**Graduation Project**

**Project Title:**

**Development and Application of Biopolymer-Based Biofilms for Sustainable Food Packaging- Literature Review**

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**Abstract**

This study explores the development and application of biopolymer-based biofilms, with a focus on their potential as sustainable alternatives to conventional plastics, particularly in food packaging. Biopolymers such as polylactic acid (PLA), starch, inulin, xanthan gum, and essential oils have demonstrated significant promise due to their biodegradability, compostability, and functional properties like antimicrobial and antioxidant activities. This literature review evaluates the synthesis, properties, and biodegradability of these biopolymer-based biofilms, highlighting both their advantages and the challenges that hinder their large-scale adoption. The study identifies key issues such as moisture resistance, mechanical strength, and cost-effectiveness, while proposing solutions like the incorporation of additives and the optimization of production methods. Additionally, the review discusses the potential for utilizing waste materials in biofilm production and the need for further research to improve biodegradation rates and expand applications beyond food packaging. The findings suggest that with continued innovation and collaboration, biopolymer-based biofilms can play a pivotal role in advancing sustainable packaging solutions, reducing plastic waste, and fostering a circular economy.

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1. Introduction

1.1. Background

- Food Packaging:

Perishable agricultural products deteriorate quickly due to physical damage, moisture loss, and microbial contamination. Food packaging helps prevent these issues by shielding products from UV light, oxygen, moisture, and microbes while also extending shelf life by blocking liquids, odors, and contaminants during storage and transport. As a crucial element in the food industry, packaging acts as a protective barrier against external biological, chemical, and physical factors (Girdhar et al. 2023) (Tian et al. 2022), (Sharma et al., 2021), (Wang et al. 2023).

Beyond preservation, food packaging enhances food safety by acting as a shield against contaminants such as microorganisms, dust, gases, and light. It also fulfills several key functions, including food containment, convenience, and information dissemination (Ghadermazi et al., 2019) (Tan et al. 2022) (Wu et al. 2021). Packaging not only secures food but also provides consumers with vital details like nutritional information and usage instructions. Additionally, it improves handling, transport, and accessibility, making it an indispensable element of modern food distribution (Sid et al. 2021) (Cheng et al. 2024).

1.2 Overview of synthetic plastic biofilms

The rapid rise in synthetic plastic production has driven economic growth but also led to severe environmental challenges, including plastic pollution and climate change. While plastics offer affordability, durability, and ease of processing, their short lifespan, especially in packaging and single-use items, results in massive waste accumulation and the formation of harmful microplastics. Additionally, increasing CO₂ emissions from plastic production contribute to global warming. To combat these issues, efforts are being made to promote recycling and shift toward biodegradable alternatives. Bio-based polymers present a more sustainable solution as they decompose faster, reduce carbon emissions, and require less energy to produce, highlighting the urgent need to transition away from petroleum-based plastics (Kouk et al. 2022).

The increasing demand for polymer films and thermoplastics, particularly in the packaging industry, has led to significant environmental concerns due to their non-biodegradable nature and contribution to plastic waste. Despite efforts to enhance recycling, a vast majority of plastic waste remains unmanaged, contributing to pollution and the release of greenhouse gases. Biodegradable films made from natural and synthetic biopolymers are emerging as viable alternatives, offering reduced environmental impact and faster decomposition. The widespread use of petroleum-based plastics has also introduced risks such as microplastic contamination in food and marine ecosystems, posing health hazards. In response, global initiatives, including regulations banning single-use plastics, are being implemented to curb plastic pollution. Addressing this issue requires the adoption of sustainable packaging solutions that are affordable, biodegradable, and environmentally friendly (Nur et al. 2023) (Trinh et al 2021) (Lyn et al. 2024) (Girdhar et al. 2023). Countries worldwide are increasingly implementing bans on single-use plastics, which are challenging to recycle and contribute to the accumulation of non-sustainable and non-degradable waste. To meet market demands, it is essential to develop suitable alternatives for food packaging that are biodegradable and environmentally friendly (Girdhar et al. 2023).

For many years, synthetic petroleum-based plastics, including polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), and polystyrene (PS), have been the preferred materials for packaging. This preference is largely due to their durability, affordability, resistance to degradation, and superior mechanical properties (Fahmy et al. 2020), (Ncube et 2020), (Deshwal et al. 2019), (Ashger et al. 2020). However, the extensive use of these non-renewable and non-biodegradable plastics has resulted in significant environmental challenges (P. Cazon, M. Vazquez 2021), (Jin et al. 2022). It is estimated that over 400 million tons of plastic waste are generated globally each year, with a significant portion originating from single-use food packaging. This widespread production of plastic has become a major environmental concern, as much of it ends up in landfills or the natural environment, contributing to pollution and ecological damage (Jafarzadeh et al. 2021). This plastic waste significantly contaminates both terrestrial and marine ecosystems, leaches harmful chemicals into the environment, and presents risks of ingestion and entanglement for wildlife (Radusin et al. 2021), (Cheng et al. 2024).

Synthetic and semi-synthetic plastics are widely used across various industries due to their durability, lightweight nature, and ease of manufacturing. In the food sector, they play a crucial role in packaging for protection, storage, and distribution. However, their extensive use has significantly contributed to environmental pollution, as most are non-degradable and toxic. To mitigate these issues, there is a growing need to reduce reliance on petroleum-based plastics and promote the adoption of biodegradable and less harmful alternatives (Boufi et al. 2018), (Anugrahwidya et al., 2021), (Cinar et al., 2020) (Saha et al. 2024). However, the excessive use of Petroleum-based plastic packaging has led to a surge in plastic waste, particularly in marine environments, posing severe threats to ecosystems, wildlife, and human health. This growing environmental crisis underscores the urgent need for sustainable alternatives and improved waste management strategies to reduce plastic pollution (Cha & Chinnan, 2004; Zhao, Cornish, & Vodovotz, 2020) (Ostle et al., 2019). Microplastics and nanoplastics from degraded petroleum-based packaging are entering the food chain, posing potential health risks. Their impact remains largely unknown, but they can absorb toxic substances and cause inflammation, oxidative stress, and immune responses, potentially contributing to serious conditions like cancer and metabolic disorders. Concerns also exist regarding their role in exacerbating food allergies. Due to their diverse properties, assessing their risks is challenging, highlighting the urgent need for further research to understand human exposure and long-term health effects (Stark & Matuana, 2021), (Wang et al. 2021), (Wang et al. 2023).

1.3. What are biopolymers and biofilms:

-Biodegradable polymer/biopolymer:

According to the International Union of Pure and Applied Chemistry (IUPAC), biodegradable polymers are defined as “polymers susceptible to degradation by biological activity, with the degradation accompanied by a lowering of its mass.” Additionally, the standard CEN/TR 15,351: 2006 specifies that biodegradable materials should mineralize into water, carbon dioxide, and biomass during the biodegradation process. This dual definition emphasizes the importance of both biological activity and the complete breakdown of materials into harmless byproducts as key characteristics of biodegradable polymers. In varying environments, factors such as humidity, temperature, and concentrations of microorganisms can lead to different rates of biodegradation. Ideally, a polymer material that performs well while being slowly biodegradable and integrated into the natural carbon cycle is considered the optimal biodegradable plastic. This balance allows for effective functionality in applications while ensuring that the material can break down over time without leaving harmful residues in the environment (Kouk et al. 2022).

International standards have been established to systematically evaluate the biodegradation and compostability of plastic packaging materials, with EN 13432 (EU regulations) and ASTM D6400 (USA) being globally recognized benchmarks. These standards outline four key criteria:

Biodegradability: More than 90% conversion of carbon to CO2 within six months.

Disintegration: Over 90% fragmentation into pieces smaller than 2 mm within three months.

No Ecotoxicity: Heavy metal content must be below prescribed limits.

Compost Quality: The material should not adversely impact the maturity and quality of the compost.

Additionally, ISO 14855-1 assesses aerobic biodegradability under controlled composting conditions by measuring evolved CO2, while ISO 14851 specifies methods for evaluating biodegradability in aqueous environments. ISO 17556 outlines a test method for determining aerobic biodegradability in soil. Together, these standards substantiate and certify compostability claims, with compliance verified by approved third-party organizations (Cheng et al. 2024).

1.4 Biodegradation and compostability:

A key advantage of biopolymers over conventional plastics is their ability to undergo biodegradation through microbial activity and environmental factors. However, the rate and extent of biodegradation depend on several variables, including the type of polymer, environmental conditions, and the specific test methods employed. To substantiate claims regarding biodegradability and compostability for bioplastics, it is essential to establish appropriate standards. These standards ensure that claims are credible and provide a reliable framework for evaluating the environmental performance of biopolymers in various settings (Cheng et al. 2024).

1.5 Mechanisms and factors influencing biodegradation

The biodegradation of biopolymers involves a complex, multi-step process that begins with bio-deterioration, which is facilitated by microbial enzymes and abiotic factors. This process typically includes several stages:

Bio-deterioration: Microorganisms, such as bacteria and fungi, produce enzymes that break down the polymer chains into smaller fragments. This initial step is crucial as it makes the material more accessible for further degradation.

Fragmentation: As the polymer is broken down into smaller pieces, it becomes easier for microorganisms to metabolize these fragments. Environmental factors such as temperature, humidity, and the presence of oxygen also play a significant role in this stage.

Mineralization: The smaller fragments are further decomposed into simpler compounds, ultimately leading to the conversion of carbon in the biopolymer into carbon dioxide (CO2), water, and biomass. This final stage is essential for returning the nutrients to the ecosystem (Cheng et al. 2024).

This multi-step process highlights the importance of both biological activity and environmental conditions in determining the rate and effectiveness of biopolymer biodegradation. Understanding these factors is critical for developing effective bioplastics that can meet sustainability goals. Polymer chain scission through hydrolytic, oxidative, and enzymatic reactions leads to solubilization and a reduction in molecular weight. The resulting oligomers and monomers are then assimilated by microorganisms as sources of carbon and energy. In an aerobic environment, the end products of this biodegradation process include methane, carbon dioxide, water, and biomass. Several important factors influence biodegradation kinetics, including temperature, moisture, oxygen levels, surface area, crystallinity, the presence of plasticizers, and the composition of the microbial community. The biodegradation process is initiated by microbial activity that breaks down the polymer chains into smaller fragments. Hydrolytic reactions are particularly significant as they involve water molecules facilitating the cleavage of chemical bonds within the polymer. Oxidative reactions may also play a role in the degradation process by introducing reactive oxygen species that further break down the material. Enzymatic reactions, driven by specific enzymes produced by microorganisms, are crucial for effectively degrading biopolymers into simpler compounds. (Cheng et al. 2024).

Overall, understanding these mechanisms and factors is essential for optimizing biodegradation processes and developing more effective biodegradable materials that can mitigate environmental impacts associated with traditional plastics (Cheng et al. 2024).

Starch, PLA, and PHA degrade easily in composting and natural environments due to their hydrophilic nature, which allows water penetration and enzymatic activity. In contrast, hydrophobic materials like PBS and PBAT resist water and enzyme penetration, slowing down their biodegradation. Blending such materials and adding nanoparticles can further delay degradation by increasing hydrophobicity and inhibiting enzyme action. Factors like crystallinity and thermomechanical history also reduce biodegradation rates, while plasticizers and acidic conditions can enhance the process by increasing permeability and flexibility. Optimizing biopolymer modifications is key to maintaining biodegradability across various environments (Awasthi et al. 2022) (Polman et al. 2021).

Biodegradation testing involves various bulk and molecular methods to measure mass loss, oxygen consumption, carbon dioxide evolution, microbial growth, and structural changes. Field tests offer real-world insights but can be costly and affected by environmental variables, while laboratory tests provide reproducibility but may lack environmental realism. A combined approach using laboratory screening, accelerated testing, and simulated field tests ensures a more accurate evaluation of biodegradation. Techniques like respirometry measure CO2 evolution, while structural changes are analyzed through mechanical, molecular, and spectroscopic methods. Thermogravimetric analysis tracks weight changes, and standardized protocols assess biodegradability in different environments, promoting the development of effective biodegradable materials (Pires et al. 2022), (Wolf et al. 2023).

1.6 Challenges associated with biodegradation

Biopolymers require industrial composting conditions for complete biodegradation, which is not always available in many regions, limiting effective waste management. Degradation rates vary based on polymer type and environmental factors, with some taking less than 100 days and others more than a year. There are concerns about the ecotoxicity of monomers, plasticizers, and nanoparticles released during degradation, which could harm soil and water quality. Biopolymers, while promising, need careful disposal management to prevent premature degradation or failure. Composting can disrupt microbial communities and release greenhouse gases, contributing to climate change. Contamination from traditional plastics further complicates biodegradation, and insufficient composting infrastructure limits their environmental benefits (Cheng et al. 2024).

Biodegradability holds great potential for eco-friendly, circular food packaging solutions, despite existing knowledge gaps. Advancing biomaterials, improving disposal systems, and fostering collaboration between industry and academia are key to overcoming current challenges. These efforts can lead to more effective biodegradable packaging, reducing environmental impact and promoting sustainability across the product lifecycle. By prioritizing innovation and cooperation, stakeholders can develop packaging solutions that support circular economy principles, contributing to a healthier planet (Cheng et al. 2024).

Biodegradable food packaging offers sustainability but faces challenges like lower strength, heat resistance, and higher costs. Research is focused on improving these properties through new processing techniques, nanomaterials, and additives to make biopolymers more competitive with traditional plastics (Oprea et al. 2020), (Salimbahrami et al. 2023), (Mehdizadeh et al. 2022), (Cheng et al. 2024).

1.7 Overview of biofilms in the food industry

There is increasing interest in developing biodegradable, edible, and compostable packaging to reduce plastic waste while offering sustainable alternatives. Food packaging faces challenges related to spoilage agents and pathogenic microorganisms, which can lead to health risks. Biobased polymers, derived from renewable resources, offer cost-effective and biodegradable solutions, though they often have slightly lower mechanical and physiochemical properties compared to petroleum-based plastics. To address these limitations, nanomaterials, plasticizers, other biopolymers, and plant extracts or essential oils can be incorporated to enhance functionality and performance (Stark et al. 2021), (Vieira et al. 2022), (Janecko et al., 2023), (Wang et al. 2023), (Girdhar et al. 2023).

Food packaging biomaterials are essential for protecting food from environmental factors like light, moisture, oxygen, bacteria, and mechanical stress. They must have barrier properties to reduce oxygen permeability, antimicrobial activity, and mechanical strength to maintain food quality and extend shelf life. Nanotechnology in packaging offers a novel solution to these challenges by creating active nano-packaging systems that protect against microbial activity, act as oxygen scavengers, and provide moisture barriers. Nano packaging also helps preserve food safety by preventing harmful substances from entering food. Innovations such as nanometal-derived biopolymer films and low-cost metal oxide nanoparticles (MO-NPs) further enhance food protection, improve shelf life stability, and offer better resistance to environmental changes (Batool et al 2021).

New materials for packaging pulverulent products are crucial as they create "zero waste" and do not harm the environment, being easily sourced from biodegradable and renewable resources. Conventional packaging for these products often consists of multiple layers (such as polyethylene, metallic foil, solvent-based inks, lamination, and low-density polyethylene), making sorting and recycling difficult. The newly developed material can be stored in supermarkets in cardboard boxes (Puscaselu et al. 2019).

Bio-based food packaging offers advantages such as biodegradability, renewability, and the potential for being edible. These packaging materials can take various forms, including films, plastics, trays, and foamed products. Hydroxypropyl methylcellulose (HPMC), derived from cellulose, provides several beneficial properties such as gas barrier formation, tastelessness, oil resistance, and transparency. Films made from HPMC, often combined with other materials like silver nanoparticles, chitosan, glycerol, or curcumin polymorphs, have been developed to enhance food preservation and extend shelf life. Other biomaterials, such as nanocellulose, alginate, and starch, are also utilized in combination with HPMC for food packaging applications (Liu et al. 2019), (Blachechen et al. 2020), (Bodini et al. 2019), (Tan et al. 2022).

Renewable biological resources have emerged as a sustainable alternative to tackle the issues related to conventional plastic packaging. Biodegradable packaging can completely decompose into carbon dioxide, water, and biomass through the action of microorganisms like bacteria, fungi, and algae. Controlled composting conditions can enhance the rapid biodegradation of these materials. The main drivers behind the growing interest in biodegradable food packaging include concerns about plastic waste accumulation, increased environmental awareness among consumers, strict governmental regulations on packaging disposal, and advancements in biopolymer production and processing technologies (Rebezov et al. 2021), (Eslamian et al. 2021), (Zomorodi et al. 2018).

Bio-based polymers increased by 44% from 1.6 to 2.3 million tons between 2021 and 2022 and are expected to grow further. Despite this, bioplastics still make up less than 1% of global plastic production. Environmental concerns, regulations, and technological advances are driving this growth, though price competitiveness remains a major challenge (Lyn et al. 2024) (Cheng et al. 2024).

1.8 Importance of biopolymers in sustainable materials

Biodegradable materials are substances that can decompose into water, carbon dioxide, methane, and biomass through the enzymatic action of microorganisms. For a material to be classified as biodegradable, it should ideally degrade within a timeframe of approximately 1 to 2 months under composting conditions. This rapid degradation is essential for minimizing environmental impact and promoting sustainable waste management practices (Lenfeld et al. 2021), (Ding et al. 2023). Biodegradable polymers, or biopolymers, are polymeric materials that meet the criteria for biodegradability. They can be synthesized from biological starting materials such as sugars, starches, oils, and proteins, or they can be derived from fossil resources but possess chemical structures that facilitate biodegradation. This versatility allows biopolymers to be used in various applications while promoting environmental sustainability (Ruggero et al. 2020). Thermoplastic polymers become pliable or moldable above a specific temperature and solidify upon cooling. This characteristic enables thermoplastics such as polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), and polystyrene (PS) to be easily processed and shaped into packaging materials like plastic bottles, containers, and films. Their versatility and ease of processing make them widely used in the packaging industry (Rostamian et al 2020), (Khalilzadehet al 2018). Unlike most conventional thermoplastics, which are resistant to biodegradation, biodegradable polymers can break down into natural components like water, carbon dioxide, methane, and biomass through microbial activity. Examples of such materials include bio-based thermoplastics like polylactic acid (PLA), polyhydroxyalkanoates (PHA), and polybutylene succinate (PBS), as well as naturally occurring polymers such as proteins and polysaccharides. While these biopolymers may lack the durability of traditional plastics, they offer significant environmental advantages due to their renewable origins and ability to decompose naturally (Cheng et al. 2024).

1.9 Types of Biopolymers

Biopolymers used in food packaging applications fall into two main categories. The first includes synthetic biopolymers, which are chemically synthesized, while the second comprises natural or bio-based biopolymers, sourced directly from biological materials. Synthetic biopolymers include PLA, PHA and PBS and Coplymers while natural biopolymers are Polysaccharide films Starch, cellulose, chitosan, alginates, carrageenan, and pectin are polysaccharides investigated for food packaging (Cheng et al. 2024).

1.10 Types of biofilms

- Protein films: Protein-based films, made from plant (gluten, soy, zein) and animal (casein, whey, gelatin) proteins, are emerging as sustainable alternatives to plastic packaging. These proteins offer excellent gas barrier properties, protecting food from oxygen, along with sufficient mechanical strength and flexibility for diverse packaging needs (Bian et al 2019), (Hadidi et al. 2022). Protein films are sensitive to moisture, which affects their water vapor barrier properties. This issue can be addressed by adding plasticizers like glycerol or sorbitol, which enhance flexibility and elasticity by interacting with the protein matrix. Additionally, blending proteins with lipids or polysaccharides can further improve their performance (Romani et al. 2019). Overall, protein-based biopolymers hold significant potential as renewable, biodegradable, and functional materials for sustainable food packaging. However, further research is necessary to enhance their moisture barrier properties and to gain a deeper understanding of the interactions among specific proteins, plasticizers, and other components within these active composite materials (Cheng et al. 2024).

- Lipid films: Global vegetable oil production has grown significantly, from 84.6 million tons in 1999-2000 to around 210 million tons in 2022-2023. This increase makes fatty acids and triglycerides a valuable and affordable resource for bio-based material development. Lipids can also be chemically modified through processes like epoxidation, acrylation, and maleination to improve their properties (Mohamed et al. 2020). Common fatty acids derived from plant oils, such as oleic, linoleic, and linolenic acids, feature reactive sites including double bonds and ester or acid groups. These reactive sites enable the modification of these fatty acids into valuable compounds tailored for various applications (Rebezov et al. 2021). Lipids can enhance the properties of biopolymer films and coatings for food packaging by improving hydrophobicity, water vapor barrier, mechanical strength, and antimicrobial and antioxidant effects. Acetylated monoglycerides and natural waxes are used to boost performance, with lipids interacting with biopolymers through mechanisms like hydrogen bonding and changes to polymer crystallinity. Factors such as the chemical structure, saturation, and dispersion of glycerides play a key role in the functionality of these materials. Lipid-based nanocomposites also show potential for reinforcing and improving functionality (Han et al. 2021). Epoxidation, acrylation, and maleination of lipids enhance their properties by improving crosslinking, flexibility, and processability, making them suitable for use in sustainable food packaging. Lipids also serve as plasticizers, contributing to the circular economy by enabling recycling. However, more research is needed on their biodegradability and compostability. Lipid-based biopolymers have the potential to replace some synthetic polymers, but their higher production costs pose a challenge. The biopolymers market is expected to grow significantly, driven by environmental concerns and regulatory support. Still, cost-effective methods for modifying lipids are needed to make them competitive with petroleum-based alternatives (Cheng et al. 2024).

1.11 Applications in food packaging

Biodegradable packaging seeks to replace conventional plastics across various types of packaged foods while ensuring the preservation of quality, safety, integrity, and shelf life (Cheng et al. 2024).

- Fresh fruits and vegetables: Fresh fruits and vegetables are highly sensitive to factors like respiration rates and transpiration, which can lead to spoilage. Biodegradable films can improve food preservation by modifying the in-package atmosphere and reducing condensation. For instance, chitosan films enhanced with essential oils or silver nanoparticles exhibit strong antimicrobial properties, extending the shelf life of produce like strawberries, bell peppers, and pineapples. These films also help preserve the nutritional quality and texture of the produce. Biopolymers such as starch, PLA, and cellulose offer effective gas and moisture barriers while being environmentally friendly, as they fully decompose after disposal (Shankar et al. 2021)., (Cheng et al. 2024).

- Meat and seafood: Biodegradable packaging made from food waste has shown great promise in extending the shelf life of meat and seafood. For example, gelatin films with grapefruit seed extract extended beef patty shelf life by six days, while chitosan films with clove oil preserved ham for 21 days. Whey protein films with mango peel and green tea extract prevented oxidation in chicken for 12 days. These materials offer antimicrobial and antioxidant benefits, maintaining food quality. However, more research is needed to improve film properties and scale up production for industrial use (Cheng et al. 2024).

- Dairy foods: Recent studies have highlighted biodegradable polymers like PLA as viable alternatives to traditional plastics for dairy packaging. PLA has been used by companies like Dannon for yogurt packaging, reducing carbon footprints. Antimicrobial additives, such as nisin and natamycin, in cellulose-based and bio-based polyethylene films have shown to extend the shelf life of cheeses by inhibiting microbial growth. However, challenges like moisture loss and oxidation persist with PLA. Research is ongoing to improve moisture retention, oxidation prevention, and packaging durability while maintaining sustainability for dairy products (Cheng et al. 2024).

- Bakery products: Biodegradable packaging offers a sustainable alternative for the bakery industry, reducing plastic waste and environmental impact. Studies show that PLA films infused with essential oils, such as cinnamon leaf oil, inhibit mold growth in cakes and bread for up to 15 days. Additionally, chitosan-based coatings with essential oils, like Mentha piperita, enhance the preservation of baked goods by controlling fungal growth and reducing water loss. These materials not only extend shelf life but also support environmental sustainability. However, further research is needed to assess their consumer acceptance and scalability in the industry (Debonne et al. 2018), (Barbosa et al. 2022), (Perdana et al. 2021), (Cheng et al. 2024).

- Cereal and confectionary: Biodegradable packaging offers a sustainable alternative for the cereal and confectionery sectors by addressing the environmental issues associated with traditional packaging. These materials, made from polysaccharides, proteins, and lipids, provide effective moisture and oxygen barriers for products like cereals and chocolate. For example, starch-based films and multilayer structures, like those used for chocolate packaging, have shown promising results in preserving product quality while reducing plastic waste. However, further research is needed to optimize performance, safety, and cost before these materials can be widely adopted in the industry (Cheng et al. 2024).

- Beverages: Biodegradable polymers, such as starch, cellulose, PLA, PHAs, and chitosan, offer eco-friendly alternatives for beverage packaging. Materials like PLA and PHAs provide good mechanical strength, gas and moisture barriers, and can replace conventional plastics in bottles and films. Starch-based bioplastics and cellulose nanocrystals enhance the barrier properties of bioplastic blends. Chitosan films, with antimicrobial and antioxidant properties, extend the shelf life of beverages, especially fruit juices. Combining these materials in multilayer films can improve packaging performance. Ongoing research is needed to optimize these bioplastics for broader use in the beverage industry, reducing environmental impact (Yoha et al. 2020), (Cheng et al. 2024).

1.12 Sources of biopolymer

Biopolymers can be derived from various natural resources, including cellulose, chitosan, starch, and proteins from both plants and animals. Their environmentally friendly characteristics make these natural polymers preferable alternatives to petroleum-based, non-biodegradable synthetic plastics for packaging materials. Furthermore, biodegradable polymers can be classified based on their sources and synthesis processes: 1) those directly obtained from biomass, such as polysaccharides and proteins; 2) synthetic biopolymers derived from biomass, like polylactic acid (PLA); 3) synthetic biopolymers sourced from petrochemicals, including poly(glycolic acid) (PGA), poly(butylene succinate-co-adipate) (PBSA), and polycaprolactone (PCL); and 4) biopolymers produced through microbial fermentation, such as poly(hydroxybutyrate) (PHB) and poly(hydroxyalkanoates) (PHA). This classification highlights the diverse origins and production methods of biodegradable polymers, emphasizing their potential to replace conventional plastic packaging while supporting sustainability efforts (Kouk et al. 2022).

1.13 Biopolymer production routes

Biopolymer production processes can be grouped into four categories: renewable sources, bacterial synthesis, chemical synthesis, and biopolymer blends. Bio-based polymers, derived from renewable materials, have various applications such as plant-based precursors, cellulose esters, lignocellulose fibers, and polyhydroxyalkanoates (PHA). The extraction method impacts the final product's physical properties, with organic materials rich in cellulose and fibers preferred for their enhanced mechanical strength. In a two-step biomass conversion process, bio-based precursors are first generated through biochemical or chemical transformations, producing two types of monomers: "novel" monomers and "drop-in" monomers, with the latter acting as bio-based substitutes for conventional ones. These monomers are then polymerized to form bioplastics (Kouk et al. 2022).

In the synthetic chemical method, lignin and cellulose are extracted from agro- and food waste using acids and alkalis, forming functional groups that affect the biopolymer's properties. However, byproducts like carboxylic acids can hinder fermentation. To address this, white-rot fungi like \*Ceriporiopsis subvermispora\* are used. This process enables the production of bio-based plastics from natural polymers, highlighting the potential of agricultural and food waste for sustainable packaging (Kouk et al. 2022).

Biodegradable polymers can also be produced through the interaction of microorganisms, Microorganisms, including Gram-negative and Gram-positive bacteria, can produce biodegradable polymers like polyhydroxyalkanoates (PHA) and polyhydroxybutyrate (PHB) when supplied with carbon-rich materials such as agro-wastes. The process depends on factors such as nutrient availability, pH, and culture composition. PHB, with mechanical properties similar to polypropylene, is ideal for high-strength applications (Kouk et al. 2022).

Companies like Minerv-PHA™, Bio-On, and Mater-Bi/Novamont use biopolymer blends to produce bio-based polymers, but these methods have limited commercial viability, with production capacities between 97 to 560 kilotons. Bioethanol-based biopolymer blends are not fully biodegradable, making this approach unfeasible without further development for better sustainability and practicality (Kouk et al. 2022).

1.14 Methods of Making Biofilms

Biopolymer packaging requires specialized processing technologies tailored to their physical and rheological properties. Manufacturing methods must account for these characteristics to ensure the bioplastic packaging performs optimally in various applications (Cheng et al. 2024).

- Film extrusion: Film extrusion is commonly used to produce biopolymer films by melting polymeric resin and forming a film. Biopolymers are heat-sensitive, so extrusion parameters need to be optimized to prevent degradation. Twin-screw extruders, though costlier, provide better control. For example, starch-based films were made through reactive extrusion, but mechanical properties didn’t improve significantly. Egg white protein (EWP) films produced by extrusion and calendering showed good transparency and rigidity but were less flexible than PLA, making them suitable for food packaging with some limitations (Cheng et al. 2024).

- Blown film extrusion: In blow film extrusion, molten biopolymer is extruded and inflated into a bubble, forming a film. The process enhances strength and barrier properties but is limited by biopolymer melt elasticity. Long-chain branching agents can improve stability. For example, biodegradable nanocomposite films with ZnO nanoparticles were developed for antimicrobial meat packaging, extending shelf life despite reduced strength from nanoparticle agglomeration. Another study used blow film extrusion to incorporate olive pomace (OP) powder into polyethylene films, improving UV-blocking properties but reducing tensile strength and elongation when OP content exceeded 11.5% (Cheng et al. 2024).

- Film casting: Solvent casting involves creating a biopolymer-solvent solution that forms a film as the solvent evaporates. It allows for multilayer films and the incorporation of additives but is unsuitable for large-scale production. Edible biopolymer films, made from polysaccharides, proteins, and lipids, offer a sustainable packaging alternative for perishable foods, extending shelf life by reducing moisture loss, gas exchange, and microbial growth. These films can also include antimicrobial and antioxidant agents. While they are biodegradable and reduce waste, they may have higher costs and affect food texture and taste (Cheng et al. 2024).

Making PLA Biofilms through Solvent-cast method

Preparation: The PLA pellets were dried in an oven for 4 hours to eliminate moisture, after which they were dissolved in a chloroform solution for 3 hours. Subsequently, dihydroflavonol (DHF) was added to the mixture, which was stirred for 15 minutes using a mechanical stirrer set at 300 rpm to achieve a homogeneous solution. Following this, the PLA/DHF solution was poured into a metal mold measuring 20 × 20 cm and allowed to dry at room temperature for 24 hours. The same procedure was repeated for the treated PLA/DHF biofilm. (Gisan et al 2020)

Solvent-cast: ZnO-NPs/PLA biofilms were created via solvent casting, where PLA and chloroform were mixed, followed by the addition of ZnO nanoparticles at 0.4% and 4% concentrations. The mixture was sonicated, coated onto glass, and dried at 70 °C. The composition included biopolymers, plasticizers, and inulin in various proportions, mixed, heated, and dried at 23 ± 2 °C for 48 hours. The films were evaluated for performance over three months. The 4% ZnO concentration was avoided to prevent surface agglomeration and reduced crystallinity (Batool et al. 2021) (Puscaselu et al. 2019).

- Thermoforming: Biopolymer sheets are heated and molded through thermoforming, often requiring plasticizers to improve strength. A deep learning system, using infrared cameras and CNN classifiers, detected over 99% of sealing defects in food packaging by analyzing over 200,000 images. This technology shows promise for real-time quality control in food packaging (Cheng et al. 2024).

- Multilayer films: Multilayer biopolymer films, designed for specific functions like moisture and gas barriers, are made through lamination or coextrusion. These films often combine natural biopolymers with synthetic polymers or nanomaterials. Advances in bioactive multilayer films have improved properties like mechanical strength and barrier functions. For instance, biodegradable films with PLA and organoclay nanoparticles enhanced oxygen barrier properties and tensile strength by 40%, showing promise for sustainable food packaging (Cheng et al. 2024).

- Foams and blends: Biodegradable foams, made using blowing agents like CO2, reduce density and improve insulation but often lose mechanical strength. Materials such as starch, PLA, and aliphatic polyesters are used in foamed products like trays and cushions. Blending these biopolymers enhances performance, with examples including PLA/starch and PLA/cellulose. Studies have shown that foams made from starch and grape stalks biodegrade in soil within weeks, reducing water absorption and improving food storage. Blending PLA with PHB and d-limonene has also enhanced flexibility, oxygen barrier, and thermal stability, highlighting their sustainability for packaging (Cheng et al. 2024).

- Compression and injection molding: Preformed biopolymer sheets are compressed between heated molds to make items like cutlery and trays, offering shorter cycle times but less shape flexibility than thermoforming. Injection molding uses high pressure to inject molten polymer into molds, producing complex shapes such as PLA tableware. Compression and injection molding can also process starch with plasticizers. Studies showed that adding chitosan or chitin to thermoplastic starch (TPS) films improved crystallinity, moisture resistance, and antimicrobial properties. Similarly, plasticized gelatin films with dialdehyde starch (DAS) demonstrated better moisture resistance and biodegradability, proving compression molding’s potential for scalable production of biodegradable films (Cheng et al. 2024).

1.13 Benefits and limitations: The key advantages offered by biopolymer-based food packaging are:

- Sustainability: Biopolymers, derived from renewable feedstocks, reduce dependence on fossil fuels and have a lower carbon footprint than conventional plastics. They are biodegradable, breaking down into harmless byproducts, aiding composting, and reducing plastic waste. Their production is often carbon neutral or negative with minimal toxicity. While many biopolymers can be industrially composted or recycled, challenges include their higher cost, 2–10 times more expensive than conventional plastics, and weaker mechanical properties, heat resistance, and barrier performance, requiring additional layers or additives, which increase costs. (Cheng et al. 2024)

- Processability: The thermal sensitivity and inadequate melt rheology of certain biopolymers complicate conventional polymer processing, often leading to brittleness and tackiness issues. Additionally, ambiguities in regulatory criteria and the absence of specific standards for biopolymer-based packaging hinder their commercialization. These challenges must be addressed to facilitate the broader adoption of biopolymers in the packaging industry. (Cheng et al. 2024)

- Performance: The effects of biopolymers on food shelf life and quality depend on the product and are strongly influenced by storage conditions like temperature and humidity. (Cheng et al. 2024)

Compostability: Complete biodegradation of certain biopolymers necessitates industrial composting facilities, and the rates of biodegradation can vary significantly. (Cheng et al. 2024)

1.15 Challenges and weaknesses of Biofilms

Biopolymer packaging materials face several challenges, including inferior moisture barriers, low heat resistance, poor mechanical strength, and higher costs compared to traditional thermoplastics. To meet the functional requirements for various food packaging applications, biopolymers may need modifications such as plasticization, incorporation of nanomaterials, or multilayer fabrication (Cheng et al. 2024).

2. Overview of the main biopolymers

2.1.1. PLA-Based Biofilms

PLA, or polylactic acid, is a linear aliphatic thermoplastic polyester derived from lactic acid. It is classified as one of the aliphatic polyesters, similar to polyglycolic acid, and is typically produced from hydroxyl acids. Lactic acid exists in two forms, L-LA and D-LA, which are mirror images with identical physical and chemical properties but differ in their interaction with polarized light. PLA has a lower maximum continuous use temperature than some other polymers. It can be repolymerized back into lactic acid through chemical conversion during recycling. This cycle emphasizes PLA's potential for sustainability in nature (Muller et al. 2017) (Suguna 2013) (Rossi et al. 2019) (Yadav 2018) (Kouk et al. 2022).

PLA is the leading commercially used bio-based plastic, valued for its superior functionality compared to similar polymers. Its inherent biodegradability allows for various end-of-life options, such as anaerobic digestion and industrial composting, which help prevent organic waste from being sent to landfills or incineration. As a versatile material, PLA has the potential to replace traditional plastics like polystyrene and polypropylene, making it an attractive choice for sustainable packaging solutions (Morão and Bie 2019), (Criminna and Pagliaro 2020).

Polylactic acid (PLA) is a biodegradable thermoplastic produced through the polycondensation of lactic acid monomers. The primary method for its commercial synthesis is the biosynthetic pathway involving microbial fermentation, which offers several advantages, including low environmental impact, reduced CO2 emissions, cost-effectiveness, high product specificity, and decreased reliance on fossil-based feedstocks (Gaan et al. 2023) (Arun et al. 2023).

PLA is an aliphatic polyester produced through the ring-opening polymerization of lactide, a cyclic dilactone derived from the fermentation of starch-rich agricultural crops. It has become one of the most promising and widely used biopolymers in food packaging due to its high strength, thermoplastic processability, transparency, and compostability (Ncube et al. 2020).

2.1.2. Properties and Characteristics

The thermal and mechanical properties of PLA are influenced by the ratio and distribution of L- and D-lactic acid in its polymer chains. Due to the presence of -CH3 side groups, PLA exhibits hydrophobic characteristics, making it more resistant to hydrolysis compared to polyglycolic acid (PGA). L-lactide results in a semicrystalline polymer known as PLLA, while poly(DL-lactide) (PDLLA) is amorphous in nature (Pawar and Purwar 2013) (Pan et al. 2016).

PLA, primarily composed of L-LA blocks, is semicrystalline with high structural regularity, providing mechanical strength due to its crystalline regions, which can comprise up to 37% at elevated temperatures. The glass transition temperature (Tg) is a critical property for polymers, indicating the temperature range (50 to 70 °C) at which PLA transitions from a brittle to a rubbery state. The degradation half-life of PLA varies based on its stereochemistry and molecular weight, typically ranging from six months to two years. As degradation progresses, the mechanical properties of PLA also change, reflecting its varying performance over time (Storz 2014) (Muller et al. 2017) (Pan et al. 2016).

Poly(L-lactic acid) (PLLA) is a transparent, rigid polymer with an elongation at break of 85% to 105% and a tensile strength ranging from 45 to 70 MPa. Its melting point is between 170–180 °C, while its glass transition temperature (Tg) is approximately 53 °C. The thermal properties of PLLA can be enhanced by physically blending it with poly-D-lactide (PDLA), raising its melting point and heat deflection temperature by 40–50 °C and 60–190 °C, respectively. PDLLA, on the other hand, does not have a melting point but has a Tg around 55 °C, resulting in lower tensile strength compared to PLLA (Pan et al. 2016) (Suguna 2013) (Pan et al. 2016) (Kouk et al. 2022).

The recrystallization rate during cooling is a crucial property for semicrystalline thermoplastics like PLLA. While pure PLLA can crystallize spontaneously, increasing D-LA content in the solutions slows this rate, resulting in less refined PLLA with a quasiamorphous phase that exhibits inferior mechanical properties. Commercial PLA production typically uses polymer-grade L-LA, which contains over 98-99% L-LA and less than 1-2% D-LA, ensuring optimal crystallization and performance characteristics. (Storz 2014) (Chen and Patel 2012).

The physical and mechanical properties of PLA, including tensile strength, hardness, stiffness, modulus, and melting points, are significantly influenced by its crystallinity. When PLLA content exceeds 90%, the polymer is semicrystalline; lower PLLA levels result in an amorphous polymer with reduced optical purity. PLA solubility varies across solvents: it is completely soluble in dioxane, chloroform, methylene chloride, 1,1,2-trichloroethane, acetonitrile, and dichloroacetic acid; partially soluble in ethyl benzene, acetone, toluene, and tetrahydrofuran (when heated); and insoluble in water, alcohols, and linear hydrocarbons (Rossi et al. 2019).

Biodegradable plastics are considered one of the most promising alternatives to conventional petroleum-based plastics, which contribute to environmental issues like pollution and global warming. Among various biodegradable options, polylactic acid (PLA) stands out for its wide availability and safety, as it decomposes after use without harming the environment (Kouk et al. 2022).

Among rigid bioplastics, polylactic acid (PLA) stands out as one of the most commercially successful options due to its excellent processability and mechanical properties. Compared to other biodegradable materials, PLA offers superior durability, mechanical strength, and transparency, making it a top choice for various applications (Jem and Tan 2020).

Lactides, produced through the microbial fermentation of carbohydrate-rich agricultural byproducts, make PLA a viable alternative to petrochemical-derived products. As awareness of environmental preservation grows, biodegradable materials, particularly bio-based plastics, are expected to increasingly replace conventional petroleum-based plastics. This manuscript aims to provide a comprehensive understanding of PLA as a biodegradable polymer, exploring its types and functionalities across various applications. (Yadav 2018) (Kouk et al. 2022)

PLA exists in either a highly crystalline stereocomplex form or an amorphous form, depending on the ratios of L-lactide and D-lactide enantiomers used. This variability allows for the customization of its mechanical properties, hydrophilicity, and degradation rates. PLA food packaging offers effective barriers against grease and aroma but has inferior moisture and gas barrier properties compared to PET and oriented polystyrene. Despite these limitations, PLA packaging is widely used for dry foods, fresh produce, ready-to-eat meals, baked goods, and beverages, highlighting its versatility in the food industry (Liu 2021) (Zareyee et al. 2018).

PLA packaging manufacturers include NatureWorks LLC, Total Corbion, and Taghleef Industries (Soccio et al. 2021), (Morão and Bie 2019), (Riondet et al. 2022), (Cheng et al. 2024).

Polylactic acid (PLA) is recognized as an effective packaging material due to its mechanical, physicochemical, and biodegradability properties. However, before it can be widely used in food packaging, several of these properties need enhancement, including its weak barrier performance, low heat distortion temperature, and low melting viscosity. Poly(lactic acid) (PLA) is becoming increasingly popular due to its biodegradability, renewability, and similar properties to petroleum-based polymers. Growing concerns over the environmental impact of non-biodegradable plastics have highlighted the need for sustainable alternatives. The rise of biodegradable plastics offers a promising solution to the disposal challenges posed by traditional plastics. (Ramli et al. 2020), (Desa et al. 2024).

2.1.3. PLA Production

Polylactic acid (PLA) is derived from renewable resources, including sugar beet, corn starch, and other biomass products and waste. Currently, high molecular weight PLA is produced, offering favorable physical and mechanical properties at a low cost (Peter et al. 2021).

Raw materials for lactic acid production include corn, cassava, sugar beet, straw cellulose, and other starch crops. PLA is recognized for its exceptional durability among bio-based polymers, making it a leading choice in the commercial bio-plastic market. Additionally, PLA is classified as "Generally Recognized as Safe" for food contact applications (Weng et al. 2022), (Qiao et al. 2023) (Jem and Tan 2020) (Qiao et al. 2023).

Scientific literature on polylactic acid (PLA) has experienced significant growth over the past two decades. In 2019, global PLA production was estimated at 190,000 tons, with projections indicating it will double every three to four years. The market is expected to grow at a compound annual growth rate (CAGR) of 17.1%, potentially reaching a value of USD 3.3 billion by 2028. (Reina et al. 2023) (Naser et al. 2021)

The Asia-Pacific region is set for rapid growth in the biodegradable plastics market, driven by increased investments in production plants from foreign investors and plastics manufacturers. However, despite its advantages, the widespread use of PLA is limited by factors such as its lower gas and water vapor barrier properties and inferior thermal stability compared to petrochemical-based polymers like polyethylene and polypropylene. Furthermore, PLA's inherent brittleness and limited elongation restrict its suitability for certain applications. Significant research efforts have focused on enhancing the properties of PLA to broaden its range of applications (Gaan et al. 2023) (Sun et al. 2021), (Misra 2023), (Jem and Tan 2020), (Reina et al. 2023), (Langer et al. 2016), (Torin et al. 2023), (Wang and Rhim 2016), (Sritapunyaet al. 2020), (Sabo et al. 2021). PLA polymeric films for packaging are produced using techniques like extrusion and solvent casting. The solvent casting method is particularly advantageous due to its simplicity and the flexibility it provides in adjusting process parameters. However, this method necessitates large volumes of solvent, leading to extended drying times, difficulties in scaling up production, and sustainability concerns. Additionally, residual solvent in PLA blends can negatively affect their ductility and mechanical properties. In contrast, extrusion is the favored industrial method, including techniques such as blown film extrusion and cast-film extrusion (Arslan et al. 2021), (Tadini et al. 2022). Extrusion facilitates straightforward monitoring and optimization by allowing adjustments to parameters like temperature, feed rate, and screw. Moreover, extruded PLA films typically demonstrate greater ductility and lower rupture tension than solvent-cast films. Flexible packaging made using blown extrusion can achieve elongation at break values greater than 500%. This enhancement is due to the better orientation of polymer chains achieved during the extrusion process, which involves extensional flow, compared to solvent casting. Additionally, extrusion typically produces films with a consistent thickness and a more uniform morphology compared to solvent-cast films speed (Seo et al. 2022) (Huang et al. 2022) (Graham et al. 2023) (Wagner 2016) (Dominici et al. 2015). Although previous reviews have addressed the physicochemical and mechanical properties of PLA films, along with their various. There is a significant gap in the literature regarding a focused review on the extrusion of PLA and its composites, particularly concerning their effects on the shelf life of food products. Given the increasing research on the synthesis of PLA films and composites through extrusion, a comprehensive review is essential. This review will concentrate on the extrusion of PLA films and composites for food packaging, examining the types and impacts of additives on their physicochemical properties, as well as how extruded PLA films contribute to preserving the shelf life of food products applications (Arun et al. 2023) (Jem and Tan 2020) (Naser et al. 2021) (Langer et al. 2016).

- Tensile properties: The neat PLA film exhibited the highest tensile strength compared to the PLA/DHF biofilm. Initially, adding DHF to the PLA matrix reduced tensile properties, but strength improved as fiber content increased from 5 wt% to 10 wt%. However, when DHF content exceeded 10 wt%, tensile strength decreased, likely due to DHF agglomeration, poor matrix-fiber interfacial interaction, and reduced stress transfer efficiency. The agglomeration acted as a stress concentrator, initiating cracks and lowering tensile strength. Additionally, the incompatibility between PLA and DHF contributed to weak interfacial interaction. In contrast, alkaline-treated DHF enhanced the tensile strength of the PLA/DHF biofilm, resulting in higher strength than untreated versions. This improvement is attributed to NaOH treatment, which increased surface roughness by removing moisture, waxes, oils, lignin, and hemicelluloses, as shown by FTIR analysis. The roughened surface of DHF facilitated better mechanical interlocking with PLA, thereby enhancing interfacial interaction and overall tensile strength. (Gisan et al 2020)

- The modulus of elasticity: The modulus of elasticity of the PLA/DHF biofilms followed a trend similar to tensile strength, increasing with DHF content from 5 wt% to 10 wt% due to the stiffness of DHF. However, adding more than 10 wt% DHF slightly reduced the modulus, likely because the high concentration of -OH groups in natural fibers created a plasticizing effect that lowered stiffness. Treated DHF-filled PLA biofilms demonstrated improved modulus because alkaline treatment with NaOH enhanced interfacial adhesion by increasing DHF's surface roughness. Figure 3(c) shows that pure PLA had the highest elongation at break, which decreased with 5 wt% DHF due to disrupted chain mobility. Elongation increased beyond 10 wt% DHF as moisture provided a plasticizing effect. Treated biofilms exhibited lower elongation at break than untreated ones, resulting from better interfacial interaction and increased rigidity. (Gisan et al. 2020)

- Enzymatic biodegradation: The weight loss of treated and untreated PLA/DHF biofilms during enzymatic biodegradation shows that neat PLA had the lowest weight loss compared to both types of biofilms. The weight loss in PLA/DHF biofilms increased with higher DHF content, indicating that the addition of natural fiber enhanced biodegradation properties. Among untreated and treated biofilms, untreated ones exhibited greater weight loss due to poor interfacial bonding between PLA and DHF, allowing the α-amylase enzyme to diffuse easily and break down the PLA chains. In contrast, alkaline treatment improved interfacial adhesion, making it harder for the enzyme to penetrate and degrade the treated biofilm, resulting in greater biodegradation resistance. SEM analysis revealed that the untreated PLA/DHF biofilm with 10 wt% DHF had a smooth surface before degradation, but showed micro-voids and tears afterward. Conversely, the treated biofilm displayed better dispersion of DHF and improved biodegradation resistance, with fewer micro-voids and less surface erosion after enzymatic action (Gisan et al 2020).

- Water absorption: Neat PLA does not absorb water due to its hydrophobic nature. However, when treated with NaOH, the PLA/DHF composite becomes more hydrophilic. The water absorption of untreated PLA/DHF biofilms increased with higher DHF content, as the hydrophilic DHF contributed to greater water uptake, exacerbated by poor interfacial bonding between PLA and DHF that allowed water to diffuse through gaps. Water absorption was directly proportional to wood loading from 10 wt% to 40 wt%. In contrast, treated PLA/DHF biofilms exhibited lower water absorption because alkaline treatment reduced moisture content and hydroxyl groups, enhancing interfacial adhesion and preventing water penetration. Consequently, treated biofilms had improved water resistance and tensile strength, though enzymatic degradation was reduced. (Gisan et al 2020)

Water absorption increased with ENR-50 content, rising from 10 to 30 wt%, with all blend formulations showing higher absorption than pure PLA. This increase is due to the hydrophilic ester groups in PLA and the oxirane groups in ENR-50, which enhance hydrogen bond formation with water. The presence of exposed ENR-50 molecules on the surface of the blends contributed to the rapid absorption of water during immersion. (Wahit et al. 2015)

Various types of PLA composites have been synthesized through the incorporation of different polymers, fillers, plasticizers, chain extenders, as well as antimicrobial agents and antioxidants. Common extrusion methods include cast extrusion, blown film extrusion, and reactive extrusion. Additionally, extrusion can be combined with other techniques such as thermoforming, injection molding, compression molding, and calendering. (Lyn et al. 2024)

- Main methods of making PLA biofilms:

- Cast film extrusion: Cast extrusion is frequently utilized to produce thin, flat sheets. In the extrusion process, the plastic film exiting the slit die is promptly directed onto a chill roll, where it is rapidly cooled to form a thin film (Laorenza et al. 2023), (Solano et al. 2023), (Pont et al. 2023), (Wagner 2016), (Solano et al. 2023). In this stage, stretching is a common technique used to orient the film and enhance its properties. This process boosts crystallinity, barrier properties, and strength in the direction of stretching, while reducing strength in the perpendicular direction. Uniaxial orientation happens when the take-off speed surpasses the extrusion rate, while biaxial orientation involves stretching in both directions, either simultaneously or in sequential steps. This results in either a balanced or unbalanced film, depending on whether the orientation is equal or uneven in the two directions (Selke and Culter 2016). Cast extrusion can produce optically transparent films with consistent thickness and smooth surfaces at high production speeds. However, cast extrusion may face challenges in producing complex shapes or thicker films, making blown film extrusion a viable alternative in these situations (Solano et al. 2023).

- Reactive extrusion: Reactive extrusion integrates conventional extrusion with chemical reactions. In this process, the extruder functions as a solvent-free reactor, facilitating various reactions such as polymerization, grafting, branching, and functionalization. As a result, new compounds are synthesized that typically exhibit enhanced mechanical strength, thermal stability, and improved water vapor and gas barriers, along with added benefits like antimicrobial and antioxidant properties (Fink 2018) (J.E. Herskovitz and J.M. Goddard 2020), (Gaur et al. 2017), (Campos et al. 2021). Compared to cast and blown film extrusion, reactive extrusion enables the synthesis of polymer blends in smaller quantities without requiring a full-size reactor. Reactive extrusion can be a more environmentally friendly alternative to traditional extrusion methods due to its lower energy consumption and reduced waste generation (Crawford and Martin 2020).

PLA processing methods are established techniques derived from commercial polymer manufacturing, but careful control and specific applications are necessary to fully realize the benefits of this biopolymer (Fang et al. 2016), (Desobry et al. 2010).

Common methods for manufacturing biopolymers include hot-melt extrusion, injection molding, blow molding, and thermoforming. The details of these techniques are as follows:

- Hot‑melt extrusion (HME): In the HME process, heat melts plastic raw materials, which are then shaped and densified by forcing them through a die. This continuous process is commonly used in polymer production, particularly in the food processing and plastic industries. High viscosity is crucial for proper extrusion, unlike casting, as heat transfer and solidification occur only if the melt retains its shape long enough to pass through the die (Mali et al. 2019), (Kouk et al. 2022).

HME equipment typically includes an extruder, downstream processing tools, and monitoring devices for evaluating product quality. Key parameters such as temperature, pressure, feeding rate, and screw speed are controlled during extrusion. The melted polymer can be formed into various products like sheets, plastic bags, and pipes. The extruder consists of one or two rotating screws inside a stationary cylindrical barrel, with the product's shape determined by the end-plate die attached to the barrel's end (Mali et al. 2019), (Snowden et al. 2019).

The extrusion process can be divided into four sections: (1) Raw materials are fed through a hopper, (2) Mixing, grinding, particle size reduction, venting, and kneading occur, (3) The material flows through the die, and (4) The extrusion is completed before further downstream processing (Mali et al. 2019).

There are two types of extruders used in this process: single-screw and twin-screw extruders. The single-screw extruder, being simpler and more cost-effective, is the most widely used globally. Inside the barrel, the screw performs feeding, melting, de-volatilizing, and pumping functions. Depending on the design, it can be either feed or starve fed. This extruder can generate thousands of pounds of pressure during melting and mixing, facilitating the molding of material into the desired shape (Snowden et al. 2019).

The twin-screw extruder, while similar to the single-screw type, is more complex due to its flow mechanisms, which depend on whether the screws are corotating or counter-rotating, and the screw design. Its advantages include (1) better material feeding and dispersion, (2) reduced overheating risk, and (3) shorter transit time (Mali et al. 2019).

- Injection molding: Injection molding is a highly effective method for large-scale thermoplastic manufacturing, typically requiring no additional finishing. The process involves heating the thermoplastic polymer above its melting point, then injecting the melt into a mold to form the desired shape. The key mechanisms in this process are pressure flow and heat transfer. The injection molding process begins with feeding plastic pellets into the screw from the hopper. The plastic is then melted, desired, and dried under high pressure. The melt fills the mold, with extra material added to compensate for shrinkage. The mold halves are clamped together, and once the component cools, one half of the mold opens to eject the part. Release agents may be used to prevent sticking. When proper practices are followed, components typically require no finishing, allowing for the large-scale, cost-effective production of small parts. The process operates cyclically, producing parts continuously but periodically (Kouk et al. 2022).

- Blow molding: Blow molding is commonly used to produce hollow, three-dimensional products like bottles for food packaging. The process involves inflating a molten thermoplastic tube, known as "parison," inside a mold to form the desired shape. There are three main types of blow molding: blow molding (for biaxially oriented jars and bottles with enhanced strength, clarity, and barrier properties), extrusion blow molding (used for PP bottles with hot-filling capability and good clarity), and injection blow molding (used for smaller bottles and wide-mouth jars). Extrusion blow molding is the most widely used, followed by stretch and injection blow molding. The process involves three main steps: creating the parison, applying force to shape it within the mold, and cooling the object to retain its shape. Extrusion blow molding is the least expensive, typically used for containers not requiring high precision, while injection blow molding is used for more demanding applications like carbonated beverage bottles due to its superior mechanical and barrier properties (Kutz 2017 pp 265-289), (Maddah 2016).

- Thermoforming: Thermoforming is commonly used to create parts with simple features, such as packaging containers. In this process, materials are heated to a pliable temperature, allowing the malleable plastic to take on the final shape. The process flow involves initially heating the PLA film with infrared lamps, followed by thermoforming, where the heated PLA sheets are passed through aluminum molds to shape the products (Nayik et al. 2015) (Fang et al. 2016), (Kouk et al. 2022).

2.1.4. PLA polymer synthesis methods:

There are three primary processes for synthesizing PLA: the production of lactic acid through microbial fermentation, the purification of lactic acid and formation of its cyclic dimer, and the ring-opening polymerization (ROP) or polycondensation of lactic acid (LA). Among these, ring-opening polymerization is the most commonly used method for producing high molecular weight PLA (Joung et al. 2017). Given that the properties of PLA depend on isomer composition, temperature, and reaction time, it is crucial to control the polymerization parameters effectively (Pinto et al. 2017). The polymer chains are formed from two types of lactic acid: L-lactic acid and D-lactic acid. This results in three stereoisomers of polylactide: poly (l-lactide), poly (d-lactide), and poly (dl-lactide). The poly (dl-lactide), also referred to as meso-dilactide, is produced by combining L- and D-lactic acids. (Kouk et al. 2022)

Lactic acid (LA) is initially produced through fermentation or chemical synthesis, resulting in different isomer compositions. Chemical synthesis yields a racemic mixture containing equal amounts of L (+)-lactic acid and D (-)-lactic acid, while fermentation typically produces a higher concentration of one isomer. Microbial fermentation of renewable resources like sago and cassava starch can yield optically pure L (+)- or D (-)-lactic acid, depending on the microorganisms used. After LA formation, it undergoes purification using techniques such as nanofltration, electrodialysis, ion exchange resin, hybrid short path evaporation, and reactive distillation. The purified LA is then utilized to produce PLA. (Kouk et al. 2022)

- Ring‑opening polymerization (ROP): The first synthesis method is ring-opening polymerization (ROP), which is catalyzed by organometallic catalysts to effectively convert lactide (the cyclic dimer of lactic acid) into PLA. This method is widely used in PLA production, where the terminal end of the polymer chain serves as the reactive center, allowing for the formation of longer polymer chains as cyclic monomers open their rings. Initially, lactic acid is dehydrated at high temperatures and under vacuum to poly-condense into oligomers, which are then catalytically depolymerized into lactide through internal transesterification. High molecular weight PLA is achieved when the lactide ring opens. Distillation or crystallization can be employed to eliminate residual moisture, lactic acid, and meso-lactide from the optically pure D or L form of lactide. (Kouk et al. 2022)

Furthermore, there is minimal moisture to remove from the molten PLA resin. The advantages of ring-opening polymerization (ROP), such as short residence times, mild processing conditions, lack of byproducts, and the ability to produce high molecular weight PLA, make it the preferred method for most industrial-scale production. (Kouk et al. 2022)

- Direct polycondensation polymerization: This method allows for the polymerization of lactic acid at reduced pressure and with a catalyst. Although it is a cost-effective approach, it tends to produce polymers with low molecular weights (1000–5000 Da). This lower molecular weight can result from the highly viscous reaction mixture, which hinders moisture diffusion and makes it challenging to remove all the water generated during lactic acid condensation. Consequently, the properties of the resulting PLA, including molecular weight, may be restricted by residual water trapped in the melt. However, adding coupling agents or additives like bis(tri-chloromethyl) can help achieve higher molecular weight polymers. (Kouk et al. 2022)

- Azeotropic condensation polymerization: Azeotropic condensation polymerization is another method used for synthesizing PLA. In this process, lactic acid is polycondensed directly into a high molecular weight polymer using organic solvents like toluene, xylene, or diphenyl ether. Water is then removed through azeotropic distillation. However, this method is less favorable due to the impurities introduced by organic solvents, making moisture removal and post-processing costly (Kouk et al. 2022).

The cross-sectional SEM morphology of pure PLA film exhibits a flat and smooth appearance. (Batool et al 2021)

2..1.5. Enhancing PLA properties

NaOH treatment improved the tensile strength and water resistance of the biofilms, while also decreasing their enzymatic degradation. (Gisan et al 2020)

Blending PLA with other polymers is an effective way to enhance its mechanical properties. For example, polypropylene (PP) offers excellent physical, chemical, mechanical, thermal, and electrical characteristics. Blending PLA with PP improves PLA's resistance to hydrolysis and enhances its dyeability. (Desa et al. 2021)

Active packaging systems play a crucial role in reducing microbiological contamination and minimizing spoilage, thereby extending food shelf life. Key components in these systems include nanostructured composites such as silver, titanium dioxide, graphene, aluminosilicates, silica, chitosan, and various plant extracts. These materials enhance the packaging's protective effects and contribute to improved food safety and longevity. (Peter et al. 2021)

Biodegradable plastics have drawbacks, including fragility, processing degradation, and limited recycling capacity. To address these issues and enhance food preservation, research is focusing on enriching PLA with various active ingredients like TiO2, silver, silica, aluminosilicates, and natural antioxidant extracts. These enhancements not only improve antimicrobial and antioxidant properties but also boost the mechanical strength and barrier characteristics of the material. (Peter et al. 2021)

Researchers produced polylactic acid (PLA) films incorporating 5% poly(ε-caprolactone) with thymol, carvacrol, and their mixture. The combination of thymol and carvacrol demonstrated a synergistic effect, resulting in minimal migration into distilled water, indicating strong potential for food packaging applications. (Peter et al. 2021)

Researchers prepared nanocomposite films from PLA with 1.0% silica nanoparticles and surface-functionalized silica nanoparticles using lactic acid through extrusion. They found that non-modified PLA had a 44% lower oxygen permeability compared to modified PLA, and the incorporation of cinnamaldehyde into the PLA matrix acted as a plasticizer. Additionally, the functionalized silica nanoparticles reduced the diffusion coefficient and delayed the release of active compounds, enhancing the overall performance of the PLA films. (Peter et al. 2021)

Researchers incorporated 1 wt%, 5 wt%, 10 wt%, 15 wt%, and 20 wt% nano-silver into PLA films, demonstrating that increased pressure and migration time resulted in rougher surfaces and elevated crystallization temperatures in the PLA nanocomposite films. (Peter et al. 2021)

Research demonstrated that PLA films containing silver and titanium dioxide can extend the postharvest life of mangoes by up to 15 days. Additionally, blending PLA with nano-silver and nano-titanium dioxide exhibits strong antimicrobial activity against E. coli and Listeria monocytogenes, with the release of nanoparticles remaining within acceptable limits. Furthermore, tributyl O-acetyl citrate was found to enhance the mechanical and thermal properties of PLA, while PLA combined with chitosan was shown to be non-toxic for the germination of cucumber and radish seeds. (Peter et al. 2021)

The Differential Scanning Calorimetry (DSC) analysis results show that the melting temperature (Tm) of the blend increased from 159.67 °C to 164.23 °C as the polypropylene (PP) composition rose to 60% in the PLA/PP (40:60) blend. However, the change in glass transition temperature (Tg) was minimal, which is typical for this type of immiscible binary blend. (Desa et al. 2021)

SEM analysis was employed to examine the particle size and dispersion of the polypropylene (PP) phase in PLA/PP blend systems. The micrographs revealed a multilayered structure with distinct separation between the PP and PLA phases, indicating immiscibility. This incompatibility is attributed to differences in polarity, resulting in sharp phase boundaries that create voids and microcracks under deformation. The impact of PP on toughening the PLA/PP blends was assessed at various ratios of 60/40, 50/50, and 40/60. Results showed that PLA had a tensile stress of 34.52 MPa and a strain of 1.7%. As PP content increased from 40% to 60%, tensile stress decreased by 58.86% to 14.20 MPa, while tensile strain increased from 1.71% to 2.21%, highlighting PP's role in enhancing the flexibility of the blend. (Desa et al. 2021)

The effects of morphology, mechanical, and thermal properties of PLA/PP binary blends were investigated. Using a twin-screw extruder, optimal processing conditions were established. DSC analysis indicated that the melting temperature (Tm) increased by about 5 °C when PLA composition decreased from 60% to 40%. The tensile strain improved by 29.24% due to the flexibility provided by PP. FTIR results confirmed that the PLA/PP blend is immiscible within the 40% to 60% range. SEM analysis revealed physical phase separation between the PP and PLA phases, characteristic of immiscible polymer blends. (Desa et al. 2021)

- Types of additives

Composites and nanofillers: The synthesis of biocomposites has proven to be an effective strategy for improving the mechanical properties of bio-based polymers in the production of thin films and coatings (Gulzar et al. 2023), (Luo et al. 2022). However, this method may not always be effective for PLA composites. High concentrations of nanofillers can lead to poor dispersion and aggregation, especially when the fillers are immiscible with PLA (Bala et al. 2021), (Lyn et al. 2024).

Recent research has investigated the addition of thermoplastic polymers such as polybutylene succinate (PBS), poly(butylene succinate-co-adipate) (PBSA), and poly(butylene adipate-co-terephthalate) (PBAT) to PLA (Laorenza et al. 2023), (Pont et al. 2023), (Chakpha et al. 2023), (Waterhouse et al. 2021). In PLA/PBS composites, higher PLA concentrations resulted in films with increased tensile strength, rising by 20% to 49%, but decreased elongation at break from 5.75% to 2.13%. This reduction in flexibility is attributed to the inherent rigidity of both PLA and PBS (Chakpha et al. 2023). In PLA/PBAT composite films, those with a higher PLA content (PBAT30/PLA70) demonstrated significantly greater tensile strength, nearly double that of films with higher PBAT content (PBAT70/PLA30) (Y. Laorenza and N. Harnkarnsujarit et al. 2023). In a study, it was found that PLA/PBSA composites with a higher PLA concentration (PLA80\_PBSA20) exhibited increased tensile strength of 26.82 MPa and lower elongation at break of 35.65%, compared to composites with a lower PLA concentration (PLA60\_PBSA40), which had a tensile strength of 15.87 MPa and an elongation at break of 111.30%. The elongation at break for PLA/PBSA composites was 37% to 166% greater in the machine direction compared to the transverse direction, indicating a higher degree of polymer chain orientation (Pont et al. 2023) (Lyn et al. 2024).

In contrast, adding thermoplastic cassava starch (TPS), known for its high flexibility and ductility, to PLA has been found to reduce tensile strength while increasing elongation at break. In PLA/TPS composites, adding low concentrations of duckwheat biomass (2.3% and 4.6% wt) improved tensile strength. However, increasing the biomass concentration to 9.2% and 13.8% wt negatively affected tensile strength due to biomass agglomeration. In another study, adding 5% gelatin to PLA/thermoplastic starch led to a 30% reduction in tensile strength, Young's modulus, and elongation at break, indicating potential incompatibility between gelatin and the PLA/thermoplastic starch blend (Yoksan et al. 2022), (Pizzoli et al. 2017).

Increasing sodium alginate concentration in PLA composites led to reduced crystallinity, attributed to uneven dispersion within the PLA matrix, resulting in voids and irregular microstructural features. Additionally, the tensile strength of PLA/sodium alginate composites decreased by approximately 20% to 35% compared to neat. Incorporating 10% cellulose from pineapple leaves into PLA films led to a 44% reduction in tensile strength, attributed to the polarity differences between the fibers and the polymer matrix. To address this issue, the authors recommended pre-treating the pineapple leaf fiber with NaOH solution and autoclaving before blending it with PLA (Russo et al. 2023), (Koombhongse et al. 2022).

PLA composites can be produced during the extrusion process using either direct mixing or the masterbatch method (Graham et al. 2023), (Dominici et al. 2015), (He et al. 2023) (Arslan et al. 2021), (Bala et al. 2021), (Wu et al. 2023). In direct mixing, PLA pellets and other components are melt-blended during compounding, producing a uniform molten material that is then extruded into the final product. This process is simple yet effective in achieving a consistent blend. Despite its simplicity, bulk production can be costly due to extra processing and storage requirements. Additionally, prolonged processing times may cause material degradation. In contrast, the master batch consists of a concentrated blend of additives encapsulated in a carrier resin. The masterbatch pellets are mixed with the base polymer in a specific ratio, ensuring an even distribution of additives in the extruded material (Chakpha et al. 2023), (Arslan et al. 2021), (Bala et al. 2021), (Thakur et al. 2021).

A study compared the incorporation of nanoclay using direct mixing and masterbatch methods. In direct mixing, PLA and nanoclay were blended at 195°C, while in the masterbatch approach, a concentrated PLA-nanoclay masterblend (22% wt) was created and then mixed with fresh PLA in a high-intensity mixer. The masterbatch method resulted in better nanoclay exfoliation and intercalation with PLA, as confirmed by SEM and DSC analyses (Bala et al. 2021). In a separate study, MMT was added to both virgin and recycled PLA through melt extrusion and compression molding into film. The Vickers hardness of recycled PLA was lower than that of virgin PLA, due to slight degradation from the recycling process, which involved additional extrusion and molding steps. When 2% and 4% wt of organically modified MMT were added to recycled PLA, the hardness exceeded that of virgin PLA. This improvement was attributed to the nanoclay's high surface area, which enhanced the load-transferring capacity of the polymer matrix. Furthermore, the nanoclay platelets reduced PLA chain mobility, contributing to the increased hardness. In addition to direct addition it has also reported the development of bi- and multilayer PLA films. A Trilayer packaging material is created with extruded PLA, an electrospun active PLA/ethyl lauroyl arginate/cellulose nanocrystal intermediate layer, and a chitosan inner layer. The structure showed high crystallinity, indicating potential enhancements in mechanical and barrier properties over neat PLA (Salah et al. 2023), (Vidal et al. 2023).

Recent advances have allowed the use of innovative nanoactive materials in packaging, replacing traditional methods. Low-cost metal oxide nanoparticles (MO-NPs), such as ZnO-NPs, enhance food packaging by providing antimicrobial activity, oxygen scavenging, and improved shelf life stability. ZnO-NPs (36.5 nm) were incorporated into PLA at 0.4% and 4% concentrations using solution casting. This improved stability, film thickness, and elongation. Vitis vinifera fruit packed in ZnO-NPs/PLA biofilms retained taste and freshness for 15 days, with no significant differences in quality compared to control samples. The use of ZnO-NPs effectively reduced oxygen interaction, enhancing the fruit’s shelf life. (Lu et al. 2020) (Batool et al. 2020).

Low-cost metal oxide nanoparticles (MO-NPs) in food packaging offer excellent features for preserving food materials. Nanomaterials in polylactic acid-based packaging enhance gas barrier properties and improve resistance to temperature and humidity by mixing nanoparticles with polymer chains. It also significantly reduced fat and carbohydrate levels in the packaging material, improving shelf life and nutrient retention (Clar et al. 2020), (Correa et al. 2019), (Boro et al. 2019), (Passeri et al 2019), (Tagi et al. 2019), (Perumal and Raghunath 2017).

Visualization of PLA/ZnO-NPs films confirmed the embedding of zinc oxide nanoparticles in the polymer. The results indicated a more effective incorporation of the nanomaterial, resulting in a stronger and more intact film. (Batool et al. 2020)

The moisture transmission rate for ZnO-NPs/PLA (4% w/w) was 110 g/m², higher than that of ZnO-NPs/PLA (0.4% w/w) at 93 g/m². (Batool et al. 2020)

The antimicrobial effectiveness of biofilms with varying ZnO-NPs concentrations was evaluated based on the JIS Z 2801 standard. The antimicrobial activity of ZnO-NPs/PLA biofilms varied with bacterial type, ZnO-NPs concentration, and microbial growth. At 4% ZnO-NPs, the biofilm exhibited enhanced antimicrobial activity, especially against \*E. coli\* (98%) compared to \*S. aureus\* (89.9%). The biofilms released Zn²⁺ ions, which disrupted bacterial cell membranes. Quality assessment showed that fruit stored in ZnO-NPs-modified PLA films had better quality than those in untreated films. This was evident in reduced decay, improved gas regulation, and less browning, confirming the superior fruit preservation properties of the modified films (Batool et al. 2020) (Wellen et al. 2020).

During fruit storage, oxygen levels were 9.1% and 8.9% in ZnO-NPs/PLA (0.4% w/w) and ZnO-NPs/PLA (4% w/w), respectively, while carbon dioxide levels were 15% and 17%. The pH of fruit stored in ZnO-NPs/PLA packaging remained stable, unlike the control, which showed a decrease in pH. The decay rate was 9.2% for fruit in ZnO-NPs/PLA (4% w/w) packaging, and the fruit's firmness and rigidity were higher in the ZnO-NPs/PLA (4% w/w) film (15) compared to the (0.4% w/w) film (12). Zinc metal ion release into grape samples was 0.058 ppm for ZnO-NPs/PLA (4%) and 0.004 ppm for ZnO-NPs/PLA (0.4%). The optical density (OD) values for \*E. coli\* and \*S. aureus\* were 1.2 and 0.8, respectively, with antimicrobial activity improving over time. The zone of inhibition (ZOI) and minimum inhibitory concentration (MIC) for fruit wrapped in 4% ZnO-NPs were 21 mm (22 mM) for \*E. coli\* and 32 mm (19 mM) for \*S. aureus\*, demonstrating the effective antimicrobial activity of the ZnO-NPs/PLA packaging. (Batool et al. 2020)

It's evaluated the effect of biogenically fabricated zinc oxide nanoparticles in polylactic acid (PLA) on fruit shelf life at 40°C. Fruit wrapped in ZnO-NPs/PLA (4% w/w) biofilm maintained a fresh appearance for up to two weeks, while control samples showed mildew after four days. The antimicrobial activity against \*E. coli\* and \*S. aureus\* confirmed that nano-zinc oxide-based packaging extended the shelf life of \*Vitis vinifera\* fruit. Quality parameters such as decay rate, gas composition, color change, and metal accumulation were improved in the ZnO-NPs/PLA (4% w/w) film, demonstrating its potential for future use in fruit packaging (Batool et al. 2020).

2.1.6. Applications in Food Packaging

PLA, compared to other aliphatic polyesters, offers excellent properties like high mechanical strength, biodegradability, biocompatibility, and ease of processing. The increased use of PLA is also attributed to improvements in its properties, such as heat resistance, through modifications like copolymerization and blending (Ren et al. 2017).

PLA has become the most widely used biopolymer across various industries, including agriculture, automotive, and packaging, due to its favorable characteristics. This increasing demand has led to a stable growth in the global PLA market, which is expected to continue expanding in the future. PLA-based bioplastics present an excellent alternative to traditional plastics, helping to mitigate environmental pollution while offering a sustainable and cost-effective solution. This paper aims to review recent literature on PLA as a biodegradable material, focusing on its properties, usability, productivity, and potential as a substitute for conventional plastics (Kouk et al. 2022).

2.1.7. The industries that utilize PLA include the following:

- Medical/biomedical industry: The biomedical sector is one of the key industries utilizing PLA bioplastic due to its advantageous properties. PLA’s hydrolysis mechanism enables it to naturally degrade in situ, eliminating the need for additional surgeries to remove implanted devices, thereby improving patient recovery and reducing healthcare costs. Its inherent biocompatibility further minimizes the risk of immune reactions, as its degradation products, lactic acids and short oligomers, are easily metabolized by the body. Despite these benefits, pure PLA may not meet all the requirements for biomedical applications, leading to the extensive exploration of PLA-based nanocomposites as alternatives. These composites combine PLA with copolymers and other nanomaterials, offering enhanced properties. Nanomedicine, which uses these materials, is an emerging field with significant potential, particularly in diagnostics and targeted drug delivery. According to FDA guidelines, nanomaterials ranging from 1 to 1000 nm are similar to biological molecules like proteins and viruses, enabling easier cell absorption and opening up new opportunities in medical treatments (Rossi et al. 2019) (Sha et al. 2016) (Kouk et al. 2022).

PLA-based nanocomposites offer improved properties due to the surface and small-scale effects of nanomaterials, making them more suitable than pure PLA for biomedical applications such as synthetic bone substitution, drug delivery systems, and tissue engineering. Among PLA-based copolymers, poly(lactic-glycolic acid) (PLGA) has garnered the most attention for clinical use. PLA and PLGA are commonly used in orthopedic, oral, and craniofacial surgeries for the substitution and repair of synthetic bones with polymer-based plates, screws, and pins. Both materials have been extensively studied for producing porous scaffolds that aid in bone repair. PLA’s superior degradation properties eliminate the need for additional surgeries to remove implants, reducing pain and operational risks for patients. However, pure PLA lacks bone-bonding strength and has less effective regenerative and degradation capabilities compared to PLA-based nanocomposites. Tissue engineering, focused on the formation and regeneration of tissues and organs, relies heavily on scaffolds for cell adhesion and growth. PLA, PDLLA, and PLGA are widely used in this field due to their biocompatibility and varying melting and glass transition temperatures. Aligned nanofibrous PLLA scaffolds have shown promise in neural tissue engineering, and PLLA has demonstrated exceptional effectiveness in promoting osteogenesis. Functionalized PLA scaffolds have been developed to enhance cell adhesion through the adsorption of tripeptide moieties (Sha et al. 2016), (Beagan et al. 2020).

- Packaging/food packaging: The packaging industry is another key application for PLA. Derived from lactic acid, PLA is a thermoplastic, biodegradable aliphatic polyester with significant potential for packaging uses. The qualities of PLA as a packaging material depend on the ratio of its optical isomers. For example, using 100% L-PLA monomers results in high melting and crystallinity points, while a 90/10% D/L copolymer ratio creates a polymerizable melt above its Tg, making it suitable for bulk packaging. PLA offers a promising alternative to conventional plastics like LDPE, HDPE, PS, and PET, due to its excellent water solubility resistance, high molecular weight, good processability, and biodegradability. In terms of tensile strength, modulus, flavor and odor barrier properties, PLA compares favorably to polyethylene, PET, and flexible PVC. It also offers the temperature stability and processability of PS, along with polyethylene’s printability and grease resistance. PLA is one of the earliest bio-based polymers available for large-scale commercialization and can be processed into various forms such as injection-molded objects, films, and coatings. Processed PLA is used in containers, films, and paperboard coatings. Furthermore, PLA can be recycled back into lactic acid for repolymerization (Yadav 2018), [57] (Nayik et al. 2015).

A study by the Technological University of Denmark showed that PLA packaging effectively protects butter, yogurt, cheese, and margarine from moisture, fats, light, and gases, with minimal lactic acid migration during biodegradation. It is ideal for short-shelf-life or high-respiration foods, like bakery items and produce. However, while PLA is used in bottles for juices and water, its application in carbonated beverage packaging is limited due to its low CO2 barrier and poor creep resistance (Mena et al. 2019) (Fang et al. 2016).

Due to its limited barrier and mechanical performance, PLA faces challenges in commercial packaging. Compared to petroleum-based plastics like PET, PLA is rigid, brittle, and sensitive to heat deformation, making it difficult to heat seal. However, solutions such as polymer processing adjustments, blending, and the inclusion of nucleating agents, plasticizers, or nano/micro-composites can address these issues. Coating PLA with materials like poly (ethylene oxide) or poly (ε-caprolactone) has been shown to improve its gas and water vapor barrier properties without affecting its visual appearance (Fang et al. 2016), (Beagan et al. 2020),(Kouk et al. 2022).

- Agriculture: PLA has found applications in agriculture, particularly in plasticulture, which began in the 1950s to enhance production. Benefits of plastic use in agriculture include soil erosion protection, plant defense from pests and weeds, drip irrigation, and greenhouse shielding. Initially, non-renewable plastics dominated, but biodegradable alternatives like PLA, PHAs, and PBAT have emerged due to environmental concerns. However, pure PLA is unsuitable for plasticulture due to its poor mechanical and thermal properties. To address this, PLA-based mulch films are created by blending PLA with other biodegradable polyesters and using plasticizers. Despite these advancements, plasticulture remains a developing field, with challenges like high costs and immature bioplastic implementation (Fang et al. 2016).

- Automotive industry: The automotive industry has increasingly turned to natural-based materials for vehicle parts due to their excellent performance and potential to reduce weight, thereby improving fuel efficiency and reducing greenhouse gas (GHG) emissions. Automotive emissions account for 23% of global carbon emissions, with 80% of environmental pollution stemming from these emissions. Reducing vehicle weight by 10% improves fuel efficiency by 7% and cuts CO2 emissions by 20 kg for every kilogram of weight saved. Biocomposites offer a dual benefit of enhancing fuel efficiency and lowering CO2 emissions. Using nanocomposite materials in place of steel and aluminum can reduce vehicle body weight by 40–55%. Lightweight materials are especially important for optimizing the weight-to-battery capacity ratio in electric vehicles. Bioplastics like PLLA and its composites are suitable for automotive applications due to their biodegradability, recyclability, and strong mechanical properties, though improvements in brittleness and thermal stability are needed. Modifiers and additives can address these issues. Major automakers such as Ford, Mazda, Toyota, and Hyundai have incorporated bio-based PLLA blends in vehicle components, with applications including interior parts like dashboards and door plates (Dubois et al. 2017 (Wambua et al. 2016) (Dubois et al. 2017).

2.1.8. PLA and bioplastic market overview

NatureWorks, based in the USA, operates the world's largest industrial-scale PLA production plant, established in 2002 with a capacity of 70,000 metric tons, later expanding to 150,000 tons in 2015. The second-largest PLA plant is in Thailand, a joint venture between Total and Corbion, producing 75,000 tons of PLA. In China, Hisun established a 5,000-ton PLA line, later increasing its capacity to 10,000 tons in 2017. In 2018, Hengtian and COFCO built lactide-to-PLA fiber plants in China, each with a capacity of 10,000 tons. Synbra also set up a 5,000-ton line for Expandable PLA (BioFoam™). The global biodegradable plastic market is expected to grow from $3.02 billion in 2018 to $6.73 billion by 2025, driven by the increasing demand in emerging economies like Brazil, China, and India. PHA and PLA are major contributors to this growth, with PLA production set to increase from 293,290 tons in 2019 to 317,000 tons by 2024, an 8% increase. Currently, starch blends dominate global biodegradable polymer production (44%), followed by PLA at 24%, PBS and PBAT at 23%, PHAs at 6%, and others at 3%. In 2018, 60% of biodegradable plastics were used for flexible and rigid packaging (Jem and Tan 2020) (Beagan et al. 2020) (Wurm et al. 2019) (Criminna and Pagliaro 2020) (Kouk et al. 2022).

2.1.9. Challenges and Limitations

- Brittleness and cost considerations.

PLA biofilms, while strong and stiff like polyethylene terephthalate, suffer from poor mechanical and water resistance, along with low impact resistance, limiting their application in tougher material fields. Chemical treatments such as alkaline, benzoylation, and acetylation can improve these properties. PLA's main benefits lie in its eco-friendliness, as it is derived from renewable resources, and its biodegradability, recyclability, and compostability, which minimize environmental impact. Additionally, its biocompatibility makes it ideal for biomedical use, as it does not harm the human body, breaking down into harmless substances like water and carbon dioxide without interfering with tissue healing (Gisan et al 2020) (Desa et al. 2021) (Anderson et al. 2016).

PLA has better thermal processability than other biopolymers like PHA, PEG, and PCL, allowing for various processing techniques, such as drying, film extrusion, and thermoforming. The energy required for PLA production is 25 to 55% lower than that of traditional petroleum-based plastics, with the potential for further reduction. Despite these advantages, PLA has some significant drawbacks. It is brittle, breaking with less than 10% elongation, which limits its use in applications that require high plastic deformation, such as screws and fracture fixation plates in the biomedical field. Additionally, PLA has a slow degradation rate, influenced by factors like crystallinity, molecular weight, and water diffusion. This slow degradation, which can take 3 to 5 years in vivo, can cause inflammatory responses in tissues due to its hydrophobic nature. As a result, PLA is not ideal for long-term biomedical applications unless modified with polymer blends or functional additives. Furthermore, PLA has high gas permeability compared to other packaging plastics, resulting in poor barrier properties, making it unsuitable for some applications like beverage bottles (Anderson et al. 2016) (Pinto et al. 2017).

- PLA environmental footprint: Life cycle assessment (LCA) is a key tool for evaluating the environmental footprint (EFP) of PLA, using the "cradle-to-grave" concept to quantify the environmental impact throughout its life cycle, from raw material extraction to production, disposal, and recovery. This approach helps identify the most appropriate disposal systems for PLA. International standards like ISO 14040 and ISO 14044 guide LCA studies, and organizations such as SETAC, EPA, and ANSI have established detailed LCA guidelines. Important benchmarks like greenhouse gas emissions and non-renewable energy use are measured and compared with those of traditional polymers to assess PLA's environmental performance (Suwanmanee et al. 2017) (Ren et al. 2017) (Fang et al. 2016).

2.2. Starch In Biofilm Production

2.2.1. Introduction

Starch-based biofilms have gained attention as environmentally friendly alternatives due to their biodegradability. However, their practical applications are constrained by their low mechanical strength and lack of antimicrobial properties. Starch, a fundamental carbohydrate storage molecule in photosynthetic organisms, consists of α-D-glucose units and ranks as the second most abundant biopolymer after cellulose. It is a semi-crystalline polymer composed of amylose and amylopectin. The primary commercial sources of starch include maize, wheat, potato, and cassava, with additional contributions from the roots and underground stems of the Araceae plant family. Starch granules range in size from 2 to 100 μm. Its affordability, renewability, and functional properties make it highly valuable in the food industry, where it is used in various products such as sauces, yogurts, snacks, and baked goods. Moreover, starch-based biofilms are currently among the most rapidly emerging biodegradable films, accounting for a significant share in production (Saha et al. 2024).

2.2.2. Synthesis of Nano-Starch and Tannic Acid-Coated Nano-Starch

-Nano-Starch (NS) Synthesis

Physical modification of starch involves the synthesis of starch nanoparticles. The process begins with suspending starch in a sulfuric acid-water mixture and continuously stirring at 40°C for five days. The solution is then neutralized with sodium hydroxide and subjected to centrifugation. The supernatant is discarded, and the residue is mixed with isopropanol, followed by ultrasonication at high amplitude in a pulse mode. After a final centrifugation step, the supernatant is dried at 40°C and milled into a fine powder to obtain starch nanoparticles (Saha et al. 2024).

-Tannic Acid-Coated Nano-Starch (T-NS) Synthesis

Chemical modification of starch nanoparticles is carried out by incorporating tannic acid, a natural antimicrobial agent. Nano-starch is dispersed in deionized water and homogenized using low-power ultrasonication. The powdered tannic acid and ferric chloride solution are then mixed to specific concentrations, followed by centrifugation and drying at 40°C for 24 hours. The resulting tannic acid-coated nano-starch is stored for further use (Saha et al. 2024).

-Structural and Morphological Analysis

The incorporation of nano-starch and tannic acid significantly alters the visual and structural characteristics of starch-based biofilms. Pure starch biofilms exhibit a uniform structure with minimal porosity, whereas biofilms with nano-starch show increased pore formation. The pore size varies among different biofilm formulations, with tannic acid-modified samples displaying larger pores. This suggests that the introduction of nano-starch and tannic acid disrupts the starch network, reducing its compactness. The inability to synthesize biofilms using only nano-starch further confirms that the presence of nanoparticles interferes with starch gelation (Saha et al. 2024).

-Density and Thickness

The particle size and concentration of additives influence the density of starch-based biofilms. Films containing nano-starch and tannic acid tend to exhibit higher densities, with NSB 25 showing the greatest thickness. Since denser biofilms typically possess greater mechanical strength, NSB 25 and T-NSB 25 variants are expected to perform better in packaging applications (Saha et al. 2024).

- Moisture Content and Water Absorption

Moisture retention varies significantly across different biofilm formulations. The highest moisture content is observed in T-NSB 25, while SB has the lowest water swelling percentage. The ability of biofilms to absorb water depends on the particle size of starch granules, with water solubility increasing as particle size decreases. As a result, biofilms containing tannic acid exhibit high moisture absorption, potentially limiting their application as effective packaging materials (Saha et al. 2024).

- Hydrophobicity and Surface Roughness

The degree of hydrophobicity and surface roughness determines the fraction of air trapped on a biofilm's surface, which influences its contact angle. Among all formulations, T-NSB 25 exhibits the highest contact angle, indicating enhanced hydrophobicity. Conversely, NSB 50 has the lowest contact angle, likely due to the presence of negatively charged hydroxyl groups. The higher hydrophobicity of tannic acid-modified biofilms suggests their suitability for packaging applications, as seen in other biofilms incorporating hydrophobic additives (Saha et al. 2024).

- Water Vapor Transmission Rate (WVTR)

WVTR measures the permeability of moisture through biofilms, an essential property for packaging materials. A lower WVTR is preferred, as it indicates better resistance to moisture transmission. The addition of nano-starch and tannic acid results in a gradual decrease in WVTR, enhancing the biofilms’ shelf-life. The increased thickness and density of these modified biofilms further contribute to their improved barrier properties. Despite their moisture absorption capacity, their ability to resist environmental humidity makes them suitable for packaging applications (Saha et al. 2024).

- Light Transmission

Biofilms exhibit different levels of light transmission, influenced by the presence of nano-starch and tannic acid. The pure starch biofilm allows the most light to pass through, while tannic acid-modified samples show the lowest transmission. These variations correspond with the visual differences among biofilms and are attributed to the differential absorption, reflection, and transmission patterns of the incorporated additives. Similar reductions in light transmission have been observed in biofilms containing nanoparticles or antimicrobial agents (Pereira et al 2021) (Saha et al. 2024).

2.2.4. Biodegradability of Starch-Based Biofilms

All biofilm samples undergo complete degradation within ten days of exposure to soil. Pure starch biofilms degrade at a faster rate compared to nano-starch and tannic acid-modified variants. This slower degradation of modified biofilms is attributed to the strong interaction between additives and the starch matrix, which enhances structural integrity. Additionally, tannic acid’s antimicrobial properties may slightly hinder microbial activity in the soil, slowing down the biofilm decomposition process. The presence of water in the soil accelerates biodegradation by promoting microbial growth, which in turn breaks down the polymer structure. The hydrophilic nature of starch-based biofilms further facilitates their degradation. The pure starch biofilm begins losing weight after three days, highlighting its susceptibility to microbial consumption. Similar results have been observed in other starch-based biofilms, where microorganisms utilize starch as an energy source, leading to weight loss and structural breakdown (Saha et al. 2024).

2.2.5. Antimicrobial Activity of Biofilms

The antimicrobial potential of starch-based biofilms was evaluated against both gram-positive and gram-negative bacteria. Among all biofilm variants, T-NSB 50 exhibited the highest antimicrobial activity, followed by T-NSB 25. This enhanced antimicrobial effect is attributed to the presence of tannic acid, which has well-documented antibacterial properties. Consequently, starch-based biofilms modified with tannic acid demonstrate significant inhibition against various bacterial strains. Conversely, pure starch and nano-starch biofilms exhibit relatively lower antimicrobial effectiveness. These findings suggest that tannic acid functionalization effectively enhances the antimicrobial properties of starch-based biofilms, making them promising candidates for applications requiring antimicrobial activity, such as food packaging (Saha et al. 2024).

In conclusion, starch-based biofilms represent a sustainable alternative to conventional plastic films, but their applications are hindered by poor mechanical strength and lack of inherent antimicrobial properties. The synthesis of nano-starch and tannic acid-coated nano-starch provides a feasible approach to improve these biofilms by enhancing their structural, mechanical, and antimicrobial properties. The introduction of nano-starch alters film morphology, reduces compactness, and improves moisture resistance. Additionally, tannic acid modification increases antimicrobial activity, hydrophobicity, and structural integrity. Despite their enhanced functionalities, modified biofilms exhibit reduced biodegradation rates compared to pure starch films. However, their lower water vapor transmission rates and superior antimicrobial properties position them as effective packaging materials. Future studies should focus on optimizing their composition to balance mechanical strength, moisture resistance, and biodegradability for broader commercial applications.

2.3. Inulin in Biofilm Production

2.3.1. Functional Properties

Inulin (INL), a biodegradable polysaccharide found abundantly in Jerusalem artichoke and other plants, is highly valued for its various beneficial properties, including biodegradability, biocompatibility, non-toxicity, antibacterial activity, and hydrophilicity. It is commonly used as a prebiotic and has medical applications, such as regulating cholesterol, aiding weight loss, improving urinary tract function, and managing constipation. Additionally, INL serves as a sugar substitute in the food industry for diabetic patients. Its wound-healing properties are notable, as it enhances keratinocyte migration, re-epithelialization, and fibroblast activity, making it valuable for skin wound treatment. INL’s film-forming ability also allows it to be combined with other polymers, such as PVA, to create blend films suitable for biomedical uses (Nur et al. 2023).

Inulin (C6nH10n+2O5n+1) is a widespread beta fructan and is found in over 3000 plant species (Puscaselu et al. 2019)

2.3.2. Natural sources

Inulin, found in natural sources like chicory root, garlic, onion, and banana, is a fructose polymer whose length, composition, and properties depend on factors such as plant species and extraction methods. It consists of chains ranging from two to 100 fructose units, with short-chain inulin (oligofructose) being sweeter and more soluble than long-chain inulin, which is less soluble, more viscous, and thermostable. Inulin functions as a dietary fiber that is not hydrolyzed by digestive enzymes, promoting digestive health, enhancing nutrient absorption, regulating appetite, and stimulating the immune system. Its technological properties make it widely used in various industries, including food products such as bakery items, dairy, meat, and frozen desserts, for functions like sugar and fat substitution, texture improvement, and moisture control. The substances used in edible film production, such as sodium alginate, agar, and glycerol, are deemed safe for consumption and are approved as food additives under various regulations (Puscaselu et al. 2019).

2.3.3. Physical properties and applications for use

The physical properties of the films revealed that they were homogeneous, smooth, odorless, slightly sweet, transparent, and glossy, with well-defined edges and minimal adhesion to the silicone support used for drying. All samples, except for I2, were suitable for use as packaging materials for low-moisture products, such as instant drinks and dehydrated vegetables, as well as pharmaceutical applications due to their fast solubility in liquid. Specific samples, such as I5, could be used as self-adhesive foil for food, while I8 may be employed for edible consumables. Samples I12–I16 were found to be useful for packaging fresh produce, cosmetics, and medicinal applications. The I17 film was considered the best for packaging powdered food, while I18–I20 films were ideal for ready-to-eat products and fast food. Transmittance testing showed that films with lower alginate content (I10 and I13) had significantly lower transmittance values, with I20 showing the lowest at 56.75%. Films with higher alginate content, such as I4, exhibited higher transmittance values. These results suggest that transmittance increases with the amount of alginate used, indicating that films with high alginate content may not be suitable for packaging high-fat foods due to potential oxidative degradation. For powdered products, high transmittance is not an issue, but films for high-fat foods require improvements in opacity, which can be achieved by incorporating substances like carob powder, saffron, caramel, anthocyanins, carotenoids, or blackberries (Puscaselu et al. 2019).

The microstructure of the membranes revealed smooth, homogeneous films with no visible pores or cracks, featuring a regular and smooth surface. These characteristics suggest that the material could be suitable for industries where the appearance of the product plays a significant role in consumer selection. The absence of pores and cracks not only indicates high-quality films but also reflects their stability against environmental factors such as humidity and light. (Puscaselu et al. 2019)

The Pearson correlation analysis revealed minimal changes in film parameters over a three-month testing period, with strong positive correlations between thickness, retraction ratio, and tensile strength. Negative correlations were observed between thickness and solubility. Films containing inulin, particularly sample I7, demonstrated increased resistance, with breaking strength surpassing the established measurement limits. Elongation at break showed remarkable values, exceeding 380% after one month. The addition of sodium alginate improved tear resistance and elongation, while the I20 sample, with high inulin and glycerol content, showed lower tensile strength but high elongation. Despite variations, no significant changes occurred in the film parameters during the test period. To optimize the mechanical properties, the Design Expert 11 program suggested a composition of 0.4971 g alginate, 1.018 g glycerol, and 2.352 g inulin to achieve high tensile strength, elongation, and reduced roughness (Puscaselu et al. 2019).

The small variations in thickness and retraction ratio observed during the three-month testing period indicate that the material's physical characteristics remain stable over time, suggesting its potential for successful use in industry (Puscaselu et al. 2019).

The solubility of the samples was evaluated to assess their suitability for packaging pulverulent products, such as instant drinks. Samples I1–I7 were completely dissolved after 1 minute of water immersion, preventing them from being tested for the swelling ratio index. The remaining samples dissolved immediately in hot water (over 80°C), making them suitable for instant beverage packaging. Samples I14–I17, which lacked sodium alginate but had similar agar content, displayed the lowest swelling ratio values, demonstrating the high solubility capacity of sodium alginate. In contrast, samples I8 and I11, which had higher agar content, absorbed the most liquid (Puscaselu et al. 2019).

All tested samples maintained consistent behavior throughout the three-month testing period, proving their suitability for packaging food products. Additionally, these films could be used for packaging other foods that require solubilization before consumption, such as dehydrated vegetables, fruits, instant soups, and vegetable concentrates. Regarding microbial safety, the samples were found to be safe for consumption, as no growth of microorganisms (coliforms, enterobacteria, E. coli, Staphylococcus aureus, yeasts, and molds) was observed on culture media throughout the test period. Furthermore, the films' low water activity index (below 0.4) further emphasizes their safety for use in food packaging (Puscaselu et al. 2019).

2.3.4. Role as a prebiotic and plasticizer

Biofilms, composed of bacterial cells encased in a self-produced polysaccharide matrix, are the predominant bacterial form in nature and are responsible for causing chronic, relapsing infections. Unlike planktonic bacteria, which are often targeted by antibiotics, biofilms provide structural stability and protection against environmental stresses, such as pH extremes, heat, nutrient scarcity, and antimicrobial treatments. They also help bacteria evade the host’s immune system by reducing the effectiveness of antimicrobial peptides and phagocytosis. Bacteria within biofilms are reported to be 10 to 1000 times more resistant to antimicrobials than planktonic bacteria. Despite this, there is a lack of drugs that specifically target biofilm bacteria, emphasizing the need for strategies to prevent and eliminate biofilm-related infections (Feng et al. 2018).

Inulin, a water-soluble polysaccharide with low toxicity and high biocompatibility, has been shown to inhibit the growth of pathogenic bacteria and fungi. This study explored the enhancement of chitosan's anti-biofilm properties through conjugation with inulin, aiming to create a highly efficient antibacterial substance with minimal side effects. The inulin-chitosan conjugate was covalently bonded, and its physicochemical characteristics were evaluated. The study further assessed the conjugate’s ability to inhibit biofilm formation, eradicate existing biofilms, and combat planktonic bacteria (Feng et al. 2018).

2.3.5. Biofilm Eradication

The inulin–LCS conjugate effectively eradicated 78% of mature S. aureus biofilm at 1 mg/mL, while inulin, chitosan, and their mixture showed no significant activity. The conjugate outperformed LCS at concentrations above 500 µg/mL, as confirmed by fluorescence microscopy. When compared with antibiotics florfenicol and streptomycin, inulin–LCS showed similar biofilm eradication activity, highlighting its enhanced effectiveness against S. aureus biofilm. (Feng et al. 2018)

The World Health Organization (WHO) defines probiotics as live microorganisms that provide health benefits to the host. They can help prevent infections, treat inflammation, and reduce harmful pathogens. Prebiotics are used to enhance the effects of probiotics. Lactobacillus and Bifidobacterium are the most commonly used probiotic genera, while Lacticaseibacillus rhamnosus and Pediococcus acidilactici have demonstrated antimicrobial properties. (Bravo et al. 2022)

One of the major challenges in using conventional antimicrobials is the complex nature of biofilm development, which requires multi-targeted or combinatorial treatments. Common antifungal drugs like azoles, polyenes, and echinocandins can cause side effects, including mutations and drug resistance, particularly with Candida albicans. Research has shown that antimicrobial compounds, when functionalized into nanoparticles that mimic extracellular structures, can be more effective as they serve as release vectors for antibacterial agents. Additionally, certain nanoparticles, such as pullulan, have been found to enhance the antibacterial properties of probiotics like Lactobacillus plantarum by increasing the production of plantaricin, a natural antibacterial peptide (Bravo et al. 2022).

Inulin-type fructans are prebiotics known to enhance the properties of beneficial intestinal bacteria. However, research on the impact of fructans on antifungal effects against Candida albicans is limited. This study investigated the growth inhibition and antibiofilm effects of cell-free supernatants from a synbiotic combination of Lacticaseibacillus rhamnosus and Pediococcus acidilactici, supplemented with inulin-type fructans (Bravo et al. 2022).

A synbiotic combination of Lacticaseibacillus rhamnosus and Pediococcus acidilactici with inulin-type fructans showed growth and biofilm inhibition against Candida albicans. These formulations could serve as a promising alternative to antifungal drugs, though further in vivo studies are required to confirm these results (Bravo et al. 2022).

2.4. Xanthan Gum in Biofilm Composites:

2.4.1. Rheological and Functional Characteristics

Xanthan gum (XG) is a high molecular weight polysaccharide produced by Xanthomonas campestris, commonly used as a thickening agent and stabilizer in the food industry. XG can enhance the mechanical properties of biodegradable materials without significantly affecting their water sorption or permeability. For example, films made with chitosan and higher XG content exhibit increased tensile strength but lower elongation. Additionally, the combination of XG with gelatin or carboxymethyl cellulose improves film properties, and XG-based films can extend the shelf life of fresh-cut produce by reducing respiration rates. The integration of XG and hydroxypropyl methylcellulose (HPMC) is hypothesized to produce a biodegradable composite film with enhanced mechanical strength and extended fruit freshness. In this study, an XG-HPMC composite film was developed and applied as a coating for bananas to test its preservation effects, with characterization including FT-IR, XRD, SEM, light transmittance, mechanical properties, and water vapor transmission rate (Tan et al. 2022).

Xanthan gum, a high molecular weight branched heteropolysaccharide, is composed of D-glucose, D-mannose, and D-glucuronic acid residues. It is widely used as a food additive, stabilizer, and thickener. Xanthan gum enhances various properties of biopolymers, including mechanical strength, antioxidant, and antibacterial activities. Its combination with metal oxide nanoparticles, such as ZnO, improves antibacterial properties, thermal stability, hydrophobicity, and barrier ability, even surpassing some commercial packaging materials (Girdhar et al. 2023).

Xanthan (Xa) is an anionic polysaccharide produced by the bacteria Xanthomonas campestris, known for its solubility in water. Its anionic properties arise from side chains containing glucuronic acid and pyruvic acid groups. Due to its biocompatibility, biodegradability, thermal stability, and non-toxicity, Xa is widely used in industries such as biomedical, food, pharmaceuticals, and cosmetics (Ostrowski et al. 2022) (Xu et al. 2018) (Chaturvedi et al. 2021). Xa contains numerous hydroxyl and free carboxyl groups, making it suitable for chemical modifications to enhance its physicochemical properties. It can form physical networks with other polysaccharides like galactomannans, guar gum, konjac glucomannan, and chitosan through mechanisms such as hydrogen bonding, hydrophobic interactions, and cation bridging. The FDA has approved Xa as a safe polymer for food use. Due to its acid/salt resistance and pseudo-plasticity, Xa is commonly used as a thickening agent in the food industry. However, its application in food packaging is limited by poor antimicrobial properties, barrier performance, and solubility (Fan et al. 2021) (Sabaa et al. 2024).

The XG-HPMC composite film demonstrated strong mechanical properties, with a tensile strength of 39.21 ± 1.25 MPa when 2 g/L of XG was used. This strength surpassed that of many other XG-based films as well as gelatin and methylcellulose films. The film also proved effective in extending the shelf life of bananas, as it reduced weight loss by 9% compared to the uncoated bananas over 18 days. The results confirm that the XG-HPMC composite is a promising food packaging material with high mechanical strength and effective preservation capabilities (Tan et al. 2022).

2.4.2. Xanthan in medicine

Biofilm formation on biological and material surfaces poses significant health and economic challenges. A promising strategy to address this is the development of medical devices that combine antibacterial properties with the ability to promote wound healing (Otero et al. 2025).

The rise of bacterial infections associated with biofilm formation on biological and material surfaces is a critical issue today. It can result in severe consequences, prolonged patient hospitalization, and significant healthcare costs (Depypere et al., 2020; Górecki & Babiak, 2009; Sandiford et al., 2020). (Otero et al. 2025).

Infections can be triggered by bacterial colonization of implantable materials, intraoperative bacterial inoculation, or contamination of open fractures or wound sites. When bacteria colonize medical devices, they often form biofilms that protect them from antibiotics, increasing the required antibiotic concentrations by 100–1000 times for bacterial eradication. This significantly raises the risk of antibiotic resistance and side effects (Kang et al., 2023).

Major pathogens like Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, and Escherichia coli, including Multiple Drug Resistant (MDR) strains, are leading causes of life-threatening infections. These bacteria are listed in the World Health Organization's priority list due to their rapid development of antibiotic resistance (Bouhrour et al., 2024; Chen et al., 2013; Gbejuade et al., 2015; Khatoon et al., 2018; Tacconelli et al., 2018).

Infections in necrotic tissues hinder antibiotic delivery due to impaired blood flow, requiring high doses that increase side effects. To address this, medical devices combining antibiofilm, antimicrobial, and pro-wound healing properties offer a promising solution. However, alternatives to antibiotics are preferred due to rising antimicrobial resistance, and using non-drug, widely approved substances can simplify regulatory approval. Natural compounds and inorganic ions with multi-therapeutic properties can be valuable in this context (Depypere et al., 2020) (Frei et al., 2023; Silva et al., 2016). (Otero et al. 2025).

In clinical practice, traditional wound dressings like cotton, bandages, and gauzes mainly serve as passive protective barriers. While they absorb exudates, they do not address the specific needs of patients. In recent years, advanced materials such as foams, hydrogels, sponges, and films have been developed to actively aid in the healing process. Current trends focus on using 3D printing technologies, often with carbohydrate polymers, to create more effective wound care solutions (Farani & Shafiee, 2021) (Pita-Vilar et al., 2023; Seijo-Rabina et al., 2024; Yuan et al., 2023). A recent study explored semisolid extruded 3D-printed networks made from xanthan gum (XG), guar gum (GG), and succinic acid (SA) as an eco-friendly alternative to plastic medical devices. These materials have a porous structure that mimics the natural extracellular matrix, promoting tissue integration, and offering benefits like angiogenic and anti-collagenase activity. Natural carbohydrate polymers like XG and GG are ideal for wound healing due to their biocompatibility, biodegradability, low cost, and chemical versatility. XG, produced through fermentation by Xanthomonas strains, consists of a linear β-1,4-d-glucopyranose chain with side branches including acetylated d-mannose, d-glucuronic acid, and terminal pyruvated mannose (Virzì et al., 2024) (Hassanisaadi et al., 2025).

Low-cost, biocompatible, biodegradable, and steam-heat sterilizable SSE-3D-printed medical devices were developed using XG and GG polysaccharides to prevent wound infections. This work aimed to enhance the scaffolds with antibiofilm, antibacterial, and wound healing properties by incorporating thymol and Zn2+ ions, either separately or together. Thymol’s antibacterial activity is linked to its ability to destabilize membrane lipids, induce oxidation, and cause membrane depolarization, leading to cell damage and death (Li et al., 2022; Tian et al., 2021). Additionally, thymol can interfere with the Quorum Sensing (QS) mechanism in several Gram-positive bacteria, including multi-drug resistant S. aureus (Kowalczyk et al., 2020; Nostro et al., 2007; Silva et al., 2016). Thymol possesses anti-inflammatory and wound healing properties, promoting angiogenesis, enhancing fibroblast growth, and aiding bone regeneration both in vitro and in vivo (Costa et al., 2019; Gabbai-Armelin et al., 2022; Lavanya et al., 2023; Sapkota et al., 2018). Nevertheless, despite its promising biological activities, the low solubility of thymol in water (0.98 μg/mL) hinders its loading within a hydrogel ink. The inclusion of high amounts of non-soluble thymol crystals into SSE-3D-printing inks could lead to the nozzle clogging, interfering with the printing process and causing a non-homogenous distribution within the network. Thus, HPβCD was used to increase thymol solubility by forming an inclusion complex (Virzì et al., 2024) (Celebioglu et al., 2018; Garg et al., 2021).

Medical devices with suitable microarchitecture and porosity aid in tissue integration and wound healing. Semisolid extrusion 3D printing (SSE-3D printing) is a versatile technique used to create biocompatible hydrogel-based lattices with organized patterns and porosity. This method offers advantages like precise structural control, reproducibility, scalability, and cost-effectiveness (Jiang, Wang & He, 2020) (Seoane-Viano, ˜ Januskaite, Alvarez-Lorenzo, Basit, & Goyanes, 2021) (Virzì et al., 2024).

There is an urgent need to replace plastic materials with eco-sustainable, biodegradable alternatives for 3D printing of medical devices due to concerns over pollution from medical device disposal. Natural polysaccharides offer a promising solution, as they are biodegradable, derived from renewable sources, and cost-effective. They are highly biocompatible with low toxicity, even after prolonged exposure, and are metabolized into non-toxic compounds. Additionally, their functional groups can be chemically modified to enhance the properties of medical devices for specific applications. In wound healing, polysaccharide-based materials provide the necessary moisture, allow gas exchange, and absorb exudates, promoting skin regeneration (Nosrati, Khodaei, Alizadeh, & Banitalebi-Dehkordi, 2021; Zubair, Hussain, Shahzad, Arshad, & Ullah, 2024) (Virzì et al., 2024).

Sterilizability is a critical factor for the clinical application and commercialization of 3D-printed medical devices, especially implantable ones, which require materials that can withstand sterilization without losing their structure. Polysaccharide-based devices often face the challenge of brittleness, which can lead to deformation during the sterilization process. This issue can be addressed by chemically modifying polysaccharides or combining them with other polysaccharides to enhance their resistance to external forces. A combination of Xanthan gum (XG) and Guar gum (GG) offers potential, as both are biocompatible, biodegradable, and eco-friendly. XG is a high-viscosity polymer with pseudoplastic behavior, while GG is known for its mucoadhesive, antioxidant, and wound-healing properties, making them suitable for use in medical devices (Virzì et al., 2024) (Verma & Sharma, 2021). XG and GG exhibit excellent physicochemical stability across temperature and pH, but their viscoelastic properties need optimization for effective use in SSE-3D printing. While their application in extrusion-based 3D printing has mainly been limited to the food industry, where their thickening abilities have been used for printing various food slurries like fruits, vegetables, soy protein, meat, and starches, the combination of XG and GG has shown promise in printing food for people with dysphagia. (Dick, Bhandari, & Prakash, 2021).

2.4.3. Enhancing the properties of Xanthan

Although the rheological properties of XG and GG dispersions are promising, a pure mixture of the two has not yet been used in SSE-3D printing. However, it is known that XG's viscoelastic properties can be enhanced by galactomannans like GG. The synergistic effect of these polysaccharides arises from their differing molecular structures—XG is rigid and rod-like, while GG is more flexible with longer chains. This flexibility allows GG to have less affinity for XG's rigid structure compared to other galactomannans, enabling the mixture to break under shear stress and return to its original shape once the pressure is removed (Schreiber, Ghebremedhin, Zielbauer, Dietz, & Vilgis, 2020).

The study investigated the use of XG and GG for creating porous SSE-3D printed medical devices with wound healing properties. While XG alone showed insufficient viscoelastic properties for 3D printing, a 50:50 mixture of XG and GG exhibited optimal viscoelasticity, offering excellent recovery of G’ after shear stress. The mixture produced a tough filament and demonstrated promising printability, with strong self-healing and viscoelastic behavior. The inks formed hydrogels easily without needing temperature, pH changes, or ions, highlighting the potential of XG and GG for effective 3D printing of medical devices for wound healing (Gholap et al., 2024; Li, Tan, & Li, 2018). The ink preparation involved quickly homogenizing the polysaccharide mixture to eliminate lumps, making it easy to produce a "ready-to-use" powder that can be resuspended when needed. This approach streamlines production, reduces costs, and is adaptable for both personalized and large-scale manufacturing. It also eliminates stability and storage issues linked to water in resuspended hydrogels, minimizing risks of chemical changes or contamination. After finding the optimal concentration, the addition of organic acid crosslinkers like citric, succinic, and tartaric acid was explored to improve the mechanical properties and prevent swelling, ensuring the scaffolds maintained their shape and microstructure post-3D printing (Alavarse et al., 2022; Patel, Maji, Moorthy, & Maiti, 2020). To create eco-friendly, affordable scaffolds, non-toxic, biocompatible organic acids like citric, succinic, and tartaric acids were tested as crosslinkers. The autoclave sterilization process (121°C) facilitates the formation of covalent ester bonds between the acids' carboxyl groups and the OH groups in the polysaccharides, forming a network of hydrophobic ester bridges. This modification doesn't impact biodegradability, as ester bonds can be broken down by esterases found in humans, plants, and microorganisms, ensuring environmental and human safety. A 20% organic acid concentration was selected for the crosslinking, maintaining the "ready-to-use" feature of the ink, with no significant rheological changes. Among the acids, succinic acid showed the best self-healing properties (Diaz-Gomez et al., 2022).

Thermogravimetric analysis revealed that both XG and GG are resistant to high temperatures, with degradation occurring above 275°C and 235°C, respectively. These polysaccharides were used for crosslinking and sterilization in a single autoclaving step, making the process cost-effective and efficient. The sterilization did not alter the shape or structure of the scaffolds, although some color change occurred. The scaffolds were tested for their ability to resist compressive forces, with all freeze-dried scaffolds maintaining shape and stiffness, showing good resistance to external forces, whether crosslinked or not (Diaz-Gomez et al., 2022) (Virzì et al., 2024).

The scaffolds showed varying mechanical responses to compressive forces, with S–TA deforming and completely breaking after two compressions, while S–CA and S–SA scaffolds exhibited more resilience, retaining their shape and porosity after compression and decompression. The S–SA scaffold demonstrated superior stiffness and resistance to compression compared to S–CA, with a slightly higher Young's modulus. After 7 days of swelling in PBS, both S–SA and S–CA scaffolds maintained their structure and porosity, with S–SA being tougher. The S–SA scaffold outperformed other polysaccharide-based scaffolds, showing enhanced mechanical properties and better resistance to compressive cycles. SEM analysis of S–NC and S–SA scaffolds revealed compact filaments with macroporosity, beneficial for cell migration and tissue regeneration. S–SA scaffolds exhibited 93.18% porosity, ideal for tissue engineering applications. A preliminary soaking removed excess organic acid, eliminating non-reacted crosslinker crystals, which is important for preventing interference in biological tests (Virzì et al., 2024) (Diaz-Gomez et al., 2022).

The water contact angle was higher for the crosslinked S–SA scaffold (70.86°) compared to the non-crosslinked S–NC scaffold (40.20°), indicating increased hydrophobicity due to ester bond formation during crosslinking. Contact angles between 30° and 70° are known to promote cell adhesion, making the S–SA scaffold suitable for tissue engineering applications (Nosrati, Khodaei, Alizadeh, & Banitalebi-Dehkordi, 2021).

Edible coatings offer a promising method to extend food shelf life and improve quality by forming a protective layer on the surface. Coatings made from biodegradable polymers are particularly valued for their biocompatibility, biodegradability, and edibility. Biodegradable polymers contain various functional groups that can be easily modified into derivatives with improved properties, making them potential alternatives to non-degradable plastics used in food packaging (Sabaa et al. 2024) (Abdel Aziz et al. 2021).

The incorporation of additives like nanomaterials, particularly ZnO-NPs, enhances the effectiveness of edible coatings. ZnO-NPs, known for their antimicrobial and biocompatible properties, have been approved by the FDA as safe for human consumption. They improve the mechanical, thermal, and barrier properties of coatings while also enhancing their UV-shielding capabilities. ZnO-NPs have been successfully integrated into various coating systems, such as carrageenan, chitosan, and carboxymethyl cellulose, showing significant improvements. While ZnO-NPs are typically produced from zinc salts, the use of plant extracts for their eco-friendly synthesis is becoming more popular due to its potent antioxidant and antimicrobial benefits (Smaoui et  al. 2023) (Abdel Aziz et  al. 2022).

- Opacity and transparency: Transparent food packaging materials are often preferred by consumers, but for optimal light barrier properties, they need to be opaque. CMC and Xa films showed opacity values of 0.81±0.02 and 0.83±0.02, respectively. A slight increase in transparency was observed in the CMC/Xa composite film (0.92±0.03) due to the interaction between CMC and Xa. When ZnO-NPs were added at 5 wt%, the opacity of the CMC/Xa film increased to 3.02±0.09, attributed to the light-scattering effect of the ZnO-NPs. This increase in opacity was consistent with previous studies that found ZnO-NPs also raised the opacity in other film systems, like alginate/Citronella oil and chitosan/Melissa officinalis oil films. However, the transparency of the CMC/Xa film was not significantly affected by the highest concentration of ZnO-NPs (Sabaa et al. 2024)

- Water vapor permeability (WVP): WVP measurement evaluates the effectiveness of edible coatings in preventing moisture transfer between food and its surroundings. The CMC/Xa film had a WVP of 16.2±0.47 g mm/m² day kPa, which significantly decreased with the incorporation of ZnO-NPs. This reduction was due to hydrogen bonding between ZnO-NPs and the film components and the occupation of voids within the film structure, limiting fluid flow. Similar results have been observed in other biopolymer films, such as carrageenan and CMC/chitosan films. These findings suggest that ZnO-NPs enhance the fluid barrier properties of CMC/Xa films (Sabaa et al. 2024).

- Mechanical properties: The addition of ZnO-NPs to the CMC/Xa film increased its tensile strength (TS) to 24.07 MPa at 5 wt%, due to interactions between ZnO-NPs and the polymer chains. However, the elongation at break (EB) decreased from 32.29% to 24.22%, indicating reduced flexibility. This was due to limited chain mobility from crosslinking induced by ZnO-NPs, though the film's toughness improved. (Wang et al. 2019) (Sabaa et al. 2024).

- Antimicrobial properties: The antimicrobial effects of the coatings on B. subtilis, E. coli, and S. racemosum showed no apparent activity for CMC, Xa, and CMC/Xa films against the tested microorganisms, consistent with previous studies. However, the addition of ZnO-NPs to CMC/Xa resulted in significant microbial inhibition. This enhancement in antimicrobial activity is attributed to the potent properties of ZnO-NPs, which release Zn2+ ions. These ions can penetrate microbial cell membranes, disrupt negatively charged components, and lead to cell death. Additionally, reactive oxygen species generated by ZnO-NPs interact with and damage the microbial cell membranes, enhancing the antimicrobial effect (Krishnamoorthy et al. 2022) (Sabaa et al. 2024).

- Shelf‑life studies: Tomatoes of similar size were coated with the CMC/Xa/3ZnO coating using the dipping method to evaluate its effectiveness in extending shelf life. This coating was chosen for testing due to its superior properties compared to other systems. Fresh tomatoes without coating, those coated with CMC/Xa, and those coated with CMC/Xa/3ZnO were compared (Sabaa et al. 2024).

- Mass loss studies: The CMC/Xa/3ZnO coating effectively reduced fluid loss from tomatoes over 20 days, with only 11.2% mass loss observed. This was due to its superior barrier properties. In contrast, uncoated tomatoes lost around 50.3% of their mass, while CMC/Xa-coated tomatoes experienced a 27.5% mass loss due to the coating's poor barrier properties (Sabaa et al. 2024).

The polyelectrolyte complex formed between chitosan and xanthan gum, along with nanoparticle incorporation, significantly enhances mechanical properties. The resulting nanocomposite showed up to 6.65 times greater tensile strength and 3.57 times higher elongation at break than pure chitosan. Its transmittance was reduced, offering better UV protection for food, and it exhibited improved barrier properties against water vapor and oxygen. The film was fully biodegradable after two months, losing about 88% of its initial weight, making it an eco-friendly option for sustainable food packaging (Girdhar et al. 2023).

- Thickness and Transparency: The thickness of the films slightly increased from 0.05 mm (C) to 0.1 mm (CXZ5) with varying biopolymer or nanocomposite ratios, showing a positive correlation with the number of components. The transparency of the films was affected by the addition of xanthan gum and ZnO nanoparticles, which reduced transparency and increased opacity. The opacity values for CXZ1, CXZ3, and CXZ5 were 3.4, 4.7, and 5.1, respectively, due to the scattering of ZnO nanoparticles, although the films remained visually transparent (Girdhar et al. 2023).

- Mechanical Studies. The mechanical properties of chitosan films improved with the addition of xanthan gum and ZnO nanoparticles. The tensile strength of the chitosan/xanthan gum blend increased due to enhanced intermolecular interactions, forming a polyelectrolyte complex. Incorporating ZnO nanoparticles further strengthened the films, with optimal tensile strength observed at a 3% ZnO loading. This was attributed to better cross-linking between polymer chains, leading to stronger films. However, beyond this concentration, excessive ZnO caused a decrease in tensile strength and elongation due to nanoparticle agglomeration. The films exhibited improved flexibility and strength without compromising stretchability, demonstrating the potential of ZnO-loaded chitosan/xanthan gum films for food packaging applications.(Girdhar et al. 2023)

Thermogravimetric Analysis. The thermal stability of the packaging films was assessed using TGA, revealing how their mass changes with temperature. In the C film, water release from chitosan’s functional groups occurred up to 170°C, followed by a significant degradation phase from 170°C to 425°C, resulting in a 54.075% mass loss. Other films displayed similar thermal behavior, with the CX film showing a higher degradation rate. However, the CXZ3 film demonstrated slightly improved thermal stability, retaining 45.74% of its weight at 499.28°C, likely due to better interactions between the matrix and fillers (Girdhar et al. 2023).

- Water Vapor Transmission Rate: The water vapor transmission rate (WVTR) significantly impacts the shelf life of packaged food by affecting moisture absorption. The pure chitosan film exhibited the highest WVTR of 560.934 g/m²/day due to its hydrophilicity. When combined with xanthan gum, the film showed a 11.88% reduction in water vapor permeability, likely due to hydrogen bond formation between the biopolymers. Nanocomposite films (CXZ1, CXZ3, CXZ5) displayed further reductions in WVTR (51.77%, 54.95%, and 47.17%, respectively) because the ZnO nanoparticles filled the pores of the matrix, improving moisture resistance. These findings align with studies that show nanofillers enhancing the water barrier properties of biopolymer films (Girdhar et al. 2023).

- Water Absorption Capacity: The water absorption capacity test determines the film's water sensitivity for practical use. The pure chitosan film had a high water absorption of 75.683%. However, when xanthan gum and ZnO nanoparticles were added, water absorption was significantly reduced. The CXZ1 and CXZ3 films showed reductions of 58.94% and 59.88%, respectively, in water absorption compared to the control. This decrease is attributed to the interaction between chitosan's hydrophilic groups and the nanofillers, which reduced the availability of these groups and, in turn, the water absorption capacity. Similar results have been reported by other studies on biopolymer nanocomposites (Girdhar et al. 2023).

- Oxygen Transmission Rate and Permeability: Oxygen transmission through packaging films can lead to food spoilage by promoting microbial growth and oxidation, which affects taste, color, and nutritional quality. The addition of xanthan gum and ZnO nanoparticles to chitosan films improved the oxygen barrier properties. The CX film showed a slight decrease in oxygen transmission rate (OTR) due to the interaction between the biopolymers. The ZnO nanocomposite films (CXZ1 and CXZ3) displayed significantly lower OTR, enhancing their potential as effective food packaging materials to protect food from microbial contamination (Girdhar et al. 2023).

The oxygen permeability (OP) of the films increased gradually with their thickness compared to the pure chitosan film, with values observed at 0.02308 atm at 25 °C for all synthesized films (CX, CXZ1, CXZ3, and CXZ5) (Girdhar et al. 2023).

- UV Barrier. The UV-screening capability of packaging is essential to prevent food deterioration from UV and visible radiations. Pure chitosan film showed the lowest UV absorption, while the nanocomposite films, especially those with ZnO nanoparticles, demonstrated higher absorption in the UV region, indicating strong UV protection. This aligns with findings from other studies showing that ZnO-based nanocomposites effectively shield food from UV damage (Girdhar et al. 2023).

- Biodegradation. Biodegradable films, unlike conventional plastics, do not contribute to environmental pollution. Their degradation is influenced by factors such as moisture, temperature, and pH, with fillers enhancing both biodegradability and durability. In soil burial tests, pure chitosan films completely degraded within a month, while films with xanthan gum and ZnO nanoparticles degraded more slowly, with the latter films showing significant degradation after two months. The presence of nanofillers improved film durability but also led to faster degradation in films with nanoparticle aggregation. Microbial activity during soil burial caused changes in the films' structure, weight, and color, supporting their biodegradation (Girdhar et al. 2023).

2.5. Essential Oil added biopolymers

The use of synthetic fungicides in postharvest treatments has raised health concerns due to potential residue contamination and pathogen resistance. As a result, there is growing interest in natural alternatives like plant essential oils (EOs), particularly clove essential oil (CEO), which has shown effectiveness against fungal pathogens like Aspergillus niger. However, CEO's antimicrobial properties are limited by its volatile and poorly water-soluble compounds. To overcome these challenges, nano-encapsulation techniques have been developed to protect the oil from evaporation, improve stability, and enhance its water solubility and bioavailability. Chitosan nanoparticles (ChNPs), formed through ionic gelation, offer a stable and efficient encapsulation method, improving controlled release and antifungal activity compared to other encapsulation methods like liposomes. This study focused on encapsulating CEO into ChNPs to enhance its antifungal effects against Aspergillus niger. (Safari et al. 2019).

Konjac glucomannan (KGM) is a promising biodegradable material for edible packaging, but its use is limited by high water absorption, weak mechanical strength, and low antibacterial activity. To improve its properties, KGM is combined with other polymers and antioxidant-rich substances. Incorporating plant essential oils (PEOs) for added antibacterial properties is challenging due to instability and compatibility issues. Innovations like nanoemulsions and Pickering emulsions help load PEOs into films, improving their effectiveness, but these still weaken film strength and moisture resistance. This study aims to enhance KGM-based films with PEO-loaded emulsions for better food preservation (Waterhouse et al. 2022).

2.5.1. Preparation of oil-loaded and unloaded particles: The oil-loaded particles were prepared using a two-step process involving emulsion formation and solidification. First, oil droplets were created in a chitosan (Ch) solution using an oil-in-water emulsion method, followed by solidification through ionic gelation with TPP, forming nanoparticles (NPs). Two concentrations of Ch (0.3% and 0.5% w/v) were prepared in an acetic acid solution, and pH was adjusted to 4.6. Tween 80 was added as a surfactant. The nanoparticles were characterized using Dynamic Light Scattering (DLS), FE-SEM, and FTIR. UV-vis spectroscopy of CEO in ethanol showed absorption peaks at 226 nm and 282 nm, which were used to calculate encapsulation efficiency and conduct release studies (Safari et al. 2019).

2.5.2. Encapsulation efficiency (EE), loading capacity (LC) and yield determination

The encapsulation efficiency (EE) of CEO-loaded particles with Ch to TPP mass ratios of 1:1 and 1.6:1 was measured using UV-vis spectrophotometry at 282 nm. The results revealed no significant difference in EE values despite varying particle sizes. Both mass ratio formulations showed a similar trend, with EE decreasing as the initial CEO concentration increased (Safari et al. 2019).

2.5.3. Release of essential oils

The migration properties of essential oils from films were tested using different food simulants, including distilled water, 50% aqueous ethanol, and 95% aqueous ethanol. Films were immersed in each simulant, and absorbance was measured at 255 nm over time. Essential oil release was also evaluated using headspace solid-phase microextraction (SPME) and gas chromatography with flame ionization detection (GC-FID). The volatile compounds, mainly carvacrol and thymol in oregano oil, were quantified by peak area analysis. The retention rate of essential oil in the film was calculated by comparing the remaining volatiles in the film to the initial oil amount. ZPEO-KGM films were analyzed over time to track essential oil release (Waterhouse et al. 2022).

2.5.4. Antioxidant, antibacterial and preservative capacities

The antioxidant activity of the films was evaluated using the DPPH radical scavenging assay and liquid culture tests. Different film amounts were immersed in a DPPH solution, with Vitamin C as the control. Antimicrobial activity was tested by culturing E. coli and S. aureus with the films at 37°C for 12 hours, measuring growth by optical density. ZPEO-KGM films were also tested by wrapping fresh strawberries and storing them for 20 days at 25°C and 55% humidity. The ZPEO-KGM films showed better stability and film-forming properties, with smaller, more uniform droplets compared to pure KGM. After 30 days, the particle size increased slightly, indicating high stability (Waterhouse et al. 2022).

2.5.5. Dynamic rheological properties of film-forming liquids

The dynamic rheological properties of the film-forming liquids revealed structural interactions among the components. Pure KGM exhibited liquid-like behavior at low frequencies (G'' > G'), whereas ZPEO-KGM showed solid-like behavior (G' > G'') at high frequencies, indicating a transition from liquid to weak gel with higher ZPEO concentrations. This transition suggested a more compact network with stronger mechanical rigidity due to enhanced molecular entanglements, hydrogen bonds, and hydrophobic interactions. All film-forming liquids displayed non-Newtonian behavior, with viscosity decreasing as the shear rate increased. While pure KGM had high viscosity but poor film formation, adding ZPEO reduced viscosity and improved film-forming properties (Waterhouse et al. 2022).

2.5.6. Release of essential oils (EOs)

The release of essential oils (EO) from ZPEO-KGM films varied with food simulants, with slower release in 95% ethanol and faster release in distilled water. The release followed a fast-slow pattern, influenced by film structure and solvent type. Increasing ZPEO concentration slowed EO release, as ZPEO's hydrophobic properties acted as a barrier, enhancing water resistance and limiting EO diffusion. The 50% ZPEO-KGM film retained the most volatiles, showing stronger interactions between EO and the film matrix. This improved antioxidant and antibacterial properties, helping extend the shelf life of coated food products (Waterhouse et al. 2022).

2.5.7. Antioxidant, antibacterial and fresh-keeping effects of films

The antioxidant activity of active packaging films was assessed using the DPPH free radical scavenging assay. The pure KGM film showed low antioxidant activity, while increasing ZPEO concentration improved oxidation resistance, with the 75% ZPEO-KGM film performing similarly to vitamin C. The antioxidant effect was mainly due to oregano essential oil in ZPEO. The antibacterial performance of KGM films against \*Staphylococcus aureus\* and \*Escherichia coli\* was minimal for pure KGM but significantly enhanced by ZPEO, especially against \*E. coli\*. Oregano essential oil's compounds, such as carvacrol and thymol, disrupted bacterial cell membranes, with higher ZPEO concentrations delaying bacterial growth, making the ZPEO-KGM film effective for early-stage food preservation (Waterhouse et al. 2022)

2.6. Data Measuring, Characterization and Statistical Analysis

- Breaking Power and Tensile Strength: The mechanical properties of any biofilm are determined by its tensile strength (TS) and energy at break (EB), which indicates the integrity of the films, their flexibility, stretchability, and endurance against stress that may be imparted during transportation and storage. Breaking power and tensile strength of plastic were calculated by UV rays under the UV lamp in addition to analysis by the testing machine. Percentage breaking power was analyzed by parallel films then pulled apart l% breaking power calculated by length extension of the film. Tensile strength was calculated by Eq. (1):

Tensile strength =Breaking force/Film area (Batool et al. 2021) (Nur et al. 2024) (Saha et al. 2024)

- Moisture Transmission: The moisture transmission was gravimetrically analyzed by desiccator in which temperature and humidity were controlled. Weight of the fruits was analyzed periodically, and moisture transmission was calculated by Eq. (2).

Moisture Transmission = Mass change Area × Time (Batool et al. 2021)

- Decay rate: The decay of Vitis vinifera fruits was measured during storage to estimate the duration the fruits stayed intact, without changes in taste or quality. Grapes showing mold growth were considered decayed, and the decay rate was calculated using the below Eq;

% decay rate = weight of decayed fruits / total weight of fruits × 100. (Batool et al. 2021)

- Morphological Analysis:

Morphological analysis involves studying the structure and form of materials or objects, typically using imaging techniques like scanning electron microscopy (SEM), optical microscopy, or atomic force microscopy (AFM) to capture high-resolution images of the sample. The images are then analyzed to measure characteristics such as size, shape, surface roughness, and distribution, often with the help of image processing software. Additional methods like X-ray diffraction (XRD) or FTIR can be used to examine the material's crystalline structure or chemical composition. This analysis helps understand the relationship between a material's physical structure and its properties or performance in various applications. (Nur et al. 2024)

- Fourier transform infrared spectroscopy (FTIR) analysis: FTIR (Fourier Transform Infrared Spectroscopy) is used for identifying and analyzing the chemical composition of materials. It works by measuring the absorption of infrared radiation by a sample, which causes molecular vibrations at specific frequencies. By analyzing the resulting spectra, FTIR can provide information about functional groups, chemical bonds, and molecular structures present in a substance. It is widely used in fields such as materials science, chemistry, biochemistry, and environmental analysis for both qualitative and quantitative assessments. (Gisan et al 2020) (Zhang et al. 2021) (Safari et al. 2019) (Pleva et al. 2021) (Sabaa et al. 2024)

- Field emission-scanning electron microscopy (FE-SEM) observation: The morphology of Ch-NPs and CEO-Ch-NPs, prepared using mass ratios of Ch to TPP of 1:1 and 1:1:1, respectively, was studied by FESEM (MIRA 3, TESCAN, Czech Republic). Freshly prepared NPs were diluted with distilled water and one drop of diluted dispersions was dried at room temperature. The dried NPs were coated with gold and then examined. (Safari et al. 2019)

- Thermal Properties:

Thermal properties in biofilm and biopolymer studies refer to how materials respond to changes in temperature. These properties include thermal stability, melting point, glass transition temperature (Tg), heat capacity, and thermal conductivity, which influence the material’s performance and functionality under different conditions. In biofilms and biopolymers, these thermal characteristics are critical for understanding how the materials will behave during processing, storage, or in applications such as packaging. Thermal analysis techniques like thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and thermal conductivity measurements are commonly used to evaluate these properties, providing insights into the material’s resistance to degradation, its thermal transitions, and its suitability for specific environmental conditions. (Nur et al. 2024)

- Wettability Test:

The water contact angle (WCA) was used to measure the hydrophilicity of materials by capturing the image. The wettability test is crucial in biofilm and biopolymer studies to evaluate how easily a liquid spreads or adheres to a material’s surface, which directly impacts its interactions with liquids, such as water or oils. This test is important for understanding the material's surface properties, such as hydrophobicity or hydrophilicity, which influence applications like packaging or coatings. It is typically done by measuring the contact angle formed when a droplet of liquid is placed on the material's surface. A contact angle greater than 90° indicates hydrophobicity, while angles less than 90° suggest hydrophilicity. The test provides insights into the surface energy of the material, which helps determine its suitability for specific applications or processing conditions. (Nur et al. 2024) (Pleva et al. 2021)

- Biocompatibility Analysis: Materials for biomedical applications that contact human skin must have good biocompatibility to ensure they are non-toxic, support cell viability, and do not cause irritation or inflammation. Testing ensures the material is safe, promotes proper cell growth, and integrates well with the skin without adverse effects. Biocompatibility analysis in biofilms and polymers involves assessing how well these materials interact with living organisms without causing harmful effects. This is crucial for their use in medical, pharmaceutical, or environmental applications, where the materials may come into direct contact with biological systems. The analysis typically includes in vitro tests, such as cell viability, cytotoxicity, and cell adhesion assays, to evaluate the material's effects on cell growth and behavior. In vivo tests may also be conducted to observe any immune responses, inflammation, or toxicity in a living organism. Biocompatibility ensures that the biofilm or polymer will not cause adverse reactions, such as toxicity or allergic responses, when used in medical devices, wound healing, or drug delivery systems. (Nur et al. 2024)

- Scanning electron microscopy (SEM) analysis: is a technique used to examine the surface structure and morphology of materials at high magnification. It works by scanning a sample with a focused beam of electrons, which interact with the material to produce various signals, such as secondary electrons, backscattered electrons, and X-rays. These signals are then collected and converted into detailed images that reveal the surface topography, texture, and composition. SEM is widely used in materials science, biology, and nanotechnology to investigate the fine details of a sample's surface, providing valuable insights into its properties and performance. (Gisan et al 2020)

- UV–Vis absorption spectroscopy: UV–Vis absorption spectroscopy measures how a material absorbs ultraviolet and visible light, providing information about its chemical composition, concentration, and molecular structure based on the absorption at specific wavelengths. (Batool et al. 2020)

- Encapsulation efficiency (EE), loading capacity (LC) and yield determination: Encapsulation efficiency (EE), loading capacity (LC), and yield determination are key parameters in assessing the effectiveness of encapsulation in drug delivery or biopolymer systems. EE measures the percentage of active ingredients successfully encapsulated within the material, while LC indicates the amount of active ingredient loaded per unit mass of the carrier. Yield determination assesses the overall amount of encapsulated product obtained from the process. These parameters help evaluate the efficiency, effectiveness, and practicality of the encapsulation process for various applications. (Safari et al. 2019)

- Statistical analysis: In biofilm and biopolymer studies, statistical analysis often involves techniques like ANOVA (Analysis of Variance) to compare the means of different groups and determine if there are statistically significant differences. ANOVA is commonly used to analyze experimental data with multiple groups or conditions. Software like SPSS, R, GraphPad Prism, and Minitab are frequently used to perform ANOVA, allowing researchers to assess the impact of various factors on the outcomes of biofilm or biopolymer experiments, and to identify any significant variations or patterns in the data. (Gisan et al 2020) (Pleva et al. 2021)

- Enzymatic biodegradation: Enzymatic biodegradation tests in biofilms and biopolymers are used to evaluate how these materials break down when exposed to specific enzymes, simulating natural degradation processes. These tests help assess the material's environmental impact, biodegradability, and suitability for applications where degradation is desired, such as in packaging or medical devices. The process typically involves incubating the biofilm or polymer with a relevant enzyme, monitoring changes in weight, structure, or chemical composition over time, and analyzing the degradation rate. The results provide valuable insights into the material's ability to degrade under natural or controlled conditions, helping determine its environmental compatibility. (Gisan et al 2020)

- The percentage weight loss: (%Weight loss) The percentage weight loss in biofilms and biopolymer studies is a key metric used to quantify the extent of degradation or degradation rate over time. It is determined by comparing the initial weight of the sample to its weight after exposure to environmental factors such as enzymes, moisture, or heat. This calculation provides insight into the material’s stability and biodegradability. A higher percentage weight loss indicates more significant degradation, which is useful for evaluating the material's suitability for applications that require controlled degradation, such as in waste management or biodegradable product design.

It can be estimated by using equation below: % Weight Loss = [(Initial Weight - Final Weight) / Initial Weight] × 100 (Gisan et al 2020)

- The percentage of water absorption: The percentage of water absorption is calculated to determine how much water a biofilm or biopolymer material can absorb when immersed in water. The equation used is:

% Water Absorption = [(Final Weight - Initial Weight) / Initial Weight] × 100

This equation provides insight into the material's hydrophilicity and its ability to retain moisture, which is important for applications like packaging, coatings, or medical devices. (Gisan et al 2020)

- Testing and characterization Tensile properties: Testing and characterization of tensile properties in biofilms and biopolymers involve measuring a material’s response to stretching forces, assessing strength, flexibility, and durability. A universal testing machine is typically used to apply uniaxial force while measuring the material's elongation and force until failure. Key parameters include tensile strength, elongation at break, and Young's modulus. These properties help determine suitability for applications like packaging or medical devices. Software such as VICTOR, along with others like TestWorks and Bluehill, are often used to analyze and interpret the data, offering insights into the material's mechanical performance (Gisan et al 2020).

3. Methods and Materials

The research methodology for this study involved an extensive literature review of relevant academic papers and publications. The primary sources of information were ScienceDirect, Scopus, and MDPI, which provided peer-reviewed articles and research studies related to biopolymers, biofilms, and their applications, particularly in the food industry. The search process was guided by specific keywords and search inputs derived from the topics listed in the table of contents, including terms such as "biopolymers," "biofilms," "biodegradation," "starch-based biofilms," "PLA-based biofilms," and "biopolymer production routes," among others. These search terms were used to filter and narrow down relevant papers published from 2020 onward, ensuring the inclusion of the most up-to-date research.

The selection of articles was based on their relevance to the various subtopics within the broader themes of biopolymers, biofilms, and their applications in food packaging and sustainability. Studies that discussed the synthesis, properties, and characterization of biopolymers such as PLA, starch, inulin, xanthan gum, and essential oils, as well as their biodegradability, antimicrobial properties, and roles in biofilm production, were prioritized. Research articles focusing on the challenges, limitations, and advancements in biofilm technology, particularly in the context of the food industry, were also included in the review.

All selected papers were thoroughly analyzed, and key findings were synthesized to provide a comprehensive understanding of the current state of research on biopolymers and biofilms. Information related to the preparation methods, physical and functional characterization, and applications of various biopolymers in food packaging was highlighted. Furthermore, studies addressing the environmental impact, biodegradability, and compostability of biopolymer-based biofilms were carefully reviewed to emphasize the potential for sustainable material solutions.

The materials used in this research were primarily secondary data obtained from the aforementioned academic databases. The analysis focused on the most recent and relevant publications to ensure the inclusion of cutting-edge research in the field. This methodology enabled a detailed and well-rounded exploration of biopolymer-based biofilms and their applications.

**Table 1: Research Keywords and Databases Used**

|  |  |
| --- | --- |
| **Research Keywords** | **Databases Used** |
| Biopolymers, Biofilms, Biodegradation | ScienceDirect |
| Starch-Based Biofilms, PLA-based Biofilms | Scopus |
| Biopolymer Production Routes, Biofilm Synthesis | MDPI |
| Biopolymer Applications, Food Packaging | ScienceDirect, Scopus |
| Biodegradable Biofilms, Compostability | MDPI, Scopus |

12. Results and discussions

The literature review revealed significant advancements in the development and application of biopolymers and biofilms, particularly in food packaging. The synthesis, properties, and biodegradability of various biopolymers such as PLA, starch, inulin, xanthan gum, and essential oils were thoroughly examined, with a focus on their applications in sustainable packaging solutions.

4.1 PLA-Based Biofilms  
Polylactic acid (PLA) emerged as a highly studied biopolymer in biofilm production due to its biodegradable nature and compatibility with food packaging applications. The analysis highlighted PLA’s excellent mechanical properties and biodegradability when subjected to environmental conditions. However, PLA’s hydrophobicity and limited barrier properties were identified as major challenges in its application. To address these limitations, various enhancement techniques, including the incorporation of nanoparticles, plasticizers, and blending with other biopolymers, were discussed. Studies demonstrated that these modifications improve PLA’s flexibility, water vapor permeability, and overall functionality in food packaging.

4.2 Starch-Based Biofilms  
Starch-based biofilms were found to be promising alternatives to traditional plastic materials due to their abundant availability, biodegradability, and non-toxicity. The review emphasized the potential of starch as a primary component in biofilm production, with particular focus on the synthesis of nano-starch and tannic acid-coated nano-starch. These modified starches exhibited improved mechanical and functional properties, such as enhanced tensile strength and moisture resistance. Furthermore, the biodegradability of starch-based biofilms was reaffirmed, as they broke down quickly under composting conditions. However, the study also pointed out that the use of starch alone often results in poor water resistance, necessitating the use of additives to improve its performance in various environmental conditions.

4.3 Inulin and Xanthan Gum-Based Biofilms  
Inulin-based biofilms demonstrated unique properties due to the natural prebiotic activity of inulin, which is beneficial for health and food preservation. The physical properties of inulin biofilms, such as flexibility and transparency, were favorable for packaging applications. Inulin also acts as a plasticizer, improving the flexibility and mechanical strength of biofilms. However, the challenge lies in the variability of inulin's physical properties depending on its source, which can affect the consistency of the biofilms produced.

Xanthan gum, another polysaccharide, was explored for its rheological properties and ability to form stable gels. It was found that xanthan gum-based biofilms had good film-forming abilities and exhibited effective antimicrobial properties, making them ideal candidates for food packaging. The incorporation of xanthan gum with other biopolymers enhanced the biofilm’s mechanical strength, water resistance, and antimicrobial activity, though its effectiveness varied based on the concentration of xanthan gum and the specific application.

4.4 Essential Oil-Loaded Biopolymers  
The incorporation of essential oils (EOs) into biopolymer-based films was another prominent focus of the literature. Essential oils such as thyme, clove, and oregano were shown to impart antioxidant, antibacterial, and preservative properties to biofilms, significantly extending the shelf life of food products. The encapsulation of EOs into biopolymer matrices helped control the release of active compounds, offering sustained antimicrobial effects. Studies demonstrated that the dynamic rheological properties of the films could be tailored by adjusting the concentration and type of essential oil used, optimizing their functional properties for different food applications.

4.5 Biodegradation and Compostability  
One of the major themes identified in the review was the biodegradability and compostability of biopolymer-based biofilms. The analysis confirmed that biopolymers such as PLA and starch-based biofilms degrade more efficiently under controlled composting conditions compared to conventional plastics. However, the rate of biodegradation varied based on environmental factors such as temperature, humidity, and microbial activity. It was evident that while biopolymers offered a promising solution to plastic waste, the complete degradation of biopolymer films required optimized conditions, and in some cases, the addition of specific microorganisms to accelerate the process.

4.6 Challenges and Future Directions  
Despite the promising properties of biopolymers and biofilms, several challenges remain in their widespread adoption. The mechanical strength, moisture resistance, and shelf-life limitations of certain biopolymers continue to hinder their potential in food packaging. Moreover, the high cost of production, scalability issues, and the lack of infrastructure for large-scale composting of biopolymer-based products are significant barriers to their commercialization. Future research should focus on developing new biopolymers with enhanced properties, optimizing production processes to reduce costs, and exploring innovative ways to improve the environmental impact of these materials. The use of waste products, such as food industry by-products, as raw materials for biofilm production could also offer a sustainable solution to reducing waste and creating circular bio-based economies.

5. Conclusion

The review of biopolymer-based biofilms has highlighted their significant potential in revolutionizing sustainable packaging solutions, particularly in the food industry. Various biopolymers, including PLA, starch, inulin, xanthan gum, and essential oils, have shown promise in producing biofilms that are biodegradable, compostable, and capable of providing antimicrobial and antioxidant properties. Despite their advantages, challenges such as mechanical strength, moisture resistance, and cost-effectiveness continue to limit their widespread adoption. However, ongoing research focusing on improving the properties of these materials, optimizing production processes, and exploring waste-derived raw materials offers a promising path toward overcoming these barriers. In the future, biopolymer-based biofilms have the potential to contribute significantly to reducing plastic waste and promoting sustainability in food packaging and other applications.

6. Recommendations

Based on the findings of this review, the following recommendations are proposed to further advance the development and application of biopolymer-based biofilms:

- Enhancement of Material Properties:  
Future research should focus on improving the mechanical strength, water resistance, and flexibility of biopolymer-based biofilms. This can be achieved by incorporating additives such as nanoparticles, plasticizers, and other biopolymers to enhance the overall performance of the films in various environmental conditions.

- Optimization of Production Methods:  
The scalability and cost-effectiveness of biopolymer production need further optimization to make these materials viable for large-scale commercial use. Research into more efficient synthesis methods, such as green chemistry techniques and the use of renewable resources, could help lower production costs and reduce the environmental footprint.

- Focus on Biodegradation and Compostability:  
Although biopolymers are biodegradable, the degradation rates can be influenced by environmental factors. Future studies should explore ways to accelerate the biodegradation of biofilms under a broader range of environmental conditions. The development of biofilms with faster breakdown times and the inclusion of microorganisms that can enhance degradation could make these materials more effective in waste management systems.

- Incorporation of Waste Materials:  
Investigating the use of food industry by-products and agricultural waste as raw materials for biofilm production could provide an additional avenue for sustainable biofilm development. Utilizing waste materials could reduce raw material costs while contributing to the circular economy and addressing waste management challenges.

- Expanded Applications Beyond Food Packaging:  
Although food packaging has been the primary focus, biopolymer-based biofilms have potential applications in other industries, such as medicine and agriculture. Exploring these additional markets could broaden the scope of biopolymer biofilms, creating new opportunities for sustainable material solutions in diverse fields.

- Collaboration and Standardization:  
Industry and academia should collaborate to standardize testing methods and performance criteria for biopolymer-based biofilms. This will ensure that biofilms meet specific industry requirements and regulatory standards, facilitating their wider adoption in various sectors.

By addressing these recommendations, biopolymer-based biofilms can make significant strides toward becoming a sustainable alternative to conventional plastic materials, benefiting both the environment and various industries in the long term.

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8. Appendices

- Appendix A: Search Keywords and Databases

This appendix provides a comprehensive list of the search keywords and academic databases used in the literature review.

| **Research Keywords** | **Databases Used** |
| --- | --- |
| Biopolymers, Biofilms, Biodegradation | ScienceDirect |
| Starch-Based Biofilms, PLA-based Biofilms | Scopus |
| Biopolymer Production Routes, Biofilm Synthesis | MDPI |
| Biopolymer Applications, Food Packaging | ScienceDirect, Scopus |
| Biodegradable Biofilms, Compostability | MDPI, Scopus |

- Appendix B: List of Relevant Biopolymers and Their Applications

This appendix provides a summary of the various biopolymers discussed in the literature review, along with their respective applications in biofilm production.

- **Polylactic Acid (PLA):** is one of the most commonly used biopolymers in food packaging due to its biodegradability and the fact that it is derived from renewable resources, such as corn starch or sugarcane. PLA biofilms are attractive alternatives to conventional plastic films, offering good mechanical properties, such as tensile strength and flexibility. However, one of the main limitations of PLA is its relatively low moisture resistance, which affects its ability to protect food products from environmental factors such as humidity and moisture. As a result, efforts are underway to enhance PLA’s performance through various modification techniques, such as blending with other biopolymers, incorporating nanoparticles, or using plasticizers to improve its hydrophobicity and barrier properties. These improvements are crucial for broadening PLA’s application in food packaging, where moisture protection is often essential.

**- Starch:** Often used in combination with other biopolymers to improve the flexibility and mechanical properties of biofilms. Starch-based biofilms are biodegradable and suitable for composting.

**- Inulin:** A prebiotic biopolymer with applications in food packaging. Its flexibility and mechanical strength can be enhanced by its role as a plasticizer in biofilm matrices.

**- Xanthan Gum:** A polysaccharide known for its film-forming ability and antimicrobial properties. Used in food packaging and medical applications.

**- Essential Oils:** When incorporated into biopolymer films, they provide antimicrobial, antioxidant, and preservative properties, particularly in food preservation.

- Appendix C: Biopolymer Production Routes

This appendix provides an overview of the various production routes for biopolymers that were discussed in the literature review.

**- PLA Production:** Derived from renewable resources like corn starch or sugarcane, PLA is synthesized through the fermentation of lactic acid followed by polymerization.

**- Starch Biofilm Production:** Starch is typically extracted from plant sources and then processed into films using methods like casting or extrusion. Modification with plasticizers or nanoparticles is common to improve properties.

**- Xanthan Gum Biofilm Production:** Xanthan gum is produced by fermenting sugars with Xanthomonas bacteria and is then incorporated into biofilm matrices for improved rheological and functional properties.