

ISOLATION AND EVALUATION OF SAPONIN EXTRACT FROM SOYMEAL AS A
NATURALLY OCCURRING COUPLING AGENT FOR FIBER-BASED BIOCOMPOSITES
FOR FOOD PACKAGING

By

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ABSTRACT

Soymeal is the prominent byproduct of soybean oil extraction and is mainly used as a high-protein source in animal feed. Saponins are also components of this soymeal. They have been associated with various health issues for livestock and are noted to negatively impact the taste and appeal to eat. The safe removal of saponins from soymeal can provide a sustainable source for saponins, and simultaneously provide a more desirable meal for livestock. The aim of this study was to investigate the feasibility of a saponin extract from soymeal as a naturally occurring coupling agent to improve the properties of fiber-based composites for food packaging. The first part of this study focused on determining the invasiveness of the saponin extraction on the meal. The isolation of saponins was proven via HPLC-MS, and a proximate composition analysis of soymeal post-extraction confirmed a nutritional content that was consistent with the initial meal. Second, this study focused on investigating the effectiveness of the saponin extract as a coupling agent. This effectiveness was shown for polylactic acid-soyhull composites based on an improvement of mechanical properties. The treatment of the filler with sulfuric acid prior to its inclusion into the polymer matrix improved the water vapor permeability, UV barrier, and thermal stability of the composites. With both treatments (saponin extract and acidification), mechanical, barrier, and thermal properties of composites were improved. This study shows that the saponin extract from soymeal has promise for potential as a coupling agent.

This thesis is dedicated to my parents, brother, and grandparents.

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CHAPTER 1

INTRODUCTION

1.1 Introduction

The need and use for plastics in food packaging has increased over 400% over the last 50 years [1]. The primary plastics used in this food packaging take upwards of 400 years to degrade biologically [2]. This poor biodegradability is cause for ecological and humanitarian concern, ranging from toxic garbage patches covering the oceans to microplastics contaminating humans' bloodstream [3].

Although a reduction or overall elimination of plastic use seems like a simple solution, plastics in food packaging offer light weights, desirable aesthetics, cheap and low processability, and provide more than capable strength and barrier properties for protection and extension of food shelf life [4 - 6]. Beyond this, the high energy requirements for alternative or reusable packaging formats often outweigh the environmental impact they may be thought to provide in the long term [7, 8]. Eliminating plastics entirely from food packaging is not currently a feasible solution to the problem and therefore, the investigation of adjusting them, or substituting for more eco-friendly alternatives has become paramount.

One of the most promising materials for the future of food packaging has been polylactic acid (PLA). PLA is a biodegradable, industrially compostable plastic derived from polymerized fermented lactic acid from crops including corn, potatoes, and sugar. It shows potential for the future of packaging materials due to its abundant and sustainable source, good strength, and ease of processability. However, it typically shows low thermal stability, minimal toughness, and modest moisture barrier properties [9]. While it can be industrially composted in days, neat PLA can still take upwards of 80 years to decompose in nature [10].

Fillers are materials that are inserted into a polymer matrix as a direct partial substitute for raw plastic [11]. They are often incorporated to reduce raw plastic use and thus cost of manufacturing, while ideally retaining performance comparative to the neat polymer. Some fillers have even been found to induce antimicrobial [12, 13], antioxidant [13], and antifungal [14] properties, improving the capacity to prevent product spoilage as a food packaging material.

Agricultural waste is defined as the unwanted or unusable materials produced from agricultural operations immediately relating to the growing of crops or the raising of animals [15]. Agricultural waste has been a popular filler source due to its low cost and chemical composition, making it more desirable for industrially produced composites [16]. Agriculturally sourced fillers have been consistently found to improve biodegradation rate [17 - 22] and have occasionally resulted in similar or improved barrier properties to water [23] and oxygen [24]. The drawback in using agricultural waste-based fillers has consistently been a reduction in tensile properties, mainly toughness and elongation [25, 26]. Typically, these decreases in mechanical properties are attributed to the reactive groups on the filler surface not interacting with the polymer main chain, causing gaps and overall disruption in the uniformity and compatibility of the matrix, thus decreasing the overall strength [27, 28].

Treatments to both the waste and the polymer have been investigated in attempt to combat these deteriorative properties. The most common treatments include the acidification or alkalization of the waste, and/or the incorporation of a coupling agent [29]. Acidification and alkalization treatments involve exposing the filler to highly acidic or basic environments, respectively, with the idea of removing impurities, changing the morphological structure of the particles, and/or otherwise promoting better adhesion into a polymer chain [30]. Various studies have concluded in the improvement in properties of organic waste composites with PLA as a result of these filler treatments [30, 31].

Coupling agents are compounds that form chemical bonds between dissimilar molecules, working to provide better adhesion and interfacial bonding between a polymer matrix and non-polymer components [32]. The typical structural requirements for coupling agents involve having two types of functional groups, one able to copolymerize with the desired polymer, and one able to strongly adhere to the foreign material used [33].

The extent and potential for coupling agents in the future of packaging is noteworthy, particularly in the future of filler-based plastics. Perhaps the main restriction in the use of coupling agents in this field is the increased cost to incorporate, their chemical origin, and the effect they can have on the biodegradability of the polymer. In the area of filler-based composites, common commercially available coupling agents such as methylene diphenyl diisocyanate (MDI), toluene diisocyanate (TDI), silane, and maleic anhydride have been known

to successfully improve mechanical properties but end up notably decreasing the biodegradability [34 - 36]. There have been investigations of naturally derived coupling agents with moderate mechanical improvements and non-disruption of biodegradability, including pine rosin, palm and sesame oils, and fatty acids including hexanoic, octanoic, decanoic, and dodecanoic acids [37, 38]. However, these are extracted invasively and unsustainably, show minute improvements, and have little potential to be applied on a higher production scale.

Saponins are triterpene glycosides naturally found in many plants, having a particularly noteworthy presence in soapwort, licorice, and soy [39, 40]. Saponins are long molecules, characterized by both largely hydrophilic and hydrophobic ends, giving them excellent surfactant and emulsification capabilities [41]. Isolated saponins have been heavily investigated in pharmaceutical and fields, and have been cited to contain anti-inflammatory, anti-cancer, antimicrobial, antioxidant, and cholesterol-inhibiting capabilities [42-46]. Based on the structural requirements above for viable coupling agents, saponins can be considered with great potential for this application as well. Saponins from soap berry have been investigated for potential as a coupling agent in the use of polypropylene composites incorporated with nanocellulose with some success [47]. Although all varieties of saponin differ based on their genus of origin, saponins derived from soy and soy related products, deemed “soyasaponins”, are believed to have equal promise for these types of applications.

Soybeans are one of the most grown crops worldwide, with global production reaching more than 370 million tons in 2021 [48]. It is estimated that more than 85% of this yield is used in the extraction of soybean oil, which is widely used for biofuel and cooking oil [49]. During this extraction process, the raw beans are subject to repeated exposure to heat and hexane immersion, removing lipids and leaving behind byproducts such as “defatted soymeal”, and “soyhulls”. In the United States, 97% of soymeal is sold directly to farmers or feed supply stores for use as a high protein animal feed for cattle, poultry, fish, and swine, while the remaining 3% is used for derived protein concentrate or soymilk [50]. Soyhulls are typically either added to the meal for their fibrous content or are disposed of. The increase in soybean oil extraction facilities has resulted in a large influx of both these byproducts, requiring additional uses for them [51].

Soymeal is considered a nutritious and growth inducing formula for these livestock due to its high digestible protein and carbohydrate content, promoting health, energy, growth, and

productivity. Although it is widely ingrained into farm animal's diets, there have been numerous findings that some aspects of it may induce adverse health effects. For instance, saponins and isoflavones in the meal have been known to cause ruptures in intestinal tracts, decreased nutrient digestion and appetite, and decreased embryonic health and development in bovine mammals and swine [52 - 56]. Saponins in feed have also been heavily attributed to inducing fatal enteritis and severely reduced nutrient digestion in fish. [57, 58]. Furthermore, the bitter flavors these compounds induce have been shown to reduce many of the animal's desire to consume the meal at all [59 - 61]. Based on these findings, a soymeal void of these compounds would provide all the positive aspects regarding its nutritional proficiency as a food source, without the negative health effects often involved. A safe and minimally invasive extraction of these compounds would result in a saponin isolate removed from the meal, and an overall healthier food product. It would also provide merit for a bountiful, sustainable, and easily accessible source for these compounds for use beyond packaging.

This thesis aims to establish a sustainable and naturally sourced coupling agent with a minimally invasive procedure that can even improve the raw material it was extracted from. It investigates the effectiveness of the proposed compound on soyhull-PLA composites. Additionally, it assesses the effect of acidification of the filler on the performance of the proposed coupling agent. This study provides a baseline for improving the properties of green plastic composites without disrupting the "green" aspect of them.

1.2 Objectives

The purpose of this study is first, to demonstrate that saponins can be safely extracted from defatted soymeal without damaging the physical or nutritional integrity of it, allowing for its continuation to be sold as an improved, high-protein food source for animals.

The second aim of this study is to determine the efficacy of saponin extract to be used as a naturally occurring coupling agent for PLA and an agricultural waste filler of crushed soybean hulls.

Finally, it is to determine the effect of acid-treatment of the soyhull filler on the resultant composite properties and the performance of the saponin coupling agent.

1.3 Hypotheses

1. Saponins can be safely extracted from defatted soymeal without damaging or otherwise interrupting the physical and nutritional integrity of the meal, leaving it fit for consumption.
2. Saponins can function as a naturally occurring coupling agent for a PLA-soybean hull composite to improve the overall mechanical properties of the composite.
3. The use of sulfuric acid (H_2SO_4) during treatment of the crushed soybean hull filler with the saponin coupling agent will improve the extent to which the saponins interact with the filler particles.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to PLA

Poly lactic acid or PLA is a promising material currently at the forefront of alternative and sustainable polymers in place of traditional polyolefins [1, 2]. Low molecular weight PLA is synthesized by either chemical synthesis or bacterial fermentation of lactic acid from sugars of crops such as corn, cassava, sugarcane, sugarbeet, and maize [1, 2].

The polymerization of higher molecular weight PLA is seen to be done in one of three separate processes: direct condensation polymerization, direct polycondensation in an azeotropic medium, or lactide formation polymerization. The latter is by far the most seen on the industrial scale. Lactide formation is achieved through the removal of water under mild conditions without solvent. Generally, L-lactide, a product of the L-lactic acid, is used in the mass production of PLA, thus the alternative names Poly (L-lactide) or Poly Lactide. [1]. PLA can be produced in a wide array of structures, ranging from fully amorphous to highly crystalline.

PLA is observed as a highly prominent plastic for the future of plastic manufacturing due to its sustainable and widely available source, ease of processing, and effective biodegradation. However, it has multiple characteristic contingencies restricting its potential for use, including low flexibility, thermal resistance, and gas barrier properties [3, 4].

2.2 Plastic Additives

With the intention of improving performance, lowering the cost of production, reducing energy use, or promoting higher biodegradability, the use of additives is often employed to polymer matrices. These include additives such as plasticizers, fillers, compatibilizers, and stabilizers [5].

2.2.1 Plasticizers

Plasticizers are often incorporated into polymer matrices for improved flexibility and malleability, traits that are sought after in the production of plastic materials. These are typically low molecular weight compounds that are compatible with the plastic resin of use [6].

2.2.2 Stabilizers

Stabilizers are compounds introduced into polymer matrices with the desired intent of increasing the plastic's resistance to certain degradation mechanisms both during processing and use. These envelop a variety of functional stabilizers, including heat stabilizers [7], light stabilizers [8], and oxidation stabilizers [9]. These additives come in a variety of forms and are most often employed in mechanical or civil engineering applications where materials may be subject to excessive conditions.

2.2.3 Plastic Fillers

Fillers are additive compounds typically in the micro or nano size that are incorporated with a polymer as a means to promote a large variety of performance enhancements, or act as a substitute for raw plastic to reduce cost [10]. Fillers can come in a near infinite range of forms and sources and can be responsible for improving properties such as thermal and UV light resistance, biodegradation, antimicrobial capability, antioxidant capacity, barrier properties, and mechanical properties, depending on the type of filler used [10]. Based on their nature of origin, fillers can generally be classified into two categories: organic and inorganic.

2.2.3.1 Organic Fillers

Organic fillers are particles or fibers incorporated into polymer matrices that have been derived from living, or what was once living matter [11]. Most organic fillers seen in recent literature have been derived from plant material, including wood and other fibrous green matter [12], and occasionally from animal material, including shells from eggs or even crustaceans [13].

2.2.3.1.1 Organic Fillers from Agricultural Waste

Byproducts from agricultural materials and processes, deemed “agricultural waste”, are the main source of organic fillers [10]. Examples of waste used as organic fillers include corn stalk [14], fruit peels [15] [16], rice husks [17], coffee grounds [18], wheat straw [19], nutshells [17], and eggshells from chickens [13]. The reasons these are common sources for fillers are abundance, and exceptionally low costs. A low-cost partial replacement in plastic without the degradation of properties can be an important economic incentive for the eventual adaptation of this technology into industry.

2.2.3.1.2 Properties of Composites from Agricultural Waste

Plastics incorporated with fillers derived from agricultural waste have consistently resulted in more brittle properties in comparison to neat plastics. This is resembled by improvements in stiffness, with reductions in percent elongation, flexural strength, and tensile strength [10, 11]. These fillers have also been acknowledged to reduce the barrier properties of the resultant composites to both water [14, 17], and to a lesser extent, oxygen [17, 20]. Fillers from agricultural sources have been attributed to decreases in thermal stability as well [17, 21]. These property reductions are generally attributed to the hydrophilic surface nature of a majority of these fillers, resulting in poor interfacial adhesion, and gaps in the hydrophobic polymer matrix [22]. While the poor inclusion into the polymer matrix for these fillers results in poorer mechanical and barrier properties, it often provides easier access for biodegradation mechanisms to decompose the material at end of life, resulting in quicker biodegradation in natural settings [23, 24].

2.2.3.1.3 Applications

The increases in stiffness and decreases in thermal stability for composites involving agriculturally sourced fillers leave them only considered for non-packaging and insignificant applications in automotive, aircraft, leisure, and electronic industries [25]. These fillers are typically able to be incorporated at higher loadings than others due to their softer and nonabrasive nature [25, 26]. This allows for a higher substitution rate, reducing the raw plastic needed for composite production. The earlier deteriorations, along with low barrier properties associated with fillers from organic nature leaves very little application in food packaging formats for them. The majority of the investigation for its potential as a food packaging material has remained in academia.

2.2.3.2 Inorganic Fillers

Inorganic fillers are particles formed or derived from materials non-associated with living matter. The majority of these fillers are derived from minerals found in nature and finely ground. the most common inorganic fillers have been calcium carbonate (CaCO_3) [27, 28], talc powders [29], and nano clays [30]. Inorganic fillers have been associated with inducing a number of effects on plastic properties, and occasionally providing new ones. Similar to organic fillers, the

use of inorganic fillers also typically induces greater stiffness, along with reductions in percent elongation, and flexural and tensile strength [28, 30-32]. This is also largely attributed to the surface chemistry of the fillers being incompatible with that of the polymer. Some inorganic fillers have however been cited to improve the thermal resistance of the resultant composite [31-32]. There are cases where inorganic fillers have been observed to have minimal effect, or even improve the barrier properties of films as well [31-32] [35]. Some less common, or chemically produced inorganic fillers have been cited to induce plastics equipped with antimicrobial activity [33-34]. Inorganic fillers have also been observed to improve the biodegradation rate of the composite [35-36]. Due to the improved properties associated with inorganic fillers in relation to organic fillers, these types of additives are seen more often in industrial and packaging settings. The abundance in nature, and relatively cheap cost results in a similar cost-based incentive for mass production and incorporation that could be seen in organic filler polymers [27, 37].

2.2.3.3 Pre-Treatment of Fillers

Due to the excessive deteriorative properties of polymers when incorporated with fillers of both organic and inorganic nature, surface treatments to the filler are often applied to promote a better relationship between them and the plastic [38]. The most common treatment for reducing the hydrophilicity of all filler particles is the removal of as much water from them as possible. These drying mechanisms are typically done via hot drying [17, 20, 39], lyophilizing [40, 41], or spray drying [40, 42]. All three have been noted to improve dispersity and porosity in comparison to non-dried fillers [40, 41]. Beyond the removal of water, various chemical treatments work to alter the filler particles themselves.

2.2.3.3.1 Surface Treatment of Organic Fillers

There are a variety of chemical treatments for organic fillers prior to their inclusion into a polymer matrix as a mechanism of improving their compatibility with the plastic.

Alkalinization is employed to reduce organic matter into more basic components, it is a common technique for the isolation of cellulose from other constituents such as lignin and hemicellulose in many agricultural sourced fillers [43-45]. The most common chemicals used in the alkalinization of filler particles are NaClO, [46] and NaOH [47]. Alkalinization has been

proven to improve the acceptance of filler particles into various polymer matrices, resulting in higher tensile and barrier properties [45, 48].

Acidification is also often employed to isolate or deteriorate cellulose structures from other contents in filler materials depending on the acid used [49]. The most common acids used in the pretreatment acidification process for fillers include HCl [50], H₂SO₄ [50], oxalic acid [51], formic acid [52], or a combination of them [51, 52]. Acidification has also been seen to improve the mechanical properties of organic filler-based composites, often increasing tensile strength and stiffness of resultant composites [53, 54].

Regardless of which treatment is used, the isolation or degradation of cellulose from the other components of many organic fillers allows for a more hydrophobic surface chemistry. The improvements in tensile properties associated with these treated fillers is largely attributed to this, cited to improve dispersion and compatibility with the polymer.

2.2.3.3.2 Surface Treatments of Inorganic Fillers

The most common surface modifiers for fillers such as CaCO₃, talc, and nano clays involve the replacement of the hydrophilic reactive groups with hydrophobic ones. This can be achieved in a number of ways or a combination of them. In industry and academia, the most common methods for this involve high intensity thermal treatments such as gamma irradiation [55], stearic acid coatings, [56-58] and phosphate ester treatments [59].

The treatment with stearic acid phosphate esters, and irradiation, have all been proven to show improvements in tensile strength and % elongation [55, 57-59]. These improvements are attributed to the altering of surface chemistry, resulting in better dispersion and compatibility of fillers within the matrix [58, 59].

2.2.4 Coupling Agents

Coupling agents envelop a wide range of compounds that work to bind dissimilar materials together in unconventional environments [38]. They have been incorporated in polymer science in the form of combining multilayer plastic films and sheets, as well as in the inclusion of other additives [60]. They work to provide a strong linkage between the polar and nonpolar substituents and thus vastly improve mechanical and barrier properties. They have been widely

used in filler-based composite resins for both organic and inorganic fillers as a means of improving resultant mechanical properties.

2.2.4.1 Chemical (Commercial) Coupling Agents

Some of the most common coupling agents used in polymer-additive relationships are silane [61], maleic anhydride [62], methylene diphenyl diisocyanate (MDI) [63] or derivatives of these [64]. Some coupling agents, such as maleic anhydride are grafted onto the polymer before filler loading [65], others such as MDI are often included with the filler simultaneously [63], where others are incorporated as treatments for the filler prior to inclusion with the polymer [66]. Regardless of the mechanism, most commercial coupling agents are revered for their notable ability to improve the mechanical properties of plastics involving fillers from all sources [61-66].

Commercial coupling agents are used in industry and are attributed to be crucial in the establishment of many specialized plastics and films today [38, 67]. The main hindrances for more extensive use of commercial coupling agents involve the price of incorporation due to the complicated mechanisms of creation, and the negative effects they have on the biodegradation of the plastics, often extending the time it takes for them to degrade naturally [61, 62]. This is largely attributed to the chemical nature of these compounds, and the complex interactions they elicit in materials affected by them, increasing the difficulty to reach groups necessary to decompose [61, 62].

2.2.4.2 Naturally Derived Coupling Agents

There have been scarce studies investigating the potential for producing and characterizing a naturally occurring coupling agent. Some existing examples of such natural coupling agents include derivations from pine rosin [67], and palm [68] and sesame [69] oils. There have also been investigations into various fatty acids, including hexanoic, octanoic, decanoic, and dodecanoic acids [70]. However, these studies have shown minimal mechanical improvements, and the complex processes required limit the potential for adoption and application at higher production scales.

2.3 Soy & Soybean Meal

Soybeans are one of the most grown crops in the world. Global production of soy reached more than 370 million tons in 2021 [71]. It is estimated that over 85% of this production is for the extraction of oil from the soybean [72]. The oil extracted is most often used for cooking oil production, or as biofuel [73, 74]. During this oil extraction process, soybeans are subject to hull cracking, removal, and separation under high heat and pressure. The exposed beans are then exposed to repeated hexane baths where a majority of the lipid contents are removed and isolated from the solid matter. The solid soybean waste that is left over is then dried, washed and “roasted” to produce a protein and carbohydrate rich soybean derivative deemed “soymeal”. In the United States, over 97% of this soymeal is sold to feed livestock, mainly cattle, swine, poultry, and fish, with the remainder used for protein isolate or soymilk [75]. This byproduct of oil extraction has a number of significant compounds within it that can be extremely beneficial outside animal feed. The proper extraction and utilization of these compounds can work to improve their investigation in science and provide increased profitability for both soybean farmers and refiners.

2.3.1 Soyasaponins

Saponins are characterized as triterpene glycosides naturally occurring in many plant species, particularly soapwort, licorice, and soy [76, 77]. Soyasaponins are the saponin types occurring naturally in soy. Soymeal has been found to contain anywhere from 0.5-1.5 wt.% saponin [78, 79]. There are over 10 different types of soyasaponin, with Soyasaponin I (Bb) being the most prevalent [80, 81]. Soyasaponins are chemically identified by the glycosylation sites on the backbone and characteristic surfactant properties. The basic components of their structure, containing both hydrophilic and hydrophobic “ends” give them these properties [82].

Beyond this, soyasaponins have been investigated to contain numerous benefits in the pharmaceutical industry, including anti-cancer, antioxidant, anti-microbial, anti-inflammatory, and cholesterol inhibiting properties [83-86]. There has been minimal investigation for a purpose for soyasaponins in material science, however their unique structure gives them much potential for a wide variety of purposes. One study investigated saponins derived from soapberry to show minute improvements in tensile, flexural, and impact strength when used to treat cellulose

nanocrystals intended for incorporation into polypropylene in comparison to unmodified nanocrystal composites [87].

2.3.2 Soy Isoflavones

Isoflavones are a group of phenolic compounds found in numbers across a variety of plant and fungal species [88]. The main isoflavone present in soy is genistein, comprising over 60% of the total isoflavone content, the remainder of the content consists of daidzein and glycitein [89, 90]. The total content of soy isoflavones in defatted soymeal is recorded to be 0.2-0.5 wt.% [89]. Soy isoflavones, when separated from the meal have also been cited to have a number of anti-inflammatory, anti-cancer, antioxidant, and antimicrobial benefits in medical settings [88].

2.3.3 Soymeal as a Food Source

Soymeal is one of the most popular feed choices for livestock such as chickens, ruminants (cattle, goats, sheep), swine, and fish [91]. Its high protein and digestible amino acid content (49-53 wt.%) make it extremely effective for livestock to build muscle, and its high carbohydrate fraction (35-39%) give it exceptional qualities as a rich energy source [92]. Despite its proficiency as a quality food source, there are various components of soymeal that can induce negative effects in some of the animals consuming it, notably, soyasaponins and soy isoflavones.

2.3.4 Nutritional Effect of Saponins & Isoflavones in Soymeal

Despite their extensive potential in a medical environment, soyasaponins and isoflavones' presence in soymeal can cause a number of negative effects on the meal itself, and on some of the animals consuming it. The effects of this on the meal quality, and on livestock that consume the meal most are individually listed below.

2.3.4.1 Soyasaponin & Isoflavone Effect on Flavor of Meal

Both saponins and soy isoflavones are cited in literature for the characteristic overpowering bitter taste they bring to products containing them. Saponins and isoflavones from food sources other than soybean meal have been known to cause undesirable taste effects on their food products, decreasing their appeal for consumption [93-96]. Most mammals, including cattle and swine, have evolved to despise bitterness for its common indication of toxicity in food

products. Mammals have been observed to prefer food sources void of these compounds for taste alone [97-99]. Chickens exhibit the same, but a less pronounced effect, and fish have not shown any preference on taste [100, 101]. Essentially, the removal of these compounds should improve the desirability of the meal as a food source for cattle, swine, and chickens, and have little to no effect on it for fish. A meal that is more appetizing for animals will result in them eating more of it, inducing greater growth and production capacity for them.

2.3.4.2 Soyasaponin & Isoflavone Effect on Health of Livestock

Soyasaponins and isoflavones have been investigated extensively on their nutritional benefit to the animals that consume them. The removal of them from a soymeal product should produce a positive effect on the overall health, productivity, and hence profitability for cattle and fish in particular.

2.3.4.2.1 Soyasaponin & Isoflavone Effect on Cattle

Soyasaponins have been found responsible for reductions in fertility and reproductive health, degradation of cell membranes and intestinal linings, reduced digestion, and reduced appetite for cattle [102-105].

Soya isoflavones have been associated with early embryonic mortality or complications requiring abortions for cattle. Furthermore, they have been observed to disrupt the function of the corpus luteum, inhibiting progesterone secretion and causing additional disorders during early pregnancy, including early embryonic death [106-108].

A soymeal void of both these compounds would likely improve the metabolic health of cattle in comparison to the existing soymeal.

2.3.4.2.2 Soyasaponin & Isoflavone Effect on Fish

There have been no positive attributes to a meal higher in saponin content in the diet of farmed fish. Soyasaponins were determined accountable for inducing acute enteritis and severely decreasing digestibility in salmon, and significant intestinal ruptures in trout [109-111].

Isoflavones in feed have been heavily correlated to inducing skewed sex reversals in fish that undergo them, providing significantly more female than male when fed with meal containing

the isoflavones [112]. They have also been cited to disrupt reproductive systems of multiple species of fish [113, 114].

Most species of farmed fish have shown a consistent inability to digest both soyasaponins and soy isoflavones. Due to this, the only effects observed from these compounds are due to their unique structures remaining intact through the body, binding to, disturbing, and rupturing tissue in the digestive and reproductive systems for these animals.

2.3.4.2.3 Soyasaponin & Isoflavone Effect on Swine

The effects of soyasaponin content in soymeal for pigs has not been extensively studied. The few papers that exist suggest little to no negative effects of their presence [115] and have even been attributed to improving immunological responses in pigs that consume feed with them [116].

Soy isoflavones have also been significantly associated with increased immune responses for swine [117, 118]. However, there are correlations with isoflavones to inhibited growth and development for piglets, and reproductive tissue damage for pregnant or weening adult swine [119].

The complete removal of both of these compounds for meal intended as feed for swine may not be advised due to the significant positive effects they can have on the animal's immune system.

2.3.4.2.4 Soyasaponin & Isoflavone Effect on Poultry

Soyasaponins in the diet of chickens have been explicitly connected to improvements in reproductive efficiency and overall health, with minimal negative effects [120, 121].

Soy Isoflavones have also been convincingly correlated to improvements in growth, antioxidant activity in tissue, egg laying production and quality, and improved immune functionality [122, 123].

A meal void of soyasaponins and isoflavones is not recommended in the diet of chickens based on the current literature. Soy isoflavones in particular are extremely beneficial to overall health and production of these animals.

2.3.5 Consequences of Removal of Soyasaponins & Isoflavones from Soymeal

Due to the mixed effects these compounds have depending on the type of animal ingesting the meal, it is difficult to assess their true value. For meal that is sold to feed cattle or fish, soyasaponins and isoflavones can be considered a waste component that can be removed and used for an alternative, more beneficial purpose. The removal of these compounds from the meal would even produce a more desirable meal for these animals, and a decreased risk of the health symptoms associated with their presence, resulting in a win-win scenario.

However, for meal being sold as feed for chickens or swine, the removal of these compounds can alter the state of the food source in a negative fashion. Due to the various positive attributes these compounds are associated with having in both these animals' diets, they could be considered essential for optimal growth, health, and productivity and thus not acceptable for removal.

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CHAPTER 3

MATERIALS & METHODS

3.1 Extraction and Quantification of Saponin Derivative from Soymeal

3.1.1 Materials

Defatted soymeal was provided by Zeeland Farm Services (ZFS) Inc. soybean processing facility (Ithaca, MI, USA). N-butanol (>99% purity), food-grade ethanol (>95% purity), and soyasaponin standard (Soyasaponin I (Bb) of >95% purity) were all purchased from Sigma Aldrich (Sigma Aldrich St. Louis MO, USA) and Cayman Chemical (Cayman Chemical Ann Arbor MI, USA). Deionized water was supplied by the Michigan State School of Packaging.

3.1.2 Extraction of Soyasaponin Derivative

Following the procedures of [1], & [2], 10 g of the defatted soymeal was subject to a 24-hour maceration in 200 mL of 60% aqueous ethanol inside 12 oz jars without agitation. Following the maceration, the meal was vacuum filtered out of the solution using a no. 1 filter paper (Whatman qualitative filter paper, grade 1, Cat no. 1001 110) and a GAST model DOA-P704-AA vacuum pump (Max Pressure 60psi, Volts: 115Amps: 4.2Hz:60) until no liquid continued to drop for at least 10 seconds.

The filtered liquid was then placed in an open 250-mL beaker and evaporated at room temperature until only solid or very viscous residue deemed “soybean molasses” remained. Following the procedure of [3], the dry residue was introduced to a 1:1 (v/v) mixture of deionized water and n-butanol each at a ratio of 5:1 (mL/g), and vortexed at 1,000 rpm until it had been evenly distributed within the mixed solution. This solution was then centrifuged at 4,000 rpm for 10 minutes, to produce a distinct two-phase system. The top phase consisted of a mostly clear butanol fraction, the bottom phase an opaquer green water fraction, with a white middle separating layer between the two.

The butanol fraction was then collected via pipette, azeotroped with 5-10 mL of water and rotary evaporated under reduced pressure to leave a secondary green residue. This residue was collected from the flask with ethanol and introduced to a second centrifugation at 4,000 rpm for

10 minutes. After the second centrifugation, the precipitate formed at the bottom was collected and evaporated into a white powder that served as the saponin concentrate derived from soymeal.

3.1.3 Quantification of Saponin in Derivation

Analysis of saponins was performed by Liquid Chromatography/mass spectrometry (LC-MS/MS) using a Thermo Q-Exactive mass spectrometer interfaced with a Thermo Vanquish UHPLC (Thermo Fisher Scientific, MI, USA). Samples were either extracted with 80% methanol (solid samples) or diluted with 80% methanol (liquid samples). 10 μ L of sample extract was injected onto a Waters Acquity UPLC BEH-C18 column (2.1x100mm) (Waters Corp. MA, USA) and compounds were separated using the following gradient: initial conditions were 99% mobile phase A (10 mM ammonium formate in water) and 1% mobile phase B (acetonitrile + 0.1% formic acid) and held for 1 min followed by a ramp to 99% B at 16 min, held at 99% B until 18 min, then return to 1% B until 20 min. The flow rate was 0.3 ml/min and the column temperature was 40°C. Compounds were ionized by electrospray operating in positive ion mode with a capillary voltage of 3.5 kV and source gas and temperature settings adjusted for a 0.3 ml/min flow rate from the UHPLC. Data were acquired using a data-dependent MS/MS method with survey scans at 35,000 resolution across m/z 100-1500, AGC target of $3e6$ and max inject time of 100 ms. The top 5 ions were chosen for MS/MS scans at 17,500 resolution, AGC target of $1e5$ and max inject time of 50 ms, dynamic exclusion of 3 seconds, and stepped normalized collision energies of 10, 30 and 60 V. Raw data were imported into Progenesis QI software for peak alignment and peak picking. Saponins peaks were identified based on accurate mass and fragmentation patterns. Quantities of all saponins were estimated using an external standard curve of soyasaponin I (Bb) obtained prior to the quantification of the samples.

3.2 Renourishment of Soymeal

The post-maceration soymeal attained after paper filtration was oven dried at 55 °C for at least 24 hours, and then the bottom fraction of the centrifuged solution mentioned in **3.1.2**, was poured back onto it, allowing for nutrient reintegration via osmosis.

3.2.1 Verification of Nutrient Consistency after Reintroduction

To verify that the bottom fraction reintroduction succeeded in replenishing the soymeal with the unnecessary extracted nutrients, a proximate composition analysis was performed. These composition analyses compared the 5 major components (protein, lipid, mineral/ash, moisture, carbohydrate) of soymeal samples both before extraction, and after the reintroduction. All soymeal samples tested were dried in a 55 °C oven for 24 hours prior to being analyzed and were done in at least 3 replications.

3.2.1.1 Protein Content

To determine the approximate protein content of the soymeal samples, a LECO FP828P Nitrogen Analyzer (LECO Corp. MI, USA) was utilized. Initially, a four-point standard curve (with each point done in triplicate) was produced with different quantities of nicotinic acid. Thereafter, at least 3 blank samples were run to ensure proper calibration. Pre- and post-extraction soymeal samples between 0.2 and 0.3 g were then placed into the machine, where the N₂ content was analyzed. To translate these N₂ values into protein constituents, a Jones' factor of 6.25 (industry standard) was utilized [4].

3.2.1.2 Mineral/Ash Content

The values for the mineral/ash content of the soymeal samples were determined using a muffle furnace method in a Thermolyne B1 Muffle Furnace 208V (Thermo Scientific, MA, USA), following the protocol of [5]. Pre-weighed soymeal samples were set in porcelain crucibles and heated at 550 °C for 24 hours, cooled in a desiccator for 1-2 hours, and re-weighed against their initial weight to determine the mineral/ash content.

3.2.1.3 Lipid Content

The determination of the lipid/phospholipid content of the soymeal samples was carried out through the use of a microwave-assisted (MA) lipid extraction method using a MARS6 Microwave Digestion System (CEM Industries, NC, USA) followed by gravimetric analysis, following the protocol of [6]. The microwave extraction parameters involve a 1-stage, 400-Watt heating on a rotating carousel at 55 °C for 15 minutes.

0.5 g of each soymeal sample type was introduced to 8 mL of 4:1 ethyl acetate: methanol (+0.1% BHT as an antioxidant), put into pressurized 55 mm tubes and set into the carousel. The extract was then transferred to clean and pre-weighed 10mL glass tubes and evaporated under nitrogen and mild heat (37 °C). These conditions were used to prevent the oxidation of the extracted lipid product as much as possible. After evaporation, the lipid residue was weighed and divided against the initial 0.5 g sample to determine lipid content.

3.2.1.4 Moisture Analysis

Moisture values for all soymeal samples were determined using an A&D MX-50 Moisture Analyzer (A&D Company, MI, USA). Samples of 1g were weighed and placed into the moisture analyzer and heated until moisture content reached a rate of change of less than 0.1% /min.

3.2.1.5 Carbohydrate Content

The carbohydrate content of soymeal samples was determined using a difference method with the equation below, as is standard in proximate composition analyses of this nature [7].

$$\% \text{ Carbohydrate} = 100\% - \% \text{ Protein} - \% \text{ Lipid} - \% \text{ Mineral} - \% \text{ Moisture}$$

3.3 Preparation of Filler for Incorporation into Polymer Matrix

3.3.1 Materials

Crushed soybean hulls were provided by the Zeeland Farm Services soybean processing facility (Ithaca, MI, USA). These were cracked off the soybean via heating, they were then dried, and separated using air filtration during production.

Sulfuric Acid (>96.4% purity) was obtained from J.T. Baker (Fischer Scientific, Hampton, NH, USA). Deionized water was obtained from the Michigan State School of Packaging.

3.3.2 Particle Size Reduction of Soybean Hull Filler

Soybean hulls were dried for 24 hours at 55 °C, milled for 15 minutes using an 8000D Dual Mixer/Mill (SPEX Sample Prep, Metuchen, NJ, 2010), and sieved through a 140 mesh (105 um) sieve until a substantial amount of material was attained for sample production. This soyhull powder was stored in a Ziplock bag in a desiccator until film preparation.

3.3.3 Treatment of Powdered Soybean Hull Filler

To provide a medium for saponins to interact with the filler particles, the procedure of [8] was followed. This procedure involved the immersion of filler particles into a water-saponin solution at a ratio of 10:90 v/v, followed by a 24-hour drying at 80 °C. The use of an acid catalyst of sulfuric acid (H₂SO₄) was investigated by inducing a pH of less than 2 during immersion.

12 total samples (3 replicates for each treatment) of 2 g of soybean hull were immersed in 26 mL of water in separate 200 mL beakers (with and without 0.05 g of saponin extract). 3 drops (0.15 mL) of sulfuric acid were then added to six of the samples (three with saponin and three without saponin) to provide the acid medium. All samples were then covered with foil and left at room temperature for 24 hours. After this, the samples were uncovered until the liquid had evaporated, and put into an oven at 55 °C for 24 hours to fully dry. After drying, the samples were crushed using a mortar and pestle to size with visibly large constituents removed and left to dry at 55 °C for an additional 12 hours. The samples were then re-weighed and adjusted for consistency.

3.4 Preparation/Fabrication of PLA/Soyhull Composites

PLA pellets were pre-conditioned in an oven at 50 °C for 24 hours before being added to a three-piece mixer (C.W Brabender Instruments Inc., NJ) rotating at 80 rpm at 180 °C for 2 minutes. Without stopping, the hull samples attained from **Section 3.2.3** were gradually introduced into the mixer over the course of 30 seconds until all powder was incorporated, and melt-blended for an additional 4 minutes. Five different sample types were produced with 3 replicates of each, including a control of neat PLA. The composition and given titles of these composites are listed in **Table 1**.

The composites were then pressed into sheets using a heated hydraulic press (PHI Model No. QL438-C, California, USA) between two 12" x 12" x 0.6" Teflon coated stainless steel sheets (McMaster-Carr, IL, USA) at 183.9 °C under constant pressure of 7.5 tons for 2 minutes. Prior to the application of pressure, the resins were pre-heated on the hot press at the same temperature for 6 minutes. After pressing, the composites were removed from the heat and left at room temperature for 15 minutes before being removed from the plates.

Table 1: Composition/title of PLA/Soyhull Composites.

Title	PLA (g)	Soyhull (g)	Saponin (g)	Total (g)	H ₂ SO ₄ Treatment
Neat PLA	19.8	0	0	19.8	N/A
No Sap No H	18	1.8	0	19.8	No
Yes Sap No H	18	1.8	0.045	19.845	No
No Sap Yes H	18	1.8	0	19.8	Yes
Yes Sap Yes H	18	1.8	0.045	19.845	Yes

3.5 Characterization of Composites

3.5.1 Mechanical Properties

The tensile properties of all composites were determined using an Instron Universal Testing Machine Model 5565 P6021 (Instron Engineering Corp. MA, USA). For preparation for tensile testing, samples were cut according to ASTM D882 [9] using a TMI Sample Cutter (RDM Test Equipment Ltd., UK) and measured for thickness at ten separate locations using a digital micrometer (TMI). The samples used for tensile testing are shown in **Figure 1**.

Samples were cut to 12.7 mm width and no less than 101.6 mm in length and tested with an initial grip separation of 50 mm and a rate of separation of 5.08 mm/min in accordance with ASTM D882 [9]. At least five samples were tested from each sheet produced.

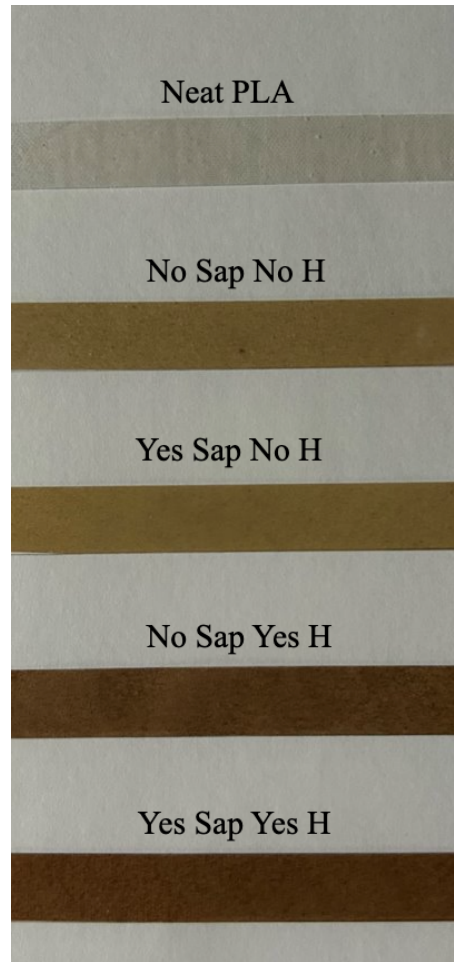


Figure 1: Image of the cut samples for tensile testing.

3.5.2 Barrier Properties

3.5.2.1 Oxygen Permeability

The oxygen transmission rates of all composites were obtained using an Ox-Tran Model 2/22 Oxygen Permeability Analyzer (Ametek, PA, USA) and then changed to oxygen permeability coefficients using the equation in [10]. Specimens from each treatment were cut from random sheet locations and masked with adhesive-backed 101.6um thick aluminum foil (McMaster Carr, IL, USA) with a testing area of 3.14 cm². Samples were tested at 23 °C and 50% RH in accordance with ASTM D3985-17 [11]. Results are presented in kg*m/m²*Pa*day. At least two samples were tested from each sheet produced.

3.5.2.2 Water Vapor Permeability

The water vapor transmission rates for all composites were determined with a Mocon PermTRAN-W Model 3/34 Water Vapor Permeability Analyzer (Ametek, PA, USA). These were then translated to permeability coefficients using the equation in [10]. Specimens from each sheet were cut and masked with adhesive-backed 101.6 μm thick aluminum foil (McMaster Carr, IL, USA) with a testing area of 3.14 cm^2 . Samples were tested at 37.8 $^{\circ}\text{C}$ and 100% RH in accordance with ASTM F1249-20. [12]. Results are presented in $\text{kg}\cdot\text{m}/\text{m}^2\cdot\text{Pa}\cdot\text{day}$. At least two samples from each sheet produced were tested.

3.5.3 Thermal Properties

3.5.3.1 Thermal Gravimetric Analysis

The thermal degradation behavior of all composites was determined using a TGA Q50 Thermogravimetric analyzer (TA Instruments, Delaware, USA) with a nitrogen flow rate of 60 mL per minute. 7-11 mg of sample was cut and put into a standard aluminum pan (TA Instruments, DE, USA) where it was subject to heating from 25 $^{\circ}\text{C}$ to 550 $^{\circ}\text{C}$ at a rate of 10 $^{\circ}\text{C}/\text{minute}$. At least 3 replications from each sheet produced was tested.

3.5.3.2 Differential Scanning Calorimetry (DSC)

The crystallinity and thermal properties of all composites were determined using differential scanning calorimetry DSC Q100 (TA Instruments, DE, USA) with a purge nitrogen gas flow rate of 70 mL/min. 5-10 mg of sample was cut and placed into a standard aluminum pan (TA Instruments, DE, USA) closed with a standard aluminum lid and then heated a rate of 10 $^{\circ}\text{C}/\text{min}$ to 210 $^{\circ}\text{C}$, down to -20 $^{\circ}\text{C}$, and back up to 210 $^{\circ}\text{C}$. The first heating was used to attain values for enthalpy of melting (ΔH_m), and enthalpy of cold crystallization (ΔH_c), the second heating was used for the determination of cold-crystallization temperature (T_c), melting temperature (T_m) and glass-transition temperature (T_g). The Universal Analysis 2000, V4.5 software (TA Instruments, DE, USA) was used to determine the values T_c , T_m , T_g , ΔH_m , and ΔH_c . At least 3 replications from each sheet produced was tested. The equation used to calculate the percent crystallinity ($\%X_c$) for PLA and the composites is below.

$$\%X_c = \frac{\Delta H_m - \Delta H_c}{(1 - \%filler) * 93} * 100\%$$

3.5.4 Morphological Analysis

Images of the composites were attained using a scanning electron microscope (SEM) (JEOL 6610LV, JEOL, MA, USA). Samples were freeze-cracked using liquid nitrogen and coated with a thin layer of platinum prior to analysis.

3.5.5 Optical Properties

3.5.5.1 Color

The color of the composite sheets was measured with a spectrophotometer (Labscan XE, Hunter Lab Associates Laboratory Inc., VA, USA) through a 2mm² view area. After calibration, the CIELAB color coordinates L*, a*, and b* were attained from the EasyMatch QC software. Ten replications for each sheet produced was tested.

3.5.5.2 Light Transmittance

The light transmittance of each composite was determined with a UV/Vis spectrophotometer (Shimadzu UV 1800 Spectrophotometer Kyoto, Japan) and the UVProve 2.61 software. Four replicate samples of equal thickness (0.32mm) from each sheet were cut and placed into the machine. The absorbance was taken from 200 nm to 800 nm with measurements taken every 0.5nm at “medium” speed. The absorbance spectra are displayed in nm vs. absorbance for the full spectrum measured.

3.6 Statistical Analysis

To perform the statistical analysis for the tensile, barrier, and color data, a two-way ANOVA test was performed with a p-value of 0.05. To perform the statistical analyses for DSC results, a linear mixed model was incorporated using the package ‘lmerTest’ in the R program. Mean separation and letter differentiation was performed using the ‘emmeans’ package in R. Post hoc comparisons of the data was conducted using Tukey’s adjustment, and a significance value of $p < 0.05$. The alpha level for all analyses was 0.05, and the p-value for multiple comparisons was corrected by Bonferroni correction.

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CHAPTER 4

RESULTS & DISCUSSION

4.1 Quantification of Soyasaponin Extract

The white precipitate remaining after centrifugation in 3.1.2 was dried and estimated to consist of >51% soyasaponin (>33% soyasaponin Bb (I), specifically). The remaining components were deemed to be largely isoflavones (genistein, daidzein, and glycitein), with a small lipid/phospholipid portion based on the HPLC/MS results. This purity was slightly lower than [1] and [2], that performed similar procedures for saponin extraction. Possible reasons for this include the derivation source, polarity of the maceration solvent, and singular partitioning.

Soy isoflavones, the other major component of the extract, are distinguished in structure as well and contain ample reactive sites to interact with the filler particles and potentially alter their surface chemistry [3].

Due to this purity level, and the chemical composition of the remaining fraction of the extract, the properties attributed to this extract cannot be isolated to soyasaponins alone.

4.2 Soymeal Nutrient Evaluation

4.2.1 Proximate Composition Analysis of Soymeal After Extraction & Reintroduction

Table 2: Proximate composition analysis of the soybean meal before the extraction and after the reintroduction process.

Constituent	Content (%)	
	Original soymeal	Improved soymeal
Protein	51.60 ± 0.69 ^a	52.09 ± 1.42 ^a
Lipid	2.91 ± 0.97 ^a	0.36 ± 0.00 ^b
Mineral/Ash	6.28 ± 0.10 ^a	6.37 ± 0.09 ^a
Moisture	1.55 ± 0.21 ^a	2.95 ± 0.00 ^b
Carbohydrate (make-up compound)	37.66 ^a	38.23 ^a
Saponin ^{1.}	1.25 ± 0.12 ^a	0.31 ± 0.00 ^b

Different lower-case letters (a, b) indicate significant differences between the columns.

Table 2 (cont'd)

¹. Saponins within the meal are considered a subcategory of the lipid and carbohydrate content and thus are already included in the overall composition value [4, 5].

The values shown in **Table 2** for the original meal are consistent with USDA standards for defatted soybean meal [6].

4.2.1.1 Protein Content

The protein content of the post-extraction meal was consistent with that of the original meal sample. The use of a less polar solvent in 60% ethanol, and the maceration temperature of 23 °C was incorporated to limit protein solubilization and prevent denaturation [7]. Due to protein content being the largest source of appeal for defatted soymeal, the retention of this content is extremely important for the value of the post-extraction meal.

4.2.1.2 Mineral/Ash Content

The mineral/ash content within the meal also remained consistent after extraction. This was mostly expected as most minerals present in soymeal are not as readily soluble in water or other polar solvents [8].

4.2.1.3 Lipid Content

The lipid composition of the meal after extraction and reintroduction procedures was notably lower (<15% of original value) than the original meal. A loss of value due to this difference in lipid content could be considered as lipids provide essential energy storage and absorption for the livestock consuming them [9]. However, due to the notion that the meal is already defatted, the initial lipid content is extremely low, and livestock are typically supplemented with their dietary lipids from other food sources regardless [10].

4.2.1.4 Moisture Content

The moisture content of the meal is higher after the extraction process in comparison to the initial meal. This moisture content likely higher due to the primary and secondary immersions in water/ethanol the post-extraction meal undergoes during the maceration, and reintroduction phases. This integrates the water further into the microstructure of the meal, making it more difficult and time consuming to evaporate. Since the drying procedure for these

samples was only at 55 °C for 24 hours, it is reasonable to assume that this additional water may not have had enough energy to leave.

4.2.1.5 Carbohydrate Content

The broad carbohydrate content of the meal is undifferentiable from the original soymeal. This is vital in the continuation of the post-extraction soymeal as an energy-rich animal feed.

4.2.2 Saponin Content

The saponin content in the initial meal was slightly higher than other values seen from defatted soymeal [5, 11]. This may be due to the growing conditions of the soybean effecting these values, or the type of defatting process the soymeal was subject to compared to others [12]. Saponin content of the post-maceration meal is less than a third than that of the initial. This proves the extraction mechanism was successful in separating a majority of the saponins existing in the meal at least in the butanol fraction. Macerating in a less polar solvent, or repeated partitioning may increase this extraction effectiveness. Regardless, a meal source with less saponins in it is expected to be less harmful to the health and production of livestock such as cattle and fish.

4.3 Characterization of PLA-Soybean Hull Composites

4.3.1 Mechanical Properties

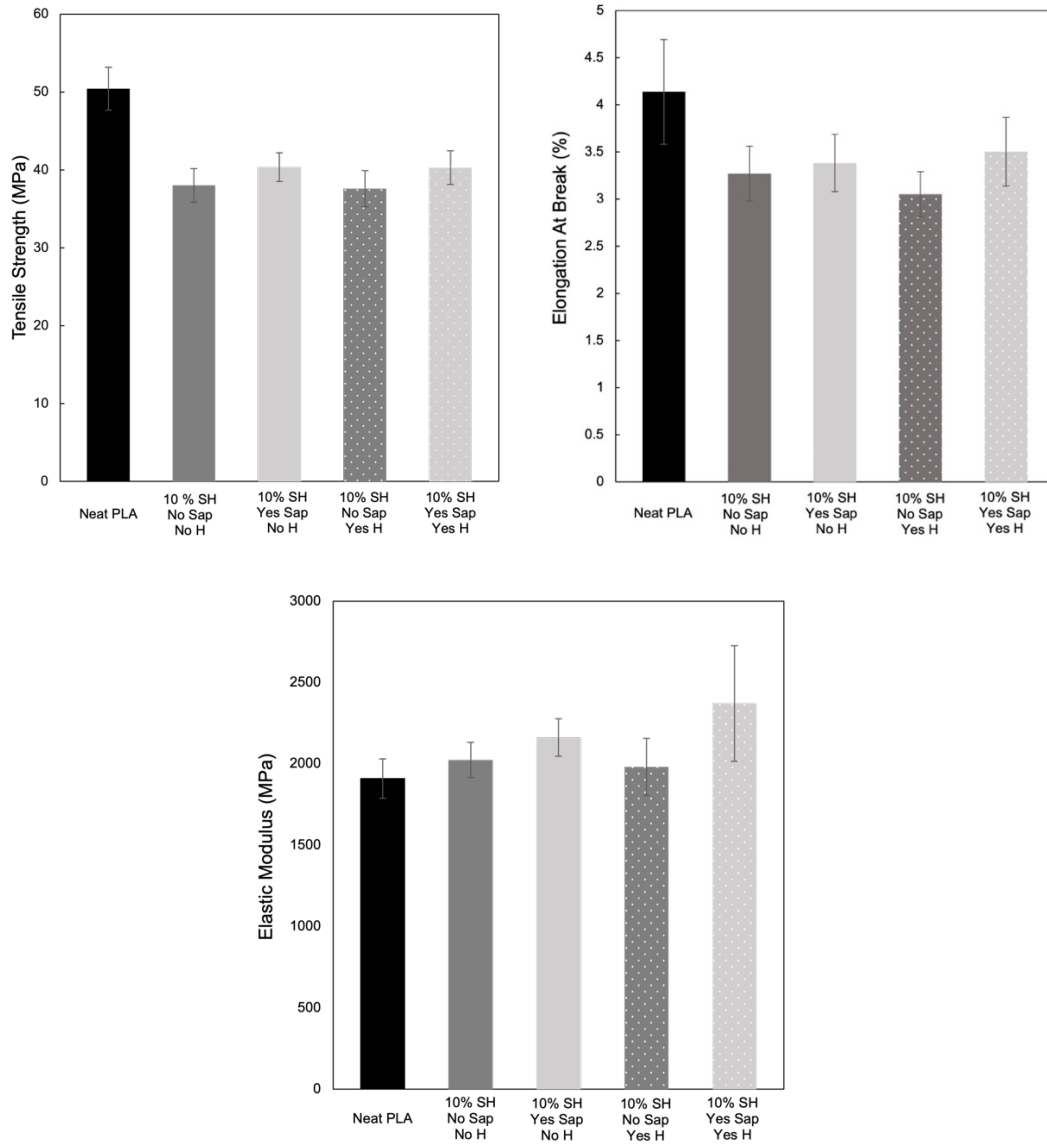


Figure 2: Tensile strength, elongation at break, and elastic moduli of all composites.

Table 3: Tensile test results for all composites.

Sample	Tensile Strength (MPa)	Elongation at Break (%)	MOE (MPa)
Neat PLA	50.41 ± 3.60 ^a	4.14 ± 0.55 ^a	1909.64 ± 120.69 ^a
No Sap No H	38.00 ± 2.18 ^b	3.36 ± 0.41 ^{bc}	2023.64 ± 108.87 ^{ab}
Yes Sap No H	40.35 ± 1.84 ^c	3.38 ± 0.30 ^b	2161.54 ± 115.00 ^b
No Sap Yes H	37.58 ± 2.33 ^b	3.05 ± 0.24 ^c	1980.54 ± 175.40 ^{ab}
Yes Sap Yes H	40.29 ± 2.17 ^c	3.50 ± 0.36 ^b	2370.49 ± 354.49 ^b

Different lowercase letters (a, b, c) indicate groupings that are statistically different from another.

The inclusion of the soyhull filler decreased the strength of the PLA composites. This is indicated by the lower tensile strength values for composites with all filler treatments compared to the neat PLA. This loss in strength was expected due to the inclusion of a mostly incompatible substance into the plastic matrix [13].

The treatment of the filler with the saponin extract improved the composites tensile strength in comparison to the non-saponin treated samples, however, this improvement was still considerably less than the values for the neat PLA. The acid treatment of the filler prior to inclusion did not affect the tensile strength of composites in comparison to the non-acid treated samples. The combination of both the saponin and acid treatments resulted in a composite with improved strength compared to the untreated and solely acid treated samples, however this level of improvement was not considered different from the solely saponin-treated filler. This indicates that the combination of the two treatments did not initiate any additional effect between the saponin and the filler in this area.

The inclusion of the soyhull filler also decreased the elongation values of composites regardless of the treatment in comparison to the neat PLA as expected [13]. All treatment variations of saponin, acid, and the combination of the two provided no improvements in this area compared to composites produced from the untreated filler. This limits the belief of a saponin-induced improvement, or that there is a significant catalysis in saponin interaction from the acid medium.

The elastic modulus of composites did not change with the inclusion of the soyhull filler. An increase in this area was expected due to the rule of mixture [14], however the loading factor of 10% may have limited the extent of this effect. The use of the saponin-treated filler resulted in a modulus significantly higher than the neat PLA, however, it was indifferentiable from the untreated filler composites. The acid treatment of the filler shows no difference in comparison to both the neat plastic and the untreated filler. The combination of the saponin and acid treatments on the filler produced composites with higher moduli than that of the neat PLA but was still indifferentiable from the untreated filler composites.

4.3.2 Barrier Properties

Table 4: Oxygen Permeability (OP) and Water Vapor Permeability (WVP) for all composites.

Sample	OP * E-18 (kg*m/m ² *Pa*s)	WVP * E-14 (kg*m/m ² *Pa*s)
Neat PLA	4.24 ± 0.29 ^a	1.85 ± 0.03 ^a
No Sap No H	4.00 ± 0.39 ^a	1.93 ± 0.04 ^b
Yes Sap No H	3.96 ± 0.40 ^a	1.92 ± 0.05 ^b
No Sap Yes H	4.17 ± 0.26 ^a	1.88 ± 0.04 ^{ab}
Yes Sap Yes H	3.87 ± 0.42 ^a	1.84 ± 0.04 ^a

Different lowercase letters (a, b) indicate groupings that are statistically significant from another.

4.3.2.1 Oxygen Permeability

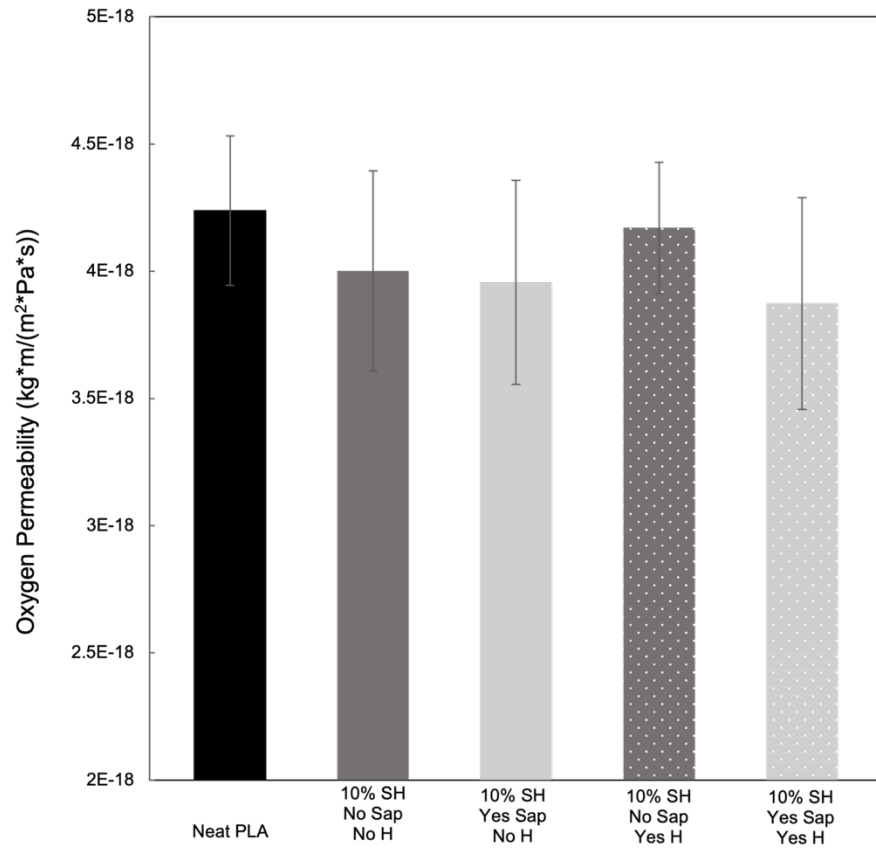


Figure 3: Oxygen permeability coefficients for all composites in kg*m/m²*Pa*s.

The inclusion of the filler regardless of the treatment had no effect on the oxygen permeability of the composites leaving comparable values to the neat PLA. This is likely attributed to the tortuosity effect that fillers have been known to induce for composites [15]. This provides a more tortuous path with more obstacles for permeants, particularly less polar ones such as oxygen. This effect in turn mitigates the otherwise negative effect fillers can have on the uniformity of the polymer structure on permeability.

4.3.2.2 Water Vapor Permeability

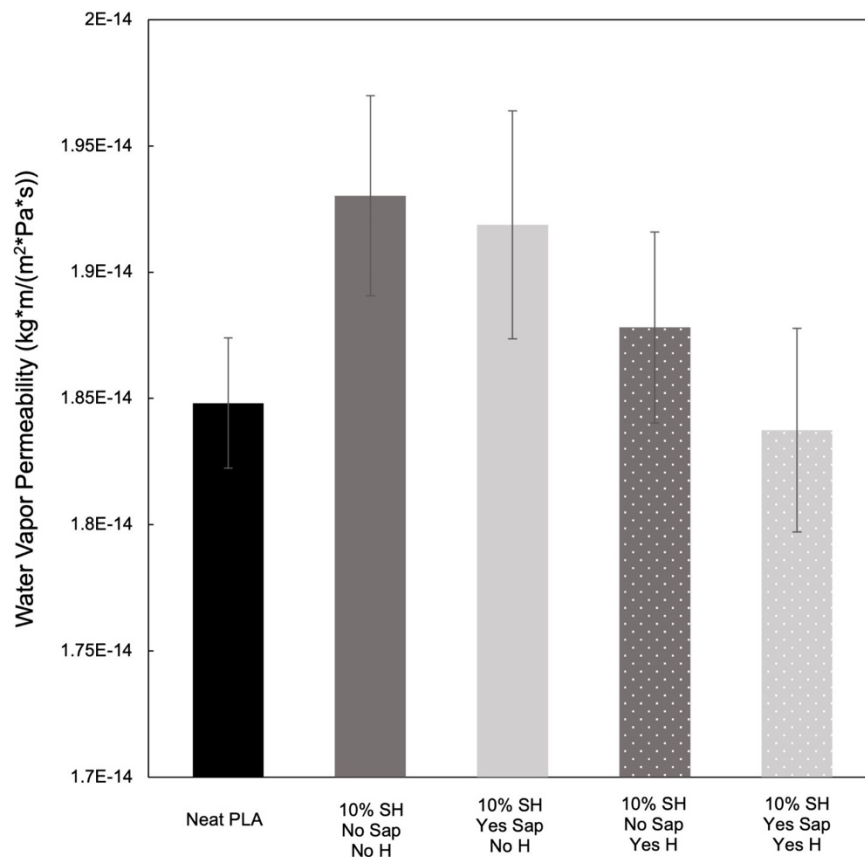


Figure 4: Water vapor permeability values for all composites in kg*m/m²*Pa*s.

The inclusion of the untreated soyhull showed statistically significant reductions in the material's barrier to water, the resultant permeability was more than 5% higher than the neat PLA. This is often seen in fillers of similar nature due to their hydrophilicity [13, 16]. The saponin treatment of the filler did not have a significant effect on the WVP of composites when compared to the untreated composites, retaining a significant deterioration in this category compared to the neat plastic. The acid treatment of the filler showed no effect on WVP values in comparison to the untreated filler-composites, yet the acid treated composites were also indistinguishable from neat PLA. The combination of the saponin and acid treatments resulted in a water vapor barrier significantly better than both non-acid treated composites and comparable to the neat PLA. Improvements in this area are often seen from from both acid-treated fillers and from coupling agents because of the modified surface chemistry involved with them [13, 16].

These improvements are generally attributed towards the tortuosity effect mentioned earlier, as well as a decreased hydrophilicity of the filler after treatment.

4.3.3 Thermal Properties

4.3.3.1 Thermogravimetric Analysis

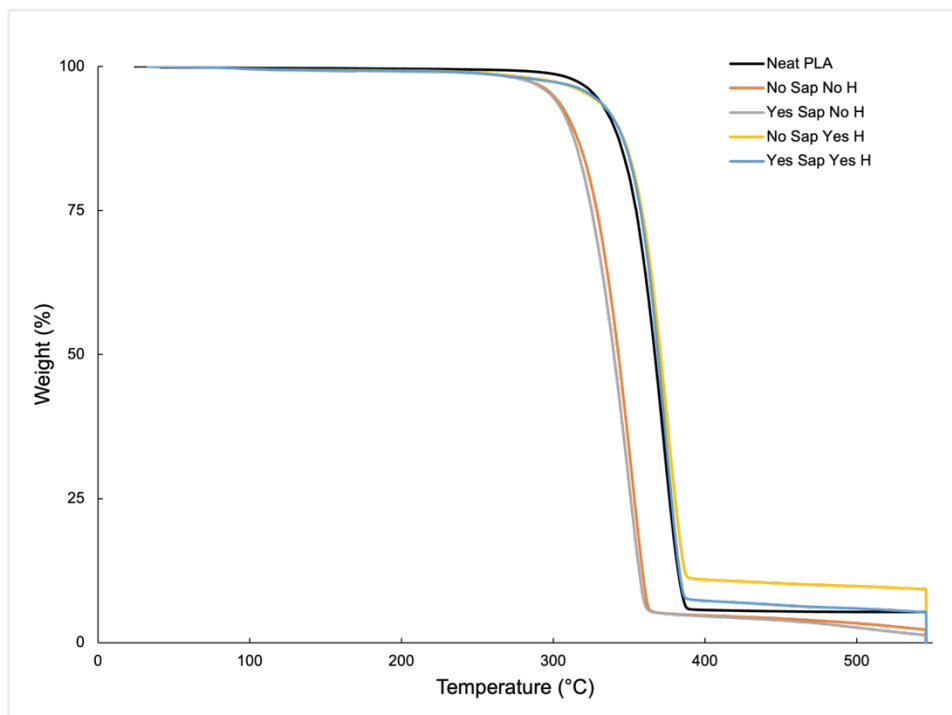


Figure 5: Displays the weight change (%) as a function of Temperature (° C) observed during Thermogravimetric Analysis for all composites.

There is an initial loss observed for the filler-composites around 100-120 °C. This loss is less than 1% of the total weight and is attributed to the loss of water that may have remained in the samples [17]. All composites showed a further increase in weight loss of an additional 1-2% between 210-290 °C that was not observed in the neat PLA. This secondary loss can be indicative of the initial loss of hemicellulose and lignin [18, 19]. The third step in weight loss is observed between 300 and 370 °C, where over 90% of the weight was lost. This is widely accepted as the degradation temperature range of PLA, and is also a result of the decomposition of cellulose in the filler-composites [20].

The weight loss was maximized at 350-355 °C for the non-acid treated composites, and at 380-385 °C for the acid treated and neat resins. The non-acid treated hulls contain large

quantities of cellulose which decompose at 270-330 °C [21], this may have initiated the weight loss at an earlier stage [22]. The hydrolysis of the cellulose in the soyhulls induced from the acid treatment likely produced higher contents of oligosaccharides in place of cellulose, which have higher degradation temperatures, thus diminishing this earlier onset degradation [19, 22]. There is no observed effect of the saponin-treatment on thermal stability of the composites.

4.3.3.2 Differential Scanning Calorimetry (DSC)

Table 5: Glass transition temperature (T_g), cold crystallization temperature (T_c), melting temperature (T_m), and the percent crystallinity (X_c) of all composites.

Sample	T_g (°C)	T_c (°C)	T_m (°C)	X_c (%)
Neat PLA	60.81 ^a	112.91 ^a	148.12 ^a	15.54 ^a
No Sap No H	60.33 ^a	112.65 ^a	147.70 ^a	20.69 ^b
Yes Sap No H	60.74 ^a	112.94 ^a	148.43 ^a	18.92 ^b
No Sap Yes H	59.56 ^a	111.22 ^b	146.98 ^b	19.49 ^b
Yes Sap Yes H	59.47 ^a	112.25 ^{ab}	146.92 ^b	19.52 ^b

Different lowercase letters (a, b) in each column indicate groupings that are statistically significant from another.

The inclusion of the soyhull filler showed no effect on the T_g of composites regardless of the treatment in comparison to neat PLA. The filler loading is not considered high enough to have a drastic effect on the transition temperature [23].

The untreated, and saponin-treated soyhull composites showed no difference in T_c in comparison to the neat control. However, the acid treatment of the filler showed a markable decrease in this value. This may be attributed to the non-washing of the acid residue off the filler prior to inclusion. Sulfuric acid has been cited in previous literature to catalyze transesterification reactions between the esters in the PLA mainchain during extrusion. This typically leads to shorter chain lengths, less overlapping, and thus less required energy for movement among them [24, 25]. The combination of both acid and saponin treatments had no effect on this value compared to all other samples produced.

The inclusion of the non-acid treated filler showed little effect on the T_m of composites compared to the neat PLA. The acid treatment of the filler showed a decrease in this value. This can also be attributed to the potential transesterification reaction the residual H_2SO_4 may have

catalyzed during extrusion, thus reducing the energy required for freedom of movement of the chains. The combination of the saponin and acid treatments showed a similar melting temperature to the acid-treated sample, statistically lower than the other treatment variations.

The presence of the filler increased the crystallinity content of the PLA; however, this increase was not greatened by either saponins or acidification. The difference of percent crystallinity with the inclusion of all treatment variations of soybean hulls indicates their viability as a nucleating agent [26, 27]. The incorporation of other coupling agents has shown improvements in X_c , this is generally attributed to the improved adhesion into the polymer, allowing for more uniformity within the matrix [28, 29].

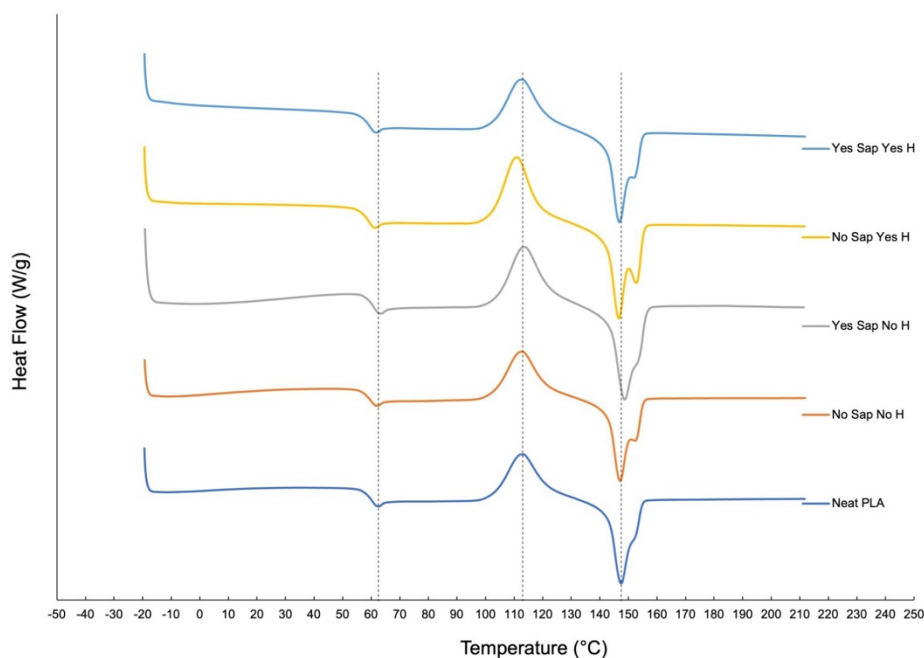


Figure 6: Heat Flow in (W/g) as a function of temperature from -20 to 215 $^{\circ}\text{C}$ for all composites. The T_g , T_c , and T_m temperature values for the neat PLA (bottom) are exemplified with vertical lines for comparison.

The establishment of a double peak in the melting temperature range for the 10% SH composites has been noted frequently in PLA composites including organic fillers [27, 28, 30]. This double peak is generally attributed to a double lamellar thickness within the composite structure. Specifically, there may be two separate crystalline phases comprised of different “perfection” levels that thereby melt at different temperatures. It is generally assumed that

whichever structure is less “perfect” is responsible for the earlier peak, where the more organized lattice is melted at a higher temperature [27, 28]. The presence of this second peak appears to diminish with the presence of saponins under both acid and non-acid treated conditions. This may be evidence of the saponins ability to provide a more uniform crystalline structure in composites.

4.3.4 Morphological Analysis

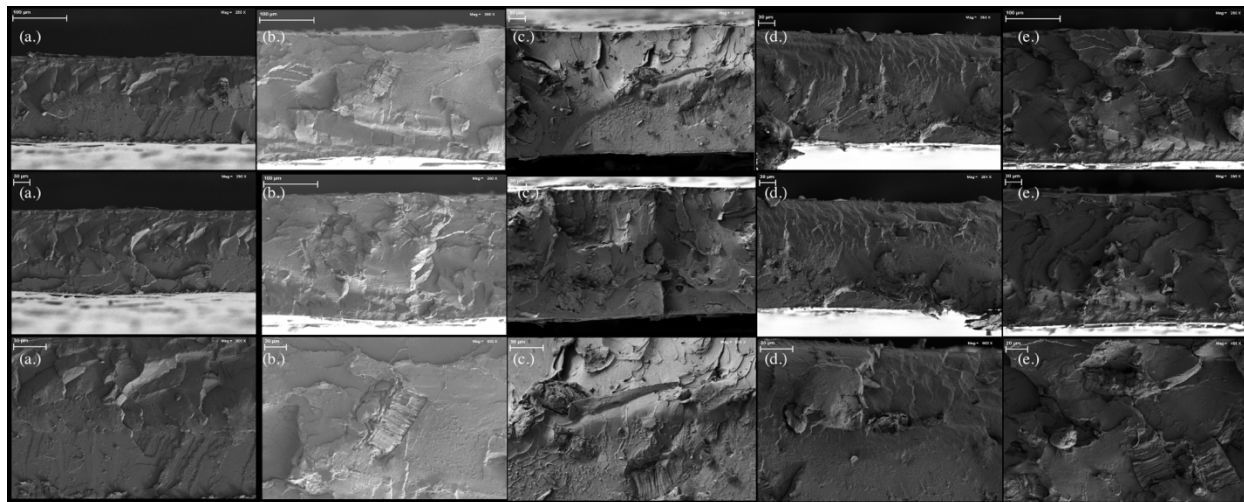


Figure 7: Displays the SEM microscopic images for all composites; a.) Neat PLA, b.) No Sap No H, c.) Yes Sap No H, d.) No Sap Yes H, and e.) Yes Sap Yes H.

Images are shown at both 250x (1st and 2nd row), and 500x (3rd row) magnitudes for visual comparison.

The images above display an estimation for the extent of adhesion for some of the filler particles given the different treatments. The presence of visible gaps between the filler particles and the matrix indicates a poor interfacial adhesion between the two, this is seen clearly in the 500x magnitude image for b.). The images in c.) provide excellent visuals for a well adhered filler into the matrix, this adhesion can perhaps explain the better mechanical properties observed in the fillers from the saponin treatment.

4.3.5 Optical Characterization

4.3.5.1 Color

Table 6: Lightness (L^*), and color coordinates a^* and b^* of the composite and neat PLA sheets.

Sample	L^*	a^*	b^*
Neat PLA	89.85 ± 0.26^a	-1.27 ± 0.00^a	4.57 ± 0.21^a
No Sap No H	77.33 ± 1.41^b	2.33 ± 0.43^b	22.25 ± 1.56^b
Yes Sap No H	76.412 ± 1.48^b	2.80 ± 0.49^b	24.27 ± 1.53^{bc}
No Sap Yes H	65.70 ± 5.14^c	7.21 ± 1.42^c	23.99 ± 2.16^c
Yes Sap Yes H	64.74 ± 2.99^c	8.38 ± 0.90^d	26.39 ± 1.17^d

Different lowercase letters (a, b) in each column indicate groupings that are statistically significant from another.

The neat PLA shows L^* , a^* , and b^* values resembling a grayish clear color, with a slight greenish-yellow tint, as expected. The addition of the soyhulls to the PLA resulted in significantly darker composites. This is seen often with filler-based composites due to differing light-reflection properties [31]. The inclusion of the soyhulls into the matrix produced significantly higher values on both the b^* spectrum and a^* spectrum. The inclusion produced sheets that were more red and yellow in appearance. The acid treatment of the filler prior to incorporation further increased this redness and yellowness in the resultant composite. This color change is likely due to the alteration of the surface chemistry of the fillers, thus disrupting the usual wavelength reflectiveness range [32].

The saponin treatment had a significant effect on the a^* and b^* values. Although it only comprised 0.25 wt.% of the entire composite, simply the presence of these compounds may have worked to alter these values. The saponin extract itself had a yellowish tint to it, providing reason for the higher b^* values observed.

4.3.5.2 Light Transmittance

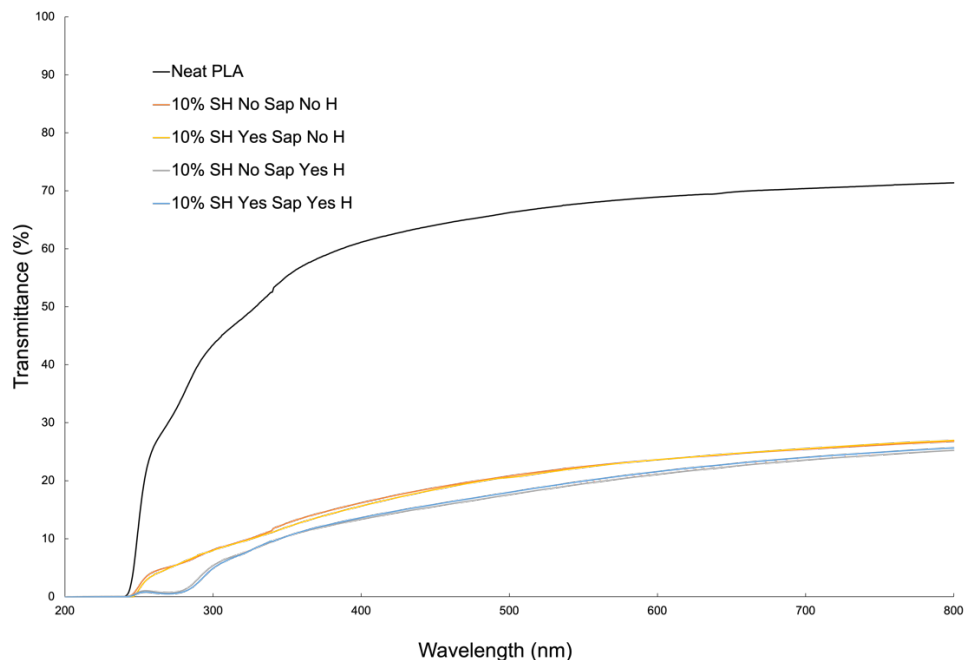


Figure 8: Transmittance of the composite sheets and neat PLA sheets through the visible, UVA, UVB, and some of UVC light spectrum.

As expected, the inclusion of a filler at a 10% loading reduced the transparency of the film through visible light by nearly 70%. This is largely attributed to the different structures within the soybean hull fillers, absorbing a vast majority of the light in the visible and UV spectrum when compared to the neat plastic. This finding is consistent with others in similar studies [31]. Although the decreased transmittance diminishes visible appeal of the composites, the continued absorbance of light in the UV spectrum bodes well for the material as a UV-blocker in light-sensitive food products.

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CHAPTER 5

CONCLUSION

5.1 Conclusion

Soyasaponins were able to be safely and effectively extracted from defatted soymeal without harming significant macromolecules of the meal including proteins, minerals, and carbohydrates. The treatment of the soyhull filler with the saponin extract prior to extrusion was able to produce PLA-soyhull composites with improved tensile strength compared to the untreated composites, providing evidence of viability as a coupling agent. The saponin treatment also provided evidence for more uniformity in composite crystallinity from the DSC curve results.

The acid treatment of the soyhulls prior to extrusion produced composites with improved water barrier, thermal degradation resistance, and UV barrier properties in comparison to the untreated composites. However, this treatment also coincided with lower T_c , and T_m values, the non-washing of the acid residue on the filler is believed to have played a significant role in these observations.

The combination of the acid and saponin treatments produced a composite with significant improvements in tensile strength in comparison to the untreated composites. However, these improvements were not notably greater than the composites including the saponin treatment alone. This treatment combination also showed improved WVP, thermal resistance, and UV barrier properties in comparison to the untreated filler composites. It also produced composites with decreased T_c and T_m , limiting the potential for some food packaging applications.

The proof of a safe extraction method of soyasaponins from soymeal and the establishment of their non-necessity in meal for some livestock provides a fully sustainable source for soyasaponins with commercial appeal for their extraction.

The potential of soyasaponins as an agent to improve tensile properties breaks significant ground in what was otherwise a minimally investigated field. The parameters used while testing including the concentration, mechanism of action, and sample production are all areas where

improvement can be made. This work provides a basis for others in discovering other naturally occurring coupling agents, testing saponins from other sources, and the overall betterment of green plastic composites.

The effect on acidification is not consistent with most literature in improving mechanical properties. However, the non-washing of the fillers after treatment gives difficulty in providing a true assessment. The acid treatment did not appear to hinder or improve the effectiveness of the saponin treatment.

5.2 Future Work

The effectiveness of the post-extraction soymeal as an improved food source for cattle and fish should be investigated for confirmation.

The evaluation of differing concentrations of the soyasaponin relative to the waste would be significant in attaining an optimal effectiveness. The investigation into evidence of the chemical reactions taking place as a result of the saponin treatment would be beneficial for fuller comprehension. Usage of the extract with fillers and plastics other than soyhull and PLA can also be investigated. The effectiveness of the saponin extract in comparison to commercially available coupling agents would provide an idea for the level of improvement. A look into the potential antimicrobial and antioxidant inducing properties of saponins on composites could be promising. Finally, the effect on the resultant biodegradability of composites after the saponin extract inclusion is necessary to prove the full viability of a truly green coupling agent.