Valorization of sorghum distillers dried grains with solubles (DDGS) as functional films using combination of suitable pretreatment with electrospinning and solvent-casting film manufacturing technologies

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Abstract

Growing environmental concerns related to petroleum-based plastics have increased interest in developing sustainable and biodegradable packaging materials derived from renewable resources. Distillers' dried grains with solubles (DDGS), a major by-product of grain fermentation in bioethanol production, represent a promising feedstock for biodegradable film manufacturing due to their high protein and fiber content. Sorghum-derived DDGS, in particular, offers additional advantages because sorghum is a drought-tolerant and widely cultivated crop with strong potential for sustainable agricultural systems. Valorization of DDGS into value-added packaging materials therefore supports both waste reduction and circular bioeconomy strategies.

The first objective of this thesis was to develop biodegradable films from sorghum DDGS using solvent casting and to evaluate the influence of chemical pretreatment, mechanical processing, and lipid removal on film performance. DDGS samples were subjected to four solvent pretreatments including water, hydrochloric acid (HCl), sodium hydroxide (NaOH), and sodium hydroxide combined with ethanol (NaOH+EtOH), together with two ball milling cycles (1× and 3×) under both defatted and non-defatted conditions. The resulting films were characterized for physicochemical, surface, and mechanical properties including color difference (ΔE), thickness, moisture content, contact angle, total phenolic content (TPC), elongation, normalized toughness and structural properties using FTIR analysis. Results demonstrated that solvent pretreatment significantly influenced several film properties, particularly color, surface wettability, and mechanical behavior ($p < 0.05$). Alkaline pretreatments generally enhanced phenolic extraction and surface hydrophobicity, while mechanical milling improved particle dispersion within the film matrix. Application studies using fresh-cut apples as a model food

system showed that DDGS-based coatings maintained stable microbial counts during storage and slowed browning kinetics.

The second objective of this thesis was to develop functional nanofibrous biodegradable films using coaxial electrospinning and DDGS-derived bioactive extracts. Core–shell nanofibers were produced using pullulan or micellar casein as shell polymers and emulsified active ingredients including thymol and DDGS extracts as the core phase. Emulsions were stabilized using Tween 20 or lecithin and processed through a coaxial electrospinning system to generate nanofibrous mats. The electrospun films were characterized for physicochemical, structural, and mechanical properties including color, thickness, moisture content, tensile behavior, and FTIR spectra. Results showed that polymer type and emulsifier significantly influenced film performance ($p < 0.05$). Pullulan-based films exhibited improved mechanical strength and lower moisture content compared to micellar casein films. Incorporation of DDGS extracts did not significantly affect color or thickness, ($p > 0.05$), but influenced moisture content and deformation behavior of the nanofibers ($p < 0.05$). In addition, selected electrospun films reduced browning kinetics of fresh-cut apples compared to uncoated samples.

Overall, this research demonstrates that sorghum DDGS can be successfully valorized into functional biodegradable films through both conventional solvent casting and advanced electrospinning technologies. The findings highlight the importance of pretreatment strategies and formulation parameters in tailoring film properties and demonstrate the potential of DDGS-derived materials for sustainable food packaging applications.

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Dedication

I dedicate this thesis to my family.

Chapter 1 - Review of Literature

Abstract

Biodegradable packaging films have gained increasing attention in recent years due to growing environmental concerns associated with petroleum-based plastics. Renewable biopolymers such as proteins, polysaccharides, and lipids derived from plant and agro-industrial sources are considered promising alternatives for sustainable packaging materials. In this chapter, an overview of biodegradable film systems and their main fabrication technologies is presented, including conventional methods such as solvent casting, extrusion, and coating, as well as advanced techniques like electrospinning. The chapter also summarizes different types of biopolymer films and the key characterization methods used to evaluate their mechanical, barrier, and structural properties. Particular attention is given to sorghum and distillers dried grains with solubles (DDGS) as promising agricultural by-products for biodegradable film production. Finally, the challenges, processing limitations, and modification strategies related to DDGS-based films and biodegradable packaging systems are discussed.

1.1 Introduction to Biodegradable Films

Food packaging is an essential component of modern food systems, ensuring safety, quality preservation, and shelf-life extension during distribution and storage. Conventional petroleum-derived plastics including polyethylene, polypropylene, and polystyrene dominate the market due to their excellent barrier and mechanical properties at low cost (Pawde et al., 2025). However, their persistence in the environment and accumulation such as microplastic waste, have intensified global demand for sustainable alternatives (Marsh & Bugusu, 2007). There has

been growing interest in biodegradable materials and their packaging that are more sustainable because of these environmental concerns.

Packaging systems are commonly classified into primary, secondary, and tertiary packaging based on their functional role in product protection and distribution (Verghese et al., 2015). Primary packaging is the layer that is in direct contact with the food product and serves as the main barrier against environmental factors such as moisture, oxygen, contamination, and physical damage; examples include films, coatings, pouches, and wraps (Ncube et al., 2020). Secondary packaging encloses one or more primary packages and provides additional protection, product grouping, and labeling or branding functions, such as cartons or overwrap films (Majid et al., 2018). Tertiary packaging is used for bulk handling, storage, and transportation, facilitating distribution and logistics through systems such as stretch wrap, shrink wrap, and pallet packaging, without direct contact with the food product (Borrelle et al., 2020).

Biodegradable and active films have gained increasing attention for fresh produce applications, where controlling moisture loss, respiration rate, and microbial growth is critical for maintaining quality during storage (Otoni et al., 2017). Biodegradable films are polymer-based materials that can be broken down by microorganisms into natural end products such as carbon dioxide, water, minerals, and biomass without leaving harmful residues in the environment (Kyrikou & Briassoulis, 2007). Biodegradable films are typically produced from renewable biopolymers such as starch, cellulose, proteins, or lipids (A. Kumar et al., 2025a). Their degradation occurs via enzymatic hydrolysis and microbial assimilation. Hydrolytic enzymes cleave glycosidic, ester, or peptide linkages, generating oligomers and monomers (Santana & Meireles, 2023). These are then metabolized into CO_2, H_2O, CH_4, or microbial biomass (Guliyev et al., 2023). The degradation kinetics depend on molecular architecture, crystallinity, amorphous

ratio, and hydrophobicity, as well as environmental conditions such as moisture, temperature, and pH (Siracusa et al., 2008). Highly crystalline polysaccharides, degrade more slowly because tightly packed chains limit enzymatic access, whereas amorphous regions are readily hydrolyzed (Adak et al., 2025). Similarly, hydrophobic proteins like zein or kafirin exhibit slower biodegradation than hydrophilic starch-based systems (Hassan et al., 2018; Taylor et al., 2015).

Active packaging refers to packaging systems that go beyond passive barrier protection and are designed to intentionally interact with the packaged food or its surrounding environment to extend shelf life, improve safety, and maintain quality (Ahari & Soufiani, 2021). These systems may incorporate components such as oxygen scavengers, moisture regulators, antimicrobial agents, antioxidant releasers, or ethylene absorbers that actively modify internal package conditions (Yam et al., 2005). Unlike conventional packaging, which functions mainly as a physical barrier, active packaging technologies dynamically control deterioration mechanisms such as oxidation, microbial growth, and moisture migration (Sarfraz et al., 2021). Because of these functions, active packaging has gained increasing importance in food systems, especially for perishable products and minimally processed foods, where quality preservation is critical (Du et al., 2023). Biopolymers extracted from food industry residues such as fruit and vegetable peels, cereal brans, and seafood processing by-products can be used as matrices or functional additives in biodegradable films, thus reducing raw material costs and waste disposal burdens while enhancing sustainability (A. Kumar et al., 2025b). These valorized biopolymers can also carry antioxidant and antimicrobial agents, improving the functional performance of the films for food preservation applications (Cvetković et al., 2025). However, optimizing extraction

methods and processing conditions remains critical to ensure consistent film quality and to fully exploit the environmental advantages of waste-derived materials.

1.2 Biodiverse Valorization and Repurposing of Waste Stream

A significant direction in biopolymer research is the valorization of agricultural and food industry by-products, which transforms low-cost residues into functional materials. This strategy is integral to circular economy principles, aiming to minimize waste, reduce dependency on non-renewable feedstocks, and enhance the overall sustainability of the food system (Marotta et al., 2025).

Previous research showed that, various residues can be used as renewable sources for film formation, soy protein from oil extraction, starch from tuber crops, lignocellulosic fibers from cereal brans, and proteins from distillers' dried grains with solubles (DDGS) have all been successfully utilized as biopolymer feedstocks (Hassan et al., 2018; Xiang et al., 2014). Among these residues, distillers' dried grains with solubles (DDGS) has gained particular attention due to its large production volume, rich protein and fiber composition, and strong potential for value-added conversion within circular bioeconomy frameworks (Buenavista et al., 2021). For example, DDGS was valorized through an integrated biorefinery approach by anaerobic co-digestion with rice husk to convert this low value ethanol by-product into higher-value outputs including biogas and bio-hythane (hydrogen–methane fuel blends) thereby shifting its use from conventional fodder toward circular bioenergy and soil amendment applications (Pavithra Bharathi et al., 2026).

In addition to DDGS, numerous other agricultural and food processing residues have been successfully valorized into value-added materials through targeted upcycling strategies.

Oilseed by-products such as soy and canola meals have been processed into protein isolates and film-forming biopolymers (Popović et al., 2020). Starch-rich residues from tuber and cereal crops have been converted into biodegradable films, coatings, and thermoplastic materials (Culqui-Arce et al., 2025). Cereal brans and lignocellulosic fractions have been further utilized as sources of dietary fiber, reinforcement fillers in biocomposites, and polysaccharide extracts for packaging applications (Sánchez-Safont et al., 2018). Fruit and vegetable processing wastes have also been upgraded into pectin, cellulose derivatives, antioxidant extracts, and antimicrobial compounds for functional food and packaging systems (Mohd Basri et al., 2021). These examples demonstrate that diverse agro-industrial side streams can be redirected from low-value disposal routes toward high-value material and functional ingredient applications.

A wide range of processing and fractionation techniques have been applied to valorize corn and cereal by-products, including mechanical, chemical, biochemical, thermal, and advanced green extraction technologies, depending on the target compounds and intended end use (Skendi et al., 2020). Supercritical CO_2 based processes have been done for valorization of corn milling byproducts which enable the extraction and conversion of DDGS, germ, and stover into higher-value products such as oils, antioxidants, biofuels, fermentable sugars, and encapsulated bioactives (Santana & Meireles, 2023).

In addition to supercritical CO_2 extraction, several other processing technologies have been widely applied for the valorization of cereal and bioethanol by-products. Solvent-based extraction and alkaline or acid fractionation methods are commonly used to recover proteins, lipids, and phenolic compounds from DDGS and other grain residues (Lu et al., 2025). Enzymatic treatments have also been employed to release fermentable sugars, bioactive peptides, and functional fiber fractions under mild processing conditions (Teixeira-Guedes et al., 2023).

Furthermore, fermentation-based bioprocessing enables conversion of agro-industrial residues into organic acids, enzymes, and biofuels through microbial transformation pathways (Adil et al., 2026).

Beyond environmental and economic benefits, by-products frequently impart intrinsic functionalities that enhance film performance. For instance, polyphenol-rich residues improve antioxidant and UV-protective properties, while fiber or protein rich fractions reinforce the polymer matrix, increasing tensile strength and reducing permeability (Skendi et al., 2020). However, the inherent variability in the composition of by-products, influenced by crop type, processing conditions, and seasonal factors, remains a key challenge for industrial standardization (Buenavista et al., 2021).

To address this, ongoing research emphasizes the importance of pretreatment, compositional analysis, and process optimization to achieve consistent film quality and mechanical performance. Overall, biodiverse valorization serves as a bridge between food waste management and sustainable material innovation, transforming underutilized biomass into high-performance, biodegradable films that align with both environmental responsibility and functional efficiency.

Biodegradable films offer multiple environmental advantages. First, they reduce dependence on fossil carbon by replacing petroleum feedstocks with renewable biomass (Cheng et al., 2024). Second, their potential for composability allows them to reintegrate into natural biogeochemical cycles, minimizing accumulation in landfills (Ramakrishnan et al., 2024; Topuz & Uyar, 2024). Edible films and coatings produced from food processing residues such as proteins, polysaccharides, and chitosan-rich by-products provide sustainability advantages because they are manufactured from food-grade biopolymers and are biodegradable after

disposal, thereby reducing persistent plastic packaging waste (Hamed et al., 2016). Beyond their environmental benefits, these coatings function as semi-permeable barriers to gases and water vapor, enabling regulation of oxygen and carbon dioxide transfer and thus helping control respiration rate and moisture loss in fresh fruits and vegetables (Rhim et al., 2013). Overall, biodegradable films present a sustainable solution to plastic pollution, though their performance still requires optimization.

1.3 Biopolymer Films

Biopolymers often form the backbone of current biodegradable packaging technologies. Their natural abundance, renewability, and molecular diversity make them versatile alternatives to synthetic plastics. These films derived from polysaccharides (e.g., cellulose, starch, alginate), proteins (e.g., gelatin, whey, zein), and lipids can be tailored for specific applications ranging from moisture barriers to active packaging carriers (S. Dassanayake et al., 2019). Importantly, the choice of polymer strongly influences not only film properties but also processing feasibility, degradation profile, and regulatory acceptance for food-contact applications (Siracusa et al., 2008).

Film production technologies significantly affect film structure and performance. Biopolymer films can form via various mechanisms depending on the molecular interactions and process conditions (Flórez et al., 2023). During film formation, polymer chains first become mobile in solution or melt state, then approach each other through diffusion and concentration increase, and finally establish intermolecular interactions that stabilize a continuous matrix (Felton, 2013). These interactions include chain entanglement, hydrogen bonding, electrostatic attraction, hydrophobic association, and in some systems covalent crosslinking, all of which determine final film cohesion and morphology (Hammann & Schmid, 2014). Process conditions

such as temperature, concentration, shear, and drying rate directly influence polymer chain alignment, packing density, and phase distribution, thereby affecting mechanical strength, barrier properties, and environmental stability (Wu et al., 2021). These include drying-induced film formation in casting and thermoplastic transformation in extrusion, both of which strongly influence polymer chain alignment and final film morphology (Azeredo & Waldron, 2016; Hassan et al., 2018). These interactions govern film integrity, flexibility, solubility, and resistance to environmental factors.

1.3.1 Polymer Crosslinking (Chemical and Physical Methods)

Crosslinking is a crucial strategy in enhancing the structural integrity, barrier properties, and environmental resistance of biopolymer films. It involves the formation of linkages between polymer chains, transforming a linear or branched structure into a three-dimensional network (Azeredo & Waldron, 2016). This network reduces polymer chain mobility, thereby improving tensile strength, reducing solubility, and limiting gas and moisture transmission. Crosslinking methods are generally divided into chemical and physical approaches, each with unique mechanisms and implications (Alavarse et al., 2022).

Chemical crosslinking refers to the formation of covalent bonds between polymer chains through reactive functional groups such as amino, hydroxyl, or carboxyl groups, producing a permanent three-dimensional network that improves mechanical strength, moisture resistance, and thermal stability (C. Liu et al., 2024). Common chemical crosslinkers include glutaraldehyde or enzymatic agents like transglutaminase, which create amide, ester, or ether linkages in protein or polysaccharide matrices (Oryan et al., 2018). For instance, glutaraldehyde crosslinking in gelatin films has been shown to increase tensile strength by more than 50% and reduce solubility due to the formation of stable Schiff base linkages. Enzymatic crosslinkers such as

transglutaminase also improve protein-based films by catalyzing ε-(γ-glutamyl) lysine bonds, yielding stronger, less water-sensitive networks compared to untreated controls (Alavarse et al., 2022). However, concerns related to the toxicity, residual reactivity, and regulatory limitations of certain chemical crosslinking agents used in food-contact materials have encouraged the development of enzymatic and naturally derived crosslinking alternatives (Azeredo & Waldron, 2016). For example, glutaraldehyde treatment in gelatin films has been shown to increase tensile strength by more than 60% and significantly decrease swelling in aqueous environments (Alavarse et al., 2022). Similarly, citric acid is often used in starch-based films to create ester linkages, improving water resistance without the toxicity concerns associated with aldehyde crosslinkers (Cao et al., 2020). Enzymatic crosslinking is particularly attractive for edible films; transglutaminase-catalyzed whey protein isolate films, for instance, exhibit enhanced elasticity and reduced solubility compared to uncrosslinked controls (Lisitsyn et al., 2021). These covalent bonds significantly enhance film strength, water resistance, and thermal stability but may involve toxic residues or require careful purification steps if synthetic agents are employed (Azeredo & Waldron, 2016).

On the other hand, physical crosslinking relies on hydrogen bonds, van der Waals forces, and ionic interactions to form cohesive polymer matrices which are often induced by changes in temperature, pH, or ionic strength (Lisitsyn et al., 2021). This mechanism is more environmentally friendly, as it avoids toxic reagents, and is often sufficient for edible films or biodegradable coatings. However, its stability decreases under humid conditions, where excess water disrupts hydrogen bonding and increases film solubility (Benbettaïeb et al., 2016). Ionic crosslinking is typically considered a form of physical crosslinking because the network structure is formed through reversible electrostatic interactions between oppositely charged groups, rather

than permanent covalent bonds (Lee & Mooney, 2012). For instance, ionic crosslinking of pectin with divalent cations improves mechanical resistance while maintaining biodegradability (Karnwal et al., 2025). Also, calcium ions can crosslink alginate films by forming “egg-box” junctions between guluronic acid residues, creating gels and films with better water stability (Cao et al., 2020). Temperature cycling can also be used to reinforce protein- or starch-based films; gelatin networks become more ordered after repeated heating–cooling cycles, while starch films undergo retrogradation that enhances crystallinity and barrier properties (Cao et al., 2020). Physical methods are generally safer and more environmentally friendly, making them suitable for food-contact applications, though they often result in films with lower resistance to humidity and mechanical stress (Karnwal et al., 2025).

The addition of glycerol increases polymer chain mobility and flexibility in protein-based films, but it can also decrease tensile strength due to reduced intermolecular interactions (Ben et al., 2022). Also, whey protein isolate films achieve their structure primarily through disulfide bonding and hydrophobic interactions, which provide good mechanical strength and oxygen barrier properties (Yoo & Krochta, 2011). In contrast, starch films are driven by hydrogen bonding and retrogradation during solvent evaporation, which enhances crystallinity and barrier properties but frequently leads to brittleness unless plasticizers are added (Karnwal et al., 2025). These mechanisms are essential for designing biopolymer films with tailored performance, whether for packaging with high barrier requirements, biomedical scaffolds requiring biocompatibility, or agricultural films that benefit from controlled solubility and environmental responsiveness.

Hybrid strategies have also been explored, combining mild chemical agents with physical treatments to balance strength, safety, and biodegradability. For example, chitosan films treated

with genipin, a natural crosslinker, and then subjected to heat treatment showed improved tensile properties and reduced solubility compared to films treated by either method alone (Kildeeva et al., 2020). Such approaches demonstrate the versatility of crosslinking strategies and highlight how tailoring the method to the polymer system and end-use application can yield optimized functional properties.

While these mechanisms are well established for purified proteins and polysaccharides, heterogeneous systems such as DDGS are less understood. The presence of residual lipids, fibers, and fermentation-induced protein modifications may alter intermolecular interactions and film-forming behavior, necessitating targeted investigation.

1.4 Manufacturing Technologies

1.4.1. Solvent Casting

The casting method is the most widely used technique for film formation at laboratory and pilot scales. Solvent casting produces uniform films through solvent evaporation (Suhag et al., 2020). The selected polymer is dissolved or dispersed in a suitable solvent; this step is referred to as solubilization (Koide et al., 2013). The prepared solution is transferred into a predefined mold or onto non-stick surfaces (V. Gupta et al., 2022). During the drying stage, sufficient time is allowed for the solvent to evaporate, resulting in the formation of a polymer film attached to the casting surface (Sharma et al., 2024). Since food-derived materials are sensitive to high temperatures, conducting casting method under relatively mild thermal conditions is advantageous (Nair et al., 2023). However, solvent-cast films often exhibit semi-crystalline domains that make them brittle and inflexible unless plasticizers are added (Ghosal et al., 2018a).

1.4.2 Extrusion

Extrusion is a thermomechanical process in which polymeric materials are melted, homogenized, and forced through a die to form continuous sheets or films. (Adak et al., 2025). It is a continuous process in which the polymer is subjected to heat and pressure, causing it to melt and be pushed through a small orifice (Prabha et al., 2021). An important advantage of extrusion is its suitability for large-scale, continuous production with high throughput and relatively low processing time, making it highly attractive for industrial film and packaging manufacturing (Giannakas, 2024). However, the disadvantage of extrusion processing is that the high temperature, pressure, and shear conditions involved can promote the degradation of heat-sensitive active compounds and their chemical stability (Del Nobile et al., 2009).

1.4.3. Coating

Coating refers to the deposition of a polymer solution or a formulation containing multiple components onto a surface, typically defined as the substrate (H. Chen et al., 2019). It differs from solvent casting mainly in the mode of application, as the coating process focuses on applying a formulated layer onto an existing surface rather than forming a standalone film through bulk solvent evaporation (Senturk Parreidt et al., 2018). Biodegradable coatings can act as semi-permeable barriers, reduce dehydration and delay senescence in fruits and vegetables without altering their intrinsic properties (Jama et al., 2026). Therefore, biopolymer-based films incorporating functional components are considered promising alternatives to conventional packaging for fresh produce preservation. While biodegradable coatings offer an environmentally friendly alternative to conventional petroleum-based packaging, they possess

several functional and economic disadvantages depending on their source material. For instance, starch-based coatings show brittle behavior and high water sensitivity (Nawab et al., 2017).

Table 1.1 Comparison of commonly used film fabrication techniques and their advantages and limitations reported in previous studies.

Fabrication Method	Process Description	Advantages	Limitations	References
Solvent casting	Polymer dissolved in a solvent, cast on a flat surface, and dried by evaporation to form a continuous film.	Simple laboratory method, uniform thickness, suitable for heat-sensitive biopolymers.	Low scalability; residual solvent issues, brittle films without plasticizers.	(Dirpan et al., 2023)
Extrusion	Thermoplastic biopolymers melted and forced through a die to form sheets; solidify upon cooling.	Industrially scalable, good control over thickness and density.	High processing temperature may denature proteins or degrade actives, limited to thermoplastics.	(Pawde et al., 2025)
Compression molding	Biopolymer powder or blend pressed under heat and pressure in a mold cavity.	Rapid production of thick, uniform films, no solvent required.	Restricted to heat-stable polymers; limited orientation control; poor	(Adak et al., 2025)

Fabrication Method	Process Description	Advantages	Limitations	References
			compatibility for active incorporation.	
Electrospinning	High voltage applied to polymer solution, charged jet forms nanofibers collected as a porous mat.	High surface area; tunable porosity; enhanced flexibility and plasticity even without plasticizers; enables encapsulation of sensitive compounds.	Sensitivity to humidity and temperature.	(Ghosal et al., 2018a)

Note. Information adapted from (Dirpan et al., 2023b), (Pawde et al., 2025), (Adak et al., 2025) and (Ghosal et al., 2018)

1.4.4 3D Printing

Three-dimensional (3D) printing is an emerging fabrication method in food packaging that enables the precise design and production of customized structures using biopolymers and other food-grade materials (Leontiou et al., 2023). This technique allows controlled deposition of materials layer by layer, making it possible to tailor package geometry, porosity, and functional properties (Jonkers et al., 2022). As a result, 3D printing shows strong potential for developing innovative, biodegradable, and application-specific food packaging systems. However, a major disadvantage of many 3D-printed products is their limited ability to tolerate post-processing

steps, as cooking losses and shrinkage can distort or destroy the intricate printed structure (Singhal et al., 2020).

Even though advanced fabrication approaches such as three-dimensional (3D) printing and precision coating methods are considered innovative in the development of biodegradable and functional packaging materials, fiber-based structuring technologies have recently gained attention because they enable the formation of micro- and nanoscale fibrous layers with high surface area and controllable structure (Reneker & Yarin, 2008; Witek-Krowiak et al., 2024). Electrospinning and its advanced configuration, coaxial electrospinning, have gained increasing attention as high-precision techniques for producing micro- and nanostructured films (Alli et al., 2025).

1.5 Electrospinning

Electrospinning is based on electrohydrodynamic principles, where charge accumulation and Coulombic repulsion within a polymer droplet under a strong electric field overcome surface tension and drive jet formation (Cho et al., 2025). It is a fiber production method where a high voltage is applied to a polymer solution that solidify into nanofibers. Electrospinning can produce defect-free fibers with uniform morphology when solution parameters such as conductivity and viscosity are carefully optimized (Y. Zhang et al., 2024a).

The electrospinning process begins with a polymer solution held in a syringe fitted with a metallic needle (Naeim et al., 2025). When a high-voltage electric field is applied, typically 10–30 kV, the polymer droplet at the tip of the needle becomes charged. As the voltage increases, electrostatic repulsion overcomes the surface tension of the liquid, causing the droplet to deform into a conical shape known as the Taylor cone (Ahmadi Bonakdar & Rodrigue, 2024a). The

clearest demonstrations of this phenomenon is to show how the Taylor cone geometry directly influences jet stability and fiber diameter (Taokaew & Chuenkaek, 2024). When the applied electric force exceeds the surface tension of the polymer solution, a charged jet is ejected from the tip of the cone and accelerates toward the grounded collector. As the jet travels through the electric field, it undergoes stretching, bending, and whipping instabilities, leading to significant elongation and solvent evaporation (Xue et al., 2019).

During this process, the polymer chains become progressively oriented, and the solvent rapidly evaporates, causing the jet to solidify into continuous nanofibers before deposition. The resulting fibers interlace randomly or in an aligned fashion depending on the collector type and processing parameters, forming a nonwoven mat (Torres-Giner, 2011). This electrohydrodynamic spinning process enables precise control over fiber morphology, diameter, and porosity key structural features that influence the mechanical strength, permeability, and overall performance of biodegradable films (Ramakrishna et al., 2006). For example, electrospinning can produce smooth continuous nanofibers, beaded fibers, ribbon-like fibers, or core–shell structured fibers depending on formulation and setup (Zander, 2013). Fiber diameters can be tuned from micrometer-scale fibers down to nanofibers in the 100–800 nm range commonly reported for protein and polysaccharide systems (Choi et al., 2004). Electrospun mats typically exhibit highly interconnected pore networks or surface porosity under controlled humidity conditions (Mailley et al., 2021).

Electrospinning offers unique advantages for biodegradable film development, such as high surface area-to-volume ratios, tailored mechanical properties, and the ability to encapsulate thermally sensitive bioactives like essential oils or antioxidants without degradation (Agarwal et al., 2013). Electrospun films are attractive for packaging because they can carry sensitive

bioactive compounds like essential oils without degradation (Wen et al., 2016). Many studies show that electrospinning films preserve antimicrobial or antioxidant activity better than films made by casting (Y. Zhang et al., 2024).

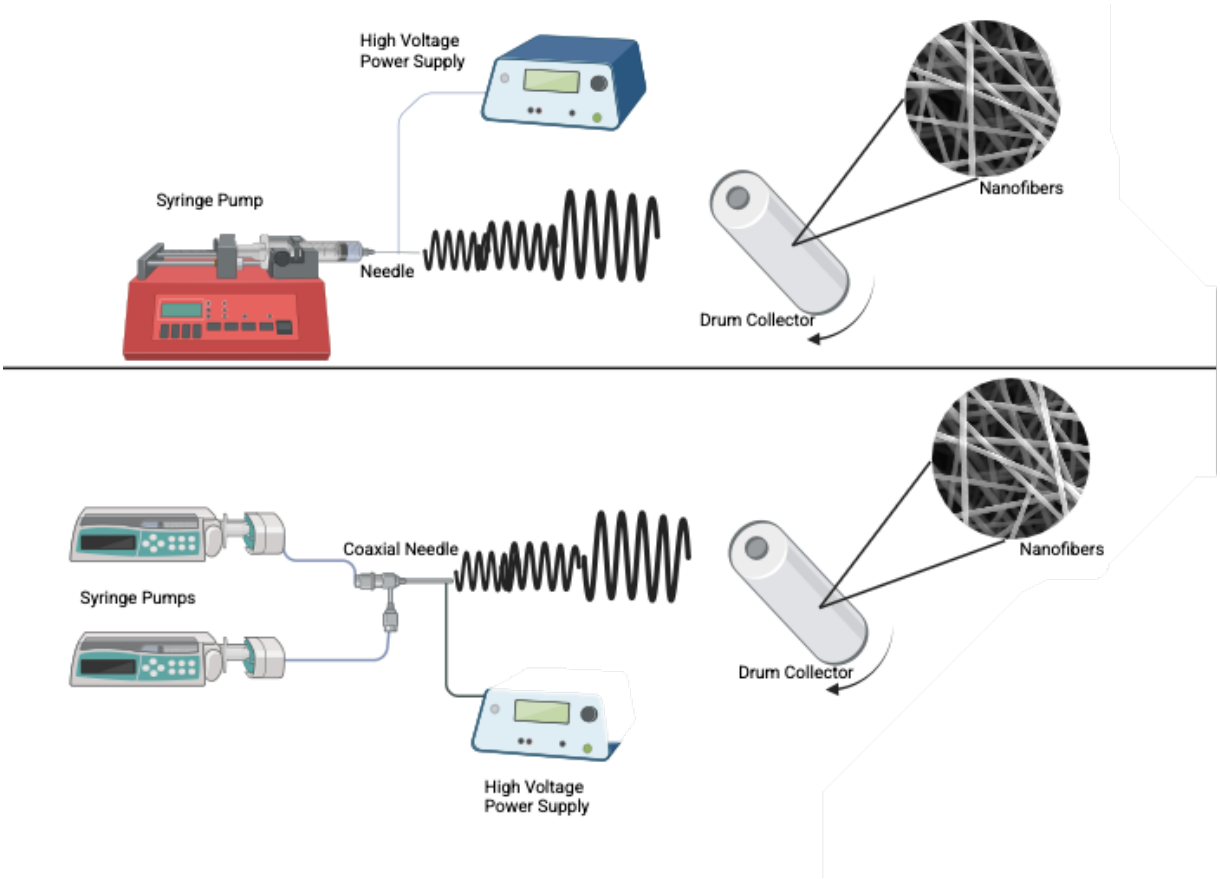


Figure 1.1 Electrospinning and Coaxial Electrospinning Process
The SEM images were obtained from the Nanoscience Instruments Electrospinning Image Gallery (Nanoscience Instruments, n.d.).

Coaxial and emulsion electrospinning can be done using a co-axial needle. This enables core–shell fiber nanostructures which are highly suitable for the encapsulation, protection, and controlled release of sensitive bioactive compounds in food packaging and biomedical systems (Yoon et al., 2018).

In coaxial electrospinning, two concentric fluids are simultaneously processed to form fibers with a distinct core and shell. For example, this configuration has been used to load orange essential oil in a zein sheath, producing antimicrobial fibrous membranes with controlled release suitable for extending the shelf life of fresh produce and other perishable (Reneker & Yarin, 2008). Coaxial electrospinning also improves processability when the core material alone is not electrospinnable (Aghababaei et al., 2024). A spinnable shell polymer can act as a carrier matrix, enabling the incorporation of low-molecular-weight, low-viscosity, or highly conductive core solutions that would otherwise form droplets instead of fibers (Rajanna et al., 2022). This is especially relevant for food by-product extracts or lipid fractions, where shell polymers such as pullulan, PVA, or proteins provide jet stability and fiber continuity (Tavana et al., 2023). Pullulan is frequently used in electrospinning due to its good spinnability and ability to form uniform, continuous nanofibers. Its flexible polymer chains and sufficient molecular weight support stable jet formation and effective encapsulation of active compounds (Ai et al., 2024). For example, pullulan provides the necessary chain length, while zein and sunflower lecithin act as mediators to stabilize the extract within the fiber through hydrogen and hydrophobic interactions (Mandura Jarić et al., 2025a). Thus, it's been highlighted that coaxial electrospinning as a versatile approach for enhancing encapsulation efficiency, stability, and controlled release of bioactive compounds in active packaging, while recent work demonstrates its use in starch-based and protein–PVA nanofiber coatings for meat freshness monitoring and antioxidant protection (Luraghi et al., 2021; Torres-Giner, 2011).

1.5.1 Polymer Selection and Solution Properties in Electrospinning

The choice of polymer and the properties of the solution, including viscosity, conductivity, and surface tension, can influence fiber formation (Frenot & Chronakis, 2003).

Biopolymers like zein, gelatin, chitosan, and starch derivatives are commonly used. They are often require blended with synthetic polymers or surfactants to optimize spinnability (C. Zhang et al., 2020). These additives improve spinnability by increasing chain entanglement and stabilizing the electrospinning jet, which helps produce continuous, bead-free fibers (Ramakrishna et al., 2006).

The key solution properties such as viscosity, electrical conductivity, and surface tension, must be optimized to ensure fiber continuity and structural integrity (Abdulhussain et al., 2023a). Viscosity affects the polymer chain entanglement necessary for stable fiber formation; low-viscosity solutions tend to produce beads or droplets, while excessively viscous ones may clog the needle (Tiwari & Venkatraman, 2012). The ideal solution viscosity typically ranges between 0.1 and 2.0 Pa.s, and polymer concentration should exceed 2.5 times the critical entanglement concentration (Sundera Murthe et al., 2019). Conductivity is essential for generating sufficient charge within the solution, influencing jet acceleration and elongation (Reneker & Yarin, 2008). Ionic additives like acetic acid or salts are often introduced to improve conductivity (Shenoy et al., 2005). Additionally, surface tension controls the formation of the Taylor cone and jet ejection; lower surface tension facilitates jet initiation but may increase bead formation if not counterbalanced by viscosity (Rosell-Llompart et al., 2018). The balance among these three parameters, viscosity, conductivity, and surface tension, is crucial for producing uniform, bead-free nanofibers suitable for high-performance film applications (Torres-Giner, 2011).

1.5.1.1. Emulsion Systems and Their Role in Electrospinning Applications

Emulsions are thermodynamically unstable colloidal systems consisting of two immiscible phases, typically oil and water, where one phase is dispersed as droplets within the other with the aid of emulsifying agents. Depending on the phase distribution, emulsions are

commonly classified as oil-in-water (O/W), water-in-oil (W/O), and more complex systems such as multiple emulsions (e.g., W/O/W), which enable encapsulation and controlled release of active compounds (Lam & Nickerson, 2013). In electrospinning applications, O/W emulsions are particularly preferred for incorporating hydrophobic bioactive compounds into hydrophilic polymer matrices (Wen et al., 2017). The stability and characteristics of emulsions are strongly influenced by the type and concentration of emulsifier, as well as the physicochemical properties of the continuous phase. Non-ionic surfactants such as Tween 20 provide high hydrophilicity and steric stabilization, leading to smaller droplet size and improved emulsion stability, which in turn enhances fiber uniformity during electrospinning (Prodromidis et al., 2024). In contrast, natural emulsifiers such as lecithin may introduce variability due to their complex composition but can contribute to biocompatibility (Thy et al., 2025).

1.5.2 Processing Parameters Affecting Fiber Morphology

The key parameters such as applied voltage, tip-to-collector distance, and flow rate directly affect fiber morphology (Chinnappan et al., 2022). High voltages enhance fiber stretching, while controlled flow rates and appropriate environmental conditions such as humidity and temperature contribute to uniform fiber structures (Ahmadi Bonakdar & Rodrigue, 2024b). The voltage applied to the system influences the intensity of the electric field; higher voltages typically produce finer fibers due to increased elongation forces, but excessively high voltages may induce instabilities or lead to fiber fusion (C. Zhang et al., 2020).

The flow rate of the polymer solution must be finely controlled to match the rate of solvent evaporation, a slow and steady flow favors uniform fiber deposition, whereas high flow rates may result in beads or incomplete drying (Z.-M. Huang et al., 2003). The distance between

the spinneret and the collector, typically ranging between 10 and 20 cm, determines the duration available for fiber elongation and solvent evaporation; short distances can cause fiber wetting, while long distances can reduce deposition efficiency (Baji et al., 2010). Moreover, the type of collector influences fiber alignment and structure: flat stationary collectors yield randomly oriented mats, while rotating drum collectors or patterned electrodes can create aligned or multilayered fiber configurations (Baji et al., 2010).

Environmental factors such as relative humidity and ambient temperature further affect fiber drying, crystallinity, and surface morphology (Ahmadi Bonakdar & Rodrigue, 2024b). For instance, high humidity can delay solvent evaporation and lead to pore formation or fused fibers, whereas low humidity supports smooth, dry fibers with more consistent diameters (Y. Zhang et al., 2024a). The interplay of these parameters must be carefully arranged to achieve optimal films for specific food or biomedical applications.

1.5.3 Electrospun Films in Food Packaging and Biomedical Applications

Electrospun biopolymer films possess a unique nanofibrous architecture characterized by high surface area, tunable porosity, and fiber-level customizability (Abdulhussain et al., 2023b). These properties make them highly attractive for advanced applications such as active food packaging, antimicrobial wraps, enzyme immobilization, and controlled release systems enabling the encapsulation of heat- and oxidation-sensitive compounds such as essential oils, polyphenols, vitamins, and antimicrobial peptides without significant degradation (Topuz & Uyar, 2024). The porous nanofiber network also supports the controlled release of these actives, which can be designed to respond to pH, moisture, or microbial activity, making electrospun films excellent for smart packaging systems (Liang et al., 2025). For example, electrospun zein and chitosan

nanofibers incorporating thymol or cinnamaldehyde have been shown to extend shelf life and reduce microbial growth on perishable products (Ansarifar & Moradinezhad, 2022).

1.5.4 Comparison of Conventional and Electrospun Biopolymer Films

While casting and extrusion are effective for certain food packaging applications, these conventional techniques lack the microstructural complexity and surface area enhancement offered by electrospinning (Zhang et al., 2020; Y. Zhang et al., 2024). They are also limited in their ability to incorporate and control the release of functional compounds, particularly heat-sensitive bioactives, which are often degraded under the high temperatures and mechanical stress of extrusion (Hadidi et al., 2022). In contrast, electrospun films consist of interconnected nanofibers, which form highly porous, light mats that support the loading of sensitive compounds and allow for tunable release kinetics (Yoon et al., 2018). Electrospun nanofiber layers added into bilayer films can help reduce water vapor and oxygen permeability and at the same time improve mechanical strength, mainly because the fibrous network creates more tortuous diffusion pathways and a tightly interconnected structure (Navaei et al., 2025). Structural modifications such as crosslinking can further enhance barrier performance, with reductions in water vapor permeability reported for genipin-crosslinked nanofibers (Kildeeva et al., 2020). Nonetheless, electrospinning faces scalability challenges, including low throughput and sensitivity to environmental conditions, which limit its industrial adoption (Maduna & Patnaik, 2024). Moreover, production is strongly influenced by factors such as ambient humidity, which can cause bead formation and inconsistent fiber morphology (Islam et al., 2025). Despite these obstacles, the superior functionality and customization potential of electrospun films make them promising materials for advanced applications, particularly in active packaging systems where controlled release is required (Cho et al., 2025b).

1.6 Types of Biodegradable Films

Biodegradable films are typically classified based on the natural macromolecules from which they are derived: proteins, polysaccharides, lipids, or a combination (R. K. Gupta et al., 2022). Each of them brings unique physicochemical and functional characteristics suited to specific applications in food packaging. The effectiveness and versatility of these films are largely influenced by their biopolymer composition and processing methods (Siracusa et al., 2008).

1.6.1 Protein-Based Films

Protein-based films offer good oxygen barrier properties, moderate mechanical strength, and high transparency when compared to films made from polysaccharide and lipid-based films (Y. A. Shah et al., 2023). They are derived from animal or plant proteins, including gelatin, whey, casein, soy protein isolate, and zein (Kaewprachu et al., 2016). These films form via denaturation and reaggregation of protein chains, leading to strong intermolecular interactions such as hydrogen bonding and hydrophobic interactions (Atef et al., 2015).

Protein-based film-forming materials are produced through a combination of recovery, extraction, and modification techniques that transform raw biological sources into functional biopolymers suitable for film applications (Purewal et al., 2023). These proteins are primarily obtained from agro-industrial streams using methods such as alkaline or acid extraction, isoelectric precipitation, filtration, and drying to isolate concentrated protein fractions (H. Chen et al., 2019). Depending on the source, proteins may also be valorized from by-products, such as gelatin from collagen-rich animal residues (skin and bones), whey protein and casein from dairy

processing streams, and soy protein isolate and zein from oilseed and corn processing by-products (Álvarez-Castillo et al., 2021; Lu et al., 2025; R. Zhang et al., 2024).

Gelatin is a protein obtained by the partial hydrolysis of collagen extracted from animal connective tissues such as skin, bones, and tendons through acid or alkaline processing followed by thermal extraction (Haug & Draget, 2009). Gelatin films are especially notable for their film-forming capacity and flexibility. However, they exhibit high water sensitivity due to the abundance of hydrophilic groups in their amino acid composition, which limits their use in high-moisture foods unless blended with polysaccharides, lipids, or crosslinking agents (Said & Sarbon, 2022).

Whey protein is a globular protein fraction obtained as a by-product of cheese production from milk, typically isolated through filtration and drying processes (Gangurde et al., 2011). Whey protein films exhibit good oxygen barrier properties under low humidity conditions due to their dense protein network structure (Ramos et al., 2012). In addition, their amphiphilic nature allows the formation of matrices capable of incorporating bioactive compounds (Oymaci & Altinkaya, 2016). For instance, whey protein films containing oregano essential oil exhibited prolonged antimicrobial activity against E. coli and Listeria monocytogenes, while maintaining desirable transparency and flexibility (Vásconez et al., 2009).

Zein, a hydrophobic prolamin, which is a class of alcohol-soluble storage proteins found in corn, can form films with good moisture resistance, though they are often brittle unless plasticized (Alsadat-Seyedbokaei et al., 2025). Zein is widely used in coatings for nuts, confectionery, and pharmaceuticals because of its inherent hydrophobicity (Poluri et al., 2025). Plasticizers such as glycerol, polyethylene glycol, or sorbitol are commonly incorporated to reduce brittleness, increase elongation at break, and improve flexibility, though they may reduce

barrier properties. Studies have also reported that blending zein with polysaccharides like pullulan or starch further improves flexibility without compromising its hydrophobic performance (Lawton, 2004).

Casein is the predominant milk protein and naturally exists as casein micelles, supramolecular aggregates of αs1-, αs2-, β- and κ-casein stabilized by colloidal calcium phosphate in an aqueous milk serum (Holt et al., 2013). These micelles exhibit a flexible, amphiphilic structure with polar phosphate groups and hydrophobic domains, enabling strong intermolecular interactions and barrier properties against non-polar molecules (Huppertz et al., 2017). However, due to their limited spinnability, casein is typically electrospun in combination with carrier polymers such as polyethylene oxide (PEO) or polycaprolactone (PCL), which improve solution viscoelasticity and enable fiber formation. For example, electrospun casein/PEO nanofibers containing 10–20 wt% PEO exhibited uniform fiber morphology and enhanced mechanical properties, with tensile strength increasing from 0.91 to 1.88 MPa upon crosslinking with tannic acid (Flores-Nieves et al., 2022). Therefore, casein is widely applied in coatings, adhesives, packaging, textiles, and biomedical materials (Yang et al., 2024).

Sodium caseinate (SC), the water-soluble form of casein, produces films and nanofibers with increased mechanical strength, hydrophilicity and film-forming ability. The film-forming ability of SC can be attributed to its molecular structure and functional group composition. SC contains multiple functional groups capable of hydrogen bonding and covalent crosslinking, which allows it to form uniform networks when processed into films or blended with other polymers (Şendil et al., 2023).

Kafirin, the major storage prolamin of sorghum, has attracted interest as a film-forming biopolymer due to its hydrophobicity, high glass transition temperature, and ability to form

cohesive matrices through disulfide bonding and hydrophobic interactions (Lisitsyn et al., 2021). Early work on films from sorghum kafirin demonstrated transparent, uniform structures with oxygen barrier properties and good compatibility with plasticizers (Muhiwa et al., 2017). Kafirin films containing citral and quercetin significantly reduced lipid oxidation and microbial growth in chilled chicken fillets, indicating that kafirin-based edible films can simultaneously improve safety and extend shelf life of perishable foods. These films were prepared by dissolving kafirin in aqueous ethanol with glycerol as a plasticizer, followed by the addition of citral and quercetin into the solution, which was then cast and dried to form uniform and flexible films (Lou et al., 2021).

1.6.2 Polysaccharide-Based Films

Polysaccharide-based films are the most widely studied biopolymer systems relative to protein and lipid-based films due to their abundance, and film-forming properties. Common polysaccharides include cellulose, starch, alginate, pectin, chitosan, and gums (Benalaya et al., 2024). Polysaccharide based films are typically hydrophilic and offer greater gas barriers but limited moisture resistance (Y. Zhao et al., 2021). Their structural diversity and chemical modifiability allow researchers to tailor film properties through functionalization or blending with other polymers (Baniasadi et al., 2026).

The structural diversity and chemical modifiability of polysaccharides arise from variations in their monosaccharide composition, glycosidic linkages, branching degree, and molecular weight, which result in a wide range of physicochemical and functional properties (Elango et al., 2023). For instance, linear polysaccharides such as cellulose exhibit strong intermolecular hydrogen bonding and crystalline regions, contributing to high mechanical strength, whereas branched structures such as starch or pectin provide greater flexibility and

film-forming ability (Ai et al., 2024; V. Kumar et al., 2014; Nawab et al., 2017). In addition, the presence of reactive functional groups, including hydroxyl (–OH), carboxyl (–COOH), and amino ($–NH_2$) groups, enables chemical modification through reactions such as esterification, etherification, oxidation, and crosslinking, allowing precise tuning of hydrophobicity, mechanical strength, and barrier properties (X. Wang et al., 2020). For example, acetylation of starch has been shown to improve water resistance and flexibility by reducing intermolecular hydrogen bonding and increasing hydrophobic character (C. Zhang et al., 2020).

Starch is a naturally occurring polysaccharide composed of amylose (linear) and amylopectin (branched) fractions, primarily found in plant storage organs such as cereal grains (e.g., corn, wheat, rice), tubers (e.g., potato), and roots (e.g., cassava) (Whistler & Daniel, 2000). Electrospinning of starch is inherently challenging due to its limited solubility, strong intermolecular hydrogen bonding, and insufficient chain entanglement, which often necessitate the use of carrier polymers, or crosslinking agents to achieve stable fiber formation (Hemamalini & Giri Dev, 2018). However, it has been demonstrated that pure starch fibers can be produced by carefully selecting appropriate solvent systems; for instance, native high-amylose starch has been successfully electrospun by dissolving starch (15% w/w) in 95% aqueous dimethyl sulfoxide (DMSO), enabling adequate chain mobility and fiber formation without the need for additional polymers (L. Kong & Ziegler, 2014). The type of starch source, such as corn, potato, or cassava, strongly influences film characteristics due to differences in amylose-to-amylopectin ratios, crystallinity, and retrogradation behavior (Karnwal et al., 2025).

Cellulose is a linear, high-molecular-weight polysaccharide composed of β-(1→4)-linked D-glucose units, forming a highly ordered structure stabilized by extensive intra- and intermolecular hydrogen bonding. Cellulose films are also thermally stable and transparent,

which makes them useful for both food packaging and pharmaceutical applications (Azeredo et al., 2017). However, electrospinning cellulose remains challenging due to its limited solubility in conventional organic solvents, which restricts adequate chain mobility and solution processability; chemically or enzymatically derivation is often required (Frenot et al., 2007).

Methylcellulose (MC) is a non-ionic, water-soluble cellulose derivative produced by substituting hydroxyl groups in cellulose with methoxy groups (Nasatto et al., 2015). MC solutions exhibit thermal gelation behavior and good film-forming capacity, which makes it suitable for edible coatings, biodegradable packaging, and electrospinning applications (L. Zhang et al., 2023). MC can be successfully electrospun into uniform nanofibers using aqueous ethanol systems, producing homogeneous nonwoven fiber mats with consistent morphology (Frenot et al., 2007). Also methylcellulose-based coatings have been successfully applied to fruits and vegetables, extending shelf life by slowing down respiration and moisture loss (Ramakrishnan et al., 2024).

Carboxymethyl cellulose (CMC) is an anionic cellulose ether derived by substituting hydroxyl groups with carboxymethyl groups, which enhances its solubility and enables the formation of viscous solutions with sufficient chain entanglement required for electrospinning for increasing hydrophilicity and film-forming ability (Hashmi et al., 2020; Rahman et al., 2021). However, due to CMC's high charge density and insufficient chain entanglement, CMC alone is difficult to electrospin, and a carrier polymer such as polyethylene oxide (PEO) is typically required to stabilize the jet and maintain viscosity (Tongdeesoontorn et al., 2011). When blended with PEO, CMC produces continuous nanofibers, but the resulting fiber morphology is strongly dependent on the substitution pattern of the CMC molecule rather than on molecular weight or degree of substitution (Basu et al., 2017).

Chitosan is a cationic polysaccharide obtained by the deacetylation of chitin, primarily sourced from crustacean shell waste, and is characterized by the presence of amino ($–NH_2$) groups that confer antimicrobial activity (Aranaz et al., 2021). Chitosan's cationic nature allows electrostatic interactions with negatively charged bacterial membranes, leading to cell leakage and growth inhibition (M. Kong et al., 2010). These properties make chitosan-based films particularly suitable for highly perishable foods such as fresh meat, poultry, seafood, and fresh-cut fruits, where microbial spoilage is a major concern (No et al., 2007). For example, chitosan-coated chicken fillets have been reported to significantly reduce microbial growth and extend shelf life from approximately 5–7 days to 10–14 days under refrigerated conditions, depending on formulation and storage parameters (Dutta et al., 2009). In addition, chitosan films enriched with natural extracts such as essential oils or plant polyphenols exhibit synergistic antimicrobial and antioxidant activity, further broadening their applications in active packaging (S. Kumar et al., 2020).

Pullulan is a linear, water-soluble polysaccharide produced by *Aureobasidium pullulans*, composed of maltotriose units linked through α-(1→6) glycosidic bonds (Singh et al., 2008). Pullulan exhibits cohesive and uniform films due to its flexible molecular chains and strong intermolecular hydrogen bonding, producing clear, glossy, and highly transparent films (Farris et al., 2014). Pullulan films are known for very low oxygen permeability because of their dense molecular packing and lack of hydrophobic regions in the polymer backbone (Mandura Jarić et al., 2025a). Specifically, increasing the concentration of pullulan, moving from an 80:20 to a 50:50 amaranth-pullulan ratio, resulted in the formation of thicker, more uniform, and non-beaded fibers while facilitating a more rapid initial release of the encapsulated curcumin due to the dissolution properties of the carbohydrate polymer (Blanco-Padilla et al., 2015). Also,

pullulan acts as a protective "wall material" for sensitive bioactive compounds like astaxanthin, making astaxanthin's radical scavenging rate dropped by over 41% under UV exposure, the pullulan-based hybrid fiber only saw a 14% decrease, demonstrating superior stability (Ai, 2024). Other than it’s physical properties, it maintains considerable intermolecular mechanical strength and possesses a thermal cracking temperature of approximately 305°C to 326°C, indicating robust stability for various processing conditions such as high-voltage (22 kV to 27 kV) electrospinning and continuous stirring at temperatures between 60°C and 70°C (Ponrasu et al., 2021).

1.6.3 Lipid-Based Films

Lipids are a diverse group of hydrophobic or amphiphilic biomolecules, including fats, oils, waxes, and phospholipids, primarily composed of long-chain hydrocarbons and characterized by their low polarity and limited solubility in aqueous systems (Turchini et al., 2022). Lipid-based films primarily consist of waxes, fatty acids, or oils, which impart good moisture barrier properties due to their hydrophobic nature (Mohamed et al., 2020). Despite their functional advantages in providing moisture barrier properties, lipids are generally not suitable as primary materials for electrospinning because they are unstable when used as for sustained release matrix (Hai et al., 2019). However, some studies have shown that at sufficiently high phospholipid concentrations, continuous fiber formation can be achieved. Additionally, the choice of solvent plays a critical role in determining fiber morphology and diameter; for example, the use of cyclohexane with azolectin, a soybean-derived phospholipid, resulted in fibers with diameters of approximately 4–5 μm, whereas the use of limonene produced significantly thicker fibers with diameters around 14.3 μm. (Jørgensen et al., 2015).

Lipid-based components such as beeswax, candelilla wax, carnauba wax, and stearic acid are especially useful in preserving the moisture content of high-water-activity foods such as fruits, vegetables, and meats by reducing water vapor transfer and slowing dehydration (Devi et al., 2024). For example, carnauba-shellac wax coated apples demonstrated reduced weight loss and extended storage life compared to uncoated controls, highlighting their effectiveness as edible coatings (Jo et al., 2014). However, lipid-based films often show weak adhesion to hydrophilic substrates and tend to be brittle, so they are commonly blended with hydrocolloids or formulated as emulsified systems to improve film uniformity and flexibility (Hassan et al., 2018).

1.6.4 Composite Films (Blended and Multi-Layered Biopolymers)

Composite films integrate two or more types of biopolymers to capitalize on the strengths of each and overcome their weaknesses (Q. Wang et al., 2022). These films can be designed either as homogeneous blends, where polymers are mixed at the molecular level, or as structured multilayer laminates, where each layer serves a specific function, such as a protein layer for oxygen barrier and a lipid layer for moisture resistance or infusion complex functional ingredient (Xu et al., 2024). Moreover, composite films improve flexibility, mechanical strength, and overall stability, making them one of the most promising approaches in biopolymer film development (Alias et al., 2022).

Blended composite films are formed by combining two or more biopolymers to overcome the limitations of individual components and achieve improved overall performance (Roy et al., 2024). These systems rely on polymer–polymer interactions, such as hydrogen bonding and electrostatic interactions, to create more cohesive and stable film matrices. For example, blending corn strach with polyvinyl alcohol (PVA) has been shown to increase tensile strength

93% compared to pure starch films (Patil et al., 2021). Also, studies have shown that incorporating chitosan or starch into gelatin films can improve moisture resistance. (Pulla-Huillca et al., 2021). Lipids are often combined with polysaccharides or proteins to produce composite films that balance barrier properties and mechanical integrity (Hassan et al., 2018). Such combinations allow the integration of complementary properties, where one polymer provides structural integrity while the other contributes to film flexibility or barrier performance (Yan et al., 2022).

Multilayer films are designed by assembling two or more distinct layers with complementary properties, enabling functional separation and enhanced overall performance (Haynie et al., 2005). In these systems, each layer can be tailored to provide specific attributes, such as mechanical strength, moisture resistance, or active compound retention (Anukiruthika et al., 2020). Similarly, multilayer films combining gelatin with ethyl cellulose have demonstrated synergistic improvements in thermal stability, water vapor resistance, and bioactive compound retention (Y. Zhao et al., 2021).

The incorporation of active agents such as antimicrobial compounds, antioxidants, or pH-responsive indicators into composite films further broadens their functionality and enables their use in active and intelligent packaging applications (Yildirim et al., 2018). For example, chitosan-based films enriched with oregano or thyme essential oils have shown strong antimicrobial effects against foodborne pathogens, while maintaining mechanical strength (Gaba et al., 2022). Recent developments also include nanocomposite films, where nanoparticles such as organo nano clays are incorporated to chitosan improve antimicrobial activity (Alias et al., 2022). Such hybrid systems illustrate how blending and layering strategies enable the design of multifunctional films that go beyond passive protection, offering responsive and value-added

functionalities. The performance of these biopolymers is strongly dependent on processing method and compositional uniformity, particularly when derived from agro-industrial by-products.

1.7 Characterization of Biodegradable Films

The performance of biodegradable films is usually evaluated using a range of characterization techniques that provide information about their strength, stability, and structural properties. These evaluations are essential to determine whether biopolymer films can be used as an alternative to conventional plastics in packaging applications.

1.7.1 Mechanical Properties

Mechanical performance is one of the most critical quality attributes of biodegradable films, since tensile strength, elongation at break, and elastic behavior determine handling resistance, flexibility, and end-use durability which are essential for maintaining structural integrity during processing as well as for protecting food products throughout their shelf life (Siracusa et al., 2008). Tensile strength is determined as the maximum stress the film can withstand before breaking, calculated by dividing the maximum force by the cross-sectional area of the sample (Behera & Hari, 2010). Elongation at break represents the percentage increase in length at the point of rupture, indicating film flexibility (Y. Zhu & Wu, 2023). Elastic behavior, often expressed as Young's modulus, is obtained from the slope of the initial linear region of the stress–strain curve and reflects the stiffness of the material (Ashby et al., 2019). These measurements are commonly performed using a universal testing machine under controlled conditions following standardized methods such as ASTM D882 (Saba et al., 2019).

Protein-based films generally tend to have better mechanical strength than polysaccharide films, since protein chains can build more compact intermolecular networks through hydrogen bonding, hydrophobic interactions, and covalent crosslinks (Khin et al., 2024). For example, whey protein isolate (WPI) films have demonstrated high tensile strength and improved elasticity relative to single-component polysaccharide films (Yoo & Krochta, 2011). Similarly, gelatin/zein nanofibers display good film-forming ability with the improved resistance against water or ethanol, compared to the pure gelatin or zein nanofibers (C. Zhang et al., 2020).

Polysaccharide-based films such as starch and alginate are often brittle due to limited chain mobility and weak intermolecular cohesion (Hassan et al., 2018). Therefore, plasticizer incorporation, protein blending, and chemical crosslinking are frequently applied to improve flexibility and toughness (Silva et al., 2009).

In agro-industrial byproduct films, protein structural integrity plays a major role in mechanical behavior. Films prepared from DDGS protein fractions displayed lower tensile strength compared to wheat gluten films, indicating that the proteins have a reduced ability to establish inter- and intra-molecular interactions, such as disulphide cross-links and hydrogen bonds, which are essential for stabilizing the gluten network (Prabhakumari et al., 2018).

Electrospun biopolymer materials behave mechanically differently than cast films, mainly because they consist of a porous fiber network with a very high surface area instead of a dense continuous structure (Ghosal et al., 2018b). As a result, electrospun mats generally show lower tensile strength than cast films, since the presence of void spaces and limited inter-fiber bonding reduces effective stress transfer across the structure. However, they can exhibit higher elongation at break, as individual fibers are able to rearrange, slide, and redistribute stress under tensile loading (S. Agarwal et al., 2013b). For instance, protein–polysaccharide electrospun

systems have shown tunable elasticity and toughness through formulation control and solvent selection (X. Huang et al., 2025; Ramakrishnan et al., 2024). In electrospun protein-based fibers, denaturation and molecular unfolding during solution preparation strongly influence chain entanglement and final mechanical performance (Mendes et al., 2017).

1.7.2 Barrier Properties

Barrier properties are essential to control gas and moisture transfer through films (Y. A. Shah et al., 2024). Water vapor barrier performance is determined through water vapor transmission rate (WVTR) and water vapor permeability (WVP) tests (Shogren, 1997). In this method, the film is sealed over a cup containing a desiccant or distilled water to create a controlled humidity gradient, and the change in mass over time is monitored. WVTR is expressed as the amount of water vapor transmitted per unit area and time ($g/m^2 \cdot day$), while WVP is calculated by normalizing WVTR with respect to film thickness and the partial pressure difference across the film, providing an intrinsic material property (Bertuzzi et al., 2007). Gas barrier properties are commonly assessed by measuring oxygen transmission rate (OTR) and oxygen permeability (OP) (Morris, 2017). In this technique, the film separates two chambers, where one side is exposed to oxygen and the other to an inert carrier gas such as nitrogen. The amount of oxygen permeating through the film is quantified using a sensor. OTR represents the oxygen flow per unit area and time ($cc/m^2 \cdot day$), whereas OP accounts for film thickness and pressure difference, reflecting the inherent gas barrier performance of the material (Abdul Khalil et al., 2019).

Polysaccharide-based films generally provide good oxygen barrier capacity but are poor at resisting water vapor due to their hydrophilic nature (Nechita & Roman (Iana-Roman), 2020). This behavior is critical in food packaging, where oxygen barrier properties are essential for

preventing oxidative spoilage, while poor moisture resistance can lead to quality deterioration such as texture softening, microbial growth, and reduced shelf life (Sahraee et al., 2019). Carrageenan films, for example, have low oxygen permeability but require lipid incorporation to reduce water vapor transmission which is particularly important for maintaining crispness and preventing moisture migration in food products. (R. Zhang et al., 2019).

DDGS-derived films have been reported to show relatively high water vapor permeability, which is attributed to heterogeneous composition and disrupted protein networks resulting from ethanol processing (Prabhakumari et al., 2018). This structural irregularity reduces matrix cohesion and facilitates moisture diffusion. Consequently, pretreatment strategies including alkali extraction, acid hydrolysis, and defatting are increasingly investigated to improve network uniformity and moisture barrier behavior in DDGS film systems.

Although single electrospun layers are typically porous and therefore show higher permeability, multilayer electrospun coatings and compressed fiber mats have demonstrated improved oxygen and moisture barrier properties (Cho et al., 2025b). Coaxial electrospinning enables core–shell architectures that incorporate hydrophobic or bioactive cores, enhancing moisture resistance and functional performance (Yao et al., 2017). The developed CP/PCO nanofiber films composed of a chitosan shell and an oregano essential oil (OEO) emulsion core demonstrated significantly improved moisture resistance (H. Wang et al., 2025).

1.7.3 Morphological Analysis

Film structure and morphology are closely linked to functional performance, since parameters such as porosity, thickness, and microstructure directly influence mechanical and barrier properties (Wihodo & Moraru, 2013). Scanning electron microscopy (SEM) is commonly used to assess film surface homogeneity, porosity, and defects (Mendes et al., 2017). Whey

protein-pectin systems, for example, showed rougher surface texture than pectin control films under SEM (Urango et al., 2025). For instance, a blend with gelatin and soy protein isolate containing 30 wt% gelatin shows a much finer and more homogeneous phase morphology compared to blends with 10% or 60% gelatin revealed on SEM analysis (Bai et al., 2013). In DDGS-based films, SEM images revealed rough surfaces with voids, attributed to protein denaturation during ethanol processing (Prabhakumari et al., 2018).

1.8 Sorghum as a Film Component and DDGS Biopolymer Applications

Sorghum, *Sorghum bicolor L.,* the botanical name for sorghum, is an African crop that belongs to the Gramineae family and is high in carbohydrates. Kansas is the US top producer and exporter of sorghum, making it the fifth most significant cereal crop in the world behind wheat, maize, rice and barley . (Kazungu et al., 2023). Besides the use of animal feed and potential human food, sorghum is also used for ethanol production in the United States. Sorghum DDGS is the dry fraction obtained after the fermentation of starch to generate ethanol. After this step, ethanol is separated by distillation, and the wet residual is centrifuged to fractionate solids from solubles. Finally, the solubles are concentrated via evaporation and dried with the solid fractions to obtain DDGS (Li et al., 2011a). The protein content of sorghum DDGS is approximately 30–40%, making it a cost-effective source of protein (Muhiwa et al., 2017).

Although DDGS has been explored as a low-cost biopolymer source, most studies focus on isolated fractions or composite systems, with limited attention to the combined effects of pretreatment, lipid removal, and processing method on film performance. Sorghum and distillers dried grains with solubles (DDGS) are abundant agricultural resources with potential for

biopolymer applications. Their compositions, rich in proteins and polysaccharides, allow them to be explored as low-cost alternatives for biodegradable films.

1.8.1 Chemical Composition and Functional Properties

Sorghum contains high levels of starch and kafirin, a hydrophobic prolamin protein that accounts for 70–90% of its protein content (Qi et al., 2019). Kafirin's hydrophobicity and tendency to form disulfide bonds make it suitable for film formation, though films tend to be brittle unless plasticizers are added (Li et al., 2011a). Sorghum wax, a by-product of ethanol and wet milling, also provides hydrophobic coatings that can serve as water barriers (Qi et al., 2019). DDGS, a co-product of ethanol fermentation, is composed of proteins (27–33%), hemicellulose (20%), cellulose (15%), and lipids (8–12%), along with small amounts of starch and lignin (Buenavista et al., 2021). These macronutrients provide both film-forming capacity and functional properties.

1.8.2 Film-Forming Potential of Sorghum-Based Biopolymers

Sorghum kafirin films have been compared to zein films and were found to have similar tensile strength but lower extensibility and weaker water vapor barriers (De Mesa-Stonestreet et al., 2010). Sorghum starch films are biodegradable and inexpensive but, like other starch-based films, they require modification or blending to reduce brittleness and moisture sensitivity (Qi et al., 2019). Sorghum wax fractions offer opportunities for coating applications, improving water resistance in multilayer packaging systems (Leguizamón et al., 2009).

Sorghum kafirin proteins form films through disulfide bonding and hydrophobic interactions, producing water-resistant but relatively stiff materials. The addition of tannins improve flexibility and antioxidant activity (De Mesa-Stonestreet et al., 2010). Sorghum starch

has also been used to produce films with good transparency, but high moisture sensitivity remains a challenge (De Carvalho et al., 2024). Protein-rich DDGS films by solvent casting show moderate tensile strength but high hydrophilicity, while hemicellulose-rich fractions improve rigidity and gas barrier properties (Prabhakumari et al., 2018). Recent work demonstrated that polysaccharides extracted from DDGS, when blended with polyethyleneimine, resulted in antimicrobial films with improved strength and stability (Galaburri et al., 2025a).

1.8.3 Agricultural and Industrial Availability of Sorghum

Sorghum is one of the world's top five cereals, widely cultivated in Africa, Asia, and the United States. In the U.S., more than 30% of sorghum grain is directed to ethanol production, generating significant amounts of sorghum DDGS as a by-product (Qi et al., 2019). This wide role as both a food and industrial crop ensures a continuous and large-scale supply of sorghum and its coproducts, making them economically attractive feedstocks for biopolymer development.

1.8.4 DDGS in Biopolymer Applications

Research on DDGS utilization in films has focused on both protein and polysaccharide fractions. Protein films prepared by solvent casting from wheat DDGS showed moderate tensile strength but high sensitivity to moisture and elevated water vapor permeability (Prabhakumari et al., 2018). DDGS has also been studied as a bio-filler in composites with synthetic polymers such as PLA and phenolic resins, where it improved biodegradability without substantially compromising strength (K. A. Rosentrater, 2006). More recently, hemicellulose-rich fractions extracted from DDGS were processed into standalone films, highlighting the potential of polysaccharides as a functional film-forming component (Xiang et al., 2014). Pretreatments such

as alkali extraction or enzymatic hydrolysis have been applied to improve solubility, protein dispersibility, and film uniformity (Huda et al., 2021). Composite films containing DDGS polysaccharides and polyethyleneimine demonstrated not only higher tensile strength but also antimicrobial activity, particularly when copper ions were incorporated (Galaburri et al., 2025a). These findings confirm that both chemical and physical modifications can significantly enhance the applicability of DDGS in film production.

1.9 Chemical and Physical Modifications for DDGS

Chemical and solvent pretreatments are widely used to modify the molecular structure and functional behavior of DDGS components before film formation (K. Liu, 2009). Hydrochloric acid treatment partially hydrolyzes protein and polysaccharide structures, reduces aggregation, and improves water solubility, which in turn helps achieve more uniform dispersion and better intermolecular interactions during film formation (Noureddini & Byun, 2010). On the other hand, alkaline pretreatment using sodium hydroxide further promotes protein unfolding and exposes reactive functional groups such as amino, carboxyl, and hydroxyl moieties, improving intermolecular interactions and film cohesion (Cookman & Glatz, 2009; J. Zhao et al., 2021).

Ethanol-based solvent systems, particularly when used with alkali, help remove lipophilic and low-molecular-weight fractions, leading to a more homogeneous film matrix with reduced phase separation (J. Gupta et al., 2016). Ethanol acts as an organic solvent that solubilizes non-polar substances such as residual lipids, pigments, and small hydrophobic molecules, which would otherwise disrupt film continuity (M. Y. Wang et al., 2004). At the same time, alkali treatment promotes protein unfolding and partial solubilization by breaking hydrogen bonds and disrupting intermolecular interactions, increasing the availability of reactive functional groups

(Zaini et al., 2019). Such combined chemical–solvent strategies have been shown to recover protein fractions with improved functional properties and facilitate more homogeneous networks in polymer films (Cookman & Glatz, 2009; U. Shah et al., 2021). These pretreatments are crucial for sorghum DDGS, where fermentation-derived protein conformations and residual oils can otherwise impede coherent film structure formation. For example, kafirin proteins extracted from sorghum DDGS using acetic acid, HCl–ethanol, and NaOH–ethanol treatments exhibited significant differences in extraction yield, purity, and molecular structure (Y. Wang et al., 2009a). Alkaline and acetic acid extraction produced substantially higher protein purity and yield compared with HCl–ethanol extraction. The lower performance of the HCl–ethanol system was attributed to insufficient disruption of denatured protein aggregates and less effective reduction of disulfide bonds at low pH, which limited solubilization of crosslinked proteins (Y. Wang et al., 2009a). The same study reported that pretreatment chemistry influenced secondary structure distribution.

Acidic and alkaline extraction methods yielded protein fractions dominated by α-helix structures, while HCl–ethanol extraction showed increased β-sheet content, due to heat and acid-induced aggregation and coagulation during processing (Y. Wang et al., 2009a). These conformational differences are important because increased β-sheet content is typically associated with stronger intermolecular packing, reduced solubility, and stiffer but more brittle film networks (Kim et al., 2025).

Ball milling acts as a mechanical pretreatment that enhances the film-forming performance of biomass and biopolymer systems without introducing extra chemical reagents (Delogu et al., 2017). Ball milling reduces particle size and disrupts rigid fibrous structures, increasing the specific surface area and improving the dispersion of components in the film-

forming solution (Zheng et al., 2018). This mechanical disruption weakens strong associations between protein and fibrous or lignocellulosic fractions, facilitating more uniform polymer distribution and greater physical entanglement during solvent evaporation (Qu et al., 2017). Ball milling can alter the secondary structure of proteins, exposing more aromatic amino acid residues, reducing the particle size of pumpkin seed protein, and increasing its solubility (Q. Zhang et al., 2024). While direct studies of ball milling on sorghum DDGS-based films are limited, evidence from biomass pretreatment and polysaccharide modification literature demonstrates that ball milling can significantly affect molecular dispersion and functional properties in complex biopolymer systems (He et al., 2023; Qu et al., 2017).

Defatting is an important modification step in many biopolymer-based systems, since residual lipids can interfere with polymer interactions and hinder the formation of uniform and continuous film structures (Alnadari et al., 2023). Residual lipids can form hydrophobic domains that disrupt biopolymer cohesion, promote phase separation, and hinder uniform network formation (Kowalczyk et al., 2024a). Lipid extraction using solvents such as ethanol improves the compatibility between protein and polysaccharide fractions by reducing hydrophobic regions, allowing closer packing of biopolymer chains and stronger intermolecular bonding (Cookman & Glatz, 2009).

The removal of lipids also enhances the accessibility of functional sites and improves wettability within the matrix, contributing to films with improved mechanical performance and reduced heterogeneity in thickness (Kowalczyk et al., 2024b). Studies on DDGS extraction and fractionation support the role of defatting in preparing more homogeneous protein-rich materials with enhanced functional properties suitable for film formation (Cookman & Glatz, 2009; Prabhakumari et al., 2018).

1.10 Obstacles in Biodegradable Films

Although biodegradable films show great potential, several challenges limit their industrial adoption. The main obstacles are associated with scalability, cost, processing issues, and additive compatibility.

1.10.1 Scalability and Cost Limitations

Large-scale industrial production is still constrained by raw material costs and energy-intensive processing requirements (Mohanty et al., 2018). Solvent casting is commonly used in laboratory studies but is not economically viable on a commercial scale (Baidurah, 2022). On the other hand, electrospinning, produces films with excellent properties but has low throughput (Ahmadi Bonakdar & Rodrigue, 2024b). Cost issues are further aggravated by the need for refined or purified proteins and polysaccharides. For instance, whey protein films provide strong barriers but require expensive extraction and processing (Yoo & Krochta, 2011). Agricultural by-products such as DDGS or sorghum offer low-cost feedstocks, but variability in chemical composition across sources reduces consistency and creates additional costs for pretreatment (Buenavista et al., 2021).

1.10.2 Processing Challenges

Processing methods often highlight the limitations of biopolymer systems. Many biodegradable films are sensitive to humidity and temperature, which restricts their application (Romruen et al., 2024). Whey protein films show excellent oxygen barrier properties but lose structural integrity under high relative humidity (Yoo & Krochta, 2011). Starch-based films are inexpensive and widely studied but are brittle and water-sensitive unless plasticizers or blending

agents are added (Mali et al., 2004). Protein–polysaccharide complexes are highly dependent on processing conditions, such as pH and ionic strength, which determine their solubility and phase behavior (Hassan et al., 2018). For example, whey protein–pectin complexes form stable structures only in narrow pH ranges (Urango et al., 2025). Similarly, films derived from DDGS require chemical or enzymatic pretreatment to improve solubility and homogeneity, which adds to the complexity of processing (Huda et al., 2021).

In polysaccharide films, lipids or essential oils added to improve water resistance may cause phase separation and weaken film structure (Y. Zhao et al., 2021). Similar issues occur with protein-based films that incorporation of bioactive compounds or nanoparticles may disrupt the polymer network and lower tensile strength (Ansarifar & Moradinezhad, 2022). DDGS-based films also face challenges with additive compatibility because the native proteins and fibers have heterogeneous molecular structures, complicating the incorporation of stabilizers or plasticizers (Galaburri et al., 2025a).

DDGS composition varies depending on processing conditions, leading to inconsistency in film properties (Belyea, 2004). High water sensitivity and relatively low mechanical strength compared to synthetic polymers further limit application potential (Buenavista et al., 2021). Additionally, defatting, fractionation, or enzymatic treatments are often required, which increases processing complexity and cost (Huda et al., 2021). However, the opportunities are significant: DDGS valorization supports circular economy principles by transforming a low-value bioethanol by-product into packaging materials (Chatzifragkou et al., 2015). With appropriate modifications, such as blending with other biopolymers, crosslinking, or nanofiller incorporation, DDGS films could achieve the mechanical and barrier properties needed for wider application (Galaburri et al., 2025a). Sorghum-based biopolymers, particularly kafirin films and

polysaccharides, also hold potential as renewable resources for packaging, coatings, and agricultural uses. These challenges motivate alternative strategies for utilizing DDGS either as a structural matrix or as a functional extract.

Although extensive research exists on purified biopolymers and electrospun systems, limited systematic work has evaluated agro-industrial by-products such as sorghum DDGS under combined chemical pretreatment, defatting, and mechanical milling conditions, particularly when processed via electrospinning and coaxial electrospinning. The interactions between pretreatment chemistry, compositional heterogeneity, and nanofiber formation remain poorly understood. This gap highlights the need for systematic investigation of DDGS-derived systems under controlled modification and processing conditions.

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Chapter 2 - Knowledge and objectives

Agro-industrial by-products and emerging crops have attracted increasing interest as renewable resources for the development of sustainable food materials. Among these, Distillers' Dried Grains with Solubles (DDGS), a by-product of grain fermentation in ethanol production, represents an abundant and underutilized material rich in proteins, fibers, and residual lipids. These components provide potential functionality to produce biodegradable films and coatings that could serve as alternatives to petroleum-based packaging materials. The goal of this study was to investigate the potential of sorghum DDGS as a renewable source for biodegradable film formation and to understand how different pretreatment strategies influence the structural and functional properties of the resulting materials.

DDGS contains a complex matrix of proteins, polysaccharides, and lipids that can influence film formation behavior. However, the presence of insoluble components and heterogeneous particle structure may limit its ability to form uniform films. Therefore, pretreatment strategies are necessary to modify the structure of DDGS and improve its film-forming performance. I hypothesize that chemical pretreatments combined with mechanical processing can alter the interactions between proteins, polysaccharides, and lipids within the DDGS matrix, resulting in improved film formation and enhanced physicochemical properties. In this study, DDGS was evaluated under two lipid conditions (defatted and non-defatted). The samples were subjected to solvent pretreatments including water, hydrochloric acid (HCl), sodium hydroxide (NaOH), and sodium hydroxide combined with ethanol (NaOH+EtOH), while two different ball milling cycles (1× and 3×) were simultaneously applied to modify particle structure and enhance dispersion of DDGS components. These processing strategies were

expected to influence the solubilization of DDGS components and the intermolecular interactions within the film matrix.

Based on this hypothesis, the objective of the first part of this research was to develop biodegradable films from pretreated DDGS using a solvent casting approach and to evaluate how chemical pretreatments, lipid removal, and mechanical processing affect the film properties. The resulting films were characterized through a series of physicochemical, mechanical, structural and antimicrobial analyses to better understand the relationship between processing conditions and film performance. These properties included thickness, moisture content, color, surface hydrophobicity, total phenolic content, normalized toughness, elongation and Fourier transform infrared spectroscopy (FTIR). Through these analyses, this study aimed to determine how modifications in DDGS composition and structure influence the overall functionality of the films.

In addition to cast films, electrospinning was explored as an alternative approach for producing nanofibrous biodegradable films incorporating DDGS-derived extracts. Electrospinning is a versatile technique capable of generating fibrous structures with high surface area and controlled morphology, which may enhance the interaction between film materials and food surfaces. However, the formation of stable electrospun fibers depends strongly on the properties of the polymer solution and the composition of the film-forming system. I hypothesize that incorporating DDGS-derived extracts into emulsion electrospun biopolymer matrices can provide additional functional properties while maintaining fiber formation stability. Prior to the main electrospinning experiments, preliminary trials were conducted to determine suitable polymer systems and active compound concentrations capable of producing stable nanofibers. Different formulations were screened to evaluate solution spinnability and fiber formation

behavior. Based on these observations, pullulan was selected as the primary polymer matrix due to its favorable electrospinning characteristics and ability to form uniform nanofibrous structures. Among the tested formulations, solutions containing 0.8% thymol produced stable electrospinning behavior and consistent fiber morphology. Therefore, pullulan-based electrospun systems containing DDGS-derived extracts were selected for further investigation.

The electrospun films were subsequently evaluated for their structural characteristics and potential functionality as biodegradable food-contact materials. In order to assess their and DDGS casting method films' practical performance, selected films were applied to fresh-cut apples as a model food system. Fresh-cut fruits are highly susceptible to surface deterioration and quality changes during storage, making them suitable systems for evaluating the effectiveness of biodegradable coatings and packaging materials. Through these experiments, the study aimed to examine how DDGS-based and electrospun films interact with the apple surface and whether they can contribute to maintaining visual and antimicrobial quality during refrigerated storage.

Overall, this research sought to provide a better understanding of how processing strategies such as chemical pretreatment, lipid removal, mechanical milling, and electrospinning influence the structure and functionality of DDGS-derived biodegradable films. The findings of this study may contribute to the development of sustainable packaging materials derived from agricultural by-products while expanding the potential applications of DDGS in food systems.

Chapter 3 - Optimization of Chemical and Physical Pretreatment of Distillers Dried Grains with Solubles (DDGS) for Solvent Casting Biodegradable Film Production

Abstract

Biodegradable packaging films are increasingly explored for sustainability, yet the potential of distiller's dried grain with solubles (DDGS) as a high-protein, fiber-rich by-product remains underutilized for film development. The objective of this study was to develop biodegradable films from sorghum DDGS and evaluate the effects of film formulation, solvent type (water, hydrochloric acid (HCl), sodium hydroxide (NaOH) and sodium hydroxide combined with ethanol (NaOH+EtOH)), defatting, and ball milling (1x and 3x milling cycle) on film performance. Films were prepared by solvent casting and characterized for physicochemical, surface, and mechanical properties including ΔE, thickness, moisture content, surface hydrophobicity, total phenolic content (TPC), normalized toughness, elongation and FTIR analysis. Statistical analysis showed that solvent pretreatment was the dominant factor influencing film properties ($p<0.05$). Solvent type was the primary factor governing film properties, significantly affecting total color difference (ΔE), surface hydrophobicity, and mechanical performance in both defatted and non-defatted systems ($p < 0.05$). In defatted films, the interaction between solvent type and milling cycle further influenced ΔE, whereas in non-defatted films, ΔE was mainly controlled by solvent type alone. Mechanical properties showed a similar trend, where solvent type and its interaction with milling significantly affected normalized toughness with NaOH-treated films exhibiting comparatively higher values under specific conditions. The effect of defatting was treatment-dependent, significantly influencing

ΔE and moisture content only in selected solvent–milling combinations, rather than across all conditions. Overall, among the tested conditions, NaOH-3× treatments provided comparatively improved mechanical performance while maintaining similar barrier properties. Film-coated samples maintained stable microbial counts during storage, suggesting that the coatings did not promote microbial growth and indicating their potential as coatings for maintaining the quality of fresh-cut fruits.

3.1 Introduction

Distillers dried grains with solubles (DDGS), a coproduct of grain fermentation, can be used as a promising renewable material for biodegradable film production (Prabhakumari et al., 2018). According to the Renewable Fuels Association, the ethanol industry produces hundreds of millions of tons of DDGS annually, using billions of bushels of corn or other grains such as sorghum (Buenavista et al., 2021). The chemical composition of sorghum is approximately 65-80% starch, 7 - 15% protein, other polysaccharides up to 10% and 1.5 - 6 % lipids depending on the variety (Frankowski et al., 2022; Hwang et al., 2002). Its compositional balance of proteins, fibers, and lipids makes it appealing for film formation, particularly when combined and compared with other biopolymers or plasticizers (Galaburri et al., 2025). Following ethanol fermentation, however, the remaining by-product sorghum DDGS exhibits a markedly different composition, reported as 30.84% protein, 8.95% lipids, and 5.27% crude fiber (Li et al., 2011b).

Most research on DDGS-based materials has focused on corn-derived DDGS, given its abundance in U.S. bioethanol production. Alternatively, sorghum is also widely used as a fermentation substrate, particularly in regions where it offers agronomic advantages such as drought tolerance and resistance to high temperatures (Leguizamón et al., 2009). Sorghum

DDGS differs from corn DDGS in its compositional profile, particularly in protein composition, as sorghum proteins are mainly composed of kafirin fractions that differ in solubility and intermolecular interactions compared to corn zeins, which may influence film-forming behavior (K. Liu, 2011). To our knowledge, studies investigating the use of sorghum-derived DDGS as a raw material for biodegradable film production remain limited.

Despite its potential, native DDGS exhibits inherent drawbacks for film formation (Chatzifragkou et al., 2016). The relatively high lipid content (9.32% of the dried mass) may interfere with the formation of a cohesive polymer matrix and limit the mobility of biopolymer chains during film formation (Marotta et al., 2025; Wang et al., 2005). Additionally, particle size heterogeneity and hydrophobic surface components often lead to poor dispersion in aqueous systems, resulting in films with lower tensile strength, uneven surfaces, and compromised barrier properties (Prabhakumari et al., 2018). These challenges can be partially overcome by applying suitable pretreatment strategies, such as chemical treatments to modify the matrix composition and intermolecular interactions, and mechanical processes to reduce particle size and improve dispersion.

Chemical pretreatments, such as acidic or alkaline, have shown promise in altering the structural and functional properties of DDGS components. Alkaline treatment, using sodium hydroxide, can promote protein unfolding, deprotonation of acidic groups, and partial removal of lignin and hemicellulose, enhancing solubility and interfacial adhesion (Lu et al., 2025). DDGS proteins can be solubilized under alkaline conditions and subsequently recovered by adjusting the pH toward their isoelectric point, a process that promotes protein aggregation and intermolecular interactions and supports the formation of a cohesive film network (Prabhakumari et al., 2018). Acid hydrolysis with hydrochloric acid can hydrolyze hemicellulose and modify

protein conformation, increasing film uniformity and reducing polymer chain entanglement (Mosier, 2005; Sun & Cheng, n.d.). The incorporation of ethanol into alkaline solutions has been explored as an effective lipid removal method, further improving matrix homogeneity and water resistance (Prabhakumari et al., 2018). Removal of lipids from DDGS prior to processing has been shown to improve hydrogen bonding, increase surface wettability, and enhance transparency and tensile properties (K. Liu, 2011). Defatting can promote the formation of a more compact and homogeneous protein–polysaccharide matrix by removing residual lipids that interfere with intermolecular interactions, which may lead to improved barrier properties and lower water vapor permeability (Prabhakumari et al., 2018; Wang et al., 2005). However, systematic comparisons of defatted and non-defatted DDGS films under varying chemical and mechanical pretreatments are limited in the literature.

Mechanical size reduction, particularly through wet milling, can be used as another effective approach to improving DDGS film performance. Reducing particle size increases the available surface area, enhances filler–matrix interaction, and promotes more uniform dispersion in film-forming solutions (Rosentrater, 2006). Combining chemical pretreatment with mechanical modification has been reported to enhance the structural disruption of lignocellulosic materials. For example, NaOH-assisted ball milling simultaneously reduces particle size and promotes lignin removal, increasing the accessibility of functional groups and facilitating improved interactions within the material matrix (H. Lin et al., 2024).

The objective of this study is to evaluate the effects of four chemical pretreatments (water, hydrochloric acid (HCl), sodium hydroxide (NaOH), and sodium hydroxide combined with ethanol (NaOH + EtOH)), wet milling treatment (single and triple ball milling cycles), and two lipid conditions (defatted and non-defatted DDGS), on the physical and mechanical

properties of DDGS-based films. The resulting films were characterized for physicochemical properties, including color difference (ΔE); thickness and moisture content, surface properties hydrophobicity, mechanical properties such as normalized toughness and elongation, structural characteristics (FTIR analysis), antioxidant potential (total phenolic content (TPC)), and antimicrobial activity.

3.2 Materials and Methods

3.2.1 Materials

DDGS were donated by Pure Field Ingredients (Russell, KS). Xanthan gum (XG), and locust bean gum (LBG) were purchased from Danisco America, USA, and glycerol was purchased from Sigma-Aldrich. HCl, NaOH, and EtOH were obtained from Fisher Scientific (Waltham, MA, USA). All chemicals were of analytical grade used without further purification.

3.2.2 Methods

Pretreatments of DDGS

DDGS samples were first divided into two portions. For one portion, lipids were removed by Soxhlet extraction using 100% hexane overnight to obtain defatted DDGS. The hexane solvent was selected to ensure majority of polar lipids remain in the DDGS bulk. The remaining portion was kept as non-defatted DDGS and used without lipid extraction. Both defatted and non-defatted DDGS samples were then subjected to simultaneous chemical and mechanical pretreatments. Briefly, DDGS was mixed with different solvents, namely distilled water (control), HCl (0.1 N) solution, NaOH (0.1 N) solution, and a NaOH (0.1 N) + 50% ethanol mixture, at a solid-to-solvent ratio of 1:3 (w/v). The mixtures were processed in a planetary ball mill (PM 100, Retsch) under identical operating conditions for either one cycle (1×) or three cycles (3×). Each cycle was 15 min. After ball milling, the suspensions were centrifuged to

separate the solid fraction. The resulting cake was collected, washed to remove residual solvent, and lyophilized. The dried DDGS materials were then used for the preparation of biodegradable packaging films. The supernatant was also collected and lyophilized for the second part of the study as discussed in the subsequent chapter. The pretreatment of DDGS was done by another student in our lab.

Film Preparation

Film-forming solutions (10 g) were prepared using a formulation containing DDGS (10 wt%, d.b.), LBG and XG (0.5 wt% each, dry basis), and glycerol (1 wt%, d.b) as plasticizers. The ingredients were dispersed in distilled water under magnetic stirring for an hour at 60-70 °C for hydration and homogeneity. The mixtures were cast into plastic Petri dishes (diameter = 92 mm) and dried at room temperature for 24 h to evaporate moisture and form films. Dried films were peeled and stored at refrigerated temperatures prior to testing.

Mechanical and Physiochemical Properties

Normalized Toughness and Elongation: Normalized toughness and elongation at break (EAB) of the films were determined using a texture analyzer (TA-XT2i, Texture Technologies Corp., USA) equipped with TA-96B tensile grips, following ASTM D882. Film strips (15 × 50 mm) were mounted between the grips and stretched in tension mode until break. The test was conducted at a pre-test speed of 1 mm/s, test speed of 1 mm/s, and post-test speed of 1 mm/s with a trigger force of 5 g and a maximum travel distance of 60 mm. Film thickness was measured prior to testing using a digital micrometer and used to calculate the cross-sectional area. Normalized toughness was calculated as the total area under the force–distance curve up to the breaking point, representing the energy absorbed before rupture, and was normalized by dividing by the corresponding film thickness to obtain normalized toughness

($N.mm_{distance}$)/$mm_{thickness}$), while elongation at break was expressed as the percentage increase in length relative to the initial gauge length.

Surface Hydrophobicity: The surface hydrophobicity of DDGS films was evaluated by contact angle measurements using an optical drop tensiometer (Attension Theta, Biolin Scientific, Phoenix, AZ, USA). Prior to analysis, the instrument was calibrated using a 4 mm diameter metal ball standard according to the manufacturer's guidelines. Measurements were performed using the sessile drop method at room temperature by depositing a 5 µL droplet of deionized water onto the film surface using a pipette. To account for surface variability, measurements were taken at five different spots on each film sample, and the average value was reported. Contact angle values were calculated from the droplet profile using the Young–Laplace equation using OneAttension software (Biolin Scientific, Phoenix, AZ, USA). The measurement protocol was adapted from Yildiz (2024).

Color Analysis: Film color (L, a, b) was measured using a colorimeter (PCE-CSM 4, PCE). The equipment was calibrated on the black and the white surface. The equipment was calibrated using standard black and white calibration plates before measurements. The L value represents lightness (0 = black, 100 = white), while a indicates the red–green axis (positive values represent redness and negative values represent greenness) and b represents the yellow–blue axis (positive values indicate yellowness and negative values indicate blueness). The color (ΔE) was calculated by using the following equation where $\Delta L, \Delta a \; and \; \Delta b$ represent the differences between the measured color parameters of the film sample and the corresponding reference values. In this study, the reference values were taken from the calibration standard, and the deviations in L, a, and b were used to calculate the overall color difference. ΔE was calculated based on the Equation (3):

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \qquad (3)$$

Film Thickness: Thickness was measured with a digital micrometer (accuracy ±0.001 mm) at five random positions per film, and average values were reported.

Moisture Content ($MC_{db(}(\%)$*:* The moisture content of the films was determined gravimetrically. Samples (0.04-0.08 g) were weighed prior to drying (initial weight, W_i) and then placed in a hot-air oven at 110 °C for 48 h. After drying, the films were reweighed (final weight, W_f), and the moisture content was calculated on a dry basis (db) and expressed as percentage using the Equation (4) below:

$$MC_{db}(\%) = \frac{W_i - W_f}{W_f} x100\% \qquad (4)$$

Total Phenolic Compound (TPC) Analysis: Total phenolic content (TPC) of the film samples was determined using the Folin–Ciocalteu colorimetric method, with gallic acid used as the standard compound. Briefly, each film sample (0.1 mg) was weighed and transferred into microcentrifuge tubes. The samples were extracted with 70% (v/v) methanol (1 mL) and stirred overnight at room temperature to ensure sufficient extraction of phenolic compounds. After extraction, the supernatants were diluted with 70% methanol to obtain 1:10 and 1:20 (v/v) dilutions for analysis. For the assay, an aliquot of the diluted extract was mixed with Folin–Ciocalteu reagent. After a short reaction period, sodium carbonate solution was added to neutralize the mixture and allow color development. The reaction mixtures were incubated at room temperature in the dark for 10 minutes, and the absorbance was measured at 765 nm using a UV–Vis spectrophotometer. A calibration curve was prepared using gallic acid solutions at different concentrations in 70% methanol. The gallic acid equivalent concentration of each sample was obtained by substituting the absorbance values into the calibration curve equation,

y=ax+b. TPC values were calculated from the standard curve and expressed as mg gallic acid equivalents (GAE) per g of film using Equation (5):

$$TPC\ \left(mg\frac{GAE}{g}\right) = \frac{y*V*Df}{m} \qquad (5)$$

where y is the gallic acid equivalent concentration obtained from the calibration curve, V is the extraction volume (mL), Df is the dilution factor, and m is the mass of the film sample (g).

FTIR Measurement: Fourier Transform Infrared Spectroscopy (FTIR): FTIR spectra were recorded using a Spectrum Two (PerkinElmer, Waltham, MA, USA) in the range of 4000–600 cm^{-1} with four scans at a resolution of 4 cm^{-1}. Spectra were used to identify functional group modifications caused by chemical pretreatments.

Antimicrobial Activity and Shelf-Life Analysis:

Preliminary Antimicrobial Screening of Films: Preliminary antimicrobial screening of the films was conducted using a well plate diffusion method. Film samples were tested against *Salmonella enterica*, *Staphylococcus aureus*, and *Listeria monocytogenes*. Antimicrobial activity was evaluated by measuring inhibition zones around the wells. Films demonstrating stronger inhibitory effects, together with favorable mechanical and physical properties, were selected for product application studies.

Product Application

Selected film samples were evaluated in a model food system using fresh Red Delicious apples. Apples were manually peeled and cut into uniform slices with an average weight of approximately 2 g and consistent thickness to ensure sample standardization. Apple pieces were coated/wrapped with the selected films and stored in an environmental chamber at 4 °C and 20% relative humidity for 7 days. Samples were analyzed at predetermined storage times (day 0, 3, and 7) for microbial quality, and color changes were monitored throughout storage.

Microbial Analysis

Red Delicious apples were peeled and cut into uniform pieces, and 2 g of sample was used for each treatment. The apple samples were wrapped with the respective film formulations and stored under refrigerated conditions. Microbial analyses were conducted on days 0, 3, and 7. Total Aerobic Mesophilic count was enumerated on Aerobic Count Petri film after incubation at 37 °C for 48 h, while yeast and mold counts were determined using Yeast and Mold Count Petri film incubated at 28 °C for 48 h. Microbial counts were expressed as log CFU/g. Color was measured as describes above over storage time. Pictures were taken in parallel with color measurements.

Statistical Analysis

Statistical analysis was performed using analysis of variance (ANOVA) with a significance level of $\alpha = 0.05$. To evaluate the effects of processing parameters, data for non-defatted and defatted DDGS films were initially analyzed separately using two-way ANOVA, considering solvent type (water, HCl, NaOH, and NaOH + EtOH) and milling cycle (1× and 3×) as independent variables. Post-ANOVA Tukey's multiple comparison test was applied to determine differences among individual treatments. The effect of lipid removal was evaluated separately by comparing non-defatted and defatted films under identical solvent type and milling cycles. These pairwise comparisons were performed using ANOVA and Tukey's multiple comparison test. All films were prepared and analyzed in triplicate. For analysis of texture, color, surface hydrophobicity, and thickness repeated measurements of the identical samples were averaged to a single mean and used in ANOVA with n = 3.

3.3 Results and Discussion

3.3.1 Physicochemical Properties of the DDGS Films

The visual appearance and homogeneity of DDGS films varied as a function of solvent type and lipid extraction as shown in Figure 3.1 and Figure 3.2. Water-treated films were heterogeneous in appearance with visible surface irregularities and uneven dispersion within the film matrix. This can be the limited solubilization of DDGS components in water, leading to the presence of insoluble particles and poor matrix cohesion. In contrast, HCl-treated films appeared more uniform and homogeneous, indicating improved matrix formation and compact structure. NaOH and NaOH+EtOH films appeared more brittle, likely due to increased protein denaturation and reduced matrix flexibility under alkaline conditions, and were more prone to cracking during handling, suggesting a more rigid film network. Additionally, defatted films generally appeared slightly less transparent, and rigid compared to non-defatted films, due to the removal of lipid fractions that may otherwise contribute to matrix flexibility. This suggests that lipid removal enhanced intermolecular interactions, resulting in a more compact but less flexible film structure.

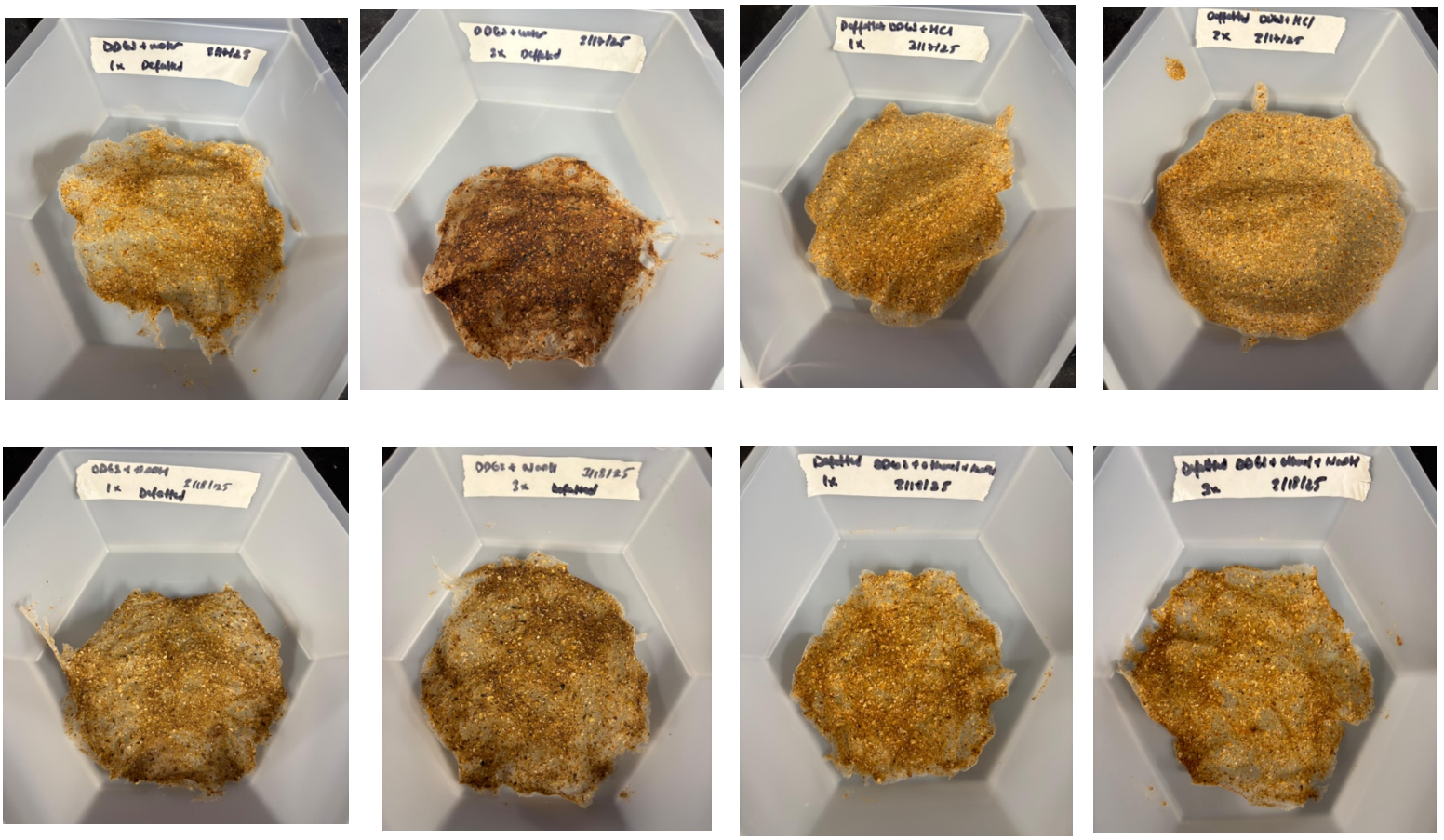


Figure 3.1 Visual appearance of defatted-DDGS films prepared with different solvents and milling cycles. From left to right: Water-1×, Water-3×, HCl-1×, and HCl-3× (top row); NaOH-

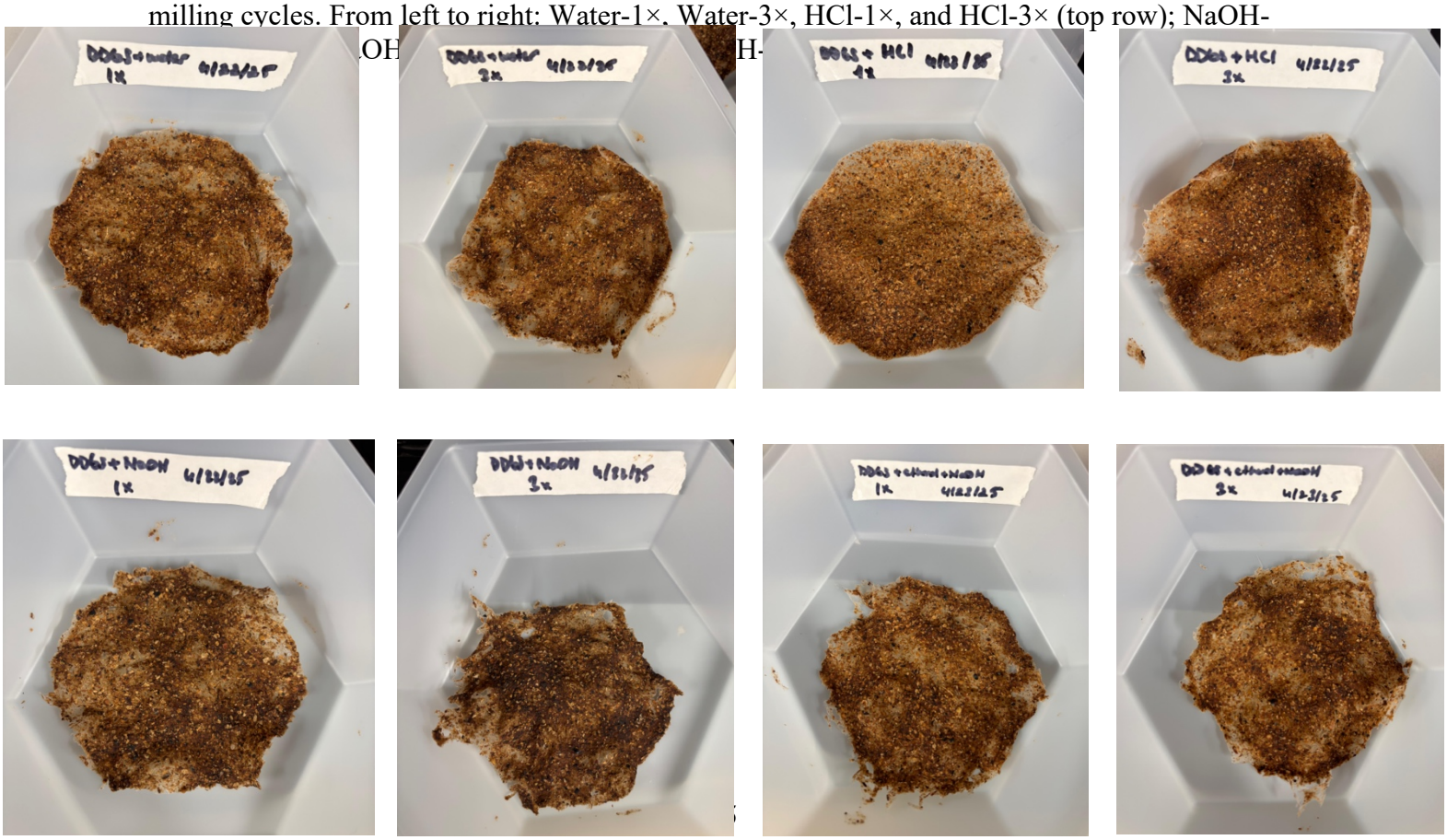


Figure 3.2 Visual appearance of non-defatted DDGS films prepared with different solvents and milling cycles. From left to right: Water-1×, Water-3×, HCl-1×, and HCl-3× (top row); NaOH-1×, NaOH-3×, NaOH+EtOH-1×, and NaOH+EtOH-3× (bottom row).

The physical and barrier properties of DDGS films are presented in Table 3.1 and discussed below.

ΔE: For defatted films, solvent type and solvent type × milling cycle interaction had a significant effect on ΔE values ($p < 0.05$); whereas milling cycle alone had no significant effect on ΔE. In non-defatted films, solvent type significantly affected ΔE values ($p < 0.05$), whereas milling cycle and solvent × milling interaction were not significant ($p > 0.05$). However, post-hoc comparisons indicated that these differences were limited to specific treatments, with most groups showing overlapping significance levels for both nondefatted and defatted. This can be attributed to solvent-dependent modifications in film composition and microstructure affecting optical properties. Liu, (2008) has reported that generally, smaller particle size fractions in corn DDGS were found to be relatively lighter (higher L value) and less red, but more yellow, compared to coarser fractions. Furthermore, lipid removal significantly influenced ΔE ($p < 0.05$). Defatting has a significant effect for all the films except for the NaOH+EtOH 1× film, where no significant difference between defatted and non-defatted films was detected ($p > 0.05$). Defatted films exhibited higher ΔE* values than non-defatted films ($p< 0.05$), indicating that removal of lipids increased the ΔE of the DDGS film matrix. This can be attributed to defatting increases the exposure of underlying protein and fiber components, leading to greater light scattering and more pronounced color differences. A similar pattern has been reported in spent-grain films, where lipid removal increased ΔE values (Qazanfarzadeh et al., 2024).

Film thickness: Solvent type significantly affected film thickness for defatted films ($p < 0.05$); however, milling cycle and the interaction of milling cycle and solvent type had no

significant effect on the films thickness ($p>0.05$). Solvent type significantly affected film thickness for non-defatted films ($p < 0.05$); however, milling cycle and the interaction of milling cycle and solvent type had no significant effect on the films thickness ($p>0.05$). Since all film formulations were prepared using identical polymer concentrations and casting conditions, similar thickness values were expected across treatments. Also, HCl- treated films showed lowest thickness showing that it also effects the moisture content of the films. Previous studies on silver carp surimi-based protein films have reported that protein unfolding under alkaline conditions enhances matrix formation and leads to increased film thickness; however, this trend was not observed in the DDGS-based films in the present study (Munir et al., 2020).

Lipid removal had a significant effect on film thickness ($p < 0.05$). Post hoc analysis revealed that this difference was only observed in the NaOH-3× films, while no significant differences were found between defatted and non-defatted films for the other treatments ($p > 0.05$). It had been demonstrated that extraction chemistry changes DDGS protein purity and associated non-protein fractions, which can modify matrix packing density and therefore film thickness (Y. Wang et al., 2009a).

Moisture content: Solvent type significantly affected the moisture content of the defatted DDGS films ($p < 0.05$), whereas milling cycle or the solvent type x milling cycle interaction were not significant ($p<0.05$). In contrast, no significant effects of solvent type, milling cycle or their interaction were observed for the non-defatted films ($p > 0.05$). Although numerical differences were observed among treatments, post-hoc comparisons indicated overlapping groups, suggesting that moisture content was not consistently distinguishable across all conditions. This behavior may be attributed to treatment-dependent exposure of polar groups and sorption sites within the film matrix. However, Gomes et al. (2025) reported that as sunflower oil

phase decreased from 5% to 0%, the sunflower protein-based (SF) emulsion and film systems exhibited higher equilibrium water sorption, while oil-rich formulations showed lower moisture uptake, consistent with lipid-induced hydrophobic effects that limit water binding. Also, ball milling is known to reduce particle size and alter protein structural organization and surface area, which can increase water interaction capacity without introducing new chemical functionalities (Qu et al., 2017).

The effect of lipid removal on moisture content was significant ($p < 0.05$), indicating a treatment-dependent influence. However, post-hoc analysis revealed that significant differences between defatted and non-defatted films were limited to specific treatments, namely HCl-1×, HCl-3×, NaOH+EtOH-1×, and Water-1× ($p < 0.05$) (Table 1). This behavior can be attributed to treatment-dependent differences in matrix composition, where lipid removal increases the availability of polar groups and enhances water binding under specific conditions.

Table 3.1 Physical properties, ΔE, thickness, and moisture content of DDGS-based films as influenced by solvent types and milling cycle for non-defatted and defatted systems. Different lowercase letters indicate significant differences among solvent types and milling conditions within the same lipid group, whereas Roman numerals denote significant differences between non-defatted and defatted films under identical treatment conditions ($p < 0.05$).

Sample	ΔE	Thickness (mm)	Moisture Content (%db)
Non-Defatted DDGS Films			
Water-1x	$18.53 \pm 4.86^{ab,II}$	$0.52 \pm 0.03^{bc,I}$	$22.72 \pm 0.35^{a,I}$
Water-3x	$21.63 \pm 1.61^{b,II}$	$0.50 \pm 0.01^{bc,I}$	$17.01 \pm 9.27^{a,I}$
HCl-1x	$16.81 \pm 5.34^{ab,II}$	$0.41 \pm 0.03^{a,I}$	$15.85 \pm 2.44^{a,I}$
HCl-3x	$16.25 \pm 1.90^{ab,II}$	$0.45 \pm 0.03^{ac,I}$	$11.08 \pm 1.16^{a,I}$
NaOH-1x	$13.61 \pm 1.53^{a,II}$	$0.53 \pm 0.01^{b,I}$	$23.74 \pm 4.95^{a,I}$
NaOH-3x	$13.79 \pm 4.47^{a,II}$	$0.56 \pm 0.02^{b,I}$	$23.18 \pm 1.59^{a,I}$
NaOH+EtOH-1x	$19.69 \pm 2.35^{ab,I}$	$0.52 \pm 0.01^{b,I}$	$14.62 \pm 0.03^{a,I}$
NaOH+EtOH-3x	$17.26 \pm 2.64^{ab,II}$	$0.53 \pm 0.01^{b,I}$	$15.03 \pm 5.15^{a,I}$
Defatted DDGS Films			
Water-1x	$25.42 \pm 3.31^{ab,I}$	$0.47 \pm 0.03^{abc,I}$	$28.70 \pm 1.54^{ab,II}$

Water-3x	$35.16 \pm 0.51^{a,I}$	$0.51 \pm 0.02^{bc,I}$	$27.53 \pm 3.88^{ab,I}$
HCl-1x	$32.88 \pm 6.94^{ab,I}$	$0.39 \pm 0.02^{a,I}$	$29.89 \pm 0.04^{ab,II}$
HCl-3x	$36.82 \pm 4.66^{b,I}$	$0.41 \pm 0.02^{ac,I}$	$40.49 \pm 7.23^{b,II}$
NaOH-1x	$21.99 \pm 1.87^{ab,I}$	$0.47 \pm 0.08^{abc,I}$	$20.66 \pm 5.63^{a,I}$
NaOH-3x	$21.44 \pm 3.77^{ab,I}$	$0.46 \pm 0.01^{abc,II}$	$22.24 \pm 8.87^{a,I}$
NaOH+EtOH-1x	$27.47 \pm 4.26^{a,I}$	$0.56 \pm 0.06^{b,I}$	$27.24 \pm 2.19^{ab,II}$
NaOH+EtOH-3x	$30.77 \pm 3.85^{ab,I}$	$0.54 \pm 0.03^{b,I}$	$27.82 \pm 2.01^{ab,I}$

TPC: Solvent type and milling cycle significantly affected TPC for both defatted and non-defatted films ($p < 0.05$), while their interaction was not significant for both non-defatted and defatted films (Table 3.2). Water-treated samples showed the lowest TPC values, while NaOH and NaOH+EtOH treatments resulted in the highest values among the solvent type. The increase in TPC with alkaline treatments can be attributed to the breakdown of lignocellulosic structures and the release of bound phenolic compounds. The addition of ethanol further enhanced the extraction of hydrophobic phenolics. Similar trends have been reported in DDGS based protein films, where alkaline and solvent-assisted treatments increase phenolic content by disrupting lignocellulosic linkages and improving the extractability of bound phenolics (K. Liu, 2011; Prabhakumari et al., 2018). Increasing milling cycle from 1× to 3× consistently enhanced TPC across all treatments, suggesting that particle size reduction improved phenolic extraction through increased surface area and greater matrix accessibility (Qu et al., 2017). Defatting had no significant effect on TPC of the DDGS films in our study. In contrast to findings reported by Liu (2011), where lipid removal was suggested to influence the retention or distribution of phenolic compounds within the matrix, defatting did not significantly affect TPC values in the present study ($p > 0.05$).

Table 3.2 Total phenolic content (TPC) of DDGS-based films as affected by solvent types and milling cycle (1× and 3×) for non-defatted and defatted matrices. Different lowercase letters (a–g) indicate significant differences among solvent types and milling cycle combinations within the same lipid condition, while Roman numerals (I–II) indicate significant differences between non-defatted and defatted films for the same treatment condition ($p < 0.05$).

Sample	Non-Defatted (GAE/g)	Defatted (GAE/g)
Water - 1x	$0.53 \pm 0.08^{a,I}$	$0.71 \pm 0.17^{a,I}$
Water - 3x	$2.03 \pm 0.08^{ab,I}$	$2.51 \pm 0.17^{ab,I}$
HCl - 1x	$4.11 \pm 0.40^{bc,I}$	$4.61 \pm 0.59^{bc,I}$
HCl - 3x	$5.38 \pm 0.40^{cd,I}$	$6.42 \pm 0.59^{cd,I}$
NaOH – 1x	$6.70 \pm 0.32^{de,I}$	$7.38 \pm 0.25^{de,I}$
NaOH – 3x	$7.40 \pm 0.32^{e,I}$	$9.6 \pm 1.02^{ef,I}$
NaOH + EtOH – 1x	$8.96 \pm 0.57^{f,I}$	$11.35 \pm 0.59^{f,I}$
NaOH + EtOH – 3x	$10.23 \pm 0.08^{f,I}$	$13.81 \pm 0.34^{g,I}$

Surface Hydrophobicity: For defatted films, solvent type, milling cycle significantly affected surface hydrophobicity ($p < 0.05$); while no significant difference observed among their interaction on surface hydrophobicity. For non-defatted films, solvent type and milling cycle also showed significant effects on surface hydrophobicity ($p < 0.05$); while their interaction was not significant on surface hydrophobicity ($p>0.05$). The highest contact angle was observed in NaOH-3× treated defatted films. This indicates that alkaline treatment combined with milling and lipid removal enhances surface hydrophobicity, because of protein structural changes and increased exposure of hydrophobic groups. These findings indicate that both solvent pretreatment and milling cycle influenced the surface characteristics of the films. The significant interaction observed in defatted films further suggests that the effect of solvent type on surface hydrophobicity depended on the applied milling cycle. In contrast, the absence of a significant interaction in non-defatted films indicates that solvent type and milling cycle acted independently in influencing surface hydrophobicity.

However, the lipid removal had a significant effect on surface hydrophobicity ($p < 0.05$). It is revealed that significant differences were observed only for Water-1×, NaOH+EtOH-1×, and NaOH+EtOH-3× films ($p < 0.05$). This behavior can be attributed to structural rearrangement and exposure of hydrophobic protein fractions at the film surface.

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Table 3.3 Contact angle (θ) of DDGS-based films as affected by solvent type and milling cycle for non-defatted and defatted matrices. Different lowercase letters indicate significant differences among solvent type and milling cycles within each lipid group, while Roman numerals (I–II) indicate significant differences between non-defatted and defatted films for the same condition ($p < 0.05$).

Sample	Non-Defatted	Defatted
Water - 1x	$51.45 \pm 7.20^{ab,I}$	$32.48 \pm 7.21^{ad,II}$
Water - 3x	$63.91 \pm 12.75^{b,I}$	$56.85 \pm 9.33^{bcd,I}$
HCl - 1x	$42.96 \pm 15.59^{abc,I}$	$30.72 \pm 12.33^{a,I}$
HCl - 3x	$51.07 \pm 13.35^{ab,I}$	$40.18 \pm 4.95^{abd,I}$
NaOH – 1x	$48.19 \pm 6.52^{ab,I}$	$61.11 \pm 15.29^{bc,I}$
NaOH – 3x	$50.09 \pm 4.54^{ab,I}$	$66.57 \pm 18.31^{c,I}$
NaOH + EtOH – 1x	$24.73 \pm 1.77^{c,II}$	$39.73 \pm 7.09^{abd,I}$
NaOH + EtOH – 3x	$33.55 \pm 4.18^{ac,II}$	$58.60 \pm 3.41^{bc,I}$

3.3.2 Mechanical Properties of the DDGS Films

The mechanical properties of DDGS-based films as affected by solvent type, milling cycle, and defatting are presented in Table 3.4.

Solvent type and their interaction significantly ($p<0.05$) affected elongation at break while milling cycle had no significant effect in non-defatted films ($p > 0.05$). In defatted films, solvent type and the interaction between solvent type and milling cycle were significant ($p < 0.05$), while milling cycle alone was not significant ($p > 0.05$). Lipid removal did not have a significant effect on elongation at break ($p > 0.05$), as no consistent differences were observed between defatted and non-defatted films. This indicates that film flexibility was more sensitive to structural rearrangements induced by solvent pretreatment and processing conditions rather than lipid content. Similar observations have been reported in kafirin-based films, where intermolecular interactions and network organization play a dominant role in determining elongation behavior (Wang et al., 2009).

For non-defatted films, solvent type, milling cycle, and their interaction did not significantly affect normalized toughness values ($p > 0.05$). In contrast, for defatted films, solvent type and the interaction between solvent type and milling cycle had a significant effect ($p < 0.05$), while milling cycle alone was not significant ($p > 0.05$). Lipid removal had a significant effect on normalized toughness ($p < 0.05$). However, this difference was only observed for the NaOH-3× treatment, while no significant differences were found for the remaining conditions ($p > 0.05$). These findings suggest that energy absorption behavior was influenced by matrix organization in defatted systems, where the removal of lipids may allow closer protein–polysaccharide interactions, although this effect was treatment-specific and not uniform across all conditions.

Table 3.4 Mechanical properties, elongation, and normalized toughness of DDGS-based films as influenced by solvent pretreatment and milling cycle for non-defatted and defatted samples. Different lowercase letters indicate significant differences among pretreatment–milling combinations within each lipid group, whereas Roman numerals represent significant differences between non-defatted and defatted films under identical processing conditions ($p < 0.05$).

Sample	Elongation (mm)	Normalized Toughness($W/mm_{thickness}$)= ($N.mm_{distance}$)/$mm_{thickness}$)
Non-Defatted DDGS Films		
Water-1x	$32.30 \pm 0.76^{a,I}$	$0.29 \pm 0.09^{a,I}$
Water-3x	$32.64 \pm 0.53^{ab,I}$	$1.01 \pm 0.11^{a,I}$
HCl-1x	$32.75 \pm 0.01^{ab,I}$	$1.01 \pm 0.24^{a,I}$
HCl-3x	$31.88 \pm 0.26^{a,I}$	$0.59 \pm 0.04^{a,I}$
NaOH-1x	$32.53 \pm 0.60^{ab,I}$	$0.78 \pm 0.06^{a,I}$
NaOH-3x	$33.96 \pm 0.11^{b,I}$	$1.15 \pm 0.14^{a,I}$
NaOH+EtOH-1x	$32.74 \pm 0.20^{ab,I}$	$0.82 \pm 0.12^{a,I}$
NaOH+EtOH-3x	$32.15 \pm 0.15^{a,I}$	$0.41 \pm 0.03^{a,I}$
Defatted DDGS Films		
Water-1x	$31.62 \pm 0.20^{a,I}$	$0.23 \pm 0.01^{d,I}$

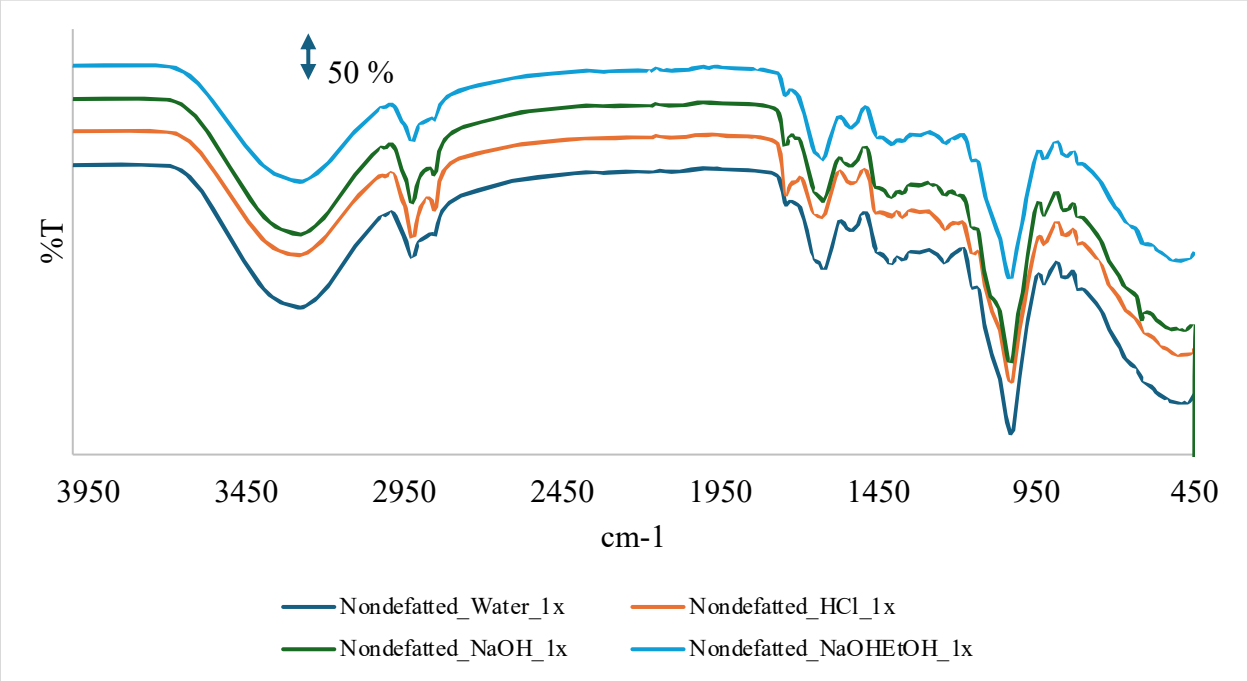
Water-3x	$32.20 \pm 0.07^{ab,I}$	$0.88 \pm 0.07^{abc,I}$
HCl-1x	$32.81 \pm 0.10^{ab,I}$	$1.029 \pm 0.22^{a,I}$
HCl-3x	$31.50 \pm 0.20^{a,I}$	$0.44 \pm 0.07^{bd,I}$
NaOH-1x	$32.56 \pm 0.64^{ab,I}$	$0.66 \pm 0.10^{bcd,I}$
NaOH-3x	$33.19 \pm 0.59^{b,I}$	$1.01 \pm 0.04^{ac,II}$
NaOH+EtOH-1x	$32.95 \pm 0.01^{ab,I}$	$1.03 \pm 0.03^{abc,I}$
NaOH+EtOH-3x	$32.63 \pm 0.10^{ab,I}$	$0.69 \pm 0.14^{bcd,I}$

FTIR: FTIR spectra of defatted and non-defatted DDGS-based films are presented in Figure 3.3.

The broad band at 3200–3400 cm^{-1} (O–H/N–H stretching) was more pronounced in defatted films, suggesting increased hydrogen bonding due to lipid removal. The peaks at 2920–2850 cm^{-1} (C–H stretching) were more intense in non-defatted films, indicating the presence of residual lipids, which contributed to relatively lower moisture content and higher hydrophobic character compared to defatted films. Similar C–H stretching features in the 3000–2900 cm^{-1} region have been reported for starch- and DDGS-polysaccharide–based films plasticized with glycerol, where the asymmetric and symmetric $–CH_2–$ stretching bands appear near 2934 and 2879 cm^{-1}, together with a $–CH_2–$ bending band around 1413 cm^{-1} (Galaburri et al., 2025). Characteristic amide I (1650 cm^{-1}) and amide II (1540 cm^{-1}) bands showed slight shifts and intensity changes, particularly in NaOH- and HCl-treated films, indicating protein structural modifications. These changes are consistent with the improved mechanical performance observed in selected alkaline-treated samples relative to Water-1× films. The 1000–1100 cm^{-1} region (C–O stretching of polysaccharides) became more pronounced with increased milling cycle (3×), suggesting improved dispersion and matrix homogeneity, which also aligns with the enhanced mechanical performance of 3x milled films than 1x ones. Similar absorption bands

have been reported in maize starch–quercetin complexes, where FTIR spectra showed characteristic peaks around 2930 cm^{-1} corresponding to C–H stretching and 1662 cm^{-1} associated with carbonyl vibrations (L. Zhang et al., 2011).

Overall, FTIR analysis confirms that solvent pretreatment and defatting primarily influenced intermolecular interactions (hydrogen bonding, lipid removal, and protein conformation), while milling cycles improved structural uniformity, supporting trends observed in mechanical, moisture, and surface properties.



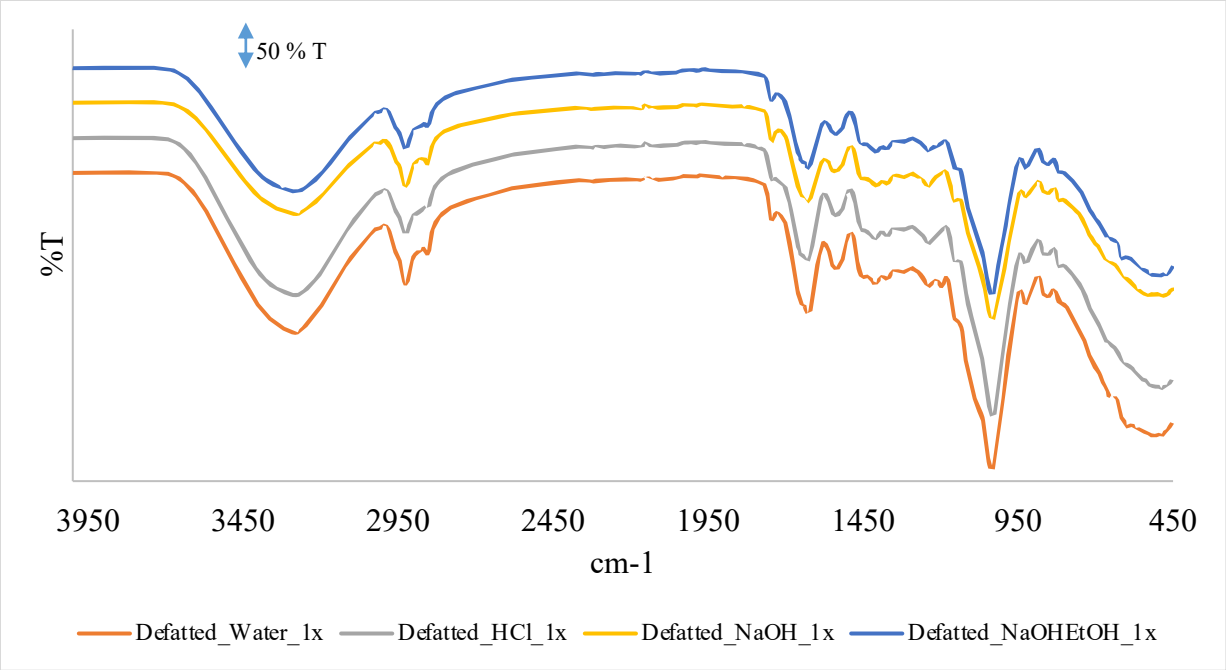


Figure 3.3 FTIR spectra of DDGS films produced using different solvent pretreatments (water, HCl, NaOH, and NaOH+EtOH) and 1x ball milling cycles with non-defatted and defatted in order.

Preliminary Antimicrobial Screening of Films



Figure 3.4 Preliminary antimicrobial activity of DDGS-based films against *Salmonella spp., Staphylococcus aureus,* and *Listeria monocytogenes* evaluated by agar diffusion method.

The antimicrobial activity of DDGS-based films was evaluated using agar diffusion (Figure 3.4). In general, no distinct inhibition zones were observed for most samples, indicating limited diffusion of active compounds into the agar. However, measurable inhibition zones were

observed for specific treatments, with defatted DDGS_Water (3×) films showing an inhibition zone of 2.5 cm and non-defatted DDGS_NaOH+EtOH (3×) films showing a zone of 2.0 cm. Despite these observations, the antimicrobial effect was not consistent across all samples, and most films exhibited only slight localized growth suppression, suggesting predominantly weak or contact-dependent antimicrobial activity. This behavior can be attributed to restricted release of bioactive compounds from the polymer matrix, which limits their diffusion into the surrounding medium

Based on the overall performance, considering antimicrobial screening together with physical and mechanical properties, non-defatted DDGS_Water-3x and DDGS_NaOH-3x films were selected for further application studies. Although no distinct antimicrobial activity was observed in the agar diffusion assay, these films were preferred due to their balanced mechanical performance and structural integrity.

3.3.3 Product Application

Color change of apples was significantly affected by both film type and storage time ($p < 0.05$), indicating that the applied treatments and duration of storage played a critical role in visual quality deterioration. ΔE was analyzed using $\ln(\Delta E)$ as a function of time to evaluate the kinetics of color change has shown in Figure 3.5.

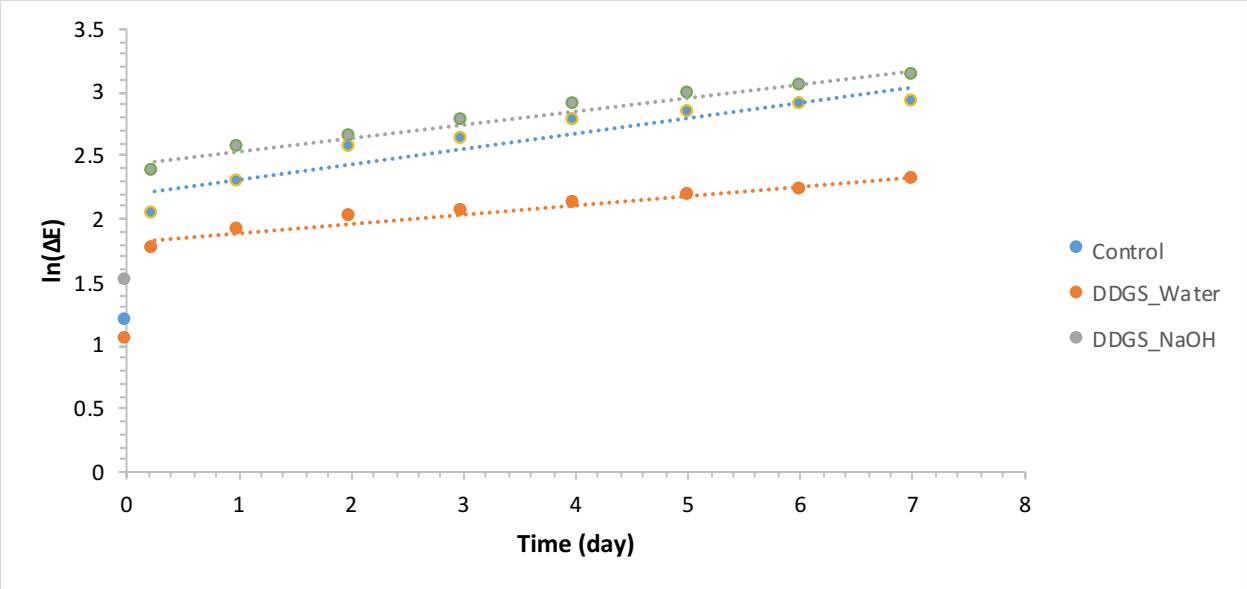


Figure 3.5 Changes in ln(ΔE) of apple samples coated with different DDGS-based films during 7 days of storage. Points represent measured values, and dotted lines indicate linear regression used to estimate the color change rate.

Table 3.5 Initial color change (0–0.25 day) and color degradation rate (slope of ln(ΔE) vs time) of apple samples coated with DDGS-based films

	Change in 0-0.25 day	Slope
Control	0.849	0.1219
DDGS_Water	0.701	0.0736
DDGS_NaOH	0.866	0.1061

The linear relationship between ln(ΔE) and storage time suggests that the browning process followed a pseudo-first-order kinetic behavior, allowing the estimation of the color degradation rate constant (k) from the slope of the fitted lines (Table 3.5). All treatments showed a gradual increase in ln(ΔE), confirming progressive browning of the apple samples during storage. However, the magnitude of the initial color change and the subsequent degradation rate differed among treatments. The largest initial increase in color difference (0–0.25 day) was observed for DDGS_NaOH (0.866), followed by Control (0.849) and DDGS_Water (0.701), suggesting that the initial interaction between the coating solution and the apple surface influenced the immediate visual response. This initial color change may also be related to the

migration of soluble compounds from the DDGS film matrix into the fruit surface. DDGS films contain soluble compounds such as phenolics, proteins, and residual pigments, which can migrate into the moist fruit matrix during storage (Prabhakumari et al., 2018). A similar phenomenon has been reported in studies using biodegradable poly (lactic acid)/ Poly (3-hydroxybutyrate)/acetyl tributyl citrate membranes enriched with oregano/lemongrass essential oils, where direct contact between the film and the fruit resulted in faster deterioration of strawberries compared to the control samples. This outcome was attributed to the uncontrolled release and leaching of active compounds from the film matrix, which adversely affected the fruit surface, leading to accelerated visual degradation and spoilage (Rusková et al., 2023a). In contrast, the kinetic rate of color degradation (slope) during storage showed a different trend. Control samples exhibited the highest slope (0.1219), indicating the fastest color deterioration, while DDGS_NaOH (0.1061) showed a moderate rate. DDGS_Water presented the lowest slope (0.0736), suggesting that despite the larger initial color change, this coating slowed down the subsequent browning rate during storage. Overall, these results indicate that DDGS-based films influenced both the immediate color response and the long-term browning kinetics of apple samples.



Figure 3.6 Visual appearance of apple samples coated with DDGS-based films during storage (day 0, 3, and 7 from top to bottom). Samples are arranged from left to right as control, non-defatted DDGS_Water (3× milling), and non-defatted DDGS_NaOH (3× milling).

Microbial stability of the apple samples was evaluated by monitoring total aerobic bacteria and yeast–mold populations during storage. These were selected as key indicators of spoilage, since aerobic bacteria reflect overall microbial load, while yeast and mold growth are primarily associated with surface deterioration and visible spoilage in fresh fruits. The initial microbial load for all samples was low, with aerobic bacteria measured at 1.50×10^1 CFU/g (log 1.88) and yeast–mold at 7.50×10^1 CFU/g, and these values remained unchanged throughout the storage period (day 0–7) for all treatments, including the control and DDGS-based films.

The films exhibited a bacteriostatic effect against both total aerobic bacteria and yeast–mold populations, indicating their potential to suppress microbial growth on the apple surface during storage. No increase in microbial counts was observed in DDGS_W or DDGS_NaOH samples, where both aerobic bacteria and yeast–mold remained constant at 1.50×10^1 CFU/g and 7.50×10^1 CFU/g, respectively, throughout storage, consistent with the trend observed in the control.

This can be explained by the release of bioactive compounds from the film matrix, which may exert antimicrobial activity by disrupting microbial cell membranes, altering permeability, and interfering with metabolic functions. Phenolic compounds, in particular, are known to cause cell membrane damage, leakage of intracellular components, and enzyme inhibition, which collectively reduce microbial growth (De Rossi et al., 2025). In DDGS-based systems, residual phenolic compounds, proteins, and other soluble fractions can contribute to antimicrobial effects (Chatzifragkou et al., 2016).

Conclusion

The present study demonstrated that sorghum DDGS can be successfully utilized as a renewable raw material for the development of biodegradable packaging films. The results showed that solvent pretreatment, ball milling cycle, and lipid removal significantly influenced several physicochemical and mechanical properties of the films. Solvent pretreatment significantly affected color difference, moisture content, total phenolic content, and surface hydrophobicity ($p < 0.05$), while milling cycle influenced structural homogeneity and total phenolic content through ball milling. In particular, alkaline pretreatments (NaOH and NaOH+EtOH) increased the total phenolic content and, in some cases, improved surface hydrophobicity and mechanical performance compared to water-treated films. Defatting also altered film characteristics by modifying intermolecular interactions and increasing hydrogen bonding within the matrix.

Product application studies using fresh-cut apples further showed that DDGS-based coatings influenced the browning kinetics of the fruit and maintained stable microbial counts during storage. Overall, the findings suggest that sorghum DDGS represents a promising sustainable feedstock for biodegradable film production, and that suitable combinations of chemical pretreatment and mechanical processing can be used to tailor film functionality for potential food packaging applications.

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Chapter 4 - Development of Functional Electrospun Nanofibers Using Micellar Casein/Pullulan with Different Emulsifiers Incorporating Thymol/DDGS-Derived Bioactives

Abstract

Electrospinning has emerged as a promising technique for producing nanofibrous films from biodegradable and renewable materials with enhanced functionality for active food packaging applications. In particular, coaxial electrospinning enables the fabrication of core–shell structures, allowing the incorporation and protection of functional ingredients. In this study, biodegradable and functional films were developed using coaxial electrospinning and pullulan or micellar casein as shell polymers, Tween 20 and lecithin as emulsifiers and emulsified active ingredients including thymol and DDGS-derived extracts as core materials. DDGS extracts were obtained from different solvent pretreatments (water, HCl, NaOH, and NaOH+EtOH) combined with ball milling and defatting to recover phenolic-rich fractions. Films were characterized for physicochemical, structural, and mechanical properties including color (ΔE), thickness, moisture content, tensile properties, and FTIR analysis. In addition, preliminary antimicrobial screening and product application tests were conducted on fresh-cut apples. Lecithin-based films produced lower moisture content and improved barrier behavior compared to Tween 20 films. Polymer type significantly affected film properties ($p < 0.05$), with pullulan films exhibiting lower moisture content, greater thickness, and improved mechanical strength compared to micellar casein films. Increasing pullulan concentration significantly enhanced mechanical strength and toughness ($p < 0.05$), indicating stronger fiber networks due to increased polymer chain entanglement. Incorporation of different DDGS extracts did not significantly affect ΔE or

thickness ($p > 0.05$), but significantly influenced moisture content and mechanical strength ($p < 0.05$). Antimicrobial activity of the films was evaluated using an agar diffusion assay, which showed clear inhibition halos. The pullulan films containing 0% and 0.8% thymol, and 0.8% DDGS_NaOH extract reduced browning. These results demonstrate that coaxial electrospinning combined with DDGS-derived bioactive compounds can produce functional biodegradable nanofiber films with potential applications in active food packaging.

4.1 Introduction

Electrospinning involves the application of a high-voltage electric field to a polymer solution, leading to jet elongation and solvent evaporation, ultimately depositing nanofibers on a collector (Y. Wang et al., 2023). Compared to solvent casting or extrusion, polysaccharide electrospun fibers exhibit greater surface area-to-volume ratio and porous network. Also they show improved ability to incorporate active compounds such as antimicrobials, antioxidants, and freshness indicators (Amorim et al., 2024). These unique properties have supported their integration into active and intelligent food packaging, where functionality beyond passive containment is required (Aman Mohammadi et al., 2020).

Food-grade electrospinning had largely focused on natural biopolymers, particularly proteins and polysaccharides, due to their biodegradability and generally recognized as safe (GRAS) status. Protein-based systems have attracted considerable attention because of their nutritional value and functional properties (A. Agarwal et al., 2022). However, proteins alone often present challenges during electrospinning. Their predominantly globular structures and limited chain entanglement reduce viscoelastic stability, leading to jet instability and discontinuous or bead-like fiber formation (Nieuwland et al., 2013). Although casein, the

dominant milk protein, has been widely studied for edible films due to its amphiphilic nature, its electrospinning remains challenging mainly due to intrinsically disordered and proline-rich protein structure (Flores-Nieves et al., 2022). Thus, proteins often require modification through pH adjustment, thermal treatment, or changes in solvent conditions to induce partial unfolding, enhance intermolecular interactions, and improve chain entanglement, thereby enabling stable electrospinning (Sullivan et al., 2014).

In contrast, most polysaccharides are suitable for electrospinning due to their linear or flexible chain structures, which promote chain entanglement and enhance solution viscosity (Z. Lin et al., 2023). Polysaccharides such as pullulan, guar gum, and starch have been widely reported to produce stable and continuous nanofibers under electrospinning (Aceituno-Medina et al., 2013; Aman Mohammadi et al., 2019; L. Kong & Ziegler, 2014). Among these, pullulan is particularly promising due to its amorphous structure, better oxygen barrier properties, and film-forming ability. Its molecular flexibility and compatibility with food-grade systems enable the formation of uniform, bead-free fibers (Min et al., 2021). For example, amaranth protein–pullulan systems produced uniform, continuous, bead-free fibers suitable for active packaging demonstrating that the presence of pullulan improved fiber uniformity and enabled the formation of continuous, bead-free structures (Aceituno-Medina et al., 2013). Similarly, guar gum can improve rheological behavior of whey protein isolate by increased solution viscosity and increased protein secondary structure with formation of α-helical structures, resulting in enhanced chain entanglement (Aman Mohammadi et al., 2019). These studies primarily highlight the role of polysaccharides in improving spinnability, fiber morphology, and functional performance of protein-based electrospinning systems.

While basic electrospinning has been widely explored, coaxial electrospinning, where two solutions are simultaneously ejected through concentric needles, with the shell providing spinnability and the core containing active agents, provides additional versatility by enabling core–shell fiber structures (Taokaew & Chuenkaek, 2024). These techniques allow incorporation of sensitive or poorly spinnable compounds into a core phase protected by a biopolymer shell. Similarly, emulsion electrospinning stabilizes immiscible droplets in a polymer matrix, which migrate into the fiber core during jet elongation (C. Zhang et al., 2020). Both approaches enhance the stability and controlled release of incorporated bioactives, making them valuable for active packaging.

Lecithin, a natural phospholipid emulsifier, is widely used to stabilize food emulsions and has been applied successfully in electrospinning to facilitate the incorporation of oils, proteins, and other compounds into fibers (Henao-Ardila et al., 2024). Lecithin stabilizes emulsions primarily through the formation of bilayer vesicles called liposomes, where its phospholipid structure enables the encapsulation of hydrophobic compounds within the lipid core while maintaining interfacial stability via steric and electrostatic interactions (Thy et al., 2025). For example, the incorporation of 2% lecithin into poly(ε-caprolactone) (PCL) scaffolds increases the specific elastic modulus by approximately 30% by reducing fiber curvature (Coverdale et al., 2017). On the other hand, Tween 20 (TW20), a non-ionic surfactant also known as polyoxyethylene sorbitan monolaurate, is widely used in food and pharmaceutical systems due to its high hydrophilicity and strong emulsifying capacity (Dimitrova & Leal-Calderon, 1999). TW20 stabilizes emulsions through the formation of micelles, where its high hydrophilic–lipophilic balance (HLB) promotes efficient reduction of interfacial tension and provides steric stabilization, leading to smaller and more uniformly dispersed droplets (Knoch et al., 2021). In

electrospinning applications, addition of TW20 has been reported to improve emulsion stability, prevent phase separation, and enhance fiber uniformity by promoting consistent jet formation (Bardoňová et al., 2022). Compared to natural emulsifiers such as lecithin, TW20 provides more controlled and reproducible emulsion systems, particularly in formulations containing low-molecular-weight or hydrophobic compounds (McClements & Gumus, 2016).

DDGS, a coproduct of bioethanol production from cereal grains, represents one of the most abundant agro-industrial byproducts, with annual production exceeding 40 million tons in the United States alone (Leguizamón et al., 2009). Previous research showed that DDGS can be incorporated into biodegradable films using aqueous or alkaline–ethanol pretreatments followed by solvent casting with plasticizers, improving mechanical and barrier properties (Prabhakumari et al., 2018). However, the potential of DDGS in electrospinning remains underexplored. DDGS extracts enriched in soluble proteins, peptides, and small molecular compounds, may serve as a valuable feedstock for emulsion-based electrospinning. Incorporating DDGS extracts into core phases not only supports waste valorization but also offers opportunities to enhance the functional activity of packaging films.

This study investigates the use of micellar casein or pullulan shell solutions combined with a different emulsifier (lecithin or Tween20) stabilized active ingredients (thymol or DDGS extracts) emulsion core as a novel approach to food-grade coaxial electrospinning. It is hypothesized that coaxial electrospun fibers produced from this system will yield continuous, uniform films with improved barrier and functional properties, while simultaneously promoting sustainable use of an abundant agro-industrial byproduct.

4.2 Materials and Methods

Micellar casein powder was obtained from (Naked Nutrition, US). Pullulan (≥95% purity) was purchased from Nutriscience Ingredients. Food-grade soy lecithin (≥95% phosphatidylcholine) was obtained from American Lecithin Company. Pure sorghum DDGS were provided by PureField Ingredients, Russel, KS, US, and the extracts were prepared by aqueous extraction and centrifugation. Analytical-grade sodium hydroxide (NaOH), glacial acetic acid, ethanol, and glycerol were purchased from Fisher Chemicals. Deionized water was used in all experiments.

Preparation of DDGS Extracts

DDGS extracts were prepared using different solvent systems and pretreatment conditions. Initially, DDGS was divided into two portions. For one portion, lipids were removed through solvent extraction, and the recovered lipid fraction was also retained and used as a DDGS-derived extract.

The remaining non-defatted portion was subjected to mechanical pretreatment via ball milling. During ball milling, DDGS was treated with various solvents, including distilled water, 0.1 N hydrochloric acid (HCl), 0.1 N sodium hydroxide (NaOH), and a 0.1 N sodium hydroxide (NaOH) + 50% ethanol (EtOH) mixture with 1:3 w/w DDGS:solvent ratio.

Following ball milling, the suspensions were centrifuged, and the supernatants were collected as DDGS extracts for further characterization and application. The detailed preparation procedure of DDGS extracts was performed by another researcher in the laboratory and is described in the previous chapter; therefore, only the collected supernatants were utilized in the present work.

Preparation of Shell Solutions

Polymers were used to form shell-solutions as summarized in Table 4.1. Micellar casein was dissolved and heated in deionized water at 5% (w/v) for 1 h. The pH was adjusted to 10 using 5% NaOH after cooling. Ethanol (1% v/v) and glycerol (1% w/v) were added to enhance solubility and spinnability. The solution was stirred overnight at room temperature until completely homogeneous. Pullulan solution (15% w/v) was prepared by dissolving in 15% (v/v) acetic acid. Glycerol (1% w/v) was incorporated as a plasticizer. The solution was stirred until clear and fully dissolved.

Preparation of Core Emulsion and Shell Formulation

Table 4.1 Shell and core solutions for the electrospinning

Single Electrospinning			
15% Pullulan+15% Acetic Acid+ 1% glycerol (control)			
5% Micellar Casein (pH=10 with 5% NaOH) +1% glycerol +1% ethanol (control)			
Coaxial Electrospinning			
Shell Solution	**Core Solution**		
	Emulsifier Type	Active Ingredient	Active Ingredient Concentration (%)
15% Pullulan+15% Acetic Acid+ 1% glycerol	4 % Tween20	Thymol	0.2
15% Pullulan+15% Acetic Acid+ 1% glycerol	4% Tween20	Thymol	0.8
15% Pullulan+15% Acetic Acid+ 1% glycerol	4% Lecithin	Thymol	0.2
15% Pullulan+15% Acetic Acid+ 1% glycerol	4% Lecithin	Thymol	0.8

5% Micellar Casein (pH=10 with 5% NaOH) +1% glycerol +1% ethanol	4% Tween20	Thymol	0.2
5% Micellar Casein (pH=10 with 5% NaOH) +1% glycerol +1% ethanol	4% Tween20	Thymol	0.8
10% Pullulan+15% Acetic Acid+ 1% glycerol	4% Tween20	DDGS Lipid Extract	0.8
15% Pullulan+15% Acetic Acid+ 1% glycerol	4% Tween20	DDGS Lipid Extract	0.8
15% Pullulan+15% Acetic Acid+ 1% glycerol	4% Tween20	DDGS Water Extract	0.8
15% Pullulan+15% Acetic Acid+ 1% glycerol	4% Tween20	DDGS HCl Extract	0.8
15% Pullulan+15% Acetic Acid+ 1% glycerol	4% Tween20	DDGS NaOH Extract	0.8
15% Pullulan+15% Acetic Acid+ 1% glycerol	4% Tween20	DDGS NaOH+EtOH Extract	0.8

The core solution was emulsions of DDGS Extracts (lipid, water, HCl, NaOH, NaOH+EtOH) or thymol stabilized by TW20 or lecithin. Emulsifier (4% w/v) was dispersed in deionized water under stirring, followed by the gradual addition of active ingredients. The emulsifier dispersed in water was mixed with the lipid core using IKA T18 basic, Ultra-Turrax at 10,000 rpm for 3 min. Emulsions were prepared by high pressure homogenizer (PandaSmart Homogenizer, GEA) for 3 cycles at 500 bars. The emulsions were stored at 4 °C until used.

Electrospinning Process

Electrospinning was carried out using a laboratory-scale electrospinning system (NLS20, NLI) equipped with both single-nozzle and coaxial configurations Figure 4.1.

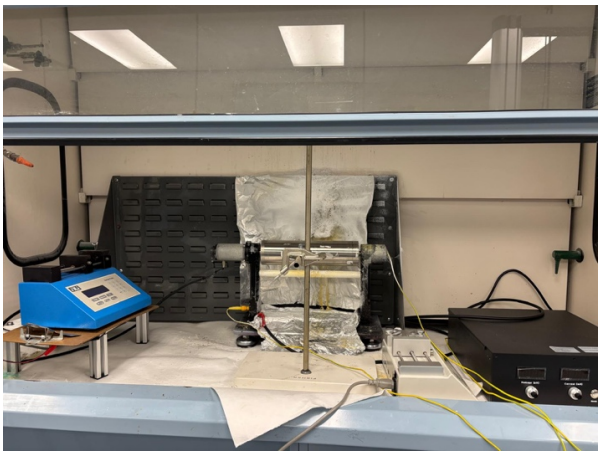


Figure 4.1 Electrospinning process

For the single electrospinning, the polymer solution was loaded into a syringe fitted with a 21G needle, and fibers were generated through a conventional electrospinning process. Preliminary trials were conducted by varying the applied voltage (12–22 kV), flow rate (0.2–1.5 mL/h), and tip-to-collector distance (8–15 cm) to obtain a stable jet and uniform fibers. For coaxial electrospinning, a concentric needle system was used to simultaneously process two different solutions. The shell solution was loaded into the outer syringe fitted with a 15G needle, while the core solution was loaded into the inner syringe fitted with a 21G needle to enable core–shell fiber formation. Similar to the single-nozzle electrospinning, preliminary conditions were screened within 12–22 kV for voltage, 0.5–2 mL/h for both shell and core flow rates, and 8–18 cm for tip-to-collector distance. The preliminary trials have been given in Appendix A. The optimized coaxial electrospinning parameters were 18 kV applied voltage, 1 mL/h shell flow rate, 0.5 mL/h core flow rate, and a 12 cm tip-to-collector distance. The optimization process was based on achieving a stable Taylor cone and continuous jet formation, along with uniform fiber morphology without

bead defects. A detailed summary of all preliminary conditions and parameter screening is provided in Appendix A.

All electrospinning experiments were conducted at 22 ± 2 °C and 35–45% relative humidity. Fibers were collected on aluminum foil-covered drum collectors, carefully removed, and stored in zip-lock bags at room temperature until further analysis.

Mechanical and Physiochemical Properties

Normalized Toughness and Elongation: Normalized toughness and elongation at break (EAB) of the films were determined using a texture analyzer (TA-XT2i, Texture Technologies Corp., USA) equipped with TA-96B tensile grips, following ASTM D882. Film strips (25 × 50 mm) were mounted between the grips and stretched in tension mode until break. The test was conducted at a pre-test speed of 1 mm/s, test speed of 1 mm/s, and post-test speed of 1 mm/s with a trigger force of 5 g and a maximum travel distance of 60 mm. Film thickness was measured prior to testing using a digital micrometer and used to calculate the cross-sectional area. Normalized toughness was calculated as the total area under the force–distance curve up to the breaking point, representing the energy absorbed before rupture, and was normalized by dividing by the corresponding film thickness to obtain normalized toughness ($N.mm_{distance}$)/$mm_{thickness}$), while elongation at break was expressed as the percentage increase in length relative to the initial gauge length.

Color Analysis: Film color (L*, a*, b*) was measured using a colorimeter (PCE-CSM 4, PCE). The equipment was calibrated using standard black and white calibration plates before measurements. The L* value represents lightness (0 = black, 100 = white), while a* indicates the red–green axis (positive values represent redness and negative values represent greenness) and b* represents the yellow–blue axis (positive values indicate yellowness and negative values

indicate blueness). The color (ΔE) was calculated by using the following equation where $\Delta L, \Delta a \; and \Delta b$ represent the differences between the measured color parameters of the film sample and the corresponding reference values. In this study, the reference values were taken from the calibration standard, and the deviations in L, a, and b were used to calculate the overall color difference. ΔE was calculated based on the Equation (3):

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \qquad (3)$$

Film Thickness: Film thickness was measured with a digital micrometer (accuracy ±0.001 mm) at five random positions per sample, and average values were reported.

Moisture Content ($MC_{db}(\%)$): The moisture content of the films was determined gravimetrically. Samples (0.04-0.08 g) were weighed prior to drying (initial weight, W_i) and then placed in a hot-air oven at 110 °C for 48 h. After drying, the films were reweighed (final weight, W_f), and the moisture content was calculated on a dry basis (db) and expressed as percentage using the Equation (4) below:

$$MC_{db}(\%) = \frac{W_i - W_f}{W_f} \; x100\% \qquad (4)$$

Total Phenolic Compound (TPC) Analysis: Total phenolic content (TPC) of the extracts was determined using the Folin–Ciocalteu colorimetric method, with gallic acid used as the standard compound. Briefly, each extract (0.1 mg) was weighed and transferred into microcentrifuge tubes. The samples were extracted with 70% (v/v) methanol (1 mL) and stirred overnight at room temperature to ensure sufficient extraction of phenolic compounds. After extraction, the supernatants were diluted with 70% methanol to obtain 1:10 and 1:20 (v/v) dilutions for analysis. For the assay, an aliquot of the diluted extract was mixed with Folin–Ciocalteu reagent. After a short reaction period, sodium carbonate solution was added to neutralize the mixture and allow color development. The reaction mixtures were incubated at

room temperature in the dark for 10 minutes, and the absorbance was measured at 765 nm using a UV–Vis spectrophotometer. A calibration curve was prepared using gallic acid solutions at different concentrations in 70% methanol. The gallic acid equivalent concentration of each sample was obtained by substituting the absorbance values into the calibration curve equation, y=ax+b. TPC values were calculated from the standard curve and expressed as mg gallic acid equivalents (GAE) per g of film using Equation (5):

$$TPC\ \left(mg\frac{GAE}{g}\right) = \frac{y*V*Df}{m} \qquad (5)$$

where y is the gallic acid equivalent concentration obtained from the calibration curve, V is the extraction volume (mL), DF is the dilution factor, and m is the mass of extract (g).

Fourier Transform Infrared Spectroscopy (FTIR): FTIR spectra were recorded using a Spectrum Two (PerkinElmer, Waltham, MA, USA) in the range of 4000–600 cm^{-1} with four scans at a resolution of 4 cm^{-1}. Spectra were analyzed to identify functional group modifications resulting from the incorporation of DDGS supernatants and lecithin emulsions.

Antimicrobial Activity and Shelf-Life Analysis

Preliminary Antimicrobial Screening of Films: Preliminary antimicrobial screening of the films was conducted using a well plate diffusion method. Film samples were tested against *Salmonella enterica*, *Staphylococcus aureus*, and *Listeria monocytogenes*. Antimicrobial activity was evaluated by measuring inhibition zones around the wells. Films demonstrating stronger inhibitory effects, together with favorable mechanical and physical properties, were selected for product application studies.

Product Application

Selected film samples were evaluated in a model food system using fresh Red Delicious apples. Apples were manually peeled and cut into uniform slices with an average weight of

approximately 2 g and consistent thickness to ensure sample standardization. Apple pieces were wrapped with the selected films and stored in an environmental chamber at 4 °C and 20% relative humidity for 7 days. Samples were analyzed at predetermined storage times (day 0, 3, and 7) for microbial quality, and color changes were monitored throughout storage.

Microbial Analysis

Red Delicious apples were peeled and cut into uniform pieces, and 2 g of sample was used for each treatment. The apple samples were wrapped with the respective film formulations and stored under refrigerated conditions. Microbial analyses were conducted on days 0, 3, and 7. Total Aerobic Mesophilic count was enumerated on Aerobic Count Petri film after incubation at 37 °C for 48 h, while yeast and mold counts were determined using Yeast and Mold Count Petri film incubated at 28 °C for 48 h. Microbial counts were expressed as log CFU/g. Color was measured as describes above over storage time. Pictures were taken in parallel with color measurements.

Statistical Analysis

Statistical analysis was performed using analysis of variance (ANOVA) with a significance level of $\alpha = 0.05$. All experiments and films were prepared and analyzed in triplicate. Results were expressed as mean ± standard deviation. Data were analyzed using analysis of variance (ANOVA) in R, followed by Tukey's HSD post hoc test. Differences among means were considered significant at $p < 0.05$.

4.3 Results and Discussion

4.3.1 Preliminary trials for the electrospinning films

Preliminary electrospinning trials were conducted using different biopolymers, including whey protein isolate (WPI), sodium caseinate, gelatin, pullulan, and micellar casein, in order to

evaluate their spinnability and fiber-forming potential under various solution and processing conditions. The experimental parameters and corresponding observations are presented in Appendix A.

Among the tested polymers, WPI, sodium caseinate, and gelatin solutions exhibited limited spinnability, often resulting in bead formation or unstable jet behavior, indicating insufficient chain entanglement and suboptimal solution properties for continuous fiber formation. In contrast, pullulan and micellar casein demonstrated stable electrospinning behavior and were able to produce more uniform and continuous fiber structures under optimized conditions.

Therefore, pullulan and micellar casein were selected as the primary biopolymers for subsequent experiments, as they showed superior film-forming ability and electrospinning performance compared to the other tested polymers.

4.3.2 Total Phenolic Content of DDGS extracts and active ingredients

TPC of DDGS extracts and DDGS itself are shown in Table 4.2. Extract type had a significant effect on TPC, with significant differences observed among the 18 groups ($p<0.05$). Among all samples, NaOH + EtOH treatments showed the highest TPC values, followed by NaOH, while water and HCl treatments resulted in lower phenolic content (Table 4.2). This indicates that alkaline conditions were more effective in releasing bound phenolic compounds.

Defatted extracts showed higher TPC values compared to non-defatted ones. This supports better solvent accessibility after lipid removal, allowing more efficient extraction of phenolic compounds. Similarly, increasing milling cycle from 1× to 3× slightly increased TPC, showing reduced particle size and increased surface area. The low TPC values observed in water-treated samples suggest that most phenolic compounds in DDGS are present in bound

form and cannot be easily extracted under neutral conditions. Acid treatment improved extraction as comparted to water-treated ones but was still less effective than alkaline treatments (Table 4.2). These results are consistent with previous studies showing that the use of NaOH alkaline extraction demonstrated a high efficacy for recovering polyphenolic compounds sorghum kernels, appearing at least equally or more efficient than traditional aqueous acetone methods; this enhanced yield is due to the alkaline solution reacting with the pericarp cell wall material, which facilitates the release of higher quantities of phenolic compounds from the grain (Blackwell et al., 2012).

Overall, NaOH + EtOH pretreatment combined with defatting and increased milling intensity resulted in the highest phenolic recovery. These differences in phenolic content and composition are expected to influence intermolecular interactions within the electrospun system and therefore affect the physical and mechanical properties of the resulting films.

Table 4.2 Total phenolic content (TPC) of DDGS extracts obtained under different chemical pretreatments, lipid conditions and milling cycles. TPC values are expressed as mg gallic acid equivalents (GAE) per g DDGS. Different letters indicate significant differences among treatments ($p < 0.05$).

Sample	Non-Defatted (GAE/g)	Defatted (GAE/g)
Water - 1x	4.23 ± 0.55^{ab}	4.41 ± 0.73^{ab}
Water - 3x	5.60 ± 0.09^{abc}	8.63 ± 0.73^{cde}
HCl - 1x	6.97 ± 0.18^{bcd}	8.94 ± 0.36^{cde}
HCl - 3x	7.75 ± 0.18^{bcd}	9.07 ± 0.54^{cde}
NaOH – 1x	10.37 ± 0.73^{de}	19.15 ± 0.36^{fg}
NaOH – 3x	11.67 ± 1.47^{e}	20.19 ± 0.36^{fg}
NaOH + EtOH – 1x	17.75 ± 1.01^{f}	22.64 ± 2.19^{gh}
NaOH + EtOH – 3x	19.12 ± 0.92^{fg}	23.81 ± 1.28^{h}
DDGS	10.10 ± 0.91^{de}	

Lipid	3.25 ± 0.54[a]

4.3.3 Electrospun films

4.3.3.1 Effect of polymer type and active ingredient concentration on film properties

Pullulan-based electrospun films appeared white, continuous, and uniform relative to micellar casein films (Figure 4.2). The fibrous mats were thicker and more opaque compared to micellar casein films, which may be related to the higher viscosity and chain entanglement of pullulan solutions. Increasing thymol concentration did not cause visible aggregation or structural collapse, suggesting successful encapsulation within the fibers. On the other hand, micellar casein films appeared thinner and more transparent compared to pullulan films (Figure 4.3). The nanofiber mats were visually less dense, which is consistent with the lower thickness and higher moisture content observed in the physicochemical measurements. Thymol incorporation did not visibly affect the film structure, indicating that the electrospinning process remained stable across different thymol concentrations.

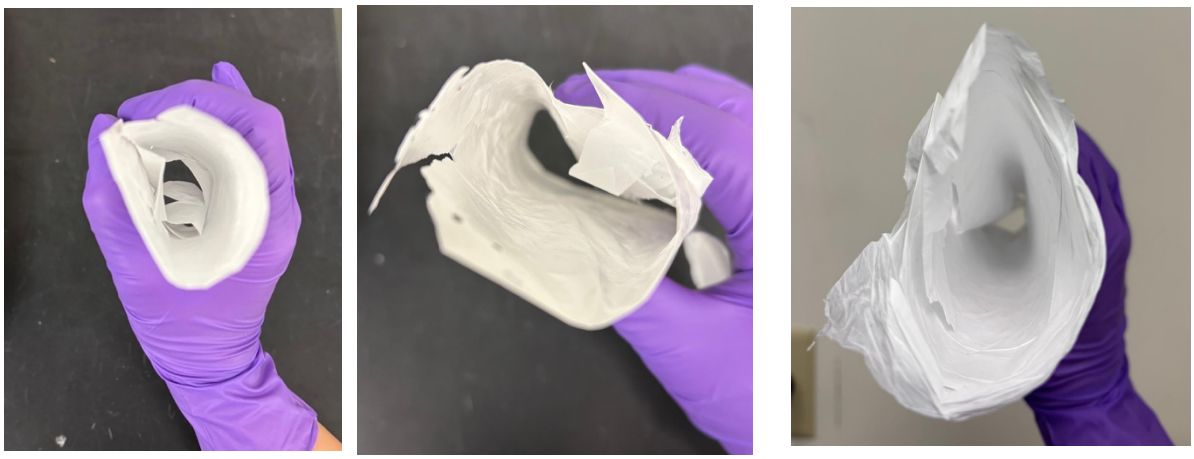


Figure 4.2 Visual appearance of electrospun pullulan films containing 4% Tween 20 with 0% (left), 0.2% (middle), and 0.8% (right) thymol.

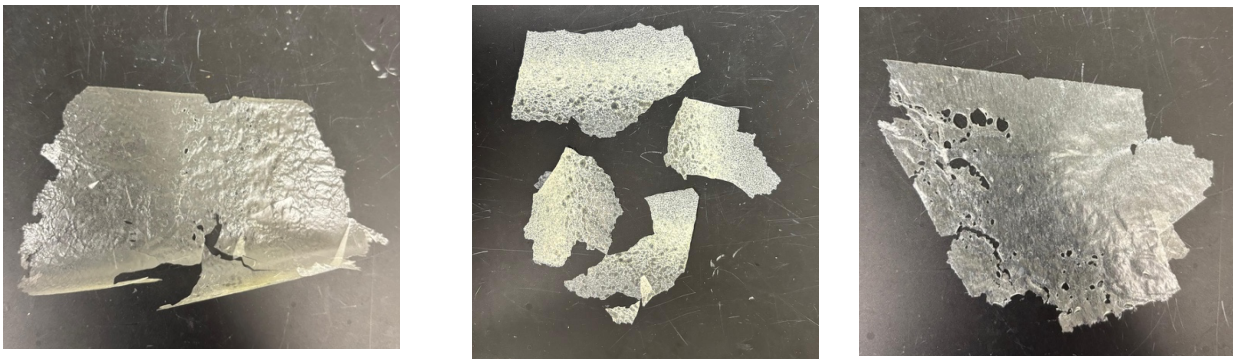


Figure 4.3 Visual appearance of electrospun micellar casein films containing 4% Tween 20 with 0% (left), 0.2% (middle), and 0.8% (right) thymol.

Physical properties of the pullulan/micellar casein films with different thymol concentrations

The physical properties of electrospun pullulan or micellar casein films with different thymol concentrations are presented in Table 4.3.

Polymer type and interaction of polymer type and thymol concentration had a significant effect on ΔE ($p < 0.05$); however, thymol concentration had no significant effect on ΔE ($p>0.05$). Pullulan-based films exhibited higher ΔE values compared to micellar casein films (Table 4.3). This can be attributed to the thicker fiber mats and higher light scattering in polysaccharide-based systems (Riviello et al., 2024). In addition, pullulan nanofiber mats are typically white in appearance, which enhances light reflection and contributes to higher ΔE values because of high L* (lightness) values (Ai, 2024). The significant interaction effect indicates that thymol influenced color differently depending on the polymer matrix. However, no visible color heterogeneity was observed, suggesting that thymol was uniformly distributed within the fiber structure rather than forming aggregates, which is consistent with previous findings for electrospun pullulan systems (Mandura Jarić et al., 2025b).

Thymol concentration, polymer type and the polymer × thymol interaction had significantly affected film thickness ($p < 0.05$). Pullulan films exhibited higher thickness values

compared to micellar casein films (Table 4.3). This behavior is related to the higher chain entanglement and viscosity of pullulan solutions, which promote the formation of thicker and denser fiber mats during electrospinning (Tomasula et al., 2016). Although thymol incorporation did not cause structural collapse, slight increases in thickness at higher thymol concentrations may be due to changes in solution properties such as viscosity and conductivity, which affect jet stretching and fiber deposition.

Polymer type had a significant effect on moisture content ($p < 0.05$), however thymol concentration and the interaction of polymer type and thymol concentration had no significant effect on moisture content ($p>0.05$). Micellar casein films showed higher moisture content than pullulan films (Table 4.3). This is expected due to the hydrophilic nature of protein-based systems (P. Gupta & Nayak, 2015). Casein contains polar amino acid residues such as serine, threonine and asparagine that can form hydrogen bonds with water, and its electrospun fiber network tends to be more porous, allowing greater water retention (Tomasula et al., 2016).

Table 4.3 Physical properties of electrospun films as influenced by polymer type (pullulan, micellar casein) and thymol concentration. Different lowercase letters indicate significant differences ($p < 0.05$).

Sample	ΔE	Thickness (mm)	Moisture Content (%db)
Pullulan			
0% Thymol (control)	56.03 ± 4.28^{ab}	0.09 ± 0.007^{a}	9.17 ± 3.36^{a}
0.2%Thymol	69.46 ± 1.17^{c}	0.21 ± 0.04^{b}	10.13 ± 0.46^{ab}
0.8% Thymol	65.93 ± 2.87^{c}	0.22 ± 0.06^{b}	12.01 ± 0.92^{abc}
Micellar Casein			
0% Thymol (control)	52.85 ± 3.99^{ab}	0.05 ± 0.009^{a}	20.95 ± 6.75^{bc}
0.2%Thymol	46.69 ± 4.57^{a}	0.07 ± 0.005^{a}	21.00 ± 0.92^{bc}

0.8% Thymol	52.42 ± 5.07^{ab}	0.07 ± 0.004^{a}	22.78 ± 6.74^{c}

Mechanical properties of the pullulan/micellar casein films with different thymol concentrations

Polymer type and thymol concentration and their interaction significantly affected elongation ($p<0.05$). This suggests that similar structural modifications within the polymer matrix governed both strength and extensibility.

Polymer type and thymol concentration had a significant effect on normalized toughness ($p < 0.05$); however, their interaction did not show any significant difference on normalized toughness ($p>0.05$). Pullulan films exhibited higher values compared to micellar casein films, indicating greater energy absorption during deformation and therefore higher toughness (Table 4.4). The increase in mechanical performance with thymol in pullulan systems suggests that thymol was effectively incorporated without disrupting polymer chain entanglement.

These findings are consistent with previous studies showing that pure pullulan formulation can maintain mechanical integrity even at higher bioactive loadings such as 15% tocotrienol fraction, as long as polymer–polymer interactions remain dominant (Wijekoon et al., 2025). In contrast, the lower normalized toughness values observed in micellar casein films indicate a less resistant and more brittle structure, can be attributed to weaker intermolecular interactions and higher moisture sensitivity.

Sample	Elongation (mm)	Normalized Toughness ($W/mm_{thickness}$= $N.mm_{distance}/mm_{thickness}$)
Pullulan		
Control	2.52 ± 0.00^{ab}	10.94 ± 3.17^{b}

0.2%Thymol	7.08 ± 0.67^{c}	11.52 ± 1.72^{b}
0.8% Thymol	4.91 ± 2.00^{bc}	7.3 ± 2.27^{ab}
Micellar Casein		
Control	0.80 ± 0.02^{a}	6.73 ± 2.27^{ab}
0.2%Thymol	0.70 ± 0.02^{a}	1.98 ± 0.06^{a}
0.8% Thymol	0.59± 0.07^{a}	2.02 ± 0.12^{a}

Table 4.4 Mechanical properties of electrospun films as influenced by polymer type (pullulan, micellar casein) and thymol concentration. Data were analyzed using two-way ANOVA followed by Tukey's test. Different lowercase letters indicate significant differences ($p < 0.05$).

FTIR: Micellar casein films exhibited characteristic amide I (1650 cm^{-1}) and amide II (1540 cm^{-1}) bands, corresponding to protein backbone vibrations. These bands were absent in pullulan films. The stronger and broader O–H/N–H band in micellar casein films explains their significantly higher moisture content (Table 4.3), due to increased hydrogen bonding capacity. Pullulan films displayed dominant carbohydrate peaks between 1000–1100 cm^{-1}, confirming the polysaccharide structure. A comparable result was reported for electrospun pullulan–whey protein isolate (WPI) nanofibers, where ATR-FTIR spectra confirmed the simultaneous presence of polysaccharide and protein components and revealed carbon–hydrogen and hydrogen-bond interactions between pullulan and WPI chains, indicating strong molecular interactions within the nanofiber matrix (Ali et al., 2023). Interestingly, thymol incorporation did not significantly shift the amide bands in micellar casein films. This suggests limited interaction between thymol and the protein matrix, which may explain why micellar casein films did not show mechanical reinforcement with thymol addition (Table 4.3). Overall, FTIR confirms that polymer chemistry primarily dictated moisture and mechanical behavior, consistent with tensile results.

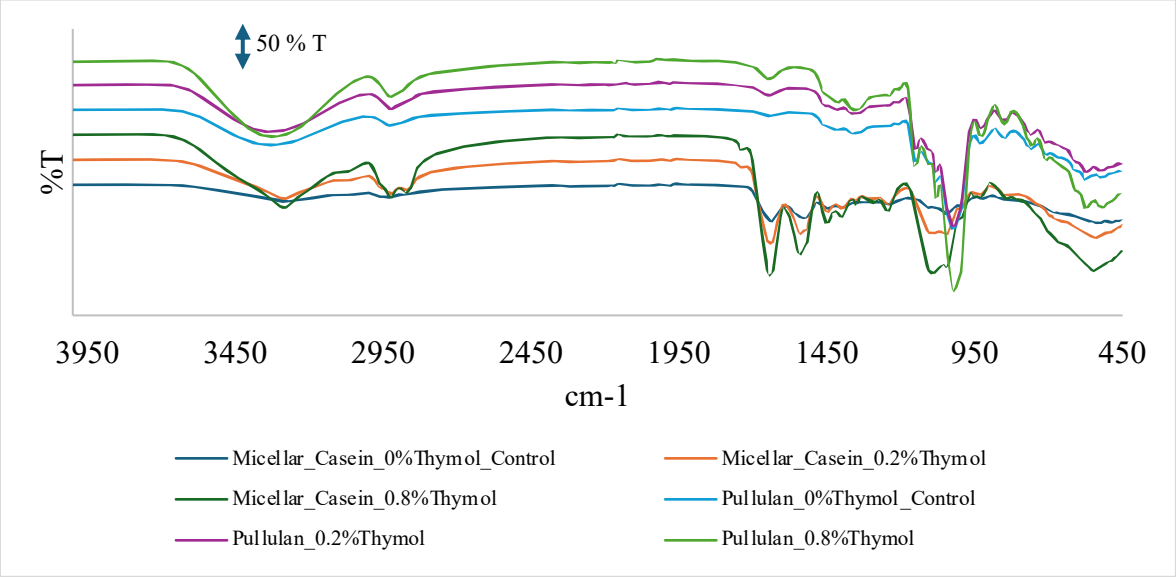


Figure 4.4 FTIR spectra of the films with different polymer types and active ingredient concentrations.

4.3.3.2 Effect of emulsifier type and active ingredient concentration on film properties

Electrospun pullulan films exhibited a continuous and homogeneous fibrous mat structure with a white and slightly opaque appearance (Figure 4.5). No visible phase separation or aggregation of thymol was observed, indicating that thymol was successfully incorporated within the electrospun fibers. Increasing thymol concentration from 0.2% to 0.8% did not visibly alter the overall structure or integrity of the films, suggesting that thymol incorporation at these levels did not disrupt fiber formation. Similar to Tween 20 films, lecithin-stabilized films showed uniform nanofiber mats without visible structural defects (Figure 4.6). The films appeared slightly opaquer compared to Tween 20 ones, which may be attributed to the presence of

phospholipid components affecting light scattering within the fibrous structure. However, no visible changes in film morphology were observed with increasing thymol concentration.

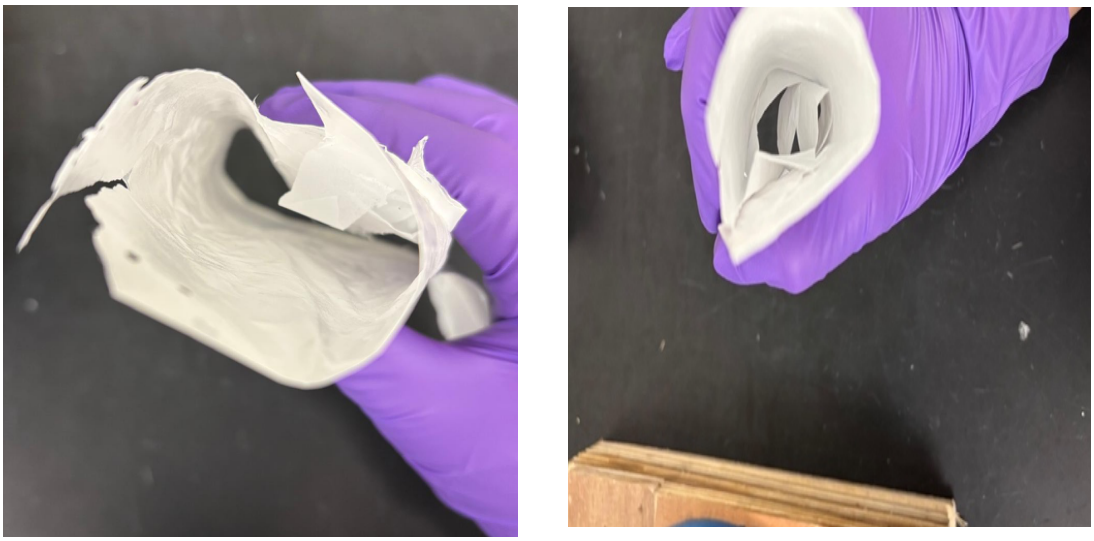


Figure 4.5 Visual appearance of electrospun pullulan nanofiber films containing 4% Tween 20 with 0.2% (left) and 0.8% (right) thymol.

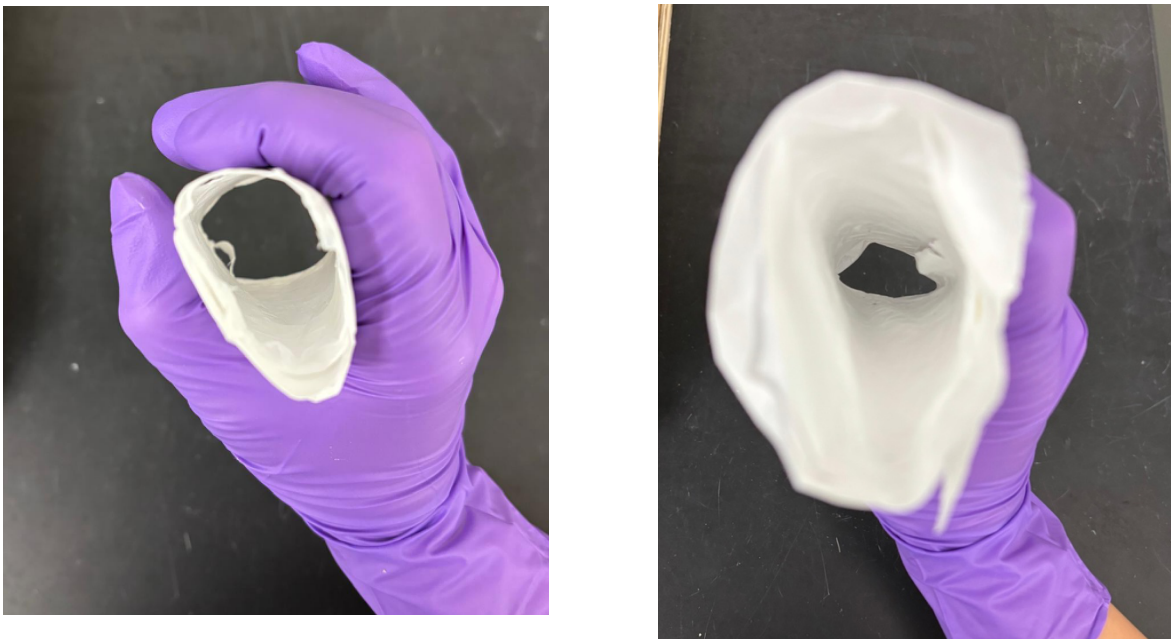


Figure 4.6 Visual appearance of electrospun pullulan nanofiber films containing 4% lecithin with 0.2% (left) and 0.8% (right) thymol.

Physical Properties of the Pullulan Films with Different Emulsifiers and Active Ingredient Concentration

The physical properties of electrospun pullulan films produced with different emulsifier types, Tween 20 and lecithin, and thymol concentrations, 0.2% and 0.8%, are presented in Table 4.5.

Thymol concentration and emulsifier type and their interaction did not have a significant effect on ΔE or thickness ($p > 0.05$). This is as expected, and the electrospinning process played a dominant role in fiber formation across all samples. Since electrospinning involves rapid solvent evaporation and jet stretching, small compositional changes such as thymol concentration were not sufficient to alter these properties (Rashid et al., 2024).

On the other hand, emulsifier type significantly affected moisture content ($p < 0.05$), whereas thymol concentration and the interaction of emulsifier type and thymol concentration had no effect on moisture content ($p>0.05$). Lecithin-containing films showed lower moisture content compared to Tween 20 systems (Table 4.5). This can be explained by the more hydrophobic nature of lecithin, which reduces water affinity within the fiber network. Similar trends have been reported in pea protein films where candelilla wax or oleic acid incorporation decreases moisture content due to increased hydrophobic interactions (Kowalczyk et al., 2024a).

Table 4.5 Physical properties of pullulan electrospun films as affected by emulsifier type (Tween 20, lecithin) and thymol concentration (0.2% and 0.8%). Different lowercase letters indicate significant differences among treatments ($p < 0.05$).

Sample	ΔE*	Thickness (mm)	Moisture Content (%db)
TW20			
0.2%Thymol	69.46 ± 1.17^{a}	0.21 ± 0.04^{a}	10.13 ± 0.46^{a}
0.8% Thymol	65.93 ± 2.87^{a}	0.22 ± 0.06^{a}	12.01 ± 0.92^{a}
Lecithin			
0.2%Thymol	69.03 ± 4.33^{a}	0.25 ± 0.01^{a}	6.98 ± 0.97^{b}
0.8% Thymol	65.39 ± 4.90^{a}	0.28 ± 0.01^{a}	6.77 ± 0.85^{b}

Mechanical Properties of the Pullulan Films with Different Emulsifiers and Active Ingredient Concentration

The mechanical properties of pullulan electrospun films with different emulsifier types and thymol concentration are given in Table 4.6. Neither thymol concentration nor emulsifier type and their interaction had a significant effect on elongation ($p > 0.05$). This suggests that the incorporation of thymol up to 0.8% did not negatively affect the mechanical integrity of the electrospun fibers. The absence of mechanical deterioration indicates that thymol was successfully embedded within the fiber structure without disrupting fiber continuity. This is important since thymol is a volatile and hydrophobic compound, and proper encapsulation is required to maintain both functionality and structure (Y. Liu et al., 2021; Min et al., 2021).

On the other hand, emulsifier type and thymol concentration significantly affected normalized toughness ($p < 0.05$) while their interaction had no significant difference ($p>0.05$). Lecithin-based films showed lower values compared to Tween 20 films, indicating lower energy required for deformation and therefore reduced toughness (Table 4.6). This can be related to a more compact and less flexible network structure formed in the presence of lecithin. The

stronger intermolecular interactions, through hydrogen bonding, can restrict polymer chain mobility. Similar trends have been reported with previous studies showing that lecithin can strengthen interfacial interactions within lecithin and gelatin co-emulsified cinnamaldehyde electrospun fibers (L. Lin et al., 2024). However, in another study with thymol incorporation within chitosan/polyethylene oxide nanofiber systems show that tensile strength and the elongation at break exhibited a sharp decrease when the crosslinker content, genipin, was increased to 1% (Z. Zhu et al., 2024).

Table 4.6 Mechanical properties of pullulan electrospun films as affected by emulsifier type (Tween 20, lecithin) and thymol concentration (0.2% and 0.8%). Different lowercase letters indicate significant differences among treatments ($p < 0.05$).

Sample	Elongation (mm)	Normalized Toughness ($W/mm_{thickness}$= $N.mm_{distance}/mm_{thickness}$)
TW20		
0.2%Thymol	7.08 ± 0.67^{a}	11.52 ± 1.72^{a}
0.8% Thymol	4.91 ± 2.00^{a}	7.3 ± 2.27^{ab}
Lecithin		
0.2%Thymol	4.85 ± 1.30^{a}	1.69 ± 0.04^{b}
0.8% Thymol	3.01 ± 0.48^{a}	1.68 ± 0.10^{b}

FTIR: All pullulan-based films exhibited a broad absorption band at 3200–3400 cm^{-1}, corresponding to O–H stretching vibrations of hydroxyl groups in pullulan. The intensity of this band was slightly reduced in lecithin-containing films compared to Tween 20 films, suggesting decreased hydrogen bonding with water. This observation is consistent with the lower moisture content measured in lecithin films (Table 4.5), indicating a more hydrophobic structure. In a similar study, electrospun nanofibers prepared from pullulan and whey protein isolate exhibited a broad O–H stretching band around 3300 cm^{-1} together with C–H stretching vibrations near 2915

cm^{-1}, which were attributed to hydrogen bonding interactions between the polysaccharide and protein components (Ali et al., 2023). The slightly increased intensity in samples containing 0.8% thymol supports successful incorporation of the hydrophobic compound into the fiber structure at this band. A similar observation has been reported for electrospun gelatin nanofibers containing cinnamaldehyde stabilized with soy lecithin, where FTIR spectra showed characteristic C–H stretching bands around 2927 cm^{-1} and ester C=O vibrations near 1742 cm^{-1} associated with phospholipid structures, confirming the incorporation of hydrophobic components within the electrospun fiber matrix without significant chemical modification of the polymer backbone (L. Lin et al., 2024).

In the fingerprint region (1200–900 cm^{-1}), strong peaks corresponding to C–O–C and C–O stretching of the pullulan backbone were observed. Tween 20 films showed marginally broader peaks in this region, which may reflect a less compact network structure. This aligns with slightly higher moisture content. In contrast, lecithin-containing films exhibited sharper peaks, suggesting improved structural cohesion, which correlates with lower moisture values.

Importantly, no new distinct peaks appeared upon increasing thymol concentration from 0.2% to 0.8%, indicating that thymol was physically encapsulated rather than chemically reacting with the polymer matrix. This supports the mechanical results where no significant deterioration in tensile properties was observed.

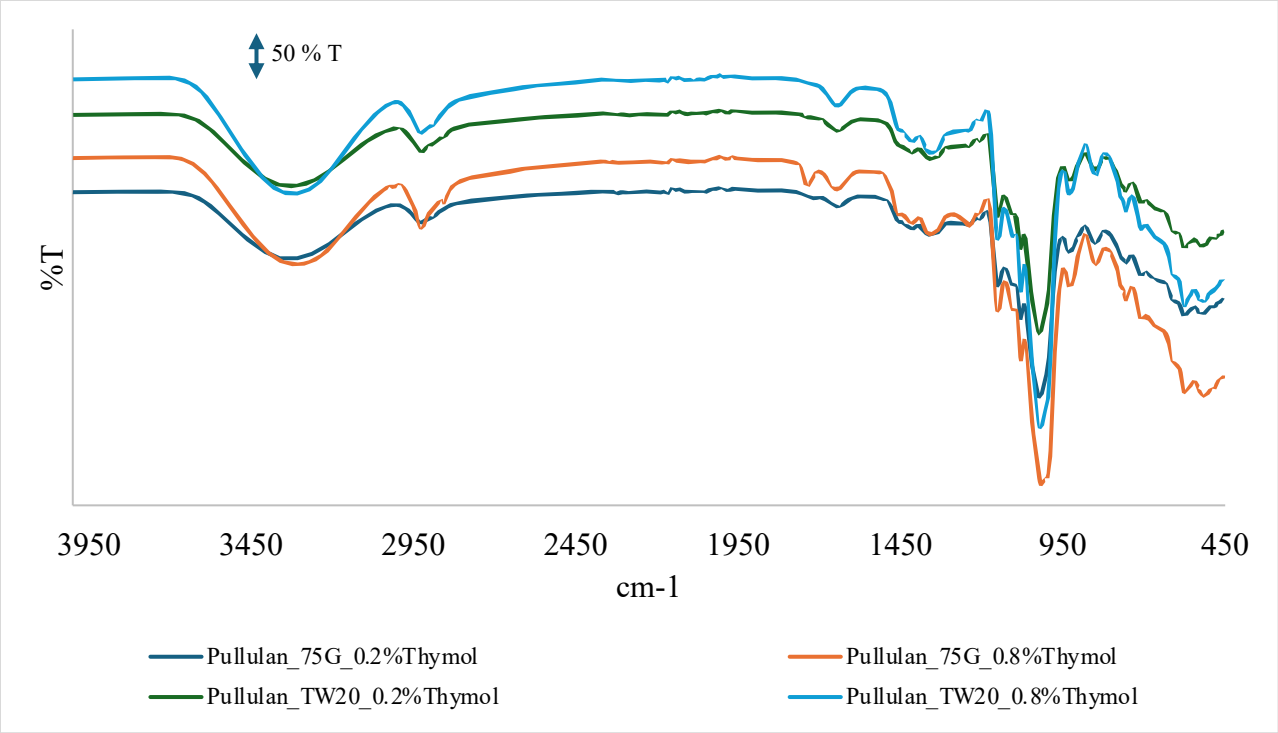


Figure 4.7 FTIR spectra of the films with different emulsifier types and active ingredient concentrations.

4.3.3.3 Effect of polymer concentration on film properties

Both films formed continuous nanofibrous mats with similar white appearance (Figure 4.8). However, films prepared with 15% pullulan appeared slightly thicker and denser compared to those produced with 10% pullulan. This observation is consistent with the increased polymer concentration, which enhances solution viscosity and fiber deposition during electrospinning.



Figure 4.8 Visual appearance of electrospun films containing DDGS lipid extract prepared with 10% (left) and 15% (right) pullulan.

Physical Properties of the pullulan films with different polymer concentrations

Pullulan concentration did not significantly affect ΔE, thickness or moisture content ($p > 0.05$). This indicates that increasing polymer concentration within this range did not alter the optical appearance or moisture-related barrier properties of the films. Since these properties are mainly governed by polymer chemistry rather than moderate concentration differences, similar values are expected for polysaccharide-based electrospun systems.

The absence of a significant difference in thickness can be explained by the similar total polymer mass deposited during electrospinning. Although different solution concentrations were used, the processed volumes were adjusted (15 mL for 10% and 10 mL for 15%), resulting in comparable fiber mat build-up. Therefore, the final thickness values remained similar between the two formulations.

Similarly, pullulan concentration had no significant effect on moisture content ($p<0.05$), suggesting that increasing pullulan concentration did not change the hydrophilic nature of the polymer matrix. This confirms that barrier properties are primarily controlled by polymer type rather than concentration.

Table 4.7 Physical properties of electrospun films prepared with different pullulan concentrations (10% and 15%). Different lowercase letters indicate significant differences ($p < 0.05$).

Sample	ΔE	Thickness (mm)	Moisture Content (%db)
10% Pullulan	106.62 ± 0.07^{a}	0.31 ± 0.06^{a}	11.74 ± 2.86^{a}
15% Pullulan	103.22 ± 3.37^{a}	0.36 ± 0.05^{a}	11.16 ± 0.49^{a}

Mechanical Properties of the pullulan films with different polymer concentrations

The mechanical properties of the films are presented in Table 4.8. Polymer concentration had a significant effect on normalized toughness ($p < 0.05$), while no significant effect was observed for elongation ($p > 0.05$). Films produced with 15% pullulan showed substantially higher values compared to 10% pullulan, indicating improved mechanical strength and energy required for deformation (Table 4.8). This dramatic increase in mechanical performance can be attributed not only to enhanced polymer chain entanglement, but also to fundamental changes in solution rheology and fiber morphology. Increasing pullulan concentration significantly increases solution viscosity, which stabilizes the electrospinning jet and reduces capillary breakup. Greater chain entanglement during electrospinning reduces bead formation, promotes more uniform fiber morphology, and enhances fiber–fiber interactions within the mat structure. As a result, the overall load-bearing capacity of the film increases, and the main improvement was observed in strength and toughness rather than flexibility. Similar trends have been reported

in cellulose acetate (CA) and poly (vinyl chloride) (PVC) electrospun films, where increasing PVC concentration from 12% to 16% improves tensile from approximately 0.006 N/Tex to roughly 0.014 N/Tex more than doubling the strength of the mat due to better fiber uniformity and reduced structural defects (Tarus et al., 2016).

Table 4.8 Mechanical properties of electrospun films prepared with different pullulan concentrations (10% and 15%). Different lowercase letters indicate significant differences ($p < 0.05$).

Sample	Elongation (mm)	Normalized Toughness ($W/mm_{thickness}$= $N.mm_{distance}/mm_{thickness}$)
10% Pullulan	7.09 ± 1.81^{a}	4.41 ± 0.97^{a}
15% Pullulan	8.07 ± 0.08^{a}	55.91 ± 4.91^{b}

FTIR: FTIR spectra of 10% and 15% pullulan films were nearly identical in peak positions, confirming that increasing concentration did not alter chemical structure but affected physical network density. However, 15% pullulan films exhibited slightly sharper and more intense carbohydrate peaks (1000–1100 cm^{-1}), suggesting increased chain entanglement and intermolecular interactions. This directly supports the dramatic increase in normalized toughness in Table 4.8. Since no chemical shifts occurred, the mechanical improvement can be attributed to physical entanglement rather than chemical modification, which is typical for electrospun polysaccharide systems. Similar FTIR behavior has been reported for electrospun poly (vinylidene fluoride) nanofibers, where characteristic bands corresponding to different crystalline phases (e.g., 761 and 840 cm^{-1}) remained present across samples prepared with different polymer concentrations, indicating that concentration mainly influences molecular organization rather than introducing new functional groups (Shao et al., 2015).

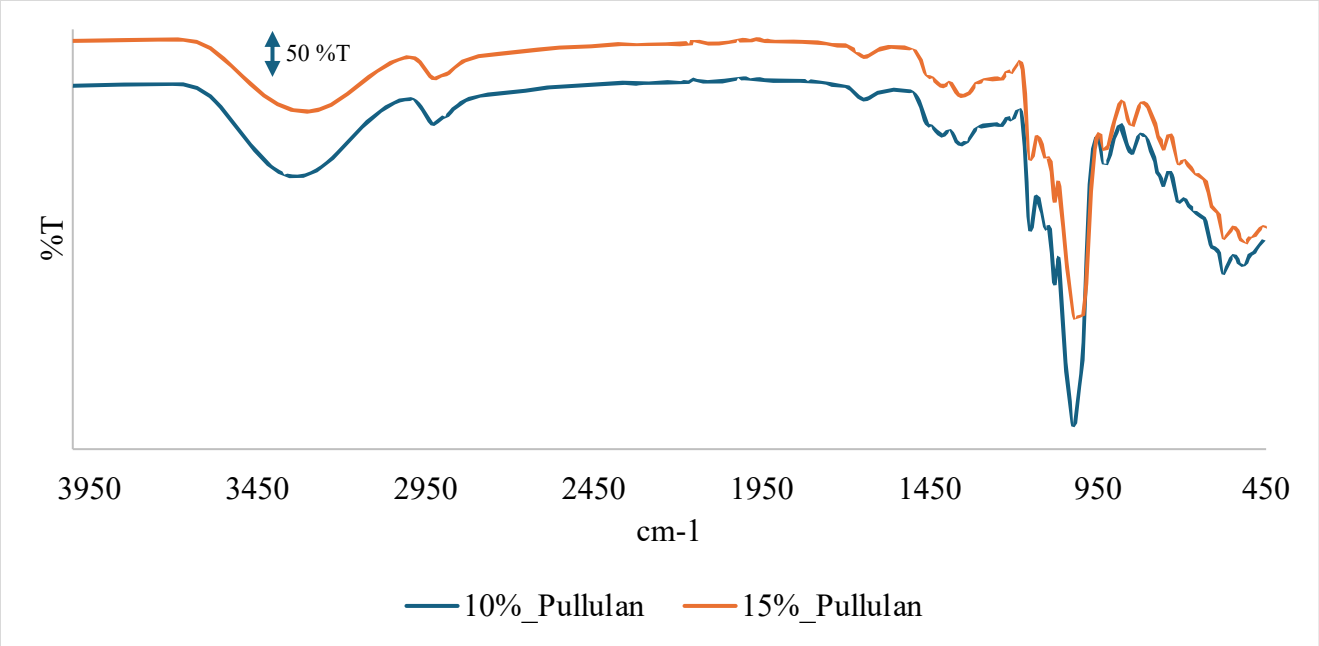


Figure 4.9 FTIR spectra of the films with different polymer concentration.

4.3.3.4 Effect of different solvent-pretreated DDGS extracts on film properties

All films exhibited uniform nanofibrous structures without visible defects, indicating that incorporation of DDGS extracts did not disrupt electrospinning (Figure 4.10-4.11). Slight differences in opacity and color tone were observed among the films. In particular, films containing DDGS lipid extract appeared slightly opaquer, while films containing water and HCl extracts appeared lighter. These variations likely reflect compositional differences among the extracts (Figure 10-11).

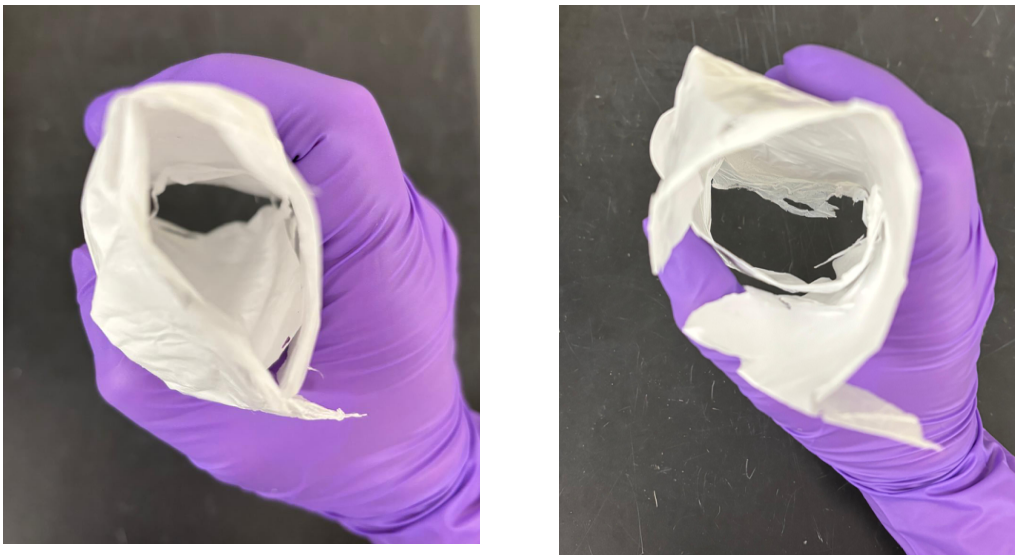


Figure 4.10 Visual aspects of the electrospun pullulan films containing DDGS_Water (left) and DDGS_ HCl (right) incorporated.

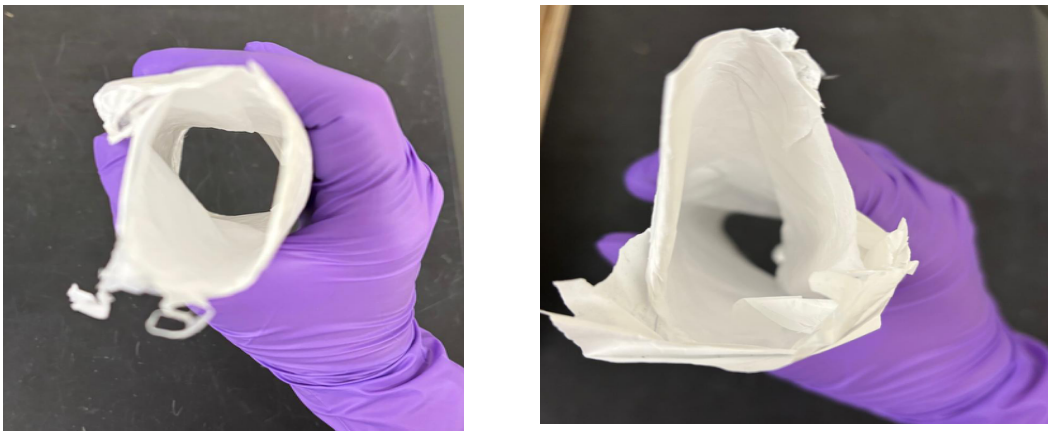


Figure 4.11 Visual aspects of the electrospun pullulan films containing DDGS_NaOH (left) and DDGS_ NaOH+EtOH (right) incorporated.

Physical Properties of the pullulan films with different DDGS extracts incorporated

The physical properties of electrospun 15% pullulan films containing different DDGS-based active ingredients are presented in Table 4.9.

The type of DDGS extract did not significantly affect ΔE or thickness ($p > 0.05$). This indicates that the incorporation of different DDGS-derived core materials did not alter the overall fiber formation or barrier structure of the films. Since electrospinning is mainly governed by the

shell polymer, the continuous pullulan matrix likely dominated fiber morphology and diffusion pathways, masking the effect of core composition on these properties.

In contrast, DDGS extract had a significant effect on moisture content ($p < 0.05$). Films containing DDGS lipid extract showed higher moisture content compared to other treatments (Table 4.9). This can be explained by the presence of hydrophobic lipid domains within the fiber structure, which can create heterogeneous regions and alter water–polymer interactions. In coaxial electrospinning systems, the chemistry of the core phase is known to influence moisture uptake behavior due to such structural heterogeneity (Yao et al., 2017). Similarly it has been reported in core–shell electrospun systems, where the shell polymer, pullulan governs diffusion while the core, gelatin and tea polyphenols mainly affects local interactions such as moisture retention (Zhou et al., 2024). Therefore, although DDGS extract type influenced internal water interactions, it did not significantly alter overall barrier performance.

Table 4.9 Physical properties of electrospun pullulan films containing different DDGS-derived active ingredients. Different letters indicate significant differences ($p < 0.05$).

Sample	ΔE	Thickness (mm)	Moisture Content (%db)
Active Ingredients			
DDGS_Lipid	103.22 ± 3.37^{a}	0.36 ± 0.05^{a}	11.16 ± 0.49^{b}
DDGS_Water	106.70 ± 0.18^{a}	0.31 ± 0.09^{a}	4.34 ± 2.82^{a}
DDGS_HCl	106.54 ± 0.09^{a}	0.36 ± 0.05^{a}	3.41 ± 1.13^{a}
DDGS_NaOH	106.16 ± 0.39^{a}	0.40 ± 0.09^{a}	6.52 ± 1.20^{a}
DDGS_NaOH+EtOH	106.42 ± 0.18^{a}	0.37 ± 0.07^{a}	3.18 ± 0.67^{a}

Mechanical Properties of the pullulan films with different DDGS extracts incorporated

The mechanical properties of the films are shown in Table 4.10. However, DDGS extract type significantly affected elongation and normalized toughness ($p < 0.05$). It shows that fracture

behavior and toughness were sensitive to the type of DDGS extract used. Films containing DDGS lipid exhibited the highest normalized toughness, indicating greater energy absorption before failure and therefore improved toughness (Table 4.10). This behavior can be attributed to the presence of hydrophobic domains within the fiber structure, which may promote energy dissipation and delay crack propagation during deformation.

It has been reported that acid and alkaline–ethanol treatments of DDGS lead to protein fractions with different molecular weight distributions and compositions, particularly affecting kafirin subfractions (Y. Wang et al., 2009b). These compositional differences influence intermolecular interactions and structural behavior, which is reflected in the variation in mechanical response observed in this study. For example, the higher elongation values observed in NaOH-treated samples suggest increased flexibility, while lower normalized toughness values in water-treated samples indicate a weaker and less resistant structure.

Table 4.10 Mechanical properties of electrospun pullulan films containing different DDGS-derived active ingredients. Different letters indicate significant differences ($p < 0.05$).

Sample	Elongation (mm)	Normalized Toughness ($W/mm_{thickness}$= $N.mm_{distance}/mm_{thickness}$)
Active Ingredients		
DDGS_Lipid	8.07 ± 0.08^{ab}	55.91 ± 4.91^{c}
DDGS_Water	4.18 ± 3.19^{a}	15.9 ± 6.82^{a}
DDGS_HCl	6.41 ± 5.32^{ab}	24.72 ± 5.05^{ab}
DDGS_NaOH	13.74 ± 3.05^{b}	33.02 ± 8.25^{b}
DDGS_NaOH+EtOH	8.55 ± 1.94^{ab}	28.09 ± 3.16^{ab}

FTIR: In films containing DDGS extracts, the overall spectral profile remained dominated by pullulan peaks, confirming that the shell polymer controlled the structural framework. However,

subtle variations were observed. Films containing DDGS lipid showed slightly enhanced C–H stretching intensity (2920–2850 cm^{-1}), confirming incorporation of lipid components. NaOH and NaOH+EtOH extracts showed minor intensity variations in the 1600–1500 cm^{-1} region, can be associated with phenolic compounds and protein fragments released during alkaline pretreatment. These differences are consistent with the TPC results reported in Table 4.3, where NaOH+EtOH treatments yielded the highest phenolic content. Despite these compositional differences, the absence of major peak shifts confirms that DDGS extracts were encapsulated rather than chemically bonded to pullulan. This explains why elongation and toughness were affected (Table 4.10). The lipid-containing films showed increased toughness, which can be attributed to hydrophobic domains acting as energy dissipation sites during deformation.

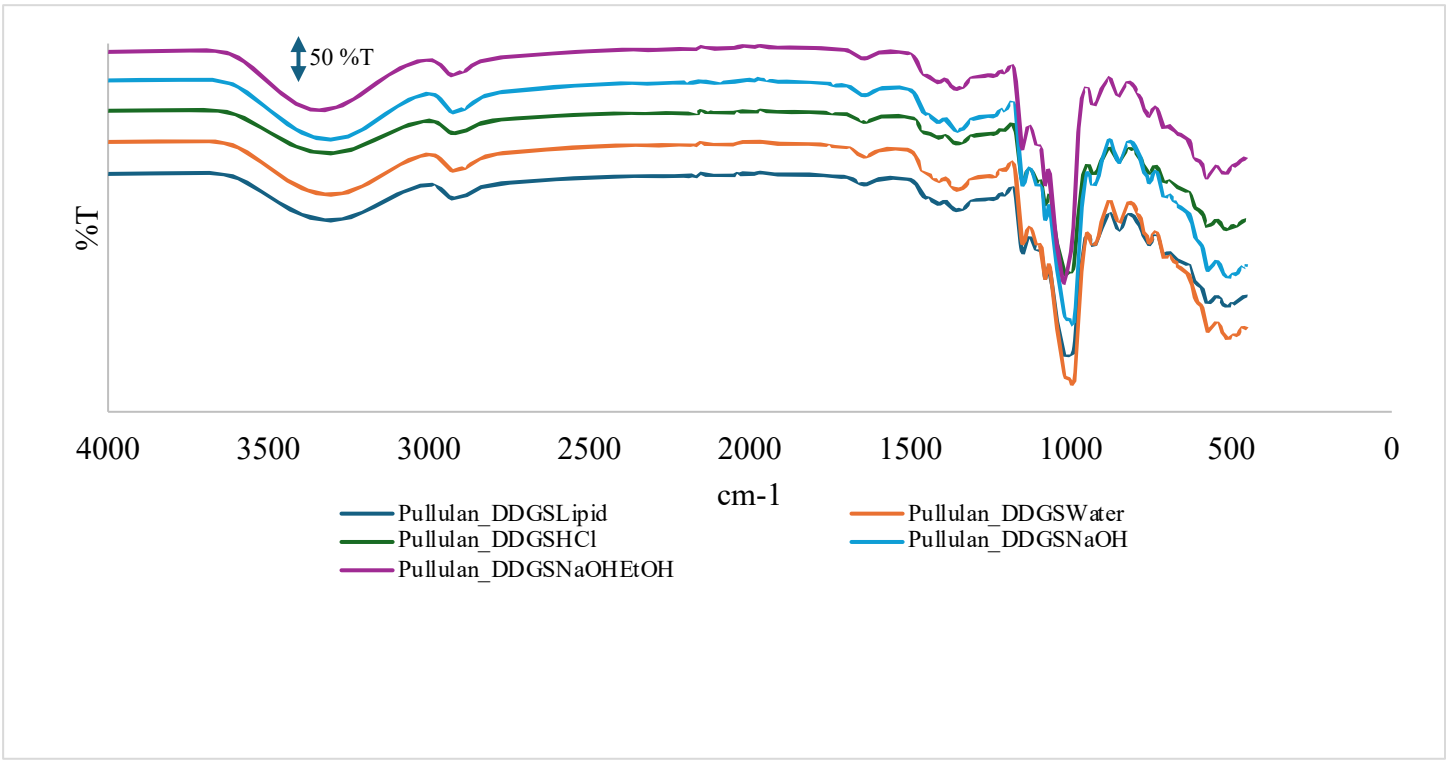


Figure 4.12 FTIR spectra of the films with different DDGS extracts.

Preliminary Antimicrobial Screening of Films

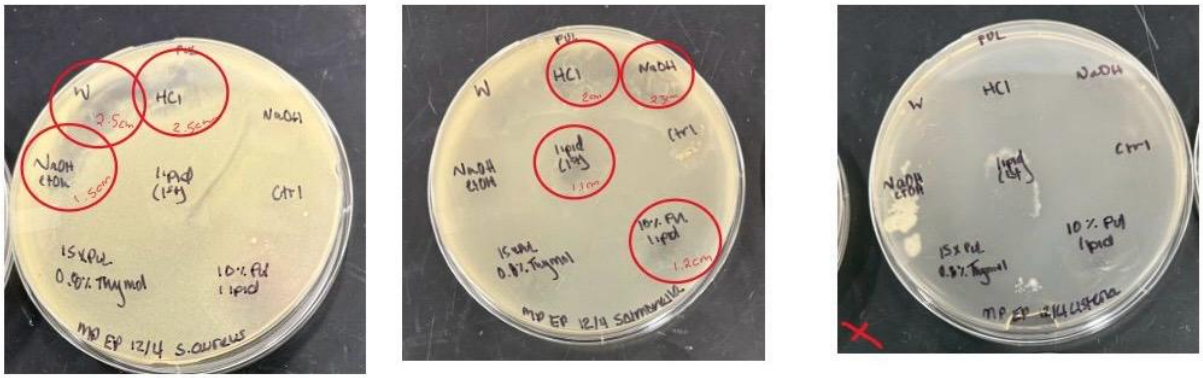


Figure 4.13 Preliminary antimicrobial screening of electrospun films against *Salmonella, Listeria monocytogenes*, and *Staphylococcus aureus* using agar diffusion assay.

Films containing different DDGS extracts (water, HCl, NaOH, NaOH+EtOH, lipid), thymol, and controls were placed on inoculated agar plates. Preliminary antimicrobial activity of the electrospun pullulan-based films was evaluated using agar diffusion assays against Staphylococcus aureus, Salmonella, and Listeria monocytogenes (Figure 4.13). For Staphylococcus aureus, inhibition zones were observed for W (water), HCl, and NaOH+EtOH extract films, with zones approximately around 20–25 mm. No inhibition was observed for NaOH and control samples. For Salmonella, inhibition zones were observed for electrospun incorporated HCl (20 mm) and NaOH (25 mm) extract films. In addition, lipid-containing films DDGS lipid with15% pullulan and 10% pullulan + DDGS lipid films showed smaller inhibition zones (11–12 mm). Other films incorporated with DDGS water, NaOH+EtOH extracts and control, did not show clear inhibition. For Listeria monocytogenes, no inhibition zones were observed for any of the tested films. During testing, pullulan-based electrospun films partially dissolved upon contact with the agar surface, which may have affected the clarity of inhibition zones.

Overall, antimicrobial activity was limited and depended on both the type of treatment and the microorganism. Among the tested samples, DDGS NaOH extract, and lipid-containing films showed relatively more consistent inhibition. Based on these results, NaOH-treated DDGS extracts, and 15% pullulan + lipid films were selected for subsequent product application studies.

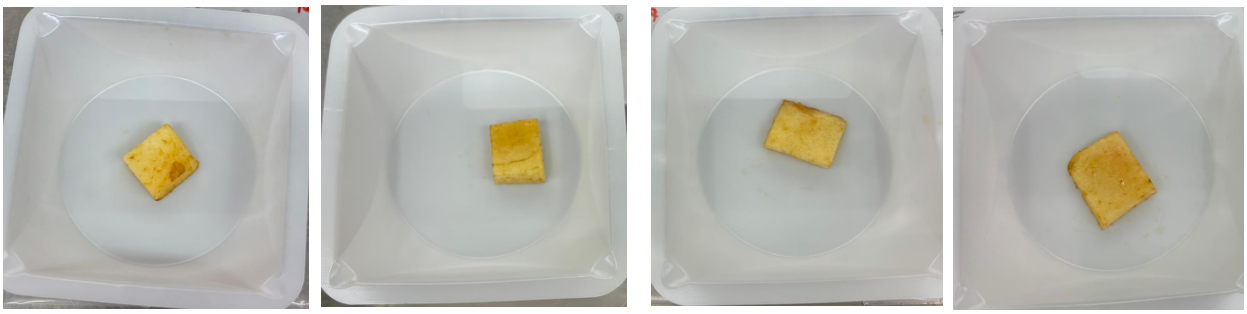
4.3.4 Product Application

Film type and storage time significantly affected on ΔE ($p < 0.05$), while no significant interaction between film type and time was observed ($p > 0.05$), indicating that the effect of treatments remained consistent throughout storage. The change of ΔE was expressed as ln(ΔE) as a function of time, and all samples exhibited an increasing trend, indicating progressive browning during storage. However, the magnitude and rate of ΔE varied depending on the applied film.
At day 0, control samples exhibited the lowest ln(ΔE) values, while film-coated samples showed higher initial color differences (Figure 4.14). This increase can be attributed to the presence of the electrospun coating layer, which may alter the optical properties of the apple surface through changes in light scattering and reflectance. Electrospun films typically form a semi-transparent fibrous network, which can influence instrumental color measurements independently of biochemical browning reactions (Blachowicz & Ehrmann, 2023; Rusková et al., 2023b).

Day 0



Day 3



Day 7

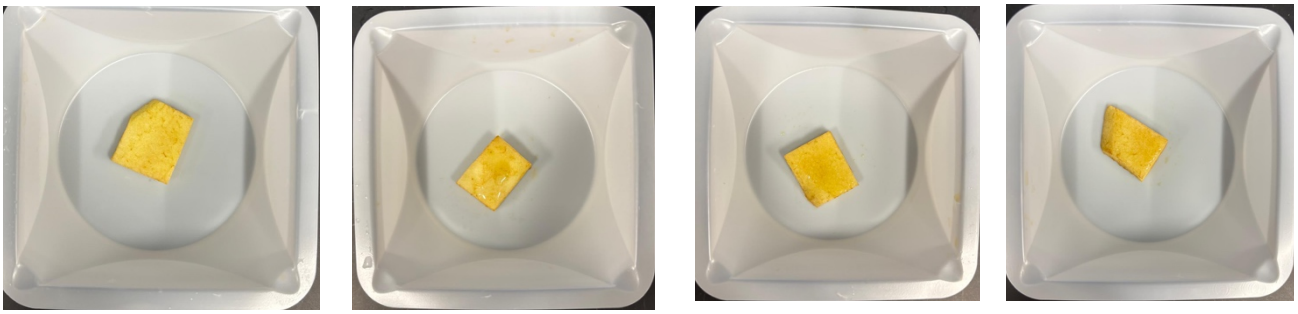


Figure 4.14 ΔE of apples day 0, 3 and 7 with the order (left to right) control, pullulan, 0.8%thymol incorporated and 0.8% DDGS NaOH extract incorporated electrospun films.

To better evaluate browning kinetics, the slope of ln(ΔE) versus time was calculated (Figure 4.15). The control samples showed the highest slope value (0.1219), indicating the fastest rate of color degradation during storage. In contrast, apples coated with electrospun films exhibited lower slopes, suggesting a reduced browning rate (Table 4.11). The lowest slope was observed for 0.8% thymol films (0.077), followed by 0.8% DDGS NaOH extract films (0.082) and pullulan films (0.093). These results indicate that the presence of electrospun coatings slowed the progression of color change compared with uncoated samples. The reduction in browning rate may be associated with the barrier properties of the fibrous coating, which can partially limit oxygen diffusion to the fruit surface. Oxygen availability plays a critical role in enzymatic browning, as polyphenol oxidase (PPO) catalyzes the oxidation of phenolic compounds in the presence of oxygen, producing brown pigments (Oms-Oliu et al., 2008). Therefore, coatings that reduce oxygen exposure may delay browning reactions.

Unlike conventional casting films, electrospun films form a porous yet uniform fibrous network that can create a semi-transparent coating on the fruit surface. This structure may influence color measurements by modifying how light interacts with the apple surface, rather than solely reflecting biochemical changes. Additionally, the glossy appearance of the films may amplify perceived color differences during instrumental analysis. The absence of a significant film × day interaction suggests that all treatments followed a similar kinetic trend over time, and the primary effect of the films was on the initial magnitude and overall level of color difference, rather than altering the rate of color change.

Overall electrospun coatings may influence instrumental color measurements through both surface and optical effects, and therefore, the observed increase in ΔE should be interpreted not only as browning but also as a consequence of film-induced changes in surface appearance.

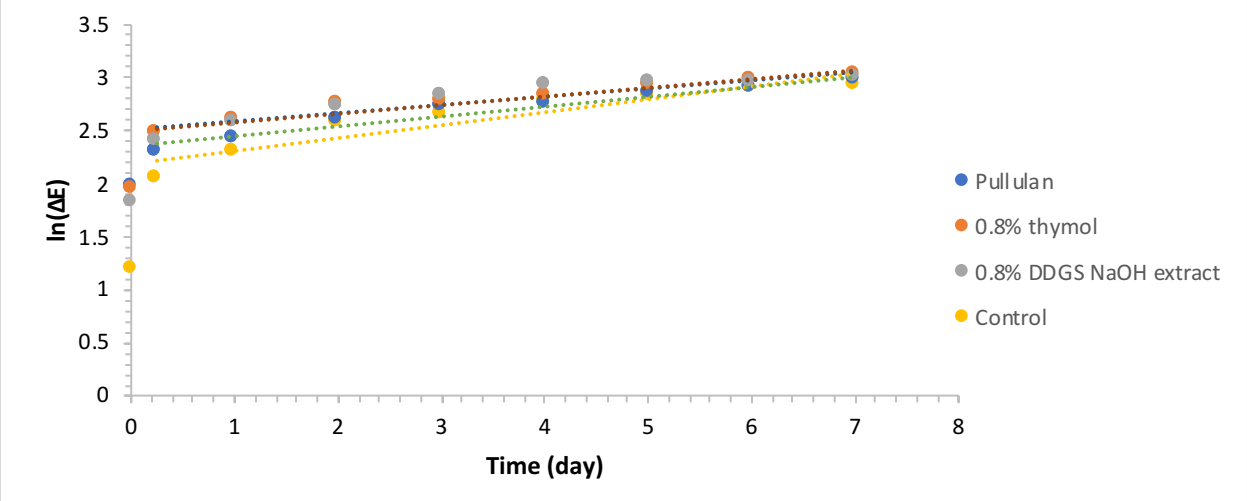


Figure 4.15 Changes in ln(ΔE) of apple samples coated with different pullulan electrospun films during 7 days of storage. Points represent measured values, and dotted lines indicate linear regression used to estimate the ΔE rate.

Table 4.11 Initial ΔE (0–0.25 day) and color degradation rate (slope of ln(ΔE) vs time) of apple samples coated with pullulan electrospun films.

Sample	Change in 0-0.25 day	Slope
Control	0.849	0.1219
Pullulan	0.341	0.093
0.8% Thymol	0.528	0.077
0.8% DDGS NaOH Extract	0.597	0.082

Microbial stability of the apple samples was evaluated by monitoring total aerobic bacteria and yeast–mold populations during storage. The results showed that microbial counts remained nearly constant throughout the storage period, indicating a bacteriostatic condition. For pullulan control and thymol-incorporated samples, aerobic bacteria (1.50×10^1 CFU/g; log 1.88) and yeast–mold (7.50×10^1 CFU/g) counts remained unchanged from day 0 to day 7. Similarly, DDGS_NaOH-incorporated films exhibited only a temporary increase at day 3 (2.20×10^1 CFU/g; log 2.04 for aerobic bacteria and 1.10×10^2 CFU/g for yeast–mold), followed by a return to initial levels at day 7.

Overall, the absence of a continuous increase in microbial counts indicates that the system exhibited a bacteriostatic effect, where microbial growth was inhibited rather than completely eliminated.

Conclusion

This study demonstrated that coaxial electrospinning can be successfully used to produce biodegradable nanofiber films using pullulan and micellar casein as shell polymers and DDGS-derived extracts or thymol as active ingredients. Polymer type was the primary factor significantly affecting film properties ($p < 0.05$), with pullulan-based films exhibiting higher thickness and mechanical strength than micellar casein. Emulsifier type showed a significant effect on normalized tougness ($p<0.05$), with Tween 20–based films exhibiting higher values

than lecithin-based films. Increasing pullulan concentration enhanced mechanical strength and toughness due to improved polymer chain entanglement ($p<0.05$). Although DDGS extract type did not significantly affect color or thickness ($p>0.05$), it influenced moisture behavior and mechanical deformation, with lipid-containing films showing greater toughness ($p<0.05$). In addition, electrospun coatings reduced the browning rate of fresh-cut apples compared to the control, indicating potential for active food packaging applications. Overall, the results demonstrate that electrospinning combined with DDGS-derived compounds can provide a sustainable approach for developing functional biodegradable packaging materials.

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Chapter 5 - Overall Conclusions

This study demonstrated that sorghum distillers' dried grains with solubles (DDGS) can be effectively utilized as a film-forming material through both solvent casting and electrospinning approaches. In the solvent-casting part, DDGS was subjected to different chemical pretreatments (water, HCl, NaOH, and NaOH+EtOH), mechanical processing (1× and 3× ball milling), and defatting, and their combined effects on film properties were systematically evaluated. The results showed that solvent type and its interaction with milling significantly influenced color, surface properties, and mechanical behavior ($p < 0.05$), while defatting exhibited treatment-dependent effects.

In the electrospinning part, functional nanofibrous films were successfully developed using micellar casein and pullulan systems incorporating thymol with different emulsifiers, as well as DDGS-derived bioactives. Polymer type and emulsifier significantly affected mechanical performance and moisture properties ($p < 0.05$), with pullulan-based systems generally exhibiting improved mechanical strength. Thymol incorporation contributed to changes in intermolecular interactions, while DDGS-derived extracts influenced deformation behavior without significantly altering key properties such as color and barrier performance ($p > 0.05$). Among the tested systems, selected NaOH-treated films in solvent casting and pullulan-based electrospun systems showed comparatively improved performance, highlighting their potential as promising formulations.

Overall, the findings indicate that DDGS, an abundant agro-industrial by-product, can be valorized into biodegradable films with tunable properties through appropriate processing and formulation strategies. These results suggest that DDGS-based films have strong potential for application in sustainable food packaging systems.

For future research, electrospinning approaches can be further improved by exploring polymer blending strategies to enhance fiber formation and structural stability. In addition, loading capacity and encapsulation efficiency of bioactive compounds should be systematically investigated to better understand and optimize functional performance. Further studies on barrier properties, controlled release behavior, and scalability are also recommended to support the practical application of DDGS-based materials in food packaging.

Appendix A - Preliminary Trials for Electrospun Films

Table A.1. Preliminary trials for the electrospinning

Polymers	Solution Properties	Electrospinning Rate
WPI		
Not a film	10% WPI+10% acetic acid+ 1% glycerol (1)	12 cm, 14.5 kV, 1 mL/h, 20 mL
Put in electrospinning but it was gelled so couldn't form a film in the end	5% WPI + 30% acetic acid (2)	8 cm, 1 mL/h, 17 kV, 5mL
Sodium Caseinate		
Not a film	Emulsion (4%sodium caseinate in coconut oil&thymol)+ (10%WPI+10% acetic acid+1% glycerol) (3)	2 mL/h (10 mL of each), 10 cm, 13 kV
Not a film	(9:1, caseinate solution:coconut oil) 4% sod caseinate (4)	8 cm, 15 kV, 20 mL, 1 mL/h
Film produced but it melted the aluminum foil	10% sodium caseinate+ 5% NaOH + 1% glycerol (5)	8 cm, 17 kV, 1 mL/h (20 mL) + 2.5 mL/h (10 mL)
Gelatin		
Not a film	5% gelatin+ 10% acetic acid+ 2.5% glycerol (6)	12 cm, 13 kV, 1 mL/h, 20 mL
Not a film	7.5% gelatin+ 10% acetic acid+2.5% glycerol (7)	10 cm, 15 kV, 1mL/h, 20 mL
Not a film	10% gelatin+ 10% acetic acid +2.5% glycerol (8)	10 cm, 15 kV, 1 mL/h, 20 mL
Not a film	7.5% gelatin+10% acetic acid+ 2.5% glycerol (9)	10 cm, 15 kV, 1 mL/h, 20 mL

Not a film	7.5% gelatin + 10% acetic acid (10)	12 cm, 14.5 kV, 2mL/h, 20 mL
Not a film	10% fish gelatin+ 20% acetic acid+ 1% glycerol (11)	13 kV, 10 cm, 1 mL/h, 20 mL
Not a film	10% fish gelatin+ 20% acetic acid (12)	10 cm, 15 kV, 1 mL/h, 16 mL
Not a film	15% fish gelatin+30% acetic acid (13)	10 cm, 15 kV, 1 mL/h, 16 mL
Not a film	15% fish gelatin+ 30% acetic acid (14)	15 mL, 2 mL/h, 10 cm, 16 kV
Not a film	10% fish gelatin+ 30% acetic acid+ 0.5% glycerol (15)	16 kV, 10 cm, 1 mL/h, 20 mL
Not a film	10% fish gelatin+ 30% acetic acid+ 1% ethanol	15.5 kV, 12 cm, 40 mL (1 mL/h+2.5 mL/h)
Brittle film, couldn't take out from the foil	15% gelatin+ 30% acetic acid (16)	10 cm, 30 mL(1mL/h+2.5 mL/h), 15 kV
Not a film	5% gelatin+ 30% acetic acid +0.5% glycerol (17)	8 cm, 17 kV, 20 mL(1 mL/h+2.5 mL/h)
Succesfull film	15 % gelatin+30% acetic acid (18)	8 cm, 17 kV, 1 ml/h, 20 mL
Brittle film	10 % gelatin+ 30% acetic acid (19)	17 kV, 8 cm, 7 mL(2 mL/h)+ 20 mL (1 mL/h)
Successful film	15 % gelatin+ 30% acetic acid+1% glycerol (20)	17 kV, 8 cm, 20 mL (1mL/h)+ 10 mL(2.5 mL/h)
Pullulan		
Successful film	10% Pul+ 30% acetic acid+ 1% glycerol (21)	8 cm, 17 kV, 20 mL, 1 mL/h

Successful film	15% Pul+ 15% acetic acid+ 1% glycerol (22)	8 cm, 17 kV, 40 mL, 1 mL/h
Successful film	15% Pul+ 30% acetic acid+ 1 % glycerol (23)	8 cm, 17 kV, 20 mL(1 mL/h)+ 10 mL (2.5 mL/h)
Successful film	15 % Pul + 30% acetic acid+ 1 % glycerol (24)	8 cm, 17 kV, 20 mL, 2.5 mL/h
Successful film	15% Pul + 30% acetic acid+ 1% glycerol (25)	8 cm, 17 kV, 20 mL, 1 mL/h (overnight)
Successful film	15% Pul+ 15% acetic acid+ 1% glycerol (26)	8 cm, 17 kV, 20 mL, 1 mL/h (overnight)

WPI Films Images

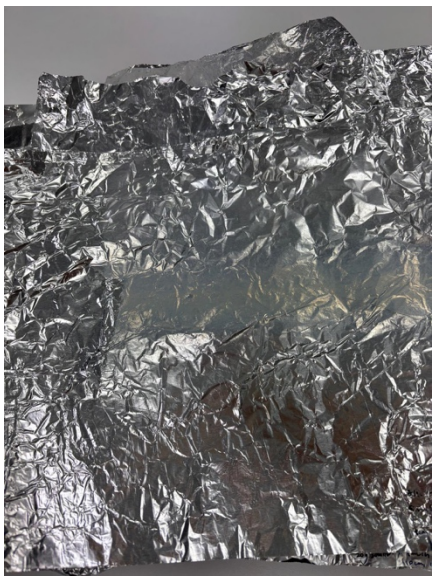


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(2)

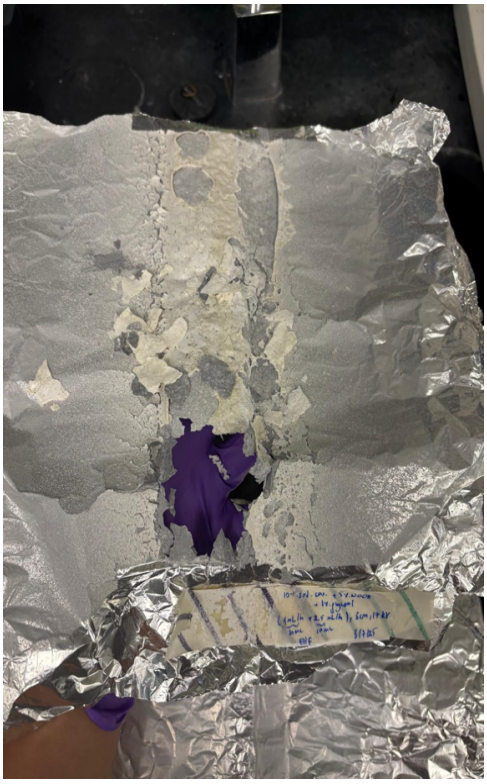
Sodium Caseinate Films



(3)



(4)



(5)

Gelatin Films



(6)



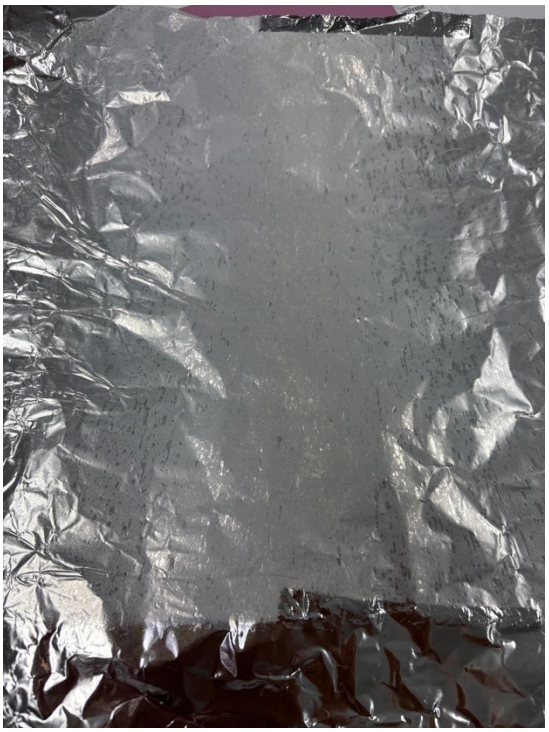
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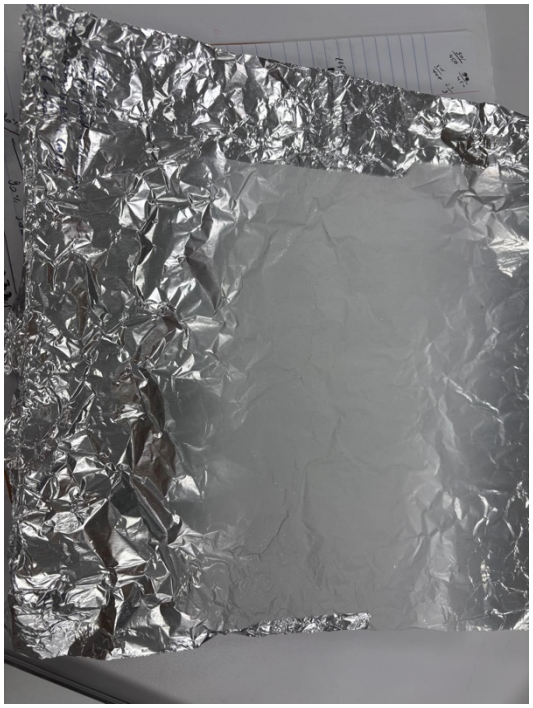
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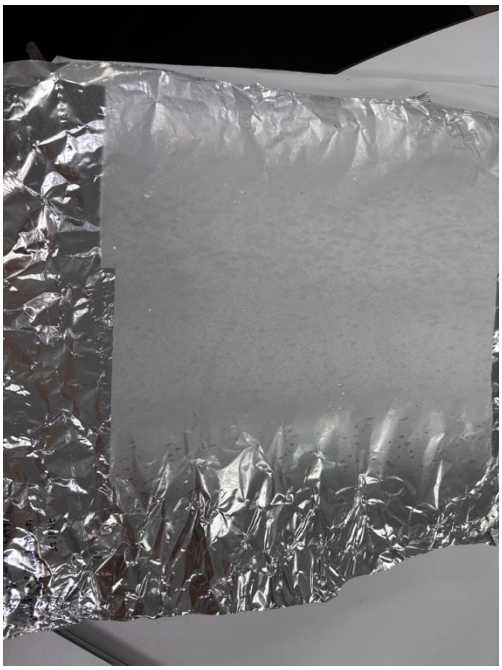
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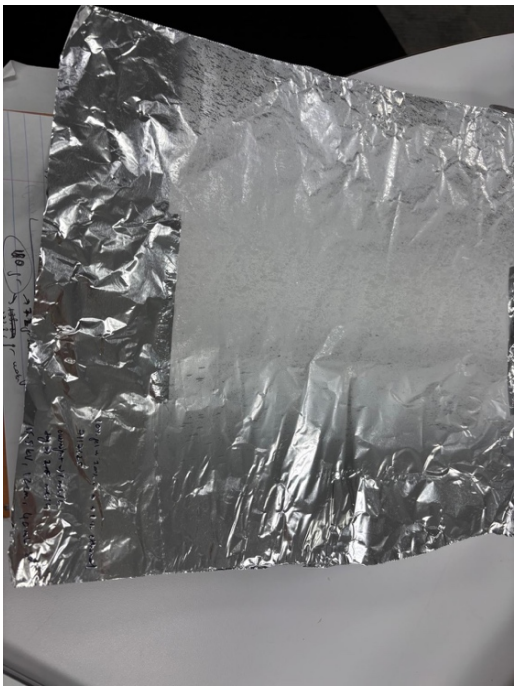
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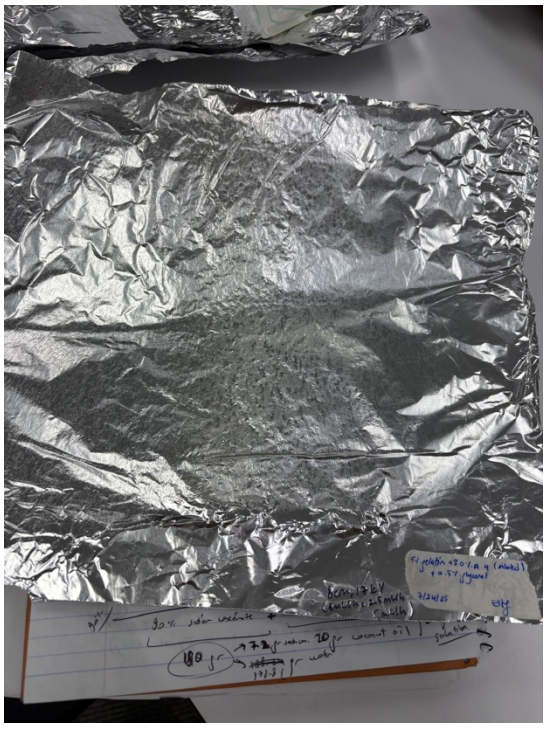
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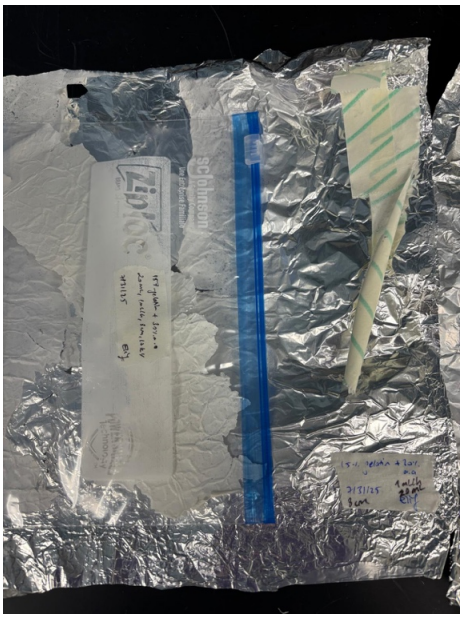
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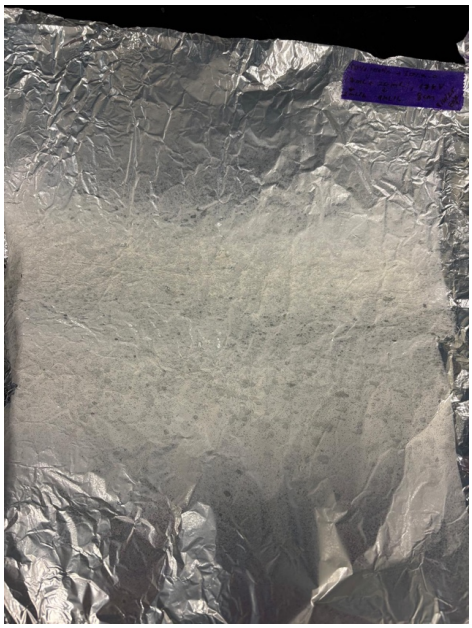
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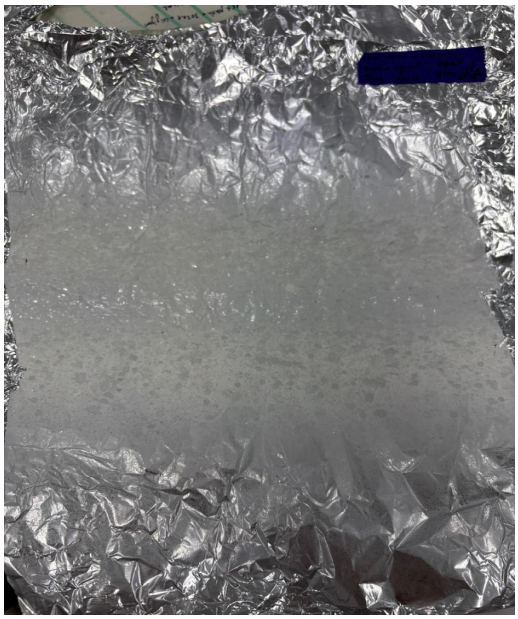
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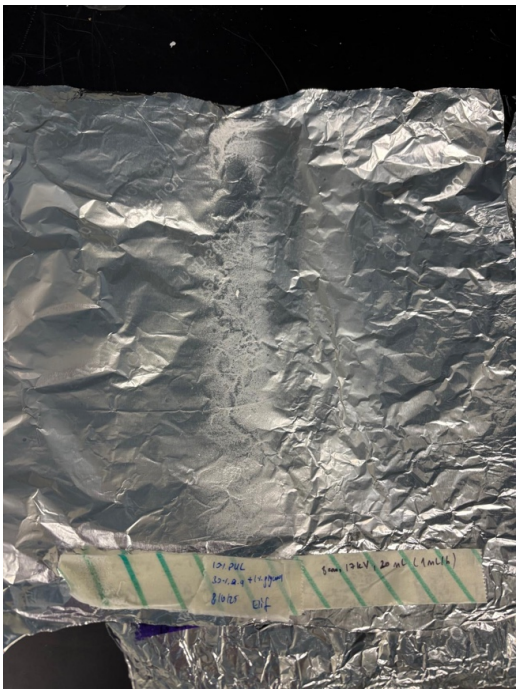


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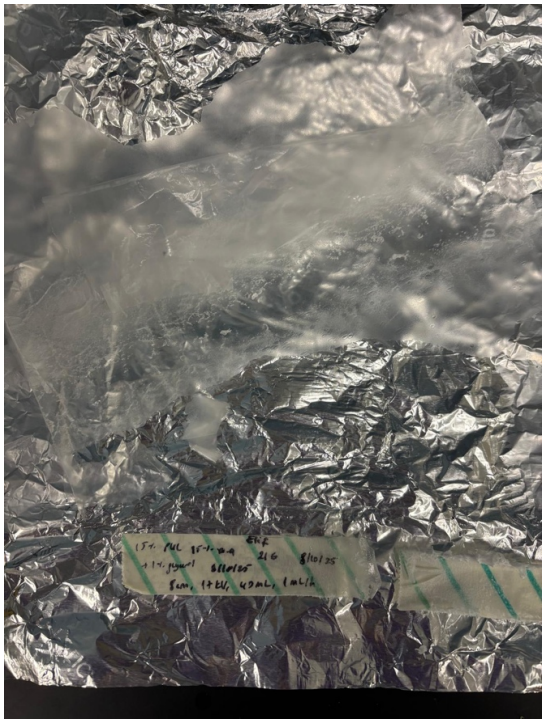


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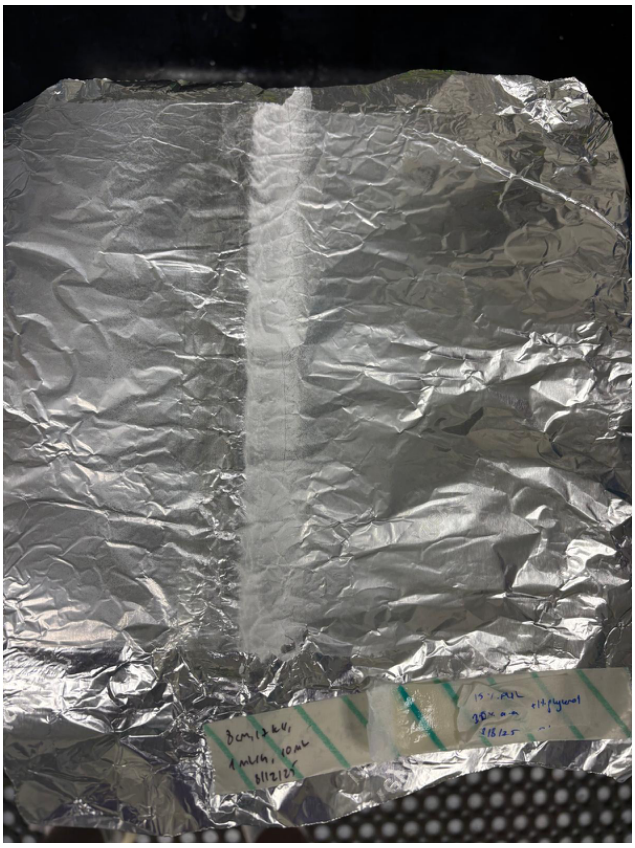
Pullulan films



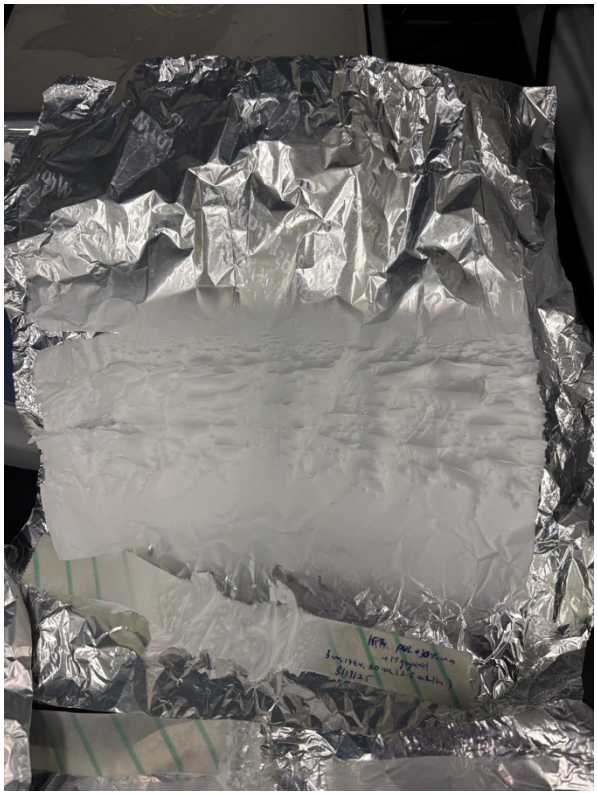
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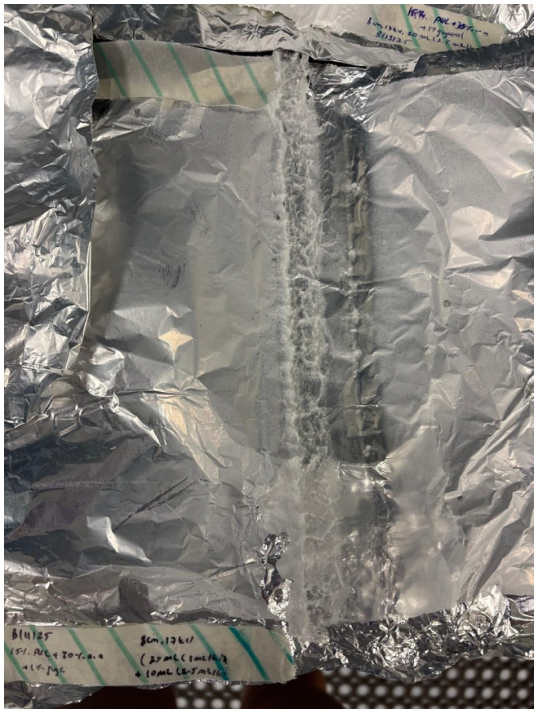
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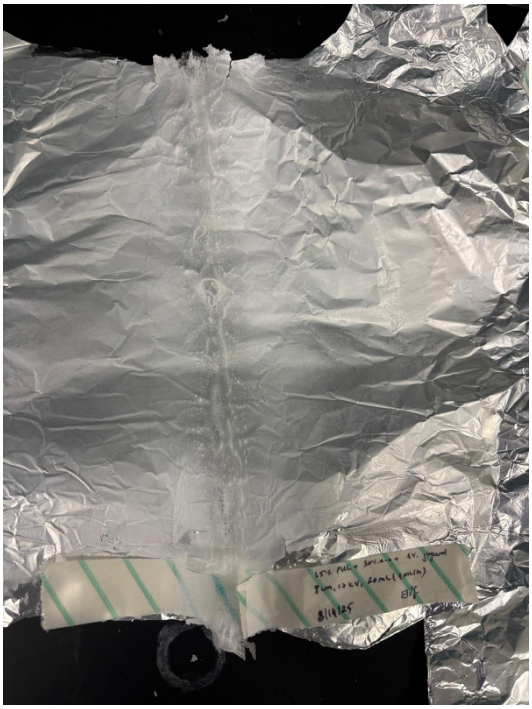
(23)



(24)



(25)



(26)