-test distribution. For the moisture content analysis, correlation analysis was also conducted (1= perfect positive correlation, 0 = no correlation, -1 = perfect negative correlation).

Results and Discussion

Initial Material Analysis

Thermal Properties of Pure Albumin Powder

An initial degradation peak in DSC was shown between 220-230°C, with a much larger peak starting between 245-250°C. Ninety three percent of the albumin powder degraded by the end of the TGA run (Figure 2.2). These results were similar to the results obtained in the work conducted by Sharma and Luzinov (Sharma S 2012). For the DSC data, the endothermic dip began at 75°C with a broad peak between 120 – 125°C. This indicated that the material had fully passed its transition phase—denaturation. An endothermic decomposition or pyrolysis peak occurred at 250°C, which exhibited the onset of degradation. Therefore, the albumin-based bioplastics were molded at 120.5°C, as this was the safe temperature of processing albumin into the plastics with as little degradation occurring as possible.

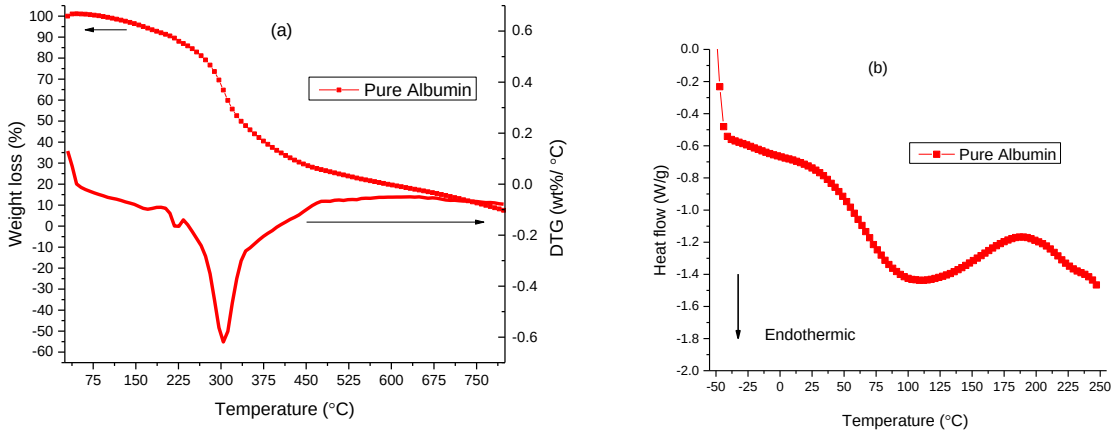


Figure 2.2: Thermographs of pure albumin powder, (a) TGA, (b) DSC.

Based on the albumin being fully denatured between 120-125°C without degradation, it was determined that the plastics were to be molded higher than this temperature, but below temperatures where degradation occurs (Figure 2.2).

Dynamic Mechanical Analysis

In the plastics with water as a plasticizer, we found that as the amount of water was increased, the initial storage modulus of the resulting plastics decreased, with the tan delta peak occurring at 70 °C (Figure 2.3(a)). This was consistent with the research conducted by González-Gutiérrez et al (González-Gutiérrez 2011). The increased water content caused an increase in the initial tan delta values, as well as caused the tan delta peaks to decease in temperature (or lowered glass transition temperature) and occurred at lower temperatures, which indicated the increased viscous heat dissipation. The shifted curves indicated that the 75/25 albumin-water formulation was the most desirable of the blends examined, as this formulation possessed the mix of a modulus that was comparable to the other water plasticized samples (and higher than the 70/30 albumin-water formulation), while possessing an elasticity (Tan δ) that was much higher than the other formulations (and equal to the 70/30 Albumin-Water formulation), as

shown in Figure 2.3(a). The same trends occurred in the albumin plastics that had glycerol as a plasticizer—the higher percent led to the higher initial tan delta and lower modulus, as well as the shifting of the tan delta peaks to the left (Figure 2.3(b)). However, at lower content of both water and glycerol, the bioplastics showed anti-plasticization and plasticization phenomenon (Galdeano, Mali et al. 2009). Based on the results, we determined that the 75/25 albumin-glycerol was the composition with the highest overall tan delta peak as well as the moderate modulus values (Figure 2.3(b)).

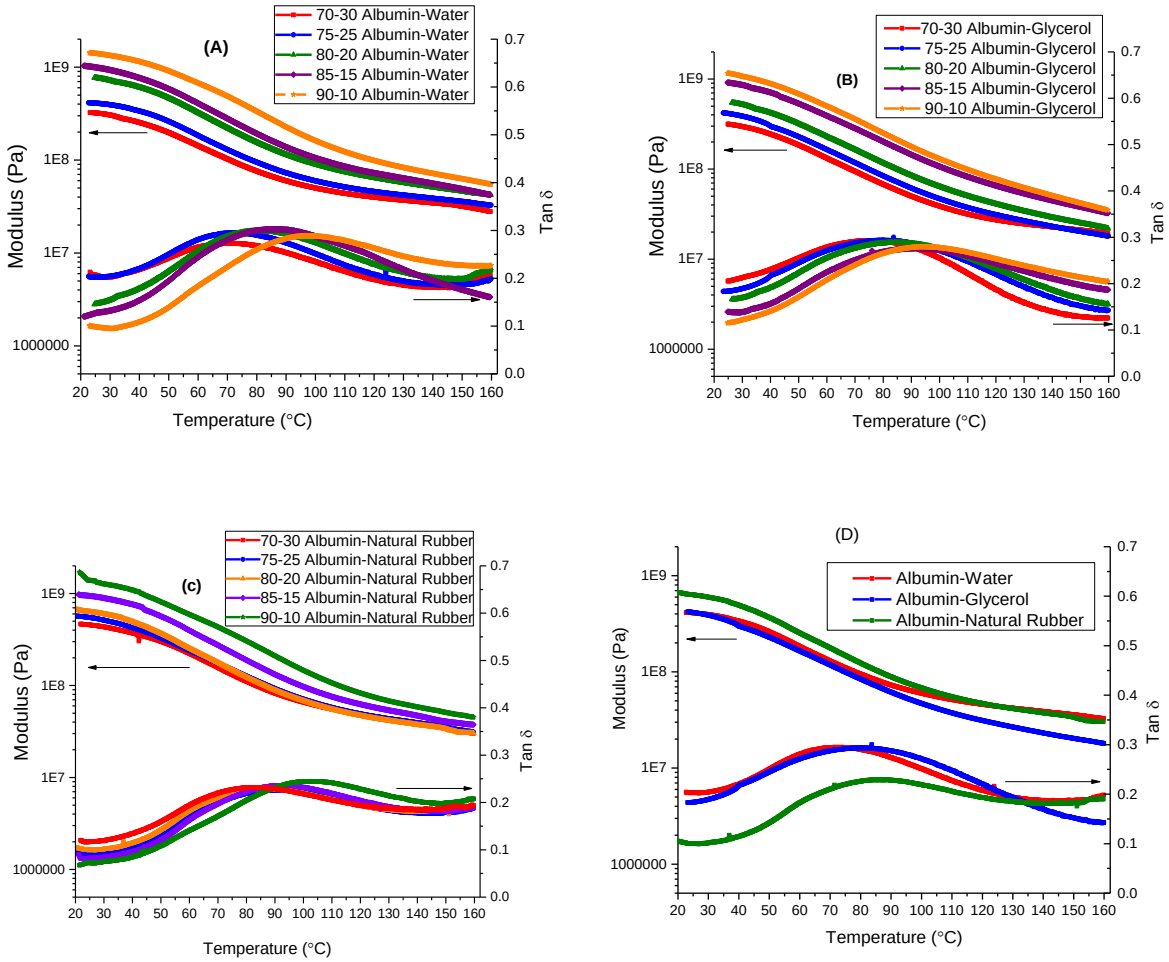


Figure 2.3: Dynamic mechanical analysis of initial albumin plastics: (a) albumin-water, (b) albumin-glycerol, (c) albumin-natural rubber, (d) optimum blends of each plastic.

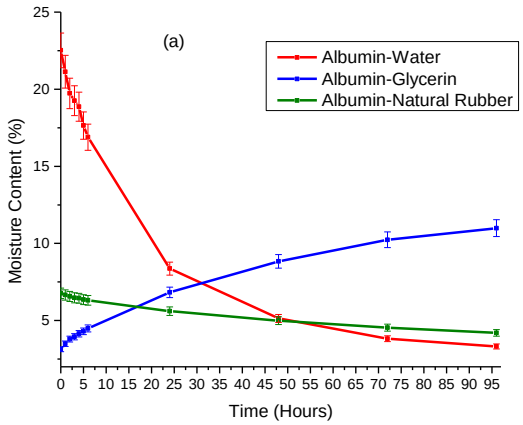
For the albumin plastics with natural rubber latex (NRL) as the plasticizer, we observed the same trends, although there was very little difference in the initial tan delta values, (Figure 2.3(c)). The 80/20 albumin-NRL formulation possessed the optimal mix of high initial modulus and tan delta as its tan delta values were comparable to the 70/30 albumin- and the 75/25 albumin-NRL. However, the 80/20 albumin-NRL bioplastics possessed a higher initial modulus while having a tan delta peak at a lower temperature than the bioplastics that contained lower weights of rubber (Figure 2.3(c)). When we compared the plastics based on the types of plasticizer used, we found that the initial modulus was similar for all three plasticizers, but the natural rubber-plasticized bioplastics exhibited the lowest initial tan delta values whereas other plasticizers (water and glycerol) showed the highest viscous heat dissipation (Pommet, Redl et al. 2005). With this analysis completed, it was determined that the optimum blends for albumin plastic production were 75/25 albumin-water, 75/25 albumin-glycerol, and 80/20 albumin-NRL (Figure 2.3(d)).

Time Study

Bioplastic Moisture Gain/Loss Analysis

The 75/25 albumin-water bioplastics demonstrated a large decrease in moisture content within 48 hours of molding, losing an average of between 15-17% of the initial moisture content (Figure 2.4). However, after the initial loss of moisture content, the plastics maintained stable moisture content after 96 hours of molding. This loss in moisture content was due to the loss of water from the bioplastic as time progressed, producing a stiffer and more brittle plastic (Van Soest 1997). While the water based bioplastics lost moisture content, the 75/25 albumin-glycerol steadily increased in moisture content after molding, reaching 10% in moisture content 96 hours after molding, as the time progression correlated (0.978) with an increase in moisture content

from the environment. The gain in moisture content was due to a combination of glycerol leaching from the bioplastics, and the plastic absorbing ambient moisture. This could render this plastic unsuitable for most applications, as the resulting water absorption would alter the properties of the plastic, reducing its tensile strength. While the water- and glycerol- based plastics underwent a comparatively large change in moisture content during the study ($p = 0.002$), the 80/20 albumin-NRL bioplastics were more stable in terms of moisture content, maintaining moisture content between 5% and 7% on average throughout the study, as it was significantly equivalent to the albumin-glycerol values ($p = 0.472$). This moisture content stability was due to natural rubber being stable and nonreactive in the environment, with the only moisture loss due to the loss of water that was in the natural rubber latex molding base (Carvalho 2003).



Plasticizer	Mean	Standard Deviation	SE of mean	95% CI of Mean	Variance	Corrected Sum of Squares	Coefficient of Variation
Water	14.2442	7.45988	2.24924	(9.23258, 19.25581)	55.64975	556.49746	0.52371
Glycerol	5.83631	2.88814	0.87081	(3.89604, 7.77659)	8.34134	83.41339	0.49486
Natural Rubber	5.90156	0.91934	0.27719	(5.2834, 6.51919)	0.84518	8.45185	0.15578

	Water	Glycerol	Natural Rubber
Correlation	-0.91936266	0.97825976	-0.98230022
P Value for Albumin-Water and Albumin-Natural Rubber	0.002025024		
P Value for Albumin-Water and Albumin-Glycerol	0.002015512		
P Value for Albumin-Glycerol and Albumin-Natural Rubber	0.472126155		

Figure 2.4: Moisture content of albumin plastics over time: (a) moisture content variation over time chart, (b) statistical analysis of each plasticizer type table.

Based on our findings in the study, it was determined that natural rubber provided the most stability in terms of moisture content, as the other plasticizers either lost moisture over time (water-based bioplastic) or gained moisture (glycerol-based bioplsatic) (Figure 2.4).

Bioplastic Dynamic Mechanical Analysis

The 75/25 albumin-water plastics showed the most significant amount of change: the modulus drastically increased after five days of conditioning (initial = 2.5 E8 Pa, 5 days = 1.8 E9 Pa), with lower initial tan delta values (initial = 0.23, 5 days = 0.09); and tan delta peak shifted to the right (Van Soest 1997) (Figure 2.5(a)). These characteristics pointed to the unbound water being released over time under ambient conditions, which reduced the ability of water to plasticize, as shown in past studies (Verbeek 2010). The change in properties of these plastics over time was most likely due to the amount of moisture loss that occurs over time, which resulted in a stiff plastic. This drastic change in the properties of the albumin-water plastics over time pointed to a lack of usability in the material.(Figure 2.5(a)). The 75/25 albumin-glycerol plastics demonstrated almost completely opposite results, though the amount of change over time was not as drastic when compared to albumin-water plastics (Figure 2.5(b)). The conditioning for the glycerol-based plastics led to the lowering of initial modulus (initial = 5.4 E8 Pa, 5 days = 2.6 E8 Pa) and a slight increase in tan delta (initial = 0.17, 5 days = 0.21), as well as the general lowering and shifting to the left of the tan delta peak. These changes in viscoelastic properties were most likely due to the gradual leaching of glycerol from the plastic, with ambient moisture taken in to replace it, weakening the hydrogen bonds within the plastic in the process (Lodha 2005). For the 80/20 albumin-NRL plastics, there was very little change in the plastic after it was allowed to condition for 24 hours, with the tan delta and modulus values essentially identical after conditioning (Figure 2.5(c)). This lack of change in properties may have been due to natural

rubber lacking the ability to react to the environment. If given enough time it will remain bonded to the albumin and maintain its basic properties (Carvalho 2003).

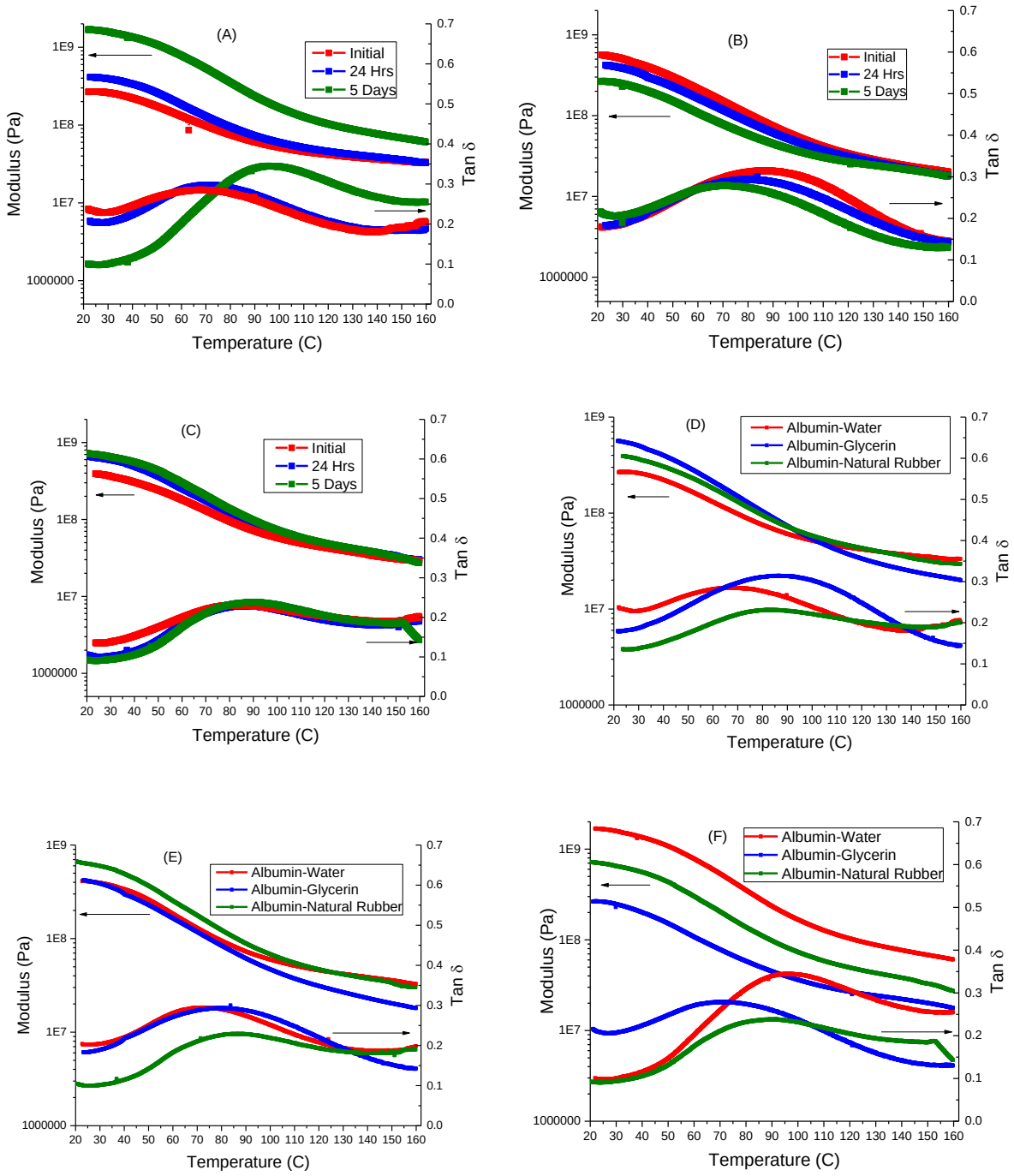


Figure 2.5: Dynamic mechanical analysis of time study on albumin plastics. (a) albumin-water, (b) albumin-glycerol, (c) albumin-NRL, (d) initial plastics, (e) 24 hour plastics, (f) 5 day plastics.

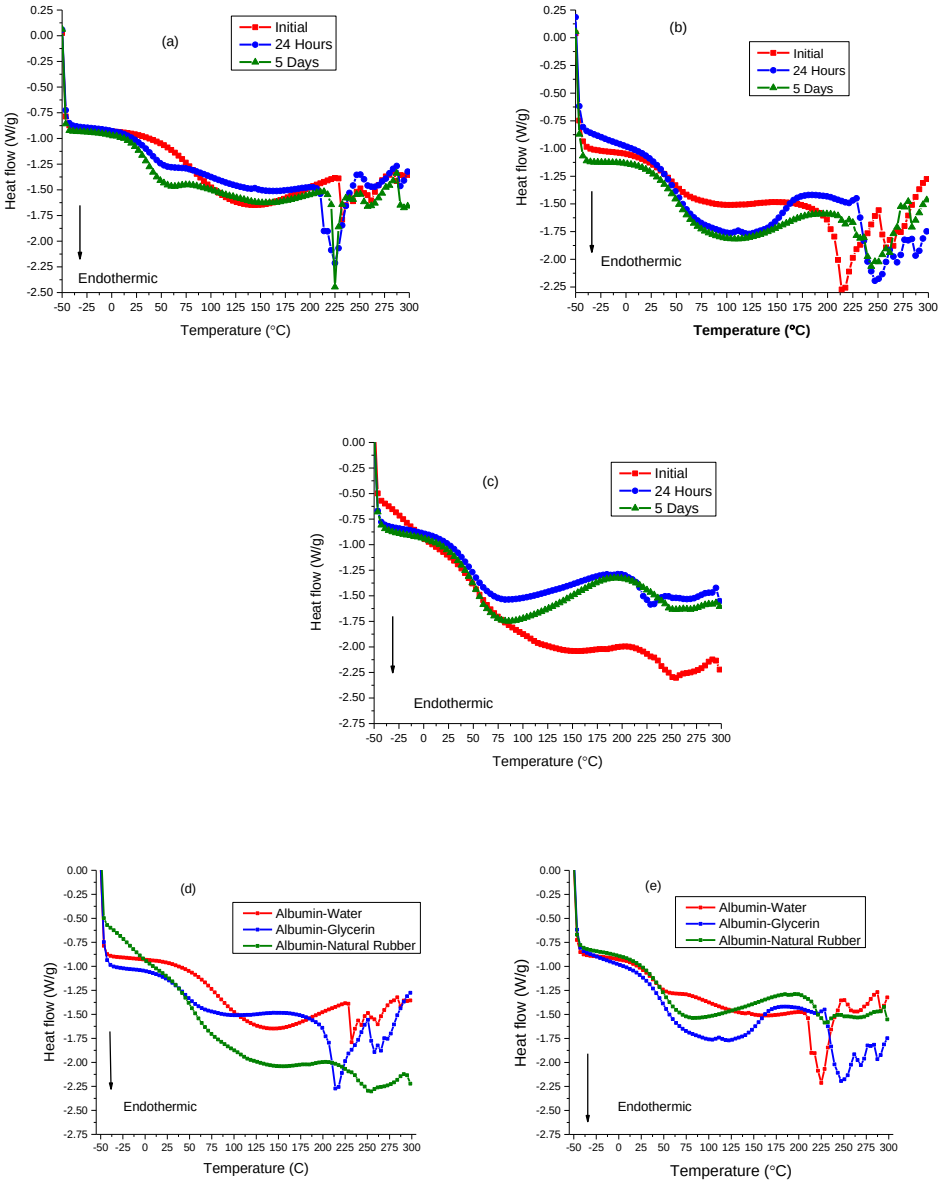
When the plasticizers are compared, the 75/25 albumin-water plastics gradually became the most brittle over time, as their modulus increased and tan delta decreased. The 75/25 albumin-glycerol underwent the exact opposite in properties, as they initially started as the most brittle of the plastics, but at the end of the experiment became the most ductile due to its loss of modulus and maintaining its initial tan delta. As for the 80/20 albumin-NRL plastics, they were the most consistent of the plastics. After the decrease of tan delta and increase of modulus after 24 hours, their viscoelastic properties normalized (Figures 2.5(d) through (f)).

Bioplastic Thermal Analysis

In the 75/25 albumin-water plastics, the glass transition temperature range of 40-60 °C was more evident in the 24 hour and five day samples (Figure 2.6a), while for all three samples, an endothermic peak was seen around 225°C. This peak in the albumin-water bioplastics may be attributed to the degradation or decomposition of protein polymers. For the 75/25 albumin-glycerol plastics, the glass transition phase of 50-110 °C was also very noticeable, with a small dip beginning at 180 °C (due to glycerol) and a larger endothermic peak between 215-220 °C for bioplastics that had been molded on the same day (Figure 2.6b). The larger endothermic peaks were at 250 °C for the 24 hour and five day samples. This shift in protein decomposition to the higher temperature could have been attributed to the absorption of moisture and reorganized polymer chains due to the displacement of unbound glycerol molecules (Chen 2005). As for the 80/20 albumin-NRL samples, a much clearer glass transition of 40-80 °C occurred with the 24 hour and 5 day samples in comparison to the initial sample, with all three samples beginning to have an endothermic decomposition peak at 225 °C (Figure 2.6c).

When the plastics are compared with each other, it was found that the natural rubber-based bioplastics underwent a much more noticeable endothermic enthalpy change initially, but

after conditioning it recovered to normal glass-transition phase, similar to the other plasticizers used (Figures 2.6(d) through 5(f)).



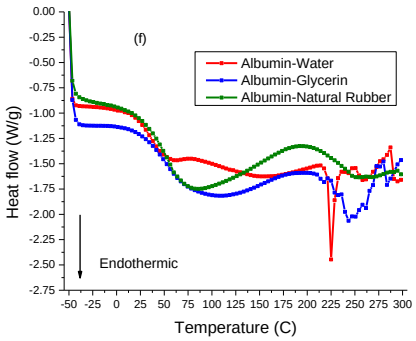


Figure 2.6: Differential scanning calorimetry of time study on albumin plastics. (a) albumin-water, (b) albumin-glycerol, (c) albumin-NRL, (d) initial plastics, (e) plastics after 24 hours, (f) plastics after 5 days.

In the thermogravimetric analyses, we found that the amount of time after molding did not have an effect on the amount of mass loss at higher temperatures, as all of the curves were similar depending on the type of plasticizer used. The 75/25 albumin-water plastics possessed one degradation peak at 300 °C, where the protein within the plastic begins to degrade, while a small drop at the beginning of each curve was due to moisture loss (Figure 2.7(a)). This moisture loss was more evident in the initial plastic, as this sample had the highest amount of moisture, resulting in a mass drop between 50 – 100 °C. For the 75/25 albumin-glycerol plastics, there was one bimodal degradation peak for all of the samples (Figure 2.7(b)). The first peak began at 225 °C, which was most likely due to glycerol degradation (flashpoint of glycerol is around 180 °C, with mass loss occurring in nitrogen gas environments at 199°C (Castelló 2009)) within the bioplastic; the peak was right shifted, most likely due to stabilization in the albumin matrix. The second peak between 300 – 325 °C was most likely due to the albumin protein itself degrading. The 80/20 albumin-NRL samples also possessed a bimodal degradation peak, although we found that the peaks were in different temperature ranges compared to one in glycerol plasticized bioplastics (Figure 2.7(c)). For instance, the first peak seen at 300 °C was the initial degradation of the protein in the plastic. However, the second peak seen at a higher temperature at 375°C was

most likely due to the natural rubber latex degradation, with rubber degrading between 350-360°C (Mathew 2001). The initial sample of natural rubber latex sample also degraded at a higher rate and a slightly lower temperature due to the water contained in the natural rubber latex molding base still being present in the plastic.

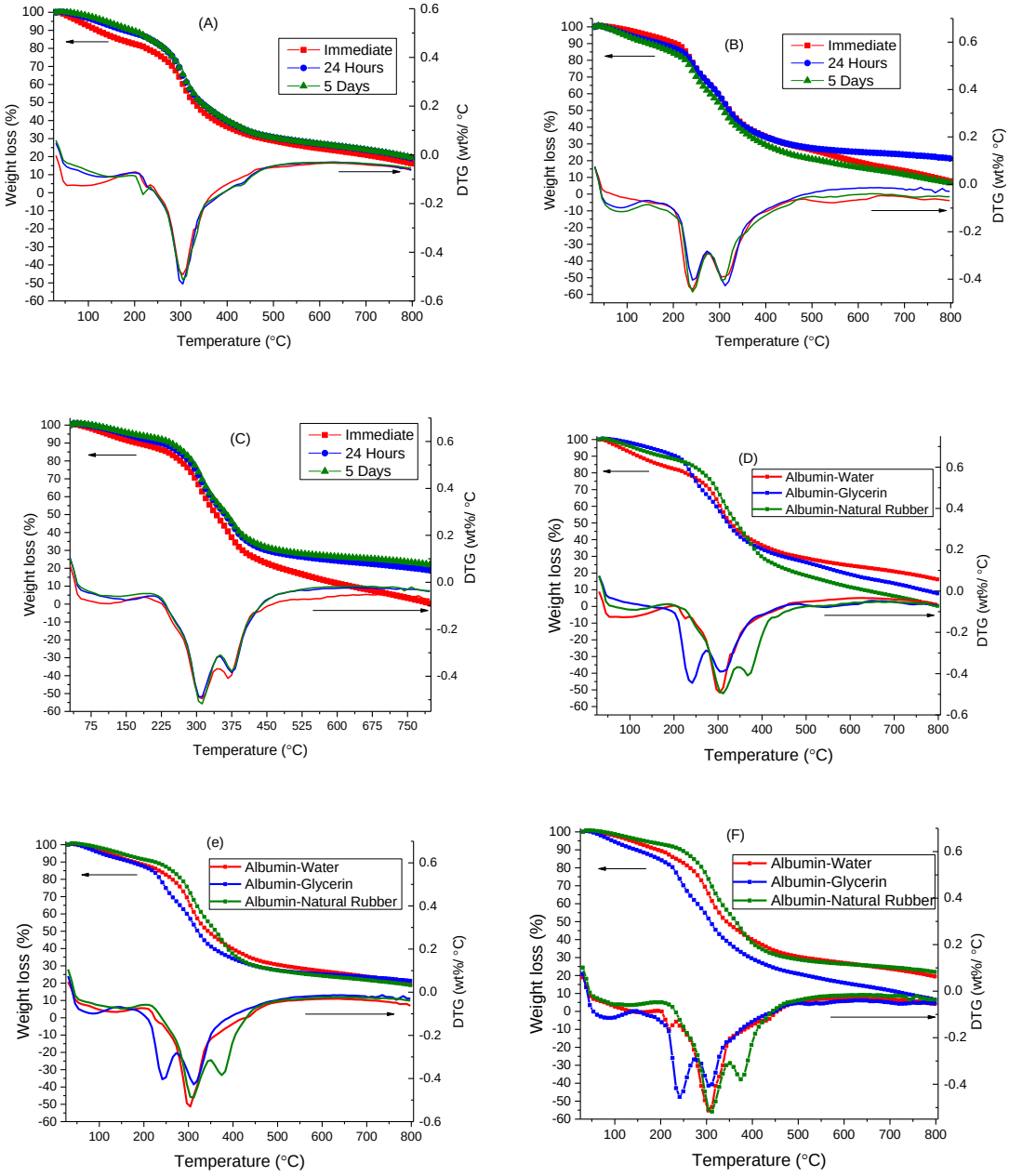


Figure 2.7: Thermogravimetric analysis of time study on albumin plastics. (a) albumin-water, (b) albumin-glycerol, (c) albumin-NRL, (d) initial plastics, (e) plastics after 24 hours, (f) plastics after 5 days.

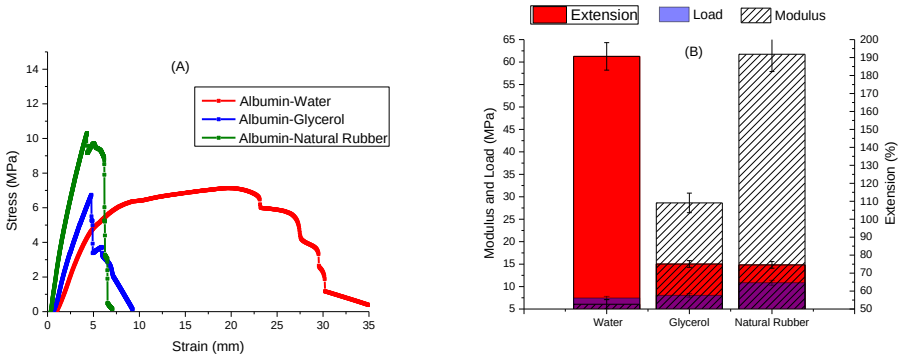
When all three plasticizers are compared, we found that as time progressed, the albumin-glycerol plastics possessed the lowest onset of degradation temperature, which may have been due to the increase of water moisture contained in the plastic leading to the weakening of protein-protein bonds in the plastic (Figures 2.7(d) through 5(f)). The albumin-water and albumin-NRL plastics showed the higher onset degradation temperatures. This was most likely due to the natural latex making crosslinks with plastic matrix and the water-based plastic becoming stiffer over conditioning time due to restructured polymer chains. Once the degradation of the plasticizer had occurred, however, it was found that the water and natural rubber plastics would reach about the same rate of mass loss, with the glycerol plastics losing almost all of their mass over the time of the experiment.

Overall, based on the DSC data, it was found that after conditioning for 24 hours the plastics maintained consistent values. As for the TGA data, it has been determined that the albumin-natural latex plastics had the highest onset degradation temperatures, while the 75/25 albumin-glycerol plastics had the lowest temperature for degradation onset.

Tensile Properties of Bioplastics

In terms of the amount of extension that occurred before breaking, the 75/25 albumin-water plastics possessed by far the highest amount of extension, extending nearly 200% on average before a ductile break; the other plastics extended around only 75% before breaking (Figure 2.8), as the glycerol and natural rubber plastics were statistically undistinguishable ($p = 0.943$). One possible reason why the water was able to facilitate a higher extension (but not load bearing) could have been due to the bonding that would occur within the structure as plasticization occurred, as found in previous research (Pommet, Redl et al. 2005, Verbeek 2010). We found that the 80/20 albumin-NRL plastics required a much higher load to break the samples

(around 12 MPa) and inherently had a much higher modulus near 60 MPa. For the 75/25 albumin-water and 75/25 albumin-glycerol plastics, the maximum loads that we observed were indistinguishable from each other, as a p-value of 0.757 illustrates. This ability for the albumin-NRL plastics to undergo a high load before plastic deformation may have been due to the natural rubber providing more load-bearing material in the structure of the plastic, counteracting any potential losses due to long-range plasticization prevention. When the rubber serves as a load bearing constituent of the plastic, it was possible for the plastic to undergo a higher amount of stress before breaking (Carvalho 2003). Comparing each of the plastic types overall, it was evident that the ductile 75/25 albumin-water and the 80/20 albumin-NRL plastics were the best types of bioplastic to use, The water-based samples allowed large amounts of extension before breaking, while the NRL-based samples were stiff and required the highest amount of load needed to break the samples. As for the brittle 75/25 albumin-glycerol plastics, there was very little benefit in terms of tensile properties. These plastics possessed neither the extension nor the strength that the other plastics possessed. The weak tensile properties of the Albumin-Glycerol plastics could have been explained through disordered conformations, as the relatively large chemical structure of glycerol prevented any long-range plasticization to occur (Aman Ullah 2011). When long range plasticization was prevented, there was a limit to how much force a plastic could have undergone. The short polymer chains were broken under stress, resulting in a violent break at a lower stress than with a long polymer chain.



Plasticizer Type	Load (MPa)	5% Error Range	Extension (%)	5% Error Range	Modulus (Mpa)	5% Error Range
Water	7.43717	0.37186	61.26078	3.06304	52.64266	2.63213
Glycerol	8.03141	0.40157	15.031	0.75155	109.10194	5.4551
Natural Rubber	10.84041	0.54202	14.824	0.7412	191.83989	9.59199

	Max Load				Extension				Modulus		
	Water	Glycerol	Natural Rubber		Water	Glycerol	Natural Rubber		Water	Glycerol	Natural Rubber
Standard Deviation	0.656	0.909	2.120		15.420	4.864	3.985		37.652	61.105	40.860
P-Value for Water and Glycerol	0.757				0.007				0.101		
P-Value for Water and Natural Rubber	0.020				0.002				0.001		
P-Value for Glycerol and Natural Rubber	0.038				0.943				0.040		

Figure 2.8: Tensile properties of time study on albumin plastics after 24 hours of conditioning. (a) stress-strain curve, (b) modulus, load, and extension chart,(c) statistical values of modulus, load, and extension.

When we examined the tensile properties obtained through this study, we found that the results were similar to the results obtained in the study by Jerez et al. In their study, they determined that the lower processing temperature of the albumin bioplastic molding process resulted in modulus values that were similar to polymers such as low density polyethylene (LDPE) and high density polyethylene (HDPE) (Jerez A 2007). This discovery made possible for the use of albumin plastics in place of LDPE and HDPE in certain applications, as the reduction of protein as a total percentage of the blend and the lower processing temperature lowers the costs of plastic production.

Based on the results, we determined that the 80/20 albumin-NRL plastics were able to undergo the greatest amount of stress, while the 75/25 albumin-water plastics were able to undergo the greatest amount of strain before breaking (Figure 2.8).

Scanning Electron Microscopy Images of Bioplastics

When the 75/25 albumin-water plastics were analyzed through scanning electron microscopy, the pictures showed that when the plastic was broken, a scratched and pitted surface was the result, pointing to the fact that the break was not clean and that the mixture of the albumin and plasticizer was fairly homogenous (Figure 2.9(a)). This scratched surface indicated the high level of stress needed to break the sample, which could also have been seen in the high extension of the albumin-water bioplastics (Figure 2.8). For the 75/25 albumin-glycerol bioplastics, the spotted surface of the broken plastic was of highest interest. These images could be evidence of the glycerol leaching from the plastic on a much smaller scale, with the moisture being removed from the sample when the SEM chamber was sealed under high vacuum (Figure 2.9(b)). As the glycerol slowly leached into the environment, pores could form inside the plastic, with moisture from the environment causing the pores to absorb water. When the SEM chamber was sealed and moisture was vacuumed from the testing chamber, cracks formed in the plastic because the moisture was being removed from the plastic. With the 80/20 albumin-NRL bioplastics, what was most evident with these pictures was the lack of homogenous mixture of the albumin and the rubber, as there were pockets of albumin and pockets of rubber throughout the whole plastic sample (Figure 2.9(c)). The jagged surface of the plastic also illustrated the amount of force that was required to break the plastic, as the break would have been sudden and drastic (Carvalho 2003). This abrupt breaking point was also illustrated in the tensile strength and modulus results (Figure 2.8); the latex was able to hold the plastic together until giving way under a high load. Based on the results of this analysis, we determined that it was glycerol leaching in the 75/25 albumin-glycerol plastics that altered the properties of the plastic, while multiple phases of material in the 80/20 albumin-NRL plastics could have been observed.

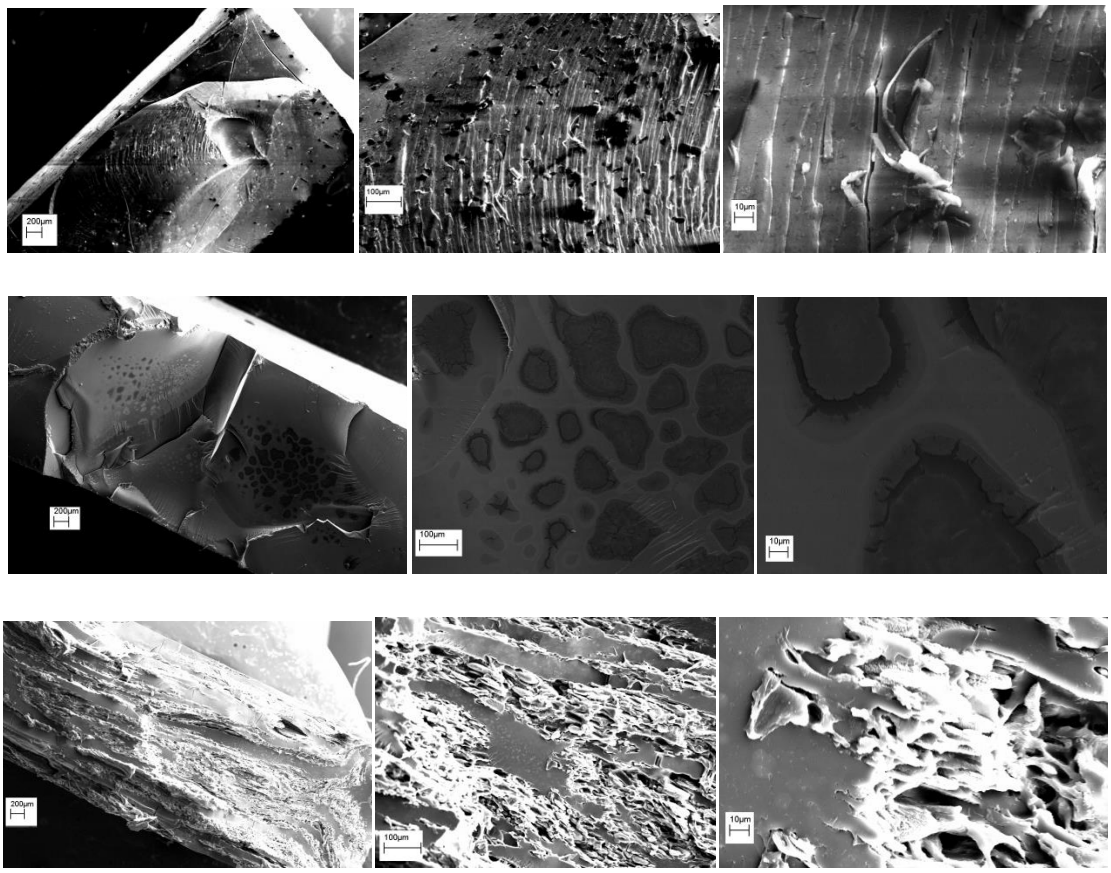


Figure 2.9: Scanning electron microscopy images of albumin bioplstics. (a)albumin-water, (b) albumin-glycerol, (c) albumin-NRL. Magnification of 20x, 100x, and 500x.

Conclusions

When comparing the amounts of plasticizer used in molding bioplastics from albumin, we found that the blending ratios that had the best combination of initial modulus and elasticity were the 75/25 albumin-water, 75/25 albumin-glycerol, and 80/20 albumin-NRL. Of these blends, we found that the 80/20 albumin-NRL bioplastic provided the best thermal, viscoelastic, and tensile properties, while the properties of the 75/25 albumin-water and 75/25 albumin-glycerol plastics were not comparable due to moisture loss in the water-based bioplastics over time, while glycerol leaching occurred in the glycerol-based bioplastics as time passed. There are multiple avenues of interest that should be examined in the light of the knowledge gained in this study. In order to determine whether the albumin-NRL (as well as albumin-water for short

term uses) plastics would be suitable for medical applications or not, further studies are needed to ensure that the plastics will inhibit bacterial growth in order to prevent post-operation infection, as well as potentially aid in the application of drugs through elution. Another area of interest requiring further research is the continued modification of the blending by altering the percentages of each component used, as well as adding different materials into the blends in order to reach the optimum properties for the other applications. As for the aspect of moisture content, it would be beneficial to examine the possible uses of other materials that would limit the amount of moisture content change that would occur with albumin plastics, as it has been demonstrated that this area has a significant effect on the overall properties of the plastic.

References

1. Mukerjee T, K. N., PLA based biopolymer reinforced with natural fibre : a review. *Journal of Polymers and the Environment* **2011,** *19*, 714-725.

2. Flieger M, K. M., Prell A, Řezanka, T, Votruba J, Biodegradable plastics from renewable sources. *Folia Microbiology* **2003,** *48* (1), 27-44.

3. Sharma S, H. J., Luzinov I., Biodegradable plastics from animal protein coproducts: Feathermeal. *Journal of Applied Polymer Science* **2008,** *110*, 459-467.

4. Jerez A, P. P., Martínez I, Gallegos C, Guerrero A, Protein-based bioplastics: effect of thermo-mechanical processing. *Rheologica Acta* **2007,** *46*, 711-720.

5. Hook A, e. a., Combinatorial discovery of polymers resistant to bacterial attachment. *Nature Biotechnology* **2012**, 1-10.

6. Widiastuti, I., Sbarski, I, Masood, SH, Creep behavior of PLA-based biodegradable plastic exposed to a hydrocarbon liquid. *Journal of Applied Polymer Science* **2012**.

7. Dorigato, A., Pegoretti, A, Biodegradable single-polymer composites from polyvinyl alcohol. *Colloid and Polymer Science* **2012,** *290* (4), 359-370.

8. Baron F, R. S., Compounds with antibacterial activity. In *Bioactive Egg Compounds* R. L.-F. o. Rainer Huopalahti, M. A., and Rüdiger Schade, Ed. Springer-Verlag Berlin: Heidelberg, 2007; pp 191-198.

9. Cegielska-Radziejewska R, L. G., Szablewski T, Kijowski J, Physico-chemical properties and antibacterial activity of modified egg white—lysozyme. *European Food Research and Technology* **2010,** *231*, 959-964.

10. Peters Jr., T., *All About Albumin: Biochemistry, Genetics, and Medical Applications*. Academic Press: San Diego, California, 1996.

11. Zilberman, A., and Elsner, J J, Antibiotic-eluting medical devices for various applications. *Journal of Controlled Release* **2008,** (130), 202-215.

12. Sue, H. J., Wang, S, Lane, J L, Morphology and mechanical behaviour of engineering soy plastics. *Polymer* **1997,** *38* (20), 5035-5040.

13. Menard, K., *Dynamic Mechanical Analysis: A Practical Introduction*. CRC Press: Boca Raton, Florida, 1999.

14. Sharma S, L. I., Water aided fabrication of whey and albumin plastics. *Journal of Polymers and the Environment* **2012,** *20*, 681-689.

15. González-Gutiérrez, J., Partal, P, and García-Morales, M, Effect of processing on the viscoelastic, tensile and optical properties of albumen/starch-based bioplastics. *Carbohydrate Polymers* **2011,** *84* (11), 308-315.

16. Galdeano, M. C.; Mali, S.; Grossmann, M. V. E.; Yamashita, F.; Garcia, M. A., Effects of plasticizers on the properties of oat starch films. *Materials Science & Engineering C-Biomimetic and Supramolecular Systems* **2009,** *29* (2), 532-538.

17. Pommet, M.; Redl, A.; Guilbert, S.; Morel, M.-H., Intrinsic influence of various plasticizers on functional properties and reactivity of wheat gluten thermoplastic materials. *Journal of Cereal Science* **2005,** *42* (1), 81-91.

18. Van Soest, J. J. G., and Knooren, N., Influence of glycerol and water content on the structure and properties of extruded starch plastic sheets during aging. *Journal of Applied Polymer Science* **1997,** *64* (7).

19. Carvalho, A. J. F., Job, A.E., Alves, N., Curvelo, A.A.S., and Gandini, A., Thermoplastic starch/natural rubber blends. *Carbohydrate Polymers* **2003,** *53* (1), 95-99.

20. Verbeek, C. J. R., van den Berg, L E, Extrusion processing and properties of protein-based thermoplastics. *Macromolecular Materials and Engineering* **2010,** *295* (1), 10-21.

21. Lodha, P., and Netravali, A. N., Thermal and mechanical properties of environment-friendly 'green' plastics from stearic acid modified-soy protein isolate. *Industrial Crops and Products* **2005,** *21* (1), 49-64.

22. Chen, P., Zhang, L., and Cao, F., Effects of moisture on glass transition and microstructure of glycerol-plasticized soy protein. *Macromolecular Bioscience* **2005,** *5* (9), 872-880.

23. Castelló, M., Dweck, J, Aranda, DAG, Thermal stability and water content determination of glycerol by thermogravimetry. *Journal of Thermal Analysis and Calorimetry* **2009,** *97* (2), 627-630.

24. Mathew, A., Packirisamy, S, Thomas, S, Studies on the thermal stability of natural rubber/polystyrene interpenetrating polymer networks: thermogravimetric analysis. *Polymer Degradation and Stability* **2001,** *72*, 423-429.

25. Aman Ullah, T. V., David Bressler, Anastasia L. Elias, and Jianping Wu, Bioplastics from feather quill. *Biomacromolecules* **2011,** *12*, 3826-3832.

CHAPTER 3

PROTEIN-BASED BIOPLASTICS AND THEIR ANTIBACTERIAL POTENTIAL[2]

[2] Alexander Jones, Abhyuday Mandal, and Suraj Sharma. 2015. Journal of Applied Polymer Science. **132**:18. Reprinted with Permission. Modified to include data on Zein bioplastics (no statistical analysis).

Abstract

The use of conventional petroleum-based plastics in many applications poses the risk of contamination, potentially causing infection when in medical applications, and contamination when used in food packaging. Nontraditional materials such as protein are being examined for their potential use in the production of bioplastics for applications that require uncontaminated materials. The proteins of albumin, soy, and whey provide possible sources of raw material for bioplastic production, as they have already been utilized in the area of edible films and low-stress applications. We conducted this study to investigate the thermal, viscoelastic, and antibacterial properties of the albumin, soy, and whey bioplastics with the use of three plasticizers—water, glycerol, and natural rubber latex (NRL). *Bacillus subtilis and Escherichia coli* were utilized as Gram (+) and Gram (-) species, respectively, for antimicrobial analysis. Albumin and whey bioplastics exhibited similar thermal and viscoelastic properties, whereas soy bioplastics had varied viscoelastic properties based on the plasticizer used. In terms of antibacterial activity, the albumin, whey, or zein plastics plasticized with glycerol were the best bioplastics, as no bacterial growth was observed on the plastics after 24 hours of inoculation of Gram + and – bacterial species. In terms of the future impact of this research, the aim will be to scale up production of the bioplastics for use in food packaging as well as biomedical applications.

Keywords

bioplastics, antibacterial, sustainability, plasticizers, food packaging

Introduction

The cost of contamination through conventional plastics in numerous applications has been examined for the material being wasted, as well as the physical harm done to individuals.

For instance, in 2002, 4.5 out of every 100 hospital admissions resulted in a hospital-acquired infection in the United States, with over 99,000 deaths being the end result (Peleg 2010). There is also a fiscal cost to hospital-acquired infections, as a sustained illness will require additional hospital visits. In a study by Gould, an outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) would result in a doubling of the cost of a hospital visit, with an overall cost between $1.5 and $4.5 billion dollars in the United States on a yearly basis (Gould 2006). Based on the findings by Neely and Maley, both MRSA and vancomycin-resistant enterococci (VRE) were able to survive at least one day when inoculated onto the surface of materials commonly used in healthcare applications, with some microorganisms being able to survive for more than 90 days (Espert 2004). It is because of these issues that materials that could provide antimicrobial properties are being examined for biomedical applications, as that would help in containing or reducing the hospital-acquired infections.

Another area in which contamination is a notable risk is food packaging, where the material is in contact with food that will be consumed. According to a review study by Lau and Wang, there are five different aspects in which traditional plastics will contaminate food: the gradual degradation of the plastic that contains the food, compounds such as benzene and bisphenol A that are incorporated in the molecular structure of the plastic; contamination caused by the environment; contamination due to the processing agents utilized in order to produce the plastics; and other contaminants that are specific to the type of monomer utilized (Lau 2000). Food contamination by traditional plastics is caused by the use of a polymer that was not incorporated in the food product itself, leading to the migration into the food. There are three interrelated stages that occur when food becomes contaminated by the plastic packaging:

diffusion that occurs within the polymer, solvation of the migrant at the food-polymer interface, and the dispersion of the migrant into the bulk of the food product (Lau 2000).

In order to determine alternative materials such as proteins to be used in plastics, thermal and viscoelastic analysis must first be conducted to determine their suitability for the given application. In a study by Sharma et al., the protein albumin from egg-white denatures at a temperature of 136.5°C ± 3°C, ensuring protein's ability to orient and form a bioplastic.(Sharma 2008) This alteration of the protein orientation was due to the breaking of hydrophobic interactions and hydrogen bonds of the protein itself, allowing the bioplastic to form. Moreover, bioplastics undergoing cyclic loading multiple times did not cause failure, a phenomenon typically associated with conventional plastics (Sharma 2008). Another protein that has been used extensively in the production of bioplastics is soy protein isolate (~90-95% protein). In a study by Paetau et al., the optimal temperature of soy plastic thermomechanical molding was between 120 and140°C, as higher temperature led to thermal degradation and affected properties during molding (Paetau 1994). The tensile and viscoelastic properties of the resulting bioplastics were highly dependent on the moisture content of the soy protein and the molding temperature. For instance, soy protein with a lower moisture content possessed greater tensile properties when molded at 120°C, whereas soy protein with a higher moisture content exhibited higher tensile properties when molded at 140°C (Paetau 1994). Whey protein, byproduct of cheese production, would also be a suitable choice for bioplastic production, as it has been used extensively in the area of edible film (Gounga 2007). For whey proteins, the minimum temperature of molding into a film was 104°C, with degradation starting above 140°C (Sothornvit 2003).

It is because of the contamination issue with traditional plastics in applications where contamination is possible that biopolymers made from proteins are being examined for their

potential use in medical applications. In a review conducted by Qiu et al., it was found that biopolymers could promote antimicrobial activity in three ways: the creation of an anti-adhesive surface, the disruption of cell-cell communication through antibacterial agents, or lysing the cell membrane to kill the bacteria (Qiu 2007). Albumin protein (not in bioplastic film) has been studied for its antimicrobial in clinical research and treatment. Albumin is able to exhibit antimicrobial properties through its enzyme, lysozyme that utilizes a lysis reaction to kill cells (Baron 2007). Another protein that could be utilized in applications that require antimicrobial properties is whey. Whey has been found to contain immunoglobulins and glycomacropeptides, constituents that bind toxins and help prevent bacterial infection (Yalcin 2006). It is also possible to promote the antimicrobial activity of protein based bioplastics through the use of additives, which possess antimicrobial activities. For instance, when additives such as grape seed extract and nisin were added to the soy protein during plastic production, the plastic inhibited microbial growth (Sivarooban 2008). In another study, wheat gluten and eggwhite bioplastics loaded with bioactive agents, formic acid and oregano essential oil demonstrated antimicrobial activity (Martínez, Partal et al. 2013). Also of note are the areas of antifouling and anti-adhesive properties of plastic surfaces to prevent microbial adhesion to the surface (Page 2009). Our objectives in this study were to determine the thermal and viscoelastic properties of albumin, soy, and whey bioplastics through the use of water, glycerol, and natural rubber latex plasticizers, and to evaluate the antibacterial properties of these bioplastics.

Materials and Methods

Materials

Albumin (purity ≥ 99%) and ultra-high molecular weight polyethylene powder (particle sizes of 53-75 microns) were obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA);

the soy protein edible (protein content ≥ 72%) was acquired from MP (Solon, OH, USA); biPro whey protein (purity ≥ 99%) was obtained from Davisco Foods Int'l, Inc. (Le Sueur, MN, USA); and zein purified protein was acquired from Acros Organics (New Jersey, USA). Plasticizers were purchased through various sources: deionized water was supplied by a water filtering system in the lab; glycerol was obtained from Sigma-Aldrich with a purity ≥99%. A 70% solid, 30% water mixture of natural rubber latex (pH = 10.8) was acquired from the Chemionics Corporation (Tallmadge, OH, USA). In a study by Tarachiwin et al. on natural rubber from *Hevea brasiliensis*, the small rubber particles (SRP) showed mean diameter less than 250 nm whereas larger rubber particles (LRP) showed mean diameter larger than 250 nm.(Tarachiwin, Sakdapipanich et al. 2005) For antibacterial analysis, various materials were purchased for testing: Bacto tryptic soy agar and broth from Bectin, Dickinson and Company (Sparks, MD, USA); Dey-Engley neutralizing broth from Remel (Thermo Scientific, Suwanee, GA, USA); agar-agar solution that consisted of granulated Agar-Agar from EMD (Gibbstown, NJ, USA) and sodium chloride from Baker (Phillipsburg, NJ, USA); and phosphate buffered saline solution from HiMedia (Mumbai, India). The bacterial species of *Bacillus subtilis* (Gram (+)) and *Escherichia coli* (Gram (-)) were provided through Dr. Jennifer Walker and the Department of Microbiology at the University of Georgia.

Thermal Analysis of Raw Material

Thermal gravimetric analysis (TGA) was performed using a Mettler Toledo TGA/SDTA851e, with material examined from 25°C – 500 °C under a N_2 atmosphere with a heating rate of 10 °C min^{-1}. Differential scanning calorimetry (DSC) was performed using a Mettler Toledo DSC821e, with materials examined from -50 °C to 250 °C under a N_2 atmosphere with a heating rate of 10 °C min^{-1}. For all sample testing, the weight of each sample

was set between 2.0 – 4.0 mg to ensure consistent results and determine optimum plastic molding conditions.

Preparation of Compression Molded Samples

The molding of bioplastic blends was performed on a 24-ton bench-top press (Carver Model 3850, Wabash, IN, USA) with electrically-heated and water-cooled platens. Stainless steel molds were used to form dog bone-shaped bioplastics for antibacterial plastic analysis. To form the plastics, protein and plasticizers were mixed manually in predetermined w/w ratios to be placed into the molds (as indicated throughout the paper). The mixture of protein and plasticizers were prepared in small batches of varying masses based on density of materials for dog bone plastics (≤ 6 g for albumin and soy, ≤ 5 g for whey, and ≤ 4 g for polyethylene and zein), while the DMA flexbars were made of 2 g of plasticized proteins. Subsequently, the mixture was filled into the flexbar and dog bone cavity of the stainless steel molds, with plungers placed on top of the molds to prevent the mixture from leaking. After covering with a plunger, the molds were then compressed for a 5-minute molding time at 120 ºC, followed by a 10-minute cooling period for the protein plastics. For the polyethylene plastics, a 20-minute compression molding time at 150 ºC followed by a 10-minute cooling period was utilized. Both the bioplastic and polyethylene samples were prepared under a pressure of at least 40 MPa, as a certain minimum amount of pressure must be applied in order to be able to mold a plastic.(Sue 1997) After the samples were cooled for 10 minutes under pressure, the pressure was released and the samples were removed. The plastic samples were conditioned at 21.1 °C and 65% relative humidity for 24 hours before characterization through dynamic mechanical analysis and antibacterial testing.

Dynamic Mechanical Analysis

DMA flex bars of the protein plastics were analyzed for their viscoelastic properties through the use of dynamic mechanical analysis (DMA)(Menard 1999) by using a DMA 8000 Dynamic Mechanical Analyzer from Perkin Elmer. The analyzer examined the viscoelastic properties of the plastics by determining both the storage and loss modulus. The two types of moduli differ by which storage modulus (E′) is an indication of the elastic region of the material where energy is stored, while loss modulus (E′′) is the amount of energy that is dissipated through heat in the viscous region. The resulting moduli were then put in ratio form (E’’/E’) to calculate tan δ, which denotes the viscoelasticity of a given material(Fried 2003). DMA was conducted from 25°C to 160°C, with a temperature ramp of 2 °C min^{-1}. The settings of the analyzer were set to dimensions of 9×2.5×12.5 mm^3 using a dual-cantilever setup at a frequency of 1 Hz with a displacement of 0.05 mm, within the range of plastic deformation. Each sample type was analyzed in duplicate.

Mechanical Properties

The mechanical properties of the conditioned bioplastics were measured by using the Instron testing system (Model 3343) interfaced with the Blue Hill software. The test was performed according to the standard test method for tensile properties of plastics (ASTM D 638-10, Type I) with a 5 mm min^{-1} crosshead speed, a static load cell of 1000 N, and a gauge length of 4 cm. Samples were run in quintuplicate (n=5) for each blend type in order to ensure precise measurement.

Scanning Electron Microscopy (SEM)

Soy, whey, and zein SEM samples (n =2 for each protein-plasticizer combination) were prepared from cryogenic DMA flex bar fracture surfaces after being placed in a conditioning

chamber (21.1°C and 65% relative humidity) for at least 24 hours. DMA flex bars were submerged in liquid nitrogen for 20 seconds; after that they were immediately broken. The samples were mounted, then sputter coated for 60 seconds with an Au/Pt mix. SEM images were recorded on a Zeiss 1450EP variable pressure scanning electron microscope. Coated samples were analyzed at 20X, 100X, and 500X for each blend type.

Antibacterial Testing of Plastics

The antibacterial properties of the conditioned plastics were measured using the ASTM E 2180-01 standard test method, in which the aqueous based bacterial inoculum remains in close, uniform contact in a "pseudo-biofilm" state with the bioplastic. For each blend type, the Gram (+) specie *Bacillius subtilis* and the Gram (–) specie *Escherichia coli* were utilized as challenge bacterial cells to determine the efficacy of bacterial growth on the plastic surfaces. After equilibration of standardized culture banks of $1\text{-}5\times10^{8}$ cells/mL through the use of dynamic light scattering analysis, 1 mL of the culture was applied to 100 mL of agar slurry for inoculation. Once inoculated, the slurry was then applied to a 9 cm^2 area of the bioplastics that had been swabbed with phosphate-buffered saline to promote adhesion by reducing surface tension. After the appropriate time of application of agar (within one hour for 0-h samples and at least 24h for 24-h samples after incubation), the agar was removed through the use of neutralizing broth, followed by sonicating and vortexing each for 1 min. The neutralizing broth containing the agar was diluted five times in a 10^{-1} dilution set, and then the dilutions were applied to tryptic soy agar plates, which were incubated for 24 h at 37 °C. After incubation, the culture plates were counted for microbial growth and averaged to determine colony forming units (CFU)/mL. Samples were run in triplicate ($n = 3$) for each protein-plasticizer combination (as well as the polyethylene plastic control sample) in order to ensure precise measurement.

Fourier-Transformed Infrared (FTIR) Spectroscopy of Plastic Sample Surface

The surface of the protein plastic samples were examined for chemical properties through FTIR analysis. Infrared spectroscopy studies were done using a Thermo-Nicolet model 6700 spectrometer equipped with a variable angle grazing angle attenuated total reflection (GATR-ATR) accessory (Harrick Scientific).

Statistical Analysis

Statistical analyses were performed by fitting a regression model. For each plastic-plasticizer blend tested, bacterial growth for 0-hour and 24-hour samples were analyzed by fitting two-way ANOVA using the statistical software of SAS and R. Box-Cox transformations were used to determine the appropriate transformations needed to satisfy the normality assumptions of the experimental errors. As the dataset has several very big and small values, Cook's distances were examined to ensure that no individual observation is an outlier that influences the conclusions.

Results and Discussion

Material Analysis

Thermal Properties of Proteins and Bioplastics

An initial degradation peak (Figure 3.1) was observed for both soy and whey between 70-80 °C, indicative of bound moisture loss, while for albumin it was between 220-230 °C (Jones 2013). Much larger degradation peaks started at different temperatures for each of the proteins: 245-250 °C for the albumin powder; 190-200 °C for soy protein; 200-210 °C for the whey protein; and 220-230 °C for the zein protein. At the end of the TGA run, 75% of the protein powders degraded, as the proteins were similar in the overall level of degradation due to the burning of the proteins (Figure 3.1a). For the DSC data, we find endothermic peaks of 70-80 °C

for all of the proteins, an indication of the denaturing of the pure protein powders (Arntfield 1981), with potential onsets of degradation after 220 °C (Ogale 2000). These results were similar to the results obtained in the work conducted by Sharma and Luzinov (Sharma 2012).

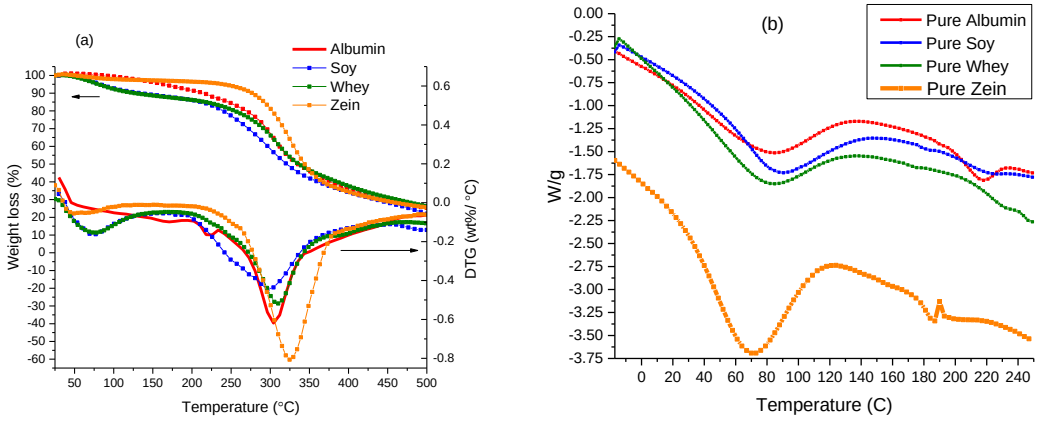


Figure 3.1. Thermographs of pure protein powders, (a) TGA, (b) DSC.

When compared to the optimum blends (Figure 3.2) of bioplastics, degradation peaks depended upon the plasticizer used, as plastics blended with water possessed similar thermal degradation peaks in comparison to plastics that did not contain any plasticizer. However, bimodal degradation peaks were witnessed in plastics prepared with glycerol and NRL, as the glycerol-based albumin and whey bioplastics possessed degradation peaks between 240-250 °C (below protein degradation peaks between 300-315 °C) while the NRL in albumin and soy bioplastics would degrade at temperatures higher than the proteins (approximately 375°C). This occurs due to the glycerol (Rahmana 2010) and natural latex (Rao 2008) that are bound within the plastics to begin degrading at temperatures that differ to glycerol or NRL that is not bound within a plastic For the DSC data, endothermic dips occurred at varying temperatures: a small peak beginning at 75 °C, with a broad peak at 120-125 °C for albumin (Jones 2013); a narrow peak starting at 50 °C and a broad peak at 85-90 °C for soy; and a narrow peak beginning at 35

°C, with a broad peak at 80-85 °C for whey protein. These peaks indicated that the material had fully denatured at lower temperatures for soy and whey (80-90 °C) due to higher bound moisture levels, whereas albumin denatured at a higher temperature between 120-125 °C. An endothermic decomposition or pyrolysis peak occurred at 250 °C for all of the proteins, which exhibited the onset of degradation, as amino acids degrade at temperatures in this region. Therefore, the protein-based bioplastics were molded at 120 °C to minimize thermal degradation while ensuring full denaturation leading to bioplastics.

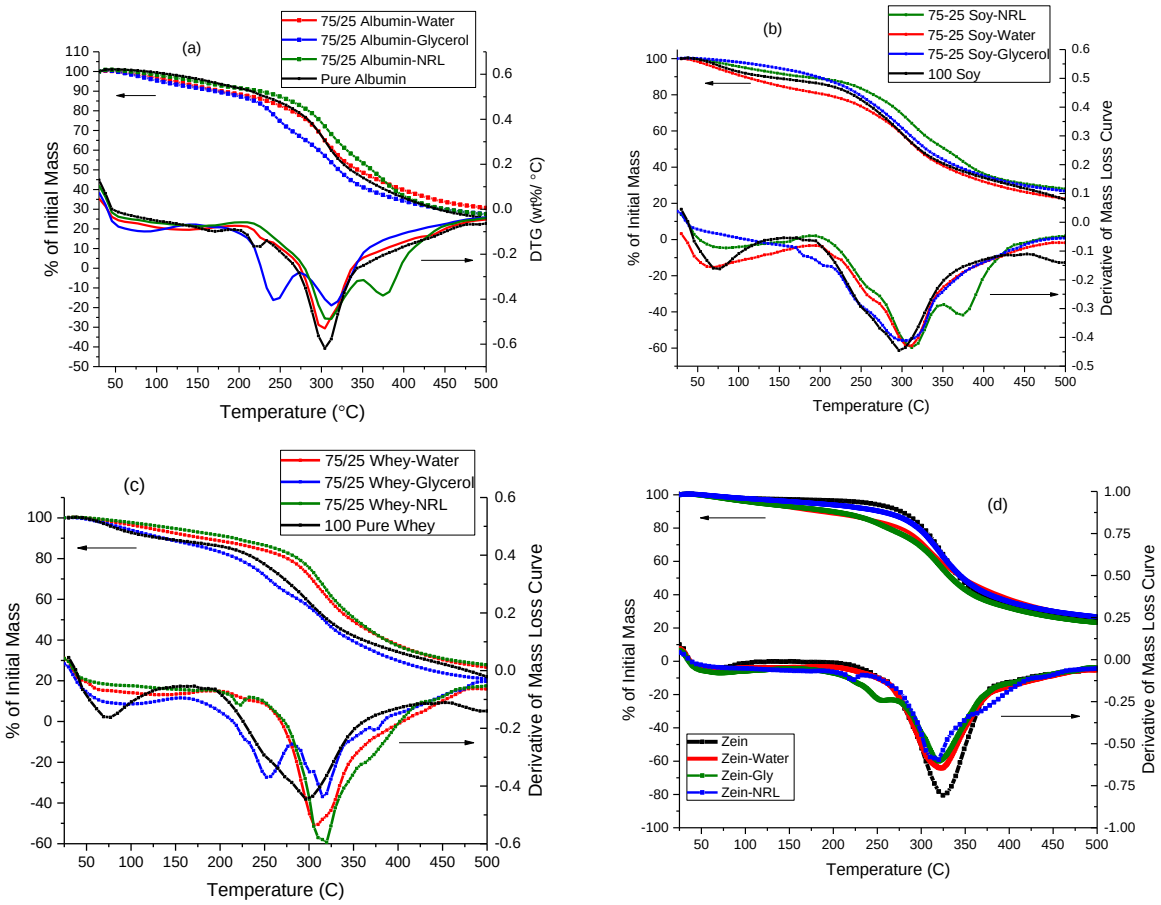


Figure 3.2. Thermogravimetric analysis of optimal protein plastic blends: (a) Albumin, (b) Soy, (c) Whey, (d) Zein.

When these results are compared to bioplastics that have been blended with plasticizers (Figure 3.3), the curves are similar in shape and peak areas unless water was utilized as a

plasticizer. In this case, endothermic peaks in albumin, whey, and zein bioplastics occurred between 220-225 °C, while in soy plastics the endothermic peaks occurred between 180-185 °C. One potential reason for this lowering of glass transition and degradation temperatures is the addition of water in the plastic increased polymer-water interactions, to the detriment of polymer-polymer interactions (Robertson 2013). Since it has been postulated that the effectiveness of plasticizers for bioplastics are highly dependent upon how they affect hydrogen bonding or hydrophobic interactions (Lunt 2001), that may be why this property is witnessed only in water-plasticized bioplastics.

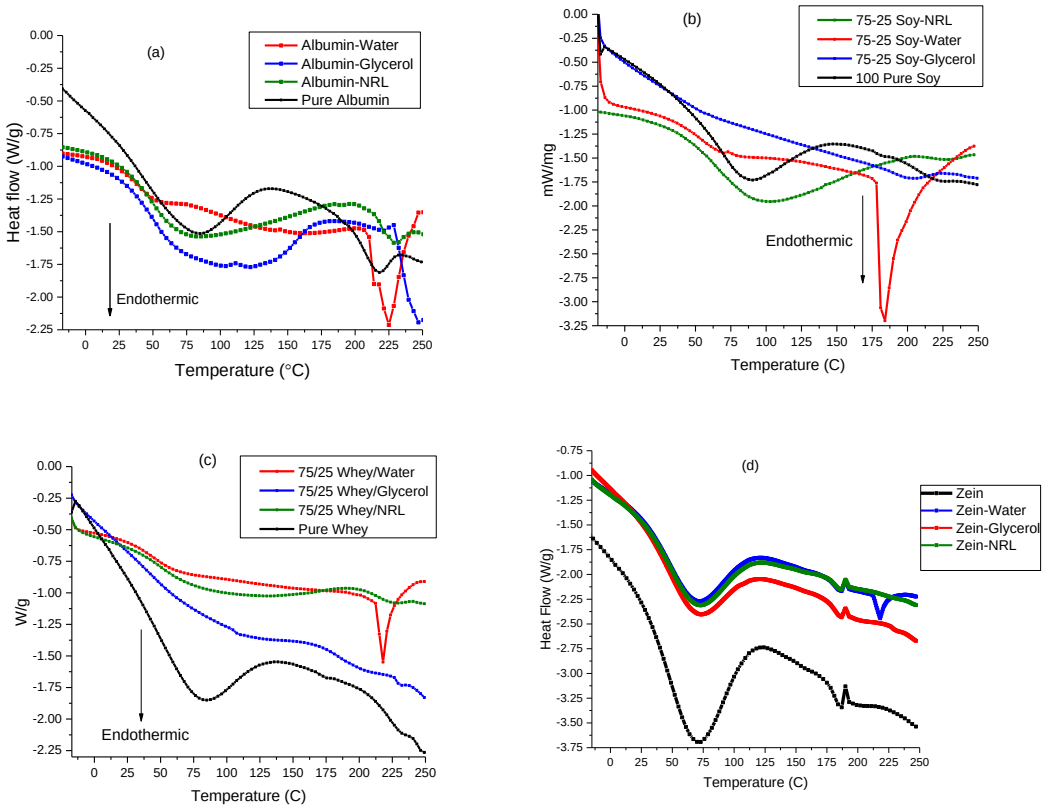


Figure 3.3. Differential scanning calorimetry of optimal protein plastic blends: (a) albumin, (b) soy, (c) whey, and (d) zein.

Dynamic Mechanical Analysis

In the albumin (Jones 2013) and whey plastics, we found that the plastics made with the plasticizers of water and glycerol had similar properties, as each had tan delta peaks occurring at lower temperatures in comparison with plastics plasticized with NRL (Figures 3.4(a) and 4(c)). While the albumin and whey bioplastics plasticized with water and glycerol possessed similar viscoelastic properties, the bioplastics plasticized with natural rubber possessed a lower initial tan delta, the tan delta peak occurring at higher temperatures, as well as a higher initial modulus. These results point to higher levels of protein-glycerol or protein-water interactions and less protein-protein interactions in the thermoplastic hydrophilic polymers (albumin or whey), thereby shifting the tan delta peaks (glass transition) to lower temperature with higher initial tan delta values as well as dropping the elastic modulus (E') than plastics that do not possess any plasticizer (Galietta 1998). Moreover, the bioplastics produced in the absence of plasticizers were stiff as evident from the higher elastic or storage modulus throughout the temperature of DMA testing. This phenomenon explains the breaking of protein-protein interactions and favoring the protein-plasticizer interaction, thereby producing flexibility in the resulting bioplastics. However, NRL seems less effective plasticizer for albumin or whey proteins as we see resulting bioplastics behaving more or less like stiff material with higher elastic modulus and lower tan delta values.

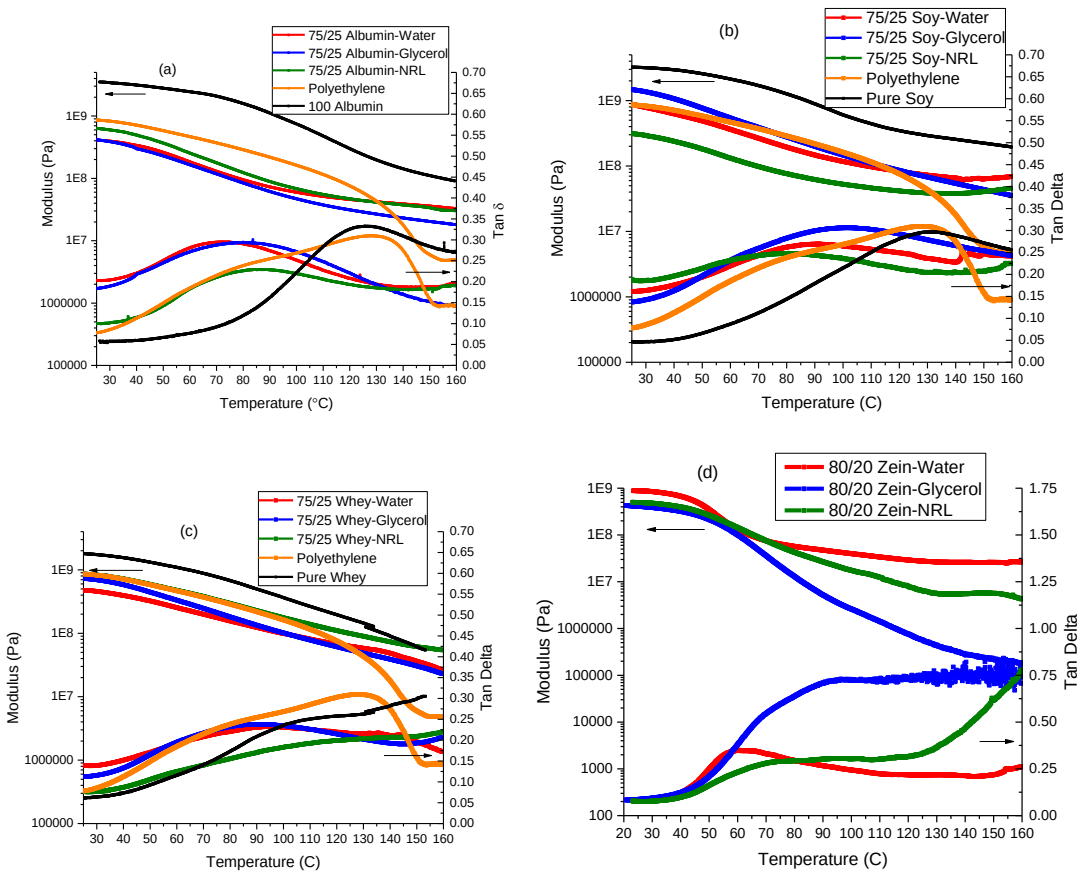


Figure 3.4. Dynamic mechanical analysis of optimal protein plastic blends: (a) albumin, (b) soy, (c) whey, and (d) zein.

The soy-glycerol and soy-water plasticized plastics displayed the highest modulus and lowest initial tan delta, as well as the highest tan delta peak temperatures as compared to their counterpart proteins, albumin and whey; these findings were consistent with the work by Zhang et al. Soy proteins possess strong intra- and inter-molecular interactions, such as hydrogen bonding, dipole–dipole, charge–charge, and hydrophobic interactions that promote stiffness or brittleness of soy plastics. Glycerol and water may be unable to break up intermolecular bonds to the same level as in whey and albumin based plastics (Zhang 2001). However, the opposite was found for the soy-NRL plastics, as they possessed the highest initial tan delta values and lowest initial modulus, differing from the albumin-NRL and whey-NRL plastics (Figure 3.4(b)). The possible explanation is that NRL plasticized soy plastics had less dispersed rubber particles (or

probably bigger phases of rubber particles), leading to a ductile material compared to NRL plasticized whey and albumin plastics. For zein plastics, it was found that the use of water as a plasticizer will lead to a plastic that will have higher initial modulus when compared to zein-glycerol and zein-NRL plastics. In terms of the tan delta values, there is a sharp increase in the tan delta of zein-glycerol plastics after 60 °C, as reaching temperatures past glass transition may induce viscous flow in materials. These phenomena were also corroborated in the tensile performance as presented in the next section.

Tensile Testing

In terms of the amount of strain placed on the plastics, the albumin-water bioplastics were able to withstand the most strain by far, extending over 70% on average before a ductile break (Figure 3.5a through d). When the plastics are compared based on protein content, the NRL plasticized albumin bioplastics failed at the stress levels over 14 MPa, while the water or glycerol plasticized failed near 8 MPa. These findings could have been due to increased hydrogen bonding that occurs during plasticization when plasticized with water or glycerol, while the NRL (because of more protein-plasticizer interaction) could serve as an additional load bearing constituent in the plastic. (Jones 2013)

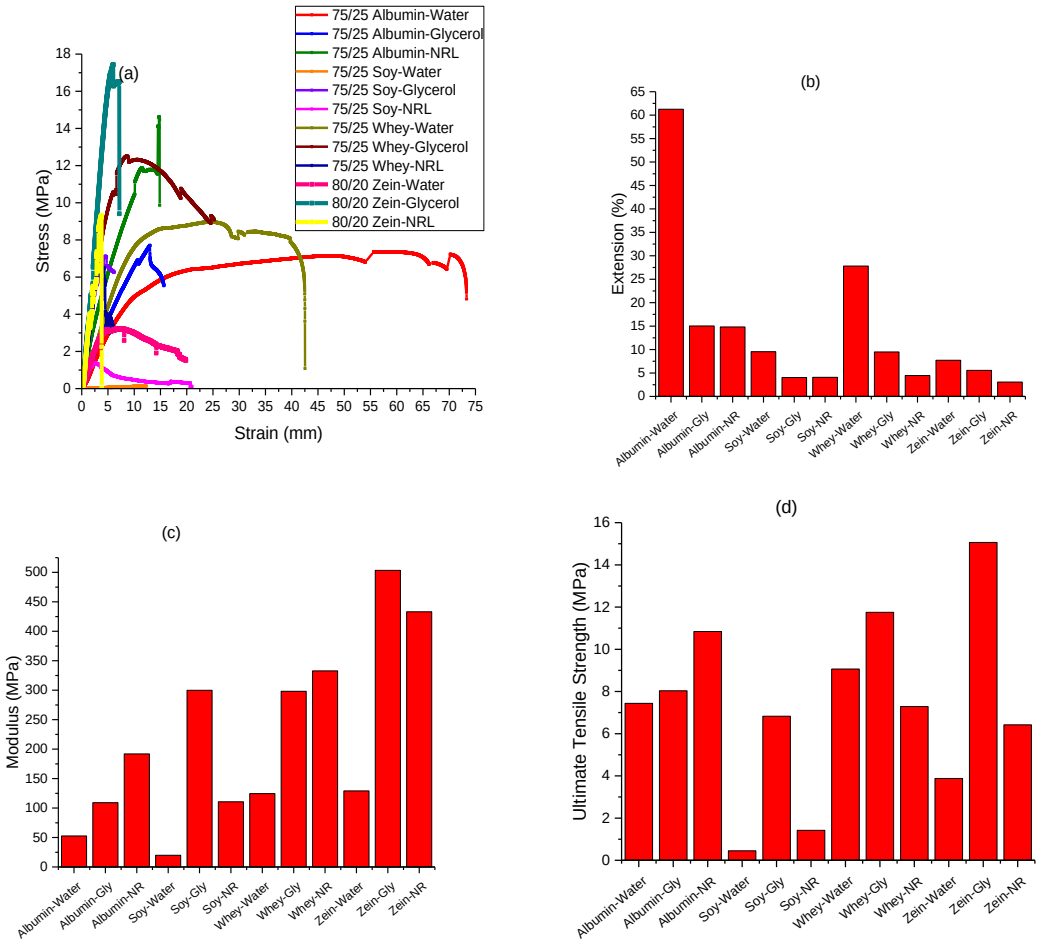


Figure 3.5. Tensile properties of optimal protein plastic blends: (a) Stress-strain curves, (b) Elongation, (c) Modulus, and (d) Ultimate tensile strength. Gly: glycerol, NRL: natural rubber latex. (e) Zein tensile properties

For soy plastics, poor tensile properties were evident, as the plastic that was able to withstand the greatest amount of load (soy/glycerol) was only 7.5 MPa with brittle fracture, consistent with the findings made by Schilling et al (Schilling 1995). This lack of ability for the soy plastics to undergo high stress or strain may be due to the soy protein lacking the ability to form a structure that possesses long range orientation when plasticizers are utilized. As for the whey plastics, the whey plastics that have been plasticized with water performed similarly to the albumin/water plastics. The whey/water plastics were able to withstand only 27.5% of strain before breaking, but able to withstand over 8 MPa of stress. For whey protein it was found that when glycerol is utilized as a plasticizer, the plastic was able to withstand 12.5 MPa of stress and

9.8% of extension before failure, an extension that was similar to observe by McHugh and Krotcha (McHugh 1994). When plasticized with NRL, the whey plastics possessed minimal tensile properties, as the protein may not be able to form a suitable structure during plasticization. As for the zein plastics, it was found that through the use of glycerol we obtain a material that will have a higher modulus (17.75 MPa) when compared to when plasticized with water (3 MPa) or NRL (9.25 MPa). The use of water as a plasticizer will result in an elongation of 19.84%, while glycerol-plasticized zein will have a higher extension (7.18%) when compared to NRL-plasticized zein plastics (3.8 %).

When the plastics are compared to each other based on elongation and modulus, we determined that the albumin plastics prepared with water possessed higher levels of elongation compared to any other plastic, but whey blended with NRL plastics possessed the highest modulus values (Figure 3.5b). In comparison, the soy plastics possessed few tensile properties that would be comparable to the other proteins, as the modulus in the soy/glycerol plastics was the only tensile property that was similarly seen in other protein plastics.

Antibacterial Testing

Influence of Bioplastic Formulations.

The above mentioned bioplastics produced using optimal level of various plasticizers were then evaluated for their antibacterial performance in comparison to a polyethylene (PE) control sample. For the polyethylene control samples a moderate level of growth (15.37%) by the Gram (-) and Gram (+) species was observed with a resulting CFU/mL value of $6.13*10^7$ after 24 h (Figures 3.6 – 8). However, the result was statistically irrelevant at the 95% level, as neither the Gram (-) nor the Gram (+) contacted plastic samples possessed an α value lower than 0.05. These findings were consistent with the analysis conducted by Seyfriedsberger et al, as the

promotion/inhibition of bacterial growth was marginal due to polyethylene not possessing any inherent properties to modify bacterial growth settings (Seyfriedsberger 2006).

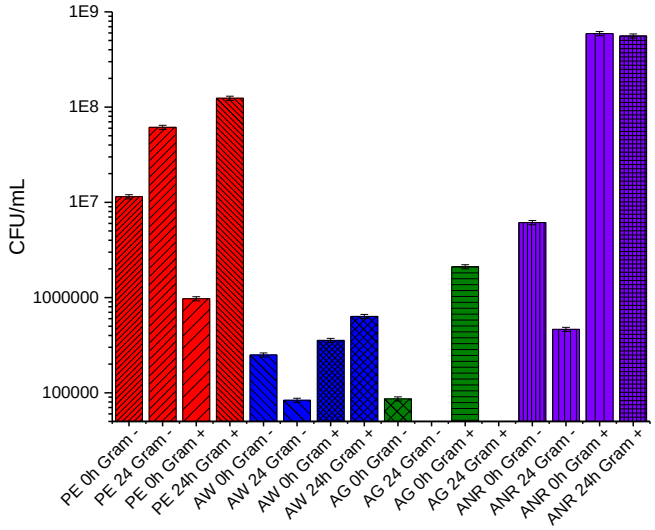


Figure 3.6. Antibacterial analysis of albumin protein plastic blends. PE: ultra high molecular weight polyethylene, AW: 75/25 albumin-water, AG: 75/25 albumin-glycerol, ANR: 75/25 albumin-NRL.

In the albumin bioplastics, we found that the plastics made with plasticizers water and NRL showed similar properties, as each were able to reduce the amount of bacterial growth by both Gram (-) and Gram (+) bacteria (Figure 6). However, only the albumin plasticized by water was statistically significant in limiting Gram (-) bacterial growth at the 95% confidence level ($\alpha = 0.013$), as the albumin-water bioplastic decreased the CFU/mL level to $8.36*10^4$ after 24 h of contact. The albumin-glycerol bioplastics in contrast possessed a strong inhibitive effect in antibacterial growth, as no growth after 24 hours occurred [Gram (-) $\alpha = 0.002$, Gram (+) $\alpha = 0.004$]. This may be attributed to bioactive property of albumin due to lysozyme enzyme (Padgett T 1998) plus the gradual leaching of glycerol from the plastic, as this creates an aqueous environment, preventing microbial adhesion and growth on the bioplastic. Evidence of glycerol leaching is illustrated in Figure 3.23 (Appendix), as the signature wavelengths of glycerol are

witnessed at 850, 925, 900, 1045, and 1117 cm^{-1} (Lodha 2005, Jiugao 2005). However, the glycerol leaching from the plastic may only be bacteriostatic in nature, as concentrations of at least 28 per cent of glycerol would be required for bacteriocidial properties (Werkman 1953).

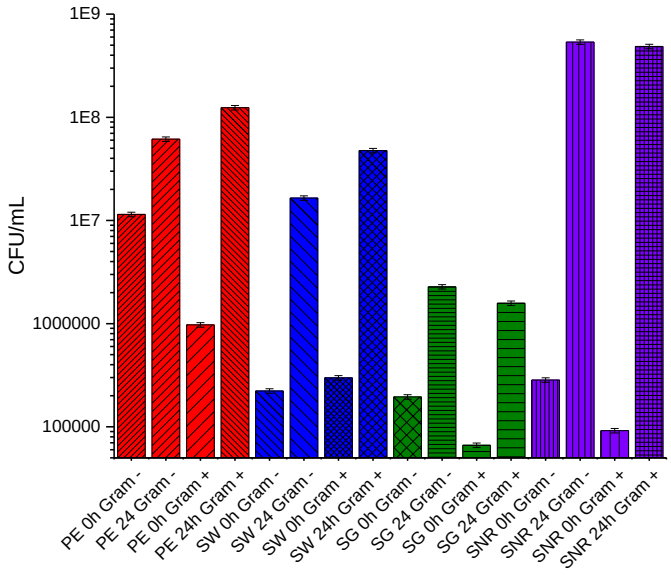


Figure 3.7. Antibacterial analysis of soy protein plastic blends. PE: ultra high molecular weight polyethylene, SW: 75/25 soy-water, SG: 75/25 soy-glycerol, SNR: 75/25 soy-NRL.

In the soy bioplastics, we found that none of the plastics were able to reduce the amount of bacterial growth by both Gram (-) and Gram (+) bacteria, as bacteria increased in growth after 24 hours on the soy bioplastics (Figure 3.7). The soy plasticized by water was even statistically significant, as it promoted Gram (+) bacterial growth at the 99% confidence level ($\alpha = 0.008$), increasing the CFU/mL to $4.76*10^7$. Of note is the soy bioplastics plasticized with glycerol, as overall lower rates of bacterial growth occurred in comparison to the soy plastics plasticized by water and NRL. We find these results were consistent with the findings of Padgett et al, as they determined that soy bioplastics to be more suitable for an edible plastic application than antimicrobial application when bacterial inhibitors are not incorporated into the plastics (Padgett T 1998, Cha 2004).

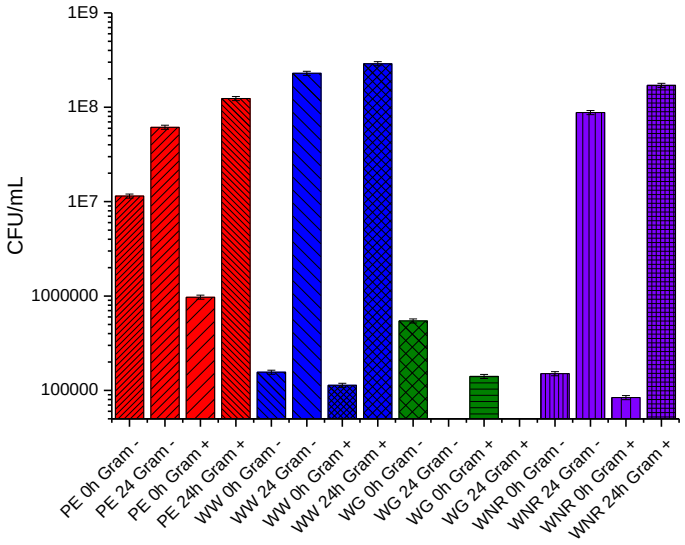


Figure 3.8. Antibacterial analysis of whey protein plastic blends. PE: Ultra High Molecular Weight Polyethylene, WW: 75/25 whey-water, WG: 75/25 whey-glycerol, WNR: 75/25 whey-NRL.

In the whey bioplastics, we found results were similar in relation to the soy bioplastics, as the plastics made with plasticizers, water and natural rubber were unable to reduce the amount of bacterial growth by both Gram (-) and Gram (+) bacteria (Figure 3.8). Statistically the results were even more drastic, as the whey plastics promoted Gram (-) and Gram (+) bacterial growth at the 99% confidence level ($\alpha < 0.001$ for water plasticized whey plastics, $\alpha < 0.002$ for natural rubber plasticized whey plastics). However, the whey bioplastics were similar to the albumin bioplastics when plasticized with glycerol, as they possessed a strong inhibitive effect in antibacterial growth, as no growth after 24 hours occurred (Gram (-) $\alpha = 0.002$, Gram (+) $\alpha = 0.019$). This antibacterial activity may be attributed to certain peptides that are contained in the structure of whey protein, as the three peptides of secretory leukocyte protease inhibitor, trappin-2, and elafin have been found to possess antimicrobial activity (Wiesner 2010). Like in the albumin-glycerol bioplastic, this also may be also due to the gradual leaching of glycerol from the plastic in the creation of an aqueous environment.

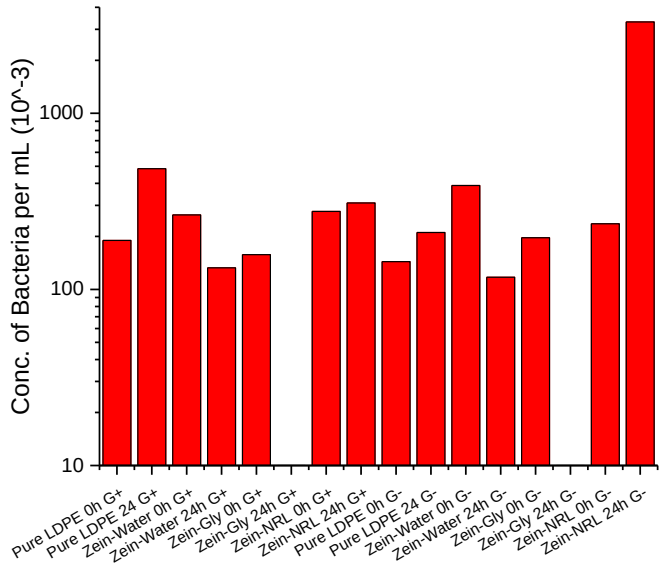


Figure 3.9. Antibacterial analysis of zein protein plastic blends. LDPE: Low Density Polyethylene, 80/20 zein-water, 80/20 zein-glycerol, 80/20 zein-NRL.

With surface antimicrobial analysis of zein bioplastics, we find that zein possesses similar antimicrobial properties as albumin when plasticized with glycerol, preventing any Gram + or Gram – growth after 24 hours of surface contact. However, there is no significant effect when plasticized with water or NRL. Like in whey and albumin plastics, this may be due to a combination of antimicrobial components that are found in zein protein (Torres-Giner 2009), as well as glycerol leaching from the plastic that could contain these compounds.

Statistical Analysis of Antibacterial Property of Bioplastics.

For the statistical analysis, response was the proportional change in count after 24 hours:

$$y = \frac{Count\ at\ 24\ h - Count\ at\ 0\ h}{Count\ at\ 0\ h} = \frac{Count\ at\ 24\ h}{Count\ at\ 0\ h} - 1$$

Mathematically, it was the same as considering $y = \frac{Count\ at\ 24\ h}{Count\ at\ 0\ h}$. We fit a linear regression model, separately for Gram (+) and Gram (-) bacteria, for the two-way layout given by

$$y_{ijk} = \eta + \alpha_i + \beta_j + \omega_{ij} + \epsilon_{ijk} \qquad (1)$$

for i =1 (Albumin), 2 (Soy), 3 (Whey); j = 1 (Water), 2 (Glycerol), 3 (NRL) and k = 1, 2, 3 were the three samples taken. Here y_{ijk} was the response corresponding to the kth sample with the ith level of protein and the jth level of plasticizer. Note that in our model we have 1 + 3 + 3 + 9 = 16 parameters (η, α_1, α_2, α_3, β_1, etc.). Here, α_i and β_j were the main effects of protein and plasticizer, respectively and ω_{ij} was the protein-plasticizer two-factor interaction effect. The term "main effect of protein" means the effect of the individual protein (albumin, soy, or whey) irrespective of the effect of plasticizer. Similar interpretation is given for "main effect of plasticizer," which means the effect of the individual plasticizer (water, glycerol, or natural rubber latex) irrespective of the effect of the protein. Moreover, ω_{ij} term denotes the individual protein-plasticizer effects. For example, ω_{11} represents the albumin-water interaction, and ω_{12} represents albumin-glycerol interaction. However, this model is overparamatized, so not all parameter values can be estimated uniquely. In order to overcome this problem, standard baseline constraints have been used (Wu 2009). In particular, we took $\alpha_1 = 0$ and $\beta_1 = 0$ so that albumin and water can be considered as baselines for comparison. The errors ϵ_{ijk} were assumed to be normal (Gaussian), identically and independently distributed with zero mean and some constant variance σ^2.

For both Gram (+) and Gram (-) datasets, after we fit the model, the residual versus fitted plot showed a clear “fanning out” pattern and the normal probability plot indicates departure from normality of errors. We considered the Box-Cox transformation and the corresponding plots (see Appendix Figure 3.9) indicated that the likelihood is maximized around $\lambda = 0$ suggesting the log-transformation. Here we considered the response as $y + 10^{-4}$, that small positive term was added to make all the responses positive. After taking the log-transformation, the improvements of the residual versus fitted plot and the normal probability plot were very

apparent. Also the Cook's distances for the log-transformed data indicated that there were no influential points (see Appendix Figure 3.14), and the assumptions of linear regression could be considered to be satisfactorily met.

Gram Negative Bacteria

The ANOVA table (given in Table 3.1) illustrated that all the main effects of protein, plasticizer, and protein-plasticizer two factor interactions were strongly significant. The multiple R^2 for this model is 99.77%, indicating a good fit. In the regression fit, it is customary to consider baseline constraints which assumes the coefficients corresponding to water and albumin to be 0 (in other words, $\alpha_1 = 0$ and $\beta_1 = 0$). With respect to that, the coefficients of others (along with their p-values) were given in Table 3.2. First we note the p-values of all the regression coefficients mentioned in Table 2 (except rubber) were very small and statistically significant. The estimate of the coefficients for soy (β_2) and whey (β_3) were 5.5 and 8.5, respectively, indicating albumin bioplastics showed fewer numbers of colonies as the coefficient of albumin (α_1) is set to 0, and that is smaller than both 5.5 and 8.5, consistent with the findings of Peters Jr. and Padgett (Peters Jr. 1996) (Padgett T 1998). Similarly the estimate of coefficient of glycerol (β_2) is negative, which confirms that it prevents the growth of colonies significantly.

Table 3.1. Two-way analysis of variance corresponding to model (1) for Gram (-) bacteria

	Df	Sum Sq	Mean Sq	F value	P value
Protein	2	327.2	163.58	1261.9	<2E-16
Plasticizer	2	513.2	256.61	1979.6	<2E-16
Protein × Plasticizer	4	171.9	42.98	331.6	<2E-16
Residuals	18	2.3	0.13		

Table 3.2. Estimated values of regression coefficients for some parameters of model (1) for Gram (-) bacteria

Coefficients	Estimate	Std. Error	t value	p-value
Soy (α_2)	5.50	0.29	18.7	3.04E-13
Whey (α_3)	8.45	0.29	28.8	< 2E-16
Glycerol (β_2)	-8.03	0.29	-27.3	4.18E-16
NRL (β_3)	0.42	0.29	1.4	0.16737

Gram Positive Bacteria.

The ANOVA table (given in Table 3.3) illustrated that all the main effects of protein and plasticizer, as well as the protein-plasticizer two factor interactions were strongly significant. The multiple R^2 for this model was 99.68%, indicating a good fit. The other results for Gram (+) bacteria were similar to those of Gram (-) bacteria (see also Table 3.4).

Table 3.3. Two-way analysis of variance corresponding to model (1) for Gram (+) bacteria

	Df	Sum Sq	Mean Sq	F value	P value
Protein	2	311.2	155.6	766.5	< 2E-16
Plasticizer	2	635.8	317.9	1565.8	< 2E-16
Protein×Plasticizer	4	193.1	48.3	237.7	2.59E-15
Residuals	18	3.7	0.2		

Table 3.4. Estimated values of regression coefficients for some parameters of model (1) for Gram (+) bacteria

Coefficients	Estimate	Std. Error	t value	p-value
Soy (α_2)	4.81	0.37	13.1	1.23E-10
Whey (α_3)	7.59	0.37	20.6	5.59E-14
Glycerol (β_2)	-9.46	0.37	-25.7	1.21E-15
Rubber (β_3)	0.76	0.37	2	0.0543

Conclusions

When comparing the thermal properties of the proteins, we found that the proteins had similar degradation rates, with soy and whey occurring at temperatures between 50-60 °C lower than albumin, which degrades 25 °C lower than zein protein. In terms of the viscoelastic properties, the albumin and whey exhibited similar properties based on the plasticizer used,

while soy plastics exhibited a greater range of properties based on the plasticizer, and zein possessing the highest modulus values when plasticized with glycerol or NRL. As for antibacterial properties, we found that plasticizing either albumin, whey, or zein with glycerol produced the bioplastic with the strongest antibacterial properties. In terms of the statistical analysis, we found that the key determinant of antibacterial properties of a given bioplastic is the protein and plasticizer. With the knowledge gained in this study, there are different areas of interest that could be further studied. To determine if albumin or whey plastics could be utilized in medical settings, various testing would have to be conducted based on the intended end use in areas such as packaging medical products (ASTM F2097 – 10: Standard Guide for Design and Evaluation of Primary Flexible Packaging for Medical Products), as well as infection testing for medical applications (ASTM F813 - 07(2012): Standard Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices). Drug elution analysis would serve as another major area of interest, as the application of drugs over a period of time would be useful in treating patients in numerous settings. For food packaging applications, the testing of water and oxygen vapor permeability properties of the plastics would be crucial to determine, as these properties would determine whether they would be suitable for such applications. The further addition of different materials to the bioplastic blends, as well as the examination of other proteins would also be useful to examine, as it would serve to determine what blends and materials should be used to produce a bioplastic with the best combination of properties based on the application.

References

1 F Baron, Réhault, S, 'Compounds with antibacterial activity', in *Bioactive Egg Compounds* ed. by Marc Anton R. L.-F. o. Rainer Huopalahti, and Rüdiger Schade (Heidelberg: Springer-Verlag Berlin, 2007), pp. 191-98.

2 D S Cha, Chinnan, M S, 'Biopolymer-based antimicrobial packaging: A Review', *Critical Reviews in Food Science and Nutrition,* 44 (2004), 223-37.

3 A Espert, Vilaplana, F, Karlsson, S, 'Comparison of water absorption in natural cellulosic fibres from wood and one-year crops in polypropylene composites and its influence on their mechanical properties', *Composites: Part A,* 35 (2004), 1267-76.

4 J. Fried, *Polymer Science & Technology*. 2nd edn Prentice Hall, 2003).

5 G Galietta, Gioia, LD, Guilbert, S, Cuq, B, 'Mechanical and thermomechanical properties of films based on whey proteins as affected by plasticizer and crosslinking agents', *Journal of Dairy Science,* 81 (1998), 3123-30.

6 I. M. Gould, 'Costs of hospital-acquired methicillin-resistant *Staphylococcus aureus* (Mrsa) and its control', *International Journal of Antimicrobial Agents,* 28 (2006), 379-84.

7 M E Gounga, Xu, S, Wang, Z, 'Whey protein isolate-based edible films as affected by protein concentration, glycerol ratio and pullulan addition in film formation', *Journal of Food Engineering,* 83 (2007), 521-30.

8 A Jones, Zeller, M A, Sharma, S, 'Thermal, mechanical, and moisture absorption properties of egg white protein bioplastics with natural rubber and glycerol.', *Progress in Biomaterials,* 2 (2013).

9 O-W Lau, Wong, S-K, 'Contamination in food from packaging material', *Journal of Chromatography A,* 882 (2000), 255-70.

10 J Lunt, Shafer, A L, 'Polylactic scid polymers from corn: Applications in the textiles industry', Cargill Dow Polymers, LLC, 2001).

11 Inmaculada Martínez, Pedro Partal, Moisés García-Morales, Antonio Guerrero, and Críspulo Gallegos, 'Development of protein-based bioplastics with antimicrobial activity by thermo-mechanical processing', *Journal of Food Engineering,* 117 (2013), 247-54.

12 T. H. McHugh, Krochta, J. M., 'Sorbitol- vs glycerol-plasticized whey protein edible films: Integrated oxygen permeability and tensile property evaluation', *Journal of Agricultural and Food Chemistry,* 42 (1994), 841-45.

13 KP Menard, *Dynamic Mechanical Analysis: A Practical Introduction* (Boca Raton, Florida: CRC Press, 1999).

14 Han I Y Padgett T, Dawson P L, 'Incorporation of food-grade antimicrobial compounds into biodegradable packaging films', *Journal of Food Protection,* 61 (1998), 1330-35.

15 I Paetau, Chen, C-Z, Jane, J, 'Biodegradable plastic made from soybean products. 1. Effect of preparation and processing on mechanical properties and water absorption', *Ind. Eng. Chem. Res.,* 33 (1994), 1821-27.

16 K. Page, Wilson, M., Parkin, I. P., 'Antimicrobial surfaces and their potential in reducing the role of the inanimate environment in the incidence of hospital-acquired infections', *Journal of Materials Chemistry* (2009), 3819-31.

17 A. Y. Peleg, Hooper, D. C., 'Hospital-acquired infections due to gram-negative bacteria', *New England Journal of Medicine* (2010), 1804-13.

18 T Peters Jr., *All About Albumin: Biochemistry, Genetics, and Medical Applications* (San Diego, California: Academic Press, 1996).

19 Y Qiu, Zhang, N, An, Y H, Wen, X, 'Biomaterial strategies to reduce implant-associated infections', *International Journal of Artificial Organs,* 30 (2007), 828-41.

20 W.A.W.A. Rahmana, Sina, L.T., Rahmata, A.R., Samadb, A.A., 'Thermal behaviour and interactions of cassava starch filled with glycerol plasticized polyvinyl alcohol blends', *Carbohydrate Polymers,* 81 (2010), 805-10.

21 V. Rao, Johns, J., 'Thermal behavior of chitosan/natural rubber latex blends', *Journal of Thermal Analysis and Calorimetry,* 92 (2008), 801-06.

22 N-L M Robertson, Nychka, J A, Alemaskin, K, Wolodko, J D, 'Mechanical performance and moisture absorption of various natural fiber reinforced thermoplastic composites', *Journal of Applied Polymer Science,* 130 (2013), 969-80.

23 C. H. Schilling, Babcock, T., Wang, S., Jane, J., 'Mechanical properties of biodegradable soy-protein plastics', *Journal of Materials Research,* 10 (1995), 2197-202.

24 G Seyfriedsberger, Rametsteiner, K, Kern, W, 'Polyethylene compounds with antimicrobial surface properties', *European Polymer Journal,* 42 (2006), 3383-89.

25 S Sharma, Hodges, J, Luzinov, I, 'Biodegradable plastics from animal protein coproducts: Feathermeal.', *Journal of Applied Polymer Science,* 110 (2008), 459-67.

26 S Sharma, Luzinov, I, 'Water aided fabrication of whey and albumin plastics', *Journal of Polymers and the Environment,* 20 (2012), 681-89.

27 T Sivarooban, Hettiarachchy, N S, Johnson, M G, 'Physical and antimicrobial properties of grape seed extract, nisin, and EDTA incorporated soy protein edible films', *Food Research International,* 41 (2008), 781-85.

28 R Sothornvit, Olsen, C W, McHugh, T H, Krochta, J M, 'Formation conditions, water-vapor permeability, and solubility of compression-molded whey protein films', *Journal of Food Science,* 68 (2003), 1985-99.

29 H J Sue, Wang, S, Lane, J L, 'Morphology and mechanical behaviour of engineering soy plastics', *Polymer,* 38 (1997), 5035-40.

30 Lucksanaporn Tarachiwin, Jitladda T. Sakdapipanich, and Yasuyuki Tanaka, 'Relationship between particle size and molecular weight of rubber from *Hevea brasiliensis*', *Rubber Chemistry and Technology,* 78 (2005), 694-704.

31 C.H. Werkman, 'Biochemical use of glycerol', in *Glycerol*, ed. by C.S. Miner, Dalton, N.N. (New York: American Chemical Society, 1953), pp. 397-401.

32 J. Wiesner, Vilcinskas, A., 'Antimicrobial peptides: The ancient arm of the human immune system', *Virulence,* 1 (2010), 440-64.

33 C. F. J. Wu, Hamada, M. S., *Experiments: Planning, Analysis, and Optimization*. 2nd edn Wiley, 2009).

34 A S Yalcin, 'Emerging therapeutic potential of whey proteins and peptides', *Current Pharmaceutical Design,* 12 (2006), 1637-43.

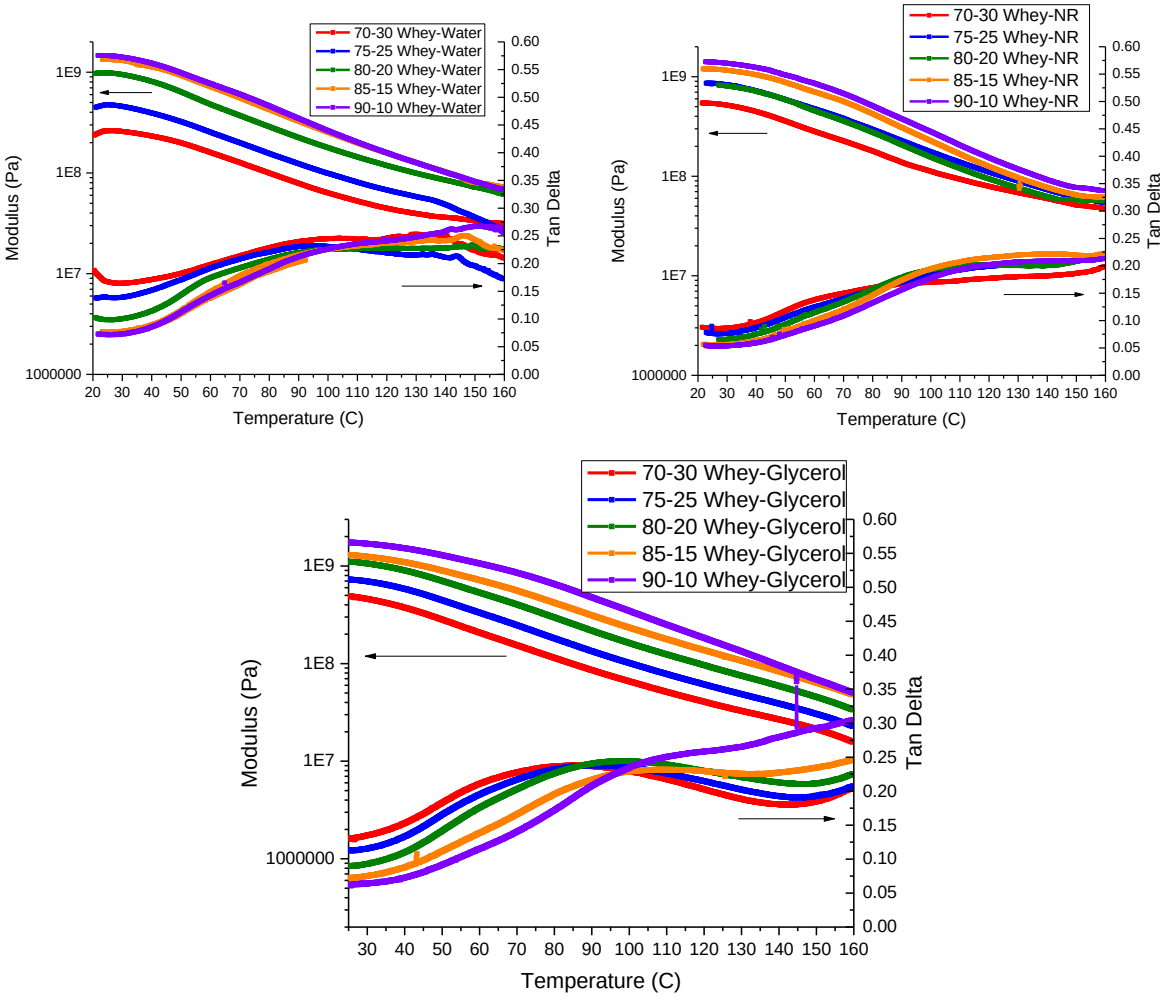
35 J Zhang, Mungara, P, Jane, J, 'Mechanical and thermal properties of extruded soy protein sheets', *Polymer,* 42 (2001), 2569-78.

36 Torres-Giner, S., Ocio, M.J., Lagaron, J.M., Novel antimicrobial ultrathin structures of zein/chitosan blends obtained by electrospinning. *Carbohydrate Polymers* **2009,** *77* (2), 261-266.

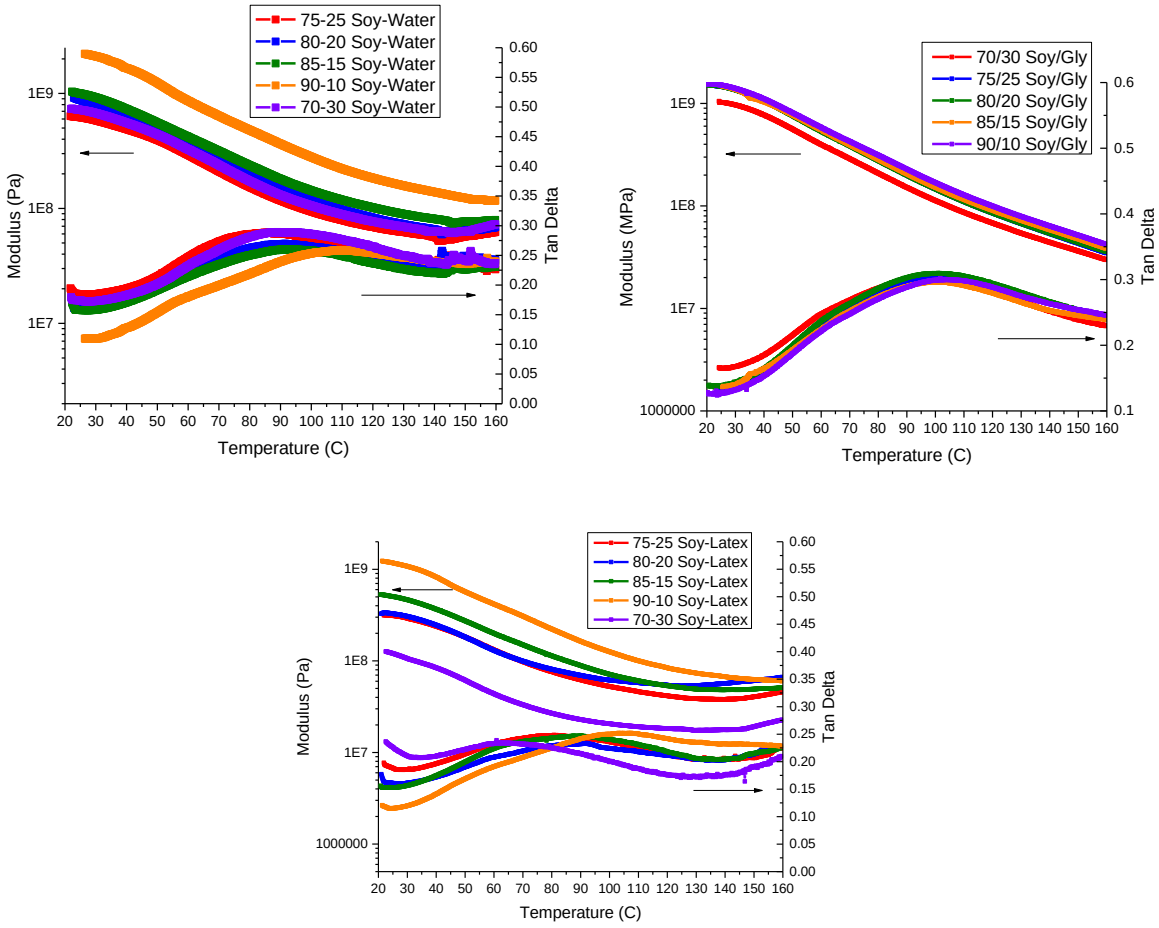
37 Jiugao, Y., Ning, W., Xiaofei, M., The effects of citric acid on the properties of thermoplastic starch plasticized by glycerol. *Starch - Stärke* **2005**, *57* (10), 494-504.

38 Lodha, P., Netravali, A.N., Thermal and mechanical properties of environment-friendly ‘green’ plastics from stearic acid modified-soy protein isolate. *Industrial Crops and Products* **2005**, *21* (1), 49-64.

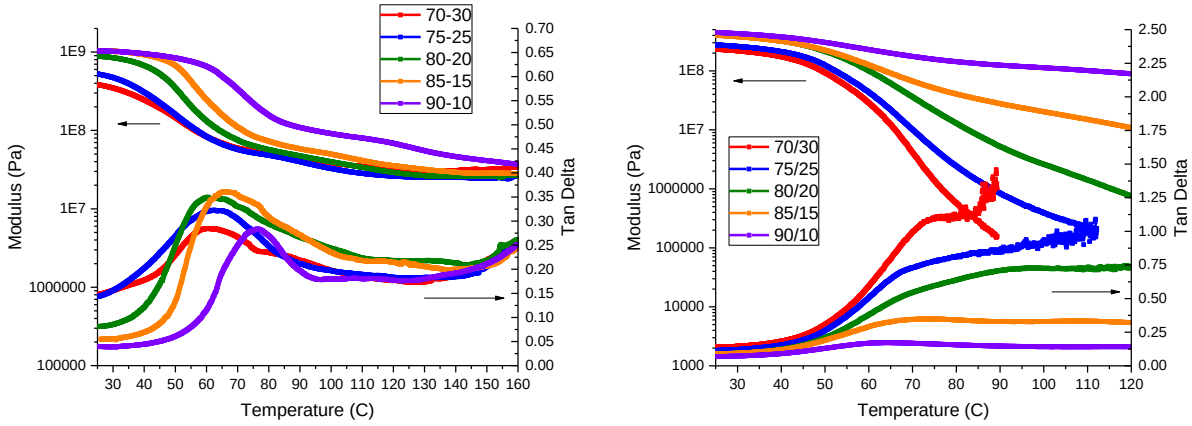
Appendix Figures

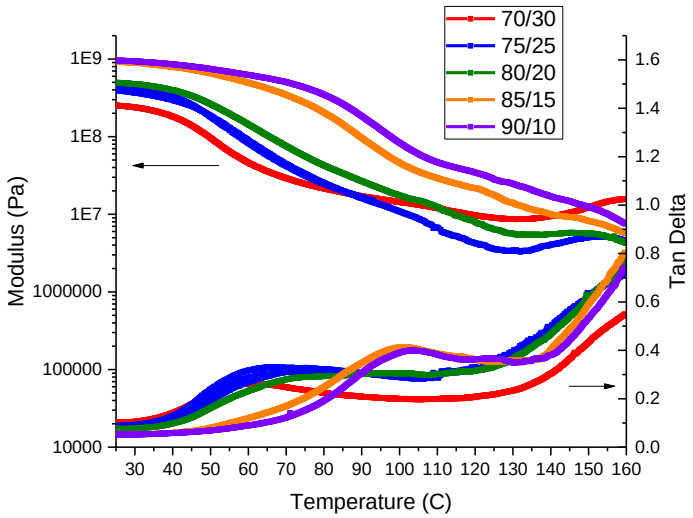


Figures 3.10-12. Dynamic mechanical analysis of whey/water, whey/glycerol, and whey/NRL plastics.



Figures 3.13-15. Dynamic mechanical analysis of soy/water, soy/glycerol, and soy/NRL plastics.





Figures 3.16-18. Dynamic mechanical analysis of zein/water, zein/glycerol, and zein/NRL plastics.

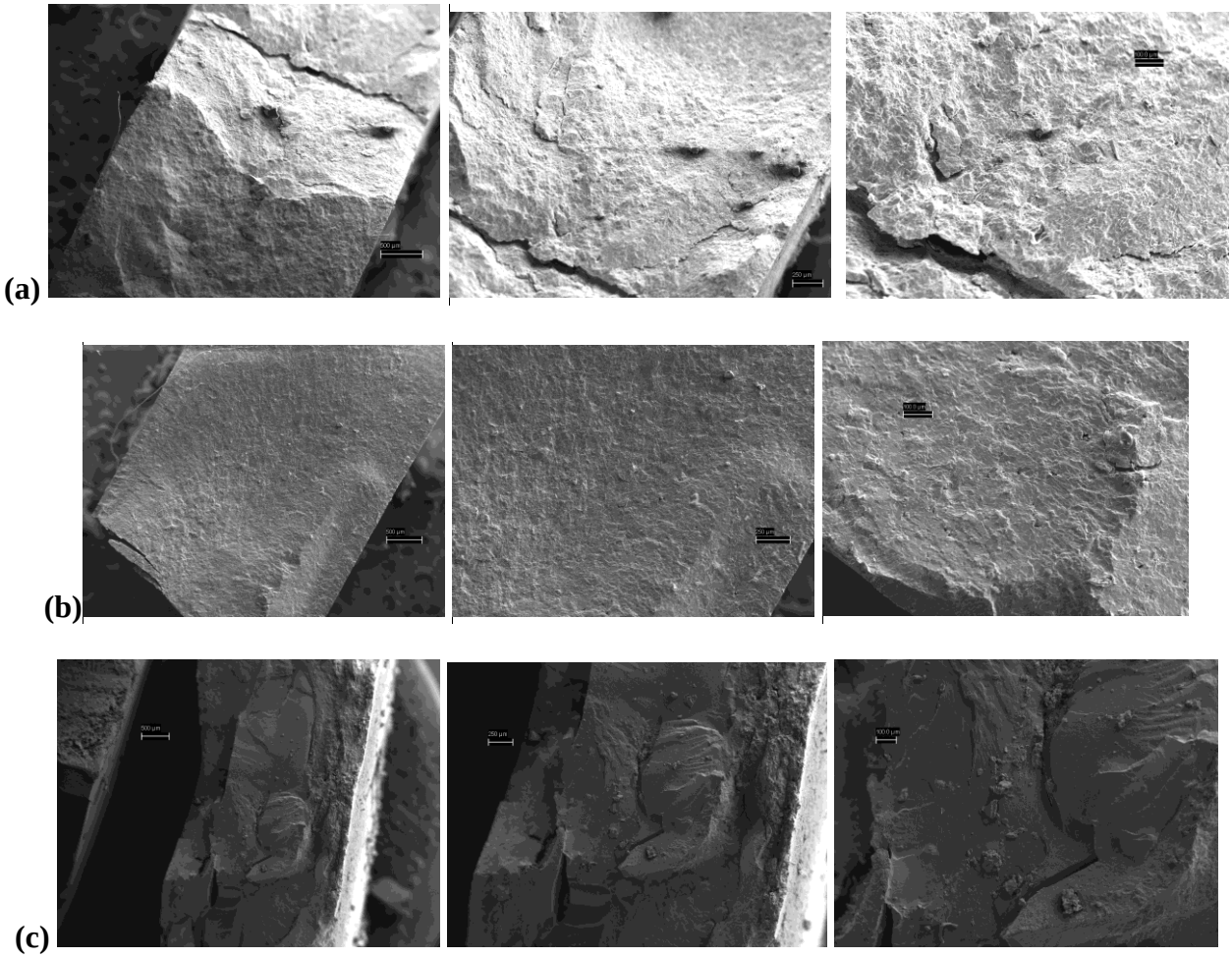


Figure 3.19: Scanning electron microscopy images of soy bioplastics. (a)soy-water, (b) soy-glycerol, (c) soy-NRL. Magnification of 20x, 100x, and 500x.

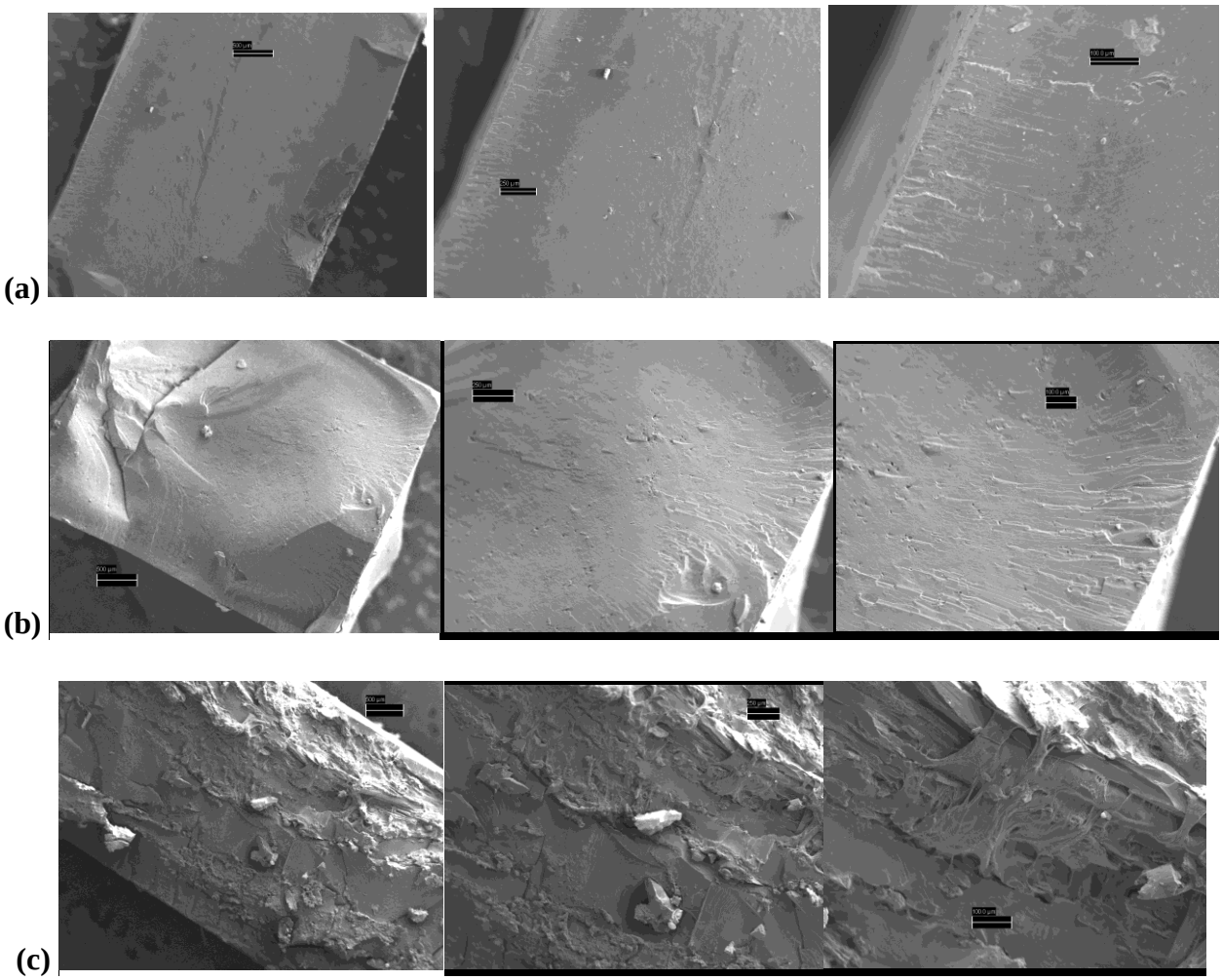
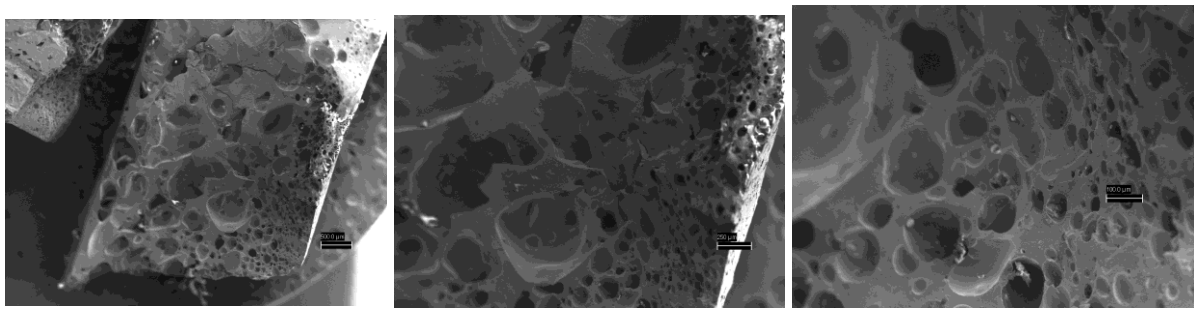
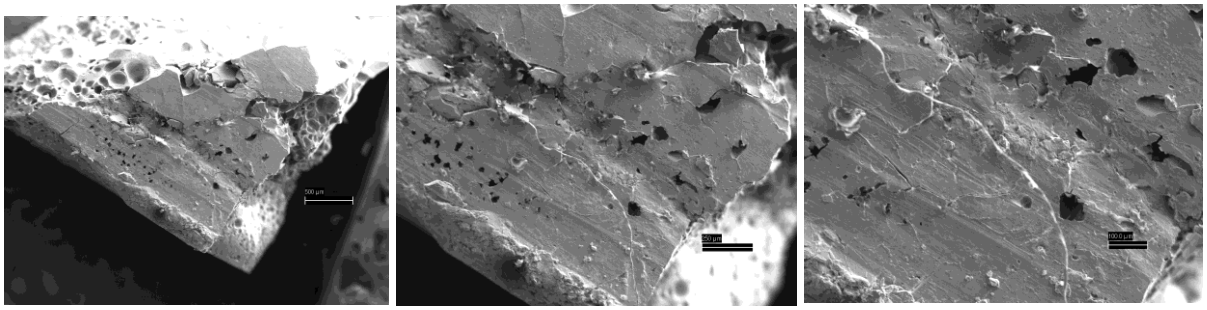


Figure 3.20: Scanning electron microscopy images of whey bioplastics. (a)whey-water, (b) whey-glycerol, (c) whey-NRL. Magnification of 20x, 100x, and 500x.





(b)

Figure 3.21: Scanning electron microscopy images of zein bioplstics. (a)zein-water, (b) zein-NRL. Magnification of 20x, 100x, and 500x.

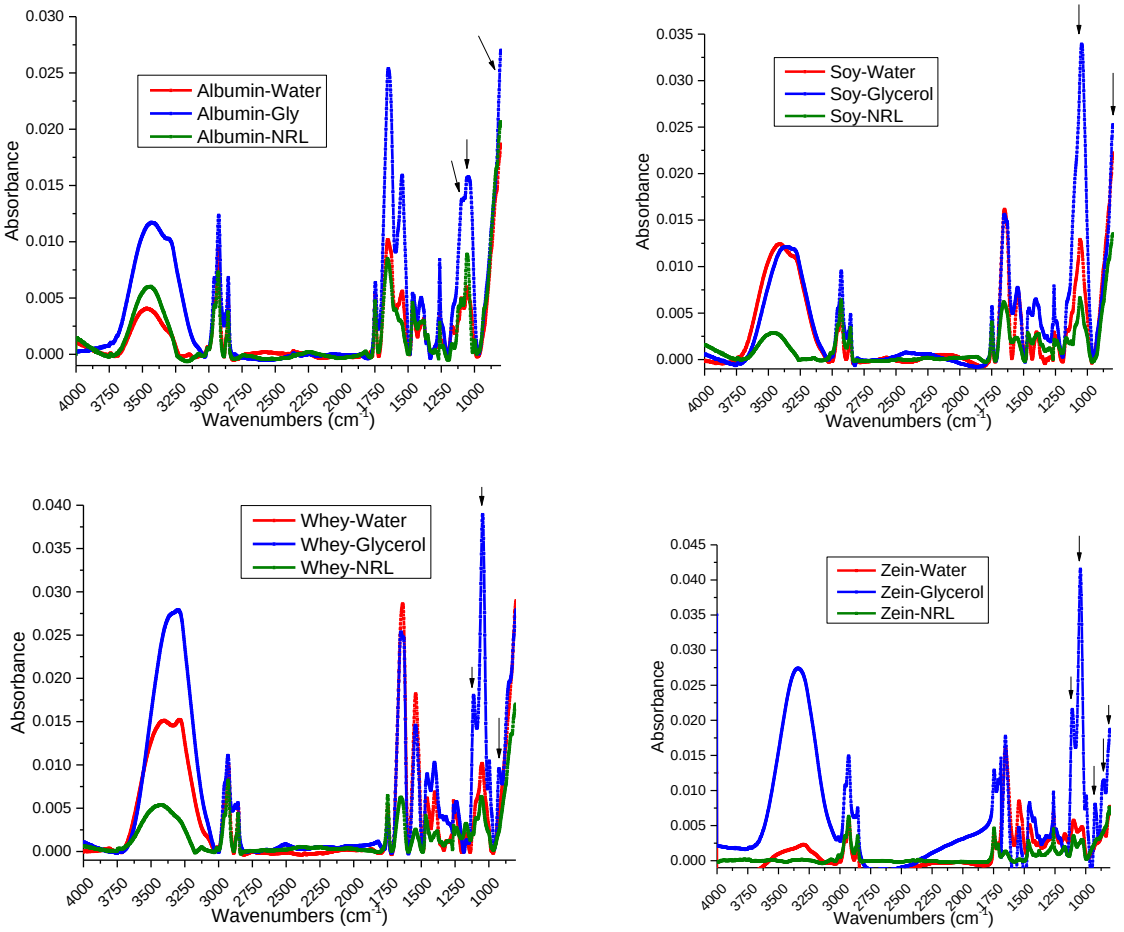
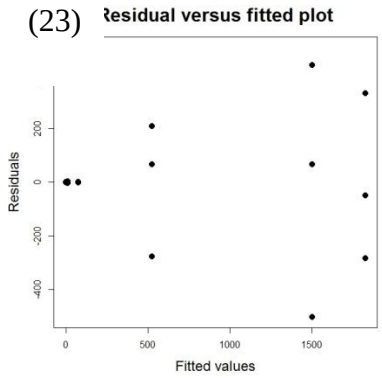
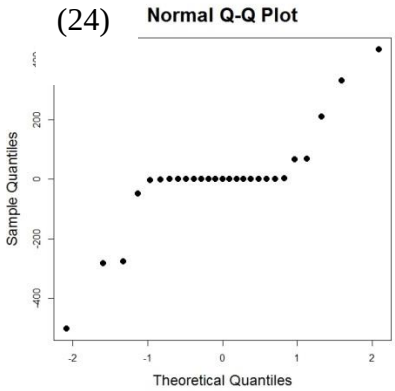
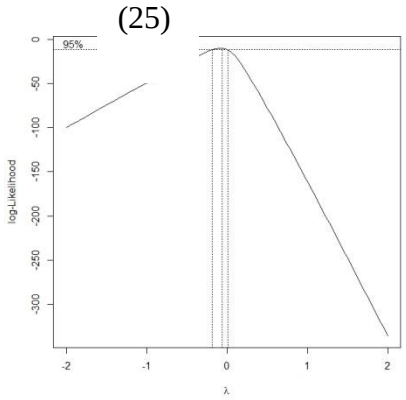


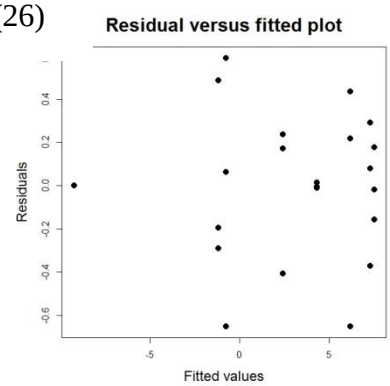
Figure 3.22: FTIR spectra of albumin, soy, whey, and zein bioplastics.

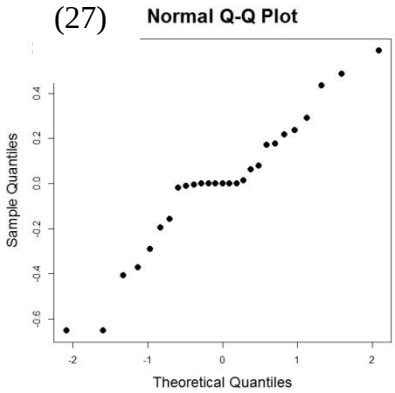
(23) 

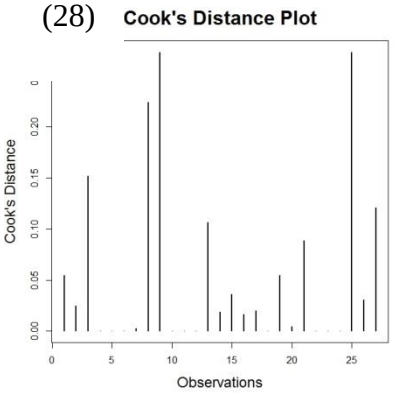
(24) 

(25) 

Figures 3.23-25. Original data for bacterial analysis.

(26) 

(27) 

(28) 

Figures 3.26-28. Log-transformed data for bacterial analysis.

CHAPTER 4

ALBUMIN AND ZEIN-BASED PLASTICS AND THEIR POTENTIAL USE IN MEDICAL AND FOOD PACKAGING APPLICATIONS[3]

[3] Alexander Jones, Abhyuday Mandal, and Suraj Sharma. To be submitted to ACS Biomaterials Science & Engineering.

Abstract

To prevent the occurrence of hospital-acquired infections and the spoilage of food, the usage of compounds to be eluted from polymer substrates serve as a potential solution. We investigated the physical, thermal, biodegradation, and drug- elution properties of the albumin and zein bioplastics that were plasticized with glycerol and blended with varying amounts of low-density polyethylene (LDPE). *Bacillus subtilis* and *Escherichia coli* were utilized as Gram (+) and Gram (-) species, respectively, for antimicrobial and drug-elution analysis, as these species are common in the human body and in food environments. With the usage of bioplastics made from thermomechanical molding of proteins such as albumin from hen egg white or zein from corn in tandem with drug eluting compounds, it could be possible to limit the amount of contamination that occurs in crucial food and medical applications that utilize only low-density polyethylene in the substrate formation.

Keywords

thermoplastics, bioplastics, antibacterial, drug elution, biodegradation, food packaging

Introduction

In medical and food packaging applications, there are many drawbacks to the continued use of conventional plastic materials, such as polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET). These petroleum-based plastics lack the inherent property of preventing the growth of bacteria when contaminated, causing potential harm to individuals. For instance, numerous strains of bacteria such as *Acinetobacter baumannii* and methicillin-resistant *Staphylococcus aureus* (MRSA) have been found to be viable on the surface of plastics for over a month (Hota 2004). In the hospital, this can lead to the contamination of other surfaces, leading

to potential cross-contamination (Schultz 2003). In the application of food packaging, this translates to foods that will potentially spoil more rapidly in comparison to food products that have been placed in a sterile environment. For example, in one study, the cultures of *Lactobacillius* species and *Brocothrix thermosphacta*, bacteria were found to be associated with the spoilage of refrigerated beef and pork that had been previously sterilized and placed in a vacuum-sealed plastic package after 30 days of refrigeration at 4 °C (Borch 1996). Another issue posed by the usage of conventional plastic materials in both medical applications and food packaging is the gradual leeching of chemicals utilized in the production process of the plastic into the material contained within the plastic. In health care settings this can result in the leeching of materials such as bisphenol A and phthalates into the body through transfusion or dialysis (Halden 2010), while in food packaging it has been found that milk in bottles made from low density polyethylene (LDPE) is contaminated with naphthalene (utilized as a dispersant during plastic production) that gradually leeches from the plastic itself (Lau 1994). One other notable drawback that is present with the use of conventional plastics is the lack of the recycling of plastics that will occur when utilized in medical and food packaging applications. In medical settings, the recycling of plastics that are used for medical procedures and laboratories is not widely practiced, as the recycling of biomedical waste will pose a health hazard due to the ease of contamination (Lee 2002). For food packaging applications, while it is possible to recycle the products, a major issue that is posed is consumer participation, as LDPE, polystyrene, and polypropylene food containers have been found to have poor recovery rates in terms of recycling (Hopewell 2009).

In order to address the issue of bacterial contamination and growth in medical and food packaging plastics, there have been multiple approaches studied. One approach is the

incorporation of additives in the conventional plastics that will lend antibacterial properties to the resulting plastic. For medical plastics, this can be done with the incorporation of compounds such as sodium ampicillin (Queiroz 2001) and ciprofloxacin (Dimalo 1994) into the polymer substrate of materials that will be utilized. As for food packaging, it is possible to incorporate common food preservatives, such as sodium benzoate and sodium nitrite (Vartiainen 2003) into the plastic that will gradually leech into the environment. Surface treatments can also be utilized in the production of antimicrobial plastics, as it has been found that coated plastics with antibacterial compounds such as nisin (Neetoo 2008) or a combination of lysozyme and silver nanoparticles (Eby 2009) will result in a plastic that possesses antibacterial properties. Another area of research interest is the modification of the plastic surface that will come into contact with the bacteria. In medical applications, this can be done through the lubrication of the plastic surface to prevent the adhesion of bacteria when in contact (MacCallum 2015), as well as nanotexturing of films with tetrahydrofuran to generate a more hydrophobic surface when the film is treated with ethanol or methanol (Loo 2012) to prevent bacterial adhesion. Hydrophobic surfaces can also be imparted onto food packaging films through the use of shrink-inducing to make a super-hydrophobic substrate, preventing bacteria from adhering to the surface due to it being structured as opposed to flat (Freschauf 2012).

To address the issues of lack of biodegradability and antimicrobial properties, the use of alternative materials such as proteins as a raw material in the production of plastics has been examined. Of particular note are the proteins of albumin from the hen egg white and the zein protein that is contained in corn. With the use of plasticizers, it is possible to utilize both of these proteins in the production of plastics for the areas of food packaging and medical applications (Gillgren 2008, Jones 2013). One possible advantage of these alternative materials is their

antimicrobial potential. For instance, albumin, whey, and zein-based bioplastics, plasticized with glycerol, did not prevented the growth of bacteria (*E. coli* and *B. subtilis*) on the surface of the plastic (Jones 2015). Zein plastic films designed for food packaging, when blended with antibacterial compounds such as lysozyme and a chelating agent disodium EDTA, caused a decrease in bacterial growth as well as antioxidant activity (Güçbilmez 2007). When these proteins are loaded with compounds such as ciprofloxacin hydrochloride, the protein are able to possess the same elution properties that are present in conventional plastics, making medical application a potential usage (Jain 2008).

Our objectives in this study are to determine the thermal and mechanical properties of albumin and zein plastics plasticized with glycerol and their thermoplastic blends with LDPE, and to evaluate the water absorption, biodegradation, antibacterial, and drug elution properties of these plastics for potential use in medical or food packaging applications.

Materials and Methods

Materials

Albumin (purity ≥ 99%) was obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA). The zein purified protein was acquired from Acros Organics (New Jersey, USA); Low density polyethylene (LDPE) powder (500 micron) was obtained from Alfa Aesar (Ward Hill, MA, USA). The glycerol used as a plasticizer was obtained from Sigma-Aldrich with a purity ≥99%. For antibacterial and drug elution analysis, various materials were purchased for testing: Bacto tryptic soy agar, tryptic soy broth, and Mueller-Hinton agar from Bectin, Dickinson and Company (Sparks, MD, USA); Dey-Engley neutralizing broth from Remel (Thermo Scientific, Suwanee, GA, USA); agar-agar solution that consisted of granulated agar-agar from EMD (Gibbstown, NJ, USA); sodium chloride from Baker (Phillipsburg, NJ, USA); and phosphate

buffered saline solution from HiMedia (Mumbai, India). The materials to be examined for elution study were the following: sodium benzoate and sodium nitrite, obtained from Carolina Biological Supply Company (Burlington, NC, USA); ampicillin (sodium salt), obtained from IBI Scientific (Peosta, IA, USA); and ciprofloxacin, obtained from TCI (Tokyo, Japan). The bacterial species of *Bacillus subtilis* (Gram (+)) and *Escherichia coli* (Gram (-)) were graciously provided by Dr. Jennifer Walker at the department of microbiology at the University of Georgia.

Thermal Analysis of Raw Material

Thermal gravimetric analysis (TGA) was performed using a Mettler Toledo TGA/SDTA851e, with material examined from 25°C – 500 °C under a N_2 atmosphere with a heating rate of 10 °C min^{-1}. Differential scanning calorimetry (DSC) was performed using a Mettler Toledo DSC821e, with materials examined from -20 °C to 250 °C under a N_2 atmosphere with a heating rate of 10 °C min^{-1}. For all sample testing, the weight of each sample was set between 2.0 – 4.0 mg to ensure consistent results and determine optimum plastic molding conditions.

Preparation of Compression Molded Samples

The molding of bioplastic blends was performed on a 24-ton bench-top press (Carver Model 3850, Wabash, IN, USA) with electrically-heated and water-cooled platens. Stainless steel molds were used to form dog bone-shaped bioplastics for antibacterial plastic analysis. To form the plastics, protein and glycerol plasticizer, to aid in processing (25% for albumin plastics, 20% for zein plastics (Jones 2013), were mixed manually in predetermined w/w ratios to be placed into the molds (as indicated throughout the paper). The mixture of protein, polymer, and additives were prepared in small batches of varying masses based on density of materials for dog bone plastics (≤ 6 g for albumin and ≤ 4 g for zein and LDPE due to zein and LDPE material

possessing less density compared to albumin), while the DMA flexbars (prepared with spacers to prevent material loss due to the ejection of material out of the mold by pressure) were made of 2 g of plasticized proteins. Subsequently, the mixture was filled into the flexbar and dog bone cavity of the stainless steel molds, with plungers placed on top of the molds to prevent the mixture from leaking. After covering with a plunger, the molds were then compressed for a 5-minute molding time at 120 ºC, followed by a 10-minute cooling period for the protein plastics. Samples were prepared under a pressure of at least 40 MPa (Sue 1997). After the samples were cooled for 10 minutes under pressure, the pressure was released and the samples were removed. To prepare the films for drug elution analysis, it was possible to mold the samples using the same process that was used to make DMA flexbars, except in this process it was not necessary not use spacers in order to make a thinner sample. In preparation of the films, it was necessary to blend the protein and drug/food preservative powders in order to ensure a consistent blend throughout the plastic. After the blending of protein and drug/food preservative, it was then possible to add in the plasticizer. When plastic molding was completed, the plastic samples were conditioned at 21.1 °C and 65% relative humidity for 24 hours before characterization through dynamic mechanical analysis, antibacterial, drug elution, and elution kinetics testing.

Dynamic Mechanical Analysis

DMA flex bars of the protein plastics were analyzed for their viscoelastic properties through the use of dynamic mechanical analysis (DMA) (Menard 1999) by using a DMA 8000 Dynamic Mechanical Analyzer from Perkin Elmer. The analyzer examined the viscoelastic properties of the plastics by determining both the storage and loss modulus. The two types of moduli differ by which storage modulus (E´) is an indication of the elastic region of the material where energy is stored, while loss modulus (E´´) is the amount of energy that is dissipated

through heat in the viscous region. The resulting moduli were then put in ratio form (E''/E') to calculate tan δ, which denotes the viscoelasticity of a given material (Fried 2003). DMA was conducted from 25°C to 120°C, with a temperature ramp of 2 °C min^{-1}. The settings of the analyzer were set to dimensions of 9×2.5×12.5 mm^3 using a dual-cantilever setup at a frequency of 1 Hz with a displacement of 0.05 mm, within the range of plastic deformation. Each sample type was analyzed in duplicate.

Mechanical Properties

The mechanical properties of the conditioned bioplastics were measured by using the Instron testing system (Model 3343) interfaced with the Blue Hill software. The test was performed according to the standard test method for tensile properties of plastics (ASTM D 638-10, Type I) with a 5 mm min^{-1} crosshead speed, a static load cell of 1000 N, and a gauge length of 4 cm. Samples were run in quintuplicate (n=5) for each blend type in order to ensure precision.

To model the mechanical properties of the thermoplastic blends produced, several models were utilized to predict the modulus and elongation of the resulting thermoplastic. For predicting the stiffness of a given blend, we have utilized Kerner-Hashin equations that assume that the material (protein or LDPE) dispersed into the phase of the plastic matrix itself will adopt a sphere-like shape. When it was assumed that there was strong adhesion between the phases in the plastic, the equation utilized was[63]:

$$E = E_1 \frac{\frac{\phi_2 E_2}{(7-5\upsilon_1)E_1+(8+10\upsilon_1)E_2}+\frac{\phi_1}{15(1-\upsilon_1)}}{\frac{\phi_2 E_1}{(7-5\upsilon_1)E_1+(8+10\upsilon_1)E_2}+\frac{\phi_1}{15(1-\upsilon_1)}}$$

where E, E_1 ,E_2 were the moduli for the binary blend of protein-LDPE, the matrix, and the dispersed phase; φ1, φ2 were the volume fractions of the matrix of protein-LDPE and the

dispersed phase; and ν_1 was the Poisson ratio for the protein-LDPE matrix. For volume fractions of the LDPE and the proteins, the density of the protein material was assigned a value of 1g/cm^3. However, if there is no adhesion between protein and LDPE, the model will change to[63]:

$$E = E_1 \frac{1}{1+\frac{\phi_1}{\phi_2}[15(1-\upsilon_1)/(7-5\upsilon_{1)}]}$$

When modeling the phase inversion region, it was necessary to utilize a Davies equation that allows for the observation of dual-phase continuity[64]:

$$E^{1/5} = \phi_1 {E_1}^{1/5} + \phi_2 {E_2}^{1/5}$$

To model the predicted elongation of a given blend, we have utilized a Nielson equation, which assumes that there was good adhesion between the protein-LDPE phases[65]:

$$\varepsilon_c = \varepsilon_0(1-\phi^{\frac{1}{3}})$$

where ε_c was the elongation to break of the thermoplastic blend, and ε_0 is the protein or LDPE that constitutes the matrix of the thermoplastic blend.

Scanning Electron Microscopy (SEM)

Albumin and whey thermoplastic SEM samples (n =2 for each protein-LDPE blend type) were prepared from cryogenic DMA flex bar fracture surfaces after being placed in a conditioning chamber (21.1°C and 65% relative humidity) for at least 24 hours. DMA flex bars were submerged in liquid nitrogen for 20 seconds; after that they were immediately broken. The samples were mounted, then sputter coated for 60 seconds with an Au/Pt mix. SEM images were recorded on a Zeiss 1450EP variable pressure scanning electron microscope. Coated samples were analyzed at 20X, 100X, and 500X for each blend type.

Water Absorption Testing of Plastics

The water absorption properties of the conditioned plastics were measured by performing the standard test method for water absorption for plastics (ASTM D 570-98 (2010) e1). After conditioning for 24 hours, the samples were dried in an oven set at 50 ± 3°C for 24 hours, cooled in a desiccator for one hour, then immediately weighed to the nearest 0.001 g. The materials were then tested for long term immersion, in which the samples were placed in water set to a temperature of 23 ± 1°C for five days, with samples being removed and blotted every 24 hours prior to weight measurement and placement back into the water bath. Samples were run in quintuplicate (n=5) for each blend type in order to ensure precise measurement.

Susceptibility of Plastics to Microbial Degradation

The susceptibility of the conditioned plastics to be degraded by microbial attack was measured by performing the standard practice for evaluating microbial susceptibility of nonmetallic materials by laboratory soil burial (ASTM G 160-12). After conditioning, the dog bone (6g for albumin, 4g for zein and LDPE) and flexbar sized (2g for all plastics) samples were placed in containers that contained a soil that was composed of equal amounts of fertile topsoil, cow manure, and coarse sand (10 to 40 mesh). The containers were then placed in an environmental chamber where the temperature would remain at 30 ± 2°C, with a relative humidity of 85 to 95%. The materials were then tested for thirty and sixty day exposure periods, after which the samples were then cleaned to remove soil collecting on the surface, documented by photography, and weighed to the nearest 0.001g to compare to samples that have not been subject to testing. Samples were run in quintuplicate (n=5) for each blend type in order to ensure precision.

Antibacterial Testing of Plastics

The antibacterial properties of the conditioned plastics were measured using the ASTM E 2180-01 standard test method, in which the aqueous based bacterial inoculum remains in close, uniform contact in a "pseudo-biofilm" state with the bioplastic. For each blend type, the Gram (+) specie *Bacillius subtilis* and the Gram (–) specie *Escherichia coli* were utilized as challenge bacterial cells to determine the efficacy of bacterial growth on the plastic surfaces. After equilibration of standardized culture banks of $1\text{-}5\times10^{8}$ cells/mL through the use of dynamic light scattering analysis, 1 mL of the culture was applied to 100 mL of agar slurry for inoculation. One minute after inoculation, the slurry was then immediately applied to a 9 cm^2 area of the bioplastics that had been swabbed with phosphate-buffered saline to promote adhesion by reducing surface tension. After the appropriate time of application of cultured agar (within one hour for 0-h samples and at least 24h for 24-h samples after incubation at 37 °C), the agar was removed from the plastic surface through both sonication (1 min) and vortexing (1 min) the plastics in 30 mL of Dey-Engley neutralizing broth. The neutralizing broth containing the agar was diluted five times in a 10^{-1} dilution set, and then the dilutions were applied to tryptic soy agar plates, which were incubated for 24 h at 37 °C. After incubation for 24 hours, the culture plates were counted for microbial growth and averaged to determine colony forming units (CFU)/mL. Samples were run in triplicate ($n = 3$) for each protein-plasticizer combination (as well as the polyethylene plastic control sample) in order to ensure precision.

Drug Elution and Zone of inhibition Study

The potential of the plastics to elute antibiotics and food preservatives to generate zones of bacterial inhibition was determined through the use of the performance standards for antimicrobial disk susceptibility tests; approved standard—eleventh edition (M02-A11) that has

been developed by the Clinical and Laboratory Standards Institute in Wayne, PA (Clinical and Laboratory Institute, 2012). The plastic blends were prepared with four levels of drug or food preservative (0, 5, 10, and 15%) using the sample procedure listed in Section 2.3, with dry drug added to the plastic blend before compression molding. After preparation, the samples were then cut into disk-sized plastics that were applied to the surface of Mueller-Hinton agar dishes that had been already inoculated with either Gram (+) specie *Bacillius subtilis* or the Gram (–) specie *Escherichia coli* at a concentration of $1\text{-}5\times10^{8}$ cells/mL. After application, the plates were then incubated for five days 30 °C, during which the zones of inhibition were measured every 24 hours to determine the change of diameter of the inhibition zone size over time. Samples were run in triplicate (n = 3) for each plastic type-additive combination (as well as the LDPE plastic control samples) in order to ensure precision.

Drug Elution Kinetics

The in vitro release of ampicillin and ciprofloxacin from the albumin bioplastics blended with varying levels of drug or food preservative (0 for blank to eliminate any albumin-based compounds, 5, 10, and 15% for drug release determination) into phosphate-buffered saline (PBS) was determined by the immersion of the bioplastics into 25 ml of PBS in centrifuge tubes. The centrifuge tubes were then placed in a 37 °C shaking bath at shaking speed of 50 rpm for five days. At 24 hour intervals, the absorption of both ampicillin and ciprofloxacin was determined by a UV-VIS spectrophotometer (Shimadzu UV-2401 PC UV-VIS Recording Spectrophotometer) at the absorbance peaks of 230 nm (Queiroz 2001) for ampicillin and 275 nm for ciprofloxacin (Jain 2008, Cazedey 2012). In order to determine concentrations of solutions, linear calibration curves were obtained by measuring the absorption of solutions with concentrations of ampicillin and ciprofloxacin, as shown in Figure 4.1. For ampicillin, the

equation derived from the linear fit is $y = 0.07912x + 0.08022$; while for ciprofloxacin it is $y = 0.63685x + 1.20162$, where *x* is equivalent to the absorption measured at the specific wavelength, and y is equal to the concentration of drug in solution.

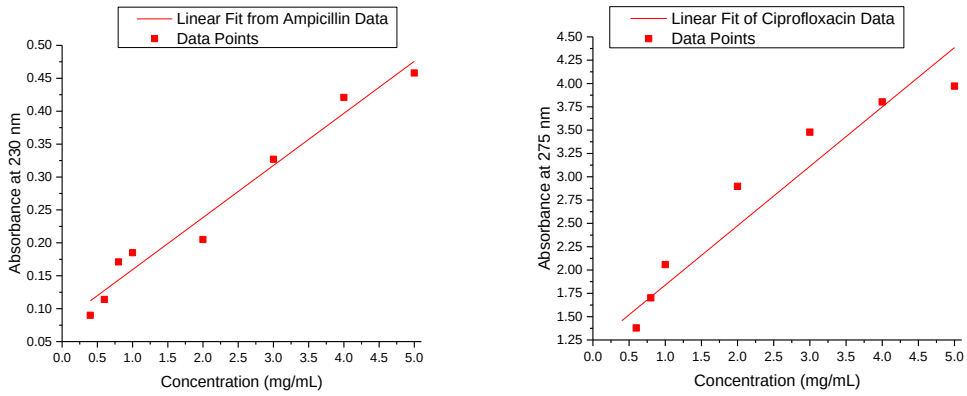


Figure 4.1: Calibration Curves of Ampicillin and Ciprofloxacin

Statistical Analysis

To compare the ability of plastics to elute drug and to determine the effect of the addition of LDPE into plastics, statistical analyses were performed by fitting a regression model. For plastic-drug/food preservative blends that contained 15% of the elution material, inhibition zones after five days were analyzed by fitting a two-way ANOVA using the statistical software of SAS and R. Box-Cox transformations were used to determine the appropriate transformations needed to satisfy the normality assumptions of the experimental errors.

Results and Discussion

Material Analysis

Thermal Properties of Protein Bioplastic and their Thermoplastic Blends

In the thermoplastics blends of albumin and zein with LDPE as shown in Figure 2, we first observe bound water loss between 60-75 °C, then initial degradation peaks between 220-230 °C and 315-325 °C, indicative of the onset of degradation of first the glycerol contained in the plastics (Castelló 2009), then the proteins in the plastic (Magoshi 1992, Wongsasulak 2007). The

albumin-based plastics will have higher degradation peaks where glycerol degradation occurs due to the plastics containing more glycerol (25%) when compared to zein plastics (20%). However, the zein plastics possess higher degradation peaks at protein degradation temperature due to zein plastics possessing more protein (80%) when compared to the albumin plastics (75%). As we increase the amount of LDPE in the resulting thermoplastic, we find that the magnitude of these initial degradation peaks decreases to the point where it is marginal, as LDPE will not degrade at these temperatures. The most prominent degradation peak observed for both types of blends is the degradation that occurs at 475 °C, as this is indicative of the onset of LDPE degradation (Park 2000). However, as we decrease the amount of LDPE contained in the thermoplastic, we find that the magnitude of mass loss is lessened to the point where it is slight for samples that have less than 10% of LDPE in their formulation. This is due to the fact that for blends that contain high levels of protein, most of the mass has already been lost at lower temperatures, so any additional mass loss change is marginal. Based on our results, we find that as the amount of LDPE is increased in the plastic formulations, the thermal degradation properties will become more similar to LDPE degradation patterns than the protein plastics.

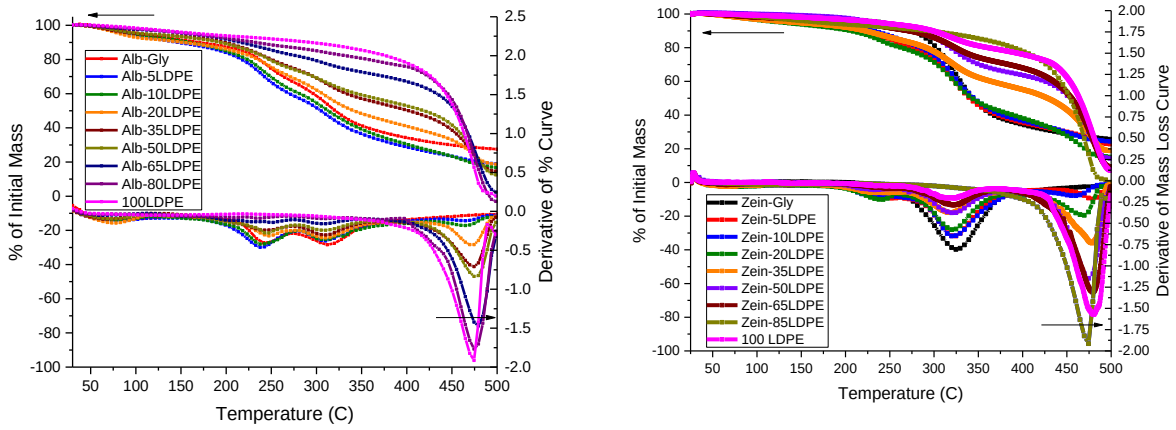


Figure 4.2: Thermogravimetric analysis of albumin and zein thermoplastic blends.

For the DSC analysis as shown in Figure 4.3, we find that there is an endothermic peak at 75-90 °C for albumin, but a much broader endothermic peak at 60-80 °C for zein-based thermoplastics. These varying endothermic peaks indicate a difference in the glass transition temperatures for the protein-based plastics, as zein possesses a lower denaturing temperature (60-80 °C) (Kim 2004) in comparison to albumin (84.5 °C) (Herald 1992). When we increase the amount of LDPE contained in the thermoplastic blend, we find that these initial endothermic peaks are less noticeable, as an endothermic peak of 115-120 °C becomes more prominent, indicative of the melting of LDPE in the thermoplastic blends (Liu 2002). After 235 °C, there is an onset of an endothermic peak for the protein-containing plastics, as this is the temperature at which thermal degradation of amino acids will occur.

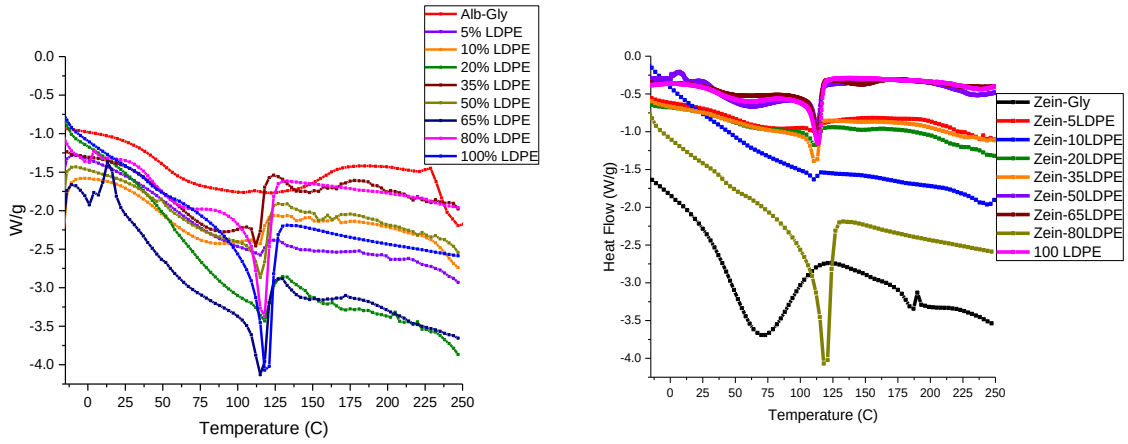


Figure 4.3: Differential scanning calorimetry of albumin and zein thermoplastic blends.

Viscoelastic Properties of Thermoplastic Blends

In the albumin plastics that have been blended with LDPE, as shown in Figure 4.4(a), we find that as we increase the amount of LDPE utilized in the blend, there is a gradual increase in the tan delta peak temperature (75 °C for Alb-Gly plastics, 90-95 °C for plastics that contain 50% or more of LDPE) as well as tan delta height, an indication of glass transition of the protein in the plastic. This could be due to the gradual increase of interactions between protein with

LDPE and LDPE-LDPE interactions, with LDPE-LDPE interactions more highly favored when the plastic is made of 50% or more of LDPE. These changes will impart viscoelastic properties on the resulting plastic that are more similar to pure LDPE plastics (Shieh 2001). These interactions result in a material that will possess a lower initial tan delta value in comparison to pure, glycerol-plasticized, albumin bioplastics. However, we find that incorporating more than 20% of LDPE in the albumin thermoplastic will result in a material that will have a lower initial modulus at 20 °C, as well as lower modulus at 120 °C. The thermoplastic blends possess lower modulus values as more LDPE is added due to LDPE-LDPE interactions produce a material that will possess a lower modulus in comparison to materials composed of material that consist of protein-protein and protein-glycerol interactions, as illustrated in Figure 4.4(b) (Averous 2000, Jones 2013).

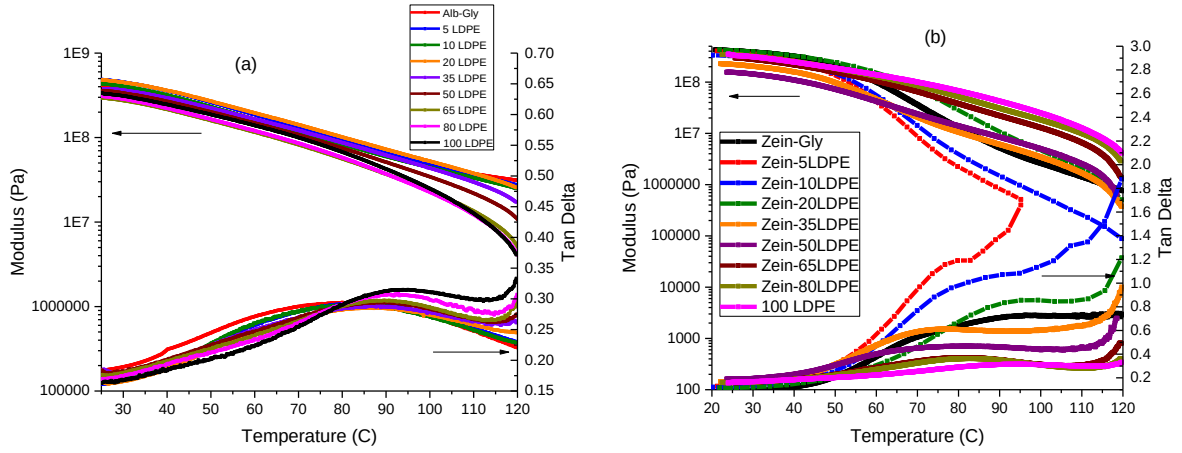


Figure 4.4. Dynamic mechanical analysis of albumin and zein thermoplastic blends.

When we analyze the zein thermoplastic blends, as shown in Figure 4.4(b), we find that there is not a significant difference in the initial modulus and tan delta of the bioplastics (from 25 °C to 40 °C for albumin, 25 °C to 60 °C for zein plastics) as we increase the amount of LDPE in the thermoplastic blends. However, as we increase temperature to a certain point (55-120 °C for 5% and 10% LDPE, 80 - 120 °C for 20% LDPE), an elevation of tan delta values as well as shift

of tan delta curves to higher temperatures occurs. This shift in tan delta values is due to the increase of protein-LDPE interactions, and a decrease in protein-protein and protein-glycerol interactions (Corradini 2004). When the amount of LDPE contained in the blend is at least 35%, we witness tan delta values that are more similar to pure LDPE than zein-glycerol plastics. This change in viscoelastic properties can be due to the increase of LDPE-LDPE interactions in the material, with an increase in storage modulus values and decrease in tan delta when compared to pure zein-glycerol plastics at temperatures above 60°C (Shin 2004). As the amount of LDPE in the plastic increases, there will be a resulting decrease in the protein-protein and protein-glycerol interactions. We also witness a dramatic increase in tan delta values for plastics that contain at least 10% LDPE in the blend at 115 °C, which is an indication of the beginning of melting of LDPE, which begins to occur between 105-115 °C (Liu 2002). We find that once there is at least 35% of LDPE in the zein thermoplastic, the resulting thermoplastic has similar viscoelastic properties to pure LDPE thermoplastics than zein bioplastics, as there is enough molecular interaction within the plastic to form a stable material.

Mechanical Properties of Thermoplastic Blends

To determine the mechanical properties of the plastics, it will be necessary to conduct tensile testing. For the albumin-based plastics, we find that the addition of LDPE of up to 65% w/w into the thermoplastic blend will increase the modulus of the resulting plastic. However, there is a decrease in the modulus of the plastic when more than 80% of the blend consists of LDPE. This increase in the modulus of the thermoplastics may be due to the fact that decreasing the amount of albumin and glycerol promotes polymer-protein interaction, increasing the ability of the material to bear a load at lower concentrations of LDPE (Yokesahachart 2011). This increased LDPE-protein interaction is lessened as more LDPE is added to the blend, as LDPE-

LDPE interactions within the thermoplastic will increase, leading to mechanical properties more similar to clean LDPE plastics, such as higher extension and lower modulus. This finding is supported by the SEM images obtained of the samples when more LDPE is added (See **Supporting Information**), as separation of the protein and LDPE phases of the plastic can be witnessed at high concentrations of LDPE. In terms of the extension of the thermoplastics, we find that maximum extensibility is achieved when the thermoplastic blend is as homogenous as possible, as only the thermoplastic that contains 80% LDPE is comparable to pure albumin-glycerol or pure LDPE plastics. This lack of relative extendibility in the thermoplastic blends may be due to extension of plastics being highly dependent upon molecular long range orientation in the plastic, with the addition of materials that break up orientation decreasing the extendibility of the resulting material (Carvalho 2003).

When compared to the Kerner and Davies models, we find that the observed modulus values of albumin-LDPE thermoplastics will be higher. This increase of modulus points to a compatibility of albumin protein with LDPE, with high levels of adhesion between the two phases and a synergistic effect when LDPE is added into an albumin protein-based matrix. As for elongation modeling, we find that the albumin-LDPE thermoplastic elongation values are close to values that are determined through the Nielson model, pointing to adhesion between albumin and LDPE phases in the plastic.

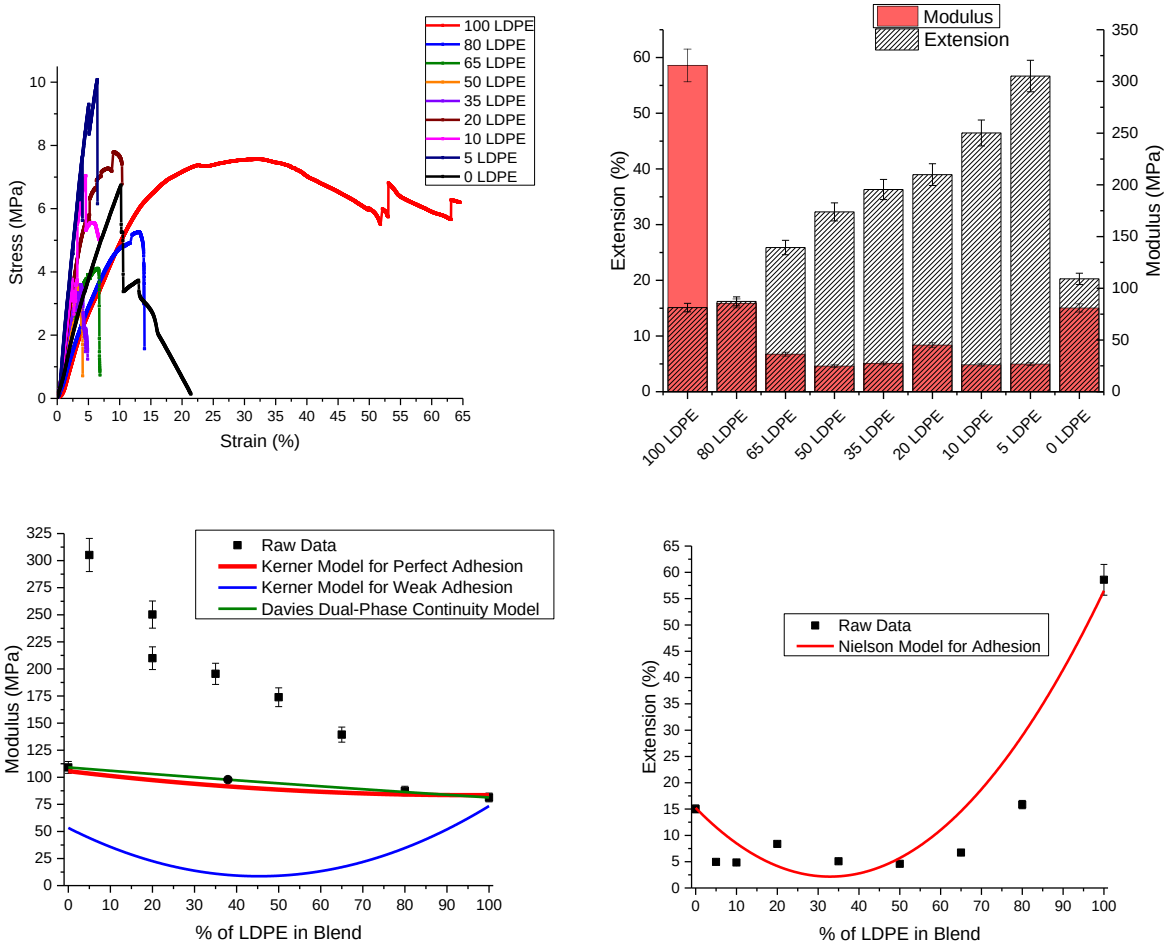


Figure 4.5. Tensile testing and modeling of albumin thermoplastic blends.

As for the zein thermoplastics, we find that as we add 5-20% of LDPE into the thermoplastic blends, there is a significant holding of high modulus of the resulting thermoplastic blends, suggesting strong protein-LDPE interaction compared to albumin-LDPE thermoplastic blends. This finding is supported by the SEM images obtained of the samples (See **Supporting Information**), as the smooth surface of the thermoplastics are broken up by scratches and pits, an indication of a non-clean break of the material when preparing the sample. It is important to notice that the neat, zein plastics showed higher modulus than neat, albumin plastics, which suggests strong protein-protein interactions. However, there is no change in the extension properties that has slightly upward trend at 50% and above of LDPE content. The significant loss

of modulus in the thermoplastic blends at 35% and above of LDPE may be due to zein being the main load bearing constituent of the thermoplastic, and with the addition of more LDPE (which exhibits low modulus) increases, the load bearing capabilities of the resulting blends will decrease (Tian 2008). In terms of the extension of the thermoplastic, there is a slight decrease in the extendibility of the thermoplastic when LDPE is added to the blend, until the blend consists of at least 50% LDPE. This lack of difference in terms of extension may be due to the immiscible nature of the zein-LDPE blend. When molding or extruding immiscible material, it is possible to produce a material that will be able to transfer stress under low deformation due to pseudo-adhesion behavior between zein and LDPE (Leclair 1996). However, when high deformation is applied, the material will be unable to withstand the same amount of strain as the pure protein or pure polymer plastics (Herald 2002, Corradini 2004).

When modeling the modulus of zein-LDPE thermoplastics through Kerner and Davies models, we find that the observed modulus values of zein-LDPE thermoplastics will be similar to the Kerner model for weak adhesion, as well as the Davies model. This decrease of modulus when material is added into a plastic matrix points to a lack of synergistic effect of zein protein with LDPE, with lower levels of adhesion between the two phases when compared to albumin-LDPE thermoplastics. After comparing the elongation data with modeling through the Nielson model, we find that the zein-LDPE thermoplastic elongation values are close to (or higher) values that are determined through the Nielson model, pointing to some adhesion between zein and LDPE phases in the plastic.

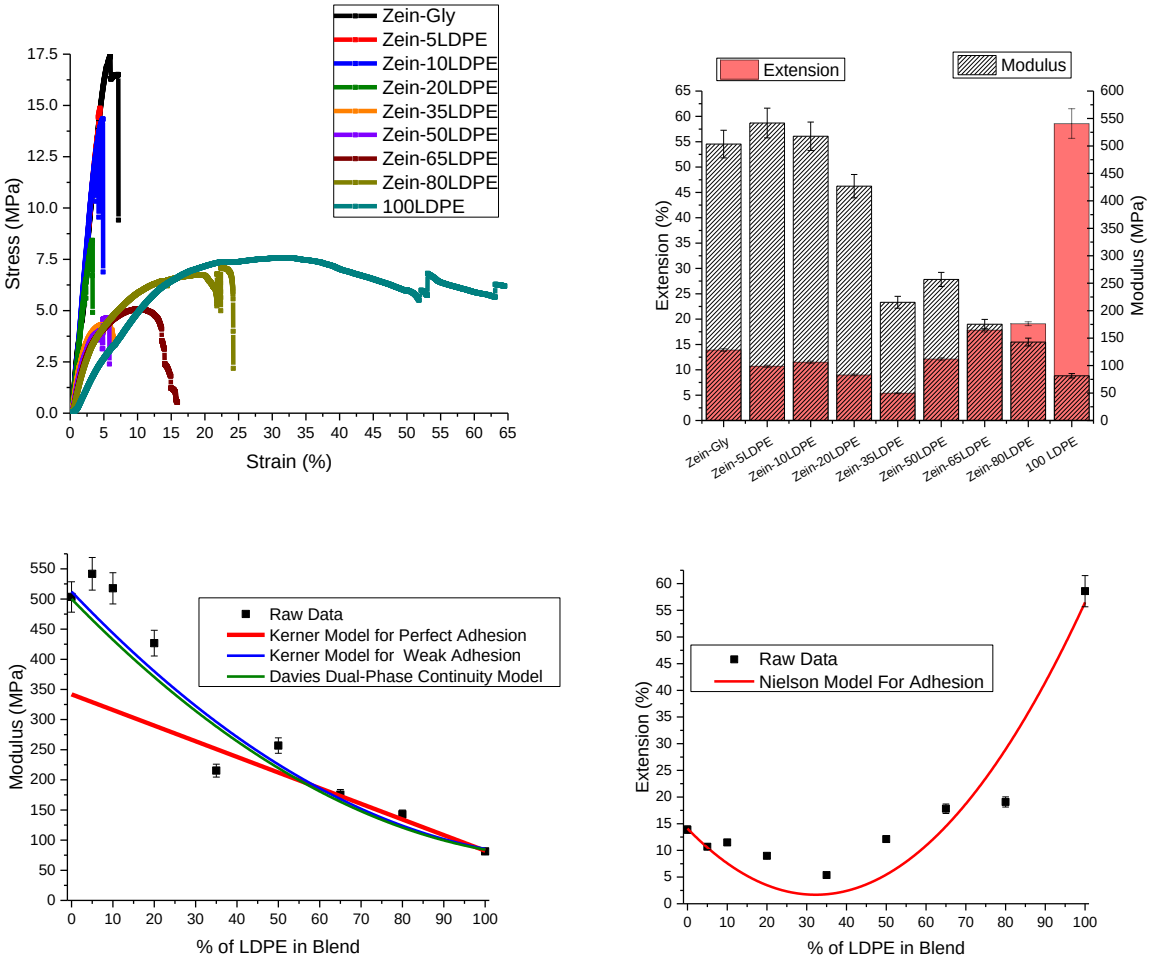


Figure 4.6. Tensile testing and modeling of zein thermoplastic blends.

Water Absorption Properties of Thermoplastic Blends

When subjected to submersion we find that albumin-based plastics exhibit loss in mass due to solubilized matter and/or structure instability (as shown in Figure 4.7(a))**,** while Zein-based plastics exhibit an increase in mass gain (as shown in Figure 4.7(b))**.** The zein plastics containing up to 5% of LDPE content end up with masses that are over 300% compared to their initial masses after seven days of water submersion, while albumin plastics will only have a mass that is 125% of their initial mass due to the amount of moisture uptake. This is due to the zein-based plastics possessing a greater ability to absorb more water due to the addition of glycerol as a plasticizer to the zein, as this will cause a substantial increase in the water absorption of the

resulting plastic when compared to unplasticized zein plastics (Parris 1997). Of note is the decrease of water absorption in thermoplastics that contain 50% LDPE or greater, as LDPE is not a material that will absorb water due to its hydrophobic nature (Nakamura 2005).

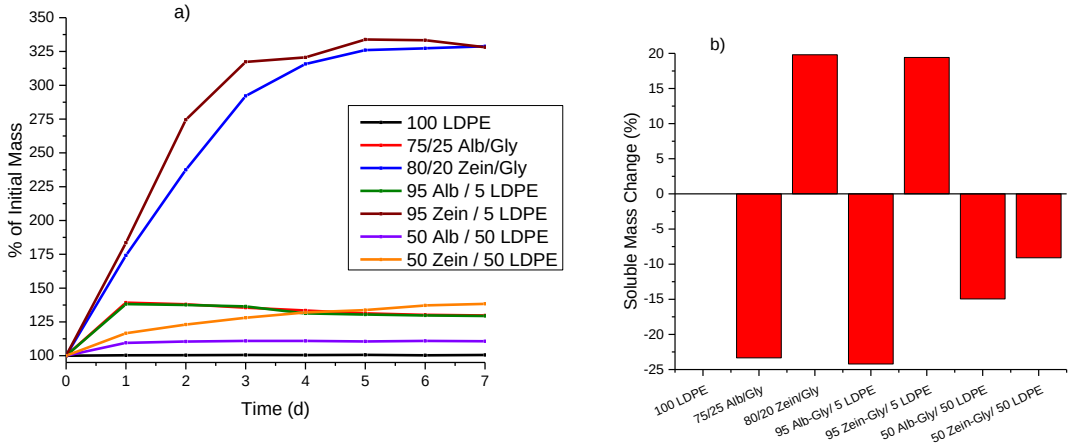


Figure 4.7 a and b. Water absorption and soluble mass change of albumin and zein thermoplastics.

When the samples are dried and weighed to measure the amount of soluble material that is lost, we find that albumin-based plastics are more susceptible to mass loss when submerged in water in comparison to the zein-based plastics. The albumin thermoplastics that contain 5% or less of LDPE loses 20% of their soluble mass, as albumin is a hydrophilic material that will interact with the water bath (Gao 2002). Like any other protein, albumin will be susceptible to soluble mass loss in water due to the affinity for proteins to fold and unfold in globular structures in water in order to interact with other molecules (Pace 2004). In contrast, the zein plastics with lower LDPE content do not lose soluble mass when submerged in water, as there is in fact a positive mass change after drying, most likely due to the large amount of water absorbed by the plastics that remained after drying procedure is followed. When we increase the amount of LDPE in the blends to 50%, we find for that both the albumin and the zein plastics there is a less drastic change in the overall mass of the dried samples. This is due to the lack of solubility of

LDPE in water, as this will result in a plastic that will be less susceptible to mass loss in the case of albumin plastics, and less able to absorb high amounts of water in the case of zein thermoplastics (Psomiadou 1997).

Biodegradability Properties of Protein-based Thermoplastics Blends

In the albumin plastics that have been subjected to microbial attack through soil burial, we find that there is a drastic decrease in the amount of material recovered after 30 days (27.66%), with no material recoverable after 60 days. If we add 5% of LDPE to the albumin plastic there is a greater loss of mass (16.36% recoverable after 30 days) potentially due to an increase in susceptibility of albumin to biodegrade due to lower protein-protein interactions within the plastic, but some samples still remain after 60 days of soil burial (7.65%). The plastics lose mass due to the ability for albumin to be broken down and consumed by bacteria that forms in the soil, as only residual amounts of LDPE can be recovered after medium term burial. LDPE is not susceptible to biodegradation due to the fact that very few strains of bacteria are able to process and consume the material (Yang 2014). In comparison, zein plastics maintain a greater level of integrity after soil burial, as sample recovery for both pure zein bioplastics and zein plastics with 5% of LDPE is possible after both 30 and 60 days. For instance, after 30 days of burial there is 48.46% of pure zein plastics left and 73.42% of zein plastics with 5% LDPE, while after 60 days there is 4.34% of for pure zein plastics and 36.18% of zein plastics made with 5% LDPE. Zein possesses this advantage of microbial attack resistance can be pointed to its hydrophobic properties (Han 2010), as it does not react to water to the same extent of albumin, preventing bacteria from having a resource that would aid in growth (Shukla 2001, Han 2010).

When we produce plastics that consist of 50% of LDPE, we find that there is a comparative lack of degradation after 60 days, as considerable amounts of mass remain for both

albumin and zein plastics (65.08% and 61.50%, respectively). Since LDPE is not susceptible to degradation by microbial attack (97.79% of initial mass remains after 60 days), more protein-based plastic mass can be recovered from the soil with higher concentrations of LDPE use (Arvanitoyannis 1998).

Figure 4.8. Plastics that have been subjected to biodegradation susceptibility analysis: (a) Albumin-Glycerol (30 Days); (b) and (c) Albumin-Glycerol-5 LDPE (30 and 60 Days); (d) and (e) Albumin-Glycerol-50 LDPE (30 and 60 days); (f) and (g) Zein-Glycerol (30 and 60 days); (h) and (i) Zein-Glycerol-5 LDPE (30 and 60 Days); (j) and (k) Zein-Glycerol-50 LDPE (30 and 60 days); (l) and (m) LDPE (30 and 60 days)

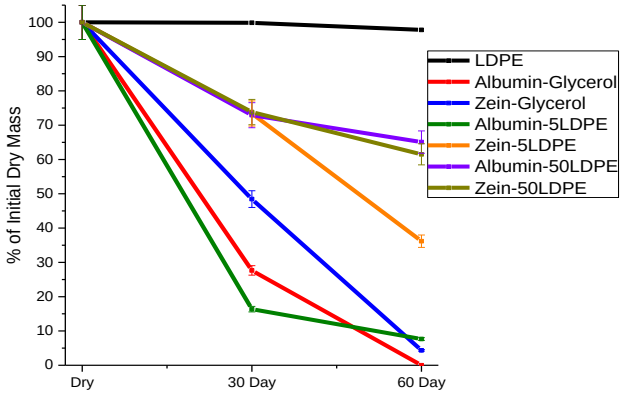


Figure 4.9. Mass change of samples analyzed for susceptibility of biodegradation through microbial attack.

Antibacterial Properties of Albumin and Zein Thermoplastics

Surface Antibacterial Testing

In order to determine if albumin or zein-based plastics would be an effective tool in the prevention of bacterial spread in certain applications, it will be necessary to conduct surface antibacterial testing. Figure 5.9 shows that after the application of inoculated agar to the surface of both albumin and zein-based plastics, we find that as the amount of LDPE in the thermoplastic increases, there is a decrease in the inhibitive effect of the plastic on surface bacteria growth. For instance, in the plastics that contained 20% LDPE there remained at least 150 CFUs/mL after the application of Gram + bacteria, while with 5% of LDPE there are less than 25 CFUs/mL recovered. Albumin-glycerol bioplastics are able to prevent the growth of bacteria on its surface after 24 hours of application for both Gram + and Gram – bacteria, due to potential glycerol leeching and antibacterial properties of the albumin and zein proteins itself (Torres-Giner 2009, Jones 2015). However, when we increase the LDPE (no antimicrobial efficacy) content to the thermoplastic blend, complete surface bacterial growth prevention on the resulting thermoplastic is not present. For instance, in the albumin plastics that contain 20% LDPE there is a 15.88%

decrease in Gram + bacterial colonies, and for zein that contains the same amount of LPDE there is a 25.23% decrease. However, when there is only 5% of LDPE in the plastics, there is a 72.79% decrease in Gram + bacterial colonies for albumin plastics, while for zein plastics there is a 96.45% decrease. These results corroborate with results found in past research on this subject, as plastics that have been incorporated with antibacterial additives such as nisin in PE-PEO films (84.6% inhibition after 3 days) (Cutter 2001) and chitosan-PEO films (3 $\log_{10}$ reduction after 24 hours) (Coma 2006, Zivanovic 2007) as complete resistance to bacterial growth on plastic surfaces of thermoplastics is not possible without the use of additives specifically designed to prevent bacterial growth (Vartiainen 2003).

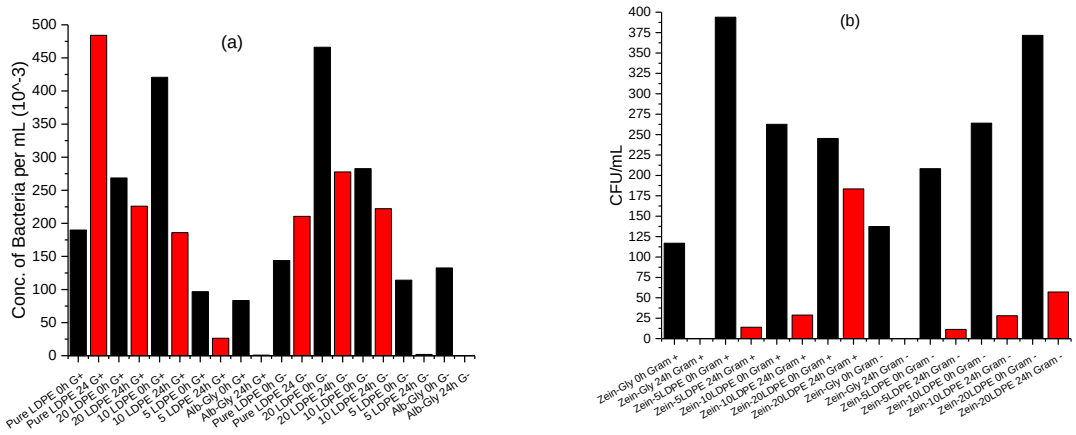


Figure 4.10. Surface antimicrobial properties of (a) albumin and (b) zein thermoplastics.

Drug Elution Properties of Thermoplastic Blends

In order for use in medical and food packaging applications, it will be necessary to impart additional antimicrobial properties into these plastics. To enhance antimicrobial properties, we have utilized two common medical drugs (ampicillin and ciprofloxacin) and two food preservatives (sodium benzoate and sodium nitrite) in the preparation of drug eluting plastics. With the ability of drug elution, it will be possible to prevent bacteria growth in a given area, as opposed to the prevention of surface bacterial adhesion.

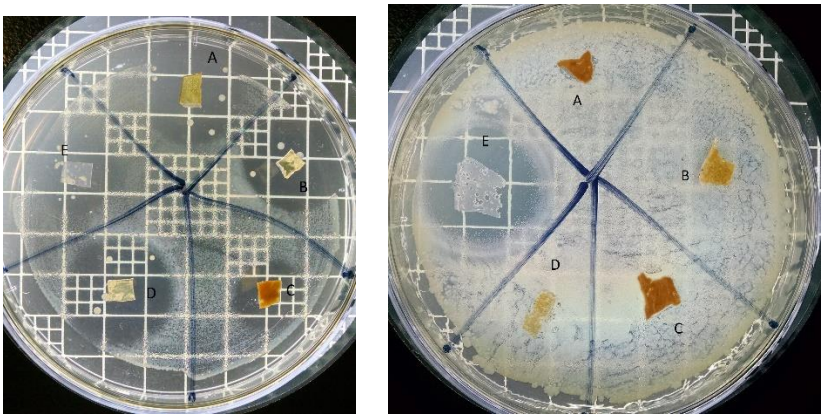


Figure 4.11 and 12. Drug elution for Gram + samples (Figure 11 A – zein-5LDPE-ciprofloxacin, B- albumin-5LDPE-sodium benzoate, C- zein-gly-ampicillin, D- albumin-gly-sodium benzoate, E- LDPE-ciprofloxacin) (Figure 12 A – zein-5LDPE, B- albumin-5LDPE-sodium nitrite, C- zein-gly, D- albumin-gly-sodium nitrite, E- LDPE-ampicillin.

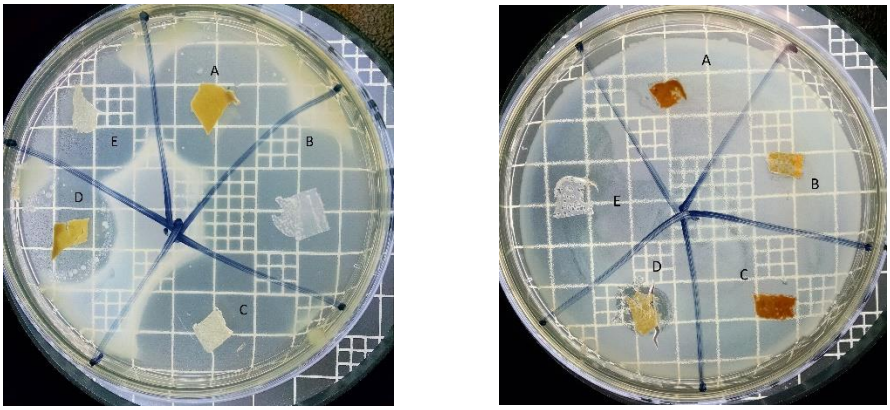


Figure 4.13 and 14. Drug elution for Gram + samples (Figure 13 A – zein-5LDPE-ampicillin, B- LDPE-ciprofloxacin, C- alb-gly-ampicillin, D- zein-gly-sodium benzoate, E- alb-5LDPE-ampicillin) (Figure 14 A – zein-5LDPE-sodium nitrite, B- albumin-5LDPE, C- zein-gly, D- albumin-gly-sodium nitrite, E- LDPE sodium benzoate).

After imparting additional antibacterial properties into the thermoplastic through the elution of additives, we find that sodium nitrite is an ineffective additive to utilize, as the plastics in which it is imbedded do not generate any zones of inhibition on inoculated petri dishes, as shown in Figures 5-11 through 14. The lack of effective antibacterial elution properties of sodium nitrite can be due to a lack of oxygen intake in the testing environment will allow

anaerobic species to continue growth due to a lack of ability to absorb the sodium nitrite, which is possible in an environment with higher levels of oxygen (Fang 1985). This lack of the inhibition zone may also be due to the potential lack of elution during the allotted time period. When we utilize sodium benzoate, we do find a gradual increase in the zone of inhibition of the plastics in which it is imbedded over time, a sign of the release of benzoic acid into the environment. Benzoic acid will be generated by the dissociation of the sodium benzoate by the bacteria, releasing sodium hydroxide as well (WHO 2000). During the dissociation of sodium benzoate, the release of benzoic acid will reduce the pH of intracellular water by over 1 pH unit (Krebs 1983), inhibiting cell growth.

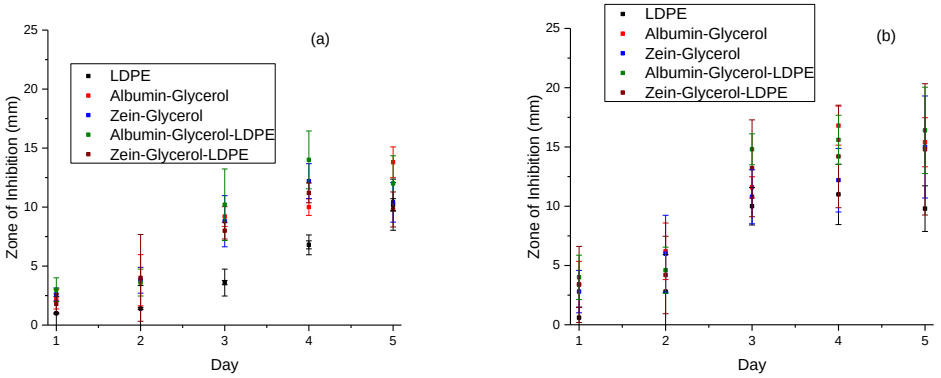


Figure 4.15. Zone of inhibition for plastics with 15% sodium benzoate: (a) Gram + and (b) Gram -.

With the utilization of antibiotics such as ampicillin and ciprofloxacin, we find that both are much more effective in terms of inhibition zones after 5 days created for both Gram + (43.4-39.2 mm for ampicillin, 42.1-37.7 mm for ciprofloxacin) and Gram – bacteria (35.2-19.4 mm for ampicillin, 38.5-41.7 mm for ciprofloxacin) when compared to sodium benzoate (15.2-8.1 mm for Gram +, 20.1-7.4 mm for Gram -) and sodium nitrite (0 mm of inhibition for both bacteria). Both of the antibiotics exhibit inhibition zones of increasing size as time passes, with the plastics that contain ciprofloxacin possessing a linear trend in terms of zone of inhibition growth after

five days. Ciprofloxacin possesses this advantage due to its ability to inhibit both Gram + and Gram – growth, as it has been designed to be effective against a wide range of bacterial organisms, as well as the ability to elute from a material easily (Unnithan 2012). While ampicillin possesses an ability to consistently inhibit Gram + bacteria growth, for Gram – bacteria we find that the zone of inhibition stays a consistent size (37.2-18.3 mm) after 5 days. This may be due to the fact that ampicillin lacks the same antibacterial effectiveness against *E. coli*, as well as the bacteria potentially gaining a resistance to the ampicillin (Reinthaler 2003).

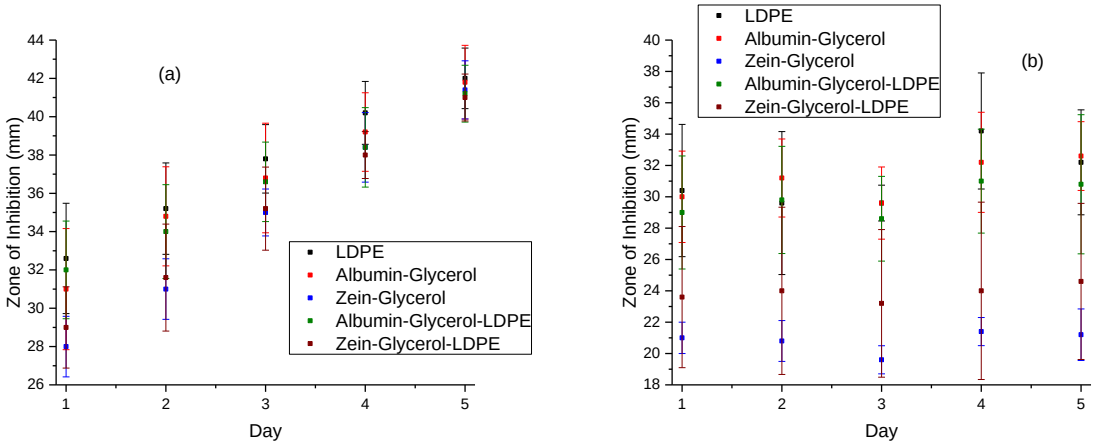


Figure 4.16. Zone of inhibition for plastics with 15% ampicillin: (a) Gram + and (b) Gram -.

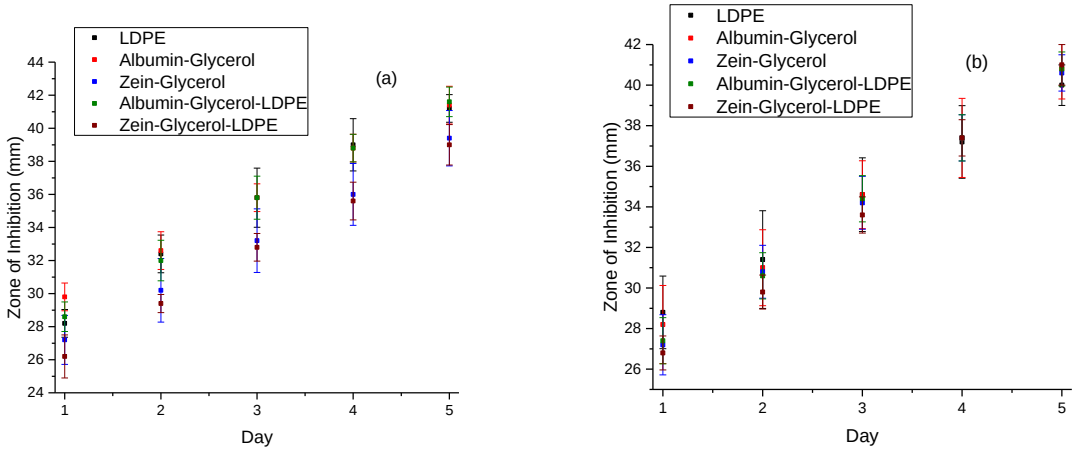
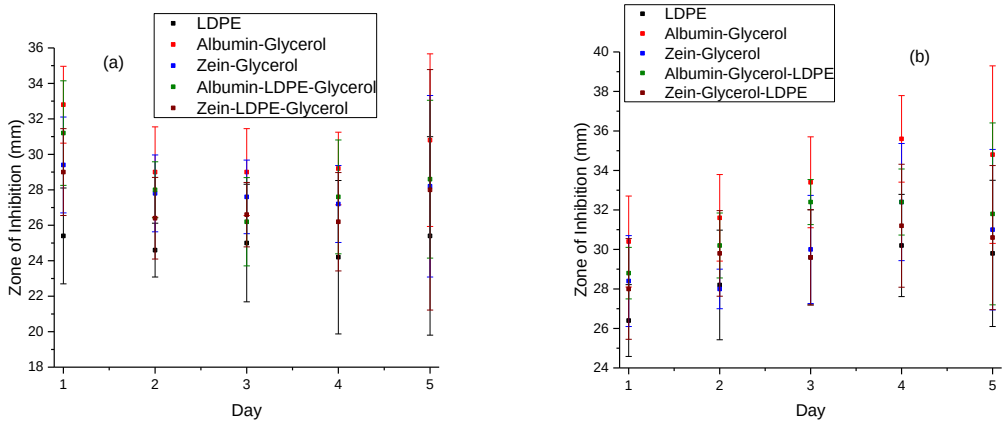


Figure 4.17. Zone of inhibition for plastics with 15% ciprofloxacin: (a) Gram + and (b) Gram -.

Altering of Drug Levels and Inhibition Zone Effects

After the initial analysis of the plastics that have been loaded with 15% of either food preservatives or antibiotics, we then adjust the amount of additive loaded into the plastics and its resulting effect on drug elution and the inhibition zones of a given plastic. When we modify the plastics to contain lesser amounts of the antibiotics, we find the overall size of the inhibition zones will decrease, as well as recording results that will have higher variability. The decrease in inhibition zone size is due to lower amounts of antibiotic that will be released from the plastic, with the potential of drug resistance formation by the bacteria if the dose of antibiotic in the environment is too low. We also find that the results that we obtain will have a higher degree of variability when we compare them to plastics that have 15% of drug loaded into the plastic. The increase in variability is due to the fact that since there is less antibiotic in the plastic, there is an increase in probability that the drug release from the plastics will not be as uniform (Reza 2003). Another finding we make is that the albumin-based plastics will result in relatively higher zones of inhibition when compared to the pure LDPE and the zein plastics, with increased levels of drug elution possible. The albumin plastics possess this ability due to their increased ability to react to the environment in comparison to zein and LDPE plastics, as albumin is more permeable in areas that contain higher moisture such as bacterial colonies (Gennadios 1996).



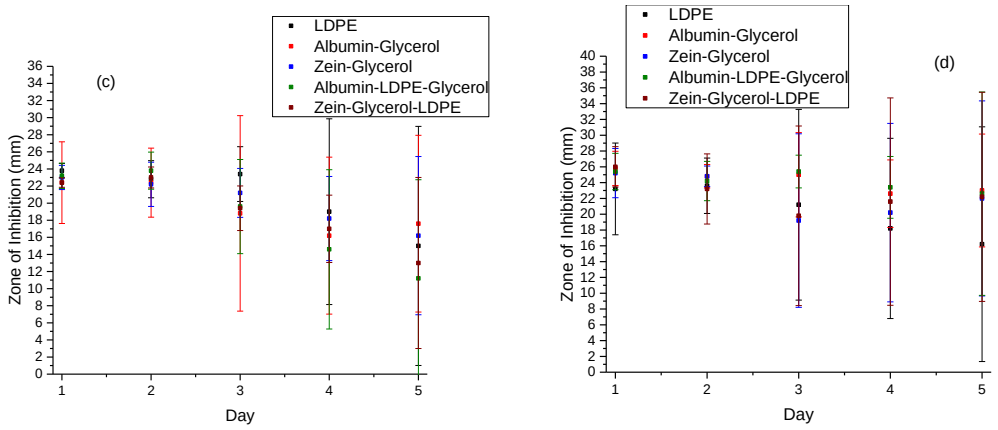


Figure 4.18. Zone of inhibition for plastics with ciprofloxacin: 10% - (a) Gram + and (b) Gram -; and 5% (c) Gram + and (d) Gram -.

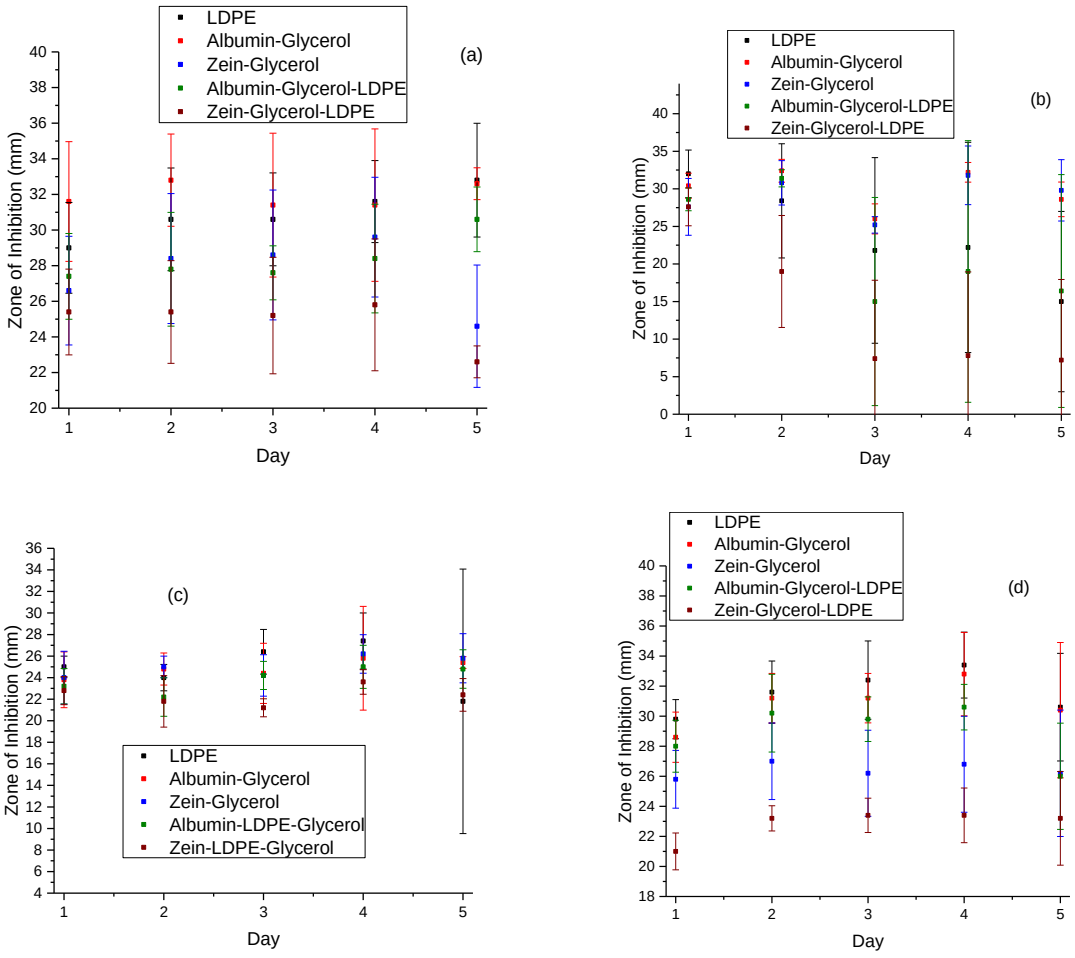


Figure 4.19. Zone of inhibition for plastics with ampicillin: 10% - (a) Gram + and (b) Gram - ; and 5% (c) Gram + and (d) Gram -.

As for the sodium benzoate plastics, we find that as we decrease the amount of food preservative in the plastic, the plastics will be unable to produce a zone of inhibition when

encountered with a Gram + bacteria. This lack of effectiveness against Gram + such as *B. subtilis* may be due to sodium benzoate being unable to generate enough benzoic acid in solution to eliminate Gram + colonies at lower concentrations (El-Shenawy 1988). We also find that much like the plastics that have been loaded with antibiotics, the sodium benzoate containing plastics will have a much higher level of variability when it comes to the zone of inhibition generated when encountering Gram – species. Just like in the thermoplastics with ciprofloxacin and ampicillin, this could also be due to the lack of even dispersion of the low amounts of food preservative in the plastic.

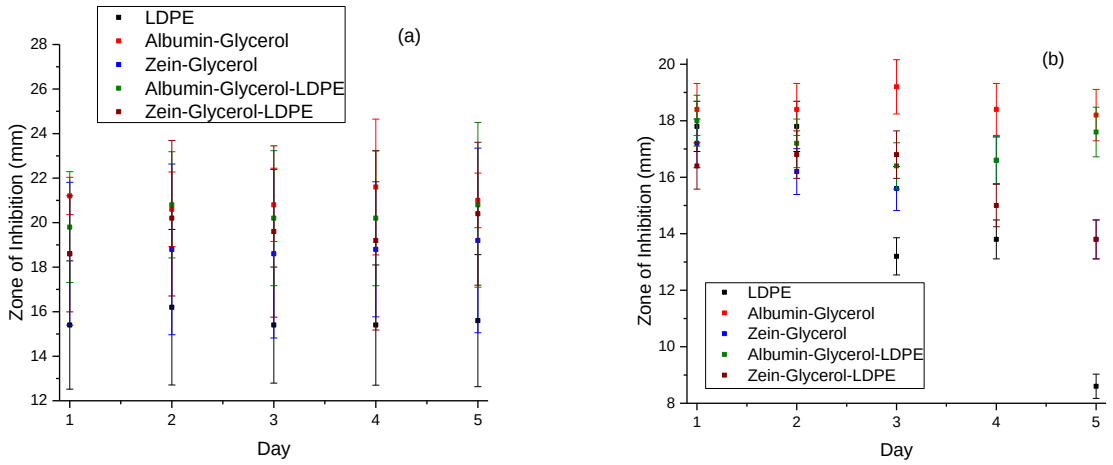


Figure 4.20. Zone of inhibition for plastics with (a) 10% and (b) 5% of sodium benzoate: Gram -.

Analysis of Elution Kinetics of Albumin Bioplastics

Through our analysis that determines that the albumin-glycerol bioplastics that contained the antibiotics of ampicillin and ciprofloxacin, we then analyze the bioplastics for the kinetics of drug elution at differing concentrations. When analyzing the albumin bioplastics that contain ampicillin, we find that the amount of ampicillin loaded into the plastic is crucial in the amount of antibiotic that will eventually be released. With the albumin that contains 15% of ampicillin, we find that it will elute more ampicillin in solution in one day than what will be eluted from the 5% samples in five days, as well as the amount to be eluted from the 10% samples after three

days. Albumin bioplastics can elute more drug due to the fact that it contains more drug, and after its initial release it still contains ampicillin than can be released over time (Liu 2010). As for the albumin bioplastics that contain ciprofloxacin, we find that the release of drug from the plastic is more gradual, as the plastic that contains 15% ciprofloxacin will only release a considerably higher amount of antibiotic after five days in solution. Albumin bioplastics that contain ciprofloxacin could need more time for effective drug elution due to the ciprofloxacin being bound to the albumin-glycerol material in a way that inhibits an immediate release when compared to other drugs (Anguita-Alonso 2006).

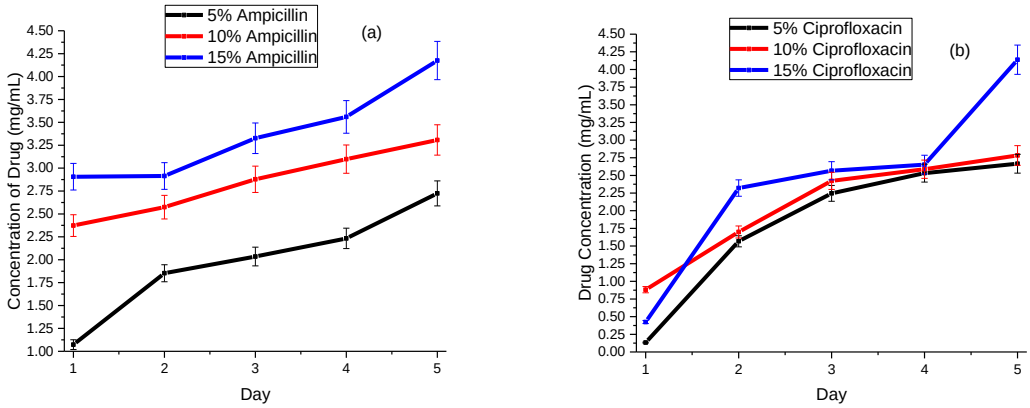


Figure 4.21. Elution rate of drug from albumin-glycerol bioplastics: (a) ampicillin and (b) ciprofloxacin

Statistical Analysis of Drug Elution Raw Data

Initial Statistical Analysis

With the statistical analysis of the drug elution experimental raw data, certain inferences can be made. We fit a regression model with the diameter of the inhibition zone as the response and different types of proteins and drugs or preservatives as explanatory variables. One standard assumption for fitting a regression model is that the errors are identically and independently distributed Normal random variables with zero mean and some constant variance. However, this assumption will not be valid since there is (almost) no inhibition for the control (no drug) and

preservative, Sodium Nitrite. As seen in Figure 4.18, the boxplots that compare the resulting inhibition for different drugs and food preservatives, we should concentrate on Sodium Benzoate, Ampicillin and Ciprofloxacin only.

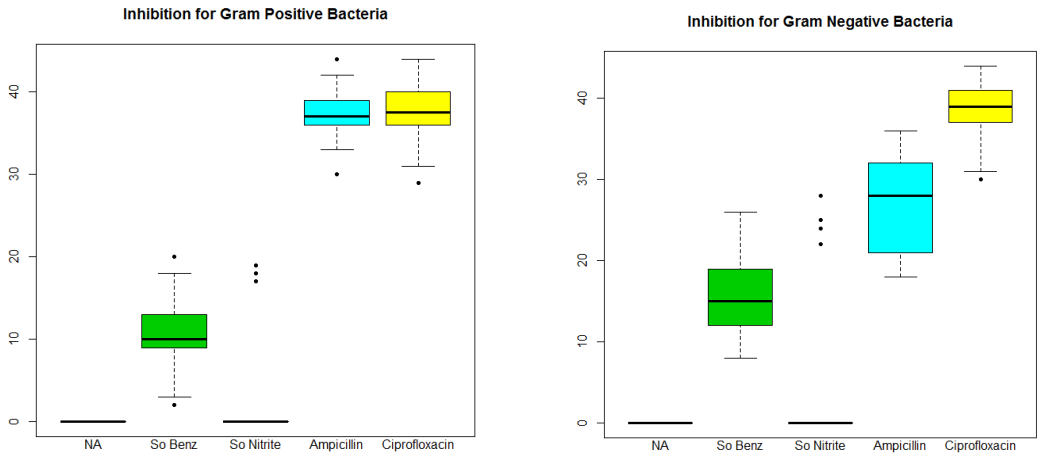


Figure 4.22: Boxplot of the inhibition data

After the elimination of Sodium Nitrite as a potential additive, it is now possible to fit a regression model with the diameter of the inhibition zone as the response and different types of proteins and three drugs/food preservatives (sodium benzoate, ampicillin and ciprofloxacin). We entertain both main effects of proteins and drugs as well as their interactions in our model, and fit two separate models for Gram + and Gram – bacteria. As shown in Tables 4.1 and 4.2, it is determined that the factors of material use and drug/preservative use are both statistically significant, as well as the interaction between the two factors, for both Gram + and Gram – bacteria. In comparing the influences of the factors on expected results, it can be seen that sum of squares corresponding to the factor of drug is 14652 out of a total of 15729, while in the Gram – bacteria it is 7684 out of the total of 9739. Clearly the type of drug/preservative use can explain most of the variation in data, so we determine that the use of additives has the greatest influence for both Gram + and Gram – bacteria.

Tables 4.1 and 4.2. ANOVA tables for examining protein, drug, and protein:drug interactions on elution properties.

ANOVA for Gram Positive Bacteria

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
factor(Protein)	5	123	25	6.478	5.03e-05
factor(Drug)	2	14652	7326	1933.569	< 2e-16
factor(Protein):factor(Drug)	10	681	68	17.964	6.76e-16
Residuals	72	273	4		
Total	89	15729			

ANOVA for Gram Negative Bacteria

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
factor(Protein)	5	260	52	5.707	0.000173
factor(Drug)	2	7684	3842	422.199	< 2e-16
factor(Protein):factor(Drug)	10	1140	114	12.526	2.77e-12
Residuals	72	655	9		
Total	89	9739			

With the examination of the regression coefficients for both Gram + and Gram – negative data, many inferences can be made. For the Gram + bacteria, we find that the combination of albumin and ampicillin results in the maximum amount of inhibition (3.6 + 34.8 + 6.4 - 4.2 = 40.6 mm), followed by zein and ciprofloxacin (3.6 + 35.8 + 6.8 - 6.0 = 40.2 mm). Ciprofloxacin with albumin and LDPE are also good, with predicted inhibition being 39.6 and 39.4, respectively. As for the Gram – bacteria, we find that the combination of albumin and ciprofloxacin would result in the maximum amount of inhibition (9.8 + 29.6 + 5.6 - 2.4 = 42.6 mm), then followed by zein and ciprofloxacin (9.8 + 29.6 + 5.6 - 4.8 = 40.2 mm) and LDPE and ciprofloxacin (9.8 + 29.6 = 39.4 mm).

Tables 4.3 and 4.4. Full regression values for examining protein, drug, and protein:drug interactions on elution properties.

Regression output for Gram + Bacteria

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	3.6000	0.8705	4.136	9.46e-05
Albumin	6.4000	1.2311	5.199	1.80e-06
Zein	6.8000	1.2311	5.524	4.99e-07
Albumin-LDPE	8.4000	1.2311	6.823	2.34e-09
Zein-LDPE	6.2000	1.2311	5.036	3.39e-06
Cellulose Disk	12.4000	1.2311	10.072	2.17e-15
Ampicillin	34.8000	1.2311	28.268	< 2e-16
Ciprofloxacin	35.8000	1.2311	29.080	< 2e-16
Albumin x Ampicillin	-4.2000	1.7410	-2.412	0.018397
Zein x Ampicillin	-7.6000	1.7410	-4.365	4.17e-05
Albumin-LDPE x Ampicillin	-9.6000	1.7410	-5.514	5.19e-07
Zein-LDPE x Ampicillin	-8.0000	1.7410	-4.595	1.80e-05
Cellulose Disk x Ampicillin	-17.2000	1.7410	-9.879	4.92e-15
Albumin x Ciprofloxacin	-6.2000	1.7410	-3.561	0.000659
Zein x Ciprofloxacin	-6.0000	1.7410	-3.446	0.000952
Albumin-LDPE x Ciprofloxacin	-10.4000	1.7410	-5.974	8.07e-08
Zein-LDPE x Ciprofloxacin	-9.2000	1.7410	-5.284	1.29e-06
Cellulose Disk x Ciprofloxacin	-20.4000	1.7410	-11.717	< 2e-16

Regression output for Gram - Bacteria

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	9.800	1.349	7.264	3.59e-10
Albumin	5.600	1.908	2.935	0.004471
Zein	5.200	1.908	2.726	0.008055
Albumin-LDPE	6.600	1.908	3.459	0.000914
Zein-LDPE	5.000	1.908	2.621	0.010697
Cellulose Disk	13.200	1.908	6.919	1.56e-09
Ampicillin	22.400	1.908	11.741	< 2e-16
Ciprofloxacin	29.600	1.908	15.515	< 2e-16
Albumin x Ampicillin	-5.800	2.698	-2.150	0.034946
Zein x Ampicillin	-16.200	2.698	-6.004	7.12e-08
Albumin-LDPE x Ampicillin	-8.000	2.698	-2.965	0.004103
Zein-LDPE x Ampicillin	-12.600	2.698	-4.670	1.36e-05
Cellulose Disk x Ampicillin	-23.800	2.698	-8.821	4.50e-13
Albumin x Ciprofloxacin	-2.400	2.698	-0.889	0.376697
Zein x Ciprofloxacin	-4.800	2.698	-1.779	0.079461
Albumin-LDPE x Ciprofloxacin	-7.800	2.698	-2.891	0.005075
Zein-LDPE x Ciprofloxacin	-6.800	2.698	-2.520	0.013945
Cellulose Disk x Ciprofloxacin	-20.000	2.698	-7.412	1.91e-10

When we compare the individual types of protein/polymer as well as the type of additive utilized, several findings are determined. Through the use of our regression model, we find that that the additive of ciprofloxacin is best for the prevention of Gram – bacteria growth. As for the type of plastic, zone of inhibitions will be greatest when albumin is utilized as the material, with zein in a close second, and LDPE with the lowest inhibition zones. When we conduct the same

type of regression analysis for the Gram + results, we find that both the additives of ampicillin and ciprofloxacin are highly effective in the prevention of bacterial growth. The regression model suggests that the combination of albumin with ampicillin as an additive will lead to the largest zone of inhibition, with any of the plastic types (albumin, zein, or LDPE) being effective in bacterial growth prevention when blended with ciprofloxacin.

Adding LDPE to Albumin and Zein

It is necessary to then examine the effect of the addition of LDPE into the plastic blends on the level of drug elution, as this will determine if the material will still be suitable for elution applications. Since we determine that the interaction is significant, it will be appropriate to consider each protein separately. However, when we compare both albumin with albumin blended with LDPE and zein and zein blended with LDPE, let us consider models without interaction and fit the model to the data points pertaining to either albumin and albumin-LDPE or zein and zein-LDPE. In the comparison between albumin and albumin-LDPE, we see that there is no significant difference between the two proteins for both Gram + and Gram - bacteria, as the p-values in the ANOVA tables shown in the Supporting Information are sufficiently big. For the zein and zein-LDPE comparison, the same inferences can be made for both Gram + and Gram -, as shown in the ANOVA tables. However, the p-value corresponding to the proteins is not too big for Gram + bacteria, but even there we can conclude that adding LDPE does not make any different at 10% level of significance. These conclusions are based on a model with drugs sodium benzoate, ampicillin and ciprofloxacin, but the conclusions will essentially not change even if the control (no drugs) and sodium nitrate samples were included in the model.

Tables 4.5 and 4.6. ANOVA tables for examining influence of LDPE addition to albumin plastics.

ANOVA Table to Compare (Albumin) & (Albumin-LDPE) for Gram + Bacteria

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
factor(Protein)	1	11	10.8	2.368	0.136
factor(Drug)	2	5116	2558.0	560.783	<2e-16
Residuals	26	119	4.6		

ANOVA Table to Compare (Albumin) & (Albumin-LDPE) for Gram - Bacteria

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
factor(Protein)	1	17.6	17.6	2.01	0.168
factor(Drug)	2	3071.7	1535.8	175.09	8.21e-16
Residuals	26	228.1	8.8		

Tables 4.7 and 4.8. ANOVA tables for examining influence of LDPE addition to zein plastics.

ANOVA Table to Compare (Zein)& (Zein-LDPE) for Gram + Bacteria

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
factor(Protein)	1	24	24.3	6.897	0.0143
factor(Drug)	2	5086	2542.8	721.755	<2e-16
Residuals	26	92	3.5		

ANOVA Table to Compare (Zein)& (Zein-LDPE) for Gram - Bacteria

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
factor(Protein)	1	0.8	0.8	0.059	0.81
factor(Drug)	2	2933.6	1466.8	103.333	4.24e-13
Residuals	26	369.1	14.2		

Conclusions

As we compare the thermal properties of the protein-based thermoplastics, we find that as we add more LDPE into the thermoplastic blend, the resulting plastics will have thermal properties more similar to LDPE plastics than pure protein plastics. In terms of the water absorption and susceptibility to biodegradation analysis, we find that the addition of LDPE will produce a plastic that is less susceptible to absorbing large amounts of water, as well as limiting the amount of material that will be biodegradable when buried in soil. As for the drug elution properties of the resulting thermoplastics, we find that pure albumin-glycerol bioplastics loaded with the antibiotics ampicillin and ciprofloxacin provide the best drug elution properties of all of the thermoplastic blends analyzed. There are multiple avenues of interest that could be followed based on the findings made in this study. Since these plastics would need to be examined for

potential use in the area of medical packaging (or medical devices themselves), it will be necessary to test these materials under methods such as ASTM F2097 – 10: Standard Guide for Design and Evaluation of Primary Flexible Packaging for Medical Products, or ASTM F813 - 07(2012): Standard Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices. If these plastics were to be utilized in food packaging, it will be necessary to test them through test standards such as ASTM F1640 – 09: Standard Guide for Packaging Materials for Foods to Be Irradiated, or ASTM E1870 – 11: Standard Test Method for Odor and Taste Transfer from Polymeric Packaging Film, to determine if the use of these plastics will decrease the quality of the food to be consumed. The examination of the elution of the drug/food preservative of these plastics over a longer period of time (7+ days) will also be necessary, as medical and food packaging will be placed in storage longer than a week before it is utilized. Utilizing other types of drugs such as amoxicillin and moxifloxacin, as well as other types of bacteria such as *Staphylococcus aureus* and *Neisseria meningitidis* should also be analyzed for additional or more specific uses of the resulting product.

References

1. Hota, B., Contamination, Disinfection, and Cross-Colonization: Are Hospital Surfaces Reservoirs for Nosocomial Infection? *Clinical Infectious Diseases* **2004,** *39* (8), 1182-1189.

2. Schultz , M., Gill , J., Zubairi , S., Huber, R., Gordin, F., Bacterial Contamination of Computer Keyboards in a Teaching Hospital. *Infection Control and Hospital Epidemiology* **2003,** *24* (4), 302-303.

3. Borch, E., Kant-Muermans, M-L., Blixt, Y., Bacterial Spoilage of Meat and Cured Meat Products. *International Journal of Food Microbiology* **1996,** *33* (1), 103-120.

4. Halden, R. U., Plastics and Health Risks. *Annual Reviews of Public Health* **2010,** *31*, 179-194.

5. Lau, O.-W., Wong, S-K., Naphthalene Contamination of Sterilized Milk Drinks Contained in Low-density Polyethylene Bottles: Part 1. *Analyst* **1994,** *119* (5), 1037-1042.

6. Lee, B.-K., Ellenbecker, M.J., Moure-Eraso, R., Analyses of the recycling potential of medical plastic wastes. *Waste Management* **2002,** *22* (5), 461-470.

7. Hopewell, J., Dvorak, R., Kosior, E., Plastics Recycling: Challenges and Opportunities. *Philosophical Transactions: Biological Sciences* **2009,** *364* (1526), 2115-2126.

8. Queiroz, A. C., Santos, J.D., Monteiro, F.J., Gibson, I.R., Knowles, J.C., Adsorption and release studies of sodium ampicillin from hydroxyapatite and glass-reinforced hydroxyapatite composites. *Biomaterials* **2001,** *22* (11), 1393-1400.

9. Dimalo, F., O'Halloran, J.J., Quale, J.M. , In vitro elution of ciprofloxacin from polymethylmethacrylate cement beads. *Journal of Orthopaedic Research* **1994,** *12* (1), 79-82.

10. Vartiainen, J., Skytta, E., Enqvist, J., Ahvenainen, R., Properties of Antimicrobial Plastics Containing Traditional Food Preservatives. *Packaging Technology and Science* **2003,** *16* (6), 223-229.

11. Neetoo, H., Ye, M., Chen, H., Joerger, R.D., Hicks, D.T., Hoover, D.G., Use of nisin-coated plastic films to control Listeria monocytogenes on vacuum-packaged cold-smoked salmon. *International Journal of Food Microbiology* **2008,** *122* (1-2), 8-15.

12. Eby, D. M., Luckarift, H.R., Johnson, G.R., Hybrid antimicrobial enzyme and silver nanoparticle coatings for medical instruments. *ACS Applied Materials and Interfaces* **2009,** *1* (7), 1553-1560.

13. MacCallum, N., Howell, C.,Kim, P., Sun, D., Friedlander, R., Ranisau, J., Ahanotu, O., Lin, J.J., Vena, A., Hatton, B., Wong, T-S., Aizenberg, J.,, Liquid-Infused Silicone As a Biofouling-Free Medical Material. *ACS Biomaterials Science and Engineering* **2015,** *1* (1), 43-51.

14. Loo, C.-Y., Young, P.M., Lee, W-H., Cavaliere, R., Whitchurch, C.B., Rohanizadeh, R., Superhydrophobic, nanotextured polyvinyl chloride films for delaying Pseudomonas aeruginosa attachment to intubation tubes and medical plastics. *Acta Biomaterialia* **2012,** *8* (5), 1881-1890.

15. Freschauf, L. R., McLane, J., Sharma, H., Khine, M., Shrink-induced superhydrophobic and antibacterial surfaces in consumer plastics. *PLOS One* **2012,** *7* (8), 1-7.

16. (a) Jones, A., Zeller, M A, Sharma, S, Thermal, mechanical, and moisture absorption properties of egg white protein bioplastics with natural rubber and glycerol. *Progress in Biomaterials* **2013,** *2* (12); (b) Gillgren, T., Stading, M., Mechanical and Barrier Properties of Avenin, Kafirin, and Zein Films. *Food Biophysics* **2008,** *3* (3), 287-294.

17. Jones, A., Mandal, A., Sharma, S., Protein-based bioplastics and their antibacterial potential. *Journal of Applied Polymer Science* **2015,** *132* (18).

18. Güçbilmez, Ç. M., Yemenicioglu, A., Arslanoglu, A., Antimicrobial and antioxidant activity of edible zein films incorporated with lysozyme, albumin proteins and disodium EDTA. *Food Research International* **2007,** *40*, 80-91.

19. Jain, D., Banerjee, R., Comparison of ciprofloxacin hydrochloride-loaded protein, lipid, and chitosan nanoparticles for drug delivery. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **2008,** *86B* (1), 105-112.

20. Sue, H. J., Wang, S, Lane, J L, Morphology and mechanical behaviour of engineering soy plastics. *Polymer* **1997,** *38* (20), 5035-5040.

21. Menard, K., *Dynamic Mechanical Analysis: A Practical Introduction*. CRC Press: Boca Raton, Florida, 1999.

22. Fried, J., *Polymer Science & Technology*. 2nd ed.; Prentice Hall: 2003.

23. Institute, C. a. L. S., M02-A11 In *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition* Clinical and Laboratory Standards Institute Wayne, PA, 2012; Vol. M02-A11

24. Cazedey, E. C. L., Salgado, H.R.N, Spectrophotometric determination of ciprofloxacin hydrochloride in ophthalmic solution. *Advances in Analytical Chemistry* **2012,** *2* (6), 74-79.

25. Castelló, M., Dweck, J, Aranda, D.A.G., Thermal stability and water content determination of glycerol by thermogravimetry. *Journal of Thermal Analysis and Calorimetry* **2009,** *97* (2), 627-630.

26. (a) Magoshi, J., Nakamura, S., Murakami, K-I., Structure and physical properties of seed proteins. I. Glass transition and crystallization of zein protein from corn. *Journal of Applied Polymer Science* **1992,** *45* (11), 2043-2048; (b) Wongsasulak, S., Kit, K.M., McClements, D.J., Yoovidhya, T. Weiss, J., The effect of solution properties on the morphology of ultrafine electrospun egg albumen–PEO composite fibers. *Polymer* **2007,** *48* (2), 448-457.

27. Park, J. W., Oh, S.C., Lee, H.P., Kim, H.T., Yoo, K.O., A kinetic analysis of thermal degradation of polymers using a dynamic method. *Polymer Degradation and Stability* **2000,** *67* (3), 535-540.

28. Kim, J. M., Whang, J.H., Kim, K.M., Koh, J.H, Suh, H.J., Preparation of corn gluten hydrolysate with angiotensin I converting enzyme inhibitory activity and its solubility and moisture sorption. *Process Biochemistry* **2004,** *39* (8), 989-994.

29. Herald, T. J., Smith, D.M., Heat-induced changes in the secondary structure of hen egg s-ovalbumin *Journal of Agricultural Food Chemistry* **1992,** *40* (10), 1737-1740.

30. Liu, C., Wang, J., He, J., Rheological and thermal properties of m-LLDPE blends with m-HDPE and LDPE. *Polymer* **2002,** *43* (13), 3811-3818.

31. Jerez, A., Partal, P., Martinez, I., Gallegos, C., Guerrero, A., Egg white-based bioplastics developed by thermomechanical processing. *Journal of Food Engineering* **2007,** *82* (4), 608-617.

32. Shieh, Y.-T., Chuang, H-C., DSC and DMA studies on silane-grafted and water-crosslinked LDPE/LLDPE blends. *Journal of Applied Polymer Science* **2001,** *81* (7), 1808-1816.

33. Averous, L., Moro, L., Dole, P., Fringant, C., Properties of thermoplastic blends: starch–polycaprolactone. *Polymer* **2000,** *41* (11), 4157-4167.

34. Corradini, E., Mattoso, L.H.C., Guedes, C.G.F., Rosa, D.S., Mechanical, thermal and morphological propertiesof poly(e-caprolactone)/zein blends. *Polymers for Advanced Technologies* **2004,** *15* (6), 340-345.

35. Verbeek, C. J. R., van den Berg, L.E., Extrusion processing and properties of protein-based thermoplastics. *Macromolecular Materials and Engineering* **2010,** *295,* 10-21.

36. Vaz, C. M., Mano, J.F., Fossen, M., van Tuil, R.F., de Graaf, L.A., Reis, R.L., Cunha, A.M., Mechanical, dynamic-mechanical, and thermal properties of soy protein-based thermoplastics with potential biomedical applications. *Journal of Macromolecular Science, Part B: Physics* **2002,** *41* (1), 33-46.

37. Carvalho, A. J. F., Job, A.E., Alves, N., Curvelo, A.A.S., Gandini, A., Thermoplastic starch/natural rubber blends. *Carbohydrate Polymers* **2003,** *53* (1), 95-99.

38. Tian, H., Wang, Y., Zhang, L., Quan, C, Zhang, X., Improved flexibility and water resistance of soy protein thermoplastics containing waterborne polyurethane. *Industrial Crops and Products* **2010,** *32* (1), 13-20.

39. Leclair, A., Favis, B.D., The role of interfacial contact in immiscible binary polymer blends and its influence on mechanical properties. *Polymer* **1996,** *37* (21), 4723-4728.

40. Herald, T. J., Obuz, E., Twombly, W.W., Rausch, K.D., Tensile Properties of Extruded Corn Protein Low-Density Polyethylene Films. *Cereal Chemistry* **2002,** *79* (2), 261-264.

41. Parris, N., Coffin, D.R., Composition factors affecting the water vapor permeability and tensile properties of hydrophilic zein films. *Journal of Agricultural and Food Chemistry* **1997,** *45* (5), 1596-1599.

42. Nakamura, E. M., Cordi, L., Almedia, C.S.G., Duran, N., Mei, L.H.I., Study and development of LDPE/starch partially biodegradable compounds. *Journal of Materials Processing Technology* **2005,** *162-163*, 236-241.

43. Gao, C., Donath, E., Möhwald, H., Shen, J., Spontaneous deposition of water-soluble substances into microcapsules: phenomenon, mechanism, and application. *Angewandte Chemie* **2002,** *114* (20), 3943-3947.

44. Pace, C. N., Trevino, S., Prabhakaran, E., Scholtz, J.M., Protein structure, stability and solubility in water and other solvents. *Philosophical Transactions B* **2004,** *359* (1448), 1225-1235.

45. Psomiadou, E., Arvanitoyannis, I., Biliaderis, C.G., Ogawa, H., Kawasaki, N., Biodegradable films made from low density polyethylene (LDPE), wheat starch and soluble starch for food packaging applications. Part 2. *Carbohydrate Polymers* **1997,** *33* (4), 227-242.

46. Yang, J., Yang, Y., Wu, W-M., Zhao, J., Jiang, L., Evidence of polyethylene biodegradation by bacterial strains from the guts of plastic-eating waxworms. *ACS Environmental Science and Technology* **2014,** *48* (23), 13776-13784.

47. Han, J., Salmieri, S., Tien, C.L., Lacriox, M., Improvement of water barrier property of paperboard by coating application with biodegradable polymers. *Journal of Agricultural and Food Chemistry* **2010,** *58* (5), 3125-3131.

48. Shukla, R., Cheryan, M., Zein: the industrial protein from corn. *Industrial Crops and Products* **2001,** *13* (3), 171-192.

49. Arvanitoyannis, I., Biliaderis, C.G., Ogawa, H., Kawasaki, N., Biodegradable films made from low-density polyethylene (LDPE), rice starch and potato starch for food packaging applications: Part 1. *Carbohydrate Polymers* **1998,** *36* (2-3), 89-104.

50. Torres-Giner, S., Ocio, M.J., Lagaron, J.M., Novel antimicrobial ultrathin structures of zein/chitosan blends obtained by electrospinning. *Carbohydrate Polymers* **2009,** *77* (2), 261-266.

51. Cutter, C. N., Willett, J.L., Siragusa, G.R., Improved antimicrobial activity of nisin-incorporated polymer films by formulation change and addition of food grade chelator. *Letters in Applied Microbiology* **2001,** *33* (4), 325-328.

52. (a) Zivanovic, S., Li, J., Davidson, P.M., Kit, K., Physical, mechanical, and antibacterial properties of chitosan/PEO blend films. *ACS Biomacromolecules* **2007,** *8* (5), 1505-1510; (b) Coma, V., Martial-Gros, A., , Garreau, S., Copinet, A., Salin, F., Deschamps, A., Edible antimicrobial films based on chitosan matrix. *Journal of Food Science* **2006,** *67* (3), 1162-1169.

53. Fang, C.-S., Post, L.S., Solberg, M., Antimicrobial effect and disappearance of sodium nitrite in *Staphylococcus aureus* cultures. *Journal of Food Science* **1985,** *50* (5), 1412-1416.

54. WHO, Concise international chemical assessment document No. 26: Benzoic acid and sodium benzoate; World Health Organization - INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY: Geneva, 2000.

55. Krebs, H. A., Wiggins, D., Stubbs, M., Studies on the mechanism of the antifungal action of benzoate. *Biochemical Journal* **1983,** *214* (3), 657-663.

56. Unnithan, A. R., Barakat, N.A.M., Pichiah, P.B.T., Gnanasekaran, G., Nirmala, R., Cha, Y., Jung, C., El-Newehy, M., Kim, H.K. , Wound-dressing materials with antibacterial activity from electrospun polyurethane–dextran nanofiber mats containing ciprofloxacin HCl. *Carbohydrate Polymers* **2012,** *90* (4), 1786-1793.

57. Reinthaler, F. F., Posch, J., Feierl, G., Wüst, G., Haas, D., Ruckenbauer, G., Mascher, F., Marth, E., Antibiotic Resistance of E. coli in sewage and sludge. *Water Research* **2003,** *37* (8), 1685-1690.

58. Reza, S., Quadir, M.A., Haider, S.S., Comparative evaluation of plastic, hydrophobic and hydrophilic polymers as matrices for controlled-release drug delivery. *Journal of Pharmacy and Pharmaceutical Sciences* **2003,** *6* (2), 282-291.

59. Gennadios, A., Weller, C.L., Hanna, M.A., Froning, G.W., Mechanical and barrier properties of egg albumen films. *Journal of Food Science* **1996,** *61* (3), 585-589.

60. El-Shenawy, M. A., Marth, E.H., Sodium benzoate inhibits growth of or inactivates *Listeria monocytogenes*. *Journal of Food Protection* **1988,** *51* (7), 525-530.

61. Liu, H., Leonas, K.K., Zhao, Y., Antimicrobial Properties and Release Profile of Ampicillin from Electrospun Poly(ε-caprolactone) Nanofiber Yarns. *Journal of Engineered Fibers and Fabrics* **2010,** *5* (4), 10-19.

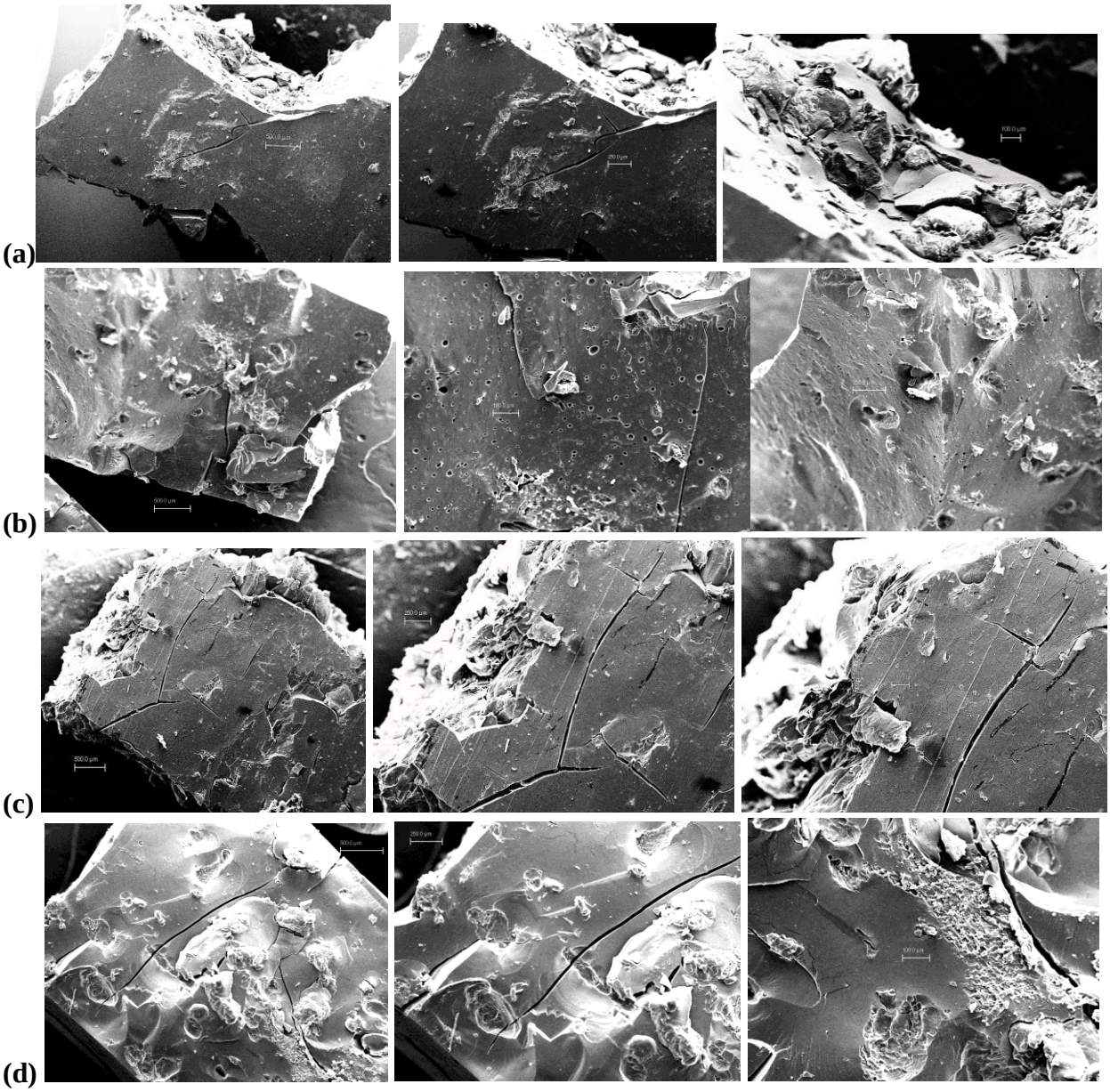
62. Anguita-Alonso, P., Rouse, M.S., Piper, K.E., Jacofsky, D.J., Osmon, D.R., Patel, R., Comparative Study of Antimicrobial Release Kinetics from Polymethylmethacrylate. *Clinical Orthopaedics and Related Research* **2006,** *445*, 239-244.

63. Kerner, E. H., The elastic and thermo-elastic properties of composite media. *Proceedings of the Physical Society, Section B* **1956,** *69* (8).

64. Sperling, L. H., *Polymeric multicomponent materials: an introduction*. Wiley-Interscience: 1997.

65. Neilson, L. E., Landel, R.F. *Mechanical properties of polymers and composites*; Marcel Dekker Inc.: New York, 1974.

Appendix Figures



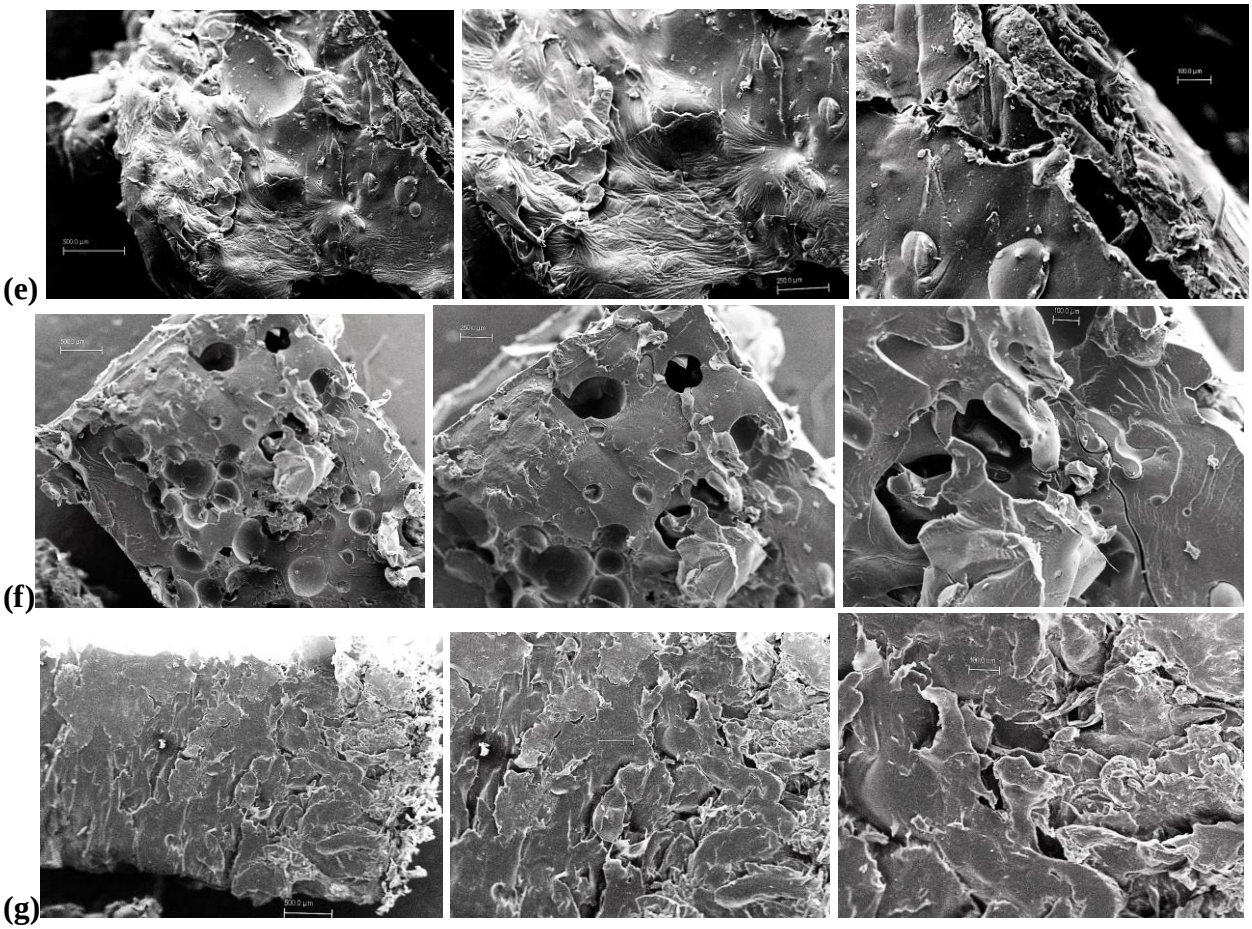
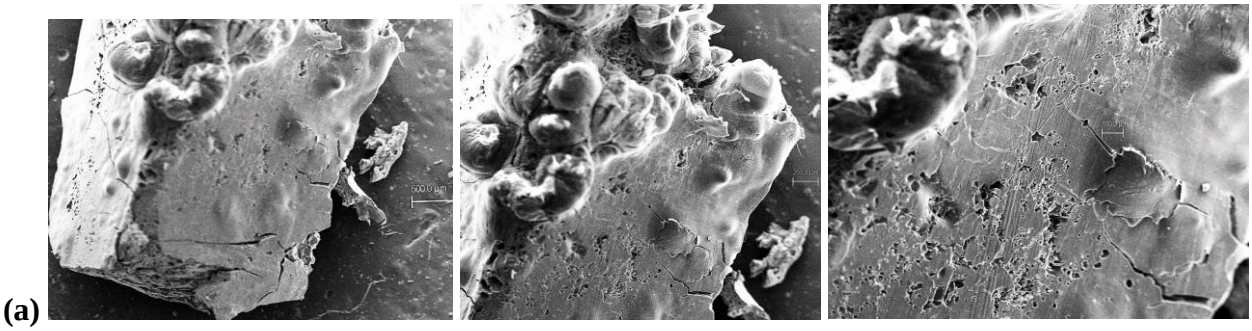
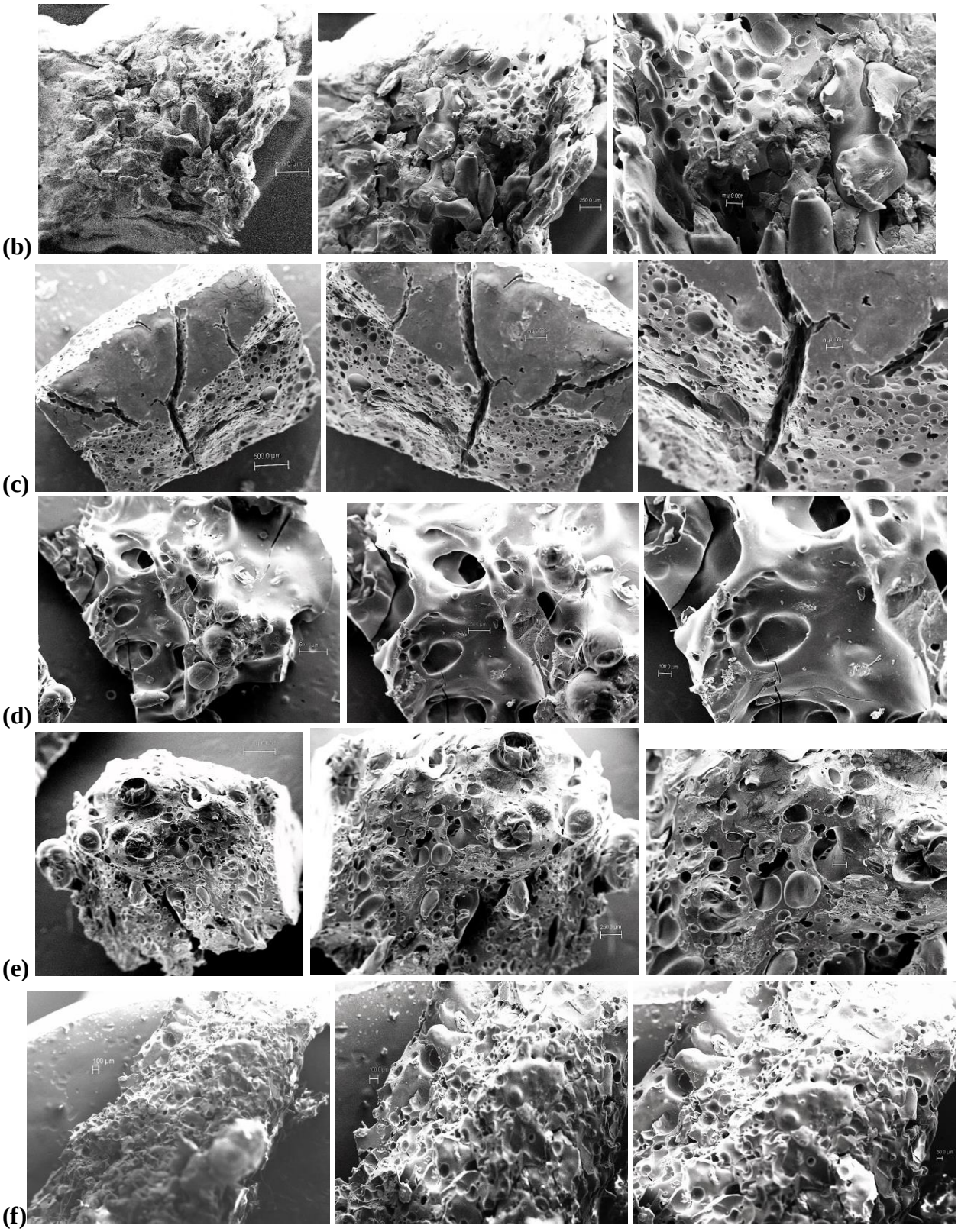


Figure 4.23: Scanning electron microscopy images of albumin-LDPE thermoplastics. (a)95/5 albumin-LDPE, (b) 90/10 albumin-LDPE, (c) 80/20 albumin-LDPE, (d) 65/35 albumin-LDPE, (e) 50/50 albumin-LDPE (f) 35/65 albumin-LDPE (g) 20/80 albumin-LDPE. Magnification of 20x, 100x, and 500x.




(b)
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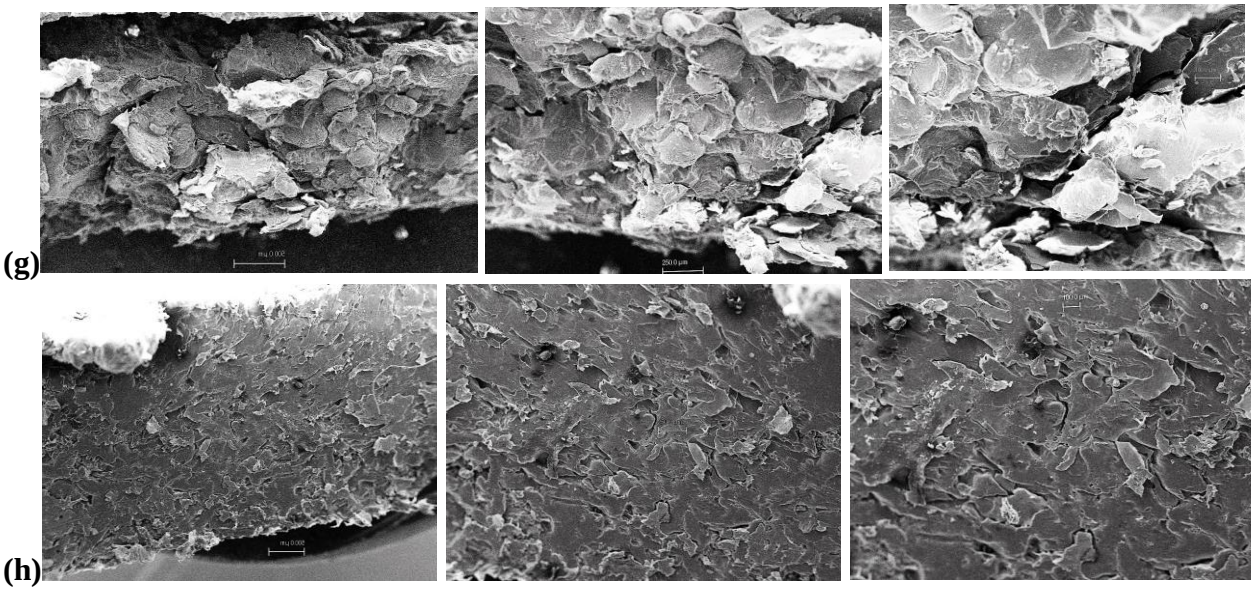

Figure 4.24: Scanning electron microscopy images of zein-LDPE thermoplastics. (a)80/20 zein-glycerol, (b) 95/5 zein-LDPE, (c) 90/10 zein-LDPE, (d) 80/20 zein-LDPE, (e) 65/35 zein-LDPE, (f) 50/50 zein-LDPE (g) 35/65 zein-LDPE (h) 20/80 zein-LDPE. Magnification of 20x, 100x, and 500x.

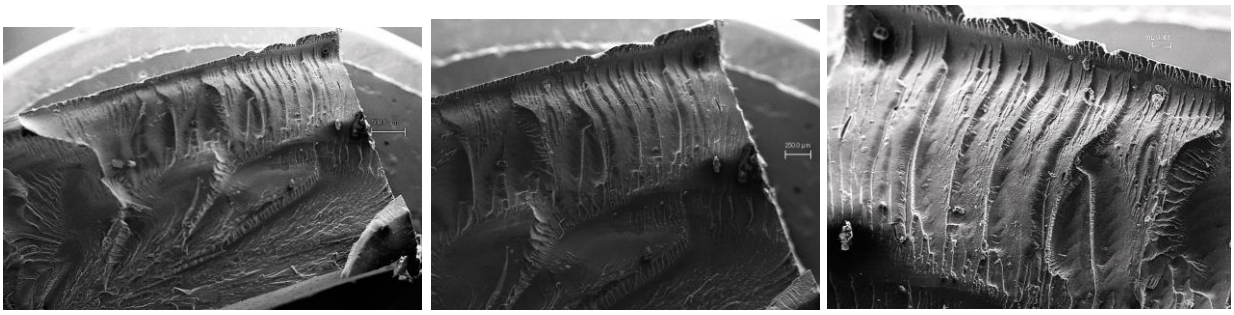

Figure 4.25: Scanning electron microscopy images of LDPE plastics. Magnification of 20x, 100x, and 500x.

CHAPTER 5

A LIFE CYCLE ASSESSMENT OF PROTEIN-BASED BIOPLASTICS FOR FOOD PACKAGING APPLICATIONS[4]

[4] Alexander Jones, Sudhagar Mani, Suraj Sharma. To be submitted to Bioresource Technology.

Abstract

There are many issues with the usage of petroleum-based plastics in food packaging applications, such as the risk of contamination due to a lack of antibacterial properties, as well as a lack of biodegradability for a product that is not commonly recycled by consumers. Several materials are being examined for usage in food packaging plastics to address these issues, such as the proteins of albumin from hen egg white and zein from corn. We conducted this study to investigate the environmental impact of producing and disposing of albumin and zein-based bioplastics for food packaging, with the results compared to that of low density polyethylene (LDPE), a common petroleum-based plastic used in food packaging. For both albumin and zein plastics, it was found that the processes of converting raw biomass into pure protein and the logistical operations that are necessary in setting up a plastic production operation are the key contributors to environmental emissions. When compared to LDPE, it was found that the albumin and zein bioplastics have lower levels of non-carcinogen emissions, but higher levels of greenhouse gas emissions, acidification, and eutrophication potential. In terms of the future impact of this research, the aim will be to examine how certain processes can be modified to lessen their environmental impact.

Keywords

bioplastics, sustainability, albumin, food packaging, zein

Introduction

A relatively new area of materials science and polymer chemistry is the examination of proteins from biomass as a potential source of raw material in the production of plastics. When proteins are utilized in the production of a given plastic, this plastic is then classified as a bioplastic (Vert 2012). With the use of proteins in the production of plastics come a change of

properties of the resulting plastic, making it more desirable for certain applications. One area in which bioplastics have been examined for use has been in the area of food packaging plastic. When it comes to determining what type of material to use to produce food packaging, there are certain properties that are examined. In terms of cost and ease of manufacture, polyethylene is the most common choice when it comes to making traditional food packaging plastics, as it is the plastic that is most easily made with addition polymerization. Food packaging plastics made from polyethylene tends to fall into two different categories: high density polyethylene (HDPE) for more durable applications like the packaging for milk and juices; and low density polyethylene (LDPE), which is more suitable for flexible lids and squeezable bottles for food storage (Marsh 2007). However, there are major downsides to the use of polyethylene in food packaging, one of which being the lack of recycling after the packaging has been used. In 2009, it was estimated that only 9.7% of food package wastes generated from HDPE out of 620,000 tons was recycled. On the other hand, negligible or limited amount of food package wastes from LDPE was recycled out of 800,000 tons of total wastes generated in 2009. (EPA 2010).

This lack of biodegradability of traditional food packaging plastics also results in the gradual leeching of plastics from land to the ocean. It has been estimated that in 2010, between 4.8 and 12.7 million metric tons of plastic waste entered into the ocean from coastal countries (Jambeck 2015). Due to the movement of ocean currents, large gyres have formed on the surface of the ocean have formed in certain locations. The oceans currents cause the formation of gyres by generating a anticyclonic rotation in an isolated area, where the surface water will be pulled into the middle of the gyre, then submerged underneath the plastic at the core of the gyre (Gross 2013). The formation of the gyres will result in the gradual shifting of concentration of plastics on the surface of the ocean, where certain areas will have a much higher concentration of plastic,

while others will have very little. For instance, in the North Pacific gyre it was determined that in certain areas of the concentration of plastics on the surface reached 10^6 per square kilometer, while outside of these areas there was very little plastic found (Law 2014). When examined further, it was found that the major convergence of plastics on the surface of the ocean will occur in subtropical latitudes, as this area is also witness to a convergence of surface currents (Law 2010). In order to limit the amount of ocean surface waste that is generated, it is necessary to examine other types of plastics that can be utilized.

Another major downside that is posed by the use of traditional plastics in food packaging is the lack of inherent antibacterial properties within the plastic to prevent spoilage. While the true cost of food spoilage hasn't been calculated accurately, it has been estimated to run into the billions of dollars a year, and that is only taking into account the lost value of the food that was to be consumed (Stratford 2006). Food spoilage is due to the microbial spoilage that will take place in food when it encounters microflora during storage. Organism types such as yeast, bacteria, and fungi are able to consume the nutrients provided by the food, and when temperature and pH conditions are favorable the food will end up becoming spoiled (Gram 2002). In order to prevent bacterial growth, the addition of food preservatives into the plastic itself has been examined as a potential answer on how to limit food spoilage when packaged. When food preservatives such as sodium benzoate or sodium nitrite are incorporated into traditional food packaging plastics at a concentration of 15%, inhibition of Gram + bacteria and fungi was determined (Vartiainen 2003). However, with the use of additives in plastic production comes with the drawback of potential leeching of said food preservatives once the plastics are disposed of in a landfill.

With these limitations in the current plastics that are utilized in food packaging applications, alternative materials such as proteins are being examined for their potential use. One protein of interest is the albumin protein that is derived from the egg of a hen, which contains the lysozyme enzyme. When the lysozyme is isolated from the albumin protein, it is determined that the enzyme will exhibit antibacterial activity against bacteria that is common in spoiled food such as *Listeria monocytogenes* (Hughey 1989). Another protein that has been examined for potential food packaging application is zein, one of the protein constituents that is derived from maize. When additives such as nisin or lauric acid are incorporated with zein when producing a plastic film, it has been found that antimicrobial effects are exhibited by the protein plastic films (Hoffman 2001). Further studies of protein-based plastics also point to antibacterial potential, as it has been found that bacteria that is common in causing food spoilage are unable to grow on the surface of albumin-based plastics (Jones 2015).

These protein-based plastics may also pose an advantage in comparison to petroleum-based plastics due to the fact that they possess the potential of biodegradation over time. This capability of biodegradability can decrease the environmental burden that will occur due to plastic use, as the CO_2 emission levels generated during biodegradable plastic composting are lower than the incineration that traditional plastics that will take place (Yuki 2012). However, the precursor steps that are necessary to produce the biodegradable plastics have been found to have a much greater environmental impact than that of traditional plastics, as it will require more energy and water to produce the raw materials of biodegradable plastics (Chaffee 2007), as well generate higher levels of photochemical ozone formation during landfill disposal (Khoo 2010). Further analysis must be conducted and improvements in the production of raw materials must be made in order to produce a viable alternative to petroleum-based plastics.

To justify the use of proteins in the production of food packaging plastics in place of traditional plastics, it will be necessary to conduct life cycle assessments of the technologies to be utilized. Based on previous life cycle assessment, it has been found that plastic food packaging has a lower environmental impact in comparison to other traditional packaging technologies such as glass (Humbert 2009), but had a greater environmental impact in comparison to materials such as recycled paper (Zabaniotou 2003). However, a gap in the life cycle assessment of food packaging is present due to the fact that materials that are produced by alternative biostocks have not been taken into account, such as plastics that are derived from protein sources. Therefore, the objectives of this study were to (1) conduct a cradle to grave life cycle assessments of protein-based bioplastics manufactured from polyethylene, albumin protein, and zein protein for food packaging applications and to (2) assess the environmental impacts of protein-based bioplastics over conventional food plastics.

Methods

Bioplastics manufacturing process

To produce a bioplastic, it is necessary to first denature a protein in order to change it from a globular form to a more linear structure. In order to perform denaturation, it will be necessary to apply both heat and pressure in order to ensure that the protein will denature in a way in which it will form a plastic at the end of the process (Sue et al. 1997). The heat at which the protein will denature varies based on the type of protein used (Jones 2015), but after heating it is crucial to maintain pressure while the material is cooling to ensure that the material will maintain its solid plastic state (Jones 2013). After cooling, a completed plastic is generated and can be modified in further processing.

Goals and scope definition

We use life cycle assessments (LCA) to compare the various inputs and outputs that are associated with the production of food packaging products in the United States. For this LCA, the analysis will be limited to the production of raw materials and the production of the food packaging, in a cradle-to-grave approach. This limitation is made in order to allow for a model that can be developed that will not depend on any steps necessary outside the scope of gathering and utilizing raw materials.

For this LCA, we compare the production of food packaging plastic from albumin from hen egg white (Alb), zein from corn protein, and low density polyethylene (LDPE). Life cycle data for the production, harvesting, transport, storage, and use of the materials to be utilized in bioplastic production will be used. This will take into account the energy and emissions associated with the various products whose use is necessary in the production of the raw materials, such as fertilizers, chicken feed, and petroleum. In this LCA, the effective functional unit will be one kilogram (kg) of plastic produced, as this will serve as a good fit in terms of comparison when compared to the standards that are used in other LCAs conducted on food packaging applications (Bohlmann 2004), (Perugini 2005).

The production and use of food packaging plastics

To begin the process of converting biostocks into bioplastics, it will first be necessary to harvest and transport the raw materials that will be necessary in the production of the plastics. For this, it is necessary to take into account all of the inputs and outputs of the production of albumin, zein from corn, and LDPE, as well the glycerol that will be used to plasticize the protein-based plastics.

Once harvested and transported, it will then be possible to convert the raw materials into something that can be utilized for packaging purposes. To begin this process, it will be necessary to convert the materials into forms that allow for the production of plastics. For the protein-based plastics, it will be necessary to denature the proteins. Protein denaturing occurs when the base structure of the protein is modified to the extent where tertiary and secondary structure is lost due to the elimination of weak stabilizing bonds throughout the protein (IUPAC 1997). This modification is possible with the use of agents such as radiation, heat, organic solvents, and strong acids and bases. For protein-based plastics, the use of both heat and pressure in the formation of plastics is a widely used method (Sue 1997); (Jones 2013); (Sothornvit 2003). It will also be necessary to mix a plasticizer in with the protein to form the plastic, and for these protein plastics glycerol will be utilized as the plasticizer based on the results of past studies. For LCA purposes, the albumin-based plastic will consist of a 75-25 mixture of albumin and glycerol, while the zein bioplastic will consist of an 80-20 mixture of zein and glycerol. As for the LDPE, it can be converted using the same process (without plasticizer), as it is able to be processed at temperatures in the range of 105 to 115 °C.

After the raw materials have been converted into plastic and become utilized by the consumer, it will be necessary to dispose of the materials. As the amount of food packaging plastic made from LDPE that was recycled was negligible (EPA 2010), for this model the LDPE plastic will be disposed of by the use of landfill. When it comes to the protein-based plastics, they pose the great advantage of being naturally biodegradable, as pure protein-based plastic films in nature will degrade completely in less than three weeks (González 2011). In LCAs this can lead to the biodegradable material having a much lower environmental impact in comparison

with petroleum-based plastics, but lack of data in this area prevents the use of definitive statements in the matter (Bohlmann 2004).

Inventory analysis

A series of cradle-to-grave scenarios were developed and utilized to determine the environmental impact of the use of each raw material in food packaging applications. Data for many of the production steps for the production and disposal of the food packaging plastics was generated through the SimaPro software library, as it has gathered pertinent data for the examination for these processes in other applications (SimaPro 2015). For more specific settings in the processes, additional information was gathered based on the purification of zein from corn (Shukla 2001) and albumin from egg white (Stevens 1991), as well as the production of glycerol to be utilized for the plasticization of protein-based plastics (Franklin 2011) and the environmental impact of biodegrading materials (Bohlmann 2004).

For egg production for albumin protein, it will be necessary to take into account the inputs of feed to allow the chickens to produce eggs, as well as the electricity and natural gas needed to incubate and transport the eggs. While the eggs themselves are main output of interest in this process, it must be taken into account that there will be air and water emission outputs as well due to the farming of the chickens. After harvesting the eggs, it will then be necessary to convert the egg whites in the egg into a protein source that can be utilized for plastic production. The process that will be utilized for this scenario will involve the precipitation of albumin from egg white through the use of ammonium sulphate, a process that can be scaled up for industrial scale operations (Chick 1913). It must be noted that there is a 6.6% percent conversion rate of albumin from the eggs, as the rest of the egg matter will consist of the shell, the yolk, and water.

When it comes to the production of corn for zein production, there are large amounts of inputs necessary for the cultivation of corn. The inputs of corn production include the land and water used to grow the crops themselves, as well as all of the fertilizers and pesticides needed in order to ensure that there will be a successful yield of corn. The use of pesticides in corn production will have the side effect of generating large amounts of air and water emissions, as this will be an undesired output of this process besides corn. When converting the corn into a zein protein, it will be necessary to dry the corn before extracting the zein with the use of a mixture of water and 2-propanol (Wilson 1984). In the conversion of the harvested corn into zein, there will be a yield of 3.9% of total mass of harvested product that will be usable zein (Shukla 2001).

In terms of the use of LDPE, there will be a large amount of avoided products that will be utilized as inputs into the system, as many elements will be gathered in the same process as producing LDPE. However, the major factor in this process that must be factored in is the large amount of emissions that will be generated along with the LDPE as an output, as there will be air, water, and land emissions (in kg) that will be a result in the production of LDPE.

For the use of glycerol as a plasticizer in the plastics made from albumin or zein, the main inputs of this process will include the kernel oils and methanol for raw materials, as well as the process and transportation energy necessary for the production to occur. The outputs of this process besides glycerol include atmospheric and waterborne emissions (in kg), and the reaction is assumed to have a conversion rate of 97.24 % (Franklin 2011).

Once the raw materials have been produced, it will then be necessary to transport all of the materials to the intended production facilities where the plastics can be made. To transport the materials by rail, it will be necessary to utilize certain inputs, such as the energy generated by

oil and the metals necessary to construct the transportation infrastructure. In terms of the outputs for this process, there will air, water, and soil emissions, as well as the final waste flows (all in kg) that are included with the main output of sending a certain amount of material a certain number of kilometers. For the purposes of this assessment, we will assume that the production of the food packaging plastics will be located 50 km from where the raw materials are produced and modified.

Before the plastics can be produced from the raw materials, it will be necessary to store the materials in a warehouse to ensure that constant production will be possible. This step in the process has very similar inflows due to the fact that it will be necessary to build the warehouse on land close to the production facilities, as well as provide energy to the warehouse for climate control. The outputs for this step in the process besides the ability to store material include the air, water, and soil emissions (in kg) that occur due to the power provided to the warehouse. With the production of plastic now possible, there are other inputs necessary besides the raw materials to allow for the production to occur. The added inputs required for plastic production will include the resources that are avoided when producing the plastics, as well as the heat and energy required for processing the raw material and form plastics with high heat and pressure. It is because of this energy use that besides the plastic produced, there will also be emissions to the air, water, and ground (in kg). The process diagram of albumin and zein-based bioplastics are shown in Figure 5.1 and 2.

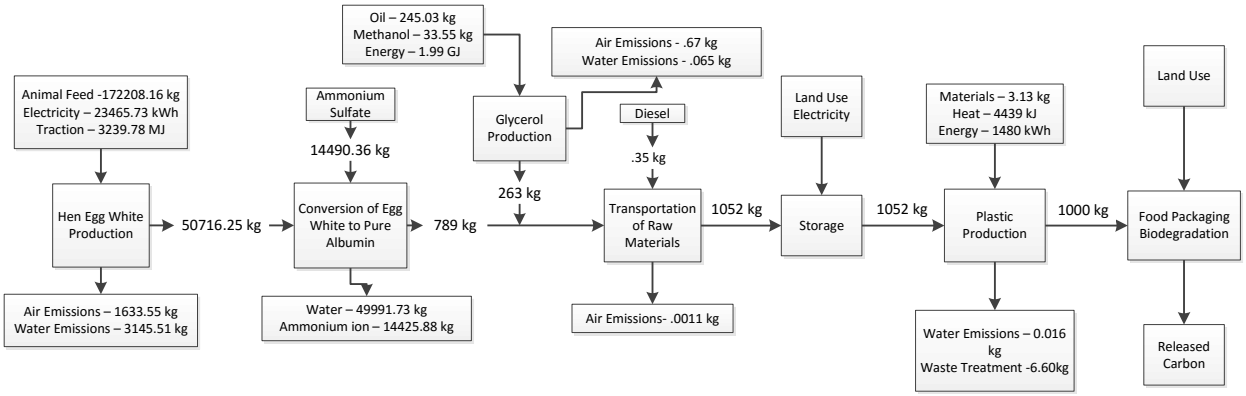

Figure 5.1. Process design of conversion of egg white to food packaging plastic and disposal.

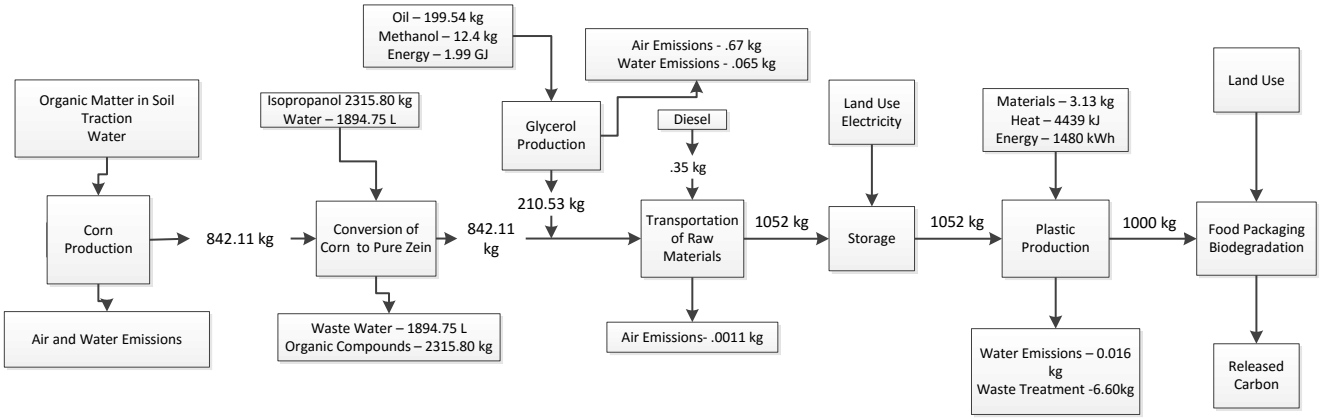

Figure 5.2. Process design of converting zein protein to food packaging plastic and disposal.

Once the plastic material has been used by the consumer, it will then be necessary to take into account the environmental impacts that will occur when the plastic is disposed of. For the LDPE plastics, landfill disposal is one of the more common ways in which the material is taken care of. In this final step of the process, land use is an important input, as well as the amount of materials that are avoided when the land is being used to store waste (in kg, MJ, or m^3). With the use of landfills there are large amounts of emissions generated (in kg), as well as trace amounts of other waste flows having an effect on environmental impact. The process diagram of the life cycle of LDPE-based plastic is illustrated in Figure 5.3.

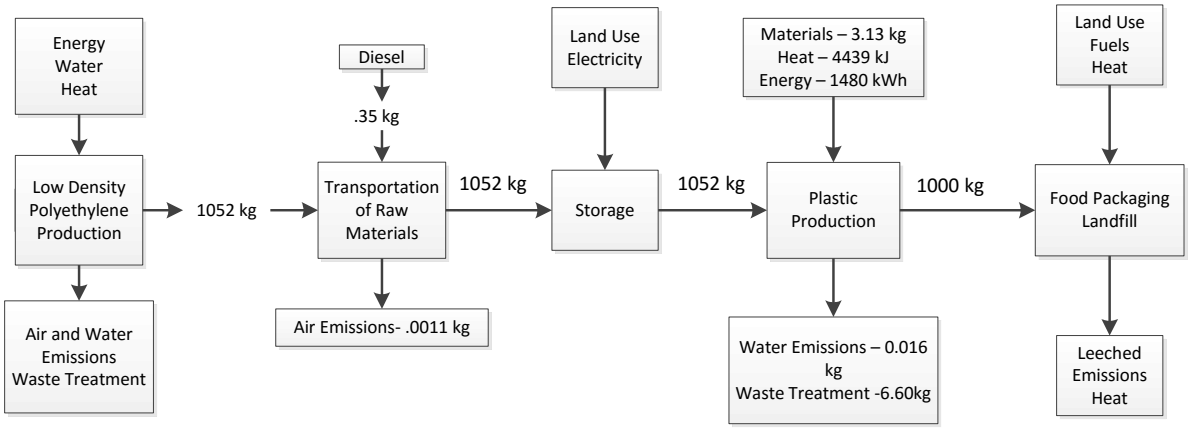

Figure 5.3. Process design of conversion of low density polyethylene to food packaging plastic and disposal.

Impact Assessment Methods

After data collection, it was then necessary to analyze the inputs and outputs of plastic production through use of the SimaPro 7.3 Life Cycle Assessment software. The data was entered into the software based on the type of plastic being produced, as certain types of plastic required much different raw materials, and separated as such. Once entered and separated, a cradle-to-grave assessment was possible through the use of both the Tool for the Reduction and Assessment of Chemical and other environmental Impacts (TRACI 2 V3.03) as developed by the EPA, as well as the Building for Environmental and Economic Sustainability (BEES) software that has been developed by the National Institute of Standards and Technology. TRACI analyzed the data and determined the various environmental impacts that each process of plastic use would have into nine separate categories: global warming (kg CO_2 eq.); acidification (H+ moles eq.); carcinogens (kg benzene eq.); non-carcinogens (kg toluene eq.); respiratory effects (kg PM 2.5 eq.); eutrophication (kg N eq.); ozone depletion (kg CFC-11 eq.); ecotoxicity (kg 2,4-Dichlorophenoxyacetic acid); and smog (g NO_x eq.). This initial analysis is crucial, as it serves as a good indicator on which steps in the plastic production will have the greatest environmental impact. After this initial analysis, the three types of plastics will be compared based on the key

impacts of global warming potential, acidification, eutrophication, and the release of non-carcinogens into the environment, as well as solid waste generated and the amount of water needed to produce one kg of packaging plastic material. These four impacts have been chosen to the fact that they indicate the levels of CO_2 emissions, environmental changes due to plastic production, as well as the unintentional leeching of materials into the environment. An example of the environmental impact that plastic production has (in this case, the conversion of plastic from raw materials and global warming gas emissions) is illustrated in Figure 4.

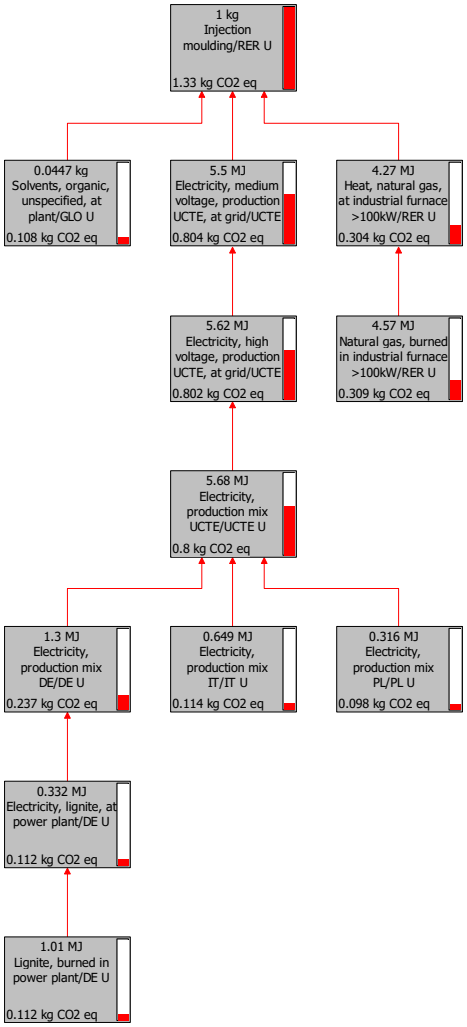

Figure 5.4. Flow chart of CO_2 emissions generated in the operation of a injection molding process (SimaPro 2015).

Sensitivity Analysis of Impact Assessment

In order to determine how sensitive the results to varying conditions, alternate scenarios have been utilized. One alternate scenario to be run will be to model how raw material yield will affect environmental impacts. In this scenario, raw material yields were elevated by 20% in one TRACI analysis and decreased by 20% in another analysis to determine how sensitive the data generated will be to agricultural yield changes. These results will be compared to both the initial protein conversion rate the LDPE plastic in terms of environmental impact, as this will indicate if said rates would be able to diminish or enhance the impacts of use of the plastics.

Another area that is crucial to examine is the rate at which raw material can be converted into protein that can be utilized in plastic production. In a similar fashion as is examined in raw material yield alternate scenarios, conversion rates were elevated by 20% in one TRACI analysis and decreased by 20% in another analysis, with the results compared to the initial rate scenarios.

Economic Cost of Plastic Production Assessment

To determine the economic feasibility of plastic production for food packaging, it will be necessary to compare the plastic types based on the cost of production and disposal. The plastics will be compared in three areas: the cost of the protein or polymer that will be converted based on market prices (Alibaba.com 2015, Alibaba.com 2015, Platts 2015); the cost of the plasticizer glycerol for protein-based plastics (Quispe 2013) as LDPE plastics do not require plasticizers for production; and the cost of landfill for LDPE plastics (Journal 2012), as protein-based plastics will biodegrade and will not incur a landfill cost. The costs will be calculated on a per metric ton of plastic to be produced and disposed of.

Results and Discussion

Initial Cradle to Grave Analysis of Albumin, Zein, and LDPE plastics

The results of the initial cradle-to-grave analysis of the production of each plastic type are shown in Figures 5.5 through 5.7. For the albumin-based bioplastic production, a key finding is that the three key processes that determine environmental impact are the harvesting of eggs for raw material, the conversion of the egg whites into albumin, and the logistical operations of transport and storage of the raw materials prior to production. The egg production and albumin purification processes dominate the environmental categories of acidification, eutrophication, and ozone depletion, as these environmental impact take into account the costs of feeding the chickens who will produce the eggs (Xin 2011), as well as the chemicals needed to convert egg whites into pure albumin on an industrial scale (Chick 1913). As for the logistics process, it dominates the other categories, as the transportation of raw material will result in the use of fossil fuels, as well as the required construction of storage facilities will require land transformation and additional emissions.

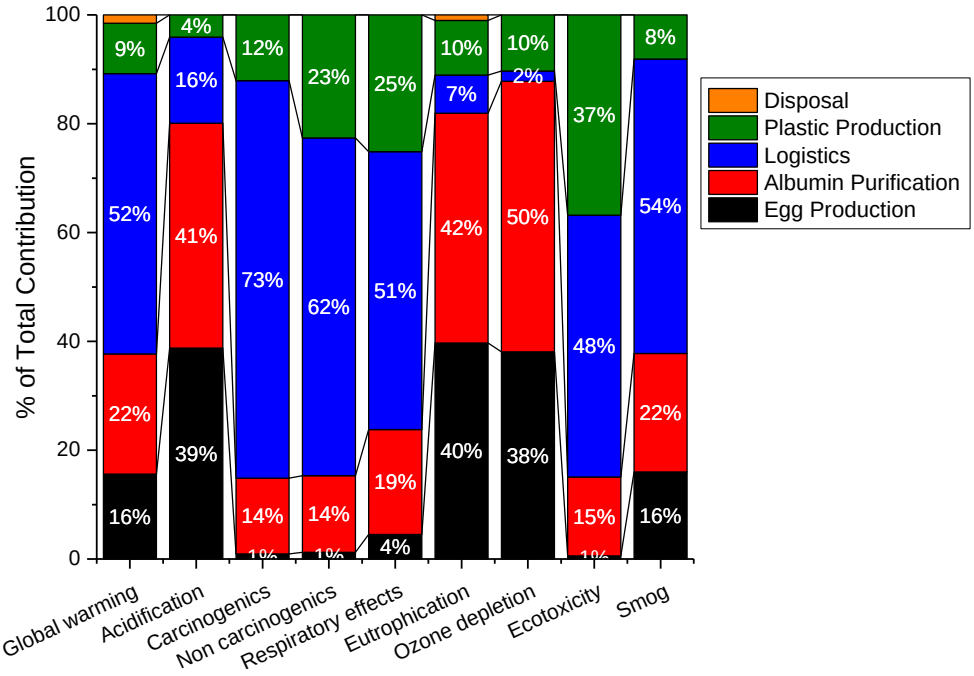

Figure 5.5. Comparison of albumin plastic use processes on the environment through the use of TRACI 2 (V 3.03).

The processes of zein extraction and logistics are the two key contributors in environmental impacts when it comes to the production and disposal of zein-based bioplastics. Zein extraction is especially impactful when it comes to the ozone depletion, smog, and eutrophication impacts, as the drying of the harvested corn (Kim 2009) and the chemical treatment of the resulting dry corn (Wilson 1984) will require additional fossil fuel use and chemical treatments that will result in harmful waste emissions. The logistics impact for zein is similar to that of the logistics of albumin, with greater impact in global warming, carcinogens, and non-carcinogens due to the transport of zein protein and resulting storage facilities.

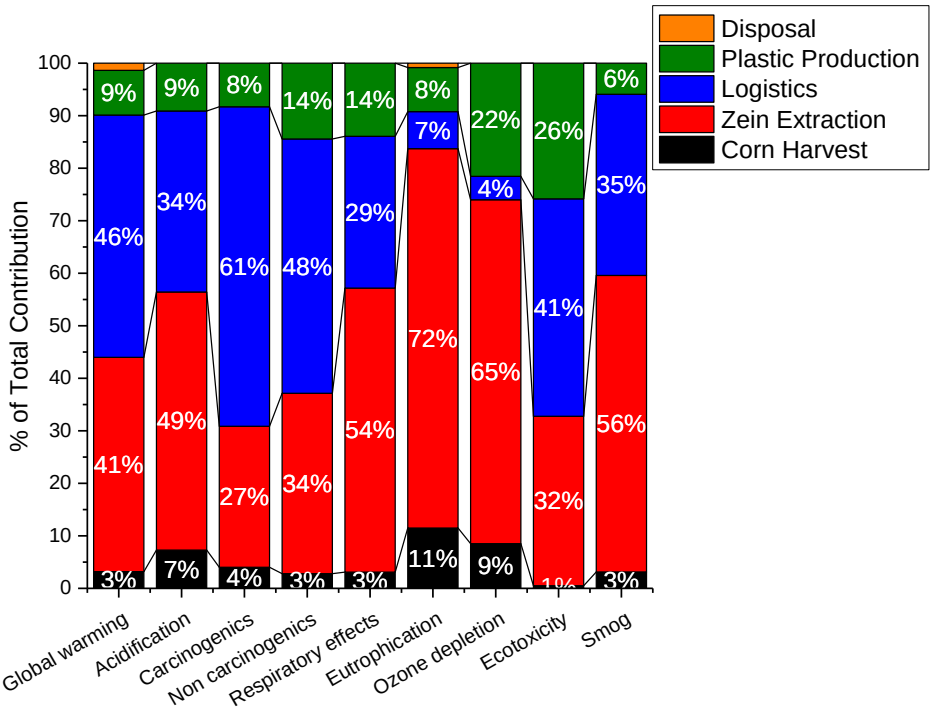

Figure 5.6. Comparison of zein plastic use processes on the environment through the use of TRACI 2 (V 3.03).

With LDPE plastic use analysis, it is found that there is no overall dominant process that will dictate the environmental impact of LDPE plastic usage. For instance, while the production of LDPE will result in higher emissions of global warming gasses, acidification potential, and smog, the eventual disposal of the LDPE plastics has a substantial impact on the amount of non-carcinogens released and eutrophication potential that is possible. These findings for LDPE disposal can be explained through the long term residence of the plastic over time in a landfill (Perugini 2005), as additional materials utilized in LDPE production will contribute to a high level of emissions over time.

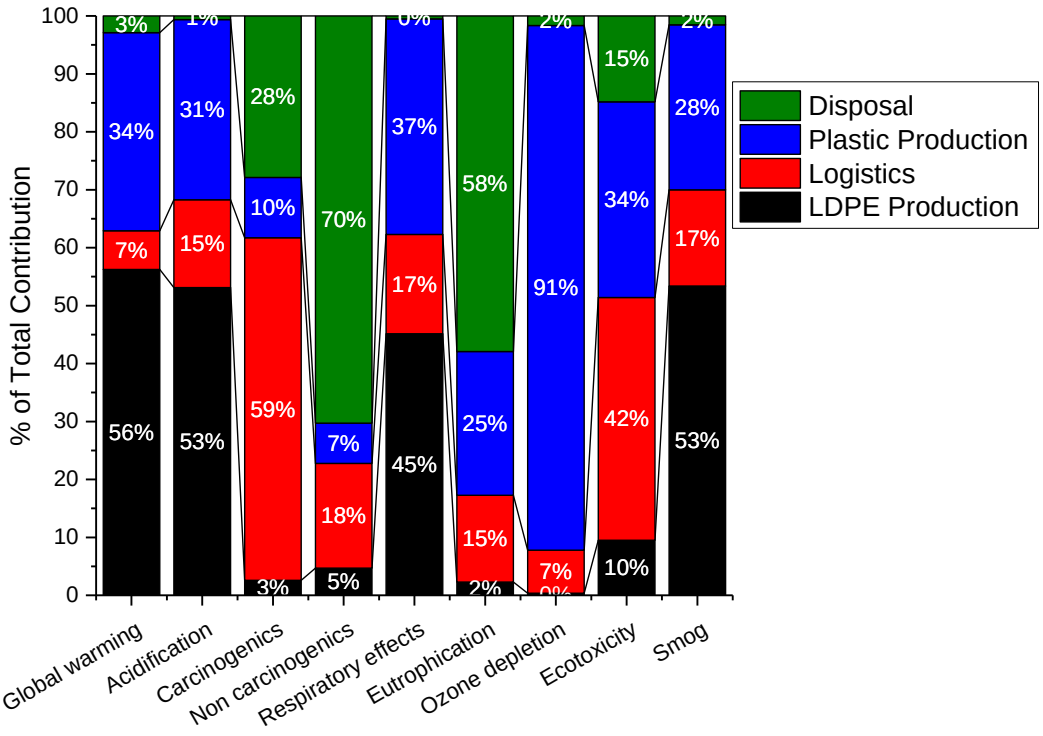

Figure 5.7. Comparison of LDPE plastic use processes on the environment through the use of TRACI 2 (V 3.03).

Comparison of Albumin, Zein, and LDPE Production in Initial Scenario

An expected finding is the lower water usage that is a result of LDPE plastic use and disposal, as shown in Table 5.1. LDPE plastics do not require large amounts of water in order to harvest its raw material, so there will be less water usage overall. However, an unexpected finding made is that LDPE plastic usage will also result in less solid waste generated, with LDPE solid waste generation half of the solid waste generated in zein plastic usage, and much lower in comparison to albumin plastic usage. The lower levels of solid waste generation can also be drawn to a lack of intensive biomass generation step, as well as a lack of process needed to convert a raw material into a usable input, or lack of use of glycerol to make a plastic (Franklin 2011).

Table 5.1. Analysis of water usage and solid waste generation in plastic production and disposal through BEES 2 (V 3.03).

Raw Material Type	Water Usage (l)	Solid Waste Generated (kg)
Albumin	9570	0.214
Zein	12800	0.002
LDPE	8740	0.001

When notable air emissions from plastic production are examined, the use of LDPE plastics is responsible for lower levels of all of the gasses, including fossil-based carbon. The decreased levels of petroleum use can be pointed to the fact that both the albumin and the zein required the use of diesel-powered tractors in order to harvest and transport raw materials from the field to a location where they can be utilized. The usage of zein plastics will end up with the highest levels of fossil fuel emissions, as natural gas required to dry the corn before zein extraction (Anderson 2011) will contribute even more to greenhouse gas emissions.

Table 5.2. Analysis of notable air emissions from plastic production through the use of TRACI 2 (V 3.03).

Notable Air Emission (in kg CO_2 eq.)	Albumin	Zein	LDPE
Carbon dioxide	2.988	0.949	3.095
Carbon dioxide, fossil	3.641	5.139	0.033
Carbon dioxide, land transformation	0.194	0.210	0.007
Dinitrogen monoxide	5.908	0.199	0.014
Methane, biogenic	7.300	3.695	0.003
Methane, fossil	1.725	0.550	0.509

As for the water emissions from plastic production are examined, the use of albumin plastics will be responsible for the lowest levels of BOD5 and COD, while LDPE will have the lowest levels of nitrate and phosphate emissions. Albumin bioplastics are able to limit BOD5 and COD emissions due to the fact there is a lack of major wastewater generation processes such as fertilizer use or plastic leeching in a landfill. In terms of the LDPE plastics, they are able to limit

the release of nitrate and phosphate into water due to the fact that fertilizer use is not necessary in LDPE raw material production, severely limiting the generation of the two emissions.

Table 5.3. Analysis of notable water emissions from plastic production through the use of TRACI 2 (V 3.03).

Notable Water Emission (in kg N eq)	**Albumin**	**Zein**	**LDPE**
Ammonium, ion	0.001	0.000	0.000
BOD5, Biological Oxygen Demand	8.31177E-05	0.015	0.002
COD, Chemical Oxygen Demand	0.000138499	0.0160	0.011
Nitrate	0.016	0.005	0.001
Phosphate	0.0118	0.0144	0.009

After comparing the three types of plastic use for greenhouse gas warming potential (GWP) and non-carcinogen (NC) emissions, it is the use of LDPE plastics that will cause the lowest levels of GWP emissions. This finding is due to the fact that there is no conversion process necessary for LDPE raw material use, as well as the lack of glycerol usage in the logistics process of LDPE plastic production. When comparing albumin and zein plastics, it is the process of converting corn into zein that will cause zein bioplastic use to emit the highest levels of GWP gasses. When the three plastic types are compared for NC emissions, the LDPE plastics will emit almost 250 kg toluene eq. more of emissions when compared to the other plastics. This is due to the usage of landfilling for the LDPE plastic, as this will lead to long term leeching of NC wastes from the plastic into the areas surrounding the landfill.

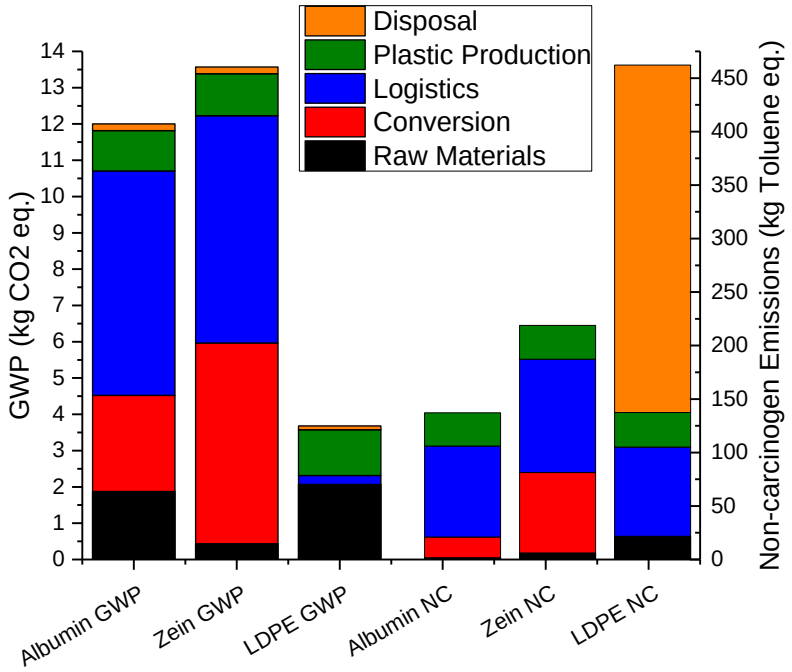

Figure 5.8. Comparison of greenhouse warming potentials and non-carcinogens between processes in plastic production.

When analyzing the plastic production processes for their potential in causing acidification and eutrophication, it is found that LDPE plastic use will result in lower potential risks in both of the emission categories. This lowered risk is due to the fact that the protein-based plastics require glycerol to be produced as a plasticizer to form a plastic, as well as a relative lack of acidification and eutrophication impacts on the use of raw materials in LDPE production. For the two protein-based plastics, it is found that albumin will have a higher acidification potential in comparison to the zein-based plastics. This increased rate of acidification potential is due to the environmental impact of raising chickens that will produce eggs, as well as the chemical process that is required to convert the egg white into pure albumin protein. When comparing the eutrophication potentials, it is the albumin bioplastic use that will have lower levels of eutrophication. While the raw material gathering process of albumin will lead to higher levels of

eutrophication, it is the of the conversion process of corn to zein that will lead to higher levels of eutrophication-causing emissions.

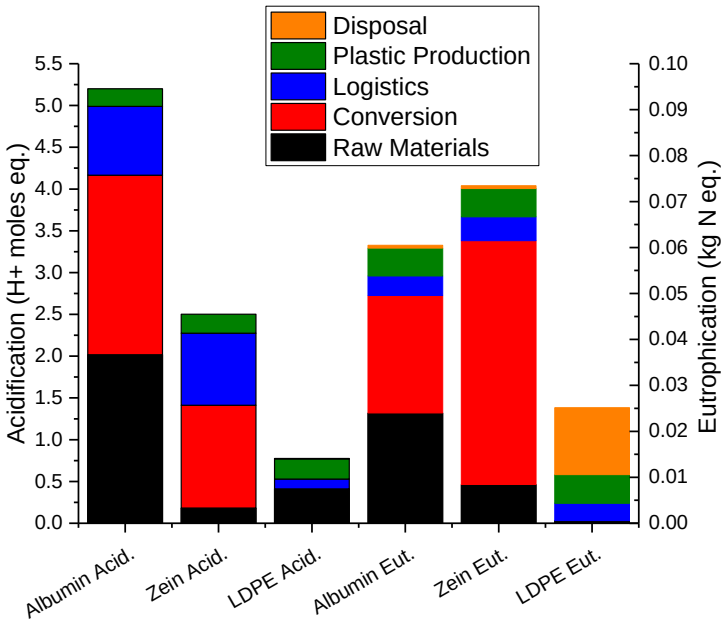

Figure 5.9. Comparison of acidification and eutrophication potentials between processes in plastic production.

Sensitivity analysis of protein-based bioplastics

Harvest Level Sensitivity

After conducting the LCA under normal conditions, we were then able to modify certain aspects of the production of protein-based plastics to determine how sensitive the LCA is to certain data modifications. For protein-based plastics, it can be possible to change the amount of raw material that could be harvested through modifying the yields from the raw material. To determine the sensitivity of the LCA data, we conducted an analysis of the production and disposal of protein-based plastics based on both an increase and decrease of possible raw material biomass yield by 20%.

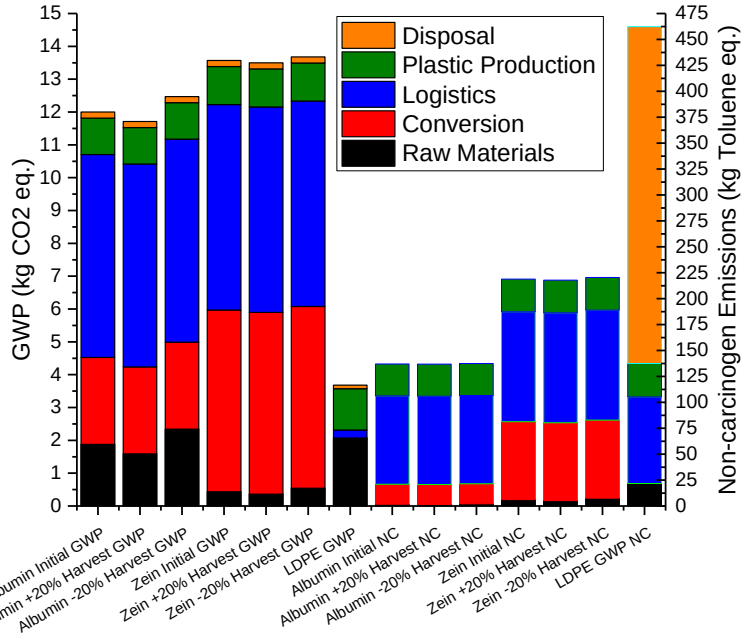

Figure 5.10. Comparison of global warming potential and non-carcinogen emissions of plastic production with bioplastics of varying harvesting rates of raw material through the use of TRACI 2 (V 3.03).

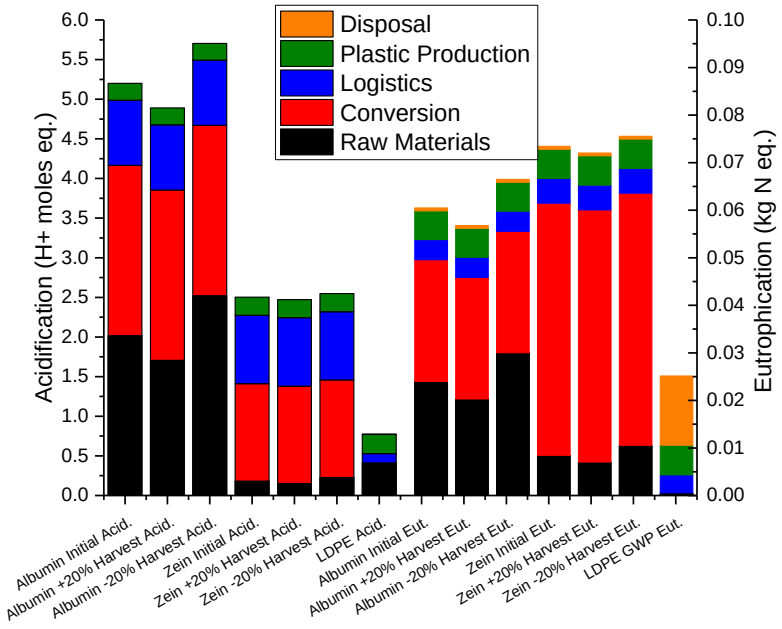

Figure 5.11. Comparison of acidification and eutrophication potential of plastic production with bioplastics of varying harvesting rates of raw material through the use of TRACI 2 (V 3.03).

With the modifying the harvest levels of raw material biomass in Figures 5.10 and 11, there is a marginal change in all four of the emission categories examined for zein protein. This is to be expected, as the harvesting of corn for zein production was not a major contributor to any of the emission categories when they are examined in the initial scenario. The same is not true for the modification of egg harvest levels and emissions, as the egg harvest process was a major contributor to the all of the emission categories to be examined. As a result, when the egg harvest levels are modified there is a noticeable change in the emissions levels, with a rise in emissions when the harvest is lower, as well as a decrease in emissions when harvest levels are increased. It must be noted that the changes in emissions levels are not substantial enough to improve its standing when compared to LDPE plastic use, as the modification of harvest levels will not decrease the emissions to an extent where bioplastic production will generate less GWP, acidification, or eutrophication causing emissions.

Protein Conversion Rate Sensitivity

One other area of interest to examine is the fact that the biomass gathered as raw material will need to be converted to a pure protein form in order to be utilized as plastic production component. This analysis is used to determine how the amount of protein gathered from a raw material biomass will have an influence on the levels of emissions that will be incurred. For this analysis, the protein conversion rates have been increased in one scenario by 20%, and decreased in the other scenario by 20%, then compared to the initial values.

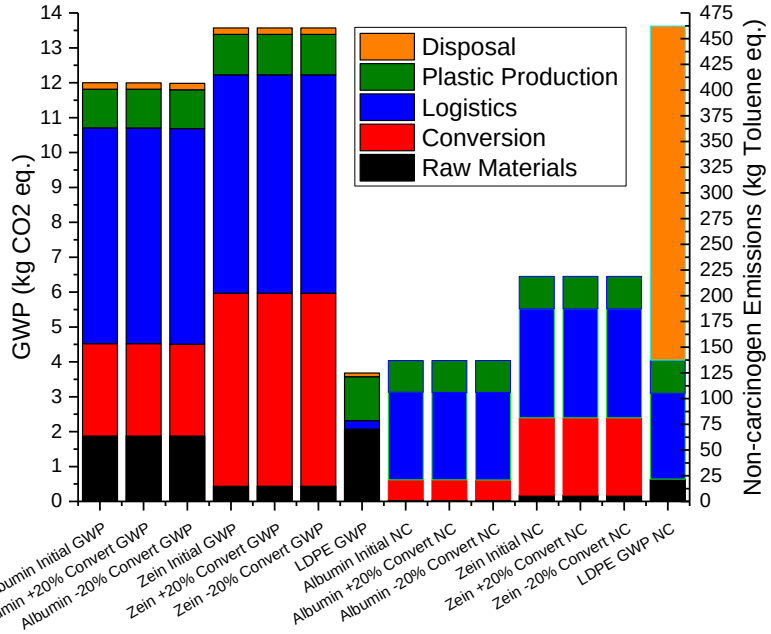

Figure 5.12. Comparison of the impact of varying levels of conversion rates on GWP and NC emissions through the use of TRACI 2 (V 3.03).

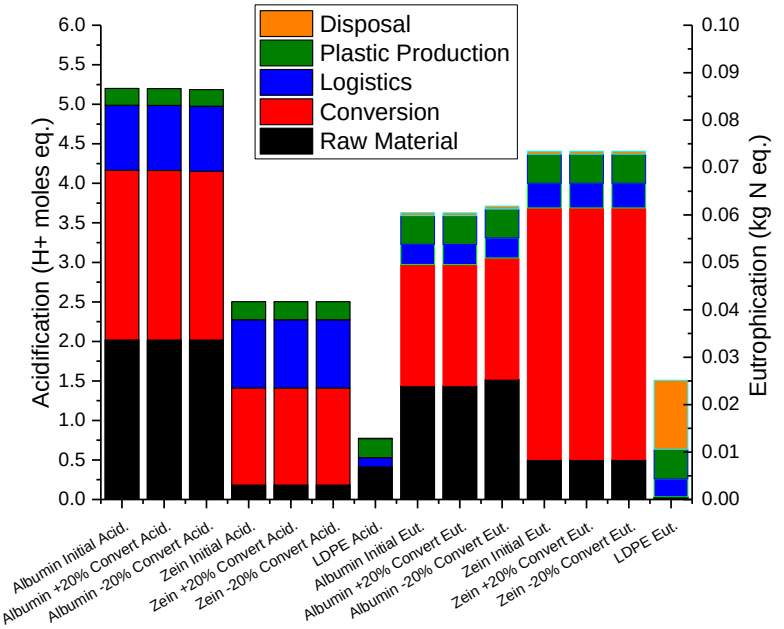

Figure 5.13. Comparison of the impact of varying levels of conversion rates on acidification and eutrophication emission potentials through the use of TRACI 2 (V 3.03).

In this analysis of modifying protein conversion yields, we find that there is no significant change in emission levels when the protein yields are increased or decreased. This finding is most likely due to the fact that it will take a much higher change in protein conversion rates in order to witness significant changes, and that it would require substantial modification of the biomass itself before conversion in order for the yield amounts to change to that extent.

Cost Analysis of Plastic Packaging Production and Disposal

When we examine the costs that are associated with the production and disposal of the plastics, we find that the LDPE-based plastics possess much lower overall costs when compared to the albumin and zein plastics. This is due to the significant difference in cost of the proteins compared to the polymer, as while LDPE costs $1.24 per kilogram (Platts 2015), albumin will cost $12.50 per kilogram (Alibaba.com 2015), and zein will cost $41.50 per kilogram (Alibaba.com 2015). The one area in which the use of protein-based plastics has a cost advantage is with disposal, as it is fully biodegradable, and therefore does not require landfill space. However, since LDPE is not biodegradable, it will be necessary to place the plastic in the landfill, with a resulting cost of $45.02 per metric ton of waste generated (Journal 2012).

Table 5.4. Analysis of differences in cost of producing and disposing one metric ton of plastic (in US $).

Process	Albumin	Zein	LDPE
Protein/Polymer Production	9,375.00	33,200.00	1,240.00
Plasticizer Cost	25.00	20.00	
Plastic Disposal			45.02
Total Cost per Metric Ton	9,400.00	33,220.00	1,285.02

Conclusions

This study examines the LCA of albumin, zein, and LDPE-based food packaging plastic containers produced in Athens, GA. For this study, environmental impacts are investigated with

a life cycle that entails cradle to grave scenarios based on the plastic produced. In the general scenario the protein-based food packaging plastics will emit more emissions that involve global warming gasses, acidification, and eutrophication, but do provide an advantage when compared to LDPE in the area of non-carcinogen emissions. When each plastic production process is examined, it is found that the harvesting of eggs, the conversion of egg white to pure albumin, and the logistics processes have the greatest environmental impact for albumin plastics, while for zein production the conversion of corn into pure zein and the logistics that are involved with zein bioplastic production have the highest environmental impacts. When alternate scenarios such as harvest yield modification, protein conversion, and production location modifications are examined, it is found that modifying these properties does not yield a drastically different end result.

In terms of additional research that can be conducted based on the results of this study, one crucial area of potential research could be the continued analysis and optimization of corn and feedstock harvesting. The environmental impact of harvesting is crucial in the environmental impact of the protein-based plastics, so any improvements in this area would be crucial in developing a more environmentally friendly plastic from alternative sources. One other major area of interest would be the analysis of how to convert biomass into pure protein form in a way that will limit environmental impacts, as this step in the process of converting biomass into plastic needs to be improved in order to make protein plastic use ecologically feasible.

References

Alibaba.com (2015). "chicken origin egg albumin/white powder with favorable price." from http://www.alibaba.com/product-detail/chicken-origin-egg-albumin-white-powder_60195863520.html?spm=a2700.7724838.35.1.5XFrCQ.

Alibaba.com (2015). "High quality ZEIN (Corn Protein) 9010-66-6." from http://www.alibaba.com/product-detail/High-quality-ZEIN-Corn-Protein-9010_1757193545.html?spm=a2700.7724838.35.1.afBnZd.

Anderson, T. J., Lamsal, B.P. (2011). "Zein extraction from corn, corn products, and coproducts and modifications for various applications: A review." Cereal Chemistry **88**(2): 159-173.

Bohlmann, G. M. (2004). "Biodegradable packaging life-cycle assessment." Environmental Progress **23**(4): 342-346.

Chaffee, C., Yaros, B. R. (2007). Final Report. Life Cycle Assessment for Three Types of Grocery Bags - Recyclable Plastic; Compostable, Biodegradable Plastic; and Recycled, Recyclable Paper. B. C. a. Associates, Progressive Bag Alliance.

Chick, H., Martin, C.J. (1913). The Precipitation of egg-albumin by ammonium sulphate. A contribution to the theory of the "salting-out" of proteins., Lister Institute.

EPA (2010). Municipal solid waste in the United States: 2009 facts and figures. U. S. E. P. Agency. Washington, DC.

Franklin, A. (2011). Cradle-to-gate life cycle inventory of nine plastic resins and four polyurethane precursors. Prarie Village, KS, Eastern Reseach Group, Inc.

González, A., Strumia, M. C., Igarzabal, C. I. A. (2011). "Cross-linked soy protein as material for biodegradable films: Synthesis, characterization and biodegradation." Journal of Food Engineering **106**: 331-338.

Gram, L., Ravn, L., Rasch, M., Bruhn, J. B., Christensen, A. B., Givskov, M. (2002). "Food spoilage—interactions between food spoilage bacteria." International Journal of Food Microbiology **78**(1-2): 79-97.

Gross, M. (2013). "Plastic waste is all at sea." Current Biology **23**(4): R135-137.

Hoffman, K. L., Han, I.Y, Dawson, P.L. (2001). "Antimicrobial effects of corn zein films impregnated with nisin, lauric acid, and EDTA." Journal of Food Protection **64**(6): 885-889.

Hughey, V. L., Wilger, P.A., Johnson, E.A. (1989). "Antibacterial activity of hen egg white lysozyme against *Listeria monocytogenes*. Scott A in Foods." Applied and Environmental Microbiology **55**(3): 631-638.

Humbert, S., Rossi, V., Margni, M., Jolliet, O., Loerincik, Y. (2009). "Life cycle assessment of two baby food packaging alternatives: glass jars vs. plastic pots." International Journal of Life Cycle Assessment **14**(2): 95-106.

IUPAC (1997). Compendium of Chemical Terminology. Oxford, Blackwell Scientific Publications.

Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andraday, R., Narayan, R., Law, K.L. (2015). "Plastic waste inputs from land into the ocean." Science **347**(6223): 768-771.

Jones, A., Mandal, A., Sharma, S. (2015). "Protein-based bioplastics and their antibacterial potential." Journal of Applied Polymer Science **132**(15).

Jones, A., Zeller, M A, Sharma, S (2013). "Thermal, mechanical, and moisture absorption properties of egg white protein bioplastics with natural rubber and glycerol." Progress in Biomaterials **2**(12).

Journal, W. B. (2012). "US landfill tipping fees reach $45 per ton; slow volume growth." Retrieved July 14, 2015, from http://www.wastebusinessjournal.com/news/wbj20121003A.htm.

Khoo, H. H., Tan, R.B.H. (2010). "Environmental impacts of conventional plastic and bio-based carrier bags Part 2: end-of-life options." International Journal of Life Cycle Assessment **15**(4): 338-345.

Kim, S., Dale, B.E., Jenkins, R. (2009). "Life cycle assessment of corn grain and corn stover in the United States." International Journal of Life Cycle Assessment **14**: 160-174.

Law, K. L., Morét-Ferguson, S.E., Goodwin, D.S., Zettler, E.R, DeForce, E., Kukulka, T., Proskurowski, G. (2014). "Distribution of surface plastic debris in the Eastern Pacific Ocean from an 11-year data set." ACS Environmental Science and Technology **48**(9): 4732-4738.

Law, K. L., Morét-Ferguson, S.E., Maximenko, N.A., Proskurowski, G., Peacock, E.E., Hafner, J., Reddy, C.M. (2010). "Plastic accumulation in the North Atlantic subtropical gyre." Science **329**(5996): 1185-1188.

Marsh, K., Bugusu, B. (2007). "Food Packaging—roles, materials,and environmental issues." Journal of Food Science **72**(3): 39-55.

Perugini, F., Mastellone, M.L., Arena, U. (2005). "A life cycle assessment of mechanical and feedstock recycling options for management of plastic packaging wastes." Environmental Progress **24**(2).

Platts (2015). "Platts Global Low-Density Polyethylene (LDPE) Price Index." Platts Global Petrochemical Index. Retrieved July 14, 2015, from http://www.platts.com/news-feature/2015/petrochemicals/pgpi/ldpe.

Quispe, C. A. G., Coronado, C.J.R., Carvalho Jr., J.A. (2013). "Glycerol: Production, consumption, prices, characterization and new trends in combustion." Renewable and Sustainable Energy Reviews **27**: 475-493.

Shukla, R., Cheryan, M. (2001). "Zein: the industrial protein from corn." Industrial Crops and Products **13**: 171-192.
SimaPro (2015). SimaPro 7.3. **7.3**.

Sothornvit, R., Olsen, C W, McHugh, T H, Krochta, J M (2003). "Formation conditions, water-vapor permeability, and solubility of compression-molded whey protein films." Journal of Food Science **68**(6): 1985-1999.

Stevens, L. (1991). "Egg white proteins." Comparative Biochemistry and Physiology Part B: Comparative Biochemistry **100**(1): 1-9.

Stratford, M. (2006). Food and Beverage Spolage Yeasts. The Yeast Handbook. G. H. F. Amparo Querol. Berlin, Springer-Verlag**:** 335-379.

Sue, H. J., Wang, S, Lane, J L (1997). "Morphology and mechanical behaviour of engineering soy plastics." Polymer **38**(20): 5035-5040.

Vartiainen, J., Skytta, E., Enqvist, J., Ahvenainen, R. (2003). "Properties of antimicrobial plastics containing traditional food preservatives." Packaging Technology and Science **16**(6): 223-229.

Vert, M., Doi, Y., Hellwich, K., Hess, M., Hodge, P., Kubisa, P., Rinaudo, M., Schué, F. (2012). "Terminology for biorelated polymers and applications (IUPAC Recommendations 2012)." Pure Applied Chemistry **84**(2): 377-410.

Wilson, C. M. (1984). "Isoelectric focusing of zein in agarose." Cereal Chemistry **61**(2): 198-200.

Xin, H., Gates, R.S., Green, A.R., Mitloehner, F.M., Moore Jr., P.A., Wathes, C.M. (2011). "Environmental impacts and sustainability of egg production systems." Poultry Science **90**: 263-277.

Yuki, S. (2012). "Life cycle assessment of biodegradable plastics." Journal of Shanghai Jianotong University (Science) **17**(3): 327-329.

Zabaniotou, A., Kassidi, E. (2003). "Life cycle assessment applied to egg packaging made from polystyrene and recycled paper." Journal of Cleaner Production **11**(5): 549-559.

CHAPTER 6

CONCLUSIONS

Screening of protein-based bioplastics

Based on the results of the screening studies we perform on the protein-based bioplastics, numerous inferences can be made. One major finding is that the properties of the bioplastic made are highly dependent upon the utilization of plasticizers, and their interaction with the protein that forms the structure of the bioplastic. When we prepare bioplastics with water as the plasticizer, we find that the thermal properties remain unchanged for the most part compared to the pure protein, but with added extension possible when under tensile stress. In plastics that contain glycerol, our analysis points to materials that will gradually leech glycerol from the plastic over time, with compounds in the protein gradually elute from the plastic as well. For bioplastics that contain natural rubber latex as the plasticizer, we observe a plastic that will partially degrade at higher temperatures due to the latex in the plastic, as well as become less reactive to the environment when compared to plastics that contain water or glycerol.

When analyzing the protein bioplastics for antimicrobial properties, we determine that it is necessary for the materials that make up the bioplastic contain bactericidal capabilities themselves. For the soy plastics, we find a complete lack of antibacterial properties, while for whey protein plastics the same inferences can be made as long as glycerol is not utilized as the plasticizer. In the albumin and zein plastics, we find a complete inhibition of bacterial growth on the plastic, which is due to a combination of glycerol leeching and compounds within the proteins that will inherently prevent bacterial growth or contamination (lysozyme in hen egg

white albumin, hydrophobic amino acids of proline and glutamine in zein). Due to these properties we analyze the albumin and zein plastics that contain glycerol as a plasticizer, as these plastics possess the potential for use in medical and food packaging applications.

Properties of albumin and zein-based Thermoplastics

With our further analysis of albumin and zein bioplastics, as well as blending of LDPE with the proteins to produce thermoplastics, numerous inferences can be made. In terms of the addition of the addition of LDPE, as more LDPE is added into the albumin or zein-based plastics, there is a resulting change in the properties of the resulting plastic, as is expected. For instance, the temperature at which substantial thermal degradation occur will increase as more LDPE is placed in the plastic, as well as a decrease in the antimicrobial properties of the plastic. At a high enough concentration of LDPE, the various properties of the thermoplastic will become more similar to pure LDPE plastics than the properties seen in pure protein-glycerol plastics. When we compare compatibilities of albumin and zein with LDPE, we find that it is albumin that possesses a greater ability to blend with LDPE to form a plastic that has improved mechanical properties. In zein plastics, we see a limited compatibility with LDPE, as there is marginal benefits to adding LDPE when properties do not change significantly, as well as diminish in other areas. As for the environmental impact that producing albumin and zein based plastics have, we find that there is more greenhouse gas emissions and eutrophication potential when protein plastics are utilized when compared to LDPE plastics. In order for these proteins to become feasible in application without having a detrimental impact on the environment, it is necessary to study alternative methods to purify raw biomass to protein that will require fewer inputs.

Future studies

Based on the findings of these studies, there are multiple avenues of research that can be followed. The study of other proteins and other alternative sources of biomass for plastic production is crucial, as this would help identify other materials that could be utilized that may possess more optimal properties than what we have encountered. For the materials we have examined in these studies, further analysis with testing properties such as water and air permeability, as well as specific medical product testing such as ASTM F813 - 07(2012): Standard Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices would help determine if such uses are possible. The addition of different types of drugs and food preservatives would also be a good area of research, as other materials could be added to these plastics that could help serve a specialized purpose such as wound healing or drug delivery. It is also necessary to analyze the methods of converting raw biomass into an environmentally friendly plastic, as we find that this is a major roadblock in developing a biomass-based plastic that has a lesser impact when compared to traditional thermoplastics like LDPE.