ADVANCING PLANT PROTEIN-BASED PROTEOPOSITE FILMS TARGETED FOR FOOD PACKAGING APPLICATIONS. A COMBINED COMPUTATIONAL AND EXPERIMENTAL APPROACH

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ADVANCING PLANT PROTEIN-BASED BIOPLASTIC FILMS TARGETED FOR FOOD PACKAGING APPLICATIONS THROUGH COMBINED COMPUTATIONAL AND EXPERIMENTAL APPROACH

By

Kristen Lorraine Patnode

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Andriy Voronov
Co-Chair

Bakhtiyor Rasulev
Co-Chair

Mohiuddin Quadir

Wenjie Xia

Approved:

10/20/2021
Date

Dean C. Webster
Department Chair

ABSTRACT

Demand for polymeric, plastic materials continues to grow each year. However, the limited supply of fossil-fuels and negative environmental impact caused by petrochemical products have led to an increased demand for bio-based plastic alternatives. While there is great interest in developing plant-based alternatives to plastic packaging products, industrial applications of such materials are limited and development of said products is time, cost, and resource consuming. For this reason, advanced, marketable plant-based bioplastics must be developed more efficiently. To achieve such a goal, this thesis outlines a combined computational and experimental approach which results in novel plant proteins-based (proteoposite) films, that demonstrate enhanced performance, developed via a time, cost, and resource-conscious, approach.

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CHAPTER 1. INTRODUCTION

1.1. Environmental Threat of Petrochemical Plastics and Plastic Production

Since the early 20th century, petrochemical plastics have found application in nearly all industries.[1] From electronics and packaging to medical and agricultural markets, the universal utilization of plastics has continued to grow ever more quickly.[2] Plastics are synthetic polymer compounds which contain a variety of performance enhancing additives.[3] The resulting products are high molecular weight materials which can be tailored to offer flexibility, strength, and/or barrier performance. Additionally, these desirable features come at a low-cost, thus further expanding their marketability.[1,3]

Although petrochemical plastics benefit human society greatly, their demerits to environmental health and depletion of finite oil and gas resources cannot be ignored. Indeed, in 2018, the United States generated 35.7million tons of plastic, of which, nearly 27million tons were disposed of in landfills (Figure 1.1), which makes up nearly 19% of all municipal solid waste in the United States.[4] Such numbers are alarming, and further highlight the need for bioplastic alternatives. Of the total produced plastics, 14.5million tons are attributed to packaging materials such as bags, sacks, pouches, etc.[4] This contributes to a significant portion of the total generated and total landfilled plastics. Therefore, targeting packaging materials for bioplastic replacement is of great interest.

Bioplastic products are designed to be biodegradable or bio-derived and demonstrate similar performance to petrochemical plastics; thereby reducing the dependence on finite virgin resources and decreasing the environmental impact.[5] Although, bioplastics sound like an ideal solution, research has yet to develop plant-based materials which demonstrate sufficiently competitive performance and cost as compared to the petrochemical alternatives.

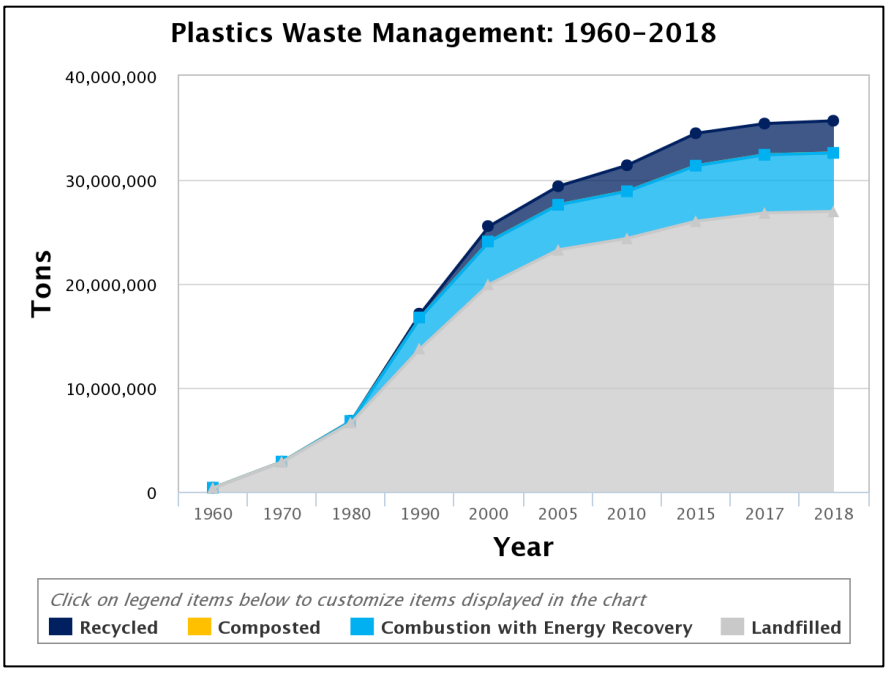

Figure 1.1. Waste management of plastics from 1960-2018 [4]

1.2. Current Developments of Bioplastic Materials

Biopolymer and bioplastics, currently occupy a very small share of the polymer market.[6] Although the use and development of biopolymers and bioplastics is an emerging and important focus, there are significant challenges that must be overcome before bioplastics can replace current petrochemical products.[7] Such challenges include: higher cost of bio-based materials, competitive uses of plant resources, and lack of desired properties such as durability, strength, and barrier performance.[6-8] Thus, in order to increase the market share of bioplastics and reduce the negative environmental impact of plastic production, new and improved bioplastic materials must be developed.

It is important to first define and understand the term "bioplastic" as this label can be ambiguous. Indeed, "bioplastic" is most commonly defined as a material composed from renewable resources and is either entirely or partially bio-derived and/or biodegradable or compostable.[6] That is to say that not all "bioplastics" are bio-based, and not all bio-based plastics

are biodegradable (Figure 1.2).[9] These differences are important to note, as advancement in bioplastic research aims to develop materials which are both bio-derived and biodegradable so as to reduce the environmental impact of both production and disposal of such materials.

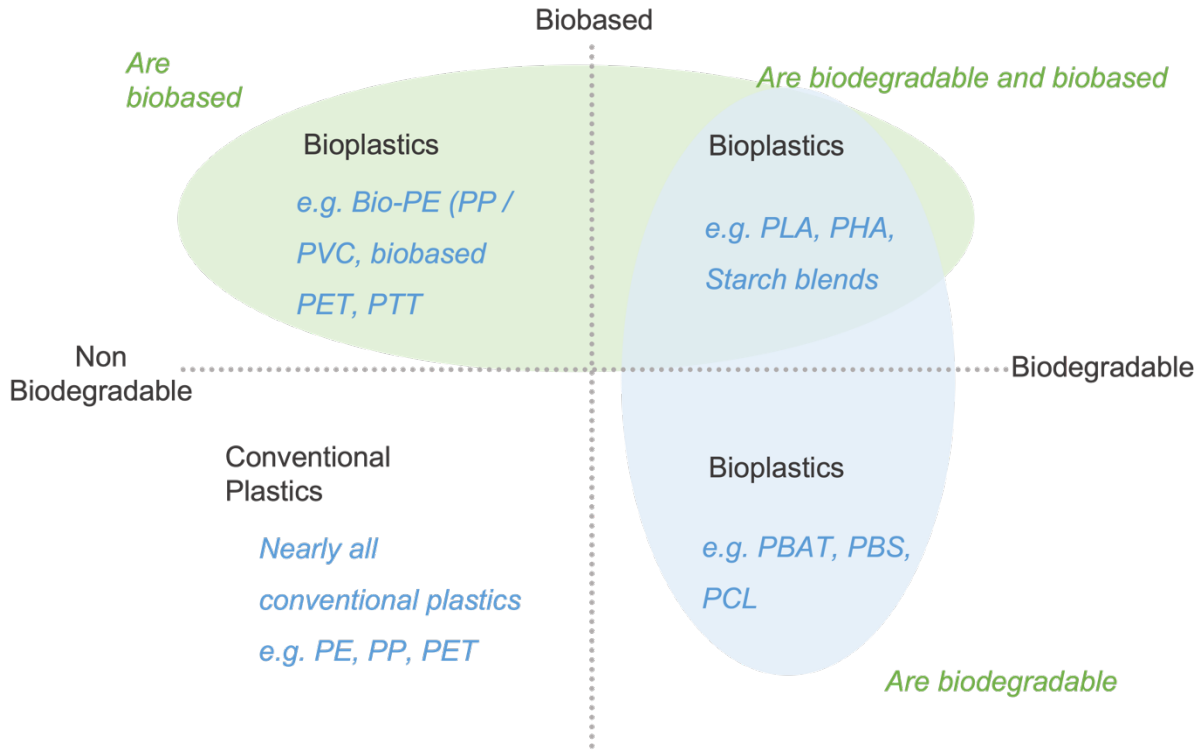

Figure 1.2. Broad Categories of Bioplastics (reproduced from Ref 9)

Since single-use applications of plastics such as packaging, and specifically food packaging, make up a large portion of the plastic waste in landfills, much research focuses on developing bio-based and biodegradable alternatives to these materials.

1.3. Protein-Based Plastics: Challenges and Opportunities

It wasn't until the mid-1900s when large scale extraction of vegetable oils and proteins was first introduced.[10] However, since the introduction of plant oils and proteins, their uses have become essential to everyday life.[10,11] Soybean, canola, and corn are some of the most popular crops which now undergo large-scale extractions to produce their respective oils and protein isolates.[12-14] While uses of each vegetable oil have been thoroughly explored and alternative

applications identified,[15-17] less work has been done to explore the material applications of these plant proteins.

1.3.1. Soybean Protein and Zein in Food Packaging Materials

Although there has been commercial and public interest in alternative uses of soy protein isolates, further investigation is required in order to develop applicable industrial materials.[18,19] Soy proteins' use as an additive has been found beneficial in numerous applications such as edible food technology, adhesives, and wood composites.[20,21] Soy protein-based materials are more limited to film applications such as food packaging. Indeed, previous work highlights the promising film forming ability of soy protein isolate.[22,23] Films prepared using soy protein demonstrate smooth, transparent films with high flexibility. However, these films are weak and do not perform well as water barriers due to the hydrophilic exterior of soy protein.[22,23] To mitigate some of these performance concerns, researchers have explored the use of various modifiers including *nano*-additives such as nanocellulose,[24] nanochitosan,[25] or nanoSiO_x.[26] While the use of such additives enhances film performance it does not address the need for cost effective alternatives to petroleum-based plastics.

Likewise, Zein from corn has received much focus as a materials resource due to its fewer number of competitive applications, and potential for higher strength.[27] Although less widely used as compared to soybean, the applications of corn oil and corn protein have seen extensive investigation.[28-30] Such work has again highlighted Zein from corn as a viable resource for food packaging preparation. Zein protein is a more hydrophobic material whose physico-chemical properties contribute to bioplastic products which demonstrate promising strength and improved barrier performance,[27] there are additional challenges which must be addressed. Previous studies

have illustrated the poor flexibility of films prepared from corn protein, which prevents further advancement of zein-based industrial materials.[31,32]

Obstacles associated with protein-based plastics include limited mechanical properties, poor water barrier performance, time required for film preparation and characterization, and cost-effective resource availability.[6,7] Although extensive work has been done studying proteins as a material for bioplastic products, this work is time and resource consuming. None of the previous literature includes a predictive modeling approach which would permit faster advancements of protein-based materials, and significantly reduce the amount of resources required, therefore also reducing the cost of development.

1.3.2. Composite Polymeric Materials from Soy Protein and Zein

After discussing the challenges that come from using either soy protein of Zein as a base material for food packaging films, it can be noted that there is potential to provide synergistic performance by eliminating each protein's specific disadvantages, if applied simultaneously with a counterpart in preparing the bioplastic materials. Indeed, soy protein films are soft, flexible materials with poor water resistance,[22,23] while Zein films are brittle films with greater moisture resistance.[31,32] Thus, there is a desire to combine the two proteins and prepare a bioplastic film from both materials. In doing so, bio-based food packaging films may be achieved which have optimum mechanical and barrier properties. Indeed, previous research has explored the possibility of combining multiple plant proteins to prepare enhanced bioplastic films,[33,34] however, a number of challenges must be addressed.

The primary obstacle faced when investigating soy-Zein composite materials is the concern of solubility. As most film formation studies utilize a solution casting approach, it is necessary for the proteins to be soluble (or dispersed) in the same solvent system. However, in the case of soy

protein and Zein, the two proteins share no common solvents in which they are miscible.[35] Indeed, while soy protein is readily soluble in water, Zein will coagulate when introduced to a 50% water solution.[36] Although Evans and Manley identified more than 50 solvents which can be used to dissolve corn protein,[36] none of the available identified materials allowed for adequate dissolution of soy protein as well. Therefore, an extensive solubility parameter study was done throughout the course of this thesis in an attempt to identify a solvent blend in which both soy and Zein proteins were soluble. Despite significant efforts, a common solvent(mixture) has yet to be identified, thus further highlighting the challenge of combining both proteins. For this reason, prior studies have instead investigated "laminated" films, wherein two independent layers are prepared utilizing separate proteins in order to achieve greater barrier performance.[34,37] However, this approach requires thermal compression as a means to laminate the films. Additionally, studies of this nature are unable to perform surface hydrophobicity measurements.[33,34,37] This may be because such laminated materials demonstrate different levels of hydrophobicity on each side of the films, and thus no number can be reported which is characteristic of the entire material. Thus, to prepare a uniform film from multiple plant proteins requires significant changes in processing techniques and limits the methods of characterizations which can be performed and considered valid. In addition to the solubility and processing challenges of combining plant-proteins in bioplastic production, the challenges of cost, time, and resources remain. For this reason, there is a need to effectively optimize the compatibility between soy and Zein proteins allowing for the advancement of bioplastic materials with enhanced mechanical and barrier properties.

1.4. Conclusions

As demonstrated by the number of previous works cited in this chapter, the demand for biobased and biodegradable plastics is continually growing. Indeed, our society increasingly calls

producers to action, asking for more environmentally friendly alternatives to current eco-damaging materials. Specifically, the desire to utilize plant proteins as a material for food packaging products has gained significant interest as proteins such as soy and Zein demonstrate good film forming abilities and promising performance. Despite their favorable characteristics, research still faces a number of challenges when preparing food packaging alternatives from plant proteins. One primary obstacle is the cost-performance analysis between bio-based products and their field performance. In order to improve industrial acceptance of protein-based packaging, the cost of production must be reduced and properties of the material enhanced. In this thesis, opportunities to advance the mechanical properties and barrier performance of novel soy-Zein proteoposite films are identified and predictive models established which allow for more cost-effective material development.

1.5. Research Objectives

This research has two primary objectives

(1) Develop/ determine the ability to utilize computational methods as a means to predict properties and performance of bioplastic plant protein-based films, both qualitatively and quantitatively.

(2) Advance the properties and performance of novel proteoposite films from soy and corn proteins by modifying with plant oil-based latexes (latex particles).

1.6. Organization of This Thesis

This thesis consists of four independent chapters. This first chapter (Chapter 1) introduces the environmental threat that petrochemical plastics pose. An investigation of prior work is illustrated here and outlines the current challenges in bioplastic development and the gaps in research surrounding plant protein-based materials. It is here also that the objectives of the research

are identified. Chapter 2 identifies the first known attempt to use protein-ligand docking as a means to qualitatively predict properties and performance of protein-based bioplastic films. This work includes an experimental component which supports the computational findings.

In the third chapter (Chapter 3) we aim to further the applicability of computational methods while simultaneously enhancing the mechanical and barrier performance of novel proteoposite films. To our knowledge, this is the first report of any such material, and we therefore established a new name to describe these soy-Zein composite films. For this reason, the novel material we call as a "proteoposite." This work illustrates the synergistic behavior of proteoposites prepared from soy protein and Zein, and modified by plant oil-based latexes. It further identifies a Quantitative Structure Property Analysis model which accurately predicts the mechanical properties of said novel materials. Finally, Chapter 4 summarizes all conclusions of this study and highlights future directions of this work.

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CHAPTER 2. COMBINED COMPUTATIONAL PROTEIN-LIGAND DOCKING AND EXPERIMENTAL STUDY OF BIOPLASTIC FILMS FROM SOYBEAN PROTEIN, ZEIN, AND NATURAL MODIFIERS[1]

2.1. Abstract

Plant-based proteins are emerging at the forefront of functional food trends, as well as sustainable component for various environmentally-friendly and sustainable polymeric materials. This study focuses on application of combined computational and experimental approach in design of plant protein-based films from soy protein and zein (corn protein). In this work, for the first time is shown the application of computational protein-ligand docking approach in design of protein-based films, by modeling the intermolecular (non-covalent) interactions of selected renewable modifiers with plant proteins, where demonstrated the effect of the incorporated modifiers on properties of protein-based films. Based on predictive modeling, we successfully prepared films based on modified both soy protein and zein protein which exhibit promising physical and mechanical behavior. Adding natural additives to plant proteins of varying chemical structure yields a broad range of protein-based films properties. By incorporation of natural plasticizers (glycerol and sorbitol) and reinforcement agent (micro-fibrillated cellulose) into the protein systems, more flexible films (elongation 2-120%) with Young's modulus of 99-400MPa that demonstrate higher surface hydrophobicity can be prepared, which confirmed the initial computational estimations. In result, we found that computational protein-ligand docking approach

[1] The material included in this chapter was co-authored by Kristen Patnode, Zoriana Demchuk, Sara Johnson, Andriy Voronov, and Bakhtiyor Rasulev. Kristen Patnode had the primary responsibilities of preparing bioplastic films, performing the computational work, and drafting and revising all versions of this chapter. Zoriana Demchuk performed a number of preliminary studies which aided in the background of this project and Sara Johnson helped with bioplastic preparation. Published article can be found at doi:10.1021/acssuschemeng.1c01202

can be used as an effective and accurate method in guiding the experiment and predicting the physical properties of a film upon incorporation of modifiers into the plant protein-based system.

2.2. Introduction

Use of petroleum-based plastics for packaging across many industries has become a standard; however, there is now increasing demand for eco-friendly and sustainable alternatives.[1] In this vein, bioplastics prepared using renewable and biobased materials are increasing in popularity.[2,3] There have been considerable research efforts to replace petroleum based plastics for biobased and, more specifically, sustainable protein-based plastic alternatives.[4,5] A common obstacle that is faced in the development of plant protein-based plastics is the inherent brittle nature and limited moisture resistance of these materials. Indeed, it is well recognized that protein-based films typically exhibit poor mechanical and water barrier properties.[6-8]

Many efforts in the development of biomaterials are focused on vegetable proteins.[6] Two popular proteins utilized in biobased film development are soy protein from soybean and zein protein from corn. The two proteins exhibit different structures as well as unique film characteristics. Soy protein is comprised of a mixture of globular proteins including 2S, 7S, 11S, and 15S fractions. Of these four globulins, the 7S and 11S make up nearly 70% of all soy protein. The 11S globulin is a homohexamer in which each protomer contains 27 strands and 7 helices which are then folded into two ß-barrel domains and two helix domains.[9] Upon denaturation of the 11S globulin, intermolecular disulfide bonds are able to form which thus affects the properties of a bioplastic film formed using soybean protein.[9,10] The 7S globulin is a trimeric protein consisting of three unique subunits which vary from 125-419 residues per unit.[11] In contrast, zein from corn is a dimer which demonstrates a high percentage of nonpolar amino acids, the three most prominent of which are glutamine, leucine, and proline. Each protomer in the dimer consists of only 33 amino

acid residues and exhibits a secondary helical structure.[10] Due to these differences in crystal structure of the proteins, research illustrates the resulting films of soy protein and zein are likewise comparatively unique.

Specifically, vegetable proteins are getting more attention in the development of biomaterials applicable to food packaging.[6,12,13] Due to the excellent biodegradability, abundance, lower cost and the inherent physico-chemical characteristics determined by types of amino acids presented in the structure, soy protein biopolymers have already received much attention and demonstrated potential for manufacturing of renewable plastics.[6,14] Soy protein possesses good film-forming capacity with great biocompatibility, and offers better characteristics in terms of barrier properties against oxygen and aroma at low or intermediate relative humidity. However, main disadvantages of soy protein–based films that need to be overcome are their high sensitivity to humidity and brittleness (lack of elasticity and, respectively, toughness).[10,14] Zein proteins, main residue from the production of corn starch, are as well shown to have promising physico-chemical properties for formation of films with good moisture and oxygen barrier performance, high glossiness and strength.[15] However, similar to soy protein, zein protein-based films do not provide balance of elasticity and strength and have very low vapor (odor) permeability. In particular, zein-based films' brittleness needs to be overcome as well as its films sensitivity to humidity needs to be diminished.[15] With this in mind, to be considered for food packaging applications, both plant protein-based films physico-chemical properties and performance need to be essentially improved by using various additives.

It is common for plasticizers and additives to be used in order to enhance flexibility and improve mechanical properties of these films.16,17 Some of the most commonly used plasticizers for these applications are glycerol and sorbitol.[18-21] Additionally, cellulose, (typically

microfibrillated or nanofiber) is a common additive incorporated into protein-based films in order to reinforce the films and improve mechanical properties.[22,23] The incorporation of additives into protein-based films needs to be investigated closely by both computational and experimental methods, in order to find which additives best improve properties of the protein-based films. In this regard an initial computational study can be very helpful to investigate possible interactions within the molecular system. It is well understood that plasticizing additives in such bioplastic systems, interact non-covalently with the plant proteins.[24,25] These physical interactions allow for the modifiers to achieve a plasticizing effect on the originally rigid matrix. A well-known and popular computational technique which explores non-covalent interactions is the protein-ligand docking approach.[26-30] Previously, our group has performed extensive research applying computational methods and specifically protein-ligand docking studies in a variety of systems, including nanoparticles,[26,27] fullerene analogues,[28,29] and organic materials.[30] By further utilizing computational models to examine the interactions between potential additives and the plant-proteins of a thermoplastic system, we aim to better guide experimental research based on computational outcomes.

Protein-ligand docking is a powerful tool to study macromolecular systems interactions with small molecules and often used in lieu of high-throughput screening.[31] This technique is widely applied in design of new effective drugs and materials.[26-31] When the structure of a macromolecular target is known, the binding of potential ligands can be simulated using protein-ligand docking technique, implemented in various software, such as AutoDock Vina, DOCK, GOLD, FlexX, or Glide.[31,32] In this technique each predicted possible docking pose is given a "score" termed the "binding affinity."[33] The predicted binding affinity is comparable with experimental values of binding affinity,[34] and often used to predict interactions between two

chemical systems. In this way, protein-ligand computational approach can provide significant insight to the influence of chemical structure and non-covalent interactions on properties and performance of an experimental system. The approach has been previously utilized in order to study protein-ligand interactions of glycerol, sorbitol, and cellulose with a variety of proteins.[35-38] However, it is worth to note, limited studies are found that examine protein-ligand docking with soy protein and zein.[39,40] To the best of our knowledge, protein-ligand docking approach has not been used previously as a tool to predict properties and performance of bioplastic films, and this study is a first work that applied this approach towards these materials.

In this work, we explore the novel application of protein-ligand docking technique as a means to estimate properties of bioplastic films made from plant proteins. We applied the computational protein-ligand docking approach to predict properties of plant protein-additive system and demonstrated that the performance of a bioplastic film is related to protein structure and its interactions with additives. The obtained protein-additive simulated results were analyzed, and experimental outcomes predicted based on computed binding affinity. Upon prediction of film properties and performance via computational study, protein-based films from soy and zein proteins with either glycerol or sorbitol as plasticizers, as well as films made using microfibrillated cellulose were prepared and predicted properties confirmed. Resulted bio-based films were characterized, including their surface hydrophobicity and mechanical properties. To the best of our knowledge, this is the first report which explores the use of computational protein-ligand docking technique as a means to predict trends in properties and performance of bioplastic films from plant proteins.

2.3. Materials and Methods

2.3.1. Materials

Soy protein isolate (91%protein, eVitamins™ Utica, MI), glycerol (MP Biomedicals, Santa Ana, CA), sorbitol (Fischer Scientific, Waltham, MA), micro-fibrillated cellulose (Exilva) (2%, Borregaard, Sarpsborg, Norway), and ethanol (96%, Sigma-Aldrich, St. Louis, MO) were used as purchased. Zein was obtained from Spectrum Chemical® and defatted via hexanes prior to film formation. The detailed procedure for the defatting of zein is reported below. All additives used were of research grade.

2.3.2. Protein-ligand Docking

In this work, a computational study of interactions nature between the proteins and additives were analyzed using the protein-ligand docking approach. All protein-ligand docking calculations were performed utilizing the AutoDock Vina program (developed by the Scripps Research Institute).[41] Two protein structures, as well as three natural additives were studied which are treated as ligands in the protein-ligand docking set up. The modifiers included glycerol, sorbitol, and cellulose. For all 3 additives, their structures were modeled and optimized by Avogadro molecular modeling software; an open source molecular builder and visualization tool (Avogadro, version 1.2).[42] The structures of each additive can be found in Figure 1.

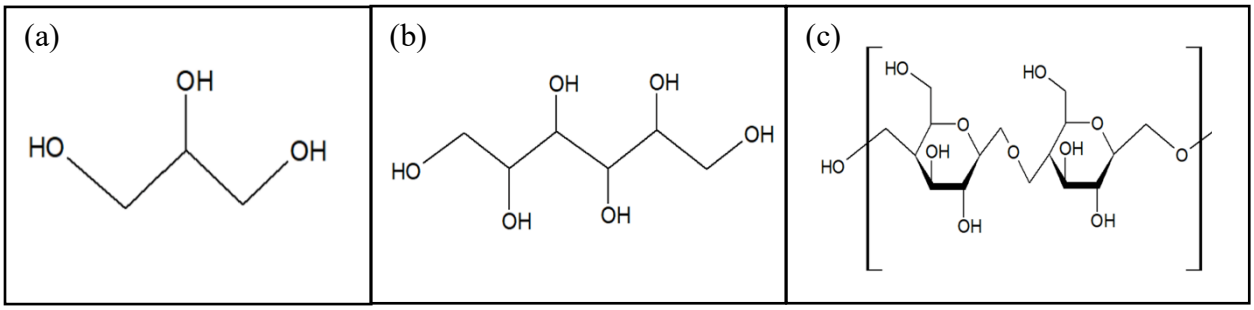

Figure 2.1. Chemical structure of each additive: a) glycerol, b) sorbitol, c) cellulose

The structures of soy protein globulins 7S (PDB ID: 3AUP) and 11S (PDB ID: 1OD5) were used for the study, and the experimentally obtained structure coordinates were downloaded from Protein Data Bank (PDB). The structure of zein protein was downloaded from the European molecular biology laboratory (EMBL-InterPro) with code Q9SYT3_MAIZE. All structures of proteins used in this study are shown in Figure 2. The binding sites on each protein structure were determined using COACH tool (Zhang Lab, University of Michigan, Ann Arbor, MI).[43,44] The AutoDock Vina code employs Lamarckian genetic algorithm (LGA) which holds the proteins as rigid and the ligands are considered flexible molecules given that there are rotatable bonds present. The protein coordinates were used as the input receptors for docking in AutoDock Vina, while the optimized models of glycerol, sorbitol, and cellulose were treated as the input ligands. All rotatable bonds of the ligands were set to be free, polar hydrogens were added to the receptors, and a search space, denoted as the "grid box", was set around each binding site of interest. Size of the grid box varied depending on the protein's binding sites' size and number of residues present in each respective binding site. All output results from the docking were visualized and analyzed using PyMol by Schrödinger (Schrödinger, LLC).[45] Reported values of binding affinity are an average of 5 calculations performed at the preferred binding pose.

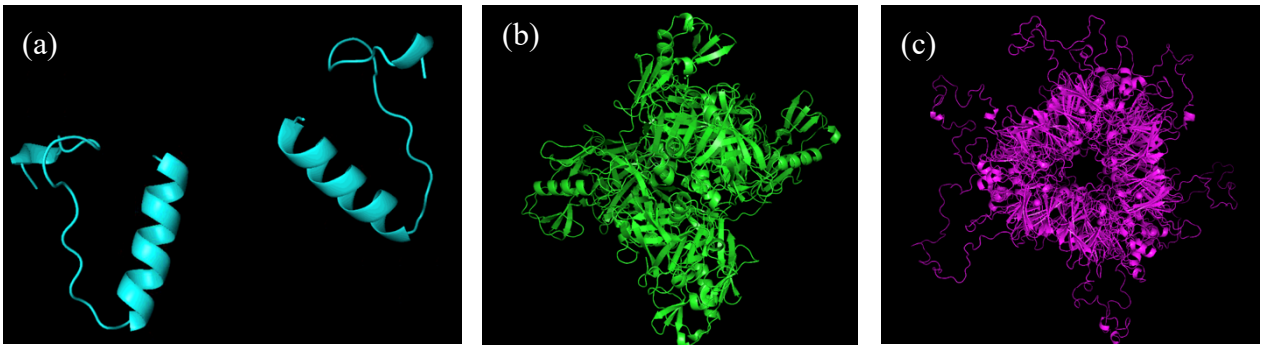

Figure 2.2. Chemical structures of (a) zein protein, (b) soy 7S globulin, and (c) soy 11S globulin

2.3.3. Soy Protein Film Preparation

Soy protein films were prepared as follows. A 10 wt% solution of soy protein dispersion (SPD) in miliQ water was first prepared and the pH adjusted to 10.5 using NaOH (5N). The dispersion was then allowed to stir via magnetic stir bar at 75ºC for 45min, sonicated for 30sec, and stored in a refrigerator. To prepare the casting solutions, additives were added in selected amounts (glycerol 50wt%, sorbitol 40wt%, Exilva 5wt%) simultaneously to the SPD and allowed to mix via magnetic stir bar at room temperature for 1h for complete homogenization. The solutions were then cast on glass using a draw down bar and allowed to dry at room temperature overnight. The resulting films were removed from glass and stored at room temperature for at least 1h prior to testing.

2.3.4. Zein Film Preparation

Protein Defatting Procedure: Zein protein was first defatted by adding hexane (5mL/g) to the protein sample and allowing to stir slowly at 60ºC for 1h. The excess hexane was removed by decantation and the process repeated for a total of 3 times and filtered by vacuum until a fine dry powder was achieved.

Protein Film Preparation: A zein protein dispersion of 10wt% in 85% ethanol was then prepared. The 85% ethanol was heated to 50ºC and the defatted zein protein added to the warm ethanol while stirring at 50ºC for 45 min. Casting solutions were prepared the same as for soy protein, then cast onto glass using a draw down bar and dried at 80ºC in an air circulating oven for 1h. Films were removed from the glass and stored at room temperature for at least 1h prior to testing.

2.3.5. Film Characterization

The water contact angle of the plant protein-based films was measured using a drop shape analyzer (DSA100, KRÜSS, Hamburg, Germany). Reported values are an average of 5 droplets.

The mechanical properties of soy protein and zein films were measured using an Instron model 5542. Tested films had a rectangular shape with constant width of 5mm. A strain rate of 5 mm/min was used and tensile stress at break, elongation at break, and Young's modulus were calculated. Reported values are an average of 5 samples.

2.4. Results and Discussion

Although formation of plant protein films is a well-established procedure, results continue to demonstrate poor flexibility and barrier properties. For this reason, computational methods are an attractive option to better understand how different natural modifiers may benefit the protein-based film system and then use finding to design bio-based films with better properties. In this regard, a computational approach, such as protein-ligand docking was used to assess the properties of investigated films. The simulation is performed in order to determine and compare the binding affinities of all three additives with zein, soy 7S globulin, and soy 11S globulin. The 7S and 11S globulin of soy protein were chosen as they account for nearly 70% of soy proteins.[10,46,47] Therefore, by performing the molecular docking on both 7S and 11S globulins and analyzing their respective results as a whole, a comprehensive understanding of the soy protein behavior upon protein-ligand docking was gathered, to use obtained results in synthesis of the films. Figure 3 shows the crystal structures of zein, soy 7S, and soy 11S with all three modifiers at their preferred binding site. It is worth noting, on zein protein, all three additives bind most favorably at the same site. Additionally, in the 7S globulin, both sorbitol and cellulose bind preferentially to the same site, and in the 11S globulin, sorbitol and glycerol show their best binding at the same binding site.

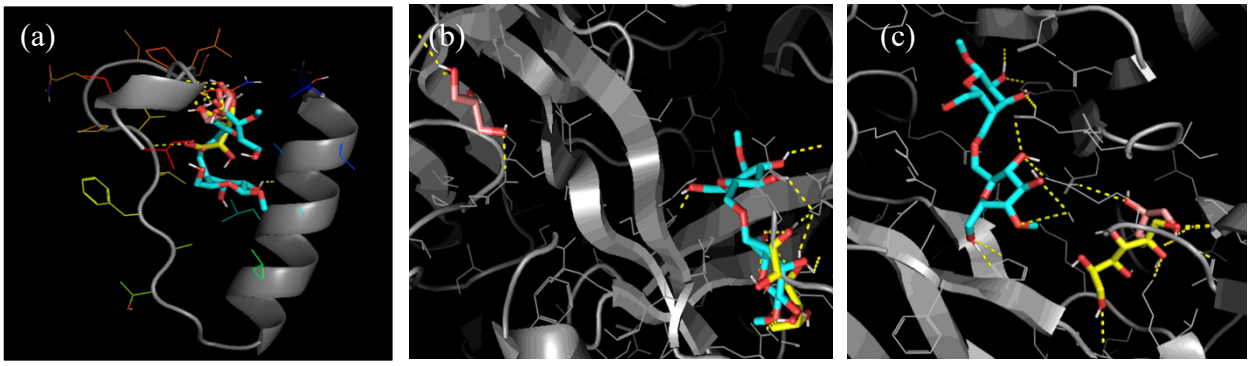

Figure 2.3. Glycerol, sorbitol, and cellulose in preferred pose on each protein (a) zein, (b) 7S, and (c) 11S. (Peach-glycerol, yellow-sorbitol, blue-cellulose)

The binding poses for glycerol, sorbitol, and cellulose on zein protein are shown in Figure 4. Also illustrated in the image are some of the relevant non-covalent interactions which are present between the ligand and the protein structure. When docking with glycerol, there are four amino acids within 4 Å, which is the desired distance for interactions to take place between heavy atoms and a ligand.[48,49] The residues within this range are all hydrophobic (ILE23, ILE24, LEU29, and ALA30) and participate in hydrogen bonding with the hydroxyl moieties on glycerol (numbers listed indicate the residue number in the amino acid sequence of the protein). Shown in Figure 4b is the binding pose for sorbitol, which has five amino acids within interacting distance, three hydrophobic residues (ALA7, ILE23, and LEU39) and two polar uncharged residues (THR3 and SER28). Many of these residues are the same as those interacting with glycerol. This suggests competitive binding takes place between each modifier on zein. Finally, there are seven amino acids within 4 Å of cellulose when docked at its preferred site (Figure 4c). Similar to glycerol and sorbitol, the hydrophobic residues interacting with cellulose are ALA7, ALA10, LEU11, ILE 23, LEU 29, and ALA 30. Additionally, the ligand interacts with the polar uncharged THR3 residue via hydrogen bonding. All three modifiers demonstrate hydrogen bonding between the ligand and the nearby residues, however, cellulose also exhibits hydrophobic interactions between the methyl group on cellulose and ALA10. Thus, as reflected in Table 1, cellulose shows the most favorable

binding with zein, which can be attributed to the greater number of hydrogen bonds as well as the combination of both hydrogen bonding and hydrophobic interactions present at the site. It is of interest to mention that the reported values in Table 1 may be considered favorable binding interactions. Indeed, in a realistic system, more than one molecule of ligand will be interacting with the protein, as there are multiple binding sites on the protein. Therefore, virtually, the binding affinity value per protein may be multiplied by the number of ligand molecules participating. Thus, a system with three glycerol molecules would demonstrate a binding score of -12.9 kcal/mol. In this sense, the binding affinity values of each ligand can be considered as significant.

Table 2.1. Docking results from AutoDock Vina for all ligands and proteins

Ligand	**Binding Affinity (kcal/mol)**	**Number of H-bonds at binding site**
	Soy 7S	
Glycerol	-4.3	3
Sorbitol	-5.7	6
Cellulose	-7.7	7
	Soy 11S	
Glycerol	-4.3	4
Sorbitol	-5.5	6
Cellulose	-7.1	5
	Zein	
Glycerol	-2.5	3
Sorbitol	-3.5	5
Cellulose	-4.7	5

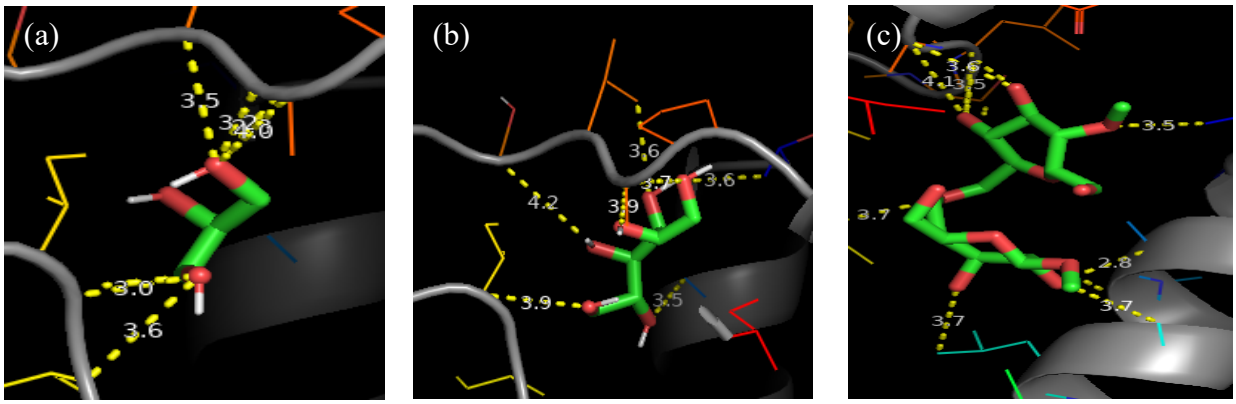

Figure 2.4. Amino acids on zein surrounding (a) glycerol, (b) sorbitol, and (c) cellulose. All modifiers are shown in their preferred pose

Figure 5 shows the poses for glycerol, sorbitol, and cellulose on the 7S globulin of soy protein. When glycerol is docked in its most favorable binding cavity (Figure 5a), there are five amino acid residues within 4Å, which are therefore able to interact with the ligand. These five residues include four partially charged residues, HIS23, ARG187, ARG363 (positively charged) and GLU366 (negatively charged) as well as one polar uncharged residue, ASN16. As seen in Figure 5b, when sorbitol is docked on the 7S globulin and in its preferred pose, there are six amino acids within 4Å of the ligand that are able to participate in non-covalent interactions. Four residues are polar uncharged (SER7, ASN43, ASN45, and SER265), one negatively charged amino acid (ASP41), and one hydrophobic residue (GLY44). All the amino acids within range of the ligand participate in hydrogen bonding. Since all of the end groups on sorbitol are hydroxyl moieties, the only interactions taking place are hydrogen bonds between the ligand and the end groups of the amino acid residues in the binding site. Lastly, in the preferred binding pose of cellulose (Figure 5c), there are nine amino acid residues within 4 Å of the ligand. One negatively charged residue (ASP41), five polar uncharged amino acids (ASN43, THR99, SER267 THR268, and THR359), two hydrophobic acids (ILE102 and MET263) and the cyclic amide, PRO101. Hydrogen bonding takes place between the ligand and all of the amino acids nearby save ILE102. The methyl end

groups on ILE102 participate in hydrophobic interactions with the free methyl groups on cellulose. Of interest to note are the similar residues which interact with both sorbitol and cellulose. Since both ligands bind most favorably at the same site, there will be competition for the binding at this pose in a multicomponent system of sorbitol and cellulose. Since cellulose has the more favorable binding affinity (Table 1), it is anticipated that cellulose would achieve successful binding in this competition for the binding site, thus preventing sorbitol from docking in its preferred position.

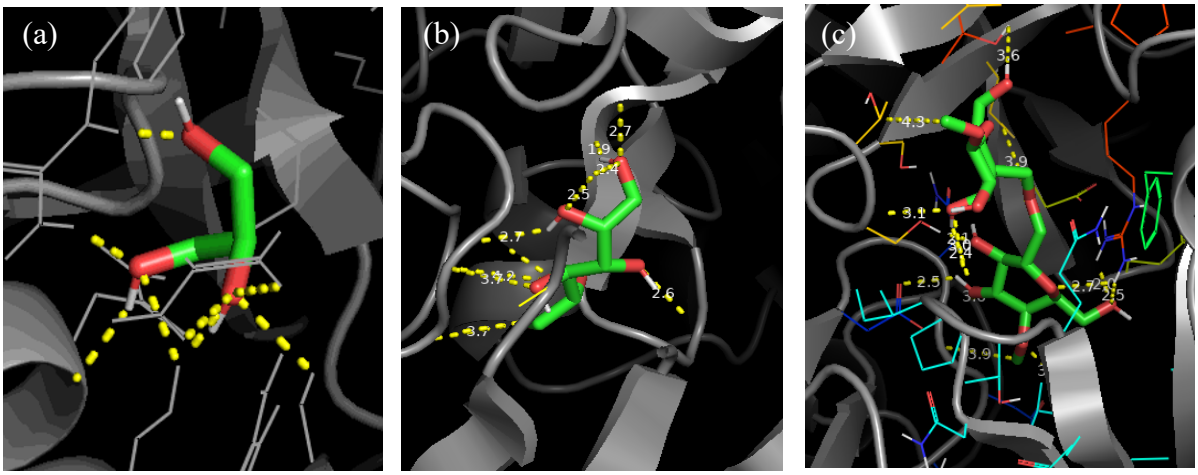

Figure 2.5. Amino acids on soy 7S surrounding (a) glycerol, (b) sorbitol, and (c) cellulose. All modifiers are shown in their preferred pose

The most favorable binding poses for glycerol, sorbitol, and cellulose on the 11S globulin of soy protein are shown in Figure 6. In Figure 6a, the preferred binding pose of glycerol is illustrated with some of the relevant non-covalent interactions highlighted. There are five amino acids within 4 Å of the ligand which are able to interact non-covalently including polar uncharged residues (THR87, GLN110, and THR326), hydrophobic amino acids (PHE81) and a positively charged residue (LYS111). When docked in its most favorable pose, sorbitol has six amino acids within interacting distance (Figure 6b) which include, positively charged LYS111 and LYS328, polar uncharged SER350, THR351, and THR357, and hydrophobic LEU 352. Finally, cellulose, when bound in its preferential position, has seven amino acids within 4 Å and therefore available

for non-covalent interactions. The residues interacting here are the positively charged HIS114, HIS330, ARG335, and ARG338, polar uncharged ASN117 and SER337, and hydrophobic PHE116. Both ARG 335 and 338 interact electrostatically with the ligand due to the positive charge on the amino acid residue and the partial negative charge on the oxygen moieties in cellulose. Furthermore, the free methyl groups on cellulose exhibit hydrophobic interactions with HIS114 and PHE116. Additionally, there are a number of hydrogen bonds present between the above listed amino acids and the ligand. Therefore, in cellulose's most favorable binding pose on 11S globulin of soy protein, all three major types of non-covalent interactions are taking place indicating better binding, which is confirmed by the binding affinity results shown in Table 1.

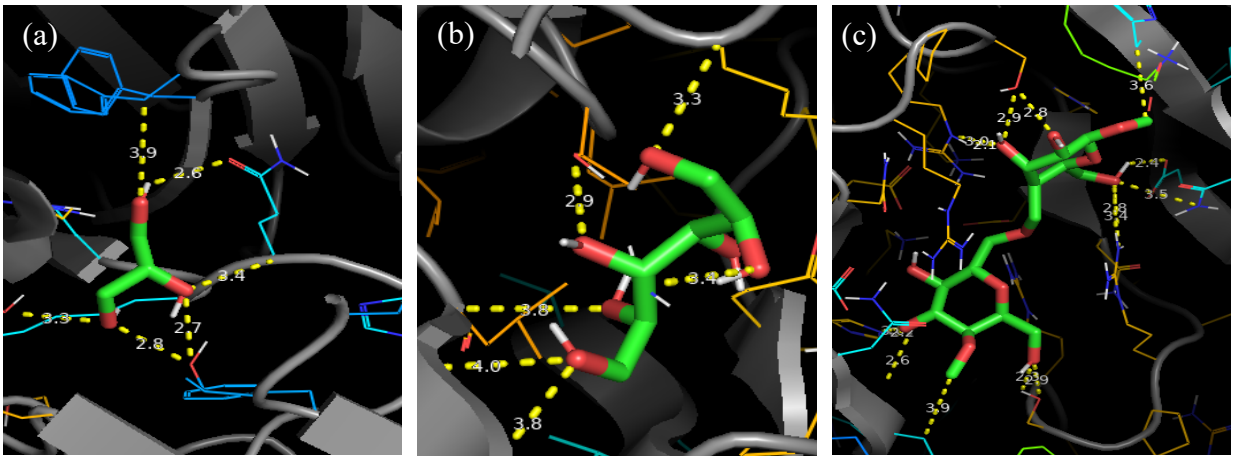

Figure 2.6. Amino acids on soy 11S surrounding (a) glycerol, (b) sorbitol, and (c) cellulose. All modifiers are shown in their preferred pose

Results from the performed docking studies provided the important information regarding which residues are participating in the binding with selected additives and specifically, the energies of binding. It is also observable what type of residuals are interacting with additives (hydrophobic, hydrophilic, H-bond donors, etc.) to predict what properties of additives are preferable for binding with the protein. To predict protein-additive interactions, ligands preferred binding pose in a multicomponent system was identified. In zein protein, all three modifiers preferably interact with the same binding site, while in soy 7S, sorbitol and cellulose bind most favorably at the same site,

and in soy 11S, glycerol and sorbitol find their preferred pose in the same site. However, since both 7S and 11S globulins are present in soy protein isolate, it can be said that all three modifiers preferably interact with the same sites in protein in a multicomponent experimental system. This suggests that in a multicomponent system, there will be competition for which modifiers are able to interact with the protein, further implying that loss or lack of synergistic effects can be evidenced. The relative binding affinity values further distinguish which modifiers will bind most preferably in the competition for specific binding positions.

In order to confirm the computational results, bioplastic films were prepared from soy and zein proteins and tested for mechanical properties and water resistance. The films were made first with protein dispersion and glycerol. They were next prepared using only sorbitol as plasticizer, and then finally films were made with both plasticizers and Exilva. All reported measurements can be found in Table 2.

Table 2.2. Mechanical properties of performance testing of bioplastic films

	Soy*				
	|Binding Affinity| (kcal/mol)	**Water Contact Angle (º)**	**Tensile Stress (MPa)**	**Elongation at Break (%)**	**Young's Modulus (MPa)**
Glycerol	3.9	75 ± 3	2.47 ± 2.18	126 ± 33	99 ± 78
Sorbitol	5.1	100 ± 2	2.93 ± 0.50	85 ± 6	154 ± 50
Plasticizers+ Cellulose	6.0	103 ± 2	4.91 ± 0.24	36 ± 7	185 ± 12
	Zein				
Glycerol	2.5	50 ± 3	2.9 ± 0.6	1.66 ± 0.15	380 ± 43
Sorbitol	3.5	56 ± 4	–	–	–
Plasticizers+ Cellulose	4.7	61 ± 3	4.1 ± 1.2	3.67 ±□□□□	271 ± 30

*binding affinity values shown are an average of binding affinity for soy 7S and soy 11S to reflect experimental situation where both globulins are present.
**Plasticizers + Cellulose in 2:8:1 glycerol:sorbitol:Exilva ratio

Interestingly, there is an increase in contact angle upon incorporation of sorbitol. Soy protein films prepared with glycerol showed a water contact angle (WCA) of 75º while films made

with sorbitol resulted in a WCA of 100º. When looking further at the protein-ligand docking results, one notes that all of the hydroxyl moieties on sorbitol are interacting with the amino acid residues at the preferred binding site. In regard to soy protein, the hydrogen bonds formed between the protein and ligand significantly disrupt the protein-protein interactions. In doing so, the hydrophobic groups within soy protein become exposed thus allowing for a higher water contact angle. Previous studies have also found that unfolding soy protein or disturbing the intramolecular interactions results in greater hydrophobicity.[50] Thus, sorbitol sufficiently disorders the protein structure to reveal the hydrophobic groups embedded in the molecule. Likewise, as Exilva is highly hydrophilic, a decrease in WCA was expected, but again there was an increase resulting in a WCA of 103º. This can be explained both by the disruption of the protein-protein interactions as well as the low amount of Exilva added. With only a 5wt% addition of Exilva, a limited effect of the hydrophilicity is observed.

The same trend is noted in films made from zein. Again, although sorbitol as a plasticizer and hydrophilic Exilva are expected to lower the WCA, there is an increase in hydrophobicity of the films instead. Zein films prepared with glycerol yield a WCA of 50º, while those formulated with sorbitol result in a WCA of 56º, and those with Exilva, 61º. This can be explained likewise to the soy protein films. As all of the hydroxyl groups in both sorbitol and Exilva interact with the protein upon binding, this creates greater exposure of the hydrophobic residues on the protein. Therefore, substantial disruption of the protein-protein interactions corresponds with more favorable binding affinity and results in higher surface hydrophobicity**.** In this way it can be observed that the relative trends in binding affinity values and the analysis of protein-ligand docking interactions effectively estimate and explain the trends in experimental water contact angle measurements.

In addition to surface wettability, the mechanical properties of the bioplastics films were determined via stress-strain analysis. In both soy and zein protein films, the tensile stress measured at break increases as binding affinity became more favorable (Table 2). However, in soy protein films, a decrease in elongation correlates with increasing absolute value of binding affinity, while zein films show a positive correlation between absolute value of binding affinity and elongation (Table 2). The differences between each protein's behavior can be explained by looking back to the protein-ligand docking analysis and considering chemical structure of the proteins.[29,31,51-53] Looking at the structures of zein and soy proteins, shown in Figure 2, some inferences can be made to explain the opposite trends in elongation for these systems. As the ligand interacts with zein protein, it significantly disrupts the protein-protein interactions, thus promoting free motion of the protein dimer. As the interactions of the ligand and the protein become more favorable, the protein is increasingly free to move and expand, thus resulting in greater elongation values when analyzed by stress-strain measures. Soy protein on the other hand, has a much bulkier structure than zein. Therefore, although the protein-protein interactions are being interrupted by the incorporated modifiers, the newly exposed residues on the protein have limited mobility. These additional functional groups may be adding steric hindrance to the already bulky system. Therefore, although there is better binding affinity and thus more significant disruption of the protein structure, a decrease in elongation is observed for those films prepared from soy protein due to the chemical structure and conformation of the macromolecule. Since toughness is a material property defined by a material's ability to withstand stress and to undergo elongation, the observed difference in protein films elongation trends can be accounted for the changes in toughness. As shown in Figure 7, opposite trends were likewise noted in toughness of soy protein and zein films, which are justified by the differences in elongation trends of the two proteins. As toughness is equal to the

area under the stress-strain curve, as the strain decreases, as seen in soy protein films, the toughness will likewise decrease. Additionally, an increase in strain, as observed in the zein bioplastics, corresponds to an increase in toughness as noted in Figure 7. Again, the relative binding affinity values and analysis of protein-ligand complexes helped to estimate and explain the trends in experimental values of tensile stress and strain for each of the bioplastics systems.

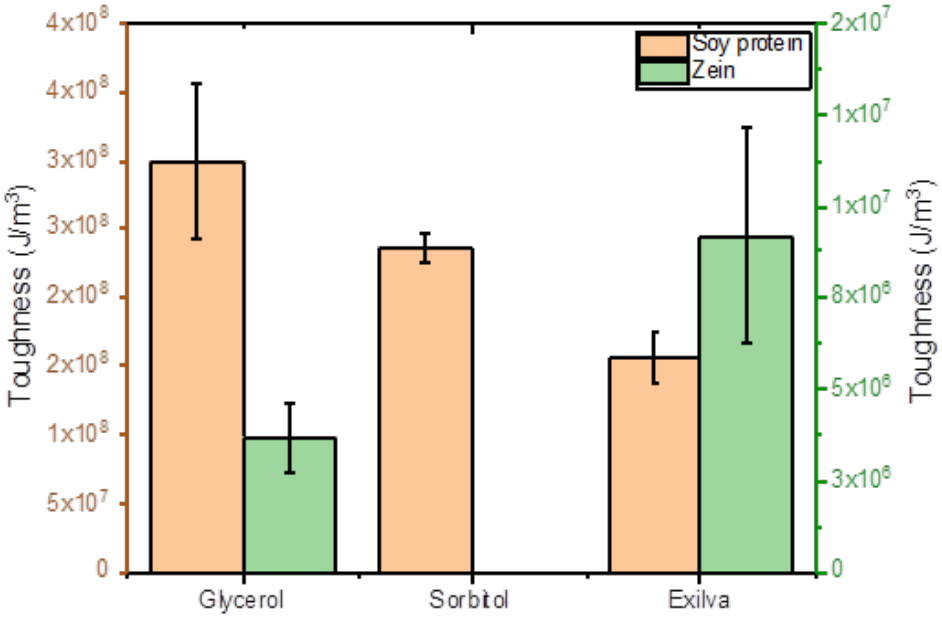

Figure 2.7. Toughness of modified soy protein and zein films with differing plasticizers/additives

Upon validation of the efficacy of this combined approach, use of protein-ligand docking may be considered for further use in virtual evaluating the effects of various alternative modifiers. While this study focused on three most prominent additives, glycerol, sorbitol, and cellulose, many more have been introduced as modifications for plant protein-based films. The use of computational approach, such as protein-ligand docking can be effectively utilized in virtual screening to evaluate the effects of new potential plasticizing and modifying additives, which may further advance the properties and performance of bioplastic films. It is our goal to investigate

further the abilities of this computational technique and apply the protein-ligand approach to more complex systems which include sustainable latex plasticizers in protein-based plastics.

2.5. Conclusions

In this work, the sustainable bio-based plant protein-based films from soy protein and zein (corn protein) were investigated by application of combined computational and experimental approaches. To best of our knowledge, it was a first attempt to use a computational protein-ligand docking approach to predict properties of bioplastic films. The feasibility of this approach in predicting protein interactions with various modifiers in bioplastic films was thoroughly examined. The experimental outcomes were compared with computed properties obtained from protein-ligand docking calculations. The binding poses of each modifier with the proteins under study were analyzed and the preferred binding sites determined. It was identified which modifiers more successfully bind to the protein, having stronger affinity to the same binding site when present in a multicomponent system like that of the protein-based films. Furthermore, the computational results were examined against experimental measurements of tensile stress, elongation and surface hydrophobicity of the bioplastics films. Trends were identified between the binding affinity values and all three experimental properties. For all systems, the strong correlations between computational data and experiments were found. In both modified soy and zein protein systems, more favorable binding affinity results in higher surface hydrophobicity and greater tensile stress. Furthermore, in zein-based films, an increase in percent elongation as modifiers of a more favorable binding were incorporated was noted, however in soy protein films, preferential binding affinity equated to a decrease in percent elongation. The study determined that applied computational protein-ligand docking approach can be successfully utilized before synthesis as an

effective method in predicting relative physical and mechanical properties of plant protein-based bioplastics upon incorporation of natural additives.

2.6. References

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CHAPTER 3. SYNERGY BETWEEN CORN ZEIN, SOY PROTEIN, AND PLANT OIL-BASED LATEXES IN BIOPLASTIC PROTEOPOSITE FILMS: EXPERIMENTAL AND COMPUTATIONAL STUDY[2]

3.1. Abstract

Plant-based proteins are attractive components which may serve as sustainable alternatives to current petrochemical products. Both soy protein and major corn protein, Zein, are of interest in food packaging applications due to their sustainability and inherent physicochemical properties. This study explores the effects of combining Zein, soy protein and plasticizing additive, plant oil-based (POBM) latex on properties of resulting bioplastic films. In looking for synergistic effects of soy protein's inherent film formation ability and Zein's higher strength, we prepare strong yet flexible soy-Zein proteoposite films. Incorporation of natural additive, POBM-latexes helps to plasticize and hydrophobize the bioplastic films, thus improve mechanical and barrier properties. Variation of the POBM-latexes' particle size further aims the ability to enhance performance of resulting bioplastic films. As a result, modified soy-Zein proteoposite films with improved moisture resistance, enhanced mechanical behavior, and greater barrier properties were developed. Machine learning-based computational models were utilized in order to find main structural factors affecting the bioplastic's properties and develop a quantitative structure-property relationship (QSPR) between the physico-chemical properties of the film components and the resulted bioplastics' properties and performance. The developed model effectively predicts experimental outcomes with >85% (R2: 0.85) accuracy. In result, it is shown that proteoposite films made of

[2] The material included in this chapter was co-authored by Kristen Patnode, Andriy Voronov and Bakhtiyor Rasulev. Kristen Patnode had the primary responsibilities of preparing proteoposite films, performing the computational work, and drafting and revising all versions of this chapter. Article is under review for publication.

two plant proteins and modified with POBM-latexes can be considered as an attractive and viable replacement for petrochemical food packaging products.

3.2. Introduction

With increasing demand for alternatives to petrochemical based products in the food packaging industry, plant protein-based thermoplastics and thermosets become a promising and attractive option. [1, 2] With international sustainability goals and a push towards a circular economy, the metric tons of plastics utilized in food packaging must be replaced or modified in order to achieve greener production.[3, 4] For this reason, studies involving plant protein-based bioplastics have gained significant popularity.[5-7] Such natural sustainable materials often demonstrate biodegradability, lower cost, are readily available, and exhibit promising physico-chemical properties. Unmodified protein-based plastics, however, lack the mechanical properties and moisture resistance necessary for effective application in food packaging. It has been well observed that protein-based films are inherently brittle and exhibit poor barrier properties. [8-10]

Two proteins commonly studied for bioplastic film formation are soy protein and Zein from corn maize.[7, 11] Having already acquired much attention, soy protein bioplastics demonstrate good film-forming ability and more flexibility as compared to alternative plant proteins.[12-14] Additionally, these soy-derived materials show promising oxygen barrier properties which have been investigated for many years.[15] The primary obstacles with soy protein bioplastics are the lack of moisture resistance and toughness. Zein-based films, however, have received less attention than its alternative plant proteins due to its significant brittleness. Although Zein contributes good moisture resistance and barrier performance, unmodified Zein protein films are exceptionally brittle and do not provide a balance in film flexibility and toughness that is adequate for food

packaging materials.[13, 16] Considering such hurdles, both soy and Zein protein-based films require modification in order to be effective as food packaging alternatives.

Previous work has explored the use of plasticizing polymers, such as polyethylene glycol as a means to advance properties of bioplastic films.[17-19] These methods successfully enhance the mechanical properties and performance of the bio-based materials; however, they do not address the need for moisture resistance, barrier properties, and sustainability. Thus, emulsion polymers (latexes) based on monomers derived from plant oils (POBMs) can be an attractive option for such modification. Such latexes are native to our groups' lab and utilize a range of POBMs in latex polymerization. Plant oil-based monomers are first prepared directly from the plant oils and then polymerized resulting in renewable latexes with tunable characteristics.[20-23] The synthesized from POBMs latex polymers are highly hydrophobic, soft and flexible which may advance properties and performance of a plant protein-based bioplastic film by plasticizing and hydrophobizing at the same time.[21, 22, 24].

Due to the time and cost-consuming nature of experiments, it is of interest to use computational methods to predict properties and performance of bioplastic films. Utilizing quantitative structure-activity/property relationships (QSAR/QSPR), relationships between structure of the film components and properties of the product can be established.[25-31] Indeed, QSAR analysis suggests that properties such as physicochemical, toxicological, and biomedicinal behavior are primarily determined from the molecular structure of a material. [25-27, 32-34] Current research efforts apply QSAR to a wide variety of disciplines to advance material behaviors by tuning the structure within a series of compounds, including linear polymers and correlate structural features of the compounds of interest with their experimental properties. The majority of QSAR/QSPR models follows the same approach. This is a 7 step approach which begins with

data selection and generation of molecular structures, then geometry optimization of the structures, descriptor generation, variable selection and/or data reduction, the development of the models, and finally model and predictability validation. [27, 35]

To date, limited number of QSAR studies have examined polymeric and macromolecular applications due to the fact that such large systems are fundamentally complicated.[36-40] For this reason, researchers have tried to identify unique descriptors that describe polymer structures[36, 37] and/or build models based on the monomeric structures (repeat units) of the desired polymer.[36-40] In recent work, Rasulev et. al.[39] report a novel mixture-QSAR approach that can be applied for complex polymeric materials, for example, polymer coatings systems. This work identified a methodology which computationally predicts experimental properties and performance of complex polymer systems with a high level of success.[39] It is worth noting that to the best of our knowledge, no work has been previously performed utilizing QSAR/QSPR to predict properties of bioplastic films. For this reason, it is of interest to apply the mixture-QSAR approach to biobased polymer film systems in order to find structure-property relationships and design the system with improved properties.

In this work, we perform a combined experimental and computational study where we modify Zein protein films with soy protein and POBM-latexes simultaneously, in order to explore the synergy between all three renewable ingredients. To our knowledge, no such material has previously been reported. We therefore developed a name to better describe the resulting novel materials; *proteoposites* in order to emphasize composite-like behavior of novel films prepared from different plant proteins. We further vary particle size of POBM-latexes to optimize mechanical properties of proteoposites. Demonstrated are the effects of soy protein and POBM-latex simultaneous incorporation on barrier properties of Zein-based proteoposite films to highlight

their efficacy as food packaging alternatives. Finally, a computational QSAR model was developed and validated which successfully predicts mechanical properties of proteoposite films. The model was used to predict an optimal film formulation, which demonstrated improved mechanics and enhanced barrier properties.

3.3. Materials and Methods

3.3.1. Materials

Zein from corn maize was obtained from Spectrum Chemical® and defatted via hexanes prior to additive incorporation. Soy protein isolate (91%protein, eVitamins ™ Utica, MI), glycerol (MP Biomedicals, Santa Ana, CA), ethanol (96%, Sigma-Aldrich, St. Louis, MO), high oleic soybean oil (Perdue Agribusiness LLC, Salisbury, MD), corn oil (PLACE), camelina oil (PLACE), *N*-(hydroxyethyl)acrylamide (HEAAm; TCI America), were used as received. All additives were of research grade.

3.3.2. Synthesis of HOSBM, CBM, and CMM

High oleic soybean monomer (HOSBM), corn oil-based monomer (CBM), and camelina oil-based monomer (CMM) were synthesized via a one-step transesterification reaction of each oil with *N*-hydroxyethyl acrylamide in the presence of 1.5wt% KOH as catalyst. A detailed description of the procedure can be found in our group's previous work[20].

3.3.3. Synthesis of Plant Oil-Based Latexes

Each of the prepared monomers (HOSBM, CBM, and CMM) was used for synthesis of POBM-based latexes via a miniemulsion process. Latexes of 20% solids content were prepared by mixing 8g of HOSBM, CBM, or CMM with AIBN (1.5wt% of oil phase) as initiator. The aqueous phase was prepared by dissolving certain amounts of surfactant (SDS, 4-8wt% of oil phase) and 0.02g NaCl in miliQ water under constant stirring. After adding the oil phase to the aqueous phase,

the pre-emulsion was formed and sonicated before the stable miniemulsions were allowed to polymerize at 75ºC for 12h under continuous stirring.

3.3.4. Protein Defatting Procedure

Zein protein was defatted prior to use in film formation by adding hexane (5mL/g) to the protein sample and stirring slowly at 60ºC for 1h. A detailed procedure can be found in our previous work.[41]

3.3.5. Soy-Zein Proteoposite Film Preparation

Soy films were prepared first. A 10wt% solution of soy protein dispersion (SPD) was prepared as described in [41]. The casting solution was prepared by incorporating 50wt% (w/w protein) glycerol and certain amounts of the POBM-latexes into 5g of the SPD and allowed to mix at room temperature for 1h so as to completely homogenize the solution. These solutions were then cast onto glass using a draw down bar and set to dry at room temperature overnight.

A 10wt% Zein protein dispersion (ZPD) was likewise prepared as previously described.[41] The casting solution was prepared by adding 40wt% (w/w protein) of glycerol to 5g of ZPD and allowed to mix at room temperature for 1h.

The Zein casting solution was then drawn down on top of the previously dried soy protein film and allowed to dry in an air circulating oven at 80ºC for 55min. The soy-Zein proteoposite films were removed from the glass and stored at room temperature for at least 12h prior to testing.

3.3.6. Film Characterization

Water contact angle of the soy-Zein proteoposite films was measured using a drop shape analyzer (DSA 100, KRÜSS, Hamburg, Germany). Reported values are and average of 5 droplets on each side of the proteoposite, for a total of 10 measurements per film.

The mechanical properties of the soy-Zein bioplastics were measured on an Instron model 5542. All tested films had a constant width of 5mm. A strain rate of 5mm/min was used, and tensile stress at break, elongation at break, and Young's modulus were calculated. Reported values are an average of 4 samples.

3.3.7. Water Vapor Transmission

Water vapor transmission of soy-Zein proteoposite films was measured gravimetrically according to ASTM E96. Film samples were mounted onto polystyrene dishes filled with water and placed in a desiccator. The film area was constant at 2.55×10^{-3} m^2 and conditions were maintained at 25ºC (±1ºC) and 50%RH.

3.4. Computational Details

3.4.1. Descriptors Generation

In this work, a computational machine learning structure-property relationship study was conducted applying QSAR/QSPR approach. For this, experimental properties of interest were used as endpoints and a set of generated theoretical structural features of bioplastic films as numerical descriptors. The set of descriptors was generated computationally in order to correlate the structure of each component in the proteoposite to changes in bioplastic film properties (i.e. endpoints). The structures of each protein were acquired from Protein Data Bank and descriptors generated from their native state. All other utilized additives were modeled by Avogadro software (www.avogadro.cc) by initial structure construction and their geometry optimization, to prepare for descriptors generation. Next, Molecular Operating Environment (MOE)[42] software was used in order to generate a set of descriptors for the investigated structures (proteins and additives). This software is capable of generating descriptors not only from the small chemical structures, but also from protein structures and was therefore utilized for the proteins as well as the additives. The

program provides various descriptors corresponding to 0D, 1D, 2D, 3D dimension structural features of investigated molecular systems,[43] and also generates protein specific indices. Additional descriptors (indicator descriptors) were added manually in order to best describe the format of the complex proteoposite system, including such properties as particle size of the POBM-latex and location of the POBM-latex within the film (POBM-latex layer position).

3.4.2. Mixed-Descriptors

Since the original generated descriptors only relate properties of single components within a film, an additional approach was employed to better describe an entire proteoposite film. Each proteoposite was treated as a mixture and the descriptors for each bioplastic film were calculated as described previously by the authors in reference [39].

3.4.3. Structure-Property Modeling (QSAR)

Upon calculation of all mixture descriptors for proteoposite films, the specific machine learning modeling was then performed. For this, a dataset of experimental and computational structural data was created. A set of 24 bioplastic films was divided into training (18 films) and test (6 films) sets, in a 3:1 training to test set ratio, in order to build a model and then test/validate it. The machine learning model (QSAR model) is a mathematical relationship between structure of a molecular system and its geometric, chemical, and physical characteristics. Thus, the goal of QSAR modeling is to find a consistent relationship between investigated physico-chemical properties of compounds and their structural properties/features, so that specific “rules” can be established which can be used to evaluate or predict the behaviors of a new, untested, system. In our study, by establishing a valid QSAR model, it is possible to predict the experimental properties of a new proteoposite.

In this study, the relationship between experimental properties (endpoints) and structural properties was developed using the following machine learning methods - variable selection Genetic Algorithm (GA)[44] and Multiple Linear Regression Analysis (MLRA). Preliminary models were developed via the GA-MLRA[45, 46] technique as included in the QSARINS software.[47, 48] The GA variable selection was applied to reduce the number of descriptors responsible for the property of interest, and find the final model. A population of 500 random models (per number of variables in a model) and 3000 iterations were used in each model development run. The MLRA was utilized to produce final QSAR models, and applied in this study.

All equations were used to calculate predictability and goodness-of-fit for each developed model can be found and are described in detail in the author's previously published works. [39, 40]

3.5. Results and Discussion

In this work, a combined experimental and computational study was performed to modify Zein protein films with soy protein and POBM-latexes simultaneously, in order to explore the synergy between all three bio-based renewable materials and be able to rationally design new ones with desired properties. As it was discussed above, while film formation of plasticized soy and zein protein-based bioplastics are well established procedures, the resulting films continue to exhibit inadequate flexibility and barrier properties. Therefore, appropriate, and sustainable modification is required. For this reason, we modified Zein protein films via soy protein and POBM-latexes.

In order to determine whether modification of Zein films with soy protein would result in synergistic effect, Zein protein control films were first prepared and then soy-Zein proteoposites formed and their properties compared to the control. Results are shown in Table 3.1.

Table 3.1. Mechanical properties and performance of bioplastic films

	Water Contact Angle (°)	Tensile Stress (MPa)	Elongation at Break (%)	Young's Modulus (Mpa)
Soy Control	34 ± 4	1.2 ± 0.1	159 ± 5.7	99 ± 18
Zein Control	50 ± 3	2.9 ± 0.6	1.7 ± 0.2	380 ± 43
Soy-Zein proteoposite	51 ± 4	10.6 ± 1.4	9.2 ± 3.3	627 ± 43

As shown by the significant increase in elongation at break, as compared to the Zein control, the inherent flexibility of soy protein is enhancing the properties of the Zein film. The advancement of stress can be attributed to the intermolecular interactions taking place between each biomacromolecule which triggers synergistic behavior within the film. As a result, films prepared demonstrate improved strength, flexibility, and maintain surface properties. Although the elongation at break of films increases more than 400%, conventional food packaging materials need to exhibit higher flexibility. Therefore, in an effort to further advance the mechanical properties of soy-Zein proteoposites, we incorporate POBM-latexes, synthesized in our lab using miniemulsion process.

Since POBM-latex polymers are highly hydrophobic and being soft and flexible act as plasticizers, we anticipate improvement in both moisture resistance and mechanical properties upon latex incorporation into the films. Soy-Zein proteoposite films were prepared with each of the three POBM-latexes; poly(HOSBM), poly(CBM), and poly(CMM), and compared with results of the original soy-Zein bioplastic film.

As illustrated in Table 3.2, the incorporation of POBM-latex enhances elongation at break of soy-Zein proteoposites. Additionally, the trend notes a decrease in tensile stress correlating with the increase in flexibility. Furthermore, the increase in elongation at break among the proteoposites

modified with three different latexes can be attributed to the varying degree of unsaturation of each POBM (Table 3.3).

Table 3.2. Mechanical properties and performance of proteoposites with POBM-latexes

Soy-Zein proteoposite with Latex Type	Latex particle size (nm)	Water Contact Angle (°)	Tensile Stress (MPa)	Elongation at Break (%)	Young's Modulus (MPa)
No latex	-	51 ± 4	10.6 ± 1.4	9.2 ± 3.3	627 ± 43
Poly(HOSBM)	100 ± 30	51 ± 4	4.1 ± 0.3	11.3 ± 3.5	300 ± 10
Poly(CBM)	100 ± 34	76 ± 3	5.2 ± 1.9	15.3 ± 4.6	306 ± 25
Poly(CMM)	100 ± 28	54 ± 1	4.0 ± 2.4	16.98 ± 3.1	419 ± 25

Due to the allylic termination, chain propagation coexists with effective chain transfer during polymerization of POBM, which is determined by the degree of unsaturation in the monomers.[21] As a result, the number-average molecular weight of resulted homopolymers decreases as follows: poly(HOSBM) > poly(CBM) > poly(CMM), corresponding to increasing degree of unsaturation in the monomers

Table 3.3. Content of unsaturated fatty acids in plant oil based monomers

Monomer from Oil	Unsaturated Fatty Acids Contents (%)		
	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
HOSBM	70-73	13-16	0-1
CBM	21-25	60-63	1-3
CMM	12-19	15-22	32-40

Poly(HOSBM) is made from the monomer with the lowest degree of unsaturation, and therefore this polymer contains longer and less entangled macromolecules (Figure 1a). As degree of unsaturation (i.e. the amount of C18:3 and C18:2 molecules in the monomer) increases, the macromolecules become shorter with and more entanglements due to dangling side fragments (Figure 1b for poly(CBM) and 1c for poly(CMM)). In shorter backbones from more unsaturated

POBMs, the dangling side fragments have greater effect on the mechanical properties. These entanglements may make the material more flexible by stretching and pulling apart, which results in greater toughness. Thus, not only does the incorporation of POBM-latexes improves the mechanical properties of soy-Zein proteoposites, understanding the monomeric structure helps better analyze results and guide future formulation. The trend is clearly observed in Figure 3.2.

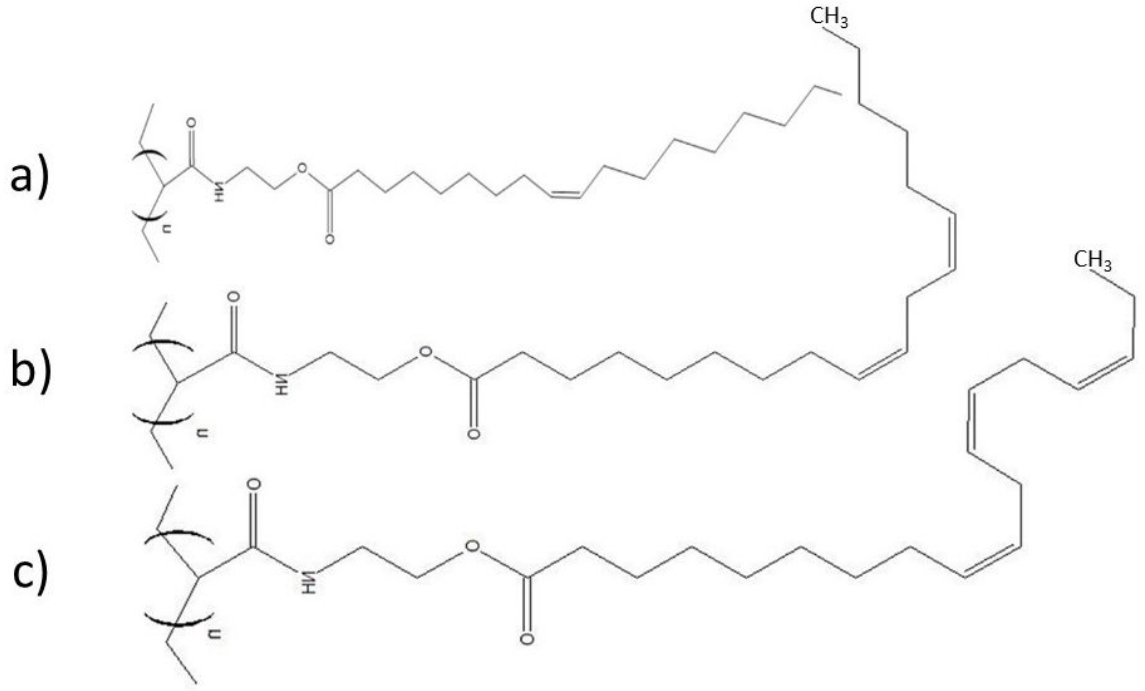

Figure 3.1. Increased number of dangling fragments in POBM macromolecules (a) poly(HOSBM), (b) poly(CBM), (c) poly(CMM)

After determining the feasibility of incorporating POBM-latexes into the proteoposite films and observing the synergistic effect, optimized films were prepared with varying particle size latexes. Each POBM-latex was prepared with particle sizes 100 (PDI=0.09) and 50 (PDI=0.23). As can be seen clearly in Figure 3.3 and Table 3.4, proteoposites prepared with the smaller particle size latex exhibit significantly higher elongation at break yet maintain surface properties.

Table 3.4. Mechanical properties and performance of proteoposite films with POBM-latexes of reduced particle size

Soy-Zein proteoposite with Latex Type	Latex particle size (nm)	Water Contact Angle (°)	Tensile Stress (MPa)	Elongation at Break (%)	Young's Modulus (MPa)
No latex	-	51 ± 4	10.63 ± 1.35	9.2 ± 3.3	627 ± 43
Poly(HOSBM)	50 ± 24	55 ± 2	8.65 ± 1.20	18.9 ± 3.1	451 ± 58
Poly(HOSBM)	100 ± 30	51 ± 5	8.09 ± 0.93	16.8 ± 2.3	423 ± 64
Poly(CBM)	50 ± 25	52 ± 4	5.33 ± 1.76	29.6 ± 8.9	371 ± 43
Poly(CBM)	100 ± 34	53 ± 2	8.04 ± 0.54	13.7 ± 6.7	364 ± 22
Poly(CMM)	48 ± 21	54 ± 3	7.49 ± 0.61	24.5 ± 5.3	423 ± 84
Poly(CMM)	100 ± 28	54 ± 2	5.90 ± 2.36	11.7 ± 5.6	418 ± 60

The effect of particle size can be explained by viewing scanning electron microscopy (SEM) images of the proteoposite films. When the particle size of the latex is smaller, the film morphology is more homogeneous due to more uniform distribution of particles throughout the proteoposite film. A more uniform distribution allows for better interaction between the proteins and the latex. As protein-latex interaction enhances, the synergy between the two materials advances. The SEM images (Figure 3.4) clearly illustrate how decrease in particle size enhances latex distribution throughout the soy-Zein proteoposite.

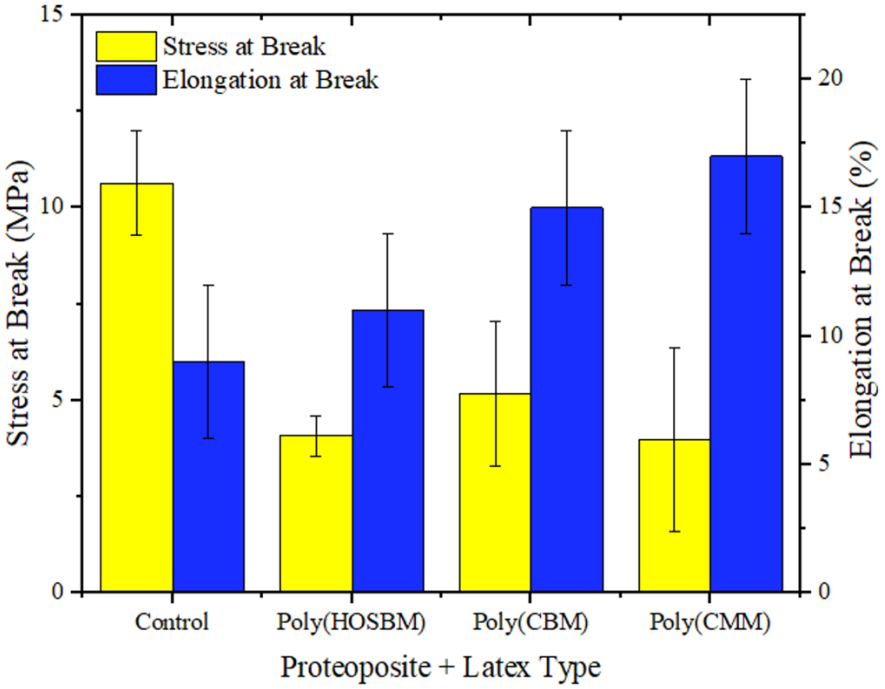

Figure 3.2. Effect of POBM unsaturation on mechanical properties of soy-Zein proteoposites

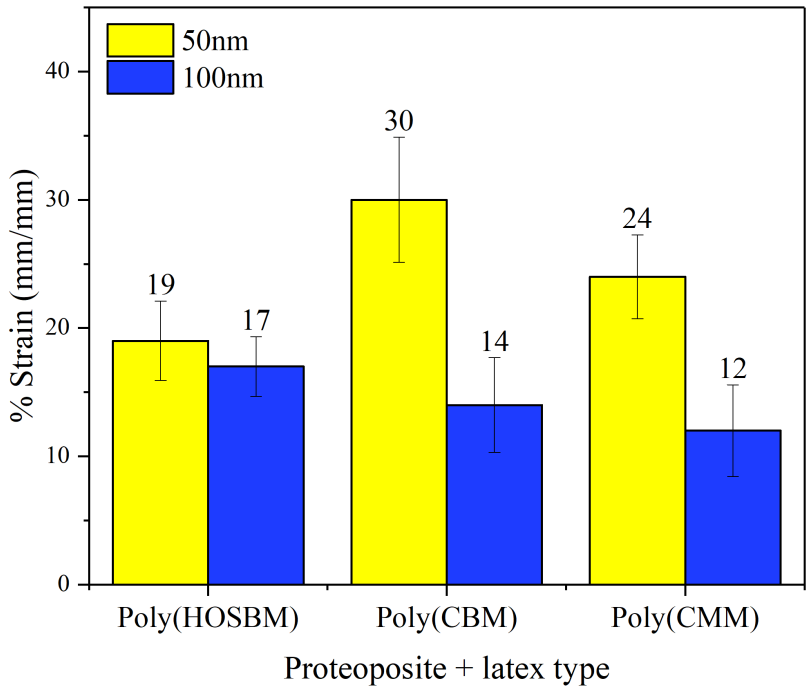

Figure 3.3. Effect of particle size of latex on elongation at break of soy-Zein proteoposites

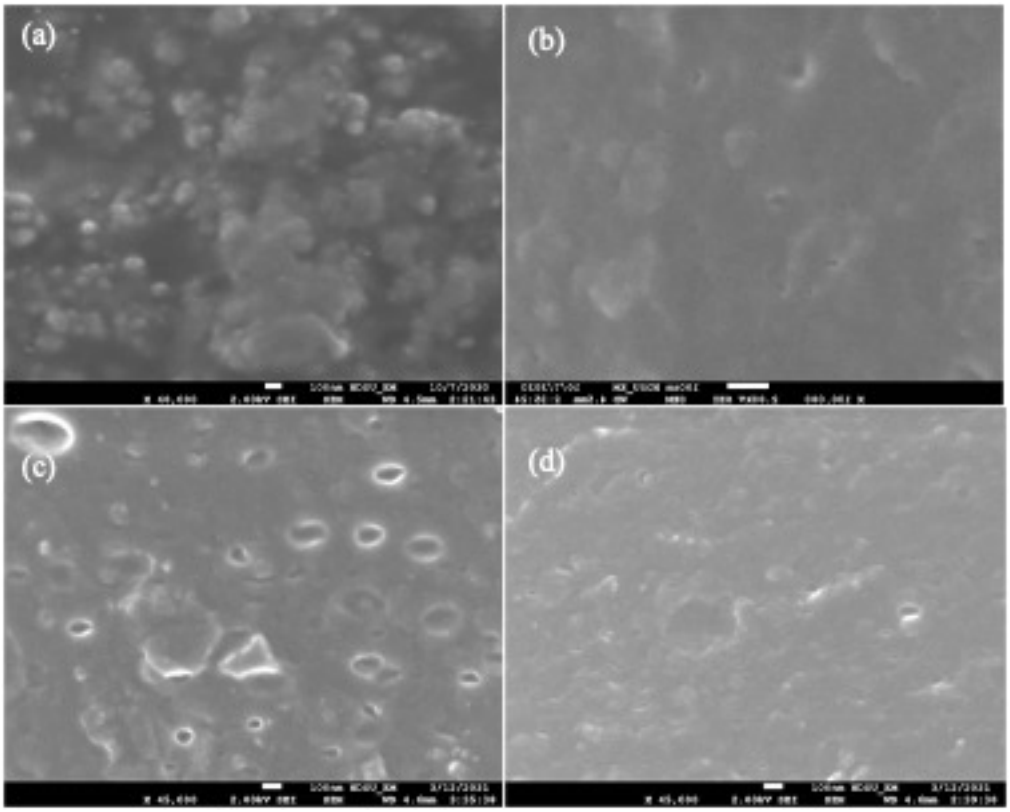

Figure 3.4. SEM images of proteoposite modified with (a,b) poly(HOSBM) and (c,d) poly(CMM) latexes with particle size 100 ± 35nm (a,c) and 50 ± 25nm (b,d)

After optimizing the proteoposite films experimentally, computational machine-learning methods were applied to develop a structure-property relationship between the components of the bioplastics and the resulting mechanical properties. The overall scheme of this study is represented in Figure 5, where the experimental work and QSAR modeling make up Step 1, Cheminformatics is Step 2, and prediction of desired properties is Step 3.[39] These steps are a regular procedure for finding the structural factors responsible for the mechanism of activity, as well as for the rational design of materials.[49-57]

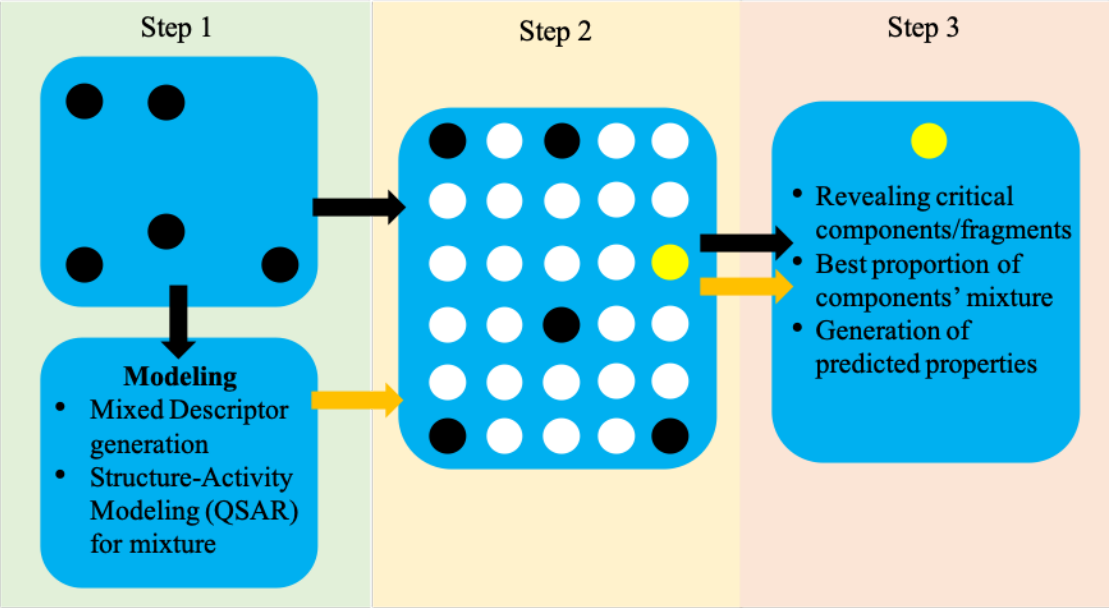

Figure 3.5. Schematic of the QSAR modeling task flow with mixed descriptors process[39]

After all initial steps on data collection and generation were complete, the QSAR modeling was applied, and a set of predictive structure-property models was built for different endpoints based on 23 soy-Zein proteoposite films. The best model which predicts the tensile stress (TS) of a proteoposite is represented below (Eq 1).

$$TS = 143.87 - 0.25(\pm 0.27)particle\ size - 976.67(\pm 112.95)npr2 \quad (1)$$

$$(n = 23, r^2_{train} = 0.84, q^2_{LOO} = 0.78, RMSE_{tr} = 0.96, F = 40.75)$$

where the structural descriptor npr2 is based on graph theory (edges and vertices) and represents the normalized ratio of principal moment of inertia about the second principal axis of rotation (PMI2) and the principal moment of inertia about a third 3 orthogonal axis of rotation (PMI3) and the second descriptor, particle size is an indicator descriptor that was included to differentiate between films prepared with latexes of varying particle size. For example, in case of particle size descriptor, samples with no latex were given the particle size value of 0, latexes of particle size 50nm were denoted as -1, and latexes of particle size 100 nm were noted as 1. The experimental results confirm a clear relationship between particle size and bioplastic film mechanics that the

QSAR model (Eq7) reveals mathematically. As per npr2 descriptor – the principal moments of inertia are eigenvalues of the mass moment of inertia eigen vector, relative to some point, which is typically the center of mass. As there are three principal axes of rotation, in three dimensions, there are always three principal moments of inertia.[58] Both PMI2 and PMI3 depend largely on the mass and surface area of each component, therefore the ratio of PMI2/PMI3 is likewise dependent on the mass and surface area of each component in bioplastic film. As seen in Eq 1, the tensile stress of soy-Zein composite films depends on the normalized ratio of PMI2/PMI3 and the particle size of the latex incorporated in the film. As it can be seen from the Eq 1, the increasing value of npr2 in bioplastic film decreases the tensile stress and vice versa.

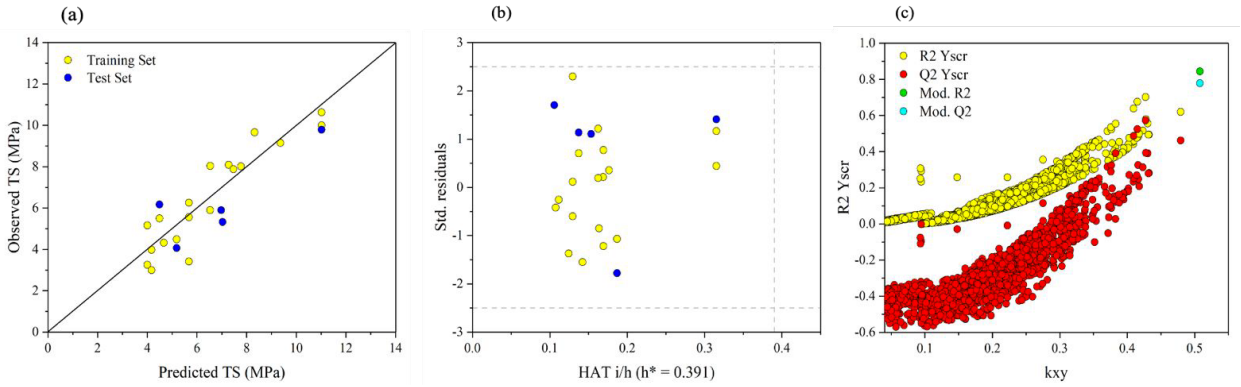

Figure 3.6. Plots for Tensile Stress model: (a) predicted vs observed correlation, (b) Williams plot, where yellow dots are the training set values and blue dots are the test set values, and (c) y-scrambling results, where blue and green dots are r^2 and q^2 of the original model, all other dots (red and yellow) are r^2 and q^2 values for simulated exp data

Figure 3.6a shows the correlation between predicted and experimental outcomes, where shown the predictive ability of the model by representing the distance of each data point (proteoposite film) from the diagonal correlation line. The Williams plot (Figure 3.6c) demonstrates the applicability domain of the model as well as the capability of the model to successfully predict the values for a similar set of bioplastic films. Finally, the y-scrambling plot (Figure 3.6b) is a validation technique which demonstrates the uniqueness of the developed model, i.e.; a good model has higher R2 (Q2) values and better separation from all other models based on

simulated data (i.e. shuffled endpoints data). The selected model demonstrates strong predictive ability (Figure 3.6a) where all test set values are well predicted and fall within the applicability domain (Figure 3.6c).

Although the obtained QSAR model illustrates strong predictive ability and good correlation (R2=0.84, Q2=0.72), it was necessary to validate the model against new experimental findings, to confirm the robustness and predictive ability of the model. In this regard, as a first step, using Eq 1, the mechanical properties for four new films were predicted. Table 3.5 shows the predicted films, their descriptor values and predicted tensile stress (MPa).

Table 3.5. Prediction of film mechanics based on QSAR model

Sample	npr2	Particle Size	Predicted TS (MPa)	Observed TS (MPa)
1	0.1377	-1	9.6164	9.8 ± 0.5
2	0.1381	-1	9.2678	9.0 ± 0.7
3	0.1380	-1	9.3188	8.4 ± 0.2
4	0.1424	-1	5.0535	4.7 ± 0.7

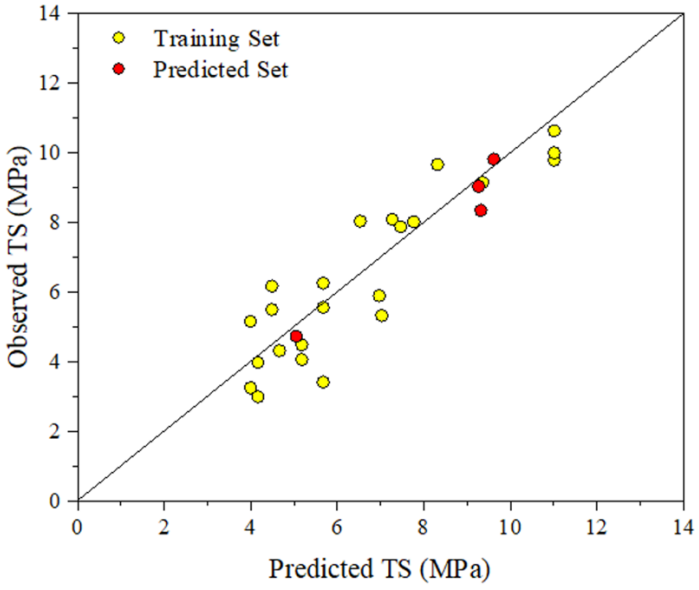

Figure 3.7. Correlation plot of QSAR model with initial dataset of 23 films and newly prepared soy-Zein proteoposites

The films were then prepared, and mechanical properties measured experimentally. As it can be seen, all bioplastic films were prepared with latexes of particle size 50nm (denoted in Table 3.5 as -1). The resulting experimental values were compared with those that the model predicted and resulting points were placed in a new correlation plot (Figure 3.7) to demonstrate the predicted/observed ratio and the success level of the model. Figure 7 illustrates the predicted values vs observed ones for the initial set of 23 bioplastic films (in yellow), as well as for newly synthesized ones (four newly prepared films, in red). It is clear that the predicted values are very close to the observed ones and therefore developed QSAR model was able to successfully predict values for the new films.

Upon validation of the QSAR model, the optimized films were tested for barrier properties in order to determine their efficacy as food packaging alternatives. By measuring the water vapor transmission of the soy-Zein proteoposite films we determine the resistance of the films toward water. Since condensation and water penetration is a common challenge in food packaging, the protein-based bioplastics should demonstrate improved resistance against water. We measured WVT using ASTME96, which is a standard method for plastic materials. Measured values of the control soy and Zein protein-based monolayers are comparable to those found in literature.[59,60] As seen in Table 3.6, the soy-Zein proteoposite with POBM-latexes shows significantly improved barrier properties, as compared to the soy and Zein control films. This decrease can be contributed both, to the increased thickness of the bioplastic films as compared to the monolayers, as well as the increased hydrophobicity which comes from the POBM-latexes.

Table 3.6. WVT values of plant protein-based bioplastic films

Sample	WVP (ng•m/m^2•s•Pa)
Zein Control	0.214
Soy Control	0.194
Soy-Zein Proteoposite Control	0.056
Proteoposite + poly(HOSBM)	0.066
Proteoposite + poly(CBM)	0.062
Proteoposite + poly(CMM)	0.076

Effective synergy between soy protein, Zein, and the POBM-latexes results in very good properties in modified soy-Zein proteoposite bioplastic films with improved barrier resistance. Additionally, as water vapor permeability accounts for the difference in film thickness, it is again confirmed that non-covalent interactions between soy and Zein proteins are responsible for the synergistic effects which improve water resistivity. In addition, the developed machine learning-based QSAR model was able to successfully predict the tensile stress for the new films.

3.6. Conclusions

In this work, the synergistic effects of soy protein, Zein, and POBM-latexes were investigated in bioplastic films and an adequate machine learning-based QSAR model developed which accurately predicts mechanical properties (tensile stress) of such proteoposite materials.

To our knowledge, this is the first investigation which modifies plant protein-based films with POBM-latexes and the first attempt to prepare predictive QSAR models of any bioplastic film and use it for predicting the films with improved properties. The efficacy of soy-Zein proteoposites was first demonstrated by comparing the properties and performance against soy and Zein protein monolayers. Furthermore, incorporation of POBM-latexes demonstrated improved mechanical and barrier properties of the protein-based films. Using the validated QSAR model, optimized soy-Zein bioplastic films were theoretically predicted and then experimentally prepared, which showed

enhanced mechanical performance and significantly improved barrier behavior. There is a notable synergy between ingredients in soy-Zein proteoposites modified with POBM-latexes making them promising and sustainable alternatives for food-packaging applications. In this way, the combined experimental and computational approach showed great results in current study and paves a way to effective rational design of new bioplastic materials.

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CHAPTER 4. CONCLUSIONS AND FUTURE WORK

4.1. Computational Conclusions

The goal of this research was to determine the feasibility of (a) preparing packaging materials (films) from plant-derived proteins combined with plant oil-based polymers, and (b) using computational methods in order to predict experimental outcomes. Throughout the course of this thesis, each chapter focused on a combined computational and experimental approach. Indeed, our findings were novel in both areas of study. In Chapter 2, it was first determined that protein-ligand docking, a computational technique, could be utilized in order to predict properties and performance of bioplastic films. This is the first time that such an application for protein-ligand docking had been identified. By comparing the predicted trends to experimental measurements, it was determined that protein-ligand docking, through the binding affinity, accurately predicts the effect various natural modifiers will have on the properties and performance of plant protein-based films.

In an effort to further the applicability of different computational methods, we moved into Chapter 3 where the use of QSAR modeling allowed us to build a predictive model for complex proteoposite (made from two different plant proteins) films. Indeed, this was the first time such complex, multilayered, films systems had been experimentally prepared and successfully modeled using machine-learning methods. Once the model was prepared, 4 additional proteoposites were prepared with various new additive compositions. The measured results were compared to the predicted values and found to match with >80% predictability. It was therefore determined that the novel, “mixed-descriptor” approach for complex proteoposite films is an effective approach to model and predict properties and performance of such bioplastic materials.

4.2. Experimental Conclusions

In addition to the computational findings of our work, novel experimental conclusions can be drawn as well. In Chapter 3 we explore the feasibility of compatibilizing two plant proteins with plant oil-based latexes in order to prepare novel polymeric materials, proteoposites. Previous studies have determined the ability to form bioplastic films from soy protein or Zein, however each of these systems fall short in mechanical or barrier properties. In our findings, by compatibilizing the two proteins, we are able to achieve bioplastic films with enhanced mechanical properties, and improved barrier performance.

Properties and performance were further enhanced by the incorporation of biobased latexes synthesized in this work from plant oil-based acrylic monomers using miniemulsion polymerization process. We were able to determine the configurational effect of entanglements based on the degree of unsaturation in the fatty acid fragments of plant oil-based macromolecules. In the latex polymers with a higher degree of unsaturation, increased elongation is noted in all proteoposite films due to the greater number of entanglements ("physical hooks") in the macromolecular backbone which are able to deform (stretch) and thus contribute to greater flexibility of the proteoposite material. In addition to the effect of latex polymer configuration, the effect of latex particle size was determined. Indeed, from SEM imaging it was determined that smaller particle size latexes were more homogenously dispersed throughout the proteoposite film which contributed to more intermolecular interactions between the proteins and the latexes resulting in enhanced mechanical and barrier properties.

4.3. Future Work

In Chapter 2, it was determined that protein-ligand docking can be used in order to qualitatively predict the properties and performance of plant protein-based bioplastic films. While

we were able to draw strong correlations between the binding affinity values and the resulting experimental properties, there is room to further improve our understanding in how protein-ligand binding affects film formation. Since the protein-ligand docking considers the system as static and in a regular setup, does not include any medium or vary specific parameters such as temperature, pressure, or pH; the complete thermodynamics of a laboratory experiment are not mimicked in this computational approach. While this difference in "set-up" did not inhibit the ability to utilize protein-ligand docking, it does raise the question of how thermodynamics of the system are contributing to the bioplastic film performance. For this reason, it would be of interest to study under molecular dynamics, a true experimental set-up, where the medium (i.e. water or ethanol) is considered along with parameters of temperature, pressure, and pH.

Likewise, in the protein-ligand docking the protein is considered to remain in its native state. While it is believed that the proteins do not undergo denaturation during the film formation process, it would be of interest to confirm the changes in protein structure. In order to study the protein conformation after film formation, it would be necessary to extract the protein from all other film components and analyze via circular dichroism spectroscopy. In this way, it would be possible to confirm the retained native structure of the plant proteins.

In Chapter 3, the incorporation of unsaturated POBM-latexes results in enhanced flexibility of the proteoposite. Since each POBM-latex maintains a certain level of unsaturation, it is possible for autoxidation to occur. An optimization study on the drying conditions of the latex-modified proteoposite would lead to better understanding of what degree of crosslinking can be achieved, the effect this crosslinking will have on mechanical and barrier properties, and how crosslinking may affect film appearance. Since crosslinked materials typically demonstrate improved barrier performance, this would be of great interest in our proteoposite systems. By increasing crosslink

density of these bioplastic films, we may improve their water and gas resistance and therefore better equip these proteoposites for applications in food packaging. Thus, it would be of interest to perform a crosslinking optimization study on latex-modified proteoposite films.

Additionally, while incorporation of plant oil-based latexes into the proteoposite films aided in the compatibility between the soy and Zein proteins, a limiting factor to this approach is the compatibility between the latex and each protein. Since the benefits of plant oil-based latexes are clearly noted in enhanced mechanical properties, it would be of interest to enhance the protein-latex compatibility. One approach is to graft POBM-latex to protein isolate thus better compatibilizing the two materials and by creating 1component responsible for increased mechanical and barrier properties. This approach would result in proteins with long hydrophobic chains attached by covalent bonds to the protein corona. These hydrophobic chains may contribute to better flexibility, higher moisture resistance, and increased strength. In this way, a single component system is achieved which still allows for synergistic behavior of the plant proteins and POBM-latexes.