Chapter 2
Review of Literature

Modern research efforts are entering on the development of novel materials that possess distinctive properties to address the expanding and diverse requirements of industrial applications and societal needs (Norrrahim et al., 2021). The growing market demand for environmentally conscious packaging alternatives has created significant research opportunities in both industrial and academic sectors regarding biopolymer film packaging. The ongoing progress in biopolymer research and development offers promising pathways toward creating environmentally responsible packaging that balances ecological concerns with market demands.

2.1. Biopolymer as basic material for food packaging

Biopolymers derive their nomenclature from Greek etymology, with "bio" referring to nature and life, while "polymer" describes their molecular structure. According to IUPAC classification, these substances are classified as macromolecules due to their structure consisting of multiple recurring molecular subunits (Karmanov et al., 2021). According to the European Bioplastics Association's classification framework, materials can be categorized as biopolymers if they are either sourced from biological materials, capable of biodegradation, or possess both characteristics. This categorization reflects current environmental priorities, with biopolymers playing an essential role in the shift toward sustainable material usage across various industries, particularly in packaging and consumer products. The association promotes biopolymer adoption as a strategy to minimize dependence on petroleum-based resources while supporting circular economic principles (George et al., 2020). Biopolymers can be grouped into several categories. In a broad sense, they include polythioesters (PTEs), polyisoprenoids, polysaccharides, polyphenols, polyanhydrides, polyoxoesters, polyamides, and nucleic acids. A more specific classification divides them into four main groups: polysaccharides, proteins and polypeptides, polymers of fatty acids, and various other organic polymers (Andreeben et al., 2019). At present, biopolymers are recognized as eco-friendly and cutting-edge packaging materials owing to their abundant availability, renewability, lack of toxicity, biodegradability, facile functionalization, and environmentally friendly nature (Niaounakis, 2015; Wang et al., 2021). However, they often have lower density, strength, modulus, and thermal stability than their petroleum-based counterparts. Ongoing research aims to improve the properties of biopolymers to make them viable replacements for many plastics applications. Biopolymers either entirely biosynthesized by living organisms or chemically derived from natural sources, and they can undergo biological degradation with ease (Chincholikar et al., 2023). Various functional groups, including hydroxyl, amide, amino, phosphate, and phenols, are present in these biopolymers. There are three primary categories of natural biopolymers: polysaccharides, polypeptides, and polynucleotides. Polysaccharides consist of simple sugar units joined by glycoside bonds. Amino acids serve as the monomers for polypeptides, while polynucleotides (such as DNA and RNA) are composed of polymers of nucleotide monomers (Kumar et al., 2023). The classification of biopolymer has been shown in **Fig. 2.1.** These biopolymers hold immense potential to substitute traditional plastics owing to their non-toxicity, compatibility with biological systems, and rapid degradation. Additionally, they exhibit exceptional physical properties when used to create films (Mary et al., 2022). Moreover, biobased polymers serve as effective carriers for bioactive components, enhancing the functionality of packaging materials and extending the shelf-life of packaged food items (Andreeben et al., 2019).

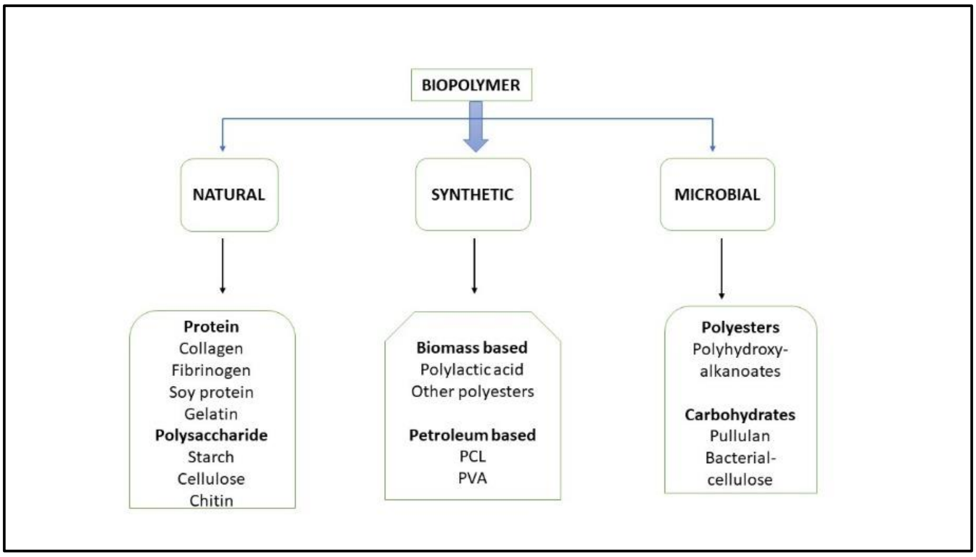

Fig. 2.1 Classification of biopolymer (Singh et al., 2021)

2.2. Starch as a packaging material

As the primary energy reservoir in living organisms, starch exists abundantly in nature, making up 60-75% of grain products by weight. At the molecular level, starch granules contain two forms of alpha-glucans - amylose and amylopectin - which together constitute 98-99% of the dry weight, with variations depending on the plant source. The linear polymer chains of amylose, connected through α-1,4-glycosidic bonds, demonstrate excellent film-forming capabilities particularly near fibers (Hoque et al., 2013). In contrast, amylopectin, which features both α-1,4-glycosidic and α-1,6-glycosidic bonds at branch points, provides enhanced thickening properties and stability during freeze-thaw cycles (Wang et al., 2020). The widespread availability, renewable nature, and biodegradable properties of starch make it an ideal candidate for various applications, particularly in bioplastics production (Gamage et al., 2022). Starch-derived films exhibit superior physical characteristics, including oxygen impermeability, and are both flavourless and transparent. However, the implementation of starch-based biopolymers faces several challenges that affect their performance. These include the necessity for modifications to enhance mechanical durability, water resistance, thermal stability, and processing efficiency for industrial use (Lauer et al., 2020). Furthermore, developing starch-based biodegradable packaging for food applications requires precise processing control and specific additive incorporation to optimize film properties (Agarwal et al., 2023). Despite these limitations, starch remains highly attractive as a biopolymer due to its abundance, cost-efficiency, and versatility in applications ranging from sustainable agriculture to food packaging (Dutta and Sit, 2022). The strong inter- and intra-molecular hydrogen bonding in starch results in an elevated near-melting temperature, which complicates the film packaging production process (Jariyasakoolroj et al., 2021). Since native starches often lack the necessary functionality for various applications, physical modifications represent a preferred approach for improving poor-quality starches cost-effectively and environmentally safely (Lim et al., 2001; da Rosa Zavareze and Dias, 2011). Starch modification techniques are employed to overcome native starch limitations and adapt it for specific food industry applications (Singh et al., 2011; Kaur et al., 2012). Among various sources, potato starch stands out as a particularly promising carbohydrate for food packaging applications (Niu et al., 2021). Its relatively large granules (25-100 μm) exhibit a B-type crystalline structure (Jagadeesan et al., 2020). Notable characteristics include high viscosity, superior paste clarity, minimal retrogradation tendency, low pasting temperature, and facile granule expansion (Zhang et al., 2020; Li et al., 2021). Potato starch contains higher phosphorous content compared to other starch sources (Zhang et al., 2020) and possesses the necessary thermoplastic properties for bio-film formation (Varatharajan, 2010). These distinctive features collectively make potato starch an exceptional choice as a functional biomaterial in food and polymer science applications

(Singh et al., 2008). The adoption of starch-based packaging in food industries aligns with increasing demands for sustainable packaging solutions, contributing to plastic waste reduction and environmental preservation. The development of starch-based materials with improved mechanical strength, water resistance, and barrier properties enables their use in challenging packaging applications, including moisture-sensitive products and perishable goods (Cheng et al., 2021). These advancements in material engineering expand the scope of starch-based packaging to encompass a wider range of products, reinforcing its position as a viable alternative to conventional packaging materials. Films made from starch-built composites or blends employed in food packaging must possess superior mechanical endurance, higher gas resistance, good adhesion to food antibacterial properties, water barrier characteristics, and oxidation resistance (Su et al., 2022). Vegetables, fruits, meat, bread, and other items have all been packaged with starch-based biodegradable polymers, which have a wide range of commercial applications (Cheng et al., 2021). The physiochemical (thickness, solubility), barrier, and mechanical properties detailed in **Table 2.1** encompass a range of factors essential for evaluating the performance and functionality of these starch-based films. Films derived from starch optimized under the right conditions acquire better mechanical and barrier characteristics, including optical characteristics, and are transparent, odorless, tasteless, and colorless. However, they suffer from the retrogradation phenomenon and have limited applications because of their hydrophilic nature. To address the retrogradation, starch modification, blending with other ingredients, enzymatic treatments etc. can be achieved aiming to improve the quality and shelf life of starch-based food products (Vu and Lumdubwong, 2016). Also, to enhance the characteristics, some additives, such as lipids, other hydrocolloids, or reinforcing agents, may be applied (Souza et al., 2021). Starch films or coatings with functional compounds are another technique to improve their qualities, and starch films can be used to illustrate active packaging in this way (Pelissari et al., 2019). Higher amylose content starch-based films and sheets perform better mechanically. Although starch does not naturally exhibit this property, proper processing can turn it into a thermoplastic substance. In general, thermoplastic starch (TPS) is created by processing a starch–plasticizer mixture in an extruder at temperatures between 140 and 160℃ under high pressure and high shear (Chakraborty et al., 2022). Table 2.1 presents the range of commercially available. biodegradable starch-based packaging materials under the brand names Mater-Bi®, Bioplast, and Biopolar, etc. It showcases diverse biodegradable packaging materials derived from various starch sources. **Table 2.2** provides a compilation of biopolymer-based functional films specifically designed for active packaging applications. The table likely includes details such as the types of biopolymers used, the functional properties incorporated into the films, and their intended applications in the field of active packaging. Due to the different structural properties of macromolecules and the ratio of amylose to amylopectin, the starch source plays an essential role in the film characteristics. Film-forming processes for the development of biodegradable starch films are shown as below:

a) Solution casting (wet process) shown in **Fig. 2.2.**

b) Melt processing (dry process) shown in **Fig. 2.3.**

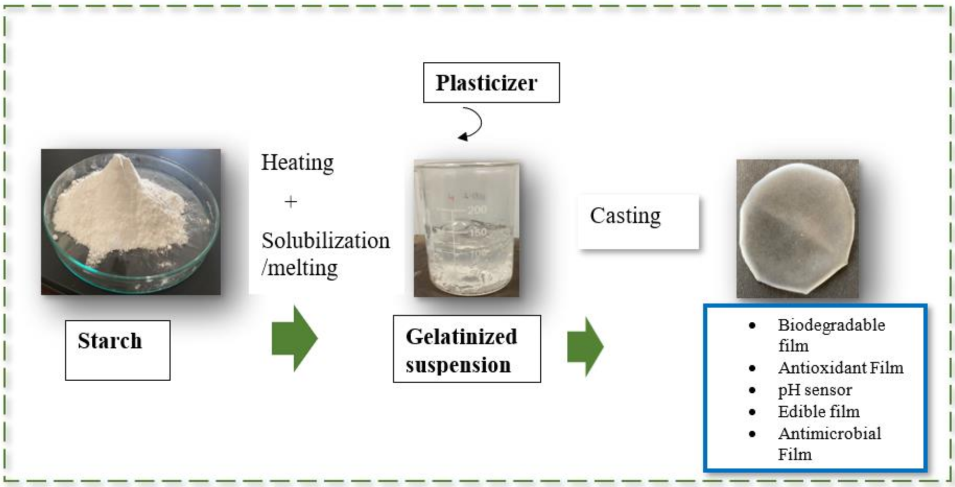

Fig. 2.2 Schematic of starch film formation by Solution Casting (wet process)

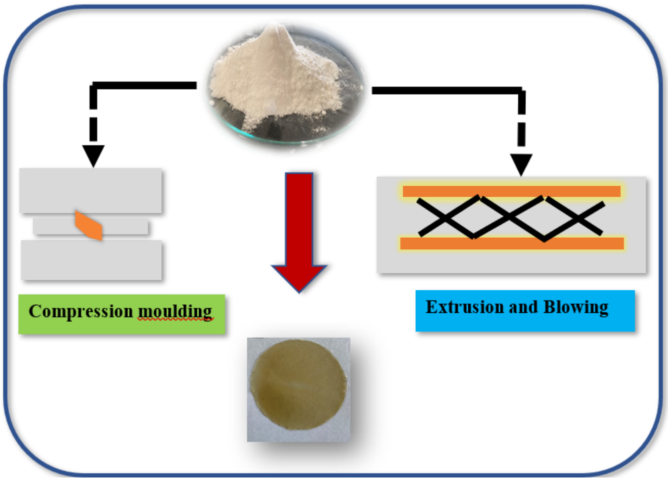

Fig. 2.3 Schematic of Starch film formation by Solution Casting (Dry process) (Versino et al., 2016)

Table 2.1 Films based on various sources of starches: Physicochemical Characterization and Mechanical Properties

S. No	Starch Source	Thickness (mm)	WS (%)	WVP (g mm/h/m^2/kPa)	TS (MPa)	References
1	Corn	0.19 ± 0.02	19.3 ± 0.1	0.40 ± 0.03	4.4 ± 0.3	Nordin et al. (2020)
2	Potato	0.17 ± 0.01	0.24 ± 0.01	0.33 ± 0.007	1.7 ± 0.1	Li et al. (2015)
3	Cassava	0.14 ±0.02	19.9±0.2	0.21±0.01	2.6 ± 0.2	Versino et al. (2014)
4	Wheat	0.22± 0.04	15.7±1.9	0.39 ±0.03	2.3 ± 0.3	Luchese et al (2018)
5	Banana	-	-	1.60 ± 0.01	4.6 ± 0.2	García-Ramón et al. (2021)

WS- Water solubility, WVP- Water vapor permeability, TS- Tensile strength

Table 2.2 Types of commercially available biodegradable packaging made of starch

Product	Source	References
Paragon®	-	Noorian et al. (2022)
Biopar®	Potato Starch	Mary et al. (2022)
Bio-P-TM®	-	Mary et al. (2022)
Bioplast TPS®	-	Mary et al. (2022)
Mater Bi®	Corn Starch	Noorian et al. (2022)
Biome EP	-	Su et al. (2022)
KU Green	Cassava Starch	Su et al. (2022)
Eco-Go	Cassava and Corn starch	Priyadarshi et al. (2019)
PLANTIC™	Corn starch-PE-PP	Priyadarshi et al. (2019)
APACK®	Starch	do Val Siqueira et al (2021)
Solanyl®	Potato Starch	Garcia-Guzman et al. (2022)
BioAgri Mulch Film	Starch-PBAT	Garcia-Guzman et al. (2022)

2.2.1. Starch modification

Starch has rapid retrogradation, weak thermal stability, and poor shear rates, its physical and chemical properties do not allow it to be used in certain types of processing (Chakraborty et al., 2022). Moreover, all starches can undergo syneresis without being influenced by the tendency of pastes to gel (Cui et al., 2021). To enhance the applications of starch in varied industries like paper, food, and textiles, different techniques of modification must be adopted. Starches are commonly modified through physical, chemical, and enzymatic methods to acquire desired industrial applications (Zia-ud-Din et al., 2017). For less susceptible coating materials to water, modifying starch can be a useful tool for adjusting the general properties of starch films. Starch alteration through thermal processes or oxidation under moisture treatment is considered the most functional choice for film production (Fatrozi et al., 2020). Potato starch films treated with heat– moisture increase their tensile strength and moisture permeability compared to native potato. Based on these findings, modified potato starch-based films can create films with a variety of characteristics that can be useful in a variety of applications (Encalada et al., 2018). To enhance mechanical properties and stability of aqueous starch-based products, starch and its derivatives undergo modification using cross-linking agents including phosphor-oxy-chloride, sodium-tri-meta phosphate, and epichlorohydrin (Vu and Lumdubwong, 2020). Oxidized starch finds widespread industrial applications due to its surface coating abilities and reduced permeability. While traditionally dominant in textile and paper industries, oxidized starch is gaining prominence in food applications owing to its advantageous properties including low viscosity, high stability, film-forming capacity, and binding characteristics (Oyom et al., 2022). Physical modification represents a chemical-free approach to altering food products (Kaur et al., 2012). This methodology employs various combinations of temperature, moisture, pressure, shear, and irradiation treatments on native starch granules to enhance water solubility and reduce granule dimensions (Alcázar-Alay & Meireles Meireles, 2015). Hydrothermal modification, a subset of physical modification, induces permanent changes in starch characteristics while preserving the granular structure (Manchun et al., 2012). A key advantage of hydrothermal modifications is their environmental sustainability, as they enhance starch functionality through simple, cost-effective processes without generating harmful waste (da Rosa Zavareze & Dias, 2012). Two primary hydrothermal modification techniques - annealing (ANN) and heat-moisture treatments (HMT) - operate at temperatures between the glass transition temperature (T_g) and gelatinization temperature. Both methods yield biodegradable films with enhanced functionality. The effectiveness of these treatments varies based on starch botanical origin, moisture content, temperature, and duration (Fonseca et al., 2021). ANN involves heating starch granules either with abundant water (below 76% w/w) or intermediate water content (40-55% w/w) at temperatures below the starch melting point. In contrast, HMT employs limited moisture conditions (below 35% w/w) at elevated temperatures (Pratiwi et al., 2018), with processing durations ranging from 15 min to 16 h (Schafranski et al., 2021). Potato starch (PS) develops desirable thermoplastic characteristics suitable for bio-film formation when combined with plasticizers such as glycerol, polyethylene glycol, and sorbitol under conditions of elevated temperature and shear pressure (Stute, 1992). PS serves as an ideal candidate for such studies due to its well-documented response to both hydrothermal treatment methods under various conditions. While numerous studies have examined the temperature effects of HMT on PS, research specifically addressing bio-film production using this technique remains limited.

2.3. Protein as a raw material for biodegradable packaging

Proteins are considered biopolymers, composed of monomeric units (amino acids) that are covalently bonded in chains to form larger molecules. Specifically, proteins are polymers in which the 20 natural amino acids are linked by amide bonds (Sid et al., 2021). The unique structural and functional properties of proteins make them valuable for the development of diverse materials such as films, fibers, foams, gels, and particles (Chen et al., 2019). It possesses advantages such as biocompatibility, biodegradability, processability, and tunable mechanical strength. Some examples of protein-based biopolymers include collagen, gelatin, silk, elastin, soy, wheat gluten, zein, whey protein, casein, and others (Mehetre et al., 2023). Protein film possesses good tensile strength, water vapor permeability, and higher antioxidant activity (Seydim et al., 2006). As a good matrix, protein-based films can be incorporated with antimicrobial and antioxidant agents to enhance the safety, stability, functionality, and shelf-life of food products by releasing or emitting their specific properties (Said and Sarbon, 2019). The process of protein incorporation into the biofilms is shown in **Fig. 2.4**. Protein-based materials demonstrate superior barrier capabilities against gases (including O_2 and CO_2) and volatile substances. Their ability to form three-dimensional macromolecular networks, reinforced by hydrogen bonds, hydrophobic interactions, and disulfide bonds, makes proteins particularly promising for biodegradable film development (Chassenieux and Jyotishkumar, 2013). Film formation is achieved through protein denaturation, which can be induced by heat, solvents, or pH adjustments (Audic et al., 2003). Research by Namratha et al. (2020) demonstrated the successful development of a composite film incorporating casein (a phosphoprotein), alginate, pectin, glycerol, and probiotic *Enterococcus faecium* Rp1, which exhibited significant antimicrobial and antioxidant properties while maintaining stable physicochemical characteristics for approximately 60 days. Casein is frequently utilized as a protein reinforcement agent in food processing applications (Han et al., 2022). Representing 80% of milk protein content, casein comprises α, β, and κ-casein components, which constitute 13%, 36%, 38%, and 10% of the total casein composition respectively (Basumatary et al., 2022). Several characteristics make casein an excellent candidate for biodegradable film production, including its biodegradability, availability, relative insolubility, high thermal stability, non-toxic nature, capacity to bind small molecules and ions, and ability to form micelles (Biswas and Sit, 2020). The coiled structure of caseinates in aqueous solutions, combined with their extensive hydrogen, electrostatic, and hydrophobic bonding capabilities between molecules, facilitates natural film formation by enhancing interchain cohesion. Studies have shown that casein-based films demonstrate reduced water vapor transmission rates, minimal water absorption across various humidity conditions, and enhanced tensile strength. However, their inherent hydrophilicity results in protein-based films being highly moisture-sensitive, leading to poor water vapor barrier properties (Dutta and Sit, 2023). To enhance the mechanical and functional properties of casein-based films for food packaging applications, researchers have explored blending with other biopolymers (Basumatary et al., 2022). The incorporation of additional biomaterials can help mitigate the moisture sensitivity of protein-based biopolymer materials (Kuorwel et al., 2011). Through modification techniques, these materials can be enhanced to compete with conventional polymer components (Bodirlau et al., 2013). The characteristics of protein films can be tailored using various methodologies, including physical, chemical, and enzymatic processes (Tomasula et al., 1995).

It is known that physical treatments exist because of their simplicity, low cost, and environmental friendliness. Ultrasound (US) comprises new sustainable and innovative methods for extracting, and processing, and typically requires less energy, time, and water than conventional methods (Chemat and Khan, 2011). Ultrasound can be characterized as sound waves that are louder than the human ear can hear, i.e., 20 kHz (Monroy et al., 2018). A cavitation bubble forms when sufficient intensity ultrasound waves pass through liquid and cause it to collapse, causing ultrasonic treatment. The cavitation bubbles collapsing creates higher temperatures with pressures for a shorter duration, where associated molecules get affected (Dey and Sit, 2017). Ultrasound modification (US) improves the elements of the film-forming solution along with their distribution, thus improving the film characteristics (Mi et al., 2023). Aside from enzymes and chemicals, heating can modify protein structure physically and has been extensively studied in various ways (Bußler et al., 2015). Protein quaternary structures are dissociated during heating, protein subunits are denaturated, and protein aggregates are formed by hydrophobic, disulfide, and electrostatic linkages (Barać et al., 2005). Moist heat treatment (autoclaving) is a process, where heat is applied with steam under pressure that leads to partial protein denaturation that decreases the degradability of protein (Seifdavati & Taghizadeh, 2012). Autoclave treatment differs as the product is exposed to extremely high temperatures (120℃) for shorter durations (1–240 s). Casein is water-soluble and therefore creates viscous gel, aids in whipping, foaming, and aeration, emulsifies, adds color, flavor, and texture, and provides several nutritional advantages to prepared foods. Understanding the impact of heat treatments on casein's functional properties is essential for optimizing its applications in the food industry, ensuring both nutritional value and desirable textural attributes in various formulations.

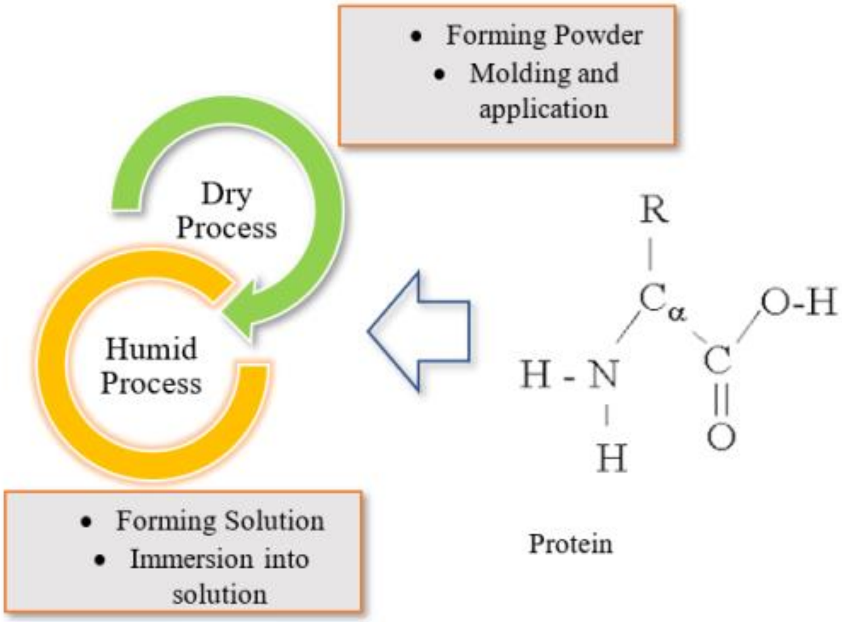

Fig. 2.4 Process of protein incorporation into films (Mohamed et al., 2022

2.4. Composite films

Composite film formation involves combining two or more matrix materials to create a monolayer film, representing an established approach to integrate various materials' beneficial properties and functionalities. Research has demonstrated diverse applications and improved properties through various composite film developments. For instance, pectin-protein composite films show superior mechanical properties and water resistance compared to single-component pectin films, making them suitable for food and pharmaceutical packaging applications (Liu et al., 2007). In sensing applications, Prussian Blue combined with N-substituted polypyrrole creates composite films effective for optical pH measurement within physiological ranges (Koncki and Wolfbeis, 1998). Advanced synthesis methods for composite films like ZnO-SiO_2 and Cu-SiO_2 enable controlled morphology applicable across different material systems (Pandele et al., 2020).

Silk fibroin-graphene oxide composite films with layered structures achieve remarkable mechanical properties, with strength and modulus characteristics approaching or exceeding those of natural nacre (Huang et al., 2013). In biopolymer-based composite films, common components include proteins, polysaccharides, and lipids. Wu et al. (2009) demonstrated the development of bio-based composite films using cellulose, starch, and lignin in ionic liquid environments, achieving notable mechanical, thermal, and gas barrier properties. Their research revealed that component ratios significantly influenced mechanical performance, with the films maintaining excellent properties in both wet and dry conditions due to component synergy.

2.4.1. Key advantages over single-material films

Composite films and coatings represent systems combining multiple biopolymers to achieve enhanced properties. Carbohydrates, proteins, and lipids serve as primary components in these developments. Various combinations have been explored, including protein-protein, carbohydrate-carbohydrate, and protein-carbohydrate for binary systems, while ternary systems incorporate protein-protein-carbohydrate combinations. These systems can be further enhanced with active ingredients, such as antimicrobial compounds, to create functional films and coatings (Dhumal and Sarkar, 2018). The combination of biopolymers (specifically proteins and polysaccharides) has emerged as an effective strategy for enhancing mechanical and water barrier properties through molecular entanglement between protein and polysaccharide molecules. Additionally, incorporating lipids with these hydrocolloids has led to the development of emulsified films (Hassan et al., 2018). The synergistic effect of combining two biopolymers, particularly protein and starch, has been noted to enhance film properties. This improvement is attributed to the formation of a continuous network resulting from strong interactions between the different polymers. Such combinations offer a promising approach to developing films with superior characteristics for various applications (Roy et al., 2024). Researchers have explored the incorporation of various bioactive compounds into protein-based films to enhance their properties. For example, whey and soy protein films have been improved by adding ingredients such as oleogel and antioxidants derived from spent coffee grounds, psyllium husk, and red grape extract (Cinnamea et al., 2016; Sukhija et al., 2016; Papadaki et al., 2023). These additions have led to enhancements in antioxidant activity, water resistance, and visual characteristics. Furthermore, studies have focused on creating composite films by combining proteins with polysaccharides. Integrating materials like chitosan, starch, and cellulose nanocrystals with different protein sources - including quinoa, whey, and faba bean proteins - has resulted in improvements to the films' mechanical strength, microstructure, and barrier properties (Sukhija et al., 2016; Robeldo et al., 2018; Rojas-Lema et al., 2021). Research conducted by Seiwert et al. (2021) demonstrated that incorporating xylan into whey protein isolate films led to substantial enhancements in both water vapor and oxygen barrier properties. Furthermore, the observed increase in melting temperatures of these composite films suggested a higher degree of compatibility between whey protein isolate and xylan within the film matrix. Comparable advancements were also noted in composite films composed of casein and egg albumen when pectin was introduced. These improvements in various properties highlight the potential benefits of combining different biopolymers in film formulations (Sood et al., 2022).

2.4.2. Materials for composite films

Research by Ochoa-Yepes et al. (2019) demonstrated the successful development of composite films combining lentil protein and starch through casting and extrusion/thermo-compression techniques. Their findings showed that protein incorporation significantly enhanced mechanical performance, with Young's modulus increasing more than fivefold from 4.1 to 22 MPa, while stress at break slightly decreased from 2.8 to 2.4 MPa. Additionally, the films exhibited improved water resistance, as evidenced by a reduction in water vapor permeability from 2.8×10^{-10} to 1.4×10^{-10} g m^{-1} s^{-1} Pa^{-1}. An innovative study examined a new formulation that combined corn starch with chitosan and incorporated the copolymer pluronic F127. The composite film showed notable improvements in both mechanical and barrier properties. Specifically, tensile strength increased from 4.2 to 6.5 MPa, while water vapor permeability showed remarkable improvement, decreasing from 21×10^{-11} to 3×10^{-14} g m^{-1} s^{-1} Pa^{-1}. These enhancements were attributed to increased matrix hydrophobicity resulting from the pluronic F127 addition (Fonseca et al., 222). Chen et al. (2020) utilized a bench-casting technique to create a zein-gelatin composite film with controlled phase separation characteristics. Through careful manipulation of the zein-to-gelatin ratio, the researchers were able to study various patterns of phase separation between these biopolymers. The resulting structure's unique properties enabled it to simultaneously incorporate both hydrophobic essential oils and hydrophilic tea polyphenols (TP), facilitated by the distinct separation between the zein and gelatin matrices within the film. Further advancing composite film development, Sood and Saini (2022) created an innovative biopolymer film combining red pomelo peel pectin with casein and egg albumin in specific ratios (50:50:0 and 50:25:25; red pomelo peel pectin:casein:egg albumin). Their research demonstrated that pectin incorporation led to enhanced structural properties, resulting in a film with improved smoothness and structural compactness.

2.5. Natural fibers as reinforcement in packaging material

The use of natural fiber as a filler for biopolymers has attracted attention as a greener alternative for material development (Salehudin et al., 2013). Natural fibers are described as composite materials because they are reinforced with hard and crystalline cellulose microfibrils embedded in an amorphous hemicellulose/lignin matrix (Kanatt (2020). Natural fibers can serve as a favorable substitute for synthetic fibers when reinforced with polymer composite materials (Arafat et al., 2021). Natural fiber reinforcement provides numerous benefits including biodegradability, environmental sustainability, widespread availability, renewability, and low mass density, among others (Thakur et al., 2014). Various natural fibers like jute, banana, flax, bamboo shoots, and corn can be incorporated into biopolymer matrices to enhance their performance characteristics (Araujo-Farro et al., 2010). These natural fibers can undergo various treatments including water-repellent applications, coupling agent modifications, surface treatments (both chemical and physical), and heat processing to prepare films (Thakur et al., 2013). The resulting films exhibit enhanced rigidity, longevity, and surface luster while maintaining strength properties comparable to synthetic polymer alternatives (Kostag et al., 2021). Several manufacturing approaches exist for creating natural fiber-reinforced composites, including hand layup, compression molding, vacuum-assisted resin transfer molding, injection molding, and pultrusion techniques (Thakur et al., 2014). While natural fibers show promise in replacing synthetic alternatives with enhanced properties, a significant challenge lies in the weak interfacial adhesion between the fiber and polymer matrix components (Kostag et al., 2021). This limitation can be addressed through appropriate treatment methods that enhance fiber-matrix adhesion and improve mechanical properties. Current research efforts focus on developing surface modification techniques to boost natural fiber compatibility with polymer matrices, with the goal of enhancing green composite performance for sustainable applications (Shi et al., 2021). Research by Sukyai et al. (2018) demonstrated that incorporating sugarcane bagasse-derived cellulose nanocrystals into whey protein food packaging films led to a 100% increase in strength and 200% increase in stiffness at 8% CNC concentration, though flexibility decreased. These improvements were attributed to the reinforcing effects of CNC and hydrogen bond formation between CNC and whey protein. A common example of natural polymer composites is cellulose-reinforced starch, though starch-based materials remain moisture-sensitive. Natural fibers serve as effective reinforcing agents for improving the properties of thermoplastic starch films (Ogunsona et al., 2018). A study by Onyeaka et al. (2022) found that adding carboxymethyl cellulose improved the moisture resistance of composite films. Specifically, when 15% carboxymethyl cellulose was incorporated, the researchers observed decreases in water vapor permeability, moisture absorption, and solubility of the films. Travalini et al. (2019) demonstrated that the introduction of cassava fibers into corn starch resulted in an enhancement of film strength by as much as 37.5%. This improvement was credited to the effective intermolecular interaction between the starch and the reinforcing component. In light of these findings, it is evident that incorporating natural materials like cellulose, lignin, creatine, and sugarcane fibers into starch-based films and composites offers a promising avenue for enhancing their chemical and physical properties, paving the way for improved bio-composite products with potential applications across various industries (Diyana et al., 2021; Liu et al., 2022).

2.6. Active packaging systems

The shift toward biodegradable packaging materials is driven by growing environmental and health considerations, moving away from synthetic alternatives. Biodegradable packaging frequently utilizes naturally-derived biopolymers, including proteins, lipids, and polysaccharides as base materials. Active food packaging technology aims to extend food product shelf life while maintaining both nutritional content and sensory qualities. These sustainable biopolymer-based materials present environmentally conscious and cost-effective alternatives to traditional plastic packaging solutions (Lee et al., 2015). There is significant demand for developing active packaging systems incorporating antimicrobial properties using Generally Recognized as Safe (GRAS) components, particularly from sustainable and economical sources, to enhance their protective capabilities (Tonyali et al., 2020). In the context of viral pandemics, developing packaging with antiviral properties has become essential. Research has shown that various natural and plant-derived substances, including green tea extract, grapefruit extract, *Hibiscus sabdariffa* extract, aloe vera, cinnamaldehyde, carvacrol, thymol, zataria, and oregano essential oils, demonstrate effective antiviral properties with potential inhibitory effects on the SARS-CoV-2 viral spike protein (Carpena et al., 2021). Incorporating natural active compounds into food packaging represents the most promising strategy for controlling microbial growth. Plant extracts are particularly valuable for biodegradable food packaging applications due to their natural origin and phytochemical properties, enabling the development of active materials that extend shelf life and enhance product value (Mir et al., 2018). Natural compounds like thymol, eugenol, and cinnamaldehyde, found in thyme, clove, and cinnamon essential oils respectively, exhibit strong antimicrobial properties due to their phenolic molecular structure. These antimicrobial and antioxidant compounds show promise when integrated into films and coatings for active food packaging applications (Carpena et al., 2021). Edible/biodegradable materials can be applied directly to food surfaces through spraying, dipping, or panning methods (Asgher et al., 2020). While recycled plastics pose contamination risks, avoiding their use leads to significant waste accumulation and environmental damage. This highlights the importance of investing in biodegradable polymers for active bio-based packaging, which addresses both food preservation and waste reduction objectives (Mir et al., 2018). Enhanced protection can be achieved by incorporating antibacterial and antioxidant components into edible and biodegradable films and coatings. This packaging approach extends food shelf life by either preventing degradation or identifying chemical and biological deterioration factors that affect the package's internal environment (Xu et al., 2023). Active packaging systems typically incorporate various components including moisture, ethylene, oxygen, and carbon dioxide scavengers, along with antioxidants and antibacterial agents presented in **Fig. 2.5**. Some of the new features of active packaging systems include gas scraping (O_2, CO_2, and ethylene), regulating moisture levels, along with preserving flavors, among others (Ghosal, 2018). Some method of active packaging that are commercially available along with their applications is shown in **Table 2.4**. It depicted examples of active packaging systems such as oxygen scavengers, moisture absorbers, antimicrobial agents, ethylene scavengers, or flavor/odor absorbers with their applications. Each system serves a unique purpose in preserving the quality and safety of packaged goods. Packing films with a variety of antimicrobial and antioxidant compounds provide strong antibacterial and antioxidant properties. For example, a number of research articles discussed the formation of antimicrobial films using various metals and their oxides as nanomaterials, such as Ag, Cu, CuO, TiO_2, and ZnO. As per recent studies, many biodegradable films with antifungal qualities have been developed, extending the shelf life of packaged foods. Similarly, other kinds of antioxidant packaging materials have also been developed utilizing plant extracts, essential oils, and naturally occurring colors such anthocyanins, curcumin, and melanin. **Table 2.5** provides a compilation of biopolymer-based functional films specifically designed for active packaging applications. The table likely includes details such as the types of biopolymers used, the functional properties incorporated into the films, and their intended applications in the field of active packaging.

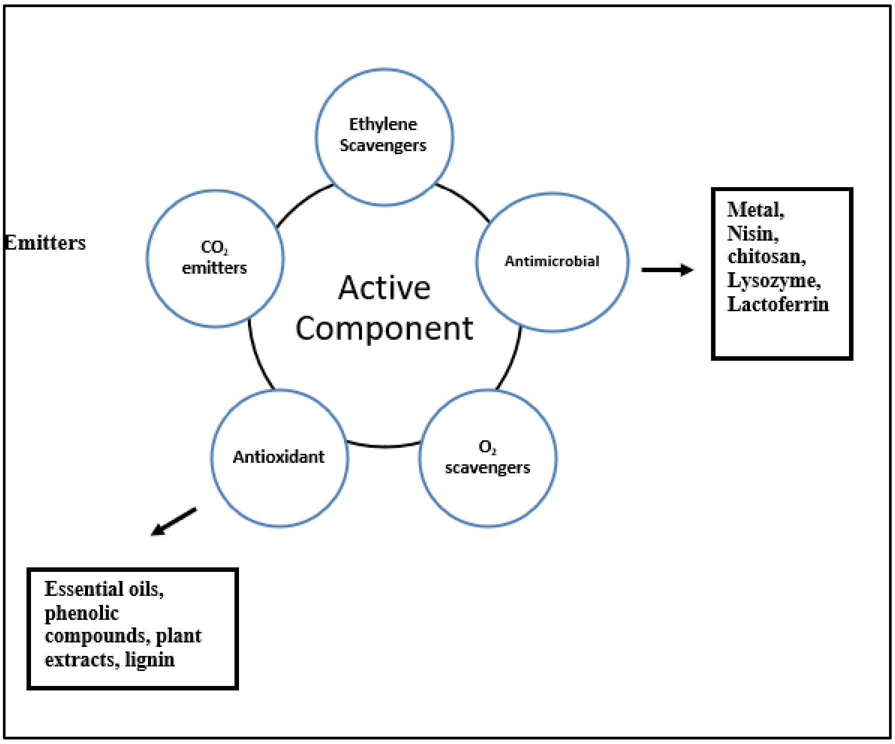

Fig. 2.5 Schematic representation of Active packaging system

Table 2.3 Types of Active Packaging Methods along with their applications

Type	Process	Active Ingredient	Major Applications	Sources
O_2 absorbers	Combination of Iron, metal or acid, catalyst, metallic salts, enzyme-based and nylon	Cucurmin, olive leaf extract	Prepared meat items, Prevent grated cheese and bakery items discoloration	Kumar and Thakur, (2020)
CO_2 absorbers/ emitters	Reinforcement with Fe_2O_3 /CaOH, $C_3Fe_2O_9$ CaO/ NaHCO3	Cinnamaldehyde	Fish and meat items	Anthierens et al. (2011)
C_2H_4 absorbers	KMn04, activated carbon and activated clays	Neem extract	Climacteric Horticulture products	Martín-Belloso et al. (2009)
Antioxidant	Corn-zein-laminated, Chitosan film	Thymol, carvacrol, and eugenol, Green tea extract	Flesh & sea foods, seeds, nuts, oils and fried products, Vacuum-packed snacks	Priyadarshi et al. (2018)
AM packaging	Film development using organic acids, silver zeolite with spices and herbs	Chitosan matrix, plant essential oils (cinnamon, oregano, lemongrass)	Raw, processed flesh, smoked fish, seafood, dairy items, fresh cut	Ahmed et al. (2022)

			fruits & veggies, Cereals & grains, bakery items, ready-to-eat foods	
Moisture absorbents	Incorporation of films using Poly Vinyl Acetate, activated clay, minerals and silica gel	resins, waxes or some insoluble proteins	Tomatoes, Mushroom, strawberries, cereals, grains, seeds, fish, and meat items	Yildirim et al. (2018)
Flavor/odor absorbers	Cellulose triacetate, acetylated paper, citric acid, Fe salt/ascorbate and activated carbon/clays	Essential oils		Yildirim et al. (2018)

Table 2.4 Examples of some commercially available active packaging systems and their application **(Ghosal, 2018)**

S. No	Active Components	Applications
1	Moisture absorber/emitters	Cheese ripening; fresh meat purge pads;
2	Oxygen absorber/emitters	MAP; protection against oxidation in meat and pastry products
3	Carbon dioxide absorber/emitters	MAP; antimicrobial
4	Volatile odor absorbers	Quality of fresh fruits
5	Ethylene absorbers and adsorbers	Controlled fruit ripening
6	Antimicrobials	Microbe control
7	Ethanol emitters	Increasing shelf life in fruits and vegetables
8	Bacteriocins	Antimicrobial
9	Organic acids	Antimicrobial
10	Chitosan	Antimicrobial
11	Natural extracts and essential oils	Antioxidant, antimicrobial

Table 2.5 Biopolymer-based functional films for active packaging applications

Film	Active ingredient	Application	References
Carboxymethyl cellulose	Carbon quantum dots (CQD)	Active packaging of lemon fruits	Riahi et al. (2022)
Kafirin	Citral and Quercetin	Controlled atmospheric storage of chicken	Giteru et al. (2017)
Zein and gelatin	Tea polyphenol (TP)	Active packaging of kiwi fruits	Xia et al. (2019)
Zein/gelatin and polyethylene	Oregano essential oil	Active packaging of longan and strawberry	Cai et al. (2022)
Kafirin and glycerol	*Lactobacillus plantarum* CIDCA 8327, and *Kluyveromyces marxianus* CIDCA 8154	Probiotics delivery	Piermaria et al. (2015)
Poly (vinyl alcohol-co- ethylene)	Gallic acid and umbelliferone	Active packaging for microbial protection	Luzi et al. (2018)
Carrageenan	Titanium dioxide nanotube and copper oxide	Active packaging of banana	Ezati et al. (2021)
Polyvinyl alcohol and gelatin	Amaranthus leaf extract (ALE)	Active packaging of fish and chicken	Kanatt (2020)
Zein and chitosan	α-tocopherol	Active packaging of mushrooms	Zhang et al. (2020)
Kafirin	Quercetin	Active packaging of cod filets	Huang et al. (2020)
Sodium alginate and chitosan	Cinnamon essential oil (CEO)	Active packaging for inhibiting mold growth	Zhang et al. (2019)
Carboxymethyl chitosan nanoparticles and sodium alginate	Zinc oxide (ZnO)	Active packaging for inhibiting bacterial growth	Wang et al. (2019)

2.7. Use of natural extracts and (essential oils) EOs in the application of active packaging systems

Biopolymer active packaging is recognized for its weaker mechanical strength with brittleness. Despite their disadvantages, natural-source polymers have gained considerable recognition. Current active food packaging research and developments have focused on the use of bio-based functional packaging materials which are made in part out of active natural compounds (Mulla et al., 2017; Lagos et al., 2019). Given that pathogen contamination is a serious risk to food safety and quality, there has thus been a significant increase in interest in antimicrobial bio-based packaging materials (Krujif et al., 2003). Natural extracts derived from green plant parts and agricultural waste provide ideal candidates for packaging applications, offering both environmental and economic benefits. These natural extracts, which possess antimicrobial, antioxidant, and anti-browning properties, effectively inhibit food spoilage factors. They contain various phenolic compounds and metabolites, including thymol and its nanomaterials, chitosan and derivatives, nisin, cinnamon, rosemary, and essential oils from garlic, clove, oregano, and thyme, as well as carvacrol and enzymes, which function as natural antimicrobial and antioxidant polymers (Suppakul et al., 2003). Essential oils (EOs), extracted from plant materials such as flowers, buds, leaves, stem, bark, and seeds, demonstrate antimicrobial, antioxidant, antitoxigenic, antiparasitic, and insecticidal properties (Roy et al., 2023). These oils contain bioactive compounds like terpenoids and phenolic acids (Tsidimou et al., 1994). When incorporated into packaging materials, they create active biopolymers with advantages including controlled antimicrobial concentrations and diffusion rates (Arafat et al., 2021). Plant-based natural sources offer extensive bioactive properties that warrant further investigation and regulation for direct food application and development of active packaging or biopolymer-based edible films. Beyond their antibacterial and antioxidant properties, EOs demonstrate food compatibility, enhancing both shelf life and flavor profiles (Carpena et al., 2021). Research has shown that rose petal essential oil exhibits antibacterial (Wu et al., 2020), antifungal (Cebi, 2021), and antioxidative properties (Qiu et al., 2022), making it promising for food packaging applications. Recent studies have analyzed antimicrobial activity in EOs from various sources including *Syzygium aromaticum* L., *Foeniculum vulgare* Miller, *Cupressus sempervirens* L., *Lavandula angustifolia*, *Thymus vulgaris* L., *Verbena officinalis* L., *Pinus sylvestris* and *Rosmarinus officinalis*. Common EO components like α-pinene, β-pinene, p-cymene, thymol, carvacrol, borneol, linalool, terpineol-4-ol, 1,8-cineole, α-terpinyl acetate, and camphor demonstrated effective bactericidal activity (0.2 μg/mL at pH 4.0) against E. coli and L. monocytogenes (Wu et al., 2020). Savory spice and herb EOs, particularly oregano, thyme, rosemary, cinnamon, and basil, along with α-tocopherol or β-carotene, show special promise for active food packaging (Carpena et al., 2021). Active packaging incorporates or immobilizes EOs within packaging films rather than directly in food, enabling dynamic food preservation (Almasi et al., 2021). EOs can be incorporated into polymeric materials primarily through extrusion and casting methods. Research supports EOs as effective active components that add value to natural extracts, suggesting their incorporation into biodegradable films could provide innovative solutions for food packaging while enhancing existing properties are listed in **Table 2.6.**

Bio-based films incorporating essential oils (EOs) have shown promising antimicrobial properties for various applications. **Table 2.7** provides a summary of various examples of bio-based films that have been incorporated with essential oils (EOs) to enhance their antimicrobial properties. These examples illustrate how different combinations of natural polymers and essential oils can be utilized to create films that not only offer environmental benefits due to their biodegradability but also provide antimicrobial protection, making them suitable for use in areas such as food packaging and preservation. Cellulose acetate films with oregano and cinnamon EOs demonstrated effectiveness against *E. coli*, *S. aureus*, and *Penicillium sp*. (Santos et al., 2016). Chitosan-based films containing thyme, clove, and cinnamon EOs exhibited antibacterial activity, particularly against gram-positive bacteria (Hosseini et al., 2009). Natural polymers like chitosan, alginates, and cellulose, as well as synthetic biodegradable polymers such as PLA, PVA, and PCL, have been used to create films incorporating EOs for biomedical applications (Cordeiro Borges et al., 2024). These films have shown potential in tissue engineering, wound healing, and as antimicrobial agents. The incorporation of EOs into biobased polymers like cellulose, starch, chitosan, and PLA has demonstrated antibacterial properties against various strains, offering an alternative to traditional antimicrobial agents (Elian et a., 2022).

Table 2.6 Essential oils and extracts from natural sources as antimicrobial agents in Food packaging applications

Active Ingredient	Packaging Application	Food Application	Source
Cinnamon essential oil cinnamaldehyde	Biopolymer like chitosan	Puree	Yildirim et al. (2018)
Carvacrol and thymol	Nanocomposites	Strawberry	Zhu et al. (2014)
Basil leaf essential oil	Fish-protein isolates/ ZnO nanocomposites	Vegetables	Kuorwel et al. (2011)
Extract of *Allium spp*	Biopolymer Matrix	Ready to eat salads	Cha and Chinnan, (2004)
Oregano (*Lippia graveolens*) essential oil	Pectin edible coatings	Tomatoes	Seydim et al. (2020)
Lemon EOs	Chitosan	Antimicrobial films in combination with thyme and cinnamon essential oils	Peng and Li (2014)
Lime EOs	Gum-based edible coating	Eliminating growth of *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* in fresh papaya	Bosquez-Molina et al. (2010)
Citrus reticulata var. tangerine EOs	Nanoemulsions based on chitosan nanoparticles	Preservation of seafoods	Rojas et al. (2007)

Table 2.7 Examples of bio-based films containing essential oils (EOs) with antimicrobial properties

Biopolymer	EO	Food product	Beneficial effect	References
Whey protein isolate	Clove	Cheese	Over 60 days of testing, the positive effects of the physical-chemical features of cheese, *E. coli*, *S. aureus*, and *L. monocytogenes* were reduced.	Ribeiro-Santos et al. (2018)
Starch	Clove and Cinnamon	Cheese	Enhance food preservation quality and anti-microbial impact	Rout et al. (2022)
Cellulose	Cinnamon and oregano	Vegetables	Cellulose acetate films incorporating oregano and cinnamon essential oils exhibit antimicrobial properties against *E. coli*, *S. aureus*, and *Penicillium*.	Santos et al. (2016)

Chitosan	Thyme, clove, and cinnamon	Fruits and vegetables	strongest antimicrobial properties against both gram-positive and gram-negative bacteria.	Hosseini et al. (2009)
Chitosan-pectin-starch	Rosemary and mint	Fish	Increased the antimicrobial activity of the film against pathogenic strains.	Gomez-Estaca et al. (2010)
Corn starch	*Zataria multiflora* Boiss or Mentha pulegium essential oils		Exhibited antimicrobial activity against *E. coli* and *S. aureus.*	Ghasemlou et al. (2013)
Cassava starch-chitosan	Oregano		Effectively inhibited *Bacillus cereus, E. coli, Salmonella enteritidis*, and *Staphylococcus aureus*	Pellisari et al. (2009)

2.8. Biodegradable active packaging systems

As global environmental issues such as greenhouse gas emissions, plastic pollution, and soil degradation become increasingly severe, innovators are rising to tackle these challenges directly. By focusing on sustainable food packaging, it can make a significant impact on these issues through innovative products and commitment to regenerative practices. Biodegradation can be defined as an end-of-life breakdown pathway for the degradation of material enforced via the degradative actions of microorganisms (typically bacteria, algae and fungi). At the end of the biodegradation process, a biodegradable plastic material should be completely degraded into smaller molecules of CO_2 and H_2O (Bhargava et al., 2020). As biopolymeric materials are typically thought to be biodegradable, many scientific articles have mostly focused on studying the influence of components on the processing qualities of the final product. According to ASTM (American Society for Testing Materials), biodegradable means "capable of decomposing into carbon dioxide, methane, water, inorganic compounds, or biomass in which the predominant mechanism is the enzymatic action of microorganisms that can be measured by standard tests in a specified period, reflecting available disposal condition" (Gamage et al., 2022). The environmental challenge posed by plastic waste stems from its extremely slow decomposition rate, leading to soil and water contamination. Replacing conventional packaging materials with biodegradable polymers derived from degradable sources (biopolymers) offers a potential solution to reduce environmental impact. These biodegradable materials can be applied directly to food surfaces through spraying, dipping, or panning techniques (Ahmed et al., 2022). While synthetic polymer materials dominate the packaging industry due to their moldability, cost-effectiveness, printability, and resistance to environmental and mechanical stresses (Bhargava et al., 2020), growing environmental and health concerns are driving a shift toward biodegradable alternatives. Natural biopolymers, including proteins, lipids, and polysaccharides, serve as foundation materials for biodegradable packaging, offering advantages such as recyclability, rapid decomposition, non-toxicity, and environmental compatibility (Zhang et al., 2020). Plant materials and their extracts contain various pigments including anthocyanins, curcumin, betalains, chlorophyll, carotenoids, tannins, as well as compounds like brazilin and quercetin, which can be utilized in developing biodegradable packaging materials (Zhang et al., 2020; Yildriz, 2022). While biodegradable plastics have inherent limitations – (polylactic acid) PLA's brittleness, (polyhydroxy alcohol) PHAs' poor barrier properties, (polyhydroxy butyrate) PHB's inadequate mechanical and thermal characteristics, (Polycaprolactone) PCL's limited hydrophilic properties, starch's weak stabilization and mechanical properties, and cellulose's processing difficulties - these can be addressed by creating blends of multiple biodegradable plastics or incorporating specific performance-enhancing additives. Plant extracts are particularly valuable ingredients for biodegradable food packaging due to their natural origin and phytochemical properties, enabling the development of active materials that extend shelf life and enhance product value (Mir et al., 2018). While recycled plastics pose contamination risks, avoiding their use results in significant waste accumulation and ecosystem damage. This situation underscores the importance of investing in biodegradable polymers for active bio-based packaging, which addresses both food preservation needs and waste reduction objectives.

2.8.1. Biodegradation mechanisms and environmental impact of biopolymeric films

According to ASTM, biodegradable means "capable of decomposing into carbon dioxide, methane, water, inorganic compounds, or biomass in which the predominant mechanism is the enzymatic action of microorganisms that can be measured by standard tests in a specified period, reflecting available disposal condition" (Gamage et al., 2022). The soil-burial test monitors the gradual decomposition of the starch and starch blends in the soil environment for days of incubation. The soil burial method is the most commonly used technique and is a time-consuming approach, but it accurately reflects the real-world conditions of biodegrading materials and provides critical information for the biodegradation process (Ahmed et al., 2022). Besides biodegradability of the starch can be examined through other approaches like in compost environment, aqueous environment, and enzymatic approaches (Gamage et al., 2022). The degradation of composite or blend films in the soil is thought to occur in two stages: (a) water diffuses into the film, causing the film to swell and microbial growth on the film; and (b) enzyme-induced and other secretory degradation, resulting in weight loss and film destruction (Julinová et al., 2020). Biodegradation is dependent not only on the chemistry of the polymer but also on the existence of the biological systems involved in the process. In a study conducted by Julinová et al. (2020) to determine the effect of different fillers on the biodegradation rate of thermoplastic starch in water and soil environments. Biodegradation was evaluated via respirometric assays determining biological oxygen demand in another study by Sen and Das (2018) for evaluating the biodegradability of starch-based antimicrobial film and its effect on soil quality. The biodegradation kept soil pH within the usual tolerance limit of plant growth and increased the organic carbon, total nitrogen, available nitrogen, and water-holding capacity of the soil. According to Su et al. (2022), the hydrophobicity of the film is usually inversely proportional to its biodegradability, whereas food packaging materials require a higher hydrophobicity. Life cycle assessment of starch-based materials for their respective applications gives crucial information regarding the environmental and ecological impact of the materials over synthetic polymers as the environmental impact of starch-based films at different stages can be evaluated such as raw material production, manufacturing, distribution, use and disposal. This analysis allows for the identification of areas where improvements can be made to reduce the environmental footprint of biodegradable-based films. Further studies on the life cycle assessment of biopolymeric materials are thereby needed to be investigated paying attention to the cost of the starch-based film for future production and research. The biodegradation behavior of composite or blended film varies depending on the biodegradation environment.

Fig. 2.6 illustrates the degradation processes of starch-based films in three environments: (A) Soil, where the film undergoes expansion and destruction, (B) Compost, where hydrolysis and oxidation lead to component degradation and film destruction, and (C) Water, where microbial attack in river water results in film breakdown. Each environment influences the degradation process differently, with microbial activity playing a key role in compost and water environments, while physical changes and oxidation dominate in soil conditions.

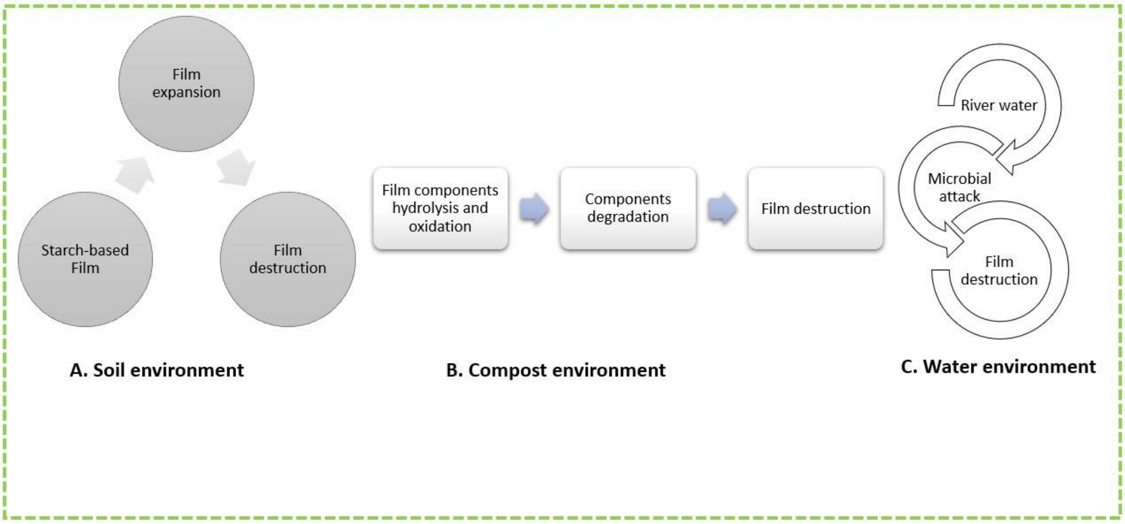

Fig. 2.6 Degradation mechanism of the starch-based composite film in different degradation environments: (A) Soil environment; (B) Compost environment; (C) water environment (Su et al., 2023)

2.9.Safety and toxicity aspect of the active packaging system

While active packaging enhances fresh produce safety and quality, several safety considerations and limitations require attention. Key concerns include the migration of active materials from packaging to produce, sachet leakage into fruits, and potential accidental ingestion of active materials by consumers (Gaikwad et al., 2020). Toxicity considerations are essential when incorporating active or intelligent ingredients into plastic films, with regulations varying by country according to Codex Commission guidelines.

Critical toxicity considerations for active materials include:

1. Mandatory food contact approval before using any active ingredient, with established migration limits (Videira-Quintela et al., 2021).
2. Active materials must not modify food composition or organoleptic properties in ways that could mask spoilage and mislead consumers (Dutta and Sit, 2022).
3. Migration of active substances and their breakdown products requires careful toxicity assessment, as with $KMNO_4$. Released substances must comply with applicable food regulations (Dainelli et al., 2008).

4. Clear labelling requirements to indicate active materials, preventing consumer confusion regarding components like O_2 scavenging sachets (Davidson and Harrison, 2002).
5. Consideration of active packaging's impact on microbial ecology and subsequent food safety implications.
6. Environmental regulations governing usage, reuse, recycling, and identification of packaging materials to ensure proper recycling or energy recovery from active packaging components (Dairi et al., 2019).
7. Thorough investigation of antimicrobial and antioxidant packaging applications across different food products with varying release levels.

Comprehensive consideration of microbial aspects is essential when implementing antimicrobial films and other active packaging techniques (Dainelli et al., 2008). Despite their potential in addressing contemporary packaging challenges, active packaging systems require additional research to enhance effectiveness, ensure regulatory compliance, and improve sustainability for broader commercial implementation.

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