Engineering Bioplastics with Biopolymers and Antimicrobials to Improve *Listeria monocytogenes* Food Safety in Ready-to-Eat Foods Over 12-Week of Storage.

By

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Abstract

Edible packaging film with chicken skin collagen to improve mechanical properties and the addition of antimicrobial compounds can provide a substitute for plastic packaging. Research was conducted on 1. Develop a plastic-alternative packaging film with biopolymer from chicken skin; 2. Incorporate antimicrobials and study their effects on the mechanical properties of the film; 3. Evaluate the efficacy of the antimicrobial film against *Listeria monocytogenes* (LM) and spoilage of ready-to-eat poultry products. The addition of lactate diacetate to edible films with collagen significantly ($p<0.05$) reduced its mechanical properties. The lactate diacetate packaging films initiated anti-listerial activities from week 4 with LM reducing by 2-3 logs at the end of 12-weeks. Edible films with chicken skin collagen and lactate diacetate offer a promising alternative for food packaging, reducing plastic waste, food waste and ensuring the food safety of meat products.

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LD Lactate diacetate

LM Listeria monocytogenes

MOX Modified Oxford Agar

PCA Plate Count Agar

MRS Mann Rogosa Sharpe

CHAPTER I.

Biomaterials Used in the Food and Packaging Industries:

LITERATURE REVIEW

1.1 Introduction

For nearly five decades, petrochemical polymers, commonly known as plastics, have found widespread use in various aspects of food packaging (Asgher et al., 2020). These polymers have achieved significant success due to their versatility and availability but face significant challenges in terms of health effects and environmental pollution (Andrady & Neal, 2009). Although these polymeric materials may appear inert, they can interact with the environment, which can lead to the migration of chemicals from plastic to food (Teuten et al., 2009).

Packaging is a complex combination of art, science and technology aimed at delivering products safely to end consumers at minimum cost (Marsh & Bugusu, 2007). In this context, food packaging is important for the preservation of both fresh and processed foods (Christyanne, 2023). It plays a critical role in ensuring that food produced in one location remains available to consumers in another location days, weeks or months after the initial harvest, production, and processing (Priyadarshi & Rome, 2020). The main purpose is to serve as protection for perishable products and, at the same time, serve as a communicator with consumers. This packaging may take the form of metal cans, glass bottles, plastic bags, or other materials designed for easy storage, protection, and consumer interest (Fisher, 2009).

Along with growing concerns about plastic pollution and the important role of packaging in food preservation, there has been a strong global interest in edible and biodegradable food packaging (Patel, 2020). As consumers increasingly demand higher quality, safer food products and longer shelf life, preference has followed natural and biodegradable materials as opposed to synthetic and non-biodegradable alternatives (Powell Young, 2021). This movement has sparked a global impact for edible, biodegradable and renewable materials as possible substitutes for petroleum-based packaging materials, setting a notable trend in the food packaging solutions market (Miguel, 2016).

Without a doubt, the environmental friendliness of such packaging, as well as its possible application in various food products, has led to an increase in industry interest in developing new solutions (Miquel,

2016). In 2013, the market for edible coatings for food products, especially fresh fruits and vegetables, boasted an impressive incorporation of over 1,000 companies with annual sales exceeding US$100 million (Pavlath & Orts, 2009). Given these figures, sustainable packaging is positioned as a central development for the food industry (Dörnyei et al., 2023).

In ancient times, people used to use nature such as leaves, shells, animal organs and other natural materials to package their products (Ncube et al., 2021). Over time, people have invented and improved materials to improve food preservation. Paper has become the primary packaging material due to its cost-effectiveness (Priyadarshi and Rome, 2020). In 1844, a historic event occurred in Bristol, England, witnessing the production of the first commercial paper bags, marking the beginning of the industrial use of paper in packaging (Risch, 2009).

Already in the 1960s, glass containers became important in the food packaging sector due to their ability to extend the shelf life of food products compared to paper containers (Heimlich, 2017). Although glass containers remain an effective means of food packaging, they also raise significant concerns about environmental degradation and pollution (Shin & Selke, 2014). During this era, synthetic polymers appeared on the market. Important information emerged from the U.S. Environmental Protection Agency's 2014 report on municipal solid waste (MSW) generation, which report the impacts of plastics (Kibria et al., 2023). The total volume of solid waste produced in 2014 amounted to 258 million tons, of which plastic waste amounted to 32.28 million tons, or 12.9% of the total volume. It should be noted that plastic packaging accounted for 43% of all plastic waste, generating 14.32 million tons (EPA, 2014).

Due to all the above factors, the industry has embarked on extensive research into biopolymers. These biopolymers not only have biodegradable properties, but in some cases have antimicrobial and antioxidant properties. (Baranwal et al., 2022). These dual functions effectively combat bacterial growth, improve appearance, and extend shelf life.

However, despite significant research efforts and products already on the market, the use of edible packaging to ensure food quality and safety remains in an evolutionary stage that requires continuous research (Petkoska et al., 2021). This technology stands out as one of the innovative solutions that meets consumer expectations.

The introduction of biodegradable materials and the replacement of synthetic additives with natural compounds are predominant (Hosseini et al., 2021). Research into new materials, their modification to enhance their properties, exploration of synergies with other processing and preservation methods, and their usefulness as biodegradable packaging reflect the contours of changing trends in edible packaging (Dang et al., 2023). These trends are intended to expand the horizon of potential applications.

The term “biodegradability” covers the process of biochemical transformation facilitated by environmental microorganisms (Havstad, 2020). These microorganisms convert substances into water, carbon dioxide and biomass. Biodegradation efficiency is determined by several factors, including environmental conditions (e.g., location and temperature), the specific material, and its intended use (Bala et al., 2022). Various environments promote the biodegradation of materials, including soils, soil surfaces, landfills, ponds, marine environments, digestion plants, home composting facilities, and industrial composting facilities (V. Afshar et al., 2023). Among them, composting or anaerobic digestion of biodegradable packaging materials represents a favorable scenario at the end of their service life (Grimaldo, 2014).

According to ISO 17088 and EN 13432, “compostable plastic” means plastic that undergoes biological degradation during composting, ultimately producing carbon dioxide, water, inorganic compounds and biomass. It is comparable to other established compostable materials (Pires et al., 2022). This decomposition process leaves toxic residues. Therefore, compost capacity involves the material properties, biodegradation rate, rate of breakdown and quality of the resulting compost (Grimaldo, 2014).

Like any new technology, biodegradable packaging poses challenges that the industry must overcome to demonstrate its food benefits, such as longer shelf life (Wu et al., 2021)as well as its positive impact on the environment and human health.

However, there are concerns about the safety of these biomaterials in food. The components used (such as polysaccharides, proteins and lipids) can be considered as nutrients for pathogenic bacteria, which poses a food safety risk (Modi et al., 2021). One solution is to create biodegradable films with antimicrobial agents (Padgett et al., 1998). These agents can attack certain spoilage microorganisms or pathogens, depending on the type of food and storage conditions.

There are several types of antimicrobials, which can be divided into synthetical chemical and natural products (Hobson et al., 2021). In the natural group, there are various sources such as animals, plants, and microbes. The most common natural antimicrobials of animal origin are lysozyme, lactoperoxidase system, lactoferrin and chitosan (Niaz et al., 2019). The most common plant sources are species and their essential oils, blueberries, hops, olives, cabbage, garlic and onions (Prakash & Kumar, 2011). The group of microbial resources includes natamycin, nisin, bacteriocins, fermentates, protective cultures and bacteriophages (Yusuf, 2018).

In the category of chemical antimicrobials, many compounds can be used, such as acetic acid, benzoic acid, benzoic anhydride, sodium benzoate, p-aminobenzoic acid, lactic acid, lauric acid, propionic acid, sorbic acid and potassium sorbate (Quintavalla & Vicini, 2002).

This review highlights the importance of biodegradable materials used in food packaging to reduce plastic pollution. Thus, discuss the different types of biomaterials that can be used to create packaging films and which of these materials can improve the mechanical and physical properties. In addition, current trends that the packaging industry is applying to address food safety issues will be explored.

1.2 Food Packaging Technology

Packaging has four main functions: containment, protection, communication, and convenience.

Containment: Refers to the ability of packaging to maintain its structural integrity during various stages of food processing such as filling, sealing, handling, transport and dispensing (Pascall et al., 2022).

Protection: Protection requirements for different foods may vary, but generally include protection from three main external influences: chemical, biological and physical (Sharma et al., 2012). Chemical protection involves minimizing compositional changes caused by environmental factors, such as exposure to gases (especially oxygen) that cause lipid oxidation, flavor changes, color changes, and vitamin degradation (Ramos et al., 2015). It also includes moisture control, which affects microbial growth, oxidation rate and texture, as well as managing changes in flavor, heat, and light. Biological control creates a barrier against microorganisms (disease and spoilage agents), insects, rodents and other animals that cause disease and spoilage (Lorenzo et al., 2018). Physical factors refer to any object that can damage the product, and also anything that food can damage.

Communication: Packaging serves as a means of conveying important information that achieves both legal obligations and marketing purposes (Schifferstein et al., 2021). Food labels must include information about the food manufacturer, ingredients (including potential allergens), pure composition, nutritional value, and country of origin (FDA, 2023). In addition, packaging graphics are necessary to convey product quality, branding, advertising aspects and sales promotion. Technology elements such as barcodes and radio frequency identification (RFID), payment processes and efficient tracking (FDA, 2023).

Convenience. A new aspect of packaging design focuses on consumer convenience, often referred to as utility or functionality. (Roberge, 2018). This includes selecting appropriate packaging sizes, ensuring ease of handling and disposal, ease of opening and dispensing, managing parts, ensuring reliability, improving product visibility and, for example, packaging design that suits consumer convenience, for oven or microwave use (Oliver, 2023).

In addition to the multifaceted factors discussed, the potential application of packaging films in food and other industries requires careful scientific research due to the following challenges:

Interactions between materials: Packaging films contain various materials: from polysaccharides, proteins, lipids to waxes. These materials interact with each other and with additional components used in their production, such as plasticizers and surfactants (Patil et al., 2023).

Complex processing technology: The use of packaging film involves a range of processing technologies, each of which imparts different properties to the final products (Wang et al., 2022).

Effect of material and processing on properties: Material selection and processing methods have a significant impact on properties such as transport characteristics, thermal behavior and mechanical strength (Stergiou et al., 2023). External factors such as relative humidity, temperature, gases, humidity, and light can affect the composition and development of the film.

Bioactive potential: Packaging films show promise as carriers of bioactive and functional compounds (Abdullah et al., 2022b). This potential application requires careful design to maximize efficacy and safety.

Integration of nanotechnology: The integration of nanotechnology opens many opportunities. This includes the development of nanoscale multilayer coatings and the introduction of nanostructures into films. (Malik et al., 2023). These innovations open opportunities for new ideas and applications.

Like any new technology, biodegradable packaging poses some challenges for the food industry, such as increased concerns about food safety, since biodegradable packaging typically contains a matrix of nutrients that can serve as food for microorganisms, accelerating their growth.

1.3 Trends in the Food Production and Packaging System

Processing and packaging are important steps in the food industry, forming an integrated path from production to consumption (Chen et al., 2020). The importance of these steps includes their end-to-end transparency, ensuring product integrity and quality (Mahalik & Nambiar, 2010). The processing phase not only involves the mechanized transformation of food products, but also includes vital cleaning and

disinfection procedures (NSF, 2023). This preventive approach is necessary to inhibit microbial growth and prevent cross-contamination within the processing plant.

Subsequently, packaging takes center stage as the culmination of any food processing. The packaging process has a noticeable impact on various properties of food products during transportation, storage and use by the end consumer (Grispoldi et al., 2022). It is important to recognize that packaging is not only functional; It is a communication channel that conveys important information about a product to consumers (GarrisonGroup, 2021).

Given this high importance, both academia and industry are working together to determine trends in packaging materials. In terms of material choices, flexible and lightweight options such as paper and fabric have gained popularity due to their minimal disposal costs. Glass and metals, known for their corrosion resistance and durability, have found their place in the packaging of high-value goods (Mahalik & Nambiar, 2010). Polymers (plastics) have many desirable properties, including transparency, flexibility, heat-seal ability, and favorable strength range (Bohlmann, 2006). Plastics have become the dominant materials in the packaging industry due to their superior properties, including strength and effective gas barrier properties, with some options also being water-resistant (Mangaraj et al., 2009).

Among the various plastic options in the packaging sector, those used are those derived from petrochemical sources such as polyethylene terephthalate (PET), polyvinyl chloride (PVC), polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyamide (Prajapati et al., 2021). However, it is important to recognize that these materials have harmful effects as they are not fully recyclable or biodegradable, creating environmental and health risks (Mahalik & Nambiar, 2010).

In fact, the production of conventional plastics largely depends on the significant consumption of energy and petroleum resources, which are destroying our planet (Evode et al., 2021). This problem poses important challenges for the future that we must address. This scenario has led to increased demand for biodegradable materials as an alternative to film and packaging production (Moshood et al., 2021).

Biodegradation, a natural process facilitated by enzymes secreted by living organisms, involves the breakdown of carbon-containing chemical compounds (Mahalik and Nambiar, 2010).

The global market for biodegradable polymers has exceeded 114 million pounds, and this figure is expected to increase at an average annual growth rate (AAGR) of 12.6%, reaching 206 million pounds in 2010 (Aruas, 2003). The essence of bioplastics development is to replicate the life cycle of biomass. To effectively perform this cycle, it is necessary to combine three essential elements that contribute to an optimal and accelerated decomposition process: humidity, temperature, and the presence of microbes (Cycoń et al., 2019).

1.4 Types of Biomaterials That Can Be Used for Packaging Manufacture

Edible coatings have emerged as an excellent solution to combat waste-related problems and offer multi-faceted benefits. They have the unique ability to protect food products, including baked goods, fruits and vegetables, from microbial contamination while simultaneously extending their shelf life (Trajkovska et al., 2021). By improving the mechanical and structural integrity of these items, edible films enhance their overall quality (Kumar and Neeraj, 2019).

Historically, edible packaging has been used to preserve the appearance and nutritional value of food products (Kumar et al., 2023). These films combine barrier properties with mechanical stability (Hvaldia et al., 2004) , thereby providing control over the dynamics of mass transfer between food components and the environment (Nilsen-Nygaard et al., 2021). Essentially, edible coatings take on the role, regulating the metabolism between the product and its environment, which ultimately contributes to increased shelf life and improved quality (Khwaldia et al., 2004).

Edible coatings, consisting of a mixture of several components including polysaccharides, proteins and lipids or combinations of them, take the form of a thin and transparent layer (Falguera et al., 2011). This layer delicately envelops the products without changing their original composition or processing methods. Importantly, this approach can support important characteristics such as moisture control, gas barrier

functions (including oxygen and CO2), sensory appeal, and overall appearance (Qiu et al., 2019). In addition, these coatings act as a powerful barrier against microbial spoilage, thereby increasing the shelf life of food products (Debofort et al., 1995).

The concept of edible films and coatings also involves careful management of mass transfer between the food product and its environment (Chettri et al., 2023). This sophisticated control mechanism allows for improved sensory properties including transparency, color retention, surface texture, Shine, and reduced stickiness (Kumar and Neeraj, 2019).

Edible coatings and films offer a promising path to reducing the demand for traditional packaging and thus reducing waste (Chawla et al., 2021). By extending the shelf life of food products and increasing the cost-effectiveness of packaging materials, these solutions mark a significant step when it comes to sustainability (Mezemir, 2017). It is presented as an environmentally friendly alternative to synthetic plastics in food packaging. The main advantage of edible films is their ability to improve the recyclability of packaging materials (Gaspar & Braga, 2023). Modern design in the food industry emphasizes the use of natural polymers and food additives (Prameela et al., 2018). Within this structure, natural components such as polysaccharides, proteins and lipids combine harmoniously with plasticizers (such as glycerol, glycol, and polyol) and surfactants to form edible films (Siracusa et al., 2008).

The structure of edible film polymers has been established in their non-toxic, biodegradable and environmentally friendly composition derived from simple plant and animal derivatives (Teixeira-Costa & Andrade, 2021). The formulation of edible coatings and films is based on a mixture of materials such as polysaccharides, proteins, and lipids. These components are carefully mixed to create an edible textured coating (Hall, 2012).

There are several components that can be used to create biodegradable films **(Table 1.1)**. The variety of recognized polysaccharides includes chitosan, pullulan, alginate, carrageenan, cellulose, starch, pectin and various gums (Kabir et al., 2022). Within the protein group, commonly used options include collagen,

gelatin, corn zein, wheat gluten, soy protein, casein, and mung bean protein (Coltelli et al., 2015). In terms of lipids, includes beeswax, polyethylene wax, carnauba wax, paraffin wax, candelilla wax, rice bran wax and jojoba oil (Kumar & Neeraj, 2019).

1.5 Polysaccharides

The film-forming properties of polysaccharides are very promising in both food and pharmaceutical industries (Nešić et al., 2019). When films are based on polysaccharides, they have a special property: oxygen permeability (Kocira et al., 2021). This biomaterial is commonly used as a coating material for various food products and drug capsules (Rinaudo, 2008). Many drug carrier systems have emerged from polysaccharides (J. Zhang et al., 2021). This innovation has the dual purpose of reducing the toxicity of anticancer drugs to normal cells and at the same time increasing their therapeutic efficacy (Saini, 2016).

It is obvious that the physiological activity inherent in polysaccharides is due to the concentrated presence of hydroxyl groups (Lin et al., 2012). This physiological activity highlights its usefulness and versatility, which contributes to its multifaceted applications, including the development of edible films due to its ability to create edible films with high oxygen and water permeability (Hassan et al., 2018).

1.5.1 Cellulose

Among the various applications of cellulose **(Figure 1.1)**, the most famous form used for film production is myofibrillar cellulose (MFC) (Shen et al., 2024). Distinguished by its economic viability, MFC represents an innovative expression of cellulose (Lavoine et al., 2012). It results from the rapid expansion of wood pulp fibers, resulting in a significant increase in surface area and the appearance of substructural microfibrils (Spence, 2011). This is achieved through the coordinated interaction of mechanical action and heat. Repeated homogenization serves as a catalyst to convert the dilute cellulose dispersion into a gel-like substance with a gel-like appearance and properties (Turbak et al., 1983).

The industry is specifically designed to produce readily available MFCs of suitable caliber for food applications (Carter et al., 2021). This availability facilitates cellulose extraction, a process that allows food

products to be developed and improved. In particular, the properties of micro fibrillated cellulose dispersions go beyond their usefulness (Osong et al., 2016).

Biodegradable MFC films act as powerful thickeners, producing viscous mixtures that have thixotropic properties (Barnes, 1997). An exceptional feature of MFC formulations is their ability to stabilize emulsions of organic liquids in water, eliminating the need for surfactants (Akhade et al., 2020). Because cellulose remains largely indigestible to humans, the thickening properties of MFC are especially important when preparing low- or no-calorie foods. MFC is an excellent option for thickening a variety of culinary products, from hot soups to various sauces, and the same is true when added to a solution to create edible films (Turbak et al., 1983). MFC's exceptional thickening efficiency in water makes it versatile. In addition, its usefulness extends to water-based paints, film formers, opacifiers and as a thickener (Turbak et al., 1983).

This versatile material finds its use as a suspending agent, film former, fiber binder and general thickener in a variety of industrial applications (Turbak et al., 1983). As highlighted above, cellulose's remarkable ability to absorb water and impart thickness makes it ideal for synergistic blends with other ingredients and water (Solhi et al., 2023). This combination culminates in films with exceptional performances suitable for various industries.

1.5.2 Starch

Starch typically consists of 20–30% amylose and 70–80% amylopectin (Cornejo-Ramírez et al., 2018). Biodegradation of starch-based polymers occurs through enzymatic action targeting the glycosidic bonds connecting the sugar groups (Yu et al., 2021). This process ends with a decrease in chain length and the release of lower molecular weight sugar units (Balat et al., 2008). In biodegradable plastics, starch integration occurs through physical mixing with its native granules or at the molecular level when combined with compatible polymers (Mahalik & Nambiar, 2010).

Starch has a wide range of uses due to its properties. It is oil resistant, flexible, oxygen impermeable, thermally stable, and water soluble (Romero-Bastida et al. 2005). A particularly important property of starch is the ability of amylose to form films (Krogars et al., 2003).

Some properties of starch, such as its tastelessness, colorlessness, odorlessness, non-toxicity, semi-permeability to CO2, biodegradability, and resistance to O2 release, do not affect the properties of food products (Dinika et al., 2020). Starch-based edible films play a critical role in reducing microbial growth, controlling enzymatic reactions, and reducing water activity in packaged foods (Majeed et al., 2023). Modified starch with special properties is used to create films and coatings (Parreidt et al., 2018).

Although these inherent properties are ideal for film formation, the high vapor permeability (VVP) and mechanical properties of starch biopolymer remain relatively low (Tiozon et al., 2021). To address these limitations, researchers have delved into incorporating additional biopolymers and additives into film production (George et al., 2020). These concerted efforts are aimed at improving both the WVT and mechanical properties of starch biopolymer-based films (Singh et al., 2022).

Various agricultural products such as wheat, rice, corn, beans and potatoes contain starch as a natural biopolymer (Tiozon et al., 2021). The specific characteristics of starch granules (their shape, structure, size, and chemical composition) vary depending on the botanical origin (Singh et al., 2022).

The two main components, amylose, and amylopectin **(Figure 1.2)**, occur in starch in trace amounts of elements such as lipids and proteins within the granules (Banks & Muir, 1980). Amylose, characterized by a linear chain structure consisting of 1.4 units of anhydro glucose, has a molecular weight ranging from 20 to 800 kg/mol. A significant portion of granular starches has an amylose content of 20 to 25% (Ganesan et al., 2018). In contrast, amylopectin has a highly branched chain configuration, connecting α-1,4 chains via an α-1,6 glycosidic linkage to 25–30 glucose units. Its molecular weight increases significantly from 5000 to 30,000 kg/mol (Singh et al., 2022).

Most starches are semicrystalline in nature, with crystallinity ranging from 15 to 45% depending on the ratio of amylose (20–25%) and amylopectin (75–80%) (Biliaderis, 2009). Crystalline domains arise from short-branched amylopectin chains, while amorphous regions are formed from branched amylose and amylopectin chains (Seidi et al., 2022). The network of hydrogen bonds connecting the starch chains blocks their dissolution in cold water. When heated in water, the crystalline architecture changes as the hydroxyl groups in amylose and amylopectin initiate partial solubilization (Quiroga, 2018). Upon further heating of starch suspensions in excess water, the formation of hydrogen bonds causes an irreversible gelatinization reaction that occurs at temperatures ranging from 65 to 90 °C depending on the type of starch (Singh et al., 2022).

The predominant methods for creating starch-based films are dry and wet (Xie et al., 2014). In the dry approach, starch mimics the glass transition temperature, taking advantage of thermoplastic properties through extrusion (Tadini, 2017). Subsequently, the low water content is used to plasticize the starch. The introduction of a plasticizer, often glycerol, into thermoplastic starch helps lower the phase transition temperature, providing a low threshold rate of decomposition (Falua et al., 2022). In contrast, the wet method begins with the solubilization of polymers followed by drying of the film-forming solution. The wet process is commonly used to cast edible preformed films (Singh et al., 2022).

The industry proposal favors the dry film production method due to its practicality and efficiency (Khan et al., 2022). Although the wet process may be simpler at the laboratory level, the dry approach is more suitable for industrial implementation (Senturk et al., 2018).

Due to the complex structure of films obtained from starch (with separation into crystalline and non-crystalline regions), improved barrier properties are observed. (Berardi et al., 2018). On the other hand, starch films have disadvantages in terms of mechanical properties: they are characterized by weakness and low tensile strength (Gutiérrez & Alvarez, 2017). However, when these films are analyzed by aspect ratio, they show high flexibility. An inherent property of starch films is their hydrophilicity (Wang et al., 2020).

This property is due to the abundance of hydroxyl groups (OH) in their structure, which makes them suitable for absorbing moisture (Zia et al., 2015)

The weaknesses of starch films, mainly attributed to amylose-induced amorphous regions, necessitate improvements in flexibility and extensibility (Muñoz-Gimena et al., 2023). To mitigate this, plasticizers and additives are introduced to counteract intermolecular forces. Alternatively, the integration of synthetic polymers represents a solution that can simultaneously improve mechanical and barrier properties while remaining biodegradable (Younas et al., 2019). In addition, the thickness of food starch films has a marked influence on their physical and mechanical properties, which are closely related to their permeability characteristics (Faisal et al., 2023).

1.5.3 Chitosan

In fact, chitin is a biopolymer like cellulose, as shown in **Figure 1.3**. This biopolymer finds its habitat in the exoskeletons of crustaceans and insects, as well as in the cell walls of fungi and yeasts (Austin et al., 1981). Compared with the function of cellulose in plant cells, chitin serves as a structural material and maintains the structure of these organisms (B. Zhang et al., 2021). The polymer itself is mainly composed of (1–4) linked 2-acetamido-2-deoxy-β-D-glucose monomers, which gives it a distinct molecular composition (Priyadarshi & Rhim, 2020).

Chitosan is the second most abundant biopolymer on Earth after cellulose and has the distinction of being the most abundant biopolymer of animal origin (Honarkar & Barikani, 2009). Its unique nitrogen content distinguishes it from other polysaccharides (Hajji et al., 2014). Chitosan occurs as a deacetylated derivative of chitin and is predominantly composed of (1-4) linked 2-amino-2-deoxy-β-D-glucose monomers (Sahoo et al., 2009).

The amino group present in chitosan has some functional properties; The electron-rich amino group captures protons, generating a positive charge that imparts various chemical, physical, and biological properties to chitosan (You et al., 2021). Its solubility, film-forming ability, viscosity, ion binding, and

antimicrobial properties make chitosan a versatile polymer that can be applied in various research fields, including food packaging (Wang et al., 2018).

There are two methods for extracting chitosan: chemical and biological (El Knidri et al., 2018). The chemical method, although faster, is usually less environmentally friendly and economical than the biological method, which provides higher yields of chitosan (Mohan et al., 2022). Chitosan can be extracted from sources such as crustaceans, insects, fungi, and cnidarians; both methods include steps such as demineralization, deproteinization and bleaching (Adetunji et al., 2023). The raw material must also undergo washing, drying and grinding (Priyadarshi & Rhim, 2020).

From chitosan, chitin can be obtained by a process of deacetylation, which is achieved by alkaline methods using NaOH solution or enzymatic approaches using the enzyme chitin deacetylase. (Younes & Rinaudo, 2015). The choice between methods depends on the desired product.

Chitosan and its derivatives find applications in many industries including food, beverages, cosmetics, pharmaceuticals, agriculture, etc. (Manigandan et al., 2018). Its antimicrobial properties and ability to form biofilms have led to its use in various sectors (Raafat & Sahl, 2009).

Film formation is of one of the most important when considering the potential of chitosan, especially in food packaging (H. Wang et al., 2018). Its barrier properties and inherent antibacterial activity contribute to improving the quality of food products (Dutta et al., 2009). Numerous studies have examined the production of edible films, membranes and coatings from pure chitosan or its mixtures. The ability of chitosan to form biofilms can be attributed to properties such as ion binding and increased packaging efficiency (Siripatrawan & Vitchayakitti, 2016). It exhibits solubility in water, and solution pH, ionic strength, and interactions with ions determine its solubility characteristics (Cavallaro et al., 2021).

For food packaging, chitosan can be used in the form of packaging films or as a direct coating of food products (H. Wang et al., 2018). The choice of production method depends on the desired result, with the solution cast film method being one of the most common (Muxika et al., 2017). Solution cast films are

showing potential in food packaging and their use has been observed to extend shelf life and maintain food quality (S. Kumar et al., 2020). However, they pose certain problems that require careful formulation development. In particular, the use of solution casting on an industrial scale remains limited due to economic and time constraints (Mijinyawa et al., 2022).

1.5.4 Gum

Gums, also called hydrocolloids or polysaccharides, are highly versatile biopolymers and are widely used as ingredients or additives in the food industry (Eghbaljoo et al., 2022). They perform various technical and sometimes nutritional functions. (Li & Nie, 2016). Its versatility is closely related to its molecular composition, which gives it special properties such as gelling agents, thickeners, humectants, emulsifiers, and stabilizers (Aires Da Silva et al., 2021). Understanding their molecular structure, thermal stability, interaction with water, and rheological behavior is critical for the research and development of applications for these polysaccharides (Zeng et al., 2022). As a result, there has been a noticeable trend towards replacing synthetic materials with natural rubbers due to their non-toxicity, affordability, safety and wide availability (Mohammadinejad et al., 2020).

Gums are classified according to their origin, behavior and chemical structure (Mirhosseini & Amid, 2012). They can come from a variety of sources, including the endosperm of plant seeds (eg, guar gum), plant exudates (e.g., tragacanth), shrubs or trees (e.g., gum Arabic, karaya gum, cashew gum), algal extracts (e.g., agar), bacteria (e.g. xanthan gum), animal sources (e.g. chitin) and others (Aires Da Silva et al., 2021). The properties and uses of these gums are closely related to their chemical structure, which may include numerous backbones and/or side chain sugars that may branch, which determines their complexity (Nieto, 2009).

Most polymers, both natural and synthetic, can degrade when exposed to thermal stress (Zia et al., 2018). This degradation has been attributed to processes such as chain depolymerization or removal of low molecular weight fragments, resulting in mass loss with increasing temperature (Riaz et al., 2021). These thermal effects are associated with physical or chemical changes and correspond to thermodynamic

phenomena (Vuillemin et al., 2019). These changes in energy and mass can be used to determine factors such as changes in crystal structure, reaction kinetics, melting and boiling points, glass transition, etc. (Kumar et al., 2020). Changes in mass related to temperature and/or time, along with continuous recording of mass during heating or cooling, are typically characterized by thermogravimetric analysis (Douglas et al., 2016).

Natural polymers are of particular interest for rheological studies (Gilbert et al., 2013). Their thickening, emulsifying, gelling, and stabilizing properties make them valuable in various industries such as food, pharmaceutical and cosmetics. (Naji-Tabasi & Razavi, 2017). These properties arise from a variety of intermolecular and intramolecular association mechanisms unique to each polymer (Wever et al., 2011). These mechanisms facilitate specific applications in various processes and products.

Gums and polysaccharides also find application in film production, which is an intensive area of research (Jiang et al., 2023). Active, functional and biodegradable packaging is an example of antimicrobial effectiveness (L. Motelica et al., 2020). In addition, chemical modification of gums to improve control of hydration, gelation and swelling opens another route to create biodegradable films that can effectively prolong the quality of food products (Alizadeh-Sani et al., 2019).

1.5.4.1 Guar Gum (GG)

Guar gum **(Figure 1.4)** is a water-soluble galactomannan extracted from the seeds of the legume plant Cyamopsis Tetragonoloba. (V. D. Prajapati et al., 2013). Chemically, it consists of a backbone consisting of mannose residues linked to β-D-1,4, with side chains of α-D-galactose residues linked to the O-6 position of every other mannose residue (Nagaraja et al., 2021). Its wide availability and excellent film-forming properties have made it a promising material for the development of biodegradable packaging films (Tripathi et al., 2019). However, guar gum-based films have certain limitations, such as lower mechanical strength and higher vapor permeability compared to petrochemical-based plastics. These disadvantages may limit the commercial application of biopolymers in some cases (Tripathi et al., 2019).

The properties of guar gum films can be influenced by the degree of methylation of the gum. (Tripathi et al., 2022). As methylation increases, apparent viscosity, intrinsic viscosity, and viscosity-average molecular weight decrease (Thombare et al., 2016). This reduction may be explained by hydrolysis of guar gum under the basic conditions used for methylation or the introduction of non-polar groups due to methylation, resulting in a decrease in the ability to form intermolecular hydrogen bonds (Thombare et al., 2016).

Comparative studies were carried out on packaging films with and without guar gum, which led to some important conclusions. For example, the inclusion of guar gum resulted in a significant reduction in the tensile strength of the films (Aydogdu et al., 2020). Control films had a tensile strength of 18.01 ± 1.6 MPa, whereas films prepared with 100% guar gum exhibited a tensile strength of 5.5 ± 1.5 MPa (Tripathi et al., 2019). Yield Strength, which indicates the rigidity of the films, decreased as the concentration of guar gum increased (Saurabh et al., 2015). The observed decrease in the mechanical properties of the films is associated with a decrease in the molecular weight of the polymer upon methylation, which was confirmed by rheological analysis (Tripathi et al., 2019). In addition, the decrease in mechanical properties may be due to a decrease in intermolecular hydrogen bonds, as indicated by IR spectra (Singh et al., 2021).

Film elongation improved significantly with increasing guar gum concentration to 75% (Saurabh et al., 2015). The elongation of the control film without guar gum was 31.5 ± 0.8%, which increased to 49.4 ± 0.9% at 75% guar gum concentration (Tripathi et al., 2019).

The addition of guar gum also had a significant effect on the vapor permeability of the films (Kirtil et al., 2021). Control films without guar gum showed a vapor permeability of 171 ± 15 g/m2/day. Films prepared with 75% and 100% guar gum showed a 17% and 40% reduction in vapor permeability compared to the control, respectively (Tang et al., 2018). This decrease can be explained by the introduction of hydrophobic methyl groups into the polymer structure, which leads to a decrease in affinity for water (Smitha et al., 2003).

Opacity, which affects the visibility of packaged products to consumers, is an important property of packaging films. (Simmonds & Spence, 2017). Opacity did not change when substituting up to 75% guar gum but decreased in films containing 100% guar gum (Tripathi et al., 2019). As a result, films made from 100% guar gum were clearer and more transparent than films made from other formulations (Tripathi et al., 2019).

1.5.4.2 Carrageenan

Carrageenan **(Figure 1.5)** are a family of linear sulfated polysaccharides found in the cell wall and extracellular matrix of several species of red algae (Khotimchenko et al., 2020). These polysaccharides have a unique chemical structure characterized by an alternating repeating sequence of disaccharides (Campo et al., 2009). This repeat sequence consists of β-D-galactose linked at the third position and α-D-galactose linked at the fourth position (Kravchenko et al., 2020). Sulfate groups are esterified at different positions of galactose units to form several isomers of carrageenan, namely κ-carrageenan, λ-carrageenan, and ι-carrageenan (kappa, lambda, and iota, respectively) (Rey & Kee, 2023). These isomers differ in the number and location of sulfate groups in the galactose units (Mendis et al., 2022).

Carrageenan are widely used in various industries due to their unique properties (Zia et al., 2017). They are widely used as thickeners, stabilizers, and gelling agents in the food industry. These polysaccharides can improve the texture and consistency of foods and related products such as sauces, dairy products, desserts and even non-food products such as cosmetics, toiletries, and toothpaste (Morris et al., 2012). The specific carrageenan isomer used depends on the desired texture and properties of the final product (Sinthusamran et al., 2017).

With their ability to form gels and provide viscosity, carrageenans play a critical role in improving the quality and stability of a wide range of products. Interactions of carrageenans. and other ingredients, as well as their response to temperature and other processing conditions, contribute to their functionality in a variety

of applications. (Zia et al., 2017). The versatility of carrageenans makes them a valuable ingredient in both food and non-food industries (Sedayu et al., 2019).

κ-carrageenan, λ-carrageenan, and ι-carrageenan, which are different isomers of carrageenan, exhibit different properties and behavior due to differences in their chemical structure (Abdou & Sorour, 2014). κ-Carrageenan, which has only one negative charge per disaccharide unit, tends to form strong and rigid gels (Derkach et al., 2018). This property is explained by the interaction of negatively charged sulfate groups with positively charged ions, leading to the formation of a gel (Derkach et al., 2018). κ-Carrageenan is known for its excellent gelling ability and ability to form solid gels in the presence of cations such as potassium (Wang et al., 2018).

On the other hand, λ-carrageenan has an average of 2.7 negative charges per disaccharide unit (Slootmaekers et al., 1991). This higher degree of negative charges promotes its behavior as a thickening and stabilizing agent rather than forming strong gels (Fontes-Candia et al., 2020). The presence of these additional excipients disrupts the formation of the gel network, resulting in a more fluid and viscous texture (Fontes-Candia et al., 2020). λ-Carrageenan's ability to increase the viscosity and stability of solutions makes it useful in a variety of applications, including food products where a creamy texture is desired. (Therkelsen, 1993).

Regarding the mechanical properties of films made from carrageenan, tensile strength and elongation at break are influenced by the carrageenan content of the film (Kassab et al., 2019). Increasing the carrageenan content generally results in an increase in tensile strength, which refers to the film's ability to extend Tensile strengths without breaking (Zhang et al., 2021). This may be due to the structuring of the film composition by carrageenan molecules, which increases its overall strength (Sedayu et al., 2020).

Elongation at break, which measures the film's ability to stretch before breaking (Chen et al., 2003), also increases with higher carrageenan content (Paula et al., 2015). This increase in elongation can be attributed to the hygroscopic nature of carrageenan, that is, its ease of absorption of water (Bharti et al., 2021). Water

absorption in carrageenan-based films acts as a plasticizer, making the films more flexible and able to stretch without breaking (Balqis et al., 2017).

However, these improved mechanical properties are often associated with increased vapor permeability (Cheng et al., 2022). Carrageenan-based films generally have higher water vapor permeability compared to other polysaccharide-based films (Long et al., 2023). This means that moisture can easily pass through the film, which can affect the film's barrier properties and its ability to protect the packaged product from moisture-related changes.

1.5.4.3 Pectin

Indeed, pectin **(Figure 1.6)** is an interesting and versatile natural polymer that has a variety of applications in the food industry and beyond (Ishwarya et al., 2022). Pectins are complex heteropolysaccharides consisting predominantly of D-galacturonic acid residues with α-(1-4) linkages (Lara-Espinoza et al., 2018). The unique structure of pectin, characterized by its branching and variable degree of esterification, gives it a wide range of functional properties that make it valuable in various applications (Khubber et al., 2023).

Pectin is known to be one of the most structurally complex polysaccharides found in nature, and its role in plant cell walls is important in maintaining cell integrity and adhesion. (Caffall & Mohnen, 2009). In the food industry, pectin has become a key ingredient in various products due to its gelling, thickening and stabilizing properties (Ngouémazong et al., 2015). It is widely used in fruit preserves, jellies, and jams to create the desired texture and consistency.

The degree of esterification (DE) of pectin is a critical parameter affecting its functionality (Ngouémazong et al., 2012). High methoxy pectin (HMP) with a DE greater than 50% is commonly used in acidic conditions to formulate gels (Liang et al., 2022). These gels form in the presence of sugar and acid, making them ideal for products such as jams and jellies. Low methoxy pectin (LMP) with DE less than 50% requires

the presence of divalent cations such as calcium to form gels (Wu et al., 2024). This property makes LMP ideal for applications such as dairy products and low pH beverages (Yousefi & Jafari, 2019).

For commercial purposes, pectin is commonly extracted from a variety of sources, including citrus peels, apple pomace, and other fruit peels and pulps (Wang et al., 2014). The extraction process involves enzymatic or acid hydrolysis to release pectin from plant material (Adetunji et al., 2017). Non-commercial pectin can also be obtained from other plant wastes such as cocoa shell, banana, and watermelon peels (Riyamol et al., 2023).

In recent years, researchers have been exploring the possibility of using pectin to develop biodegradable films for food packaging and other applications (Nastasi et al., 2022). The gelling and film-forming properties of pectin make it a promising candidate for creating edible films that can help extend the shelf life of food products while reducing the environmental impact of traditional packaging materials (Chaiwarit et al., 2020).

Thus, pectin is an unusual biopolymer with many properties and benefits that make it a promising candidate for a variety of applications, including food packaging. Its renewable, biodegradable, and biocompatible nature, as well as its film-forming ability, have stimulated interest in the use of pectin as an environmentally friendly alternative to synthetic polymer-based packaging materials (Nešić et al., 2017).

However, as mentioned above, pure pectin films have some limitations such as water solubility, high water permeability, poor water resistance and low mechanical property values (Espitia et al., 2014). These weaknesses have led to the study of strategies to improve the performance of pectin-based films, especially in challenging environments such as high-humidity environments and for high-moisture food packaging (Maftoon, 2006).

Mixing pectin with other materials such as plasticizers such as glycerin or bioactive compounds such as gamma-aminobutyric acid (GABA) has been studied to improve the properties of pectin films (Meerasri & Sothornvit, 2020). These additives can affect the vapor permeability (PVP), mechanical properties and

barrier properties of the films. For example, the inclusion of GABA and glycerol had different effects on the WVT, tensile strength (TS) and elongation at break (EB) of pectin films (Heba et al., 2022). Inclusion of GABA decreased WVT and improved mechanical properties, while inclusion of glycerol had the opposite effect on these properties (Huang et al., 2021).

The combination of pectin with other nanoparticles such as chitosan nanoparticles (CSNPs) has also shown promise in improving the properties of pectin-based films. (Garavand et al., 2022). The addition of CSNPs to pectin films resulted in improved mechanical and physical properties, including increased tensile strength and elongation (Lorevice et al., 2016). These improvements can be attributed to stronger interactions between polymer matrices, resulting in a synergistic effect on film performance (El Miri et al., 2016).

Additionally, the degree of esterification (DE) of pectin, whether high methoxy (HMP) or low methoxy (LMP), can influence its properties and potential applications. Depending on the DE, pectin can exhibit different behaviors, making it suitable for various food applications as emulsifiers and stabilizers (Freitas et al., 2021).

In the context of food packaging, pectin-based biofilms have demonstrated the potential to extend the shelf life of fruits by reducing oxygen uptake and preventing changes in texture and color (Roy et al., 2023). This is important to reduce food waste and improve the quality of food during storage.

Overall, the versatile nature of pectin and its potential for modification and combination with other materials make it a valuable candidate for the development of biodegradable films for food packaging and other applications. Ongoing research will likely lead to more innovative ways to optimize pectin-based films and improve their performance in different environments and foods (Mellinas et al., 2020).

1.5.4.4 Pullulan

Pullulan **(Figure 1.7)** is an important polysaccharide with unique properties and a wide range of applications (Prajapati et al., 2013). It is produced by the yeast Aureobasidium pullulans through aerobic fermentation (Ravella et al., 2010). The chemical structure of pullulan consists of regularly repeating α-D-

glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl units forming a copolymer with a linear structure. (Singh et al., 2017). This structure can also be considered as a sequence of α-(1→6)-linked maltotriose units (Singh et al., 2017).

The various properties of pullulan gum make it a valuable material for various purposes, from basic research to industrial applications (Singh et al., 2008). Its linear structure and well-defined composition have led to its use as a model substance for the study of polysaccharides (Pérez et al., 2000). In addition, pullulan has attracted attention due to its lack of carcinogenic, mutagenic, and toxicological activity, making it a safe and harmless material for various applications (Vasile & Baican, 2021).

The water solubility of pullulan as well as its film-forming properties make it an ideal candidate for applications in foods, pharmaceuticals, cosmetics, packaging, etc. (Farris et al., 2014). Pullulan films can be used as coatings, encapsulation materials, and controlled release vehicles for various active substances (Najafi et al., 2021).

In addition, pullulan has the ability to form transparent and flexible films, making it a popular choice for edible films and coatings (Khanzadi et al., 2015). These films can improve the quality of food products, extend their shelf life, and improve the appearance of food products. Due to its lack of taste and odor, pullulan is particularly useful in applications where the sensory properties of the final product are important (Kraśniewska et al., 2019).

The biocompatibility and non-toxicity of pullulan have also led to its use in pharmaceutical and biomedical applications (Ramesan & Sharma, 2007). It can serve as a drug delivery vehicle by providing controlled release of active compounds (Teixeira et al., 2023). Its ability to encapsulate various substances as well as its biodegradability make it a promising material for these applications.

Thus, the structure of pullulan is a unique combination of amylose and dextran due to the coexistence of α-(1→4)- and α-(1→6) linkages in its complex structure (Singh & Saini, 2014). This arrangement of bonds contributes to the structural flexibility of pullulan and its higher solubility (Chen et al., 2017). α-(1→6)

bonds in particular are responsible for pullulan's ability to form flexible chains, allowing it to mimic several properties of both natural and synthetic polymers derived from petrochemical sources (Tabasum et al., 2018).

Pullulan is characterized as a non-hygroscopic white powder that is easily soluble in both cold and hot water (Singh et al., 2023). It has a number average molecular weight, and the viscosity of its aqueous solutions correlates with its molecular weight (Singh et al., 2008). In particular, pullulan is non-toxic and non-mitogenic, odorless, tasteless, and safe for consumption (Rai et al., 2021). In solution form it exhibits stability over the entire range of possible pH values. When burned, pullulan does not release harmful gases and when discarded, it is biodegradable by microorganisms, making it environmentally friendly (Ali et al., 2020).

The dry adhesive properties of pullulan and its ability to retain foam when dissolved in water contribute to its versatility in a variety of applications (Elangwe et al., 2023). In addition, pullulan has demonstrated good oxygen barrier properties, making it suitable for food packaging where maintaining food freshness and shelf life is critical (Khan et al., 2020).

The behavior of pullulan in solution has been studied extensively, focusing on its hydrodynamic and molecular properties. (Lazaridou et al., 2003).

1.5.4.5 Locust Bean Gum (LBG)

This polysaccharide is commonly known as locust bean gum or locust bean gum **(Figure 1.8).** It is extracted from the seeds of the tree Ceratonia siliqua L., native to the Mediterranean region (Rasheed et al., 2019). Locust bean gum is a neutral polysaccharide consisting of linear chains of β-D-mannopyranosyl residues linked by (1-4) linkages (Simões et al., 2011). These linear chains contain α-D-galactopyranosyl groups linked as single-unit side chains via (1-6) linkages (Simões et al., 2011).

An important property of locust bean gum is its ability, when mixed with water, to form highly viscous solutions over a wide range of pH levels and temperatures (Maier et al., 1993). This characteristic makes it

very valuable in the food industry, where it is often used as a thickener, stabilizer, and dispersant (Barak & Mudgil, 2014). The viscosity of its solutions helps improve the texture, mouthfeel, and stability of various food products (Khoobbakht et al., 2024).

Due to its natural origin and functional properties, locust bean gum is commonly used in the formulation of food products such as dairy products, beverages, baked goods, sauces, dressings and more (Petitjean & Isasi, 2022). Its ability to improve the texture and stability of these products makes it a popular choice among food manufacturers seeking to achieve desired quality in their offerings (Tosif et al., 2021).

Importance of locust bean gum in the context of edible films and coatings for the food industry:

Polysaccharide materials . The above-mentioned materials such as starch and starch derivatives, cellulose derivatives, alginates, carrageenan, chitosan, pectinates and various plant and microbial gums mixed with locust bean gum can be used as polysaccharides in the development of edible films and coatings. Since this combination can create an integrated matrix for the formation of coatings (Nehra et al., 2023).

Film formation. Edible films and coatings made from these polysaccharides play a vital role in controlling mass transfer between food components and between food and the environment (Han, 2014). This can lead to improved food quality and longer shelf life.

Barrier properties: Locust bean gum, as a hydrophilic polymer including many of the polysaccharides mentioned, can form strong interactions through mechanisms such as hydrogen bonds and electrostatic forces (Izydorczyk et al., 2005). These interactions contribute to the films' ability to act as a barrier against the penetration of gases such as oxygen (O2) and carbon dioxide (CO2), which can affect the quality and shelf life of food products.

Sensitivity to moisture . However, the effectiveness of these interactions may be affected by moisture absorption (Al-Maharma & Al-Huniti, 2019). When these hydrophilic films absorb moisture, they can alter interchain interactions that contribute to barrier properties (Schäfer et al., 2018). This means that as relative humidity increases, the barrier properties to gases such as O2 and CO2 may decrease.

1.5.4.7 Xanthan Gum

Hydrocolloids such as xanthan gum **(Figure 1.9)** offer a promising avenue in the search for effective biodegradable materials (Patel et al., 2020). Research has shown that the addition of hydrocolloids has a noticeable effect on various properties of starch-based materials (Jiang et al., 2020). It has been observed that the addition of hydrocolloids improves the viscosity of starch gels, affects the gelatinization and retrogradation processes of starch, and increases the viscosity and viscoelasticity of starch solutions (Cristina de Melo, 2011).

Xanthan gum, obtained from the aerobic fermentation of Xanthomonas campestris, serves as an exopolysaccharide and has long been used as a stabilizer in the food, cosmetic and pharmaceutical industries (Kang & Pettitt, 1993). Interestingly, the addition of xanthan gum to the film formation process reduces the stress at which the film breaks. This result suggests the formation of a potentially weaker matrix during the film production process (Cristina de Melo, 2011).

In contrast, the addition of xanthan gum was associated with an increase in the strain at break of the resulting films (Melo et al., 2011). This effect can be attributed to the intrinsic properties of xanthan gum, which has the ability to form stable three-dimensional networks when dispersed in water (Kumar et al., 2018). The helical structure of xanthan gum supports the hypothesis that its inclusion in the system promotes higher breaking stress. Thus, although the mechanical properties of starch films are affected by the presence of xanthan gum, the elongation properties of the films tend to be improved (Cristina de Melo, 2011).

Additionally, the chemical structure of xanthan gum has accessible and soft areas that make it easier to interact with other components (Sworn, 2021). This feature allows the formation of strong gels and films in combination with other ingredients, which ultimately improves the mechanical properties of the resulting materials (Sworn, 2021). The compatibility of xanthan gum with various structures highlights its potential to contribute to the development of stronger and more functional biodegradable films (Jabeen & Atif, 2023).

1.5.4.8 Alginates

Alginate **(Figure 1.10)** gums obtained from algae and brown algae, especially giant algae, are characterized by the composition of α-D-mannuronic acid and β-L-guluronic acid (Abka-Khajouei et al., 2022). These gums have the unique property of solubility in both cold and hot water (Eghbaljoo et al., 2022). Compared to other gums, alginate gums do not inherently form gels; However, they can form gels in the presence of specific divalent cations such as Ca2+ and Mg2+ (Zheng et al., 2021). In particular, these rubbers are not thermally reversible, meaning they do not melt when heated (Ferrero et al., 1996).

Alginate gums **(Figure 1.10)** have a variety of applications due to their multifunctional properties, including thickening, suspending, emulsifying, stabilizing, gelling, and forming protective films (Qin et al., 2018). In particular, the strength of the resulting gel is influenced by the presence of higher levels of Ca2+ ions and the content of G-blocks in the alginate structure (Hu et al., 2021). The ionic cross-links inherent in the alginate structure play a fundamental role in binding to other molecules, promoting the formation of films with different functionalities. (Manzano et al., 2019).

1.6. Proteins

Proteins, composed of α-amino acids as their main components, have complex structures and behaviors that are influenced by their primary, secondary, tertiary and quaternary arrangements (Fülöp et al., 2006). Proteins, typically ranging in length from 100 to 500 amino acid residues, adopt different conformations along their polymer chains dictated by the sequential arrangement of amino acids (primary structure) (Nolan & Margoliash, 1968). These structures are formed by various forces, including van der Waals interactions, hydrogen bonds, electrostatic and hydrophobic forces, and disulfide bonds between amino acid units, resulting in the formation of various secondary structures (Chetel et al., 1985).

These secondary structures are further organized to form the overall architecture of the protein, leading to the formation of globular, fibrous, or random protein structures (tertiary protein structure) (Stollar & Smith,

2020). Moreover, proteins can interact with each other to create complex assemblies that provide unique structural and biological functions (quaternary structure) (Krochta, 2002).

Modification of protein structures (secondary, tertiary, or quaternary) can be caused by various physical and chemical agents such as heat, pressure, shear, irradiation, lipid interfaces, acids, alkalis, enzymes, cross-linking agents and metals. ions (Lacroix and Cooksey, 2005).

Derived from animal sources, protein film-forming materials contain a variety of compounds, including collagen, gelatin, keratin, egg white protein, myofibrillar protein, casein, and whey protein (Gennadios et al., 1994). Although these protein-based films have moderate mechanical properties compared to synthetic films, they are excellent as an oxygen barrier under certain humidity conditions (Chen et al., 2019). However, its hydrophilic nature contributes to relatively high vapor permeability (Thawien, 2012). However, this hydrophilicity provides an excellent barrier against flavors and lipids, slowing down the absorption of flavors (Wang & Arntfield, 2017). In addition, this property can be strategically used for the controlled release of encapsulated compounds, making protein-based films promising vehicles for the delivery of active compounds (Balaguer et al., 2012).

Some proteins are water soluble, and their solubility is determined by internal interactions within the protein network (Chou & Morr, 1979). The final characteristics of protein films are influenced not only by the origin and type of protein, but also by the processing conditions used (Grimaldo, 2014).

1.6.1 Collagen and Gelatin

Gelatin, a hydrolyzed form of collagen **(Figure 1.11)**, is widely used as a food ingredient or additive in the food industry and is classified as “generally regarded as safe” (GRAS) (Baziwane & He, 2003). The complex family of collagens includes 26 different types, the variations of which are associated with the specific amino acids present in each chain (Gorgieva & Kokol, 2011). The origin of collagen, whether it is beef, fish or chicken, influences the type of collagen, such as type III, which may contain traces of cystine. In contrast, type I collagen lacks cystine (Rýglová et al., 2017). Extraction of gelatin from collagen involves

acid and alkaline treatment to produce different isoelectric points, which contribute to different properties of the resulting food product (Rehman, 2016), (Francis, 2000).

Gelatin exhibits a wide range of properties that enhance various nutritional properties, including gelation, foaming, film formation, thickening, water retention, emulsification, stabilization, adhesion and more (Ahmad et al., 2023). The collagen unit consists of a triple helical structure consisting of three alpha chains, each of which carries a sequence of amino acids (Fietzek & Kühn, 1975). Among these sequences are ternary peptides that present XY configurations of glycine, with proline often occupying in the X position and hydroxyproline the Y position. A typical sequence includes hydroxyproline, proline, and glycine (Brodsky & Persikov, 2005). Glycine residues are critical for maintaining the tight turns that allow collagen structure to be flexible. Intermolecular and intramolecular cross-links within collagen, formed by covalent and hydrogen bonds, respectively, maintain the stability of the molecule (Karim and Bhat 2008).

The film-forming ability of gelatin is promising when combined with other ingredients (Ramos et al., 2016). During the drying process, gelatin films exhibit severe shrinkage, which requires the introduction of polyhydric alcohols to improve the adhesion and flexibility of the dried films (Lu et al., 2022).

Gelatin can be obtained from a variety of animal sources, including pork skin, tendon and bone, beef, fish, and chicken (Liu et al., 2015). The viscosity of chicken skin gelatin is higher than that of pork, but lower than that of beef (Sarbon et al., 2013). In comparison, chicken gelatin exhibits water retention capacity equivalent to pork gelatin but less than beef gelatin (Almeida & Lannes, 2013). Chicken gelatin has higher fat-binding capacity than pork and beef, and its emulsifying ability is also better (Rasli & Sarbon, 2015). Although the stability of chicken gelatin is less than that of pork gelatin, it is equal to that of bovine gelatin. In particular, the foaming ability of chicken gelatin is weaker than that of pork and beef gelatin (Mrázek et al., 2019).

1.6.2 Wheat Proteins: Gliadin and Glutenin

Wheat gluten can be divided into monomeric gliadins, which contribute to gluten viscosity, and glutenin, which provides elasticity (Žilić, 2013). The form of gliadins is characterized by a heterogeneous assembly of individual polypeptide chains with a molecular weight of 30 to 50 kDa, linked by hydrogen bonds and hydrophobic interactions (Cornell and Hoveling, 1998).

The term “gluten” refers to a cohesive, protein-based viscoelastic substance that is formed as a by-product during the separation of starch from wheat flour (Zhang et al., 2023).

The production of gluten films can be carried out by two methods: solvent-based methods and thermoplastic methods (Rudnik, 2012). Casting methods require solvent compositions covering basic or acidic conditions, combined with alcohol and disulfide bond reducing agents. This complex system is required due to the insolubility of gluten proteins in water (Guilbert et al., 2002).

Wheat gluten proteins (gliadin and glutenin) **(Figure 1.12)** can be formed into thermoplastic films by compression molding, resulting in films with high tensile strength and water retention (Zubeldía et al., 2015). Wheat gluten is emerging as a cost-effective and widely available substance derived from renewable resources, which is currently attracting widespread attention due to its potential applications in the field of thermoplastics (Patil et al., 2021).

Gliadin-based films have remarkable transparency, low mechanical strength, and structural integrity when exposed to water. Therefore, they can be mixed with other components to be used in specialized industries such as packaging materials (Hernandez-Muñoz et al., 2003). Cast gliadin films were thermally treated (Hernández Muñoz et al., 2004d) and blended with other renewable polymers with improved mechanical properties, such as chitosan.

Even lower quality gluten, which is of poor quality for improving flour in bread baking, can find application in the production of plastic films (Mangavel et al., 2002). This is due to its desirable properties, which include viscoelasticity, adhesiveness, thermoplasticity and commendable film-forming properties (Gautam

& Kumar, 2015). However, gluten is insoluble in water, which is compounded by the problem that glutenin poses during processing due to its tendency to aggregate under shear and heat (Naqash et al., 2017). On the other hand, gliadins have lower molecular weight (MW) and exhibit high solubility and stability in ethanol solutions over a long period of time (Rani et al., 2023). Several functional aspects of gliadin-derived films, such as mechanical strength, water barrier and water resistance, require improvement (Grimaldo, 2014).

1.6.3 Soy Protein Isolate (SPI)

Soy protein isolate (SPI) **(Figure 1.13)** has been introduced as a popular and profitable option for protein-based films due to its wide availability, cost-effectiveness, and high protein content of over 90%. (Tan, 2023). In addition to mechanical properties, the ability of protein films to modulate gas permeability is critical to meeting your specific packaging needs (Atta et al., 2022). In particular, films made from wheat gluten isolate and soy protein have demonstrated remarkable effectiveness in preventing oxygen penetration (Wittaya, 2012). However, it is worth noting that the ability of protein films in general to act as a barrier to water vapor is somewhat limited (Gennadios et al., 1994).

Soy protein contains various amino acids, including glutamic acid, arginine, lysine, cystine and aspartic acid, which have polar groups (Del Valle, 1981). These groups provide valuable sites for cross-linking and hydrogen bonding, which serve to improve the mechanical properties of soy protein polymers (Chabba and Netravali, 2005).

Its inherent advantages such as renewability, biocompatibility, biodegradability and film-forming properties make soy protein isolate (SPI) a promising material for packaging applications (Hadidi et al., 2022). However, the usefulness of SPI-based films is limited because they do not have strong mechanical properties and are easily affected by humidity (Ni & Dumont, 2017).

Researchers are currently working to create an innovative type of milk packaging film that has unique edible properties (Daniloski et al., 2021). These protein-based films demonstrate powerful abilities to prevent food spoilage, representing a potential replacement for plastic packaging (Ali et al., 2023). This

development is particularly important as some plastic packaging materials have raised concerns about their ability to leach substances into encapsulated foods.

1.6.4 Casein Protein

Recent developments have highlighted the ability of milk proteins, particularly casein **(Figure 1.14)**, to be a promising candidate for the development of edible and biodegradable packaging films (Mishra et al., 2022). Recent data from the USDA shows that casein-based films outperform traditional plastic materials by up to 500 times in terms of oxygen barrier capacity, a critical aspect of food preservation (Soares et al., 2018). This improvement can be explained by the fact that proteins form a more closely interconnected structure during polymerization (Jin et al., 2020). These protein-based films also offer better properties than current starch-based edible packaging options, especially for protecting light-sensitive foods (Ramkumar, 2016).

These innovative protein-based films have remarkable oxygen blocking capabilities, significantly reducing the risk of food spoilage (Hassan et al., 2018). Such innovations can help solve food waste problems that arise during the distribution stages of the food supply chain (Xiong et al., 2019). Researchers refined the technology after an initial test using pure casein produced an effective film; however, it has encountered problems in terms of handling and rapid dissolution when exposed to water (Reuters, 2016).

1.7. Lipids

Two major categories of materials used in packaging films, namely polysaccharides and proteins, have commendable mechanical and optical properties (Khalil et al., 2019). However, its susceptibility to moisture leads to a decrease in vapor barrier characteristics. In contrast, two other categories, lipids and polyesters, exhibit notable water vapor barrier properties (Kester & Fennema, 1989).

Lipids can be divided into polar and non-polar groups, with waxes being classified as non-polar lipids (Buckner, 1993). Waxes do not contain polar components such as hydrocarbons. This results in complete insolubility in water with an inability to form surface monolayers (Hadley, 1989). Therefore, waxes are the

most effective lipid barriers as they effectively prevent the penetration of water vapor into edible films (Yousuf et al., 2022). Numerous studies highlight the reduction in water vapor transmission due to the high hydrophobicity of these lipid compositions, making waxes a preferred option in this context.

On the other hand, triglycerides **(Figure 1.15)** are insoluble in water but can be distributed at interfaces to form stable monolayers (Phillips & Hauser, 1974). Its hydrophobicity is influenced by its structure: long-chain triglycerides remain insoluble in water, while short-chain triglycerides exhibit partial solubility (Prajapati et al., 2012). The addition of components such as palmitic acid, stearic acid, lauric acid and stearyl alcohol to edible films improves moisture barrier properties, which means improved resistance to water vapor (Gennadios et al., 1994).

The classification of monoglycerides is determined by their chain length, placing them in class II or III (Small, 1968). Class II lipids are insoluble in water; However, water can dissolve in the hydrophilic segment of its structure, causing swelling (Cullis & Hope, 1991). Consequently, monoglycerides exhibit emulsifying properties. In the case of low water content, reverse micelles with inverted polar heads are formed (Krog et al., 2004). On the contrary, higher water content promotes the formation of conventional micelles, where the polar groups face the aqueous phase (Furkan Aydin, 2017).

Monoglycerides find use in edible films as emulsifiers, effectively stabilizing emulsion films (Rhim & Shellhammer, 2005). In addition, they serve as agents for improving adhesion between components having different hydrophobicity. While polysaccharides and proteins impart favorable mechanical properties through polymeric interactions, lipids impart desirable barrier properties to food materials (Sedayu et al., 2019).

Thus, the fatty acids used for these purposes include waxes, non-hydrogenated vegetable oils and fatty alcohols (Furkan Aydin, 2017).

1.7.1 Bee Wax (B&W)

The water vapor permeability (WVT) of edible films composed of polysaccharides can be improved by incorporating hydrophobic substances such as beeswax (BW), carnauba wax, paraffin and butyric acids (Shahidi & Hossain, 2022). Among them, BW are often used to produce edible films using emulsion technology. BW mainly consists of hydrocarbons, alcohols, and extended acids (Wu, 1999). Creating a stable lipid emulsion in the solution used for film formation can effectively prevent WVT of the final film (Bozdemir, 2003).

Beeswax **(Figure 1.16)** exhibits a higher level of hydrophilicity and compatibility with glycerol (Zhang et al., 2022). Therefore, the presence of hydrogen bonds between different components of the film structure provides increased resistance to water vapor penetration (Zhang et al., 2022).

Coatings that include lipids generally provide greater stability but can also create sensory issues and impart a waxy feel to packaged goods (Yousuf et al., 2022). For this reason, current research is focused on the development of composite edible films and coatings, seeking a balance between stability and sensory properties (Furkan Aydin, 2017).

1.8. Plasticizer

Plasticizers play a crucial role in changing the intermolecular forces within films, which leads to increased mobility of polymer chains and improved mechanical properties (Eslami et al., 2023). However, the introduction of plasticizers negatively affects the barrier properties, leading to an increase in mass transfer through the films (Jost et al., 2014). Plasticizers commonly used in the production of edible films include polyethylene glycol 200 (PEG 200), glycerin (Gly), propylene glycol (PG) and sorbitol (S) (Bozdemir, 2003).

1.9. Advanced Strategies to Achieve Film Functionality

To improve the mechanical properties of packaging films, the industry is exploring combinations of different components to consider their individual properties.

Attempts to improve the water resistance of biopolymers often include strategies such as the use of emulsified membranes (Galiano et al., 2018). One approach involves the introduction of lipids and waxes to improve moisture barrier properties (Bourlieu et al., 2007). In addition, methods such as the formation of composite and multilayer films have been used (Zhang et al., 2019).

The combination of lipids and biopolymers through the production of multilayer or emulsion films has gained importance as a key strategy for improving water resistance (Arnon-Rips & Poverenov, 2016). This technology plays a vital role in improving the overall water resistance of the resulting films. In bilayer composite films, a polysaccharide or protein layer is coated with a lipid or wax solution to create a second lipid layer (Pérez-Gago & Rhim, 2014). Bilayer films have superior water resistance compared to emulsion films, which can be attributed to the vertical resistance to water penetration of successive lipid layers.

Gas concentrations are closely related to aspects of food quality such as aroma, taste, color, texture and nutritional value (Barrett et al., 2010). Thus, the gas barrier properties of biopolymer-based films are important for food preservation. Biopolymer-based films have favorable gas barrier properties compared to most plastic films due to the inherent polarity of hydrocolloid polymers (proteins and polysaccharides) (Abdullah et al., 2022b). These films have good gas barrier properties for O2 and CO2.

Another trend applicable to the food industry, and a response to concerns raised about the food safety implications of biodegradable packaging, is the development of antimicrobial biodegradable packaging (Motelica et al., 2020). Antimicrobial food packaging is essential to ensure food safety, extend shelf life and maintain freshness. By inhibiting the growth of harmful microorganisms and pathogens, it helps reduce food waste and improve overall food quality (Duncan, 2011). Its adaptability to different types of food makes it a versatile solution, and in the global food distribution chain, it ensures the integrity and safety of products during transport and storage (Nayak & Waterson, 2019). Ultimately, antimicrobial packaging not only protects consumer health by minimizing the risk of foodborne illness, but also increases confidence in the safety and quality of biodegradable packaged food products (Adeyeye, 2019).

1.10. Conclusion

The use of regular plastic for food packaging has caused big problems for the environment and our health. That's why people are looking for better options. Edible and biodegradable packaging made from natural materials are becoming popular because people want safer food and less waste. But there are still challenges to solve, like making sure the food stays safe and the packaging works well. Scientists are working on new ways to make these kinds of packaging better, like adding special coatings or making them fight bacteria. This is all part of the journey towards making food packaging more sustainable and better for everyone.

1.11. References

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Table 1. 1 Components that can be used to create edible coatings (Zhang, 2023).

Polysaccharides	Squirrels	lipids
➢ Cellulose ➢ Starch ➢ Chitosan ➢ Gums: - Pectin - Guar gum - Locust bean gum - Xanthan gum - Pullulan - Alginate - Carrageenan	➢ Collagen ➢ Gelatin ➢ Wheat gluten ➢ Soy protein ➢ Casein	➢ Bee wax ➢ Polyethylene wax ➢ Carnauba wax ➢ Paraffin candle ➢ Candelilla wax ➢ Rice bran wax ➢ Jojoba oil

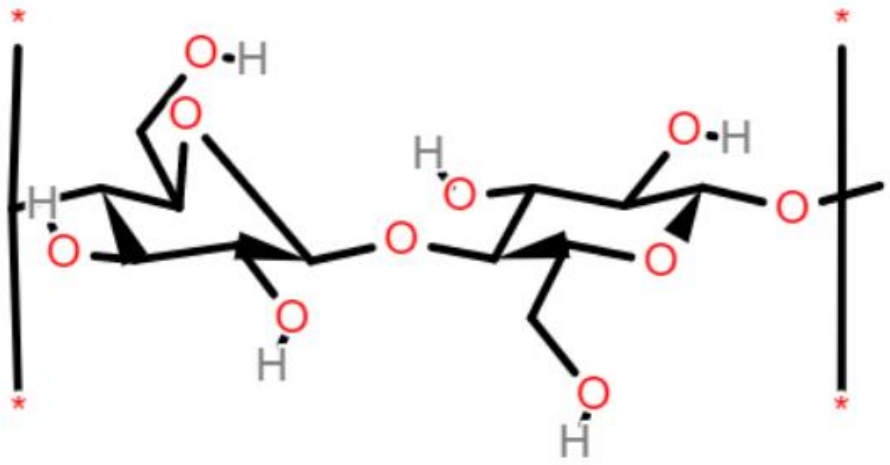

Figure 1.1 Chemical structure of cellulose (ChemSpider, 2023).

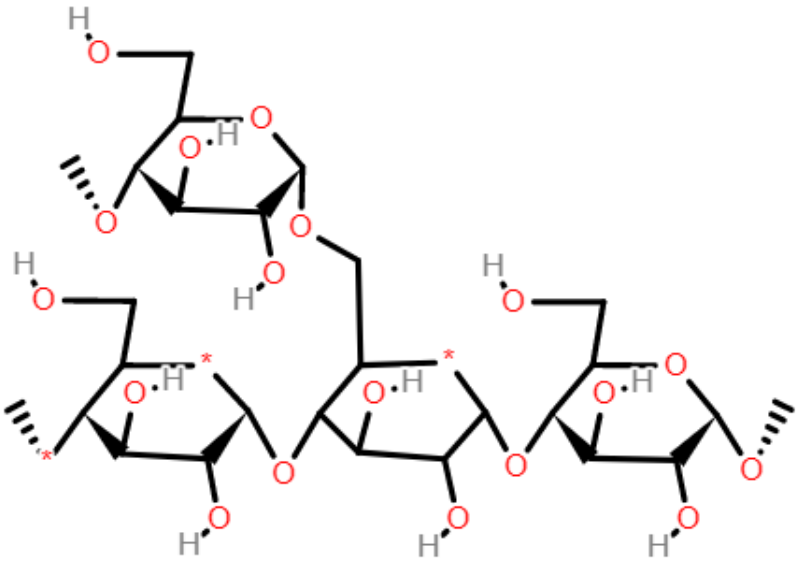

Figure 1.2 Structure of starch (amylose and amylopectin linked by glycosidic bonds) (ChemSpider, 2023).

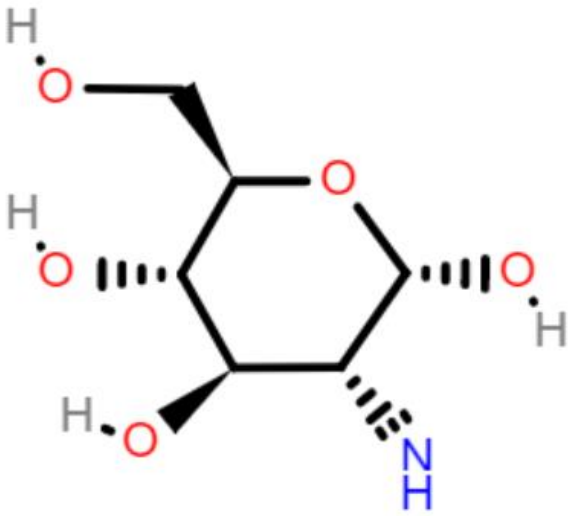

Figure 1.3 Comparison of structures of chitosan monomer (glucosamine) (ChemSpider, 2023).

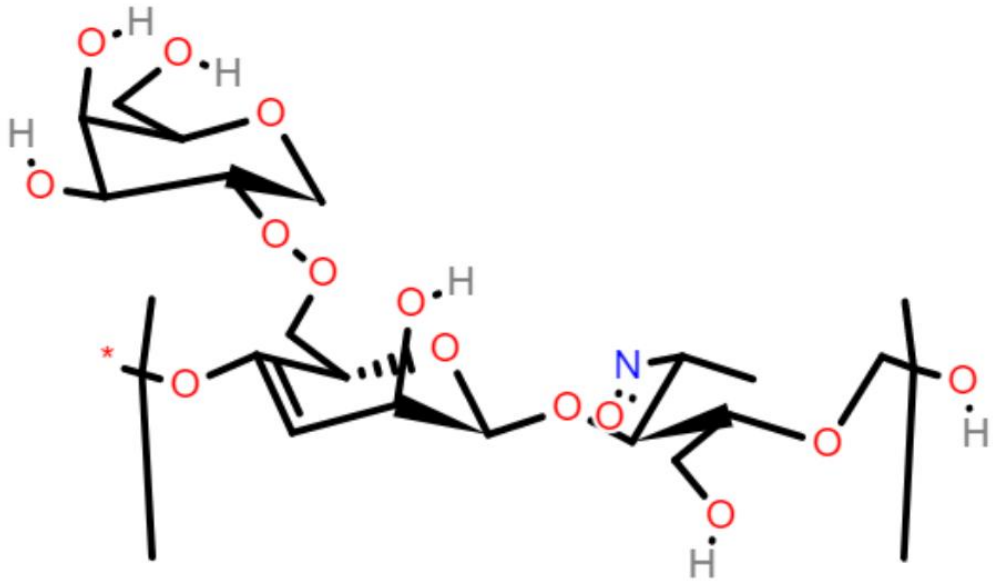

Figure 1.4 Chemical structure of guar gum (ChemSpider, 2023).

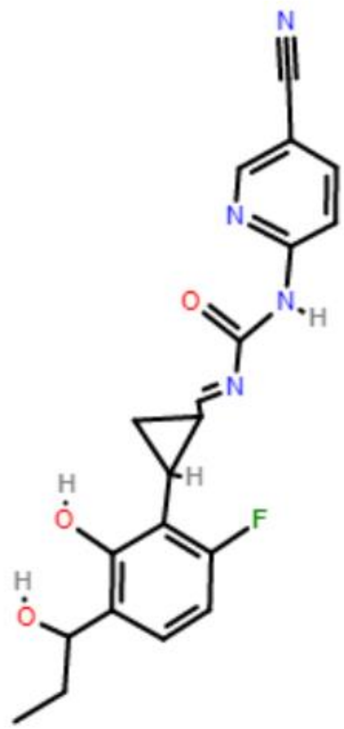

Figure 1.5 Chemical structure of carrageenan (ChemSpider, 2023).

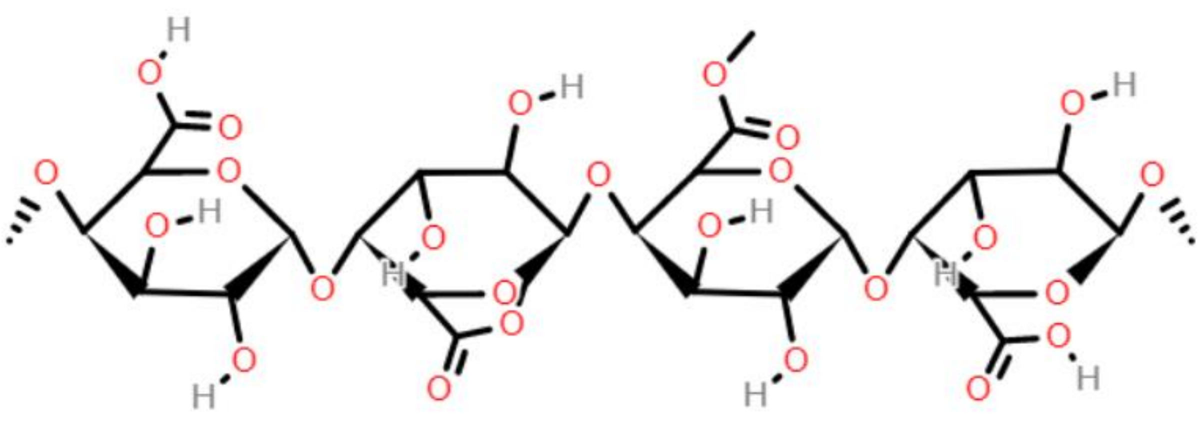

Figure 1.6 Chemical structure of pectin (ChemSpider, 2023).

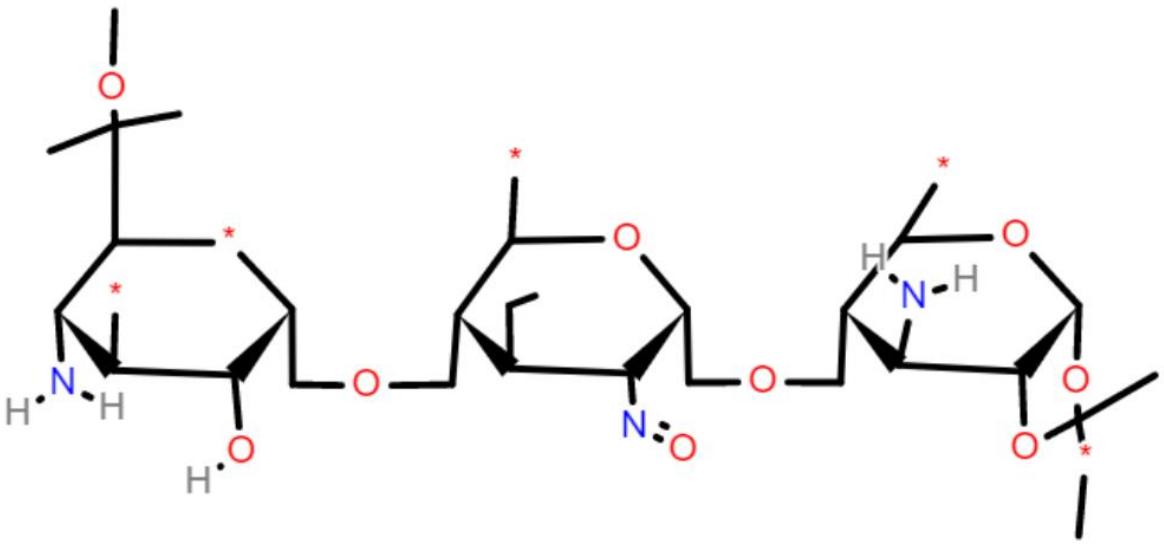

Figure 1.7 Chemical structure of pullulan (ChemSpider, 2023).

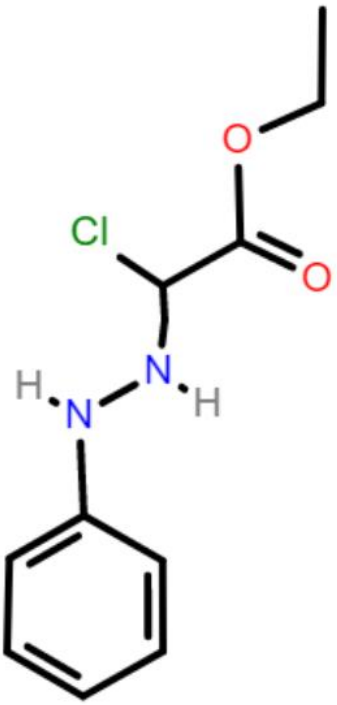

Figure 1.8 Chemical structure of locust bean gum (ChemSpider, 2023).

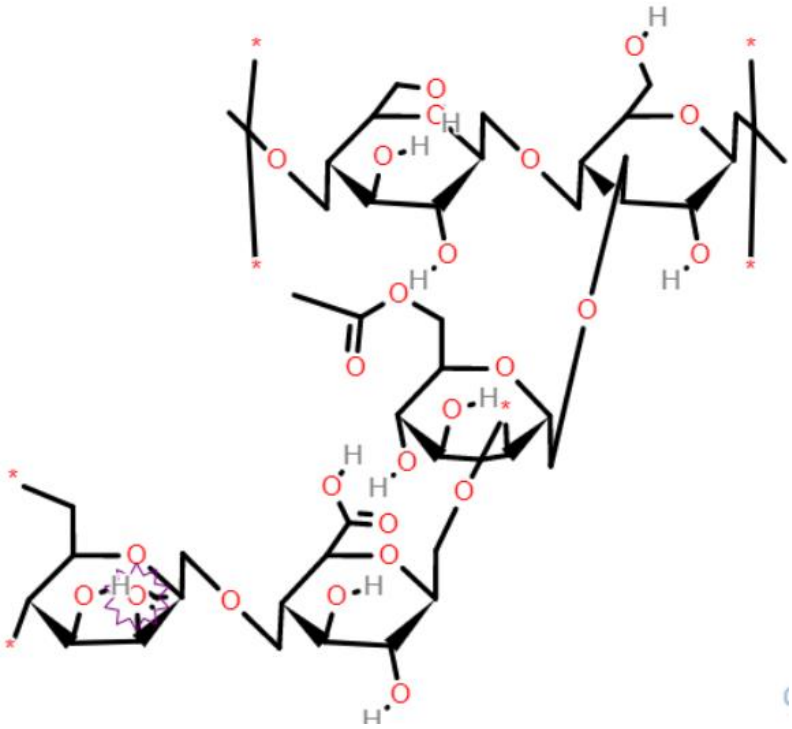

Figure 1.9 Chemical structure of xanthan gum (ChemSpider, 2023).

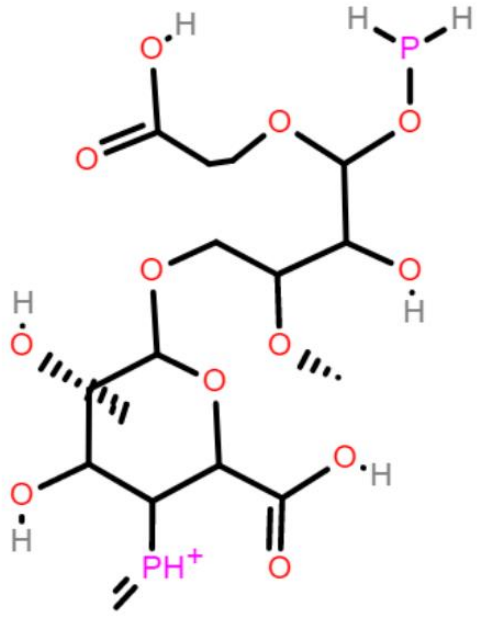

Figure 1.10 Chemical structure of alginate (ChemSpider, 2023).

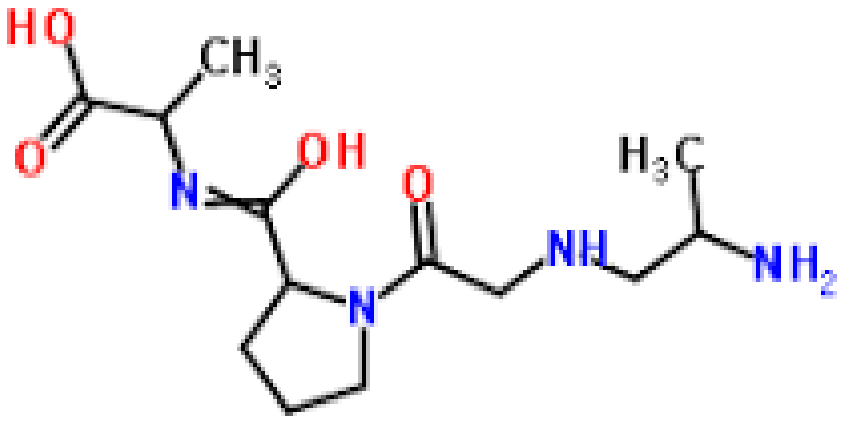

Figure 1.11 Chemical structure of collagen (ChemSpider, 2023).

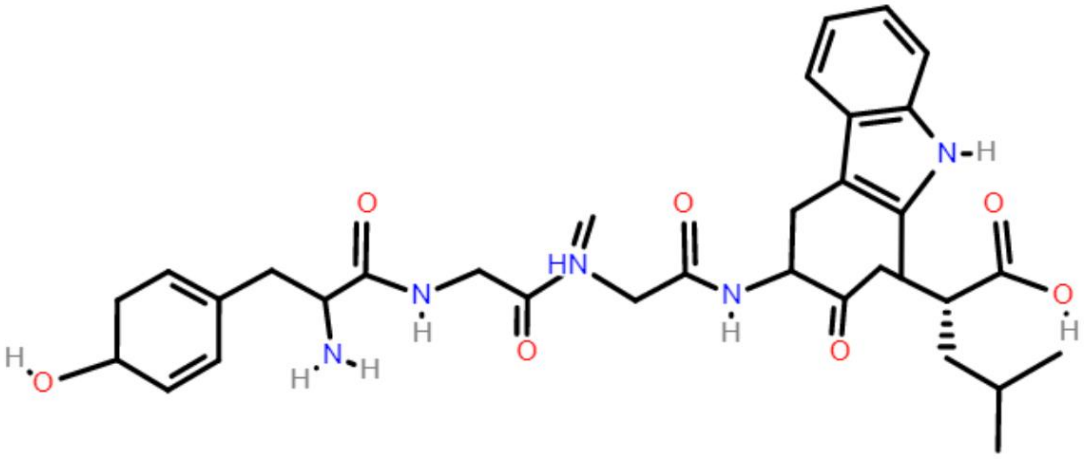

Figure 1.12 Chemical structure of gluten (ChemSpider, 2023).

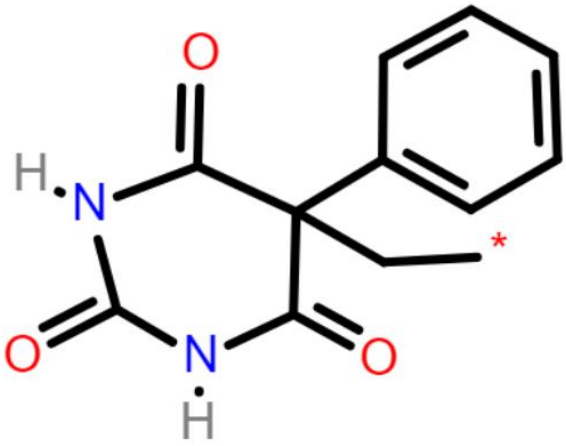

Figure 1.13 Chemical structure of soy protein (ChemSpider, 2023).

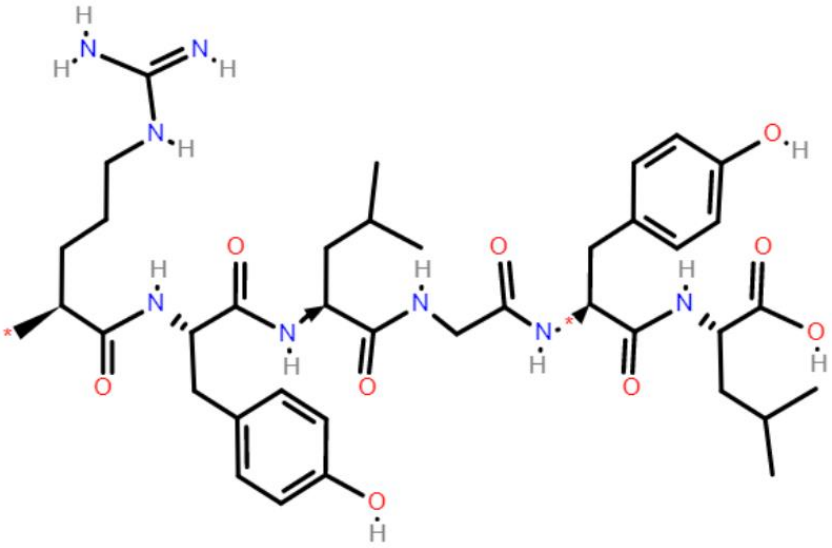

Figure 1.14 Chemical structure of casein protein (ChemSpider, 2023).

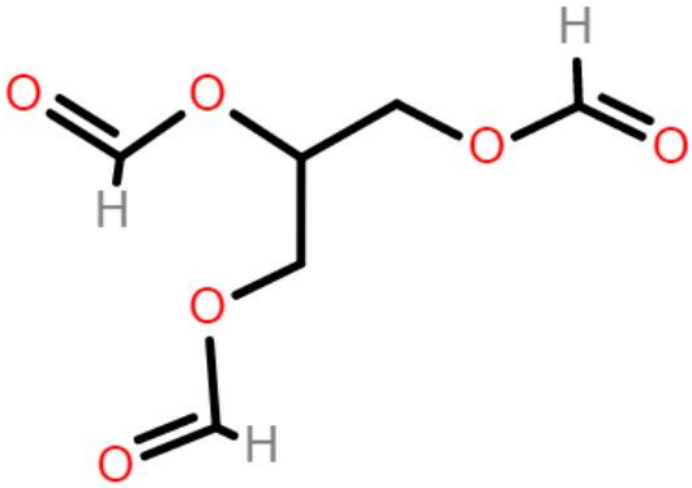

Figure 1.15 Chemical structure of triglycerides (ChemSpider, 2023).

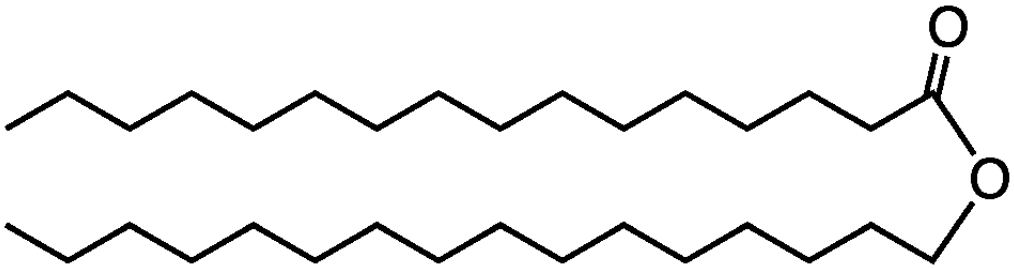

Figure 1.1 Chemical structure of beeswax (ChemSpider, 2023).

CHAPTER II

Engineering a Sustainable Packaging Film using Poultry Collagen for the Food Industry

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2.1 Abstract

Sustainable packaging is seen as the next step in the food industry. Packaging made using biodegradable materials have been studied but the incorporation of new bioactive molecules recovered from existing food is scarce. Poultry processing products such as chicken skins are sold at a very low price to the rendering industry and the research using chicken collagen in packaging is limited. The collagen was converted into gelatin to be used in the development of biodegradable packaging films. A total of eight film formulations with fifteen repetitions (n=120) were tested. Treatment 1 has 2% of chicken gelatin (CG) and 0% of nanocellulose (NC), treatment 2 has 2% CG; 1% NC, treatment 3 has 2% CG; 3% NC, treatment 4 has 2% CG; 3% NC, treatment 5 has 3% CG; 0% NC, treatment 6 has 3% CG; 1% NC, treatment 7 has 3% CG; 3% NC, treatment 8 has 3% CG; 4% NC. Thus, all the treatment contains the same percentage of starch (1%), glycerol (6%), and water (85-91%). In addition, films with 0% CG were formulated as control, however, this formulation did not form a film and could not be tested. To create the film, starch and CG in different beakers were dissolved in water (50% water for CG and 50% for starch) (45°C for CG and 85°C for starch) for 30 min, then the mixture was combined and mixed at 45°C (30 min). NC was added to the solution and mixed at 45°C for 15 min. Finally, glycerol was added and mixed at 45°C for 15 min. Tensile strength, yield strength, elongation, punching force, solubility, and water vapor Transmission (WVT) were analyzed. Data was analyzed using ANOVA with significant differences in the means at $p<0.05$ (Tukey's HSD). The highest value for yield strength and punching forces were 0.88 Mpa and 12.08 N/mm respectively (3% CG and 4% NC). The lowest value for WVT was 0.00077 and 0.00084 g/m.h.Kpa (2% CG, 4% NC and 3% CG, 1% NC). The highest tensile strength was 0.295 and 0.269 Mpa (3% CG, 3% NC and 3% GC, 4% NC). Thus, the highest elongation was 41.57% (3% CG and 0% NC). In conclusion, chicken gelatin combined with nanocellulose can form films and could be considered used for ready to eat (RTE) products.

2.2. Keywords: Biodegradable film, plastic, chicken, byproducts, mechanical properties.

2.3. Introduction

Packaging plays a central and critical role in food processing (Berk, 2009). Packaging has four principal functions: containment, protection, convenience, and communication (Geddes, 2021). In the context of packaging, protection refers to the role of packaging in safeguarding the product from external factors that could potentially damage or degrade it. Containment refers to the function of packaging in securely holding and enclosing the product. Convenience refers to how comfortable the customer feels using a determinate product. Lastly, communication is the information that package gives to the customers and how to provide this information (Geddes, 2021).

Food packaging mainly uses plastics, leading to a large percentage of global plastic waste leakage. In the world 400.3 million metric tons in 2022 are generating of plastic waste, from which the packaging industry generates 150 million metric tons (Alves, 2023).

The packaging industry has a huge responsibility for reducing the millions of tons of plastic waste, looking for plastic alternatives and moving towards sustainable. Before people started using plastic materials for packaging, they used biobased materials, such as leaves, animal organs among others. Now, because of the problems caused by plastics that don't break down, there's a renewed interest in using natural materials for packaging again (Tian et al., 2021).

There are various natural ingredients being studied for use in making bioplastics, such as starch, glycerol, and nanocellulose. Starch and glycerol are commonly used ingredients in the base formulation of biodegradable packaging film. Starch is a complex carbohydrate derived from plants, and it finds wide application in the food industry as a thickening agent, stabilizer, and texturizer. Thus, it is used in the production of biodegradable packaging materials because it helps to form structure and bind other substances (Perez et al., 2009). Glycerol is a colorless and odorless liquid derived from fats and oils (Berger & Schnelder, 1992). It is widely utilized in the cosmetics and personal care industry as a moisturizer and humectant due to its ability to attract and retain moisture (García et al., 2014). Glycerol functions as a

plasticizer in biodegradable packaging films offering flexibility and elasticity (Tryznowska and Łukasz, 2018).

There is a need to investigate novel ingredients that can be incorporated in the biobased packaging films to improve their functionality and mechanical properties that could be an alternative for plastic materials. Nanocellulose is a nanomaterial that plays a crucial role in the formulation of some biodegradable films due to its advantageous mechanical properties such as tensile strength, Yield Strength, and punching force, affordability, and biocompatibility. This study specifically concentrates on cellulose nanofibers (CNF), a type of nanocellulose derived from plant cell walls (Cuscuta reflexa plant). CNF offers unique properties such as stiffness and strength and is chosen as the focal point among various nanocellulose types (Cellulose nanocrystals (CNCs), Cellulose nanofibrils (CNFs), and Bacterial cellulose (BC)) (Gopakumar et al., 2019). In addition to nanocellulose, we investigated chicken skin collagen as a biomaterial in packaging films.

The U.S. is a leading producer of poultry with approximately 47 billion pounds of meat in 2023 (USDA ERS, 2023). With such high production volumes, the industry is continuously innovating to handle byproducts such as skin, heads, necks, blood, organs, feathers, and certain carcasses, to extract biomolecules of economic significance. One such biomolecule is collagen, a protein found in chicken skin, bone, cartilage, and other connective tissue (Wang, 2021) which can be used in the production of artificial skin production, bio-curatives, gelatin, and jelly thus adding value to the poultry byproducts and creating economically viable, consumer acceptable options (Santana et al., 2020).

Structurally, collagen is a rod-shaped protein characterized by the presence of three substantial and repetitive polypeptide chains forming a three-dimensional triple helix structure (Suparno & Prasetyo, 2019). The amino acid profile and the tissue source dictate the characteristics and functionality of collagen (Shoulders & Raines, 2009). Collagen can be extracted by different methods; physical, chemical, and biological treatment (Karuppannan et al., 2021). Typically, physical treatment is categorized into mechanical and thermal treatment while chemical treatment uses hydrolysis of strong acids or alkalis, and

the biological method utilizes enzymes and microorganisms (Chen et al., 2023). However, the type of extraction will depend on the intended purpose and the source material available for extraction.

Collagen has been used as a one fundamental component in bioplastics (Brigham, 2018), because offers biodegradability and biocompatibility with other biocomponents. Collagen is a polymer made up of amino acids, and form peptide bond between chains (Traub et al., 1969), enhancing the mechanical properties making it suitable for packaging applications (Irastorza et al., 2021).

With this innovative approach, this packaging could possibly solve plastic waste, and food waste, making this study different from others, because the use of an innovative component like chicken collagen extracted from chicken skin to make a biodegradable packaging.

Considering the significance of food packaging and the potential for enhancing the utilization of poultry byproducts and improvement on the mechanical properties in materials for packaging industry, the objective of this study was to develop a film packaging solution using chicken gelatin and nanocellulose specifically for Ready to Eat products (RTE), to substitute the utilization of plastic in the food industry and instead utilize byproducts that contribute to food waste.

2.4. Materials and Methods

2.4.1 Biomaterials:

Packaging films were prepared using chicken collagen (Essentia Protein Solution, Ankeny, Iowa 50021 U.S.), nanocellulose (CNF) at a concentration of 3% (University of Maine, Maine, U.S.), corn starch (Grain Processing Corporation, Oregon, U.S.), glycerol (Fisher bioreagents, Ottawa, Canada), and deionized water.

2.4.2 Packaging film formulations:

All packaging film formulations had the same percentage of starch (1%), and glycerol (6%) while other ingredients varied **(Table 2.1).** These formulations were derived from multiple trial-and-error experiments involving varying concentrations of ingredients.

2.4.3 Process of Packaging Film Preparation

The ingredients were weighed as per the formulations **(Table 2.1)**. The starch and chicken collagen to were mixed separately by initially adding half the water volume, and dissolving starch at 85°C for 30 min while stirring at 3,000 rpm and chicken collagen with the remaining half of the water at 45°C for 30 minutes on a hot-plate stirrer (Thermo Scientific Climatic Stirring Hot Plate, 7x7 inches ceramic) **(Figure 2.1)**. The starch and collagen solutions were combined and mixed at 45°C for another 30 min while stirring at 3000 rpm. Further, nanocellulose was added to the mixture and stirred for 15 min. As the stirring continued, glycerol was added, and the mixture was held at 45°C for an additional 15 min. The mixture was then allowed to cool at room temperature (20°C) for 15 min from which 20 g was poured into a 15 cm diameter petri dish, and dried 72 h at 35°C in a convection incubator (Thermo Electron Corporation, U.S.) resulting in a biobased packaging film **(Figure 2.1).**

2.4.4 Physical and Mechanical Analysis.

For each analysis, 15 repetitions per treatment (films per formulation) were tested as a rectangle shape film with a size of 6 cm (L) x 2.5 cm (W). The following tests were performed on all 120 (8 formulations x 15 repetitions) film samples: thickness, Tensile strength, punching force, yield strength, solubility, elongation, and water vapor permeability.

2.4.4.1 Thickness

Thickness analysis was conducted with an electronic micrometer (Chicago Brand, 0.000050 reading) with 0.001 mm of accuracy. Five points were randomly selected and measured on each film sample (Jain, 2022) (6 cm x 2.5 cm) and the average per film was calculated and thickness was reported in millimeters (mm).

2.4.4.2 Punching force

Punching force was measured using the TA.XTPlus Texture Analyzer (Texture Technologies Corp., Hamilton, MA/Stable Micro Systems, Godalming, Surrey, UK) equipped with a 5 kg load cell, a ½ in stainless steel ball probe (TA-18), and the indexable film extensibility rig (TA- 108s5i). Data was acquired using the Exponent Stable Micro System Software Package (version 6, 1, 11, 10, Visual Components, Inc.). The pre-test, test and post-test speed were 1, 0.5, and 10 mm/s, respectively with the force parameters set at 0.981 N (force) and 0.049 N (trigger force). The distance between film and attachment was 40 mm and the punching force formula is described below (Cesar, 2018):

$$(h) = \frac{Fp}{L} = N/mm$$

Where h is the value of punching force (N/mm), Fp is the force peak before break (N), and L is the average thickness of the film (mm).

2.4.4.3 Tensile Strength

Tensile strength was measured using the ASTM D882 method (American Society for Testing and Materials, 2023) on the TA.XTPlus using the same settings as Punching Force (above). Tensile strength was calculated using the following formula:

$$Tensile\ Strength\ (MPa) = \frac{Tensile\ force\ (Texturometer)}{Area\ (m2)}$$

2.4.4.4 Deformation

Deformation (%) was analyzed as described in the ASTM D882 method (American Society for Testing and Materials, 2023) using a texture analyzer (Texture Technologies Corp, TA. XTPlus, New York, U.S) using the settings for Punching Force, a peak distance of the film was determined by applying the force of the equipment.

$$\%\ Deformation = \frac{Final\ length - Initial\ length}{Initial\ length} * 100$$

2.4.4.5 Yield Strength at Breaking Point

Yield Strength (stress/strain) was calculated using the Tensile strength and deformation (%) data as described in the ASTM E111-17 method (American Society for Testing and Materials, 2017a).

$$Yield\ Strength = \frac{Tensile\ Strength\ (MPa)}{Deformation\ (mm)} = MPa$$

2.4.4.6 Solubility Index

Solubility index was calculated using a previously published method (Cesar, 2018), where 120 circular samples (15 mm diameter; 8 formulations x 15 replications) were cut and weighed. The samples were submerged into a conical tube with 50 ml of water for 24 h at room temperature (25°C) After 24 h, the samples were reweighed. The solubility percentage was measured with the following formula:

$$\%\ Solubility = \left(\frac{wi\text{-}wf}{wi}\right) \times 100$$

Where wi is the initial weight (g) and the wf is the final weight (g) of the film packaging.

2.4.4.7 Water Vapor Transmission

Water Vapor Transmission analysis was conducted as described in the ASTM E96-80 method, (American Society for Testing and Materials, 2017b). Briefly, Conical tubes (50 mL) with 25 mL of water at room temperature (25°C) were used for the analysis. Films were cut in circles (200 mm diameter) and placed on the top of the conical tubes. The tubes were stored at room temperature (25°C) inside of desiccators for seven days. The weight of each tube was recorded every day to determine the mass of water going through the film. The following formula was used to calculate water vapor permeability (WVT):

$$WVT = C\left(\frac{X}{A\,\Delta P}\right) = (g/m.h.kpa)$$

Where the C represent the slope (g/h); A is the permeability area (m2); X is film´s thickness (m); ΔP is the pressure used, which was the difference between the silicate gel (0 kPa at 25 °C) and pure water vapor (3.167 kPa at 25 °C inside of tubes).

2.4.5 Statistical Analysis

To assess the statistical significance of the differences among the groups, an analysis of variance (ANOVA) was performed. Subsequently, Tukey's post-hoc analysis was employed to identify specific pairwise comparisons and determine significant differences between the groups.

A multiple linear regression analysis was conducted on the results obtained from ANOVA test, considering all the significant parameters observed during experiments. A multiple linear regression equation was proposed to estimate values for physical and mechanical properties depending on the concentration of chicken gelatin and nanocellulose on films (Latha et al., 2013).

The multiple regression equation used:

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\ldots+\beta_nX_n+\varepsilon$$

Y is the dependent variable; β_0 is the y-intercept; β_1, β_2,…,β_n are the regression coefficients for the independent variables X_1,X_2,….X_1,X_2,…,X_n; ε represents the error term (Latha et al., 2013).

2.5 Results and discussion

2.5.1 Mechanical and physical properties

Thickness values ranged from 0.310 mm to 0.319 mm which were not significantly different compared to the range described in the literature. Packaging film thicknesses are 0.007–0.125 mm (Mangaraj et al., 2019), however the thickness value will depend on the components on the matrix. Biopolymer films used

for applications such as food packaging or biodegradable bags can have thicknesses in the range of 10 to 100 micrometers (Abdullah et al., 2022).

Thickness of the film directly impacts various mechanical properties, including punching force, Tensile strength, Yield Strength, water vapor permeability and, gas transmission (Zhang et al., 2018; Saito et al., 2022) and affect shelf-life of food (Siracusa & Ingrao, 2017). The thickness values vary based on the components and their quantity utilized in the matrix and the manufacturing process, including extrusion or coextrusion methods (Schrenk & Alfrey, 1978). In the current study, the film was cast in petri plates and the thickness was controlled by pipetting exact amounts of formulations and drying for a specified amount of time.

Tensile strength analysis is a mechanical test conducted on packaging materials to determine their maximum load-bearing capacity before rupturing or tearing occurs (Dahle et al., 2003). During this test, the material exhibits elastic behavior until a certain point and eventually ruptures (Sun et al., 2013). In this study, the highest recorded value for the Tensile strength analysis was 0.471 MPa, corresponding to formulation 8 which involved 3% chicken gelatin and 4% nanocellulose **(Figure 2.2)**. Overall, the Tensile strength values indicate that reducing the percentages of chicken gelatin and nanocellulose in the materials leads to a proportional decrease in the recorded Tensile strength values. This is explained by both chicken gelatin and nanocellulose contain functional groups that can participate in hydrogen bonding (Shen et al., 2023). Hydrogen bonds form when a hydrogen atom, covalently bonded to a more electronegative atom, interacts with another electronegative atom (Desiraju, 2011). In the case of gelatin and nanocellulose, hydroxyl (-OH) groups in nanocellulose and amino groups in chicken gelatin can engage in hydrogen bonding, creating a strong connection between the two materials (Heise et al., 2021; Li et al., 2023). Researchers have reported the tensile strength of cellulose-based packaging films (0 to 4% concentration) to range from 0.16 - 26.04 Mpa (Rhim et al., 2006) and those with 1% of concentration of cellulose aligns with the findings of this study. Compared to this study, Zhao et al. (2021) reported a much higher tensile (0.72 MPa) for starch glycerol films.

Deformation analysis measures the extent to which a material can be stretched as a percentage of its original dimensions before reaching the point of fracture (Lim & Hoag, 2013). This parameter, also known as percent elongation, encompasses both plastic and elastic deformation up to the breaking point. To calculate percent elongation, the material's final length is compared to its original length, quantifying the increase in length before fracture occurs (Chambers, 2020). This phenomenon is often referred to as strain, representing the percentage by which length can increase prior to breaking (Chambers, 2020).

In this study, the highest elongation values were observed for treatments 1, 5, 6, 7, and 8 falling within the range of 20.37% to 25.36% **(Figure 2.3).** These treatments included combinations of 2% chicken gelatin and no nanocellulose, as well as 3% chicken gelatin with 0%, 1%, 3%, and 4% nanocellulose, respectively **(Figure 2.3).** Research commonly suggests that higher percent elongation, coupled with good tensile strength, indicates superior material quality (Aziz et al., 2003). Comparatively, the values reported in this study (20.37% - 25.36%) exceed those of other polysaccharide-protein-based films (Mihalca et al., 2021). For instance, cellulose films demonstrate elongation of 7.67% - 18.5%, hemicellulose films range from 15.49% - 20.61%, starch films exhibit 1.65% - 35%, chitosan packaging varies from 12% - 58%, and polysaccharide gums show values between 1.10% and 35% (Zhao et al., 2021). In the current study, the formulations with highest elongation are the ones with high amount of chicken gelatin than other components (nanocellulose, chitosan and cellulose) because the gelatin, derived from collagen, exhibits unique properties, including high flexibility and stretchability (Gómez-Guillén et al., 2011). When incorporated into packaging films, chicken gelatin may contribute to increased elongation, making the films more flexible and resistant to deformation (Liu et al., 2015; Suderman et al., 2018).

The highest ($p<0.05$) punching force (13.023 N/mm) was for films made with formulation 8 which has 3% chicken gelatin and 4 % nanocellulose **(Figure 2.4)**. This analysis is commonly employed in industries such as manufacturing (Schober technologies, 2023), materials testing (Iqbal et al., 2016), and engineering design (Segal, 2004) to understand how materials respond to applied forces and to optimize processes involving punching or perforating actions (Stable Micro Systems, 2023), and is applicable to the packaging

industry. The values from this study are lower compared to synthetic plastics (351 N/mm) but higher comparing with starch-based materials (3.6 N/mm), because the nanocellulose, being a nanomaterial derived from cellulose, can have both amorphous and crystalline regions (Dufresne, 2013). The arrangement of amorphous and crystalline regions affects the overall structure and porosity. Collagen's structure and its combination with nanocellulose can result in a matrix with varying degrees of porosity (Walters & Stegemann, 2014). Thus, the ability of collagen to absorb and retain water can influence the tightness and porosity of the matrix (Sionkowska & Kozłowska, 2013).

Yield Strength, also known as the elastic modulus, is the ratio of the applied stress to a material along the longitudinal axis and the resulting deformation or strain measured on the same axis (SpecialChem, 2022). When a Tensile strength is applied to an object, the material undergoes extension. This behavior is described by the relationship between stress and strain within the deformation region. The extent of material deformation is influenced by factors such as length, thickness, and crystallinity (Popli & Mandelkern, 1987). Elongation is defined as extension per unit length, with all measurements being lengths, hence devoid of units (Xometry, 2023). In contrast, stress is defined as force per unit area of the material (Pa or MPa) (William & Merle, 2011). Yield Strength serves as a measure of a material's stiffness, utilizing the measurement of tensile stress and strain (elongation) (Tranoudis & Efron, 2004). In the context of this study, the highest ($p<0.05$) Yield Strength values were recorded as 1.61 MPa and 1.93 MPa, corresponding to treatments 7 and 8 involving 3% chicken gelatin along with 3% and 4% nanocellulose, respectively **(Figure 2.5)**. Comparatively, Zhao et al. (2021) reported a Yield Strength with a higher value of 4.9 MPa for starch film. This is explained by nanocellulose, being a nanomaterial with high strength and stiffness, can reinforce the matrix (Lee et al., 2014), leading to an increase in Yield Strength. However, gelatin, derived from collagen, is known for its flexibility and stretchability (Liu et al., 2015). When combined, nanocellulose and chicken gelatin may introduce a more flexible and less strength component to the matrix, potentially leading to a decrease in Yield Strength.

Solubility refers to the extent to which a material or substance can dissolve in a given solvent (Britannica, 2022). It quantifies the maximum amount of solute that can dissolve in a solvent at equilibrium (Helmenstine, 2020). In this study, there were no significant differences observed among all the formulations **(Figure 2.6)**. This phenomenon can be attributed to the inherent hydrophilic nature of the film (Hanani et al., 2014), which arises from the integration of various compounds such as gelatin, starch, glycerol and nanocellulose. Consequently, these compounds readily interact with water, making the film susceptible to dissolution.

However, this hydrophilic property of gelatin, starch, glycerol and nanocellulose presents certain challenges (Shojai & Langroudi, 2009). As most food items contain moisture, the moisture content in the food can interact with the film's hydroxyl groups leading to dissolution during storage (Cao et al., 2007). On the other hand, this property might offer a potential advantage in terms of faster degradation in water, improving sustainability.

Water vapor Transmission analysis quantifies the amount of water vapor mass that can permeate a material within a specified time frame, expressed in units of mass per area per time (Keller & Kouzes, 2017). It offers a comprehensive assessment of how effectively a thin material layer acts as a barrier to permeants (Lewis & Weaver, 2004). In this study, no statistically significant differences were observed among treatments, with WVT values ranging between 0.00101 and 0.00108 g/m.h.kPa **(Figure 2.7).**

In the context of packaging, a lower water vapor permeability is generally preferable (Yahiaoui et al., 2015). Water vapor permeability (WVT) signifies a material or packaging's capacity to allow water vapor (moisture) to traverse it (Su et al., 2010). A lower water vapor permeability indicates that the material or packaging is less permeable to water vapor, while higher permeability signifies greater water vapor transmission (Gennadios et al., 1994). In comparison to other films crafted from polysaccharides, including chitosan with values ranging between 2.5 and 8.78 g/m.h.kPa (Verónica Trejo et al., 2001), this study shows promising results.

Relative to a plastic sample (Polyvinyl chloride) with a WVT of 4.94E-05 g/m.h.kPa, the WVT of the film formulations in the current study are much higher. In the food industry, a low WVT is preferred to prevent food spoilage (Figlewicz et al., 2014).

2.5.2 Multiple regressions analysis

Table 2.2 outlines the coefficients corresponding to the percentage of chicken gelatin and nanocellulose for each analysis. These coefficients serve as a basis for predicting values related to both mechanical (such as Tensile strength, deformation, punching force, and Yield Strength) and physical properties (including solubility, thickness, and WVT). By utilizing this information, it becomes possible to optimize values during formulation and processing, thereby achieving desired outcomes across all properties (Vercellis, 2011).

2.6. Conclusion

Treatments without chicken collagen did not form film. However, biodegradable chicken film containing different percentages of chicken collagen and nanocellulose showed different physic-mechanical properties. An increase in the amount of chicken gelatin and nanocellulose increased the Tensile strength, punching force, and yield strength values. Consequently, biodegradable packaging film potentially substituting conventional plastics and thereby minimizing the food wastage.

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Table 2. 1 Packaging film formulations.

Formulation	Chicken Gelatin (%)	Nanocellulose (%)	Starch (%)	Glycerol (%)	Water (%)
1	2	0	1	6	91
2	2	1	1	6	90
3	2	3	1	6	88
4	2	4	1	6	86
5	3	0	1	6	89
6	3	1	1	6	89
7	3	3	1	6	87
8	3	4	1	6	85

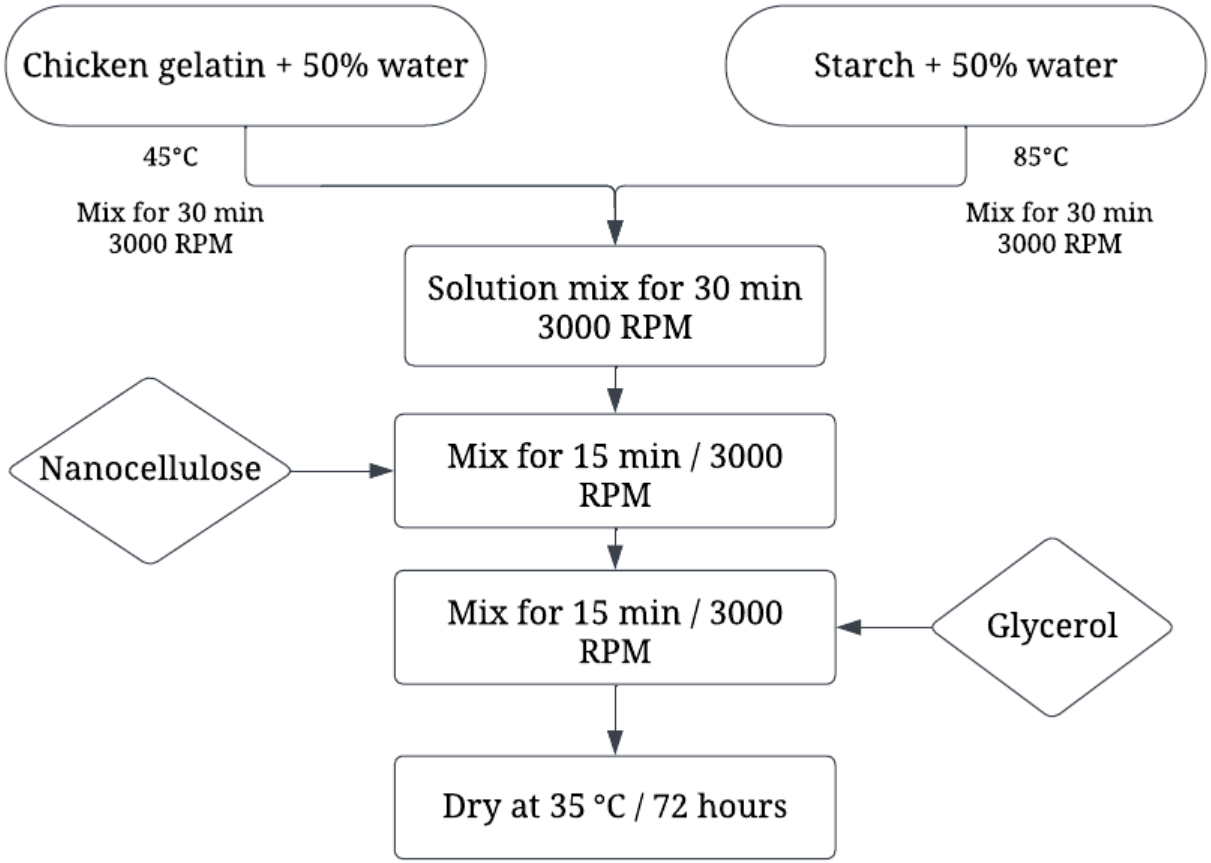

Figure 2. 1 Flow diagram of Packaging Film Preparation

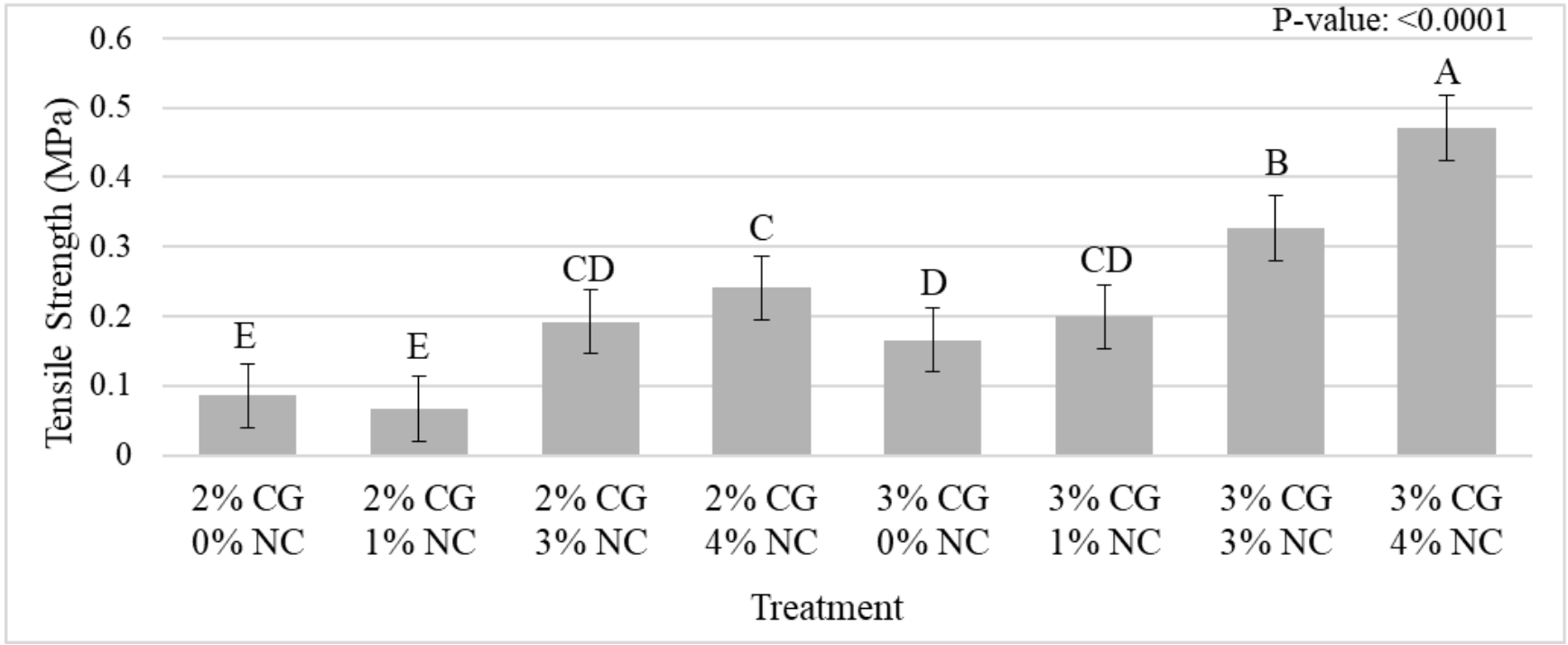

Figure 2. 2 Tensile strength of films (MPa) results among treatments. Different letters denote statistically significant differences at a significance level of $P < 0.05$ between the treatments. The letter "A" indicates the best treatment based on the comparison. CG: Chicken Gelatin; NC: Nanocellulose.

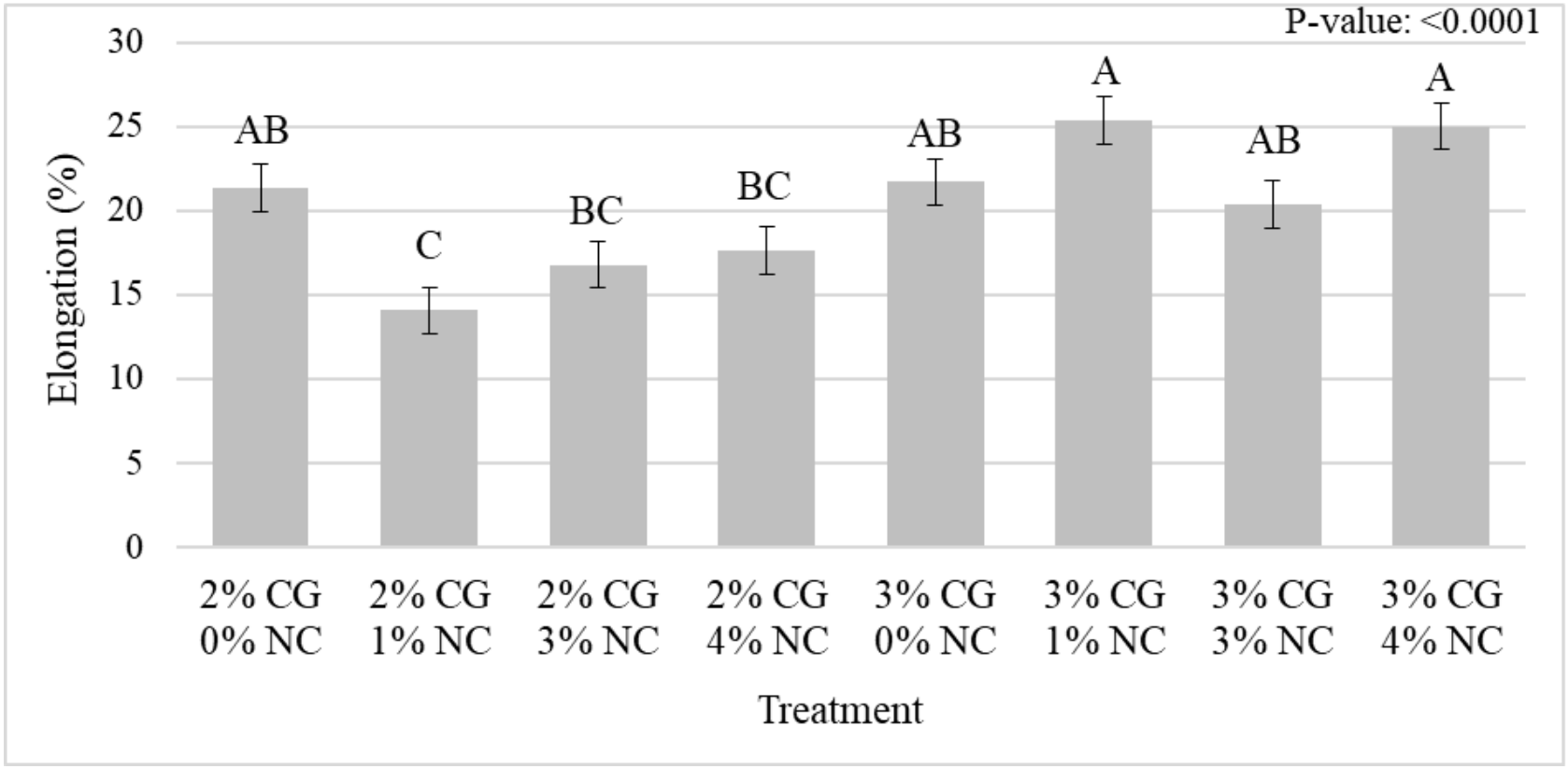

Figure 2. 3 Elongation of films (%) results among treatments. Different letters denote statistically significant differences at a significance level of $P < 0.05$ between the treatments. The letter "A" indicates the best treatment based on the comparison. CG: Chicken Gelatin; NC: Nanocellulose.

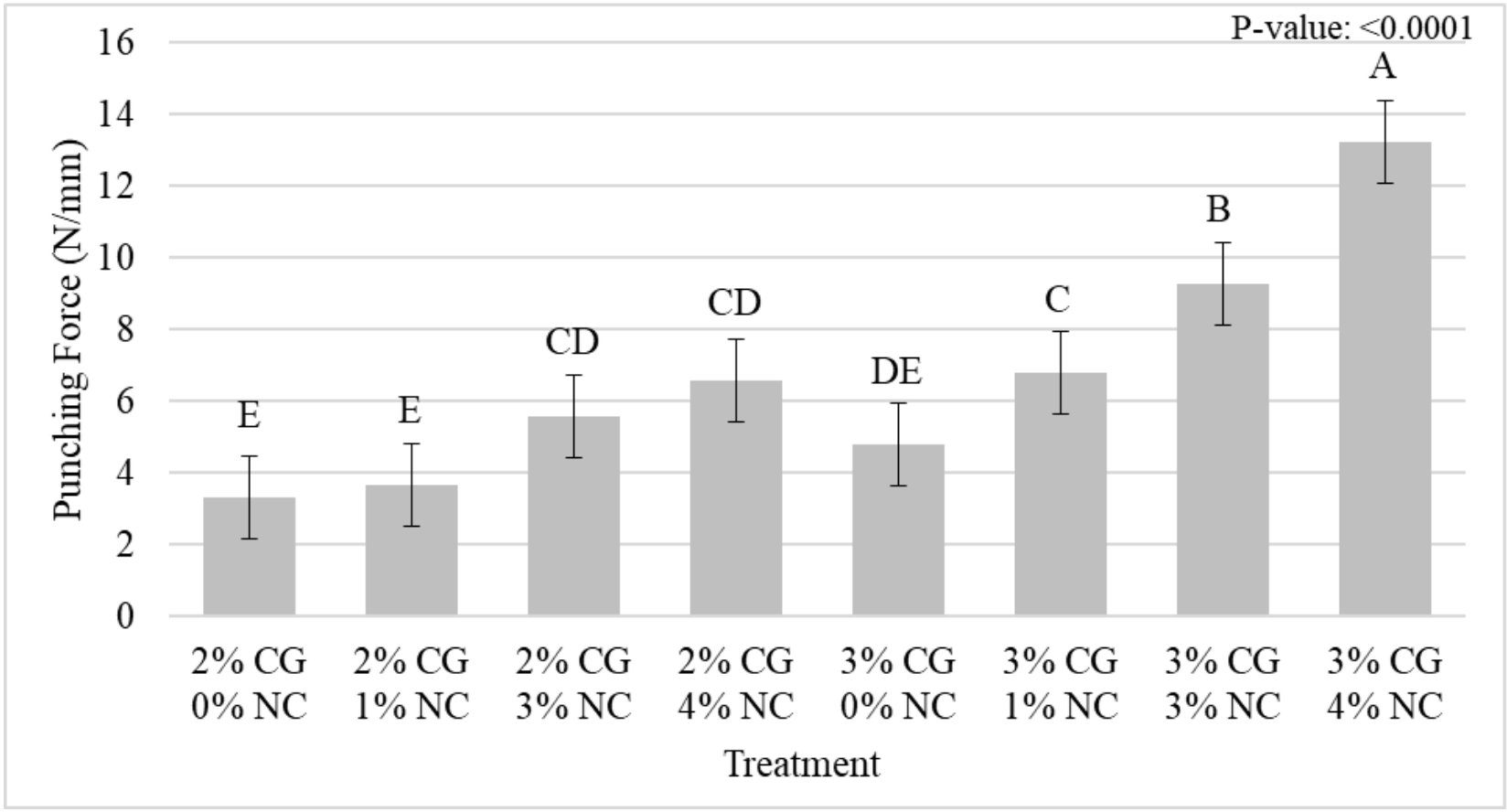

Figure 2. 4 Punching force of films (N/mm) results among treatments. Different letters denote statistically significant differences at a significance level of $P < 0.05$ between the treatments. The letter "A" indicates the best treatment based on the comparison. CG: Chicken Gelatin; NC: Nanocellulose.

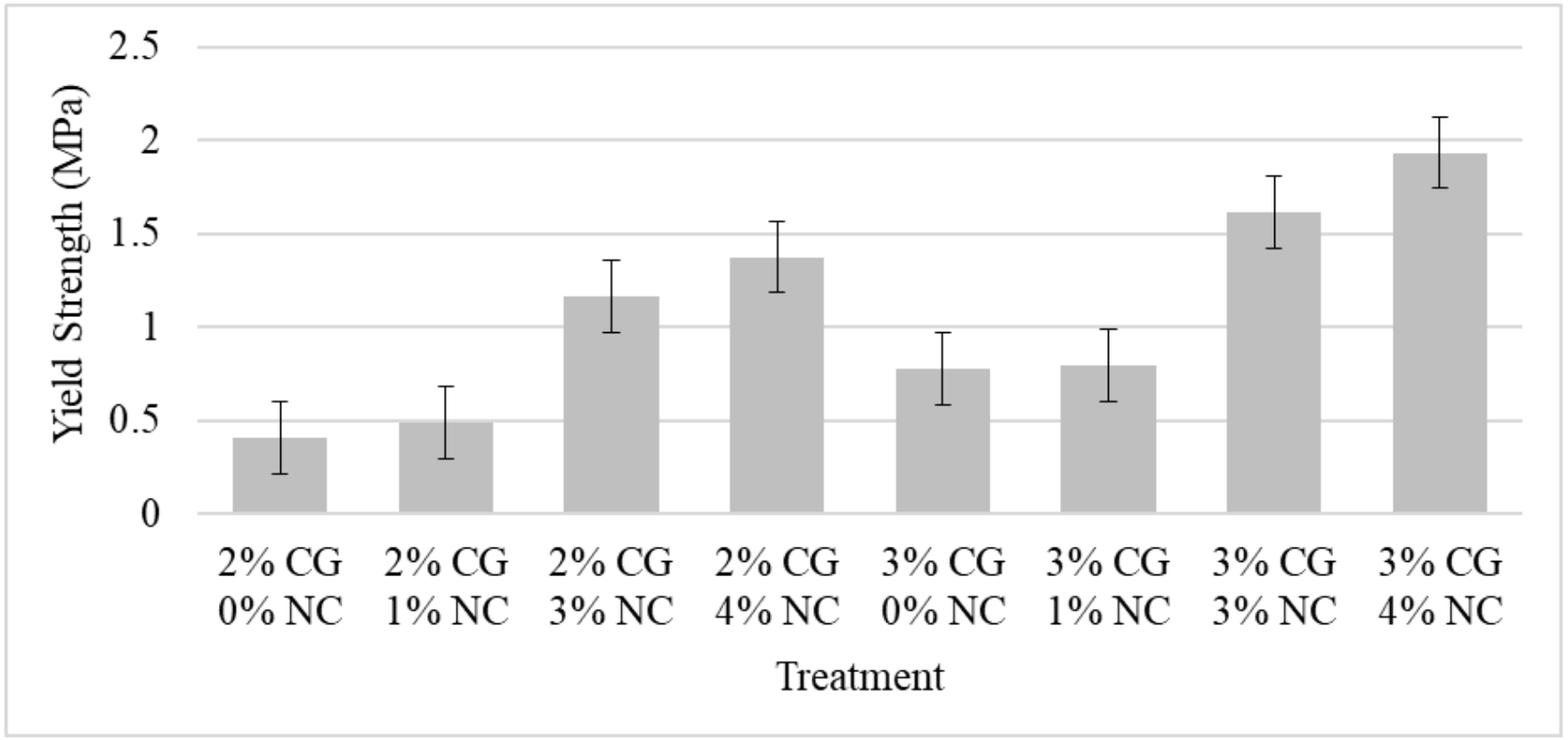

Figure 2. 5 Yield strength of films (Mpa) results among treatments. Different letters denote statistically significant differences at a significance level of $P < 0.05$ between the treatments. The letter "A" indicates the best treatment based on the comparison. CG: Chicken Gelatin; NC: Nanocellulose.

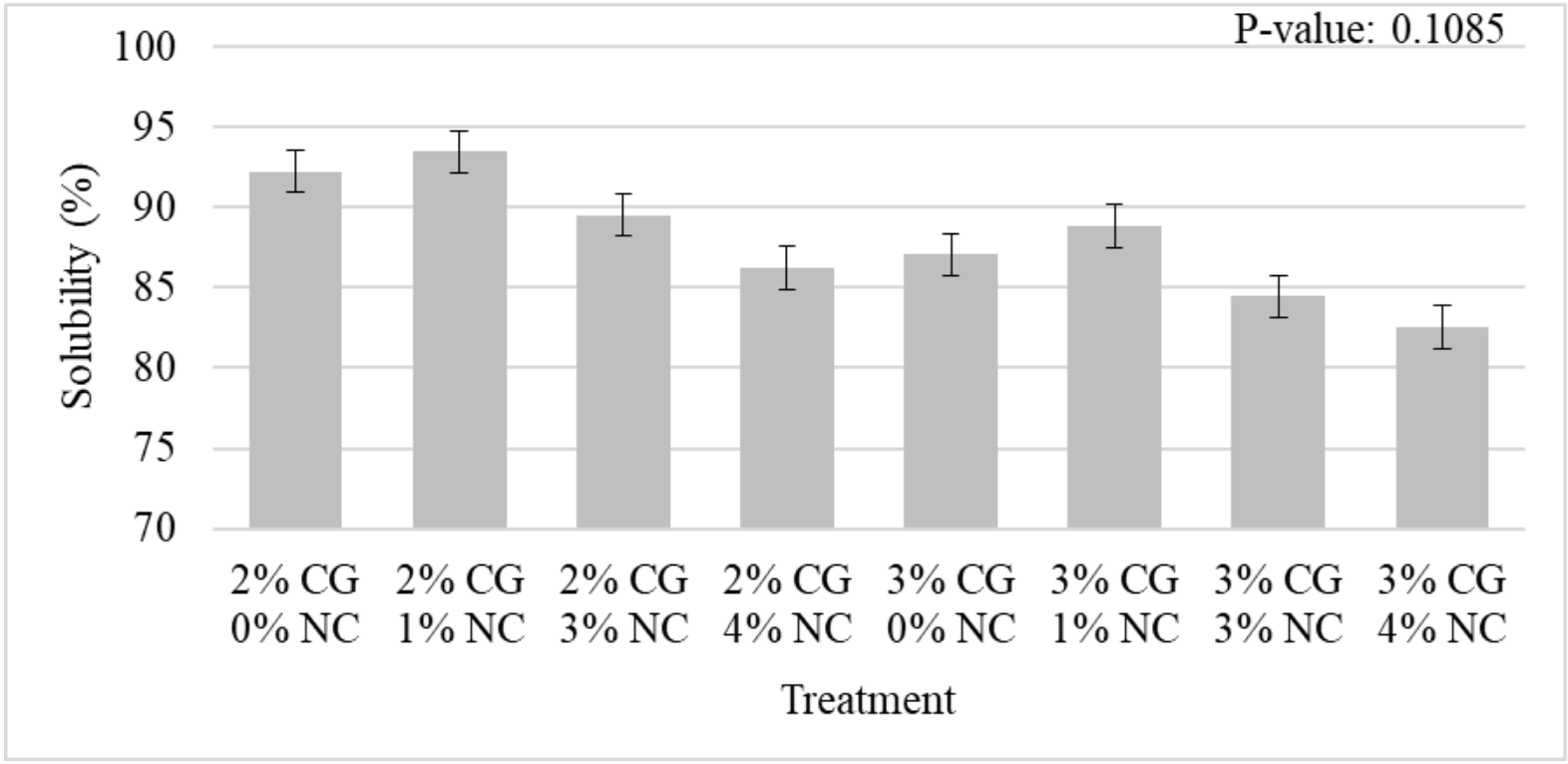

Figure 2. 6 Solubility of films (%) results among treatments. CG: Chicken Gelatin; NC: Nanocellulose.

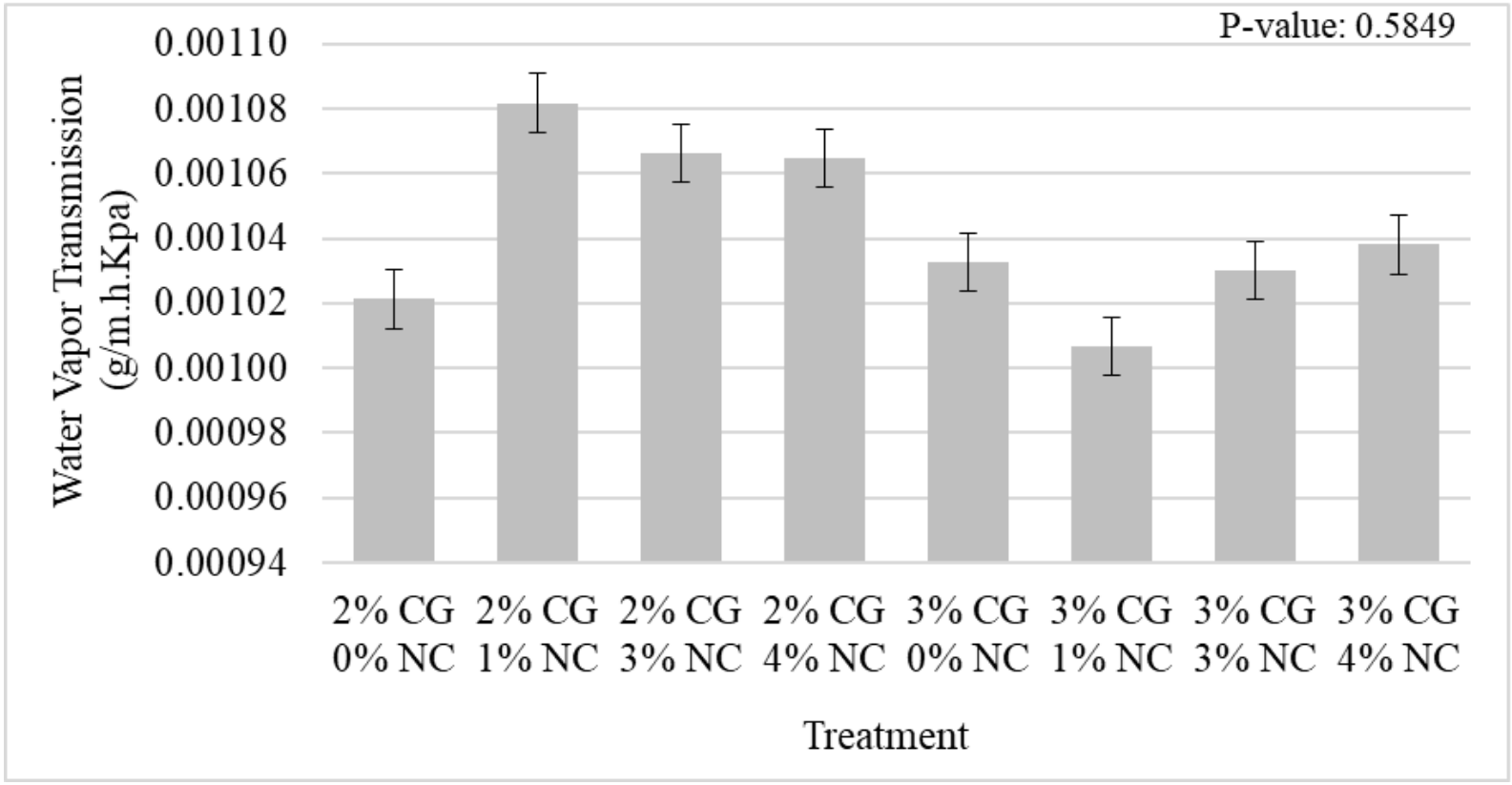

Figure 2. 7 Water Vapor Permeability of films (WVT) (g/m. h. Kpa) results among treatments. CG: Chicken Gelatin; NC: Nanocellulose.

Table 2. 2 Multiple linear regression coefficients to determine prediction equations.

	β_0	β_1 (CG)	β_2 (NC)	R-square
Tensile Strength (MPa)	-0.139630	0.101500	0.041390	0.798473
Elongation (%)	6.797620	5.528800	-0.205620	0.396716
Punching force (N/mm)	-0.336570	1.711900	0.844730	0.777614
Yield Strength (Mpa)	-0.554590	0.419400	0.286860	0.768081
Solubility (%)	102.776480	-4.762050	-1.443990	0.097689
Water Vapor Transmission (g/m.h.Kpa)	0.001110	-0.000034	0.000006	0.047899

Where: β_0 is the y-intercept; β_1, β_2,β_n are the regression coefficients for the independent variables X_1,X_2….X_1,X_2,…,X_n.

CHAPTER III

Development and Validation of a novel biobased packaging film incorporated with antimicrobial compounds against *Listeria monocytogenes* and spoilage microorganism in deli meat over 12-week of refrigerated storage.

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3.1 Abstract

Food packaging is contributing to environmental pollution and is a concern among customers pushing the food industry to innovative, sustainable, and biodegradable plastic-alternative packaging materials. Incorporating biomolecules from agricultural waste to improve mechanical properties and antimicrobials to improve food safety and shelf life in these innovative packaging can increase their market acceptance. Research was conducted on 1. Develop a plastic-alternative packaging film with biopolymer from chicken skin; 2. Incorporate antimicrobials and study their effects on mechanical properties of the film; 3. Evaluate the efficacy of the antimicrobial film against *Listeria monocytogenes* and spoilage of ready-to-eat products. Bioplastic films with a base formulation of 3% chicken gelatin+4% nanocellulose, starch (1%), glycerol (6%), and water (85%) were developed with either 0, 1, or 3% lactate diacetate (LD) as an antimicrobial. The LD was incorporated on the dried cast films. Mechanical properties were analyzed for Tensile strength, yield strength, elongation, and punching force. For antimicrobial analysis, films with three antimicrobial treatments (0, 1, and 3%) and one control treatment (without any packaging) were tested. RTE bologna was inoculated with ~6 logs of *Listeria monocytogenes*. The antimicrobial side of the film was laid on top of the inoculated deli surface, vacuum packaged, stored (4°C), and sampled weekly for 12 weeks (3 samples x 2 trials, n=240). Deli+film was stomached (Buffer Peptone Water 1:1w/w, for 1 min), serially diluted, and spread plated (0.1 mL) on Modified Oxford Agar and incubated at 37ºC for 24 hours. For spoilage analysis, uninoculated beef bologna was packaged with the same treatments and parameters as stated above and conducted at 0 hours and then weekly 12-weeks (3 repetitions x treatment x trial, n=96). The uninoculated samples were spread plated on plate count Agar (PCA) and incubated aerobically (48 h at 37 °C). A separate set of PCA and Mann Rogosa Sharpe (MRS) agar plates incubated anaerobically (72 h at 37 °C) to anaerobic plate and lactic acid bacteria counts, respectively. Colonies were counted and reported as log CFU/g. Data was analyzed (ANOVA) with significant mean differences at $P \leq 0.05$. Addition of LD to edible films with collagen significantly reduced its mechanical properties. The LD bioplastic acted as a bacteriostat, a bactericide (decrease of ~2 logs CFU/g), and bacteriostat for weeks 0-4, 5-7, and 7-12,

respectively. Our research demonstrates that antimicrobial packaging films can be developed using biobased materials and that they can be highly effective in controlling Listeria monocytogenes in RTE meat products during prolonged storage period.

3.2 Keywords: Food packaging, antimicrobial, ready to eat products, lactate diacetate, *Listeria monocytogenes.*

3.3 Introduction

Listeria monocytogenes is a major concern in the food industry, especially in ready to eat products (RTE), as there is no heat treatment or other antimicrobial step between production and consumption (Jordan & McAuliffe, 2018). Listeriosis can cause hospitalizations and death, especially to the people immunocompromised, pregnant women and elderly people (Kendall et al., 2003). Studies estimates that compared to the other foodborne pathogens, listeriosis is the third leading cause of death with a case fatality rate of 20%, about 260 deaths per year (Silk et al., 2013). Approximately one-quarter of pregnancy-associated listeriosis cases result in fetal loss or death of the newborn (Silk et al., 2013). Due to the fatal nature of the pathogen, the USDA-Food Safety Inspection Service (USDA-FSIS) has a zero-tolerance policy towards *Listeria monocytogenes* on RTE foods such as deli meat.

Achieving a zero-tolerance of *Listeria monocytogenes* in RTE foods is highly challenging due to the ubiquitous distribution of the pathogen and its ability to survive and grow in different stress conditions (ethanol, acid, oxidative stress, high temperatures among others) (Chan & Wiedmann, 2008). Once *Listeria monocytogenes* enters the processing plant through worker shoes, clothes, air, raw material among other modes, it establishes in the processing environment, food contact and non-food contact surfaces and spreads through cross contamination on surfaces and foods (Zhang et al., 2022). Some of the RTE foods associated with *Listeria monocytogenes* contamination are deli meats, soft cheeses, and smoked seafood (Todd & Notermans, 2011). Since *Listeria monocytogenes* may not always cause noticeable changes in the taste, smell, or appearance of contaminated food (Henriques & Fraqueza, 2015), consumers cannot detect its

presence, and the pathogen can go unnoticed until someone becomes ill (Donnelly, 2001). These problems not only risk to the public health but can also have significant economic implications for the food industry due to food safety recalls (Ivanek et al., 2005).

The USDA-FSIS has prescribed Alternatives such as post-process lethality steps and/or incorporation of antimicrobials to control *Listeria monocytogenes* in RTE meat products to reduce the public health risk (USDA-FSIS, 2014). Anti-listerial compounds such as sodium lactate and diacetate are generally added as an ingredient in the product to effectively control the pathogen growth during storage of RTE meats (Morey et al., 2014). However, consumers are demanding clean label products without addition of antimicrobials as well as reduction/elimination of synthetic ingredients (Asioli et al., 2017). Packaging films incorporated with antimicrobials can be developed as a post-process alternative to control the surface-contaminated *Listeria monocytogenes* while reducing/eliminating the incorporation of antimicrobials in the product thus satisfying both food safety and consumer demands (Han, 2014).

An antimicrobial packaging system is composed of three principal factors: food, packaging, and the space between them (Jung, 2005). The antimicrobial agent can be put into one of the three components. However, consumer demand for clean labels may lead to companies not adding antimicrobial in the product (Gilchrist & Edgren, 2020). On the other hand, using gases in the space between packaging and food is cost prohibitive (Siracusa, 2012) leaving the option to incorporate antimicrobial in the packaging. Antimicrobial packaging technology presents a unique opportunity for both, the scientists and industry, to reduce the growth of pathogens and spoilage microorganisms and decrease health risk and improve the safety and quality of food products (Mousavi et al., 2018). Antimicrobial packaging can be developed using biobased materials which will not only ensure food safety but also reduce plastic packaging waste (Ma et al., 2020). Several studies utilize components such as chitosan, nanocellulose, and essential oils for their biodegradable antimicrobial properties against pathogens. In our present study, we've developed a bioplastic incorporating polysaccharides (nanocellulose and starch), protein (chicken gelatin), and a novel antimicrobial agent (lactate diacetate) targeting *Listeria monocytogenes*. This innovative formulation offers improved

mechanical and antimicrobial properties suitable for ready-to-eat products, distinguishing it as a unique contribution to existing research in the field.

The current study focuses on engineering packaging film with biobased materials, incorporating antimicrobial, analyzing the mechanical properties as well as validating the antimicrobial effects *on Listeria monocytogenes* survival on a RTE meat.

3.4. Materials and Methods

3.4.1 Engineering packaging films

The packaging films were prepared using the following materials: chicken collagen (Essentia Protein Solution, Ankeny, Iowa 50021 U.S.), nanocellulose at a concentration of 3% (University of Maine, Maine, U.S.), corn starch (Grain Processing Corporation, Oregon, U.S.), glycerol (Fisher bioreagents, Ottawa, Canada), Lactate diacetate [White distilled vinegar and buffering agents (Sodium carbonate, sodium bicarbonate, and/or sodium hydroxide) and silicon dioxide (anti-caking agent)] (KEMIN industries, U.S) and deionized water.

The formulations which demonstrated highest mechanical properties during the development phase were selected as base formulations for the current research. The base formulation (control) contained 1% starch, 6% glycerol, 3% chicken gelatin (CG), 4% Nanocellulose (NC), and 85% deionized water (DW). A pre-mix of sodium lactate + sodium diacetate was incorporated in the base formulation at either 1 or 3% of total weight **(Table 3.1).**

3.4.2 Process of the antimicrobial film preparation

The ingredients were weighed as per the formulations **(Table 3.1).** The starch and chicken collagen to were mixed separately by initially adding half the water volume, and dissolving starch at 85°C for 30 min while stirring at 3,000 rpm and chicken collagen with the remaining half of the water at 45°C for 30 minutes on

a hot-plate stirrer (Thermo Scientific Climatic Stirring Hot Plate, 7x7 inches ceramic) (Figure 1). The starch and collagen solutions were combined and mixed at 45°C for another 30 min while stirring at 3000 rpm. Further, nanocellulose was added to the mixture and stirred for 15 min. As the stirring continued, glycerol was added, and the mixture was held at 45°C for an additional 15 min. The mixture was then allowed to cool at room temperature (20°C) for 15 min from which 20 g was poured into a 15 cm diameter petri dish, and dried 72 h at 35°C in a convection incubator (Thermo Electron Corporation, U.S.) resulting in a biobased packaging film.

Initial experiments were conducted to directly dissolve the antimicrobial in the solution to cast the films. However, the films with the incorporation of antimicrobial agent (Lactate diacetate) had lower mechanical properties than control (without antimicrobial) potentially due to the solubilized sodium lactate and diacetate increasing the ionic concentration and repelling biomolecules such as starch and collagen and preventing them from forming a matrix. Thus, preliminary studies against *Listeria monocytogenes* were tested but there was not reduction due to the lactate diacetate were into the solution, and the complex matrix did not allow the antimicrobial release and kill the bacteria.

A new method of antimicrobial incorporation was tested wherein the film formulation was poured in the petri dish, allowed to dry and at around 72 h, when the film was formed but had a sticky surface, the solution of lactate diacetate was sprinkle homogeneously on the surface and the film dried for another 72 h at 35°C such that the antimicrobial will be embedded in the uppermost layer of the film. Such a mechanism would allow for the antimicrobial to transfer onto the surface of the product and effectively kill the pathogen.

3.4.3 Physical and Mechanical Analysis.

For each analysis, 15 repetitions per treatment (films per formulation) were tested as a rectangle shape film with a size of 6 cm (L) x 2.5 cm (W). The following tests were performed on all 60 (4 formulations x 15 repetitions) film samples: thickness, Tensile strength, punching force, yield strength, solubility, elongation, and water vapor permeability.

3.4.3.1 Thickness

Thickness analysis was conducted with an electronic micrometer (Chicago Brand, 0.000050 reading) with 0.001 mm of accuracy. Five points were randomly selected and measured on each film sample (Jain, 2022) (6 cm x 2.5 cm) and the average per film was calculated. The thickness results were reported in millimeters (mm).

3.4.3.2 Punching force

This analysis was made using the TA.XTPlus Texture Analyzer (Texture Technologies Corp., Hamilton, MA/Stable Micro Systems, Godalming, Surrey, UK) equipped with a 5 kg load cell, a ½ in stainless steel ball probe (TA-18), and the indexable film extensibility rig (TA- 108s5i). Data was acquired using the Exponent Stable Micro System Software Package (Visual Components, Inc.), version 6, 1, 11, 10. The pre-test speed was 1 mm/sec, test speed was 0.5 mm/sec, and post-test speed was 10 mm/sec and force parameters of 0.981 N (force) and 0.049 N (trigger force) were used. The distance between film and attachment was 40 mm and the punching force formula is described below (Cesar, 2018):

$$(h)=\frac{Fp}{L}=N/mm$$

Where h is the value of punching force (N/mm), Fp is the force peak before break (N), and L is the average thickness of the film (mm).

3.4.3.3 Tensile Strength

Tensile strength was completed using the ASTM D882 method (American Society for Testing and Materials, 2023) on the TA.XTPlus. Using the same settings as Punching Force (above). Tensile strength was calculated by the following formula:

$$Tensile\ force = \frac{Tensile\ force\ (Texturometer)}{Area\ (m2)} = MPa$$

3.4.3.4 Deformation percentage

This analysis is described by the ASTM D882 method (American Society for Testing and Materials, 2023). Using a texture analyzer (Texture Technologies Corp, TA. XTPlus, New York, U.S), using the settings for Punching Force, a peak distance of the film was determined by applying the force of the equipment.

$$\%\ Deformation = \frac{Final\ length - Initial\ length}{Initial\ length} * 100$$

3.4.3.5 Yield Strength

Yield strength (stress/strain) is described by the ASTM E111-17 method (American Society for Testing and Materials, 2017a). Using the data from previous Tensile strength and deformation calculations, yield strength was calculated with the following formula:

$$yield\ strength = \frac{Tensile\ force\ (Mpa)}{Deformation\ (mm)} = Mpa$$

3.4.3.6 Solubility Index

Solubility index was calculated using a previously published method (Cesar, 2018), where 45 circular samples (15 mm diameter; 1/treatment x 8 treatments x 15 replications) were cut and weighed. The samples were submerged into a conical tube with 50 ml of water for 24 h at room temperature (25°C) After 24 h, the samples were reweighed. The solubility percentage was measured with the following formula:

$$\%\ Solubility = \left(\frac{wi - wf}{wi}\right) \times 100$$

Where wi is the initial weight (g) and the wf is the final weight (g) of the film packaging.

3.4.3.7 Water Vapor Transmission

This analysis is described by ASTM E96-80 method, (American Society for Testing and Materials, 2017b) Conical tubes (50 mL) with 25 ml of water at room temperature (25°C) were used. Films were cut in circles shaped 20 mm in diameter and placed on the top of the conical tubes. The tubes were stored at room temperature (25°C) inside of desiccators for seven days. The weight of each tube was recorded every day to determine the mass of water going through the film. The following formula was used to calculate water vapor permeability (WVT):

$$WVT = C\left(\frac{x}{A\,\Delta P}\right) = (g/m.h.kpa)$$

Where the C represent the slope (g/h); A is the permeability area (m2); X is film´s thickness (m); ΔP is the pressure used, which was the difference between the silicate gel (0 kPa at 25 °C) and pure water vapor (3.167 kPa at 25 °C inside of tubes).

3.4.4 Preparation of *Listeria monocytogenes* culture

Listeria monocytogenes was resuscitated from frozen stock through sequential culturing in brain heart infusion (BHI) broth (10 mL) and streaking on modified Oxford agar (MOX). The final inoculum was prepared by diluting an 18 h (35 °C) *Listeria monocytogenes* culture in BHI, to obtain a final inoculum concentration of 10^6 CFU/mL.

3.4.5 Antimicrobial packaging film validation against *Listeria monocytogenes* and spoilage microorganisms:

The deli meat (Beef bologna commercially manufactured, contained sodium diacetate as anti-listerial compound) was inoculated *Listeria monocytogenes* inoculum (0.1 mL) by distributing it over the bologna surface and spreading the culture using a sterile spreader. The inoculated bologna was placed in a refrigerator (4ºC) for 30 min to allow bacterial attachment. Further, the packaging film was placed on the bologna such that the antimicrobial side was in contact with the *Listeria monocytogenes* inoculated bologna

surface. There treatments in the study were as follows: 1. Inoculated control without any biobased packaging film (Control or C); 2. Inoculated control with the biobased film made without antimicrobials (Control film or CF); 3. Biobased film with 1% lactate diacetate (1% LD); and 4. Biobased film with 3% lactate diacetate (3% LD).

The bologna with or without the packaging film was introduced to a vacuum bag (5" x 10") and vacuum packaged in a vacuum machine (KOCH equipment, Kansas City, U.S.), using a pressure of 7 Kpa with a 4% vacuum. All the samples were stored in the fridge at 4°C for twelve weeks.

For spoilage analysis, uninoculated beef bologna was packaged with the same treatments and parameters as stated above.

Sampling was conducted at 0 hours and then weekly 12-weeks. On each sampling day, random packages (n=96, three repetitions per treatment per trial per week) were procured from the refrigerated storage and analyzed for *Listeria monocytogenes* and spoilage microorganisms. The bologna with the biobased packaging film was aseptically placed in a sterile whirl pack with a filter and then Buffer Peptone Water (BPW) was added (1:1 w/w) to it and stomached for 1 min at 300 rpm. Serial dilutions (1:10) were prepared in BPW and spread plated on MOX which were incubated for 24 h at 37 °C. Colonies exhibiting typical *Listeria monocytogenes* morphology were counted and reported as log CFU/g. The uninoculated samples were spread plated on plate count Agar (PCA) and incubated aerobically (48 h at 37 °C) to analyze aerobic plate counts. A separate set of PCA plates incubated anaerobically (72 h at 37 °C) to analyze aerobic and anaerobic plate counts. Samples were also spread plated on Mann Rogosa Sharpe (MRS) agar and incubated under anaerobic conditions (72 h at 37 °C) to analyze lactic acid bacteria counts.

3.4.6 Scanning Electron Microscopy

Packaging film samples (1 x 1 sq. cm) with and without lactate diacetate analyzed under a scanning electron microscope (SEM).The samples were placed in stubs and covered with gold using the EMS Q150 sputter coating device (Electron Microscopy Sciences, Hatfield, PA 19440, USA). The coated samples were placed

in the Zeiss EVO 50 Scanning Electron Microscope (SEM) (Carl Zeiss Microscopy, LLC, White Plains, NY 10601 USA). Various pictures were taken using 73x and 778x magnification, 20 kV voltage, 50% brightness, and 30% contrast.

3.4.7 Statistical Analysis

For the *Listeria monocytogenes* study, there were four packaging treatments with three samples analyzed per sampling time during the 12-week storage (day 0, 24 h, 48 h, and then weekly for 12 weeks). The entire study was repeated as two independent trials (n=336). The spoilage study, the parameters were similar except that the sampling was conducted on day 0, weeks, 4, 8 and 12 of storage (n=96).

Data was analyzed using analysis of variance (ANOVA) with Tukey HSD ($P \leq 0.05$) to determine statistical differences between the treatments each week.

3.5. Results and discussion

3.5.1 Mechanical and physical analyses: Mechanical analysis was performed on all the biobased films (control film, 1, 2 and 3% LD).

Tensile strength can be defined as the maximum stress that a material can bear before breaking when it is allowed to be stretched or pulled (Pal et al., 2022). Tensile strength analysis showed that the control films have the higher values (0.47 Mpa) ($P \leq 0.05$) compared to the 1, 2, and 3% Lactate diacetate (LD) films (0.19, 0.16, 0.09 Mpa, respectively) **(Figure 3.1)**. Similarly, punching force analysis, testing the containment property of the packaging (Aguirre et al., 2021), demonstrated that the control films had the highest value 13.23 N/mm ($P \leq 0.05$) compared to the other films **(Figure 3.2)**. However, the lactate diacetate treatments containing 1 and 2%, have similar values (4.55 and 4.65 N/mm, respectively). The treatment containing the highest amount of lactate diacetate (3%) has the lowest value ($P \leq 0.05$) (2.63 N/mm). The same pattern was repeated for the Yield Strength, as the latter is related to tensile and punching force **(Figure 3.3).**

However, the values for the elongation percentage ($P > 0.05$) and solubility ($P > 0.05$) were not affected by the addition of lactate diacetate **(Figure 3.4)**. Referring to the water vapor permeability (WVT) the control contained the lowest values compared to the treatments with lactate diacetate. However, there was no difference between the different percentage of lactate diacetate.

Adding antimicrobial agents to packaging materials has a notable positive impact on microbial safety, shelf-life, and quality, especially for perishable foods (Bastarrachea et al., 2011). However, these agents often introduce complications to the packaging material. Depending on the type of antimicrobial agent used, they can bring about significant alterations in crucial properties such as mechanical strength, permeability, volatility, and optical and thermal characteristics, and even affect the physical appearance of the packaging material (Bastarrachea et al., 2011; Kuorwel et al., 2014).

The increasing amount of lactate diacetate decreases mechanical values because the mode of action of sodium lactate diacetate is primarily related to its ability to lower the pH of the environment (Abou-Zeid et al., 2007). Lactate diacetate is a salt derived from lactic acid and acetic acid (Mohammed Shafit & Williams, 2010). When it is added to a solution or a product, it undergoes hydrolysis, releasing lactic acid and acetic acid (Jensen et al., 2003). In an acidic environment, gelatin gets denaturalized because the protein starts unfolding from complex to simple structure (Fink et al., 1994). When this occurs the matrix of the film is being affected and reduces the mechanical strength.

After the mechanical and physical analysis, the antimicrobial and shelf analysis were performed taking the lowest and the highest concentrations of the antimicrobials (1 and 3 % of lactate diacetate).

3.5.2 Antimicrobial packaging film validation against Listeria monocytogenes: *Listeria monocytogenes* levels remained similar for four weeks, irrespective of the treatment indicating the bacteria was in a lag phase and was dormant. The bacteriostatic effect observed in the control samples can be due to the presence of anti-listerial compounds (Sodium Diacetate) in the commercially bought bologna. We had hypothesized that 1 and 3% films will release the antimicrobials within the first 48 h of storage

exhibiting some level of anti-listeria activity. Contrary to our hypothesis, the films did not release the antimicrobial as is evidenced from the *Listeria monocytogenes* population compared to the controls.

Although the controls showed no growth or death of *Listeria monocytogenes* after week 4, the 1 and 3% LD films demonstrated significantly lower ($P \leq 0.05$) *Listeria monocytogenes* counts from week 5 compared to the controls indicating bactericidal activity **(Figure 3.8)**. At the end of week 7, the *Listeria monocytogenes* counts in the 1 and 3% LD films were 2 logs lower compared to the controls ($P \leq 0.05$). The delayed release of LD from the films could be due to time taken for the diffusion of water from the product into the film surface. The movement of water dissolved the LD embedded and dried on the film's surface and once in a solution form, LD exhibited its anti-listeria properties. Lactate diacetate, like many antimicrobial agents, often requires water to be effective (Yilmaz Atay, 2019). The mechanism of action of lactate diacetate involves the release of lactic acid and acetic acid when it comes into contact with water (Bangar et al., 2022).

Two key factors affecting how much and how quickly moisture moves are the water activity equilibrium (related to thermodynamics) and the factors influencing the rate of diffusion (associated with the dynamics of mass transfer) (Labuza & Hyman, 1998). Managing the starting moisture level and how moisture moves within food is crucial for ensuring the quality and safety of food products (Slade et al., 1991). It's ideal for food manufacturers to create products with specific moisture levels to ensure the production of a safe product with the best possible shelf life (Wason et al., 2021). It should be noted that the water holding capacity of the RTE both naturally due to proteins and enhanced by addition of ingredients such as salt, phosphates, starches and hydrocolloids can influence the movement of water into the film. Our observations can guide researchers and the packaging industry to develop better methodologies to incorporate antimicrobials in the film to observe *Listeria monocytogenes* kill in the early stages of storage.

From week 7 to 12, the lactate diacetate continued to be acted as a bacteriostatic preventing the growth of the *Listeria monocytogenes*, entering in the stationary phase **(Figure 3.8)**. Lactate diacetate, through its

ability to lower the pH of the environment by releasing lactic acid and acetic acid, creates an acidic condition that inhibits the growth of bacteria.

It is interesting to note that at week 9, *Listeria monocytogenes* in the control samples started to grow indicating a reduction in the listerostatic ability of the anti-listeria compounds in the bologna. Moreover, from week 7 onwards, the 1% LD samples exhibited approximately 1-log growth, yet lower than the controls, indicating some alteration in its anti-listeria efficacy. However, the 3% LD samples did not exhibit any growth after week 7 indicating that the film released the antimicrobial and then probably had a sustained release of LD to continue the suppression of the pathogen throughout the storage study.

5.3 Antimicrobial packaging film validation on spoilage microorganisms: There was low to no growth of spoilage microorganisms on the bologna irrespective of the treatments until week 8. After week 8, there was a significant increase in the spoilage microorganisms as exhibited in **Figures 3.9, 3.10, and 3.11.** It is interesting to note that the 3% LD film samples had approximately 0.6 log CFU/g lower bacterial counts in APC, AnPC and LAB indicating potential antimicrobial effect against spoilage microorganisms.

3.6. Conclusion

Our research demonstrates that antimicrobial packaging films can be developed using biobased materials and that they can be highly effective in controlling *Listeria monocytogenes* in RTE meat products during prolonged storage period. Researchers must carefully select the antimicrobials considering their contribution to the ionic strength of the film formulation as it can affect the film formation. As evidenced from our research, the process of incorporating the antimicrobial and its release should be thoroughly assessed to obtain optimum and sustained elimination of *Listeria monocytogenes.*

3.7. Future Research

Potential future research in the realm of biodegradable packaging could involve conducting biodegradability analyses alongside soil analyses aimed at evaluating the impact of these materials on soil microorganisms. This approach would be understanding how biodegradable packaging interacts with soil ecosystems, particularly focusing on the microbial communities involved in the degradation process. By assessing the decomposition of biodegradable materials in soil environments and the role of microorganisms in this process.

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Table 3. 1 Packaging film formulations.

Formulation	Chicken Gelatin (%)	Nanocellulose (%)	Lactate diacetate (%)	Starch (%)	Glycerol (%)	Water (%)
1	3	4	0	1	6	85
2	3	4	1	1	6	85
3	3	4	2	1	6	85
4	3	4	3	1	6	85

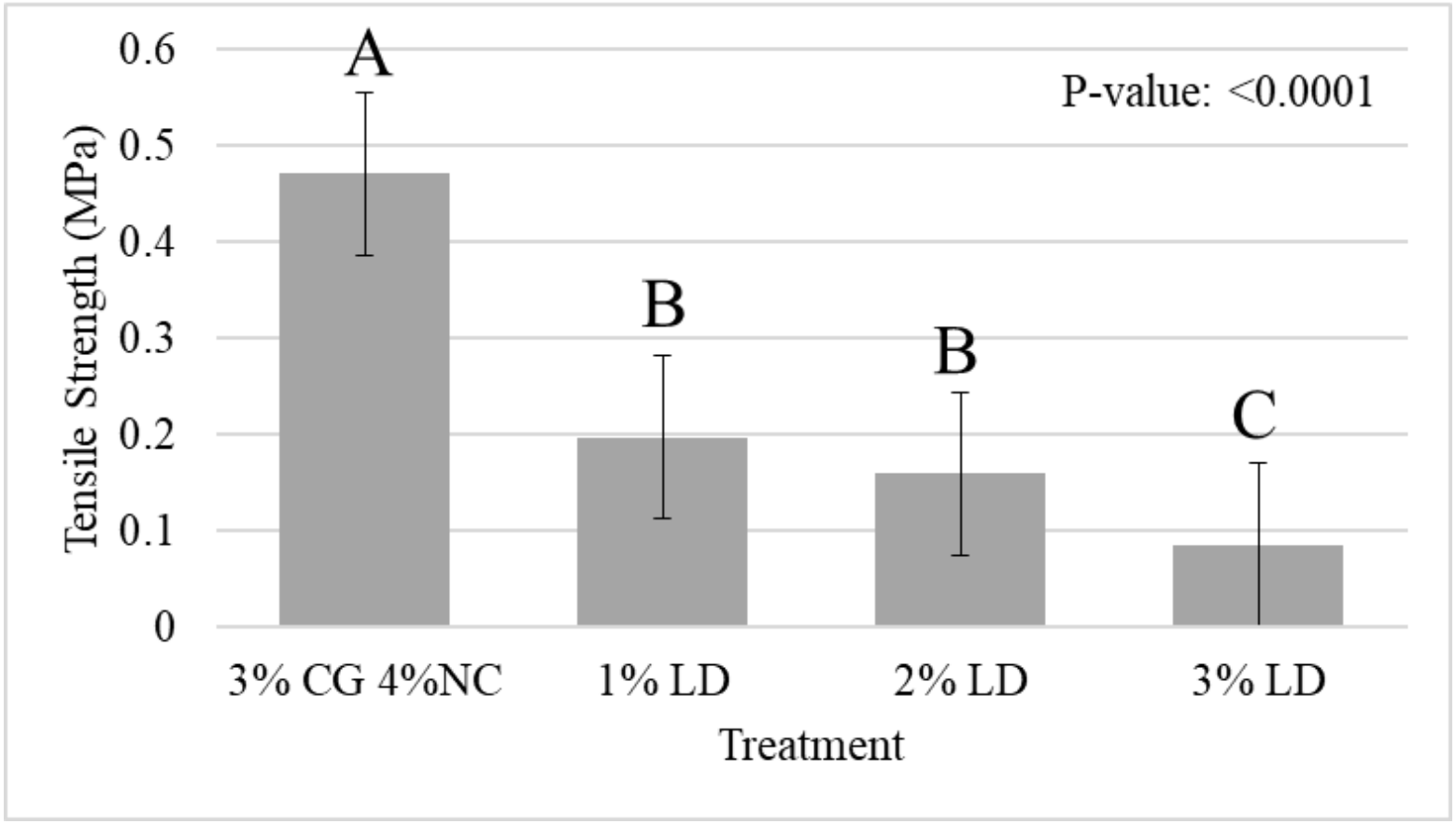

Figure 3. 1 Tensile strength of films (MPa) results among treatments. Different letters denote statistically significant differences at a significance level of $P < 0.05$ between the treatments. The letter "A" indicates the best treatment based on the comparison. CG: Chicken Gelatin; NC: Nanocellulose; LD: Lactate Diacetate.

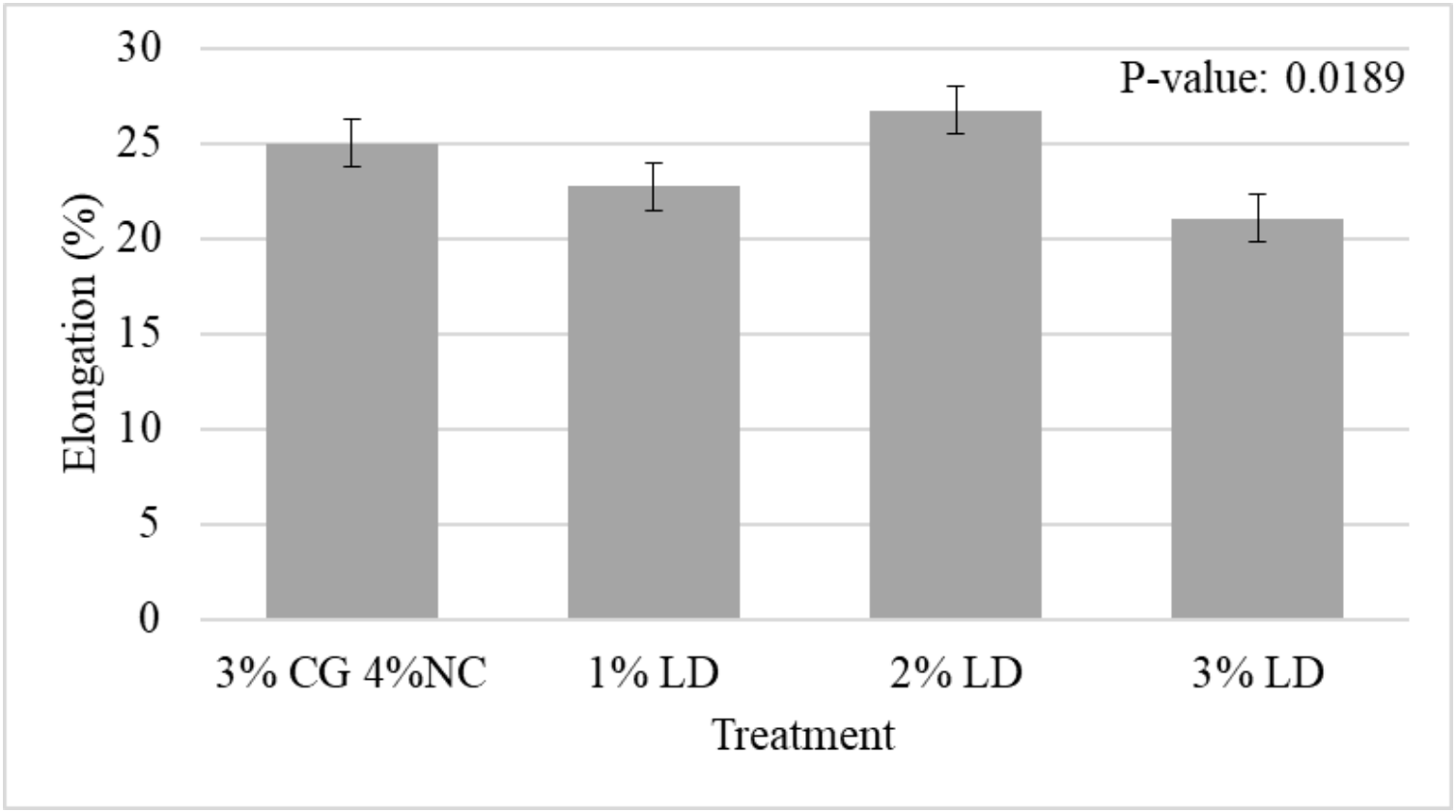

Figure 3. 2 Elongation of films (%) results among treatments. CG: Chicken Gelatin; NC: Nanocellulose; LD: Lactate Diacetate.

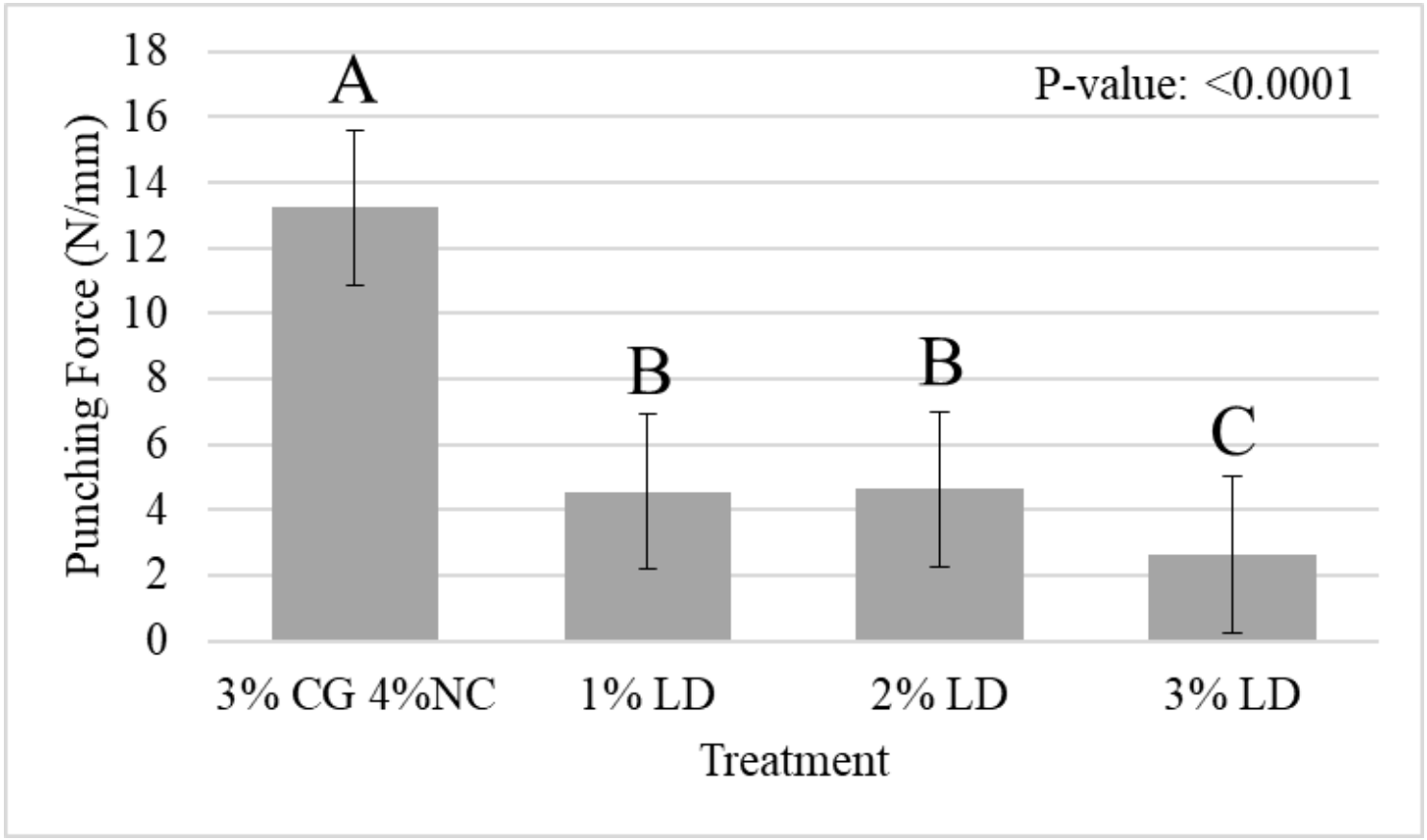

Figure 3. 3 Punching force of films (N/mm) results among treatments. Different letters denote statistically significant differences at a significance level of $P < 0.05$ between the treatments. The letter "A" indicates the best treatment based on the comparison. CG: Chicken Gelatin; NC: Nanocellulose; LD: Lactate Diacetate.

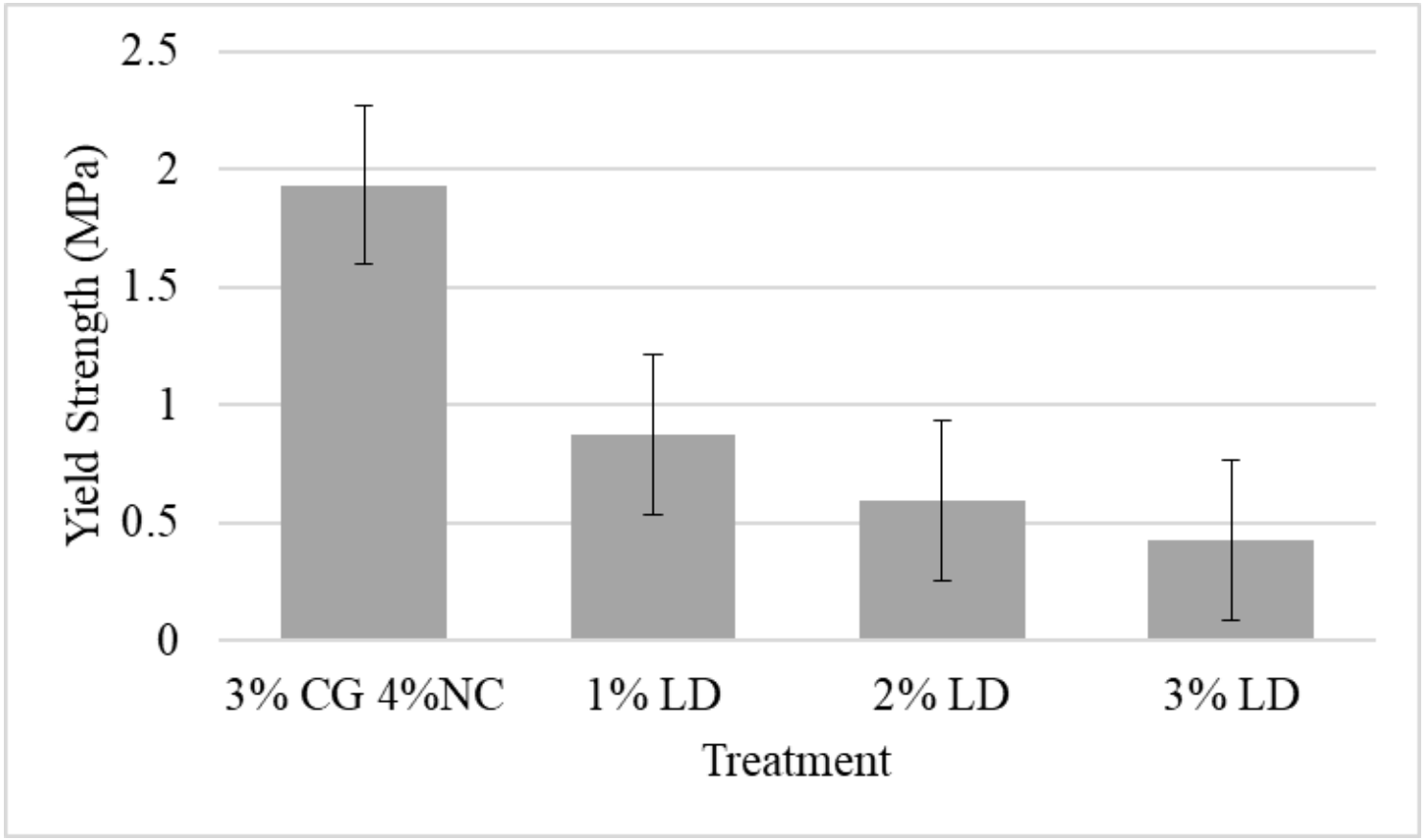

Figure 3. 4 Yield strength of films (MPa) results among treatments. Different letters denote statistically significant differences at a significance level of $P < 0.05$ between the treatments. The letter "A" indicates the best treatment based on the comparison. CG: Chicken Gelatin; NC: Nanocellulose; LD: Lactate Diacetate.

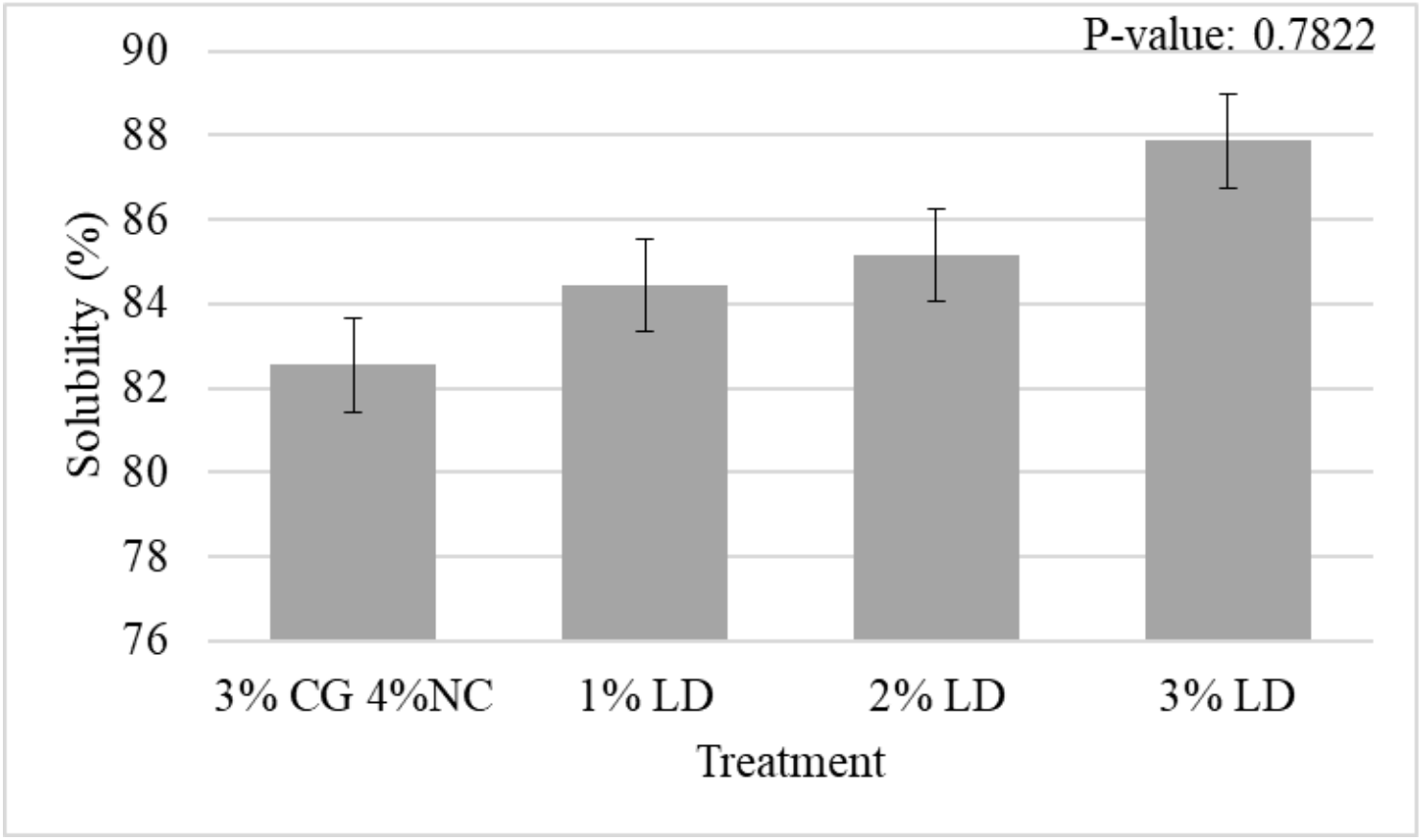

Figure 3. 5 Solubility of films (%) results among treatments. CG: Chicken Gelatin; NC: Nanocellulose; LD: Lactate Diacetate.

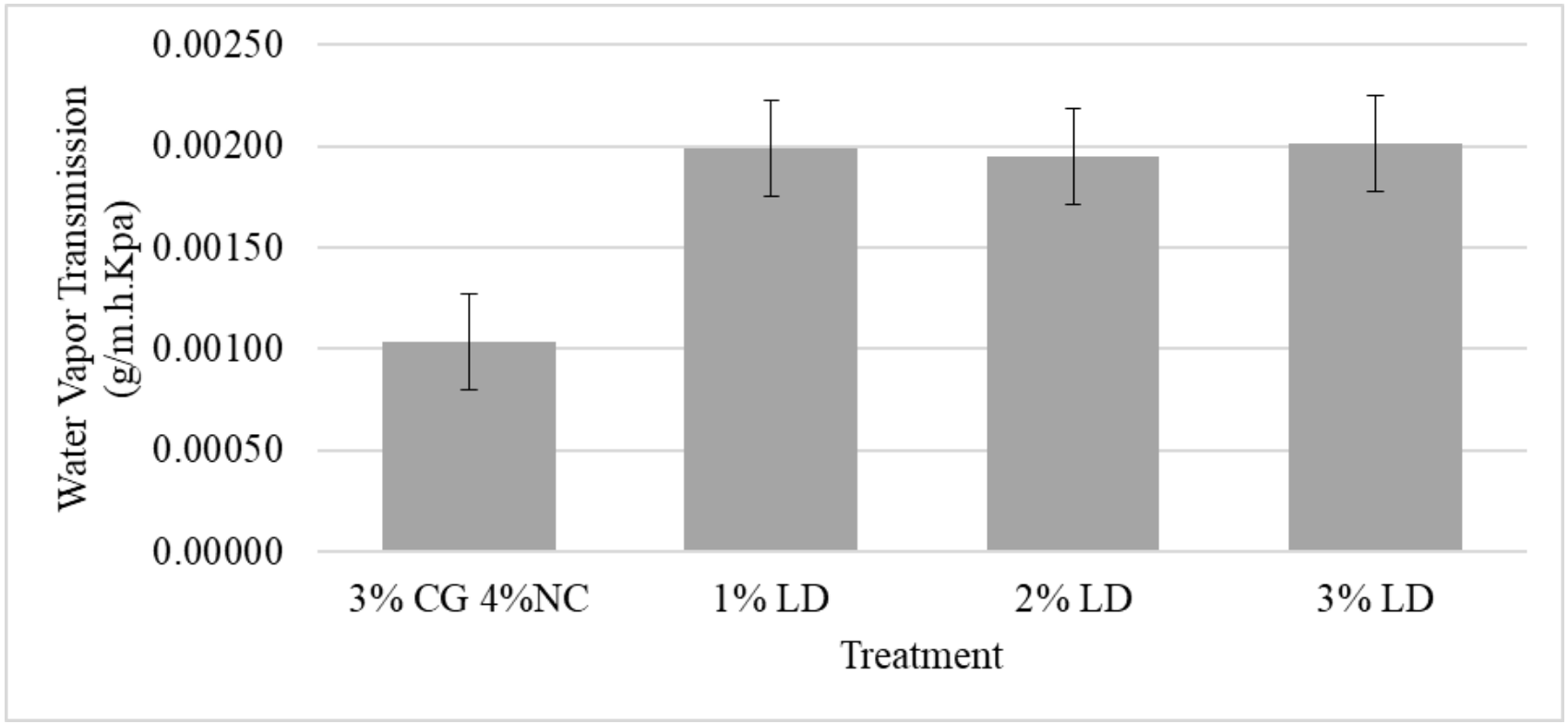

Figure 3. 6 Water Vapor Permeability of films (WVT) (g/m. h. Kpa) results among treatments. Different letters denote statistically significant differences at a significance level of $P < 0.05$ between the treatments. The letter "A" indicates the best treatment based on the comparison. CG: Chicken Gelatin; NC: Nanocellulose; LD: Lactate Diacetate.

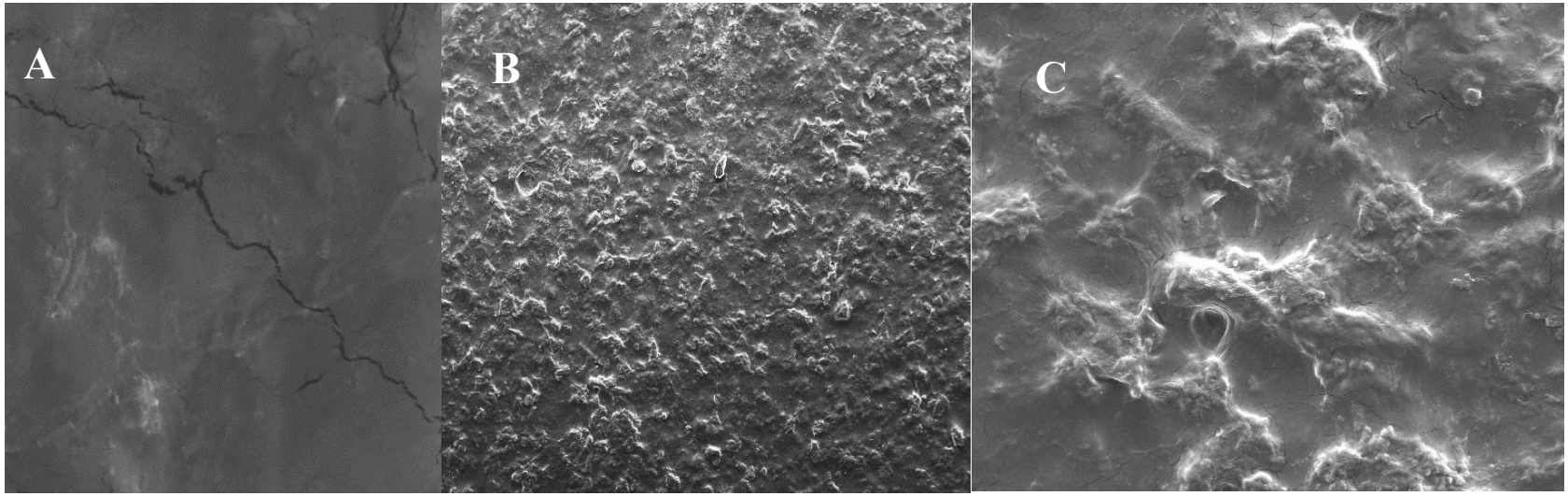

Figure 3. 7 Control film without antimicrobial (73x magnification); B: LD packaging film (73x magnification); C: LD packaging film (778x magnification).

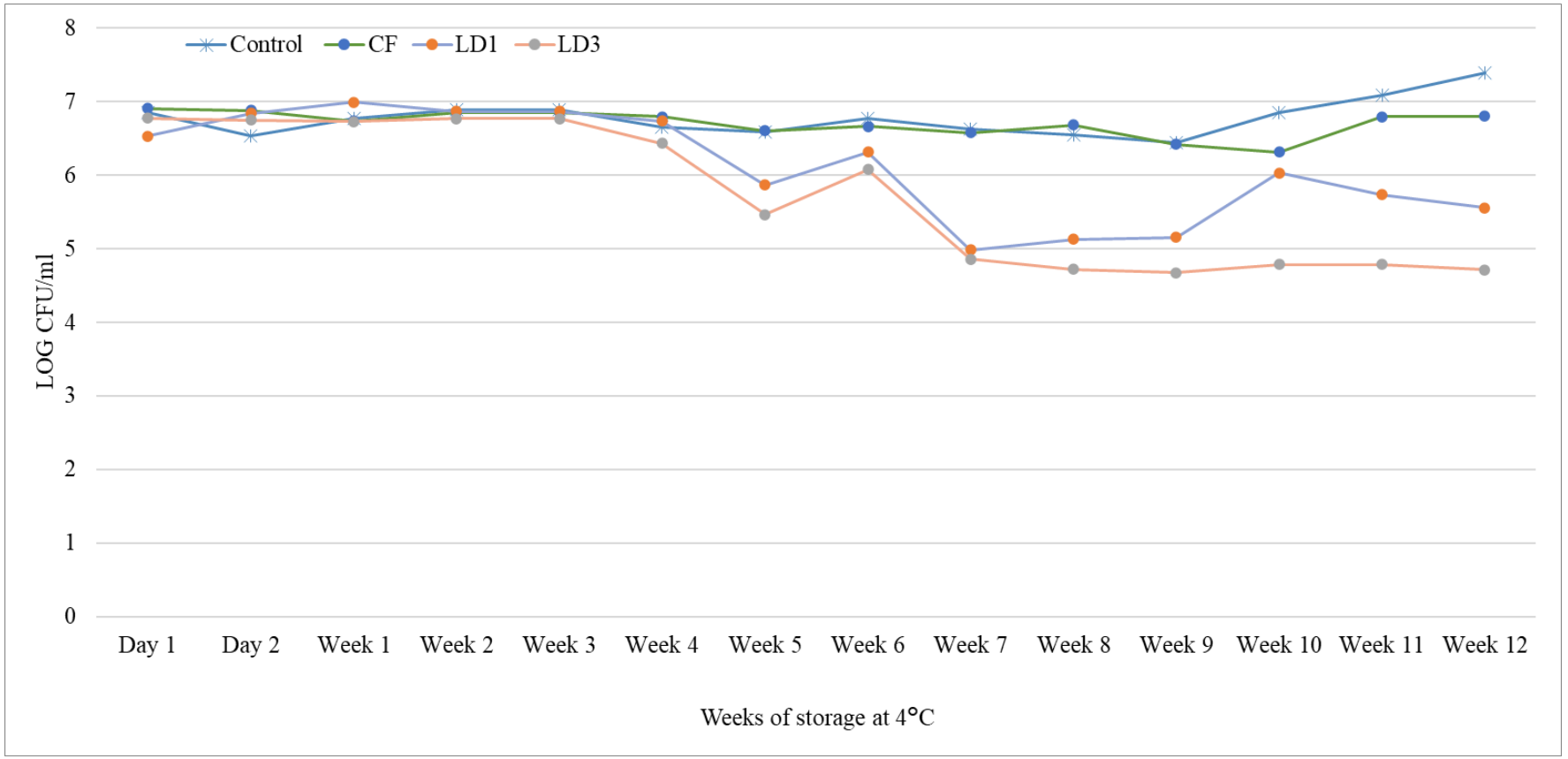

Figure 3. 8 Antimicrobial analysis against Listeria monocytogenes of different treatments. CF: Control with packaging film and without antimicrobial; LD1: Lactate diacetate 1%; LD3: Lactate Diacetate 3% of concentration. With beef bologna at 4°C for 12 weeks of storage.

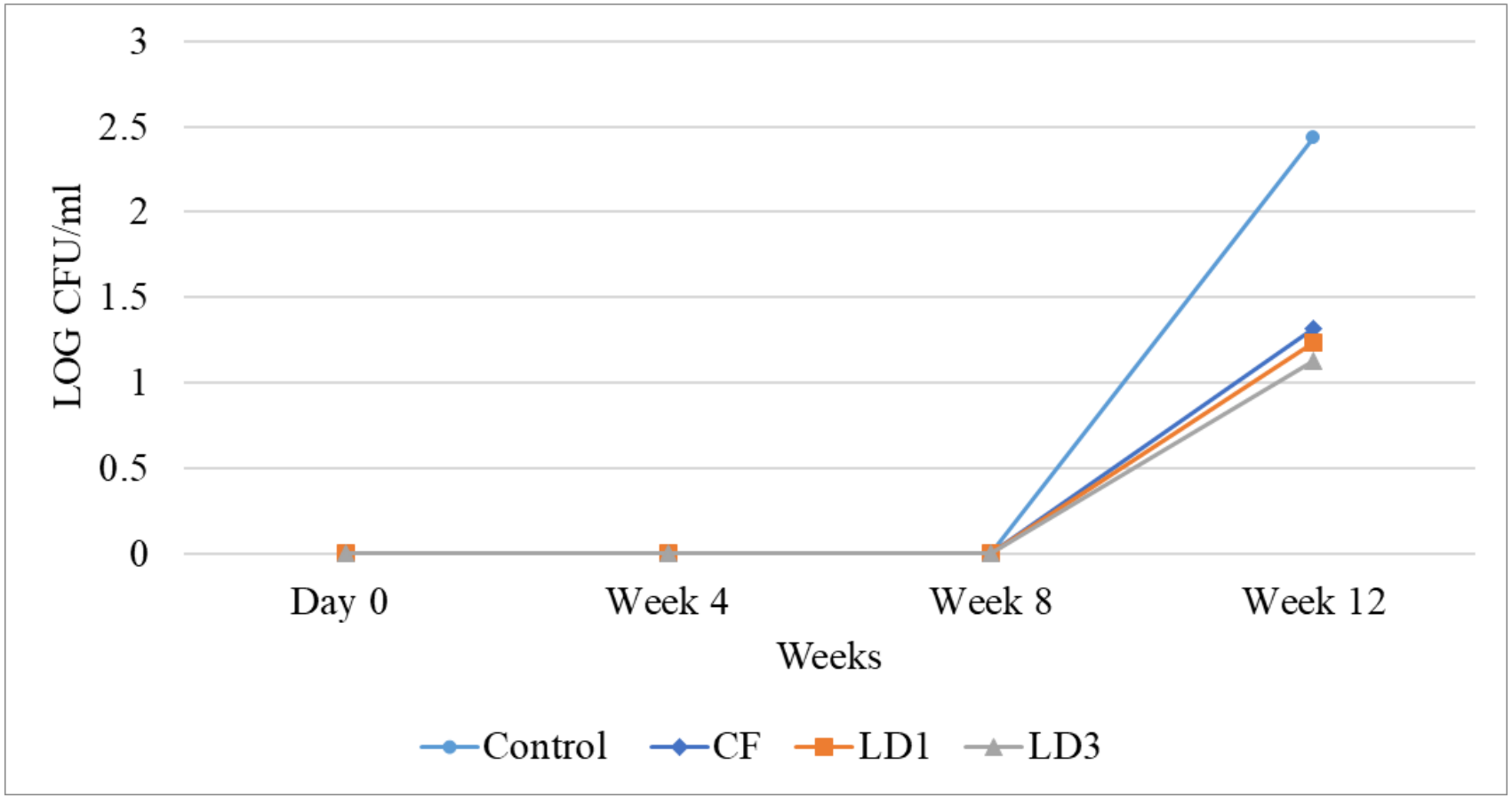

Figure 3.9 Shelf analysis against aerobics spoilage bacteria of different treatments. CF: Control with packaging film and without antimicrobial; LD1: Lactate diacetate 1%; LD3: Lactate Diacetate 3% of concentration. With beef bologna at 4°C for 12 weeks of storage.

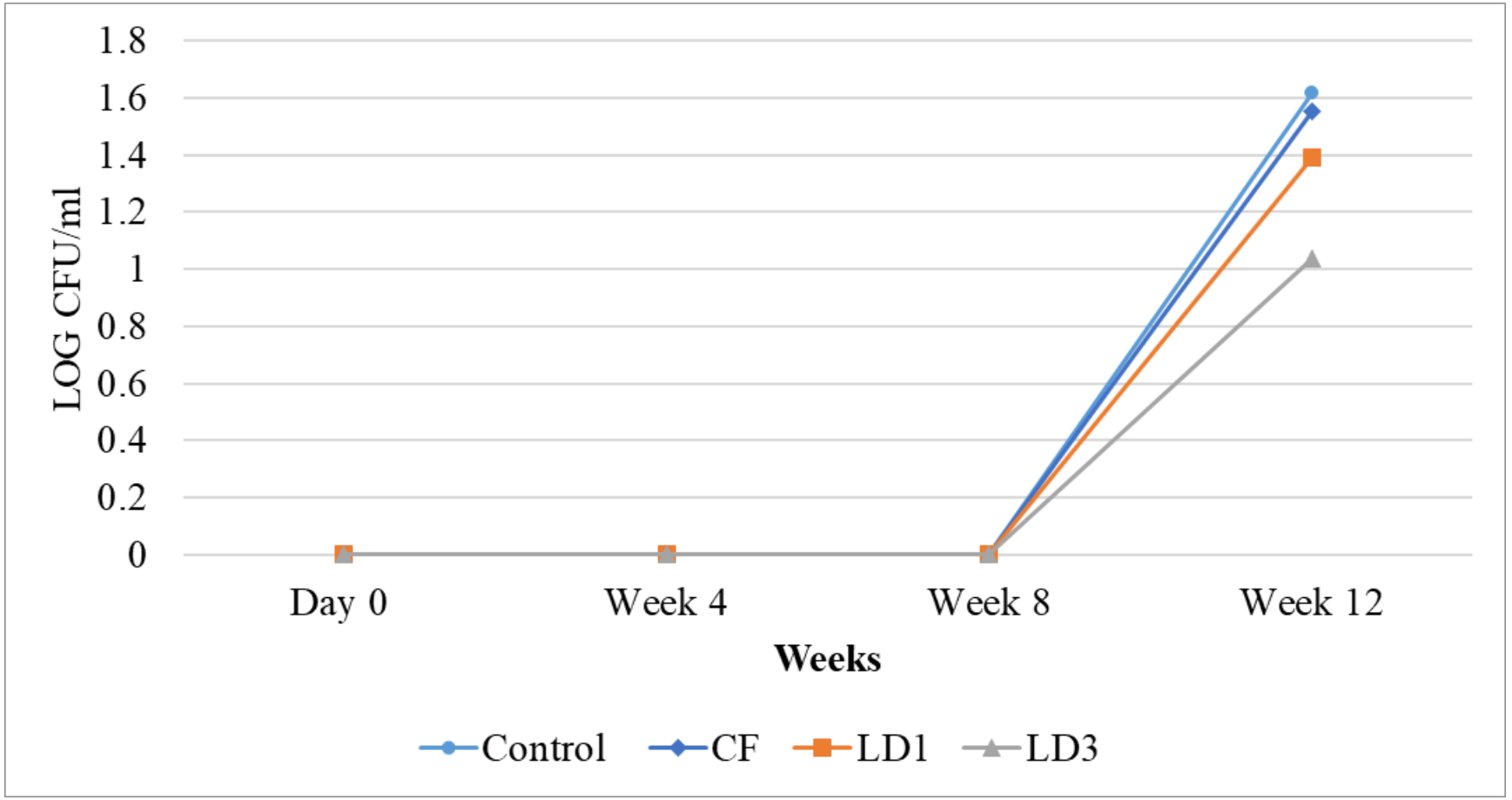

Figure 3.10 Shelf analysis against anaerobics spoilage bacteria of different treatments. CF: Control with packaging film and without antimicrobial; LD1: Lactate diacetate 1%; LD3: Lactate Diacetate 3% of concentration. With beef bologna at 4°C for 12 weeks of storage.

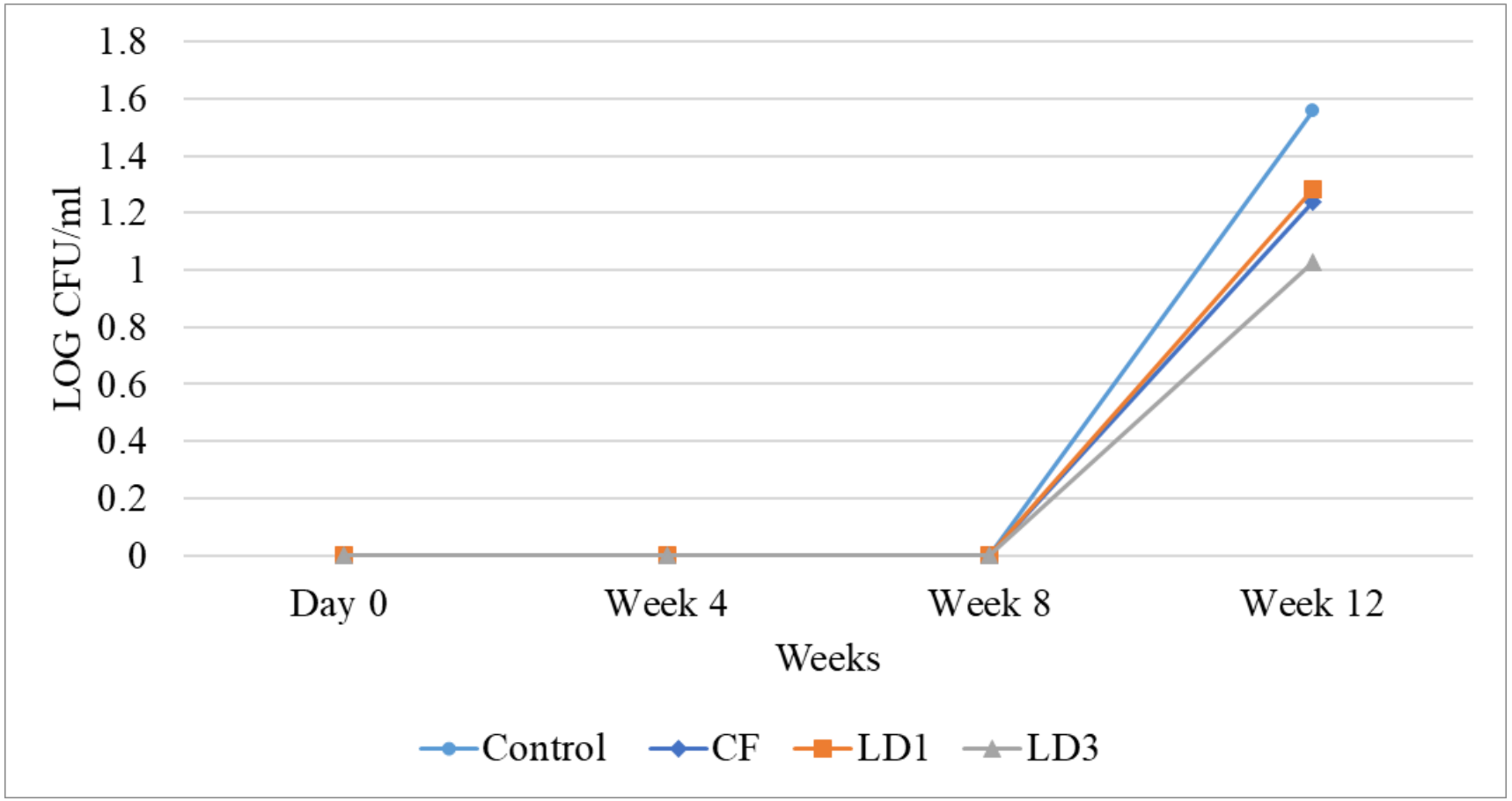

Figure 3.11 Shelf analysis against lactic acid bacteria of different treatments. CF: Control with packaging film and without antimicrobial; LD1: Lactate diacetate 1%; LD3: Lactate Diacetate 3% of concentration. With beef bologna at 4°C for 12 weeks of storage.